✓ FINAL REPORT

DETERMINATION OF THE RATE AND EFFICIENCY
OF NITROGEN TRANSFER FROM PHYTOPLANKTON TO ZOOPLANKTON
USING NITROGEN-15 AS A TRACER

by

Virginia Harris

NOTICE
This report was prepared as an account of work sponsored by the United States Government. Neither the United States nor the United States Energy Research and Development Administration, nor any of their employees, nor any of their contractors, subcontractors, or their employees, makes any warranty, express or implied, or assumes any legal liability or responsibility for the accuracy, completeness or usefulness of any information, apparatus, product or process disclosed, or represents that its use would not infringe privately owned rights.

FOR WORK ACCOMPLISHED UNDER
ATOMIC ENERGY COMMISSION CONTRACT E(11-1)3222
NITROGEN METABOLISM IN THE SEA

Oswald A. Roels
Principal Investigator
Biological Oceanography Group
LAMONT-DOHERTY GEOLOGICAL OBSERVATORY
OF
COLUMBIA UNIVERSITY
Palisades, New York 10964

NOTICE

PORTIONS OF THIS REPORT ARE ILLEGIBLE. It has been reproduced from the best available copy to permit the broadest possible availability.

MASTER

23 February 1976

427 attached

DISTRIBUTION OF THIS DOCUMENT IS UNLIMITED

DISCLAIMER

This report was prepared as an account of work sponsored by an agency of the United States Government. Neither the United States Government nor any agency Thereof, nor any of their employees, makes any warranty, express or implied, or assumes any legal liability or responsibility for the accuracy, completeness, or usefulness of any information, apparatus, product, or process disclosed, or represents that its use would not infringe privately owned rights. Reference herein to any specific commercial product, process, or service by trade name, trademark, manufacturer, or otherwise does not necessarily constitute or imply its endorsement, recommendation, or favoring by the United States Government or any agency thereof. The views and opinions of authors expressed herein do not necessarily state or reflect those of the United States Government or any agency thereof.

DISCLAIMER

Portions of this document may be illegible in electronic image products. Images are produced from the best available original document.

DETERMINATION OF THE RATE AND EFFICIENCY
OF NITROGEN TRANSFER FROM PHYTOPLANKTON TO ZOOPLANKTON
USING NITROGEN-15 AS A TRACER

by

Virginia Mae Harris

Dissertation Committee:

Professor O. Roger Anderson, Sponsor
Professor Warren E. Yasso

Approved by the Committee on the Degree of Doctor of Education

Date _____

Submitted in partial fulfillment of the
requirements for the Degree of Doctor of Education in
Teachers College, Columbia University

1976

ABSTRACT

The rate of assimilated nitrogen transfer from phytoplankton to zooplankton was measured under field conditions for natural mixed zooplankton populations using a nitrogen-15 tracer technique. The variability in rates, which ranged from 0.261 to 1.792 gram-atoms of phytoplankton nitrogen/gram-atoms of zooplankton nitrogen/24 hours, are thought to reflect a variability in the age frequency distribution of the zooplankton population.

The data were used to calculate assimilation efficiencies which were found to range from 25.4 to 66.1%. The efficiency of 66.1% was taken to be the most accurate measurement for reasons discussed in the text.

ACKNOWLEDGMENTS

I wish to extend special thanks to Dr. Roger Anderson and Dr. Oswald Roels for their guidance and encouragement during the course of this project. I also wish to thank Mr. Julian Hillegas, Dr. Gurdial Sharma and Dr. Gregg Rice for their expertise and willing advice on many matters, and Dr. Calvin Long and Mr. Richard Devine for their technical assistance. In addition, I wish to acknowledge the fellowship support of the Jessie Smith Noyes Foundation and the financial assistance of the Atomic Energy Commission under contract E(11-1)3222.

TABLE OF CONTENTS

1. Introduction, Rationale, and Statement of Objectives.....	1
2. Review of Literature.....	3
3. Experimental Design.....	8
3.1. Preliminary Studies.....	8
3.1.1. Shipboard.....	8
3.1.2. Laboratory.....	9
3.2. Large-Scale <u>In Situ</u> Nitrogen Transfer Experiments (Polytank Experiments).....	11
4. Results.....	27
4.1. Preliminary Nitrogen Transfer Experiments.....	27
4.2. Large-Scale <u>In Situ</u> Nitrogen Transfer Experiments (Polytank Experiments).....	34
5. Discussion.....	46
6. Conclusions.....	49
7. References cited.....	50
8. Appendix.....	52
8.1. Method for Chlorophyll <u>a</u> Analysis.....	53
8.2. Method for Nutrient Sampling.....	55

TABLES

1	Summary of Cruise Dates and Stations.....	14
2	Preliminary Experiments: Kjeldahl Nitrogen and Nitrogen-15 Content of Samples.....	29
3	Preliminary Experiments: Phytoplankton Cell Density Data.....	30
4	Preliminary Experiments: Phytoplankton Cells Removed by Zooplankton during Experiment.....	31
5	Preliminary Experiments: Efficiency of Nitrogen Transfer from Phytoplankton to Zooplankton.....	32
6	Preliminary Experiments: Rate of Assimilated Nitrogen Transfer from Phytoplankton to Zooplankton.....	33
7	Polytank Experiment 1: Kjeldahl Nitrogen and Nitrogen-15 Content of Samples.....	36
8	Polytank Experiment 1: Chlorophyll <u>a</u> , Temperature and Secchi Disc Data for Station and Polytank.....	37
9	Polytank Experiment 1: Inorganic Nutrient Analysis Data for Station and Polytank.....	38
10	Polytank Experiment 2: Kjeldahl Nitrogen and Nitrogen-15 Content of Samples.....	39
11	Polytank Experiment 2: Chlorophyll <u>a</u> , Temperature and Secchi Disc Data for Station and Polytank.....	40
12	Polytank Experiment 2: Inorganic Nutrient Analysis Data for Station and Polytank.....	41
13	Polytank Experiment 3: Kjeldahl Nitrogen and Nitrogen-15 Content of Samples.....	42
14	Polytank Experiment 3: Chlorophyll <u>a</u> , Temperature and Secchi Disc Data for Station and Polytank.....	43
15	Polytank Experiment 3: Inorganic Nutrient Analysis Data for Station and Polytank.....	44
16	Summary of Rates of Assimilated Nitrogen Transfer and Efficiency of Nitrogen Transfer from Phytoplankton to Zooplankton for all Experiments.....	45

FIGURES

1	Location and Names of Four Stations which were Sites of Nitrogen-15 Experiments.....	12
2	360-Gallon Polytank with Drainage Spout and Supporting Metal Framework Used as the Incubation Vessel for the Large- Scale Nitrogen-15 Transfer Experiments.....	16
3	Research Vessel ATLANTIC TWIN Used for Preliminary Experiments and Large-Scale Polytank Experiments 1 and 2.....	18
4	Research Vessel COMMONWEALTH Used for Large-Scale Polytank Experiment 3.....	20
5	360-Gallon Polytank with Drainage Sleeve and Cod-end Sieve Attached during Collection of Zooplankton and Phyto- plankton Samples at Experimental End-point.....	24

1. INTRODUCTION, RATIONALE, AND STATEMENT OF OBJECTIVES

Many measurements have been made of primary production in the marine ecosystem. However, measurements of primary production alone do not enable an assessment of production at higher levels in the food chain. In theory, nitrogen should provide a satisfactory measure of production in ecosystems. Nitrogen is a major structural component of cells and its ratio to carbon and phosphorus is relatively constant. In fact, nitrogen might even be better than carbon or phosphorus as a measure of population growth since the latter are not only structural elements but are continuously turned over in the energy processes of organisms. Nitrogen is also thought to be a limiting factor in production in the sea and this offers an additional reason for the study of its flow through the marine ecosystem.

A number of studies have been done concerning the rate of nitrogen fixation and assimilation by natural phytoplankton populations. However, very little is known about the rates and efficiencies of nitrogen transfer to higher trophic levels in the marine food chain.

This study is concerned with the measurement of the rate and efficiency of nitrogen transfer from the first to the second trophic level, or, from the producer to the primary consumer level. The specific objectives of the study are to measure the rate and efficiency of assimilated nitrogen transfer from phytoplankton to zooplankton in the

natural marine environment, using ^{15}N -labelled phytoplankton as a "tracer" and a large-volume (1 m^3) experimental container as a means of maintaining the natural community during the course of the experiment.

These objectives may be stated more clearly in mathematical form. Thus, the rate of nitrogen transfer from phytoplankton to zooplankton may be expressed as:

Gram-Atoms Phytoplankton N/Gram-Atoms Zooplankton N/24 Hrs

This rate is calculated from the total Kjeldahl nitrogen content of the zooplankton at the end of the experiment and from the ^{15}N -content of the zooplankton at the end of the experiment.

The efficiency of nitrogen transfer (ENT) may be expressed as a percentage:

$$\text{ENT} = \frac{\text{Gram-Atoms of } ^{15}\text{N in Zooplankton}}{\text{Gram-Atoms of } ^{15}\text{N in Phytoplankton Consumed}} \times 100$$

2. REVIEW OF LITERATURE

Qualitative descriptions of the cycles of elements which are important to life are by now largely complete in outline form; however, the details of the quantitative aspects of these cycles are not well known. In the past, this lack in quantitative data has been due to the difficulty of following, or tracing, an individual element within its natural cycle. Studies of the rates of movement of a particular element have depended upon the determination of material-balances, a method which is not applicable in all cases. Today, the isotopes of the elements carbon, hydrogen, nitrogen, sulfur, and oxygen which constitute organic matter provide the necessary "tools" to follow organic molecules directly.

This literature review will be limited to a discussion of past work that has been done concerning one specific area of study in the marine ecosystem, the transfer of matter from the primary producers (phytoplankton) to the primary consumers (herbivores) and to the methods used in these studies.

One of the first definitive laboratory studies on the assimilation of phytoplankton by zooplankton was done by Marshall and Orr (1955a) using the copepod, Calanus finmarchicus. They cultured a number of phytoflagellates and diatoms in media containing radioactive phosphorus and fed the ^{32}P -labelled phytoplankton to the copepod.

After 24 hours of incubation, the copepods, eggs and fecal pellets were removed and measured for radioactivity. It was assumed that the sum of the ^{32}P in these three fractions gave the total ^{32}P ingested. Thus, the quantity of ^{32}P digested was obtained by subtracting the amount in the fecal pellets from the total. The assimilation efficiency, or percentage of the ingested food that is digested and absorbed is obtained from the ratio of ^{32}P digested to the total ^{32}P ingested. Marshall and Orr found that for four species of diatoms tested, the percentage assimilation was in all cases greater than 50% and usually greater than 80%. Likewise, for the phytoflagellates, the assimilation efficiencies were again generally high. It was also found that these percentages were not affected by changes in the concentration of food. It was recognized by the authors that these findings refer only to the phosphorus-containing part of food and may not apply to other dietary constituents. However, these assimilation efficiencies were confirmed in other experiments by Marshall and Orr (1955b), using cultures labelled with ^{14}C instead of ^{32}P . Thus, assimilation efficiency ranged from 53 - 78% in ^{14}C experiments with the flagellate Cryptomonas sp., compared with 51 - 89% in cultures labelled with ^{32}P . Likewise, in experiments with the diatom Skeletonema costatum, the values obtained were 60 - 75%, compared with those of 54.5 - 87.0% for the earlier study.

Data for phosphorus assimilation by the younger stages of C. finmarchicus showed as high assimilation efficiencies as those found for the adults (Marshall and Orr, 1956). With a diet of Ditylum, late naupliar and young copepodite stages assimilated 77.8 - 95.9% of the food; with Syracosphaera, assimilation was higher than 98%; and copepodite stage I, feeding on Skeletonema, assimilated 48.9 - 91.5%. In a similar study, Berner (1962) found assimilation efficiencies of 52.0 - 97.5% for the copepod Temora longicornis (Muller) which was fed ^{32}P -labelled Skeletonema.

Since phosphorus and carbon are major constituents of the algal cell, it was concluded by Marshall and Orr (1955a) that the major part of the organic material ingested by Calanus was assimilated. However, further work showed that the same high assimilation efficiency could not be expected for all dietary constituents. Corner and co-workers (1967) studied the assimilation of nitrogen by C. finmarchicus using Skeletonema as the feed culture. Nitrogenous compounds taken up over a 24 hour period by the copepods were estimated as the difference in levels of particulate nitrogen (determined by Kjeldahl analysis) ^{available to the animals} at the beginning and end of the experiment. The difference between this value and the quantity found as fecal pellets, expressed as a percentage of the total amount removed, gave the assimilation efficiency. The values found were in the range 57.5 - 67.5%

when Skeletonema as fed at concentrations representing 35 - 270 ug particulate N/l. No correlation was found between the level of available food and the proportion assimilated, a finding which was in agreement with those of Marshall and Orr (1955a) in their experiments with 32p.

In the study by Marshall and Orr (1955a) and the study by Corner et al. (1967), a quantitative collection of fecal pellets was done. Conover (1966a) eliminated the need for a quantitative collection of fecal pellets by using a "ratio" method for estimating assimilation efficiency. The method is based on the assumption that only the organic material of food is affected by the process of digestion. Therefore, the percentage assimilation of the organic fraction of food can be calculated simply by obtaining the ratio of ash-free dry weight : dry weight for a sample of the food and a sample of the feces.

This "ratio" method was used by Conover (1966b) to study the assimilation efficiency of C. hyperboreus feeding on Thalassiosira fluviatilis. The average value for assimilation efficiency remained approximately 70% over food concentrations ranging from 100 to 1900 ug C/l. Thus, no correlation was found between assimilation efficiency and either the food level or the quantity of food ingested. In experiments done by Conover using the method at sea, it was found that mixed zooplankton feeding on natural particulate matter assimilated an average of 67% of the

ingested food.

Butler et al. (1970) studied C. finmarchicus under both laboratory and field conditions. From measurements of nitrogen and phosphorus excretion as well as N:P ratios in the animals, phytoplankton, and fecal pellets, it was calculated that even in a high concentration of natural phytoplankton, 77% of ingested phosphorus and 62.5% of ingested nitrogen was assimilated. It should be noted that these values are close to those observed in the laboratory experiments described above.

3. EXPERIMENTAL DESIGN

3.1 PRELIMINARY STUDIES

Three large-scale (1 m^3) in situ experiments were done in December 1972, April 1973, and September 1973 (Poly-tank experiments 1, 2, 3, respectively). However, before this study was attempted on the large-volume scale, three preliminary laboratory-scale studies were done during a cruise to Bermuda in February 1972. These preliminary studies were done to test the feasibility of using a ^{15}N -tracer technique to quantitatively measure the transfer of nitrogen from phytoplankton to zooplankton. The experimental design of the preliminary experiments included a shipboard and a laboratory phase which are described in detail in the following sections.

3.1.1. Shipboard

Zooplankton were collected while on station (Fig. 3, Ta. 1) by means of surface net tows using a double or single 79μ net and $79\text{--}202\mu$ cod-end sieves. One 10-minute single net tow was made for Experiment 1. The zooplankton collected in this tow were transferred to a 20-liter polyethylene carboy, fed 490 ml of ^{15}N -labeled Coccolithus huxleyi at a density of 1.5×10^6 cells/ml, and brought to a volume of 20 liters with 0.33μ glass-fiber filtered sea water. Two 5-minute single-net tows were made for Experiment 2. The zooplankton were transferred as above, fed 480 ml of ^{15}N -labeled C. huxleyi at a density of 8.7×10^5 cells/ml, and brought to a volume of 20 liters. Two five minute double-net tows were made for Experiment 3. The

zooplankton were transferred as before, fed 480 ml of ^{15}N -labelled C. huxleyi at a density of 1.7×10^6 cells/ml, and brought to a volume of 10 liters with filtered sea water.

In each experiment a 10-ml aliquot of the C. huxleyi feeding culture was taken immediately prior to feeding, refrigerated and returned to the laboratory for cell count and frozen on glass-fiber filters for later analysis of total Kjeldahl nitrogen and ^{15}N content. The experimental carboys were kept in reduced light intensity on board ship and delivered to the laboratory within 3 hours of experimental incubation.

3.1.2. Laboratory

Laboratory cultures of Coccolithus huxleyi were labeled with ^{15}N by growing them in f/4 medium (Guillard and Ryther, 1962) containing $\text{Na}^{15}\text{NO}_3$ as explained hereafter. The "percentage of ^{15}N media" refers to that percentage of NaNO_3 in the medium which is $\text{Na}^{15}\text{NO}_3$.

The culture for Experiment 1 was grown in a 60% ^{15}N medium for 12 hours. For Experiment 2, the phytoplankton were grown in a 60% ^{15}N medium for 12 hours and then transferred to an 80% ^{15}N medium in which they grew for 20 hours. For Experiment 3, the phytoplankton were grown in a 60% ^{15}N medium for 12 hours and then transferred to an 80% ^{15}N medium for 43 hours. These cultures were the phytoplankton inoculum for the ^{15}N experiments.

3   Upon return to the laboratory, the experimental carboys were kept in reduced light intensity at room temperature and

aerated constantly. An initial phytoplankton cell count was done and cell density was monitored throughout the period of incubation. Experiments 1, 2, and 3 were terminated after 30, 18, and 6 hours of feeding time, respectively. Phytoplankton cell counts were made at the end of each experiment. The zooplankton were collected by passing the carboy contents through a 79 μ net, resuspending the collected organisms in filtered sea water, and filtering them onto a Gelman glass-fiber filter. The carboy was rinsed three times with a small volume of the carboy filtrate to ensure removal of all zooplankton. The zooplankton samples were frozen for subsequent analysis for total Kjeldahl nitrogen and ^{15}N content. Following removal of the zooplankton, three 1-liter volumes of carboy filtrate were glass-fiber filtered to obtain samples of the ^{15}N -labeled Coccolithus huxleyi which remained in the carboy at the end of the experiment. These three phytoplankton filters were frozen for subsequent analysis for total Kjeldahl nitrogen and ^{15}N content. The three 1-liter filtrates resulting from the phytoplankton filtration were frozen for similar analysis.

Cell counts were made using a Speirs-Levy eosinophil counting chamber and Kjeldahl nitrogens were determined using a standard digestion technique followed by steam distillation of the ammonia into boric acid. An aliquot of the Kjeldahl distillate was used for the determination of total nitrogen on a Technicon AA-II AutoAnalyzer and a second aliquot was used, after isotopic dilution and redistillation into hydrochloric acid, to determine the ^{15}N content by mass spectro-

metry.*

The rate and efficiency of assimilated nitrogen transfer from phytoplankton to zooplankton were calculated from these data.

3.2. LARGE-SCALE IN SITU NITROGEN TRANSFER EXPERIMENTS (Poly-tank experiments)

Following the successful completion of the three preliminary laboratory-scale nitrogen transfer studies, plans were made to scale-up the experimental design to the 1 m³ scale. This larger scale enabled us to make an in situ study of nitrogen transfer with minimal disturbance of the natural populations.

Three large-scale experiments were done: one in December 1972, one in April 1973, and one in September 1973 (Poly-tank experiments 1, 2, 3, respectively). A description of the experimental design of these experiments follows.

EXPERIMENTAL DESIGN

The experiments were done on station on the Continental Shelf off New York (Fig. 1, Ta. 1). The Continental Shelf station was selected because the communities of this region are largely composed of phytoplankton and zooplankton. There were two reports available in the literature (Curl, 1962; Ryther and Yentsch, 1958) concerning the biological background of the location and these two reports were used to estimate the

---

* Mass spectrometric determinations were made courtesy of Dr. Calvin Long at the College of Physicians and Surgeons, Columbia University. The analytical method gives an error of ± 0.0012 mg-at N on a sample containing 0.5 mgN (all samples for mass spectrometric analysis were prepared by isotopic dilution so that they contained 0.5-1.0 mgN).

Figure 1 Location and names of the four stations which
were sites of ^{15}N experiments

"Bermuda"	32°14'N	64°37'W	Preliminary experiments 1,2,3
"Shelf 1"	40°27'N	73°09.5'W	Polytank experiment 1
"Shelf 2"	40°01'N	73°23.2'W	Polytank experiments 2,3

TABLE 1 SUMMARY OF CRUISE DATES AND STATIONS

Experiment	Date	Location	Station Coordinates
Preliminary Expt 1	2/11/72	Sargasso Sea off Bermuda: 9.5 miles east of Gibbs Hill Light (Bermuda) in 2100 meters of water	Lat. 32°14'N Long. 64°37'W
Preliminary Expt 2	2/12/72	Sargasso Sea off Bermuda: 9.5 miles east of Gibbs Hill Light (Bermuda) in 2100 meters of water	Lat. 32°14'N Long. 64°37'W
Preliminary Expt 3	2/13/72	Sargasso Sea off Bermuda: 9.5 miles east of Gibbs Hill Light (Bermuda) in 2100 meters of water	Lat. 32°14'N Long. 64°37'W
Polytank Expt 1	12/12/72- 12/13/72	Continental shelf off New York: 30 miles east of Ambrose Light (N.Y.) in 33 meters of water	Lat. 40°27'N Long. 73°09.5'W
Polytank Expt 2	4/18/73- 4/19/73	Continental shelf off New York: 30 miles southeast of Ambrose Light (N.Y.) in 78 meters of water	Lat. 40°01'N Long. 73°23.2'W
Polytank Expt 3	9/20/73- 9/21/73	Continental shelf off New York: 30 miles southeast of Ambrose Light (N.Y.) in 78 meters of water	Lat. 40°01'N Long. 73°23.2'W

expected phytoplankton and zooplankton standing stocks for the station.

The incubation vessel was specially designed for these experiments and consisted of a 360-gallon polytank with drainage spout and supporting metal framework (Figs. 3,4,5).

The stepwise experimental procedure was as follows:

1. When on station, a sample of the surface water was filtered for nutrient analysis (for method, see Appendix), and the polytank was filled with one cubic meter of seawater containing the natural standing stocks of phytoplankton and zooplankton. A 50-gallon barrel was used to fill the polytank (Figure 4).

2. Immediately after the polytank was filled, a fluorometric chlorophyll a measurement was made (for method, see Appendix), and a 200-ml aliquot was glass-fiber-filtered for nutrient analysis (for method, see Appendix).

3. The ^{15}N -labelled phytoplankton culture, used as the inoculum for the experiment, was grown in the laboratory during the week preceding the experiment. The labelling was accomplished by culturing the phytoplankton in Guillard and Ryther's (1962) f/4 medium which contained ^{15}N -labelled NaNO_3 as the only nitrogen source.

4. While the polytank was being filled, a chlorophyll a measurement was made on the ^{15}N -labelled phytoplankton inoculum culture(s). A 5-ml aliquot of the inoculum culture was preserved in glutaraldehyde for later cell count, and another aliquot of the culture was glass-fiber filtered at 3" Hg and frozen immediately for later Kjeldahl analysis and ^{15}N

Figure 2   360-gallon polytank with drainage spout and supporting metal framework used as the incubation vessel for the large-scale ^{15}N transfer experiments.

50-gallon barrel used in filling polytank is seen at right of polytank.

Figure 3 : Research Vessel ATLANTIC TWIN used
for preliminary experiments and
large-scale Polytank Experiments
1 and 2

Polytank

5

Figure 4 Research Vessel COMMONWEALTH used for
large-scale Polytank Experiment 3

analysis.

5. Based on the chlorophyll a concentrations of the polytank and of the ^{15}N -labelled phytoplankton inoculum culture, a volume of the inoculum culture was measured such that the phytoplankton inoculum represented 33% of the total nitrogen available for grazing.

The selection of the 33% enrichment of the phytoplankton standing stock with the ^{15}N -labelled phytoplankton inoculum culture was based on the need to obtain at least a one atom % label of ^{15}N in the zooplankton fraction to enable analysis with the mass spectrometer.

The nitrogen content of the herbivore population of the station was estimated from the study done by Curl (1962). From the three preliminary experiments, a rate of nitrogen transfer was known and this rate was used to determine the expected nitrogen transfer. It was calculated that one-tenth of the transferred nitrogen had to be ^{15}N , to attain the one atom % label in the zooplankton fraction to enable us to do a mass spectrometric analysis.

Therefore, it was necessary that the phytoplankton standing stock be enriched with ^{15}N -labelled phytoplankton such that at least one-tenth of the material grazed was 100% ^{15}N -labelled.

To allow a margin of safety in the mass spectrometric analysis, it was decided to enrich the phytoplankton standing stock with ^{15}N -labelled phytoplankton such that one-third of the material grazed was 100% ^{15}N -labelled.

6. The phytoplankton inoculum was added to the polytank

and a 24-hr incubation period followed.

7. Immediately after inoculation, a sample of the phytoplankton fraction was filtered for later Kjeldahl and ^{15}N analysis.

8. The polytank was aerated continuously during incubation and water-cooled.

9. After inoculation, twelve hours thereafter, and at the twenty-fourth hour of incubation, three parameters were measured: chlorophyll a, inorganic nutrient analysis, and temperature.

10. After twenty-four hours of incubation, the polytank contents were passed through a cod end sieve to collect the 79-202 μ zooplankton fraction and the 202-1000 μ zooplankton fraction (Figure 6). Each mesh with its sample of particulate nitrogen was rinsed with glass-fiber filtered (Gelman GF/A) seawater to resuspend the sample. The sample was then filtered at 3" Hg onto a Gelman GF/A filter, rinsed with 40 ml of sodium sulfate solution (isotonic with seawater) and with 20 ml of distilled water and immediately frozen. In addition, 12 liters of polytank filtrate containing the phytoplankton fraction was collected by catch samples while the polytank was being sieved and filtered at 3" Hg onto glass-fiber filters (Gelman GF/A). The same rinsing procedure was followed as for the zooplankton samples. The phytoplankton filters and final filtrates were frozen immediately.

All of the filtered samples were analyzed for Kjeldahl

Figure 5   360-gallon polytank with drainage sleeve and cod end sieve attached during collection of zooplankton and phytoplankton samples at experimental end-point.

nitrogen using a standard digestion technique followed by steam distillation of ammonia into boric acid. An aliquot of the Kjeldahl distillate was used for the determination of total nitrogen on a Technicon AA-II AutoAnalyzer and a second aliquot was used, after isotopic dilution and redistillation into hydrochloric acid, to determine the ^{15}N content by mass spectrometry.* All nutrient samples were analyzed for inorganic nutrient content using a Technicon AA-II three channel AutoAnalyzer. Standard manifolds were used for nitrate, nitrite, ammonia, phosphate and silicate determinations. Each method was calibrated and checked for linear response using a series of replicate standards before samples were analyzed, and replicate standards were run once every thirty samples during analysis to maintain accurate calibration. Precision of the analyses of replicate standards was maintained throughout at $\pm 0.25 \mu\text{g at l}^{-1}$ or better for all methods.

From the analyzed data, the rate of assimilated nitrogen transfer and efficiency of nitrogen transfer (both as previously defined herein) from phytoplankton to zooplankton were calculated.

---

* Mass spectrometric determinations were made courtesy of Dr. Calvin Long at the College of Physicians and Surgeons, Columbia University. The analytical method gives an error of $\pm 0.0012 \text{ mg-at N}$ on a sample containing 0.5 mg N (all samples for mass spectrometric analysis were prepared by isotopic dilution so that they contained $0.5\text{--}1.0 \text{ mg N}$).

4. RESULTS

The results for the three preliminary laboratory-scale experiments and the three large-scale in situ polytank experiments will be given in separate sections.

4.1. PRELIMINARY NITROGEN TRANSFER EXPERIMENTS

Tables 2, 3, and 4 contain all observed data in each of the experiments (Experiments 1, 2, and 3 took place on February 11, 12, and 13, 1972, respectively).

The data obtained from the three experiments are summarized in the first three columns of Table 2. The fourth column is used to tabulate the ^{15}N as a percentage of the total nitrogen for each sample taken.

The observed values of phytoplankton densities and volumes are presented in Table 3.

In Table 4, the total number of cells in the carboy at the beginning and end of each experiment is tabulated in the first two rows. In the third row, the difference between these two cell counts (cells consumed) is recorded and the fourth row contains the duration of each experiment.

The efficiency of nitrogen transfer from phytoplankton to zooplankton is calculated according to the procedure outlined in the footnote to Table 5.

Table 6 presents the rate of assimilated nitrogen transfer from the phytoplankton to the zooplankton. This rate was calculated from the rate of ^{15}N uptake by the zooplankton

THIS PAGE
WAS INTENTIONALLY
LEFT BLANK

PRELIMINARY EXPERIMENTS

TABLE 2. KJELDAHL NITROGEN AND NITROGEN-15 CONTENT OF SAMPLES

Preliminary Experiment Sample	Cells/ Filter	Kjeldahl Nitrogen ($\mu\text{g N}$)	^{15}N Content ($\mu\text{g } ^{15}\text{N}$)*	$^{15}\text{N}\%$ of Total Nitrogen**
Phytoplankton Inoculum, Exp. 3 ***	1.7×10^7	12.54	7.01	55.91
Phytoplankton Filter #1, Exp. 1	1.4×10^7	117.85	2.56	2.18
Phytoplankton Filter #2, Exp. 1	1.4×10^7	93.47	1.04	1.12
Phytoplankton Filter #3, Exp. 1	1.4×10^7	102.25	1.12	1.10
Average of three	(1.4×10^7)	(104.52)	(1.57)	(1.51)
Phytoplankton Filter #1, Exp. 2	1.3×10^7	42.35	6.70	15.84
Phytoplankton Filter #2, Exp. 2	1.3×10^7	38.09	7.55	19.82
Phytoplankton Filter #3, Exp. 2	1.3×10^7	32.53	6.91	21.24
Average of three	(1.3×10^7)	(37.65)	(7.05)	(18.74)
Phytoplankton Filter #1, Exp. 3	6.4×10^7	120.29	40.16	33.39
Phytoplankton Filter #2, Exp. 3	6.4×10^7	125.73	47.50	37.78
Phytoplankton Filter #3, Exp. 3	6.4×10^7	117.69	43.67	37.11
Average of three	(6.4×10^7)	(121.23)	(43.77)	(36.10)
Zooplankton Filter, Exp. 1	-	3685.10	20.32	0.55
Zooplankton Filter, Exp. 2	-	3236.56	35.38	1.09
Zooplankton Filter, Exp. 3	-	13247.62	72.79	0.54
Carboy Filtrate, Exp. 1	-	2.7	-	-
Carboy Filtrate, Exp. 2	-	0.5	-	-
Carboy Filtrate, Exp. 3	-	4.1	-	-

* "Carboy Filtrate" samples were not analyzed for ^{15}N content since total N content was below the minimum required for mass spectrometric analysis.

** $^{15}\text{N}\%$ of cellular N = $(^{15}\text{N}/\text{total Kjeldahl N}) \times 100$.

*** We do not have data on "Phytoplankton Inoculum" samples for experiments 1 and 2 as the sample for Exp. 1 was lost in shipment and that for Exp. 2 was lost during Kjeldahl digestion. Nevertheless, the experiments are still valid as all calculations can be based on data obtained for the phytoplankton samples taken at the end of the experiments.

PRELIMINARY EXPERIMENTS
TABLE 3. PHYTOPLANKTON CELL DENSITY DATA

	Preliminary Experiment 1	Preliminary Experiment 2	Preliminary Experiment 3
Density of cells in phytoplankton inoculum culture (cells/ml)	1.5×10^6	8.7×10^5	1.7×10^6
Volume of phytoplankton inoculum culture added to experimental carboy (ml)	490	480	480
Experimental volume in carboy (ml)	20,000	20,000	10,000
Density of phytoplankton cells in carboy at beginning of experiment (cells/ml) *	3.7×10^4	2.1×10^4	8.2×10^4
Density of phytoplankton cells in carboy at end of experiment (cells/ml)	1.4×10^4	1.3×10^4	6.4×10^4

* These densities of Coccolithus huxleyi are higher than the natural density of C. huxleyi found to be 2700 cells/liter at the surface waters in the Sargasso Sea off Bermuda in January 1958 by Hulburt et al. (1960). The selection of C. huxleyi as the phytoplankton species to be fed to the zooplankton was based on our findings in previous years that this is the predominant phytoplankton species in the surface waters at the Bermuda station. Higher than naturally occurring phytoplankton densities were used in the ^{15}N carboy experiments to ensure enough transfer of ^{15}N from the phytoplankton to the zooplankton to enable mass spectrometric analysis.

PRELIMINARY EXPERIMENTS

TABLE 4. PHYTOPLANKTON CELLS REMOVED BY ZOOPLANKTON DURING EXPERIMENT

	Preliminary Experiment 1	Preliminary Experiment 2	Preliminary Experiment 3
Phytoplankton total cell count at beginning of experiment (cells) *	7.4×10^8	4.2×10^8	8.2×10^8
Phytoplankton total cell count at end of experiment (cells)	2.8×10^8	2.6×10^8	6.4×10^8
Phytoplankton total cell count removed by zooplankton (cells) **	4.6×10^8	1.6×10^8	1.8×10^8
Duration of experiment (hours)	33	22	7

* See Table 2 for supplementary data on cell counts.

** Phytoplankton total cell count removed by zooplankton is equal to the phytoplankton total cell count at the beginning of the experiment minus the phytoplankton total cell count at the end of the experiment.

PRELIMINARY EXPERIMENTS

TABLE 5. EFFICIENCY OF NITROGEN TRANSFER FROM PHYTOPLANKTON TO ZOOPLANKTON

	Preliminary Experiment 1	Preliminary Experiment 2	Preliminary Experiment 3
Efficiency of nitrogen transfer *	39.1	40.7	61.8

- *The efficiency of nitrogen transfer for each experiment was obtained by
- (1) Calculating the $\mu\text{g } ^{15}\text{N}$ per phytoplankton cell for each experiment. (The averaged phytoplankton filter data for each experiment given in Table 2 was used for this calculation.)
  - (2) Calculating the $\mu\text{g } ^{15}\text{N}$ removed by the zooplankton during the duration of the experiment by multiplying the $\mu\text{g } ^{15}\text{N}$ per phytoplankton cell (calculated in 1) by the phytoplankton total cell count removed by the zooplankton during the duration of the experiment (Table 4).
  - (3) Taking the ratio of the $\mu\text{g } ^{15}\text{N}$ of the zooplankton sample of each experiment (Table 2) to the $\mu\text{g } ^{15}\text{N}$ removed by the zooplankton during the duration of the experiment (calculated in 2).
  - (4) Expressing this ratio as a percentage.

PRELIMINARY EXPERIMENTS
TABLE 6. RATE OF ASSIMILATED NITROGEN TRANSFER FROM PHYTOPLANKTON TO ZOOPLANKTON

	Preliminary Experiment 1	Preliminary Experiment 2	Preliminary Experiment 3
µg phytoplankton N/µg zoo- plankton N/24 hours.	0.256	0.064	0.052

4.2. LARGE-SCALE IN SITU NITROGEN TRANSFER EXPERIMENTS (Poly-tank experiments)

Tables 7 through 15 contain all observed data in each of the polytank experiments. Tables 7, 8 and 9 refer to Polytank Experiment 1; Tables 10, 11 and 12 to Polytank Experiment 2; and Tables 13, 14 and 15 to Polytank Experiment 3.

The data for Kjeldahl nitrogen and nitrogen-15 content along with the nitrogen-15 percentage of total nitrogen are given in Tables 7, 10 and 13.

The observed values for chlorophyll a, temperature and secchi disc data for the stations and polytank are presented in Tables 8, 11 and 14.

The data for inorganic nutrients for the stations and polytank are given in Tables 9, 12 and 15.

Table 16 presents a summary of the rates of assimilated nitrogen transfer from phytoplankton to zooplankton along with the values for efficiency of nitrogen transfer for all experiments (including the preliminary experiments). The rate of nitrogen transfer was calculated by taking the ratio of the ^{15}N content of the 79-1000 μ zooplankton fraction at the experimental end-point to the total Kjeldahl nitrogen content of the zooplankton (79-1000 μ fraction) after correction had been made for the fact that the phytoplankton available for grazing were less than 100% ^{15}N -labelled. This correction was made to allow comparison among the experimental results. The efficiency of nitrogen transfer from phytoplankton

to zooplankton was calculated by taking the ratio of the ^{15}N content of the 79-1000 μ zooplankton fraction at the end-point of each experiment to the ^{15}N content of the phytoplankton removed by the zooplankton over the 24-hour incubation period and expressing this ratio as a percentage. For Polytank Experiments 1 and 3, the quantity of phytoplankton nitrogen removed by the zooplankton was derived from the decrease in chlorophyll a in the polytank over 24 hours using a conversion ratio of nitrogen:chlorophyll a of 15:1 (Strickland, JDH In: Chemical Oceanography, ed. J.P. Riley and G. Skirrow, 1965, p. 516). For Polytank Experiment 2, the quantity of phytoplankton nitrogen removed by the zooplankton was based on the observed decrease in Kjeldahl nitrogen in the polytank phytoplankton fraction over the 24-hour experimental period.

TABLE 7. POLYTANK EXPERIMENT 1
KJELDAHL NITROGEN AND NITROGEN-15 CONTENT OF SAMPLES

SAMPLE	Cells/Filter	Kjeldahl N ($\mu\text{g N}$)	^{15}N Content ($\mu\text{g } ^{15}\text{N}$)	^{15}N % of Total Nitrogen**
<u>Monochrysis</u> Inoculum	8.50×10^7	33.31	9.54	28.64
<u>Skeletonema</u> Inoculum*	-	-	-	-
Polytank Phytoplankton, 24 hr	-	81.28	5.01	6.16
Polytank Zooplankton, 24 hr 79-1000 μ	-	844.09	17.38	2.06

* Sample lost by accident during Kjeldahl digestion.

** ^{15}N % of total nitrogen = $(^{15}\text{N}/\text{total Kjeldahl N}) \times 100$.

TABLE 8. POLYTANK EXPERIMENT 1
 CHLOROPHYLL a, TEMPERATURE AND SECCHI DISC DATA
 FOR STATION AND POLYTANK

SAMPLE	Chlorophyll <u>a</u> (mg/m ³)	Temperature (°C)	Secchi Disc (m.)
Station, surface water	0.228	9.0	7.9
<u>Monochrysis</u> inoculum culture	40.296		
<u>Skeletonema</u> inoculum culture	29.083		
Polytank, before inoculation	0.228		
Polytank, 2 hr	0.306		
Polytank, 24 hr	0.254		

TABLE 9. POLYTANK EXPERIMENT 1

INORGANIC NUTRIENT ANALYSIS DATA FOR STATION AND POLYTANK

SAMPLE	$\gamma\text{At N/l}$ $\text{NO}_3^- + \text{NO}_2^-$	$\gamma\text{At N/l}$ NO_2^-	$\gamma\text{At Si/l}$ SiO_2	$\gamma\text{At P/l}$ $\text{PO}_4^{=}$	$\gamma\text{AtN/l}$ NH_3
Station, surface water	2.34±0.55	0.62±0.10	1.35±0.60	0.81±0.19	3.06±0.09
Polytank, 2 hr	3.44	0.65	1.95	0.76	1.62
Polytank, 24 hr	3.02	0.70	2.25	0.81	2.23

TABLE 10. POLYTANK EXPERIMENT 2

KJELDAHL NITROGEN AND NITROGEN-15 CONTENT OF SAMPLES

SAMPLE	Cells/Filter	Kjeldahl N ($\mu\text{g N}$)	^{15}N Content ($\mu\text{g } ^{15}\text{N}$)	^{15}N % of Total Nitrogen *
<u>Monochrysis</u> Inoculum	9.84×10^8	2047.90	496.67	24.25
<u>Skeletonema</u> Inoculum	3.02×10^8	1075.62	422.97	39.32
Polytank Phytoplankton, 2 hr	-	956.94	-	-
Polytank Phytoplankton, 24 hr	-	600.38	23.78	3.96
Polytank Zooplankton, 24 hr 79-202 μ	-	9938.03	766.23	7.71
Polytank Zooplankton, 24 hr 202-1000 μ	-	2021.38	82.54	4.08
Polytank Zooplankton, 24 hr Total 79-1000 μ		11959.41	848.77	7.10

* ^{15}N % of total nitrogen = (^{15}N /total Kjeldahl N) x 100

TABLE 11. POLYTANK EXPERIMENT 2
 CHLOROPHYLL a, TEMPERATURE AND SECCHI DISC DATA
 FOR STATION AND POLYTANK

SAMPLE	Chlorophyll <u>a</u> (mg/m ³)	Temperature (°C)	Secchi Disc (m)
Station, surface water	0.113	7.9	11.0
<u>Monochrysis</u> Inoculum culture	67.824	-	
<u>Skeletonema</u> Inoculum culture	15.330	-	
Polytank, before inoculation	0.114	7.9	
Polytank, 2 hr	0.088	7.9	
Polytank, 12 hr	0.083	8.9	
Polytank, 16 hr	0.179	11.9	
Polytank, 20 hr	0.237	13.2	
Polytank, 24 hr	0.417	14.7	

TABLE 12. POLYTANK EXPERIMENT 2
INORGANIC NUTRIENT ANALYSIS DATA FOR STATION AND POLYTANK

SAMPLE	γ At N/l $\text{NO}_3^- + \text{NO}_2^-$	γ AT N/l NO_2^-	γ At Si/l SiO_2	γ At P/l PO_4^{3-}	γ At N/l NH_3
Station, surface water	0.38±0.50	0.14±0.09	2.05±0.82	1.53±0.13	1.04±0.22
Polytank, before inoculation	0.50	0.14	3.08	2.08	0.77
Polytank, 2 hr	1.50	0.27	2.87	1.85	1.65
Polytank, 12 hr	1.13	0.18	12.30*	2.14	1.38
Polytank, 16 hr	1.25	0.14	1.23	1.40	0.88
Polytank, 20 hr	1.00	0.18	2.66	1.53	1.16
Polytank, 24 hr	1.38	0.16	2.87	1.30	0.99

* Contamination suspected

TABLE 13. POLYTANK EXPERIMENT 3
KJELDAHL NITROGEN AND NITROGEN-15 CONTENT OF SAMPLES

SAMPLE	Cells/Filter	Kjeldahl N ($\mu\text{g N}$)	^{15}N Content ($\mu\text{g } ^{15}\text{N}$)	^{15}N % of Total Nitrogen *
<u>Monochrysis</u> Inoculum	1.74×10^9	2007.14	2007.14	100
Polytank Phytoplankton, 24 hr	-	394.46	43.74	11.09
Polytank Zooplankton, 24 hr 79-202 μ	-	2660.54	100.38	3.77
Polytank Zooplankton, 24 hr 202-1000 μ	-	6049.13	151.79	2.50
Polytank Zooplankton, 24 hr TOTAL 79-1000 μ		8709.67	252.17	2.90

* ^{15}N % of total nitrogen = ($^{15}\text{N}/\text{total Kjeldahl N}$) x 100

TABLE 14. POLYTANK EXPERIMENT 3
 CHLOROPHYLL a, TEMPERATURE AND SECCHI DISC DATA
 FOR STATION AND POLYTANK

SAMPLE	Chlorophyll <u>a</u> (mg/m ³)	Temperature (°C)	Secchi Disc (m.)
Station, surface water	0.085	20.0	13.0
<u>Monochrysis</u> inoculum culture	70.332	-	
Polytank, before inoculation	0.085	20.0	
Polytank, 3 hr	0.229	20.0	
Polytank, 14 hr	0.420	17.0	
Polytank, 24 hr	0.171	19.0	

TABLE 15. POLYTANK EXPERIMENT 3
INORGANIC NUTRIENT ANALYSIS DATA FOR STATION AND POLYTANK

SAMPLE	$\gamma\text{At N/l}$ $\text{NO}_3^- + \text{NO}_2^-$	$\gamma\text{At N/l}$ NO_2^-	$\gamma\text{At Si/l}$ SiO_2	$\gamma\text{At P/l}$ $\text{PO}_4^{=}$
Station, surface water	1.44±0.50	0.30±0.10	1.35±0.60	0.48±0.12
Polytank, before inoculation	0.88	0.17	1.50	0.62
Polytank, 3 hr	1.31	0.18	1.80	0.57
Polytank, 14 hr	1.81	0.24	1.50	0.80
Polytank, 24 hr	1.69	0.30	2.40	0.54

TABLE 16. SUMMARY OF RATES OF ASSIMILATED NITROGEN
TRANSFER AND EFFICIENCY OF NITROGEN TRANSFER
FROM PHYTOPLANKTON TO ZOOPLANKTON FOR ALL EXPERIMENTS

EXPERIMENT	Rate of Assimilated Nitrogen Transfer *	Efficiency of Nitrogen Transfer(%)**
Preliminary		
Experiment 1	0.265	39.1
2	0.064	40.7
3	0.052	61.8
Polytank		
Experiment 1	0.334	33.1
2	1.792	66.1
3	0.261	25.4

* μg phytoplankton N/ μg zooplankton N/24 hours

** The ratio of the ^{15}N content of the zooplankton at the end-point of each experiment to the ^{15}N content of the phytoplankton removed by the zooplankton during the duration of the experiment expressed as a percentage.

5. DISCUSSION

A summary of the rates of assimilated nitrogen transfer and efficiency of nitrogen transfer from phytoplankton to zooplankton for all experiments is given in Table 16.

The results for the rates of assimilated nitrogen transfer obtained for the Preliminary and Polytank Experiments are not completely comparable due to the difference in the methods of zooplankton collection used in each experimental situation. In the Preliminary Experiments, zooplankton were collected by means of net tows and it is certain that many of these animals were dead or moribund as a result of the mechanical manipulation during collection. In the Polytank Experiments, zooplankton were collected using a "dipping" barrel (Figure 3) and therefore no physical damage of the zooplankton is suspected.

The lower transfer rates obtained in the Preliminary Experiments are probably due to the presence of a large percentage of dead organisms in the experimental population. These non-feeding animals are included in the total zooplankton nitrogen fraction determined at the experimental end-point and therefore would decrease the calculated transfer rate.

The variability of transfer rates obtained for the Polytank Experiments are thought to reflect differences in the age frequency distribution of the herbivorous population. The rates, 0.334 and 0.261 gram-atoms phytoplankton

nitrogen/gram-atoms zooplankton nitrogen/24 hours, obtained for Polytank Experiments 1 and 3, respectively, were measured during the months of December (Experiment 1) and September (Experiment 3), when it is expected that the zooplankton populations consist largely of adult animals in a metabolic state of nitrogen balance. The rate of 1.792 obtained for Polytank Experiment 2 was measured in April when the zooplankton population is expected to contain many juvenile organisms which would be actively growing and therefore incorporating much greater quantities of nitrogen than the adults.

The assimilation efficiencies for the Preliminary Experiments ranged from 39.1 to 61.8% with an average of 47.2%. For the Polytank Experiments, the efficiencies ranged from 25.4 to 66.1% with an average of 41.5%.

Conover (1966b) found an assimilation efficiency ranging from 17.7 to 80.6% with an average of 67.0% during field studies using mixed zooplankton populations. Butler and co-workers (1970) found an assimilation efficiency of 62.5% for C. finmarchicus under both laboratory and field conditions.

The assimilation efficiencies for the ^{15}N transfer experiments were calculated based on a derived estimate of the quantity of phytoplankton nitrogen removed by the zooplankton during the experimental period for all the experiments except Polytank Experiment 2. In the three Preliminary Experiments, the estimates of phytoplankton nitrogen

removed by the zooplankton was based on cell count data; for Polytank Experiments 1 and 3 it was based on chlorophyll a data. The calculation of assimilation efficiency for Polytank Experiment 2 was based on direct measurements of phytoplankton nitrogen content (using Kjeldahl analysis) at the beginning and end of the experiment and therefore this efficiency is considered to be the most accurate value of assimilation efficiency for these experiments.

The assimilation efficiencies for all of the ^{15}N transfer experiments ranged from 25.4 to 66.1% (average 44.4%) and fell within the range of 17.7 to 80.6% (average 67.0%) found by Conover (1966b). In addition, the assimilation efficiency for Polytank Experiment 2 of 66.1%, which is the most accurate efficiency measurement of the transfer experiments is in close agreement with the average assimilation efficiency of 67.0% measured by Conover (1966b) under field conditions.

6. CONCLUSIONS

The rate of assimilated nitrogen transfer from phytoplankton to zooplankton can be measured under field conditions for natural zooplankton populations using a ^{15}N tracer technique. In three successive experiments done at different times of the year, the transfer rates were found to be 0.334, 1.792, and 0.261 gram-atoms phytoplankton nitrogen/gram-atom zooplankton nitrogen/24 hours. The variability in the transfer rates is thought to reflect a variability in age frequency distribution of the experimental zooplankton populations.

The assimilation efficiencies ranged from 25.4 to 66.1% and were in agreement with assimilation efficiencies for natural zooplankton populations reported in the literature.

7. REFERENCES CITED

- Berner, A. (1962). Feeding and respiration in the copepod Temora longicornis (Muller). J. mar. biol. Ass. U. K. 42: 625-640.
- Butler, E. I., Corner, E. D. S. and Marshall, S. M. (1970). On the nutrition and metabolism of zooplankton. VII. Seasonal survey of nitrogen and phosphorus excretion by Calanus in the Clyde Sea-Area. J. mar. biol. Ass. U. K. 50: 525-560.
- Conover, R. J. (1966a). Assimilation of organic matter by zooplankton. Limnol. Oceanogr. 11: 338-345.
- _____ (1966b). Factors affecting the assimilation of organic matter by zooplankton and the question of superfluous feeding. Limnol. Oceanogr. 11: 346-354.
- Corner, E. D. S. and Newell, B. S. (1967). On the nutrition and metabolism of zooplankton. IV. The forms of nitrogen excreted by Calanus. J. mar. biol. Ass. U. K. 47: 113-120.
- Curl, H., Jr. (1962). Standing crops of carbon, nitrogen, and phosphorus and transfer between trophic levels in continental shelf waters south of New York. Rapp. P-v. Reun. Cons. Perm. Int. Explor. Mer. 153: 183-189.
- Guillard, R. R. L. and Ryther, J. H. (1962). Studies on marine planktonic diatoms. I. Cyclotella nana Hustedt and Detonula confervacea (Cleve) Gran. Can. J. Microbiol. 8: 229-239.
- Marshall, S. M. and Orr, A. P. (1955a). On the biology of Calanus finmarchicus. VIII. Food uptake, assimilation and excretion in adult and stage V Calanus. J. mar. biol. Ass. U. K. 34: 495-529.
- _____ (1955b). The Biology of a Marine Copepod, Calanus finmarchicus (Gunnerus). Edinburgh: Oliver and Boyd.
- _____ (1956). On the biology of Calanus finmarchicus. IX. Feeding and digestion in the young stages. J. mar. biol. Ass. U. K. 35: 587-603.
- Riley, J. P. and Skirrow, G., eds. (1975). Chemical Oceanography, Vol. 1. New York: Academic Press.

Ryther, J. H. and Yentsch, C. S. (1958). Primary production of continental shelf waters off New York. Limnol. Oceanogr. 3: 327-335.

Strickland, J. D. H. and Parsons, T. R. (1972). A Practical Handbook of Seawater Analysis. Ottawa: Fisheries Research Board of Canada.

8. APPENDIX: Methods for chlorophyll a
analysis and nutrient sampling.

8.1. CHLOROPHYLL a METHOD*

PROCEDURE

1. Place a Gelman GF/A filter on millipore filter funnel (use forceps).
2. Add up to 2 ml of 1% Mg_2CO_3 solution (SHAKE WELL FIRST) dropwise to cover entire surface of filter.
3. Filter x ml of culture at 3" Hg.
NOTE: x = 500 ml of surface seawater
= 5 ml of dense culture
4. Fold GF/A filter into a cone (use forceps) and insert into homogenizing tube (use glass rod).
5. Cover filter with 90% Acetone and homogenize (rinse teflon homogenizer to remove sample - use 90% Acetone).
6. Transfer entire contents of homogenizing tube to a 10 ml graduated centrifuge tube.
7. Make up volume in centrifuge tube to 10 ml.
8. Centrifuge for 5 minutes at top speed.
9. Decant supernatant into clean fluorometer cuvette.
10. Read against a 90% Acetone blank in fluorometer (ZERO FLUOROMETER WITH BLANK).
11. Note window and reading.
12. Add 2 drops of 5% HCl to sample cuvette, mix, and read again.

---

* Method based on that outlined in Strickland and Parsons, 1968.

CALCULATION of mg chl a/m³

$$\text{mg chl } \underline{a}/\text{m}^3 = [(\text{window factor}) (R_B - R_A)]/V$$

Where R_B = reading before acid was added

R_A = reading after acid was added

V = volume of sample filtered in liters

8.2 NUTRIENT SAMPLES - METHOD

1. Place a Gelman GF/A filter in filtration funnel.
2. Prime filter by filtering 50 ml of 2x-glass distilled water through filter. Discard this rinse.
3. Filter several small aliquots of sample at 3" Hg, rinse collection flask and discard.
4. Filter about 400 ml of sample water at 3" Hg.
5. Rinse sample bottles with several small aliquots of filtered sample, then transfer about 200 ml of sample to the glass bottle and refrigerate immediately. Transfer about 200 ml of sample to the polyethylene bottle and freeze immediately.