PHYSICAL, CHEMICAL, AND BIOLOGICAL OCEANOGRAPHIC OBSERVATIONS OBTAINED ON EXPEDITION SCOPE IN THE EASTERN TROPICAL PACIFIC NOVEMBER - DECEMBER 1956



UNITED STATES DEPARTMENT OF THE INTERIOR FISH AND WILDLIFE SERVICE

EXPLANATORY NOTE

4

The series embodies results of investigations, usually of restricted scope, intended to aid or direct management or utilization practices and as guides for administrative or legislative action. It is issued in limited quantities for official use of Federal, State or cooperating agencies and in processed form for economy and to avoid delay in publication. UNITED STATES DEPARTMENT OF THE INTERIOR, Fred A. Seaton, Secretary Fish and Wildlife Service, Arnie J. Suomela, Commissioner

PHYSICAL, CHEMICAL, AND BIOLOGICAL OCEANOGRAPHIC OBSERVATIONS OBTAINED ON EXPEDITION <u>SCOPE</u> IN THE EASTERN TROPICAL PACIFIC NOVEMBER - DECEMBER 1956

By Robert W. Holmes and other members of the Scripps Cooperative Oceanic Productivity Expedition

> Part 1. Methods and Station Data. Part 2. Scientific Reports.

"This work was financed by the Bureau of Commercial Fisheries under Contract No. 14-19-008-2485, with funds made available under the Act of July 1, 1954 (68 Stat. 376), commonly known as the Saltonstall-Kennedy Act."

Special Scientific Report--Fisheries No. 279

Washington, D. C. November 1958 The Library of Congress has cataloged this publication as follows:

Scripps Cooperative Oceanic Productivity Expedition, 1956. Physical, chemical, and biological oceanographic observations obtained on Expedition SCOPE in the eastern tropical Pacific, November-December 1956, by Robert W. Holmes and other members of the Scripps Cooperative Oceanic Productivity Expedition. Washington, U. S. Dept. of the Interior, Fish and Wildlife Service, 1958.

117 p. map, diagrs., tables. 27 cm. (U. S. Fish and Wildlife Service. Special scientific report : fisheries, no. 279)

Includes bibliographies.

1. Pacific Ocean. 1. Hohnes, Robert W. 11. California. University. Scripps Institution of Oceanography, La Jolla. (Series).

SH11,A335 no. 279 551.466 59-60425

Library of Congress

The Fish and Wildlife Service series, Special Scientific Report--Fisheries, is cataloged as follows:

U. S. Fish and Wildlife Service. Special scientific report: fisheries. no. 1-(Washington, 1949-

no. illus., maps, diagrs. 27 cm.

Supersedes in part the Service's Special scientific report.

1. Fisherles-Research.

SH11.A335

639.2072

59-60217

Library of Congress

[2]

ABSTRACT

This SCOPE report describes the methods employed, lists in tabular form the results obtained, and includes a series of papers which discuss the results of a preliminary analysis of certain of the biological observations which were obtained on a cruise to the Eastern Tropical Pacific accomplished by the University of California, Scripps Institution of Oceanography, under Contract No. 14-19-008-2485 with the Department of Interior, U. S. Fish and Wildlife Service. Scientific personnel, equipment, and financial support for the data analysis have been largely provided by the University of California, Scripps Institution of Oceanography and the Inter-American Tropical Tuna Commission.

Information was obtained on the vertical and horizontal variations in temperature, salinity, dissolved oxygen, inorganic phosphorus, nitrite, alkalinity, pH, chlorophyll "a", primary production, bacterial abundance, and zooplankton standing crop. A nearly continuous record of incident solar radiation was obtained and was accompanied by daily measurements of the attenuation of blue-green light in the ocean. Water samples and fine-mesh net-hauls were collected for the subsequent analysis of phytoplankton abundance and species composition. The distribution of vertebrates was also studied with special emphasis on oceanic bird distribution.

Nine scientific papers which are the result of an analysis of certain of the SCOPE data are included in Part 2 of this report. They are: Possible application of a bacterial bioassay in productivity studies, by William Belser; SCOPE measurements of productivity, chlorophyll "a", and zooplankton volumes, by R. W. Holmes, M. B. Schaefer, and B. M. Shimada; Size fractionation of photosynthesizing phytoplankton, by Robert W. Holmes; Diurnal variation in the photosynthesis of natural phytoplankton populations in artificial light, by Robert W. Holmes and Francis T. Haxo; Attachment of marine bacteria to zooplankton, by Galen E. Jones; Preliminary studies of bacterial growth in relation to dark and light fixation of C1402 during productivity determinations, by G. E. Jones, W. H. Thomas, and F. T. Haxo; The effects of organic and inorganic micronutrients on the assimilation of Cl4 by planktonic communities and on bacterial multiplication in tropical Pacific sea water, by Galen E. Jones and William H. Thomas; The vertebrates of SCOPE, November 7 - December 16, 1956, by Robert Cushman Murphy; The alcohol-soluble and insoluble fractions of the photosynthetically fixed carbon in naturally occurring marine phytoplankton populations, by William H. Thomas.

CONTENTS

Page	Э
PART 1. METHODS AND STATION DATA, by Robert Holmes 1	
Introduction	
Procedure at noon stations	
Procedure at in situ productivity stations	
Procedure between stations	
Continuous observations	
Methods	
Incident solar radiation	
Submarine daylight	
Salinity, temperature, depth	
Surface current by Geomagnetic Electrokinetograph (GEK). 7	
рН 7	
Alkalinity	
Nitrite	
Inorganic phosphorus	
Dissolved oxygen	
Chlorophyll "a"	
Primary production	
Zooplankton standing crop	
Bacteria	
Noon station data \ldots \ldots \ldots \ldots \ldots \ldots \ldots 11	
Observations between noon stations	
GEK observations	

Page

PART 2. SCIENTIFIC REPORTS	53
Possible application of a bacterial bioassay in productivity studies. William Belser	55
SCOPE measurements of productivity, chlorophyll "a", and zooplankton volumes. R. W. Holmes, M. B. Schaefer, and B. M. Shimada	59
Size fractionation of photosynthesizing phytoplankton. Robert W. Holmes	69
Diurnal variation in the photosynthesis of natural phytoplankton populations in artificial light. Robert W. Holmes and Francis T. Haxo	73
Attachment of marine bacteria to zooplankton. Galen E. Jones	77
Preliminary studies of bacterial growth in relation to dark and light fixation of Cl ⁴ O ₂ during productivity determinations. G. E. Jones, W. H. Thomas, and F. T. Haxo	79
The effects of organic and inorganic micronutrients on the assimilation of Cl ⁴ by planktonic communities and on bacterial multiplication in tropical Pacific sea water. Galen E. Jones and William H. Thomas	87
The vertebrates of SCOPE, November 7 - December 16, 1956. Robert Cushman Murphy	101
The alcohol-soluble and insoluble fractions of the photosynthetically fixed carbon in naturally occurring marine phytoplankton populations.	
William H. Thomas	113

PART 1. METHODS AND STATION DATA

By

Robert W. Holmes

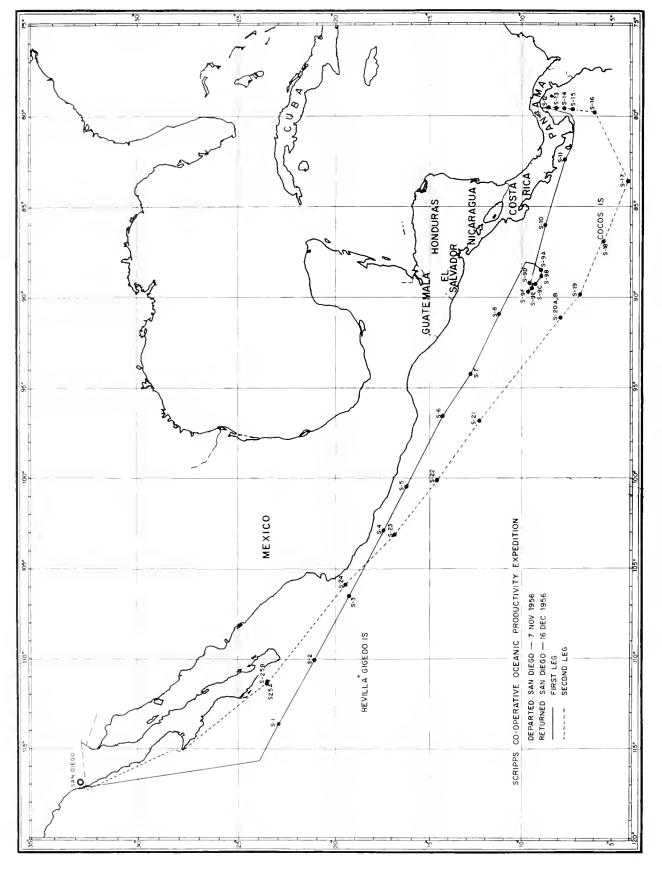


FIGURE 1. Track of the M/V Stranger on SCOPE.

INTRODUCTION

SCOPE (Scripps Cooperative Oceanic Productivity Expedition) was a cooperative biological oceanographic survey of the eastern tropical Pacific conducted during November and December 1956. The expedition was designed to examine regional variations in primary production in tropical areas of interest to the American tuna fisheries and to provide a basis for subsequent studies to explain the biological effects of variations in the oceanic circulation and the influences thereof on the distribution and behavior of the tunas. Participating in this endeavor were scientists from the Scripps Institution of Oceanography, the Inter-American Tropical Tuna Commission, and the American Museum of Natural History. This study was made possible by the establishment of a contract (No. 14-19-008-2485) between the University of California, Scripps Institution of Oceanography and the Department of Interior, U. S. Fish and Wildlife Service. Personnel and additional financial support were provided by the University of California, Scripps Institution of Oceanography and the Inter-American Tropical Tuna Commission.

In addition to fulfilling the objectives listed above, some effort was devoted to a study of the biological methods employed and to a study of certain fundamental biological problems which have a bearing on the productivity of ocean waters. Thus, certain studies on the standing crop and nutritional requirements of bacteria, on the solubility of phytoplankton protoplasm, and on the distribution of organic growth factors were included in the observational program. Studies of this nature will eventually contribute to a better understanding of events at low trophic levels in the food chain and help us understand the interchange and interaction between the chemical environment, the phytoplankton and phytoplankton production.

The expedition departed from San Diego on the M/V Stranger on November 7th, 1956 and returned to San Diego on December 17th, 1956. The track is illustrated in Figure 1.

The following is a list of scientific personnel participating in the expedition:

Robert W. Holmes, expedition leader, Assistant Research Biologist, Scripps Institution of Oceanography, University of California

Dr. William H. Brandhorst, Scientist, Inter-American Tropical Tuna Commission

Dr. Francis T. Haxo, Assistant Professor, Scripps Institution of Oceanography, University of California *

Dr. Galen E. Jones, Assistant Research Biologist, Scripps Institution of Oceanography, University of California

Robert J. Linn, Senior Marine Technician, Scripps Institution of Oceanography, University of California

Dr. Robert C. Murphy, Lamont Curator Emeritus of Birds, American Museum of Natural History

Park Richardson, Laboratory Technician, Scripps Institution of Oceanography, University of California

Dr. Milner B. Schaefer, Director, Inter-American Tropical Tuna Commission

Dr. Bell M. Shimada, Senior Scientist, Inter-American Tropical Tuna Commission

Dr. William H. Thomas, Assistant Research Biologist, Scripps Institution of Oceanography, University of California**

* Panama to San Diego ** San Diego to Panama

Not all of the data and material collected on the expedition have been analyzed. The phytoplankton standing-crop samples have not been examined nor have many of the possible interrelationships between the biological, chemical and physical observations been studied. This work is presently being carried out by Robert W. Holmes. The information on the distribution of oceanic birds is presently being incorporated into a monograph on tropical oceanic birds by Dr. Robert Cushman Murphy.

Certain of the data obtained on the expedition, and included in this report, have been presented at scientific meetings. Drs. G. Jones and W. Belser presented papers at the Detroit, Michigan, meetings of the Society of American Bacteriologists in April-May 1957 which included information obtained on SCOPE.

PROCEDURES AT NOON STATIONS

At approximately local noon of each day, weather permitting, a station was occupied. The general procedure at these stations was as follows:

- 900 ft. BT lowering and general weather observations including barometer reading, dry- and wet-bulb air temperatures, wind direction and speed, sea and swell observations, and sky condition.
- Collection of surface water sample for trailing bottle productivity studies.
- 3. Submarine photometer lowering.
- 4. 50 m. Surface, vertical phytoplankton net haul using a 40-cm. truncate net, with a mesh size of 32µ.
- 5. Plastic sampler cast to 100 m. Water samples collected were used in photosynthetic studies in the shipboard incubator and for the determination of chlorophyll "a" concentrations. A small aliguot from each depth was also preserved for subsequent phytoplankton analysis.
- 6. J-Z sampler cast for bacterial abundance studies.
- 7. Nansen bottle cast to approximately 700 m.-The water samples were employed for oxygen, salinity, alkalinity, inorganic phosphorus, pH, and nitrite determinations.
- Oblique zooplankton meter-net tow to a depth of approximately 300 m.

PROCEDURE AT IN SITU PRODUCTIVITY STATIONS (S-9 SERIES, S-20, S-25A, and S-25B)

Shortly after arrival at these stations a

surface parachute drogue was released and all subsequent observations were taken alongside the drogue.

The sampling program was rather variable but consisted of a series of observations, casts, etc., similar to those taken at each noon station. At the S-9 stations several hydrographic casts were made with the Nansen bottles very closely spaced.

The area in which the S-9 station series were located is referred to in this report as "the Dome" or as the thermal anticline region. This is a large region lying off the west coast of Costa Rica characterized by an intense, shallow thermocline. This is an area of high productivity in which the characteristics of upwelled water are absent.

. PROCEDURES BETWEEN STATIONS

While underway, between noon stations, 900-ft. BT lowerings were made every three hours (0000, 0300, 0600, 0900, 1200, 1500, 1800, and 2100 hours) accompanied by routine weather observations. Surface chlorophyll "a" and inorganic phosphorus determinations were frequently made at 0600 which was also the usual time that the morning trailing bottle productivity experiment began.

CONTINUOUS OBSERVATIONS

- 1. Sea-surface temperature was continuously recorded with a Taylor thermograph.
- Incident solar radiation was measured by a 10-junction Eppley pyrheliometer combined with a Speedomax 0-10 mv recorder.

METHODS

1. Incident solar radiation:

A gimbals-mounted Eppley 10-junction pyrheliometer was placed above all superstructure on the aftermast of the M/V<u>Stranger</u>. The signal from the pyrheliometer was fed into a 0-10 mv Speedomax recorder and was recorded on chart paper travelling at the rate of two in. per hour.

The integration yielding the daily radiation total was performed with a planimeter and day length was computed from the Speedomax trace. The value for the daily total is given, together with other data, in the tables containing the noon station observations.

2. Submarine daylight

Two different filters and submarine photometers were utilized in the measurement of submarine daylight. The transmission characteristics of the two filters (Chance OB-10 and Wratten No. 45) are given in Figure 2. Since the transmission of the Wratten 45 changed somewhat during its use, a third curve is given which was made with this filter immediately after the return of the expedition. The darkening of the 45 filter was assumed to be a gradual process and, as there was no significant shift in the spectral characteristics, all readings made with this filter are believed to be comparable.

Both of the photometers employed were essentially identical, the only important difference being that the collector plate (abraded translucent plastic) in the first instrument (used from Station S-1 to S-4) was elevated above the instrument housing in such a manner that the flat plate collector was not shadowed and had an angle of acceptance of 180° . In the second instrument, used throughout the remainder of the expedition, the angle of acceptance of the collector was somewhat less than 180° owing to the fact that a shoulder on the photometer housing rose a few millimeters above and around the collector plate.

With a single exception reducing screens were not employed. The photometer was lowered in the water until the output of the Photronic cell (Weston 856, Type RR) in the photometer was less than 1000 μ a (usually at about 2 m. depth). The output of a gimbals-mounted deck cell, likewise filtered with a Wratten 45 filter, was noted at the same moment as the output of the submerged cell was recorded. This process was repeated at successive depths until the output of the submarine cell was too low to be measured with the microammeter. Due to fluctuations in ambient light and disturbance caused by waves and swell, readings at various depths were not made until an apparent equilibrium had been reached. In cases where wave action was particularly disturbing and/or fluctuations in ambient light were very marked, simultaneous readings of the output of the deck and underwater cell were repeated and an average of these values was used.

The current generated by the Photronic cell in the photometer was measured with a damped Rawson multimeter (0-50, 0-100, 0-200, 0-500, 0-1000 μ a) which possessed an internal resistance (on all scales) of 100 ohms. The output of the deck cell was measured with a 0-1 milliampere meter (internal resistance: 50 ohms).

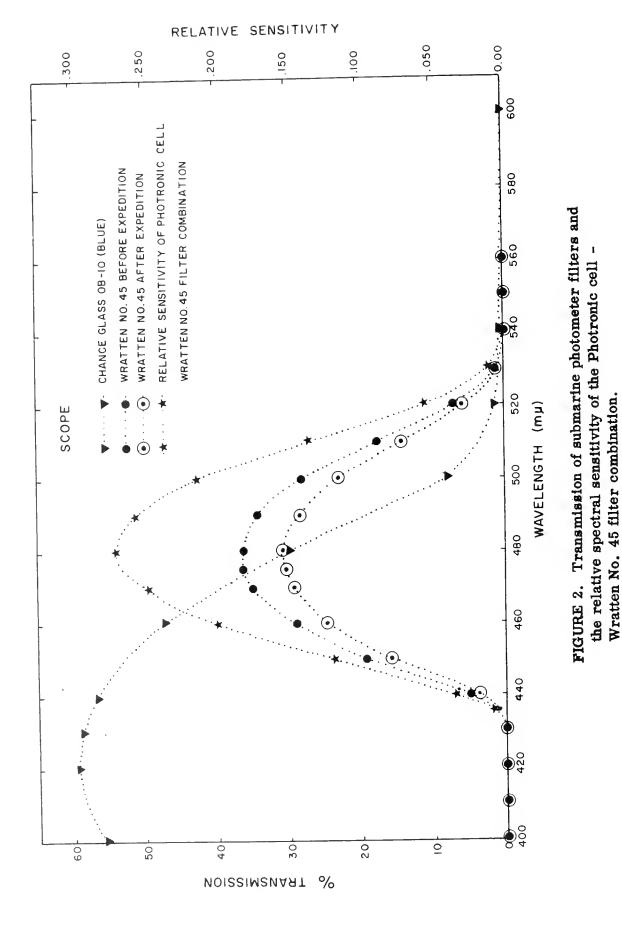
The data presented in the tables have been corrected in the following manner: all of the submarine photometric data (μ a) have been corrected for departures in linearity of response of the photronic cell, and these values in turn adjusted to a constant but arbitrary incident radiation value (deck cell reading). The "true" instrument depth has likewise been computed from wire-angle measurements and length of wire out.

The diffuse attenuation coefficient per unit distance (meter), k, and the percent transmission per unit distance (meter), T, have been calculated for each depth interval using the following formulas:

$$k = \frac{\ln I_{\lambda Z_{1}} - \ln I_{\lambda Z_{2}}}{Z_{2} - Z_{1}}$$
$$T_{\lambda} = e^{-k \lambda}$$

where I is the corrected output of the submerged cell, Z is the depth in meters, and refers to the spectral sensitivity of the Photronic cell-color filter combination employed (see Figure 2).

3. Salinity, temperature, depth



- 6 -

Two or more chlorinity determinations were made with each sample, employing the Knudsen method, and these were converted to salinity.

Temperature was measured with standard reversing thermometers, and the necessary corrections (index, etc.) were carried out at the Scripps Institution of Oceanography to give in situ values. When corrected temperatures of paired protected thermometers differed by more than 0.06° C, both values appear in the tables. The temperatures listed at 0 depth are actually at an average depth of 1.5 m. below the sea surface.

Depths are based on readings of paired (protected and unprotected) reversing thermometers.

Nansen bottle spacing was determined by the thermal structure of the water and an attempt was made to place the bottles at equal-temperature intervals rather than at equal-depth intervals.

Only values at observed depths appear. As it will be some time before all station curves are drawn, it was decided to submit the data in the present form.

.4. Surface current by Geomagnetic Electrokinetograph (GEK)

All measurements were made with neutrally buoyant cable. The conversion from measured electrical potential to surface current was by the formula E = -VH S where E is the measured potential, V the surface current, H the vertical component of the earth's magnetic field, and S the interelectrode distance. No corrections, therefore, have been made for "depth of current," "electrode droop," or "windage on electrodes."

5. pH

The pH of samples was determined with a Beckman G pH meter employing glass and calomel electrodes. The values given are for in situ conditions and are accurate to 0.02 pH units.

Corrections have been made using the tables of Harvey (The Chemistry and Fertility of Sea Waters, Cambridge Univ. Press, 224 pp., 1955).

6. Alkalinity

Alkalinity was determined using the method of Anderson and Robinson (Industrial and Engineering Chemistry, Analytical Edition, Vol. 18, p. 767, 1946). These are reported for atmospheric pressure and are accurate to 0.01 millival/1.

7. Nitrite

Nitrite measurements were made with a Beckman DU Spectrophotometer employing the method of Bendschneider and Robinson (Jour. Mar. Res., Vol. 11, p. 87, 1952). The data are reported in μ gm at/1 NO -N, and the accuracy ranges from 5 to 10°/°.

8. Inorganic phosphorus

Phosphate concentrations were measured using the method of Wooster (Jour. Mar. Res., Vol. 10, pp. 91-100, 1951). Duplicate samples were not analyzed.

9. Dissolved oxygen

Dissolved oxygen measurements were made using the Winkler technique according to the directions of Wooster (Methods in chemical oceanography...employed in the California Cooperative Sardine Research Program. Scripps Inst. Oceanogr., Tech. Rept., 27 pp.).

10. Chlorophyll "a"

The water samples used for the determination of chlorophyll "a" content were collected from the surface with a plastic bucket; subsurface samples were collected with a Van Dorn-type plastic sampler. The water sample, 3.0-6.0 l. in volume, was shaken after the addition of a small amount of magnesium carbonate, and filtered through

a 47-mm. type HA, plain, white Millipore filter. The filter membranes were dried in a vacuum desiccator and then extracted with 3 mls. of 90% acetone (glass redistilled) in the cold (ca. 10°C) and dark for approximately 10-12 hours. The sample was then centrifuged until clear. The supernatant was next decanted into a volumetric flask or cylinder and the remaining precipitate in the tube resuspended with 1-2 mls. of 90% acetone, centrifuged, and the supernatant combined with that obtained previously. Recentrifugation of the combined extracts was frequently necessary to reduce turbidity. This extract was finally diluted to 6 ml., and its optical density was measured in a 10-cm. semimicro-absorption cell at 750, 665, 645, and 630 mµ with a Beckman model DU spectrophotometer. Turbidity corrections were made on the basis of the sample transmission at 750 mµ and the concentrations of chlorophyll "a" have been calculated from the equations of Richards with Thompson (Jour. Mar. Res., Vol. 11, No. 2, pp. 156-172, 1952).

11. Primary production

The C¹⁴ method was employed in these studies to determine the rate of carbon fixation by the phytoplankton. The C¹⁴ solution was prepared and standardized in the manner described by Steemann Nielsen (Jour. du Cons., Vol. 18, No. 2, pp. 117–140, 1952) with the exception that glass redistilled water rather than artificial sea water was used as the solvent. The C¹⁴ solution employed was filtered through an HA Millipore filter and put in 1-ml. glass ampules which were autoclaved. The radioactivity of the samples was measured with an NMC-PC#1 proportional counter.

In situ surface productivity was measured using samples dipped from the sea surface with a plastic bucket at either sunrise or local noon. The samples were placed in clean, well aged, 250-ml. Pyrex bottles inoculated with C^{14} , and trailed astern of the vessel, just under or on the top of the sea surface, until local noon or sunset, respectively. The samples were filtered immediately and placed in a vacuum desiccator for drying. The in situ vertical measurements of productivity were carried out in the following manner. A water sample was collected at each desired depth with the plastic Van Dorn-type sampler shortly before daylight. The samples were transferred to clean, well aged, 250-ml. Pyrex bottles and the C^{14} solution injected with a plastic hypodermic syringe and stainless steel needle. The samples were resuspended at or slightly before dawn, at approximately the depth (t 1 m.) at which they were collected, on a weighted rope supported by a free-floating glass buoy (14 in. in diameter) enclosed in a cord netting and attached to a bamboo pole bearing a flag at its top. The surface sample was attached to the side of the glass buoy, just under the sea surface. The samples were collected at noon, local time, and were promptly filtered and dried for counting.

The samples incubated on shipboard were inoculated with ${\rm C}^{14}$ in the same manner as the in situ and trailing bottle material. The incubator itself was similar to that employed by Steemann Nielsen (Jour. du Cons., Vol. 18, No. 2, pp. 117-140, 1952). Temperature control was achieved by circulating subsurface sea water through the water bath at a rate of 4-6 1. per minute. The temperature in the bath fluctuated somewhat but never exceeded the sea-surface temperature by more than 2.3°C, and usually by less than 1°C. Temperatures less than that of the sea surface were not observed in the incubator. The samples were illuminated by a bank of 10 daylight-type fluorescent lamps. The lamp bank was moveable and was the means employed in keeping the intensity of light at the bottles at 1000 foot-candles.

The data presented in these pages have not been corrected for dark-bottle uptake, the isotope effect, or for phytoplankton respiration. In our experience the darkbottle uptake averages 10-13% of the uptake in the illuminated bottles when the experimental period does not exceed eight hours, although dark uptake may exceed this if the bottles are not washed carefully. This value of $10-13^{\circ}/_{\circ}$, which must be substracted from light-bottle uptake, is nearly equal to the $10^{\circ}/_{\circ}$ positive correction suggested by Steemann Nielsen (1952). The data have not been corrected for phytoplankton respiration losses during the hours of darkness. The total CO₂ concentration of sea water has been assumed to equal 90 mg/l. and all of the productivity calculations have been made using this value.

12. Zooplankton standing crop

Measurements were made of the standing crop of zooplankton by means of plankton net hauls, using gear and techniques comparable to those presently employed by the California Cooperative Oceanic Fisheries Investigations. At each station an oblique tow was made with a one-m. (mouth diameter) plankton net made of 30XXX silk grit gauze in the body and 56XXX silk grit gauze in the rear section and cod-end bag. The net was lowered from the surface to a depth of approximately 300 m. (450 m. wire length) at a rate of 50 m. per minute while the vessel was slowly underway and retrieved at a rate of 20 m. per minute. The duration of a single haul, therefore, was about 32 minutes, on the average. An Atlas flow meter was mounted in the mouth of the net to record the volume of sea water filtered by the net. Flow meters were calibrated before and after the cruise.

Zooplankton collections were preserved in $4^{\circ}/_{\circ}$ buffered formalin. Ashore, the collections were filtered and the total "wet" volumes of plankton obtained at each station were measured by displacement. The volume of water sampled by each haul was determined by a method described by the South Pacific Fishery Investigations of the U. S. Fish and Wildlife Service and the displacement volumes were then converted into terms of the volume of organisms, in cu. cm., collected from each 1000 cu. m. of sea water strained.

 Techniques used in the abundance determination of heterotrophic micro-organisms (bacteria)

Sea-water samples were collected from various

depths in the water column with sterile rubber bulbs attached to J-Z water samplers (ZoBell, Marine Microbiology: A monograph on hydrobacteriology, Chronica Botanica, 1946). The contents of the J-Z samplers were transferred to sterile 200-ml. prescription bottles immediately after arriving at the surface. The bacterial counts were determined by plating 0.1- to 5.0-ml. aliquots of the water samples in duplicate in sterile, plastic, disposable, petri dishes (Falcon Plastics, Culver City, California). The medium had the following composition: peptone (Difco), 5.0 g; yeast extract (Difco), 1.0 g; Fe₃PO₄, trace; agar, 15.0 g; aged sea water (75°/°), 1,000 ml. as defined by Oppenheimer and ZoBell (The growth and variability of sixty-three species of marine bacteria as influenced by hydrostatic pressure, Jour. Mar. Res., Vol. 11, No. 1, pp. 10-18, 1952). The sterile agar medium was cooled to 42°C ± 2°C and was poured into the seeded plates on a table suspended from the ceiling of the lounge (below decks). The suspended table was weighted underneath to provide stability and steadied with the aid of a second person. Such a free-swinging table proved sufficient to compensate for the roll of the ship in calmto-moderate seas. The plates were incubated at 31°C ± 1°C, for three days or longer before reading on a Quebec colony counter. The high temperature of incubation employed is not customary for marine bacteria. This temperature was the lowest possible aboard ship in the tropics without a refrigerated incubator. This temperature was not too high for surface forms since surface seawater temperatures were almost as high.

The results of the bacterial counts taken with the J-Z samples on the return trip from Panama are reported in the noon station data tables.

On the cruise from San Diego to Panama bacterial counts were made from water collected in plastic Van Dorn samplers. This sampler was used since Wood (Heterotrophic bacteria in the marine environment of eastern Australia. Australian Jour. Mar. and Freshwater Res.,

Vol. 4, No. 1, pp. 160-200, 1953) reported nonsterile Nansen bottles produced almost the same counts as sterile samples and since other determinations were made from these same water samples. By the end of the trip to Panama it was evident that the bacterial counts in water collected in the plastic samplers were much higher than those obtained in sterile J-Z samplers. Several direct comparisons were made at the same place, depth, and time and 10^3 to 10^4 more cells were taken from the plastic samplers. The resulting contamination in plastic samplers apparently developed from a bacterial film on the sides of samplers due to their constant use. This was concluded after observing the gradual increase in bacterial numbers after each use. These figures are not presented.

M/V <u>Stranger</u>; SCOPE; November 10, 1956; 2050, 2110¹ GCT; 22°57.0'N, 113° 34.5'W; 1800 fm; wire angle, 0°, 0°; temp., 76.0°F dry, 72.0°F wet; weather, 02; clouds, 6, amt., 8; sea, 2; swell, 330°, 3 ft, 8 sec.

OBSERVED

Depth (m)	т (°С)	S (°/)	0 (m1/1)	$PO_{\mu}-P$ (µgm at/l)	NO ₂ -N (µgm ² at/l)	рH	Alk (millival/l)
0 30 40 55 65 109 138 202 290 389 483 735	24.22 24.16 23.90 17.32 13.61 11.96 11.35 10.68 9.46 8.46 7.34 5.51	34.40 34.39 34.14 33.82 33.31 34.42 34.58 34.58 34.52 34.59 34.49 34.49	4.42 4.39 4.41 3.36 3.31 1.01 0.99 0.37 0.13 0.16 0.10 0.14	0.62 0.56 0.62 1.29 1.39 2.34 2.48 2.96 2.97 3.03 3.18		8.19 8.21 8.09 8.06 7.82 7.82 7.76 7.73 7.70 7.73 7.73 7.73	2.34 2.34 2.33 2.32 2.34 2.34 2.34 2.36 2.37 2.37 2.40 2.41

BIOLOGICAL OBSERVATIONS

Productivity

Dept (m)		Bacteria (no/ml)	<u>in situ</u> (mg C/m ⁹ /day)	incubator mg C/m ³ /hr	B- ²	ט- 3	P- ⁴
0	.125	-	-	-	0	0	0
25	.245	-	-	-	-	-	-
50	•334	-	-	-	-	-	-
100	.108	-	-	-	-	-	-
	Zooplankton Volume	e: 49 ml/10	00 m ³ total, 49	ml/1000 m ³	small.	•	

Incident Radiation Daily Maximum: 0.622 cal/cm²/min. Daily Total: 116 cal/cm². Day Length: 10.32 hrs.

¹All the times given in the station headings are messenger time(s). Biotin: for explanation of symbols see p. 56 , footnote No. 3. Uracil: Purine: (see p.54):

M/V <u>Stranger</u>; SCOPE; November 11, 1956; 2105, 2120 GCT; 21°07.0'N, 110°03.0'W; 1700 fm; wire angle, 3°, 5°; temp., 85.0°F dry, 78.0°F wet; weather, 02; clouds, 2, amt., 2; sea, missing; swell, confused.

OBSERVED

Depth (m)	Temp. (°C)	S (‰)	02 (ml/l)	$PO_{\mu}-P$ ($\mu \text{ gm at/l}$)	NO_2-N (µ gm at/l)	рĦ	Alk (millival/l)
0 15 35 50 65 85	28.12 27.65 27.22 26.17 20.96 17.72	34.62 34.63 34.60 34.51 34.45 34.39	4.49 4.30 4.35 4.27 2.64 1.93	0.48 0.55 1.13 1.50 1.76	0.0 0.0 tr. 0.5 0.1 tr.	8.20 8.20 8.20 8.19 8.04 7.96	2.38 2.36 2.36 2.36 2.35 2.34
118 196 243	13.36 12.26 11.76 11.43	34.11 34.78 34.76	2.08 - -	1.86 2.58 2.55	0.0 0.0 0.0	7.92 7.73 7.77	2.33 2.36 2.36
290 388 482 734	10.66 8.89 7.74 5.52	34.70 34.58 34.52 34.53	0.06 0.25 0.13 0.44	2.66 2.71 2.78 2.70	0.0 0.0	7.73 7.71 7.72 7.73	2.37 2.37 2.37 2.41

BIOLOGICAL OBSERVATIONS

Productivity

Depth (m)	Chlorophyll (mg/m ³)	Bacteria (no/ml)	$\frac{\text{in situ}}{(\text{mg C/m}^3/\text{day})}$	Incubator (mg C/m ³ /hr)	B-	U-	P-
0	.157	-	3.7	0.048	-	-	-
25	.367	-	-	0.072	-	-	-
50	.217	-	-	0.20	-	-	-
100	.231	-	-	0.030	-	-	-

Zooplankton Volume: 37 ml/1000 m³ total, 37 ml/1000 m³ small. Incident Radiation Daily Max: 1.59 cal/cm²/min. Daily Total: 395 cal/cm². Day Length: 10.60 hrs.

Station 2 (Cont.)

SUBMARINE DAYLIGHT (480 mu)

Depth (m)	Corr. Sub. Read. (µa)	k/m	°/oTm
7 12 22 37 72 122	640 530 390 228 48.3 6.6	- .0376 .0307 .0358 .0443 .0408	96.3 97.0 96.5 95.7 96.0

M/V <u>Stranger</u>; SCOPE; November 12, 1956; 2104 GCT; 19°17.0'N, 106°32.0'W; 1600 fm; wire angle, 10°; temp., 82.0°F dry, 77.1°F wet; weather, 02; clouds, 8, amt. 3; sea, 3; swell, confused.

OBSERVED

Depth (m)	Temp. (°C)	S (‰)	02 (m1/1)	Р0 4- Р (µgm at/l)	NO ₂ -N (µgm at/l)	Нq	Alk (millival/l)
0	28.56	34.57	4.47	0.63	-	8.21	-
23	28.20	34.47	4.47	0.48	-	8.22	-
33	27.33	34.40	5.61	0.52	-	8.24	-
47	22.45	34.48	3.84	0.96	-	8.13	-
56	20.22	34.51	2.51	1.39	-	8.02	-
74	14.98	34.55	0.64	2.17	-	7.83	-
137	12.65	34.79	-	2.38	-	7.76	-
188	11.85	34.81	0.21	2.36	-	7.76	-
234	, 11.35	34.74	0.10	2.36	-	7.75	-
	11.07						
279	10.58	34.72	0.07	2.54	-	7.72	-
375	9.31	34.61	0.07	2.62	-	7.72	-
471	7.88	34.54	0.06	2.74	-	7.74	-
721	5.64	34.52	0.07	2.90	-	7.71	-

BIOLOGICAL OBSERVATIONS

Productivity

Depth (m)	Chlorophyll "a" (mg/m ³)	Bacteria (no/ml)	(mg c/m ³ /day)	Incubator (mg C/m ³ /hr.)	B-	U-	P-
0	.204	-	2.1	0.78	0	0	0
25	.246	-	-	0.66	-	-	-
50	.812	-	-	0.22	-	-	-
100	.135	-	-	0.024	-	-	-

Zooplankton Volume: 32 ml/1000 m³ total, 32 ml/1000 m³ small. Incident Radiation Daily Max: 1.38 cal/cm²/min. Daily Total: 451 cal/cm². Day Length: 10.85 hrs.

Station 3 (Cont.)

SUBMARINE DAYLIGHT (480 mm)

Depth (m)	Corr. Sub. Read. (µa)	k/m	% ⊤/m
12 22 32 42 52 62	509 262 106 58.8 19.1 7.4	.0664 .0905 .0589 .1124 .0949	93.6 91.3 94.3 89.4 90.9

M/V <u>Stranger</u>; SCOPE; November 13, 1956; 2017 GCT; 17°27.0'N, 102°53.0'W; 2210 fm; wire angle, 6°; temp., 84.5°F dry, 77.0°F wet; weather, 02; clouds, d, amt., 2; sea, 1; swell, 340°, 3 ft, 10 sec.

OBSERVED

Depth (m)	Temp. (°C)	(°/••)	02 (ml/l)	$PO_4 - P$ (µgm at/1)	NO ₂ -N (µgm at/l)	ЪĦ	Alk (millival/l)
0	29.38	3 4.34	4.33	0.58	-	8.19	-
24	29.02	34.29	4.42	0.45	-	8.19	-
42	25.50	34.40	4.17	0.64	-	8.17	-
52	21.78	34.34	3.56	0.97	-	8.12	_
56	20.14	34.47	2.08	1.37	-	8.00	-
80	15.27	34.67	0.09	2.56	-	7.7 ⁸	-
121	13.17	34.86	0.05	2.46	-	7.79	-
169	12.37	34.87	0.08	2.60	-	7.79	-
241	,11.36	34.80	0.10	2.66	-	7.74	-
	12.14	-					
286	11.00	34.77	0.08	2.84	-	7.75	-
386	9.68	34.70	0.10	2.79	-	7.74	-
480	8.05	34.61	0.10	3.12	-	7.74	-
731	6.01	34.57	0.08	3.29	-	7.68	-
706							

BIOLOGICAL OBSERVATIONS

Productivity

Depth (m)	Chlor op hyll "a" (mg/m ³)	Bacteria (no/ml)	$(\frac{\text{in situ}}{(\text{mg c/m}^3/\text{day})})$	Incubator (mg C/m ³ /hr. <u>)</u>	В-	U-	P-
0	.118	-	4.5	0.13	-	-	-
25	.130	-	-	0.17	-	-	-
50	.588	-	-	0.23	-	-	-
100	.582	-	-	0.11	-	-	-

Zooplankton Volume: 76 ml/1000 m³ total, 54 ml/1000 m³ small. Incident Radiation Daily Maximum: 1.15 cal/cm²/min. Daily Total: 452 cal/cm². Day Length: 10.95 hrs.

M/V <u>Stranger</u>; SCOPE; November 14, 1956; 1804 GCT; 16°15.5'N, 100°28.0'W; 2400 fm; wire angle, 9°; temp., 83.0°F dry, 77.0°F wet; weather, 02; clouds, 6 and 8, amt., 2; sea, 2; swell, confused.

OBSERVED

Depth (m)	Temp. (°C)	S (‰)	02 (ml/l)	PO_4-P (µgm at/l)	NO ₂ -N (µgm at/l)	рĦ	Alk (millival/l)
0 8 15 24 43 52 66 190 285 384 480 728	29.22 29.22 29.18 29.16 26.80 22.19 18.56 12.81 • 12.12 10.89 9.35 7.96 5.91	33.61 33.60 33.61 33.68 34.24 34.45 34.45 34.87 34.83 34.73 34.67 34.62 34.55	4.32 4.34 4.42 4.31 4.27 1.70 0.31 0.19 0.07 0.14 0.13 0.14 0.11	0.46 0.40 0.36 0.37 0.52 1.68 2.02 2.54 2.54 2.54 2.68 2.92 3.22 3.22		8.22 8.25 8.25 8.23 8.20 7.97 7.84 7.82 7.72 7.72 7.72 7.72 7.72 7.72	2.34 2.32 2.34 2.34 2.36 2.36 2.37 2.37 2.37 2.38 2.37 2.39 2.40 2.40

BIOLOGICAL OBSERVATIONS

Productivity

Depth	Chlorophyll	Bacteria	,		B-	U-	P-
(m)	(mg/m^3)	(no/ml)	(mg C/m ³ /day)	Incubator (mg C/m ³ /hr.)			
0	0.213	-	-	0.15	-	-	-
5	-	-	-	0.16	-	-	-
10	-	-	-	0.13	-	~	-
15	-	-	-	0.079	-	-	-
25	0.162	-	-	0.056	-	-	-
40	-	-	-	0.10	-	-	-
50	1.02	-	-	-	-	-	-
75	-	-	-	0.033	-	-	-
100	0.337	-	-	-	-	-	-

Zooplankton Volume: 87 ms/1000 m³ total, 85 ml/1000 m³ small. Incident Radiation Daily Maximum: 1.55 cal/cm²/min. Daily Total: 377 cal/cm². Day Length: 11.00 hrs.

M/V <u>Stranger</u>; SCOPE; November 15, 1956; 2138, 2155 GCT; 14°17.0'N, 96°34.0'W; 1900 fm; wire angle, 30°, 35°; temp., missing; weather, 02; clouds, missing; sea, 2; swell, missing.

OBSERVED

Depth (m)	Temp. (°C)	S (‰)	02 (m1/1)	$PO_{l_{\mu}}-P$ ($\mu \text{ gm at/l}$)	NO ₂ -N (µ gm at/1)	pH	Alk (millival/l)
0 19 27 30 59	27.67 26.68 25.88 24.36 21.78	33.42 33.78 33.95 34.04 34.16	4.55 4.36 3.84 3.40 3.64	0.65 0.65 0.78 1.12 1.36	- - - -	8.25 8.23 8.19 8.15 8.12	- - - -
73 82 108 141 207 276 345 536	21.02 20.86 19.05 13.71 12.54 11.53 10.50 7.75	34.27 34.25 34.48 34.84 34.82 34.79 34.76 3 ¹ .60	3.56 3.46 2.29 0.31 0.24 0.08 0.09 0.07	1.50 1.46 1.77 2.24 2.35 2.24 2.67 2.99		8.09 8.08 8.00 7.95 7.87 7.80 7.77 7.77	

BIOLOGICAL OBSERVATIONS

Productivity

Depth (m)	Chlorophyll "a" (mg/m ³)	Bacteria (no/ml)	<u>in situ</u> (mg C/m ³ /day)	Incubator (mg C/m ³ /hr.)	B-	U-	P-
0	.577	33	-	0.57	-	0	0
10	-	8	-	-	-	-	-
25	.920	11	-	-	-		+
50	.613	49	-	-	-	-	-
75	-	35	_	-	-	-	-
100	.840	38	_	_	-	-	-
150	_	49	-	-	-	-	-

Zooplankton Volume: 325 ml/1000 m³ total, 314 ml/1000 m³ small. Incident Radiation Daily Maximum: 1.55 cal/cm²/min. Daily Total: 449 cal/cm². Day Length:11.25 hrs.

M/V <u>Stranger</u>; SCOPE; November 16, 1956; 1942 GCT; 12°41.0'N, 94°15.0'W; 2200 fm; wire angle, 5°; temp., 84.0°F dry, 78.8°F wet; weather, 02;clouds, 8, amt., 3; sea, 2; swell, 340°, 4 ft. 7 sec.

OBSERVED

Depth (m)	Temp. (°C)	(°/°°)	02 (m1/1)	PO4-P (µgm at/l)	NO ₂ -N (µgm at/1)	pH	Alk (millival/l)
0 12	27.93 27.66	33.62	4.61 4.65	1.00 0.88	-	8.19 8.21	-
15	24.72	33.94	3.29	1.19	-	8.11	-
17	20.48	34 . 56	1.77	1.83	-	7.99	-
19	19.16	34.57	1.99	1.83	-	8.00	-
	16.92	34.65	0.31	2.49	-	7.83	-
29 58	13.78	34.83	0.04	2.54	-	7.82	-
114	12.52	34.82	0.07	2.32	-	7.84	-
193	11.55	34.76	0.10	2.60	-	7.80	-
287	10.04	34.70	0.10	2.80	-	7.78	-
386	8.44	34.61	0.14	2.99	-	7.75	-
477	7.46	34.59	0.12	3.17	-	7.76	-
729	5.5 ⁸	34.54	0.11	3.01	-	7.73	-

BIOLOGICAL OBSERVATIONS

Productivity

(m) (mg/m^3) (no/ml) $(mg C/m^3/day)$ (mg C/m ³ hr.)	
0.380 546 - 1.1 0 0	0 0
10 - 59	
25 3.76 12	
50 1.12 12	
75 - 19	
100 - 56	

Zooplankton volume: 192 ml/1000 m³ total, 192 ml/1000 m³ small. Incident Radiation Daily Max: 1.30 cal/cm²/min. Daily Total: 447 cal/cm². Day Length: 11.20 hrs.

M/V Stranger; SCOPE; November 17, 1956; 1935 GCT; 11°13.0'N, 90°55.0'W; 1940 fm; wire angle, 20°; temp., 81.5°F dry, 76.2°F wet; weather, 01; clouds, 8, amt., 6; sea, 3; swell, 110°, 5 ft, 6 sec.

OBSERVED

Depth (m)	Temp. (°O)	(°/)	0 ₂ (ml/l)	$PO_{4}-P$ (µ gm at/1)	NO2-N (μ gm at/1)	рH	Alk. (millival/l <u>)</u>
0 15 19 28 51 87 120 277 374 470 718	27.58 27.54 27.41 27.26 22.06 17.02 13.67 13.04 12.26 10.56 8.42 7.44 5.60	33.27 33.26 33.28 34.42 34.87 34.92 34.92 34.92 34.61 34.61 34.57	4.71 4.75 4.84 4.51 2.94 1.03 0.30 0.35 0.35 0.08 0.08 0.10 0.14	0.70 0.71 0.66 0.79 1.22 2.14 2.23 2.17 2.24 2.42 2.57 2.89 2.90	0.1 0.1 0.1 0.8 0.2 t t t 0.0 0.1 1.3 1.0 0.1	8.24 8.21 8.22 8.21 8.03 7.89 7.86 7.87 7.86 7.77 7.75 7.74 7.78	2.31 2.31 2.29 2.29 2.39 2.36 2.37 2.36 2.37 2.36 2.38 2.43 2.40 2.40

BIOLOGICAL OBSERVATIONS

Productivity

Depth (m)	Chlorophyll "a" (mg/m ³)	Bacteria (no/ml)	$\frac{in}{(mg}\frac{situ}{C/m^3}/day)$	Incubator $(mg C/m^3 hr.)$	В-	U-	P-
0	.582	-	-	-	-	-	-
25	.980	-	-	-	-	-	-
50	.762	-	-	-	-	-	-
100	.118	-	-	-	-	-	-

Zooplankton Volume: 125 ml/1000 m³ total, 125 ml/1000 m³ small. Incident Radiation Daily Max: 1.59 cal/cm²/min. Daily Total: 448 cal/cm². Day Length: 11.43 hrs.

November 18, 1956 08°56' N 88°30' W

NO CAST

BIOLOGICAL OBSERVATIONS

Productivity

Depth (m)	Chlorophyll "a" (mg/m ³)	Bacteria (n o/m l)	<u>in situ</u> (mg C/m ³ /day)	Incubator (mg C/m ³ hr.)	B-	u-	p-
0 9 15 50 100	.336 .426 .330 .300 .112	- - - -	13.0 - -	2.0	- - -	-	- - -

Zooplankton Volume: 95 ml/1000 m³ total, 95 ml/1000 m³ small. Incident Radiation Daily Maximum: 1.42 cal/cm²/min. Daily Total: 511 cal/cm². Day Length: 11.51 hrs. OBSERVED

M/V <u>Stranger</u>; SCOPE; November 19, 1956; 0159 GCT; 08°56.0'N, 88°29.5'W; 2100 fm; wire angle, 15°; temp., 78.0°F dry, 73.2°F wet; weather, 02;clouds, 3, amt., 3; sea, 4: swell, confused.

	UDSERVED									
Depth (m)	Temp. (°C)	(°/••)	02 (ml/l)	$PO_{\mu}-P$ (µgm at/l)	NO2-N (µgm at/l)	рH	Alk (millival/l)			
0 5	25.72 (25.99 (25.72	33•37 33•39	4.27 4.26	1.19 1.05	0.2 0.1	8.19 8.20	2.27 2.30			
9 12 22 27	24.60 22.18 18.28 17.04	33.60 34.07 34.65 34.69	4.02 3.26 1.78 1.38	1.33 1.70 2.11 2.19	0.2 0.2 0.5 0.5	8.15 8.08 7.99 7.88	2.31 2.32 2.36 2.35			
63 111 202 308	13.97 13.00 11.94 10.78	34.92 34.90 34.85 34.75	0.70 0.48 0.54 0.21	2.16 2.24 2.39 2.53	t t 0.1 t	7.91 7.86 7.83 7.79	2.36 2.37 2.37 2.37			
413 514 767 1035 1559	9.49 7.97 5.70 4.47 3.08	34.70 34.63 34.59 34.61 34.61	0.10 0.10 0.30 0.83	2.69 2.97 2.96 3.08	t 0.2 0.1 0.0 0.0	7.76 7.75 7.73 7.82 7.85	2.38 2.38 2.40 2.41 2.44			

BIOLOGICAL OBSERVATIONS

Productivity

Depth (m)	Chlorophyll "a" (mg/m ³)	Bacteria (n o/ml)	<u>in situ</u> (mg C/m ³ /day)	Incubator (mg C/m ³ hr.)	В-	U-	P-
0	-	-	6.4	0.77	-	-	_
2	-	-	-	0.70	-	-	-
10	-	-	-	0.50	-	-	-
27	-	-	-	0.30	-	-	-
40	-	-	-	0.33	-	-	-

Zooplankton Volume: 95 ml/1000 m³ total, 95 ml/1000 m³ small. Incident Radiation Daily Max: 140 cal/cm²/min. Daily Total: 535 cal/cm². Day Length: 11.58 hrs.

- 22 -

M/V Stranger; SCOPE; November 20, 1956; 1942 GCT; 09°15.5'N, 89°18.0'W; 1820 fm; wire angle, 0°; temp., 79.5°F dry, 76.2°F wet; weather, 02; clouds, 4-5; amt., 6; sea, 2; swell, 060°, 3 ft, 4 sec.

OBSERVED

$\begin{array}{c ccccccccccccccccccccccccccccccccccc$	Depth (m)	Temp. (°C)	(°∕₀₀)	02 (m1/1)	PO ₄ -P (µgm at/1)	NO ₂ -N (µgm at/1)	рH	Alk (millival/l)
141 11.98 34.86 0.71 2.42 0.0 7.92 2.38	6 8 11 13 15 17 19 21 23 28 30 49 73 97	24.81 24.65 24.41 22.66 20.92 17.74 - 16.42 15.98 15.22 14.48 14.27 13.61 12.70 12.17	33.64 33.71 33.68 34.24 34.71 34.77 34.78 34.79 34.78 34.78 34.78 34.78 34.79 34.78 34.79 34.79 34.79 34.79 34.79 34.79 34.79	4.11 4.05 3.91 3.39 2.82 1.53 1.25 1.26 1.01 0.87 0.88 0.79 0.66 1.13 1.16	1.04 0.96 1.03 1.20 1.40 1.79 1.78 1.89 1.87 2.04 2.17 2.04 2.15 2.20 2.27	0.1 0.1 0.2 0.2 0.3 0.3 0.1 0.4 0.5 0.5 0.5 0.5 0.4 0.1 t	8.18 8.16 8.15 8.06 7.97 7.93 7.93 7.92 7.92 7.92 7.92 7.91 7.90 7.95	2.32 2.31 2.32 2.34 2.36 2.37 2.37 2.37 2.37 2.37 2.37 2.37 2.37

BIOLOGICAL OBSERVATIONS

Productivity

${\tt Depth}$	Chlorophyll	Bacteria			B-	U-	P-
(m)	"a" (mg/m ³)	(no/ml)	(in situ (mg C/m ³ /day)	Incubator (mg C/m ³ hr.)			
0	.191	-	13	0.70	-	-	-
5 8	-	-	11	0.98	-	-	-
8	.231	-	13	0.91	-	-	-
10	-	-	15	-	-	-	-
12	.381	-	14	-	-	-	-
14	-	-	10	-	-	-	-
16	.228	-	-	-	-	-	-
18	-	-	4.5	0.56	-	_	~
20	.446	-	-	-	-	-	-
22	-	-	4.8	-	-	-	-
27	-	-	3.2	-	-	-	-
30	.302	-	-	-	-	-	_
50	.319	-	1.7	0.16	-	-	-
100	.188	-	0.29	-	-	-	-

Water Column Productivity: 0.332 mgC/m²/day

Zooplankton Volume: 250 ml/1000 m³ total, 250 ml/1000 m³ small

Incidental Radiation

Daily Maximum:	1.83 cal/cm ² /min.
Daily Total:	1.83 cal/cm ² /min. 399 cal/cm ² .
Day Length:	11.49 hrs.

	SUBMAR	INE DAYLIGHT	(425 mµ)
Depth (m)	Corr. Sub Read. (µa)	k/m	•/•T/m
2 12 22 32 41	382.0 188.0 72.4 25.6 6.3	.0798 .0954 .103 .155	93.2 90.9 90.1 85.6

Station 9D

M/V Stranger; SCOPE; November 21, 1956; 2005 GCT; 09°34.0'N, 89°13.5'W; 1800 fm; wire angle, 0°; temp., 80.0°F dry, 76.8°F wet; weather, 01; clouds, 4 and 8, amt.4; sea, 1; swell, 110°, 2 ft, 9 sec.

OBSERVED

Depth (m)	Temp. (°C)	(°/)	02 (ml/1)	$PO_{4}-P$ (µgm at/l)	NO ₂ -N (µgn at/l)	рH	Alk (millival/l)
0	(^{25,51} (25,29	33.64	4.11	1.14	0.1	8.12	-
5	24.98	33.63	4.11	1.23	0.1	8.16	-
5 8	24.90	33.64	4.09	1.11	0.1	8.16	-
10	24.83	33.84	4.03	1.46	0.1	8.16	-
12	24.74	33.67	4.01	1.28	0.1	8.16	-
14	24.53	33.68	3.98	1.18	0.1	8.14	-
16	23.82	33.80	3.60	1.23	0.2	8.15	-
18	22.63	33.96	3.29	1.36	0.1	8.11	-
20	20.65	34.30	2.54	1.70	0.2	8.05	-
22	18.86	34.51	1.89	1.85	0.2	7.99	-
24	18.11	34.69	1.67	1.83	0.3	7.99	-
27	17.18	34.72	1.13	2.04	0.3	7.93	
30	16.82	34.73	1.18	2.04	0.3	7.93	-
49	14.36	34.86	0.98	2.20	tr.	7.94	-
73	13.31	34.87	0.74	2.53	0.0	7.94	-
77	12.86	34.87	0.48	2.60	tr.	7.88	-
145	12.30	34.87	0.44	2.21	0.0	7.85	-

BIOLOGICAL OBSERVATIONS

Productivity

Depth (m)	Chlorophyll "a" (mg/m ³)	Bacteria (no/ml)	$\frac{\ln \operatorname{situ}}{(\operatorname{mg} C/m^3/\operatorname{day})}$	Incubator (mg C/m ⁵ hr.)	B-	U-	P-
0 5 8 10 12 14 16 18 20 22 27 30 50 100 200	. 308 . 310 . 340 . 342 . 335 . 458 . 280 . 092		3.2 8.1 4.6 3.7 2.5 7.3 6.2 - 10 9.4 - 3.0	0.38 0.52 0.79 - - - 0.85 - - - - - - - - - - - - - - - - - - -	1+1+1 0 - 1+1 0 - 1+1 0 0 0	0 - 0 - 0 - 0 - 1 + 1 0 0 0 0	00,0101010000

Station 9D (cont.)

Water Column Productivity = 0.402gmC/m ² / day.	
Zooplankton Volume: 135 ml/1000 m ³ total, 135 ml/1000 m ³ small	•
Incident Radiation	

SUBMARINE DAYLIGHT (480 mm)

Depth (m)	Corr. Sub. Read. (µa)	k/m	•/.T/m
2 12 23 37 46	202 167 135 69.1 40.2 29.8 15.6	.0380 .0425 .0744 .0773 .0332 .0719	96.3 95.8 92.8 92.6 96.7 93.0

M/V Stranger; SCOPE; November 23, 1956; 1332 GCT; 09°41.0'N, 89°44.5'W; 1700 fm; wire angle, 3°; temp., 79.0°F dry, 77.2°F wet; weather, 02; clouds, 6, amt., 5; sea, 1; swell, 180°, 3 ft, 6 sec.

OBSERVED

Depth (m)	Temp. (°C)	(°/°)	02 (ml/l)	PO ₄ -P (µgm at/l)	NO ₂ -N (µgm at/l)	рH	Alk (millival/l)
0	25.44	33.64	4.20	1.06	-	-	-
4	25.36	33.63	4.02	1.14	-	-	~
8	25.22	33.63	4.16	1.16	-	-	-
10	25.14	33.68	4.02	1.04	-	-	-
12	24.99	33.68	3.99	1.04	-	-	-
13	24,87	33.68	3.94	1.14	-	-	-
16	24.56	33.71	3.82	1.05	-	-	-
18	24.26	33.75	3.74	1.12	-	-	-
20	23.49	33.81	3.56	1.20	-	-	-
22	22.45	34.00	3.17	1.36	-	-	-
24	18.77	34.31	2.41	1.57	-	-	-
26	17.75	34.60	1.58	1.78	-	-	-
	17.04						
30	15.20	34.79	0.94	1.96	-	-	-
50	13.58	34.87	0.37	2.17	-	-	-
75	13.01	34.92	0.49	2.12	-	-	uha
9 9	12.76	34.92	0.51	2.18	-	-	-
148	12.16	34.87	0.56	2.15	-	~	-

BIOLOGICAL OBSERVATIONS

Depth	Chlorophyll "a"	B act eria	in situ	Incubator	B-	U-	P-
(m)	(mg/m^3)	(no/ml)	$(mg^{C}/m^{3}/day)$	$(mg C/m^3 hr.)$			
0	.130	-	7.0	0.59	-	-	_
2	-	-	7.3	0.70	-	-	-
5	-	-	7.7	0.44	-	-	-
8	-	-	5.6	0.58	-	-	-
10	.276	-	5.7	-	-	-	
12	-	-	4.4	0.46	-	-	-
14	-	-	12.0	-	-	-	-
18	-	-	14.0	-	-	-	-
20	.343	-	-	-	-	-	-
22	-	-	11.0	0.74	-	-	-
30	.387	-	5.0	-	-	-	-
50	.284	-	1.4	0.20	-	-	-
100	.011	-	-	-	-	-	-

Water Column Productivity = 0.320gmC/m²day Incident Radiation Daily Max: 1.43 cal/cm²/min. Daily Total: 482 cal/cm². Day Length: 11.45 hrs.

Station 9F (Cont.)

SUBMARINE DAYLIGHT (480 mm)

Depth (m)	Corr. Sub. Read. (µa)	k/m	°/_T/m
7 12 22 31 41 50 60 66 71	967 708 335 159 82.2 40.8 12.6 4.3 3.2	.0623 .0748 .0828 .0659 .0778 .117 .179 .0590	- 93.9 92.8 92.0 93.6 92.5 88.9 83.6 94.3

M/V <u>Stranger</u>; SCOPE; November 24, 1956; 1912 GCT; 08°42.0'N, 86°01.0'W; 1650 fm; wire angle, 3°; temp., 80.0°F dry, 75.6°F wet; weather, 02; clouds, 6, amt., 7; sea, 3; swell, 120°, 3 ft. 6 sec.

OBSERVED

Depth	Temp.	s	02	PO ₄ -P	NO ₂ -N	ΡĦ	Alk
(m)	(°C)	(°/••)	(m1/1)	(µgm at/1)	(µgm at/l)		(millival/l)
0 20 30 37 42 51 96 142 196 290 388 484 736	26.86 26.79 25.94 23.00 19.54 16.92 13.14 12.54 11.94 10.72 9.06 7.85 5.78	32.65 32.68 33.11 33.93 34.49 34.74 34.83 34.83 34.83 34.67 34.65 34.60	4.56 4.55 4.20 2.74 1.91 1.30 0.74 0.62 0.55 0.30 0.08 0.10	0.65 0.52 0.67 1.37 1.80 1.97 2.18 2.25 2.33 2.40 2.79 2.84 2.96	tr. tr. 0.1 0.3 0.5 1.0 tr. 0.1 0.0 tr. 0.7 0.0 tr.	8.24 8.23 8.09 7.98 7.94 7.89 7.89 7.86 7.78 7.76 7.75 7.86	

BIOLOGICAL OBSERVATIONS

Productivity U-P-Chlorophyll Bacteria В-Depth "a" (mg/m³) in situ Incubator (mg C/m³/hr.) (m) (no/ml) $(mg C/m^3/day)$ 28.0 0 .420 1.0 .414 1.2 -10 -25 .554 0.89 _ 50 1.0 -.759 _ 100 .227 0.32

Zooplankton Volume: 166 m¹/1000m³ total, 166 ml/1000m³ small. Incident Radiation Daily Max: 0.377 cal/cm²/min. Daily Total: 121 cal/cm². Day Length: 11.20 hrs.

SUBMARINE DAYLIGHT (480 mu)

De p th (m)	Corr. Sub. Read (µa)	k/m	°/.T/m
2	224		
7	167	.0587	94.3
12	107.5	.0871	91.6
22	64.9	.0509	95.0
32	31.6	.0719	93.0
42	12.04	.0968	90.8
5 2	3.20	.132	87.6

M/V <u>Stranger</u>; SCOPE; November 25, 1956; 1932 GCT; 07°37.0'N, 82°25.5'W; 600 fm; wire angle, 15°; temp., 74.8°F dry, 73.8°F wet; weather, 20; clouds, 9, amt., 7; sea, 2; swell, 120°, 2 ft., 3 sec.

OBSERVED

Depth (m)	Temp. (°C)	(°/)	02 (ml/l)	PO4-P (µgm at/l)	NO_2-N (µgm at/1)	рĦ	Alk (millival/l)
0	26.72	29.40	-	0.44	0.0	8.27	-
15	26.76	29.70	-	0.41	0.0	8.25	-
30	2 5. 82	31.82	-	0.98	0.0	8.22	-
44	23.04	33.48	-	1.00	1.5	8.12	-
53	18.70	34.51	-	1.69	0.2	7.96	-
59	17.03	34.78	-	1.84	0.1	7.92	-
91	15.22	34.88	-	2.21	tr.	7.88	-
127	14.14	34.93	-	2.11	tr.	7.84	-
205	12.68	34.89	-	2.32	0.0	7.80	-
300	11.12	34.82	-	2.58	0.0	7.76	-
406	9.32	34.69	-	2.82	0.0	7.71	-
507	7.97	34.65	-	3.06	0.0	7.71	-
769	5.83	34.59	-	3.09	0.0	7.71	-

BIOLOGICAL OBSERVATIONS

Productivity

Depth (m)	Chlorophyll "a" (mg/m ³)	Bacteria (no/ml)	(mg C/m ³ /day)	Incubator (mg C/m ³ /hr.)	B-	U-	P-
0	.517	-	-	0.59	-	-	-
10	.526	-	-	0.51	-	-	-
25	.734	-	-	1.3			
50	1.20	-	-	1.0	-	-	-
100	.186	-	-	0.15	-	-	-

Zooplankton Volume: 104 ml/1000m³ total, 104 ml/1000m³ small. Incident Radiation Daily Maximum: 0.977 cal/cm²/min. Daily Total: 83.2 cal/cm². Day Length:

11.43 hrs.

SUBMARINE DAYLIGHT (480 mu)

Depth (m)	Corr. Sub. Read. (μa)	k/m	°/.T/m
2	10	-	78.8
7	33	.238	

M/V <u>Stranger;</u> SCOPE; December 1, 1956; 1835 GCT; 05°59.0'N, 79°48.8'W; 1700 fm; wire angle, 13°; temp., 82.3°F dry, 75.8°F wet; weather, 02; clouds, 4, amt. 6; sea, 1; swell, confused.

OBSERVED

Depth (m)	Temp. (°C)	s (°/)	0 ₂ (m1/1)	$PO_{4}-P$ (µgm at/1)	NO ₂ -N (µgm ² at/l)	рĦ	Alk (millival/l)
0	26.68	28.30	4.52	0.30	tr.	8.24	-
6	26.50	28.38	4.57	0.20	0.0	8.24	-
18	26.62	30.61	4.40	0.18	tr.	8.24	-
29	25.94	33.11	4.35	0.31	0.0	8.23	-
39	24.32	33.61	4.10	0.50	0.1	8.16	-
43	23.03	33.93	3.42	0.73	0.7	8.12	-
52	19.43	34.66	2.13	1.16	0.4	8.01	-
94	14.34	34.96	0.85	1.60	0.0	7.92	
190	13.16	34.97	0.59	1.58	0.0	7.88	-
283	11.94	34.88	0.33	1.92	0.0	7.7 9	-
379	9.37	34.72	0.24	2.02	tr.	7.76	-
472	8.42	34.69	0.17	1.68	0.0	7.76	-
720	6.09	34.60	0.57	2.18	0.0	7.78	-

BIOLOGICAL OBSERVATIONS

Productivity

De pt h	Chlorophyll "a"	Bacteria	in situ	Incubator	В-	U-	P-
(m)	(mg/m^3)	(no/ml)	$(mg \ C/m^3/day)$	(mg C/m ³ /hr.)			
0	.329	75	13.0	0.38	0	0	0
10	.272	3		0.98	0	÷	-
25	.364	8	-	0.30	-	-	-
50	.491	1	-	0.23	0	Ŧ	0
75	-	1	-	0.26	#	0	0
100	.101	-	-	0.14	-	-	-
			0	-			

Zooplankton Volume: 95 ml/1000 m³ total, 95 ml/1000m³ small. Incident Radiation

Daily Max: 2.13 cal/cm²/min. Daily Total: 437 cal/cm². Day Length: 11.60 hrs.

SUBMARINE DAYLIGHT (480 mu)

Depth (m)	Corr. Sub. Read. (µa)	k/m	°/ _o T/m
2 7 12 22 31 41 49 54 59 67	817 576 440 265 175 74.9 44.8 33.0 24.0 12.6	- .0538 .0507 .0461 .0848 .0642 .0611 .0636 .0795	- 93.3 94.8 95.0 95.5 91.9 93.8 94.1 93.8 92.3

- 31 -

M/V <u>Stranger</u>; SCOPE; December 2, 1956; 1949 GCT; 04°09.0'N, 83°34.0'W; 1700 fm; wire angle, 0°; temp., missing; weather, 02; clouds, 8, amt., 5; sea, 1; swell, slight.

OBSERVED

Depth (m)	Temp. (°C)	(°/)	02 (ml/l)	PO4-P (µgm at/l)	NO ₂ -N (µgm at/1)	рH	Alk (millival/l)
0 9	27.02 26.42	32.96 32.95	4.43 4.50	0.46 0.40	tr. tr.	8.19 8.21	2.24 2.24
9 18	26.33	33.01	4.48	0.43	tr.	8.22	2.24
27	25.54	33.36	4.18	0.59	0.1	8.20	2.25
30	24.45	33.64	3.94	0.71	0.2	8.15	2.28
37	20.64	34.31	2.78	1.05	0.5	8.07	2.31
49	18.50	34.70	2.15	1.26	0.7	8.00	2.34
97	14.76	34.97	1.46	1.82	tr.	7.93	2.35
197	13.22	34.96	0.63	1.66	0.0	7.84	2.35
292	11.74	34.88	0.33	1.87	tr.	7.80	2.35
394	9.34	34.70	0.14	2.13	tr.	7.74	2.36
490	8.05	34.67	0.22	2.27	tr.	7.75	2.37
743	5.86	34.61	0.69	2.32	tr.	7.82	2.38

BIOLOGICAL OBSERVATIONS

Productivity Depth Chlorophyll Bacteria В-U-P- (mg/m^3) in situ Incubator $(\text{mg C/m}^3/\text{day})$ $(\text{mg C/m}^3/\text{hr.})$ (m) (no/ml)23 .196 0 6.1 0.35 0 0 0 5 6 -_ ++ 0 0 -10 5 .215 _ 0.37 ₩ + 0 2 25 .261 0.47 0 _ H + 50 .633 23 0.36 0 # + 52 75 _ $^+$ 0 0 _ -+ 100 .105 0 0.070 0

Zooplankton Volume: 139 ml/1000m³ total, 139 ml/1000m³ small. Incident Radiation Daily Max: 1.80 cal/cm²/min. Daily Total: 433 cal/cm². Day Length: 11.86 hrs.

SUBMARINE DAYLIGHT (480 mu)

Depth (m)	Corr. Sub. Read. (μa)	k/m	°/.T/m
2 7 12 22 32 41 51 69.5 77	921 727 607 416 288 135 63.8 27.7 16.7 13.2	.0473 .0360 .0377 .0367 .0841 .0749 .0834 .0632 .0335	95.4 96.2 96.4 91.9 92.8 92.0 93.9 96.7

M/V Stranger; SCOPE; December 3, 1956; 2049 GCT; 05°28.5'N, 86°57.0'W; 700 fm; wire angle, 5°; temp., 79.8°F dry, 76.0°F wet; weather, 02; clouds 8, amt. 6; sea, 2; swell, 210°, 3 ft. 5 sec. OBSERVED

				ODSERVED			
Depth (m)	Temp. (°C)	S (°∕₀₀)	02 (m1/1)	$PO_{\mu}-P$ (µgm at/l)	NO ₂ -N (µgm at/l)	рĦ	Alk (millival/l)
0 15 30 41 47 56 91 124 199 292 391 485	26.42 26.10 25.95 24.05 21.70 18.05 16.76 14.96 13.16 12.41 11.06 8.97	33.17 33.28 33.73 34.36 34.94 35.03 34.96 34.97 34.90 34.79 34.70	4.45 4.51 4.56 4.03 3.19 2.13 1.93 1.18 0.52 0.43 0.21 0.12	0.30 0.28 0.30 - 0.88 1.18 1.28 1.57 1.72 1.80 2.00 2.28	tr. 0.0 0.2 0.5 0.4 tr. tr. tr. 0.0 0.0 0.0	8.22 8.25 8.26 8.20 8.00 8.00 7.96 7.86 7.84 7.78 7.78	2.27 2.27 2.27 2.29 2.33 2.36 2.36 2.36 2.36 2.36 2.36 2.36
736	6.07	34.59	0.44	2.49	0.0	7.80	2.39

BIOLOGICAL OBSERVATIONS

Product	ivity
---------	-------

Depth	Chlorophyll "a"	Bacteria	in situ	Incubator	B-	U-	P-
(m)	(mg/m^3)	(no/ml)	$(mg C/m^3/day)$	$(mg C/m^3/hr.)$			
0	.169	114	3.8	0.24	+	0	0
10	.226	23	-	0.30	-	-	-
25	.329	19	-	0.34	-	-	-
50	.425	6	-	0.46	-	-	-
75	-	161	-	-	-	-	-
100	.230	8	-	0.11	-	-	-
			3				

Zooplankton Volume: 114 ml/1000m³ total, 114 ml/1000m³ small. Incident Radiation Daily Max: 1.94 cal/cm²/min. Daily Total: 279 cal/cm². Day Length, 11.45 hrs.

SUBMARINE DAYLIGHT (480 mµ)

Depth (m)	Corr. Sub. Read (μa)	k/m	°/.T/m
2 7 12 22 30 41 51 61 70	877 763 690 418 268 123 61 37.2 15.9	- .0278 .0201 .0501 .0555 .0708 .0701 .0494 .0944	- 98.0 95.1 94.6 93.2 93.2 95.2 91.0

M,V <u>Stranger</u>; SCCPE; December 4, 1956; 1956 GCT: 06°-6.0'N, 89°52.0'W; 1900 fm; wire angle, 10°; temp., 82.5°F dry, 77.1°F wet; weather, 02; clouds, 8, amt., 3; sea, 1; swell, 270°, 3 ft, 6 sec.

				0202200			
Depth (m)	Temp. (°C,	S (°,••,	02 (m1/1)	PCP (ugm_at/l)	N02-N (µgm. at/1)	₽Ħ	Alk (millival/l)
1 0 0 0 1 1 0 0 0 1 0 0 0 1 0 0 1 0 0 0 1 0 0 0 1 0 0 0 1 0 0 0 0 1 0	2 2 2 2 2 2 1 1 1 1 1 1 1 2 2 2 2 2 2 2		0,1-00000000000000000000000000000000000		tr.0 tr.0 tr.2 0 1 0 tr.0 0 tr.0 0 tr.0 0 tr.0 0 tr.0 0 tr.0 0 tr.0 0 tr.0 0 tr.0 tr.	1 2 2 2 2 3 3 4 4 5 5 5 5 5 5 5 5 5 5 5 5 5	2.22 2.21 2.23 2.30 2.33 2.33 2.35 2.35 2.35 2.35 2.35 2.35

OESERVED

BIOLOGICAL OBSERVATIONS

Productivity

Depth	Chlorophyll "s"	Bacteria	in situ	Incubator	Э-	U-	P-
(=,	(======================================	(no/ml)	$(mg C, m^3/day)$	$(mg C/m^3/hr.)$			
Ċ	.155	53	5	0.19	-	-	-
10	5	<u>).</u>	-	0.12	-	-	-
25	.212	1	-	0.23	-	-	-
うこ	. ~ 03	(I T	-	0.076	-	-	-
~5	-	10	-	_	-	-	-
100	.2-0	(*)	-	0.082	-	-	-
7005 ar	to To uno.			100 m ³ amali			

Zooplankton Volume: 97 ml/1000 m⁻ total, 96 ml/1000 m⁻ small. Incident Radiation Daily Max: 1.36 cal/cm⁻/min. Daily Total: 536 cal/cm⁻². Day Length: 11.72 hrs.

SUBMARINE DAYLIGHT (480 mi)

Depth (m)	Corr. Sub. Read. (1a)	Z/=	°/.T/m
7 22 22 20 50 50 50 50 70 70	889960090 7906090 7906090 7000900 17000900 1000900 1000900 1000900 1000000	- 0411 0315 06956 07705 06765 07442 0984	- 96.03 932.12 932.56 932.5 932.5 95.6

Station 20A

M/V Stranger; SCOPE; December 5, 1956; 1620 GCT; 07°50.0'N, 91°17.0'W; 1900 fm; wire angle, 7°; temp., 79.8°F dry, 76.1°F wet; weather, 02; clouds, 8, amt., 5; sea, 2; swell, 060°, 4 ft, 5 sec.

OBSERVED							
Depth (m)	Temp. (°C)	(°/)	0 ₂ (m171)	P04-P (µgm at/l)	NO ₂ -N (µgm at/l)	ΡĦ	Alk . (millival/l <u>)</u>
0 2 4 6 8 10 12 4 16 17 21 23 27 348 94	25.68 25.70 25.72 25.63 25.48 25.16 24.49 19.25 18.30 17.42 16.19 15.70 - 13.90 12.90	33.19 33.17 33.17 33.15 33.29 33.93 34.59 34.59 34.59 34.79 34.79 34.79 34.83 34.83	4.29 4.14 4.28 4.20 4.08 3.77 1.58 1.330 1.61 1.58 1.559	0.72 0.64 0.66 0.67 0.76 1.42 1.52 1.57 1.68 1.57 1.85 1.85 1.85	0.1 0.1 0.1 0.1 0.1 0.1 0.1 0.2 0.2 0.2 0.2 0.2 0.2 0.2 0.2 0.2 0.2	8.10 8.11 8.11 8.11 8.09 8.06 7.89 7.81 7.81 7.78 7.78	2.26 2.25 2.26 2.26 2.27 2.27 2.27 2.27 2.3334 2.334 2.334 2.334 2.335 2.35 2.35 2.35

BIOLOGICAL OBSERVATION

Productivity

Depth	Chlorophyll "a"	Bacteria		Incubator	B-	U-	P-
(m)	(mg/m^3)	(no/ml)	<u>in situ</u> (mg C/m ³ /day)	(mg C/m ³ /hr.)			
0	.129	97	11	0.96	++	0	0
4	.334	37	31	-	-	-	-
8	.277	37	6.0	1.1	-	-	-
10	-	-	13	-	-	-	-
12	.382	25	8.5	-	-	-	-
14	-	-	4.8	-	-	-	-
15	.290	-	-	-	-	-	-
16	-	16	-	-	-	-	-
18	.415	-	3.7	-	-	-	-
20	_	18	_	-	-	-	-
24	-	20	2.1	0.32	-	-	-
25	.454	-	-	-	-	-	-
30	-	6	0.38	-	_	-	-
50	.265	4	0.33	0.11	-	-	_
100	.052	55	-	-	-	-	-

Water Column Productivity: 0.270gmC/m²/day. Incident Radiation Daily Max: 1.64 cal/cm²/min. Daily Total: 516 cal/cm². Day Length: 11.66 hrs.

Station 20B

M/V <u>Stranger</u>; SCOPE; December 6, 1956; 0206 GCT; 07°52.0'N, 91°19.0'W; 1900 f.; wire angle, 15°; temp., 78.1°F dry, 74.8°F wet; weather, 02; swell, 050°, 3 ft, 5 sec.

OBSERVED

Depth (m)	Cemp.	(°/••)	0 ₂ (ml/l)	$PO_{h}-P$ (µgm at/1)	NO2-N (µgn at/1)	pĦ	Alk (millival/l)
0	25.60	33.24	4.20	0.73	0.1	8.20	2.29
6	25.62	33.24	4.22	0.68	0.1	8.20	2.28
15	23.35	33.65	3.36	0.93	0.1	8.16	2.30
18	19.31	34.51	1.68	1.34	0.2	8.05	2.34
21	17.66	34.67	1.41	1.19	0.2	8.00	2.36
49	13.99	34.78	0.58	1.71	0.1	7.94	2.36
98	12.70	34.88	0.37	1.77	tr.	7.89	2.37
. 194	11.31	34.83	0.80	1.72	0.0	7.92	2.37
2,25	10.58	34.78	0.80	1.79	0.0	7.91	2.37
379	9.56	34.71	0.26	2.17	tr.	7.88	2.37
468	8.39	34.70	0.17	2.29	0.0	7.86	2.38
705	6.32	34.61	n.46	2.42	0.0	7.85	2.39
9 5 1	5.03	34.60	0.46	2.43	-	7.91	2.41
1411	3.42	34.64	1.25	2.28	-	7.90	2.45
1915	2.46	34.69	1.98	2.19	-	7.90	2.43

BIOLOGICAL OBSERVATIONS

Productivity

Depth	Chlorophyll	Bacteria			В-	U-	P-		
(m)	"a" (mg/m ³)	(no/ml)	$\frac{\sin \operatorname{situ}}{(\operatorname{mg} C/\operatorname{m}^3/\operatorname{day})}$	Incubator (mg C/m ³ /hr.))				
0	.345	-	4.5	-		-	-		
Zooplankton Volume: 146 ml/1000m ³ total, 143 ml/1000m ³ small.									
SUBMARINE DAYLIGHT (480 mu)									
	De p tl (m)	h Corr	. Sub. Rea d . (μa)	k/m	°/ _° T/m				
	2 7 12 17 24		981 682 576 308 197	.0727 .0337 .125 .0631	93.0 96.7 88.2 93.9				

M/V <u>Stranger</u>; SCOPE; December 7, 1956; 2139, 2209 GCT; 12°17.0'N, 96°50.0'W; 2200 fm; wire angle, 20°, 25°; temp., 80.7°F dry, 75.2°F wet; weather, 02; sea, 3.

Depth (m)	Temp. (°C)	S (°/°))	02 (ml/l)	PO_4-P (µgm at/1)	NO_{2-N} (µgm at/l)	рH	Alk (millival/l <u>)</u>
0 8 31 52 67 129 205 300 402	25.44 25.40 23.82 21.70 19.98 18.03 16.89 12.64 11.60 10.22 (8.74 (8.64	33.61 33.61 33.93 34.23 34.43 34.54 34.54 34.65 34.89 34.81 34.74 34.65	4.56 4.55 4.48 4.24 3.92 2.59 1.86 0.29 0.11 0.13 0.95	0.56 0.62 0.59 0.79 0.95 1.21 1.52 1.49 1.88 2.01 2.21	0.1 0.2 0.6 0.5 0.8 0.0 0.1 0.1 1.2	8.23 8.23 8.18 8.13 8.06 7.98 7.95 7.80 7.77 7.75 7.74	
498 736	- 7.18 5.44	34.61 34.56	- 0.11 0.10	- 2.29 2.36	- 0.6 0.0	- 7.76 7.77	-

BIOLOGICAL OBSERVATIONS

Productivity

Depth	Chlorophyll	Bacteria			В-	U-	P-
(m)	"a" (mg/m ³)	(no/ml)	$\frac{\text{in situ}}{(\text{mg C/m}^3/\text{day})}$	Incubator $(mg C/m^3/hr.)$			
0	Lost	7	-	-	++	0	0
10	.877	14	-	-	-	-	-
25	.905	48	-	1.25	++	0	0
50	1.40	34	-	0.95	-	-	-
75	-	18	-	-	-	-	-
100	Lost	183	-	-	-	-	-
Zooplar	nkton Volume:	208 ml/1000m ³	total, 204 ml/10	000m ³ small.			

SUBMARINE DAYLIGHT (480 mm)

De pt h (m)	Corr. Sub. Read. (µa)	k/m	°/.T/m
2	580	-	-
7	356	.0976	90.7
12	230	.0873	91.6
21	65.2	.126	88.1
26	26.5	.180	83.5

M/V <u>Stranger</u>; SCOPE; December 8, 1956; 1935 GCT; 14°37.0'N, 100°09.0'W; 2000fm; wire angle, 30°; wind, calm; temp., 85.2°F dry, 77.3°F wet; weather, 02; clouds, 1, amt., 1; sea, 1; swell 110°, 2 ft, 10 sec.

Depth (m)	Temp. (°C)	(°/)	02 (ml/l)	$PO_{l_4}-P$ (µgm at/l)	NO ₂ -N (µgm at/l)	Нq	(Alk (millival/1)
0 4 8 16 26 39 49 76 144 214 293 373 596	29.48 25.17 24.78 23.96 22.48 20.95 20.10 16.21 12.85 12.00 11.10 10.00 6.96	33.97 34.00 34.02 34.18 34.28 34.45 34.45 34.72 34.87 34.83 34.83 34.76 34.70 34.57	4.56 4.47 4.53 4.47 4.11 4.05 1.53 0.32 0.14 0.09 0.09 0.09 0.09	0.62 0.65 0.67 0.78 0.97 1.10 1.41 1.86 1.85 1.92 2.00 2.15 2.38	0.1 0.2 0.2 0.4 0.5 0.2 0.2 0.2 0.0 0.0 1.6 0.8 0.4	8.21 8.20 8.19 8.18 8.13 8.06 7.94 7.85 7.85 7.85 7.85 7.80 7.77 7.77	- 2.31 2.31 2.31 2.31 2.31 2.33 2.34 2.35 2.35 2.35 2.35 2.35 2.37 2.38

BIOLOGICAL OBSERVATIONS

Productivity

Depth	Chlorophyll "a"	Bacteria	in situ	Incubator	B-	U-	P-
(m)	(mg/m^3)	(no/ml)	$(mg C/m^3/day)$	$(mg C/m^3/hr.)$			
0	0.816	75	-	3.5	-	-	-
10	1.13	13	-	2.8	-	-	-
25	0.800	13	-	0.32	-	-	-
50	0.529	5 8	-	0.15	-	-	-
75	-	37	-	-	-	-	-
100	0.298	198	-	0.034	-	-	-

Zooplankton Volume: 233 ml/1000 m³ total, 233 ml/1000 m³ small.

SUBMARINE DAYLIGHT (480 mµ)

Depth (m)	Corr. Sub. Read. (µa)	k/m	°/ _° T/m
2 7 12 22 32 42	759 506 282 102 45.6 20.4	.0810 .116 .101 .0805 .0804	- 92.2 88.9 90.3 92.3 92.3

OBSERVED

M/V <u>Stranger</u>; <u>SCOPE</u>; December 9, 1956; 2019 GCT; 16°52.0'N, 103°06.0'W; 1580 fm; wire angle, 0°; temp., 81.2°F dry, 76.8°F wet; weather, 02; Clouds, 1, amt.1; sea, 2; swell, 310°, 2 ft, 6 sec.

OBSERVED

Depth (m)	Temp. (°C)	(°/)	0 ₂ (m171)	$PO_{4}-P$ (µgm at/1)	NO ₂ -N (µgm at/1)	рĦ	Alk (millival/l)
0 10 35 52 63 75	29.04 28.29 28.46 28.46 24.07 20.97 18.64	34.05 34.06 34.24 34.33 34.34 34.54 34.54 34.54	4.42 4.34 4.45 4.40 3.90 1.98 0.89	0.38 0.40 0.38 0.39 0.62 1.19 1.58	tr. 0.0 0.0 0.5 0.2 0.1	8.25 8.23 8.24 8.23 8.16 8.02 7.90	2.31 2.30 2.31 2.32 2.32 2.32 2.32 2.33
120 197 291 390 485 737	13.64 12.06 10.98 9.68 8.28 6.05	34.85 34.83 34.76 34.69 34.61 34.56	0.13 0.09 0.09 0.10 0.11 0.10	1.92 1.91 1.99 2.16 2.26 2.32	0.9 1.6 1.4 1.0 0.8 tr.	7.79 7.79 7.75 7.74 7.75 7.72	2.33 2.35 2.35 2.37 2.37 2.37 2.38

BIOLOGICAL OBSERVATIONS Productivity

Depth	Chlorophyll	Bacteria			B-	U-	P-
	"a" _		in situ	Incubator			
(m)	(mg/m^{2})	(no/ml)	$(mg C/m^3/day)$	$(mg C/m^3/hr.)$			
0	0.109	38	1.8	0.48	-	-	-
10	0.148	66	-	0.28	-	-	-
25	0.164	65	-	0.15	-	-	-
50	0.385	23	-	0.25	-	-	-
75	-	65	-	_	-	-	-
100	0.400	51	-	0.067	-	-	-
		-		-			

Zooplankton Volume: 58 ml/1000m³ total, 33 ml/1000m³ small

SUBMARINE DAYLIGHT (480 mµ)

	. Sub. Read. (µa)	k/m	°/. T/m
7 12 22 32 42	745 520 510 368 270 181 102 42.5 19.0 11.6	- .0367 .0391 .0326 .0306 .0404 .0574 .0875 .0805 .0451	- 96.4 96.2 96.8 96.9 96.0 94.4 91.6 92.3 95.6

,

M/V <u>Stranger</u>; SCOPE; December 10, 1956; 1826 GCT; 19°30.0'N, 105°52.0'W; 2050 fm; wire angle, 5°; wind, 040°, force 4; temp., 79.8°F dry, 76.3°F wet; weather, 03; clouds, 6, amt. 5; sea, 3; swell, 350°, 10 ft; 7 sec.

Depth (m)	Temp. (°C)	(°/)	02 (ml/l)	PO4-P (µgm at/l)	NO_2-N (µgm at/l)	рĦ	Alk (millival/l)
0	26.92	34.65	4.45	0.39	tr.	-	2.34
6	26.94	34.86	4.46	0.41	0.0	-	2.31
18	26.88	34.65	4.44	0.40	0.0	-	2.32
24	26.46	34.77	4.36	0.45	0.0	-	2.32
30	22.38	34.36	4.34	0.66	tr.	-	2.30
43	18.99	34.42	1.88	1.30	0.7	-	2.29
80	14.84	34.74	0.09	1.84	0.0	-	2.31
131	12.80	34.84	0.12	1.88	2.3	-	2.32
196	11.80	34.80	0.12	1.92	2,5	-	2.32
290	10.63	34.74	0.13	2.06	1.8	-	2.33
388	9.41	34.67	0.09	2.14	0.8	-	2.33
484	8.08	34.60	0.10	2.25	0.2	-	2.34
733	5.91	34.58	0.09	2.36	0.0	-	-

BIOLOGICAL OBSERVATIONS

Productivity

Depth	Chlorophyll "a"	l Bacteria	in situ	Incubator
(m)	(mg/m^3)	(no/ml)	$(mg C/m^3/day)$	$(mg C/m^3/hr.)$
0	0.139	24	2.1	0.59
10	0.176	19	-	0.41
25	0.239	12	-	0.53
50	0.523	19	-	0.12
75	-	30	-	-
100	0.254	9	-	0.18
Zooplan	kton Volume:	77 ml/locom^3	total, 77 ml/100	Om ³ small.

SUBMARINE DAYLIGHT (480 mµ)

Depth (m)	Corr. Sub. Read. (µa)	k/m	°/ _° T/m
2	773		
7	656	0.0394	96.1
12	520	0.0465	95.4
22	340	0.0425	95.8
32	155	0.0786	92.4

Station 25A

M/V <u>Stranger</u>; SCOPE; December 12, 1956; 2108 GCT; 23°31.0'N, 111°22.0'W; 270 fm; wire angle, 3°; wind, 340°, force 3; temp., 70.8°F dry, 65.0°F wet; weather, 02; clouds, 0; sea, 3; swell, 370°, 3 ft.

Depth (m)	Temp. (°C)	(°/)	0 ₂ (m1/1)	PO_4-P (µgm at/1)	NO ₂ -N (µgm at/l)	рĦ	Alk (millival/l)
0 5 15 30 45 53	23.82 23.76 23.73 23.54 23.49 (22.38 (22.48	34.70 34.70 34.66 34.68 34.70 34.59	4.46 4.70 4.67 4.66 4.53 4.41	0.46 0.38 0.40 0.38 0.37 0.50	0.0 tr. 0.0 0.0 tr. 0.3		2.36 2.35 2.35 2.34 2.34 2.34
82 142 196 242 289 390 486	15.34 13.23 11.92 11.38 10.56 8.88 7.80	33.95 34.61 34.71 34.72 34.69 34.59 34.54	3.88 0.39 0.15 0.10 0.15 0.13 0.12	0.82 1.79 1.83 1.86 1.88 1.96 2.02	0.1 tr. 0.0 tr. tr. 0.0 tr.	- - - -	2.29 2.33 2.34 2.34 2.34 2.34 2.34 2.34

OBSERVED

BIOLOGICAL OBSERVATIONS Productivity

			IIOuucuiviuy				
Depth	Chlorophyll "a"	Bacteria	in situ	Incubator	B-	U-	P-
(m)	(mg/m^3)	(no/ml)	(mg C/m ³ /day)	$(mg C/m^3/hr.)$			
0	Lost	-	6.1	1.2	-	-	-
10	0.557	-	3.3	0.62	-	-	-
20	-	-	4.9	-	-	-	-
25	Lost	-	-	-	-	-	-
30	-	-	5.4	-	-	-	-
40	-	-	0.96	-	-	-	-
50	0.698	-	0.67	0.093	-	-	-
70	-	-	0.29	-	-	-	-
90	-	-	_	0.041	-	-	-
100	Lost	-	0.29	-	-	-	-
		, 3	. ,	3			

Zooplankton Volume: 57 ml/1000m³ total, 47 ml/1000m³ small Water Column Productivity: 0.185 gC/m²/day

Station 25A (Cont.)

SUBMARINE DAYLIGET (480 mµ)

Depth	Corr. Sub. Read.		
(m)	(µa)	k/m	°/ _o T/m
2 7 12 22 32 42 51 61 71	708 582 436 253 146 94.7 51.0 21.2 11.6	- 0.0392 0.0578 0.0544 0.0549 0.0433 0.0688 0.0878 0.0603	96.2 94.4 94.7 94.6 95.8 93.4 91.6 94.1

Station 25B

M/V Stranger; SCOPE; December 13, 1956; 0238 GCT; 23°31.5'N, 111°19.0'W; 310 fm; wind, calm; temp., 72.9°F dry, 65.6°F wet; weather, 02; sea, 1; swell, 300°, 2 ft.

OBSERVED

Depth (m)	Temp. (°C)	(°/)	0 ₂ (m1/1)	$PO_{j_4}-P$ (; gm at/l)	NO_2-N (µgm at/1)	рĦ	Alk (millival/l)
0	23.62	-	4.73	0.37	-	-	-
5	23.62	-	4.70	0.35	-	-	-
10	23.63	-	4.57	0.38	-	-	-
14	23.60	-	4.79	0.37	-	-	-
20	23.51	-	4.77	0.39	-	-	-
25	23.50	-	4.70	0.38	-	-	-
30	23.43	-	4.63	0.42	-	~	-
35	-	-	4.79	0.40	-	-	-
40	23.33	-	4.75	0.38	-	-	-
45	-	-	4.75	0.52	-	-	-
49	-	-	4.77	0.46	-	-	-
53	-	-	4.73	0.51	-	-	-
59	-	-	4.45	0.64	-	-	-
73	16.10	-	4.84	0.50	-	-	-
98	13.58	-	2.96	1.10	-	-	-
144	12.96	-	0.42	1.83	-	-	-

BIOLOGICAL OBSERVATIONS

Productivity U-P-Depth Chlorophyll Bacteria Вin situ Incubator "a" (mg/m³) $(mg C/m^3/day)$ $(mg C/m^3/hr.)$ (no/ml)(m) 7.6 0.467 32 88 0 _ _ _ 4.2 0.437 10 _ _ 20 16 3.1 _ _ -_ 25 0.437 _ -_ --_ 23 7.0 30 _ _ _ -----28 _ 40 _ 1.2 --------.... _ _ 50 27 2.1 0.795 -.... 70 0.23 -_ 13 _ --28 -90 _ ---_ 100 0.219 _ _ _ _ _

Water Column Productivity: 0.220gmC/m²/day

Station 25B

(Cont.)

SUBMARINE DAYLIGHT (480 mu)

Depth (m)	Corr. Sub. Read. (µa)	k/m	°/ _° T/m
2 7 12 22 32 41 47 58 69	644 506 376 200 103 48.5 24.5 11.7 5.4	0.0482 0.0594 0.0621 0.0674 0.0793 0.1138 0.0672 0.0764	95.3 94.2 94.0 93.5 92.4 89.2 93.5 92.6

(lm)										
plankton Volume 1(ml) Smal		20	26		34 10					
Zooplankton Volume Total(ml) Small(ml)		50	50		34 10					
Production incubator ay) (mgC/m3/hr)		0.16	0.22	0.096				0.54		0.49
$\frac{1}{(mgC/m^{3}/\tilde{d}_{ay})}$		ۍ. ع		0.76		с. С		°.		с. С
Chloro- phyll"a" (mg/m ³)	0.134 0.134	0.109	.043	.059	.070	.152		.198	.218	.160
$\begin{array}{c} \text{S} & \text{PO}_{\text{H}}\text{-P} \\ \text{($^{\circ}/_{\circ\circ}$)} & (\mu \text{gm-at/1}) \end{array}$	77.0	0.64			0.68	0.67	0.53*	.664	.027	.586
S (°/)	33 .6 8 33.61	33.99 33.98		34.22 33.98	33.84 33.99 34.58	34.56	34.37 34.34	34.72		34.71 34.41
Surface Temp(°F)	66.2 66.2 60.2 7 60.2 7	67.6 66.9 68.7 68.7	6666 67.1 67.1 6666 66.1 7 6666 66 66 66 66 66 66 66 66 66 66 66	70.0 69.1 6	70.9 70.9 70.9 70.9	76.6	75.2 76.5 77.7		82.0 82.0 82.0	82.0 81.9
Long°W	116°26' 116°26' 116°26' 116°29'	116°13' 116°13' 116°09' 116°01'	115 58	115°52' 115°51'	115 42 115 42	114.261	112°56' 112°24' 111'52'	110°21	109,201	100°56° 107°25°
Lat'N	33°01' 29°27' 28°18' 28°18'	27°23' 26°50' 26°17' 25°17'	25°23'	20 20 20 20 20 20 20 20 20 20 20 20 20 2	233 233 233 233 233 233 233 233 233 233	23°18'	22 36 1 22 20 1 22 05 1	21,34	20°281	19°46'
Date	Nov.8,56 Nov.8,56 Nov.3,56 Nov.9,56 Nov.9,56	Nov.9,56 Nov.9,56 Nov.9,56	Nov.9,56 Nov.9,56 Nov.9,56	Nov.9,56 Nov.9,56 Nov.0,56	Nov.10,56 Nov.10,56 Nov.10,56	Nov.10,56 Nov.10,56	Nov.11,56 Nov.11,56 Nov.11,56	Nov.11,56 Nov.11,56	Nov.12,56 Nov.12,56	NOV.12,56 NOV.12,56 NOV.12,56
Time (GCT)	1400 2000 2300 2200		650 805			1000	0200 0800 0800	0041	0200	
BT No.	й‡слир 		0-13		61200 45 -	0-25	 		5 5 F	

BT No.	Time (GCT)	Date)	Lat'N	Long°W	Surface Temp(°F)	S (°/°°)	S PO4-P (°/) (μgm-at/l)	Chloro- phyll _{"a"} (mg/m ³)	$\frac{1}{(mgC/m^3/day)} \frac{1}{(mgC/m^3/day)}$	cubator /m ³ /hr)Tot	Zooplankton Volume al(ml) Small(ml)
	0100 0400 0700 12000 1300	Nov.13,56 Nov.13,56 Nov.13,56 Nov.13,56 Nov.13,56 Nov.13,56	18°57' 18°57' 18°26' 18°26' 17°54' 17°54'	105°59' 105°27' 104°53' 104°21' 103°50' 103°24'	888888 4833.58 433.58 54 54 54 54 54 54 54 54 54 54 54 54 54	34.58 34.66 34.36	.576	.124	8.2	0.59	
+005 ++++	0100 0400 10000	Nov.14,56 Nov.14,56 Nov.14,56 Nov.14,56	17°08' 16°52' 16°37' 16°21'	102°12' 101°40' 101°08' 100°37'	84.6 84.6 83.1 84.7	34.33	478	711.			
	2200 0100 0400 0700	Nov.14,56 Nov.15,56 Nov.15,56 Nov.15,56	15°01' 15°44' 15°30'	100°00' 99°28' 98°59'	85.8 85.1 95.1 95.1	33.64 *		.148 *			
	1000 1300 1600	Nov.15,56 Nov.15,56 Nov.15,56				33.96		.843	32.	6. 0	
- - - - - - - - - - - - - - - - - - -	*Sample da -1 0100 -2 0400 -3 0700	data entered 00 Nov.16,56 00 Nov.16,56 00 Nov.16,56	5	TIC H	position 82.4 82.6 82.9	33.69	.460	912.			
6-5 6-5 6-5	1200 1500	Nov.16,56 Nov.16,56		95°10° 94°42'	81.0 81.0	33.57	.680	.344		ъ.t	
7-7 7-2 7-3	0000 0300 0300	Nov.17,56 Nov.17,56 Nov.17,56	12°34' 12°20' 12°07'	93°43' 93°16' 92°49'	82.5 83.1 82.6	33.16	.592	412.			
	1200 1500	Nov.17,56 Nov.17,56		91°26'	82.8 81.0	32.60	.485	.519	23	lost	

BAT. No.	Time (GCT)	Date	Lat N	Long ^w	Surface Temp(°F)	(°/••)	Р04-Р (µgm-at/1)	Chloro- phyll ₃ "a" (mg/m ³)	$\frac{1n}{(mgC/m^3/day)(mgC/m^3/hr}$	$\frac{1n}{(mgC/m^3/day)(mgC/m^3/hr)} \frac{2000}{Total(m1)} \frac{1}{Total(m1)} \frac{1}{Tot$
8888 2-1 2-1	0000 0300 0900 0300	Nov.18,56 Nov.18,56 Nov.18,56 Nov.18,56		90 251 89 241 88 53	80.1 78.4 77.5	33.57 33.40	.781	.352		
88888888888888888888888888888888888888	2200 2200 11400 11500 11500 11700 2100 22000 22000	Nov.18,56 Nov.18,56 Nov.18,56 Nov.18,56 Nov.18,56 Nov.18,56 Nov.18,56 Nov.18,56	10,16 10,16 09,40 09,17 09,08 09,17 08,58	88 88 88 88 88 88 88 88 88 88	77777777777777777777777777777777777777	33.64	1.01	.320	14.	0.
- 47 -	0400 0500 0600 0700 0800 0900	Nov.19,56 Nov.19,56 Nov.19,56 Nov.19,56 Nov.19,56		8888833 88888888 888888888888888888888	78.3 78.1 78.1 77.9 77.9 77.9					
0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0		Nov. 19,56 Nov. 19,56 Nov. 19,56 Nov. 19,56 Nov. 19,56 Nov. 19,56		888 447 1 888 441 1 888 44	2.5.5.5.4.4.2.2.2. 2.1.1.5.4.4.2.2.2.2.2.2.2.2.2.2.2.2.2.2.2.2.2					
99999999999999999999999999999999999999	1700 1815 2000 2200 2200 2200 0130 0130 0130 0130	Nov.19,56 Nov.19,56 Nov.19,56 Nov.19,56 Nov.19,56 Nov.20,56 Nov.20,56	00000000000000000000000000000000000000	888.511 888.511 888.555 888.556 888.556 89.59 89.01 89.01 89.03	777.0 777.0 777.0 76.8 76.8					

۵.

<pre>ion Zooplankton incubator volume (mgC/m³/hr) Total(ml) Small(ml)</pre>		0.27		۲۲.								0.1	
Production (mgC/m ³ /day) (mg		5.9		2.3			0,11		6.5	14.6		0.81	
Chloro- phyll,"a" (mg/m ³)	.221	.260	.152	.208	.159				.232	.345	.527	tttl5.	
PO ₄ -P (μgm-at/l)	456	.557	.380	.163	.423	.631			.736		. 423	-t27	
S (°/°) (₁	33.09	33.08	33.21	32.75	32.81		33.20	33.40	33.22	33.30	33.15	33.10 33.10 33.17	33.39
Surface Temp(°F)	7.97 7.97 7.92	78.8	79.5 78.9 8.9	78.8	80.8 79.9	77.9 20.9	77.9	76.6	C.01	- Cl C - O G		00000000000000000000000000000000000000	0.02
Long°W	84°06' 84°33' 85°02'	86°031 86°131	87°11' 87°37' 88°15'	$m \cap n$,61°09	91°11' 91°14'	91°221° 91°221°	91 ° 52'	000 000 000 000 000 000 000 000	03°30'	01.10 07.10	95°16'	96° 30 '
Lat'N	04°20' 04°31' 04°33'	04,58,00,05,00,00,00,00,00,000,000,000,000	05°38' 05°48' 06°04'	06°34' 06°34'	07°08' 07°27'	07°47'	07.53	191°80	190,60	191.60 91.60	10,51,	100 110 100 111 100 111 100 111 100 111	15°03'
Date)	Dec.3,56 Dec.3,56 Dec.3,56	nîmîmî	Dec.4,56 Dec.4,56 Dec.4,56	Dec.4,56 Dec.4,56 Dec.4,56	Dec.5,56 Dec.5,56		Dec.5,56 Dec.6,56	Dec.6				Dec.7,56 Dec.7,56	Dec.
Time (GCT)	0000 0300	U9U0 1200 1500	0000 0300 0900	0900 1200 1500	0000 0000	0900 1200		0000	1200	1800		0000	
BT No.	1-7-1 1-7-2	17-5 17-5	18-1 18-2 18-2	18-1 18-5 18-5	19-1 19-2	-49-	50-1 50-1 50-1 50-1 50-1 50-1 50-1 50-1	20-6		20-02 20-10 20-10	11-02 21-02	20-15 20-15 20-15	20-18

BT Time (GCT) No.	r) Date	Lat N	M° Shiol	Surface	S (°/°)	$\frac{POl_{l}-P}{(\mu\mu\mu-at/1)}$	$\frac{Chloro-}{phyll_{mg/m}^{n}}$	$\frac{1}{\left(m_{efC}/m^{3}/da_{y}\right)} \frac{Production}{\left(m_{eC}/m^{3}/da_{y}\right)} \frac{1}{\left(m_{eC}/m^{3}/\ln^{2}\right)}$	$ \frac{1}{(\mathfrak{m}_{s}^{2}C/\mathfrak{m}^{3}/d\mathfrak{s}_{y})} \frac{\mathfrak{P}roduction}{(\mathfrak{m}_{s}^{2}C/\mathfrak{m}^{3}/d\mathfrak{s}_{y})} \frac{1}{(\mathfrak{m}_{s}^{2}C/\mathfrak{m}^{3}/\mathfrak{m}_{y})} \frac{1}{(\mathfrak{m}_{s}^{2}C/\mathfrak{m}^{3}/$
	0 Dec.8,56 0 Dec.8,56 0 Dec.8,56	120021 120144 130051	96°591 97°33 98°05	77 - 77 76 - 8 82 - 4	33.68	.665			
21-4 0400 21-5 1200 21-6 1500		13°47' 14°12'	90,911 11,00	0 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2	33.68	.303	.251	6.2 0.91	ľ
		14.53. 15.051 15.251	100°33' 100°49' 101°21'	88888 8399 8399 8399 8399 8399 8399 839	33.56 33.78 33.80	.829	.172		
22-5 1200 22-6 1600	0 Dec. 9, 56	16°32'	102.431	83.1 83.3	34.14	.373	.146	0.46	ę
-23-1 0100 -23-2 0400 -23-3 0700	0 Dec.10,56 0 Dec.10,56 0 Dec.10,56	17°11' 17°35' 17°59'	103°25' 103°50' 104°15'	85.0 85.0 85.0 85.0 85.0 85.0 85.0 85.0	34.13	• 433	.139		
23-5 1000 23-5 1300 23-6 1600			105°29' 105°29'	81.9 81.7 80.2	34.52	.332		1.8	~
			106°24' 106°49' 107°14'	78.1 78.1 77.4	34.65 34.76	·513	.229		
24-5 1000 24-5 1300 24-7 1900	0 Dec.11,76 Dec.11,76 Dec.11,56 Dec.11,56	21,00 21,33 21,33 21,33 21,00	108 03 108 03 108 52	10.00	34.72 35.08	,467	.371.	0.39	6
			109 20 109 48 110 11	71.6 71.1 71.6	34 .6 9 34 .6 4	.738	.290		
			'70'ILL	73.0					

SCOPE

SURFACE CURRENT VELOCITY AND DIRECTION (GEN OBSERVATIONS)

Lat °N	Long W	Direc- tion	Velocity cm/sec.	-25 33	long "*	Direc-	Velocity oz,sec.
24 24 10 10 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0	2343254429117221102123532733512753055555555555555555555555555555555555	223330000012332233000012559953023319812884038000432866 4724461806574968490125599530233198128884038000432866	27.5 94.6 34.2 47.6 45.2 69.5 78.2 32.6 27.7-questionable 25.2 85.9 50.7 26.4 54.6 29.3 28.0 36.9-questionable 3.4 3.5 9.5 29.5 12 10.5 29.5 12 10.5 59.5 12 10.5 85.5 81.4 80.1 52.1 42.3 55.5 81.4 80.5 85.5 81.4 80.1 52.1 40.5 85.5 81.4 80.1 52.1 10.5 85.5 81.4 80.1 10.5 81.1 10.5 10.5 10.5 10.5 10.5 10.5 10.5 1	21°26'			

PART 2. SCIENTIFIC REPORTS

POSSIBLE APPLICATION OF A BACTERIAL BICASSAY

IN PRODUCTIVITY STUDIES

b∵.

William Belser *

An increasing number of reports in the literature, demonstrating requirements for growth factors by various marine algae (Lewin, 1954, Provasoli and Pintner, 1943a, Sweeney, 1954), the effects of external nutrilites on feeding responses (Loomis, 1953, Collier, 1950), and the possible implication of organic micronutrients in the discontinuous distribution of marine plants and animals (Lucas, 1939, Provasoli, 1956, Margalef, 1956, Wilson, 1956) has led to the formulation of this program. Many of these organic materials are required in extremely small amounts, and might be expected to be present in sea water in very low concentration. Previous attempts to isolate and characterize some of them have been moderately successful (Johnston, 1955, Provasoli and Pintner, 1953b), although somewhat cumbersome. Relatively high salt concentrations in sea water preclude direct chromatography of the organic materials, and require their preisolation, either by absorption or desalting. Dealing with materials present in micrograms-perliter quantity presents a formidable task.

With these facts in mind, I have considered the possibility of establishing a series of biochemical mutants with a wide spectrum of nutritional requirements, which might be employed directly as bioassay organisms in sea water. Attempts to train Escherichia coli, in which many mutants are already in culture, to grow in sea water were time consuming and impractical. Therefore, a number of marine bacteria were screened for desirable characteristics, and Serratia marinorubrum (ZoBell, 1944) was selected as the most suitable of these organisms for this purpose. S. marinorubrum is an easily distinguishable red pigmented organism, which will grow well in a medium composed of inorganic salts, with glycerol as the sole source of carbon. In addition, it shows a wide range of salt

* Public Health Service Research Fellow of The National Cancer Institute.

tolerance, growing in media with the salinity of fresh water, as well as in threefold concentrated sea water. This heterosmotic feature suggests the value of the organism for bloassay of rivers and lakes, as well as the ocean, in tide pools, estuaries and seasonally landlocked sloughs.

To date, several mutants have been obtained by ultraviolet irradiation of <u>S. marinorubrum</u>. One of these requires biotin, and will respond to concentrations in the order of 1 to 5 mmg,ml. (see Table 1). The second mutant has a specific requirement for uracil, and responds to concentrations between 10 and 100 mmg,ml. The third mutant thus far obtained has a nonspecific purine requirement, and will grow when supplied with any of the purine bases or their ribosides. The most sensitive response obtained with this mutant is to hypoxanthice in the range from 10 to 100 mmg,ml.

In a preliminary field total designed to test the bioassay system, some 30 sea water samples were tested. These samples were taken for me on SCOPE in waters off the coast of Mexico and Central America. The results of these tests showed guite definitely that the bioassay system has merit, since a fairly wide distribution of biotin was observed, with sporadic occurrence of uracil, and only one instance of purine (see Table 2). Controls failed to show any evidence of either reversion of any of the mutants or contamination of the water samples

MATERIALS AND TECHNIQUES

The technique for mutant induction involves the irradiation of cultures in the logarithmic phase of growth and screening for mutants after incubation. This has been done by minimal enrichment and delayed enrichment techniques. The mutants, so isolated, are identified with regard to their specific re-

Growth Requirements of Mutant	Concentration for Optimum Growth	Growth Limiting Concentration Range	Lower Limit Detectable
Purine*	10 - 2.5	.705	.05
Biotin	5 - 1.0	.04002	.002
Uracil	5 - 1.0	1.008	.08

RESPONSE OF MUTANTS TO VARYING CONCENTRATIONS OF THEIR SPECIFIC NUTRILITES

Concentrations expressed in μ g/ml.

* Purine response was measured using hypoxanthine as growth factor. The mutant responds to all four purine bases.

quirements, and their optimal and limiting substrate levels established by the optical density method of recording growth response. For use in testing sea-water samples, these are first filtered through Whatman No. 2 paper to remove any large particles and then autoclaved. The water samples are next divided into a number of replicate samples containing glycerol as added carbon source, and are then inoculated with the mutants. Positive tests may be measured in the Beckman model DU spectrophotometer for quantitative estimation of nutrient concentration, following a suitable period of incubation. Each test of unknown sea water is accompanied by reversion tests on the mutants, as well as viability controls.

Attempts are in progress to standardize the technique of testing sea-water samples so that any technician, either biologist or nonbiologist, may use it at sea. This would permit efficient utilization of shipboard facilities in that a technician in chemistry, for example, could carry out these tests along with the chemical work, and leave space for some other member of the scientific team, by making it unnecessary for a biological technician to go along for this purpose.

EXPERIMENTAL RESULTS

The results of the tests on sea-water samples are presented in Table 2 along with other SCOPE data to show correlations.

In view of the scant nature of these data and the small number of samples examined, it would be presumptuous to attempt to draw any conclusions from this test. The major purpose of the test was to assess the validity of the bioassay system, and it seems to indicate that the syste has merit. There is an indication that soluble organic materials are present and are distributed both laterally and vertically in discontinuous fashion. In comparing the bacterial counts and the chlorophyll concentration with the occurrence of organic material, it was encouraging to note that presence of bacteria seems to be inversely correlated, while there is direct correlation between algal production and growth factor occurrence. Whether the algae are present because growth factors are present, or vice versa, and whether high bacterial numbers occur at the expense of external growth factors are problems which will have to await further experimentation. A considerable amount of data would

RESULTS OF TESTS OF SEA-WATER SAMPLES WITH THE MUTANTS

Date of Sample	Depth (meters)	Sterility control	в_	U ⁻	<u>р</u> _	Bact. c o unt	Depth chloro. DETN.	mgm/m ³ chlorophyll "a"
11/10/56	Surface	0	03	0	0	2	Surf.	0.125
11/12/56	Surface	0	0	0	0	_	Surf.	0.204
11/15/56	Surface	0	++	0	0	34	Surf.	0.577
11/16/56	Surface	0	0	0	0	546	Surf.	0.380
11/21/56	Surface	0	Ŧ	0	0	-	Surf.	0.308
11	5	0	Ŧ	0	0	-	8	0.310
tt	10	0	0	0	0	-	12	0.340
tt	14	0	I	0	0	187	16	0.342
17	18	0	Ŧ	0	0	4200	20	0.335
17	22	0	÷	-	0	383	-	_
**	27	0	đ	ਰੋ	0	3200	30	0.458
11	52	0	0	0	0	-	-	-
**	100	0	0	0	0	-	100	0.280
11	200	0	0	0	0	-	200	0.092
12/1/56	Surface	0	0	0	0	75	Surf.	0.329
11	10	0	0	Ŧ	++	3	10	0.272
11	50	0	0	Ŧ	0	l	50	0.491
				•			75	No RDG
**	75	0	#			1	100	0.101
12/2/56	Surface	0	0	0	0	23	Surf.	0.196
11	5	0	-++-	0	0	6	5	
**	10	0	++	+	0	5	10	0.215
11	25	0	#	+	0	2	25	0.261
11	50	0	++	÷	0	23(8)	50	0.633
**	75	0	+	0	0	5	75	
**	100	0	Ŧ	0	0	2	100	0.105
12/3/56	Surface	0	+	0	0	114	Surf.	0.169
12/5/56	Surface	0	++	0	0	97	Surf.	0.129
12/7/56	Surface	0		0	0	7	Surf.	
	25	0	#	0	0	14	25	0.905

1) Growth controls where specific supplement was added were all ++++ growth. Reversion controls were all negative.

2) Glycerol $(0.2^{\circ}/_{\circ})$ added to all samples as carbon source.

^{3) ++++ =} non limiting concentration (optimal growth)
 ++,+, -, 0 = limiting concentrations; moderate, slight, very slight,
 and no growth respectively.

have to be gathered and processed before any definite conclusions could be drawn, but at this writing these tests certainly present a possible approach to an exciting aspect of primary production in the sea.

BIBLIOGRAPHY

- Collier, A., et al. 1950. A preliminary note on naturally occurring organic substances in sea water affecting the feeding of oysters. Science, Vol. 111, pp. 151-152.
- Johnston R. 1955. Biologically active compounds in the sea. Jour. Mar. Biol. Assn. U. K., Vol. 34, pp. 185-195.
- Lewin, R. A. 1954. A marine <u>Stichococcus</u> sp. which requires vitamin B₁₂ (Cobalamin). Jour. Gen. Microbiol., Vol. 10, pp. 93-96.
- Loomis, F. 1953. Glutathione stimulation of feeding response in Hydra. Unpublished.
- Lucas, C. E. 1947. The ecological effect of external metabolites. Biol. Rev., Vol. 22, pp. 270-295.

Margalef, R. 1956. Temporal succession and spatial heterogeneity in natural phytoplankton. Proc. Sym. Perspectives in Mar. Biol. (in press), U. of Calif. Press.

- Provasoli, L. 1956. Growth factors in marine organisms. Ibid (in press).
- Provasoli, L., and I. J. Pintner. 1953b. Assay of vitamin B₁₂ in sea water. Proc. Soc. Protozoologists, Vol. 4, No.10.
- Sweeney, B. M. 1954. <u>Gymnodinium splendens</u>, a marine dinoflagell- ate requiring vitamin B₁₂. Am. Jour. Bot, Vol. 41, pp. 821-824.
- Wilson, D. P. 1956. Some problems in larval ecology related to the localized distribution of bottom animals. Proc. Sym. Perspectives in Mar. Biol. (in press), U. of Calif. Press.
- ZoBell, C. E. 1944. A list of marine bacteria including descriptions of sixty new species. Bull, Scripps Inst. Oceanogr., Vol. 5, pp. 239-292.

SCOPE MEASUREMENTS OF PRODUCTIVITY, CHLOROPHYLL "a", AND

ZOOPLANKTON VOLUMES

by

R. W. Holmes, M. B. Schaefer, and B. M. Shimada

The productivity, chlorophyll "a", and zooplank- and varied from 6 to 10 minutes. The results ton volume data obtained on SCOPE have not yet been examined in detail. However, some aspects of sampling variability, and certain of the more in light incident in different samples on the obvious relationships among these quantities, have been examined and are, in some instances, compared with similar data and relationships obtained in 1955 on Eastropic Expedition (Holmes, (standard error) of each determination due to Schaefer, and Shimada, 1957).

SAMPLING VARIABILITY

Measurements of C^{14} uptake and of chlorophyll "a" are subject to sampling variability due to the nature of the distribution of phytoplankton organisms in the sea. The question, therefore, arises as to how representative of a general area is a single sample taken from that area.

In order to investigate sampling variability over a relatively small area, as a first approach replicate giving the very high value of 680 to studying this problem, on November 22nd, 1956 cpm. which may be aberrant. in the vicinity of 09°25' N, 89°31' W, we collected samples from a grid of nine stations on a square pattern, the station spacing being three miles. The station arrangement is shown in Figure 3. These stations were visited in the order shown, between 0915 and 1202. At each station were taken three replicate surface samples for the determination of ${\rm C}^{14}$ uptake and a single surface sample for the determination of chlorophyll "a".

 $\rm C^{14}$ uptake was determined in a 250-ml. aliquot of each replicate, using 0.9 $\mu \rm C$ of $\rm C^{14},$ and incubating each sample for four hours in the shipboard incubator at the prevailing sea-surface temperature, and at an illumination of approximately 1000 foot-candles. The incubated samples were filtered through one-inchdiameter HA Millipore filters, which were dried in a desiccator and subsequently counted in a proportional counter (Nuclear Chicago PC-1). The counting time was of a duration to give a total of at least 1000 counts in each instance,

are given in Table 3 in counts per minute, the uptake (count) being corrected for variations assumption that, over the range of intensity of illumination employed, the uptake is proportional to the illumination. The error the statistical variability of counting is in each case, not over five counts per minute.

For each set of replicate samples, we show in Table 3 the mean and standard deviation. It may be observed that the values of standard deviation are all rather similar, and are not correlated with the means, except for Station 9-SC-7 where the value of the standard deviation is very much larger than that at any of the other stations. The large variation at this station appears to be due to the single

An analysis of variance of the nine sets of three replicates (Table 4), including the suspect sample, indicates that the variance among station means is no greater than could be expected to occur by chance in the light of the variability among replicates within stations. The grand mean of 27 observations is 306.5 cpm., with a standard deviation of 90.9.

Now it may be seen that the value of 680 cpm., deviating by 374 cpm., from the mean of all observations, is a deviation of over four standard deviations from the mean value, and thus is very unlikely to be a chance event. It appears that this sample is somehow quite aberrant and should be discarded from the analysis. Omitting this sample (Table 4) decreases all variance components very greatly. The analysis of variance with this sample omitted still indicates no difference among stations that could not be expected by chance

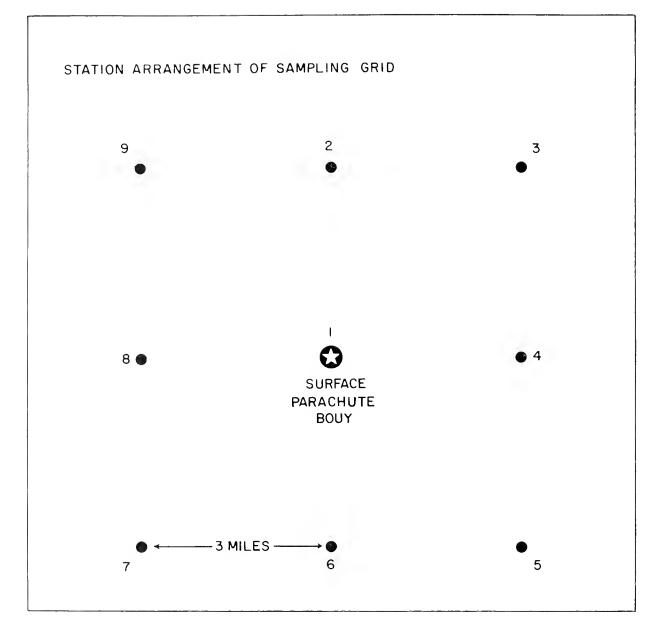


FIGURE 3. Station arrangement in sampling grid

TABLE	3
	-

Station	Count	ts per m Replicat 2		Mean	Variance (mean square)	Standard deviation
9-SG-1 9-SG-2 9-SG-3 9-SG-4 9-SG-5 9-SG-6 9-SG-7 9-SG-8 9-SG-9	Gra clu	297 308 275 217 487 324 255 260 284 and mean and mean and mean	5 G- 7	256 279 292 279 389 270 408 297 288 306.5	1,911 785 273 3,004 7,558 2,375 55,777 1,052 26 8,266 2,791	43.7 28.0 16.5 54.8 86.9 48.7 236.2 32.4 5.1 90.9 52.8

TABLE 3 c^{14} uptake in replicate samples from nine stations of sampling grid

TABLE 4

ANALYSES OF VARIANCE OF C14 UPTAKE AT STATIONS OF SAMPLING GRID

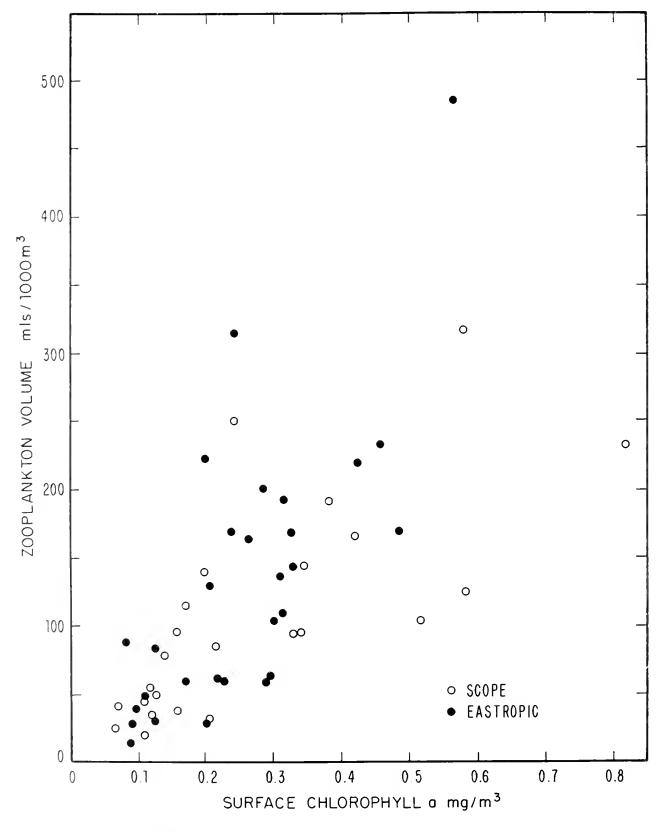
Source of variation	Degree of freedom	Sum of squares	Mean square	Variance ratio
All observations: Total Among stations Within stations	26 8 18	214,090 69,384 145,525		1.073
Omitting station 9-SG replicate 3: Total Among stations Within stations	-7 25 8 17	69,781 35,232 34,549	2,791 4,404 2,032	2.17

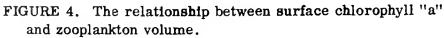
TABLE	5
-------	---

Station (Chlorophyll "a"
No.	mg/m ³
9-SG-1	0.424
9-SG-2	0.429
9-SG-3	0.365
9-SG-4	0.400
9-SG-5	0.404
9-SG-6	0.334
9-SG-7	0.400
9-SG-8	0.451
9-SG-9	0.463
Meas Standard deviation	

CHLOROPHYLL "a" CONTENT OF SURFACE SAMPLES TAKEN AT THE NINE STATIONS OF SAMPLING GRID

•





from the within-station variability. With the aberrant sample discarded, we have a grand mean of 292 cpm. with a standard deviation of 52.8 cpm.

There is then, no evidence of heterogeneity among the nine stations. Any single sample should give a fair estimate of the productivity of this 6 mile square area, with, however, a standard error of 52.8 cpm. which is $18^{\circ}/_{\circ}$ of the mean value encountered.

Chlorophyll "a" was determined from a single 6 liter surface sample at each station. The results are given in Table 5.

Since we have only a single chlorophyll sample from each station, we cannot examine the question of heterogeneity of this constituent among stations. The degree to which any single sample may be expected to represent the mean value within the area may, however, be judged from the standard deviation among the nine samples of 0.048 mg/m^3 , which is $12^{\circ}/_{\circ}$ of the mean value.

Superimposed upon sampling variability, of course, would be that due to the inherent diurnal periodicity in photosynthesis and chlorophyll (see p. 82). However, such a periodicity is not evident in these particular data.

SURFACE CHLOROPHYLL "a" ZOOPLANKTON VOLUME RELATIONSHIPS

In figure 4 surface chlorophyll "a" and zooplankton volume data obtained on both SCOPE and EASTROPIC are illustrated. While there is some scatter, the observations reveal that a positive relationship exists between these two quantities. The correlation might have been improved had we a sufficient number of vertical chlorophyll "a" profiles for integration and comparison with the zooplankton volumes.

Similar data from mid to high northern and high southern latitudes have frequently shown an inverse correlation, or none at all. The lack of extreme variation in this relationship indicates that there may exist in these tropical waters a situation more closely approaching a steady state condition than is found in other waters. Furthermore, the general agreement among surface chlorophyll "a", surface productivity, and nooplankton volume shows that any one of these will serve to indicate the general level of the other two.

PRODUCTIVITY PER UNIT CHLOROPHYLL "a" IN SUR-FACE PHYTOPLANKTON

The relationship between photosynthesis and chlorophyll "a" concentration has been studied and discussed by a number of investigators - see for instance Glendenning et al. (1956), Rabinowitch (1956), Ryther (1956). Few of these observations include data for marine phytoplankton species, but it is of interest to note that for those studied the ratios are quite similar to those observed in some land plants and algae.

The SCOPE data available for this comparison are of two types: a) the photosynthetic rate obtained with <u>surface</u> samples inoculated with C^{14} and incubated for four hours under constant light (1000 \pm 40 foot-candles) at approximately the temperature of the sea surface, and b) the rate based on C^{14} inoculated samples trailed astern of the vessel at the <u>surface</u> for about six hours (sunrise to noon, and noon to sunset). Chlorophyll "a" determinations were made with water samples collected at, or nearly at the same time, as the samples for the photosynthesis studies.

The amount of C^{14} fixed per hour at 1000 footcandles in surface water samples is presented as a function of surface chlorophyll "a" concentration at each station in figure 5. While there is considerable scatter, the data appear to fall into two discrete clusters. Best-fit lines "drawn" by eye through these two groups yield the following rates: 7.3 and 2.5 mg C/hr/mg chlorophyll "a", or, 26.8 and 9.2 mg $CO_2/hr/mg$ chlorophyll "a", respectively. Ryther and Gertsch (1957) give an average value of 3.7 mg C/hr/mg chlorophyll "a" for natural populations at 1500 foot candles. This

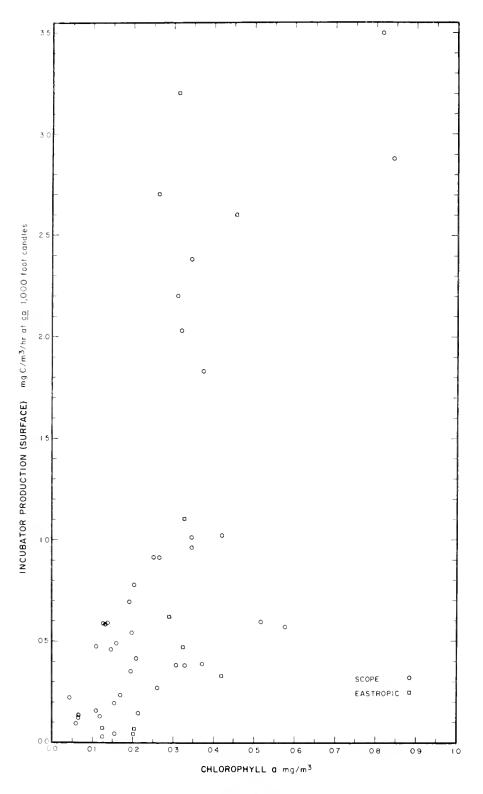
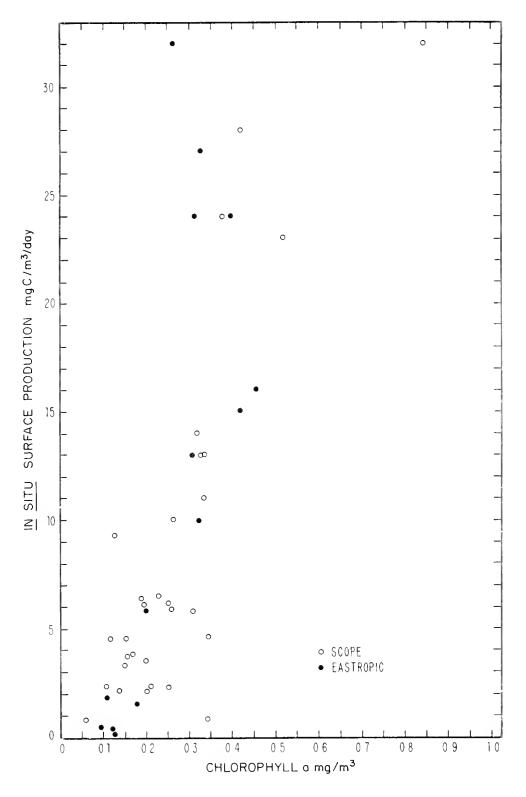
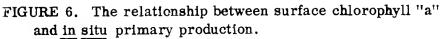


FIGURE 5. The relationship between surface clorophyll "a" and incubator production.





latter value is very similar to the average value reported above for the group exhibiting the low assimilation factor when the difference in light intensities between the two sets of experiments are equated. Talling (personal communication) has observed similar rates in cultures of the marine diatom, <u>Chaetoceros affinis</u>.

The high values (ca. 20 mg C/hr/mg chlorophyll "a") observed at a few stations are somewhat anomalous and difficult to interpret. The stations where such high values were observed were all located in the region of the thermal anticline. These higher photosynthetic rates may be associated with differences in the species composition and/or some difference in the physiological state of the organisms.

The "average" in situ rates (see Fig. 6) observed on the expedition on a per-hour basis (assuming a 12-hour day) are somewhat lower than the rates observed in the incubator, averaging about 4.2 mgC/hr/mg chlorophyll "a". Again, the highest ratios are observed at stations in the region of the thermal anticline off Costa Rica. The lower "average" value may be the result of inhibition of photosynthesis during the brightest portion of the day.

As mentioned above, there is considerable variability among individual values. Some of the variability is doubtless associated with the techniques employed but much of it is certainly quite real. A study of the possible effect of differences in species composition of the phytoplankton on the productivity-chlorophyll ratio will be examined in the near future.

These data yield further confirmation of Ryther's suggestions (1956) that it should be possible to estimate productivity from the concentration of chlorophyll "a" in sea water. However, the precision of such an estimate from chlorophyll "a" concentration would be rather poor in the Eastern Tropical Pacific. Furthermore, it appears that departures from the "average" value of carbon assimilation per unit chlorophyll "a" that is observed in the surface water become more pronounced in samples collected deeper in the photic zone.

BIBLIOGRAPHY

- Clendenning, K. A., T. E. Brown, and H. C. Eyster. 1956. Comparative studies of photosynthesis in <u>Nostoc muscorum</u> and <u>Chlorella pyrenoidosa</u>. Can. J. Bot., Vol. 3¹, pp. 9¹3-966.
- Holmes, R. W., M. B. Schaefer, and
 B. M. Shimada. 1957.
 Primary production, chlorophyll, and zooplankton volumes in the Eastern Tropical Pacific Ocean. Inter-Am. Trop. Tuna Comm., Bull., Vol.2, No. 4.
- Manning, W. H., and R. E. Juday. 1951. The chlorophyll content and productivity of some lakes in northern Wisconsin. Trans. Wis. Acd. Sci., Arts, and Let., Vol. 33, pp. 363-393.
- Rabinowitch, E. I. 1956. Photosynthesis and related processes Vol. 2, Part 2, Kinetics of photosynthesis, pp. 1211-2088, Interscience Publishers, N. Y.
- Ryther, J. H. 1956. The measurement of primary production. Limm. and Ocean., Vol. 1, No. 2, pp.79-93.
- Ryther, J. H., and C. S. Yentsch. The estimation of phytoplankton production in the ocean from chlorophyll and light data. Limn. and Ocean., Vol. 2, No. 3, pp. 281-286.
- Steemann Nielsen, E. 1952. The use of radio-active carbon (C¹⁴) for measuring the organic production in the sea. J. du Conseil., Vol. 18, No.2, pp. 117-140.

Ъy

Robert W. Holmes

To estimate the size ranges of photosynthesizing phytoplankton in tropical waters, three simple experiments were performed on SCOPE. In each experiment a surface water sample of 0.5 or 1.0 liter was inoculated with approximately 20 μc of C^{14} and placed just below the sea surface for incubation. After 2-3 hours' incubation an aliquot was taken from the sample and passed through a series of filters in the following order: a disk of No. 20 bolting silk (mesh size 106µ x 106µ by measurement), a disk of nylon bolting material (mesh size 30µ x 30µ by measurement), an AA Millipore filter (pore size specified by the manufacturer as $0.8\mu \pm 0.05\mu$), and lastly an HA Millipore filter (pore size likewise specified as 0.45µ ± 0.02µ). In addition in 2 experiments another aliquot was filtered directly through an HA Millipore filter. The pieces of netting and filters were dried and counted in the usual manner (see p. 7). The results of these experiments are given in Table 6.

From these data it can be readily seen that the activity of organisms retained by the bolting silk and nylon bolting material represented a small fraction of the total activity. In two out of three experiments only about one-half of the total activity was retained by the AA Millipore filter.

It is difficult to believe that all of the activity passed by the AA Millipore filter was contained in bacterial cells. Dark-bottle $C^{\perp 4}$ fixation in experiments of 6 hours' duration in these same waters usually averaged $10^{\circ}/_{\circ}$ and never exceeded $18^{\circ}/_{\circ}$ of the lightbottle uptake. It seems more plausible to suggest two alternative explanations. Extremely small photosynthesizing organisms (less than about $l\mu$) may have been present in these waters and passed through the AA filter and/or the bulk of material passed by the AA Millipore filter may have been cell fragments produced by the rupture and disintegration of some of the cells as they impinged upon the membrane-filter surface during filtration. Unfortunately the

water samples collected for the purpose of flagellate enumeration and identification have not yet been examined carefully but it appears from a cursory examination that the smallest naked flagellate visible in these samples are between 1-1.5 μ in "diameter." Organisms smaller than those observed in the fixed material may exist in the sea but may not have been preserved adequately enough to permit enumeration or identification. However, it seems unlikely that a significant portion of the total photosynthesizing biomass could have been such organisms.

It seems more plausible that the material passing through the AA millipore filter was largely in the form of protoplasmic fragments released from fragile cells which ruptured on the filter surface. That small naked flagellates do disintegrate as a result of filtration has been observed by the author and by Dr. W. Rodhe (personal communication) by comparing the flagellate abundances on cleared Millipore filters with those in unfiltered samples. Confirmation of this fragmentation hypothesis has also been observed by the author and Dr. R. Lasker (unpublished results) who used radioactive bacteria-free cultures of Chlamydomonas sp. Of nine aliquots, three were filtered through AA Millipore filters, three through HA, and three through PH. No essential, difference in the activities of the HA and PH filter membranes was observed whereas the activity in the AA Millipore filter averaged 12-19°/. less than that observed on the HA or PH filters. The Chlamvdomonas employed in this study was quite healthy and the cells averaged about 8µ in "diameter." A somewhat greater difference was observed in another experiment when the filtrate of a norbacteriafree culture from the AA filter, was passed successively through an HA and PH filter --here the AA filter passed about 32°/o of the activity retained by all three filters. This apparent difference in retention is probably the result of fragmentation caused by the filtration through the AA Millipore filter and the passage of some bacteria less than 0.8 μ in size.

Exp. # 1 Nov. 20, 1956

<u>ryb.</u>	II^{\perp}	110	V • 4	20, 19.	<u></u>								
No.				Fi	ltration	pro	cedu	re			Filter activity c/m	°/。 o total activi	
_	500	mls	fi	ltered	through	HA	Mill	ipore			1058		
1 2 3 4		mls mls "	of "	" filtra "	" ate from "		2 t 3		AA Milli HA Milli	pore	42 32 478 596	3.7 2.8 41.6 51.9	•
									TOTAI	L	1148	100	
Exp.	<i>H</i> 2	De	c. 2	2, 1956	5	·····							
No.				Fil	ltration	pro	cedu	re			Filter activity c/m	°/。 o total activi	
1 2				ltered	through	No.	20	boltin	d through	1	8	0.5	
3	250	11	11	17	from	No.	2	11	nylon through		18	1.1	
<u>)</u> +	250	11	11	77	from	No.	3 f	iltere	Millipore d through	ı	1438	90 .	
				· · · · · · · ·		· · · · · ·		НА	Millipore TOTAI		<u>134</u> 1598	<u>8.4</u> 100	
Exp.	// 3												
No.	<u> </u>			Fil	ltration	pro	cedu	re			Filter activity c/m	°/。 o total activi	
-	250	mls	fil	ltered	through	HA I	Mill	ipore			117	-	
1 2	" 250	" mls	of	" filtra	" ate from				g silk d through	1	0	0	· · · · ·
3					11	No.		11	nylor through	ı	0	0	
Ц	"	11	**	n	11	No.		11	Millipore through	2 1	71	55.5	
		<u> </u>		·				HA	Millipore		<u>57</u>	44.5	
									TOTAL	L	128	100	

These observations are apparently at variance with those reported by Steemann Nielsen (1952). The experiment designed by Steemann Nielsen (1952) is difficult to interpret because all of the necessary information is not given. Nevertheless, it appears that Steemann Nielsen filtered aliquots of tropical surface phytoplankton through filters of varying porosity, the coarsest having a maximum pore size slightly in excess of l μ . In these two experiments no difference in retention was observed between the various filters and Steemann Nielsen concluded that all important autotropic organisms in these samples were larger than l μ .

It would seem from the SCOPE experiments that the conclusion of Steemann Nielsen cannot be applied universally. In two of the SCOPE experiments only about half of the radioactive material was retained on the AA filter, with pore size of 0.8 μ . There can be little doubt that the amount of material retained on filters of this porosity will vary with the population composition and perhaps its physiological condition. While the bulk of the photosynthesizing biomass appears to be in the size range of 1-30 μ , if an assessment of the total activity in a water sample is desired, it would seem advisable to employ filters with a maximum pore size somewhat less than 0.5 μ .

BIBLIOGRAPHY

Steemann Nielsen, E.
 The use of radio-active carbon (C¹⁴)
 for measuring organic production in
 the sea.
 J. du Cons., Vol. 28, No. 2, pp.117-140,
 1952.

DIURNAL VARIATION IN THE PHOTOSYNTHESIS OF NATURAL PHYTOPLANKTON POPULATIONS IN ARTIFICIAL LIGHT

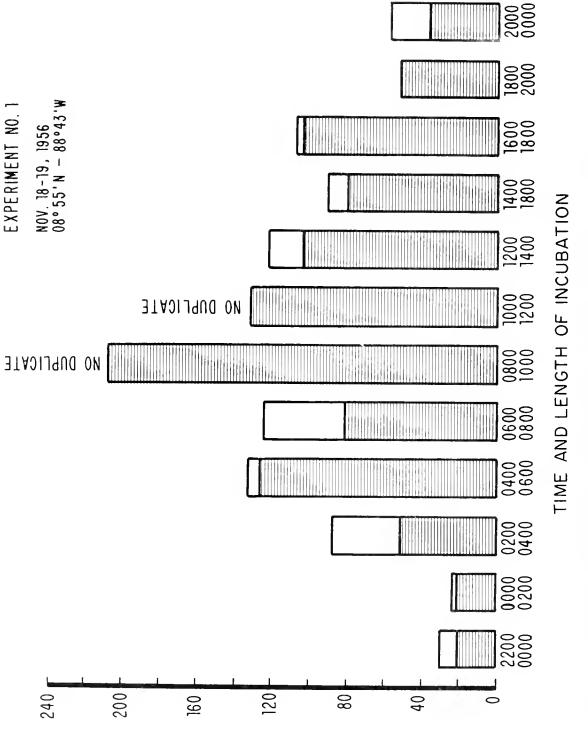
Robert W. Holmes and Francis T. Haxo

Evidence for the existence of a daily periodicity in photosynthesis of marine phytoplankton has been presented in two recent papers (Doty and Oguri, 1957, and Yentsch and Ryther, 1957). These authors observed a diurnal periodicity in photosynthesis in surface-water samples collected at intervals throughout the night and day, illuminated under constant light, and kept at a constant temperature. The rate of photosynthesis in the water samples began to increase during the early morning hours and reached a maximum at about 0800 hours. This was followed by a rapid decrease. At about 1800 hours a low level of photosynthesis was reached and was maintained until about 2400 hours when the predawn rise began culminating in the 0800 maximum.

Two preliminary experiments are described below which were designed to study this rhythm in the eastern Pacific. The techniques employed were similar to those of Doty and Oguri (1957). Surface samples were collected at two-hour intervals (three-hour intervals during the second experiment) alongside a free-floating surface buoy to which was attached, just below the sea surface, a regulation U.S. Navy parachute. The samples were collected in a large plastic bucket and two 250-ml. aliquots immediately inoculated with 1 μc of C^{14} and placed in the shipboard incubator. These samples were subjected to constant illumination (about 1000 foot-candles) from daylighttype fluorescent lights and kept at a temperature slightly exceeding (about 1°C) the sea-surface temperature for approximately two hours. After the incubation period the samples were filtered through 1-in. HA Millipore filters. The filters were then dried and counted in the normal manner (see p. γ). The data were corrected for any slight deviation in the duration of the incubation period. In the second experiment, dark-bottle uptake was subtracted from the uptake in the illuminated bottles. The results of these two experiments are illustrated in figures 7 and 8.

The results of both experiments clearly indicate that the photosynthesis of samples collected between 1800 and 0200 hours was less than that observed during the remainder of the 24-hour period. The difference between the maximum and minimum uptake varied by a factor of 5-8. This is somewhat less extreme than that observed by Doty and Oguri (1957) and greater than that reported by Yentsch and Ryther (1957). In the first experiment (Fig. 7) the daily maximum occurred between 0800 and 1000 hours while in the second experiment (Fig. 8) a maximum was observed between 1200 and 1400 hours. Unfortunately, the 0900 samples were lost in this second experiment for the daily maximum might have occurred at about this time.

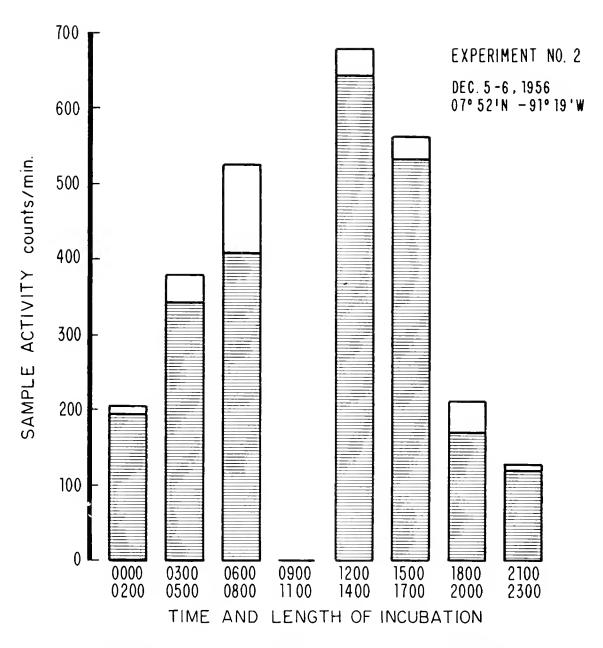
The time of the photosynthesis maximum cannot be defined with certainty since the C14 measurements were discontinuous, representing averaged rates of uptake for 2-3 hour incubation periods of samples collected at 2-3 hour intervals. The data show that the photosynthetic activity of surface waters varies diurnally. Such a periodicity may be associated with concomitant changes in phytoplankton standing crop or may be a manifestation of an inherent photosynthetic rhythm. Samples collected in Experiment No. 1 to assess the first of these possibilities have not yet been examined. In the second experiment chlorophyll "a" determinations made at the beginning of each 3-hour incubation period indicated a fairly constant chlorophyll "a" content. These preliminary results differ from those of Yentsch and Ryther (1957) and B. M. Shimada (personal communication) who have observed a diurnal periodicity in chlorophyll "a" quite similar to the photosynthetic periodicity.

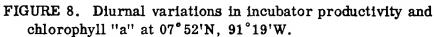


SAMPLE ACTIVITY counts/min.

FIGURE 7. Dfurnal variations in incubator productivity

ovserved at 08°55'N, 88°43'W.





BIBLIOGRAPHY

- Doty, M. S., and M. Oguri. 1957. Evidence for a photosynthetic daily periodicity. Limm. and Ocean., Vol. 2, No.1, pp.37-40.
- Yentsch, C. S., and J. H. Ryther, 1957. Short-term variationsin phytoplankton chlorophyll and their significance. Limm and Ocean., Vol. 2, No. 2, pp. 140-142.

ATTACHMENT OF MARINE BACTERIA TO ZOOPLANKTON

by

Galen E. Jones

The abundance of marine bacteria living free in the open sea is low (ZoBell, 1946). The reasons for the small numbers of microorganisms in the sea have been considered by various workers as summarized by Orlob (1956). Dilute amounts of organic matter on solid surfaces cause marine bacteria to concentrate on these surfaces (Stark et al., 1938; Heukelekian and Heller, 1940). In addition, many marine bacteria demonstrate definite attachment propensities (ZoBell, 1946). If marine bacteria attach to the surface of living organisms there is the opportunity for a symbiosis between the bacteria and the phytoplankton or zooplankton whereby the metabolic products from both groups might benefit each other; commensalism whereby one of the members of the association is benefited; or antagonism where one or both members may be inhibited by the products of the other.

This investigation was conducted to obtain informa-associated with the radiolarians was certainly tion concerning the numbers of bacteria attached considerably higher than exist free in the to plankton as opposed to those living free in the water as estimated by other bacterial counts the water. It can be assumed

METHODS

A meter-net tow was taken at a depth of 20 m. with a new, clean net at 08°55' N latitude, 88°47' W longitude. One species of zooplankton predominated: the red radiolarian, Castanidium cf. longispinum Haecker. Few other zooplankters existed in this sample. Immediately after the net was pulled aboard, about one g. wet weight of these packed radiolarians was transferred to a prescription bottle containing 45 ml. of sea water. Another sample of the radiolarians was transferred to a bottle containing 10 ppm. of the surface active agent, Tween 80, in 45 ml. of sea water. Tween 80, a relatively nontoxic surface active agent for marine bacteria (Jones, 1957) was used in an effort to remove bacteria from the plankton. It was estimated that the wet weight of the scoop of radiolarians placed in the Tween 80 was about two-thirds of that in the sterile sea water. Both of these samples were diluted 1/100 with sterile sea water. These samples were shaken vigorously for one minute.

RESULTS

Inocula were taken from 1/100 dilution as follows: 0.1, 0.5 and 1.0 ml. These were plated by the pour-plate technique into a peptone-yeast extract medium (Oppenheimer and ZoBell, 1952). The plates were incubated for 11 days at 29-31°C. The results appear in Table 7.

Little quantitative information can be derived from this particular experiment since the inoculum was not weighed and the amounts of plankton in the bottles were not estimated as equal. If the estimate of twothirds as many radiolarians in the bottle shaken with 10 ppm. Tween 80 can be assumed correct, the Tween 80 had little effect on dislodging additional bacteria from the zooplankters. However, the number of bacteria considerably higher than exist free in the water as estimated by other bacterial counts recorded in this cruise. It can be assumed from this experiment that there are between 50,000 and 100,000 bacteria per gram of wet radiolarians which is about 10³more bacteria than are generally present in sea water.

This experiment should be taken only as indicative of attachment of bacteria to marine plankton since only one group of organisms, was tested because the actual numbers of bacteria free in the water was not measured at the same place and time. However, the order of magnitude of bacteria found associated with the radiolarian, <u>Castanidium cf. longispinum Haecker</u>, when compared with the bacteria generally found in similar waters strongly suggests intimate association.

ACKNOWLEDGEMENT

The author would like to thank Mr. William R. Riedel, Scripps Institution of Oceanography, for his identification of the radiolarian, Castanidium cf. <u>longispinum Haecker</u>.

BACTERIAL COUNTS FROM WET PACKS OF RADIOLARIANS (0.5 to 1.0 g.) COLLECTED AT 08°55' N LATITUDE, 88°47' W LONGITUDE:

Dilution	Shaken with	n o Tween 80	Shaken with 10) ppm Tween 80
	Plate count	Bacteria/ml	Plate count	Bacterial/ml
1:45000	3	135,000	2	90,000
1:9000	3	27,000	1	9,000
1:4500	12	54,000	10	45,000
Average		72,000		48,000
0				,

BIBLIOGRAPHY

- Heukelekian, H., and A. Heller. 1940. Relation between food concentration and surface for bacterial growth. Jour. Bacteriol., Vol. 40, pp.547-558.
- Jones, G. E. 1957. The effects of organic metabolites on the development of marine bacteria. Bacteriol. Proc., pp. 16.
- Oppenheimer, C. H., and C. E. ZoBell. 1952. The growth and viability of sixty-three species of marine bacteria as influenced by hydrostatic pressure. Jour. Mar. Res., Vol. 11, No. 1, pp. 10-18.
- Orlob, G. T. 1956. Viability of sewage bacteria in sea water. Sewage and Industrial Wastes, Vol. 28, No. 9, pp. 1147-1167.
- Stark, W. E., Janicé Stadler, and Elizabeth McCoy. 1938. Some factors affecting the bacterial population of freshwater lakes. Jour. Bacteriol., Vol. 36, pp.653-654.
- ZoBell, C. E. 1946. Marine Microbiology: A Monograph on Hydrobacteriology. Chronica Botanica Co., Waltham, Mass.

- 78 -

PRELIMINARY STUDIES OF BACTERIAL GROWTH IN RELATION TO DARK AND LIGHT FIXATION OF C¹⁴0, DURING PRODUCTIVITY DETERMINATIONS

by

G. E. Jones, W. H. Thomas, and F. T. Haxo

The technique of studying productivity by use of radioactive carbon $(C^{\perp 4})$ has been widely employed in recent years (Steemann Nielsen, 1951, 1952, 1954; Ryther and Vaccaro, 1954; Ryther, 1956b). Steemann Nielsen (1952), using the green alga, Scenedesmus quadricauda, showed that ${\rm C}^{14}$ assimilation in the dark was very small (about $l^{\circ}/_{\circ}$ of maximal fixation in the light). When this method is used in a natural ecosystem, such as in the pelagic waters of the open ocean, a complex of factors and organisms must be considered. Both phytoplankton and zooplankton assimilate carbon dioxide in the dark. Chemosynthetic bacteria fix carbon dioxide as their sole source of carbon and even heterotrophic bacteria fix some of their carbon as carbon dioxide via the Wood-Werkman reaction (Wood and Werkman, 1938, 1940; Wood et al., 1941; and Utter and Wood, 1951). Consequently, mixed populations collected from nature might be expected to fix a greater percentage of CO_2 in the dark than the 1°/. reported by Steemann Nielsen (1952). The complicating effects of the presence of bacteria have been recognized, although as yet unsatisfactorily assessed, in productivity measurements.

One of the first methods of estimating productivity was the "light and dark bottle" experiments of Gaarder and Gran (1927). When this technique is used, water samples from various depths are dispensed in bottles and lowered to different depths for time intervals of a day or more. Oxygen production in dark (wrapped with masking tape to prevent the entrance of light) and light bottles is determined by measuring the quantities of oxygen before and after the incubation period. A modification of this method was used in the productivity measurements of Riley (1938, 1939, 1941a, 1941b). Riley's rather large estimates for productivity in the Sargasso Sea were criticized by Steemann Nielsen on the grounds that the bactericidal effect of sunlight inhibited the bacteria in the light bottles, causing a considerable difference in oxygen content between the light and dark bottles (Steemann Nielsen, 1952). It was

correctly pointed out by Steemann Nielsen that the added surface of the containers would promote bacterial growth to a far greater degree than in pelagic sea water under natural conditions in both light and dark bottles (ZoBell and Anderson, 1936). However, while the bacterial activities in the bottles are increased, most of the wave-lengths shorter than 3500 Å which are most inhibitory toward bacteria are absorbed by glass bottles (Vaccaro and Ryther, 1954). Also, those solar radiations transmitted at a depth of 10 inches did not affect the growth of marine bacteria as compared with bacterial development in the dark. Ten inches is the depth employed by Riley in his experiments. Steemann Nielsen later (1955) presented experiments suggesting that antibiotics produced by the plankton algae in the light decreased the bacterial activity. An antibiotic from Chlorella, chlorellin, has been reported (Pratt et al., 1944).

It was the purpose of the following experiments to assess the numbers of bacteria developing in light and dark bottles containing seawater samples from the tropical Pacific Ocean over different periods of time ranging up to 40 hours and estimate their influence on the carbon dioxide fixed by the total population. Any influence of the planktonic population on the marine bacteria was also noted.

METHODS

The 250-ml. glass-stoppered reagent bottles used in these experiments were cleaned as follows: thoroughly washed with a detergent ("Tide"), rinsed three or four times with sea water, filled with $10^{\circ}/_{\circ}$ HCl for at least 5 to 10 minutes and rinsed five or six times with sea water. The surface sea-water samples were collected in a plastic bucket (cleaned as above) and dispensed into the reagent bottles. The bottles were always rinsed with the sea-water sample before filling. Radioactive NaHCl¹⁴O₃ (0.9µc) was added to each bottle. The dark bottles were very carefully covered with black tape to exclude all light. An attempt to obtain dark bottles by spraying with black paint failed to produce light-tight bottles since very small holes in the painted surface permitted light to pass. The bottles were incubated in an illuminated water bath (fluorescent lighting through a glass bottom) in a random distribution. Illuminance was measured with a Weston 856 YE photocell connected to a 0-100-microammeter having a 50-ohm internal resistance. The meter was calibrated against a Weston model 756 laboratory illumination meter. Illuminance measured at the glass bottom of the water bath was 1100 to 1550 foot-candles. The average illuminance in the bottles was about 80°/. of this figure. This average illuminance was about 33°/. of saturation if saturation illuminance is taken to be 3200 foot-candles (Steemann Nielsen, 1952), or was 53°/o of saturation if Ryther's (1956) average value for 14 different phytoplankton species (2000 foot-candles) is used.

Just before the bottles were placed in the water bath (zero hour) and after each interval of time, an appropriate aliquot of the sea-water sample was removed with a sterile pipette and plated in duplicate for each bottle in sterile plastic petri dishes on a peptone-yeast extract agar (Oppenheimer and ZoBell, 1952). The bottles were shaken thoroughly before the sample was removed for the pour-plate determination. The plates were poured with agar at $42 \stackrel{+}{_{-}} 2^{\circ}C$ on a suspended table which was steadied in moderate seas with the aid of another person. One person could operate the suspended table in a calm sea, whereas pouring plates was impossible in a heavy sea. The plates were incubated in the dark at 31 ± 1°C for three days and then examined with a Quebec colony counter for the heterotrophic marine bacterial count.

The water sample from each bottle was filtered through a Millipore HA filter (0.45 ± 0.02 micra., carried out using surface sea water from Millipore Filter Corporation, Watertown, Mass.) which retained all of the plankton and most of the bacteria in the samples. The filters were washed with nonradioactive sea water, dried in a desiccator over silica gel, and the radioactive count determined in a proportional flow counter (Nuclear Measurement Corporation, PC-1).

RESULTS

Experiments were conducted to determine the increase in bacterial numbers, and C14 assimilation over an 18-hour period in bottles that were cleaned and in bottles that were cleaned and sterilized. Surface sea water was collected at 17°54' N latitude, 103°50' W longitude (BT Station 3 - 5) and incubated in light bottles for 0, 1, 2, 4, 8, and 18 hours at 30 1 1°C (sea-surface temperature, 28.8°C) in the illuminated (1250 + 150 foot-candles) water bath. The results of this experiment appear in Table 8.

The data from a similar experiment for surface sea water collected at 11°41' N latitude, 91°52' W longitude (BT Station 7 - 5), incubated under identical conditions for 0, 2, 4, 6, 8, 13, and 18 hours is presented in Table 9.

While C¹⁴ assimilation was slightly higher in the autoclaved bottles throughout most of the experimental period, this difference is not significant. Bacterial growth was lower in the autoclaved bottles in the early parts of the experiments (up to 13 hours), but was greater at 18 hours. The reason for the higher bacterial population in autoclaved bottles at the end of the experiment is not clear, but may be due to release of nutrients by autoclaving the bacteria originally present, or to possible "antibiotic" activities of the original bacteria. However, autoclaving does not appear to be necessary in carrying out a production determination, since the C¹⁴ values are not significantly different during the customary experimental period (8 hours or less).

An experiment to determine bacterial increases and carbon dioxide assimilation for a more prolonged period using the C^{14} fixaction method in both light and dark bottles was 19°08' N latitude, 105°29' W longitude (BT Station 23 - 6). The 250-ml. reagent bottles were filled completely with sea water and NaHC¹⁴O₃, (4 μ c) was added carefully with a syringe. Immediately after filling with sea water, the bottles were sampled for their bacterial counts by the pour-plate technique.

- 80 _

COMPARISON OF RINSED AND AUTOCLAYED BOTTLES IN TERMS OF BACTERIAL NUMBERS AND ${\rm C}^{140}{}_2$ ASSIMILATION

Time, hours	Rinsed b Counts/minute		Autoclave Counts/minute	d bottles Bacteria/ml
0 1 2 4 8 18	78 78 220 375 622	210 1,700 3,500 3,100 13,400 74,000	- 61 122 289 478 1,500	79 700 900 1 ,2 00 22,000 490,000

TABLE 9

COMPARISON OF RINSED AND AUTOCLAYED BOTTLES IN TERMS OF BACTERIAL NUMBERS AND ${\rm C}^{14}{\rm O}_2$ Assimilation

Time, hours	Rinsed b Counts/minute		Autoclaved Counts/minute	
0 2 4 6 8 13 18	55 101 111 126 238 291	930 2,200 8,500 79,000 890,000 750,000 1,900,000	45 54 122 149 248 309	610 1,300 3,300 28,000 38,000 130,000 4,600,000

TABLE 10

BACTERIAL DEVELOPMENT AND C¹⁴ UPTAKE IN DUPLICATE LIGHT BOTTLES CONTAINING SURFACE SEA-WATER SAMPLES FROM 19°08' N LATITUDE, 105°29' W LONGITUDE

Time, hours	Bacte	ria/ml	Count/minute				
·	Sample 1	Sample 2	Average	Sample 1	Sample 2	Average	
0 2 4 8 16 24 37.5	3,200 6,600 7,800 62,000 680,000 940,000 6,400,000	3,600 6,700 9,600 40,000 890,000 1,000,000 6,800,000	3,400 6,600 8,700 51,000 780,000 970,000 6,600,000	174 314 559 950 1,118 1,334	168 324 676 912 752 1,421	171 319 618 931 935 1,378	

Since there was a lag in time of 1.5 hours between the time the zero hour bacterial counts were plated and the time all of the bottles were inoculated with ${\tt NaHC}^{14}{\tt O}_3$ and placed in the water bath, this should be taken into account in following the bacterial populations. The illuminance in the water bath was 1400 ± 140 foot-candles. After 2, 4, 8, 16, 24, and 37.5 hours of incubation at 25 ± 1°C, two bottles were removed and the contents were plated in duplicate for bacteria, and filtered to determine the ${\rm C}^{1\,4}$ uptake by the organisms over the particular time span tested. The results for the bacteria/ml and the ${\rm C}^{14}$ assimilation showing the values for the replicates are tabulated for the light bottles (Table 10) and the dark bottles (Table 11). The average C^{14} uptake was 171 counts/minute in the light during the first two hours, with approximate doubling after four hours and again after eight hours. After eight hours, the C¹⁴ assimilation increased by a factor of 1.5X during the next eight hours and 1.3X during the last 20 hours of the experiment. Meanwhile, the bacteria were in the lag phase of growth for the first four hours, after which they entered logarithmic growth, tapering off somewhat after 16 hours. It is interesting to note that the development of the bacteria in the dark and the light was very similar.

During the first four hours the C^{14} fixation was considerably suppressed in the dark compared to the light. After two hours, 37 counts/minute. were recorded which increased to 51 counts/minute after four hours. At this point, however, the C^{14} uptake more than doubled during the next two time intervals (up to 16 hours). After this time, the C^{14} fixation proceeded at about the same rate in the dark and in the light.

The replication of the bacterial counts and C^{14} fixation was quite good with two exceptions. After 24 hours of C^{14} uptake in the light, the duplicate bottles did not agree. The higher figure is more consistent with the other results. There was considerable disagreement in the duplication of the 37.5-hour count/minute in the dark but the average figure appears reasonable (Table 11).

DISCUSSION

The necessity for maintaining dark-bottle controls during productivity measurements by the C¹⁴ method becomes evident upon examination of the data in Tables 10 and 11. When employing radioactive C^{14} as an index of assimilation of CO2 and productivity, darkbottle controls have not always been considered important as a correction factor (Steemann Nielsen, 1952). In these experiments, dark-bottle C¹⁴ fixation became very significant after eight hours of incubation (half as much Cl4 fixation in the dark as in the light after eight hours). As shown from Tables 10 and 11, the error (dark bottle fixation/light bottle fixation) which would be incurred, if the dark bottles were not considered, would be 21.6°/. after two hours, 15.5°/. after four hours, 17.6°/. after eight hours, 31.6°/. after 16 hours, 46.8°/. after 24 hours, and 48.6°/. after 37-1/2 hours. Thus, even during the customary incubation period (up to eight hours), the error might be expected to fall between 15 and 22°/..

The effect of bacteria on the total C^{14} fixation is still somewhat uncertain. However, the bacterial populations were very similar in both the light and dark bottles and their influence could be compensated for by using dark-bottle controls. (The light source in these experiments was artificial, not sunlight, however.) This conclusion is supported by the results of Vaccaro and Ryther (1954). There was no indication in our studies of an "antibiotic" effect wuch as that reported by Steemann Nielsen (1955) for the fresh-water green alga, <u>Chlorella pyrenoidosa</u>, and the marine diatom, <u>Thalassiosira nana</u>.

Some calculations are presented for estimation of the magnitude of bacterial C^{14} uptake by the Wood-Werkman reaction. If one assumes that an average marine bacterium is a short rod (1 micron long by 0.5 micron in diameter), the volume of one bacterium would be 2.0 x $10^{-1.3}$ cc. At the end of 37.5 hours of incubation, the volume of all recorded bacteria (6 x 10^9 cells/1) would be 1.2 x 10^{-3} cc/1. If 80° , of this volume is considered as

BACTERIAL DEVELOPMENT AND C¹⁴ UPTAKE IN DUPLICATE DARK BOTTLES CONTAINING SURFACE SEA-WATER SAMPLES FROM 19°08' N LATITUDE, 105°29' W LONGITUDE

Time, hours	Bacte	ria/ml	Count/minute			
	Sample 1	Sample 2	Average	Sample 1	Sample 2	Average
0	3,200	3,600	3,400			
2	5,600	5,100	5,400	44	30	37
4	8,500	7,900	8,200	59	43	51
8	58,000	59,000	59,000	139	79	109
16	660,000	390,000	520,000	345	242	294
24	1,600,000	1,700,000	1,700,000	425	451	438
37.5	5,200,000	5,700,000	5,500,000	872	467	670

TABLE 12

BACTERIAL FIXATION OF CARBON DIOXIDE CARBON IN THE DARK IN SURFACE SEA WATER COLLECTED AT 19°08' N LATITUDE, 105°29' W LONGITUDE

Time, hours	CO_2/C in dark $\mu g/l$	CO_2/C fixed by bacteria $\mu g/l$	$^{\circ}/_{\circ}$ of CO ₂ /C fixed by bacteria in the dark
2	0.18	0.0055	3
4 8	0.25 0.52	0.0088 0.066	4
16	1.41	0.55	39
24 37.5	2.0 3.2	1.65 6.6	206

moisture content (Porter, 1946) then 2.4 x 10^{-4} cc/l would be the dry volume or, multiplying by the average specific gravity, -1.1 (Ruffilli, 1933), the dry weight of the bacterial cells would equal 2.64 x 10^{-4} g/l. If 50° / of the dry weight of the bacterial cells is considered as carbon (Porter, 1946), then 1.32 x 10^{-4} g/l is the calculated weight of total carbon. Since it has been estimated that about 5° / of cell carbon of heterotrophic bacteria may be fixed by the Wood-Werkman reaction, approximately 6.6 x 10^{-6} g/l of C¹⁴ could have been fixed by the heterotrophic bacteria at the end of the 37.5-hour period in this experiment.

The total CO_2/C fixed in any of the sets of bottles may be calculated as follows: Total CO_2/C fixed/L = Total CO_2/C present in mg/l x counts/min/250 ml recovered x 4 \div Count/min added x 4

In this experiment 5,222,500 counts/minute (as measured with our apparatus) of NaHC¹⁴O₃ were added to each 250-ml. reagent bottle. The total carbon dioxide carbon in the surface seawater sample was approximately 25 mg/l. Calculating the total carbon dioxide fixed in each set of bottles in this manner and comparing these values with the estimates of the amount of C¹⁴ fixed by heterotrophic bacteria for each period, an estimation of the percentage of CO₂/C fixed by the bacteria can be obtained, as shown in Table 12 for each test period in the dark.

Steemann Nielsen (1952) has estimated that the amount of organically bound C^{14} is not a completely accurate measure of the gross production by photosynthesis since $C^{14}O_2$ is actually assimilated at a rate 6°/. slower than $C^{12}O_2$. In addition, Steemann Nielsen (1952) applies a correction of 4°/. of the photosynthetic intensity at optimum light intensity in a fourhour experiment for the loss of C^{14} through the respiration of substances produced during the experimental period. Thus, a 10°/. correction is applied. Steemann Nielsen neglects the negative correction due to C^{14} assimilation in the dark which he estimates at 1°/., as mentioned previously. However, in these experiments the dark fixation of C^{14} was 15 to 20 times the dark fixation reported by Steemann Nielsen (1952). The correction for isotopic fractionation and respiration were not applied in these calculations.

Various considerations which may affect the calculations presented in Table 12 should be mentioned. For example, the size of marine bacteria is variable (ZoBell and Upham, 1944). An increase in the length of the rod-shaped cells from one micron to two micra would double the importance of the bacteria in the foregoing calculations. However, since marine bacteria are generally very small, the values used are considered reasonable. In addition, the error in the pour-plate technique may be considerable. It has been estimated that only 1 to 10°/. of the bacteria present in a sample are recorded by this method (ZoBell, 1946), which would increase their importance in these calculations by at least a factor of 10. Little is known of the abundance or importance of chemosynthetic bacteria in the marine environment which utilize carbon dioxide as their sole source of carbon.

The various influences of bacteria, phytoplankton, zooplankton and other components in the marine ecosystem on C^{14} assimilation may perhaps be elucidated by studies on pure cultures and simple mixed populations. The uptake of C^{14} by various members of the marine population in pure cultures and in natural mixtures should provide much additional information on the actual uptake of C^{14} by these organisms as well as offer more definitive results concerning the effect of their mutual interrelationships. These experiments have been planned.

SUMMARY

Determinations of bacterial increases and 1 C¹⁴O₂ fixation in surface samples of tropical Pacific sea water contained in cleaned as well as in cleaned and autoclaved 250-ml. reagent bottles incubated in the light indicated that both the bacterial populations and the C^{14} uptake were slightly lower in the autoclaved bottles during the first few hours of incubation. The rate of increase of both the bacterial populations and the C14 uptake was greater in the autoclaved bottles after the first few hours and by the end of the 18-hour incubation period their values were higher. However, complete sterilization of the bottles is not considered necessary for determinations of productivity during the eight-hour period generally employed, since the differences were not great.

2. Dark-bottle fixation of C^{14} varied between 15.5 and 21.6°/. of light-bottle fixation during test periods up to eight hours and up to almost 50°/. by 37.5 hours, indicating that such controls are necessary for estimating productivity. 3. Bacterial C^{14} fixation was calculated as

3. Bacterial C^{14} fixation was calculated as varying between 3 and 13°/. of the total dark fixation during the first eight hours of incubation. The importance of bacteria would, of course, be proportionally less in the light.

4. The bacterial counts as determined by the pour-plate technique were essentially the same in both the light and dark bottles, and there was no indication that either light or antibiotics produced by the phytoplankton were acting adversely on the bacterial populations.

BIBLIOGRAPHY

- Gaarder, T., and H. H. Gran. 1927. Investigation of the production of phytoplankton in the Oslo Fjord. Rapp. Prov. Verb. Cons. Perm. Int. Explor. Mer., Vol. 42, pp. 3-48.
- Oppenheimer, C. H., and C. E. ZoBell. 1952. The growth and viability of sixty-three species of marine bacteria as influenced by hydrostatic pressure. Jour. Mar. Res., Vol. 11, No. 1, pp.10-18.

- Porter, J. R. 1946. Bacterial Chemistry and Physiology. J. Wiley and Sons, Inc., New York, pp.355.
- Pratt, R., T. C. Daniels, J. J. Eiler,
- J. B. Gunnison, W. D. Kumler, J. F. Oneto, and
- L. A. Strait. 1944. Chlorellin, and antibacterial substance from <u>Chlorella</u>. Science, Vol. 99, pp. 351-352.
- Riley, G. A. 1938. Plankton studies. I. A preliminary investigation of the plankton of the Tortugas Region. Jour. Mar. Res., Vol. 1, pp. 335-350.
- Riley, G. A. 1939. Plankton studies II. The western North Atlantic, May-June, 1939. Jour. Mar. Res., Vol. 2, pp. 145-162.
- Riley, G. A. 1941a. Plankton studies. III. Long Island Sound. Bull. Bingham Oceanogr. Coll., Vol. 7, No. 3, pp. 1-93.
- Riley, G. A. 1941b. Plankton studies. IV. Georges Bank. Bull. Bingham Oceanogr. Coll., Vol. 7, No. 4, pp. 1-73.
- Ruffilli, D. 1933. Studies on the specific gravity of bacteria. Biochem. Zeit., Vol. 263, pp.63-74.
- Ryther, J. H., and R. F. Vaccaro. 1954. A comparison of the oxygen and C¹⁴ methods of measuring marine photosynthesis. Jour. Cons. Int. Explor. Mer., Vol. 20, No. 1, pp. 25-34.
- Ryther, J. H. 1956a. Photosynthesis in the ocean as a function of light intensity. Limn. and Oceanogr., Vol. 1, No.1, pp.61-70.
- Ryther, J. H. 1956b. The measurement of primary production. Limnology and Oceanography, Vol. 1, No. 2, pp. 72-84.

- Steemann Mielsen, E. 1951. Measurement of the production of organic matter in the sea by means of carbon-14. Nature (london), Vol. 167, p. 684.
- Steemann Nielsen, E. 1952. The use of radioactive carbon (C...) for measuring the organic production of carbon in the sea. Jour. Cons. Int. Explor. Mer., Vol. 18, No. 2, pp. 117-140.
- Steemann Nielser, E. 195-. Cn organic production in the oceans. Jour. Cons. Int. Explor. Mer., Vol. 19, No. 3, pp. 309-328.
- Steemann Nielsen, E. 1955a. The production of antibiotics by plankton algae and its effect upon bacterial activities in the sea. Marine Biology and Coeanography Suppl. to Vol. 3 of Deep-Sea Reserch, pp. 281-286.
- Steemann Nielsen, E. 1935b. An effect of antibiotics produced by plankton algae. Nature (lordon), Vol. 176, p. 553.
- Utter, M. F., and H. G. Wood. 1951. Mechanism of fixation of carbon dioxide by heterotrophs and autotrophs. Advances in Enzymol., Vol. 12, pp. 41-151.
- Vaccaro, R. F., and J. H. Ryther. 1954. The bactericidal effects of sunlight in relation to "light" and "dark" bottle photosynthesis experiments. Jour. Cons. Int. Explor. Mes., Vol. 20, No. 1, pp. 18-24
- Wood E. G., and C. H. Workman. 1938. The utilization of CO₂ by the propionic acid bacteria. Eiochem. Jour., Vol. 32, pp.1262-1271.
- Wood, H. G., and C. H. Werkman. 1940. The relationship of bacterial utilization of 302 to succinic acid formation. Biochem. Jour., Vol. 34, pp. 129-138.

Wood, H. G., C. H. Werkman, A. Hemingway, and A. O. Nier. 1941. Heavy carbon as a tracer in heterotrophic carbon dioxide assimilation. Jour. Biol. Chem., Vol. 139, pp.365-376.

- ZoBell, C. E., and D. Q. Anderson. 1936. Observations on the multiplication of bacteria in different volumes of stored sea water and the influence of oxygen tension and solid surfaces. Biol. Bull., Vol. 71, pp. 324,-342.
- ZoBell, C. Z., and H. C. Upham. 1944. A list of marine bacteris including descriptions of sixty new species. Bull. Scripps. Inst. Oceanogr., Vol. 5, No. 2, pp. 239-292.
- ZoBell, C. E. 1946. Marine Microbiology: A Monograph on Eydrobacteriology. Chronica Botanica Co., Waltham, Mass.

THE EFFECTS OF ORGANIC AND INORGANIC MICRONUTRIENTS ON THE ASSIMILATION OF C¹⁴ BY PLANKTONIC COMMUNITIES AND ON BACTERIAL MULTIPLICATION IN TROPICAL PACIFIC

SEA WATER

Ъy

Galen E. Jones and William H. Thomas

Predatory concepts of life in the sea were emphasized throughout the early history of marine biology. However, various line of evidence prompted Lucas (1947, 1949, 1955) to propose that microorganisms in the sea can interact in a nonpredatory manner by means of external metabolites. In this way certain organisms might influence the activities of others by producing essential nutrients or by removing or excreting inhibitory substances. Certain marine phytoplankton require growth factors such as thiamin, cyanocobalamin, and biotin (Provasoli and Pintner, 1953; Lewin, 1954; Sweeney, 1954; Droop, 1957; and Johnston, 1955). Requirements for amino acids, purines, pyrimidines, and other growth factors have also been shown for some marine bacteria (Ostroff and Henry, 1939; MacLeod et al., 1954; Jones, 1957). Some marine algae contain a great diversity of growth factors (Ericson, 1953a and b; Ericson and Carlson, 1953) and could presumably supply such factors to other marine organisms. The literature on the existence of such factors in sea water has been reviewed by Vallentyne (1957).

The present paper reports experiments performed at sea on the effects of small concentrations of added organic substances on bacterial growth and Cl⁴⁰O₂ assimilation by organisms in pelagic sea-water samples. The effects of additions of certain inorganic substances on these processes were also studied.

METHODS

The radioactive C¹⁴ method for measuring organic productivity (Steemann Nielsen, 1951, 1952) was utilized to determine the amount of C¹⁴ assimilation by microorganisms in light and dark bottles containing pelagic surface sea water when microquantities of organic and inorganic nutrient pools were added. Reagent bottles of a 250-ml. capacity were thoroughly cleaned with detergent, rinsed with 10°, ECl,

and finally with sea water (five or six times immediately before use). All of the samples were surface sea water collected in a clean plastic bucket. After a 220-ml. sample of sea water was added to the bottles, the number of marine heterotrophic bacteria in the sample was determined using the pourplate method and peptone-yeast extract agar (Opperheimer and ZoBell, 1952). Similar enumerations were made at the end of the period of incubation with C^{14} . Plates were poured On a suspended table which was adequate to compensate for the roll of the ship in the Iderate seas experienced on this cruise. The bacterial plates were incubated for three days in the dark at 30 1 1°C before examining for bacterial numbers with a Quebec colony counter. Appropriate dilutions were made with sterile water blanks when high counts were anticipated. Uninoculated control plates were maintained in all cases to check the sterility of the medium, the sterility of the disposable plastic petri dishes employed and the incidence of contamination due to handling aboard ship. These uninoculated plates were sterile in most cases.

Organic constituents were added as pools to certain sea-water samples to give the following final concentrations:

Vitamin pool 1 (V-1) ug/100 ml of sample

folic acid	1.0
thiamin chloride	10.0
riboflavin	5.0
pyridoxine hydrochloride	5.0
calcium pantothenate	50.0
nicotinaride	50.0
choline hydrochloride	100.0
inositol	100.0
para-aminobenzoic acid	5.0
Diotin	0.05

Vitamin pool 2(V-2)	µg/100 ml of sample
pyridoxal phosphate	5.0
pyridoxamine dihydrochloride	5.0
cyanocobalamin	0.015
vitamin A	10.0
acetylcholine chloride	10.0
ascorbic acid	10.0
carotene	10.0
nicotinic acid	50.0
Yitamin pool 3 (Y-3)	µg/100 ml. of sample
calciferol	10.0
tocopherol	10.0
rutin	10.0
menadione	10.0

These vitamin pools were prepared in 50°/. ethyl alcohol and added in 0.1-ml. amounts per 100 ml of sample sea water. Pools of purine and pyrimidines (pp) (adenine, adenosine, adenylic acid, guanine, guanosine, uracil, cytidylic acid thymine, xanthine, and hypoxanthine), essential amino acids (EAA) (valine, isoleucine, leucine, threonine, phenylalanine, trytophane, lysine, arginine, histidine, and methionine), and nonessential amino acids (NEAA) (glutamic acid, aspartic acid, serine, proline, cystine, glycine, alanine, and tyrosine) were made up in double distilled water in one-mg. amounts per 100 ml. of sample (final concentration). The purine and pyrimidine pool as well as the nonessential amino acid pool was sterilized in the autoclave, whereas the essential amino acid pool was passed through an ultrafine Morton sintered-glass filter.

Organic complexes were added to some samples to give the following final concentrations: soil extract (from Scripps garden soil) (Sweeney, 1951) 1 ml/100 ml of sample; yeast extract (Difco) 0.001 g/100 ml.

Inorganic substances were added to give the following final concentrations: $KN0_3...10 \ \mu gm$ at $N0_3/L$ of sample; $K_2HP0_4...1 \ \mu gm$ at $P0_4/L$ of sample; PI Metals...3 ml/100 ml of sample.

The PI metal stock solution (Provasoli et al, 1957) furnished micronutrients and EDTA in the following final concentrations:

Constituent	mg/100 ml of sample
Na ₂ EDTA Fe	3.0 0.03
В	0.6
Mn	0.12
Zn	0.015
Cu	0.00012
Co	0.0003

The 250-ml. glass-stoppered reagent bottles were incubated in a water bath which was illuminated (fluorescent lighting from a battery of long bulbs at an illuminance of 1250 ± 150 foot-candles) through a glass bottom. The temperature of the water bath was maintained as close to the temperature of the surface sea water as possible. Black bottles (prepared by careful covering with black masking tape) were incubated with the light bottles as controls to distinguish dark uptake of C14 from photosynthetic fixation. After the bottles were incubated in the presence of added nutrients for approximately four hours, the NaHC1403 was added to the water samples from a sterile ampule and the samples were incubated for two to four additional hours.

At the end of the incubation period, equal volumes of water (2.0 ml) were removed from each sample bottle after shaking and plated in duplicate, as described above, to determine the numbers of heterotrophic marine bacteria. In some experiments the zero-hour count was subtracted from the final bacterial population to give the bacterial increase.

The water remaining in each bottle was filtered through an HA Millipore filter $(0.45 \pm 0.02 \text{ microns})$, Millipore Filter Corporation, Watertown, Nass.) which retained all of the larger particulate matter; the filter was then washed with more than 100 ml of sea water and dried in a desiccator for at least 24 hours over silica gel. The radioactivity on the filter pad was measured in a proportional flow counter (Nuclear Measurement Corporation, PC-1).

RESULTS

In the first experiment, the $C^{14}O_2$ assimilation and bacterial numbers in surface sea water from a poorly productive area (30°Ol' N latitude, $116^{\circ}49'$ W longitude) west of Baja California were determined in both light and dark bottles. The samples were incubated for seven hours in the illuminated water bath at $18 \pm 1^{\circ}C$. The results are shown in Table 13.

The $C^{14}O_2$ assimilation in the light was 1.6 times that in the dark and the bacterial increase was almost four times that in the dark. In this experiment the photosynthesetic activity of the phytoplankton apparently stimulated the bacterial population, presumably because of the metabolic by-products of the marine algae. The importance of the dark-bottle controls was emphasized by the 63°/. dark fixation in this experiment. In the rest of the experiments dark-bottle controls were utilized wherever possible.

In the next experiment the effect of inorganic nutrients on $C^{14}O_2$ fixation and bacterial populations was determined in poorly productive water just north of the Alijos Rocks west of Baja California (26°50' N latitude, 116°13' W longitude; Station BT - 0 - 7). NO_3 , PO4, and PI metals were added to one pair of bottles. Pairs of these inorganic additives were added to other sets of bottles containing the seawater sample. Soil extract was added in another set of bottles to the NO_3 , PO_4 , and PI metals as a final treatment. Light- and darkbottle controls containing no additions to the sea-water sample were incubated at 20 to 24°C for a total of six hours with the treated samples. After four hours 5,222,500 counts/minute of NaHCl 40 3 were added to each bottle from sterile ampules. The results of this experiment appear in Table 14.

Little or no effect on $C^{14}O_2$ assimilation due to the addition of the inorganic elements was observed in this water mass. However, the populations were permitted to adjust to the conditions in each set of bottles for only four hours before adding the NaHC¹⁴O₃, so that the period allowed for the uptake of C^{14} may not have been long enough to accentuate the differences in fixation in the different treatments, particularly in a poorly productive area such as that under study. The results in Table 14 regarding the bacteria are not particularly informative. PI metals and PO₄ appear to be the most stimulatory to bacterial development in this water, but there is no way to determine from these results whether the increase in bacterial populations is due to by-products from the phytoplankton or to direct stimulation from the inorganic substances. In this case, the large number of bacteria in the dark compared with the light supports the results of Steemann Nielsen (1955a and b).

The organic pools were compared with the inorganic additions in the following experiment. A surface sea-water sample from a poorly productive area south of Alijos Rocks off Baja California (21°34' N latitude, 110°49' W longitude; BT - 1 - 5) was treated in duplicate with the following nutrient additions: vitamin pool 1, 2, and 3; purine and pyrimidine pool; KN03; K2HPO4; and PI metals. The surface sea-water temperature was 28.4°C. A combination of all of the nutrient additions mentioned above was added to one set of bottles, and all of the vitamin pools, the purines and pyrimidines, and the inorganic additions were subsequently removed from the combined pool, one at a time, in different experimental samples. Finally, another treatment contained KNO3, KoHPO4 and 0.025 ml of Hoagland-Arnon's (1950) trace-element solution. Twelve of the bottles were selected at random and tested for the initial bacterial numbers in the bottles. To each bottle 5,222,500 counts/minute of NaHCl⁴O₃ were added after four hours of incubation at 30 $\frac{1}{2}$ l°C and the bottles in-1°C and the bottles incubated for an additional three hours. The bottles were harvested in the usual way and the bacterial counts determined. The results are presented in Table 15.

The vitamin pools exerted an inhibitory effect on the $C^{14}O_2$ assimilation of the phytoplankton in the light. Where the vitamin pools were deleted from the bottles and the purine and pyrimidine pool and the inorganic additions (KNO₃, K₂HPO₄ and PI metals) were added to the water sample, the $C^{14}O_2$ fixation was 1.5 times as great as the untreated control during the seven hours incubation. Wherever the vitamins were present $C^{14}O_2$ uptake was depressed.

C¹⁴ ASSIMILATION AND BACTERIAL INCREASE IN LIGHT AND DARK BOTTLES COLLECTED AT 30°01' N LATITUDE, 116°49' W LONGITUDE AFTER 7 HOURS INCUBATION AT 18 + 1°C.

Treatment	Bacterial increase/ml *	Counts/minute
Light bottles Dark bottles Increase due to light	1,700 455 1,245	619 <u>389</u> 230
* Average bacterial count at	zero hour was 250 bacteria/ml.	

TABLE 14

c¹⁴0₂ ASSIMILATION AND BACTERIAL NUMBERS AFTER 7 HOURS INCUBATION AT 20 - 24°C IN THE PRESENCE OF INORGANIC ADDITIONS IN WATER COLLECTED AT 26°50' N LATITUDE, 116°13' W LONGITUDE: STATION BT - 0 - 7.

Treatment	Bacteria/ml*	Counts/minute
No additions - light No additions - dark NO PO ₄ , PI metals - light NO ₃ , PO ₄ , - light PO ₄ , PI metals - light NO ₄ , PI metals - light NO ₃ , PO ₄ , PI metals, soil extract - 3, * Average bacterial count (14 b	2,060 4,050 3,960 2,900 5,000 3,320 - light 3,450	240 45 276 209 260 228 240 60 bacteria/ml.

EFFECTS OF SMALL CONCENTRATIONS OF INORGANIC AND ORGANIC ADDITIONS ON BACTERIAL MULTIPLICATION AND C¹⁴ ASSIMILATION IN WATER COLLECTED AT 21°34' N LATITUDE, 110°49' W LONGITUDE; STATION BT - 1 -5, INCUBATED 7 HOURS AT 30 [±] 1°C.

Treatment	Light Bottles Bacterial increase/ml*	c/min	Dark Bottle Bact.increase/ml*	
No additions	5,250	211	4,140	97
Vitamin pools 1, 2, 3 Purine and pyrimidine pool Inorganic pool	29,640	127	24,350	102
Purine and pyrimidine pool Inorganic pool	15,660	308	23,040	109
Vitamin pools 1, 2, 3 Inorganic pool	12,540	141	10,200	158
Vitamin pools 1, 2, 3 Purine and pyrimidine pool	30,540	128	-	130
KNO_3 , K_2HPO_4 , H and A trace elements	11,220	153	-	-

* Average number of bacteria/ml before incubation was 2400 \pm 600.

The purine and pyrimidine pool had the most marked stimulatory effect on the bacterial multiplication in the various treatments. When the purine and pyrimidine pool was removed from the complete complement of additives, the bacterial increase in both the light and dark bottles were little more than twice the number of those in the untreated controls. Increases of sixfold were recorded (Table 15) when the purine and pyrimidine pool was present. The bacteria did not appear to be inhibited by the vitamin pools. In fact, the vitamins stimulated bacterial development somewhat in all cases. The inorganic additions failed to promote more than a twofold increase in the number of bacteria.

In the experiment reported in Table 15, C^{14} assimilated by the phytoplankton was inhibited by the vitamin pools. Since these vitamin pools were prepared in 50°/. ethanol to preserve their sterility, the possibility that the alcohol per se was depressing the C^{14} uptake by the plants was considered. The final concentration of ethanol was $0.15^{\circ}/.$

In the following experiment, the ethanol was removed from the vitamin pools by hot-air evaporation at 35°C. After the alcohol was removed, the original volume of the pools was reconstituted by adding distilled water. A surface sea-water sample was collected in the same manner as before at 14°27' N latitude, 98°58' W longitude; (Station BT - 5 - 7). The temperature of the surface sea water was 28.0°C. Control bottles to which no additions were made were prepared in three different ways: 1) cleansed and rinsed with 95°/. ethanol, followed by five or six rinses with the sample sea water, 2) cleaned with a detergent, rinsed with sea water, rinsed with 10°/. HCl, rinsed five or six more times with sea water and autoclaved for 15 minutes at 15 lbs. pressure, 3) bottles rinsed five or six times with sample sea water. All of the bottles to which nutrients were added were prepared as described in the first method with 95°/. ethanol followed by five or six rinsings with sample sea water.

The same additions were made in this experiment as in the last experiment (Table 15) except that the final inorganic treatment (KNO3, KoHPOh, and Hoagland and Arnon's trace elements) were not repeated. These bottles were incubated in the illuminated water bath for four hours at 28 ± 2°C. After four hours both the light and dark bottles were removed and 5,222,500 counts/minute NaHC140, were added. The bottles were returned to the water bath for two more hours of incubation (total of six hours), after which the final bacterialassay plates were poured and the contents of the bottles filtered for C14 uptake. The results are presented in Table 16. The initial bacterial numbers, which varied between 640 and 1,250 bacteria/ml, were subtracted from the final count to determine the bacterial increase/ml.

These results confirm the conclusion of the previous experiment (Table 15) regarding the inhibition of photosynthesis by the vitamin pools. The removal of the ethanol from the vitamin pools made little difference on the $C^{14}O_2$ assimilation of the phytoplankton in the presence of these pools. Consequently, it may be concluded that the vitamin pools themselves exerted some inhibitory effect on C^{14} uptake by phytoplankton in the light. Where the vitamin pools were omitted and the purine and pyrimidine pool as well as the inorganic additions were added, photosynthesis was almost doubled compared to the untreated control (Table 16).

The dark fixation in the treated bottles was 1.5 to 2.0 times greater than that in the untreated control. The greatest dark fixation was in the sample containing the purine and pryimidine pool plus the inorganic additions as in the light. The bacterial increase in this treatment was considerable, 41,000 bacteria/ml. as compared with 12,400 bacteria/ ml. in the control dark bottles.

The most marked bacterial increase in this experiment was that resulting from the combined treatment (vitamin pools, purine and pyrimidine pool, and inorganic additions), 82,000 bacteria/ml in the light and 28,000 bacterial/ml

EFFECTS OF SMALL CONCENTRATIONS OF INORGANIC AND ORGANIC ADDITIONS ON BACTERIAL MULTIPLICATION AND C¹⁴ ASSIMILATION IN WATER COLLECTED AT 14°27' N LATITUDE, 96°58' W LONGITUDE (BT - 5 - 7) AND INCUBATED FOR 6 HOURS AT 28 \pm 2° C.

Treatment	Light Bottles	c/m		Dark Bottles	
	Bact. increase/ml		Bact. ind	rease/ml	c/m
No additions (alcohol rinsed)	20,500	569		12,400	91
No additions (autoclaved)	13,300	654		-	-
No additions (rinsed only	21,000	610		-	-
Vitamin pools 1, 2, 3 Purine and pyrimidine pool Inorganic pool	82,000	152	2	28,000	144
Purine and pyrimidine pool Inorganic pool	26,600	976	1	+1,000	208
Vitamin pools 1, 2, 3 Inorganic pool	24,000	121		34,400	143
Vitamin pools 1,2, 3 Purine and pyrimidine pool	54,000	119	6	65,000	167

C¹⁴ FIXATION AND BACTERIAL INCREASES/ml IN A SURFACE SEA-WATER SAMPLE COLLECTED AT 09°04' N LATITUDE, 89°13' W LONGITUDE AFTER 8 HOURS INCUBATION AT 25 ± 1°C IN THE LIGHT.

Treatment	Bacterial increase/ml *	Counts/minute	
No additions	59,000	273	
Combination of all	1,960,000	392	
Vitamin pool 1 (V-1)	55,500	308	
Vitamin pool 2 (V-2)	59,500	261	
Vitamin pool 3 (V-3)	64,500	162	
Purine and pyrimidine (pp)	764,500	335	
Essential amino acids (EAA)	256,000	425	
Non-essential amino acids (NEAA)) 182,000	400	
Yeast extract	2,165,000	-	
Soil extract	339,000	281	
Tween 80	334,000	779	

TABLE 18

 c^{14} ASSIMILATION AND BACTERIAL INCREASES/ml in Light and Dark Bottles treated with various organic growth factors in surface sea water collected at 09°38' N LATITUDE, 89°35' W LONGITUDE, AFTER 8 HOURS OF INCUBATION IN 25 \pm 1° C.

Treatment	Light Bottl Bact. increase/		Dark Bottles Bact. increase/ml*	c/m
No additions Biotin Thiamin Cyanocobalamin Methionine Cystine Tween 80	18,000 26,000 26,000 28,000 17,000 15,000 42,000	264 253 288 354 280 135 221	32,000 26,000 24,000 18,000 9,000 16,000 65,000	97 81 76 113 41 70 206
Tween 80		221	65,000	

in the dark. Thus, while the presence of vitamins depressed $C^{14}O_2$ uptake by the phytoplankton in the light, bacterial multiplication was enhanced. This may be due to the action of the vitamins on the bacteria directly or to the release of organic substances from the phytoplankton.

The various methods for preparing the bottles in the otherwise untreated samples had no appreciable affect on the $C^{14}O_2$ assimilation by light-incubated phytoplankton in the samples, although the autoclaved bottles showed a slightly increased $C^{14}O_2$ uptake and about one-third fewer bacteria.

An experiment of the same type was prepared to determine the effect of the individual pools and other complexes on $C^{14}O_2$ fixation and bacterial populations. Surface sea water was collected at 09°04' N latitude, 89°13' W longitude (Station BT - 9 - 24), dispensed in 250-ml glass stoppered reagent bottles, and treated as follows: no treatment (control), combination of all that follows, vitamin pool 1 (V-1), vitamin pool 2 (V-2), vitamin pool 3 (V-3), purines and pyrimidines (pp), essential amino acids (EAA), nonessential amino acids (NEAA), yeast extract $(0.001^{\circ}/_{\circ})$, soil extract $(1.0^{\circ}/_{\circ})$, and Tween 80 $(0.001^{\circ}/_{\circ})$. The temperature of the surface sea water was 24.7° C. The bottles were incubated at $25 \pm 1^{\circ}$ C in the illuminated water bath for four hours before 1,246,300 counts/minute of NaHCl 40 3 were added. The bottles were returned to the water bath and incubated for a total of eight hours. Owing to lack of space in the water bath, dark bottles were not included in this experiment. The results of the bacterial increases per ml and the $C^{14}O_{2}$ assimilation in the light appear in Table 17.

None of the vitamin pools stimulated either C^{14} uptake or bacterial development to any extent. Vitamin pool 3 (V-3) decreased the C^{14} fixation by more than 1/3 of the untreated control. The surface active agent, Tween 80, (a complex mixture of polyoxyethylene ethers of mixed partial oleic ethers of sorbitol anhydrides) increased C^{14} fixation by a factor of

three over the untreated sample, the most pronounced stimulation of C^{14} assimilation in any of the treatments. The bacterial increase was also appreciable for this treatment (Table 17).

The amino acid pools stimulated $C^{14}O_2$ fixation in the light by a factor of about 1.5, and the essential amino acids promoted a slightly greater increase. These amino acid pools also stimulated bacterial development; the essential amino acids proved about 1.3 times as effective as the nonessential amino acid pool (Table 17).

The purine and pyrimidine pool enhanced C^{140}_2 fixation only slightly but stimulated the bacterial numbers by a factor of thirteen compared to the untreated controls. These results appear consistent with those of previous experiments.

The soil extract had no effect on C¹⁴ uptake but increased the bacterial numbers by sevenfold. The treatment containing yeast extract was so cloudy and turbid by the end of the eight-hour incubation period that, it could not be filtered. Consequently, no $C^{\perp 4}O_2$ fixation data exist for this treatment. The bacteria increased by a factor of 36 compared to the untreated control which was by far the greatest increase in the bacterial population except where yeast extract was present in the combined treatment. Although the combined pools showed considerable stimulation compared to the untreated controls, the values for C^{140} , fixation and bacterial increases were lower than some of the individual treatments (Table 17). This effect was attributed to the inhibitory properties of the vitamin pools.

Finally, an experiment was conducted to determine the effects of selected individual substances from the pools on C¹⁴O₂ fixation and bacterial development. Surface sea water at 25.7°C was collected at 09°38' N latitude, 89°35' W longitude (Station BT - 9 - 36) and dispensed in the 250-ml glass-stoppered reagent bottles as in the other experiment. The following additions were made to paired bottles in the same concentrations as in the pools: no additions, biotin $(0.05\mu g/100 \text{ ml})$, thiamin $(10.0 \ \mu g/100 \ ml)$, cyanocobalimin $(0.015 \ \mu g/100 \ ml)$, methionine $(1 \ m g/100 \ ml)$, cystine $(1 \ m g/100 \ ml)$, and Tween 80 $(1 \ m g/100 \ ml)$. Bacterial plates were poured as usual with the yeast extract-peptone agar both before and after the eight-hour incubation period. After 4.5 hours of incubation at 25 \pm 1°C in the illuminated water bath, 5,222,500 counts/minute of NaHCl⁴03 were added to each bottle.

The results of this experiment, using both light and dark bottles, are presented in Table 18.

Little can be concluded from this experiment. The most stimulatory addition for $C^{14}O_2$ dixation in the light was 0.15 mµg/ml of cyanocobalamin (vitamin B₁₂) which increased $C^{14}O_2$ uptake by a factor of 1.3 compared to the untreated control in the light. The only other significant difference from the untreated control was where 10 µg/ml of the sulfurcontaining amino acid, cystine, was added, which depressed the $C^{14}O_2$ fixation by 49°/o in the light.

The addition of 10 μ g/ml Tween 80 doubled the lark fixation. Methionine inhibited the dark uptake of C¹⁴ by 58°/ $_{\circ}$.

The bacterial numbers were not influenced by the organic growth factors to any appreciable extent, but the addition of Tween 80 increased the bacterial population by a factor of 2.5 as compared with the untreated control in the light. Most of these supplements stimulated bacterial development in the light as compared to the untreated control. The bacterial development in the dark was greatest in the intreated control and in the presence of Fween $\delta 0$.

DISCUSSION AND CONCLUSIONS

In the series of experiments presented, an attempt was made to determine whether certain organic substances (NO_3 , P_1O_4 , and trace elements) and certain organic pools (vitamin, amino acids, purines and pyrimidines, Tween 80, yeast extract, soil extract, etc.) would

"trigger" increases in the $C^{14}O_2$ assimilation processes of the phytoplankton or in the bacterial populations in tropical Pacific sea water. These experiments were carried out immediately after water samples were collected.

Vitamin pools in the concentrations employed did not stimulate $\text{C}^{1\,4}$ fixation by the phytoplankton samples from the tropical Pacific Ocean. In most cases the C1402 uptake was considerably inhibited by the vitamin pools in the light (Tables 15-17). It is interesting to note in Table 17 where the vitamin pools were tested separately for their effect on the uptake of C¹⁴ by the phytoplankton, that vitamin pool 1 was slightly stimulatory compared to the untreated control, vitamin pool 2 exerted little influence, and vitamin pool 3 was inhibitory. One of the constituents of vitamin pool 3 is menadione. Dam (1944) has demonstrated that this vitamin is inhibitory to photosynthesis in Chlorella due to direct toxic action on the cells. This fact coupled with the observation that various individual vitamins such as thiamin and cyanocobalamin actually stimulated C1402 fixation by the phytoplankton (Table 18) to a small extent, suggests that the vitamins as a group are not inhibitory to C1402 fixation in the light but that various inhibitory constituents of the vitamin pools may mask the effects of other members of the pools. Vitamin pools were not inhibitory to bacterial development in any case. However, where the individual vitamin pools were added to separate bottles (Table 17), there was almost no stimulation from any of the pools as compared to the untreated control. In other experiments there was some indirect information suggesting that vitamins were stimulatory to bacterial development (Tables 15 and 16). Generally, the vitamin pools were not as stimulatory as the other organic pools tested (amino acids, purines and pyrimidines).

The purine and pyrimidine pool enhanced the development of marine bacteria and increased the $C^{14}O_2$ uptake of the phytoplankton (Tables 15-17). Purines and pyrimidines in natural waters have received little attention from previous investigators, but some evidence exists suggesting the limited distribution of the pyrimidine, uracil, and an unidentified purine in pelagic sea water (Vallentyne, 1957;

Belser, 1957). The significance of these compounds in the ecology of the sea is strongly implied by the experiments presented in this paper.

Amino-acid pools stimulated both C¹⁴O₂ uptake by the phytoplankton and bacterial development (Table 17). Some marine bacteria have been shown to require certain amino acids (Ostroff and Henry, 1939; MacLeod et al., 1954; Jones, 1957). In addition, Fogg (1952) reported that the blue-green alga, <u>Anabaena cylindrica</u>, produced equal amounts of extracellular and intracellular polypeptide nitrogen. It is highly probable that proteinaceous compounds in the sea exert considerable influence on the mutual interrelationships between marine phytoplankton and bacteria.

The great increase in $Cl^{14}O_2$ assimilation by the phytoplankton in the presence of $0.001^{\circ}/_{\circ}$ of the surface active agent, Tween 80, (Table 17) which was not confirmed by a later experiment (Table 18) will require further consideration. It is of interest to note that the bacterial numbers were increased markedly in both experiments in the presence of the Tween 80. Inorganic additions did not appear to enhance $Cl^{14}O_2$ fixation or bacterial development appreciably. This may be due to a lack of organic growth factors in tropical Pacific sea water rather than to a limitation of inorganic nutrients.

The exposure of the phytoplankton in the seawater samples to the NaHCl403 for short periods of time (2 to 4 hours) may not have been sufficient to allow appreciable differences in C1402 assimilation to take place in all cases. If the cells were deficient in one or more of these nutrients, it might take some time for uptake to be reflected by the photosynthetic mechanism. For example, it takes about 24 hours for Scenedesmus cells to recover from nitrogen deficiency to the extent of containing the amount of protein found in normal cells (Thomas and Krauss, 1954). However, in the present work short experimental periods were chosen so that photosynthesis could be measured without measuring phytoplankton growth. In subsequent experiments of this

type the times could be varied.

The importance of dark-bottle controls for all treatments in experiments of this type is evident from an examination of any of the values obtained for dark fixation compared with light fixation. This conclusion is supported by that of Jones et al. (this volume).

These experiments provide preliminary information from the natural environment which can be used for future detailed culture and photosynthetic experiments in the laboratory. For instance, the development of culture media for pelagic phytoplankton and marine bacteria might be facilitated by the inclusion of some of these substances, especially purines, pyrimidines and amino acids, in the media. Specific effects of these substances on the photosynthetic mechanisms of the phytoplankton and requirements by the bacteria may be determined in pure culture.

ACKNOWLEDGEMENTS

The authors would like to express their sincere appreciation to Dr. William L. Belser, Scripps Institution of Oceanography, for his help in formulating the organic constituents tested and for critically reviewing the manuscript. In addition, the authors would like to thank Mr. Donald W. Lear and Mr. Harold L. Scotten, Scripps Institution of Oceanography, for their help in preparing for the cruise.

BIBLIOGRAPHY

- Belser, W. L. 1957. The use of auxotrophic mutants of a marine bacterium for the bioassay of organic micronutrients in the sea. Bacteriol. Proc., pp. 30.
- Dam, H. 1944. Vitamin K in unicellular photosynthesizing organisms. Amer. Jour. Bot., Vol. 31, pp. 492-493.

Droop, M. R. 1957. Auxotrophy and organic compounds in the nutrition of marine phytoplankton. Jour. Gen. Microbiol., Vol. 16, pp. 286-293. Ericson, L. E. 1953a. On the vitamin B_{12} , folic acid-, and folinic acid groups of factors, and on the occurrence of these vitamins and of niacin, pantothenic acid and amino acids in a number of marine algae. Thesis, Uppsala University, Sweden, pp. 1-79.

- Ericson, L. E. 1953b. Further studies on growth factors for Streptococcus faecalis and Leuconostoc citrovorum in marine algae. Arkiv för Kemi, Vol. 6, No. 8, pp. 503-510.
- Ericson, L. E., and Blenda Carlson. 1953. Studies on the occurrence of amino acids, niacin and pantothenic acid in marine algae. Arkiv for Kemi, Vol. 6, No. 49, pp. 511-522.
- Fogg, G. E. 1952. The production of extracellular nitrogenous substances by a blue-green alga. Proc. Roy. Soc., B, Vol. 139, pp. 372-397.
- Hoagland, D. R., and D. I. Arnon. 1950. The water culture method of growing plants without soil. Calif. Agr.Expt.Sta.Circ., Vol. 347, Revised edition.
- Johnston, R. 1955. Biologically active compounds in the sea. Jour. Mar. Biol. Assoc. U.K., Vol. 3⁴, pp. 185-195.
- Jones, G. E. 1957. The effects of organic metabolites on the development of marine bacteria. Bacteriol. Proc., pp. 16.
- Jones, G. E., W. H. Thomas, and F. T. Haxo. Preliminary studies of bacterial growth in relation to dark and light fixation of $C^{14}O_2$ during productivity determinations, (this volume).
- Lewin, R. A. 1954. A marine <u>Stichococcus</u> sp. which requires Vitamin B₁₂ (cobalamin). Jour. Gen. Microbiol., Vol. 10, pp. 93-96.

- Lucas, C. E. 1947. The ecological effects of external metabolites. Biol. Rev., Vol.22, pp.270-295.
- Lucas, C. E. 1949. External metabolites and ecological adaptation. Symp.Soc.Expt.Biol., Vol.3, pp.336-356.
- Lucas, C. E. 1955. External metabolites in the sea. Mar. Biol. and Oceanogr. Suppl. to Vol. 3 of Deep-Sea Res., pp. 139-148.
- MacLeod, R. A., E. Onofrey, and M. E. Norris. 1954 Nutrition and metabolism of marine bacteria. I. Survey of nutritional requirements. Jour.Bacteriol., Vol.68, pp.680-686.
- Oppenheimer, C. H., and C. E. Zobell. 1952. The growth and viability of sixty-three species of marine bacteria as influenced by hydrostatic pressure. Jour.Mar.Res., Vol.1, No.1, pp.10-18.
- Ostroff, Rose, and B. S. Henry. 1939. The utilization of various nitrogen compounds by marine bacteria. Jour.Cellular Comp.Physiol., Vol.13, pp.353-371.
- Provasoli, L., and Irma J. Pintner. 1953. Ecological implications of in vitro nutritional requirements of algal flagellates. Ann. N.Y. Acad.Sci., Vol.56, No. 5, pp.839-851.
- Provasoli, L., J. J. A. McLaughlin and M. R. Droop. 1957. The development of artificial media for marine algae. Archiv. für Microbiol., Vol.25, pp.392-428.
- Steemann Nielsen, E. 1951. Measurement of the production of organic matter in the sea by means of carbon-14. Nature (London), Vol.167, p. 684.
- Steemann Nielsen, E. 1952. The use of radioactive carbon (Cl⁴) for measuring the organic production of carbon in the sea. Jour.Cons.Int.Explor.Mer., Vol.18, No.2, pp.117-140.

- 98 -

- Steemann Nielsen, E., 1955a.
 The production of antibiotics by
 plankton algae and its effect upon
 bacterial activities in the sea.
 Mar.Biol. and Oceanogr.Suppl. to
 Vol.3 of Deep-Sea Res., pp.281-286.
- Steemann Nielsen, E., 1955b. An effect of antibiotics produced by plankton algae. Nature (London), Vol.176, p. 553.
- Sweeney, Beatrice M., 1951. Culture of dinoflagellate <u>Gymnodinium</u> with soil extract. Amer.Jour.Bot., Vol. 38, No.9, pp.669-677.
- Sweeney, Beatrice M., 1954. <u>Gymnodinium splendens</u>, a marine dinoflagellate requiring vitamin B12. Amer.Jour.Bot.,Vol.41,No.10,pp.821-824.
- Thomas, W. H., and Krauss, R. W. 1955. Nitrogen metabolism in <u>Scenedesmus</u> as affected by environmental changes. Plant Physiol., Vol. 30, pp.113-122.
- Vallentyne, J. R. 1957. The molecular nature of organic matter in lakes and oceans, with lesser reference to sewage and terrestrial soils. Jour.Fish.Res.Bd. Canada,Vol.14, No. 1, pp. 33-82.

-

THE VERTEBRATES OF SCOPE

NOVEMBER 7 - DECEMBER 16, 1956

By

Robert Cushman Murphy 1/

American Museum of Natural History

My journal included observations on all vertebrates except the small and larval fishes taken in net hauls. Collecting of sea birds from a skiff was undertaken at oceanographic stations whenever weather permitted, resulting in the acquisition of 50 specimens. The birds, collected primarily for identification, have not yet all been studied for subspecific determination. For the purpose of this report specific status is in most cases adequate. A later publication will include data on taxonomy, habits, and stomach contents.

FISHES

The bulk of the fishes captured, and now at the Scripps Institution, are outside my province. The following notes are restricted to larger or otherwise readily observable species.

Ginglymostoma cirratum. Nurse shark. An example about 1.5 m. in length swam under and around the skiff shortly after daybreak of Nov. 14, 45 miles SW of Acapulco (surface water 29.6°C).

Prionace glauca. Blue shark. Observed several times in the "Dome" area, S of latitude 10°N and 200 or more sea miles W of Costa Rica. On Nov. 22, at 09°05'N, 89°3C° W, a young example only 65 cm. in length rubbed persistently against the flanks and bottom of the skiff until it was hauled aboard by the tail (surface water 25.7°C).

1/ Dr. Murphy's participation in the cruise b was made possible through a gift to the American f Museum of Natural History from Mr. Edgar J. Marston of La Jolla, together with a grant from the Council of the Scientific Staff of the Museum.

Carcharias. Sharks of this genus or type were observed almost daily in tropical waters. They frequently assembled around the <u>Stranger</u> when she was on station. In common with certain other oceanic fishes, they were strongly drawn toward any flotsam large enough to cast a shadow. Bamboo poles and glass netfloats proved sufficient to serve as an attraction.

On Nov. 22, in the position noted under the foregoing species, a <u>Carcharias</u> 2 m. in length rubbed and banged the skiff for ten minutes, sweeping its tail along the gunwale and splashing showers of water all over the craft. Such behavior is sometimes interpreted as an effort to remove ectoparasites but it may represent merely a thigmotactic drive.

<u>Galeocerdo tigrinum</u>. Tiger shark. On Nov. 16, at $12^{\circ}47^{\circ}$ N, $94^{\circ}15^{\circ}$ W, about 215 miles off the head of the Gulf of Tehuantepec, a young and spotted example, less than 2 m. in length, clung for some time to the vicinity of the skiff (surface water 28° C).

Mobula. Jumping ray. On Nov. 14, at $16^{\circ}16'$ N, 100°27' W. roughly 45 miles SW of Acapulco, a ray about 60 cm. in lateral extent jumped and somersaulted six times just ahead of the skiff (surface water 29.6°C).

Other rays, not identified, were frequently seen during the cruise.

Manta birostris. Giant ray; manta. The specific name may possibly be open to question, but the ray was indistinguishable in the field from the Atlantic form.

- 101 -

The manta was encountered seven or more times. The northernmost record was on Dec. 14, about 60 miles W of Punta San Juanico, Baja California (surface water less than 23°C).

The southernmost was in the shore waters of Cocos Island, where two followed or kept in close touch with the skiff for fully half an hour. These approached within oar's length and the larger was at least 4 m. in breadth (surface water 26.4°C). The dorsal aspect of their upcurled fin-tips, as seen in the air, was blackish, but as soon as they submerged a meter or more the color reflected through the water became a pale tan, extraordinarily reducing visibility.

<u>Coryphaena hippurus</u>. Dolphin: dorado. Commonly encountered nearly everywhere S of 15°30' N, where on Dec. 13 the surface temperature was below 23°C. Numerous dolphins were captured. Their stomachs contained squids, flying fishes, and parasitic nematodes.

The largest example measured 150 cm. in standard length and was taken at 09°16' N, 89°18' W, on Nov. 20 (surface water 25.3°C). The position is in the "Dome" area, 200 miles W of Costa Rica. With this and with two other dolphins I confirmed observations made by Benjamin Franklin in the Atlantic Ocean on Sept. 2, 1726.

Franklin's account, which is unknown to most ichthyologists, relates to his first return from England to Philadelphia. His journal states:

"We caught a couple of dolphins and fried them for dinner... These fish make a glorious appearance in the water; their bodies are of a bright green, mixed with a silver colour, and their tails of a shining golden yellow; but all this vanishes presently after they are taken out of their element, and they change all over to a light grey. I observed that, cutting off pieces of a just-caught, living dolphin for bait, those pieces did not lose their lustre and fine colours when the dolphin died, but retained them perfectly."

The repetition of this experiment showed that skin overlying severed chunks of myomeres from the back remained dark blue after the same area on the dying fish had turned almost white. In like manner, sections from the belly retained their pristine silvery yellow hue, with pale blue spots, after the same part on the body of the fish had faded.

The dermal chromatophores are under combined control of hormones and the parasympathetic nervous system. Proximal severing of the fibers evidently leaves the hormonal influence unopposed.

Exococoetidae. Flying fish. Observed throughout the cruise. An example of <u>Cypselurus californicus</u> flew aboard <u>Stranger</u> during the night of Nov. 7 at 30°34'N, not far from San Diego, and another, 40 cm. in standard length, on Dec. 15, just N of Cedros Island, Baja California. The surface water at the first of these localities was 19.1°C.

Flying fish were conspicuous above the bank surrounding the Alijos Rocks, Nov. 9; off the Gulf of Tehuantepec, Nov. 16; and at 09°46' N, 93°30' W, 300 miles from the continent, Dec. 6. Weather had much to do with observation because the fishes emerged most actively during strong winds.

An example of <u>Cypselurus nigricans</u>, 18 cm. in standard length, flew aboard within sight of the Gulf of Dulce, Panama, on Nov. 25 (surface water 26.4°C).

REPTILES

Pelamis platurus. Sea snake. The northernmost specimen was taken under a light in the evening of Nov. 15, at about 14° N, 96°10' W. This was in a zone of upwelling to leeward of the Gulf of Tehuantepec. The surface temperature was only 25°C, whereas a few hours earlier and to the north it had been 29°C. Another was captured Dec. 1 in the Gulf of Panama (surface water 27°C).

Sea snakes were most conspicuous off western Panama, near Coiba Island, on Nov. 25, as many as ten at once sometimes being within sight

The distribution of this species, the only sea snake along the Pacific coast of America, is graphically correlated with the major oceanic circulation. The normal range extends from no more than latitude 02°S (or even nearer the equator, at La Plata Island, Ecuador) northward to about 23°N, at the mouth of the Gulf of California. Seasonal countercurrent development sometimes leads to a slight transgression of these limits but the range is, in any case, latitudinally asymmetrical, like that of many other marine organisms inhabiting the warm zone between the Peru and California currents.

Chelonia. Sea turtles. Turtles sighted during the cruise of <u>Stranger</u> probably included four species, namely <u>Chelonia mydas</u> (green) <u>Eretmochelys imbricata</u> (hawksbill), <u>Caretta caretta</u> (loggerhead), and <u>Lepidochelys olivacea</u> (Pacific Ridley). The last two, both of loggerhead type, were undoubtedly the commonest, and the only turtle captured and certainly identified was <u>Lepidochelys</u>. Identification of turtles in the water at various distances offers difficulties because of the changes in the margin of the carapace that take place with age and growth.

The example of Lepidochelys olivacea was taken on Nov. 23 at 09°41' N, 89°44' W, about 220 miles from the nearest land. Its carapace was 51 cm. in length. On the left side of its snout it bore a large barnacle, not yet identified.

All other examples seen from shipboard had best be listed merely as "turtles". They were noted as especially abundant on seven different days of the cruise, namely Nov. 13, 14, 16, 23, 25, and Dec. 1 and 14. Turtles were seen also on many additional days in both coastal and Ofshore areas between latitudes 26° N and 04° N. The total range of surface temperatures throughout these waters and dates was 24.8° to 29.7°C.

Sea turtles, like many oceanic fishes, show great interest in flotsam. I repeatedly saw them change course to approach the skiff or one of the ship's floating bamboos supporting a radar reflector. They would then nudge or rub against the hard objects for long periods.

My most interesting observations concern the ecological importance of turtles as resting

such as off the semiarid Pacific coast of Mexico, turtles may offer birds the commonest and most used resting "islets". We repeatedly saw boobies of two species, as well as certain other birds, perched upon their backs. Evidently such stowaways do not incommode the surfaced turtles. This matter is referred to further in the account of the birds.

BIRDS

<u>Gavia</u> <u>immer</u>. Loon. One seen Dec. 16 off the coast of Baja California near the United States border.

Fulmarus glacialis rodgersi. Pacific fulmar. A gray-phase female collected Dec. 12 at 23°31' N, 111°22' W. This is 30 miles offshore, halfway between Santa Margarita Island and Cape San Lucas (surface water 24°C). The specimen probably represents the southernmost record of the fulmar in any ocean.

Puffinus creatopus. Pink-footed shearwater. Observed, always singly, on six days during the cruise between the coastal waters of southern Baja California and the vicinity of Cocos Island. A casual representation of this southern-hemisphere breeder north of the equator during the normal nesting season is to be expected.

<u>Puffinus griseus</u>. Sooty shearwater. A case akin to that of the preceding species. Single birds twice noted, once on Nov. 13, off southern Mexico (surface water 29.7°C), and again on Dec. 13, off Cape San Lazaro, Baja California.

The sooty shearwater nests in the antiboreal zone and usually passes rapidly across the tropics on its migrations between higher latitudes of the opposite hemisphere.

Puffinus gavia opisthomelas. Black-vented shearwater. Many seen feeding, in company with other petrels and terns, about 40 miles off Punta San Telmo, SE of Manzanillo, Mexico, Nov. 13. The birds were in a natural oily "slick" on the ocean.

places for sea fowl. In seas of sparse flotsam, On the return voyage, when we were bound north-

ward from Sebestian Viscaino Bay and were within sight of the San Benitos Islands, where this shearwater nests, scores came close to the ship on Dec. 15.

This is a "fluttering" shearwater, an apt vernacular name originating in New Zealand from where the topotypical race was described. Other subspecies inhabit the Mediterranean Sea.

Puffinus puffinus auricularis. Townsend's shearwater. Distinguishable from the preceding species chiefly by its style of flight, rather than by the blacker shade of its dorsal surface, this shearwater was seen, in company with wedgetailed shearwaters, near 09°46' N, 93°30' W, on Dec. 6.

Townsend's shearwater is a weakly marked race of the European Manx shearwater. The specific distribution is cosmopolitan.

Puffinus lherminieri subalaris. Galapagos shearwater. One of the surprises of the cruise was the abundance and wide distribution at sea of the Galapagos race of Audubon's shearwater. As a species, <u>lherminieri</u> has world-wide tropical range. The subspecifics character of <u>subalaris</u> are strongly marked, notably in the corneous nature of the nasal tubes. Identification was confirmed by the capture of an adult male with greatly enlarged gonads at 11°13' N, 90°55' W, on Nov. 17. The position is south of the Guatemala-Salvador boundary, nearly 150 miles from land.

This was the only Procellari-form bird that showed curiosity, or what might be called a "playful" interest, in the vessel. On many occasions single birds or groups performed swift and repetitious flight maneuvers around the craft. The Nov. 17 example was one of the two that flew close to the bulwarks of <u>Stranger</u> many times at dizzying speed, enabling me to make several not too successful photographs.

Thereafter we saw these small shearwaters on many dates along our course. They were abundant off the coast of western Panama on Nov. 24-25. During the night of Nov. 30, when we were bound outward through the Gulf of Panama toward Cape Mala, hundreds of them fluttered about within range of the ship's lights, and after dawn of Dec. 1 seven of the birds in a compact group flew up from astern many times, swept to within arm's reach of the rail, and then swung off widely to drop astern and come up again.

We last saw this species near Cocos Island, and in waters toward the NW, Dec. 1-6. The northernmost records were in the neighborhood of 11° N. Although the Galapagos Islands are still the only known breeding grounds, it is quite possible that this shearwater may prove to be also a resident of Cocos Island.

Dr. Bell Shimada and other members of the scientific group on <u>Stranger</u> informed me that on an earlier cruise of the M/V <u>Spencer F. Baird</u> in these same waters during late November or early December large numbers of shearwaters, which they believed to be this form, had descended on the decks, sung in pairs, and even copulated. The men had to toss the birds into the air to get rid of them. The identification is almost assuredly correct because no other shearwater of the area would be at the peak of its reproductive cycle at this season. Other instances are known in which petrels in a breeding state have adopted ships as convenient "islands".

<u>Puffinus pacificus chlororhynchus</u>. Wedgetailed shearwater. On the American side of the Pacific this petrel nests only at the Revillagigedo Islands. It ranges southward through the warm ocean waters to the Pacific coast of Colombia, where I collected specimens on the Askoy Expedition.

Aboard the <u>Stranger</u> the following was observed on three dates: a flock on the morning of Nov. 17, near 11°N, 90°55' W; many, all of the white-breasted phase, on Dec. 6, at 09°46' N, 93°30' W; and birds of both dark and lightbreasted plumage phases on Dec. 16, off northern Baja California (surface water <u>circa</u> 20°C). Now and then wedge-tails crossed the bow of the ship at close range, but never when collecting proved possible.

Oceanodroma tethys kelsalli. Galapagos storm petrel. Seen frequently throughout the cruise and represented by six specimens in the

- 104 -

collection. These establish the race as that breeding at the Galapagos Archipelago. A slightly smaller form nests on islets along the coast of Peru.

Two males at the peak of breeding condition were taken on Nov. 10 at $22^{\circ}57^{\circ}$ N, $113^{\circ}3^{\downarrow}$ W, a few miles N of the isolated Alijos Rocks and nearly 200 miles W of Cape San Lazaro, Baja California. Thereafter the species was encountered all along our course to the "Dome" area, Nov. 19-23. We met it again outside the Gulf of Panama, in the waters around Cocos Island, and for a thousand miles toward the NW until Dec. 8. The range of surface temperatures throughout these areas and dates was $2^{\downarrow}.1^{\circ}$ to 29.6° C.

Oceanodroma leucorhoa. Leach's petrel. Two specimens collected, but which of the four subspecies currently recognized along the Pacific coast of North America they represent has not yet been determined. It is likely that the typical and most northerly race migrates farther southward than any of the other three.

The pattern of distribution of Leach's petrel during the cruise of <u>Stranger</u> closely matched that of the preceding species. The first specimen flew aboard S of the Alijos Rocks during the night of Nov. 9. Thereafter the species was logged on Nov. 10, 11, 19, 20, 21-23, to the "Dome" area. Later, Dec. 2-4, we found it at 04°09' N, 83°34' W, in waters around Cocos Island, and for about 200 miles northwestward.

Probably surface temperatures have little significance in relation to the winter distribution of this storm petrel.

Loomelania melania. Black petrel. This species nests at Los Coronados and San Benitos islands W of Baja California and at islands of corresponding latitudes within the Gulf of California. It migrates southward to the ocean off northern Peru, but avoids the cool waters of the Peru Current. Its winter range S of the equator appears to be determined, indeed, by surface temperatures dependent upon current-countercurrent controls. Cape San Lazaro, seen to have been bypassed by

The black petrel was logged very frequently between Nov. 11, at 21°07' N, 109°56' W (S of the Alijos Rocks) to western Panama and the Gulf of Panama. Later we found it at our southernmost station (01°09' N, 83°31' W), around Cocos Island, and N toward the continent to the latitude of Cape San Lazaro on Dec. 13. The amplitude of surface temperature among all the observations was about 24°C to 29.6°C.

Five specimens were collected. They had heavy deposits of subcutaneous fat, as befits birds in the early stages of a long migration. In fact, they were the fattest of the four species of storm petrels obtained on the expedition.

A female shot on Nov. 14, at 16°16' N, 100°27' W, had her stomach and gullet crammed with lantern fishes of a uniform 40-mm. length.

Of all Pacific storm petrels of my personal acquaintance, this one is most persistently given to following vessels. The birds accompanied <u>Stranger</u> for days on end, sweeping widely across the wake and apparently profiting from the artificial turbulence of the water rather than from food cast overboard.

<u>Halocyptena microsoma</u>. Least petrel. Like the preceding species, this tiniest of petrels nests in the Mexican Pacific and Gulf area and migrates southward into equatorial waters.

It was observed and occasionally collected along our course between Nov. 14 and Dec. 16. There were periods of days in which none was seen, but these appeared to have nothing to do with latitude or with distance from the continental coast. The northernmost record was near Cape San Lazaro, Baja California, Dec. 14. Surface temperatures on all days on which the species was noted ranged from about 24° to 29.6°C. the many ornithologists who have visited islands W of Mexico. I find no mention of them in available texts. They prove, however, to be the probable northernmost breeding station in the Eastern Pacific of three wide-ranging tropical ocean birds, namely this species, the masked booby, and the American man-o'-war bird.

The tropic-birds at Alijos Rocks were engaged in active courtship, twos and threes joining in swift pursuit flight and keeping up an excited trill of their boatswain whistles. A male collected was at the physiological peak of breeding. It disgorged a 25-cm. fish (<u>Colalabis saira</u>).

Surface temperatures in the neighborhood of the Alijos Rocks were as low as $20.6^{\circ}C$. The tropic-birds appeared to be nesting on each of the three stacks of the group.

<u>Pelecanus occidentalis</u>. Brown pelican. This is a continent-hugging species, of little interest in an oceanographic campaign. It has reached only one group of remote oceanic islands- the Galapagos - where the resident colony is isolated and racially endemic.

We encountered two subspecies, <u>californicus</u> of the northerly and relatively arid coast, and <u>carolinensis</u> of the moist tropical Middle American coast of both Atlantic and Pacific. Nothing was learned about distribution boundaries or possible intergradation of these two forms.

Sula dactylatra. Masked booby. This largest of the tropical pelagic boobies is to a great extent a flying fish-eater. White adults, dark young, and birds in transitional plumage were seen regularly after we had reached the newly discovered nesting station at Alijos Rocks. The species avoids forested islands and continental coasts. It was not present at Cocos Island, for example, although common enough over the surrounding ocean within a distance of a few hours' sail.

Discovery of the Alijos colony, where breeding boobies appeared to be confined to the easternmost of the three stacks, rounds out our knowledge of the nesting stations in the Eastern Pacific. These extend from the Alijos Rocks S to San Ambrosio Island, off northern Chile, and include Malpelo (Colombia) and La Plata (Ecuador). The LaPlata colony is the only one within sight of the continent. Malpelo has by far the largest booby population.

At sea this booby showed marked curiosity regarding conspicuous flotsam such as our skiff and the radar reflector above bottlefloats. The birds would swoop around them again and again, and even attempt to alight. As noted above, the masked booby also makes regular and prolonged use of sea turtles as rafts for resting on the ocean. Substantial flotsam, such as logs, is used in the same way, but it is likely that turtles offer the most plentiful opportunities for perching throughout vast areas off soundings.

At any rate, on Nov. 15, some 90 miles off the Gulf of Tehuantepec, I saw eight of these boobies standing peacefully on turtles. Again, on Dec. 8, much farther off the coast, six more were observed resting in the same manner. Lone turtle-perchers were noted on numerous other occasions, in some instances apparently sleeping, with the bill tucked among the feathers of the back.

<u>Sula sula</u>. Red-footed booby. This is the only tree-and shrub-nesting member of its family. In breeding and feeding habits it occupies a somewhat different ecological niche from other boobies inhabiting the same area, thus avoiding or reducing interspecific competition.

Like the masked booby, the red-foot is an offshore and pelagic bird, rarely found near continental coasts. We entered its stronghold only at Cocos Island, a well-populated nesting station, and found it at sea only within 400 miles of that island, chiefly toward the NW. It was the only booby that followed the ship, played around the mastheads, and alighted on the superstructure. Approaching Cocos, one was caught on a fishhook. Others were collected at the island.

Throughout the tropical oceans this species has several plumage phases, the taxonomic

significance of which is not yet well understood. The Cocos Island population, however, comprises only uniformly grayishbrown birds, and we saw no other type on our voyage.

<u>Sula leucogaster</u> <u>brewsteri</u>. Brewster's booby. The case of this booby and the next poses interesting biological and biogeographical problems. Both are subspecies of the cosmopolitan brown booby, and both are confined to the west coast of America and outlying islands. From the topotypical brown booby the two races differ in a similar manner, notably in that the heads of adult males have pale or whitish feathering. The physical distinctions between the subspecies <u>brewsteri</u> and <u>etesiaca</u> are slight but are constant and readily recognized.

Physiologically, however, the differences between these two races may be relatively profound because <u>brewsteri</u> lives in an area of high aridity, whereas <u>etesiaca</u> extends from some unknown point N of western Panama southward to the coast of Colombia. It includes also Cocos Island. Whether there is a hiatus between the coastal ranges of the two races is yet unknown.

We first met Brewster's booby on Nov. 12, midway off the mouth of the Gulf of California. The birds were flying in pairs or in groups of three. Next day the first specimen was collected. Thereafter examples were observed, sometimes standing on the backs of turtles, as far as waters off the Gulf of Tehuantepec. On the return voyage we saw this booby again off the entrance of the Gulf of California on Dec. 10.

<u>Sula leucogaster</u> etesiaca. Columbian booby. The presence of boobles of this type in the "Dome" area was inconclusive because of the difficulty of discriminating, without specimens in hand, between etesiaca and brewsteri.

When we approached the coast of western Panama, large flocks of Colombian boobies became a familiar sight. Sometimes they were feeding with other sea birds, such as cormorants, jaegers, and terns. A particularly large

concentration was passed on Nov. 25 off the Islas de Ladrones, where they doubtless nest. Later we found them in the Gulf of Panama and along our course toward Cocos. On Dec. 1 a female in breeding state was collected at 05°59' N, 79°48' W.

While approaching Cocos on Dec. 3, we met a movement of Colombian boobies 50 miles from the Island. At Cocos they were nesting principally on the outlying islets, particularly on Manuelita or Nuez, where their nests, with eggs and young in all stages, were underneath tall shrubs in which red-footed boobies were nesting. Although confined to the ground for nidification, the Colombian boobies perch freely on good-sized branches of trees, but perhaps never on twigs.

The surface water at Cocos proved of slightly lower temperature than that in the range of <u>brewsteri</u>, far northward.

<u>Phalacrocorax penicillatus</u>. Brandt's cormorant. The cormorants are all coastbound birds in the part of the world under consideration. This species was noted along the coast of Baja California.

<u>Phalacrocorax olivaceus</u>. Bigua cormorant. Observed in western Panama and in the Gulf of Panama.

Phalacrocorax pelagicus. Baird's cormorant. Although named pelagicus, this species is also confined to the narrow continental platform. It was noted only within a few miles of San Diego and the Coronados Islands.

Fregata magnificens. American man-o'-war bird. This species is common to both Atlantic and Pacific sides of tropical and subtropical America but, except at the Galapagos, it is replaced by the following species as an offshore bird in the Pacific. Our most seaward records were made near the breeding station of Alijos Rocks, on Nov. 9. This is presumably the northern limit of the nesting range on the Pacific coast. Two adult males were collected here.

Thereafter we saw this species regularly to the Gulf of Panama, always interested in

- 107 -

aggregations of other sea birds and of fish and porpoises at the surface. On the voyage to the Cocos Island we left it far behind, but picked it up again off Cape San Lazaro, Baja California, on Dec. 13.

Fregata minor. Pacific man-o'-war bird. This species, sometimes called the greater man-o'war bird (although it is smaller than magnificens), occurs also in the Indian and South Atlantic oceans. It nowhere reaches American continental shores.

Both <u>magnificens</u> and <u>minor</u>, however, occur at the Galapagos Archipelago, although perhaps never at the same island. It has long been a question as to which species is resident at Cocos, a matter not solved until the visit of Stranger.

On Dec. 3, we were met by scores of \underline{F} . minor, all in immature plumage, some tens of miles E by S of Cocos. They mingled with our escort of red-footed boobies, both astern and circling the masts.

The adults at Cocos Island mostly soared high above the hills and treetops. At times one would swoop toward the water to harry a foodladen booby. This ultimately enabled me to shoot an adult breeding male. The species was not seen elsewhere.

Casmerodius albus egretta. American egret. At noon on Dec. 1 one flew, out of gunshot, past my skiff at 05°59' N, 79°48' W. The position is on the open ocean about 90 miles S of Cape Mala, Panama.

Anas platyrhynchos. Mallard. A female duck, apparently a mallard, alighted and then took off from the ocean, close to <u>Stranger</u>, on Nov. 20, at 09°16' N, 89°18' W, about 200 miles off the Costa Rican coast. On Nov. 17, 12 similar ducks passed high above us at an equal distance from the nearest land (El Salvador).

Aythya affinis, Lesser scaup. Not observed at sea, but on Nov. 28 a flock took off from Gatun Lake, Canal Zone, in front of the Barro Colorado Island Laboratory. The species has apparently not previously been recorded from Barro Colorado.

Phalaropus fulicarius. Red phalarope. Observed along course, both near the coast and far offshore, between Nov. 19 and Dec. 14. The northernmost record was off Point San Juanico, Baja California. Red phalaropes were usually met with either in pairs or in small flocks. One was once seen standing on the back of a turtle. Our only specimen, which was extremely fat, was taken on Dec. 8 at 14°37' N, 100°09' W.

Lobipes lobatus. Northern phalarope. More abundant than the foregoing species and likewise usually found in either pairs or flocks. It still ranged as far north as the ocean off San Diego on the last day of our voyage, Dec. 16. One was collected off the Gulf of Tehuantepec on Nov. 15.

Both species of phalaropes showed a predilection for oily "slicks" on the ocean.

Stercorarius pomarinus. Pomarine jaeger. The commonest of its family throughout the cruise. Seen everywhere, and almost daily, between San Diego and Panama, and in the waters NW of Cocos Island. In the Gulf of Panama it was parasitizing the laughing gulls. An example was collected on Nov. 14.

<u>Stercorarius parasiticus</u>. Parasitic jaeger. Less common than the pomarine jaeger, but presumably as widely distributed. A specimen collected to represent this species, however, has proved to be the next.

Stercorarius longicaudus. Long-tailed jaeger. A very young, practically fledgling, male jaeger, shot on Nov. 14 at 16°16' N, 100°27' W, has turned out to be longicaudus. The species was not noted elsewhere.

Catharacta skua chilensis. Chilean skua. Two brightly cinnamon skuas, seen at close range from <u>Stranger</u> on Nov. 15, over a "slick" off the Gulf of Tehuantepec, assuredly were of this form, with which I became well acquainted in Peru and Chile. The skuas were in company with Sabine's gulls, boobies, and storm petrels. Larus occidentalis. Western gull. Seen wouthward from San Diego to a distance of 90 miles off the Gulf of Tehuantepec. Along the shores of Baja California this species appeared in alternate with bands of the California gull.

Larus californicus. California gull. Encountered only along the coast of Baja California. Off the broad entrance to the Gulf of California, Dec. 10-12, we met many immature examples which behaved like veteran pensioners of ships and were content to wait hours for jettisoned garbage. At one time I counted 250 around Stranger. White, adult California gulls were mostly seen farther northward.

Larus atricilla. Laughing gull. Common in the Gulf of Panama. First seen in considerable numbers immediately after we had rounded Cape Mala on Nov. 26. On Dec. 1 several followed us out to the high sea for about 100 miles S of Balboa. This gull was also common in Gatun Lake, Canal Zone, mingling with small flocks of black terns.

The first specimen of the laughing gull, however, was collected far off the coast of Baja California, at 22°57' N, 113°34' W, on Nov. 10. A second was taken at 05°59' N, 79°48' W, Dec. 1.

Larus pipixcan. Franklin's gull. Adults still wearing full summer plumage were seen off western Panama on Nov. 25. The two collected were both young birds, taken at sea on Nov. 23 at 09°41' N, 89°44' W. The position is in the "Dome" area, about 240 miles W of Costa Rica. The longitude, which is far to westward of South America, passes through the Galapagos Islands, to which Franklin's gull is a regular winter visitor.

Larus heermanni. Heermann's gull. Observed between Cedros Island, Baja California, and San Diego, Dec. 15-16.

Xema sabini. Sabine's gull. Seen occasionally and usually at long range, southward to Panama. A female was collected on Dec. 12 at 23°31' N, 111°22' W. The position is W and a little N of Cape San Lucas. Sterna hirundo. Common tern. Small terns were seen on many dates, but the only certain identification is based upon a female of this species collected on Nov. 15 at $14^{\circ}17^{\circ}$ N, $96^{\circ}34^{\circ}$ W, off the Gulf of Tehuantepec.

Thalasseus maximus. Royal tern. Common in the Gulf of Panama, Dec. 26-30.

Chlidonias niger. Black tern. First observed off the Gulf of Dulce, western Panama, Nov. 25. Common in the Gulf of Panama and on Gatun Lake, where it mingled with laughing gulls.

The black tern in its winter range clings closely to tropical coasts and flotsam-filled waters. It never dives and it rests mostly on floating vegetation. Therefore it is always most abundant where rivers flow to the ocean through forested areas. It is very rarely found out of sight of land.

Anous stolidus. Brown noddy. An adult female with slightly enlarged ovaries was collected on Manuelita Islet, off the northern point of Cocos Island, on Dec. 3. On Dec. 8 at 14°37' N, 100°09' W, about 190 miles south of Acapulco, I saw a small flock of this species.

Megalopterus <u>minutus</u>. Black noddy. Black noddies came aboard <u>Stranger</u> early in the morning of Nov. 23. Later in the same day an adult male was collected at 09°^h1' N, 89°^h4' W, which is in the "Dome" area, about 240 miles W of Costa Rica.

On Dec. 3 several examples were seen flying in and out of a sea cave on Manuelita or Nuez Islet, Cocos Island.

Land Birds. A considerable number of land birds alighted on <u>Stranger</u> in various parts of the cruise. Some of them could be only approximately identified:

Spectyto cunicularia. Burrowing owl. Nov. 13, more than 40 miles off Petacalco Bay, Mexico.

Large flycatcher. Nov. 25, W of Coiba Island, Panama.

Empidonax. Small flycatcher. Nov. 9, at the Alijos Rocks.

Hirundo rustica erythrogaster. Barn swallow. Gulf of Panama, Dec. 26 and 30.

Petrochelidon pyrrhonota. Cliff swallow. Nov. 11 at 21°07' N, 109°56' W, midway across the mouth of the Gulf of California.

<u>Hylocichla</u>. Thrush (resembling a hermit thrush). Nov. 24, about 80 miles off the coast of Costa Rica.

Vermivora peregrina. Tennessee warbler. A young bird, sex indeterminable, came aboard on Nov. 10 at 22°57' N, 113°34' W.

Vermivora ruficapilla. Nashville warbler. Severl flew aboard W of Coiba Island and off the Gulf of Panama, Nov. 24 and 25. One younger one of undetermined sex was found in the ship's laboratory and was preserved.

Ammodramus. Sparrow. Nov. 14, 16°16' N, 100°27' W, off Acapulco.

MAMMALS

Zalophus californianus. California sea lion. About a dozen were on and around the middle Alijos Rock on Nov. 9. The other two stacks of this group could be scaled only by winged creatures. Otherwise we saw sea lions only at the Coronados Islands, and on the channel buoys of San Diego.

Mirounga angustirostris. California sea elephant. One adult bull seen swimming off the northern end of Cedros Island, Baja California, where there is said to be a small colony.

Rhachianectes glaucus. Gray whale. One surfaced near Stranger among the Coronados Islands on Dec. 16.

Physeter catodon. Sperm whale. This species was several times sighted at long range and recognized by the character of its spout.

On Dec. 6, near 09°46' N, 93°30' W, nearly

400 miles west of Costa Rica, we sighted three sperm whales and <u>Stranger</u> followed them at reduced speed, finally approaching within 30 m. Two cows and a calf lay side by side. The adults were each about 12 m. in length, and the calf, which stuck to the left flank of its mother, seemed only three to four m. shorter. All three whales sounded together and came up a quarter-mile to the right of their former course. Later in the same day two more sperm whales were watched at a distance of a mile or more.

<u>Globicephalus</u>. Blackfish. I have no way of knowing whether the blackfish seen on several occasions represented the species <u>scammoni</u> or <u>macrorhynchus</u>. They appeared not infrequently around the ship all the way from northern Baja California to waters outside the Gulf of Panama.

Delphinus bairdi. Porpoise. Schools of porpoises, indistinguishable to me from D. delphis of the Atlantic, were presumably this species. Large groups were encountered as follows: Nov. 12, 19° N, 106° W, two schools; Nov. 17, 11°13' N, 90°55' W; Nov. 24, 08°42' N, 86° W; Dec. 6, near 09°46' N, 93°30' W; Dec. 14, off Point San Juanico, Baja California; Dec. 15, E of Cedros Island.

In the evening of Dec. 14, when porpoises were showing great activity close to <u>Stranger</u>, the EDO of the sonar equipment was turned on to receive their communications. Porpoises signal in a language of high frequencies beyond the range of human ears. But the EDO pulled this down to 8000 cycles and the result was like a dawn chorus of birds in May. Whistles, piping, chattering, and musical squeals came out of the depths in a cheerful medley.

<u>Prodelphinus</u> graffmani. Spotted porpoise. On Nov. 26 a school of porpoises, indistinguishable to my eyes from P. <u>plagiodon</u> of the Atlantic, accompanied the vessel on two occasions in the Gulf of Panama.

Mesoplodent whale. On Nov. 10, near 22°57' N, 113°34' W, which is about 125

miles SW of Santa Margarita Island, Baja California, an unidentified mesoplodent overtook and passed <u>Stranger</u>. It was approximately 10 m. long and seemed to have a pronounced neck constriction; it produced no visible spout during several rises. t

THE ALCOHOL-SOLUBLE AND INSOLUBLE FRACTIONS OF THE PHOTOSYNTHETICALLY

FIXED CARBON IN NATURALLY OCCURRING MARINE PHYTOPLANKTON POPULATIONS

by

William H. Thomas

Chemical analyses of phytoplankton cells may give information valuable in determining the nutritive value of such cells to their predators. Such analyses are most easily carried out with laboratory-cultured cells, but such cells may not truly represent those occurring in nature. This paper reports experiments made at sea with naturally occurring populations in which determinations of the alcohol-soluble fraction of marine phytoplankton were made. These determinations may give an indication of the gross chemical composition of photosynthesizing cells.

Because of the small numbers of algal cells present per unit volume of pelagic water, attempts to harvest these cells for purposes of chemical analyses by the usual chemical means would be extremely time consuming. Fortunately the use of the C^{14} technique of labeling the organic matter produced by phytoplankton greatly increases the sensitivity of a chemical extraction procedure so that only relatively small volumes of water need to be handled.

In these experiments a sample of surface water was taken with a plastic bucket. Aliquots of this sample were added to 250-ml. ground-glass-stoppered bottles, C^{14} was added to each bottle, and the bottles were illuminated at 1200-1400 foot-candles at the surface sea-water temperature in a glassbottomed water bath. After incubation, aliquots of the water were filtered through HA Millipore filters (0.45 μ pore size) to determine the total activity fixed. Another aliquot was filtered through a sintered-glass filter. The residue on this filter was extracted with boiling $80^{\circ}/_{\circ}$ ethanol. An aliquot of the combined extracts was

evaporated on a steel planchet to determine the extractable activity. A linear relationship between volume of extract and activity showed that self-absorption corrections were not necessary when volumes no larger than 0.50 ml. were evaporated on the planchets. The proportion of extractable material in the cells was determined by dividing the activity extracted by the total activity in the cells. The assumption is made that after this long period of incubation all chemical entities in the cells are labeled with Cl4 to the same extent and that the ratio of extractable activity to total activity truly represents the proportion of alcohol soluble in all materials. It is further assumed that no losses of C14 occurred during evaporation of the extract on the planchets.

Considering the speed of the photosynthetic cycle as shown by Buchanan <u>et al</u>. (1952), (steady-state labeling of sugar phosphates after about 15 minutes) the first assumption seems reasonable (cf. also Fogg, 1956). Compounds appearing in the extract would include sugars, amino acids, sugar phosphates, pigments (less their protein moieties) and lipids. Substances remaining behind would include proteins, polysaccharides, and nucleic acids.

An initial preliminary experiment was performed at Station 0-1, 50 miles west of Baja California ($30^{\circ}01'$ N latitude and $116^{\circ}49'$ W longitude). In this experiment only four bottles were incubated (for eight hours) and the activity recovered in the extract was only 92 cpm. above background in 10 ml. of extract. The total activity on the millipore filter was 619 cpm. Thus about $15^{\circ}/_{\circ}$ of the activity was extracted. Because

Data:				Calculations:
Sample	net cpm	average cpm	cpm/liter of pool	cpm/liter of pool on glass filter = 2584 - 218 - 2366
MP-1-1 MP-1-2 MP-1-3 E-1-1 E-1-2	646 59 59 50 70 70 70 70 70 70 70 70 70 70 70 70 70	646 54.5 47	2584 218 403	$^{\circ}/^{\circ}$ extracted = $\frac{403}{2366} \times 100 = 17.0^{\circ}/_{\circ}$
EXPERIMENT 2:	г 2:			Calculations
Data:				
MP-2-1 MP-2-2 MP-2-2 MP-2-3 B-2-1 B-2-2 B-2-2	849 18 21 99	849 19.5 95.5	3396 78 546	cpm/liter of pool on glass filter - 3396 - 78 - 3318 °/• extracted = $\frac{546}{3318}$ x 100 = 16.4°/•

0

1

TABLE 19

EXPERIMENT 1:

)			
Data:				Calculations:
Sample	net cpm	average cpm	cpm/liter of pool	cpm/liter of pool on glass filter = 7178 - 1908 - 5270
MP-3-1 MP-3-2 MP-3-2	1864 500 151	18 6 4 477	7178 1908	°/. extracted = 856 x 100 = 15.9%.
E-3-1	69 65	65	856	
EXPERIMENT 4:	P 4:			
Data:				Calculations:
MP-4-1 MP-4-2 MP-4-2 MP-4-3 E-4-1 E-4-2 E-4-2	1626 453 332 63 62	1626 392 625	6260 1568 603	cpm/liter of pool on glass filter = 6260 - 1568 = 4692 % extracted = <u>603</u> x 100 = 12.8%

TABLE 20

EXPERIMENT 3:

of the difficulty in determining the activity of an extract of such a low specific activity, and because no replication of the plating was made, this result was only considered preliminary. It served to establish the range of activity to be expected in two further experiments.

The next experiment was performed at Station 5-9 in the Central American thermal anticline 80 miles off Costa Rica (9°28' N latitude, 89°18' W longitude) on November 21, 1956. Sixteen 250-ml. bottles of surface sea water were incubated with 2,887,000 cpm. of C14 per bottle for eight hours, and at 1200-1400 fc. and 25°C. After incubation the contents were pooled in a plastic bucket, and 250 ml. of this pool were immediately filtered through a Millipore filter (MP-1-1) to determine the total activity fixed. Then 1750 ml. were filtered through an F-porosity sintered-glass filter (maximum pore size 5 µ). Two 250-ml. portions of the filtrate from this last filtration were then filtered through Millipore filters (MP-1-2 and MP-1-3) to determine the portion of the activity not retained by the glass filter. The residue on the filter was washed twice with non-radioactive sea water and then was extracted four times with boiling 80°/. ethanol. The combined extracts were dried with a hot-air stream at 35°C and then taken up in 7.5 ml. of twice-distilled water to yield extract E-1. This whole process was then repeated on the rest of the pool to give Millipore pads PM-2-1, MP-2-2 and extract E-2 which had a final volume of 5 ml. derived from 1750 ml. of pool. The glass filter used in this last extraction had a maximum porosity of 1.2 μ . Two 0.50-ml. aliquots of each extract were evaporated on steel planchets at 35°C under a hot-air stream and an infrared lamp (E-1-1, E-1-2; E-2-1, E-2-2). The results of these experiments are shown in the Table 19.

The next experiment was performed at Station S-10, 130 miles off Costa Rica ($8^{\circ}42'$ N latitude, $86^{\circ}00'$ W longitude) on November 24, 1956. Fourteen 250-ml. bottles of surface sea water were incubated with 5,774,000 cpm. of Cl⁴ per bottle for five hours at 1200-1400 fc. at 26°C. After incubation the contents of one bottle (260 ml.) were filtered through a Millipore filter (MP-3-1). The contents of six bottles (1580 ml.) were filtered through

an M-porosity sintered-glass filter (maximum pore size 14μ). Two 250-ml. portions of this filtrate were filtered through Millipore filters (MP-3-2 and MP-3-3). The residue on the glass filter was washed and extracted in the same manner as in experiments 1 and 2. The combined extracts were made to 10.4-ml. final volume without drying and re-extracting with water to yield extract E-3. The whole process was repeated with the seven remaining bottles to yield Millipore pads MP-4-1, MP-4-2, and MP-4-3 and extract E-4 which was derived by filtration of 1575 ml. of the original water through an M-porosity glass filter and which had a final volume of 7.6 ml. Two 0.50-ml. aliquots of each extract were plated on planchets as in experiments 1 and 2. The results of these experiments are shown in Table 20.

These experiments show that about 15°/. of the cellular carbon in naturally occurring phytoplankton populations is alcohol soluble. A similar proportion of soluble to insoluble material (expressed on a dry-weight basis) is found generally in those few algae which have been investigated (cf. Fogg, 1953). It is striking that there is little variation in the percentage of extractable material in phytoplankton from the three areas. Presumably nutrient conditions might be different in the various areas and the cells might reflect this by having differing proportions of soluble material.

It can be inferred from the data, if the assumption is made that the alcohol-soluble material is also soluble in sea water, that when an algal cell dies $15^{\circ}/_{\circ}$ of the cell material would be immediately released to the water and would serve as food for bacteria and other hetrotrophic organisms. A portion of this material might also become a part of that more resistent dissolved organic material which accumulates in the ocean. It is also probable that only $85^{\circ}/_{\circ}$ of the material produced by phytoplankton has any chance at all of reaching the bottom and becoming a part of the organic material in sediments.

If a phytoplankton cell is eaten before it dies, then some $15^{\circ}/_{\circ}$ of its material is immediately available for incorporation into the body tissues of the animal which eats it.

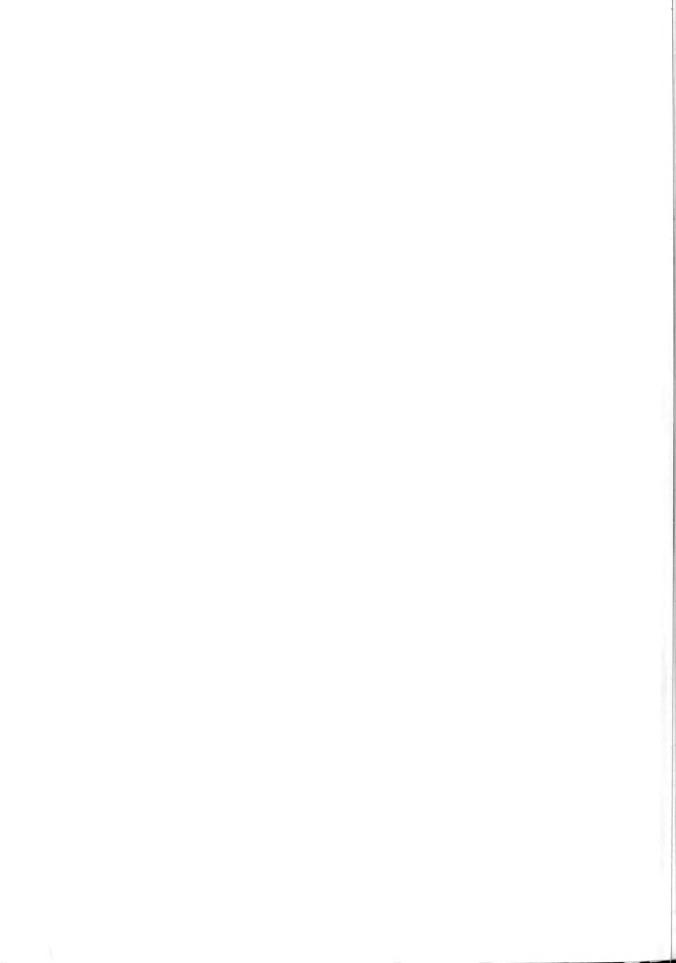
Some $85^{\circ}/_{\circ}$ of the cell material would have to be broken down by digestive enzymes in the gut of the animal before incorporation. Also fecal pellets are probably wholly made up of this insoluble material.

REFERENCES

Buchanan, J. F., J. A. Massham, A. A. Benson,
D. F. Bradley, M. Calvin, L. L. Dans,
M. Goodman, P. M. Hayes, V. H. Lynch,
L. T. Norris, and A. T. Wilson. 1952.

The path of carbon in photosynthesis XVII. Phosphorus compounds as intermediates in photosynthesis. <u>In</u> McElroy, W. D. and B. Glass, editors. Phosphorus Metabolism, Volume II, pp. 440-459. Johns Hopkins Univ. Press, Baltimore.

- Fogg, G. E. 1956. Photosynthesis and formation of fats in a diatom. Ann. Bot. (N.S.), Vol. 20, pp. 265-285.
- Fogg, G. E. 1953. Metabolism of Algae. John Wiley and Sons, Inc., New York.





. .

