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FINAL REPORT

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NITROGEN METABOLISM IN THE SEA

by

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CONTENTS

FACE	1
INTRODUCTION AND STATEMENT OF OBJECTIVES	3
PRELIMINARY STUDIES	4
LARGE-SCALE IN SITU NITROGEN TRANSFER EXPERIMENTS	5
Experimental Design	6
Results	9
REFERENCES	10

INTRODUCTION AND STATEMENT OF OBJECTIVES

A number of studies have been done concerning the rate of nitrogen 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.

Our study was 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 were 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 can be expressed as:

$$\text{Gram-Atoms Phytoplankton N} / \text{Gram-Atoms Zooplankton N} / 24 \text{ Hrs}$$

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) can be expressed as a percentage:

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

PRELIMINARY STUDIES

Three large-scale (1 m^3) in situ experiments were done in December 1972, April 1973, and September 1973. 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 measure, quantitatively, the transfer of nitrogen from phytoplankton to zooplankton. In these preliminary experiments, zooplankton were collected by means of surface net tows and transferred to a five-gallon carboy containing 20 liters of glass-fiber-filtered (0.33μ mesh) seawater. The carboy was then inoculated with ^{15}N -labelled phytoplankton which were grown in the laboratory in Guillard and Ryther's (1962) f/4 medium, containing ^{15}N -labelled NaNO_3 as the only nitrogen source. An initial phytoplankton cell count was done and cell density was monitored throughout the period of incubation. The inoculated carboy was incubated for a specific number of hours after which the zooplankton fraction and an aliquot of the phytoplankton fraction

and cell-free filtrate were collected.

The phytoplankton and zooplankton fractions were analyzed for Kjeldahl nitrogen content using a standard method, and ^{15}N content was determined by mass spectrometry. The rate and efficiency of nitrogen transfer were calculated from this data. The results of these preliminary studies showed that the rate of nitrogen transfer from phytoplankton to zooplankton (as defined earlier herein) is approximately 0.06, and that the efficiency of nitrogen transfer (as defined earlier herein) is approximately 40%.*

LARGE-SCALE IN SITU NITROGEN

TRANSFER 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 m^3 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. A discussion of the methods and results of these experiments follows:

*For a more detailed account of these preliminary experiments, see Technical Progress Report to AEC (COO-3222-02): 1972, pp. 26-29; 179-184; 203-206.

EXPERIMENTAL DESIGN

The experiments were done on station, approximately 30 miles south of Ambrose Light (New York) on the Continental Shelf.* 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 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.

The stepwise experimental procedure was as follows:

1. When on station, the polytank was filled with one cubic meter of seawater containing the natural standing stocks of phytoplankton and zooplankton.
2. Immediately after the polytank was filled, a fluorometric chlorophyll a measurement was made, a 500-ml aliquot was preserved for cell count and taxonomy, and a 200-ml aliquot was glassfiber-filtered for nutrient analysis.
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 f/4 medium which contained ^{15}N -labelled NaNO_3 as the only nitrogen source.

*See 000-00000000

4. While the polytank was being filled, a chlorophyll a measurement was made in the ^{15}N -labelled phytoplankton inoculum culture. An aliquot of the inoculum culture was preserved for later cell count, and another aliquot of the culture was filtered for later Kjeldahl analysis and ^{15}N 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 (see pp. 4-5), 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. One-half hour after inoculation, every four hours thereafter, and at the twenty-fourth hour of incubation, four parameters were measured: phytoplankton cell count (done on a preserved sample), chlorophyll a, inorganic nutrient analysis, and temperature.

10. After 24 hr, the polytank contents were sieved to collect the 79-202 μ fraction and the 202-1000 μ fraction. A sample of the polytank phytoplankton was filtered and the filtrate was saved.

All of the filtered samples plus filtrates were analyzed for Kjeldahl nitrogen (using a standard method) and for ^{15}N content (using mass spectrometric methods), preserved samples were examined for cell count and taxonomy,

and nutrient samples were analyzed for inorganic nutrient content.

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

RESULTS

Results for the December 1972 experiment indicate that the rate of nitrogen transfer from phytoplankton to zooplankton (as earlier defined herein) in the natural environment is approximately 0.10, and that the efficiency of nitrogen transfer (as earlier defined herein) is approximately 11%.

Analysis of samples for the April 1973 and September 1973 experiments was recently completed and will be presented along with a more detailed account of all preceding experimental data in the doctoral thesis of Virginia Harris. A copy of the doctoral thesis will be sent to the AEC (ERDA) when completed (1975).

REFERENCES

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