"ARTIFICIAL UPWELLING"
PROGRESS REPORT / 1974

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"ARTIFICIAL UPWELLING"
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PART A—ST. CROIX "ARTIFICIAL UPWELLING" MARICULTURE

Summary Progress Report: 1974

Our work during the past year was centered primarily around the optimization of our system for producing food for bivalve molluscs, and upon selecting bivalves with the greatest economic potential for commercial-scale "artificial upwelling" mariculture. Other work covered the beginnings of a hatchery operation and continued investigations into multiple uses of the effluent from our bivalve tanks for production of valuable plants and animals.

The progress made in our various projects this year is summarized here; the detailed reports on these projects are located in the main body of the report under the same headings.

ENGINEERING

The major engineering activity during 1974 was the design and construction of a Hatchery/Wet Lab. This building will house a Hatchery in which we can spawn new batches of shellfish as they are needed for experiments, an Experimental Shellfish Area in which quantitative studies of shellfish feeding and growth can be accomplished, and a Pilot Shellfish Area in which designs for a commercial-scale system for rearing bivalves to market size will be tested.

Algal cultures from ten elevated 550-gallon tanks (and from the two 12,000-gallon pools) adjacent to the Hatchery/ Wet Lab can be fed to juvenile larval shellfish in any desired combination or concentration (by diluting with deep water) at sites throughout the building. This facility will enable us to find the optimal diets, flow rates and food concentrations for maximum shellfish growth and food utilization throughout their life cycle, from spawning through growth to market size and development of ripe gonads.

Five additional 550-gallon algal reactors were installed for studies of the relationships between deep water, flow rate (nutrient supply), light intensity, and growth rate of a diatom used as food for juvenile bivalves. A recording Eppley pyranometer was installed for measurement of solar radiation during algal growth experiments.

Monthly SCUBA dives over the inshore section of three deep-water pipelines installed in 1972, and weekly water analyses of the water delivered by the pipes, indicate that the pipelines are still in excellent shape.

CHEMISTRY

A second channel was added to our Technicon Auto-Analyzer II, enabling us to process a greater volume of samples for dissolved nutrients.

A preliminary study was undertaken to determine the efficiency of deep-water nutrient utilization by diatoms grown in our two 12,000-gallon pools as food for bivalves.

Tests with a Monitek 250 turbidimeter indicated that measurement of forward scattering by particulate matter in water would be a suitable means of monitoring fluctuating properties in our deep-water supply, as well as the cell density from our algal cultures and shellfish tanks.

ALGAL CULTURE

Since mid-1974, we have used the diatom, *Chaetoceros curvisetus* (STX-167) as one of three algal foods for bivalve shellfish. This diatom is maintained in nearly unialgal culture for periods of 2 to 4 weeks in unenriched deep water cultures with a turnover of 1.1 dilutions per day in a 13,000-gallon pool. Enriching the deep water with a chelated iron-trace metals-vitamin mixture does not influence the longevity of cultures of this diatom as it does for the other species used. Wall growth and competition with other species of algae is apparently eliminated in cultures of this diatom because of its high efficiency of nitrate and silicate utilization. Calculations from experiments in outdoor continuous cultures with neutral density screens indicate that an optimal pool depth for this diatom is 4.1 meters at a turnover rate of 1.2 dilutions per day.

Three of five species of naked flagellate algae tested for tolerance of warm temperatures were found to grow well at temperatures up to 32°C. One of them was successfully used (in a mix with three diatoms) to rear larvae of the little-neck clam (*Tapes semidecussata*) to the juvenile stage.

Reliable growth of flagellate algae is necessary for successful operation of a shellfish hatchery in the tropics.

We found that the screening of algal species for successful growth in unenriched deep water could be accomplished in small-scale laboratory, semi-continuous cultures, thus simplifying this task.

Laboratory studies at our home base in Palisades, N.Y. on nitrogen uptake and assimilation and growth by *Chaetoceros simplex* (STX-105) in deep water, and of "resting cell" formation by the pennate diatom, *Amphora coffaeformis*, isolated from deep water were completed. These studies give important insights into the physiology of the primary steps of the food chain.

SHELLFISH CULTURE: PELECYPOD

Nine species of bivalves were studied during 1974 for their suitability in the St. Croix mariculture system. Five species were harvested at market size during the year. An analysis of six species grown to market size in our system indicates that four species have the greatest potential for commercial-scale mariculture (Japanese little-neck clams, *Tapes semidecussata*; Southern hardshell clams, *Mercenaria campechiensis*; F_1 clams, *M. mercenaria* ♂ x *M. campechiensis* ♀; and Pacific oysters, *Crassostrea gigas*). The European oyster, *Ostrea edulis*, requires further testing. The bay scallop, *Argopecten irradians*, grew rapidly to market size but has low priority for further investigation in our system on economic grounds.

The Kumomoto strain of Crassostrea gigas, valued for the half-shell trade, was introduced into our system and is growing well.

A comparative growth study of Mercenaria campechiensis, M. mercenaria, and the F_1 cross of these two clams in our system and in natural environments in St. Croix, Florida, Virginia, New York, and Hawaii, was completed this year. This study showed that M. campechiensis and the F_1 clams reached market size in our system earlier than in any of the natural environments, and M. mercenaria again did poorly in our system.

Adult Blue Mussels (Mytilus edulis) were brought to St. Croix in August of this year for spawning studies. Initial attempts to spawn them failed, probably due to their emaciated condition. Another attempt will be made after a period of "fattening" in our system.

A third batch of European oysters (Ostrea edulis) introduced to St. Croix this year, grew well initially but began to die off as they approached market size. The die-off may have been associated with a change in algal diet; this will be investigated in future experiments.

The Japanese little-neck clam (Tapes semidecussata) was reared to market size and three batches of larvae were reared to the juvenile stage in our system this year. The ability to spawn this clam at will and its very rapid growth at high densities in our tanks makes it a prime candidate for commercial mariculture based on artificial upwelling.

Analyses for mercury and pesticides (chlorinated hydrocarbons) in shellfish reared in our system showed insignificant amounts of these pollutants. In contrast, mangrove oysters from local waters showed significant contamination by mercury.

A preliminary test of a method to reduce hydrogen sulfide production in sand substrate containing clams showed some success and will be further tested. The method involves circulating water beneath a porous-bottom tray containing the sand and clams.

SHELLFISH CULTURE: GASTROPODS

Juvenile Queen Conch (*Strombus gigas*) have been maintained on a strictly algal diet in the effluent from our bivalve-rearing tanks for 14 months. The algal scum which grows spontaneously in an open tank is an adequate diet for their growth.

CRUSTACEAN CULTURE

A few Spiny Lobster (*Panulirus argus*) are still being maintained on a diet of bivalves culled from our system, to get a measure of their long-term growth rates.

The remainder of the juvenile North American Lobsters (*Homarus americanus*) brought to St. Croix in September 1973, died by August of this year, of unknown causes.

A brood of Brine Shrimp (*Artemia salina*) were reared to the adult, reproductive stage in a preliminary experiment to compare batch-feeding and continuous-flow methods of

feeding these animals diatoms. The information gained will be useful in designing a semi-automated system for rearing brine shrimp in our system.

SEAWEED CULTURE

Growth of *Hypnea musciformis*, a carageenin-producing red seaweed, grew about three times faster in the effluent from a clam tank than in reef water from Tague Bay (its natural environment) and about five times faster than in 870-m deep water. Growth rate was positively correlated with ammonia concentration in the water supplied to the tanks, but there was no correlation with nitrate or phosphate concentration. *H. musciformis* appears to have a high potential as a source of carrageenin and to clear the effluent from a bivalve shellfish-rearing operation.

Papers Published and/or Presented at Meetings in 1974

The following is a list of papers published and/or presented during 1974. Reprints were sent to the National Sea Grant Depository and Technical Information Service, as well as to the standard NOAA/Sea Grant mailing list.

Roels, O.A., 1974. Mariculture—The contribution of the sea to the world protein supply. Hoffman-La Roche Lecture at University of Montreal (Jan. 21) and at University of Manitoba (Jan. 22). Unpubl.

Roels, O.A., 1974. The sea as a source of protein. Agriculture-Canada, Food Research Institute, Ottawa (Feb. 7), Research Branch Seminars. Unpubl.

Haines, K.C., 1974. Management of marine shellfish through artificial propagation and restocking. Presented at Conference on the Virgin Islands Environment: "Our Troubled Environment—Can We Save It?" Caribbean Research Institute, College of the Virgin Islands, St. Thomas, USVI (May 11). Unpubl.

Roels, O.A., 1974. The St. Croix "Artificial Upwelling" project. International Training Program, Lecture Series, Duke University Marine Laboratory, Beaufort, N. Carolina (May 28). Unpubl.

Roels, O.A., 1974. Power, fresh water, and food from cold deep-sea water. The Sigma Xi Lectures, Olin Branch of RESA, New Haven, Conn. (June 18). Unpubl.

Sunderlin, J.B. and W.J. Tobias, 1974. Growth of the European oyster, *Ostrea edulis* Linné, in the St. Croix "Artificial Upwelling" mariculture and in natural waters. Presented at 66th Joint Annual Convention SINA/NSA, Philadelphia, Pa. (June 16-20). Submitted for publication in 1974 Proceedings.

Haines, K.C., M.W. Farmer, P.W. McDonald and O.A. Roels, 1974. Continuous outdoor culture of *Chaetoceros curvisetus* Cleve in unsupplemented deep water. 37th Ann. Mtg. Am. Soc. Limnol. Oceanogr., Univ. Washington, Seattle, Wa., Abstract (June 23-28)

Roels, O.A., 1974. Artificial upwelling: power, fresh water and protein from the sea. The Sigma Xi Lectures, Roche Research Chapter, Nutley, N.J. (Oct. 15). Unpubl.

Roels, O.A., 1974. Biological productivity of the oceans: The Virgin Islands experiment, a case study of artificial upwelling. Columbia University, Graduate School of Business: Ocean Resources Management Program, Arden House, Harriman, N.Y. (Nov. 21). (Unpubl.)

Roels, O.A., 1974. Artificial upwelling. Thomas J. Watson Research Center, IBM, Yorktown Hgts., N.Y., Seminar Series (Nov. 26). Unpubl.

Tobias, W., 1974. A new lobster larvae trap. Submitted for publication in Aquaculture: Brief Notes.

Malone, T.C., C. Garside, K.C. Haines and O.A. Roels, in press. Nitrate uptake and growth of Chaetoceros sp. in large outdoor continuous cultures. Submitted for publication in Limnol. Oceanogr.

1 ADMINISTRATION

1.1 Project Staffing: 1974*

Principal Investigator:
Roels, Oswald A. (Ph.D.)

Co-Investigators:
Aust, Leo G. (M.S., Engineering)
St. Croix Station Manager

Garside, C. (Ph.D.)
Chemistry

Haines, Kenneth C. (Ph.D.)
Project Chief Scientist; Algal Culture

Malone, Thomas C. (Ph.D.)
Phytoplankton Dynamics

Sunderlin, Judith S. (M.S.)
Shellfish Culture

Van Hemelrijck, L. (M.E.E.)
Engineering

Supporting Staff: St. Croix:
Berg, David
Mariculture Technician

Boatswain, G.
Facilities Upkeep

Davidson, Joan
Laboratory Assistant

Farmer, Mary W. (M.S.)
Graduate Student; Algal Culture

Forbes, Milton (Ph.D.)
Shellfish Hybridization (Faculty Associate)

MacDonald, Paul (B.S.)
Water Chemistry

*alphabetical order within categories.

Rodde, Kenneth (B.S.)
Shellfish Culture

Wallace, Michael
Mariculture Technician

Supporting Staff: New York:

Amos, Lynn M.
Administrative Assistant

Anderson, O. Roger (Ed.D.)
Electron Microscopy (Faculty Associate)

Berger, G. (B.S.)
Library and Lab Assistant

Hammond, L.
Laboratory Assistant

Kostyk, N. (M.S.)
Analytical Chemistry

Lui, N.S.T. (Ph.D.)
Nitrogen Metabolism of Phytoplankton
(Visiting Sr. Res. Associate)

McClain, I.
Darkroom and Lab Assistant

Pasek, B. (B.S.)
Purchasing Secretary

Picard, G. (Ph.D.)
Algal Culture (achieved Ph.D. 1974)

Trout, M.E. (B.S.)
Laboratory Manager

1.2 Consultants

Menzel, R.W. (Ph.D.)
Professor, Florida State University
Tallahassee, Fla.
Shellfish

Castagna, M.
Laboratory Director, Eastern Shore Lab.,
Virginia Institute of Marine Sciences
Shellfish

Chanley, P.
Shelter Island Oyster Farms, Inc.
Greenport, L.I., N.Y.
Shellfish

Budge, W.W.
Pacific Mariculture, Inc.
Pescadero, California
Shellfish

Hirota, J. (Ph.D.)
Aquatic Sciences Corp.
Honolulu, Hawaii
Shellfish

Guillard, R.R.L. (Ph.D.)
Woods Hole Oceanographic Institution
Woods Hole, Mass.
Phytoplankton

In addition to those above, the following have given us the benefit of their advice and collaboration: Dr. D.F. Othmer (Polytechnic Institute of New York: sea thermal power and desalination processes); Mr. W. Lodge (Consultant to Biological Oceanography at Lamont: Phase 2 planning and fund-raising); Dr. C. Angell (Fundacion La Salle, Venezuela: phytoplankton); Dr. F. Ott (Virginia Institute of Marine Sciences: phytoplankton); Dr. C.E. Epifanio (University of Delaware: phytoplankton); Mr. R. Eissinger (International Shellfish Enterprises: phytoplankton); Dr. J. Gonzalez (Puerto Rico Nuclear Center: phytoplankton); Dr. R. Tuttle (Harvard University: phytoplankton); Mr. H. Norve (Genue Products, Nova Scotia: seaweeds); Dr. Harold J. Humm (University of S. Florida, St. Petersburg: seaweeds).

1.3 Facilities (Current)

The Lamont-Doherty Marine Biology Station on St. Croix has expanded during 1974 to occupy all of the buildings on Estate Rust-op-Twist, on the North Shore about three miles west of Baron Bluff. The additional leased space includes the remainder of the warehouse and the Manor House, which will be renovated to make three additional apartments for transient and permanent staff. At this time, the complex houses five permanent staff members (two with families).

The laboratory area of approximately half an acre is about 1400 ft back from the shore. On the lower floor of the renovated building are the laboratory, including a new dust-free culture transfer room, business office, kitchen, office for the Chief Scientist, and storeroom, comprising approximately 1,200 sq.ft. On the smaller second floor are two apartments for resident scientists. In a 582 sq.ft. section of the warehouse are our two diesel-powered emergency generators, a workshop, and tool and spare parts storage.

The shore area (approximately 40,000 sq.ft) contains the mariculture complex which includes: (a) the onshore terminus of the three 3-inch deep-water pipelines; (b) pump house with two 42-gpm glass-lined centrifugal pumps installed in parallel (each with its own motor), one 360-gal. priming tank, two graphite vane pumps to supply aeration, and a recording device (Eppley pyranometer) for continuous readout

of solar radiation; (c) the shellfish hatchery and wet lab for shellfish feeding and growth experiments (described in detail in section 2.3); (d) deep-water constant head device for constant pressure in the entire deep-water distribution system; (e) two 12,000-gal. capacity concrete pools for algal culture as food for shellfish; (f) six 200-gal. poly-ehtylene tanks, four of which are used for algal culture in our continuous food-production system; two are used for experimentation; (g) four 200-gal. wood-and-fiberglass tanks in which our prime populations of shellfish are housed; (h) two 150-gal. polyethylene tanks for experimental use; (i) four 200-gal. wood-and-fiberglass tanks for miscellaneous uses; (j) ten 550-gal. concrete tanks for comparative algal growth studies; (k) submersible pumps to move the algae-rich water from the pools to the shellfish tanks; (l) assorted tanks, wood-and-fiberglass or PVC, used in the lobster and seaweed programs; (m) one shipping container for storage of tools and supplies for the beach and pipeline work; (n) a cottage which is the residence of the junior mariculture technician.

The facilities at the Biological Oceanography department at our Palisades, N.Y. home base have been described in detail in earlier reports and proposals.

2 ENGINEERING

2.1 Deep-Water Pipelines

The three pipelines installed in 1972 are still in excellent shape. Monthly SCUBA inspection dives by our beach technicians have revealed no wear or corrosion on any part of the pipelines, and only minor wear on their supporting cables. The only maintenance work required has been occasional replacement of the anchor lines for the buoys marking the offshore end of the inshore sections of the pipe. In addition, weekly nutrient and salinity analyses from the individual pipes allow us to monitor the integrity of the pipelines below the depth limits of our divers (see Appendix III for the year's nutrient and salinity data).

2.2 Pump House

The de-aerating chamber for the deep water was not installed as planned, because we have not needed de-aerated water for the lobster larvae studies which were delayed until a lobster biologist can be added to our staff.

A continuously recording Eppley pyranometer has been installed for use in our algal productivity studies.

Filters were installed on the graphite vane aeration pumps to trap the graphite particles which eventually clog the metering valves. The pumps, which have run continuously for three to four years, are being rotated through a factory service program.

2.3 Algal Reactors

Six more 550-gal. algal reactors have been set up; five have shade-screens of varying light opacities (46, 30, 20 and 1% light transmission). The shaded reactors are used by Miss Mary Farmer (predoctoral student) in experiments on the effects of light intensity on phytoplankton nutrient uptake and productivity in continuous-flow deep-water cultures (see Appendices I and II for details). The ten reactors will be used during 1975 to provide food for shellfish larvae in the hatchery, and juveniles in the experimental shellfish area (see section 2.4).

2.4 Hatchery/Wet Lab

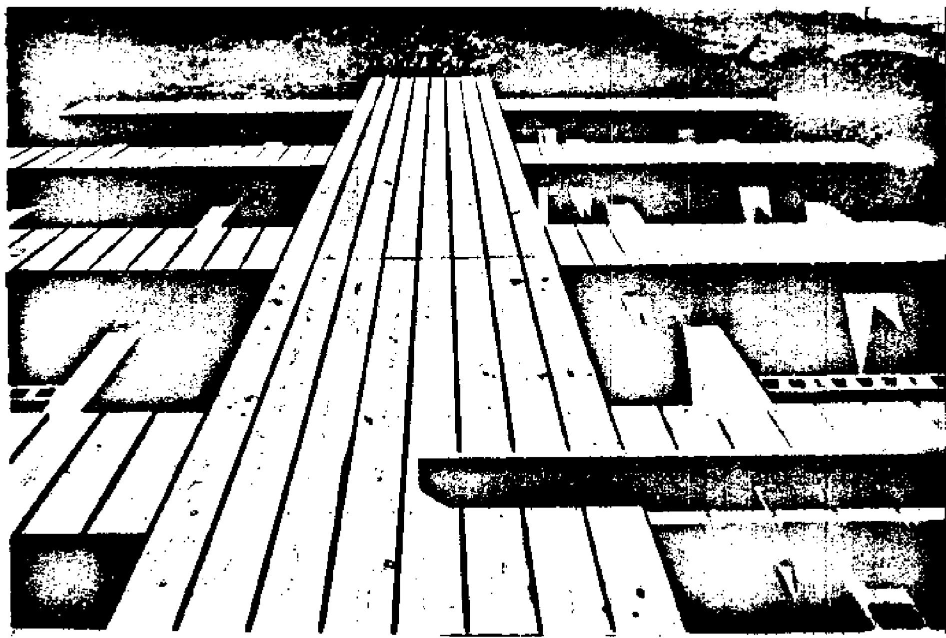
This year will see the completion of our hatchery/wet lab, consisting of a roofed area, 20 x 60 ft. At the end of the hatchery/wet lab is a 16 x 28 x 7 (high) ft. supporting structure for the ten algal reactors in which food for the hatchery will be grown (Figures 2:1A-C). All ten reactors will be piped to receive deep water for growing algae and five will also be piped to receive algal culture from the two 12,000-gal. pools, so that algal culture can be diluted with deep water and thus provide varied algal-cell densities (with equal flow rates) to juvenile shellfish in the experimental shellfish area of the building. Also included in the hatchery /wet lab is a pilot shellfish area, where designs for a small-scale automated shellfish-rearing tank will be tested.

Figure 2:1 (A). Reactor-supporting structure with decking in place, ready to accommodate the ten reactors for growing algae.

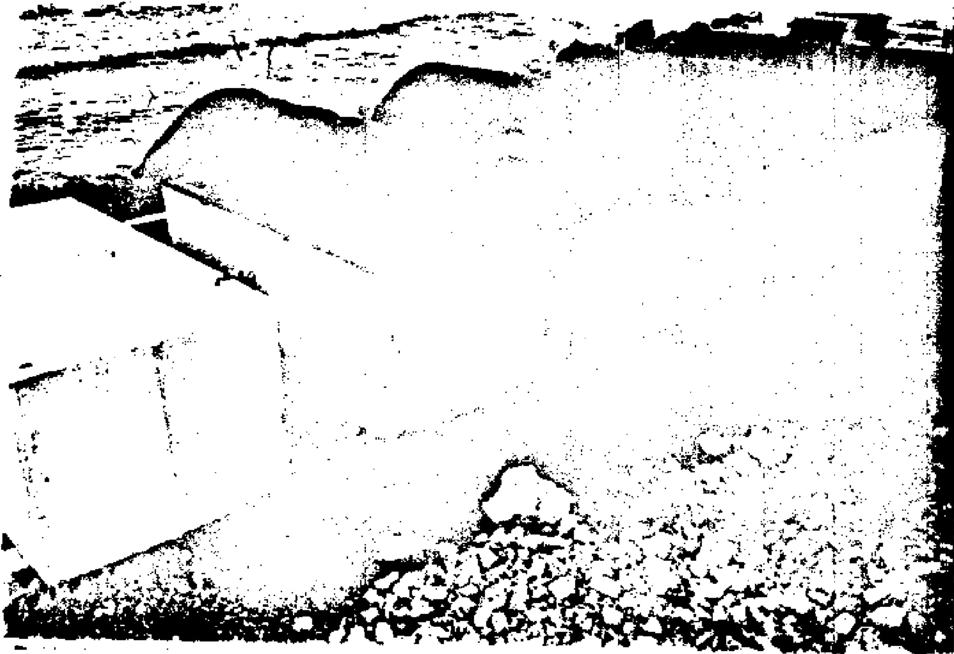
(B). Five of the ten algal reactors (presently in use as described in section 2.3) to be installed on the reactor-supporting structure (A).

(C). Interior view of the Hatchery/Wet Lab during installation of distribution system for algal cultures grown in the reactors. (The temporary tank in the background contains a batch of *Tapes semidecussata* spawned on St. Croix.)

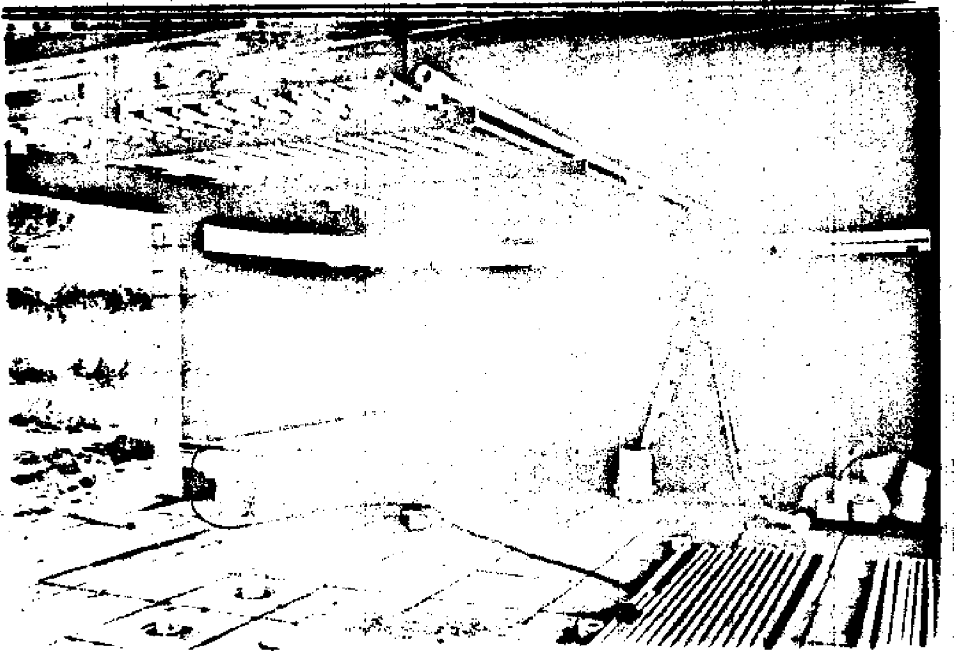
A



B



C



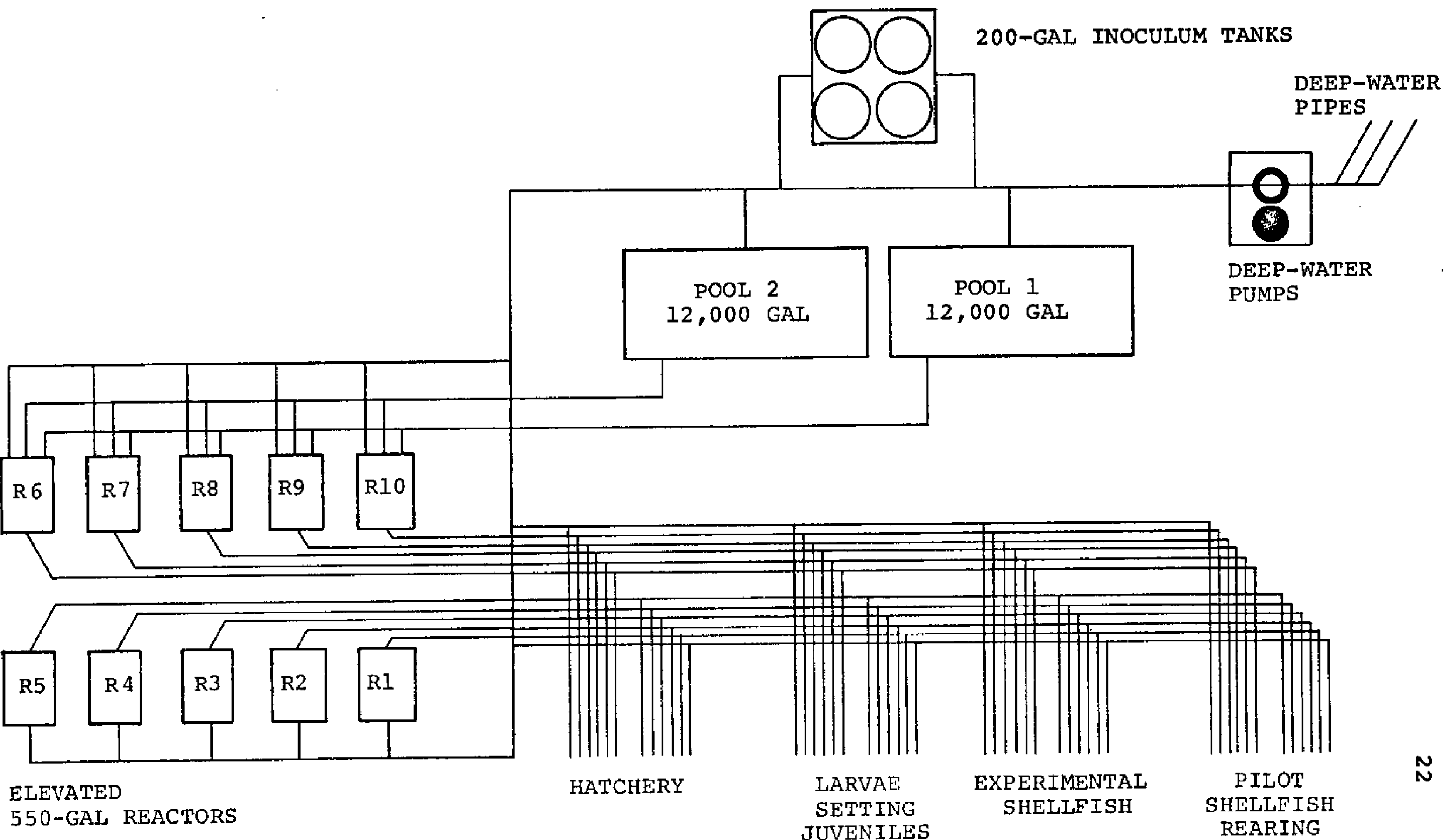
The hatchery/wet lab will have tap-offs for deep water, air, and algal culture from each of the ten reactors at 10-ft intervals through the length of the building. At the same intervals, there are entries to an under-floor drainage system leading to an external percolation field, where waste sea water is filtered through sand before returning it to the sea.

A diagram of the facilities of the St. Croix mariculture system is given as Figure 2:2.

2.5 Reverse Osmosis

An experimental reverse osmosis test, using deep water (proposed by E.I. duPont Co.) was not funded. The exceptional clarity (low particulate content) of our deep water supply has the potential of making reverse osmosis an effective method of providing fresh water.

Figure 2:2. Diagram of the facilities of the St. Croix mariculture system.


200-GAL INOCULUM TANKS
DEEP-WATER PIPES
DEEP-WATER PUMPS
POOL 2
12,000 GAL
POOL 1
12,000 GAL
R6
R7
R8
R9
R10
R5
R4
R3
R2
R1
ELEVATED
550-GAL REACTORS
HATCHERY
LARVAE SETTING JUVENILES
EXPERIMENTAL SHELLFISH
PILOT SHELLFISH REARING

3 CHEMISTRY

3.1 Weekly Determination of Deep-Water Quality

As in the previous year, we monitored the nutrient content and salinity of our three pipelines at weekly intervals. Another channel was added to our Technicon AutoAnalyzer II, so that two nutrients can be analyzed simultaneously (this has expanded our analytical capability for studies such as algal utilization of nutrients). Salinity is titrated manually.

The means and ranges of nutrient concentrations and salinity are given for the three pipelines in Table 3:1 over the period January 1 to December 31, 1974. The values for the same period in 1973 are also given, for comparison. (The data are given in Appendix III.)

3.2 Potential Method (Forward Light Scattering) for Monitoring Deep Water Pipelines

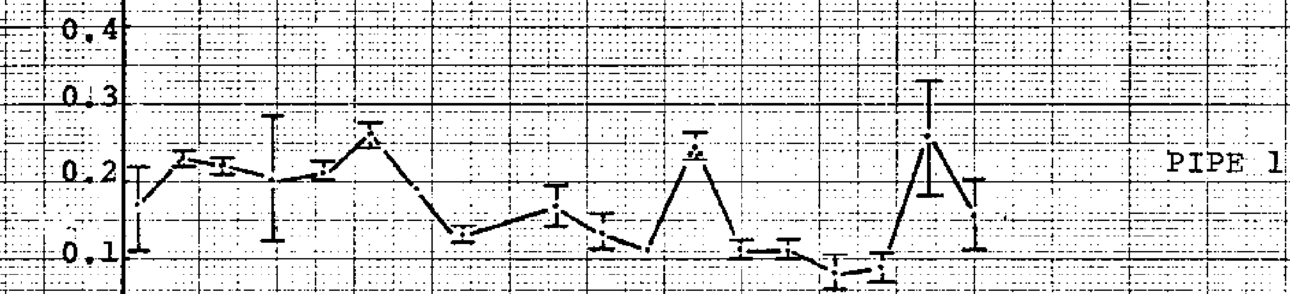
Discrete measurements of deep water turbidity were done on the Monitek Model 250 Turbidimeter to evaluate the method for monitoring the deep-water pipelines. The instrument measures the amount of forward scattering caused by particulate matter in the water sample. Duplicate samples were taken every three hours for 54 consecutive hours from each deep-water pipe. Turbidimeter readings are shown in Figure 3:1.

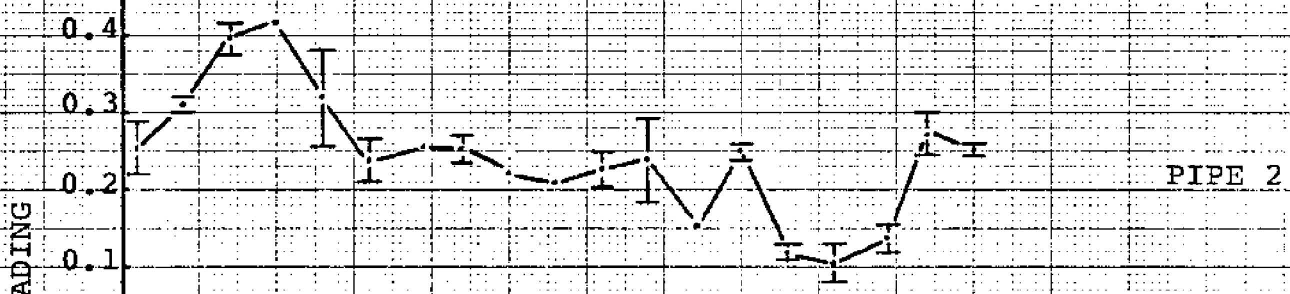
During the time of study, Pipe 1 had the least turbid

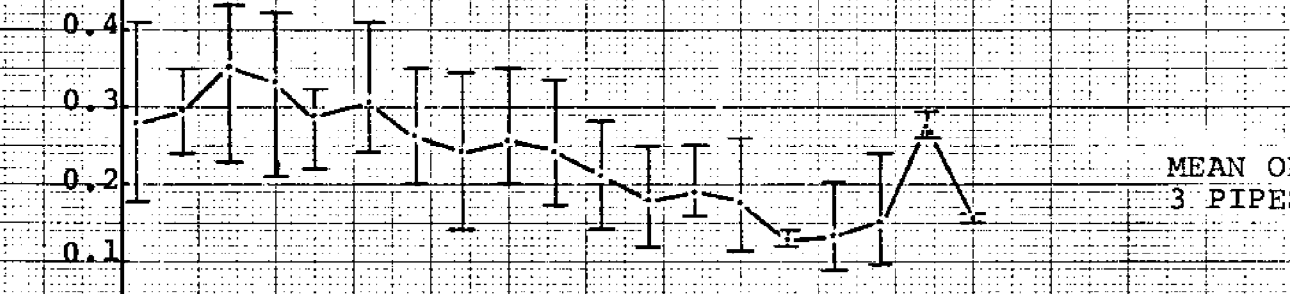
TABLE 3:1. MEANS AND RANGES OF NUTRIENT CONCENTRATIONS AND SALINITY FOR THE THREE PIPELINES

	1974		1973	
	MEAN ± 2σ	RANGE	MEAN ± 2σ	RANGE
μg-at N/liter NO_3+NO_2	31.28 ± 0.27	29.08 -32.94	31.42 ± 0.31	27.75 -33.60
μg-at NO_2-N/liter	0.20 ± 0.04	0.00 - 0.36	0.21 ± 0.05	0.02 - 0.72
μg-at NH_3-N/liter	0.68 ± 0.12	0.30 - 1.55	0.75 ± 0.13	0.25 - 2.70
μg-at PO_4-P/liter	2.14 ± 0.06	1.79 - 2.44	2.12 ± 0.10	1.74 - 2.35
μg-at SiO_4-Si/liter	19.91 ± 0.21	14.77 -28.40	21.29 ± 0.37	11.92 -40.68
Salinity ($^{o}/oo$)	34.859± 0.007	34.778-34.943	38.845± 0.008	34.744-35.010

Figure 3:1. Turbidimeter reading versus time for the three deep-water pipes and for the mean of three pipes. Vertical bars represent the range of two duplicate samples for Pipes 1-3. For the mean of three pipes, vertical bars represent the range of the pipes. The turbidimeter scale readings are ppm; a gelled diatomaceous earth standard was used for calibration.







461510
K+E 10 X 10 TO THE CENTIMETER 18 X 25 CM
KEUFFEL & ESSER CO. MADE IN U.S.A.

water and Pipe 3 the most turbid water. The water in all three pipes generally became less turbid during the course of the experiment. No attempt was made to determine the cause of the turbidity; higher readings may have been related to the presence of silt, detritus, or plankton, or any combination of these agents. It was concluded that the turbidimeter provides a sensitive measurement of turbidity in discrete samples from the pipelines. An in-line detector might be an effective device for continuous monitoring of the pipelines.

3.3 Short-Term Observations of Biological and Chemical Properties of Deep Water

The dissolved nutrient (N, P, Si) content, salinity, and chlorophyll *a* content of deep water pumped from each of the three pipelines were observed at three-hour intervals over a 24-hr period. The results of this study, undertaken at weekly intervals in November 1973, are being prepared for publication.

3.4 Test of a New, Automated Method for Ammonia Analysis

A new automated method for ammonia analysis, a modification of a method found in Strickland and Parsons (1968), was studied this year. The method is reputed to exhibit greater sensitivity to ammonia than the Berthelot hypochlorite method used previously, and amino acids are also determined. Basically, the analytical method consists

of the oxidation of ammonia to nitrite, employing sodium hypochlorite as the oxidizing reagent. The nitrite is then coupled to N-1-Naphthylenediamine dihydrochloride to form a red-purple azo dye which is analyzed spectrophotometrically. The oxidation is carried out in a very basic solution in a heated water bath while the complexing is done in an acid solution. Excess hypochlorite is removed utilizing sodium arsenite as a reducing agent.

Our tests showed that a precipitate (calcium and magnesium hydroxides) formed at the point of injection of the oxidizing reagent from seawater which was made basic. The precipitate buildup eventually disrupted flow continuity and had to be removed with a 5% HCl wash. The method did not give a linear response to standards and reproducibility of duplicate samples was not acceptable. Therefore, the new method was not adopted and we continue to use the automated Berthelot (phenol-hypochlorite) reaction for the determination of ammonia nitrogen.

3.5 Preliminary Study of Nutrient Utilization by Continuous Cultures of Diatoms in 10,000- and 12,000-Gallon Outdoor Pool Cultures

The purpose of this study was to compare the efficiency of nutrient (N, P, Si) utilization by two of the species of diatoms grown as food for bivalves.

Cultures of *Bellerochea spinifera* (clone STX-114) and *Chaetoceros curvisetus* (clone STX-167) in continuous-flow, 10,000- and 12,000-gallon pools (Pools 1 and 2, respectively)

were sampled for dissolved nitrate, phosphate, and silicate over a 24-hr period every four hours. Samples for intracellular nitrate, Lowry protein and particulate nitrogen analysis were also taken. The sky was overcast during the daylight hours of the sampling period. The results given here are incomplete; many of the analyses have not been finished.

Cells, while continuing to divide slowly, were irregular in shape and appeared to be "unhealthy" (Pool 1, containing STX-114, was scrubbed the day sampling ended due to invasions of flagellates). Nevertheless, dissolved nitrate and phosphate values showed that both STX-167 and STX-114 stripped 100% NO_3-N and approximately 95% PO_4-P. SiO_4-Si was not utilized as efficiently as nitrate or phosphate.

4 ALGAL CULTURE

4.1 Continuous Outdoor Culture of *Chaetoceros curvisetus* in Unenriched Deep Water

We have been utilizing the diatom *Chaetoceros curvisetus* (clone STX-167) as one of three foods for bivalve shellfish since mid-1974. The diatom is grown in a 13,000-gallon pool with a dilution rate of 1.1 per day, to produce 15,000 gallons of culture per day in unenriched deep water.

Cultures of this diatom can be maintained in a nearly unialgal state for periods up to one month in duration. The apparent reason for this is the diatom's high efficiency of nitrate and silicate utilization, which virtually eliminates competition with other algal species with presumably lower efficiencies of nutrient uptake. The results of a nutrient uptake and growth study in 550-gallon outdoor tanks at various flow rates (unpublished data) showed that at dilution rates of 0.5 and 1.0 per day, concentrations of nitrate and nitrite, phosphate, and silicate remain constant day and night; at higher dilution rates (1.25 and 1.5 per day) nitrate and phosphate uptake were decreased during the night. The generally higher nutrient concentrations at the two higher dilution rates favored wall growth of contaminating organisms, but no wall growth was observed at the two lower dilution rates.

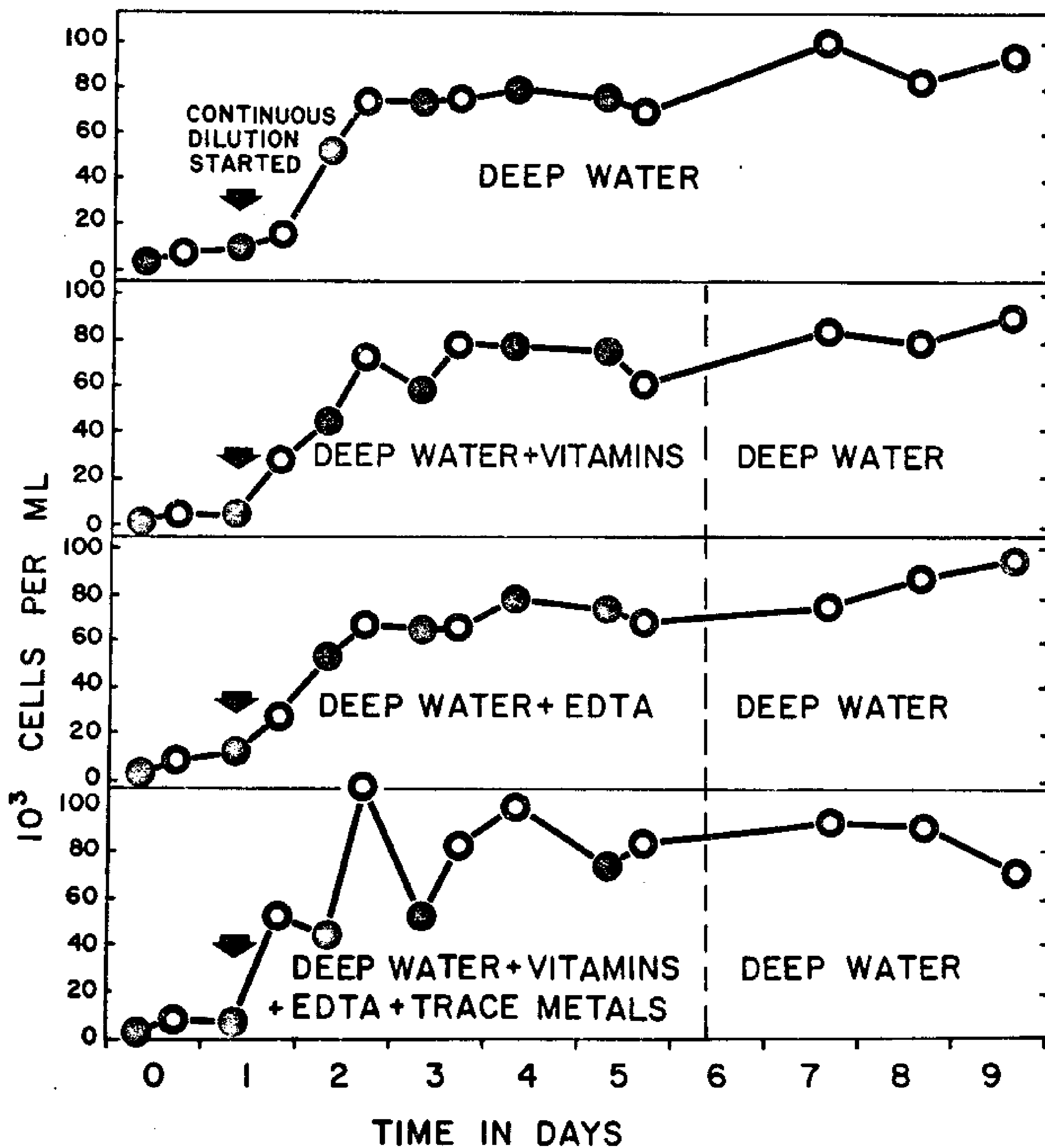
Cell density, and cell carbon and nitrogen per liter decreased at dilution rates over 1.0 per day, and for this

reason we chose a dilution rate for our 13,000-gallon pool close to 1.0 per day.

4.1.1 Growth of Four Species of Diatoms in Enriched *vs* Unenriched Deep Water

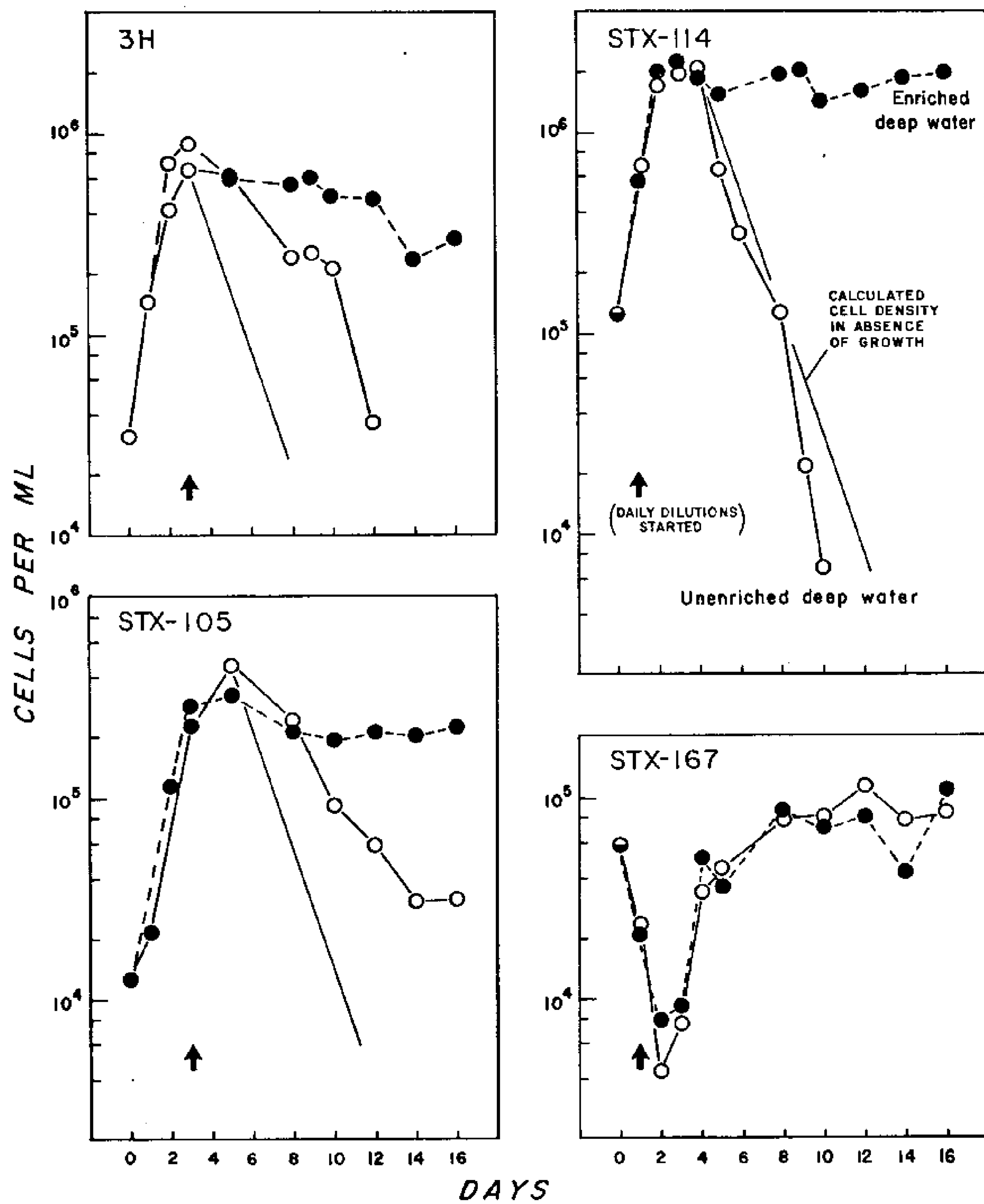
Enrichment of deep water with a chelated iron-trace metal-vitamin mixture (or its components) had no beneficial effect on growth of *Chaetoceros curvisetus* in either outdoor continuous cultures (0.5 dilutions per day; Fig. 4:1) or in indoor semi-continuous cultures (Fig. 4: 2). The indoor semi-continuous cultures show the beneficial effect of the enrichment on growth of three other species of diatoms used in our mariculture system.

Figure 4:1. Growth of *Chaetoceros curvisetus* (clone STX-167) in unenriched deep water and in deep water enriched with a chelated iron-trace metals-vitamin mixture or components of the mixture. The culture was diluted continuously at a rate of 0.5 dilutions per day; enrichments were metered in continuously using an intravenous dripper. The enrichments were terminated at the time indicated by the dashed line.



CONTINUOUS
DILUTION
STARTED
DEEP WATER
DEEP WATER+VITAMINS
DEEP WATER
DEEP WATER+ EDTA
DEEP WATER
DEEP WATER+VITAMINS
+EDTA+TRACE METALS
DEEP WATER
10³ CELLS PER ML
TIME IN DAYS
0
20
40
60
80
100
0
1
2
3
4
5
6
7
8
9

Figure 4:2. Growth of four diatoms: *Bellerochea spinifera* (STX-114), *Chaetoceros simplex* (STX-105), *Chaetoceros curvisetus* (STX-167), and *Thalassiosira pseudonana* (3H) in semi-continuous indoor cultures, using unenriched deep water or deep water enriched with a chelated iron-trace metals-vitamins mixture. The cultures were diluted once daily by adding 25 ml of new medium to 25 ml of culture, to give a dilution rate of 1.0 per day. Cultures were maintained at 23-27°C with 12 hr light/12 hr dark illumination from "daylight" fluorescent bulbs. The values plotted are the average cell densities from duplicate cultures: Open circles, unenriched deep water; closed circles, enriched deep water. The lines showing the calculated cell density expected in the absence of growth are extrapolated from the peak yields of the unenriched deep water cultures. (In addition to the dilutions indicated on the graphs, clones STX-105 and 3H were also diluted one additional time, on Day 1 or 2.)
The decline in cell numbers of the STX-167 cultures during the first 2 days was caused by the use of an inoculum from a stationary-phase culture; the inocula of the other three diatoms were taken from exponentially-growing cultures.


3H
STX-114
STX-105
STX-167
Enriched deep water
Unenriched deep water
CALCULATED CELL DENSITY IN ABSENCE OF GROWTH
(DAILY DILUTIONS STARTED)
CELLS PER ML
DAYS
0 2 4 6 8 10 12 14 16

4.2 Optimization of Algal Yield

4.2.1 Abstract

The pool depth for optimal yield of *Chaetoceros curvisetus* in continuous outdoor deep-water cultures was calculated. The data used for the calculations were obtained from experiments at two dilution rates (0.25 and 1.2 dilutions day^{-1}) in which incident light intensity reaching the culture surface was regulated by neutral density screens. The greatest calculated yields of cells and cell carbon were at a simulated depth of 4.1 meters and a dilution rate of 1.2 day^{-1}. Maximum calculated net carbon fixation was more than double that estimated for natural upwelling areas. Additional experiments in real (rather than simulated) culture depths are recommended to more accurately predict optimal culture depth.

4.2.2 Introduction

The depth of a pool used for the continuous culture of algae in a mariculture system affects (1) the intensity of light reaching the bottom of the culture, and (2) the productivity of algae per unit surface area. Thus, the depth of the pool affects production in space while the rate of flow of a continuous culture affects production in time. In a mariculture system, the production of algae should be close to optimal, both in space and in time, to obtain maximal yield.

One approach to determining optimal pool conditions is

to operate a series of continuous cultures using shades to reduce the light intensity reaching the cultures, thus roughly simulating different pool depths. By running a series of experiments at different flow rates, the optimal combination of flow rate and pool depth ought to be determined. Two of a series of four such experiments have been conducted (Appendices I and II) and some preliminary calculations are presented.

4.2.3 Materials and Methods

In each experiment, continuous cultures of *Chaetoceros curvisetus* (STX-167) were grown in reactor vessels of 2-m^3 volume (2.5 m^2 surface area, 0.8 m depth) using unenriched deep water as medium. Shading was provided by neutral density screens so that cultures were exposed to the following degrees of incident light intensity: 100, 46, 30, 20, and 3%. In the first experiment, dilution rate was 0.25 day^{-1} and in the second experiment, 1.20 day^{-1} for all five reactors. A dilution rate of 1.20 day^{-1} is close to the maximum dilution rate at which cultures of this diatom can be maintained before washout occurs (unpublished data).

Samples for several parameters were collected at sunrise and sunset (see Appendices I and II for details). Incident light intensity was recorded continuously with a pyrheliograph (Belfort Instrument Co., Baltimore, Md.). Temperature and visible light penetrating the cultures were

measured at sampling times. The rate at which light decreased with depth of culture was expressed as the extinction coefficient (k) and was calculated from the formula

$$I_z = I_o e^{-kz}$$

solving for k,

$$k = 2.3 \frac{(\log I_O - \log I_Z)}{z}$$

where e = base of natural log, I_o - light penetrating the surface of the culture, I_z = light reaching the bottom of the culture, z = depth of the culture (0.8 m), and 2.3 is the factor for conversion of natural logs to base 10 logs. Watertight Weston photocells were used to measure underwater light intensities.

Net carbon fixation (as calculated from fixation of radioactive carbon), cell production and extinction coefficients will be discussed here. Net nitrogen fixation can also be used for calculations similar to the ones to be presented when the analyses are complete.

Simulated pool depths were based on the following assumptions (1) The percentage of incident light penetrating to half the culture depth represents the mean light intensity to which the cells were exposed. (2) The extinction coefficient, k, is constant for a given culture: depth (z) multiplied by k represents the extinction of light, and e^{-kz} is the fraction of the light reaching a depth of z meters. Actually, k is not constant, it decreases somewhat more rapidly in the first meter of water, but sophisticated measurements would

be required to find the rate at which k decreases in the reactors. (3) Shading reduces the light reaching the surface of the culture and the bottom of the culture by the same percentage. Thus, if 46% of incident light reaches the surface of the culture, then 0.46 e^{-kz} reaches depth z. (4) Using the percentage of light reaching depth z, one can back-calculate what the depth would be if the amount of light reaching the surface was 100%.

Calculations were done as follows: percent light reaching the surface of each of the reactors (I_{100}) was multiplied by $e^{-0.4k}$ (using the measured k of the culture) to find the percentage light transmitted to half the culture depth ($I_{\%}$). Then, assuming 100% I reached the surface of the culture ($\ln I_{100} - \ln I_{\%}$), was divided by k to find the simulated mid-depth. Twice this value gives the simulated pool depths used in Table 4:1.

4.2.4 Results and Discussion

Simulated depth of pools, dilution rates, and daily net fixation of carbon and cell production per unit volume and per unit area are given in Table 4:1. Greatest production of cells and greatest net carbon fixation was at a simulated depth of 4.1 m and at a dilution rate of 1.2 day^{-1}. Overall production was greater at the faster dilution rate at comparable simulated depths (Fig. 4:3)

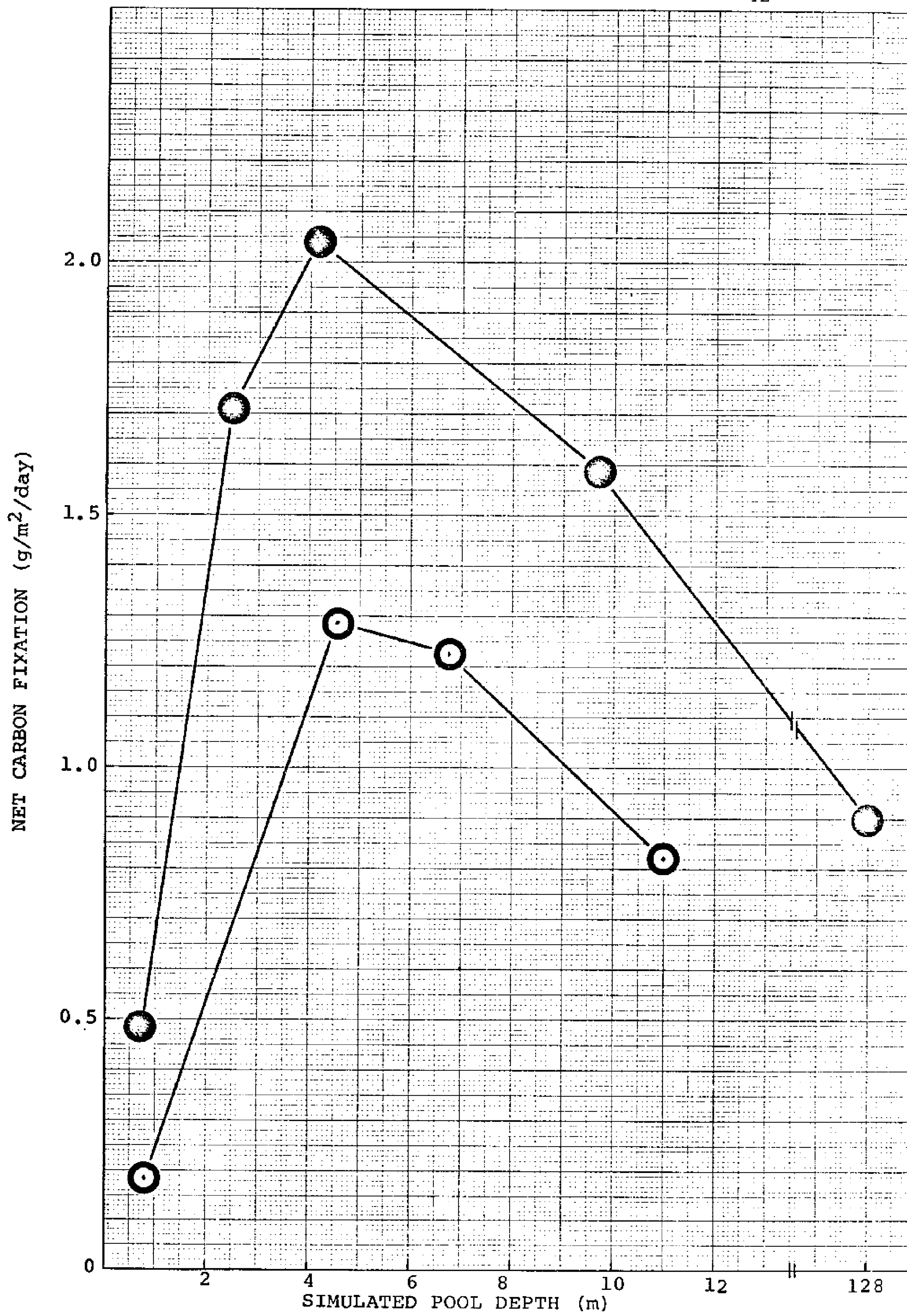
Net fixation of carbon is more than twice as great as the 0.822 $g/m^2/day$ estimated for natural upwelling areas (Ryther, 1969).

TABLE 4:1. ESTIMATION OF OPTIMAL POOL DEPTH AND DILUTION RATE FOR CONTINUOUS CULTURES OF CHAETOCEROS CURVISETUS (STX-167)

DILUTION RATE	PERCENT OF INCIDENT LIGHT	EXTINCTION COEFFICIENT (k)	SIMULATED DEPTH OF POOL (m)	MEASURED NET CARBON FIXATION ($g/m^3/day$)	CALCULATED NET CARBON FIXATION ($g/m^2/day$)	MEASURED CELL PRODUCTION ($10^{10}/m^3/day$)	CALCULATED CELL PRODUCTION ($10^{10}/m^2/day$)
0.25	100	0.256	0.80	0.232	0.185	1.96	1.57
	46	0.360	5.08	0.252	1.280	1.90	9.65
	30	0.397	6.78	0.180	1.220	1.85	12.54
	20	0.525	16.98	0.086	0.596	1.46	10.19
	3	0.717	10.92*	0.074	0.808	0.89	9.72
1.2	100	0.770	0.80	0.610	0.488	5.52	4.42
	46	0.883	2.52	0.682	1.719	7.61	19.18
	30	0.732	4.14	0.493	2.041	5.45	22.60
	20	0.372	9.52	0.166	1.58	2.24	21.32
	3	0.061	128.26*	0.007	0.898	0.34	43.6

*Deeper than the 1% light depth.

Figure 4:3. Net carbon fixation per square meter per day by *Chaetoceros curvisetus* (STX-167) in outdoor continuous culture using unenriched deep water as culture medium. Filled circles = 1.2 dilutions day^{-1}. Open circles = 0.25 dilutions day^{-1}. Net carbon production shown as a function of simulated pool depth (see text for details).


NET CARBON FIXATION (g/m²/day)
2.0
1.5
1.0
0.5
0
2
4
6
8
10
12
128
SIMULATED POOL DEPTH (m)

Theoretical calculations designed to estimate optimal depth for productivitiy of phytoplankton usually show that the shallower the water column, the greater the production, presumably reaching a maximum at a very thin layer little deeper than a chain of diatoms (Murphy 1962, 1971; Takahashi and Parsons, 1972). The present experiments show, however, a decrease in production at a depth of two meters or less. The result could demonstrate light inhibition or it could be an artifact of the assumptions used for the calculations, especially the assumption of a constant k. The extinction of light in seawater beneath a screen providing a given light intensity will be much greater, and of different spectral quality, than beneath water providing the same intensity. To predict optimal depth more accurately, productivity experiments at various real pool depths need to be done, and are planned.

4.2.5 Conclusions

On the basis of two outdoor continuous culture experiments with *C. curvisetus*, an optimal depth (4.1 m) and dilution rate (1.2 day^{-1}) have been predicted. Completion of the series of experiments will more precisely define optimal dilution rate, but further experiments at real (not simulated) pool depths are recommended to predict optimal depth.

Note: At a simulated depth of 128.26 m calculated production of cells exceeded that at 4.1 m due to the large volume of the culture. Net carbon fixation per cell was exceedingly low at this light intensity and any cell counting error would be magnified by the pool volume. The value is therefore discarded as improbable unless further experiments confirm it.

4.2.6 References

Murphy, G.I., 1962. Effect of mixing depth and turbidity on the productivity of fresh-water impoundments. Trans. Amer. Fish. Soc. 91:69-76.

Murphy, G.I., 1971. Clarifying a production model. Limnol. Oceanogr. 16:981-983.

Ryther, J.H., 1969. Photosynthesis and fish production in the sea. Science 166:72-76.

Takahashi, M. and T.R. Parsons, 1972. Maximization of the standing stock and primary productivity of marine phytoplankton under natural conditions. Ind. J. Mar. Sci. 1:61-62.

4.3 Screening of Algae to be Used in Feeding Shellfish Larvae

It became obvious that if we were to spawn our own batches of shellfish for experiments on St. Croix, we would require species of naked flagellates that are both good foods for shellfish larvae and able to tolerate warm temperatures (up to 32°C). The two species of flagellates used by most investigators for rearing larvae are the chrysomonads, *Isochrysis galbana* and *Monochrysis lutheri* —both of which are known to not tolerate warm temperatures very well.

We compared the growth rates of five clones of flagellates, suspected to be tolerant of high temperatures, with the growth rates of *Isochrysis galbana* and *Monochrysis lutheri*. The species tested are listed in Table 4:2 .

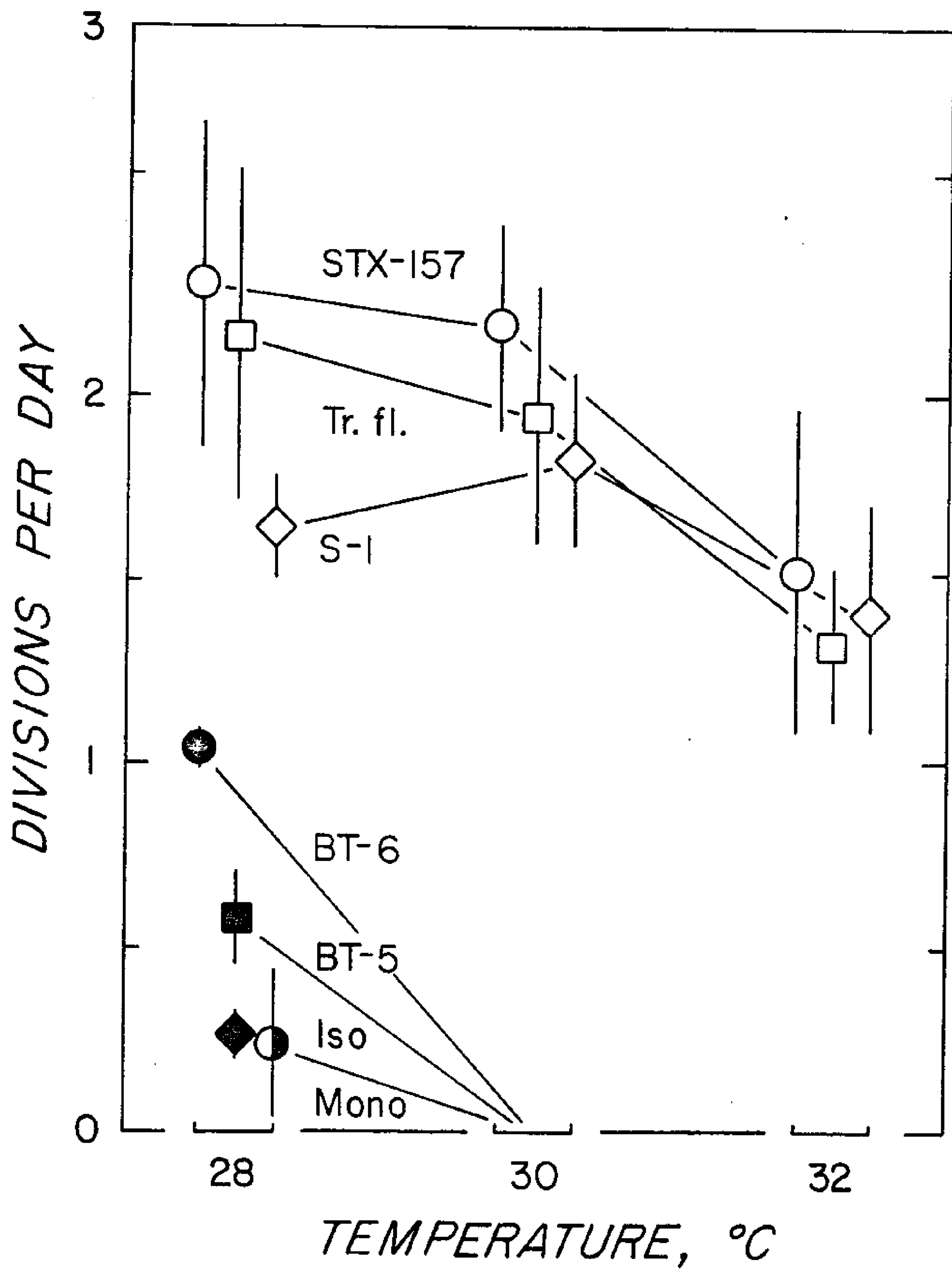
The results (Fig. 4:4) indicate that the two "low-temperature" clones (*Isochrysis* and *Monochrysis*) grew very slowly at 28°C (ca. 0.25 divisions per day) and did not grow at 30°C or higher, even when the temperature was raised very slowly, at the rate of 0.5°C per day. Two other clones (BT-5 and BT-6) grew somewhat faster than *Isochrysis* and *Monochrysis*, but also would not grow at 30°C.

Clones STX-157, Tr.fl. and S-1 averaged growth rates of ca. 1.5 to 2 divisions per day over the range tested

TABLE 4: 2. ALGAL CLONES STUDIED

CLONE	SPECIES	ORIGIN
<u>Chrysophyceae:</u>		
BT-5	Unknown	R. Guillard, Sargasso Sea
BT-6	*Coccolithus* (Emiliana) *huxleyi*	R. Guillard, Sargasso Sea
Iso	*Isochrysis galbana*	M. Parke via R. Guillard
Mono	*Monochrysis lutheri*	M. Droop via R. Guillard
Tr.fl.	Undetermined flagellate	R. Guillard, Sargasso Sea
<u>Cryptophyceae:</u>		
S-1	Undetermined flagellate	R. Guillard, Sargasso Sea
STX-157	*Rhodomonas* sp. (coral symbiont)	K.C. Haines, Tague Bay, St. Croix

Figure 4:4. Growth rates of six clones of naked flagellate marine algae at elevated temperatures. The values plotted are the means of at least three measurements; the vertical bars give the 90% confidence limits. In cases where no growth was observed at 30°C, growth resumed when the cultures were returned to 25°C.


3
2
1
0
DIVISIONS PER DAY
STX-157
Tr. fl.
S-1
BT-6
BT-5
Iso
Mono
28
30
32
TEMPERATURE, °C

(28-32°C). These three clones will be tested as foods for shellfish larvae in our hatchery. If they are suitable foods for a variety of shellfish larvae, we can confidently raise these algae in outdoor cultures year-round without fear of growth being inhibited by high temperatures. In a preliminary trial, one of these clones (S-1) was successfully used in conjunction with three diatoms to rear larvae of the clam, *Tapes semidecussata*, to the juvenile stage in our system.

4.4 Screening of Algae to be Used in Feeding Juvenile Shellfish

Twenty clones of algae that are known to be valuable foods for juvenile shellfish (or have the potential for growing in unenriched deep water) were to be tested for their ability to grow in unenriched deep water in outdoor continuous cultures. To shorten the time needed for the process of testing these clones, a test was made to see if small-scale laboratory semi-continuous cultures would give results similar to those in continuous cultures outdoors.

The results of this test (given in Fig. 4:3 in section 4.2) show that only Chaetoceros curvisetus (STX-167) of the four clones tested, grew as well in unenriched deep water as in enriched deep water; this confirmed our observations in 12,000-gallon outdoor cultures, where only C. curvisetus could be maintained in unenriched deep water for more than a week. Cultures of this diatom have lasted for more than a month with 0.5 to 1.0 dilutions per day in unenriched deep water.

We will therefore test promising clones for their ability to grow in unenriched deep water in small-scale laboratory cultures, where duplication is easily achieved and several species can be tested simultaneously.

4.5 Culture Collection and New Algal Clones

During 1974 cultures were received from, sent to, or exchanged with the following investigators outside our group:

Dr. C. Angell
Fundacion La Salle, Venezuela

Dr. R. Guillard
Woods Hole Oceanographic Institution

Mr. Paul Chanley
Shelter Island Oyster Co., Greenport, New York

Dr. F. Ott
Virginia Institute of Marine Science

Dr. C.E. Epifanio
University of Delaware

Mr. R. Eissinger
International Shellfish Enterprises

Dr. J. Gonzalez
Puerto Rico Nuclear Center

Dr. R. Tuttle
Harvard University

New cultures isolated during the past year include a non-chainforming diatom (*Chaetoceros* sp.) and what may be a new species of coccolithophore. These two organisms were isolated from a dense bloom in a 32°C, 32.5^{o}/oo, below-sea level ditch near Rust-op-Twist, St. Croix. Several species of diatoms were isolated from a bloom resulting from mixing of deep-sea sediments with deep water during a cruise off

Hawaii to study the environmental impact of manganese-nodule mining.

4.6 The Effect of the Light/Dark Cycle on Coupling Between Nitrate Uptake and Assimilation and Growth Rate in the Diatom, *Chaetoceros simplex* (STX-105) in Nitrogen-Limited Continuous Cultures

A study of the relationship between rates of nitrate supply, nitrate uptake, nitrate assimilation and growth rate in *Chaetoceros simplex* (STX-105) in laboratory cultures was completed during 1974 by a predoctoral student (Gaston Picard). The results of this study will give us insights into the nitrogen metabolism of this diatom in the St. Croix mariculture system. A progress report summarizing Mr. Picard's data analysis to date is found in Appendix IV.

4.7 Ultrastructure and Cytochemistry in *Amphora coffaeformis* (Ag.) Kutz.

This study of a pennate diatom derived from St. Croix deep water has important implications for the St. Croix "Artificial Upwelling" mariculture system because it contributes to an understanding of the cellular mechanisms for formation and revival of diatom "resting cells" found in deep water. Similar studies on diatom species used as foods for bivalves on St. Croix should give us control of algal life cycles in our mariculture system. An abstract of this study is presented here; the manuscript prepared for publication is found in Appendix V.

Abstract: The ultrastructure of logarithmic-growing cells

and of resting cells (capable of enduring unfavorable environments) in laboratory cultures of *Amphora coffaeformis* isolated from deep ocean water (870 m) was examined using electron and light microscopy. The acid phosphatase activity (lysosomal marker enzyme), chlorophyll *a* and lipid content were assessed at weekly intervals of resting cell formation during cold-dark treatment, simulating deep ocean water. Approximately four weeks are required to complete resting cell formation. During the first week, the cytoplasm undergoes extensive transformation and lysosomal activity is observed. Large vacuoles disappear and small ones develop, the mitochondria become fewer and one or more massive mitochondria appear; the cytoplasm becomes densely granular. During the second and third week, the cytoplasm continues to contract, lipid bodies begin to develop and the plastid becomes densely stained. At the fourth week, the mature resting cell is formed containing a small and compressed nucleus, one or more massive mitochondria, a well-formed plastid, and granular cytoplasm containing occasional lipid droplets. The variation in chemical constituents correlates with the microscopic structure of the cells. The fine structure of cells during growth resumption when exposed to light at 25°C is presented. Previous reports of viable, chlorophyll-containing cells at great depths in the ocean may be explained by the results reported in this paper.

4.8 Alternative Methods for Monitoring Algal Cell Concentration

Measurements of fluorescence and of turbidity were tested as alternatives to manual cell-counting for monitoring the growth of phytoplankton and the stripping efficiency of shellfish. Both methods can be automated and could be used for continuous monitoring of a large-scale mariculture system.

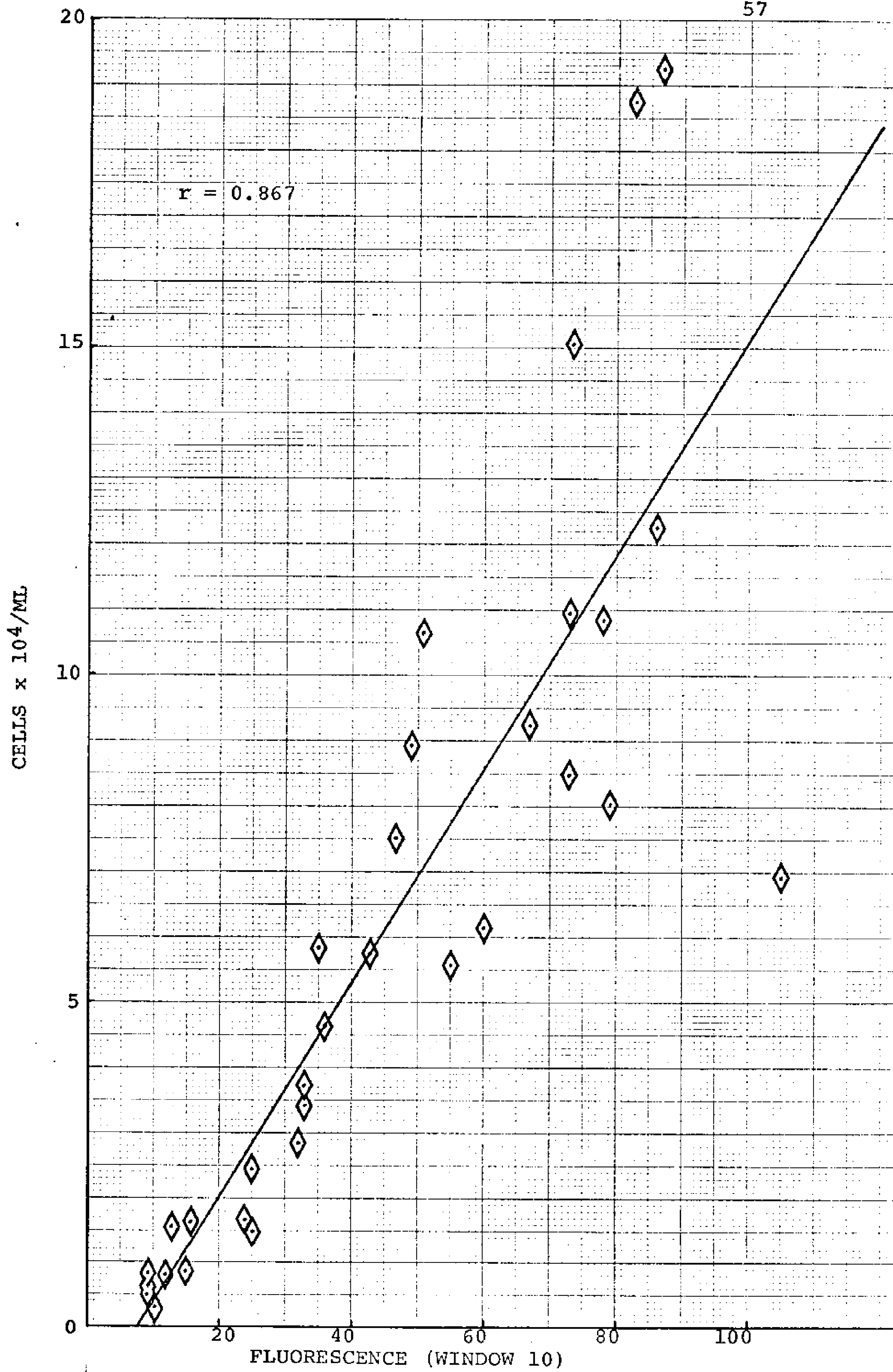
The fluorescence values are relative and represent the reading obtained from an *in vivo* sample of pool culture or tank effluent using the high sensitivity door of the Turner Model III fluorimeter. Turbidity was measured in ppm against a gel suspension standard on the Monitek Model 250 turbidimeter.

Both fluorescence and turbidity were tested for correlation with cell density in the pools and in shellfish-tank effluents. Turbidity was tested also for correlation with particulate nitrogen and particulate organic carbon.

The fluorescence vs. cell density curve in Figure 4:5 represents samples taken at 0800 for four consecutive days. The correlation was significant ($r = 0.867$) but scatter of data points increased in the denser samples.

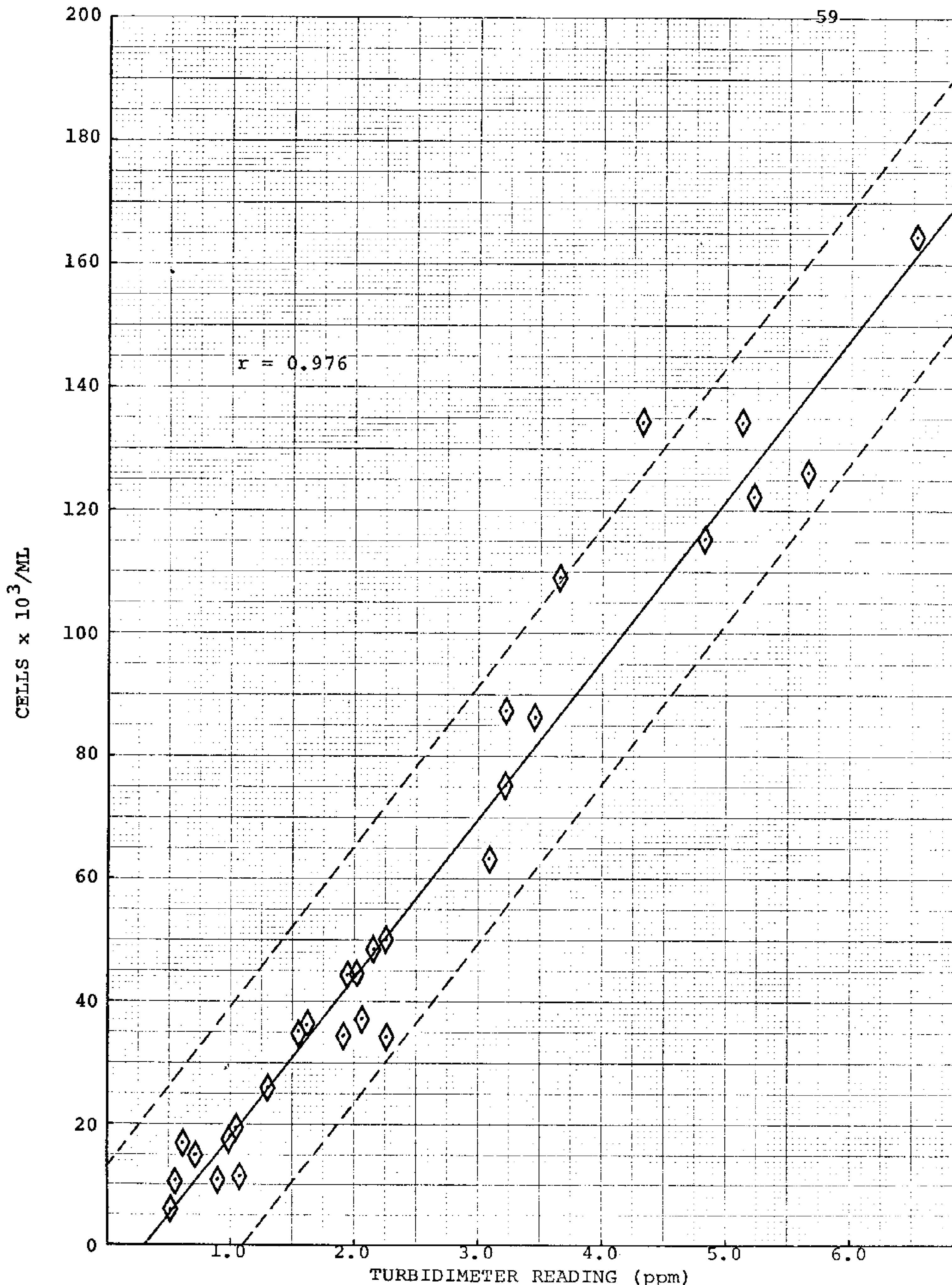
The turbidity vs. cell density curve in Figure 4:6 represents samples taken at 0800 for five consecutive days. The correlation was significant ($r = 0.976$) and with one

Figure 4:5 . Relative fluorescence of *in vivo* samples from the pools and animal-tank effluents vs. cell density of the same samples. Fluorescence readings were made on Window 10 of the Turner Model III fluorimeter; cell counts were done on a Speirs-Levy eosinophil counter.



r = 0.867
CELLS x 10^4/ML
FLUORESCENCE (WINDOW 10)
20
15
10
5
0
20
40
60
80
100

Figure 4:6. Turbidity of *in vivo* samples from the pools and animal-tank effluents vs. cell density of the same samples. Turbidity readings were done on the Monitek Model 250 turbidimeter; cell counts were done on a Speirs-Levy eosinophil counter. The regression line (solid) was calculated by least-squares regression. Dashed lines represent the regression of y on x.

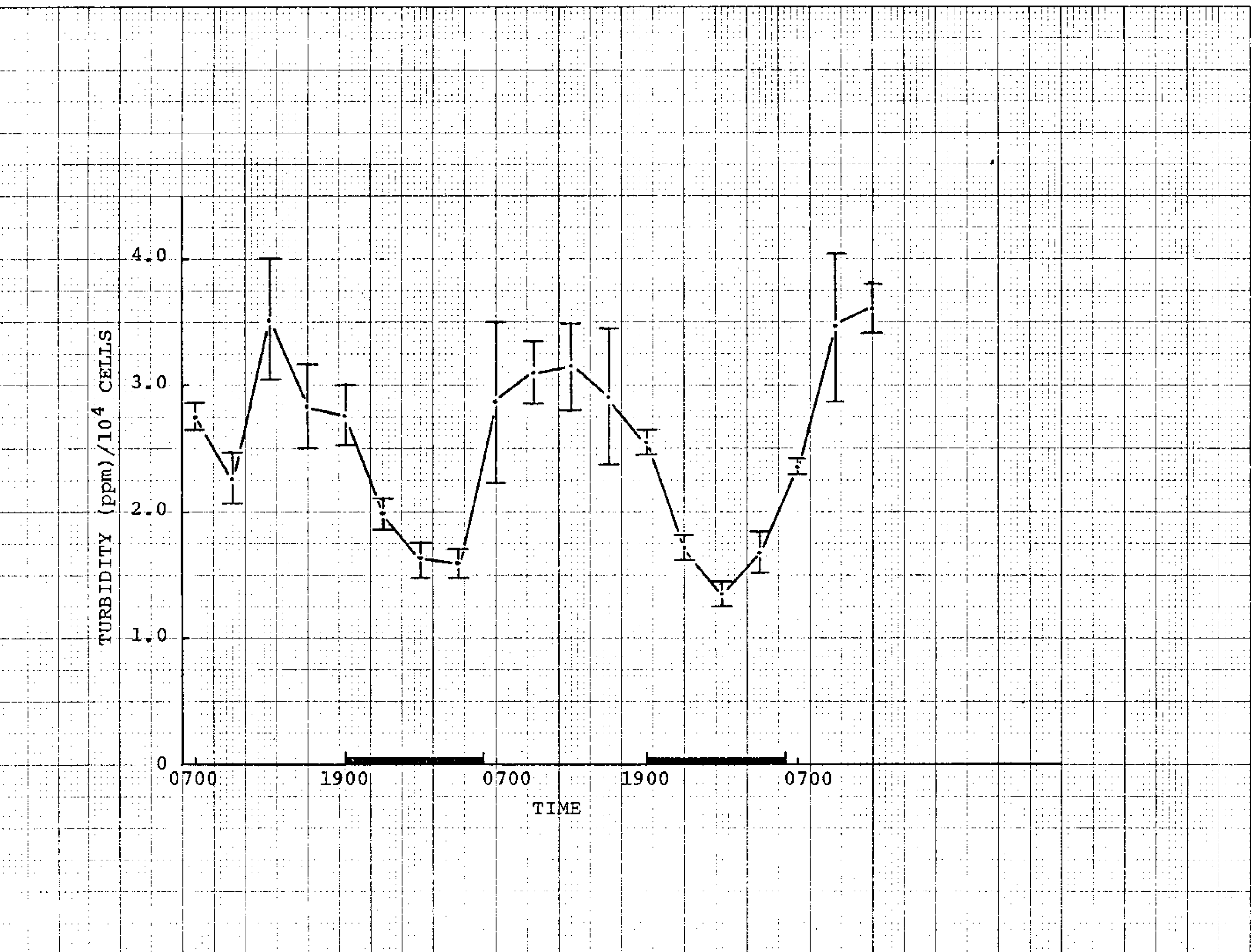

r = 0.976
CELLS x 10^3/ML
200
180
160
140
120
100
80
60
40
20
0
1.0
2.0
3.0
4.0
5.0
6.0
TURBIDIMETER READING (ppm)

exception the data points fell within the 95% confidence limits. Turbidity vs. particulate nitrogen and carbon were also significant (r = 0.835 and 0.876, respectively) but less so than turbidity vs. cell density.

Further investigation showed that the correlation between turbidity and cell density varied with time of day (Fig. 4:7) and to some extent with cell size or species (not shown).

It was concluded that measurements of turbidity could be an acceptable alternative to manual cell-counts for monitoring algal growth and shellfish stripping efficiency.

Figure 4:7 . Ratio of turbidity reading to cell density vs. time of day in a continuous culture of STX-167.



46 1470
TURBIDITY (ppm)/10^4 CELLS
4.0
3.0
2.0
1.0
0
0700
1900
0700
1900
0700
TIME

5 SHELLFISH CULTURE : PELECYPODS

Growth studies to select species of bivalve shellfish adaptable to the St. Croix mariculture system were continued during 1974; nine species of bivalves were studied. More species were harvested from our system than were introduced during the year, as shown in Tables 5:1 and 5:2. With completion of the hatchery building during 1974, we will be able to provide our own seed populations (from brood stock reared on St. Croix) for future experiments as they are needed, rather than as they are available from commercial hatcheries.

The results of studies with the different species are given in the following sections.

5.1 *Argopecten irradians* (Bay Scallops)

A second population of bay scallops, *Argopecten irradians*, was introduced to the St. Croix system on June 19, 1973, to repeat growth experiments run on a population of scallops in 1972. After 8.5 months, the average height of the population was 51 mm (market size). At market size the scallops were grown at densities of 25 to 30/ft^2. The survival rate was good (73%) until March 1974, but shortly thereafter mortality increased because scallops were becoming senescent, and by June 1974 the entire population had died. Figure 5:1 illustrates the leveling off of growth

TABLE 5:1. SHELLFISH POPULATIONS INTRODUCED TO THE "ARTIFICIAL UPWELLING" MARICULTURE SYSTEM IN ST. CROIX DURING 1974

NUMBER	SPECIES	ORIGIN	DATE	PURPOSE
8,000 (POST-SET)	_Tapes semidecussata_	Spawned in St. Croix	4-2-74	HATCHERY WORK & GROWTH STUDIES
3,000	_Crassostrea gigas_	Pacific Mari-culture, Inc. (W.W. Budge)	4-5-74	ALGAL FEEDING STUDY
24,000 (POST-SET)	_Tapes semidecussata_	Spawned in St. Croix	5-9-74	HATCHERY WORK & GROWTH STUDIES
2,000 to 3,000	_Crassostrea gigas_ (Kumomoto strain)	Bay Center Mari-culture, Inc.	7-1-74	GROWTH STUDIES
125	_Mytilus edulis_	N.Y. Fish Market	8-6-74	ACCLIMATION & SURVIVAL STUDIES & HATCHERY WORK
320,000 (POST-SET)	_Tapes semidecussata_	Spawned in St. Croix	9.7-74	HATCHERY WORK & GROWTH STUDIES

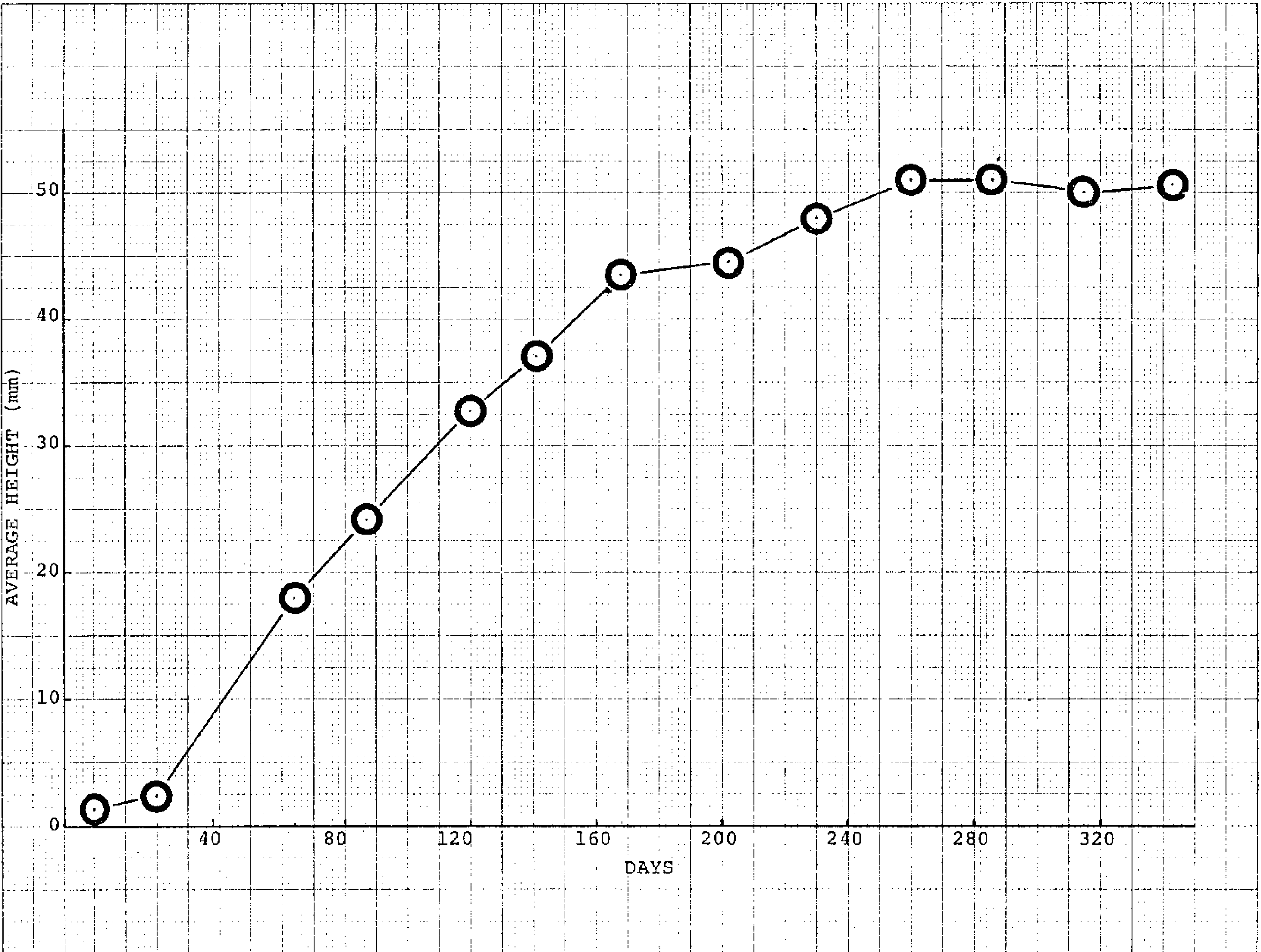
TABLE 5:2. SHELLFISH POPULATIONS HARVESTED FROM THE "ARTIFICIAL UPWELLING" MARICULTURE SYSTEM IN ST. CROIX DURING 1974

NUMBER	SPECIES	ORIGIN	REASONS FOR HARVEST	DATE INTRODUCED	DATE HARVESTED
34	F_1 clams (*M. mercenaria* ♂ X *M. campechiensis* ♀)	Chanley, P., Shelter Island Oyster Co.	market size	04-24-73	01-01-74
34	*M. campechiensis*	as above	as above	04-24-73	01-01-74
3	*Argopecten irradians*	Castagna, M., VIMS-E	as above	06-19-73	01-08-74
1	*Ostrea edulis*	Budge, W.W., Pacific Mariculture Inc.	as above	08-15-73	01-22-74
51	*M. campechiensis*	Chanley	as above	04-24-73	01-30-74
16	F_1 clams	as above	as above	04-24-73	01-31-74
34	*Argopecten irradians*	Castagna	as above	06-19-73	03-07-74
5	*Ostrea edulis*	Budge	taste test	08-15-73	03-21-74
25	F_1 clams	Chanley	as above	04-24-73	03-21-74
10	*Argopecten irradians*	as above	as above	06-19-73	03-21-74
7	*Argopecten irradians*	as above	meat/shell ratio	06-19-73	05-01-74

TABLE 5:2 (continued)

NUMBER	SPECIES	ORIGIN	REASONS FOR HARVEST	DATE INTRODUCED	DATE HARVESTED
376	*M. campechiensis*	Chanley	market size	04-24-73	05-22-74
324	F_1 clams	as above	as above	04-24-73	05-28-74
210	*Tapes semidecussata*	Budge	shell/meat ratio, taste	08-15-73	06-11-74
48	F_1 clams	Chanley	taste	04-24-73	06-11-74
48	*M. campechiensis*	as above	as above	04-24-73	06-11-74
32	*M. campechiensis*	as above	as above	04-24-73	11-07-74
24	*M. campechiensis*	as above	as above	04-24-73	11-09-74

Figure 5:1. Leveling-off of growth in *Argopecten irradians* (Bay Scallop) as the animals approached market size.


46 1470
AVERAGE HEIGHT (mm)
0
10
20
30
40
50
40
80
120
160
200
240
280
320
DAYS

as the scallops approached market size. The flattened curve is directly influenced by the death of the market-size bay scallops.

5.2 Crassostrea gigas (Pacific Oysters)

5.2.1 Comparative Feeding Study of Juvenile *C. gigas*

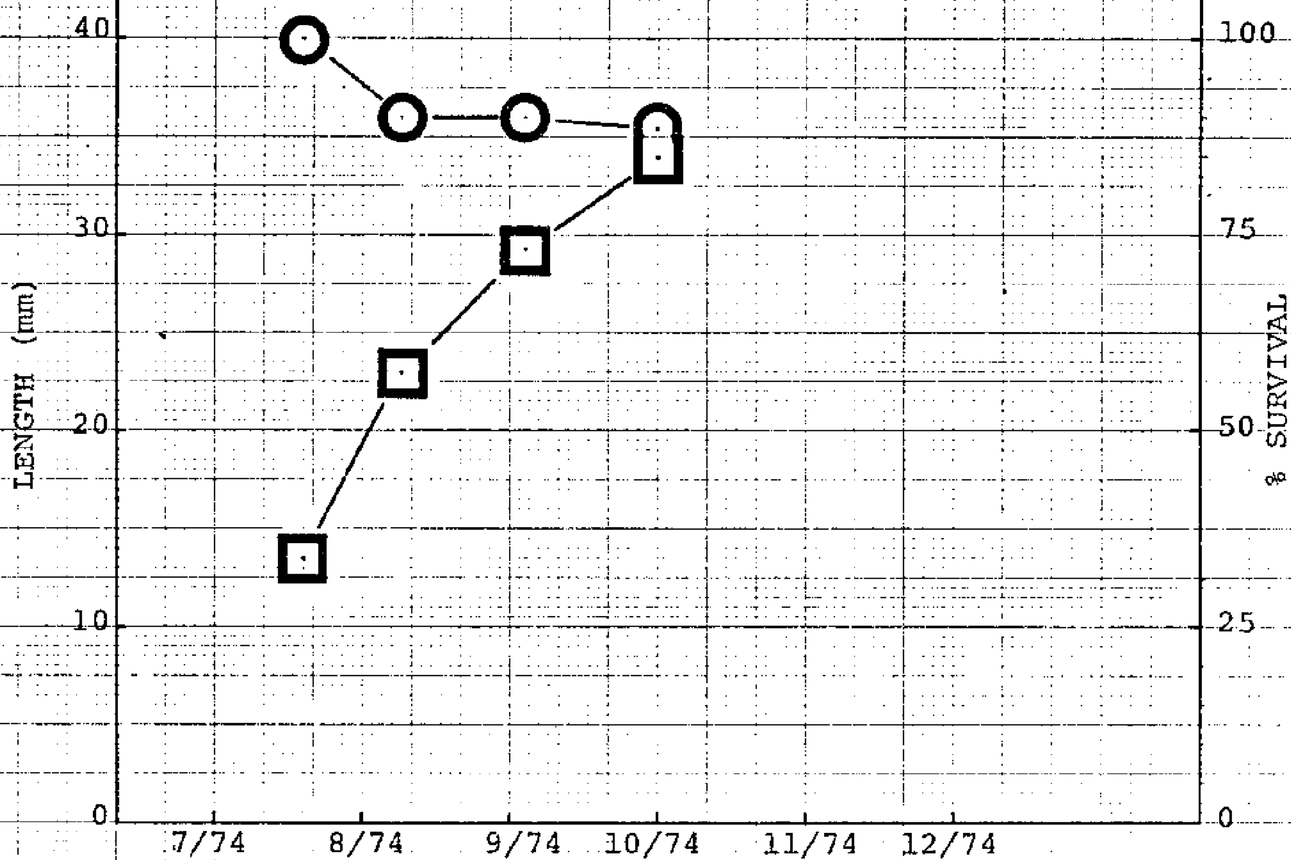
In April 1974, a batch of *C. gigas* from Pacific Mariculture, Inc., was introduced for a study on the effects of different algal diets on growth of this species. Survival and growth in all experimental treatments were poor and the experiment had to be discontinued. We were not able to repeat the experiment because Pacific Mariculture, Inc. closed its hatchery, due to poor water quality at their Pigeon Point, California, location. Our results with this batch of *C. gigas* may have been related to the difficulties Pacific Mariculture, Inc. had in rearing seed oysters.

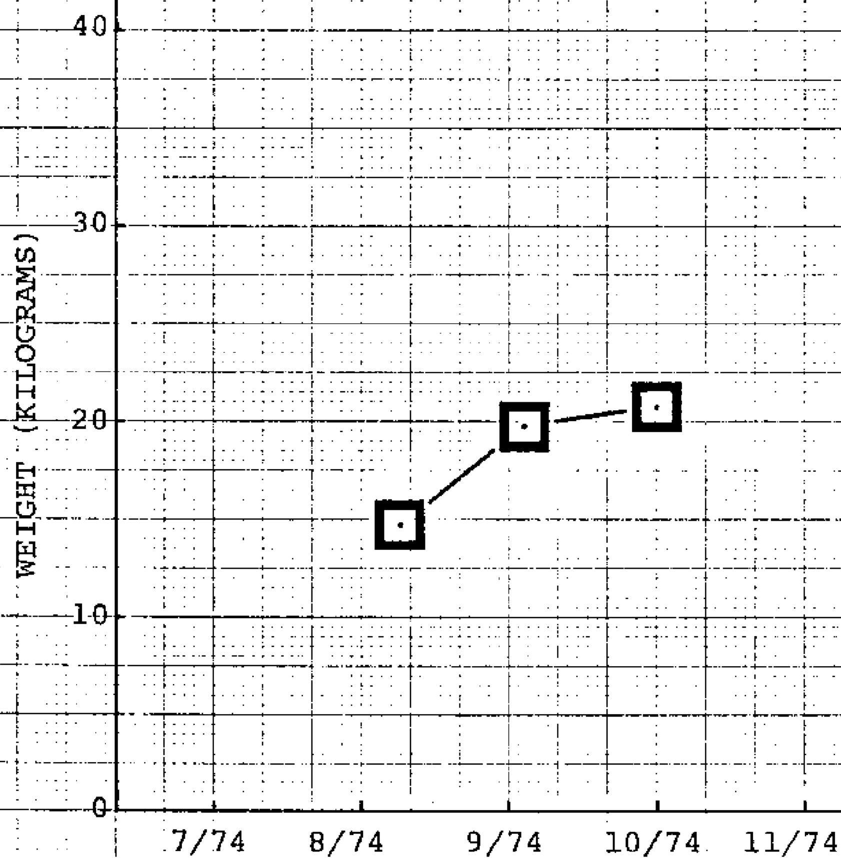
5.2.2 The Kumomoto Strain of *C. gigas*: Growth Studies on St. Croix

The Kumomoto is a "gigas-type" of oyster but is smaller and slower growing than the West Coast Pacific oyster, *Crassostrea gigas*. Two populations of *Crassostrea gigas* were reared in St. Croix; market-size oysters were obtained in 12 to 16 months. These oysters initially grew slowly but after two months in the system, growth began to speed up. Kumomoto oysters are widely used

Figure 5:2. Percentage survival and increase in length for a group of 141 randomly selected spat of *Crassostrea gigas* (Kumomoto strain).

Figure 5:3. Increase in weight of the total population of *Crassostrea gigas* (Kumomoto strain) on cultch.





46 1470
K&E 10 X 10 TO THE INCH • 7 1/2 X 10 INCHES KEUFFEL & ESSER CO. MADE IN U.S.A.

in the "half-shell" trade on the West Coast. Since C. gigas growth in St. Croix was favorable, it is important to test the mariculture potential of the Kumomoto oyster. The Kumomoto will be evaluated for growth rate, survival, taste and reproductive capabilities.

Two- to three-thousand Kumomoto spat (on cultch) from Bay Center Mariculture Company, Willapa Bay, Washington, arrived in St. Croix on July 1, 1974. At monthly intervals, growth (increase in length and in weight) and survival were monitored. Figure 5:2 shows % survival and increase in length for a group of 141 randomly selected spat. Figure 5:3 shows increase in weight of the total Kumomoto population on cultch.

5.3 Mercenaria campechiensis, M. mercenaria, and F_1 Clams (M. mercenaria ♂ x M. campechiensis ♀)

An experiment to compare growth of these three clams in the St. Croix mariculture system with growth in a variety of natural environments (St. Croix, Florida, Virginia, New York, and Hawaii) was completed in 1974. The results of this study will be published (see ms. Appendix VI); the following is an abstract of that paper.

Abstract: The growth of hard shell clams (Mercenaria mercenaria Linné and Mercenaria campechiensis Gmelin) and their F_1 cross in a controlled experimental environment was compared to growth in uncontrolled natural environments. The clams were spawned by Paul Chanley of Shelter Island Oyster Company, Inc., Greenport, New York. In the controlled

environment of the artificial upwelling mariculture system on St. Croix, U.S. Virgin Islands, pollutants and predators were absent and fouling was minimal. Salinity was 34.75 to 34.95°/oo and water temperature varied between 22° and 30°C during the experiment. Natural environments in temperate, subtropical and tropical waters were selected as sites for comparative studies: Southold, in Long Island, N.Y.; Wachapreague on Bradfords Bay, Virginia; Alligator Harbor on the Gulf Coast of Florida; Kupeke Pond in Pukoo, Molokai, Hawaii; and Salt River Inlet, St. Croix.

Clams from each population—*M. mercenaria*, *M. campechiensis* and F_1 cross—were planted at Southold, N.Y., and at both St. Croix sites. Only *M. campechiensis* and F_1 clams were sent to Virginia and Florida and F_1 clams and *M. mercenaria* were sent to Hawaii. Increase in length, "wet" weight and survival were measured at the different locations from April 1973 through May-June 1974. *M. campechiensis* and F_1 clams reached market size (greater than 25.5 mm thick) in 6.5 to 13 months in the St. Croix artificial upwelling system. These clams did not reach market size in Southold, N.Y.; Wachapreague, Virginia or Salt River Inlet, St. Croix. The F_1 clams in Molokai, Hawaii and Alligator Harbor, Florida were close to market size when the experiment was terminated after 13 months. Survival and growth of *M. mercenaria* was poor in the artificial upwelling controlled environment, in Salt River Inlet, St. Croix and in Molokai, Hawaii.

The results of this study indicate that both the F_1 clams and *M. campechiensis* do very well in the St. Croix mariculture system. However, because of the superior keeping quality of the F_1 clam under refrigeration, this clam has a greater commercial potential for the "half-shell" trade.

5.4 *Mytilus edulis* (Blue Mussel)

The blue mussel, *Mytilus edulis*, was brought to St. Croix for acclimation and spawning studies after earlier attempts to induce gametes discharge failed at the Tallman Island station in Queens, New York.

One-hundred and twenty-five mussels arrived on August 6 and were separated into two groups of 62 and 63 individuals. One group was kept in refrigerated water at 5°C (temperature on arrival was 8.5°C), while the other was allowed to reach laboratory room temperature. The salinity of both groups was increased 3 ppt every two days from an initial level of $22^{o}/oo$, to local water values ($34.8^{o}/oo$). All mussels were batch-fed algal diets from pool and/or polytank cultures in the mariculture system. During their acclimation period, five mussels were lost from the warm temperature group.

In early September, the fully acclimated mussels were moved from the laboratory and placed on-line in the culture system, receiving effluent from other shellfish tanks. After

30 days in the system, mortality reduced the population by 52% to a total of 27 individuals. Two mussels were sacrificed for internal inspection at this point and were found in an emaciated condition. The visceral mass was greatly reduced and the gonads contained few sex products. Nonetheless, 22 individuals were selected from this group for artificial spawning experiments, by means of temperature shock. These were removed from culture water of 29.2°C and placed into a container of deep water and diet algae at a temperature of 23.2°C. After a period of one hour, several mussels were actively pumping with valves open, but had not discharged gametes. After an additional hour, with no success, the mussels were returned to their tanks. This group continues to be diminished by mortality and presently consists of 10 individuals.

By contrast, the refrigerated group has demonstrated good survival, despite being batch-fed algae only once a day. The physical condition of these animals, however, was no better (superficially) than that of the warm-temperature group. From this, the primary factor of the high mortality in the acclimated group would appear to be the effects of temperature, perhaps after a preliminary weakening by a subadequate diet.

Current plans are to increase the temperature of the refrigerated group to 16-18°C and batch-feed twice a day

in an effort to increase glycogen reserves. At the same time, the gradual change in temperature should induce gonadal development, and enable another attempt at artificially inducing spawning.

5.5 *Ostrea edulis* (European Oyster)

Two previous populations of *Ostrea edulis* were grown to market size in the St. Croix mariculture system in 13 to 16 months. The third population of the European oyster was introduced to the St. Croix mariculture system on August 15, 1973. The first and second populations were introduced in April, 1972 and October, 1972, respectively. All three groups of juvenile oysters were obtained from Mr. W.W. Budge, Pacific Mariculture, Inc., Pescadero, California. The growth of these three populations is reported in the paper by Sunderlin *et al*. (Appendix VII). Additional data for the third experiment are given in Table 5:3.

The third *O. edulis* population in the St. Croix mariculture system was involved in a comparative growth study and in September 1973, 5,000 juveniles were shipped to the following locations: Florida (R.W. Menzel); Virginia (M. Castagna); New York (P. Chanley); and Salt River Inlet, St. Croix (J.B. Faile). After a week, all of the oysters sent to Florida and Virginia had died and no *O. edulis* survived in Salt River Inlet (St. Croix) after 55 days. The only comparative growth data available for this experiment are from Greenport, New York and Rust-op-Twist (the mariculture

system), St. Croix (Table (Table 5:3).

The O. edulis in the St. Croix mariculture system grew rapidly until mid-February 1974, when the larger oysters began to die, hence the irregular length measurements thereafter. One factor that may have contributed to the mortality was a change in the diet. In early 1974, Chaetoceros cf. simplex (clone STX-105) did not grow well in the pools on the beach and it was finally omitted from the production schedule in May 1974. Chaetoceros curvisetus (STX-167) was substituted for STX-105 in June 1974. The two previous O. edulis populations were fed STX-105 along with Thalassiosira pseudonana (clone 3H) and/or Bellerochea spinifera (STX-114) and the problem of larger oysters dying off, as they approached market size, was not experienced.

Presently, there are 115 O. edulis in the mariculture system—not all of these are quite market size (>76 mm in length), but they are being maintained and will be used as brood stock in the hatchery. Feeding experiments will be run, in the experimental shellfish area, on a variety of algal species and different combinations of these algal cultures will be fed to juveniles and to oysters approaching market size.

5.6 Tapes semidecussata (Japanese Little-Neck Clams)

This clam has excellent commercial mariculture potential because of its fast growth rate (7 to 11 months to reach market size), high survival (60%), and tolerance

TABLE 5:3. AVERAGE GROWTH OF <u>OSTREA EDULIS</u> POPULATIONS IN ST. CROIX AND IN SOUTHOLD, NEW YORK

DATE	RUST-OP-TWIST ST. CROIX	SOUTHOLD NEW YORK
08-15-73	3.19 mm	-
10-03-73	15.86	-
12-04-73	51.15	-
01-16-74	60.17	-
01-22-74	62.29	-
02-19-74	66.30	-
03-19-74	64.79	-
04-17-74	68.63	-
05-14-74	69.24	-
06-06-74	68.76	-
07-11-74	-	43.53 mm
07-16-74	65.95	-
08-22-74	67.71	-
08-29-74	-	53.7
10-15-74	66.3	-
SURVIVAL (%)	72.5	80.3

of high stocking densities (up to $180/ft^2$). This species was reared to market size (>38 mm in length) during 1974, and the life cycle has been completed on St. Croix. The first batch of larvae resulted from a spontaneous spawning in April 1974, and the second and third batches from controlled spawnings in May and September 1974. The April 1974 batch spawned spontaneously before reaching market size (at 23 mm) in October 1974—less than seven months after they were spawned themselves. It may be possible, through selective breeding of fast-growers, to select for clams that will reach a larger size before spawning. The details of the growth studies and larval development are given in the manuscript, being prepared for publication, given here as Appendix VIII.

5.7 Comparison of Mercury and Chlorinated Hydrocarbons in Bivalve Shellfish Grown in the St. Croix Mariculture System and in Natural Waters

In collaboration with Dr. Robert J. Reinold of the University of Georgia, samples of meat from bay scallops (*Argopecten irradians*) and Pacific oysters (*Crassostrea gigas*), grown in our "Artificial Upwelling" mariculture system at Rust-op-Twist, St. Croix, were analyzed for chlorinated hydrocarbons (Dieldrin, DDT, DDE, TDE and PCB's) and for mercury. Mangrove oysters (*C. rhizophorae*) from Salt River, St. Croix and Coral Bay, St. John were also analyzed.

The results of these analyses (Table 5:4) show that there were insignificant amounts of mercury (<0.02 ppb) in the scallops and oysters grown in our mariculture system. In contrast, the mangrove oysters from natural waters on St. Croix and St. John had significant concentrations of mercury (39 and 58 ppb, respectively). Chlorinated hydrocarbons were not present in significant concentrations (>5 ppb) in animals from the mariculture system, nor in the oysters from natural waters.

These results point out one of the great advantages of mariculture systems utilizing artificially upwelled deep water: the lack of man-derived pollutants, such as mercury, in the deep water. They also indicate the value of our mariculture system for baseline studies of contamination of marine food chains by man's pollutants.

5.8 Test of a Method for Eliminating Hydrogen Sulfide Production in Sand Substrate for Clam Culture

Anoxic sand substrate in a tank containing juvenile Japanese little-neck clams (*Tapes semidecussata*) may have been the cause of increased mortality in a population of this clam. Production of hydrogen sulfide in anoxic sand substrate makes for very unpleasant work when monitoring growth of clams grown under those conditions. We tested the feasibility of exposing the upper and lower surfaces of the substrate to water flow in the tank, thereby allowing for greater interstitial water flow in the substrate. This

TABLE 5:4. CHLORINATED HYDROCARBONS AND MERCURY IN BIVALVE SHELLFISH FROM THE "ARTIFICIAL UPWELLING" MARICULTURE SYSTEM, AND FROM NATURAL WATERS (SPRING, 1973)[1]

SPECIES	SOURCE	CHLORINATED HYDROCARBONS (ppb)	MERCURY (ppb)
Argopecten irradians (Bay scallop)	Artificial Upwelling Mariculture System	n.s.[2]	n.s.[3]
Crassostrea gigas (Pacific oyster)	as above	n.s.	n.s.
Crassostrea rhizophorae (Mangrove oyster)	Salt River, St. Croix	n.s.	39
	and Coral Bay, St. John		58

[1]From Reinold, R.J., 1974. Chlorinated hydrocarbon pesticides and mercury from Puerto Rico and the U.S. Virgin Islands, 1972-1973 (Submitted to Pesticide Monitoring Journal)

[2]not significant, <5 ppb

[3]not significant, <0.02 ppb

treatment should have had the effect of decreasing the rate of accumulation of fecal material and possibly avoiding the development of reducing conditions. The lower surface of the substrate was exposed to water flow by suspending the sand in a Nestier tray with a 2-layer plastic window screen liner; a thin layer of glass wool was sandwiched between the screens to retain the fine sand. Control trays were lined with polyethylene sheeting.

The screened trays maintained higher pH's and dissolved oxygen concentrations in the sand substrate through the first four weeks, but the glass wool layer in the screened trays became clogged after four weeks, so that there was no difference in pH and O_2 measurements thereafter. After four weeks, hydrogen sulfide production was evident in both the experimental and control trays.

We hope to repeat this experiment using a screen mesh size that will not clog easily, but which will still retain the sand.

The details of this experiment are given in Appendix IX.

5.9 Economic Comparison of Six Pelecypod Molluscs for Artificial Upwelling Mariculture

The economic values of six species of bivalve shellfish were compared on the basis of their meat production per square foot of surface area of the tanks in which they were grown. The comparison was based on results from

our St. Croix mariculture system which has not been optimized for production of each mollusc. Each species was grown in a culture tank having a volume of 200 gallons and a surface area of 16 ft^2; the flow rate of algal culture to the culture tanks varied from tank to tank. Using the growth rates, stocking densities and percent meat values of the six species listed in Table 5:5 and the flow rates of algal culture to the tanks containing each species, we calculated the yearly productivity of shellfish meat that could be expected if 36,000 gallons per day of algal culture grown in our system was used to grow only one species of shellfish at the indicated stocking densities.

The analysis is presented in Table 5:5 which indicates that four species have the greatest potential economic value; they are listed here in decreasing order of value:

Tapes semidecussata

F_1 clams

Mercenaria campechiensis

Crassostrea gigas

Argopecten irradians is considered to be too low in value, relative to the other species, to merit further investigation. The European oyster, *Ostrea edulis*, is intermediate in value and merits additional study to increase its growth under different experimental conditions.

The analysis presented here represents our first

experience with several of the species. Higher production rates can be expected as additional experience with the behavior of the different species of molluscs is gained (in experiments in progress, we are finding that _Tapes semidecussata_ grows faster as the turnover time in the tank is decreased).

TABLE 5:5. ECONOMIC COMPARISON OF SIX PELECYPOD MOLLUSCS FOR ARTIFICIAL UPWELLING MARICULTURE

	TAPES SEMIDECUSSATA (Japanese Little Neck Clam)	MERCENARIA CAMPECHIENSIS (Southern Clam or Quahog)	F_1 CLAMS (cross of M. CAMPECHIENSIS & M. MERCENARIA)	CRASSOSTREA GIGAS (Pacific Oyster)	OSTREA EDULIS (European Oyster)	ARGOPECTEN IRRADIANS (Bay Scallop)
MARKET SIZE	>38 mm in length	>25.5 mm thick	>25.5 mm thick	>30 gm in weight	>76.5 mm in length	>50.5 mm in height
MONTHS TO REACH MARKET SIZE	7-11	6.5-13	6.5-13	12-16	12-16	7-10
DENSITY PER SQ.FT. (AT MARKET SIZE)	120	50	50	15	9	25
% SURVIVAL	64	48	42	67	70	73
UNIFORMITY OF SIZE	+	+	-	+	+	+
% MEAT (SHUCKED WET WEIGHT)	39	28	31	39	22	(muscle only) 11
CALCULATED PRODUCTIVITY PER YEAR* - POUNDS OF MEAT HARVESTED PER SQ.FT.	3.02	2.19	2.66	2.09	1.54	0.44
GROSS VALUE** ($/ft^2/YR) ($/acre/YR)	$7.54 $328,878	$5.47 $238,273	$6.65 $289,674	$5.23 $227,819	$3.84 $167,270	$1.10 $47,916
RELATIVE GROSS VALUE	100	72	88	69	51	15

*Based on an algal culture supply of 36,000 gallons/day and calculated from data obtained from growth in 16 sq.ft. tanks.
**Using value of $2.50/lb. of meat.

6 SHELLFISH CULTURE : GASTROPODS

6.1 *Strombus gigas* (Queen Conch)

Commercial fishing interests in the Queen Conch, *Strombus gigas*, and its importance to the local economy (Randall, 1964) prompted a preliminary investigation into the culture potential of this animal in October, 1973; the investigation is still in progress.

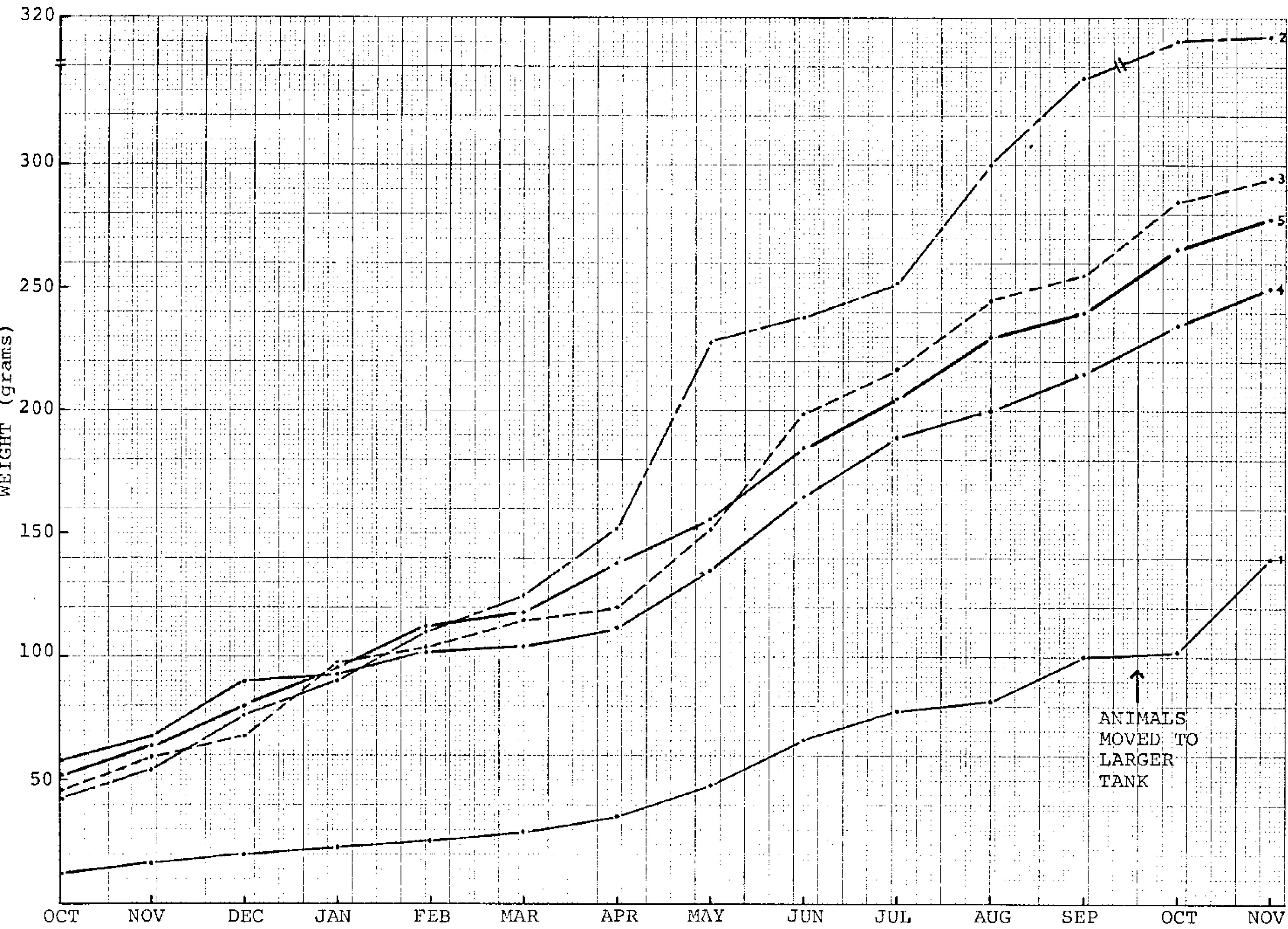
Initially, five individuals, all juveniles, were placed into two 1 ft x 1 ft x 1.5 ft rearing tanks which were left open to the sunlight and which received culture media effluent from other shellfish containers. The subsequent algal growth appeared first to be in excess of, and later adequate for, animal grazing demands to September, 1974. In mid-September, the conch were transferred to a 12 ft x 2 ft x .5 ft culture flume to allow for the increasing food requirements. The grazing area in this container should be more than adequate to sustain continued growth for some time.

The increases in size (length and weight) through November 1974 are given in Figures 6:1 and 6:2 for the five animals. All conch are still in the juvenile stage (i.e., shell lip formation about the operculum has not begun.

6.2 Abalone

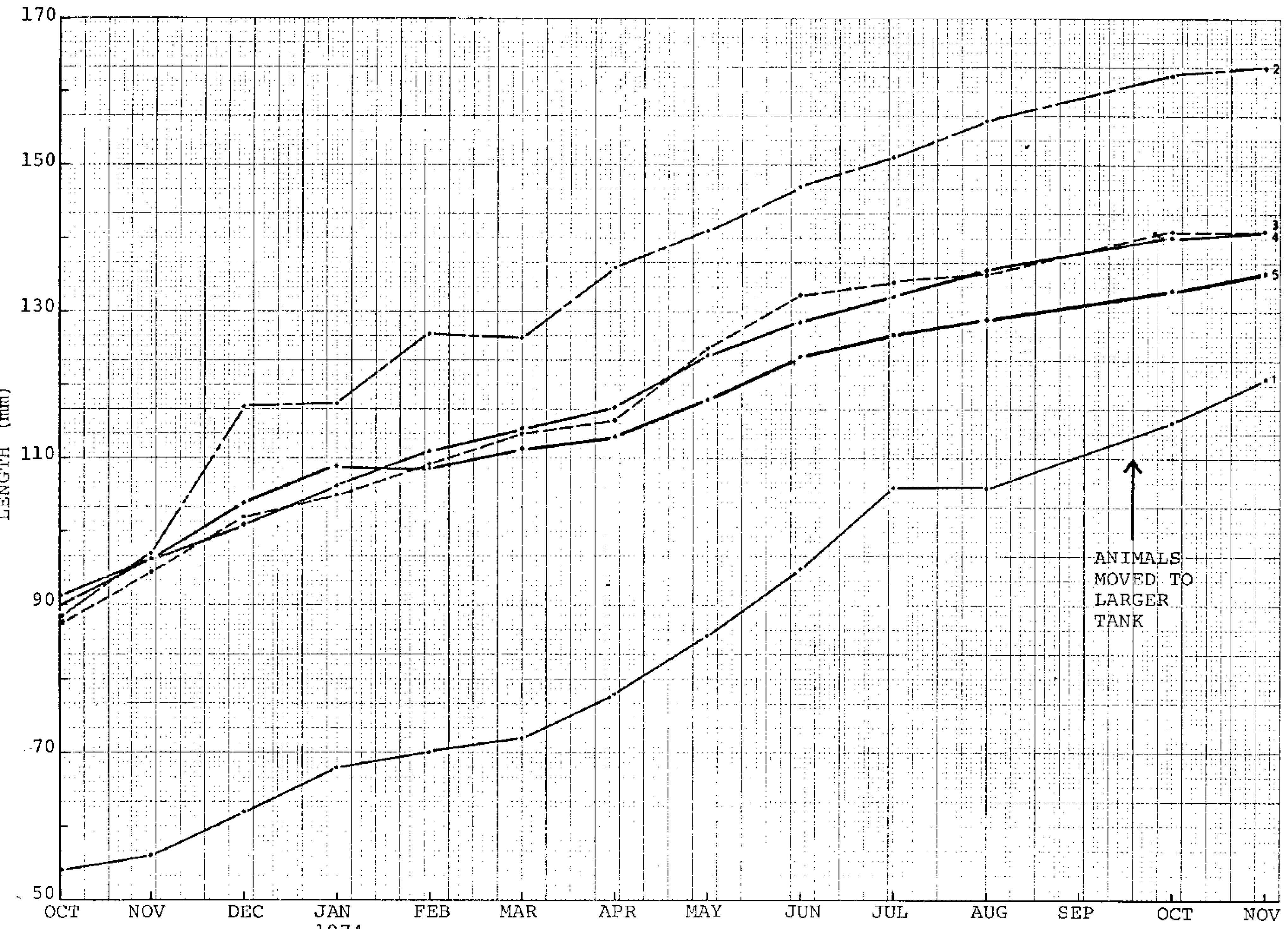
We hope to receive juvenile specimens of a warm-water abalone for growth experiments on St. Croix. These

Figure 6:1. Growth of *Strombus gigas* (Queen Conch): increase in weight (grams).



46 1510
WEIGHT (grams)
320
300
250
200
150
100
50
OCT
NOV
DEC
JAN
1974
FEB
MAR
APR
MAY
JUN
JUL
AUG
SEP
OCT
NOV
1
2
3
4
5
ANIMALS
MOVED TO
LARGER
TANK

Figure 6:2. Growth of *Strombus gigas* (Queen Conch): increase in length (mm).



10 X 10 TO THE CENTIMETER
KEUFFEL & ESSER CO.
461510
LENGTH (mm)
170
150
130
110
90
70
50
OCT
NOV
DEC
JAN
1974
FEB
MAR
APR
MAY
JUN
JUL
AUG
SEP
OCT
NOV
ANIMALS
MOVED TO
LARGER
TANK
1
2
3
4
5

are being obtained from Dr. Martin Ortiz of the Instituto Nacional de Pesca, Chiapas, Mexico.

7 CRUSTACEAN CULTURE

7.1 Growth of *Panulirus argus* (Spiny Lobster)

Of the five original *Panulirus argus* lobsters introduced into the culture system in June 1972, three individuals continue substantial growth (Fig. 7:1). The other two lobsters, identified as 3A and 2B of this group died on 7-10-72 and 10-25-73, respectively.

On August 8, 1973, three additional *P. argus* juveniles were acquired from Salt River and of which only one survives (as at 11-14-74), numbered 3A in Figure 7:1. Those numbered 3B and 3C died on 6-02-74 and 10-25-73, respectively. Up until November, 1974, only carapace length measurements were recorded on these juveniles, therefore Figure 7:1 represents the initial weight measurements for 3A.

Until May, 1973, all *Panulirus* were fed diets of selected bivalve meats of known weights in proportion to their individual sizes. After this point, the animals were placed on a maintenance diet which varied per individual in number of culled bivalves (frozen or live), and local beach crabs. It is interesting to note that the greatest surge in growth rates occurred after the experimental diets were lifted. A summary of weight measurement is presented in Table 7:1.

Figure 7:1. Growth of *Panulirus argus* (Spiny Lobster)

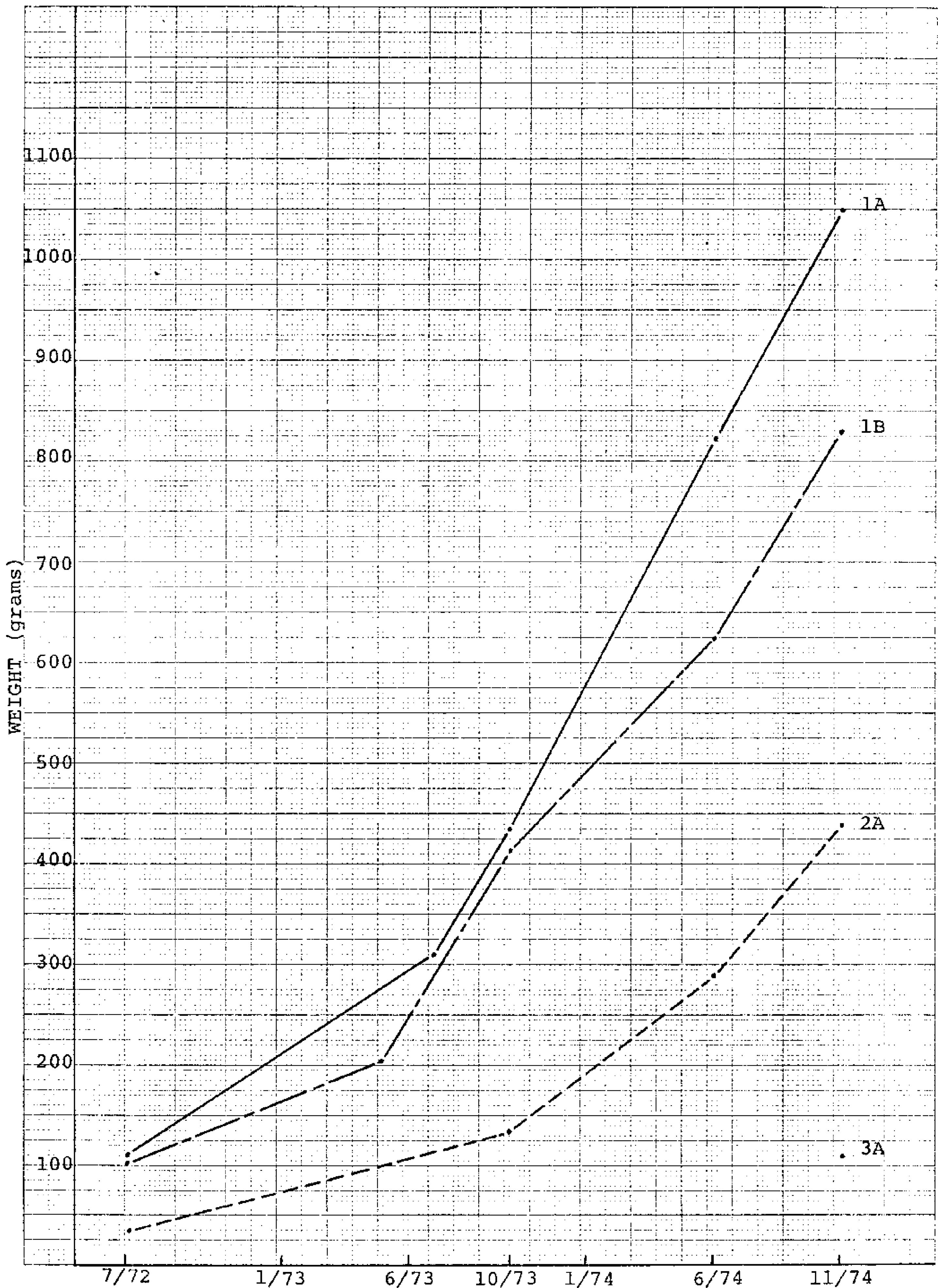

WEIGHT (grams)
1100
1000
900
800
700
600
500
400
300
200
100
7/72
1/73
6/73
10/73
1/74
6/74
11/74
1A
1B
2A
3A
461510
10 X 10 TO THE CENTIMETER
KEUFFEL & ESSER CO.

TABLE 7:1. GROWTH AND MORTALITY OF PANULIRUS ARGUS

DATE	ANIMAL NO.	WEIGHT (g)	CARAPACE LENGTH (mm)	MORTALITY
06-27-72 (INITIAL)	1A	110		
	1B	105		
	2A	35		
	2B	58		
	3A	10		
07-10-72	-	-	8.8	3A
11-24-72	1A	220		
	1B	142		
	2A	45		
	2B	63		
10-25-73	1A	435		
	1B	415		
	2A	135		
	2B	116	50.9	2B
	3A	-		
	3B	-		
	3C	-	14.0	3C
06-02-74	-	-	23.3	3B
06-11-74	1A	850		
	1B	650		
	2A	290		
	3A	-	23.0	
10-13-74	1A	1050		
	1B	850		
	2A	440		
	3A	110		

7.2 *Homarus americanus* (North American Lobster)

On September 5, 1973, 41 juvenile *Homarus americanus* were received for rearing studies in the culture system. Of these, 34 were lost by November, presumably from a combination of shipping stress and inability to adapt to a new environment. The average size on arrival was approximately 6 to 7 mm.

The diet of these animals initially consisted of brine shrimp, then minced shellfish meat culled from the system; however, all individual were lost by August 1974, the largest measuring 27 mm. Originally, the growth of this group of juveniles was to have been compared with that of individuals hatched in May 1973 from berried females held in the culture system. Unfortunately, all of the hatched group were lost by the end of June, 1973.

Table 7:2 presents a record of the remaining seven juveniles from the September 5 group. No new attempts were made to rear larvae or select new experimental diets for *Homarus americanus* in the remainder of 1974.

7.3 *Artemia salina* (Brine Shrimp)

A preliminary experiment was undertaken to determine if brine shrimp would grow to maturity and reproduce in the St. Croix mariculture system. The primary goal was to discover what technical problems would be encountered in designing a semi-automated system for rearing brine shrimp.

Nauplii hatched in deep water were fed in two different

TABLE 7:2. MORTALITY RECORD OF *HOMARUS AMERICANUS* JUVENILES

IDENTIFICATION NUMBER	DATE OF DEATH	CARAPACE LENGTH (mm)
C-23	11-05-73	decomposed
C-4	11-18-73	10.1
C-3	01-08-74	decomposed
C-12	01-10-74	decomposed
C-15	06-01-74	25.0
C-18	07-18-74	22.5
C-9	08-28-74	27.3

ways: (1) daily batches of the diatom *Thalassiosira pseudonana* (3H), and (2) continuous flow of the diatom *Chaetocerus curvisetus* (STX-167). The brine shrimp were grown in outdoor, unshaded, aerated 30-gallon tanks; the diatoms were grown in continuous outdoor cultures of 550 to 12,000 gal. volume. The brine shrimp grew very well on the two algal diets and were copulating by day 35. We did not attempt to rear successive generations on the same algal diets.

It became obvious during the course of the experiment that batch-feeding required too much handling (sieving) of the animals; toward the end, the feeding activity of the animals was so great that two feedings per day were necessary to keep diatoms available to the animals at all times. These problems were not encountered with the continuous flow system, where the flow could be easily adjusted to suit the food requirements of the animals. However, flow rates were difficult to regulate using gravity flow, and the overflow from the brine shrimp tank became clogged very easily.

The experience gained in this preliminary experiment will be useful in designing a semi-automated system for growing brine shrimp.

8 SEAWEED CULTURE

8.1 *Hypnea musciformis*

We tested the possibility of using the carrageenin-producing red alga, *Hypnea musciformis*, to remove waste nutrients from shellfish-rearing tanks in the St. Croix mariculture system. Species of *Hypnea* are harvested commercially, but the supply in nature is never abundant, therefore the genus is not a major source of carrageenin (H. Norve, Genue Products, Halifax, Nova Scotia, personal communication).

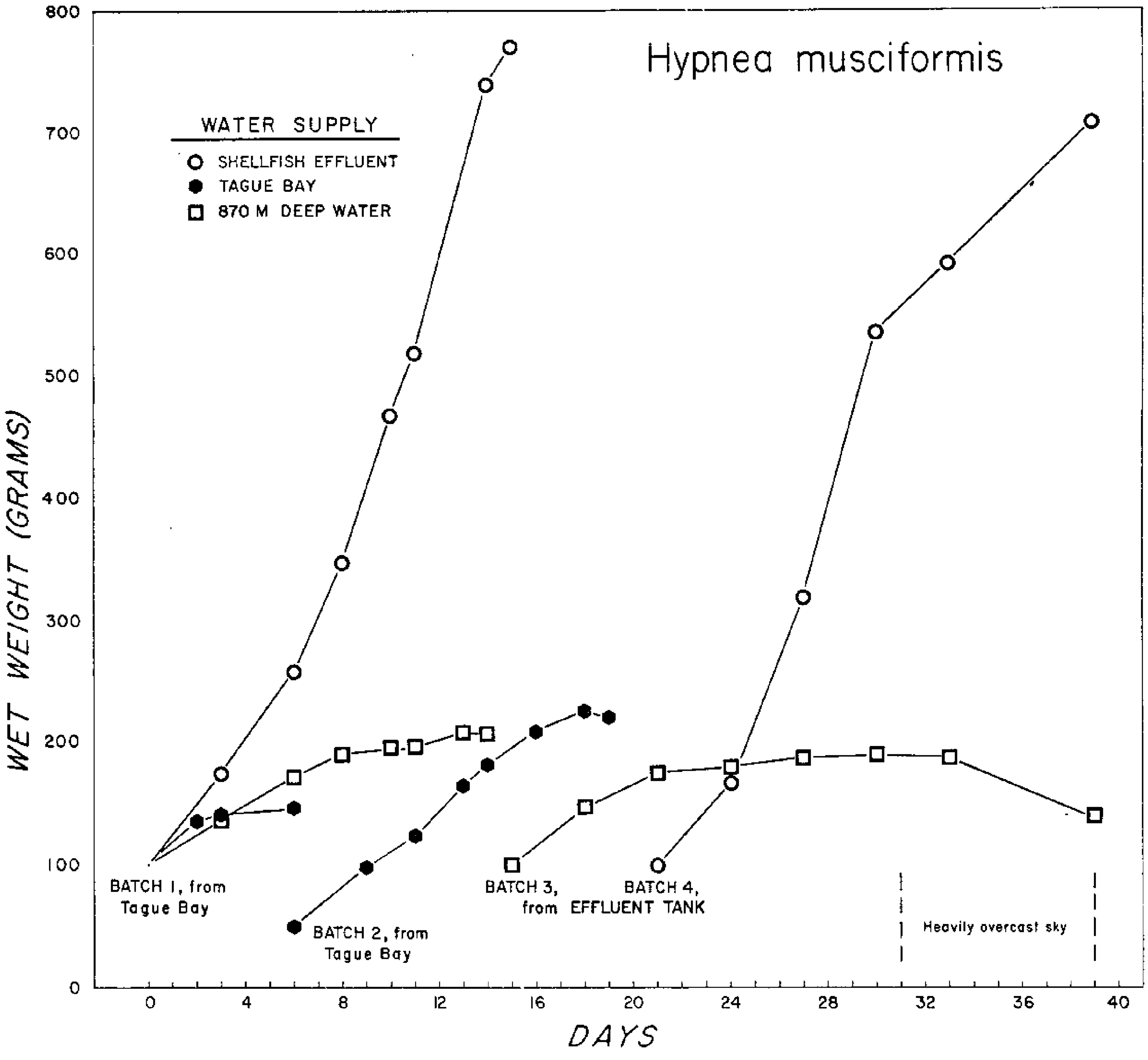
Growth of *Hypnea* in the effluent from a shellfish tank was compared with growth in 870-m deep water and with growth in surface water from Tague Bay. The alga was collected from 1 to 2-ft depth near the Fairleigh Dickinson University dock on Tague Bay. The 36-liter aerated tanks were supplied with 2.4 liters of water per minute, to give a turnover rate of 96 times per day; this high flow rate was chosen because we did not want growth of the alga to become nutrient-limited. The aeration was adjusted to keep the mass of algae constantly rolling in the tanks. The "shellfish effluent tank" received the flow from a tank in which juvenile clams (*Tapes semidecussata*) were being reared on a diet of three species of diatoms grown in deep water.

The deep-water tank received untreated 870-m deep water. Temperatures and flow rates were checked twice daily, at 0800 and 1400 hr. Growth was measured as the increase in wet weight of the alga. Mrs. Nancy Ogden kindly made the measurements of growth in the tank receiving Tague Bay water, at the Fairleigh Dickinson West Indies laboratory. Temperature variation could not be controlled and ranged from 23.2 to 30.0°C; the average tank temperatures were: effluent, 26.1°C; deep water, 24.3°C; Tague Bay water, 29.0°C. A few water samples for dissolved nutrient analyses (nitrate, nitrite, ammonia, and phosphate) were collected in mid-afternoon from the water supplies to the tanks and from their effluent streams.

Carrageenin analyses of plants raised in the tanks receiving the three different water supplies are underway (courtesy of Genue Products, Copenhagen).

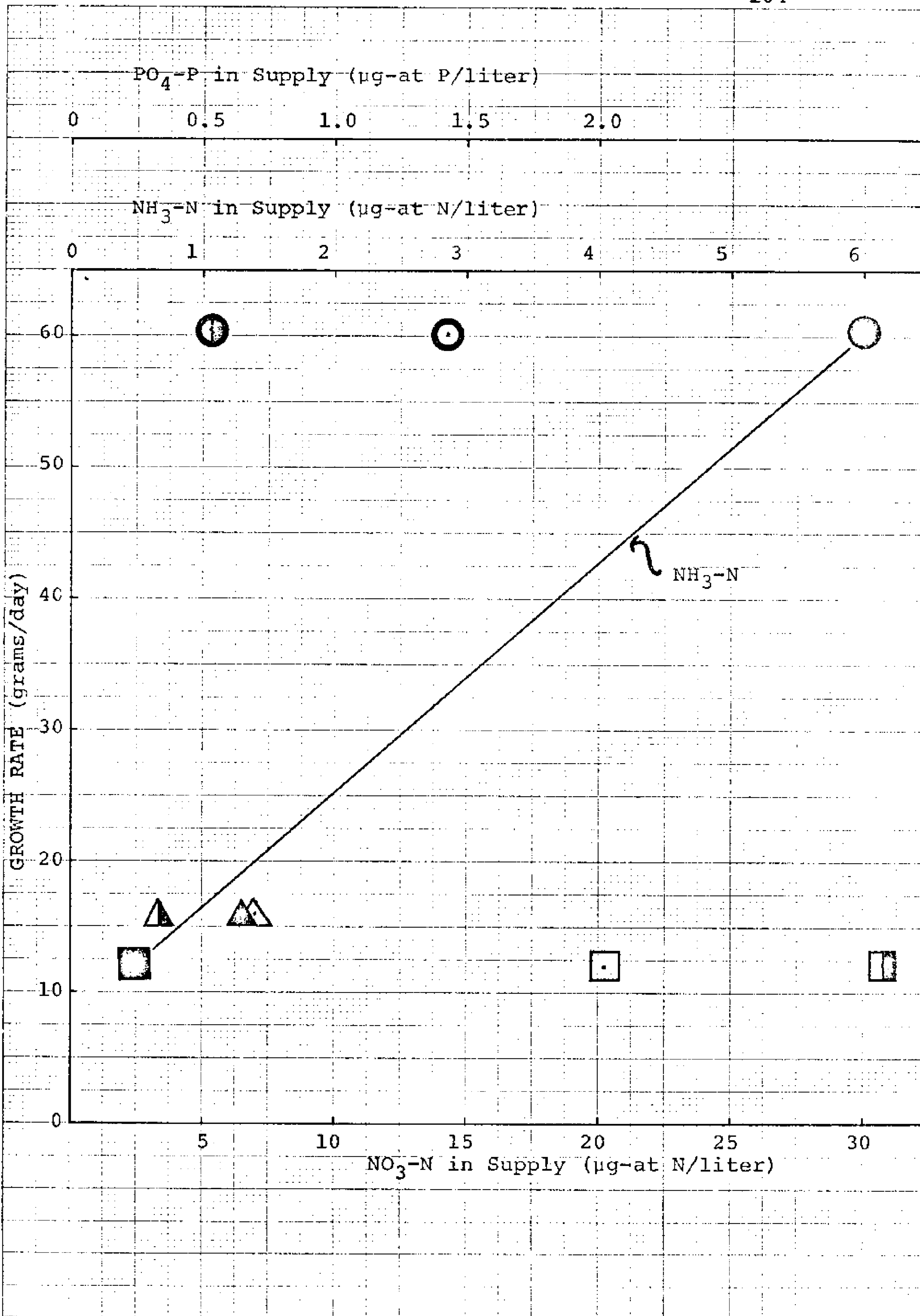
Growth of Hypnea in shellfish-tank effluent was much faster than in deep water or Tague Bay water (Fig. 8:1). The mean maximum growth rates observed (calculated from the steepest slopes of the growth curves in Fig. 8:1) were 60.2, 16.1 and 12.2 grams of the alga per day in the effluent, Tague Bay, and deep-water tanks, respectively. The growth rates were proportional to the mean ammonia concentrations in the water supplied to the three tanks, but there was no correlation between growth rate and nitrate or phosphate concentration (Fig. 8:2). This suggests that

Figure 8:1. Growth of *Hypnea musciformis* in effluent from a shellfish tank, in 870-m deep water, and in Tague Bay water.



Hypnea musciformis
WATER SUPPLY
SHELLFISH EFFLUENT
TAGUE BAY
870 M DEEP WATER
BATCH 1, from Tague Bay
BATCH 2, from Tague Bay
BATCH 3, from EFFLUENT TANK
BATCH 4,
Heavily overcast sky
WET WEIGHT (GRAMS)
0
100
200
300
400
500
600
700
800
DAYS
0
4
8
12
16
20
24
28
32
36
40

Figure 8:2. Plot of *Hypnea musciformis* growth rates vs. concentrations of ammonia, nitrate and phosphate in the water supplies to the three tanks.
Key: Circles, effluent tank; triangles, Tague Bay tank; squares, deep-water tank; closed symbol, ammonia; half-closed symbol, nitrate; open symbol, phosphate.
The values plotted are means.



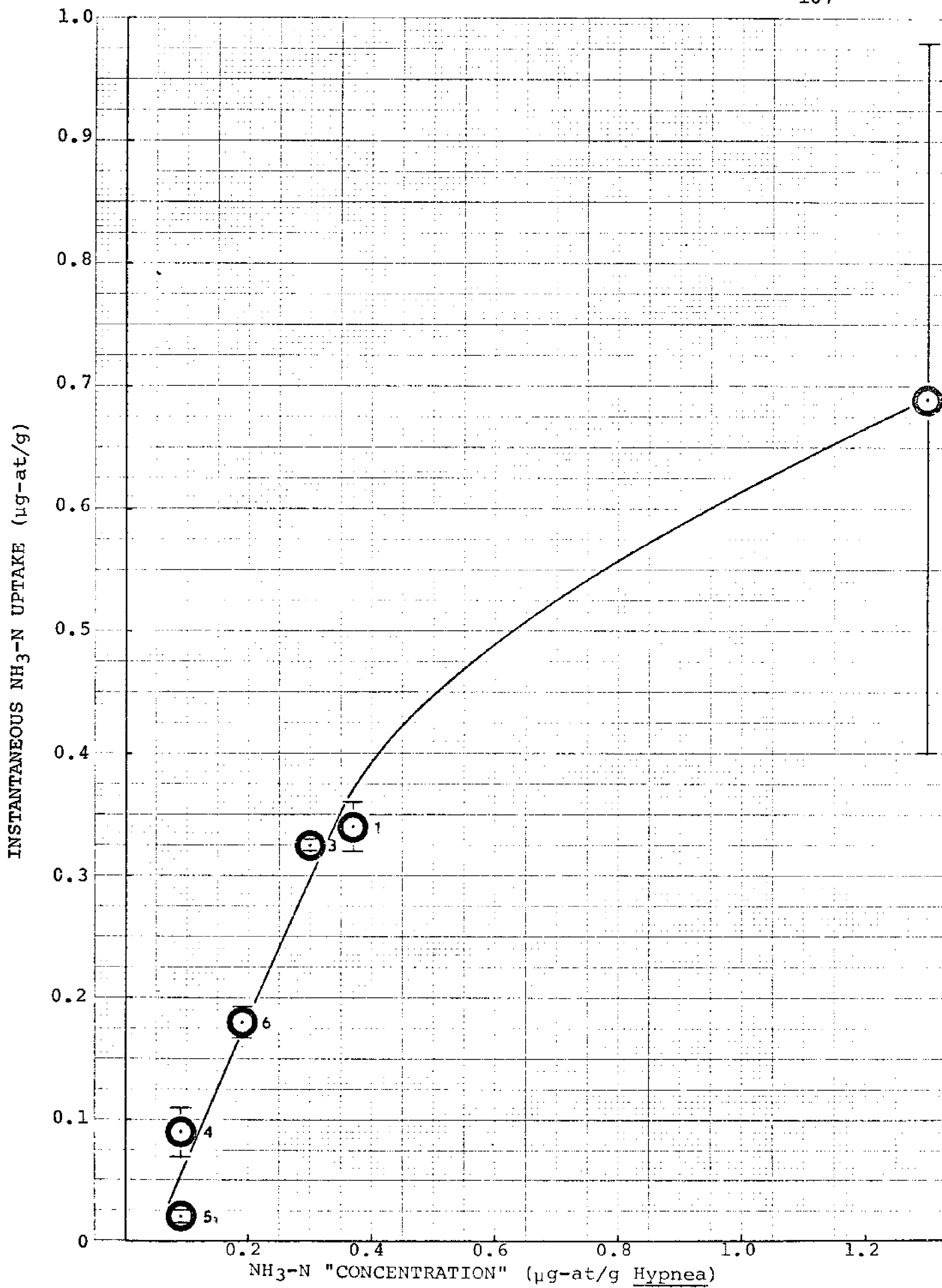
PO_4-P in Supply (μg-at P/liter)
0
0.5
1.0
1.5
2.0
NH_3-N in Supply (μg-at N/liter)
0
1
2
3
4
5
6
GROWTH RATE (grams/day)
60
50
40
30
20
10
0
NH_3-N
5
10
15
20
25
30
NO_3-N in Supply (μg-at N/liter)

the lower growth rates in deep water and in Tague Bay water were due to ammonia limitation. In addition, the decreases in weight of the _Hypnea_ in the deep-water and Tague Bay tanks after reaching a maximum at about 200 grams were accompanied by bleaching and disintegration of the alga; this indicated that the nutrient supply was not adequate to maintain the alga in a healthy condition.

The instantaneous ammonia uptake per unit weight of _Hypnea_ in the effluent tank was directly related to the ambient ammonia "concentration," defined as the total amount of ammonia in the tank divided by the weight of _Hypnea_ in the tank (Fig. 8:3). The maximum instantaneous uptake efficiency (_ca_ 50% uptake) was observed at an ammonia "concentration" of 0.2 to 0.4 μg-at NH_3-N per gram of _Hypnea_ (Fig. 8:4). The decreased uptake at the highest ammonia concentration may have been caused by the turnover rate being too fast for maximum uptake at that concentration.

Enrichment experiments are planned to verify the suggestion that ammonia concentration limits growth of _Hypnea_ in deep water, rather than some other factor, such as insufficient chelated trace metals or vitamins. Other experiments will test for optimum turnover time and depth in the tanks for maximum seaweed growth.

Figure 8:3. Relationship between instantaneous ammonia uptake by *Hypnea musciformis* and ammonia concentration in the tank receiving shell-fish effluent. The values plotted are means; the bars indicate the ranges. The ranges for ammonia concentration fall within the line symbols. The numbers next to the plotted values indicate the sequence in which the values were obtained on different days during the growth of the alga.



INSTANTANEOUS NH3-N UPTAKE (μg-at/g)
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
1
3
6
4
5
0.2
0.4
0.6
0.8
1.0
1.2
NH3-N "CONCENTRATION" (μg-at/g Hypnea)

Figure 8:4. Instantaneous ammonia uptake efficiency for *Hypnea musciformis* at different ammonia concentrations in the tank receiving shellfish effluent. (Values plotted as for Fig. 8:3.)

46 1470

8.1.1 Value of *Hypnea musciformis* for Commercial-Scale Seaweed Farming

The growth rate of *Hypnea musciformis* in the effluent from a shellfish tank under the conditions reported in section 8.1 averaged 13.5 grams dry weight per day in a 1.47 ft^2 tank. Extrapolated to a commercial-scale operation, this yearly production is equal to 1.46×10^8 grams dry weight per acre per year, or 160.7 tons dry weight per acre per year. This production is much higher than the projected 77 tons dry weight per acre per year for *Chondrus crispus* in Nova Scotia (H. Norve, Genue Products, personal communication).

The gross value of commercial-scale *Hypnea musciformis* production, assuming that it is equal in value to that of other carrageenin-producing species ($650 per ton, dry), would be $104,455 per acre per year. Genue Products has projected that for a gross yearly sales of $50,094 per acre for *Chondrus crispus* they could afford pond construction costs of $2-3 per square foot (H. Norve, personal communication). Inasmuch as our projected yield of *H. musciformis* is twice that of *C. crispus*, pond construction costs for growing *H. musciformis* could be considerably higher than $2 to $3 per square foot.

The calculated value and production per unit of pond surface would probably increase as techniques for growing

Hypnea musciformis are optimized. For example, deeper ponds and slower flow rates would lower pond construction and water pumping costs, respectively.

8.2 Other Seaweed Species

Arrangements have been made to ship the agarophyte, *Gracilaria verrucosa*, from North Carolina, and the carrageenin-producer, *Eucheuma isiforme*, from the Florida Keys, with the help of Dr. W.W. Kirby-Smith of Duke University Marine Laboratory, and Dr. C.J. Dawes of the University of South Florida, Tampa.

PART B—TALLMAN ISLAND "EFFLUENT MARICULTURE"

Introduction

A major source of pollution in the Hudson and East Rivers, New York and Raritan Bays, and parts of Long Island Sound near the New York metropolitan area is undoubtedly the treated and untreated sewage released daily into these waters.

Recently, New York City embarked on a program to improve the treatment of the approximately 1.5 billion gallons of sewage discharged daily into the surrounding estuarine waters. New treatment facilities are being constructed to handle the 560 million gallons per day that are now discharged raw, or with only primary treatment, while many of the secondary treatment plants presently in operation are being upgraded to produce effluents of greater clarity and lower biological oxygen demand. As a result of this program, there will be an improvement in the transparency of the estuarine receiving waters resulting from the reduction in suspended solids discharged into the waters surrounding the city. However, the overall nutrient load discharged in the sewage will remain essentially unchanged. High nutrient concentration, coupled with greater light penetration, may result in the development of eutrophication.

However, the potentially harmful or eutrophic nutrients of this sewage effluent are also potentially very beneficial

as a source of nutrients for food or protein production. The quality of treated water can be immensely improved through the application of tertiary "effluent mariculture" techniques and aquatic food-chain management. The resulting benefits from such an improvement will be: (1) prevention of eutrophication and reduction of water pollution; (2) production of large quantities of protein (food) which is essentially needed in the world today. We foresee that the shellfish protein produced by recycling of this otherwise wasted resource will be excellent for animal-feed purposes (see section 9). In view of the shortage on the world market of skimmed milk protein, soybean and other sources of protein for animal feed, we foresee that the produce of "effluent mariculture" may largely replace the now scarce and extremely expensive fishmeal; (3) economic benefits through the production of protein, agar, carrageenan, and other possible biological products. These economic benefits should keep the cost of such tertiary treatment of sewage to a minimum.

Our objectives are, therefore, to minimize the eutrophic nature of the sewage discharge by optimizing its useful biological capabilities. These objectives are being achieved in the following phases:

Phase I: In January, 1972, a laboratory-scale effluent mariculture system was set up in the Chlorination Building of the Tallman Island Pollution Control Plant (TIPCP) in

Queens, N.Y. In collaboration with the City of New York, and with the support of the Rockefeller Foundation and a small grant from the New York State Science and Technology Foundation, we have demonstrated the technical feasibility of "effluent mariculture" to strip nutrients from secondarily treated sewage at the Tallman Island Pollution Control Plant prior to its discharge into the East River. The phytoplankton utilize 95 to 100% of the nitrogen and phosphorus contained in the secondary sewage effluent, and by converting these nutrients into cell material, strip the effluent of its nutrient load. Phytoplankton are then filtered from the effluent by filter-feeding shellfish, resulting in a potentially usable protein source. Nitrogenous excretory products of the shellfish (Hammen _et_ _al_., 1966) return some of the previously stripped nutrients to the effluent. Therefore, a final polishing step was provided. Carrageenan- and agar-producing seaweeds (_Chondrus_ _crispus_ and _Gracilaria_ _verrucosa_) were grown in the effluent from the shellfish tanks, thereby stripping most of the remaining nutrients from the effluent and producing another potentially usable end-product. For a detailed report concerning Phase I, see "ARTIFICIAL UPWELLING—PROGRESS REPORT/1973" dated January 31, 1974.

Phase II: In November 1973 the project facilities were relocated from the Chlorination Building to their present site at TIPCP. We are presently working to scale up Phase I

to optimize the system previously developed, to acquire a clear assessment of the efficiency and the cost of operating the system so that it will become possible to make a realistic evaluation of the technical and economic feasibility of Phase III. It is estimated that this phase will take two years, i.e., 1974 and 1975.

Phase III: In Phase III we plan, with public support (New York City Department of Water Resources, New York State Department of Environmental Conservation, and the federal (NSF/RANN) government) to test the technical and economic feasibility of large-scale effluent aquaculture and to determine whether emphasis should be given to tertiary sewage treatment or to nutrient recycling and animal-feed production.

1 ADMINISTRATION

1.1 Present Facilities

The Tallman Island "Effluent Aquaculture" laboratory is located at the Tallman Island Pollution Control Plant in Queens, New York. This site was assigned to us by the New York City Pollution Control Board in August, 1973. The laobratory is located on Powell Cove of the East River, just west of the Whitestone Bridge, near the confluence of the East River and Long Island Sound.

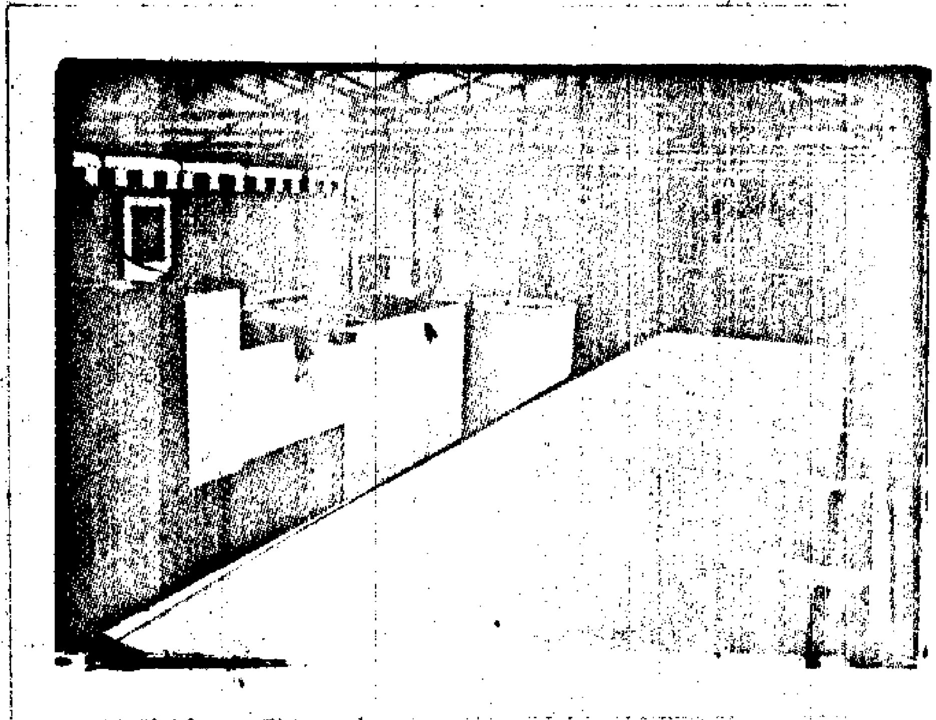
The facilities include three 10 x 50 ft. trailers, provided by the New York City Water Pollution Control Board and a 28 x 48 ft. greenhouse which was constructed by us to contain the mariculture system (Photos 1 and 2). One trailer is attached to the greenhouse and contains a temperature-controlled phytoplankton culturing room, a chemistry laboratory and a log room (Photos 3 and 4). The second trailer contains offices and a conference room, while the third trailer, which lacks electrical or plumbing facilities, serves as a storage area for laboratory equipment and construction materials.

The laboratory trailer is fully equipped with the following facilities for phytoplankton culturing and maintenance, chemical analyses, and monitoring of environmental parameters: Lab-Con-Co. UV transfer hood, Wild phase contrast microscope, Bausch and Lomb dissecting microscope,

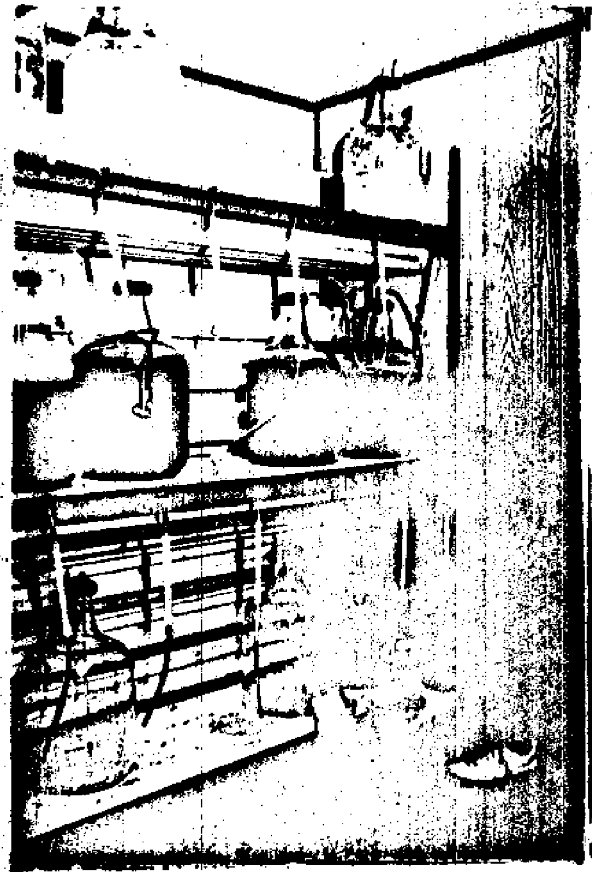
Photos 1 through 4. Exterior (1) and interior (2) views of the new "greenhouse" facility constructed at our Tallman Island project. A trailer is attached to the greenhouse and contains (3 and 4) temperature-controlled phytoplankton culturing room, chemistry laboratory and log room.

1

2



3



4

shelving units equipped with banks of fluorescent lights (Photo 3), Millipore vacuum pump and filtration apparatus (Photo 4), two torsion balances, Right-a-Weight analytical balance, New Brunswick Scientific electric autoclave, Blue M electric standard convection oven, International light plant growth photometer, A.O. Instrument refractometer, Beckman pH meter, Y.S.I. oxygen meter. The laboratory trailer is also equipped with facilities for pumping East River water directly into the lab.

The greenhouse is equipped with the following facilities for effluent mariculture (Photo 2): a Clow Corporation extended aeration secondary treatment plant (STP) having an output capacity of 1,500 gallons/day, a Vanton, Flex-E-Liner pump used to bring raw sewage into the STP, two 1-1/2-hp Conde air compressors which supply all of the air required for the STP, phytoplankton, shellfish and seaweed tanks, eight 500-gallon capacity rectangular concrete tanks (five to be used for the culturing of phytoplankton and three for seaweed), two 500-gallon capacity polytanks for storage of East River water, an I.T.T. Marlow pump used to bring river water into the polytanks (with accompanying polytank float switch and electric timer set to the tide cycles), a Sethco twelve cartridge 15μ filter for filtration of East River water prior to storage. In addition to these existing facilities, the following are being installed: two additional 500-gallon polytanks for storage of East River

water, a 9-1/2' x 3' x 5' high shellfish rack consisting of five shelves that can accommodate 80 removable trays for cultivation of shellfish, a wet table with fresh water supply for inspection and cleaning of shellfish, 8 constant-level reservoirs, one of which will contain a supply of secondarily treated effluent, another will contain river water (both to gravity-feed into the phytoplankton reactors), five of which will contain the effluent from each of five phytoplankton reactors to gravity-feed into the shellfish trays, and the eighth will contain the effluent from the shellfish trays to gravity-feed into three seaweed reactors.

1.2 <u>Staffing : 1974</u>

Oswald A. Roels, Ph.D.
Principal Investigator

Orville Terry, Ph.D. (collaborator)
Seaweeds

Gerald L. Hamm, M.S.
Station Manager; Shellfish

Virginia Harris, M.A.
Doctoral Candidate; Phytoplankton, Water Chemistry

Gregg E. Rice, Ph.D.
Plant Physiology, Phytoplankton

Dwayne Maxwell, Ph.D. (collaborator)
Phytoplankton

Ludo Van Hemelrijck, MEE
Consulting Engineer

Tom Dorsey, Ph.D.
Postdoctoral Fellow; Water Chemistry

Mark Brenner, B.A.
Shellfish

Paul McDonald, B.A.
Analytical Chemistry

Frederick L. England, Jr., BE
Engineering

David C. Boardman, B.S.
Graduate Student

Rory Cavalieri, B.S.
Graduate Student

Jay Rodriguez
Undergraduate Student

In addition, the New York City Department of Water Resources, which operates the Tallman Island Pollution Control Plant, contributes its support through consultations concerning aspects of engineering and water analysis.

2 ENGINEERING

2.1 Present Layout

Planning for Phase II facilities and operations began in May 1973. Site assignment, facilities and service aids to be furnished by the New York City Water Pollution Control Board were secured. In August 1973, two 10 x 50 ft trailers provided by the NYC Pollution Control Board were placed in their present location and utility and service facilities were installed. A third trailer, which lacks utility and service facilities, was added in October 1974.

Detailed "greenhouse" construction plans were finalized in November 1973 and sent to contractors for bidding. Actual construction was begun in April 1974 and completed in August 1974. The greenhouse (Photo 1) is 28 x 48 ft with a peak height of 16 ft. The framework is constructed of 2-1/2" galvanized steel pipe that is welded and braced together. The structure adjoins the laboratory trailer, forming one wall of the greenhouse. The remaining walls and roof are constructed of sheets of corrugated fiberglas which transmits 70% of incident light. There are two 10 x 10 ft removable panels at each end of the greenhouse to permit passage of large equipment. The greenhouse is fully equipped with the facilities and associated plumbing necessary for effluent mariculture (see section 1.1). The floor is covered with gravel, enabling easy access to under-

ground plumbing. During winter months, the greenhouse will be heated with fuel oil units; during summer, an automatically operated fan-jet ventillation system prevents build-up of heat.

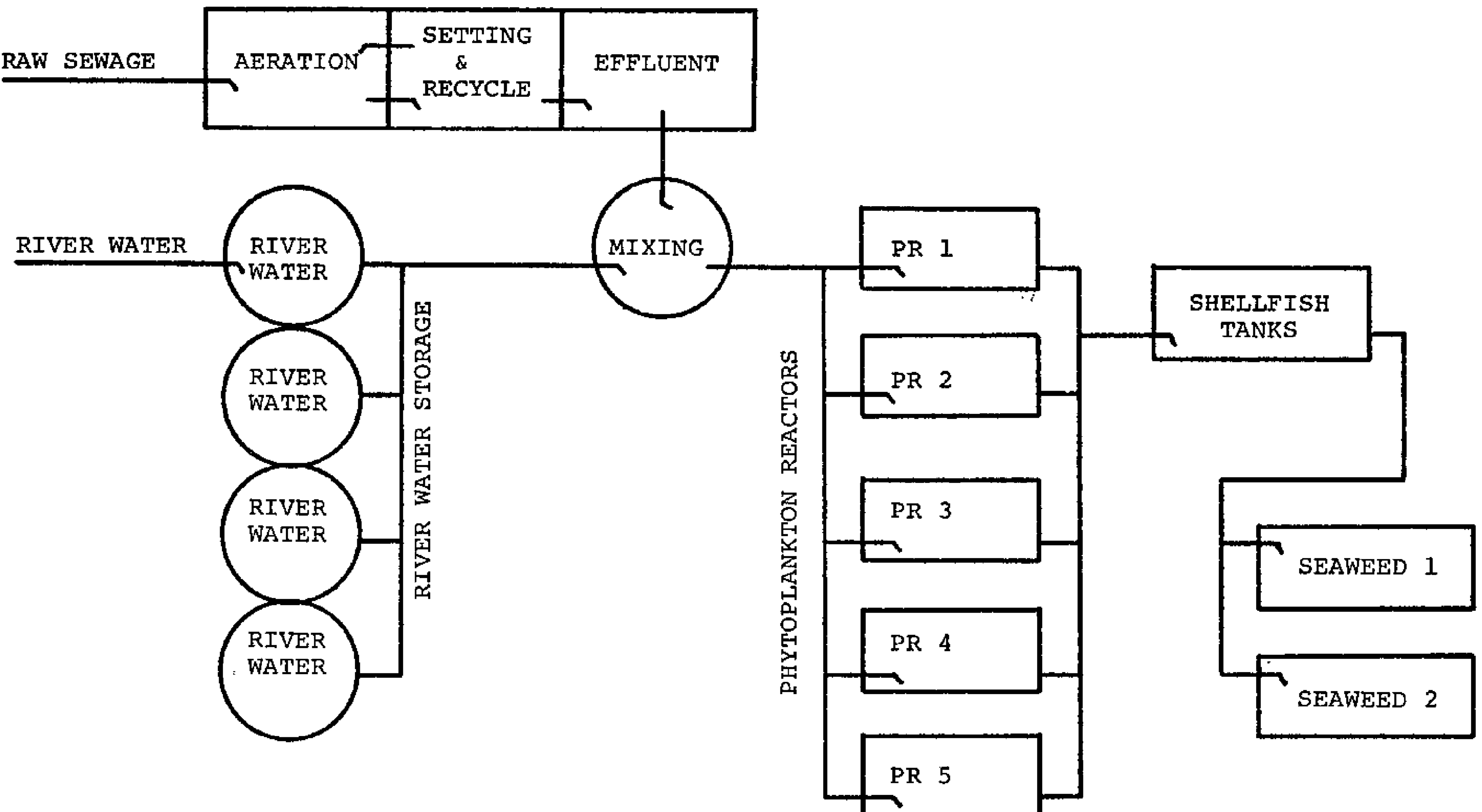
The adjoining laboratory trailer is fully equipped with the facilities necessary for phytoplankton culturing and chemical analyses (see section 1.1).

2.2 Flow Engineering

The continuous flow effluent aquaculture system will have the capacity to process up to 1,500 gallons/day of sewage. A schematic diagram of the flow within the aquaculture system is shown in Figure 2:1.

Untreated sewage is intercepted at Manhole No. 3, the main inlet of the Tallman Island Pollution Control Plant, 750 ft from the aquaculture site. A six-inch by 18-ft long piece of perforated PVC pipe serves as a filter at the manhole intercept to prevent large particles from entering and clogging the one-inch PVC suction pipe which carries sewage to the aquaculture system. The raw sewage flows continuously into the aeration compartment, the first of three compartments of the Clow secondary treatment plant (STP) where the sewage is aerated in contact with activated sludge. From here it enters the settling tank in which the activated sludge settles out (to be recycled to the aeration The secondarily treated effluent enters the final effluent holding tank from which it is distributed to the aquaculture system.

Figure 2:1. Flow diagram of the Tallman Island aquaculture system.


SECONDARY TREATMENT PLANT
RAW SEWAGE
AERATION
SETTING & RECYCLE
EFFLUENT
RIVER WATER
RIVER WATER
RIVER WATER
RIVER WATER
RIVER WATER
RIVER WATER STORAGE
MIXING
PHYTOPLANKTON REACTORS
PR 1
PR 2
PR 3
PR 4
PR 5
SHELLFISH TANKS
SEAWEED 1
SEAWEED 2

The secondarily treated effluent is pumped from the effluent holding tank into an overhead constant-level tank from which it is gravity-fed into five 500-gallon phytoplankton reactors. The flow rate of effluent entering the phytoplankton reactors is regulated by capillary tubing of different diameters.

River water is pumped at high tide into 500-gallon reservoirs from which it is distributed to the aquaculture system. The river water pump is housed in a separate pump-house located alongside the Powell Cove bulkhead. The pumping system is fully automated, containing a float switch in the river water reservoirs and an electric timer that is set to the tide cycles. The river water is passed through a Sethco filtering system, containing twelve 15μ cartridge filters, before reaching the storage facility. The river water is then pumped from the 500-gallon reservoirs into an overhead constant-level tank, from which it is gravity-fed into five 500-gallon phytoplankton reactors. The flow rate of river water entering the reactors is controlled by capillary tubing of different diameters.

Mixing of the river water and secondarily treated effluent occurs in the phytoplankton reactors. In Figure 2:1 mixing is shown to take place prior to entering the phytoplankton tanks merely to simplify the representation.

The algal cultures growing in each of the give phyto-plankton reactors is pumped into five overhead constant-

level tanks from which they are gravity-fed into the shellfish tanks. Again, flow is controlled by means of different diameter capillary tubing.

The effluent from the shellfish tanks is pumped into an overhead constant-level tank from which it is gravity-fed into the seaweed tanks. Flow is controlled by capillary tubing. Finally, the effluent from the seaweed tanks is released into Powells Cove of the East River to complete the cycle of tertiary sewage treatment.

3 CHEMISTRY

3.1 East River Water and Effluent Chemistry

Our effluent aquaculture system seeks to provide tertiary treatment for sewage by a biological method prior to its discharge into the estuary: nutrients are stripped from secondarily treated sewage by phytoplankton, which—in turn—are filtered from the effluent by shellfish. Shellfish excretory products, and any remaining nutrients from the secondary effluent are stripped by racks of agar- and carrageenan-producing fixed algae.

It is obviously of the utmost importance to document the nature and magnitude of the nutrient loads carried by secondarily treated sewage and by receiving waters and to optimize their utilization in our aquaculture system. During Phase I of our project, weekly measurements were made of the nutrient levels in the secondarily treated effluent from TIPCP and of those prevailing in the East River from November 1972 to October 1973 (see "ARTIFICIAL UPWELLING: PROGRESS 1973," Part B).

In April 1973 a massive program of reconstruction and upgrading was begun at the TIPCP and the sewage treatment processing was switched from secondary to primary treatment. At this time we started to operate a small-scale secondary treatment system to supply the laboratory's needs. Weekly sampling was therefore extended to include both TIPCP's

primary effluent and our own secondary effluent (see "ARTIFICIAL UPWELLING: PROGRESS 1973," Part B for data).

Reconstruction of the Chlorination Building at TIPCP, which contained the Phase I effluent aquaculture facilities, was begun in November 1973, forcing a relocation of the project's facilities to the present Phase II location (see section 1.1). However, we have continued to monitor nutrient levels of the TIPCP primary effluent and of East River water on a weekly basis from October 1973 to present and the results of this study are reported in this section.

In October 1974 the Phase II secondary treatment plant was put into operation. The influent and effluent of this plant will be chemically analyzed regularly for nutrient content as soon as the plant is running efficiently (see section 3.2).

3.1.1 Materials and Methods

River water and effluent samples were analyzed for levels of nitrate, nitrite, ammonia, orthophosphate, and silicate. River water samples were filtered through a Gelman glass fiber filter (0.3μ mesh) and the filtrate was stored in both glass and polyethylene bottles. The samples in glass bottles were stored at 5°C; samples in polyethylene bottles were frozen at -10°C. Nitrate, ammonia, and orthophosphate analysis was done on the samples stored in glass; nitrite and silicate analysis was done on the frozen samples. Effluent samples were filtered in the same way and then diluted 1:9 with glass-fiber filtered glass

distilled water before bottling, to bring the nutrient concentrations into the range for the analytical techniques used.

Water samples were analyzed using an AutoAnalyzer II, according to standard Technicon procedures. Nitrite is determined by treating the sample with a solution of sulfanilamide and the diazonium ion is coupled with N-(1-naphthyl)-ethylenediamine to give a pink azo dye. Nitrate is reduced to nitrite by passing the sample through a column of copperized cadmium filings. Nitrite is determined as above. Ammonia is determined by treating the sample in alkaline citrate with sodium hypochlorite and phenol to produce a blue indophenol dye. Orthophosphate and silicate are determined by reaction with ammonium molybdate followed by appropriate reduction to form phosphomolybdenum blue and a silicomolybdenum complex. Interferences between the two are avoided by the use of appropriate complexing agents and specific reducing agents.

3.1.2 Results and Discussion

Mean values and ranges for nitrate, nitrite, ammonia, orthophosphate, and silicate concentrations in primary effluent and East River water over a sampling period from October 1973 to October 1974 are presented in Table 3:1.

Nitrate and nitrite are present in both primary effluent and river water at comparable concentrations. Ammonia, orthophosphate, and silicate concentrations in the effluent are, respectively, 40, 20, and 5 times the concentrations in river water.

TABLE 3:1. RANGES AND MEAN VALUES
(in micro-equivalents/liter) FOR WEEKLY NUTRIENT
SAMPLING AT THE TALLMAN ISLAND POLLUTION CONTROL PLANT
QUEENS, NEW YORK CITY

IN PRIMARILY TREATED EFFLUENT:

Nutrient	Mean[1]	Range[2]	Period
Nitrate	37	5-125	10/18/73
Nitrite	10	2-55	to
Ammonia	1537	212-6720	10/24/74
Orthophosphate	95	60-388	
Silicate	126	8-508	

IN EAST RIVER WATER:

Nutrient	Mean[1]	Range[2]	Period
Nitrate	31	8-107	10/18/73
Nitrite	6	1-42	to
Ammonia	37	1-103	10/24/74
Orthophosphate	5	3-9	
Silicate	23	7-37	

[1] Mean of nutrient values is the arithmetic average of all values recorded for a nutrient over a particular sampling period.

[2] Range of nutrient values represents the single highest, and the single lowest value recorded over a particular sampling period.

3.2 Evaluation of our Secondary Treatment System

Regulation and evaluation of the secondary treatment plant (STP) is achieved by using several tests: visual and olfactory inspections are done daily along with suspended solids, settleable solids, pH and dissolved oxygen determinations. These results are used for day-to-day regulation of the plant. Weekly chemical oxygen demand (COD) and monthly biochemical oxygen demand (BOD) tests are also being done to evaluate the quality of the secondary treatment. These tests will enable us to compare the functioning of our secondary treatment plant with the City's pollution control standards.

The secondary treatment plant has been operating and monitored since October 1974. In November 1974 it was necessary to drain and clean the STP due to the development of a septic condition. An adjustment in the rate of aeration has since corrected this problem. Several problems with the sewage influent filtering and pumping systems have also had to be corrected (see section 2.2). These engineering problems are reflected in the results of the chemical analyses that have been made, which indicate that the STP is not yet functioning at peak efficiency.

3.2.1 Visual & Olfactory Inspection

A daily visual and olfactory inspection of the STP effluent is done to determine if it is clear and free of solids and odor. At this time the mechanical

operation of the STP is checked to ensure proper functioning of the sewage pump, air pump, sludge recycling apparatus and effluent weir.

3.2.2 Suspended Solids

Samples for the determination of suspended solids were taken daily from four locations in the STP: influent, aeration compartment, sludge return pipe, and effluent. The samples were individually filtered through pre-dried, pre-weighed glass-fiber filters. The filters were dried again and weighed to determine the weight of suspended solids. A sample of 50 ml was usually sufficient to obtain significant values in mg/l. Occasionally, it was necessary to filter as much as 400 ml, especially when dealing with relatively pure effluent.

Sixteen suspended solids determinations were done between November 13 and December 6, 1974. The mean percentage removal of suspended solids over this period has been 73% with the range of 26% to 90%.

3.2.3 Settleable Solids

The daily sample for settleable solids is taken from the aeration compartment of the STP according to the following procedure: 500 ml of the mixed liquor of the aeration compartment are collected and immediately transferred to a 500-ml graduated cylinder. The cylinder is set aside for 30 minutes after which the volume occupied by the settled solids is read. This volume, divided by five, is the percentage of settleable solids.

Thirty-four settleable solids tests were done between October 9, 1974 and December 6, 1974. The percentage of settleable solids has continuously been ≤ 1. This low percentage is due to the discontinuous operation of the sewage pump. This problem has recently been corrected and now that the sewage pump is operating on a continuous basis, the sludge will rapidly build up and this build-up will be reflected in an increase in percentage of settleable solids.

3.2.4 Dissolved Oxygen and pH

Dissolved oxygen and pH are measured daily in four areas of the STP: influent, aeration compartment, settling tank and effluent. Dissolved oxygen is measured in parts per million, using a YSI Model 51A oxygen meter. pH is measured using a Beckman Model 72 pH meter.

During the period November 13 to December 6, 1974, dissolved oxygen in the aeration compartment had a mean value of 9.1 ppm with a range of 8.6 to 10.8 ppm. During the period October 9 to December 6, 1974, mean values for pH in the influent, aeration compartment, and effluent were, respectively, 7.3, 7.3 and 7.4.

3.2.5 Chemical Oxygen Demand

COD is determined according to the Jeris method, which is a rapid and satisfactory test. The procedure for this method follows:

(1) Add approximately 0.3 g of mercuric sulfate powder to a 500-ml Erlenmeyer flask.

(2) Pipet 5.0 ml of the sample into the flask.

(3) Blanks are run by substituting 5.0 ml of distilled water for the sample and continuing with steps 4 through 7.

(4) Swirl each sample for approximately 15 seconds and then add 25.0 ml of the dichromate acid solution (automatic pipet).

(5) Swirl each sample with the acid solution. Handling the flask by the neck, place it on a preheated hotplate and heat to 165±1°C (temperature is critical). A thermometer should be placed in the flask and frequent swirling should be employed. The temperature is read by tilting the flask so that the bulb of the thermometer is submersed (be careful not to exceed the temperature range and discard the sample if you do).

(6) Cautiously add approximately 300 ml of distilled water and place in water-bath to cool to ambient temperature.

(7) After cooling, add 5 drops of Ferroin indicator solution (1.10-phenanthroline ferrous sulfate) and titrate with ferrous ammonium sulfate.

(8) Standards are run by adding 25.0 ml of the dichromate standard and 20 ml of concentrated sulfuric acid to a 500-ml Erlenmeyer flask and continuing with steps 6 and 7.

(9) Calculations are as follows:

$$N = \frac{(0.050)(25)}{\text{average of step 8}}$$

where N = normality of the ferrous ammonium sulfate

$$\text{COD (mg/l)} = \frac{N \times 800 \times (\text{avg. blanks} - \text{avg. sample})}{\text{volume of sample}}$$

(10) The chemicals used are:

(a) Dichromate acid solution, 0.05N

5 grams $K_2Cr_2O_7$(dry for 2 hr, 103°C)

20 grams Ag_2SO_4

1 liter of concentrated H_2SO_4

1 liter of concentrated H_3PO_4

(b) Ferrous ammonium sulfate, 0.05N

20 grams $Fe(NH_4)_2(SO_4)_2 \cdot 6H_2O$) dissolve in
) distilled
5 milliliters conc. H_2SO_4) water

Add distilled water to make 1.00 liter

(c) Dichromate standard, 0.050N

2.4518 grams $K_2Cr_2O_7$ primary standard grade (dry for 2 hr, 103°C)

Dilute to 1.00 liter with distilled water.

Five COD tests were run between October 9 and December 6, 1974. The mean percentage removal of COD was 65.5 with a range from 50.4 to 74.8%.

3.2.6 Biochemical Oxygen Demand

The biochemical oxygen demand is determined according to the standard five-day BOD method outlined in the 12th edition of Standard Methods for the Examination of Water and Wastewater, pages 415-420.

A single BOD determination was made on November 27, 1974 which showed a 81.4% removal of BOD.

3.3 Evaluation of our Aquaculture System

When the Tallman Island aquaculture system is fully operable it will be necessary to sample for nutrient concentrations at each level of the system. Nitrate, nitrite, ammonia, orthophosphate and silicate concentrations will be determined. Weekly measurements of these nutrients in the influent and effluent of the secondary treatment plant and in the East River water will serve as a guide to quality control of the water used for aquaculture purposes. This information is needed to regulate the ratio of sewage : river water mixtures (and hence nutrient concentrations) that are found to support the best phytoplankton growth. The best ratio of sewage : river water will be determined by growth rate studies for each phytoplankton species in different sewage-river water mixtures.

Weekly measurements of nutrient concentrations will also be made in the influent and effluent of the phytoplankton tanks, shellfish, and seaweed tanks. This data will enable us to calculate the nutrient-stripping efficiencies at each level of the aquaculture system and of the overall system.

In addition to nutrient analysis, it will be necessary to run periodic tests to determine the concentrations of

trace metals in the raw sewage, secondarily treated sewage, East River water, and at each trophic level in the aquaculture system. A preliminary study of trace-metal concentrations in each of these components has been done and the results are reported in section 7.

4 PHYTOPLANKTON STUDIES

After the termination of our successful Phase I aquaculture system at Tallman Island, our phytoplankton work has primarily been limited to laboratory investigation of the growth dynamics of different species of phytoplankton in various media and under various culturing conditions. Our early large-scale continuous culturing of phytoplankton is to be resumed at our new Tallman Island Phase II effluent aquaculture facilities Seven species of phytoplankton (Nitzschia, Skeletonema, Nannochloris, Dunaliella, Cyclotella, Monochrysis, Isochrysis) are being maintained in stock cultures and in semi-continuous carboy cultures. In addition, new species are being screened for incorporation into the Phase II aquaculture program.

4.1 Comparison of the Growth Rates of Five Phytoplankton Species in Four Types of Media

Extensive growth rate studies have been done for each of the five phytoplankton species (Nitzschia, Skeletonema, Dunaliella, Cyclotella, Nannochloris) that were cultured during Phase I of the aquaculture project. The objectives of these studies were to determine growth rates and nutrient-stripping efficiencies of each species when grown in several different sewage-containing media. The four growth media tested were 100% raw sewage, 33% sewage (67% river water), 10% sewage (90% river water), and

f/4 medium (Guillard and Ryther, Can. J. Microbiol. 8:231, 1962) which served as a control for comparisons of quality of growth.

Each of the five phytoplankton species was inoculated into two sets of each of the four types of media contained in Erlenmeyer flasks. Initial cell counts and nutrient concentrations (nitrate, nitrite, ammonia, orthophosphate, and silicate) were determined immediately after inoculation for each of the forty experimental flasks. These flasks were placed over a bank of fluorescent lights and daily cell counts were made from each culture flask for a period of seven days, or until a plateau was reached. The growth rate and the maximum cell density attained by each species in the various media was determined from the cell count data.

At the termination of the experiment, 50 ml of the medium in each flask was filtered through a glass-fiber filter. The filtrate was then analyzed for the remaining nutrient concentrations, while the C and N content of the filtered cells was determined on a Hewlett-Packard CHN analyzer. The nutrient concentration at the beginning and end of the experiment allowed calculation of the particular stripping efficiency while the N content of the cells allowed a N-balance sheet (or comparison between N lost from the medium and that gained by the cells) to be made.

Results of this work showed that none of the five phytoplankton species tested were capable of growing on 100%

sewage. Whether this lack of growth resulted from some toxicity, or from low salinity is not known. The best growth by all five species, both in terms of growth rates cell densities and stripping rates, were attained on the 33% sewage medium and the f/4 control. For all practical purposes, the cell densities attained on the 33% sewage and f/4 media were identical. Likewise, the growth rates and stripping efficiencies within both media were very similar within each species tested. On the other hand, the 10% sewage gave much poorer results with only moderate to poor growth and stripping by all five species. Table 4:1 exemplifies the results of the research for the green alga, *Dunaliella tertiolecta*.

This type of investigation is being extended further to establish the specific sewage concentration most suited to each phytoplankter's growth potential. However, it has been established by this work (and work to be described) that substrates of high sewage concentration can support the growth of specific phytoplankton species very well.

4.2 Selection of Species

We are also presently in the process of isolating and screening those new species of phytoplankton which are most adaptable to our aquaculture system. Those organisms capable of attaining high densities, growth rates, and stripping rates on high concentrations of sewage will optimize the efficiency and output of the system. To this end, we have isolated and are investigating phytoplankton

TABLE 4:1. GROWTH OF *DUNALIELLA TERTIOLECTA* IN FOUR TYPES OF MEDIA

SAMPLE	MEDIUM	GROWTH	FINAL CELL DENSITY (cells/ml)	DOUBLING TIME (hrs.)	INITIAL [N] (μg/ml)	FINAL [N] (μg/ml)	PARTICULATE N/CELL (μg/ml)	FINAL PARTICULATE N + DISSOLVED N (μg/ml)
1	f/4	GOOD	$3x10^6$	9.4	7.1	.082	9.2	9.28
2	f/4	GOOD	$4x10^6$	9.5	7.1	.073	10.2	10.27
3	33% SEWAGE	GOOD	$3.5x10^6$	7.5	9.8	.078	7.07	7.15
4	33% SEWAGE	GOOD	$3.9x10^6$	7.7	9.8	.115	8	8.12
5	10% SEWAGE	POOR	$6.5x10^5$	12.3	2.6	.35	2.55	2.9
6	10% SEWAGE	POOR	$1x10^6$	13.8	2.6	.055	3.14	2.7
7	100% SEWAGE	NONE	-	-	20.3	18.7	-	18.7+
8	100% SEWAGE	NONE	-	-	20.3	19.5	-	19.5+

which grow very well in raw sewage (NH_3 concentration greater than 1200 μeq/liter). Chlorella, Scenedesmus, Ankistrodesmus, Anacystis, Oocystis and several unidentified green flagellates have all been found capable of growing on 100% sewage and are undergoing further testing.

4.3 Comparison of Growth and Cellular Composition of Dunaliella tertiolecta in Ammonia-Containing Versus Nitrate-Containing Media

We are also engaged in a series of laboratory experiments to compare the effects of ammonia and nitrate as sole nitrogen source on the growth and cellular composition of Dunaliella tertiolecta in continuous culture under light intensity-photoperiod combinations representative of an effluent aquaculture system in New York City. This study focuses on the relative advantages of phytoplankton culture in highly, or poorly nitrified secondary sewage effluent, the effects of the seasonal variation in photosynthetically active radiant flux on intracellular carbohydrate, protein, and lipid content, and the effects of photoperiod on diurnal changes in these cellular components.

Predictive mathematical models are being constructed, based on the experimental results, which will allow us to obtain estimates of the applicability of effluent aquaculture tertiary treatment or protein production systems to various area availability, volume throughput systems. The models will also yield estimates of annual shellfish production in

these systems, and permit comparison of continuous and intermittent flow patterns of shellfish feeding.

Preliminary results indicate that under light limiting conditions, cells grown in ammonia medium have U_{max} that are 15 to 30% higher than cells grown in nitrate medium under identical conditions of light intensity and photoperiod. Thus, secondary treatment processes that result in nitrification of the sewage effluent should be avoided if effluent aquaculture is to be used as a method of tertiary treatment. In addition, it has been noted that the low light intensities and short photoperiods characteristic of fall and winter months in New York City result in a decrease in U_{max} and in the intracellular carbohydrate and protein content of *Dunaliella tertiolecta* and an increase in the intracellular lipid content. As a result, the overall caloric production of the phytoplankton phase of an effluent aquaculture system does not change markedly over the year although the carbohydrate:protein:lipid ratio does. Finally, based on experimental carbon fixation data, it appears that carbon limitation will be a major problem in an effluent aquaculture system. Specific applications of these results to particular effluent aquaculture situations in the New York City area must await completion of the mathematical models.

4.4 Growth of Facultative Heterotrophs on Sewage Effluent

We are also initiating work on *Ochromonas malhamensis* and other facultative heterotrophs to determine

if they can grow on high sewage concentrations and if they might not be more advantageous than strict autotrophs to a secondary sewage effluent aquaculture program. Facultative heterotrophs may be helpful in overcoming two inherent problems in the phytoplankton growth in sewage effluent: (a) light is generally limiting to phytoplankton growth and thus allows only slow growth and turnover rates unless huge phytoplankton pool areas are provided as absorption surfaces, and (b) the amount of inorganic carbon in the effluent is insufficient, relative to the N and P concentrations, to allow the rapid stripping of the majority of the inorganic N and P from the system.

Facultative heterotrophs, which can assimilate organic food sources as well as fixing their nutrition requirement photochemically, could obtain part of their carbon requirement from organic sources, thereby making the C/N and C/P ratios within the effluent more biologically acceptable. Likewise, these organisms may have much lower light requirements than the autotrophs, lessening the difficulties associated with light limitations. What must be determined is if facultative heterotrophs are compatible with the system—do they grow well, and does the stripping of inorganic N and P remain high despite any heterotrophic assimilation which might occur.

5 SHELLFISH STUDIES

5.1 Attempt to Spawn *Mytilus edulis*

Following the removal of our original stock of *M. edulis*, 135 new animals were collected on May 13, 1974 at the Crestwood Yacht Club's Whitestone Marina for further growth and spawning studies. These *M. edulis*, maintained in a wet table of filtered river water, were fed one carboy (20 liters) of *Dunaliella* sp. culture, metered over a period of two days by means of a peristaltic pump. Many of these *M. edulis* spawned overnight on the nights of May 13 and 14. An effort was made to save some of these larvae but none lived more than two days. By June 5, 1974, 75 shellfish had died, and the remainder (565 grams) were transferred to the refrigerator and divided into two 10-liter Nalgene pans each containing approximately seven liters of 15-μ filtered river water. Aeration was provided and an attempt to control the temperature at approximately 8°C was started. The water in each pan was changed three times each week and daily feedings of 1 to 1-3/4 liters/pan of high-density *Dunaliella* culture was initiated. On August 7, 1974, another pan was added, containing 35 *M. edulis* (485 g) from the Whitestone Marina, raising the shellfish numbers to 82 animals.

The death rates decreased as soon as the shellfish were placed in the refrigerator and has averaged 1.5 shellfish per week since June 5, 1974, which appears to be a normal rate of attrition. Infrequent examinations showed glycogen accumulation after the first month, and the average mussel

increased in weight from 8.83 g when placed in the refrigerator (June 8, 1974) to 14.24 g on December 6, 1974.

Beginning in August when the mussels began gaining weight a visual inspection showed some gonadal tissue development and glycogen accumulation. This glycogen and gonadal development continued to progress with the discovery of almost completely developed sperm within the gonads of two sacrificed male *M. edulis*. Final preparation for an attempted spawning of the cultured *M. edulis* are now being made.

Conclusions

It was concluded from the shellfish spawning programs at the Tallman Island aquaculture laboratory that the quality of river water available to us is not sufficient to promote a high rate of larval production and survival. For this reason arrangements were made with the New York State Department of Environmental Conservation to establish a shellfish hatchery at their Flax Pond Marine Research Laboratory in Oldfield, New York (Long Island). At this facility large quantities of unpolluted seawater, essential for success in shellfish spawning are readily available.

5.2 Collection of Brood Stock

Approximately 800 blue and ribbed mussels were recently collected from the Whitestone Marina docks and the mudbanks of Powell Cove near Tallman Island on the East River. These shellfish are maintained in a 500-gallon reactor with a continuous flow of approximately 700 ml/min

of 15-μ filtered river water, containing an average algal cell density of 5 x 10^5 cells/ml. These mussels are being maintained in healthy condition for later transfer to the Flax Pond facility (see section 6). They will be used as brood stock for spawning experiments that will provide a year-round supply of mussel larvae to the Tallman Island aquaculture system.

In addition, arrangements have been made to obtain *Rangia cuneata* from the Virginia Institute of Marine Science. These euryhaline clams will likewise be transferred to the Flax Pond hatchery to be used as brood stock in spawning experiments.

6 FLAX POND SHELLFISH HATCHERY AND SEAWEED PHYSIOLOGY LABORATORY

On October 2, 1974, we received permission from Dr. S. A. Schwartz, Director of Research, New York State Department of Environmental Conservation, to establish our shellfish hatchery and seaweed physiology laboratory at their Flax Pond Marine Research Laboratory, off Shore Road, Oldfield, Long Island, N.Y. We then began implementing plans for the project by installing equipment needed to supplement the facilities already available. Installation of equipment continues with a completely functional shellfish hatchery schedule to begin spawning experiments early in January, 1975.

The objective in establishing a hatchery at Flax Pond is to produce large quantities of experimental plant (macro-algae) and animal (shellfish) stocks on a year-round basis for our managed marine food chain at Tallman Island and for other aquaculture projects. Such aquaculture programs require the periodic removal and hence restocking of the particular commercially valuable organisms. To this end, a hatchery capable of supplying the necessary replacement organisms on a year-round basis will be established at a facility that fulfills the demanding controlled conditions (of water quality, temperature control, and food control) required for spawning and raising larvae or young seaweeds.

In addition to the benefit of determining the ideal conditions for producing certain young shellfish and seaweed in large quantities, other expected benefits from this wetlands laboratory are an increased knowledge of the physiology of reproduction in these seaweeds and shellfish. Application of this increased knowledge will enable us to achieve more frequent and larger harvests of shellfish and seaweed in our aquaculture systems.

7 TRACE ELEMENT ASSAYS

In cooperation with the New York State Department of Environmental Conservation, a preliminary study was done to determine trace element concentrations in East River water, sewage, phytoplankton (grown in different media), and in locally collected shellfish and seaweed. The following reports the methods and results of this study.

7.1 Preparation and Preservation of Samples

Samples were prepared and preserved in the following manner:

Water Samples

1. East River Water: A polyethylene bottle was used to collect a one-liter sample. The bottle was rinsed with distilled water and reagent grade nitric acid three times. The East River water was filtered through a glass-fiber filter, using an all-glass filtering apparatus which had also been thoroughly washed with distilled water and reagent grade nitric acid. The glass-fiber filter had been pre-washed with distilled water and nitric acid. One liter of filtrate was transferred to the pre-washed polyethylene bottle, and 10 ml of reagent grade nitric acid was added as a preservative.

2. Tallman Island Sewage: One liter of sewage was collected, prepared and preserved in the same manner as the East River water; 10 ml of nitric acid (reagent grade) again serving to prevent adhesion.

Phytoplankton

At the same time the East River water sample was being collected, water was taken for culturing of the plankton samples. Six 20-liter carboys were specially prepared by repeated washings with distilled water and reagent grade nitric acid. Pre-washed glass-fiber filters were used to filter the water.

Three species of phytoplankton were cultured: *Nannochloris* sp., *Dunaliella tertiolecta* and *Nitzschia* sp., one each in the normal F/2 medium, and one each in F/2 medium minus the EDTA and trace metals that are normally added to support growth. A total of six bottles were prepared, all of which were autoclaved.

Although we expected considerably less growth in the carboys without the EDTA and trace metals, all carboys proceeded to grow rapidly.

After 10 to 14 days' growth the phytoplankton was centrifuged in a Sorval centrifuge, Head GSA. The pellets were then freeze-dried and placed in airtight containers.

Bottle	EDTA + Trace Metals	Medium	Silicate*	Organisms	Autoclaved (Date)	Centrifuged (Date)
1	No	F/2	Yes	Nitzschia	03/25/74	04/09/74
2	Yes	F/2	Yes	Nitzschia	03/26/74	04/10/74
3	No	F/2	No	Nannochloris	03/26/74	---**
4	Yes	F/2	No	Nannochloris	03/27/74	04/11/74
5	No	F/2	No	Dunaliella	03/28/74	04/15/74
6	Yes	F/2	No	Dunaliella	03/28/74	04/16/74

*Sodium silicate was added to carboys containing Nitzschia because diatoms require this for growth.
**This bottle was accidentally broken by an assistant before it was to be centrifuged.

The formula for the EDTA plus trace metals used in the carboys is:

Primary Stock:

$CuSO_4 \cdot 5H_2O$: weigh out .98 g, dissolve in 100 ml distilled water

$ZnSO_4 \cdot 7H_2O$: weigh out 2.2 g, dissolve in 100 ml "

$CoCl_2 \cdot 6H_2O$: weigh out 1.0 g, dissolve in 100 ml "

$MnCl_2 \cdot 4H_2O$: weigh out 18.0 g, dissolve in 100 ml "

$Na_2MoO_4 \cdot 2H_2O$: weigh out .63 g, dissolve in 100 ml "

Secondary Stock:

EDTA : weigh out 4.36 g, dissolve in 900 ml of distilled water

$FeCl_3$: weigh out 3.15 g, add to EDTA solution

Add one ml of each primary stock to solution

Bring solution to one liter with distilled water.

Thirty ml of secondary stock was added to carboys #2, 4 and 6.

Shellfish (Mussels)

Two species of mussels were collected, *Modiolus demissus* and *Mytilus edulis*. All mussels were scrubbed to remove all superfluous material. The mussels were opened and the meat separated from the shell. All samples were freeze-dried, ground separately in a sintered alumina mortar, and stored in airtight containers.

Seaweeds (Fucus)

One species of seaweed, *Fucus*, was collected from the river, freeze-dried, ground in an acid-rinsed sintered alumina mortar, and stored in an airtight container.

NOTE

In every phase of the sample collection and preparation, great care was taken to avoid any metal contamination. All storage and preparation vessels were rinsed with reagent grade nitric acid. Only rinsed glass and plastic instruments were used in the preparation of samples

LIST OF SAMPLES

East River Water: One bottle containing one liter of river water, plus 10 ml of reagent grade nitric acid.

Sewage : One bottle containing one liter of sewage plus 10 ml of reagent grade nitric acid.

Phytoplankton : Five airtight vials, two *Nitzschia*, two *Dunaliella*, and one *Nannochloris*.

Shellfish : *Mytilus edulis* shells (two vials), 27.0 and 31.1 grams;

Mytilus edulis meat (one vial), 5.5 grams;

Modiolus demissus shells (two vials), 26.0 and 24.0 grams;

Modiolus demissus meat (two vials), 3.5 and 4.0 grams.

Seaweed : *Fucus* (one vial), 15.5 grams.

7.2 Results

The mussel, plankton and water samples were analyzed in the New York State Department of Environmental Conservation's Rome Pollution Laboratory (Rome, N.Y.), with the following results:

Trace Pesticide Analyses

Sample	% oil	o,p DDE	p,p' DDE	o,p DDT	p,p' DDT	PCB
Modiolus	12.64	ND	0.21	0.18	0.17	1.49
Mytilus	22.42	ND	0.47	0.78	0.38	4.33
Fucus	7.28	ND	ND	ND	ND	0.20

All other samples were found to be free of DDT and PCB. DDT results are in ppm, PCB results are in ppm as Aroclor 1254.

Metal Analyses (results in ppm)

Sample	Cd	Cr	Cu	Pb	Zn	Hg
Modiolus (1)	5.78	3.43	33.72	6.03	77.99	0.25
Mytilus (2)	14.51	7.74	18.86	32.27	261.91	--
Fucus	1.19	9.07	36.40	10.86	194.74	0.14
Dunaliella (3)	2.01	3.59	37.85	2.28	256.95	--
Dunaliella (4)	8.22	ND	29.02	4.27	324.20	--
Nitzschia (5)	14.34	17.13	29.38	8.91	265.15	--
Nitzschia (6)	15.96	18.19	38.60	15.05	1034.91	--
Nannochloris (7)	3.86	11.86	20.08	5.01	383.84	--
Modiolus shells	6.91	6.33	13.26	13.32	28.78	--
Mytilus shells	7.71	7.76	9.06	9.17	28.41	--

(1) *Modiolus demissus* flesh: Tallman Island; (2) *Mytilus edulis* flesh: Tallman Island; (3) *Dunaliella tertiolecta* with EDTA and metals; (4) *D. tertiolecta* without EDTA and metals; (5) *Nitzschia* with EDTA and metals; (6) *Nitzschia* without EDTA and metals; (7) *Nannochloris* with EDTA and metals.

Water Samples (results in ppb)

Sample	Cd	Cr	Cu	Pb	Zn	Hg
East River Water	46.8	44.8	56	ND	84	--
Tallman Island Sewage	8.2	ND	40	ND	258	--

There were not enough samples to run mercury and other metals except on the two samples where mercury values are shown.

8 NUTRITIONAL TESTS

In cooperation with the Department of Nutrition of Laval University, Quebec, Canada, a study was done to compare four diets (including Mytilus edulis) for chickens. The salt water mussel, Mytilus edulis, which was locally collected near the Tallman Island aquaculture laboratory, was a constituent of one of the diets. The following reports the methods of preparation of the Mytilus edulis used in one diet and the results comparing the growth rates of chickens receiving the various diets.

M. edulis were collected on May 8, 1974 at the C.Y.O. Whitestone Marina, located on the south shore of the East River, in Queens, N.Y. The mussels were cleaned to remove extraneous matter, towel-dried and weighed. The meat plus liquid was separated from the shell, each fraction was weighed separately, and the percentage of the total fresh weight of each fraction was calculated. Samples of both the meat + liquid and the shell fractions were freeze-dried and weighed and the percentages dry weight were calculated. Sub-samples of the freeze-dried meat and shell fractions were oven-dried at 75°C to a constant weight and then analyzed for total nitrogen content using a Hewlett-Packard CHN analyzer. Based on wet weight, the Mytilus edulis sample consisted of 52.6% meat plus liquid and 47.4% shell.

Nitrogen analysis of a freeze-dried sub-sample of the *Mytilus edulis* used as feed in the nutritional study gave the following results:

SAMPLE	WET WT. (g)	DRY WT. (g)	%DRY WT.	% N	% PROTEIN*	PROTEIN** (g)
Meat	100.0	20.5	20.5	10.23	67.8	13.9
Shells	100.0	54.5	54.5	0.87	5.5	3.0

*% N x 6.25

**Calculation based on dry weight.

The details of these tests are given in a paper prepared for publication (manuscript given here as Appendix X).

SOME EFFECTS OF SHADING ON MARINE PHYTOPLANKTON IN OUTDOOR CONTINUOUS CULTURE:

I. DILUTION RATE OF 0.25/DAY

Preliminary Report
on Dissertation Research
Project

Mary Farmer

August 19, 1974

INTRODUCTION

The long-term purpose of this study is to investigate the interaction between effects of incident light intensity and of rate of nutrient supply on growth of marine phyto-plankton. The approach is to establish one environmental condition as a growth rate-limiting condition and to study the effects of other environmental factors under that condition. Thus, the effect of decreasing the rate of nutrient supply will be examined when light is limiting to growth, and the effect of decreasing the percentage of incident light reaching the cells will be examined when a nutrient (usually nitrogen) is limiting to growth.

The experiments are to be conducted in outdoor continuous culture using large-volume culture vessels, called reactors. Each experiment is to be conducted with all reactors at a single dilution rate and with each reactor at a different degree of incident light intensity.

This preliminary report covers the first experiment described in the thesis proposal (Farmer, 1974). Cultures were run at a dilution rate of 0.25/day for two reasons: (1) to try for a nitrogen-limited condition, even at the lowest percentage of light intensity, and (2) to assure no washout of cells at the lowest percentage of light intensity.

MATERIALS AND METHODS

Reactors: The five reactors were epoxy-lined cement tanks each with a volume capacity of 2000 liters and a culture surface area of 2.52 m^2.

Shades: Four of the reactors were covered with neutral density screens of "Prop-a-lite" flameproof fabric (Jacob Brothers, 8928 Sepulveda Blvd., Sepulveda, Ca. 91343) on redwood and PVC frames to provide the following percentages of incident light intensity reaching the surface of the culture: 46, 30, 20 and 3.

Light meters: A pyrheliograph (Belfort Instrument Co., 4 N. Central Ave., Baltimore, Md. 21202) was mounted on the beach at an altitude higher than the reactors to record continuously the incident light. Watertight photocells were installed in each reactor for readings at the surface and at the bottom to calculate the attenuation coefficient, k, of the culture.

Culture medium: Antarctic Intermediate Water, called "deep water", was piped from a depth of 800 m to an artificial upwelling mariculture system and to the reactors. The concentrations of dissolved nutrients in deep water at

the time the experiment was begun are given in Table 1.

TABLE 1. CONCENTRATIONS OF DISSOLVED NUTRIENTS IN DEEP WATER, 26 JULY 1974

NUTRIENT	CONCENTRATION (μg-at/ℓ)
Nitrate	30.91
Nitrite	0.01
Phosphate	2.05
Silicate	22.43

Inoculum: The marine diatom, Chaetoceros curvisetus (clone STX-167, isolated by K.C. Haines) was used to inoculate the reactors. The diatom will grow in unsupplemented deep water in outdoor continuous culture at a maximum dilution rate of 1.4/day (unpublished data). The diatom was grown in f/4 medium in 200-gallon polytanks on the beach for two days. On the afternoon of the second day, 20 liters of this culture was inoculated into 1000 liters of deep water in each of the reactors.

Experimental Design: All reactors were unshaded at inoculation time (1600 hr, day 0). The cultures were allowed to stand in batch conditions overnight. At sunrise on day 1, filling with deep water was begun at the rate to be used for continuous flow: 348 ml/min, or a turnover of reactor volume of 0.25 times/day. At sunrise on day 2, the reactor was 3/4 full and at sunrise on day 3 the reactors were

completely full. Continuous flow was begun and shades were added to reactors 2-4 so that light intensities reaching the cultures were as given in Table 2.

TABLE 2. PERCENTAGE OF INCIDENT LIGHT (I) REACHING CULTURE SURFACE

REACTOR NUMBER	% I
1	100
2	46
3	30
4	20
5	3

Sampling was conducted at sunrise and sunset, beginning on day 1 and ending on day 10 for the following analyses: cell density, chlorophyll a and phaeopigments, CHN, cellular nitrate and dissolved nitrate, nitrite, phosphate, and silicate. Samples from day 7 were saved for ammonia analysis. Incident light intensity was recorded continuously on the pyrheliograph. Surface and bottom light readings on the photocells and culture temperatures were recorded at each sampling time.

On day 8 photosynthesis experiments were conducted by incubation of samples with radioactive carbon (^{14}C). At sunrise, suplicate light bottles and a dark bottle containing

125 ml of sample were enriched with 2.6 ml of deep water. This is the proportion of deep water that would be added to the reactors during the two-hour incubation period. Samples were inoculated with 10 μCi of ^{14}C. Bottles were incubated for two hours at the light intensity at which the cells had been growing, e.g., the bottles from reactor 1 were incubated at 100% I, the bottles from reactor 2 at 46% I, etc. The incubation chamber was an open trough cooled with deep water. Bottles were put in 6-ft long acrylic tubes open at both ends for access to cooling water and covered with the same material as the reactor shades.

The ^{14}C-incubation was to be repeated at noon with samples from all reactors incubated at all light intensities, but a rain storm brought the incident light intensity from approximately 0.9 ly/min to less than 0.08 ly/min for at least two hours (Fig. 1) so the noon incubation was done on day 10. (There had been time to start the incubation of the bottles from reactor 1 before the rain began on day 8. The results are given in the Results section and compared with the noon results from day 10.)

On day 11, a nutrient-uptake experiment was conducted by enriching aliquots from each reactor with deep water in a ratio of 1:3. Glass-stoppered bottles were incubated at the light intensity at which the cells had been growing. Duplicate bottles were removed from the incubation trough at 0, 2, 5, 7 and 8 hours for dissolved nitrate and phosphate analyses.

Figure 1. Pyrheliograph tracing of incident light intensity for days 7 through 11. One square centimeter of chart paper represents approximately 0.08 ly/min. The sudden drop in light intensity on day 8 was caused by a rain storm.

7/2
7/3
7/4
7/5
7/6
6/30
7
8
9
10
11
DAY

RESULTS

Temperature of the cultures, incident light intensities, and extinction coefficients are given in Table 3. Average temperature decreased with increasing shade, although all reactors cooled overnight so that the sunrise temperature was 25°C every day for all reactors.

Most days of the experiment were largely overcast, as can be seen in the sample pyrheliograph tracing shown in Figure 1. The darkest day (average I=0.485 ly/min) followed by two days the brightest day (average I=0.776 ly/min) during continuous flow. The brightest day of the entire experiment (average I-0.786 ly/min) was day 0, the day of inoculation. At inoculation time (1600 hr) the I was approximately 0.1 ly/min.

Extinction coefficients showed an increase with increase in shade and was therefore more closely correlated with chlorophyll concentration than with cell density.

Cell densities are shown in Figure 2 and listed in Tables 4A-E. On day 2, all reactors were contaminated by small flagellates at which time they constituted approximately 90% of the cell density, but not of biomass. Cell volume of the small flagellates was estimated to be approximately 10% of the cell volume of STX-167. Percentage of flagellates decreased thereafter but remained present throughout the experiment.

Chlorophyll a, paheopigment, and acid ratio mean values are listed in Table 4, and chlorophyll a/cell is shown in Figure 3. No diurnal periodicity was seen in any of the

TABLE 3

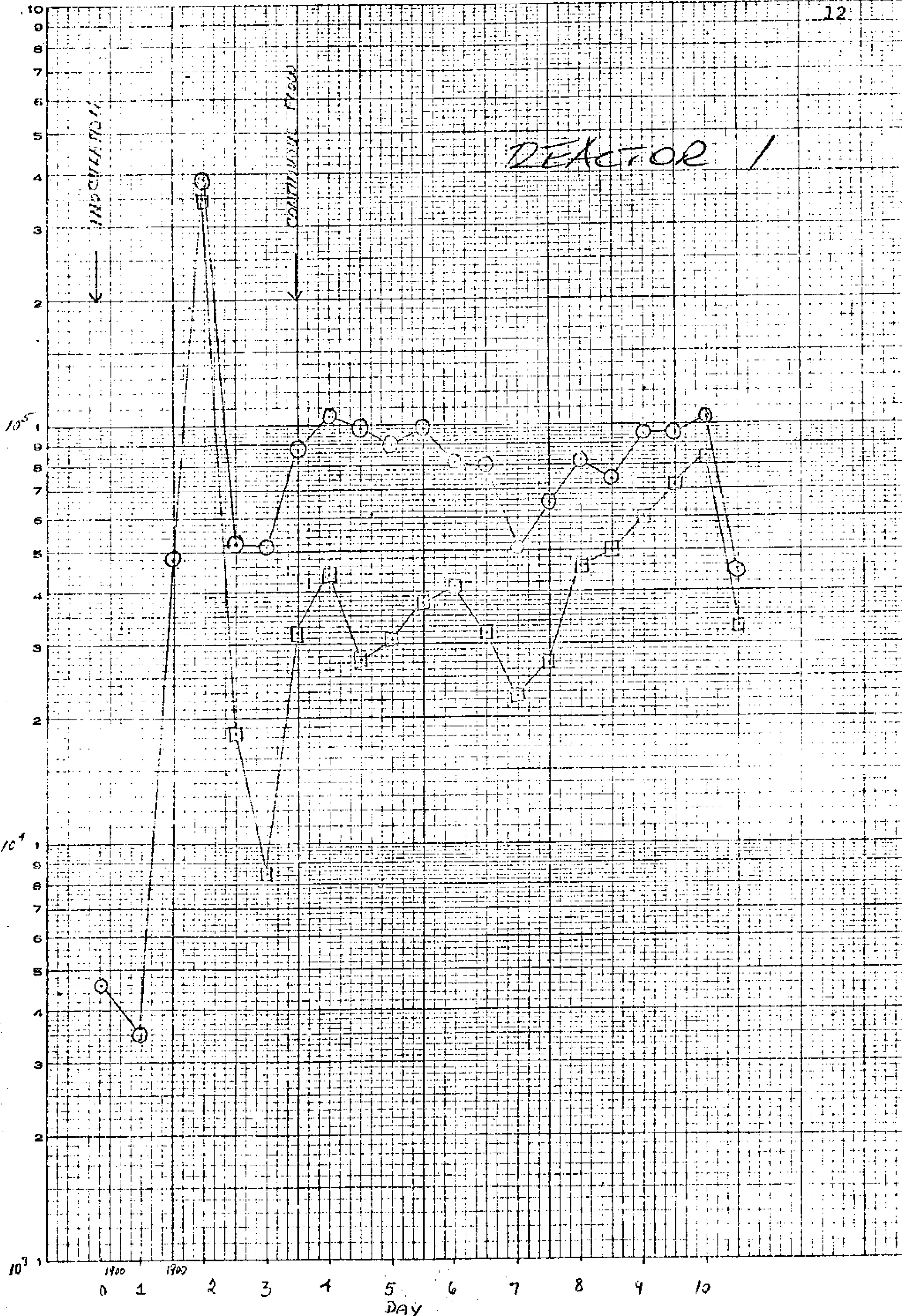
INCIDENT LIGHT INTENSITIES

Day	Mean I (ly/min)	Total I (ly/12 hr)
6/26	0.786	566.00
6/27	0.528	379.96
6/28	0.620	446.60
6/29	0.713	513.23
6/30	0.656	472.03
7/01	0.688	495.69
7/02	0.776	558.93
7/03	0.490	352.76
7/04	0.485	349.23
7/05	0.667	479.92
7/06	0.612	440.48

EXTINCTION COEFFICIENTS		TEMPERATURES	
Reactor	Mean	Mean	Range
DW	0.002		
1	0.256	27.4	25.0-29.5
2	0.360	27.0	25.0-28.5
3	0.397	26.9	25.0-28.2
4	0.525	26.9	25.0-28.2
5	0.717	26.5	25.0-27.8

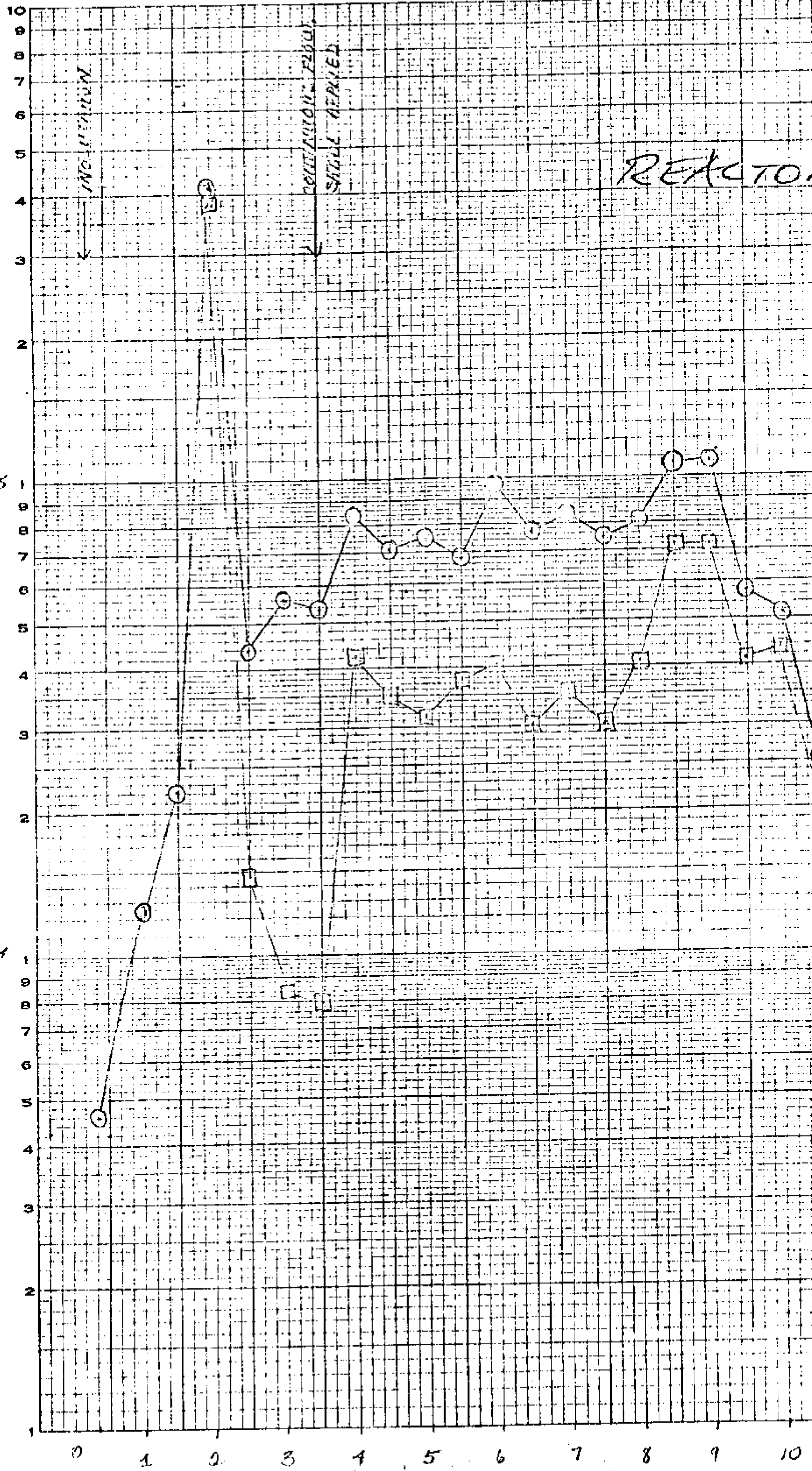
pigment analyses. The mean chlorophyll *a*/cell increased with increasing shade, as shown in Figure 4.

Figure 2. Cell densities of outdoor continuous cultures inoculated with *Chaetoceros curvisetus* (STX-167). Circles = total cell number; squares = number of cells other than STX-167. Darkened circles and squares represent samples taken at the end of the dark period.

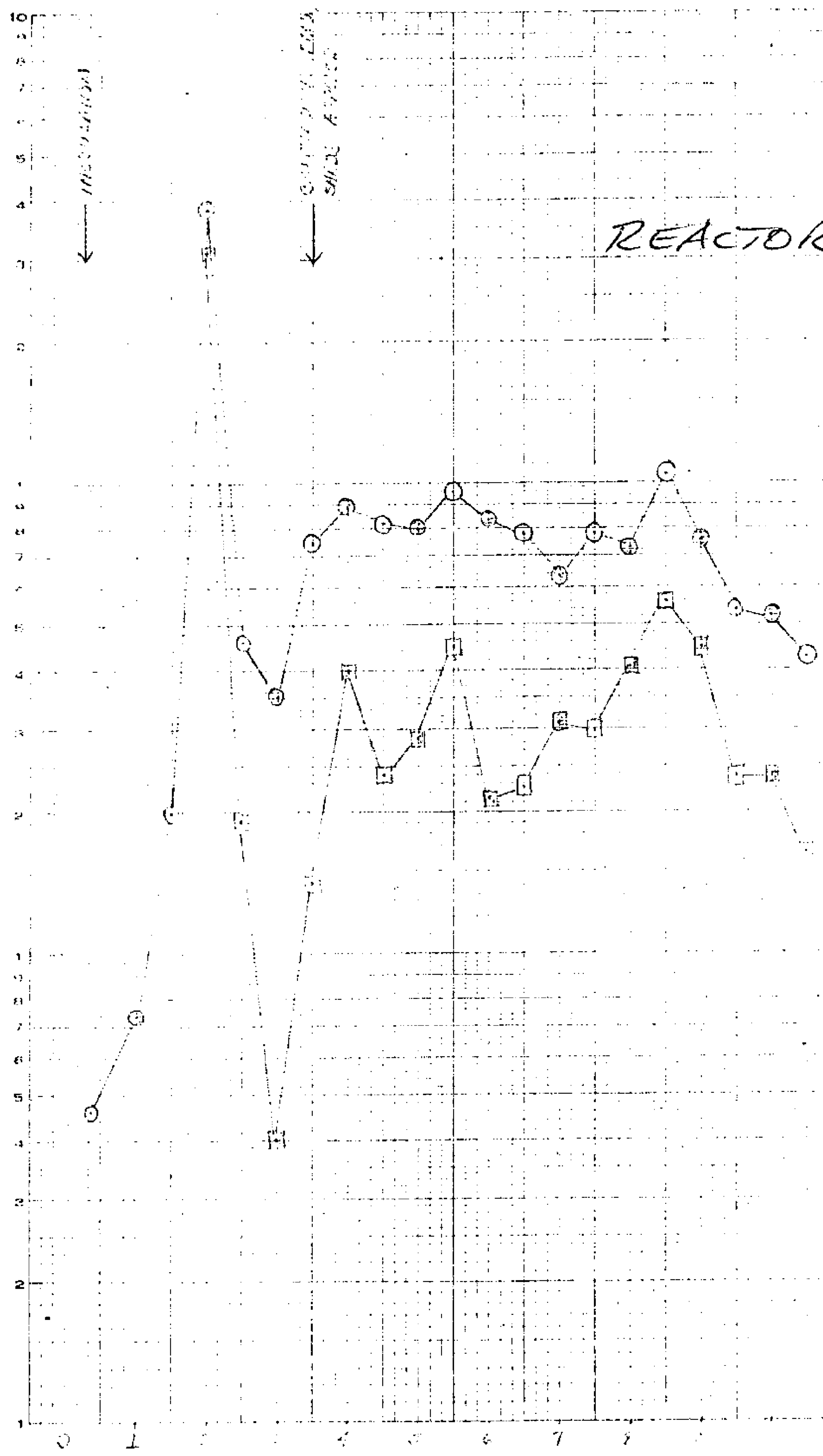


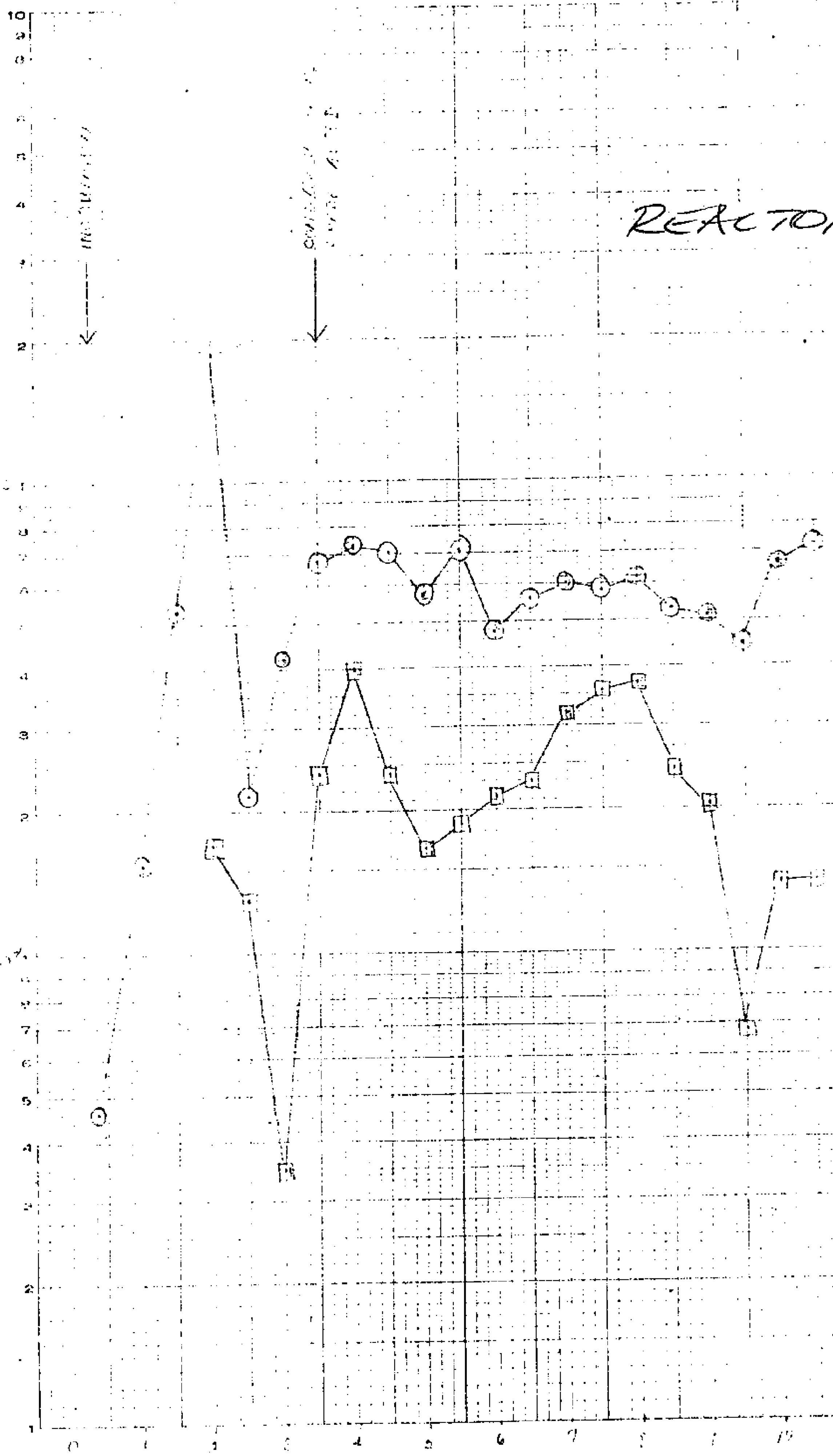
□ : SPECIES OTHER THAN SIX-767
12
REACTOR 1
INOCULATION
CONTINUOUS FLOW
10^5
10^4
10^3
1900
1900
0 1 2 3 4 5 6 7 8 9 10
DAY
EUGENE DIETZGEN CO.
MADE IN U. S. A.
NO. 340-L312 DIETZGEN GRAPH PAPER
SEMI-LOGARITHMIC
3 CYCLES X 12 DIVISIONS PER INCH

[□]: SPECIES OTHER THAN ...



EUGENE DIETZGEN CO.
MADE IN U. S. A.
NO. 340-L312 DIETZGEN GRAPH PAPER
SEMI-LOGARITHMIC
3 CYCLES X 12 DIVISIONS PER INCH


REACTOR 3
NO. 340-L312 DIETZGEN GRAPH PAPER
SEMI-LOGARITHMIC
3 CYCLES X 12 DIVISIONS PER INCH
EUGENE DIETZGEN CO.
MADE IN U. S. A.



NO. 340-L312 DIETZGEN GRAPH PAPER
SEMI-LOGARITHMIC
3 CYCLES X 12 DIVISIONS PER INCH
EUGENE DIETZGEN CO.
MADE IN U.S.A.

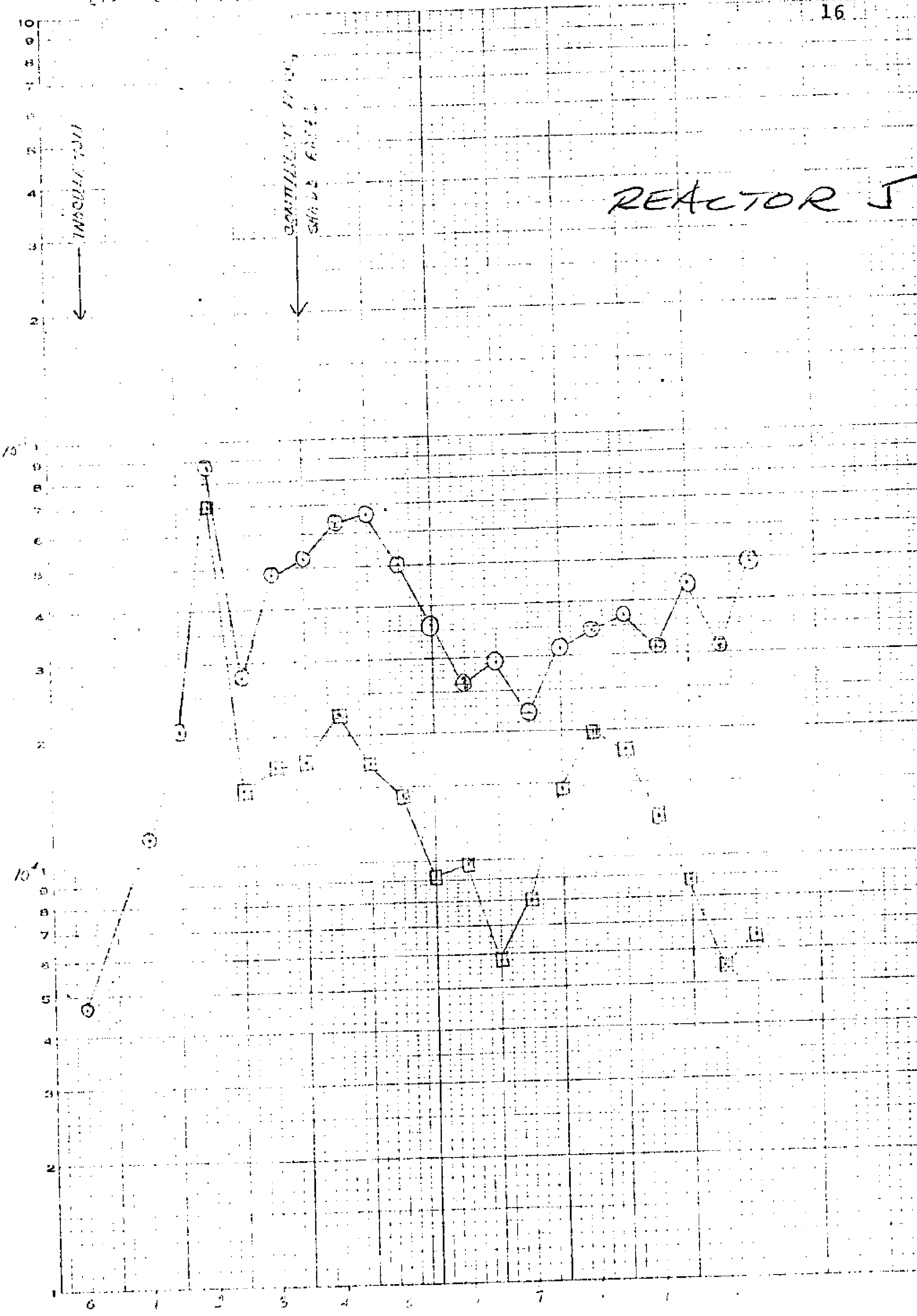

REACTOR J
NO. 340-L312 DIETZGEN GRAPH PAPER
SEMI-LOGARITHMIC
3 CYCLES X 12 DIVISIONS PER INCH
EUGENE DIETZGEN CO.
MADE IN U. S. A.

TABLE 4. CHLOROPHYLL AND CELL COUNT SUMMARY*

REACTOR No.	CHLOROPHYLL a (μg/ℓ)	PHAEOPIGMENTS (μg/ℓ)	ACID RATIO	CELL NUMBER (10^4/ml)	CHLOROPHYLL a/CELL (μg Chla/10^7 cells)
1	5.52	3.17	1.50	7.83	0.73
2	9.27	4.42	1.54	7.58	1.27
3	10.72	5.91	1.51	7.39	1.51
4	16.08	7.66	1.54	5.84	2.79
5	24.80	8.17	1.56	3.54	7.08

*Values represent means of days 5-10.

Figure 3. Chlorophyll *a*/cell (μg chlorophyll *a*/10^7 cells) in five outdoor continuous cultures.

FIG. 3

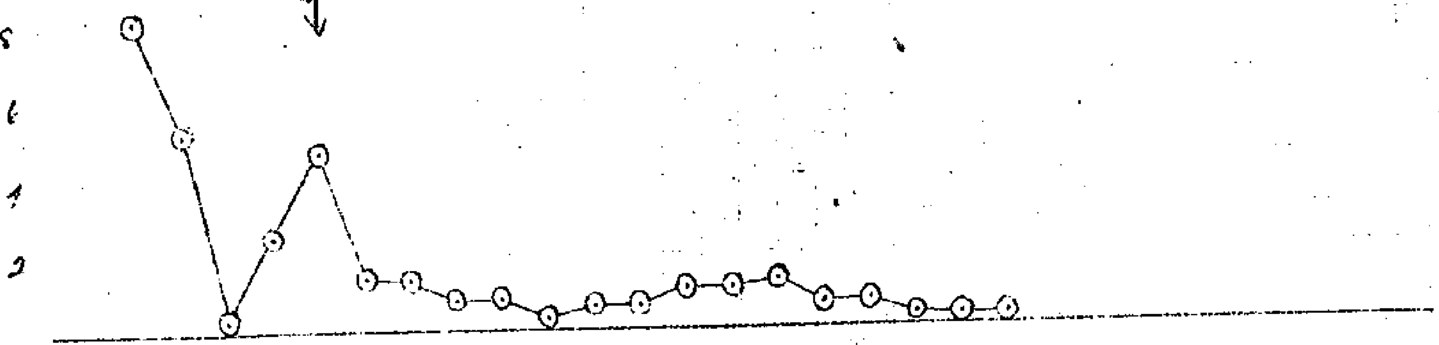
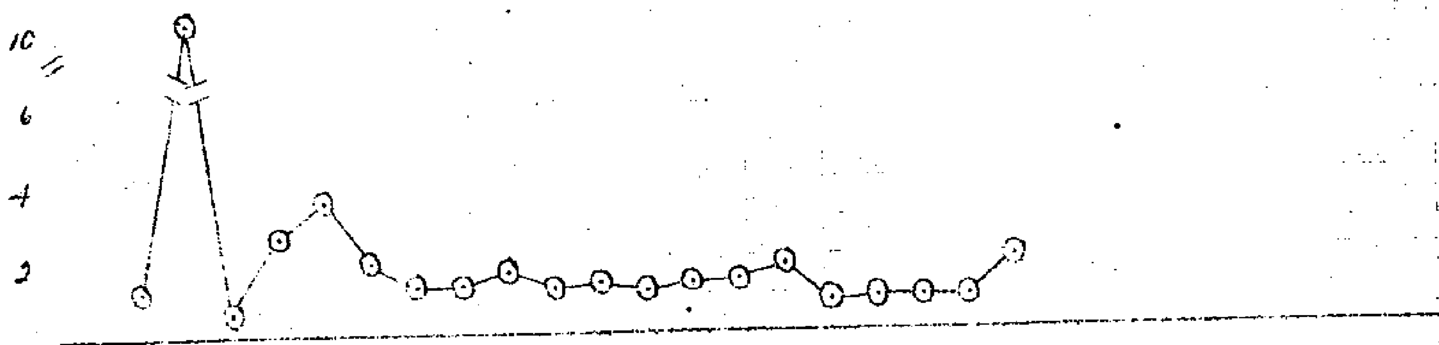
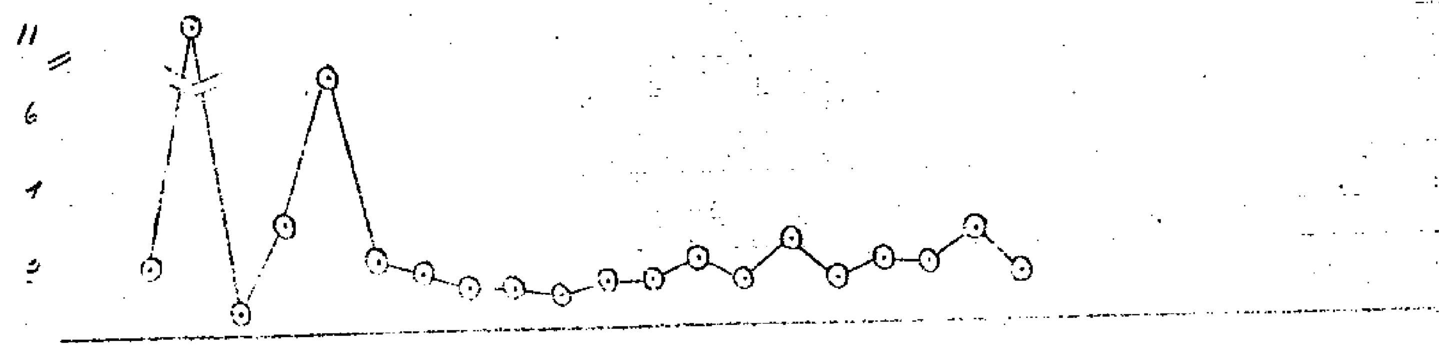
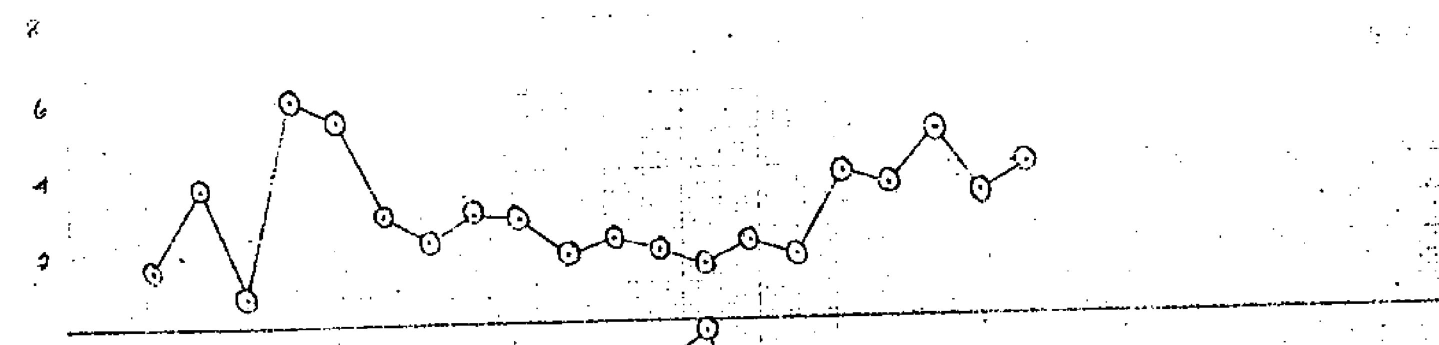
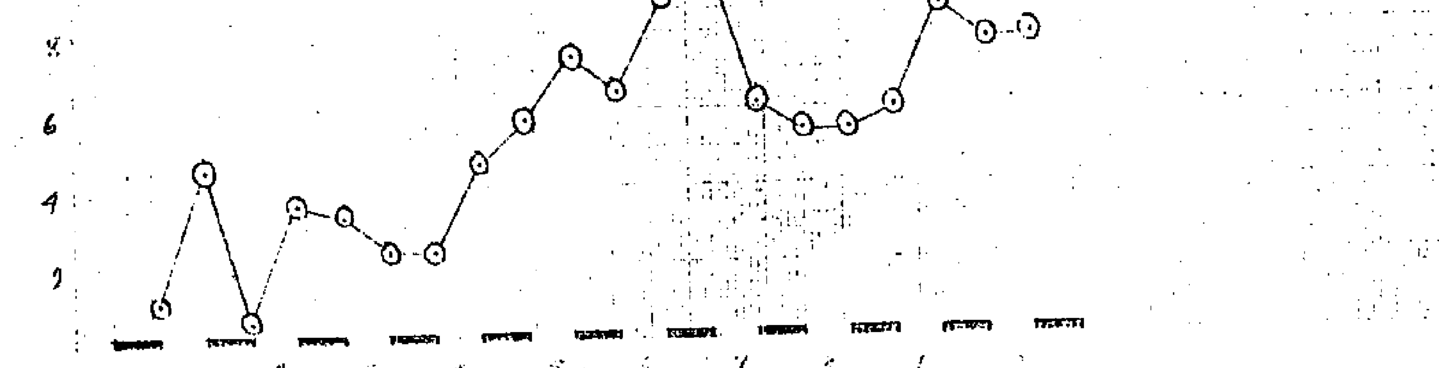
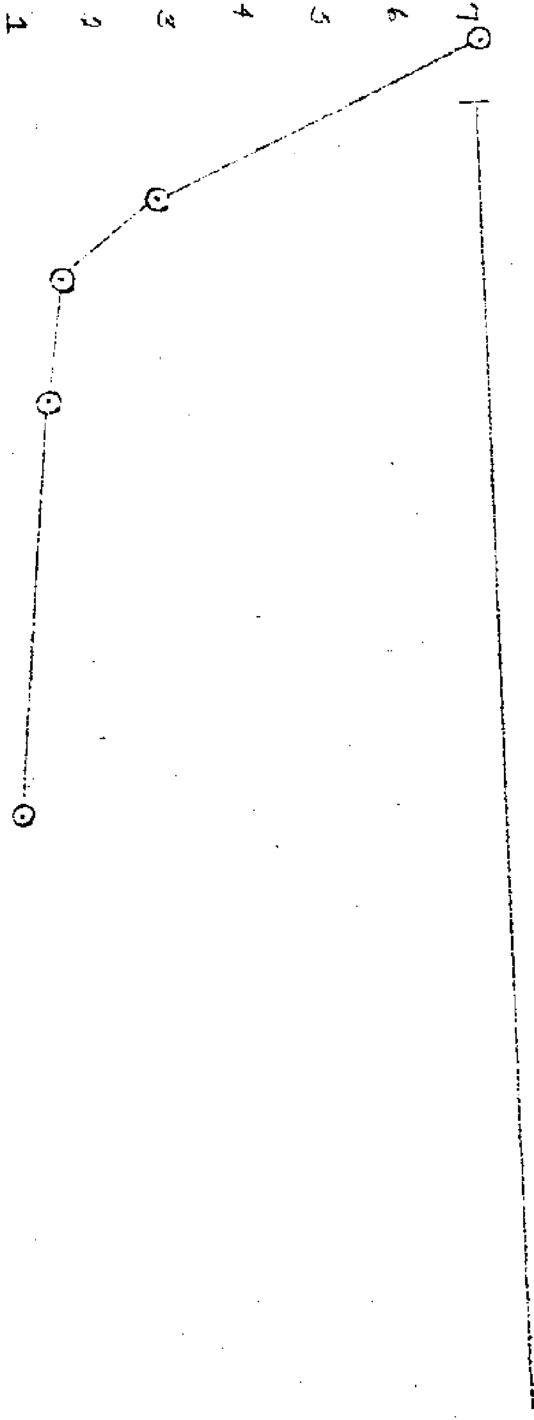
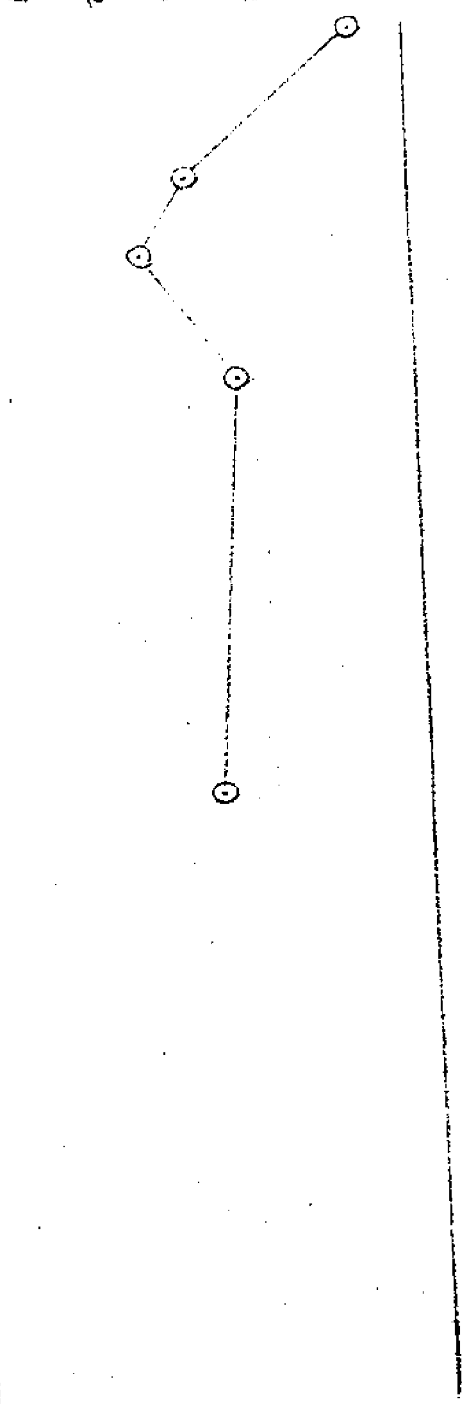






Figure 4. Mean cellular composition of cells grown in outdoor continuous culture at a dilution rate of 0.25/day and at five different light intensities. Composition shown as a function of light intensity. (PN and POC values to be filled in when analyses complete.)





PN

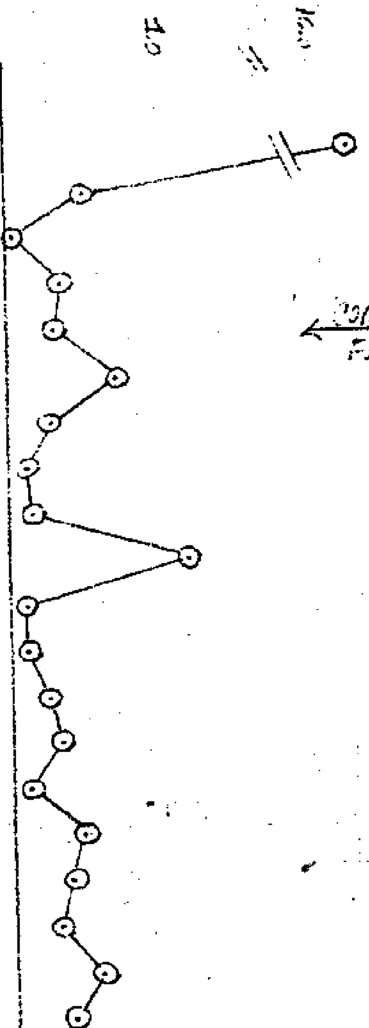
POC

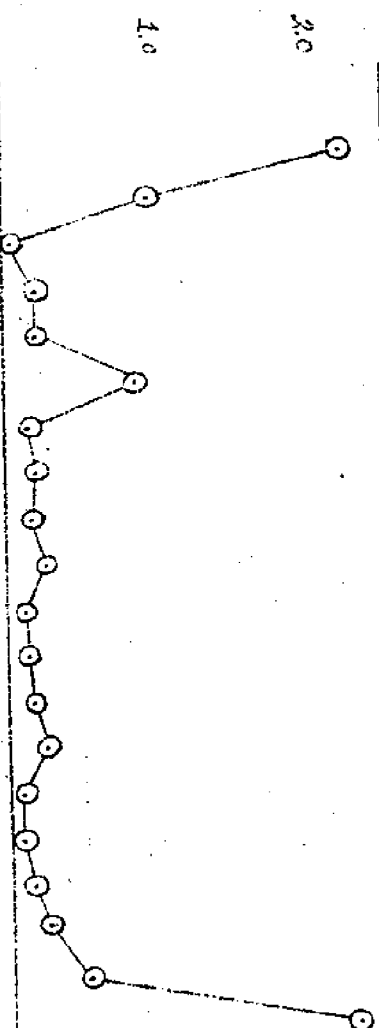
Dissolved nutrient concentrations in the medium are shown in Figure 5 and 7 through 9. Nitrate was below the limit of detection at all degrees of light intensity except the lowest after continuous flow was begun (Fig. 5). It accumulated to approximately 50% of deep-water concentration at the 1%I culture and toward the end of the experiment began to show a diel periodicity. Cellular nitrate (Fig. 6) showed no trend except the mean value was highest at the lowest I (Fig. 4). Note that in the reactors in which cells seemed to be washing out on days 9 and 10 (Fig. 2), the cellular nitrate content increased, especially in reactor 2.

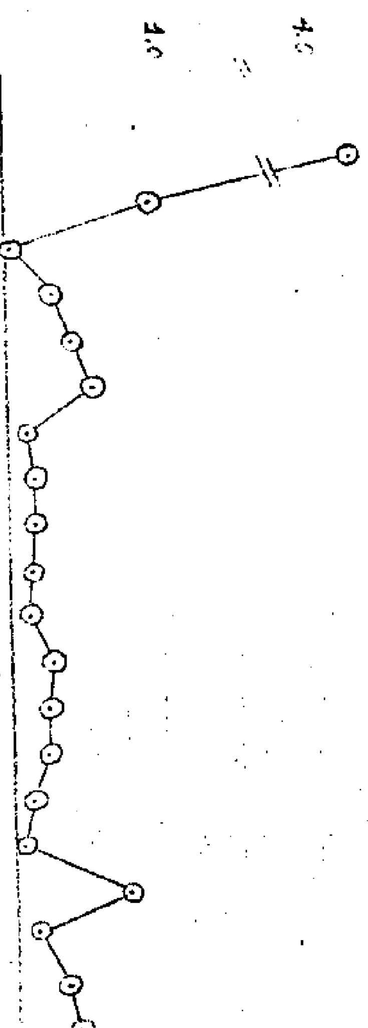
Figure 5. Concentration of nitrate in the culture medium of five outdoor continuous cultures run at a dilution rate of 0.25/day and at five different light intensities.

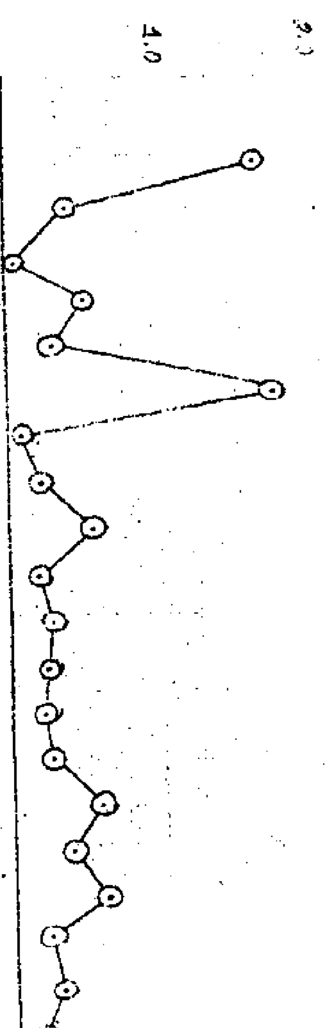
FIG. 5

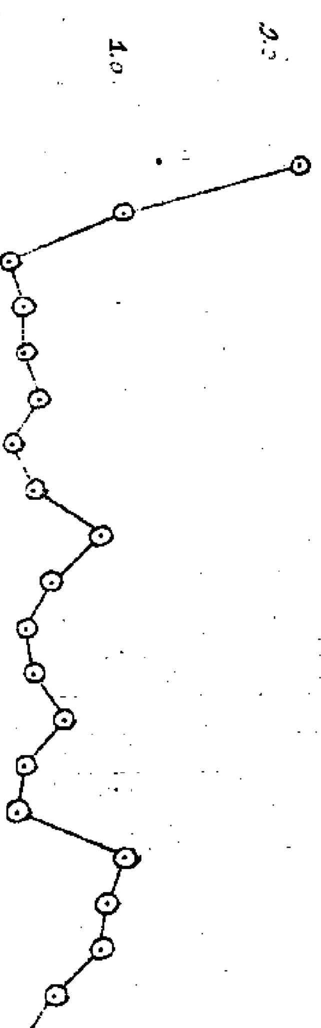
Figure 6. Cellular nitrate concentration (μg-at NO_3-N /10^9 cells) in outdoor continuous cultures run at 0.25/day dilution rate and at five degrees of light intensity.


1.0
REACTOR 1
100%I


2.0
1.0
REACTOR 2
46%I


1.0
REACTOR 3
30%I


1.0
REACTOR 4
20%I


1.0
REACTOR 5
1%I

Nitrite accumulated in the medium during days 1 and 2 (Fig. 7) while cellular nitrate (Fig. 6) decreased in the cells and while dissolved nitrate (Fig. 5) was being removed from the medium.

Ammonias from day 7 have not yet been run.

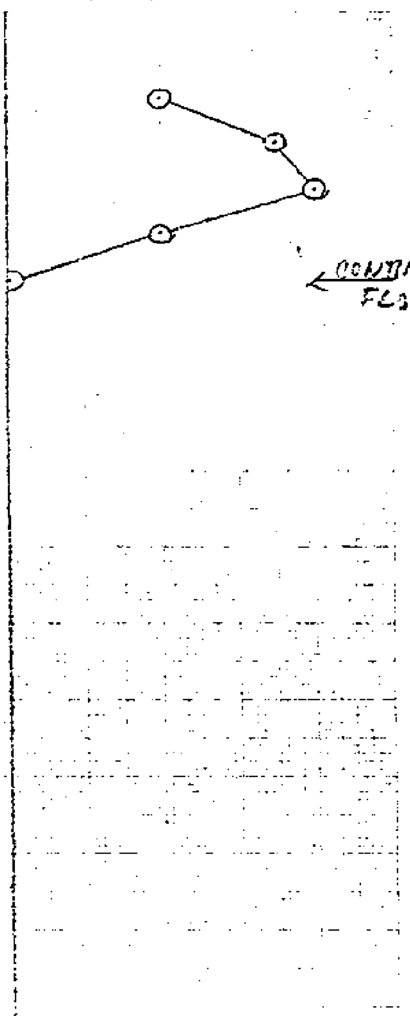
Dissolved phosphate was taken up in all reactors at less than 0.5 μg-at PO_4-P/l (Fig. 8) except in the darkest reactor, where the concentration in the medium remained slightly greater than 0.5 μg-at PO_4-P/l.

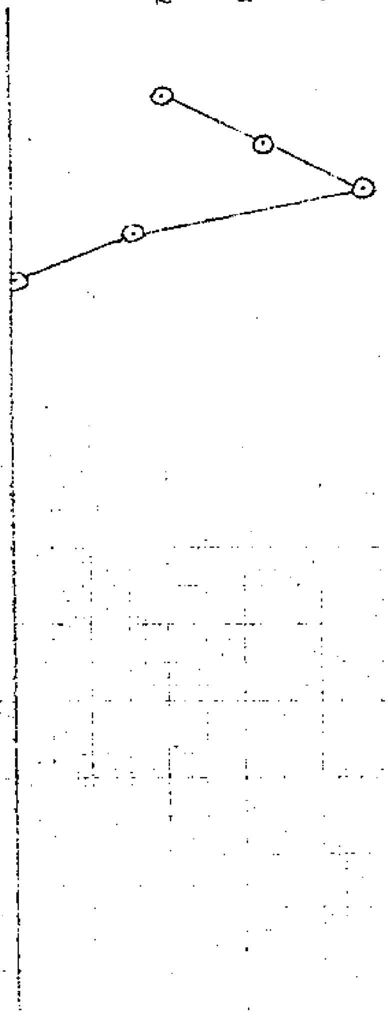
In the darkest reactor, reactive silicate concentration in the medium showed diel periodicity during days 8-10 (Fig. 9). (Silicate values for day 7 will be determined after ammonias are run on the samples.) The accumulation of silicate in reactor 1 is related to a low proportion of diatoms and a high proportion of flagellates in the culture (Fig. 2A).

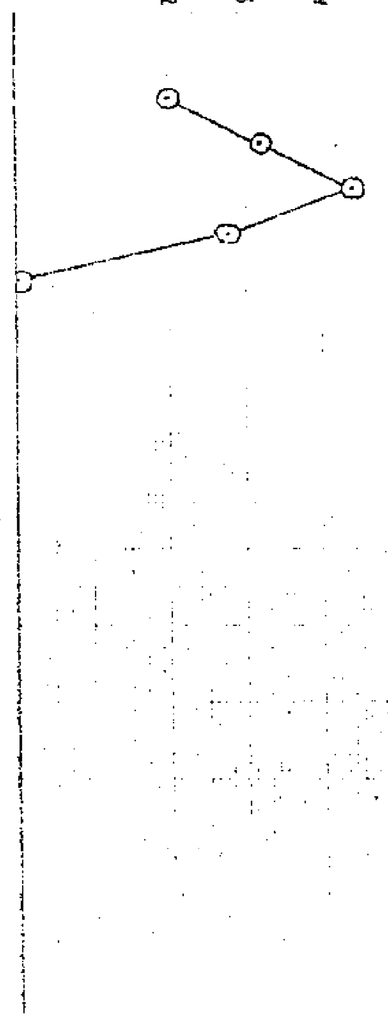
CHN values have not yet been determined.

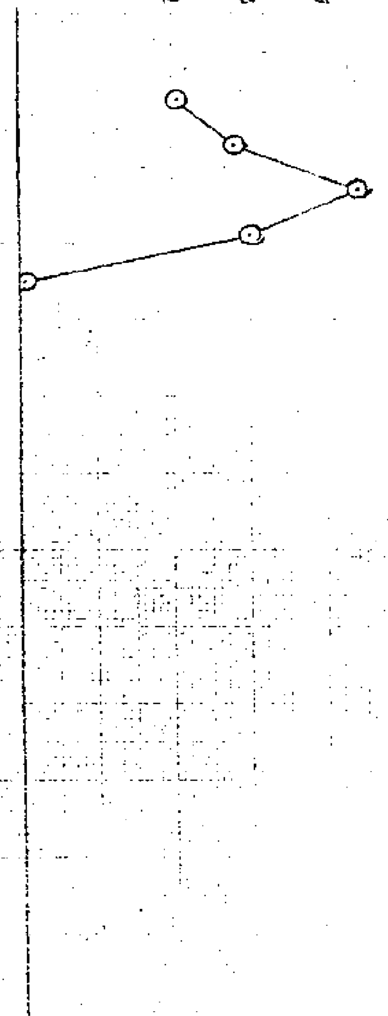
Uptake of both nitrate and phosphate over an 8-hr period from deep water-enriched samples was greatest at the lowest light intensity (Fig. 10). Uptake of nitrate but not of phosphate was inhibited at 100%I (unless any value lower than maximum means inhibition).

Figure 7. Concentrations of nitrite in outdoor continuous cultures run at a dilution rate of 0.25/day and at five degrees of light intensity. Nitrite analyses were not performed on samples when the nitrate concentration was undetectable.


μg-at NH₄-N/l
0.1
0.2
0.3
0.4
CONTINUOUS FLOW
REACTOR 1
100%I


0.1
0.2
0.3
0.4
REACTOR 2
46%I


0.2
0.3
0.4
REACTOR 3
30%I


0.1
0.2
0.3
0.4
REACTOR 4
20%I

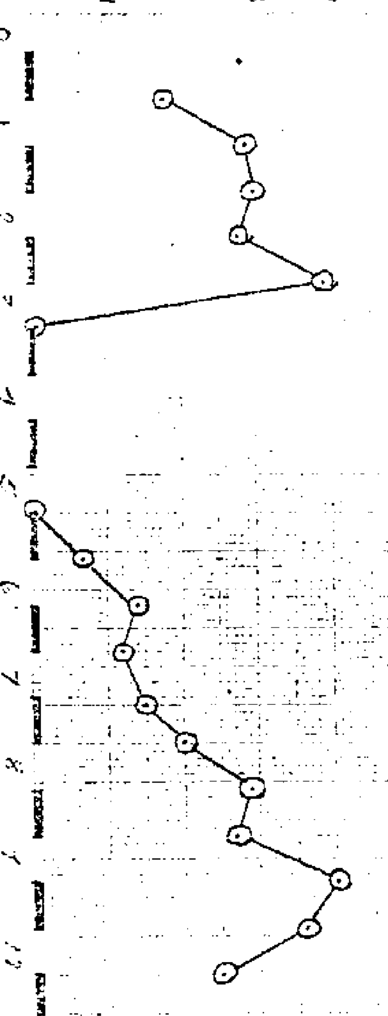

0.1
0.2
0.3
0.4
6
7
8
REACTOR 5
1%I

Figure 8. Concentrations of phosphate in outdoor continuous culture run at a dilution rate of 0.25/day and at five degrees of light intensity.

2.0
1.0
R1 100% I
2.0
1.0
R2 46% I
2.0
1.0
R3 30% I
2.0
1.0
R4 20% I
2.0
1.0
R5 1% I

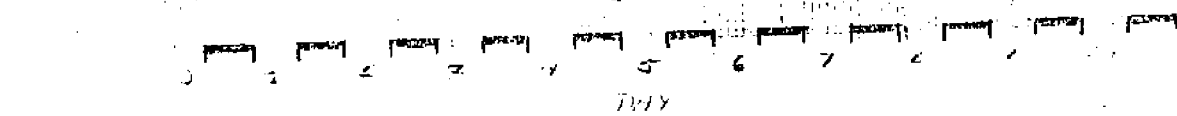

6
7

Figure 9. Concentrations of reactive silicate in outdoor continuous cultures run at a dilution rate of 0.25/day and at five degrees of light intensity.

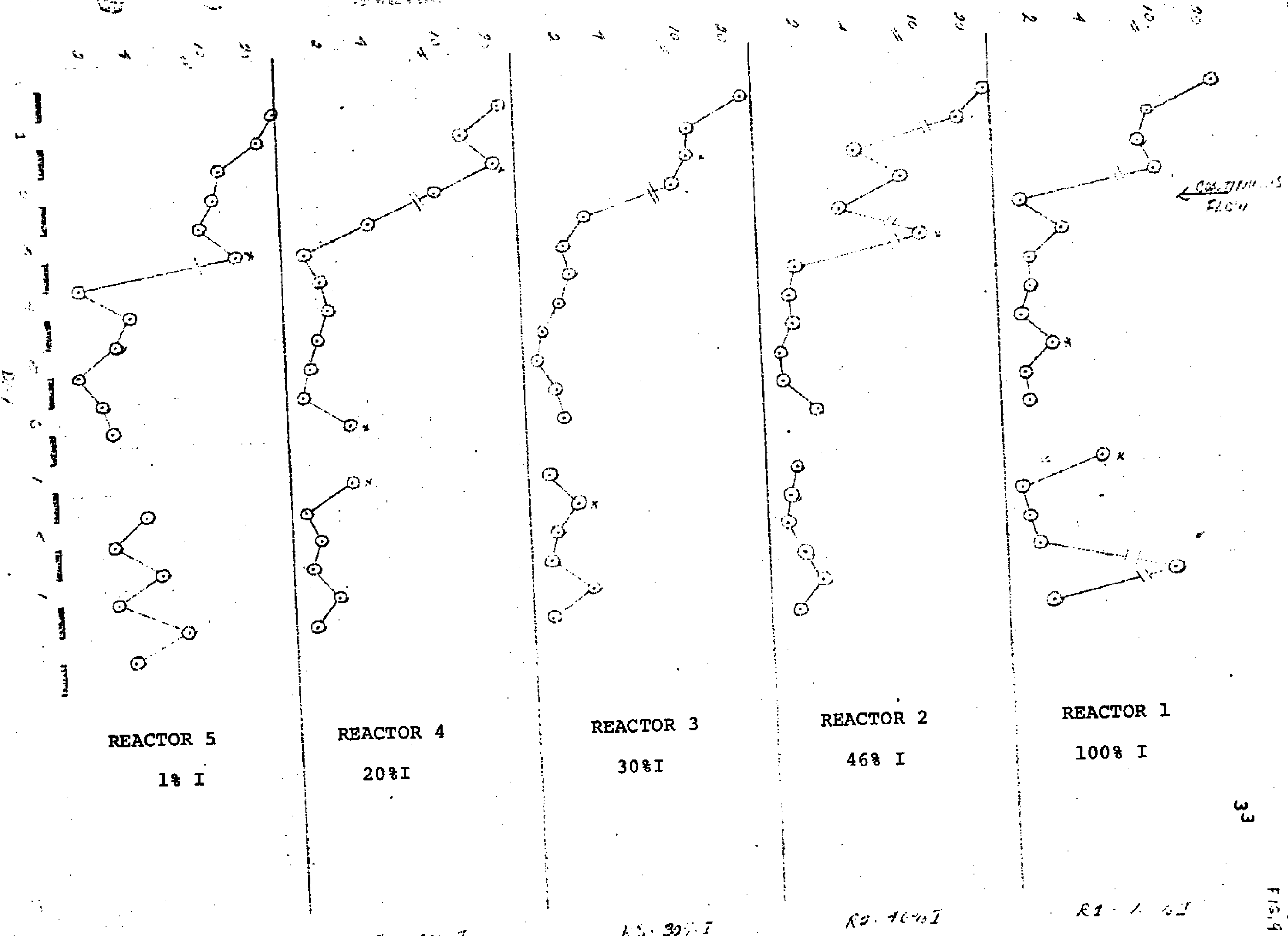
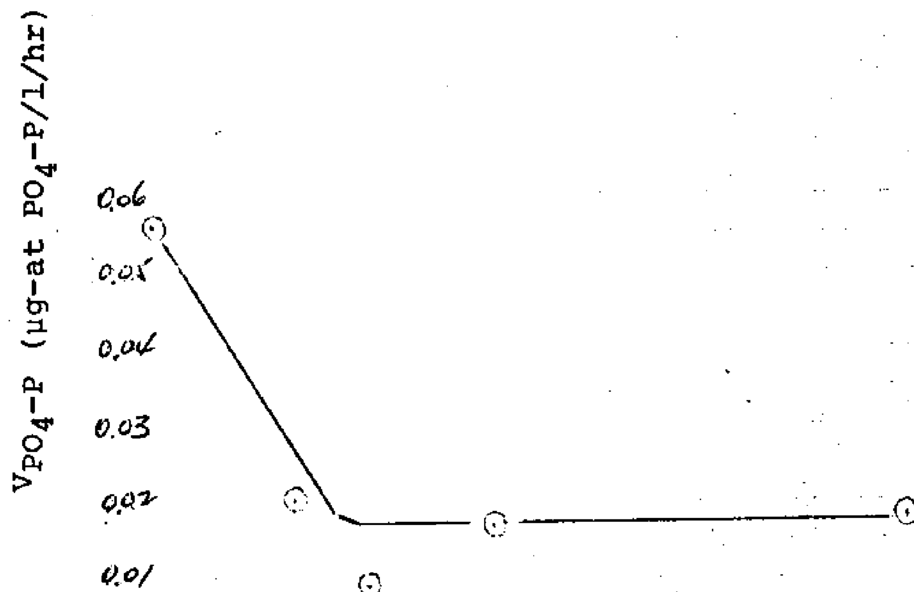
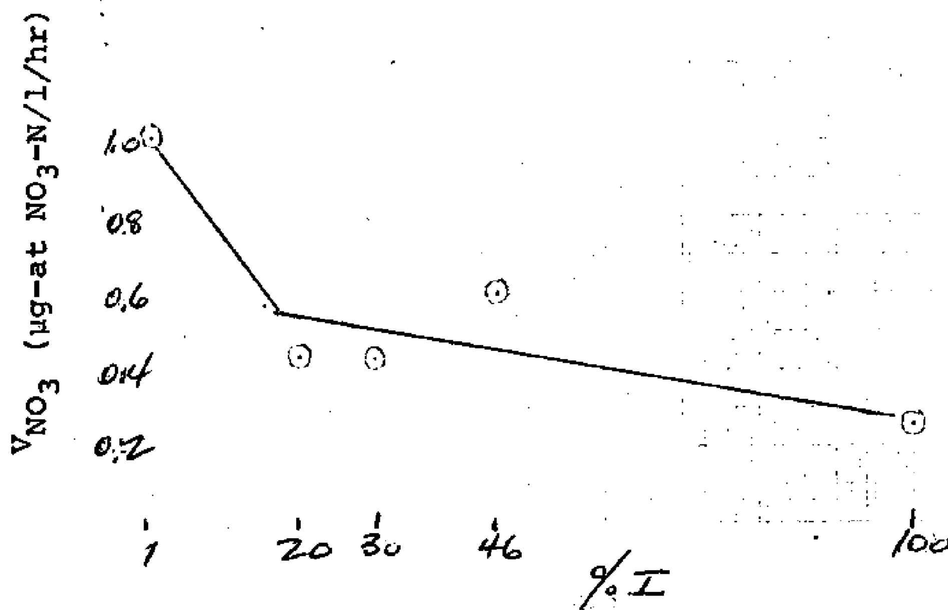

REACTOR 1
100% I
REACTOR 2
46% I
REACTOR 3
30%I
REACTOR 4
20%I
REACTOR 5
1% I
3[3]

Figure 10. Uptake rates of phosphate (upper) and nitrate (lower) as a function of light intensity. Rates in μg-at/l/hr.


V_{PO_4-P} (µg-at PO_4-P/l/hr)
0.06
0.05
0.04
0.03
0.02
0.01


V_{NO_3} (µg-at NO_3-N/l/hr)
1.0
0.8
0.6
0.4
0.2
1
20
30
46
100
% I

Uptake of radioactive carbon was inhibited at 100% I and maximal at 46% I regardless of the light intensity at which the cells had been growing (Figs. 11-12).

The P vs I curves (Fig. 11) show that when cells that have been growing at 1% I are placed in greater light intensities, the rate of photosynthesis is greater than for cells grown at any other light intensity.

Rate of photosynthesis/unit chlorophyll (Fig. 12) is almost identical for cells grown at the two lowest light intensities.

Inhibition of both photosynthesis/volume and photosynthesis/chl. a at 100% I increases greatly for cells previously grown at the lower light intensities.

Figure 11. Rate of uptake of carbon by a volume of culture as a function of percentage of incident light intensity (P vs I curves). The light history of the cultures is given as follows: open circles = 100%; closed circles = 46%; open squares = 30%; closed squares = 20%, and x's = 3% of incident light. The percentage light intensity at which the cultures had been growing is the z-axis, and the percentage light intensity of incubation is given along the abscissa.

PvsI curves

3

30

100

50

100

Figure 12. Rate of uptake of carbon by a unit of chlorophyll *a* as a function of incident light intensity (assimilation number vs I curve). Key and light ordinates as given for Figure 11.

ABSORPTION NO. VS I CURVES

15

10

5

50

100

% I of incubation

% I of quinine

Discussion

At a dilution rate of 0.25 in outdoor continuous culture of STX-167 the following observations were made:

(1) Flagellates tended to dominate the cultures numerically, except the last two days at the lowest I, where STX-167 averaged 69% of the total cell density. The flagellates in these cultures were much smaller than STX-167 and do not require silicate for growth. Since smaller cells often have lower k_s values than larger cells (Eppley and Renger, 1974), they have an advantage in severely nutrient-limited conditions. The further advantage of no silicate requirement may give the edge to the flagellates so that an azenic culture of STX-167 at this low dilution rate is not possible.

(2) Inhibition of uptake of ^{14}C and of nitrate was seen at 100% I. For ^{14}C the inhibition occurred regardless of the light intensity at which the cells had been previously grown.

(3) Limitation of total cell growth rate in all but the darkest reactor may have been by nitrate, since it was not detected in the medium. However, the relatively low silicate values, coupled with the appearance of high numbers of flagellates, suggest that silicate may have limited the growth rate of STX-167 in all but the darkest reactor. Light may have been limiting in the darkest reactor after day 5 when nitrate began to accumulate in the medium. However, silicate values began to demonstrate

periodicity on day 7 or 8, suggesting that silicate might have limited growth at least part of the time.

(4) The excretion of nitrite after inoculation and the simultaneous uptake of nitrate while cellular nitrate decreased is very puzzling. This phenomenon may suggest something about how a cell readjusts its physiology when exposed to a new medium and new environmental conditions.

In conclusion, this experiment has demonstrated light inhibition of both photosynthesis (^{14}C uptake) and rate of nitrate uptake, but not necessarily of rate of growth, when cells are grown in a severely nutrient-depleted environment.

The experiment provides a baseline from which the effects of light intensity on less severly nutrient-limited cells may be compared. The effects of nutrient supply on light-limited cells can then be studied to establish some of the interrelationships between light and nutrient effects on growth of marine phytoplankton.

REFERENCES

Eppley, R. W., and E. H. Renger. 1974. Nitrogen assimilation of an oceanic diatom in nitrogen-limited continuous culture. J. Phycol. 10: 15.

Farmer, M. 1974. Light and nutrients: Effects on growth of a marine diatom. PhD Research Proposal, Dept. Biology, City College, CUNY, New York, N.Y.

Thomas, W. H., and A. N. Dodson. 1972. On nitrogen deficiency in tropical Pacific oceanic phytoplankton. II. Photosynthetic and cellular characteristics of a chemostat-grown diatom. Limnol. Oceanogr. 17: 515.

SOME EFFECTS OF SHADING ON MARINE PHYTOPLANKTON IN OUTDOOR CONTINUOUS CULTURE:

II. DILUTION RATE OF 1.20/DAY

Preliminary Report
on Dissertation Research
Project

Mary W. Farmer

December 3, 1974

Introduction

The long-term purpose and plan of work of this study are described in part I (Some Effects of Shading on Marine Phytoplankton in Outdoor Continuous Culture. I. Dilution Rate of 0.25/Day) of this report.

This section describes outdoor cultures run at a dilution rate of 1.20/day for a period of six days of continuous flow.

Materials and Methods

The materials and methods in this section are identical to those in the first section with the following exceptions.

Flow rate: At sunrise on day 1, filling of reactors with deep water was begun at 418 ml/15 sec, or a turnover of reactor volume of 1.20 times/day. At 1700 on day 1 reactors were full. Continuous flow was begun and shades were added to reactors 2-5 so that light intensities reaching the cultures were given in section I.

Carbon uptake: On day 4 photosynthesis experiments were conducted by incubation of samples with radioactive carbon (^{14}C). One set of samples was incubated in appropriately shaded acrylic tubes as in section I; a second set was incubated by suspending the bottles in the reactor cultures. Two liters of sample from each reactor were mixed with 200 ml of deep water and the mixture was poured into 250-ml glass stoppered bottles for inoculation with 10 µCi of ^{14}C. Samples were incubated for two hours in the late afternoon at a mean incident light intensity of 0.14 ly/min. On day 5 a similar experiment was run for two hours in the morning at a mean incident light intensity of 1.18 ly/min. Samples were incubated only in the reactor vessels. On day 7 data for photosynthesis versus light intensity (P vs I) curves were collected by incubating samples from each reactor at each of the five light intensities. Incubation was for two hours in the early afternoon at a mean incident light intensity of 0.41 ly/min.

At the end of each incubation 1.0 ml of 37% formaldehyde solution
was added to each flask. Samples were filtered onto 0.25 mm HA
0.45 μ Millipore filters and stored in scintillation vials in a
vacuum dessicator. The vials were filled with 21 ml toluene cocktail
the day before counting on a Nuclear Chicago scintillation counter.

Nutrient uptake: On day 8 the deep water flow was shut off
and cultures were enriched with inorganic nutrients. An error in
calculations necessitated scratching the experiment. Within two
weeks an identical experiment was conducted with samples for cell
number and chlorophyll a at sunset only for days 1 through 7. On
day 8 the nutrient uptake experiment was conducted.

At sunrise deep water flow was shut off and each reactor was
enriched with 10 liters of deep water spiked with nutrients to the
following concentrations: 74.6 mg-at NO_3-N; 3.71 mg-at PO_4-P; and
48.1 mg-at SiO_4-Si. This enrichment brought the concentrations in
the reactors to approximately that of deep water for nitrate, phos-
phate, and silicate. No enrichment was added to reactor 5, which
had an initial high concentration of nutrients.

At 1100 hr the cultures were assumed to be well mixed. Aliquots
of the cultures were taken for four dark bottles and four bottles
inoculated with 1.0 ml formalin for controls. Samples were taken
at this time and every three to five hours thereafter until 0200 hr
the following day for dissolved nutrient analyses. Samples for cell
counts were taken at the beginning and the end of the experiment.

Results

Temperatures of the cultures, incident light intensities, and
extinction coefficients are given in table 1. Average temperature
decreased with increasing shade, although all reactors cooled
overnight so that the sunrise temperature was 24.5 + 0.3 for all
reactors throughout the experiment.

TABLE I OUTDOOR CONTINUOUS CULTURE AT [illegible] DAY DILUTION RATE

Day	Date	Mean I (ly/min)	Total I (ly/12 hr)
1	9/20	0.604	435.09
2	9/21	0.609	438.
3	9/22	0.682	491
4	9/23	0.630	453
	(1600-1800)	0.136	16.3 (2hr)
5	9/24	0.760	547
	(0900-1100)	1.179	141 (2hr)
6	9/25	0.706	508
7	9/26	0.314	226
	(1400-1600)		
8	9/27	0.786	566
Mean days 5-7		0.593	427

EXTINCTION COEFFICIENTS

Reactor	Mean	Range
DW	0.002	
1	0.770	
2	0.833	
3	0.732	
4	0.372	
5	0.061	

TEMPERATURES

R	Mean	Range
1	25.9	24.2 - 28.2
2	25.3	24.2 - 26.8
3	25.3	24.2 - 26.5
4	25.2	24.2 - 26.2
5	25.0	24.2 - 25.9

The brightest day of the experiment was day 5, when temperature also reached a maximum for all reactors at the end of the day. The most overcast day was day 7, the day of the P vs I curve experiment.

Extinction coefficients were maximal in reactor 2 where chlorophyll a/liter was also maximal (table 2).

TABLE 2

CHLOROPHYLL AND CELL COUNT SUMMARY - 1.20 DAY^{-1} DILUTION RATE

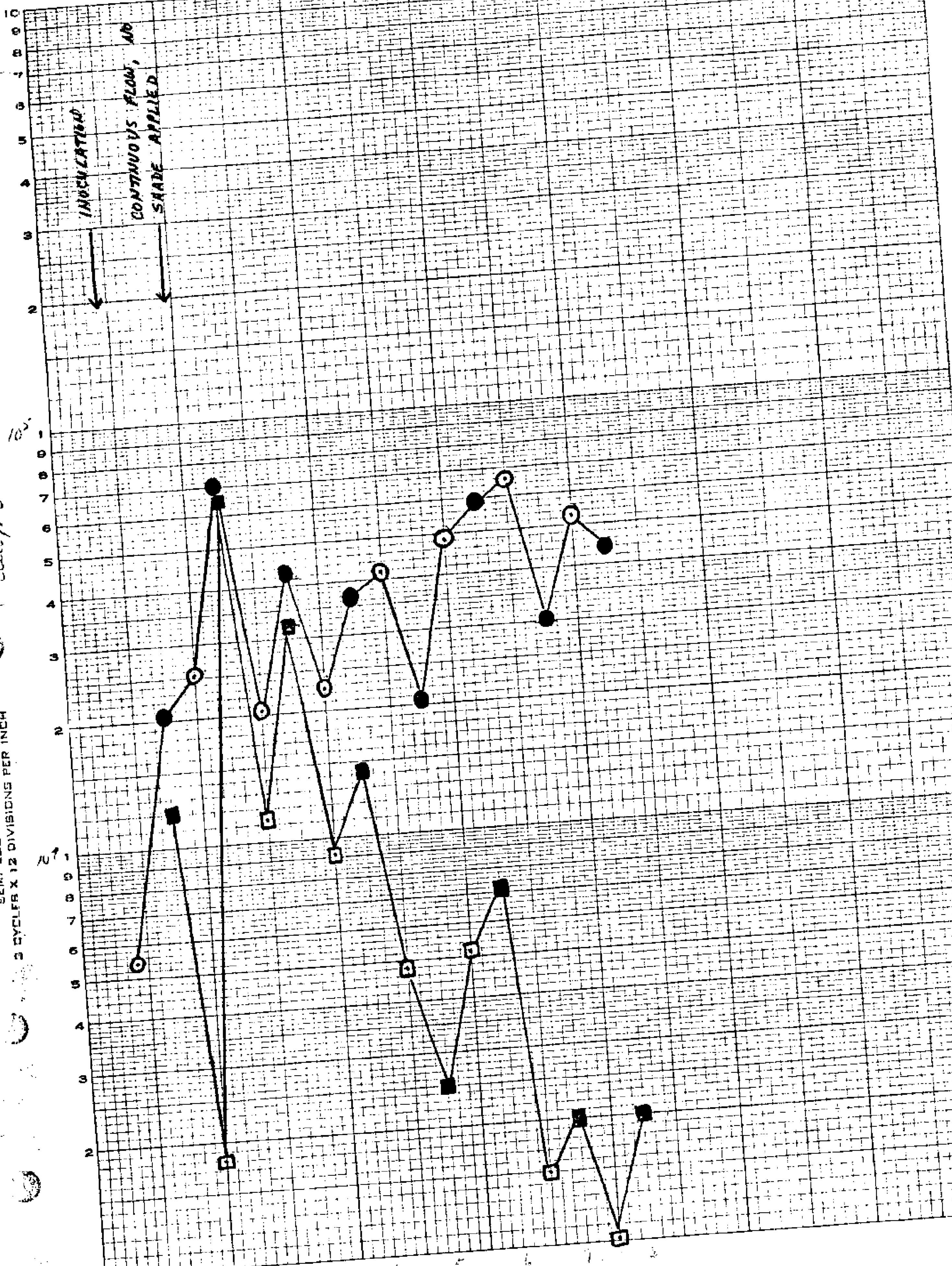
Reactor	Chlorophyll a ($\mu g/l$)	Phaeopigments ($\mu g/l$)	Acid Ratio	Cell Number ($10^4/ml$)	Chlorophyll a/cell (μg chl a/10^7 cells)
1	24.07	7.97	1.59	4.60	5.97
2	28.56	11.81	1.58	6.34	4.76
3	24.24	8.37	1.60	4.54	5.59
4	10.93	3.07	1.63	1.87	6.28
5	0.44	0.96	1.25	0.29	1.58

Values represent means of days 5-7

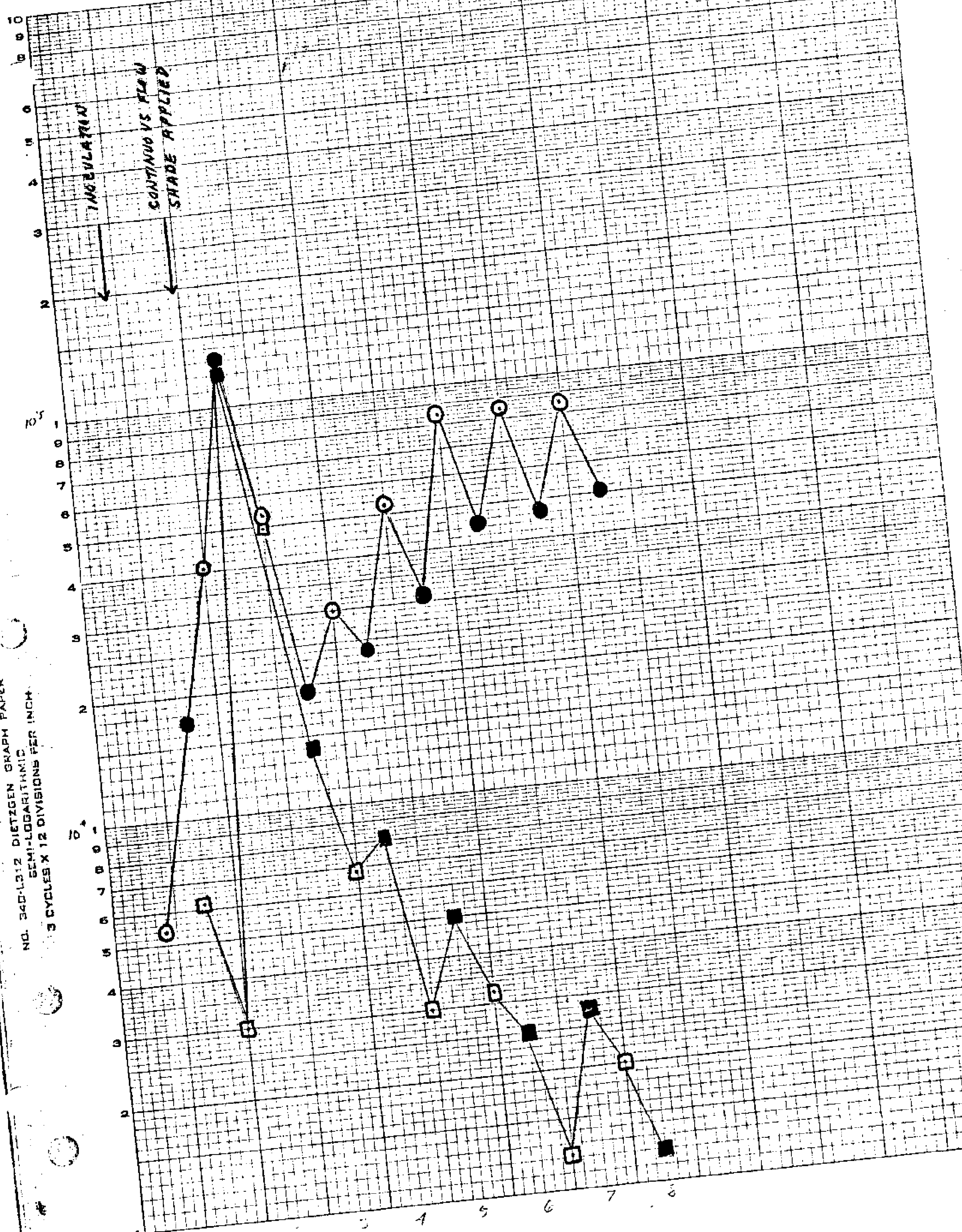
Cell densities are shown in figure 1A-E and the mean cell densities for each reactor are listed in table 2. At sunrise on day 2 the reactors contained 94-98% small flagellates. Percentage of flagellates decreased thereafter and virtually were washed out of all reactors, more quickly in reactors 3-5 than in reactors 1 and 2.

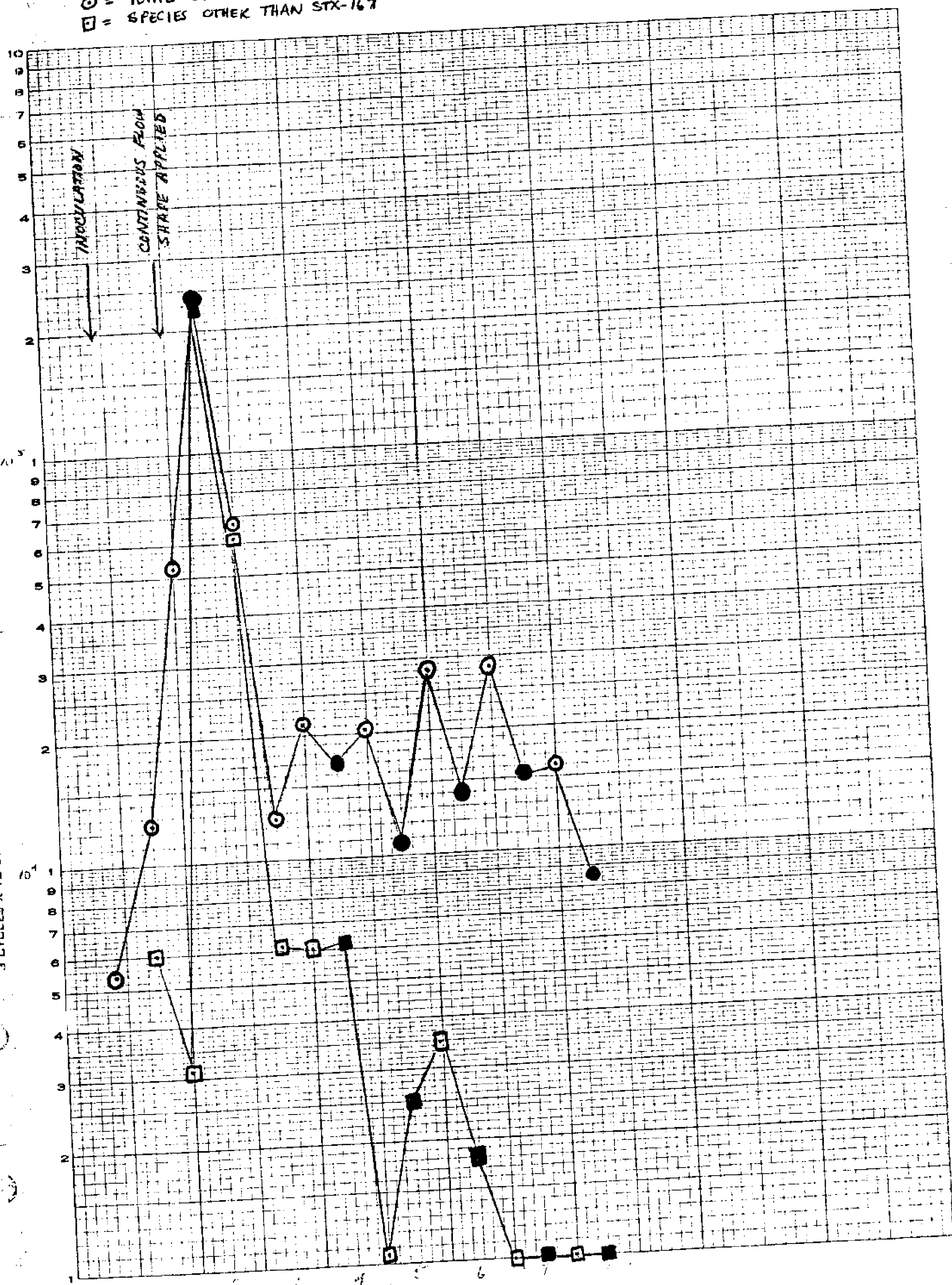
Mean chlorophyll a, phaeopigment, and acid ratio values are listed in table 2. Chlorophyll a/cell for this experiment and sunset values for the duplicate experiment described in the nutrient uptake section are shown in figure 2. Diurnal periodicity was suggested during days 5-7 for reactor 2 (46% of incident light intensity). The mean chlorophyll a/cell was highest at 20% I (figure 3).

Fig. 1. Cell densities of outdoor continuous cultures inoculated with Chaetoceros curvisetus (STX-167). Circles = total cell number; squares = number of cells other than STX-167. Darkened circles and squares represent samples taken at the end of the dark period.


⊙ = TOTAL CELL NO.
⊡ = SPECIES OTHER THAN STX-167
INOCULATION
CONTINUOUS FLOW, NO SHADE APPLIED
CELLS/ML
10^5
10^4
NO. 340-L312 DIETZGEN GRAPH PAPER
SEMI-LOGARITHMIC
3 CYCLES X 12 DIVISIONS PER INCH
EUGENE DIETZGEN CO.
MADE IN U. S. A.

12





⊙ = TOTAL CELL NO.
⊡ = SPECIES OTHER THAN STX-167
INOCULATION
CONTINUOUS FLOW
SHAPE APPLIED
NO. 340-L312 DIETZGEN GRAPH PAPER
SEMI-LOGARITHMIC
3 CYCLES X 12 DIVISIONS PER INCH
MADE IN U.S.A.

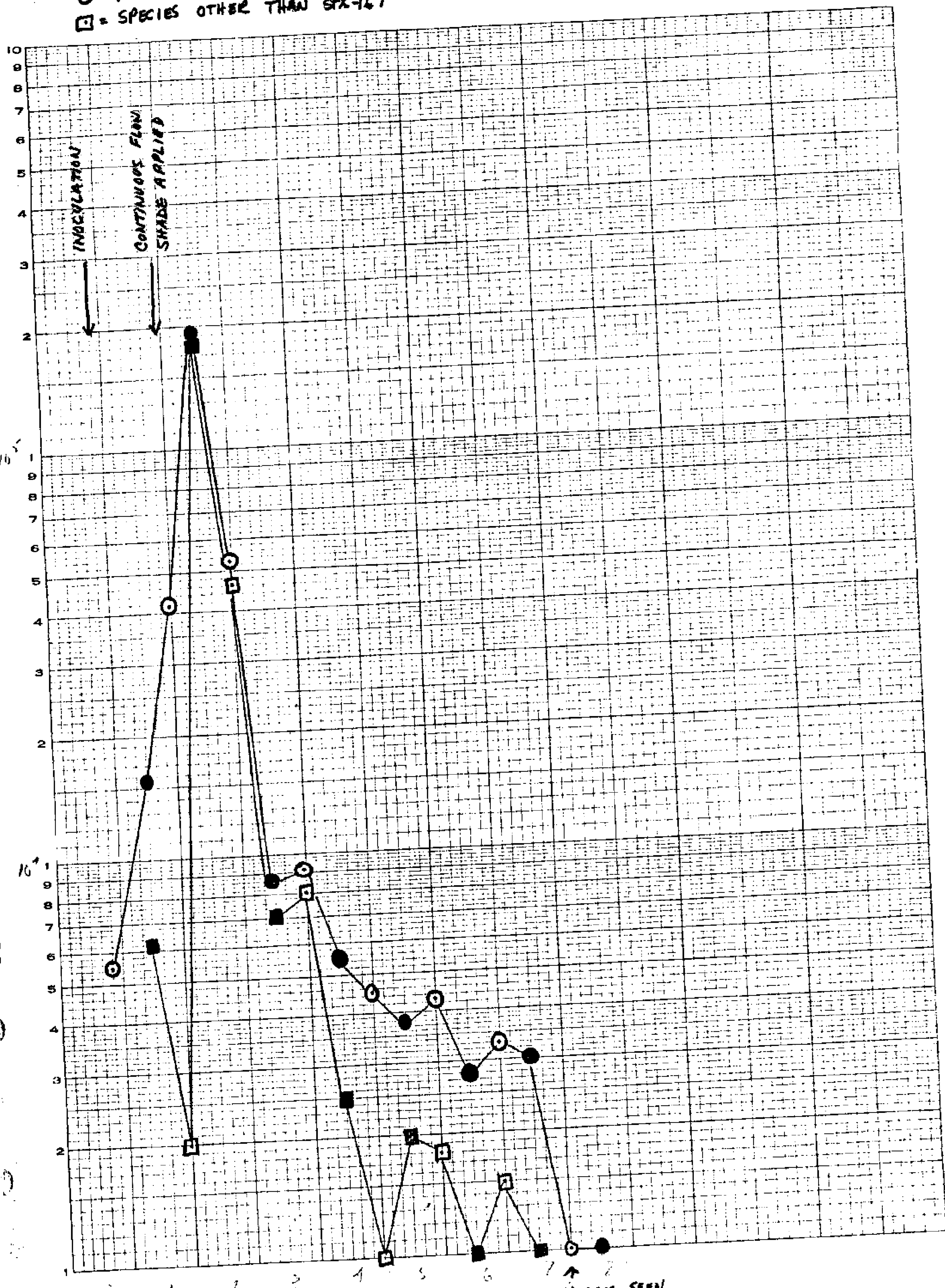
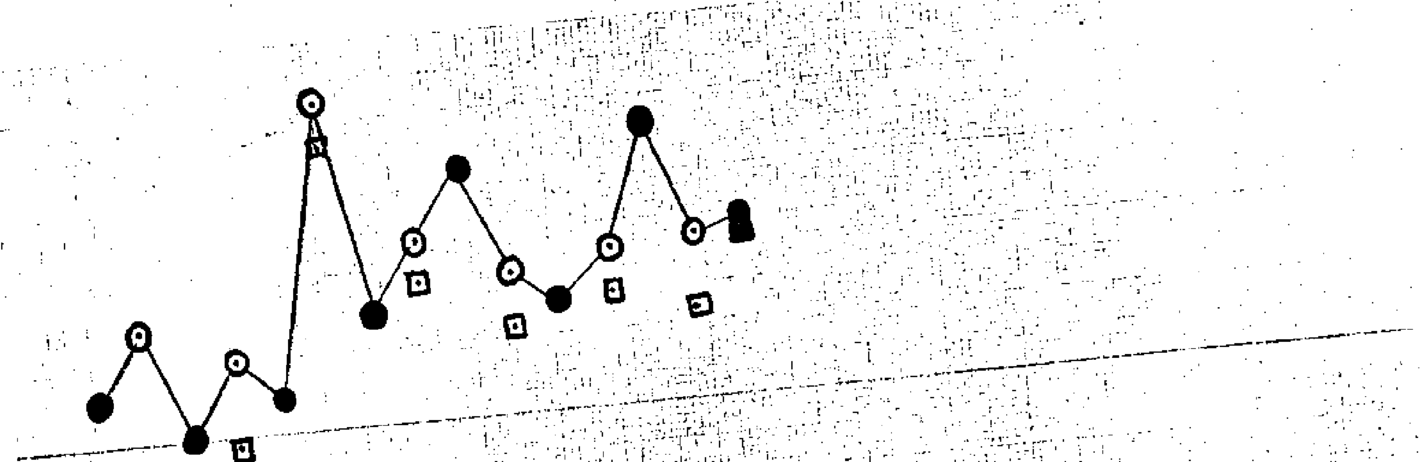
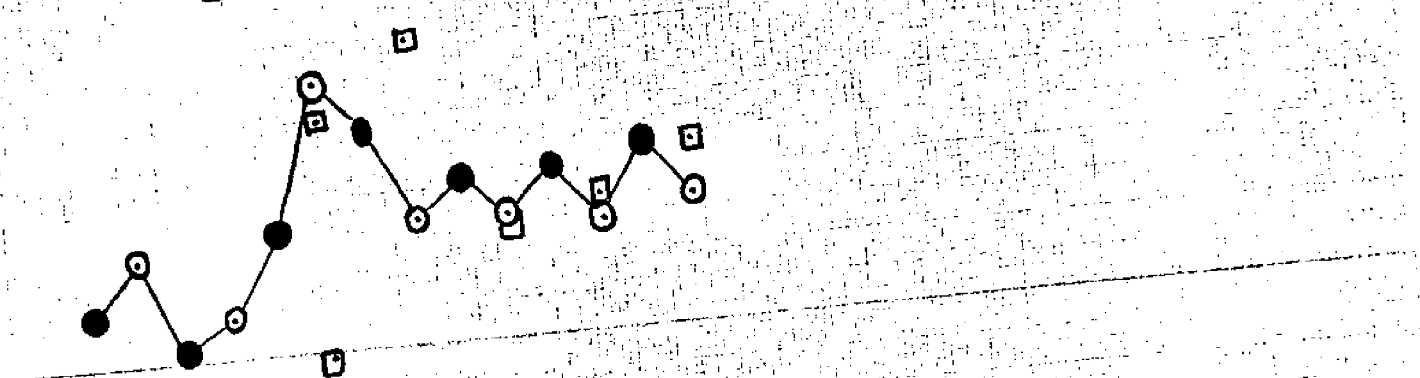
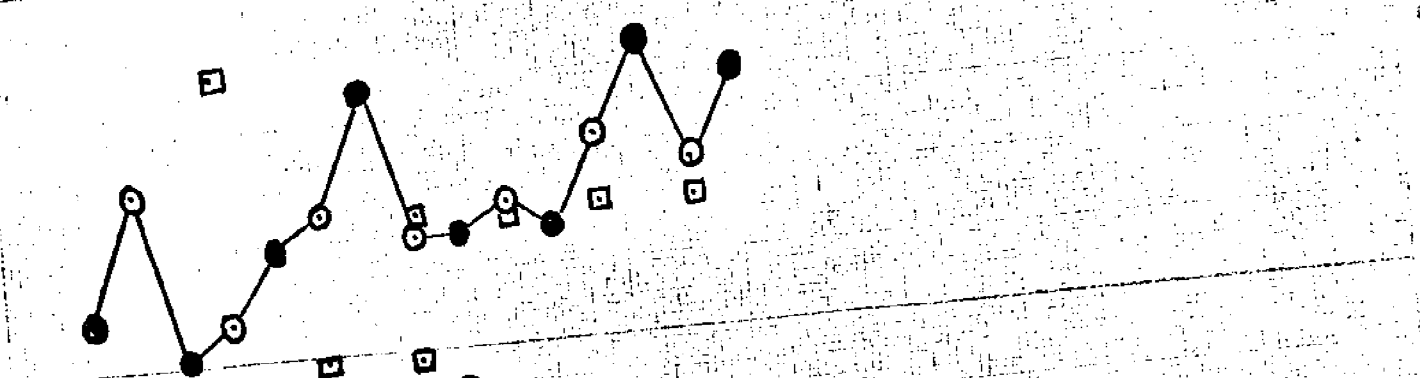

⊙= TOTAL CELL NO.
⊡ = SPECIES OTHER THAN STX-147
INOCULATION
CONTINUOUS FLOW
SHADE APPLIED
SEEN
NO. 340-L312 DIETZGEN GRAPH PAPER
SEMI-LOGARITHMIC
3 CYCLES X 12 DIVISIONS PER INCH
EUGENE DIETZGEN CO.
MADE IN U. S. A.

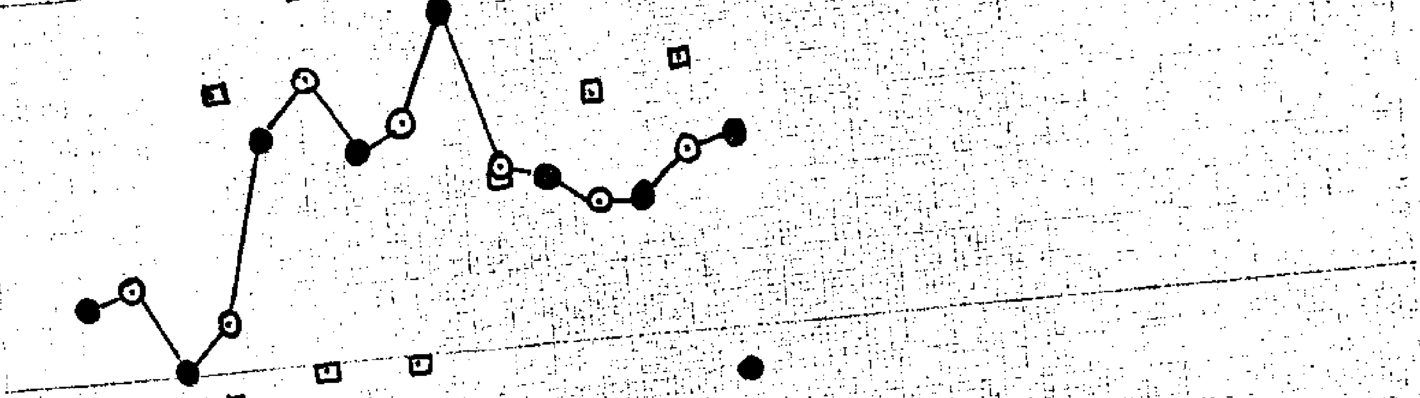
Fig. 2. Chlorophyll a/cell (µg chlorophyll a/10^7 cells) in outdoor continuous cultures of STX-167. Incident light intensities are given along the right-hand margin. Dilution rate was 1.2/day.

FIG 2









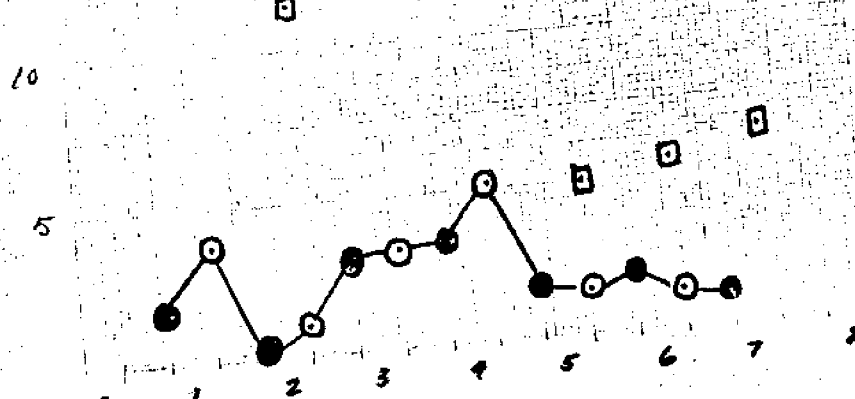
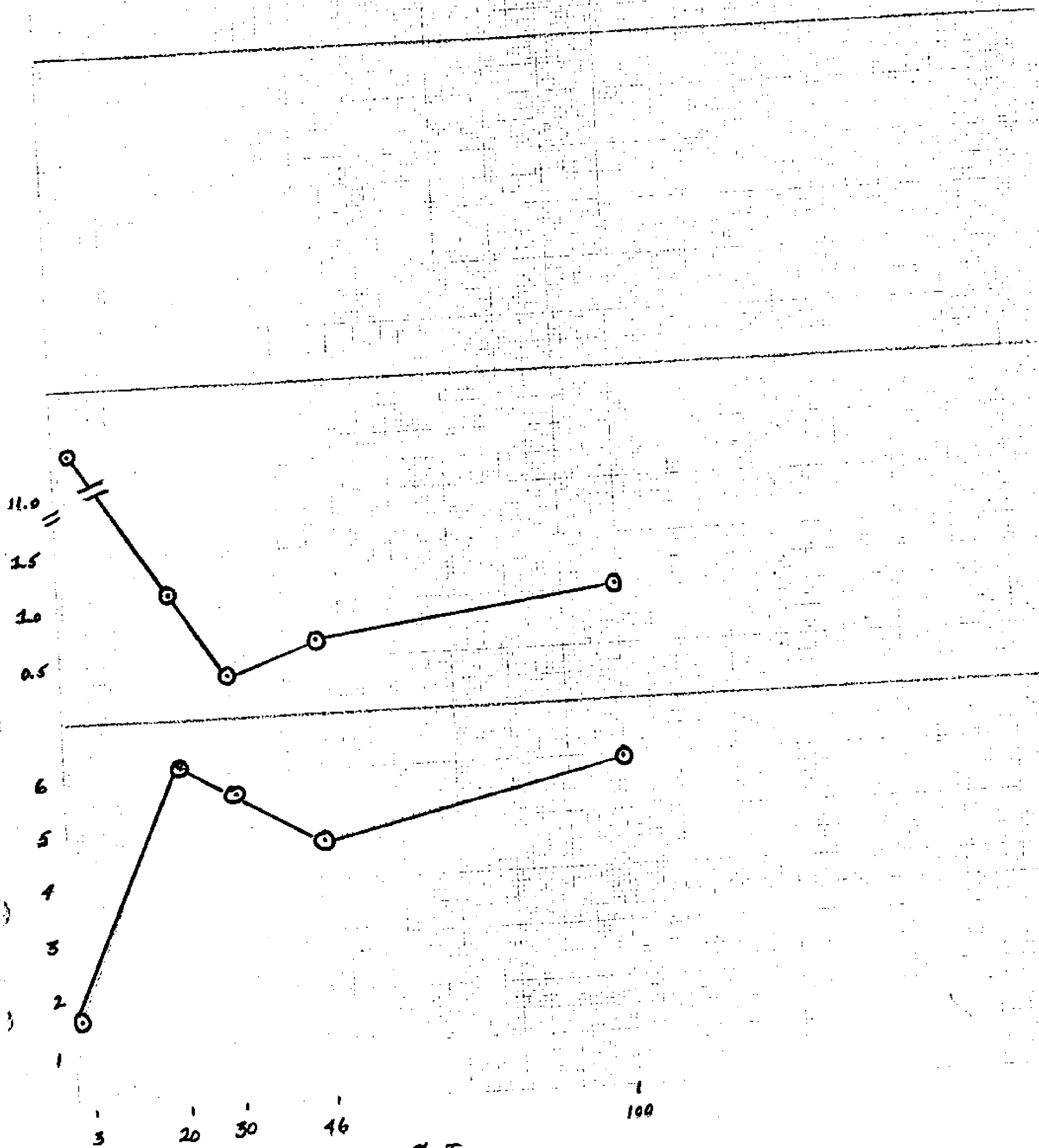


Fig. 3. Mean cellular composition of cells grown in outdoor continuous culture at a dilution rate of 1.20/day and at five different light intensities. Composition shown as a function of light intensity. (PN and POC values to be filled in when analyses complete)

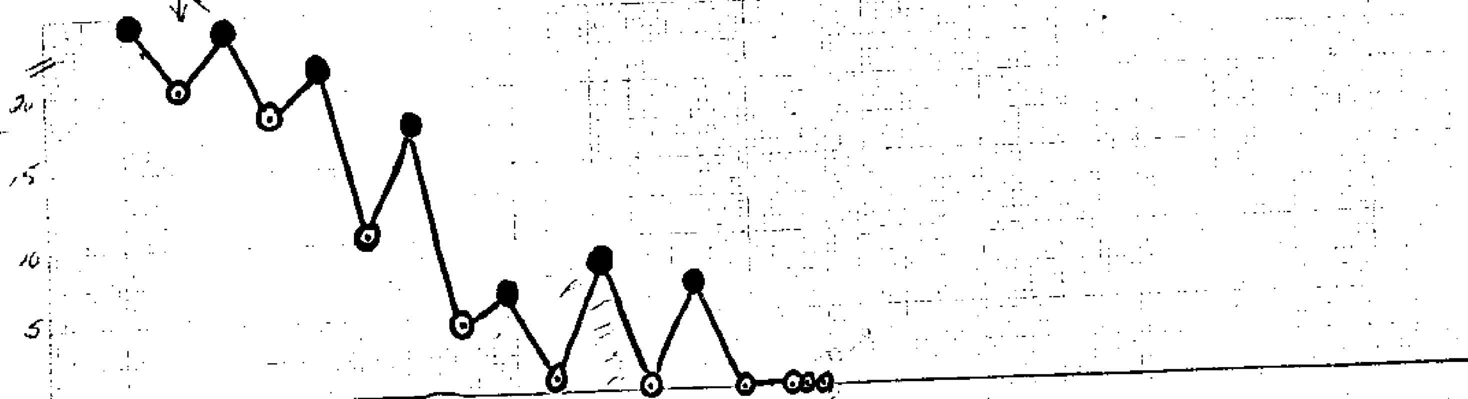
FIG 3

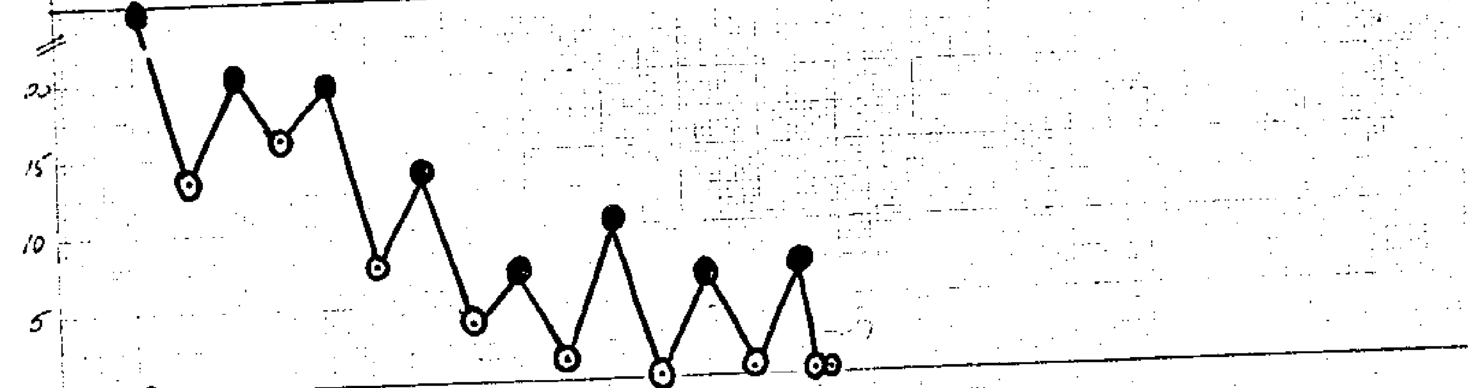


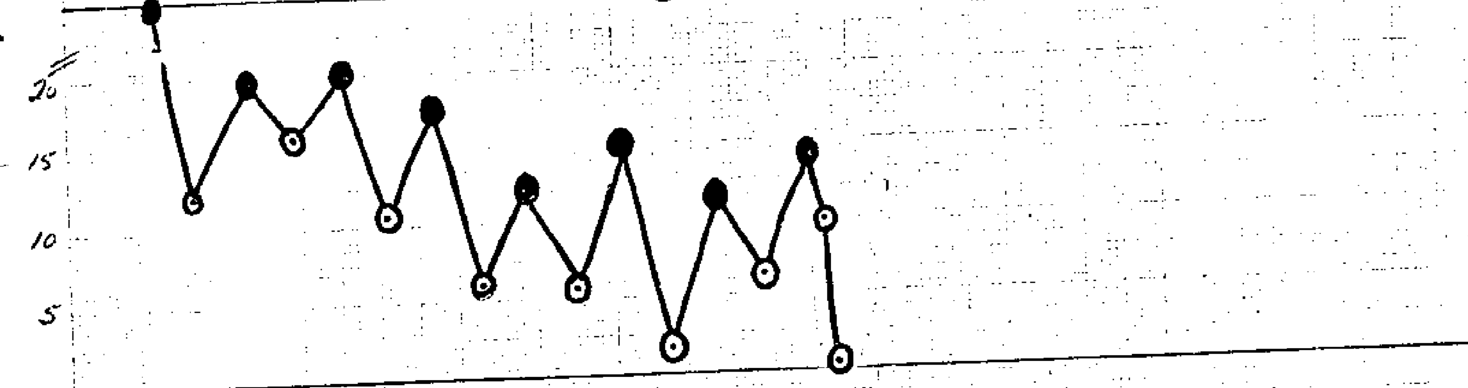
Dissolved nutrient concentrations are shown in figures 4-8. Nitrate showed a diel periodicity in all cultures but the most shaded. Concentrations approached the limit of detection by the end of the daylight period on days 5-7 for the first two reactors. Cellular nitrate (figure 9) showed periodicity best in reactors 1 and 3 and the concentration increased when cell numbers appeared to be washing out of the system (see figure 1).

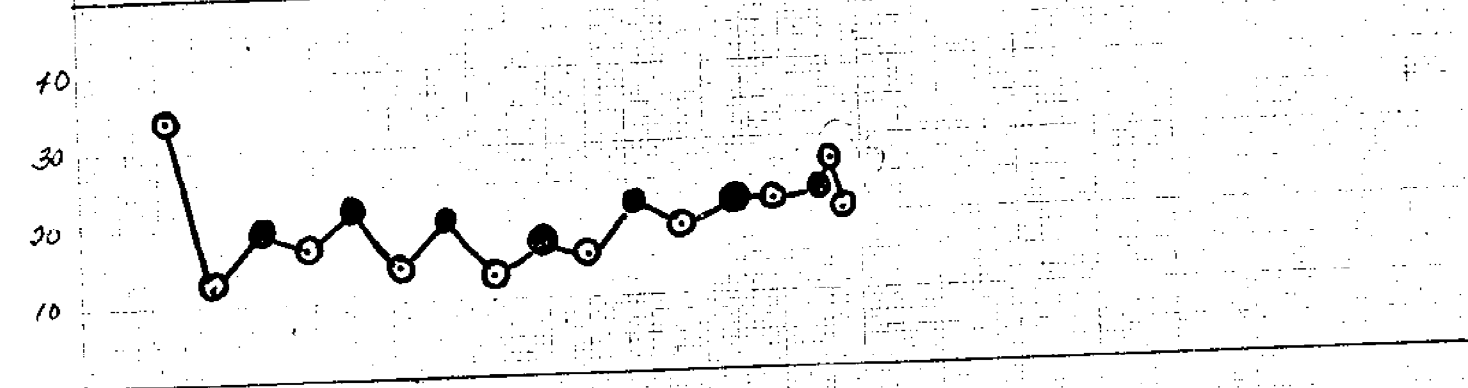
Fig. 4. Concentration of nitrate in the culture medium of five outdoor continuous cultures run at a dilution rate of 1.20/day and at five different light intensities.

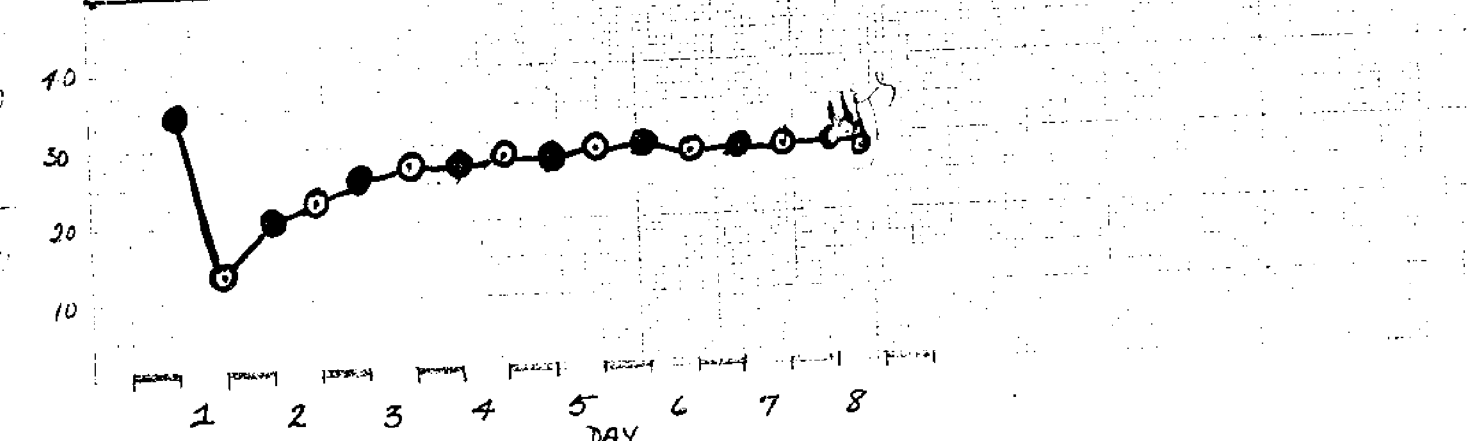
FIG 7







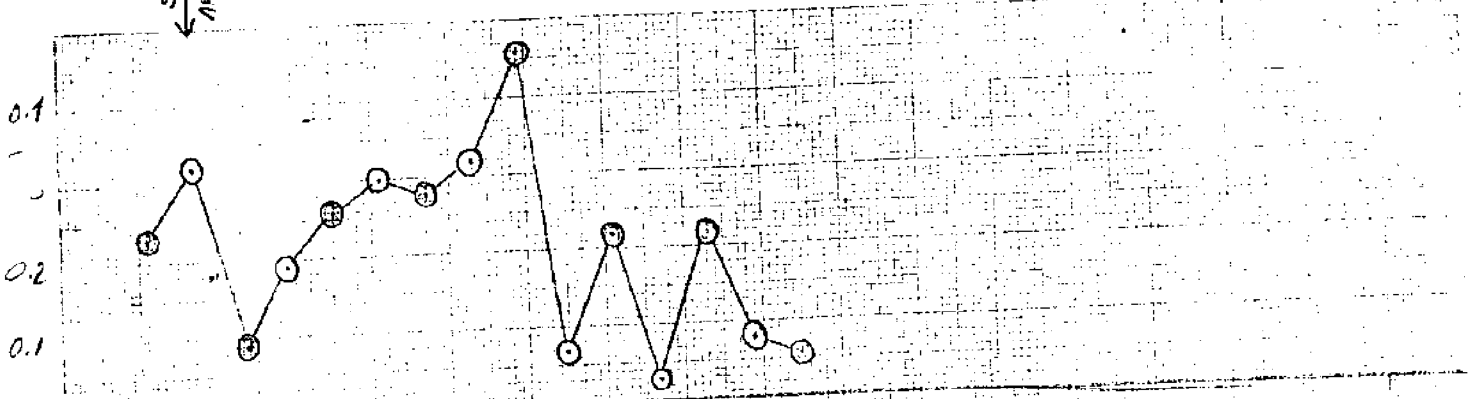


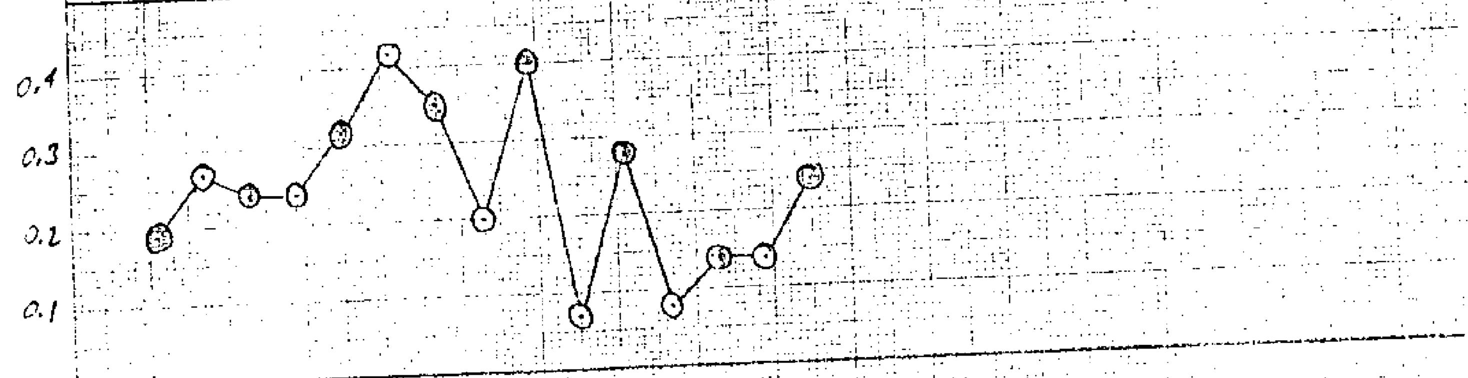


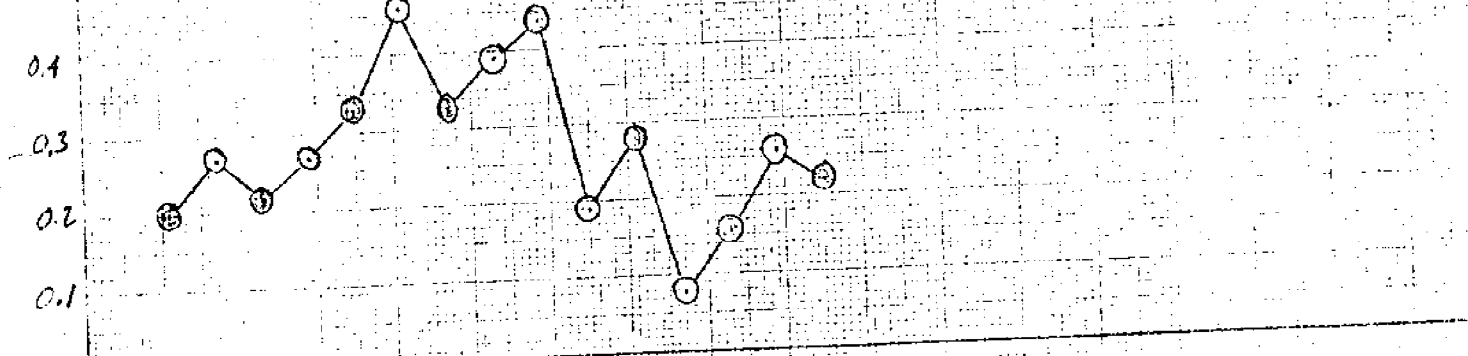
Nitrite and ammonia accumulated in the medium until continuous flow was begun (figures 5 and 6) while cellular nitrate concentration (figure 9) decreased and while nitrate was being removed from the medium. During days 5-7, nitrites, like nitrates, were higher at sunrise than at sunset for reactors 1-3. The trend was reversed in reactor 4, where mean nitrate concentration was greater than in reactors 1-3, and no trend was seen in reactor 5, where mean nitrate concentration was near that of deep water. In general, concentrations of nitrite and ammonia followed similar patterns in all cultures throughout the experiment.

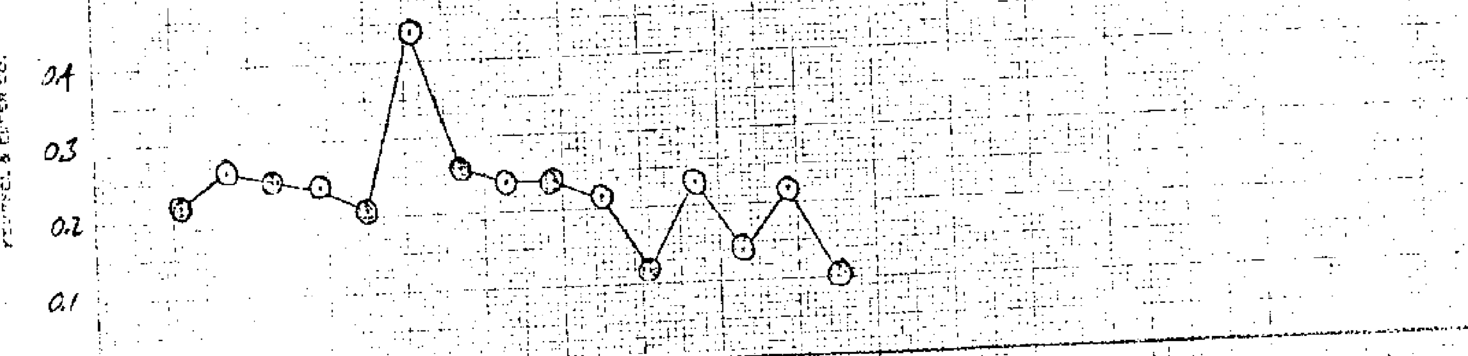
Fig. 5. Concentration of nitrite in the culture medium of five outdoor continuous cultures run at a dilution rate of 1.20/day and at five different light intensities.

FIG 5









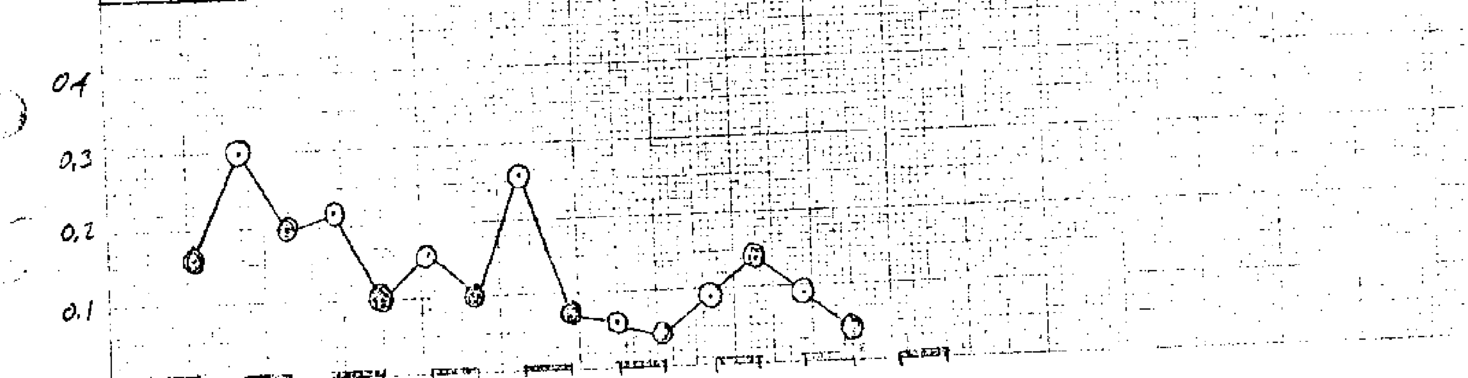


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Fig. 6. Concentration of ammonia in the culture medium of five outdoor continuous cultures run at a dilution rate of 1.20/day and at five different light intensities.

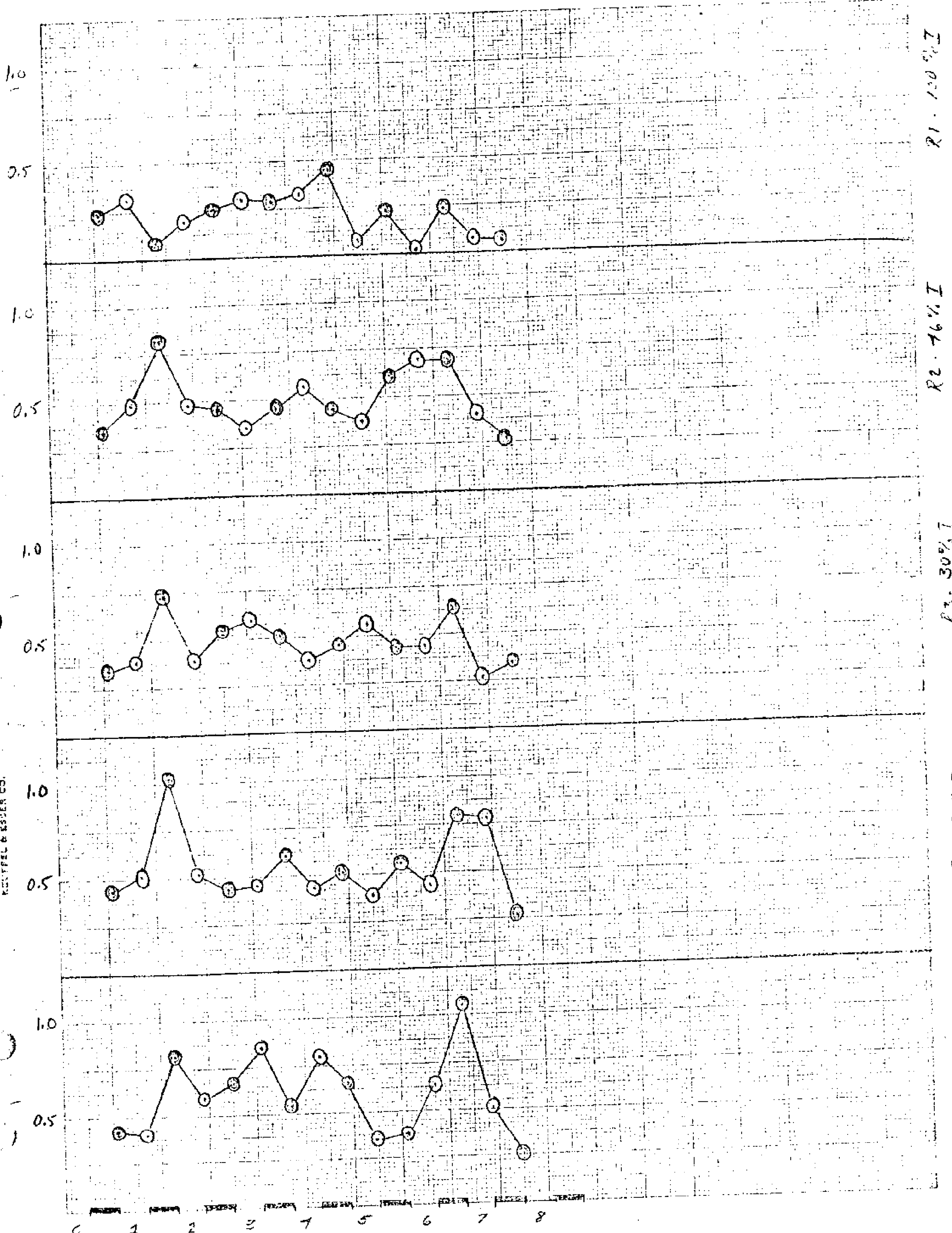


FIG 6
AMMONIA
R1 · 100% I
R2 · 46% I
R3 · 30% I
R4 · 20% I
R5 · 3% I
1.0
0.5
0
1
2
3
4
5
6
7
8

Dissolved phosphate was taken up in all reactors without periodic trends (figure 7). Concentration remained near deep water value in reactor 5.

Strong periodic trends in reactive silicate were seen in reactors 1-4 for most of the experiment and for days 6-7 in reactor 5 (figure 8).

Rates of uptake of nitrate and silicate in the batch culture at the end of the experiment are compared with rates of uptake as calculated during continuous flow in figure 10. Uptake per volume decreased with decreasing light. Uptake per cell, however, increased with decreasing light (figure 11).

Fig. 7. Concentration of phosphate in the culture medium of five outdoor continuous cultures run at a dilution rate of 1.20/day and at five different light intensities.

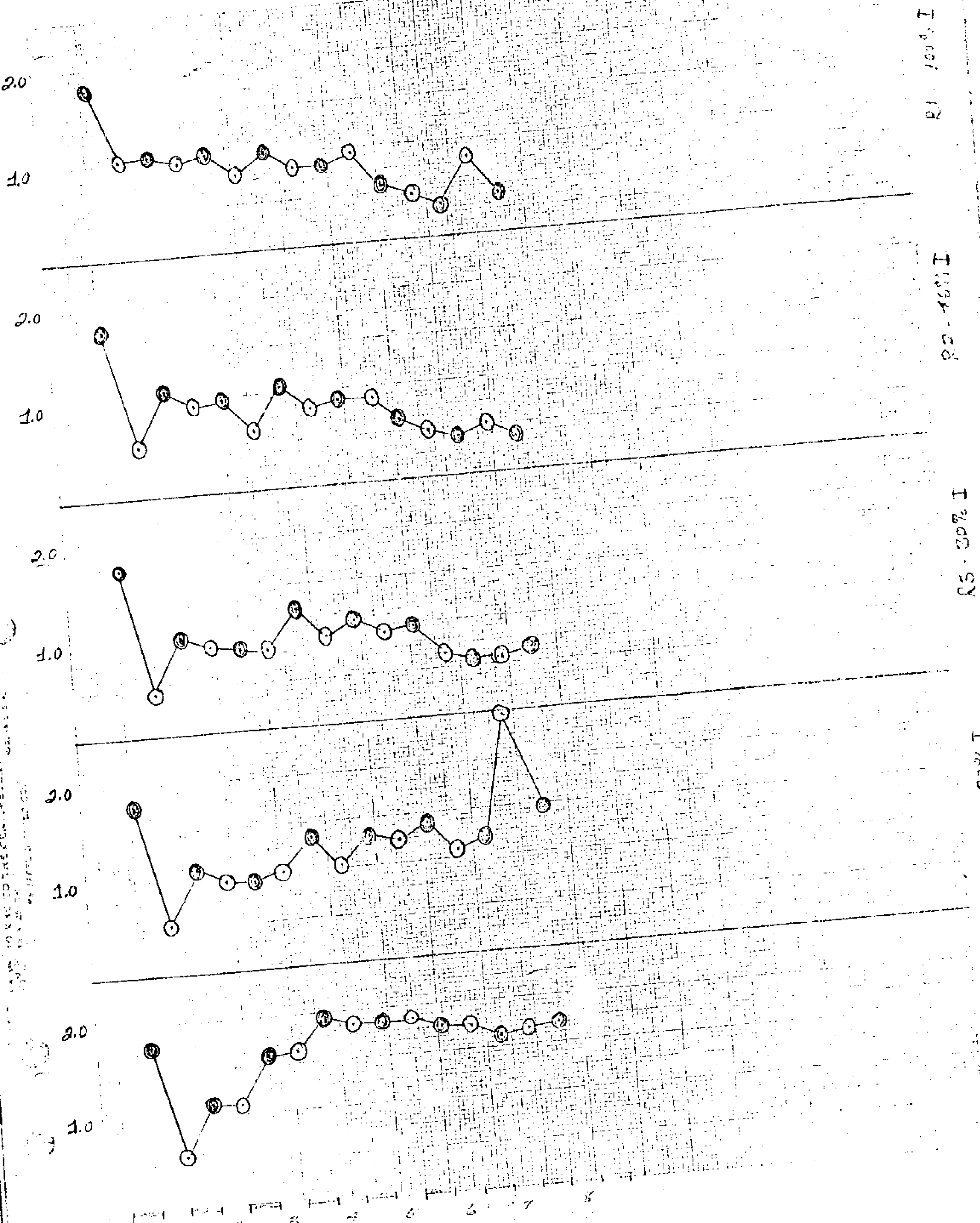
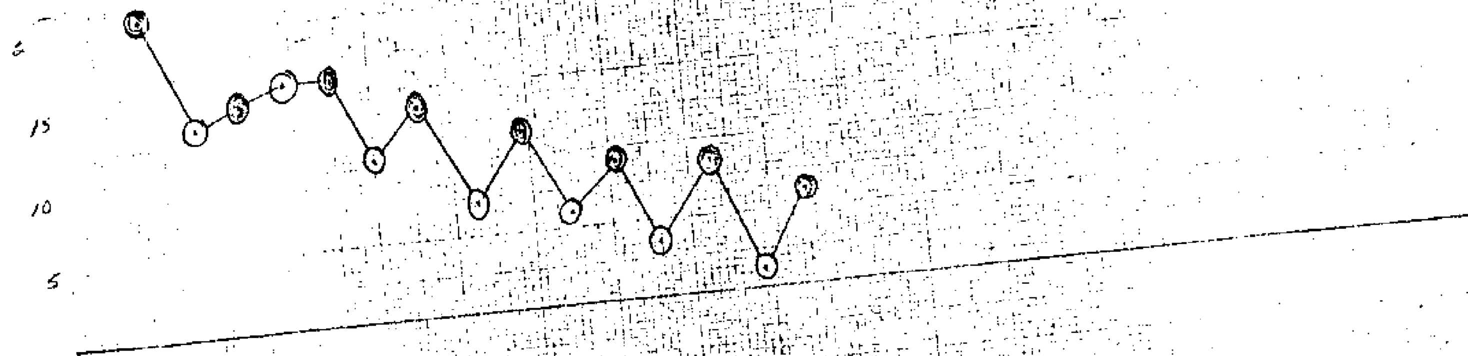
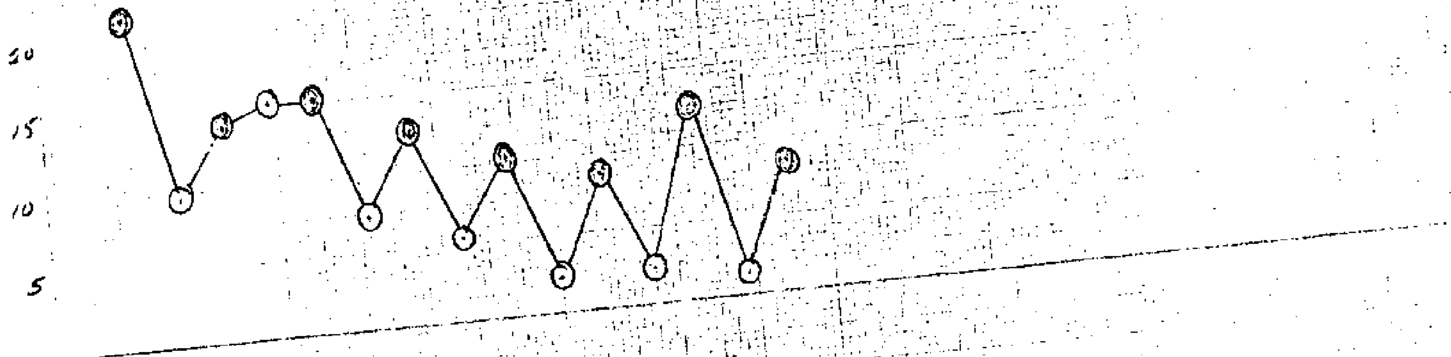
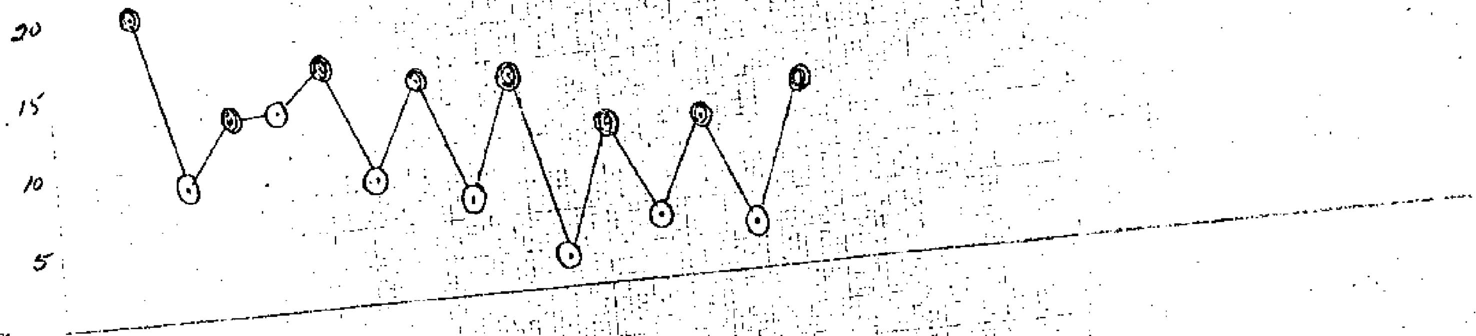

Phosphates
1.2 dilution rate
FIG 7
2.0
1.0
R1 100% I
2.0
1.0
R2 40% I
2.0
1.0
R3 30% I
2.0
1.0
R4 20% I
2.0
1.0
7
8

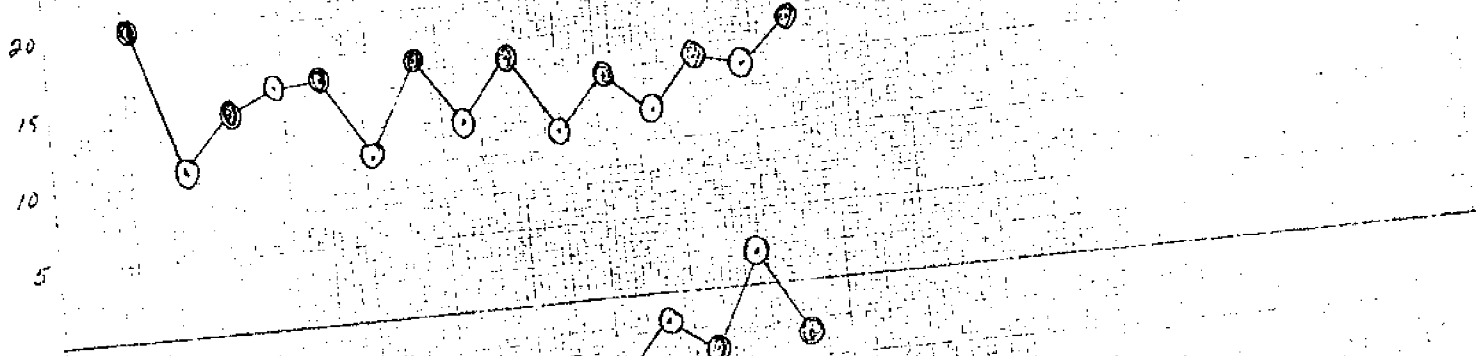
Fig. 8. Concentration of reactive silicate in the culture medium of five outdoor continuous cultures run at a dilution rate of 1.20/day at five different light intensities.

FIG 8 SILICATES









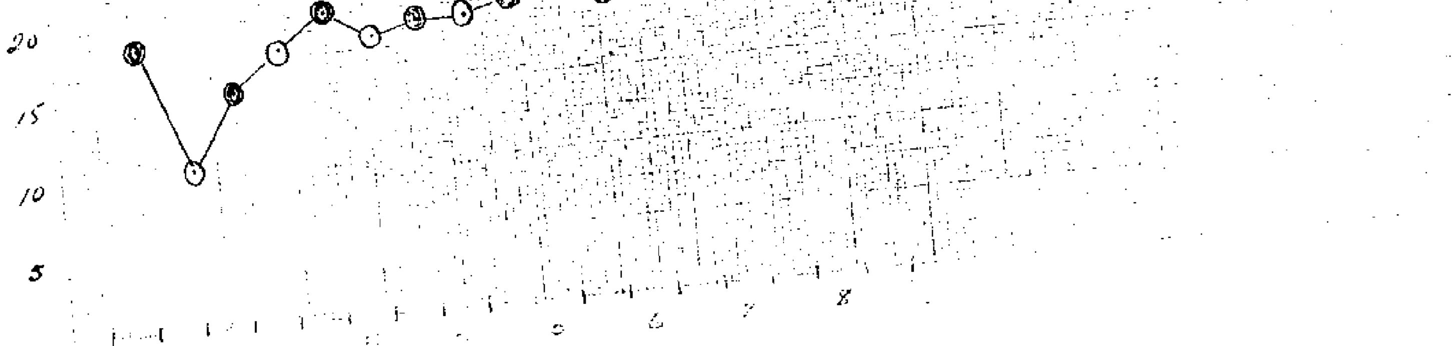


Fig. 9. Cellular nitrate concentration (µg-at NO_3-N/10^9 cells) in outdoor continuous cultures run at 1.20/day dilution rate and at five degrees of light intensity.

FIG 9

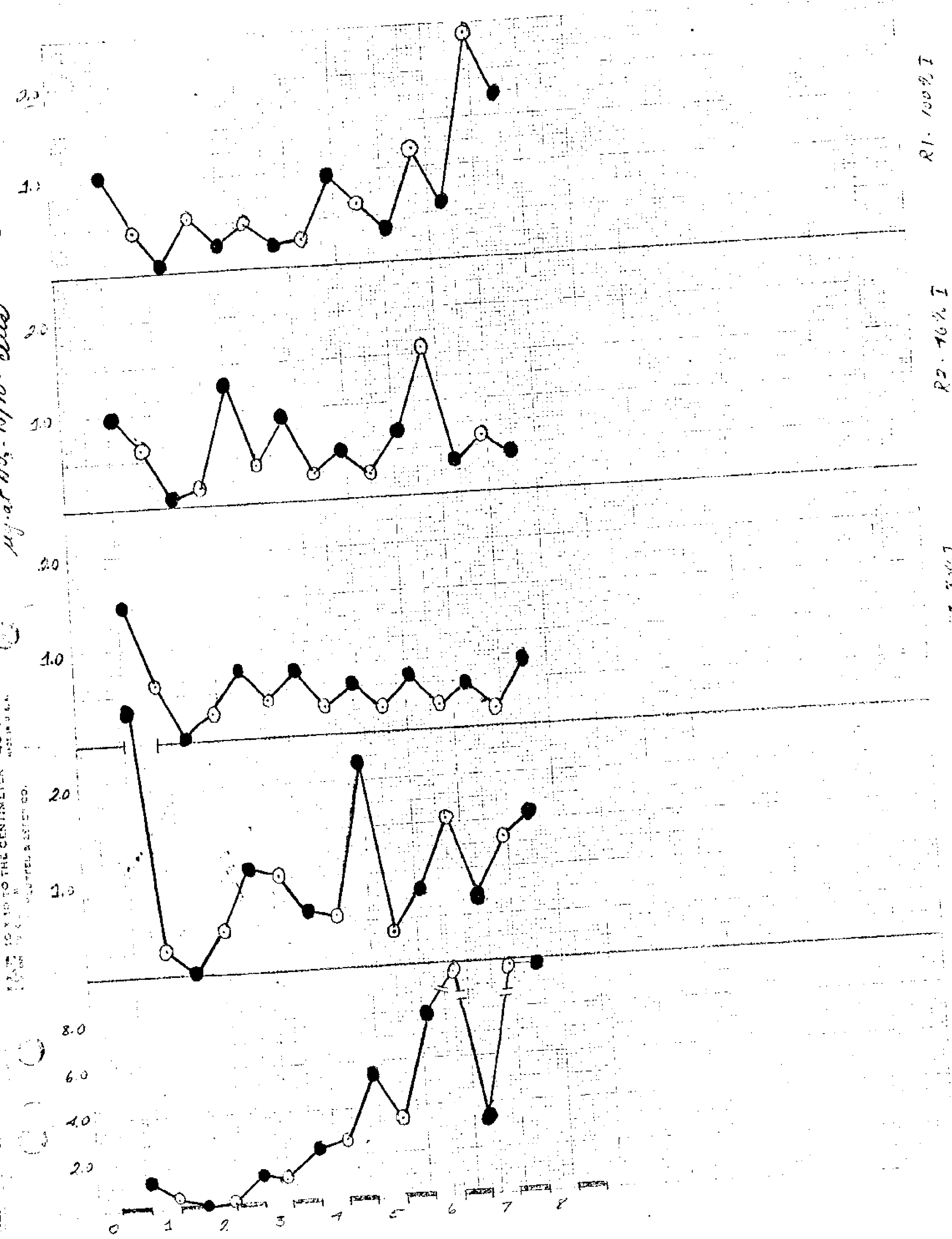
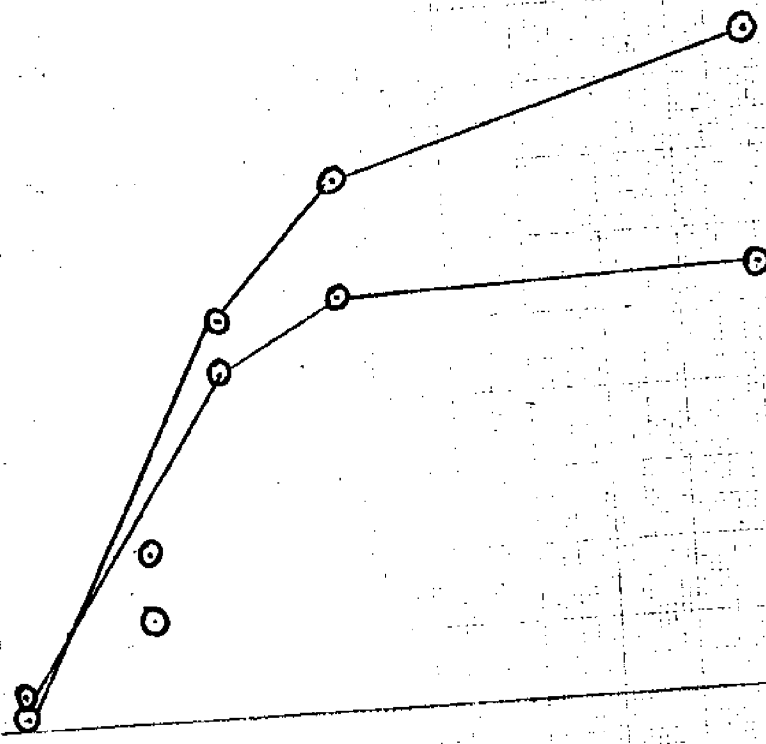


Fig. 10. Uptake rates of nitrate (upper) and silicate (lower) as a function of light intensity. Open squares and triangles = uptake rates calculated from the batch culture experiment run on day 8. Closed squares and triangles = rates calculated from concentrations in the medium during days 5-7 of continuous flow.

UPTAKE OF NO_3 AND SiO_4 AS A FUNCTION OF INCIDENT LIGHT INTENSITY

⊙ = BASE UPTAKE
⊙ = CONTINUOUS UPTAKE



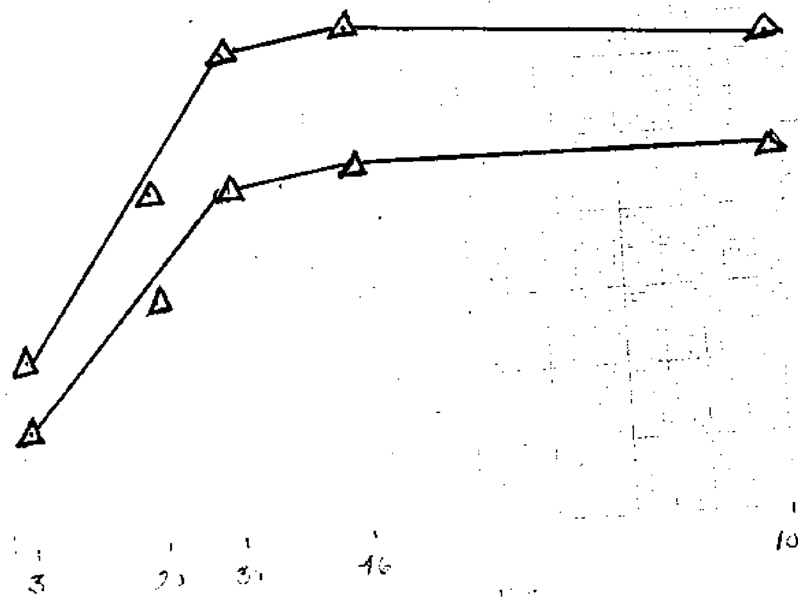


Fig. 11. Uptake per cell of nitrate (upper) and silicate (lower) as a function of light intensity. Open and closed squares and triangles represent conditions described in legend to figure 10.

UPTAKE/CELL OF NO_3 AND SiO_4
AS A FUNCTION OF INCIDENT LIGHT INTENSITY

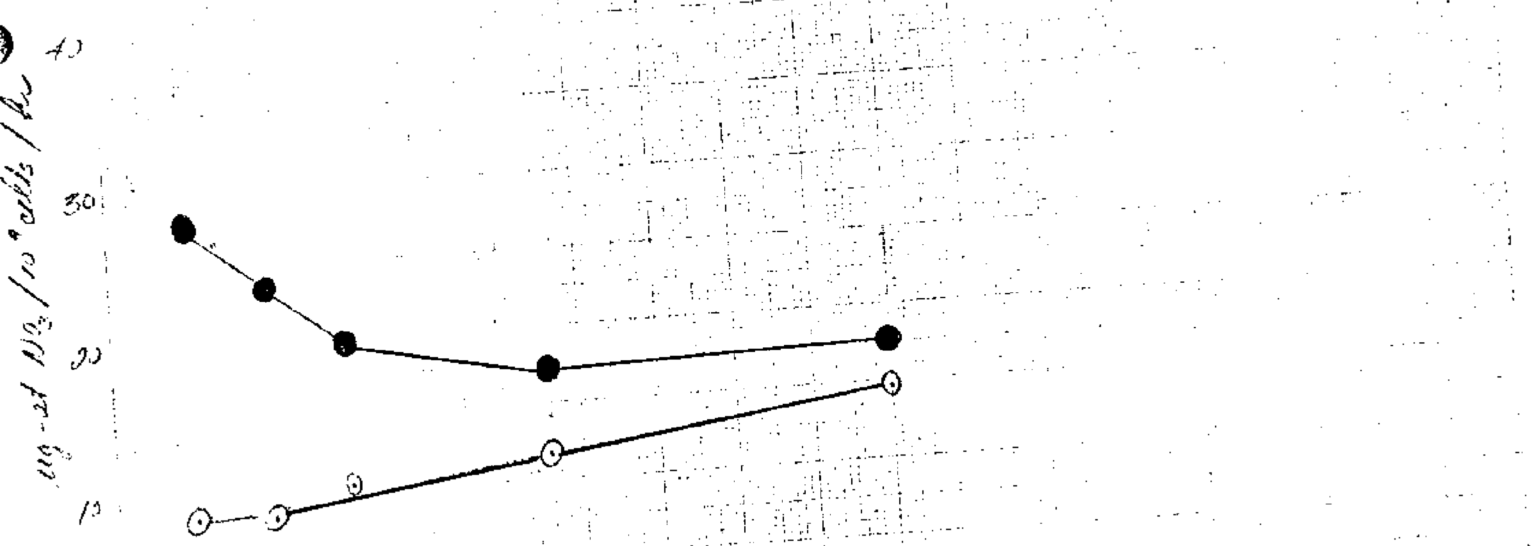


FIG 11

Incubation of glass stoppered bottles suspended in reactor cultures had similar rates of carbon uptake to bottles incubated in the trough except at 100% incident light, where temperature differences may have been influential (figure 12). Temperature in the trough (23° C) was close to the mean for the shaded reactors (24.5° C) but was much cooler than the temperature in the unshaded reactor (26.8° C) at the time of incubation.

Uptake of radioactive carbon per unit volume and per unit chlorophyll a was linear with degree of shading in reactors 2-5 (figure 13). Uptake appeared to be inhibited at 100% incident light.

Fig. 12. Uptake of radioactive carbon by samples incubated in reactors compared to samples incubated in acrylic tubes in a trough. See text for details.

Incubation in

⊡ =

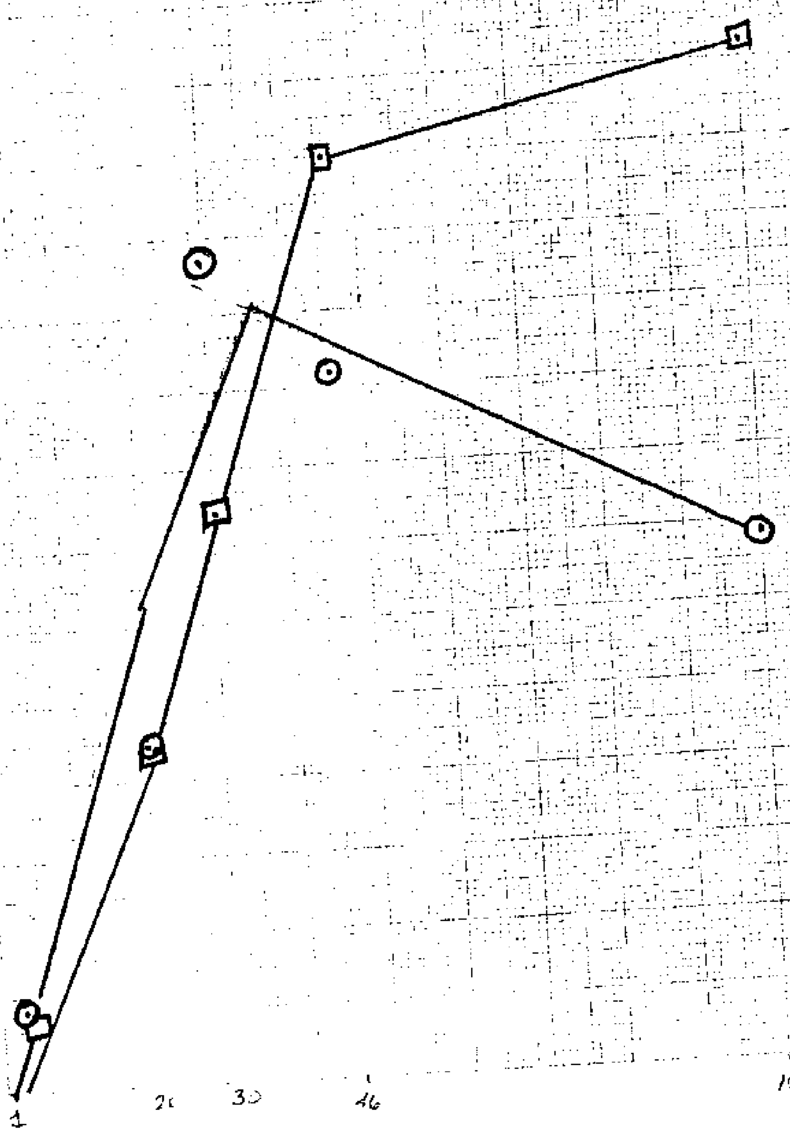


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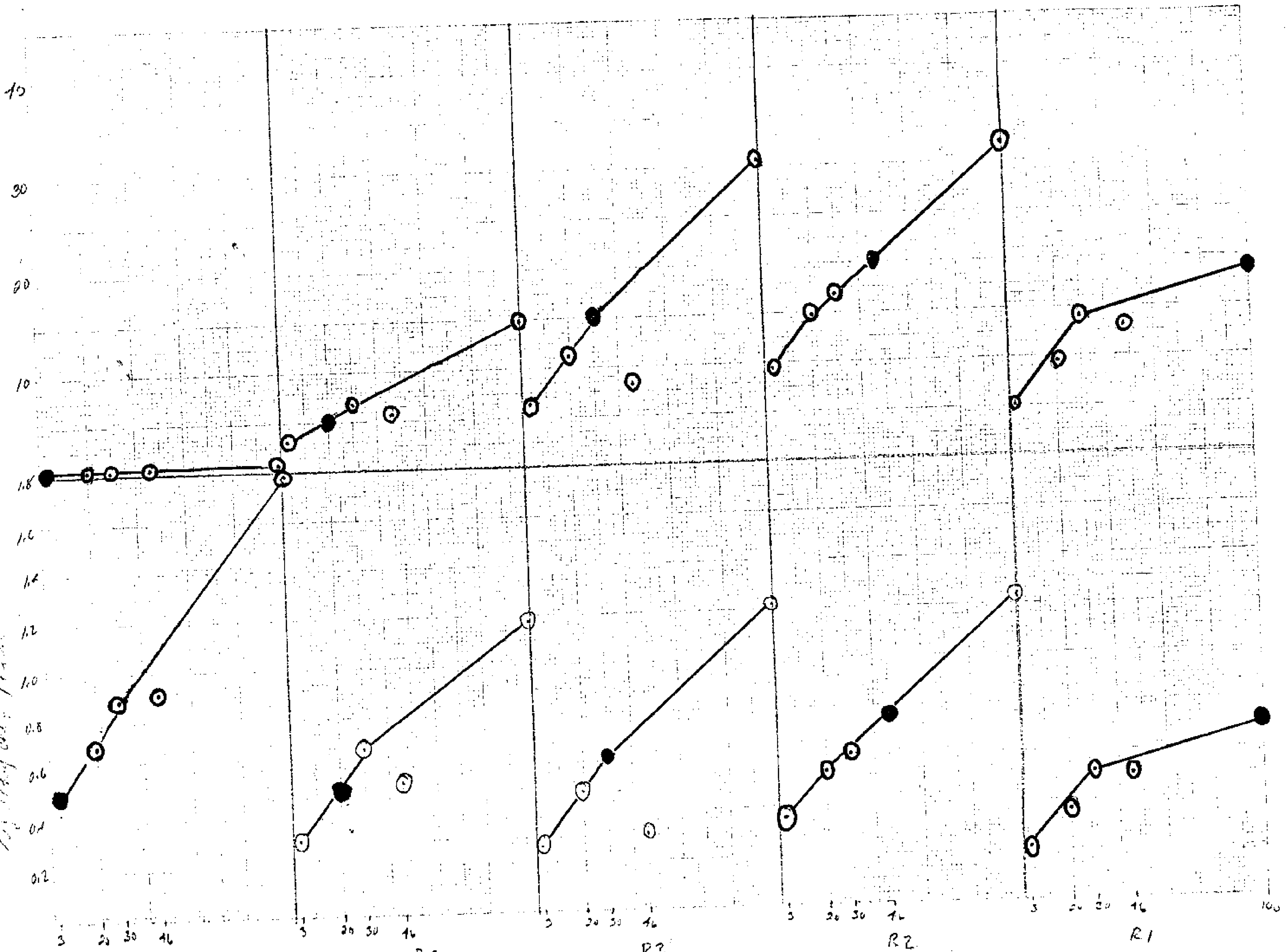
Fig. 13. Rate of uptake of carbon by a volume of culture (P vs I curves) and by a unit of chlorophyll *a* (assimilation numbers) as a function of incident light intensity.


1:20 dilution rate
mean incubation I = 0.4
10 X 10 TO THE CENTIMETER
KEUFFEL & ESSER CO.
40
30
20
10
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
3 20 30 46
R4
3 20 30 46
R3
3 20 30 46
R2
3 20 30 46 100
R1

Discussion

At a dilution rate of 1.2 in outdoor continuous culture of STX-167 the following observations were made:

(1) Diatoms were maintained in all but the most shaded culture at this flow rate whereas flagellates tended to wash out of all cultures. This is in contrast to the findings at a dilution rate of 0.2/day, where flagellates tended numerically to dominate the cultures.

Biomass, as measured by cell density and chlorophyll a, was greatest at 46% of incident light and least at 3% of incident light.

(2) No inhibition of uptake of nutrients was noted at 100% incident light. Uptake of ^{14}C appeared to be inhibited in the cultures grown at incident light although cells grown in shaded cultures and incubated in 100% incident light showed no evidence of inhibition. The incubation was conducted, however, on a very overcast day.

(3) The shape of the P vs I curves suggest that uptake of ^{14}C was light limited in all shaded cultures, perhaps because the day was overcast. Uptake of nitrate and silicate by cultures shaded to 30% or less of incident light appeared to be light-limited throughout the experiment. Uptake per cell of these nutrients increased at the lower intensities, however, so the low volume uptake was probably due to low cell numbers.

Since uptake of carbon was the only cellular rate measured that decreased with decreasing light, and since cell number also decreased with decreasing light, it would appear that cell division is more closely linked to uptake of carbon than to uptake of inorganic nutrients.

(4) Apparent excretion of nitrite after inoculation and the simultaneous uptake of nitrate while cellular nitrate decreased -- as observed in the previous experiment, was confirmed. Ammonia appeared to be excreted at the same time.

On the basis of these two experiments one might predict that:

(A) Increase in nutrient supply cannot prevent light inhibition of ^{14}C uptake although it can prevent ~~inhibition of~~ light inhibition of nutrient uptake. An experiment at the fastest possible flow rate for this species (1.4/day) should confirm this finding.

(B) Specific rate of uptake of ^{14}C at a given I may actually decrease with increased nutrient supply rate because of the correlated decrease in residence time in the culture vessel. An experiment at a dilution rate intermediate between these two experiments (0.70/day) should verify this supposition.

APPENDIX III

WATER CHEMISTRY : NITRATE, NITRITE, AMMONIA, PHOSPHATE, SILICATE AND SALINITY DETERMINATIONS IN ST. CROIX WATER SAMPLES

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WATER CHEMISTRY : NITRATE, NITRITE, AMMONIA, PHOSPHATE, SILICATE AND SALINITY DETERMINATIONS IN ST. CROIX WATER SAMPLES

STX REF 1085	DATE SAMPLED	NO_3+NO_2 (µgat N/l)	NO_2 (µgat N/l)	NH_3 (µgat N/l)	PO_4 (µgat P/l)	SiO_4 (µgat Si/l)	SALINITY (°/oo)	PIPE NO.
429	01-03-74	31.61	0.16±0.01	0.46±0.25	2.21	18.31±0.50	34.846±.004	1
430		31.56	0.16	0.63	2.15	18.36	34.851	1
431		32.18	0.18	0.52	2.25	20.25	34.831±.006	2
432		32.08	0.17	0.54	2.23	20.40	34.840	2
433		31.68	0.19	0.63	2.16	18.42	34.829±.004	3
434		31.83	0.19	0.67	2.20	18.46	34.834	3
435	01-10-74	31.69	0.23±0.04	0.69±0.16	2.18±0.07	18.28±0.06	34.889±.006	1
436		31.88	0.21	0.60	2.10	18.23	34.880	1
437		32.44	0.21	0.79	2.19	19.88	34.834±.002	2
438		32.37	0.24	0.60	2.22	19.75	34.831	2
439		32.21	0.19	0.60	2.15	17.91	34.788±.010	3
440		31.67	0.23	0.67	2.16	18.24	34.803	3
441	01-17-74	31.94	0.23	0.69	2.14	17.30	34.889±.010	1
442		31.64	0.23	0.67	2.16	17.32	34.874	1
443		32.94	0.24	0.69	2.27	19.56	34.831±.006	2
444		32.82	0.23	0.69	2.23	19.36	34.840	2
445		32.27	0.23	0.79	2.21	18.12	34.834±.004	3
446		32.21	0.26	0.84	2.17	17.90	34.840	3
447	01-24-74	31.33±0.34	0.24±0.06	0.60±0.32	2.20	19.33±0.25	34.889±.012	1
448		31.35	0.19	0.60	2.21	19.04	34.907	1
449		32.06	0.18	0.51	2.28	20.80	34.858±.006	2
450		32.01	0.18	0.63	2.24	20.68	34.869	2
451		31.72	0.18	0.62	2.20	14.84	34.884±.004	3
452		32.03	0.18	0.55	2.20	19.39	34.878	3

WATER CHEMISTRY : NITRATE, NITRITE, AMMONIA, PHOSPHATE, SILICATE AND SALINITY DETERMINATIONS IN ST. CROIX WATER SAMPLES

STX REF 1085	DATE SAMPLED	NO_3+NO_2 (µgat N/l)	NO_2 (µgat N/l)	NH_3 (µgat N/l)	PO_4 (µgat P/l)	SiO_4 (µgat Si/l)	SALINITY (‰)	PIPE NO.
453	01-31-74	30.34	0.19	0.63	2.12±0.08	16.44	34.915	1
454		30.88	0.21	0.70	2.16	16.89	34.909	1
455		32.50	0.19	0.71	2.25	20.20	34.866	2
456		32.47	0.17	0.82	2.24	20.17	34.872	2
457		30.20	0.18	0.73	2.14	16.56	34.892±.012	3
458		30.30	0.17	0.78	2.15	17.74	34.907	3
459	02-07-74	31.04	0.18	0.75	2.19	17.22	34.847±.006	1
460		31.49	0.18	0.70	2.19	17.25	34.838	1
461		32.24	0.18	0.77	2.28	20.95	34.809	2
462		32.03	0.16	0.82	2.28	20.66	34.809	2
463		31.48	0.16	0.72	2.23	19.12	34.818±.004	3
464		31.78	0.18	0.97	2.25	19.15	34.812	3
465	02-14-74	31.50±0.52	0.21±0.05	0.81±0.09	2.27±0.04	17.18	34.852±.010	1
466		31.79	0.25	1.98(?)	2.26	19.05	34.838	1
467		32.57	0.18	0.80	2.36	21.12	34.809±.006	2
468		31.84	0.19	0.83	2.33	21.14	34.818	2
469		31.89	0.24	0.79	2.28	19.11	34.809±.002	3
470		31.09	0.21	1.17	2.22	18.83	34.812	3
471	02-21-74	31.09	0.21	0.94	2.28	18.21	34.887±.006	1
472		30.97	0.20	0.97	2.27	18.10	34.879	1
473		32.19	0.23	0.82	2.33	20.84	34.867±.004	2
474		32.22	0.20	0.89	2.30	20.80	34.862	2
475		31.64	0.22	1.14	2.29	18.65	34.867	3
476		31.39	0.22	0.88	2.27	18.70	34.863	3

WATER CHEMISTRY : NITRATE, NITRITE, AMMONIA, PHOSPHATE, SILICATE AND SALINITY DETERMINATIONS IN ST. CROIX WATER SAMPLES

STX REF 1085	DATE SAMPLED	NO_3+NO_2 (μgat N/1)	NO_2 (μgat N/1)	NH_3 (μgat N/1)	PO_4 (μgat P/1)	SiO_4 (μgat Si/1)	SALINITY (°/oo)	PIPE NO.
477	02-28-74	30.92	0.19	0.85	2.30	17.75	34.885	1
478		30.68	0.23	1.08	2.22	17.55	34.879	1
479		32.09	0.23	0.87	2.42	20.20	34.885±.008	2
480		32.68	0.23	0.92	2.32	20.35	34.873	2
481		31.41	0.25	0.89	2.28	18.50	34.862±.004	3
482		31.22	0.18	0.98	2.28	18.50	34.867	3
483	03-07-74	29.76±0.52	0.26±0.05	0.85±0.09	2.15±0.04	18.56±0.25	34.825±.006	1
484		32.15	0.22	1.08	2.32	18.70	34.819	1
485		31.74	0.24	0.87	2.41	20.94	34.802±.002	2
486		31.18	0.27	1.22	2.48	21.10	34.805	2
487		31.96	0.23	0.97	2.28	20.07	34.805	3
488		31.57	0.24	1.06	2.29	19.10	34.805	3
489	03-14-74	31.31±0.15	0.20±0.04	0.35±0.15	2.30	18.55±0.27	34.856±.002	1
490		31.31	0.21	0.33	2.29	18.23	34.859	1
491		31.49	0.20	0.33	2.38	21.32	34.859±.004	2
492		31.46	0.21	0.41	2.73(?)	21.12	34.853	2
493		31.27	0.20	0.40	2.33	18.01	34.850±.010	3
494		31.27	0.23	0.43	2.52	18.20	34.836	3
495	03-21-74	31.07	0.24	0.14	2.28	18.74	34.859	1
496		31.27	0.22	0.49	2.30	18.94	34.859	1
497		31.51	0.24	0.42	2.35	21.21	34.830±.010	2
498		31.82	0.20	0.46	2.41	20.42	34.844	2
499		31.62	0.23	0.82	2.34	19.28	34.839±.006	3
500		32.07	0.22	0.41	2.41	19.23	34.830	3

WATER CHEMISTRY : NITRATE, NITRITE, AMMONIA, PHOSPHATE, SILICATE AND SALINITY DETERMINATIONS IN ST. CROIX WATER SAMPLES

STX REF 1085	DATE SAMPLED	NO_3+NO_2 (µgat N/l)	NO_2 (µgat N/l)	NH_3 (µgat N/l)	PO_4 (µgat P/l)	SiO_4 (µgat Si/l)	SALINITY (°/oo)	PIPE NO.
501	03-29-74	31.52	0.25	0.39	2.33	18.79	34.873±.010	1
502		31.62	0.21	0.38	2.37	18.89	34.887	1
503		31.72	0.21	0.45	2.40	20.11	34.830±.006	2
504		31.69	0.21	0.44	2.44	20.09	34.839	2
505		31.92	0.24	0.44	2.38	18.84	34.859±.002	3
506		31.74	0.21	0.45	2.35	18.83	34.862	3
507	04-04-74	31.74	0.23	0.44	2.15	18.65	34.875±.006	1
508		31.70	0.21	0.43	2.17	18.72	34.868	1
509		32.19	0.21	0.49	2.19	20.15	34.833±.004	2
510		32.17	0.22	0.42	2.14	20.16	34.839	2
511		31.91	0.24	0.46	2.10	18.81	34.847±.010	3
512		31.96	0.23	0.49	2.11	18.79	34.833	3
513	04-11-74	31.80±0.68	0.21	0.42	2.07±0.07	18.59	34.865±.006	1
514		31.91	0.22	0.57	2.03	18.17	34.873	1
515		33.37	0.20	0.92	2.11	20.39	34.830±.002	2
516		33.09	0.21	0.51	2.08	20.20	34.827	2
517		32.18	0.21	0.47	2.09	18.68	34.830	3
518		32.18	0.23	0.48	2.03	18.73	34.830	3
519	04-18-74	31.30	0.23	0.48	2.03	17.41	34.879±.004	1
520		31.10	0.24	0.54	2.01	16.88	34.873	1
521		32.50	0.20	0.37	2.11	20.92	34.830±.006	2
522		32.34	0.25	0.37	2.08	20.48	34.839	2
523		31.47	0.24	0.30	2.04	18.24	34.839±.004	3
524		31.52	0.27	0.30	2.02	18.34	34.844	3

WATER CHEMISTRY : NITRATE, NITRITE, AMMONIA, PHOSPHATE, SILICATE AND SALINITY DETERMINATIONS IN ST. CROIX WATER SAMPLES

STX REF 1085	DATE SAMPLED	NO_3+NO_2 (µgat N/l)	NO_2 (µgat N/l)	NH_3 (µgat N/l)	PO_4 (µgat P/l)	SiO_4 (µgat Si/l)	SALINITY (°/oo)	PIPE NO.
525	04-25-74	31.03	0.29±0.05	0.91±0.03	1.97	17.77±0.04	34.867±.004	1
526		31.68	0.24	1.08	2.00	17.38	34.873	1
527		32.60	0.25	0.78	2.12	22.23	34.859±.004	2
528		32.47	0.29	0.95	2.13	2.23	34.853	2
529		31.64	0.28	0.77	2.02	19.46	34.844±.008	3
530		31.65	0.30	1.08	2.20	19.39	34.833	3
531	05-02-74	30.45±0.20	0.30	0.95	2.07±0.05	17.31	34.827±.012	1
532		30.05	0.30	0.95	2.00	17.36	34.844	1
533		31.60	0.30	1.03	2.16	20.31	34.830±.006	2
534		31.63	0.28	1.01	2.14	20.27	34.839	2
535		30.67	0.28	1.07	2.15	17.90	34.830±.010	3
536		30.62	0.25	1.24	2.10	17.85	34.844	3
537	05-09-74	30.02	0.30	1.26	2.02	18.68	34.887±.010	1
538		30.30	0.33	1.33	1.92	18.77	34.902	1
539		31.19	0.29	1.29	2.12	22.96	34.856±.012	2
540		31.49	0.28	1.13	2.10	22.77	34.839	2
541		31.42	0.31	1.27	2.09	20.18	34.887±.010	3
542		31.27	0.28	1.23	2.07	18.58	34.873	3
543	05-16-74	29.32	0.32	1.18	1.92	17.50	34.879±.004	1
544		29.35	0.29	1.41	1.90	16.53	34.873	1
545		31.49	0.31	1.55	2.10	22.07	34.876±.006	2
546		31.39	0.28	1.39	2.13	23.20	34.859	2
547		29.84	0.29	1.39	2.01	17.42	34.896±.006	3
548		29.84	0.31	1.16	1.97	16.02	34.887	3

WATER CHEMISTRY : NITRATE, NITRITE, AMMONIA, PHOSPHATE, SILICATE AND SALINITY DETERMINATIONS IN ST. CROIX WATER SAMPLES

STX REF 1085	DATE SAMPLED	NO_3+NO_2 (µgat N/l)	NO_2 (µgat N/l)	NH_3 (µgat N/l)	PO_4 (µgat P/l)	SiO_4 (µgat Si/l)	SALINITY (°/oo)	PIPE NO.
549	05-24-74	30.52±0.29	0.23±0.04	0.47±0.09	2.01±0.11	18.91±0.31	34.892	1
550		30.62	0.23	0.51	1.98	19.73	34.892	1
551		31.70	0.21	0.51	2.07	20.72	34.867±.008	2
552		31.85	0.24	0.51	2.12	22.61	34.878	2
553		31.31	0.21	0.51	2.02	19.84	34.892±.002	3
554		31.41	0.23	0.50	2.03	20.18	34.889	3
555	05-30-74	30.97	0.24	0.52	2.00	19.54	34.821±.010	1
556		30.92	0.24	0.52	1.98	19.35	34.835	1
557		31.75	0.22	0.64	2.08	19.60	34.786±.006	2
558		31.80	0.24	0.61	2.08	22.18	34.778	2
559		31.26	0.23	0.77	2.06	20.31	34.835	3
560		31.26	0.23	0.66	2.07	17.84	34.826	3
561	06-06-74	30.83±0.10	0.23	0.52	1.88±0.03	14.77	34.845±.004	1
562		30.76	0.22	0.71	1.93	16.90	34.851	1
563		31.74	0.24	0.74	2.08	21.45	34.814±.010	2
564		31.74	0.27	1.01	2.01	22.45	34.828	2
565		31.27	0.24	1.00	1.96	19.70	34.814±.002	3
566		31.27	0.24	0.73	1.99	19.34	34.817	3
567	06-13-74	30.67	0.25	0.85	1.94±0.04	18.41	34.845±.008	1
568		30.80	0.29	0.63	1.91	17.57	34.857	1
569		32.22	0.36	0.59	2.08	22.01	34.900±.006	2
570		31.89	0.29	0.79	2.03	22.06	34.908	2
571		31.48	0.27	0.61	1.98	21.47	34.842±.002	3
572		31.88	0.32	0.55	1.98	19.80	34.845	3

WATER CHEMISTRY : NITRATE, NITRITE, AMMONIA, PHOSPHATE, SILICATE AND SALINITY DETERMINATIONS IN ST. CROIX WATER SAMPLES

STX REF 1085	DATE SAMPLED	NO_3+NO_2 (µgat N/l)	NO_2 (µgat N/l)	NH_3 (µgat N/l)	PO_4 (µgat P/l)	SiO_4 (µgat Si/l)	SALINITY (°/oo)	PIPE NO.
573	06-20-74	31.13±0.22	0.22±0.09	0.50	2.06±0.03	18.86±0.30	34.850±.004	1
574		31.26	0.25	0.52	2.13	18.86	34.844	1
575		31.93	0.24	0.51	2.22	20.44	34.864	2
576		31.58	0.21	0.71	2.21	21.39	34.864	2
577		31.53	0.25	0.66	2.08	19.31	34.859±.006	3
578		31.48	0.23	0.75	2.10	19.26	34.850	3
579	06-27-74	30.93	0.25	1.30±0.13	2.08	15.91	34.836	1
580		31.08	0.25	0.63	2.10	17.74	34.831	1
581		31.83	0.26	0.66	2.10	21.10	34.828	2
582		31.73	0.23	0.61	2.13	19.87	34.813	2
583		31.53	0.23	0.83	2.04	16.92	34.856	3
584		31.53	0.22	0.79	2.04	18.20	34.856	3
585	07-05-74	31.43	0.21	0.66	2.07	17.02	34.882	1
586		31.33	0.22	0.69	2.06	18.36	34.888	1
587		31.91	0.22	0.69	2.05	20.82	34.876	2
588		31.93	0.20	0.94	2.12	18.80	34.885	2
589		32.03	0.20	0.74	2.24	19.65	34.885	3
590		32.03	0.21	0.78	2.08	18.52	34.885	3
591	07-17-74	30.03±0.10	0.21	0.75	2.11±0.08	17.19	34.914	1
592		30.19	0.20	0.81	2.13	18.08	34.908	1
593		31.32	0.19	0.84	2.32	19.61	34.911	2
594		31.55	0.20	0.75	2.31	19.66	34.903	2
595		31.19	0.21	1.13	2.21	18.29	34.882	3
596		31.03	0.25	0.96	2.20	18.79	34.885	3

WATER CHEMISTRY : NITRATE, NITRITE, AMMONIA, PHOSPHATE, SILICATE AND SALINITY DETERMINATIONS IN ST. CROIX WATER SAMPLES

STX REF 1085	DATE SAMPLED	NO_3+NO_2 (μgat N/l)	NO_2 (μgat N/l)	NH_3 (μgat N/l)	PO_4 (μgat P/l)	SiO_4 (μgat Si/l)	SALINITY (°/oo)	PIPE NO.
597	07-25-74	30.37	0.07±0.05	0.50±0.06	2.06	23.74±0.05	34.857	1
598		29.37	0.07	0.44	2.09	15.97	34.868	1
599		30.94	0.08	0.43	2.29	21.84		2
600		30.76	0.10	0.42	2.19	25.57		2
601		31.11	0.09	0.44	2.08	25.11		3
602		30.11	0.06	0.41	1.99	24.27		3
603	08-01-74	29.95	0.10	0.45	2.13	18.60	34.891	1
604		30.16	0.10	0.37	2.19	22.62	34.899	1
605		31.17	0.08	0.46	2.23	23.50	34.882	2
606		31.11	0.10	0.45	2.28	23.75	34.885	2
607		30.69	0.09	0.46	2.17	19.72	34.862	3
608		30.69	0.10	0.54	2.20	25.62	34.871	3
609	08-08-74	30.90	0.12	0.36	2.20	25.17	34.842	1
610		31.06	0.09	0.43	2.21	19.56	34.842	1
611		30.98	0.11	0.36	2.24	22.83	34.822	2
612		30.95	0.10	0.48	2.23	24.85	34.831	2
613		31.17	0.10	0.43	2.20	24.60	34.871	3
614		31.17	0.18	0.43	2.23	21.38	34.859	3
615	08-15-74	29.98±0.11	0.05±0.01	0.34±0.15	2.18	22.51	34.899	1
616		30.22	0.07	0.37	2.20	19.78	34.885	1
617		31.14	0.03	0.42	2.25	23.69	34.828	2
618		31.10	0.08	0.30	2.31	23.79	34.828	2
619		31.77	0.00	0.32	2.21	19.33	34.885	3
620		31.24	0.09	0.37	2.23	21.31	34.891	3
621	08-22-74	30.87	0.05	0.34	1.93±0.04	23.22	34.865	1
622		31.03	0.05	0.37	1.93	20.31	34.865	1
623		31.03	0.08	0.30	1.97	24.30	34.828	2
624		31.00	0.05	0.40	1.96	22.91	34.836	2
625		31.00	0.11	0.35	1.96	23.74	34.862	3
626		31.01	0.09	0.40	1.96	24.04	34.865	3

WATER CHEMISTRY : NITRATE, NITRITE, AMMONIA, PHOSPHATE, SILICATE AND SALINITY DETERMINATIONS IN ST. CROIX WATER SAMPLES

STX REF 1085	DATE SAMPLED	NO_3+NO_2 (μgat N/l)	NO_2 (μgat N/l)	NH_3 (μgat N/l)	PO_4 (μgat P/l)	SiO_4 (μgat Si/l)	SALINITY (°/oo)	PIPE NO.
627	08-29-74	30.67	0.00	0.35	1.96	20.82±0.22	34.919	1
628		30.93	0.07	0.42	2.00	20.37	34.908	1
629		31.10	0.01	0.44	2.05	25.40	34.839	2
630		31.04	0.00	0.48	2.06	23.51	34.836	2
631		30.94	0.02	0.38	2.05	21.46	34.879	3
632		31.15	0.03	0.56	2.06	21.41	34.879	3
633	09-05-74	30.31	0.16±0.05	0.45±0.10	2.04	20.09	34.879	1
634		30.28	0.18	0.60	2.02	20.54	34.873	1
635		31.31	0.15	0.58	2.14	23.89	34.816	2
636		31.27	0.15	0.58	2.14	23.99	34.828	2
637		31.20	0.16	0.70	2.10	21.09	34.833	3
638		31.19	0.16	0.66	2.11	24.01	34.836	3
639	09-12-74	29.83±0.10	0.15±0.05	0.58±0.10	2.02±0.06	17.67±0.13	34.943	1
640		29.83	0.20	0.67	2.09	19.27	34.937	1
641		31.16	0.18	0.63	2.14	21.58	34.903	2
642		31.11	0.16	0.67	2.13	22.46	34.903	2
643		30.41	0.21	0.92	2.02	18.73	34.908	3
644		30.51	0.17	1.09	2.10	19.32	34.914	3
645	09-19-74	30.26	0.16	0.84	2.08	18.62		1
646		30.30	0.15	0.89	2.01	20.29		1
647		31.04	0.15	0.81	2.13	21.78		2
648		31.04	0.15	0.67	2.11	21.47		2
649		30.67	0.15	0.63	2.09	19.83		3
650		30.74	0.15	0.68	2.09	19.52		3
651	10-03-74	29.72	0.16±0.01	0.48±0.06	1.85±0.04	19.44±0.10	34.923	1
652		30.20	0.16	0.41	1.92	22.17	34.923	1
653		30.50	0.24	0.39	1.98	23.40	34.920	2
654		29.94	0.18	0.42	1.92	23.74	34.923	2
655		30.46	0.18	0.48	1.91	20.61	34.897	3
656		29.87	0.18	0.51	1.90	21.01	34.903	3

WATER CHEMISTRY : NITRATE, NITRITE, AMMONIA, PHOSPHATE, SILICATE AND SALINITY DETERMINATIONS IN ST. CROIX WATER SAMPLES

STX REF 1085	DATE SAMPLED	NO_3+NO_2 (µgat N/l)	NO_2 (µgat N/l)	NH_3 (µgat N/l)	PO_4 (µgat P/l)	SiO_4 (µgat Si/l)	SALINITY (°/oo)	PIPE NO.
657	10-10-74	30.42	0.20	0.49	1.82	19.77	34.920	1
658		30.26	0.29	0.58	1.91	20.35	34.915	1
659		29.91	0.16	0.57	1.94	23.63	34.909	2
660		29.76	0.23	0.42	1.92	24.12	34.909	2
661		30.84	0.18	0.52	1.94	21.64	34.900	3
662		30.89	0.17	0.36	1.99	21.73	34.909	3
*663-675: no samples								
676	10-24-74	29.08	0.19	0.44	1.82	18.60	34.932	1
677		29.13	0.20	0.56	1.84	18.35	34.929	1
678		29.11	0.19	0.48	1.84	20.39	34.909	2
679		29.11	0.18	0.42	1.79	18.40	34.920	2
680		30.84	0.17	0.42	1.95	24.29	34.883	3
681		30.84	0.17	0.38	1.96	22.68	34.886	3
682	10-31-74	29.83	0.16	0.52	1.84	20.13	34.929	1
683		30.20	0.18	0.55	1.83	21.61	34.943	1
684		30.67	0.14	0.54	1.95	21.70	34.886	2
685		31.45	0.17	0.46	2.00	24.72	34.886	2
686		30.91	0.19	0.64	1.91	21.00	34.883	3
687		31.19	0.19	0.45	1.93	23.43	34.900	3
688	11-07-74	30.64	0.18	0.59	1.90	25.07	34.903	1
689		30.63	0.18	0.64	1.88	18.81	34.897	1
690		30.78	0.19	0.53	1.91	24.55	34.803	2
691		30.71	0.20	0.52	1.93	23.46	34.803	2
692		31.26	0.21	0.58	1.92	23.76	34.803	3
693		31.26	0.20	0.45	1.92	28.40	34.817	3

APPENDIX IV

EFFECTS OF THE LIGHT AND DARK CYCLES ON THE COUPLING BETWEEN NITRATE UPTAKE, ASSIMILATION AND CELL GROWTH RATES BY *CHAETOCEROS SIMPLEX*, STX-105, A MARINE PLANKTONIC DIATOM, IN A CONTINUOUS N-LIMITED CULTURE.

Progress Report
on Dissertation Research
Project

Gaston Picard

August 19, 1974

OBJECTIVES

A. General

1: Determine the relationship between the growth rate and the processes of nitrate uptake, nitrate assimilation and photosynthesis during N-limited growth of *Chaetoceros* sp. (STX-105) in continuous steady-state culture[1] using St. Croix deep-sea water as the medium[2] with a 12-hour light and 12-hour dark cycle.

2: Evaluate the effects of dilution rates on the following parameters: (i) nutrients in the growth medium, (ii) nitrate uptake kinetics, (iii) intracellular nitrate, (iv) intracellular "protein" (determined by Lowry), (v) cellular particulate nitrogen, (vi) the activities of nitrate reductase and glutamate dehydrogenase, (vii) the specific growth rates calculated from the following population characteristics: chlorophyll *a*, particulate organic carbon (POC), particulate nitrogen (PN), and cell number, (viii) photosynthetic rate.

B. Specific

1: Compare the parameters of nitrate uptake kinetics, the maximal specific uptake rate (Vmax) and the half saturation

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[1] Steady state culture is defined as repetitive nearly-identical cycles of cell densities in function of time.

[2] The growth medium used in this study was deep-sea water from St. Croix enriched with: Fe, 1 μM; EDTA. 1 μM; vitamin B_{12} (Guillard medium f) and trace metals, f/100).

constant for uptake (K_s^u), determined by three techniques: (i) short-term uptake of nitrate with nitrogen-depleted culture (Eppley *et al.*, 1969b), (ii) nitrate uptake using known preconditioning of the cells (Caperon and Meyer, 1972b), (iii) calculated uptake rate during continuous culture by measurement of the differences between the inflow and outflow of nitrate (Eppley *et al.*, 1971), using the same preconditioned population.

2: Describe the relationship between growth rates and nitrate reductase and glutamate dehydrogenase activities.

3: Determine the effect of the dilution rate on the phasing of nitrate uptake, enzymic nitrate reduction and growth rate.

4: Test the hypothesis that the cellular organic nitrogen (q_o) is constant with different growth rates.

5: Find the dilution rates for maximum nitrate utilization (nitrate transformed into organic cellular nitrogen) and growth rate.

6: Determine the relationship between photosynthetic rates and dilution rates.

RATIONALE

It is well known that the processes of nutrient uptake and nutrient assimilation (growth) are separated in time or uncoupled. For example, at low initial nutrient concentration, the nutrient is nearly exhausted from batch cultures by the time growth rates can be determined. Growth rate in phytoplankton does not depend on current environmental nutrient concentrations, but rather upon the nutrient experience during a period of time preceding the time at which the growth rate determination is made (Caperon, 1969).

Caperon and Meyer (1972a, b) have shown that different preconditioning states of the population affect the population size and uptake kinetics of phytoplankton, and that continuous cultures are the best means by which the past nutrient history of the cells can be controlled.

There is evidence that the diurnal variation of light induces a rhythmic effect on many cellular processes such as cell division, pigment synthesis, photosynthetic rate, nutrient uptake and enzymic assimilation rates (Eppley *et al.*, 1967; Paasche, 1967, 1968; Eppley *et al.*, 1970; Eppley and Coatsworth, 1966, 1968; Eppley et al., 1971). A 12-hour light and 12-hour dark cycle will obviously be closer to natural conditions than the continuous illumination used by Caperon (1969) and Caperon and Meyer (1972a, b) in their studies.

Chaetoceros sp. (STX-105) will be grown at 25°C in deep-

sea water (870 m) obtained from an "artificial upwelling" mariculture system on St. Croix, U.S. Virgin Islands (Roels and Gerard, 1970). The nutritional requirements of Chaetoceros were unknown and have been investigated. The growth medium used throughout this study will consist of an enriched deep-sea water, with nitrate as the limiting nutrient. The significance of the results will be enhanced by simulating as much as possible, the conditions of the St. Croix mariculture station, since it represents an ideal field-test system.

An understanding of the relationship between rates of nitrate supply, nitrate uptake, assimilation and growth rate, over a 24-hour period of light and dark cycles, will give important insight into the population dynamics of phytoplankton.

MATERIALS AND METHODES

A. Organismes

The marine diatom, *Chaetoceros* sp. (STX-105), was used in this experiment. The cultures were axenic. The organism is only presently identified as *Chaetoceros* sp. (STX-105), but is very similar to *Chaetoceros simplex* (K.C. Haines, personal communication).

B. Culture Apparatus

The basic system is best represented by a schematic (Fig.1).

(a) Introduction of fresh medium to the vessel culture is showed as #1. The medium was pumped in by a peristaltic pump which can deliver a constant flow at several delivery rates.

(b) Port #2 was used for aeration and sampling by means of a two-way stopcock (#4). The air was initially bubbled through a 0.1 M solution of $ZnCl_2$ to remove traces of ammonia.

(c) The overflow (#3) maintained a constant volume of 8 liters in the chemostat.

(d) The culture vessel (carboy) had a 10 liters capacity (#5) and could be opened for cleaning.

(e) The culture vessel was kept at a constant temperature, by submergence in a Plexiglas container (#6) in which water continuall circulated at 26^{0}C.

(f) . The culture medium was mixed by air bubbling.

(g) Light was supplied by cool white fluorescent tubes (#7) at an intensity of approximately 0.4 LY/min , for a period of 12 hours (0900 - 2100) each 24 hours.

C. Medium

The basic medium for the culture of *Chaetoceros*, was artificially upwelled water from 870 M depth, pumped off the North Coast of St-Croix (Roëls and Gerard, 1970). The water has been stored at $4^{0}C$ for 2-4 months, in 55 gallons opaque polyethylene containers prior to use.

To insure an axenic and homogeneous culture the sea water was filters to remove any particulates and after addition of the nu nutrients the medium was steam-sterilized. Ferric ions was sterilized separately and added aseptically.

The minimum enrichment of the deep sea water was investigated (Picard *et al.*, unpubl.) to insure that nitrogen was the limiting nutrient. The concentrations of major nutrients in deep sea water (D.S.W.) and the nutrient concentration used for the enrichment are given in Table 1.

D. Experimental design.

The growth chamber was inoculated with an axenic culture of *Chaetoceros* sp. (STX-105) in the exponintal phase of growth. The growth medium was the same as described in Table 1. The system was run initially as a batch culture for 2-3 days. The pump was then turned on to the desired flow rate.

Based on the work of Malone *et al.* 1974 (in press), the following dilution rates were selected:

30% turnover/day	-	100ml/hr.	
60% turnover/day	-	200ml/hr.	
87% turnover/day	-	290ml/hr.	
120% turnover/day	-	400ml/hr.	(not done)

The same inoculum was used for the three chemostat runs (30, 60 and 87 %) which had duration of 20, 15 and 15 days, respectively. Sterility tests were conducted at the begining and the end of the third dilution rate (87 %) and were negative. The test consist of adding one drop of the medium, sampled aseptically, to sterile broth medium. On addition no wall effects of bacterial contamination were seen for the entire period of the experiment.

Samples were collected at 0900, 1300, 1700, 2100, 0100 and 1500 for the 30 % turnover rate. In order, to cover the 24 hrs cycle at 2 hrs interval, the sampling time for the 60 and 87 % turnover rates was alternated every day between 0900, 1300, 1700, 2100, 0100, 0500, and 0700, 1100, 1500, 1900, 2300, 0300.

The following parameters were measured:

1- pH: The hydrogen ion concentration was mesured with a radiometer pH meter.

2- Cell counts; Cells were counted with a Speirs-Levy counting chamber. Each determination represented an average number of 3700 cells per count (MIN: 3400 - MAX: 4000).

3- Chlorophyl _a_ : Chl _a_ was measured within one hour after sampling in 90 % acetone extract by fluorometry (Strickland and Parsons, 1972

4- Protein: Cellular protein determination was made by the Lowry method (Lowry, 1951). Fifty ml of culture were centrifuged down to 0.2 ml and resuspended in distilled water to a volume one milliliter. The samples were immediately frozen until analysis.

5- Dissolved nitrate and ammonia: The concentrations of nitrate and ammonium ions was measured in the incoming medium and the growth chamber at intervals of 24 and 2 hours respectively (Strickland and Parsons, 1972).

6- Primary productivity: The primary productivity was determined by the C^{14} tracer technique. The light and dark bottles used had a volume capacity of 65 mls. Each essay was made by adding 5 u Curie of C^{14} ($NaHCO_3$) to a 60 ml sample of the culture. The Bottles (light and dark) were incubated for 30 minutes in the same water bath of the growing chamber. The samples bottles (light and dark) were then filtrated on to a 0.45 Millipore filter paper. Any residual inorganic C^{14} on the membrane was removed by holding the membrane for 20 secondes over hydrochloric acid fume. The organically bound C^{14} was then counted without drying (Anderson and Zeutchel, 1970) using a liquid scintillation counter.

The unit ugC. $hr^{-1}.l^{-1}$ was obtained by:

$$ug\ C^{14}.\ hr^{-1}.l^{-1} = \frac{L.B - D.B}{Tot.\ Activity} \cdot \frac{mg\ C}{l} \cdot 1000 \cdot \frac{1}{t} \cdot \frac{100}{99} \cdot 1.06$$

where:

L.B = Count per minute (C.P.M) of the light bottle.

D.B = Count per minute (C.P.M) of the dark bottle.

Total Activity = C.P.M of the 5 u Curie ampoule.

mg C/l = Total Co_2 per liter in the medium. This value was obtained from the determination of the alkalinity of the medium according to the method of Strickland and Parsons, 1092.

1000 = Transformation of mg to ug.

t = is equal to the incubation time in hour.

$\frac{100}{99}$ = Counting efficiency, as determined empirically by a quenching curve.

1.06= Isotopic discrimination factor.

The 0900 sample was incubated in the light, while the 2100 sample was incubated in the dark.

7- Photosynthetic capacity: Same as the primary productivity measurements except that the samples was incubated in a "light-box" illuminated by only 4 fluorescent tubes but covered with aluminium paper so that the light intensity was approximatively 95 % of the chemostat light. This measurment allowed an estimate of photosynthetic capacity at different intervals at the dark cycle.

8- Total organic nitrogen and carbon: Total cellular nitrogen (PN) and carbon (POC) were analysed by combustion (CHN Analyser, Hewlett-Pachard Corp.). Samples of 200 ml were collected on a glass fiber filter paper, previously incenerated at 450^0C for one hour.

9- Nitrate reductase: The activity of the enzyme NADH- dependent nitrate reductase, was determined by the method of Eppley *et al*. (1970).

10- Intracellular nitrate: Cellular nitrate ions was measured according to the technique of Eppley and Coatsworth (1968).

11- Cellular free amino acid : The cellular free amino acid were obtained from a 95 % ethanol extraction (Jeffries *et al*.,196)

12- Polysaccharides: Samples of 25 ml were centrifuged and kept frozen until analysis. Not yet analysed.

13- Cell size: Samples of 25 ml were preserved with one drop of indol solution. Not yet analysed

14- Nitrate uptake kinetics: At each particular dilution rate at steady state (1200 hr), The rate of nitrate uptake was determined by three techniques: (1) stopping the pump, adding nitrate up to 5 - 8 ug- at NO_3-N/l, and the nutrient will be measured as it is depleted from the cultures; the frequency of the measurements will be higher at the near-zero concentrations (Caperon and Meyer, 1972 b), or continuously, using an Autoanalyzer 11 (Technicon Corp.); (2) at the time the sampling period is finished for technique 1, the N-depleted cells will be used to determine the uptake rate of nitrate by the technique of Carpenter and Guillard (1970), that is short-term uptake when the cells are incubated for 15 to 45 minutes with different concentrations of nitrate; (3) the uptake rate of nitrate will be determined at steady state, estimated by the nitrate removed by the population at 4-hr intervals.

The frequency of the measurements of those parameters is showed in Table 11.

RESULTS

Nutrient concentration

Nitrate was removed almost completely for the three chemostat runS(Fig. 2). The mean residual nitrate concentration for each turnover rate was:

Turnover rate	Mean (NO_3^-) uM
30 %	0.25 $\pm$ .05
60 %	1.13 $\pm$ .05
87 %	.38 $\pm$ .05

The higher value for the 60 % turnover rate could be partially attributed to a shorter photoperiod, du to a power failure , on day four of that run. Immediately following that longer dark period, three successives high nitrate concentrations were observed. From that point on, the residual nitrate concentrations were higher. The same pattern was also exhibited by the activity of the enzyme nitrate reductase.

The residual nitrate in the growth chamber for the three turnover rates as shown in fig. 2 exhibit irregulation (or small peak) which occur sporadically in all three curves. These irregularities although showing no obvious pattern seem to be more frequent as the dilution rate increases. This phenomenon might be related to the auxospore formation by the diatoms (Davis et al., 1973), which has to occur more and more often as the turnover rate increases.

The mean of the residual nitrate concentration in the growth

chamber in Fig.3. At 30 % turnover rate there is no significant change. However, there is an remarquable increase for 60 % and 87 % turnover rate, during the two hours preceding and succeding 0900 (see Fig.3). Following that maximum , there is a gradual decrease to a minimum approximatively at 0300 - 0500. This pattern agrees with data for intracellular nitrate as we will see later on.

The residual nitrite concentrations were consistenly low without significant diurnal variations. The mean values for each turnover rate was:

Turnover rate	Mean (NO_2^-) uM
30 %	0.16 $\pm$ 0.05
60 %	0.19 $\pm$ 0.05
87 %	0.22 $\pm$ 0.05

Ammonia data are not yet analysed

pH:

Diurnal variations of the pH (Fig.4) are more pronounced as the turnover rate increases. There is an increase starting with the beginning of the photoperiod , reaching a maximum between 1900 and 2100 (the end of the light period). A gradual decrease of the pH occurs during the night , since the pH of the incoming medium is 7.6.

The pH variation is merely a result of the primary productivity.

In addition a similar diel variation was observed (not shown) for the alkalinity of the deep sea water (100.76 mg CO_2/1). The original alkalinity was never restored after autoclaving the medium and even following extensive bubbling (24 - 48 hrs) with Co_2 saturated air, The alkalinity was 45 mg Co_2 /1.

Population size:

1- Cell number:

The 30 % turnover rate was the most stable (Fig. 5). However as the turnover rate increased to 60 % there is a broad increase from 0300 to 0900. The same pattern is folowed with 87 % turnover rate except that the increase in cell number is shifted back in the dark period 2300 and they peak at 0300 where a gradual wash out begins and the population recovers only at 1100.

The cell size distribution curve are not available at this point, but microscopic observations definively reveals that as the turnover rate increases; 1) The cell size decreases 2) There is more diurnal differences in cell size 3) The number of chains formed decreases.

The only time where the cell number was approximatively the same for the three turnover rates was during the second half of the photoperiod.

2- Cellular protein:

The amount of inorganic nitrogen fixed into protein nitrogen for each turnover rate is showed in Fig. 5. Again the 30 % rate was the most stable. The amplitude of diurnal variation increase as the turnover rates increased. The minimum was reached in the second half of the dark period. This is an indication that the amount of energy required for protein

synthesis is limiting. Probably, only the essential structural and functional enzymes are synthetised in a limited quantity. This might be the reason why such cells do not take up nitrate so efficiently at 0900, until they resume a larger cell volume. Overall, the trend is similar to the cell number , but, opposite.

3- Chlorophyl a:

There is no significant trend of diurnal variation for Chl a (Fig. 5). This is true for all three dilution rates experimented. This indicates that the diatom *Chaetoceros* sp. STX-105, can synthesize Chl a over both the light and dark cycles.

4- Particulate organic nitrogen:

Diel variation of particulate organic nitrogen (Fig. 6) shows a very small increase in the second half of the light period, for the three turnover rates. It is interesting to compare it with the protein data; as the turnover rates increases the protein content decreases regularly in the dark period while the particulate organic nitrogen exhibit a slight decrease. This indicates that as the dilution rates increased the inorganic nitrogen is still taken up but used differently i,e. probably more non-protein nitrogen is used for photosynthesis and nucleic acid.

5- Particulate organic carbon:

The amount of bound carbon produced is showed in Fig. 6. Of all the population sizes this one has the greatest diurnal variability. For each turnover rate the maximum is reached

at the end of the photoperiod, indicating its dependence on photosynthesis. However the particulate organic carbon maximum (Fig. 6) lags the maximum rate of photosynthesis (Fig. 4) by 4 - 5 hours.
It should be emphasize that the temporal variation of each population size measured was not exactly in phase from day to day . Therefore the diurnal variations reported in Fig. 5 and 6 are dampened since they resulted from averaged cycles. The mean population size are also plotted in function of the turnover rate (Fig. 7). The cell numbers are quasi-identical for the three dilution rates. The protein content of the cultures decreases linearly as dilution rates increases. The amount of Chl a increases from 30 to 60 % but much more for 87 %. This increase in Chl a is probably an adjustment of the cell to the increasingly shorter light energy received by those cells. Actually there seens to be an inverse reaction between the particulate organic carbon and the chlorophyl a . Finally, the particulate organic nitrogen reached a maximum at the 60 % turnover rate, but, exceed the supply of inorganic nitrate; thus implying that ammonia is utilized and will have to be taken into account for a complete nitrogen budget.

Cellular characteristic:

A summary of the cellular characteristics is given in Table 3 The protein content decrease with increasing turnover rates, while the maximim P.N. is at 60 %. The percentage of particulate organic nitrogen on the form of protein decrease drastically as

the turnover rates increase i.e. 57, 42 and 37 %.

Nitrate per 10^7 cells represent 0.27 %, 0.35 % and 0.39 % of the total organic nitrogen, for turnover rate of 30, 60 and 87 % respectively. The diurnal variation is showed in Fig. 8. There is an accumulation of cellular nitrate at 0300 and during the first 2 hours of light, except for the 30 % turnover rate.

The pattern of cellular nitrate is in relatively good agreement with the activity of the enzyme nitrate reductase (Fig. 8). The specific activity of the enzyme is also given in Fig. 9. The diel variation practically desapear when the specific activity is expressed in terms of cellular protein. Therefore this indicates that the diurnal variation could be due to different amounts of total enzymatic protein rather than induction mechanisms either from the sabstrate or light or both. However the increase nitrate reductase activity (Table 111) seems to come from a change of velocity since the protein decreases, as the turnover rates increases.

Particulate organic carbon (Table 111) decreases as turnover rates increases; conversaly the primary productivity and photosynthetic capacity increases, and their diel periodicity is shown in Fig. 4.

The results of the nitrate uptake kinetics are not completed and will be reported later. The determination of polysaccharides content, cell size distribution curves and free amino acid of the algal population are not done and will also be reported later. However, the determination the enzyme glutamate dehydrogenase as the ammonium dependent oxidation of pyridine nucleotide (NADH), does not work. The only technique available for phytoplankton

samples was published by Eppley et al 1970. Several unsuccesful essays were made. The measure of the fluorescence of NAD formed was invariable with respect to the abscence or presence of the substrates. A personal communication with Dr.T.T.Packard who is working actually on this technique,imform me that they still dont have a reliable G.D.H enzyme essay.

DISCUSSION

Effects of turnover rates on nitrate utilization

Based on the nitrate concentrations in the incoming medium and in the growth chamber, the percentage of nitrate removal was very high (nitrate uptake éfficiency). Expression of the 1) cellular nitrate 2) ug at Prot-N and 3) particulate organic nitrogen as percentage of the supplied nitrate cOncentration are shown below.

Turnover Rate %	Uptake Efficiency %	Cell Nitrate %	Assimilation ug at Prot-N %	Efficiency based ug at PN-N %
30	92.2	0.24	50.4	91.8
60	96.4	0.32	43.7	105.7
87	98.8	0.39	37.1	100.2

It is obvious that more nitrate is transformed into protein at 30 % turnover rate. However a portion of the assimilation particulate nitrogen a 30 % is excreted (difference between uptake efficiency and assimilation efficiency based on P.N). On the other hand the 60 % and 87 % trunover rates are more efficient for the assimilation fo nitrate, based on particulate organic nitrogen but not based on protein. It would be interesting to have the free amino acid concentrations in order to estimate the importance of that nitrogen pool. Since assimilation efficiency based on PN is still high at 60 % and 87 % and the protein content decrease, other forms of nitrogen must be increasing.

Effects of turnover rates on nitrate reductase activity (N.R.), maximum velocity of NO_3^- uptake (Vm) and uptake rate at steady state.

In order to compare those rates they must be expressed with the same units, in occurrence uM No_3^- (or No_2^-) $.hr^{-1}.1^{-1}$. The maximum velocity of nitrate uptake is taken from short term uptake experiment (Carpenter and Guillard,1970), but the cells were starved for 5 hours, in order words, the cells were preconditioned at each turnover rate and the pump (alimenting fresh medium to the growth chamber) was stop 5 hours prior to the experience. This lead to cells extremely starved allowing only for very very short incubation time in presence of the substrate (No_3^-). Only the maximum velocity of nitrate uptake (Vm) from those experiments was reasonably estimated.

The nitrate reductase activity used for this comparison is the mean value for each turnover rate. The uptake rate of nitrate for the three turnover rates at steady-state (U) were calculated from the mean data of uptake efficiency.

Turnover rate	30 %	60 %	87 %
Vm. uM $No_3^-.1^{-1}.hr^{-1}$.	149	158	115
U. uM. $No_3^-.1^{-1}.hr^{-1}$.	0.39	0.759	1.129
N.R. uM No_2^-.formed.$1^{-1}.hr^{-1}$.	3.42	6.01	6.33
% $(\frac{N.R.}{U} x100)$	877	792	561
% $(\frac{N.R.}{Vm} x100)$	2.3	3.8	5.5

From the data above, Vm, seems to be at a maximum value for turnover rates of 30 % and 60 %, and show a decrease at 87 % which could indicate a lesser degree of starvation. If this is true than it goes against Caperon & Meyer (1972) which found the opposite trend. More experiments are needed for this point.

The rate of nitrite formation by the enzyme nitrate reductase (in vitro) is 2.3%, 3.8% and 5.5% of the corresponding Vm values for the three dilution rates. However, the N.R. rates of No_2^- formation are 877% 792 and 561 % the values of nitrate uptake rates, 0.39. 0.759 and 1.129 respectively. This might lead to the conclusion that the activity of the enzyme nitrate reductase is not a limiting step in the metabolic pathways of nitrate utilization, for the three turnover rates experimented and, indicates the same for higher dilution rates. The main objection to this interpretation is that an IN VITRO enzymic test is used to simulate the IN VIVO conditions. Fig.10 shows the activity of nitrate reductase versus the actuel rate of nitrate uptake for the three turnover rates at steady-state. The rate of nitrate uptake merely represent a dynamic supply of substrate (No_3^-) to the enzyme. The enzyme activity is almost saturated at 60 % dilution rate which correspond to an uptake rate of 0.756 uM $No_3^-.l^{-1}.hr^{-1}$.

We know that the uptake can be much greater than the rate of uptake of nitrate at 60 % turnover rate. Therefore it seems that present experimental conditions, reaches a saturation plateau at a definite rate of nitrate uptake. From that rate of nitrate uptake and up the enzyme activity would increase very slowly. The determination of N.R. at 120 % turnover rate will probably reinforce this observation.

Effects of turnover rates on utilization of carbon:

The phasing between primary productivity (Fig. 4) and the particulate organic carbon (Fig. 6) is about 4 to 6 hours. As the dilutions rates increases, the discrepency between those two processes is greater. Therefore the fate of the carbon taken up can be evaluated in terms of organically bound carbon and excreted compounds containing carbon:

Turnover rate	30 %	60 %	87 %
Mean P.O.C. ug $C.l^{-1}.24\ hrs^{-1}$.	6500	6070	5090
P.O.C. washed out = Production	1950	3642	4428
C^{14}. uptake "	2624	4332	4846
Excess taken up "	674	690	418
% of the excess taken up. (Excreted)	25.7	15.9	8.6

Therefore, as the turnover rate increases, the percentage of carbon excreted decreases. The next dilution rate will be very interesting, as if the carbon uptake will be deficient or not.

Fig.11, shows the relationship between the light exposure and percentage of carbon excretion. If this curve holds, by extrapolation to 12 hours of light (~~120~~ 105 % turnover rate), there should be enough carbon taken up for the next dilution rate.

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...peron, J. and Meyer, J. (1972,b)
of marine phytoplankton.
their role in nutrient lim...
phytoplankton. Deep-Sea Re...

Caperon, J. (1969) Time lag in popula... ...
of Isochrysis galbana to a variable ...
environment. Ecology 50(2):188-192.

Caperon, J. (1968) Population response of Isochrysis
galbana to nitrate variation at limiting
concentrations. Ecology 49(5):866-872.

Davis, O.C., Harisson, P.J. and Dugdale, R.C. (1973)
Continuous culture of Marine Diatons Under
Silicate Limitation,
I. Synchronized lye cycle of Skeletonema costatum
J. Phycol. 9: 175-180

Eppley, R.W. and Coatsworth, J.L. (1966) Culture of the
marine phytoplankter Dunaliella terticlecta, with
light-dark cycles. Arch. Mikrobiol. 55:66-80.

Eppley, R.W. and Coatsworth, J.L. (1968) Uptake of nitrate
by Ditylum brightwellii. Kinetics and mechanisms.
J. Phucol. 4:151-156.

Eppley, R.W., Holmes, R.W., and Paasche, E. (1967) Periodicity
in cell division and physiological behavior of
Ditylum brightwellii, a marine planktonic diatom,
during growth in light-dark cycles. Arch. Mikrobiol.
56:305-323.

Eppley, R.W., Packard, T.T. and MacIsaae, J.J. (1970)
Nitrate-reductase in Peru current phytoplankton.
Marine Biology 6:195-199.

Eppley, R.W. and Rogers, J.N. (1970) assimilation of Ditylum bric plankton diatom. J. Phycol.

Eppley, R.W., Rogers, J.N. and McCarthy, [illegible] saturation constants for uptake [illegible] ammonium by marine phytoplankton. [illegible] 14:912-920.

Eppley, R.W., Rogers, J.N., McCarthy, J.J. and S[illegible] Light/dark periodicity in nitrogen assi[illegible] the marine phytoplankters Skeletonema co[illegible] Coccolithus huxleyi in N-limited chemost[illegible] J. Phycol. 7:150-154.

Paasche, E. (1968) Marine plankton algae grown with [illegible] dark cycles. 2:Ditylum brightwellii and Ni[illegible] turgidula. Physiol. Plant. 21:66-77.

Paasche, E. (1967) Marine plankton algae grown with light-dark cycles. 1: Coccolithus huxleyi. Physiol. Plant 20:946-956.

Roels, O.A., and Gerard, R.D. (1970) Artificial upwelling. In: Food-Drugs from the Sea Proceedings 1969, H.W. Youngken, Jr. (ed.), Marine Technology Society, Washington, D.C., pp. 103-112.

Strickland, J.D.H. and Parsons, T.R. (1972) A Manual of Sea Water Analysis, 3rd edition, Bull. Fish. Res. Board Can., Ottawa, Ontario.

Table I: Nutrient concentration and enrichment concentration of deep-sea water (D.S.W.) from St. Croix.

NUTRIENTS	DSW CONCENTRATION	ENRICHMENT CONCENTRATION
NO_3^-	31.5 μM	–
NO_2^-	0.14 μM	–
NH_4^+	μM	–
PO_4^{-3}	1.95 μM	
SiO_4^{-3}	22.5 μM	
Fe^{+3}	–	1 μM
EDTA (or natural chelator in DSW)	–	1 μM
B_{12}	–	1.8×10^{-4} μM
Trace Metal Mixture:		
Cu^{+2}	–	7.86×10^{-10} M
Zn^{+2}	–	1.53×10^{-9} M
Co^{+2}	–	8.50×10^{-10} M
Mn^{+2}	–	1.83×10^{-8} M
Mo^{+6}	–	5.20×10^{-10} M

Table 11: Number of 24 hrs. cycles where the different parameters were measured.

Parameters to be determined	Number of cycle
pH	All
Cell count	All
Chl a	All
Protein	All
Dissolved nitrate and ammonia* ions *	All
Primary productivity	3-4
Photosynthetic capacity	3-4
Total organic nitrogen and carbon	3-5
Nitrate reductase	3-4
Intracellular nitrate	1
Cellular free amino acid *	1
Polysaccharide *	1
Cell size *	1
Nitrate uptake kinetics †	1 (1200 only)

*: Samples collected but not analysed.

†: To be completed.

Table 111: MEAN CELLULAR CHARACTERISTICS OF THE CHAETOCEROS POPULATION FOR EACH TURNOVER RATE.

	39 %	60 %	87 %
ug Chl a / 10^7 cells	0.26	0.27	0.37
ug at NO_3^-N / 10^{10} cells	0.805	1.016	1.245
ug at Prot-N / 10^7 cells	0.17	0.14	0.12
ug at PN-N / 10^7 cells	0.30	0.33	0.32
ug at POC-C / 10^7 cells	5.67	5.09	4.33
Primary productivity:			
ug at C. 24 hr^{-1} / 10^7 cells	2.29	3.63	4.12
Photosynthetic capacity:			
ug at C. 24 hr^{-1} / 10^7 cells	2.66	4.82	5.77
Nitrate reductase:			
ug at NO_2^- formed hr^{-1} / 10^7 cells	0.036	0.060	0.066

C/N	18.9	15.42	13.53
PN-N/Chl a	16.15	17.11	12.11
POC-C/Chl a	261.69	226.22	140.43
Prot-N/PN-N	0.57	0.42	0.37
(ug NO_3^--N/PN-N) %	0.27	0.35	0.39

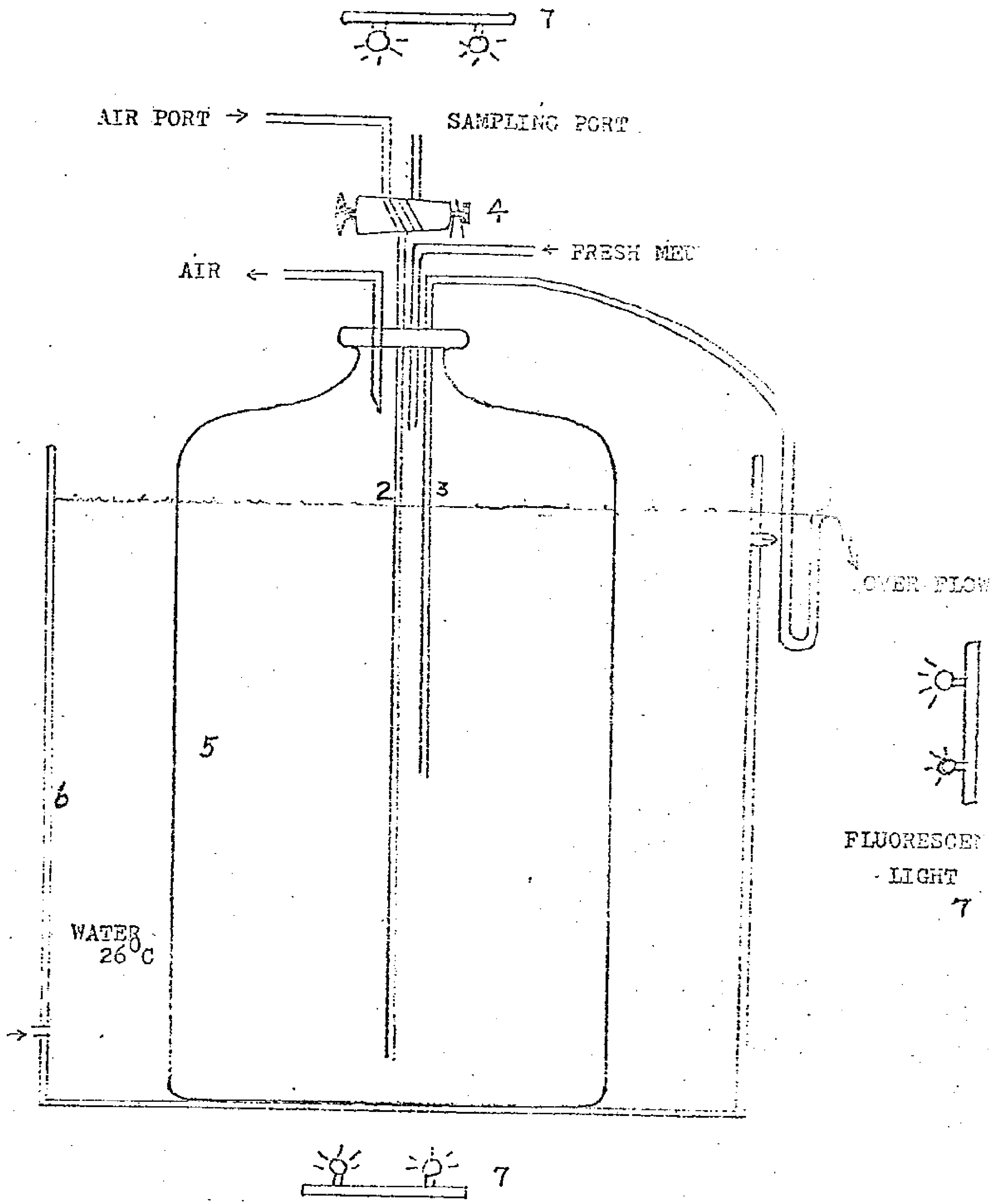
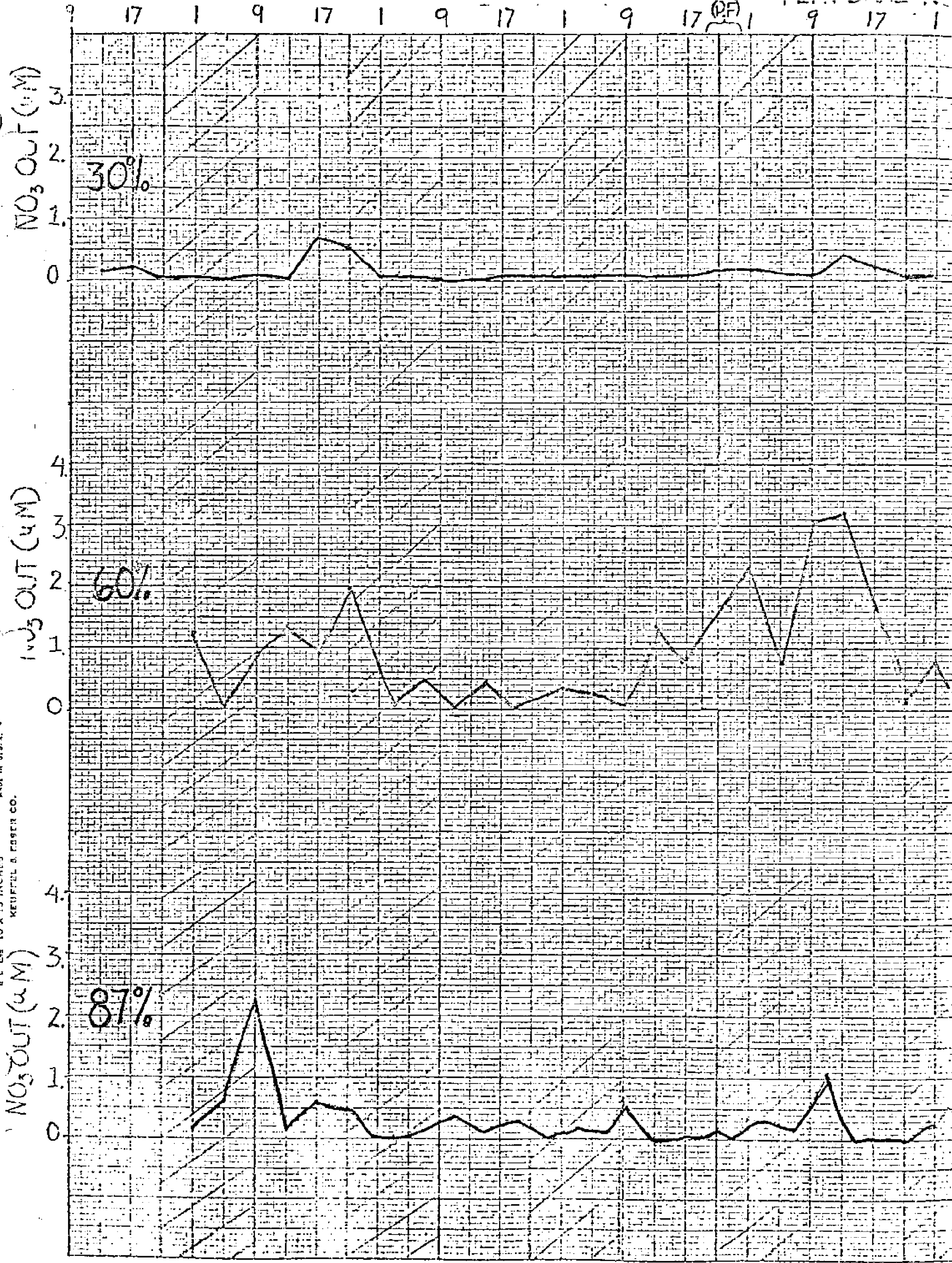
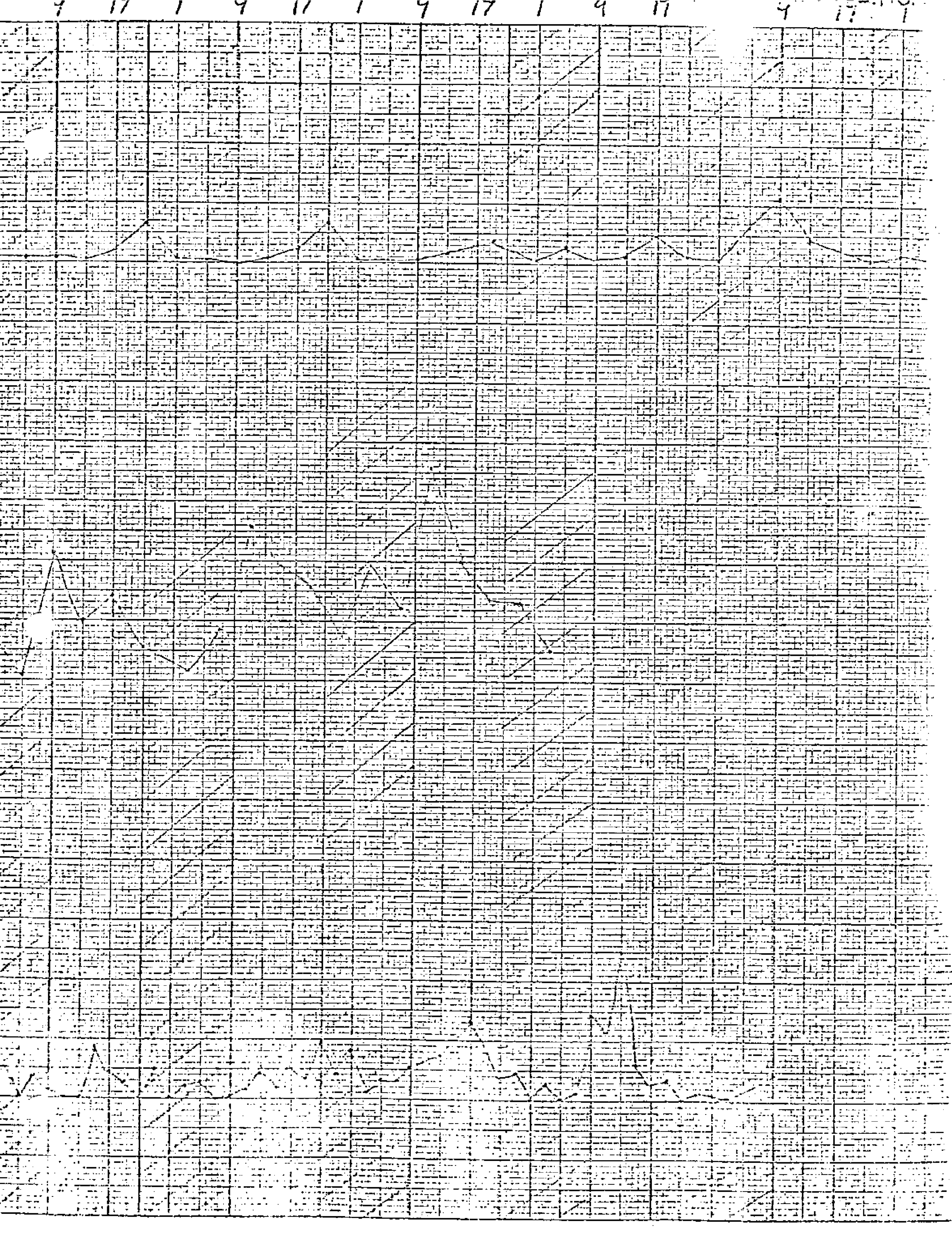


FIGURE 1: SCHEMATIC DESCRIPTION OF THE CULTURE APPARATUS



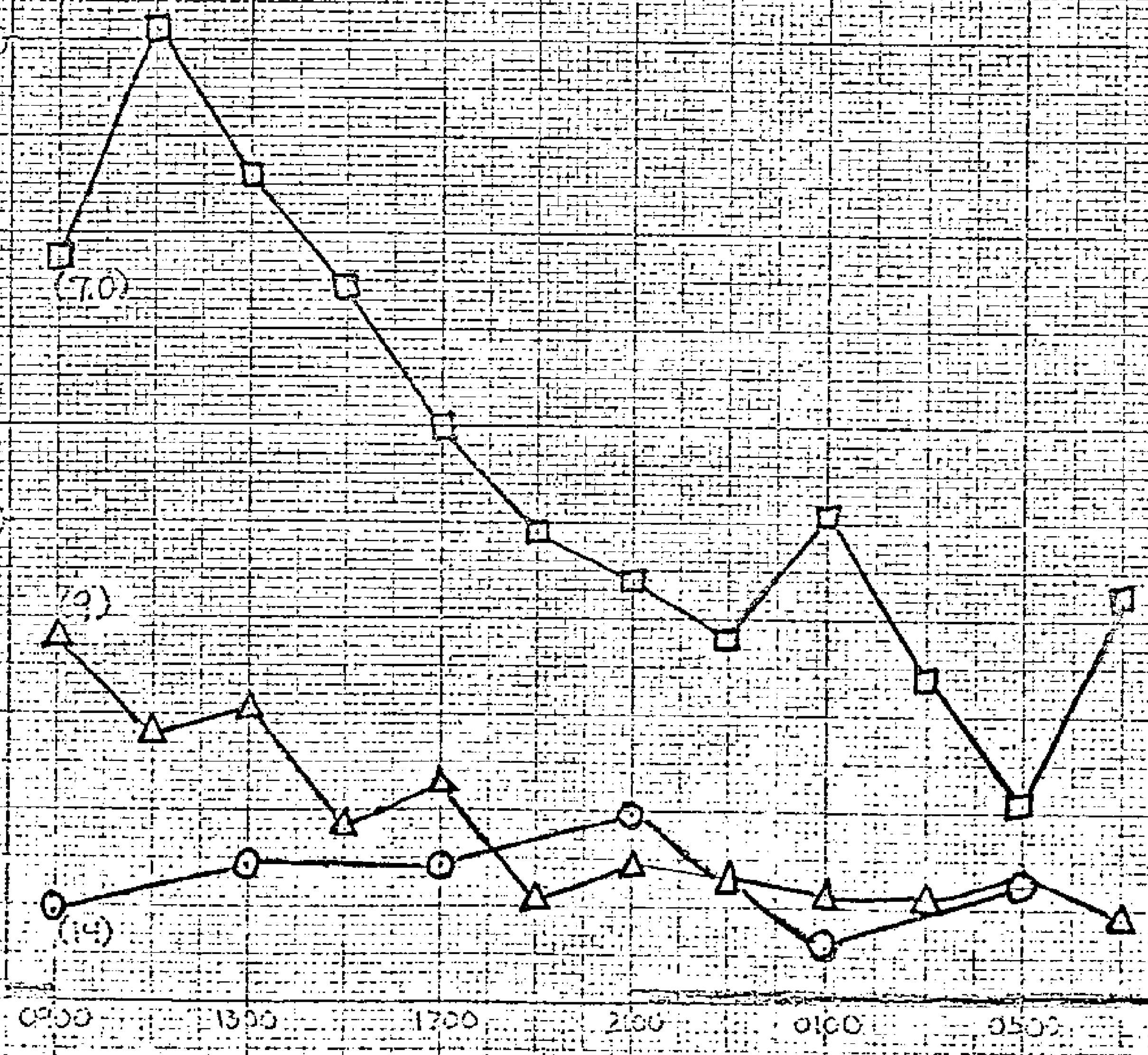
9 17 1 9 17 1 9 17 1 9 17 (DF) 1 9 17 1
NO3 OUT (uM)
30%
3.
2.
1.
0
NO3 OUT (uM)
60%
4.
3.
2
1.
0
NO3 OUT (uM)
87%
4.
3.
2.
1.
0.


9 17 1 9 17 1 9 17 1 9 17 9 17 1

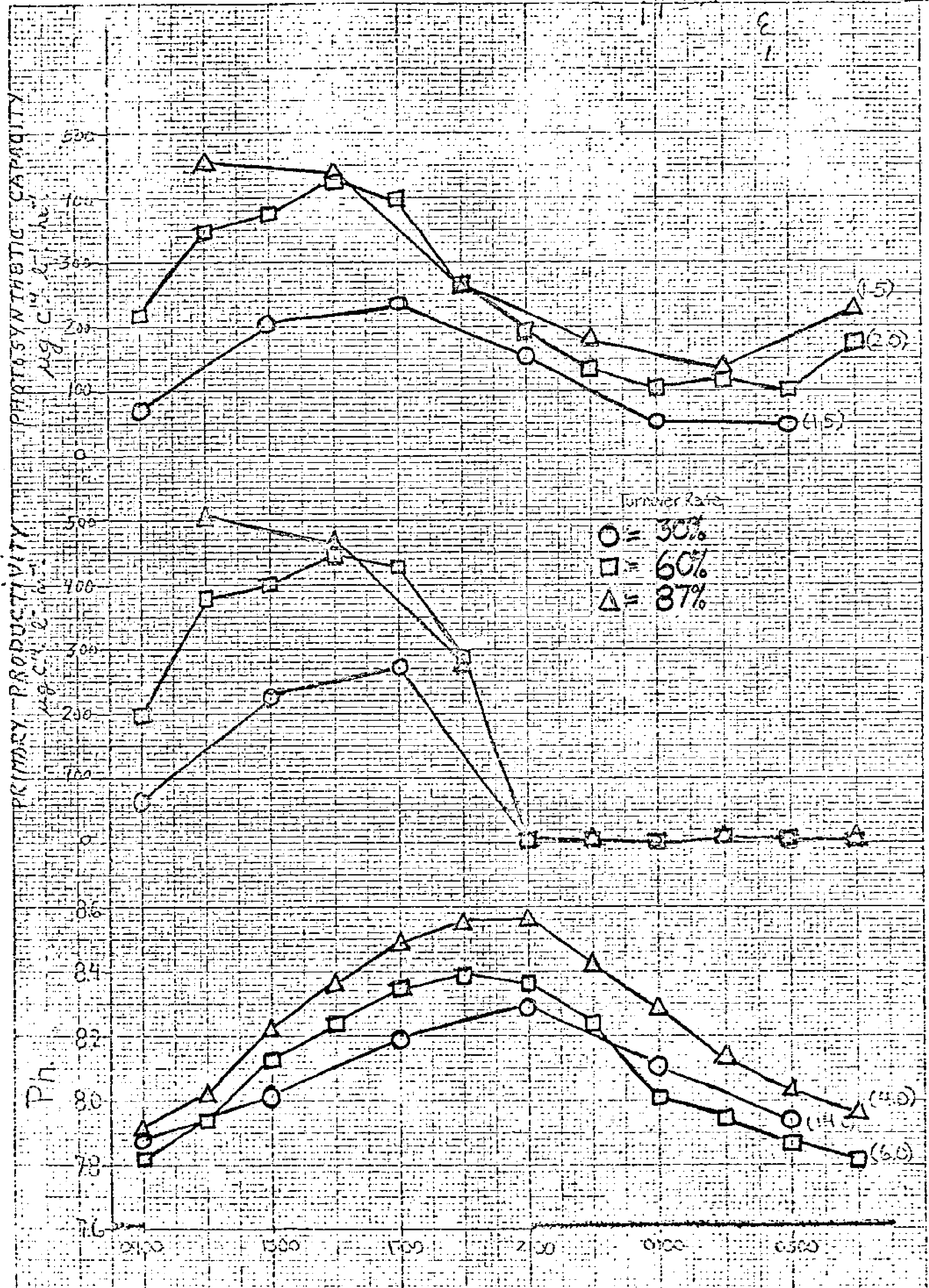
17 1 9 17 1 9 17 1 9

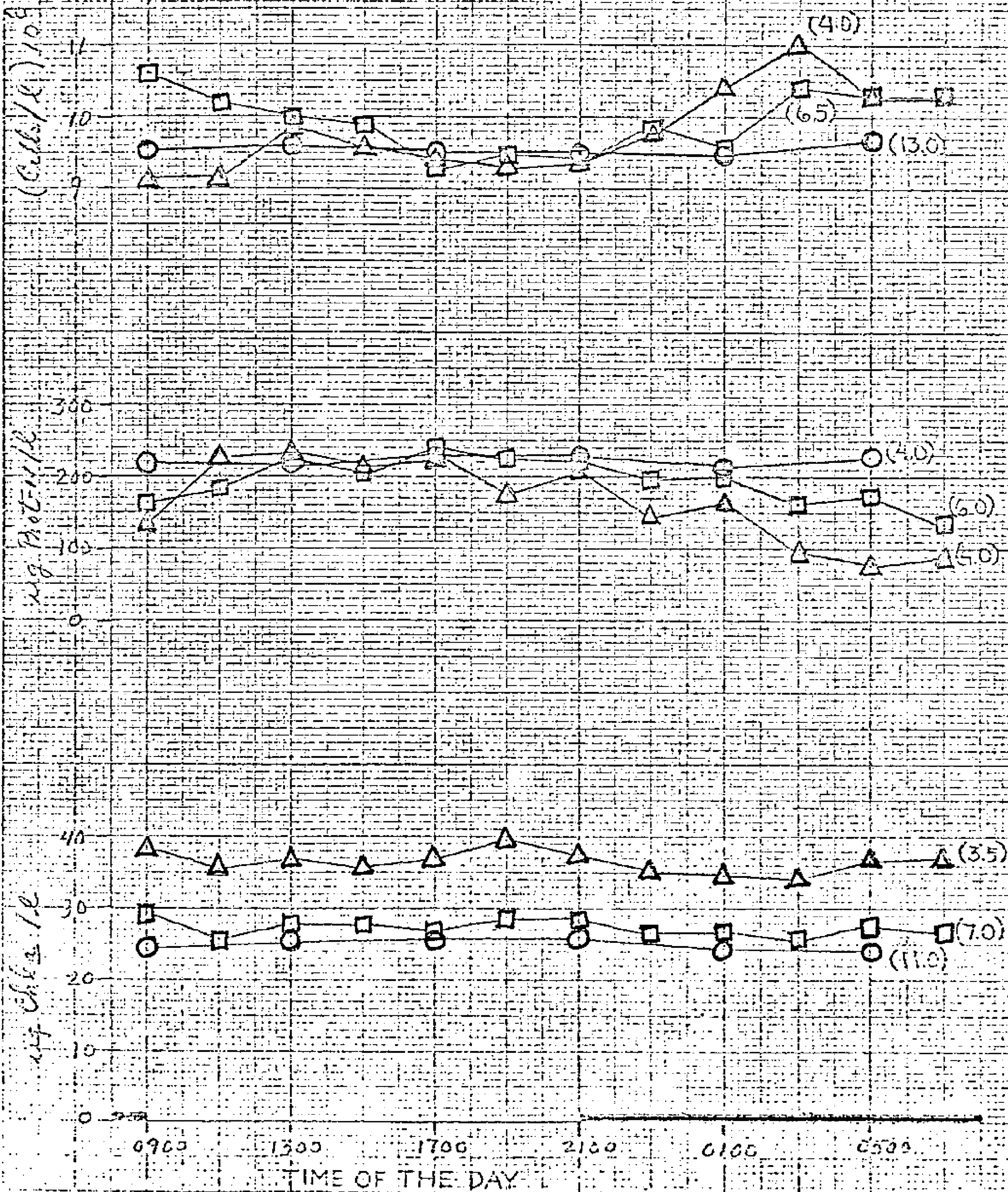
MEAN OF THE RESIDUAL NITRATE CONCENTRATION
IN THE GROWTH CHAMBER VERSUS TIME OF THE DAY

Turnover Rate
○: 30%
□: 60%
Δ: 87%



SQUARE 20 x 20 1 INCH
AS-0010-0


PHOTOSYNTHETIC CAPACITY
PRIMARY PRODUCTIVITY
Ph.
Turnover Rate
○ = 30%
□ = 60%
△ = 87%
(1.5)
(2.0)
(4.0)
(6.0)
500
400
300
200
100
0
8.6
8.4
8.2
8.0
7.8
7.6
2100
1300
1700
2100
0100
0500



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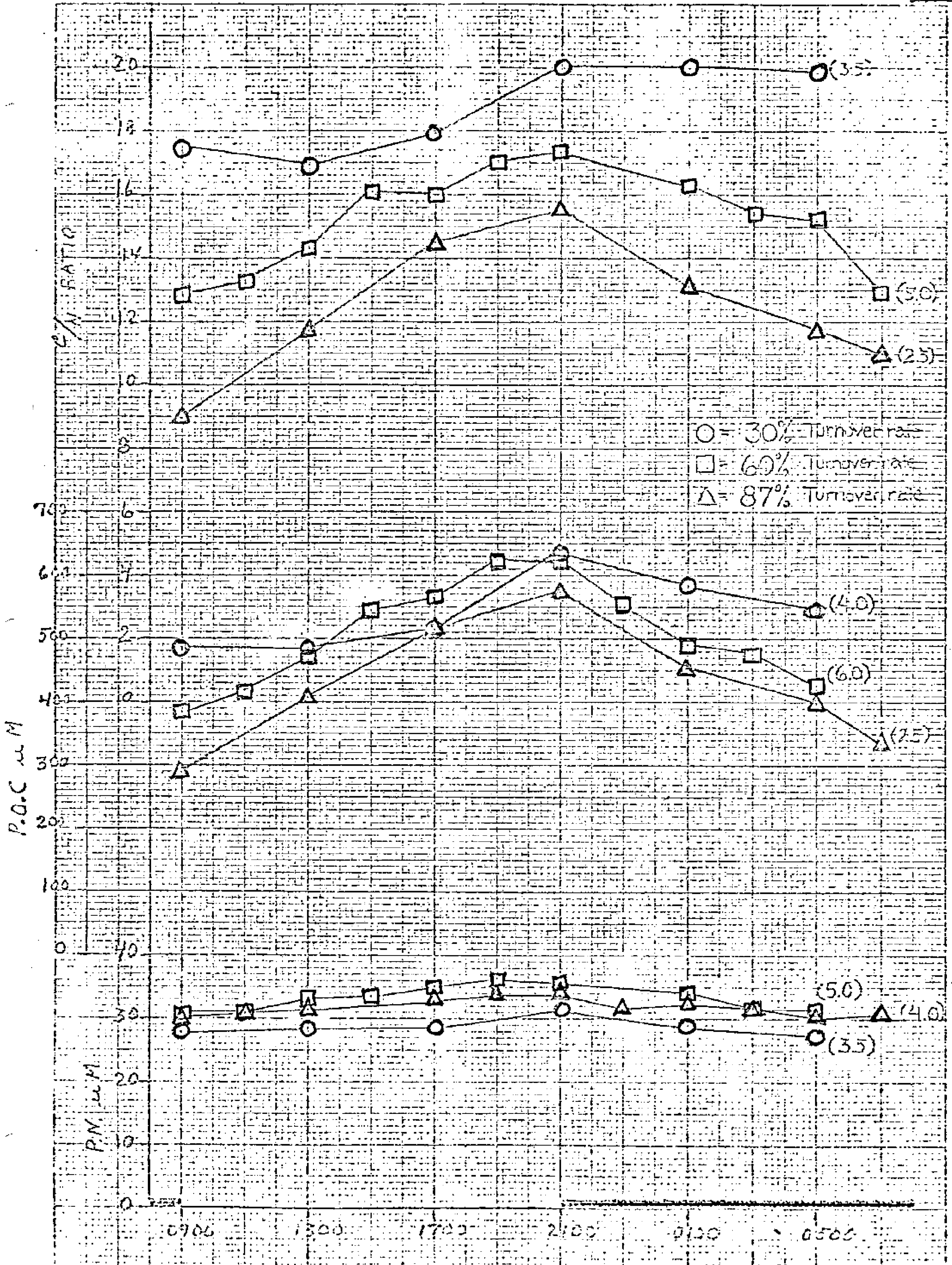
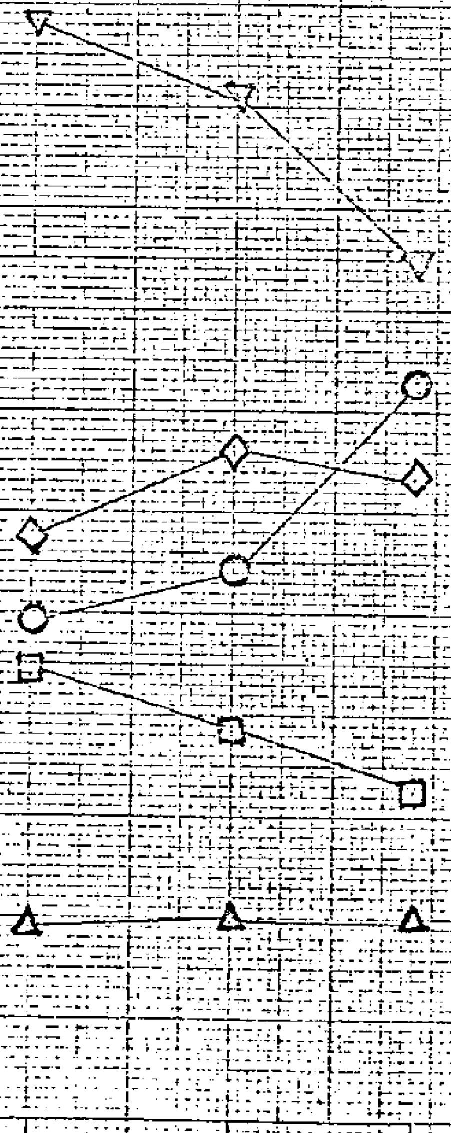
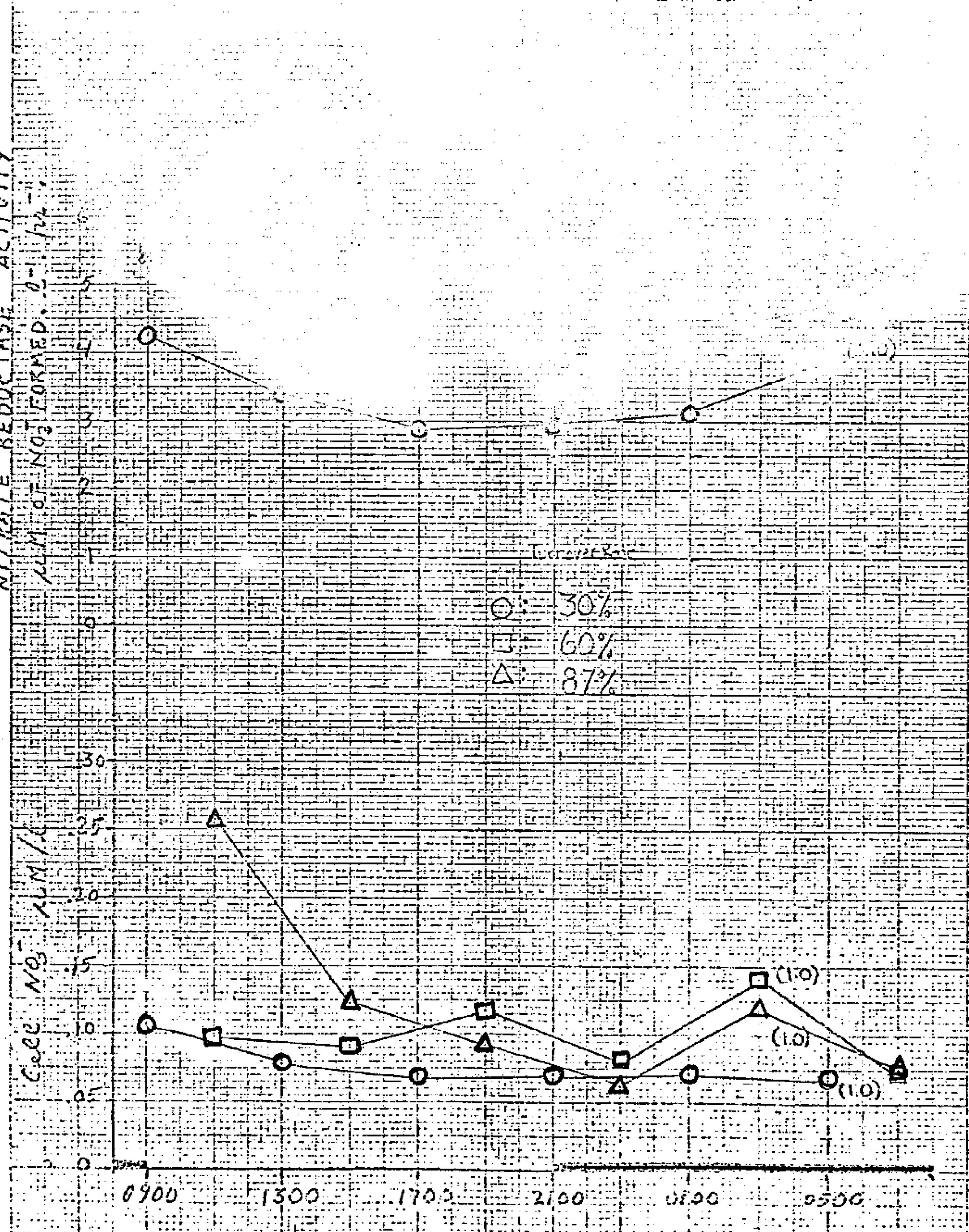


TIME OF THE DAY
Fig. 6
20
18
16
14
12
10
8
6
4
2
0
C/N RATIO
(3.5)
(5.0)
(2.5)
○ = 30% Turnover rate
□ = 60% Turnover rate
△ = 87% Turnover rate
700
600
500
400
300
200
100
0
P.O.C μM
(4.0)
(6.0)
(2.5)
40
30
20
10
0
P.N. μM
(5.0)
(4.0)
(3.5)
0900
1300
1700
2100
0100
0500



SQUARE 20 × 20 1 INCH AS-0510-08

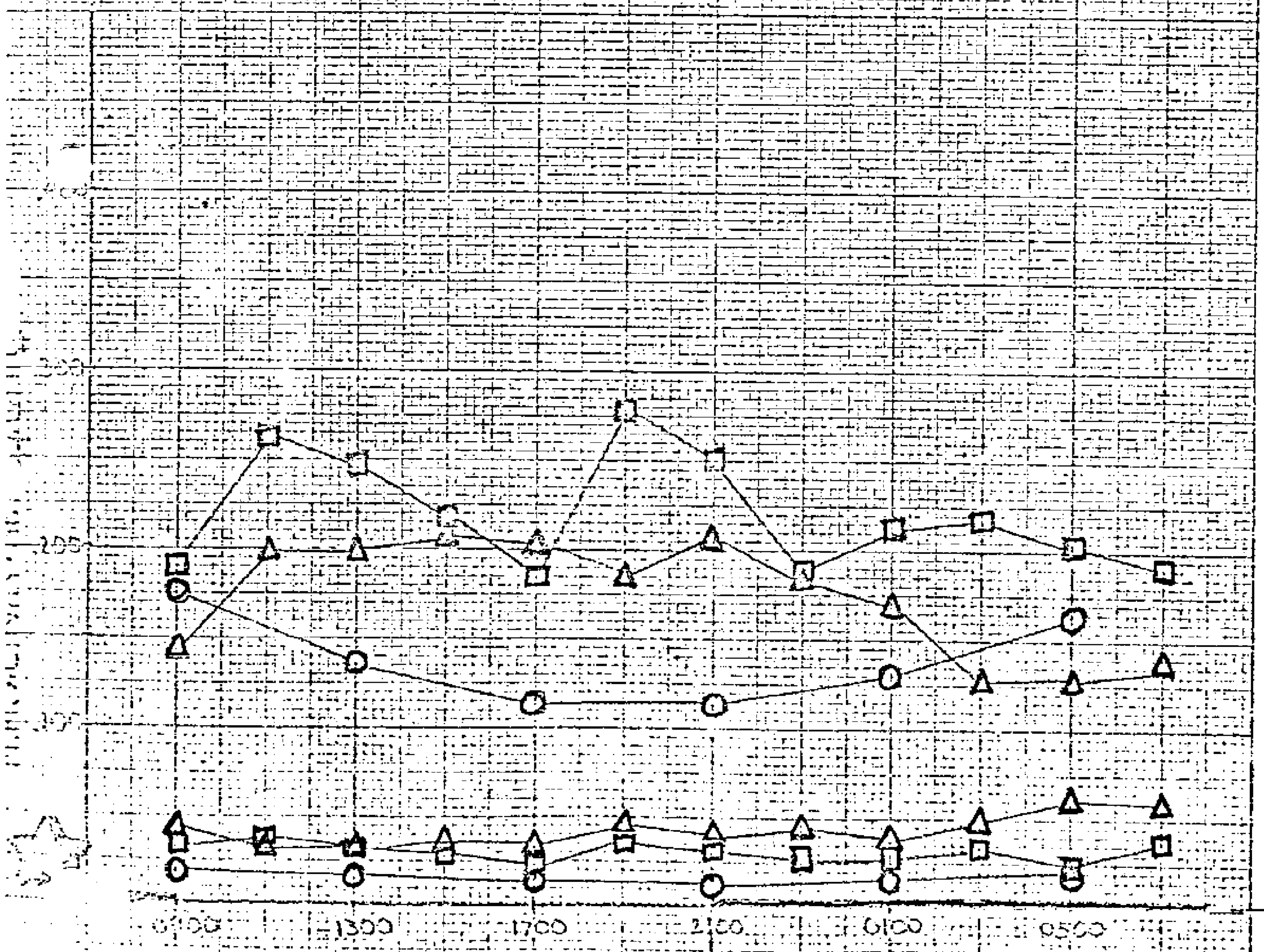


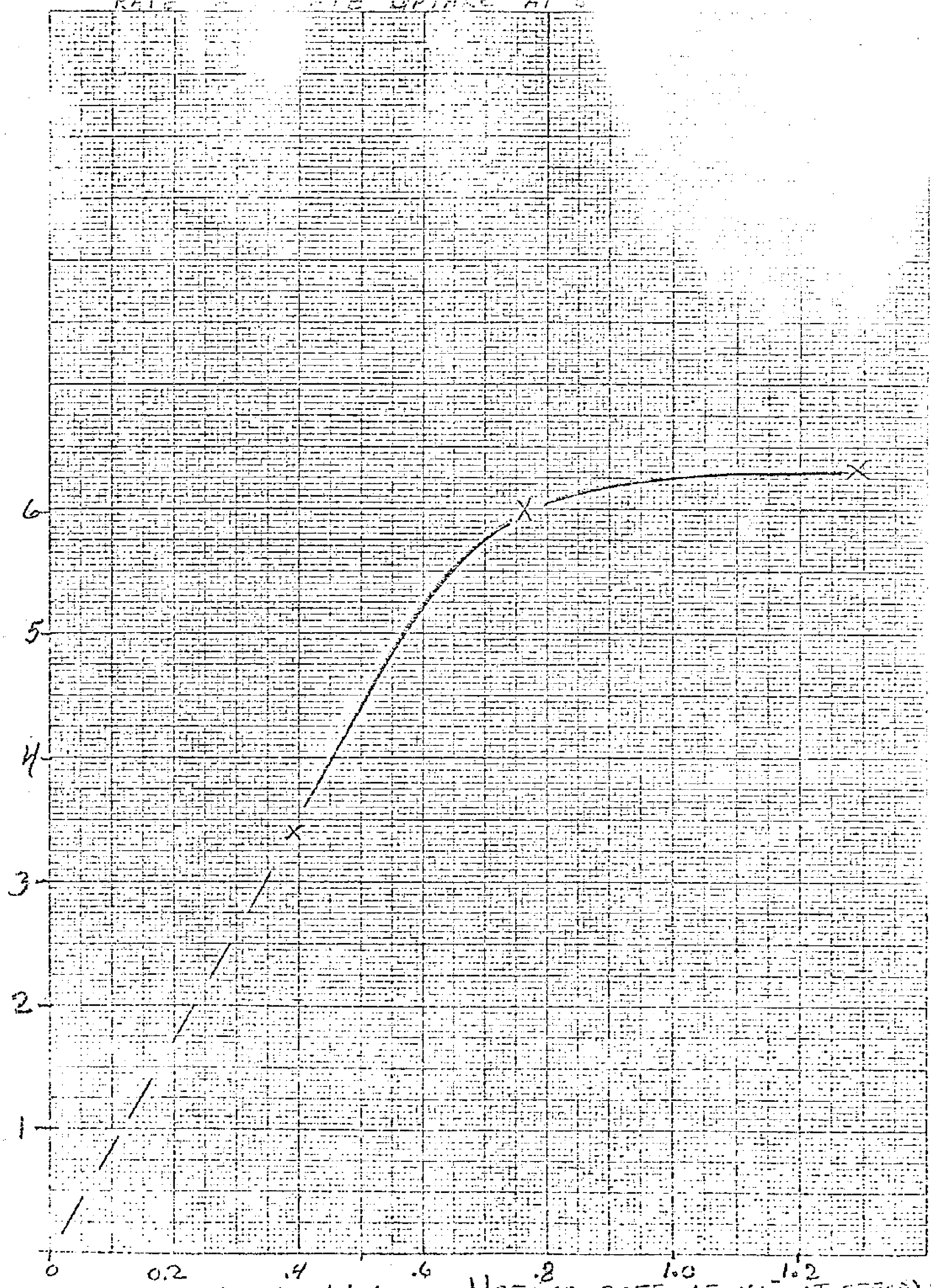
NITRATE REDUCTASE ACTIVITY
Turgor Rate
○: 30%
□: 60%
△: 87%
(1.0)
(1.0)
(1.0)
(1.0)
0900
1300
1700
2100
0100
0500
.30
.25
.20
.15
.10
.05
0

Fig 9

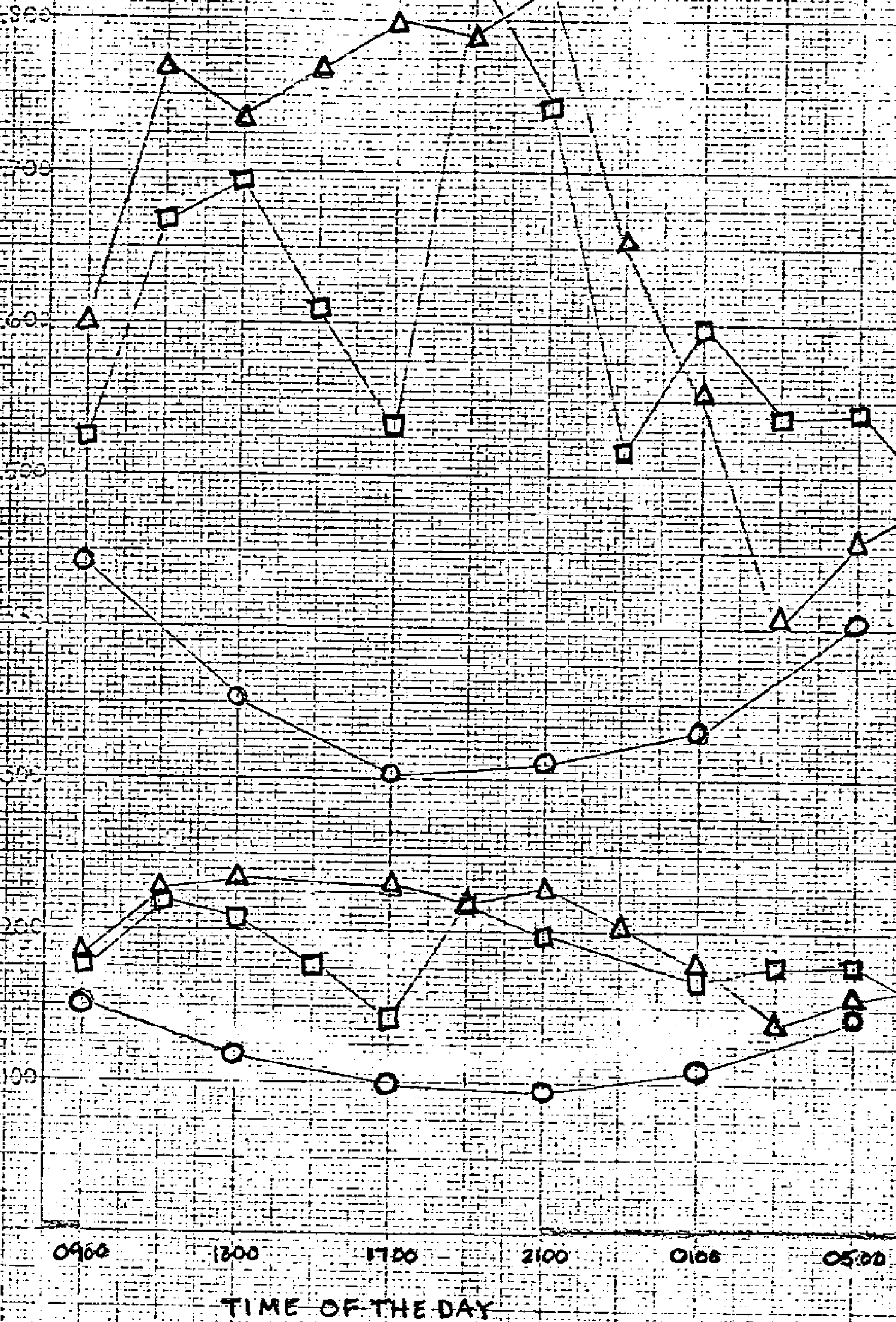
Turnover Rate
○: 30%
□: 60%
△: 87%

N.R. activity·hr⁻¹·µg Protein-N

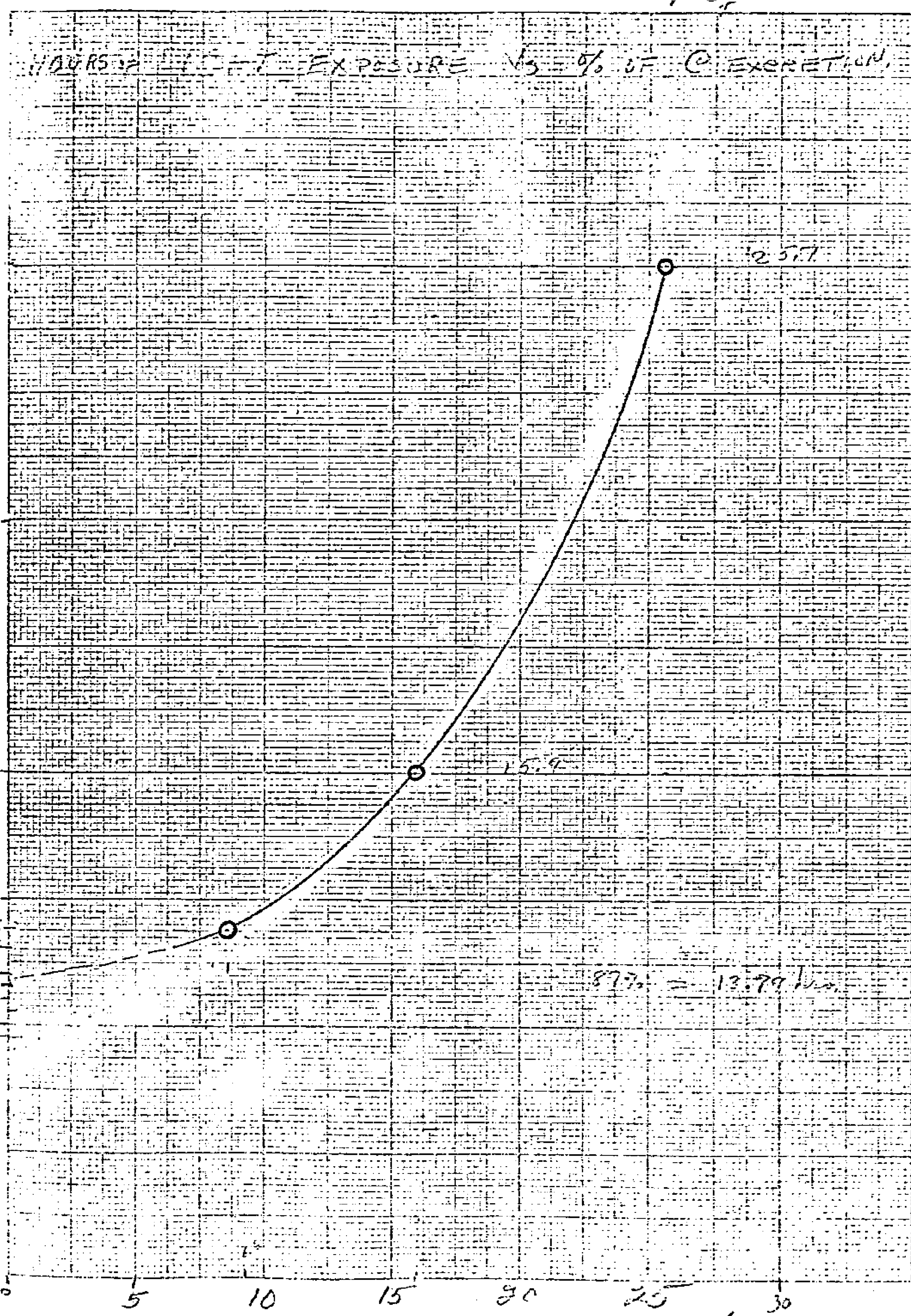




6
5
4
3
2
1
0
0.2
.4
.6
.8
1.0
1.2



0900
1300
1700
2100
0100
0500
TIME OF THE DAY
100
200


EXPOSURE Vs % OF EXCRETION
25.7
15.9
87% = 13.79
TURNOVER
15
12
5
10
15
30

APPENDIX V

THE ULTRASTRUCTURE AND CYTOCHEMISTRY OF RESTING CELL FORMATION IN *Amphora coffaeformis* (Ag.) Kutz.

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Palisades, New York 10964

Key Words:

Amphora

Resting Cell

Ultrastructure

Spores

Short title:

Diatom Resting Cells

The Ultrastructure and Cytochemistry of

Resting Cell Formation in Amphora Coffaeformis (Ag.) Kutz.

SUMMARY

The ultrastructure of logarithmic-growing cells and of resting cells (capable of enduring unfavorable environments) in laboratory cultures of Amphora coffaeformis isolated from deep ocean water (870 m) was examined using electron and light microscopy. The acid phosphatase activity (lysosomal marker enzyme), chlorophyll a and lipid content were assessed at weekly intervals of resting cell formation during cold-dark treatment, simulating deep ocean water. Approximately 4 weeks are required to complete resting cell formation. During the first week, the cytoplasm undergoes extensive transformation and lysosomal activity is observed. Large vacuoles disappear and small ones develop, the mitochondria become fewer and one or more massive mitochondria appear; the cytoplasm becomes densely granular. During the second and third week, the cytoplasm continues to contract, lipid bodies begin to develop and the plastid becomes densely stained. At the fourth week, the mature resting cell is formed containing a small and compressed nucleus, one or more massive mitochondria, a well-formed plastid, and granular cytoplasm containing occasional lipid droplets. The variation in chemical constituents correlates with the microscopic structure of the cells. The fine structure of cells during growth resumption when exposed to light at 25 C is presented. Previous reports of viable, chlorophyll-containing cells at great depths in the ocean may be explained by the results reported in this paper.

The Ultrastructure and Cytochemistry of

Resting Cell Formation in _Amphora coffaeformis_ (Ag.) Kutz.

INTRODUCTION

Although marine diatoms are abundant and contribute significantly to the fertility of the oceans, remarkably little is known about the fine structure and chemistry of their life cycle. Considerable research has been done on the ultrastructure of diatom frustules (17, 32) and the biochemistry and mechanism of frustule formation (3, 8, 9, 21-23, 28-31). Silicon deposition during frustule formation occurs in vesicles enclosed by a specialized membrane called the silicalemma (28). The origin of silicon used in frustule formation is unknown. It may be secreted directly into the vesicles by the silicalemma or the Golgi apparatus may produce secretory vesicles containing silicon deposits that contribute to the formation of the silicalemma and incipient frustule (34). Electron-dense deposits observed in Golgi vesicles during frustule formation in _Pinnularia nobilis_ are not silicon since they resisted hydrofluoric acid treatment (6).

Some pioneering research on pyrenoid structure was performed by Gibbs who described the presence of two chloroplasts in _Nitzschia angularis_ each containing a granular pyrenoid possessing several internal lamellar-containing discs (11). Pyrenoids in _Achnanthes brevipes_, however, contain a crystalline matrix composed of 5 nm diameter subunits (18). Some comparative studies on pennate diatom fine structure (not including _A. coffaeformis_) have been performed (5, 7),

REFERENCES

1. Barka, T. & Anderson, P.J. 1965. Histochemistry: Theory, Practice and Bibliography, Harper and Row, N.Y. 121.

2. Bogarad, L. 1966. The biosynthesis of chlorophylls in Vernon, L.P. and Seely, G.R. (Eds.) The Chlorophylls, Academic Press, N.Y.

3. Desikachary, T.V. 1954. Electron microscope study of diatom wall structure III. Isthmia nervosa Kutz. Amer. J. Bot. 41:616-19.

4. Dodge, J.D. 1973. The Fine Structure of Algal Cells. Academic Press, N.Y., 81-103.

5. Drum, R.W. 1963. The cytoplasmic fine structure of the diatom, Nitzschia palea. J. Cell Biol. 18: 429-40.

6. __________ 1966. Electron microscopy of paired Golgi structures in the diatom Pinnularia nobilis. J. Ult. Res. 15: 100-7.

7. __________ & Pankratz, H.S. 1964. Pyrenoids, raphes, and other fine structures in diatoms. Amer. J. Bot. 51:405-18.

8. __________ _ ____________. 1965. Locomotion and raphe structure of the diatom Bacillaria. Nova Hedw. 10:315-17.

9. Diveltz, N.E. & Colvin, J.R. 1968. The structure of the diatom Thalassiosira fluviatilis. Can. J. Microbiol. 14:1049-52.

10. Gianetto, R. & DeDuve, C. 1955. Tissue fractionation studies IV. Comparative study of the binding of acid phosphatase, B-glucuronidase and cathepsin of rat-liver particles. Biochem. J. 59:433-8.

11. Gibbs, S.P. 1962. The ultrastructure of the pyrenoids of algae, exclusive of the green algae. J. Ult. Res. 7:247-61.

12. Goldfischer, S., Carasso, N. & Favard, P. 1963. The demonstration of acid phosphatase activity by electron microscopy in the ergastoplasm of the ciliate Campanella umbellaria. J. Microscop. 2:621-8.

13. Gomori, G. 1952. Microscopic Histochemistry: Principles and Practice. University of Chicago Press: Chicago, 189.

14. ________ 1956. Histochemical methods for acid phosphatase. J. Histochem. Cytochem. 4:453-61.

15. Granick, S. 1948. Protoporphyrin 9 as a precursor of chlorophyll. J. Biol. Chem. 172:717-27.

16. Guillard, R.R.L. & Ryther, J.H. 1962. Studies of marine planktonic diatoms. I. Cyclotella nana Hustedt, and Detonula confervacea (Cleve) Gran. Can. J. Microbiol. 8:229-39.

17. Helmcke, J.G. & Krieger, W. (Eds). 1962-1970. Diatomeenschalen im Elektronenmikroskopischen Bild, Cramer, Weinheim. Teil I-VII.

18. Holdsworth, R.H. 1968. The presence of a crystalline matrix in pyrenoids of the diatom, Achnanthes brevipes J. Cell Biol. 37:831-7.

19. Holmes, R.W. 1966. Short-term temperature and light conditions associated with auxospore formation in the marine centric diatom Coscinodiscus concinnus. Nature 209:217-18.

20. Lewin, J.C. & Guillard, R.R.L. 1963. Diatoms. Ann. Rev. Microbiol. 17:373-414.

21. ________ & Lewin, R.A. 1960. Auxotrophy and Heterotrophy in marine littoral diatoms. Can. J. Microbiol. 6:127-34.

22. ________________ & Philpott, D.E. 1958. Observations on Phaeodactylum tricornutum. J. Gen. Microbiol. 18:418-26.

23. Lewin, J.C. Reimann, B.E., Busby, W.F. & Volcani, B.E. 1966. Silica shell formation in synchronously dividing diatoms. In Cameron, I.L. & Padilla, G.M. (Eds.) Cell Synchrony, Academic Press, N.Y. 169-83.

24. Malone, T.C., Garside, C., Anderson, R. & Roels, O.A. 1973. The possible occurrence of photosynthetic microorganisms in deep-sea sediments of the north Atlantic. J. Phycol. 9:482-8.

25. Manton, I., Kowallik, K. & Stosch, H.H. von. 1969. Observations on the fine structure and development of the spindle at mitosis and meiosis in a marine centric diatom (Lithodesmium undulatum). I. Preliminary survey of mitosis in spermatogonia. J. Microscop. 89:295-320.

26. ________________________ 1969. Observations on the fine structure and development of the spindle at mitosis and meiosis in a marine centric diatom (Lithodesmium undulatum). II. The swelling phase and early meiotic spindle in male gametogenesis. J. Cell Sci. 5:271-98.

27. ________________________ 1970. Observations on the fine structure and development of the spindle at mitosis and meiosis in a marine centric diatom (Lithodesmium undulatum). III. The later stages of meiosis I in male gametogenesis. J. Cell Sci. 6:131-57.

28. Reimann, B.E.F. 1964. Deposition of silica inside a diatom cell. Exp. Cell Res. 34:605-8.

29. Reimann, B.E.F., Lewin, J. & Volcani, B.E. 1965. Studies on the biochemistry and fine structure of silica shell formation in diatoms. I. The structure of the cell wall of Cylindrotheca fusiformis Reimann and Lewin. J. Cell Biol. 24:39-55.

30. ________________________ 1966. Studies on the biochemistry and fine structure of silica shell formation in diatoms. II. The structure of the cell wall of Navicula pelliculosa (Bréb.) Hilse. J. Phycol. 2:74-84.

31. Reimann, B.E.F. & Volcani, B. 1968. Studies on the biochemistry and fine structure of silica shell formation in diatoms. III. The structure of the cell wall of Phaeodactylum tricornutum Bohlin. J. Ult. Res. 21:182-93.

32. Ross, R. and Simms, P.A. 1972. The fine structure of frustules in centric diatoms: a suggested terminology. Br. Phycol. J. 7:139-163.

33. Round, F.E. 1971. Observations on girdle-bands during cell division in the diatom Stephanodiscus. Br. Phycol J. 6:135-143.

34. Stoermer, E.F., Pankratz, H.S., & Bowen, C.C. 1965. Fine structure of the diatom Amphipleura pellucida. II. Cytoplasmic fine structure and frustule formation. Amer. J. Bot. 52:1067-78.

35. Stoltze, H. J., Lui, N.S.T., Anderson, O.R. & Roels, O.A. The influence of the mode of nutrition on the digestive system of Ochromonas malhamensis. J. Cell Biol. 43:396-409.

36. Strickland, J.D.H. & Parsons, T. 1968. A practical handbook for seawater analysis. Fish. Res. Bd. Canada Bull. 167.

37. Wood, E.J.F. 1966. Perspectives in marine microbiology In Droop, M.R. & Wood, E.J.F. (Eds.) Advances in Microbiology of the Sea, Academic Press, N.Y. 1 & 14.

FIGURE LEGENDS

Fig. 1 A cleaned frustule of A. coffaeformis (ag.) Kütz. obtained from a logarithmic growing culture. Length = 19 µm. X 4,700

Fig. 2 A longitudinal section through a cell from a logarithmic-growing culture exhibits two prominent vacuoles, a centrally located nucleus (N) and a single plastid (P) that extends almost the whole cell length. X 7,960

Fig. 3 The nucleus (N) possesses a prominent nucleolus, and the surrounding cytoplasm contains vesicles (Ve), mitochondria (M), and abundant deposits of ribosomes. X 27,600

Fig. 4 A transverse section through a light-grown cell contains a large plastid and pyrenoid. The pyrenoid is enclosed by a thin membrane and possesses an internal oval membrane resembling a thylakoid membrane. The thin membrane appears to form a ridge (thin beak-like extension) at each end of the long axis of the pyrenoid. A reserve body (B), mitochondria (M), and vacuoles occur in the cytoplasm. X 18,000

Fig. 5 A cell from a culture placed in the dark at 7 C for 1 week exhibits a remarkable cytoplasmic transformation. The cytoplasm becomes densely granular and begins to contract. Numerous small vacuoles occur and the plastid stroma becomes densely stained. X 11,400

Fig. 6 An enlarged segment of Fig. 6 shows cytoplasmic details. A large mitochondrion (M) is present, some of the vacuoles enclose islands of cytoplasm suggesting autophagy through lysosomal activity. The nucleus (N) is compressed against the plastid in this cell. X 21,250

Fig. 7 Electron-dense Gomori reaction product (R) in small vacuoles at the perimeter of the perinuclear cytoplasm indicates lysosomal activity, thus supporting the hypothesis that lysosomes assist in cytoplasmic transformation. One week of cold-dark treatment. X 15,700

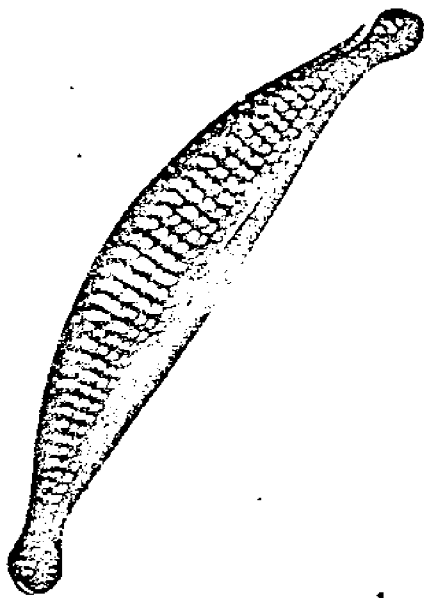
Fig. 8 Reaction product (R) also occurs in the vacuoles at the perimeter of the cytoplasm in developing resting cells. X 36,000

Fig. 9 Absence of reaction product in this NaF-treated control cell substantiates the enzymatic origin of the electron-dense cytochemical marker in Figs. 7 and 8. X 20,100

Fig. 10 A longitudinal section of a resting cell at two weeks development exhibits large lipid droplets, a densely granular cytoplasm, a plastid and beneath it a single large mitochondrion. Light microscopic examination of cells stained with Sudan IV confirms the presence of lipid in the cells at approximately the same position as the droplet (L) in this electronmicrograph and the one in Fig. 11. X 10,140

Fig. 11 A cross-section of a recently divided cell gives additional evidence of increased lipid deposition in reserve bodies at two weeks development time. The cytoplasm is densely granular, but the plastids appear fully formed. Resting cells at the third and fourth week resemble these cells except the cytoplasm is reduced in volume. X 12,480

Fig. 12 A resting cell at four weeks of cold-dark treatment presents a densely granular cytoplasm that has become condensed and in this plane of section contains no vacuoles. The cytoplasm, although condensed, is clearly intact. X 16,100



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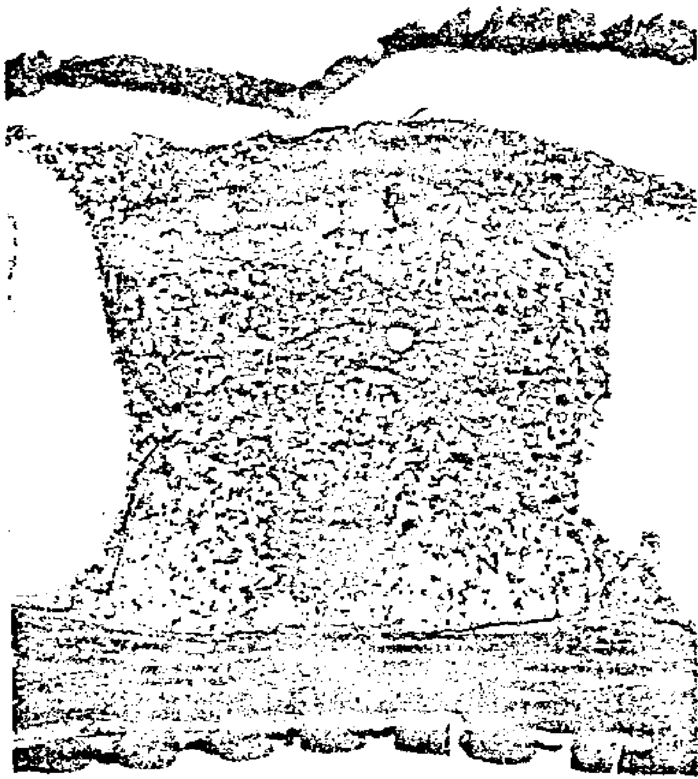

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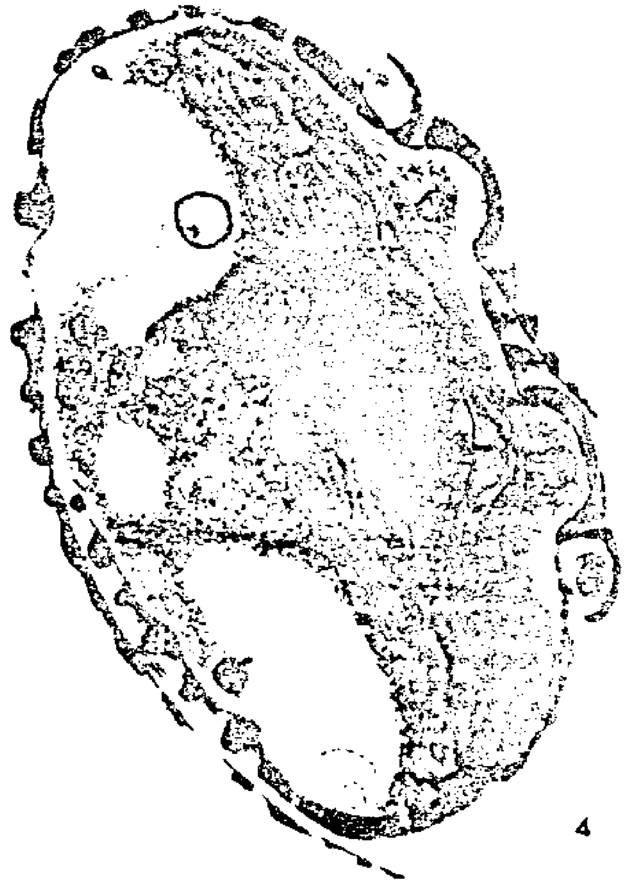

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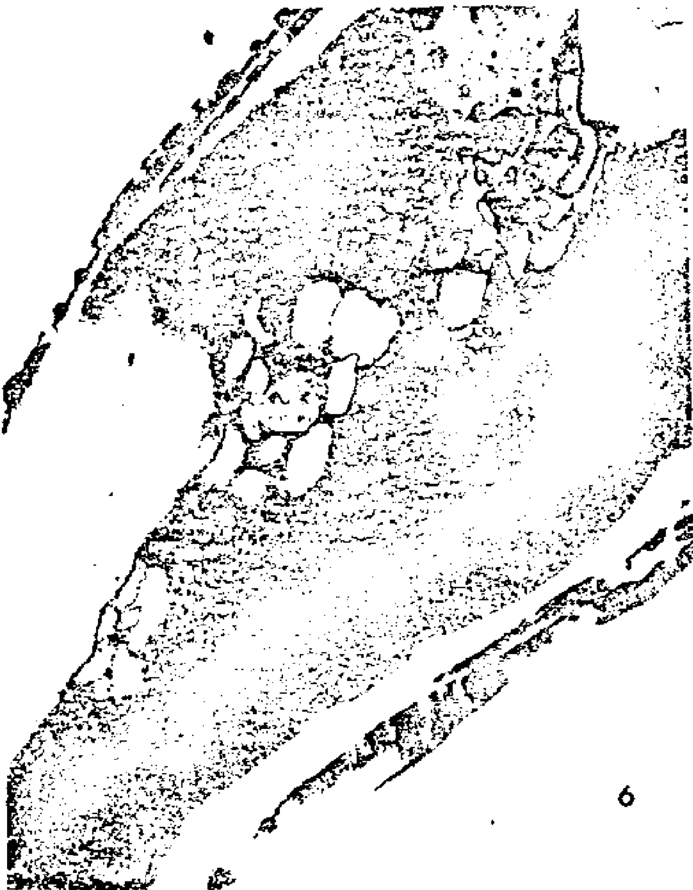
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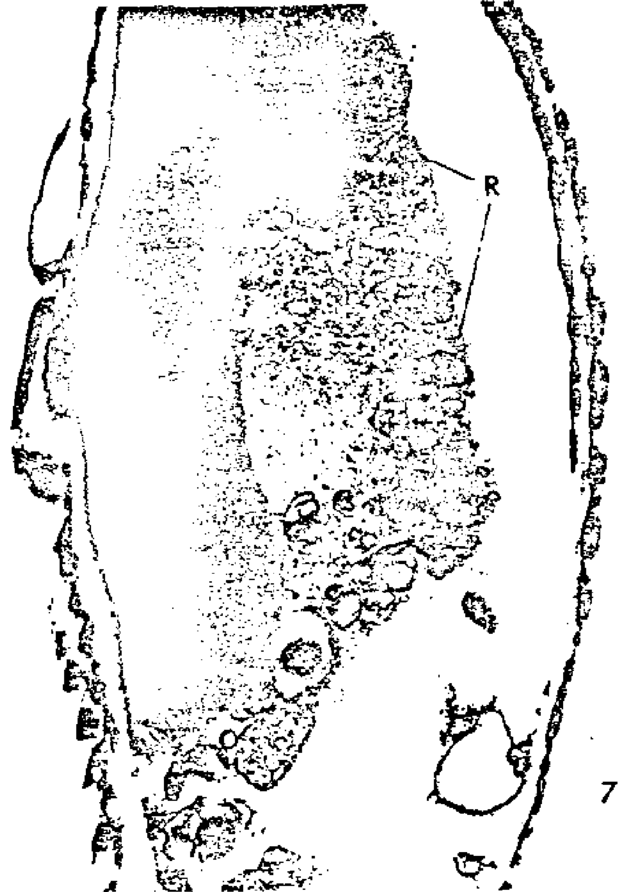
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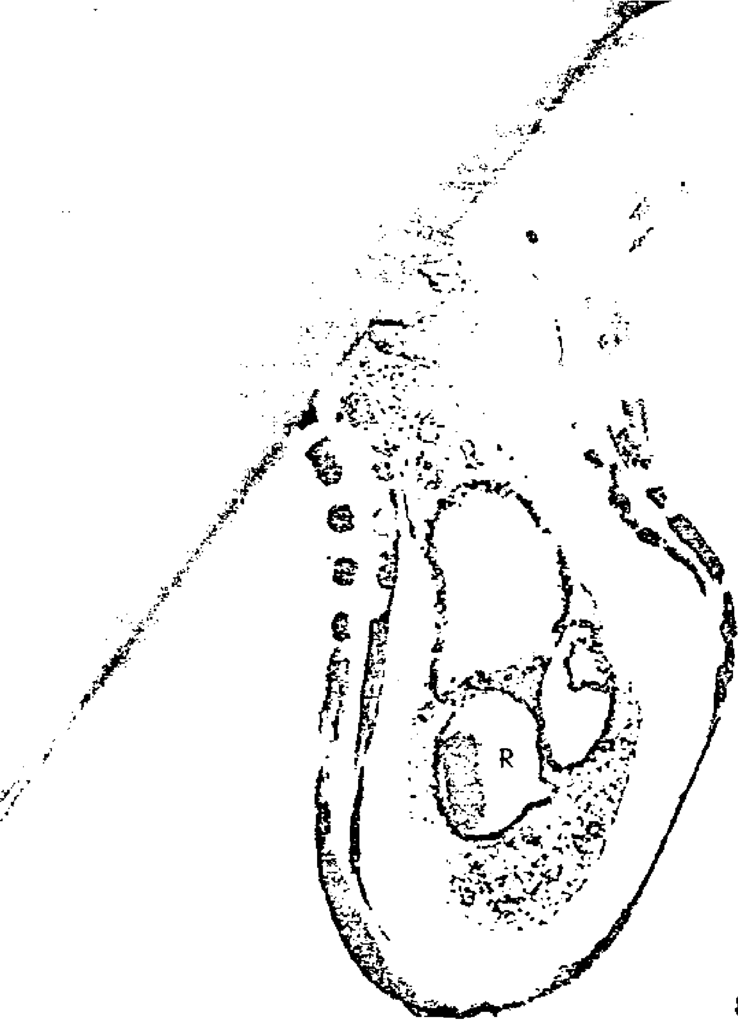
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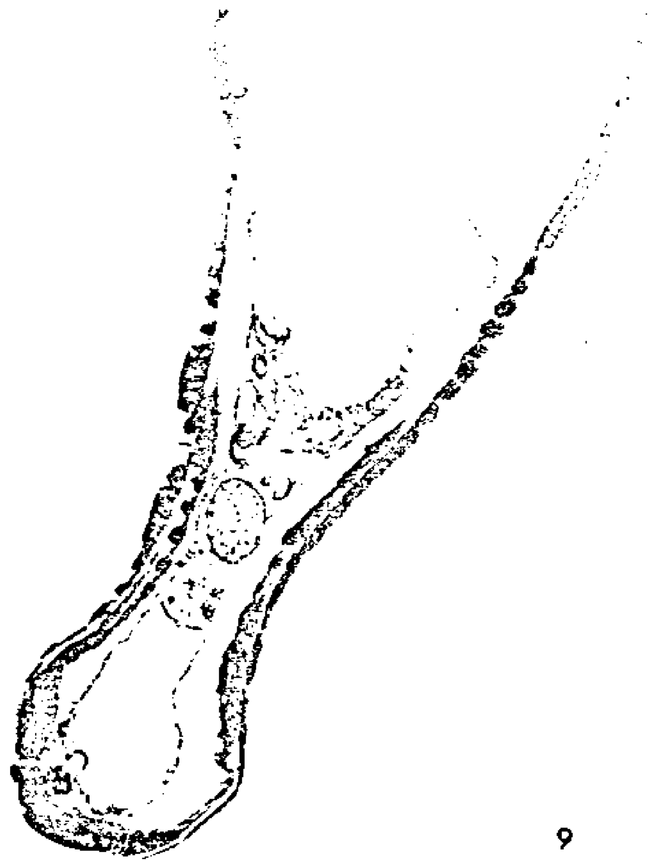
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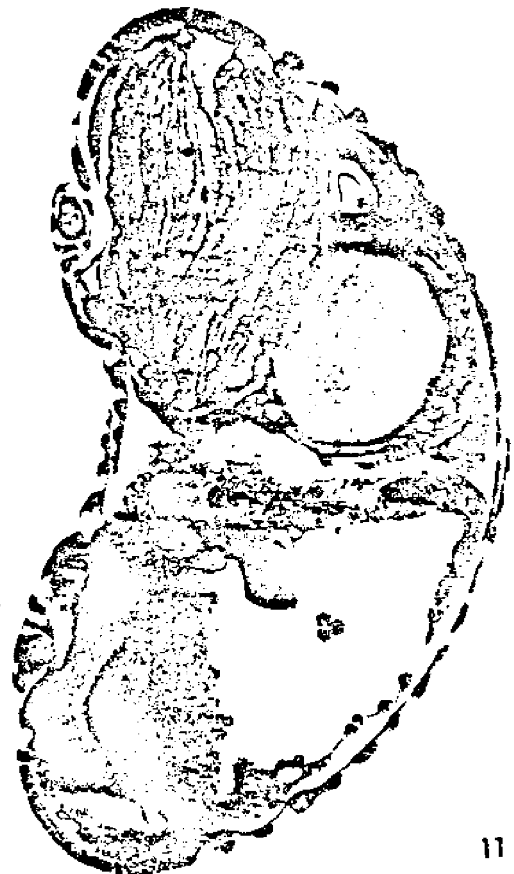
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and a lesser number of studies on centric diatoms have been reported such as the study of Diveltz and Colvin who described the theca morphology and cytoplasmic organelles of Thalassiosira fluviatilis (9).

Additional research remains to be done on the structure and chemistry of diatoms during stages of development in their life cycle. The fine structure of dividing cells and wall formation have been examined (33) and a comprehensive series of studies on spindle development at mitosis and meiosis in Lithodesmium undulatum were performed by Manton and coworkers (25-27). Little is known about the fine structure of diatoms during spore or resting cell formation. Environmental factors such as nutrient concentration, temperature and light intensity can clearly influence diatom growth and regulate stages of development in their life cycle (16, 19). During summer growth, some cells sink into deep water below the euphotic zone where they become dormant. Vertical mixing in late winter brings resting cells and nutrient-rich water to the surface thus initiating a diatom bloom.

Amphora coffaeformis is one of the diatom species obtained from deep water that resumes growth when placed in favorable environmental conditions (24). Research is reported here on the fine structure, cytochemistry, and chemical composition of cells during stages of resting cell formation and their fine structure during resumption of growth.

MATERIALS AND METHODS

Cell cultures. *Amphora coffaeformis* was isolated from deep ocean water pumped from 870 m to the surface at the Lamont-Doherty Artificial Upwelling Project on the north shore of St. Croix (U. S. Virigin Islands). Samples were collected on sterile HA Millipore filters and cultured in Guillard's f/4 medium (16) under fluorescent illumination at 25 C. Unialgal cultures were isolated by streaking agar plates and subculturing colonies. Axenic cultures for chemical analysis were obtained by mild antibiotic treatment (16). Cultures were maintained at 25 C in f/4 medium prepared in deep water obtained at the St. Croix Project and were illuminated with cool white fluorescent tubes with radiant energy flux of 198 $\mu W/cm^2$ for blue emission, 400 to 500 nm, and 204 $\mu W/cm^2$ for red emission, 600 to 700 nm.

Resting cell formation was induced by placing logarithmic growing cultures containing approximately 5×10^5 cells/ ml in total darkness at a temperature of 7 C, which is the temperature of water at 870 m depth from which the diatoms were collected. Approximately 4 weeks were required under these conditions to obtain resting cell formation. Resting cells remain viable for at least two months (longest duration investigated in this study) in the dark at 7 C.

Electron Microscopy -- Cell Structure. Cell cultures were fixed at 4 C for 45 min in 5% glutaraldehyde solution pH 8.0 prepared in 0.06 M cacodylate buffer. The glutaraldehyde-fixed cells were rinsed in cacodylate buffer pH 8.0 and post-fixed for 5 hrs at 4 C in 2% osmium tetroxide pH 8.0 prepared in 0.06 M cacodylate buffer. The fixed cells were centrifuged at 593 x g for 5 min to form a pellet which was resuspended in a slurry of 0.8% agar near room temperature, solidified at 4 C, and small cubes approximately 1 mm^3 in volume were removed for further treatment.

The agar-embedded cells were post-stained for 45 minutes in 3% uranyl acetate prepared in 10% ethanol-aqueous solution, dehydrated in a graded ethanol series, cleared in propylene oxide, and embedded in epon 812. Ultrathin sections were obtained using a Porter-Blum MT-2 ultramicrotome fitted with a Dupont-Sorvall diamond knife. The sections were collected on uncoated copper grids and stained for 5 min with Reynolds' lead citrate. Sections were examined with a Philips EM 200 electron microscope operated at 60 kV accelerating voltage.

Electron microscopy -- cytochemistry. Cytochemical staining was performed using the Gomori technique (12, 13, 14). Glutaraldehyde-fixed cells as described above were centrifuged at 593 x g for 5 min to obtain a pellet. The pellet was washed once in deionized water and thereafter washed 3 times with intermittent centrifugation using 30 ml of 0.05 M acetate buffer, pH = 5.0. Before the final wash, the cell suspension was divided into two parts and sedimented separately. One pellet served as the treatment sample and was suspended in 30 ml of Gomori acid phosphatase medium containing lead nitrate and sodium-β-glycerophosphate (Fisher Scientific Co., Pittsburgh, Pa.). Incubation was at 10 C for 60 min (35). The remaining pellet (used as a control) was treated with 0.01 M NaF to inhibit enzyme activity before incubation in the Gomori mixture under the same conditions as the first pellet. Freshly prepared Gomori medium was kept at 37 C for 1 hr and filtered before use (12). After the samples were incubated, the cells were washed in deionized water, embedded in agar and prepared for observa-

tion in the electron microscope as explained in the foregoing paragraph with the exception that no uranyl acetate stain was used.

Light microscopy. Cells were fixed in 4% glutaraldehyde pH 7.0 and stored at 4 C. A Sudan IV stain prepared in Herxheimer's solvent was used to identify lipid reserves (1).

Chemistry. Acid phosphatase (E.C. 3.1.3.2 orthophosphoric monoester phosphohydrolase) was determined with β-glycerophosphate substrate at pH 5, according to the method of Gianetto and De Duve (10), but incubation was performed at 25 C (the temperature of the cell culture) for 60 min. Enzyme activities are expressed in milliunits/mg protein and also in milliunits/cell, one unit being the amount of enzyme necessary to release 1 μM of product/min under the assay conditions. Lipid and chlorophyll a assays were performed according to the methods published by Strickland and Parsons (36). Cells to be used in lipid assays were washed two times in saline solution (35 ‰ NaCl) to remove residual culture medium and the final pellet was resuspended in 0.25 M sucrose. Chlorophyll a assays were performed by collecting cells on a glass fiber filter followed by homogenization and acetone extraction.

RESULTS

Cell Ultrastructure. The frustule of a logarithmic-growing cell is shown in Fig. 1. A longitudinal section (Fig.2) contains two prominent vacuoles, a centrally located nucleus (N), and below it a single elongated plastid (P). There are few small vacuoles in logarithmic-growing cells. The nucleus (Fig. 3) contains a prominent nucleolus and is surrounded by cytoplasm containing a rich concentration of vesicles approximately 65 nm in diameter. Some of the perinuclear vesicles contain electron-

dense deposits and may belong to part of the Golgi apparatus. Dividing cells frequently contain Golgi bodies, but growing non-dividing cells seldom exhibit them. Thin strands of endoplasmic reticulum and scattered mitochondria (M) occur at the periphery of the perinuclear cytoplasm. The mitochondrial cristae are tubular as commonly found in unicellular organisms. Ribosomes of approximately 22.3 nm diameter are abundant in the peripheral cytoplasm. The thylakoids of the plastid stain heavily and thus some of their fine structure is obscured. Close examination of lightly-stained plastids reveals that each thylakoid (approximately 44 nm thick) contains three lamellae. Osmiophilic granules, 67 to 178 nm in diameter, occur infrequently between the thylakoids. The cytoplasm is bounded by a plasma membrane directly adjacent to the theca. A transverse section (Fig. 4) exhibits a prominent pyrenoid surrounded by a thin membrane enclosing a finely granular matrix of more electron-dense quality than the surrounding plastid stroma. A membrane, 20 nm thick, forms a circular profile within the pyrenoid and in some sections assumes a semicircular or less frequently linear form transversing the pyrenoid. A reserve body (B) lies near the plastid and several mitochondria (M) are scattered in the peripheral cytoplasm. The profile of the thecae and their intersection are clearly visible. The thickness of the theca varies from 18 nm at the thinnest point to 180 nm at the thickest point.

Resting cell ultrastructure. A profound change in cytoplasmic organization occurs when logarithmic-growing cells are placed in total darkness at 7 C simulating deep ocean conditions. After 1 week, the cytoplasm becomes densely granular; the large vacuoles disappear and are replaced by smaller vacuoles (Fig. 5). Few lipid bodies are observed in the cytoplasm and the plastid stroma becomes densely stained.

The numerous small mitochondria (1.6 µm x o.2 µm) observed in the light-grown cells disappear and one or more large mitochondria (M), 2 µm diameter, begin to develop(Fig. 6). The nucleus (N) is reduced in volume and becomes compressed against the plastid or in some cases the nucleus remains globose, but contains a very thin deposit of chromatin. The nucleolus is seldom present. The plastid is surrounded by an array of irregularly-shaped vacuoles, some containing small islands of cytoplasm suggesting incipient autophagy. The extensive cytoplasmic transformation including a decrease in its volume and disappearence of organelles during resting cell formation suggests lysosomal activity. Evidence for lysosomal activity in small vacuoles at one week of cold-dark treatment is presented in Figs. 7 to 9. Electron-dense reaction product (R) of the cytochemical stain occurs in small vacuoles at the periphery of the perinuclear cytoplasm (Fig. 7) and at the perimeter of the cell (Fig. 8). Control cells treated with NaF do not contain reaction product (Fig.9), thus substantiating its enzymatic origin.

Within the second to third week of resting cell development, massive lipid droplets (L) appear within a densely granular cytoplasm (Figs. 10 and 11). Light microscopic examination of cells at 1 through 4 weeks of resting cell development revealed the presence of large reserve bodies that were stained by Sudan IV thus indicating they contained lipid. Some of the cells contained refractile bodies that could be chrysolaminarin reserve bodies (20). The percentage of cells containing reserve bodies at each weekly interval was respectively 34, 43, 30 and 17. The bodies were smaller on the average at 1 week of development than those at the second and third week. By the fourth week,

those cells still containing reserve bodies possessed decidedly smaller ones than were observed in the previous two weeks. Moreover, the amount of lipid contained in resting cells as presented in the Table and described in the chemistry section, correlates with the percentage of cells containing reserve bodies and with their relative size as observed by microscopy.

In general, the ultrastructure of resting cells at the fourth week is very much like that observed during the second and third week with the exception that the cytoplasm becomes more contracted in some cells (Fig. 12), and the vacuolor space decreases. The plastid, a few massive mitochondria, and the degenerate nucleus are the most obvious organelles in a resting cell (Figs. 6, 10, and 11). Light and electron microscopic examination of resting cell formation under cold-dark treatment shows that the process reaches a stable state at the fourth week. Some cells that do not become stable at this time continue to decline in cytoplasmic volume and they appear unable to resume growth when exposed to favorable conditions.

Growth resumption. Resting cells will resume growth when placed in the light at 25 C, but the onset of growth resumption varies within a culture and among cultures. In some cases, renewed growth occurs after 2 days in the light although lag periods as long as 2 weeks have been observed, whereupon cells become active and establish a rich culture containing motile and dividing cells within 4 days.

Figures 13 to 15 show stages in growth resumption. After 2 days, the cytoplasm begins to reestablish itself (Fig. 13). The nucleus, containing a distinct nucleolus, is expanded and numerous small vacuoles develop as the cytoplasm begins to swell. Reserve bodies begin to degenerate and the cell looks very much like those observed during the first week of resting cell formation. The massive mito-

chondria disappear as small ones increase in number. It is not possible to determine how the small mitochondria are formed, but some large mitochondria appear to bud-off smaller segments at this stage of cell activation. Cell division commences in some cases and the daughter cells produce Golgi bodies, establish a silicalemma, and secrete silicon within the frustule-forming vesicles (Fig. 14). It is clear that the vesicles are suspended within the cytoplasm and that a plasma membrane lies external to the silica-containing vesicles during these early stages of frustule formation. The electron-dense deposits in the Golgi resist HF treatment and therefore are not silicon. After 4 days in the light some cells appear to be fully advanced toward normal growth (Fig. 15). The cytoplasm is somewhat more granular than usually observed in logarithmic-growing cells, but the plastid is fully developed. Light microscopic examination of cells at this stage also shows that the cytoplasm is rich and dense.

Chemistry. Acid phosphatase activity (lysosomal marker enzyme), chlorophyll a, and lipid content of logarithmic-growing cells and of resting cells during weekly intervals in dark-cold treatment are presented in the Table. After 1 week of dark-cold treatment there is a marked decrease in all of the chemical constituents as compared to light-grown cells. Thereafter, in the second week, there is a clear increase in acid phosphatase activity, chlorophyll a, and lipid content. During the third and fourth weeks, acid phosphatase activity decreases and there is an appreciable variation in activity between samples in these last stages of resting cell formation as indicated by an increase in error variance. Chlorophyll a content per cell, however, increases to a final level nearly equal to that of the logarithmic-growing cells. Concurrently, cellular lipid content decreases and may be a sign

that the lipid reserves are being used partly as precursors in chlorophyll synthesis and to maintain cell metabolism in the last stages of resting cell formation.

The Tabular data, showing a marked decrease in chemical constituents after 1 week of cold-dark treatment, correlate with electron microscopic evidence (Figs. 2 and 5) that shows a massive reorganization of cytoplasm. The respiratory and photosynthetic organelles are transformed including: (1) a decrease in small mitochondria and the development of a few large mitochondria, (2) formation of a densely-staining plastid stroma, (3) loss of the large vacuoles, and (4) an elaboration of numerous small vacuoles which exhibit lysosomal enzyme activity. An increase in lipid during the second and third week also correlates with microscopic evidence of lipid accumulation (Figs. 10 and 11). Moreover, the large chlorophyll a content found in the third and fourth weeks is consistent with the microscopic observation that resting cells retain their plastids and that some algae are known to synthesize chlorophyll by enzyme activity in the dark (2, 15). Chemical analyses were not performed on resting cells during light-induced growth. Resting cells develop at different rates and therefore the cultures contained a mixture of cells at various stages of development, thus precluding representative sampling for bulk chemical analysis.

DISCUSSION

The existence of viable diatoms and other algae at great depths in the ocean has been known for many years (24, 37), but the mechanism of their survival and their chemical composition have not been elucidated.

Moreover, the presence of appreciable quantities of chlorophyll in the aphotic zone to 5000 m where little or no photosynthesis can occur has not been satisfactorily explained in many cases (37).

The research reported here presents evidence that diatoms form resting cells when subjected to cold-dark conditions simulating deep ocean water and that an appreciable quantity of cholorophyll, approaching that of light-grown cells, is synthesized during resting cell development. Both the existence of dormant viable diatoms and the presence of chlorophyll at great ocean depths can be explained by these findings. The presence of chlorophyll a in resting cells raises the obvious question of its role in diatom survival. It is clear that chlorophyll cannot absorb appreciable quantities of light at the great depths where viable diatoms have been found and the suggestion that chlorophyll could utilize bioluminescence to facilitate low-energy-requiring pathways of carbon assimilation is unlikely (37). An alternate explanation is offered that chlorophyll a is stored in resting cells as a reserve pigment to provide an immediate photoabsorption advantage when the cells are returned to favorable growth conditions. Those cells capable of early growth resumption have a competitive advantage in exploiting surface waters, particularly at the beginning of a phytoplankton bloom. Laboratory studies reported here show that resumption of growth can occur as early as two days after exposure to light at 25 C. Moreover, some cells exhibit longer dormancy periods thus possibly serving as reserve cells in case the early growth is thwarted by an unfavorable reversal in the environment.

The ultrastructure of light-grown cells of *A. coffaeformis* is similar to that of other pennate diatoms. The chloroplasts contain thylakoids composed of 3 lamellae as commonly observed in the Bacillariophyceae (4, 11). The pyrenoid is enclosed by a thin membrane as also observed in *Acnanthes minutissima*, *Cyclotella meneghiana*, *Cymbella affinis*, *Nitzschia sp.*, and *Surirella ovalis* (7). Golgi apparatus formation occurs predominantly during cell division. The presence of dense organic deposits in Golgi vesicles as observed in other dividing diatoms (6) suggests that these "organic plates" serve as a template for silicon deposition in the cisternae formed by the silicalemma when frustule formation commences. The silicalemma occurs within the cytoplasm of the daughter cells and, therefore, may be a fusion product of Golgi vesicles that align at the equatorial plane after cell division is completed.

The ultrastructure of resting cells and the correlated chemical composition present evidence of a remarkably ordered sequence of events leading to stable resting cell formation within 4 weeks after cold-dark treatment has started for a culture containing approximately 10^5 cells/ ml. The formation of one or more massive mitochondria while smaller ones disappear, the retention of the photosynthetic apparatus, a decrease in nuclear material, and the formation of lipid reserves all suggest that the resting cell is a parsimonious assemblage of organelles prepared to resume metabolism and growth upon return to favorable environmental conditions, The origin of the large lipid droplets formed during the second and third week of resting cell development is not known, but many membranous organelles disappear during the first week and their

lipids may be transformed into reserve lipid droplets. Although evidence for chrysolaminarin reserve bodies was presented, many of the storage bodies in *A. coffaeformis* resting cells appear to contain lipid. They stain with lipid soluble dyes and their number correlates with the lipid content of the cells. Evidence for lysosomal participation in cytoplasmic transformation and condensation is presented including cytochemical staining and cellular acid phosphatase assays. The increase in acid phosphatase activity during the second week of resting cell formation, when cytoplasmic condensation is proceeding most rapidly, further supports the lysosomal hypothesis. The relatively large amount of acid phosphatase activity in logarithmic-growing cells is expected since cell division is attended by a certain amount of lysosomal activity.

Resumption of growth is amazingly rapid for some of the resting cells when exposed to light at 25 C. Numerous mitochondria reappear within 2 days and the previously condensed cytoplasm exhibits an increase in volume and vacuolization. At this time, the nucleus is clearly reestablished and evidence of cell division is accompanied by frustule formation in the daughter cells. After 4 days, large vacuolated cells appear and gliding motion is exhibited.

Amphora coffaeformis is a remarkably versatile organism, adjusting rapidly to adverse environments, efficiently establishing resting cells to endure long periods of unfavorable growth, and promptly reestablishing itself when exposed to favorable growth conditions. This versatility and endurability may explain the widely recognized success of diatoms in exploiting ocean environments.

Table

Acid phosphatase activity, chlorophyll a, and lipid content in light-grown cells and in resting cells at weekly intervals of development *

Treatment time	Acid phosphatase activity		Chl. a	Lipid
weeks	milliunits/ mg protein	milliunits/ cell x 10^{-7}	µg/ cell x 10^{-9}	µg/ cell x 10^{-7}
0	12.9±0.04	4.48±0.16	5.5±.8	145
1	4.8±0.18	1.36±0.20	2.1±.4	77
2	11.5±4.9	3.18±1.3	4.0±.7	96
3	9.6±4.6	2.57±1.3	4.6±.9	85
4	8.9±5.0	1.64±1.0	5.1±.1	38

* Acid phosphatase assays and lipid assays were made on the same set of duplicate samples. Chlorophyll a assays were performed with a separate set of duplicate samples. Treatment was darkness at 7 C. Zero weeks treatment is for light-grown cells as described in the section on cell culture under Materials and Methods. The standard error of the mean is reported as a ± value after the column entry. The variability in acid phosphatase activity between samples increases markedly during the last three weeks of resting cell development. A clear minimum in all chemical constituents occurs at the first week which correlates with electron microscopic evidence (Fig 5) that the cells are in transition stages from active growth to resting cell development. Thereafter, in the the second week when resting cell development is well underway, an appreciable increase in all constituents occurs.

ACKNOWLEDGEMENTS

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COMPARATIVE GROWTH OF HARD SHELL CLAMS (*MERCENARIA MERCENARIA* LINNÉ AND *MERCENARIA CAMPECHIENSIS* GMELIN) AND THEIR F_1 CROSS IN TEMPERATE, SUBTROPICAL AND TROPICAL NATURAL WATERS AND IN A TROPICAL ARTIFICIAL UPWELLING MARICULTURE SYSTEM[1]

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ABSTRACT

The growth of hard shell clams (*Mercenaria mercenaria* Linné and *Mercenaria campechiensis* Gmelin) and their F_1 cross in a controlled experimental environment was compared to growth in uncontrolled natural environments. The clams were spawned by Paul Chanley of Shelter Island Oyster Company, Inc., Greenport, New York.

In the controlled environment of the artificial upwelling

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mariculture system on St. Croix, U.S. Virgin Islands, pollutants and predators were absent and fouling was minimal. Salinity was 34.75 to 34.95^{o}/oo and water temperature varied between 22° and 30°C during the experiment. Natural environments in temperate, subtropical and tropical waters were selected as sites for comparative studies: Southold on Gardiners Bay, New York; Wachapreague on Bradfords Bay, Virginia; Alligator Harbor on the northwest Gulf Coast of Florida; Kupeke Pond in Pukoo, Molokai, Hawaii; and Salt River Inlet, St. Croix.

Clams from each population—*M. mercenaria*, *M. campechiensis* and their F_1 cross—were planted at Southold, New York and at both St. Croix sites. Only *M. campechiensis* and F_1 clams were sent to Virginia and Florida, and F_1 clams and *M. mercenaria* were sent to Hawaii. Increase in length, "wet" weight and survival were measured at the different locations from April 1973 through May-June, 1974.

M. campechiensis and F_1 clams reached market size (greater than 25.5 mm thick) in 6.5 to 13 months in the St. Croix artificial upwelling system. These clams did not reach market size in Southold, New York, Wachapreague, Virginia, or Salt River Inlet, St. Croix. The F_1 clams in Molokai, Hawaii and in Alligator Harbor, Florida were close to market size when the experiment was terminated after 13 months. Survival and growth of *M. mercenaria* was poor in the artificial upwelling controlled environment, in Salt River Inlet, St. Croix and in Molokai, Hawaii.

INTRODUCTION

Hard shell clam culture in the United States is quite advanced

primarily because American biologists (Victor L. Loosanoff and his associates in Milford, Connecticut) developed and perfected hatchery techniques for rearing quahogs (Bardach, Ryther and McLarney, 1972). By controlling the water temperature, the spawning season of quahogs has been extended and in most hatcheries clams can be induced to spawn throughout the year.

Seed clams are planted on the bottom in shallow water and allowed to grow until they are harvested for market. Predators have been controlled in experimental culture areas by various techniques. Castagna spreads crushed oyster shells, crushed stone or pea gravel over the clam beds and finds this method satisfactory for protecting juvenile clams from *Callinectes sapidus* and other predators. Menzel uses plastic-coated wire mesh to fence in clam plots or wire cages to cover the planted bottom.

The expense of keeping clam seed in a hatchery until optimum planting size is reached, is a major problem in applying clam-rearing techniques to a commercial-size operation in natural environments. Hatcheries prefer to sell 1 to 3 mm seed while growers prefer to plant 12.5 mm or larger. If 1 to 3 mm seed is planted, mortality is usually very high (80-90%) unless precautions are taken against predators (methods used by Castagna and Menzel). Planting the larger seed (12.5 mm or larger) is more successful, since that size is less vulnerable to predators (Bardach *et al*., 1972).

Presently, the quahog fishery (*M. mercenaria*) is centered in the north-eastern United States with most clams being harvested in New York. In the northern waters, quahogs reach marketable size in five to eight years. Bardach *et al*. (1972) note the southward

shift in the clam industry to take advantage of the faster growing rates possible in warmer waters.

Menzel (1971) discussed the potential of clam culture in the Caribbean. His observations indicate that hatchery-reared hybrids (*M. mercenaria* X *M. campechiensis*) might be the best to use since the F_1 clams have the adaptability of the southern parent to tropical waters, have a good growth rate and have the good out-of-water storage qualities of the northern parent. *M. campechiensis* starts gaping within 24 hr when kept under refrigeration.

In the artificial upwelling mariculture system in St. Croix, U.S. Virgin Islands, 870 m deep water is pumped into ponds onshore, where planktonic algae (diatoms) are grown as food for filter-feeding shellfish in a controlled food chain. The deep water is free of man-made pollutants and diseases and predators harmful to shellfish. Therefore, even the smallest juvenile clams (1 mm) can be planted in the system without threat of predators or tidal currents carrying them away.

This paper reports the results of a comparative growth experiment with three clam populations, *M. mercenaria*, *M. campechiensis* and their F_1 cross in five natural environments and in the artificial upwelling mariculture system.

MATERIALS AND METHODS

The hard shell clams—*M. mercenaria*, *M. campechiensis* and their F_1 cross (*M. mercenaria* ♂ X *M. campechiensis* ♀)—used in this study were produced in January and February 1973 by Paul Chanley at the Shelter Island Oyster Company, Inc., Greenport, New York. The *M. campechiensis* brood stock were sent from Florida by R.W. Menzel

and the M. mercenaria came from Long Island. In April-May 1973 the juvenile clams reached 1 mm in size and were shipped to the experimental sites.

(1) The Artificial Upwelling mariculture system, Rust-op-Twist, St. Croix, is a controlled experimental environment where deep water is pumped continuously from 870 m depth into 45,000-liter concrete pools in which unialgal cultures of planktonic diatoms are grown. The pools are scrubbed every one to four weeks and reinoculated with a different diatom so that a mixed diet is fed to the clams. Three species of diatoms are cultured: Bellerochea spinifera Harg. and Guill., Chaetoceros cf. simplex Ostif. and Thalassiosira pseudonana Hasle and Heim. The algal cultures in the pools (10^5 to 10^6 cells per ml) are pumped continuously into the clam tanks at metered rates based on the feeding activity of the animals. The yearly temperature range in the clam tanks is 22° to 30°C. The salinity varies between 34.75 and 34.95°/oo. Details of the system are given by Baab et al. (1973).

(2) Salt River Inlet, St. Croix, is a natural inlet on the north shore of St. Croix where the salinity range was 33.7 to 37.6°/oo and the temperature fluctuated between 25° and 32°C. The tidal range between the highest and lowest observed levels was 38 cm (Forbes, 1973, unpublished manuscript).

(3) Kupeke Pond, Pukoo, Molokai, Hawaii, where a commercial mariculture operation is run by Aquatic Sciences Corp.

(4) Alligator Harbor, Franklin County, Florida, where the clams were kept in flowing sea water in the laboratory until September when they were planted in plastic screen-covered, sand-filled containers. When the clams reached 15 mm in length, they were planted in the bottom and covered by open-bottom cages. The water temperature ranged from 9° to 31°C during the experiment; salinity varied from 26 to 32°/oo. The normal tidal range is 50 cm and at mean low water the clams were covered by about 5 cm of water.

(5) Bradfords Bay, Wachapreague, Virginia, where the clams were held in the laboratory in trays with flowing sea water from May to October. They were then planted in screen trays on a tidal flat. The water temperature ranged from 13° to 18°C and the salinity was 28 to 29.5°/oo (data were obtained from three observations during the experiment).

(6) Southold, New York, where the clams were planted in early May in trays filled with fine sand. The trays were stacked on an empty bottom tray, strapped together and wrapped with 2.6-cm plastic netting. These trays were put overboard in the Shelter Island Oyster Company's domed canal for the summer. Temperature ranged from -1° to 26°C and the salinity varied from 24 to 30°/oo. The tidal range is approximately 1.2 m. (The "domed canal" is located on Southold Bay, Southold, New York.)

Clams from each population, M. mercenaria, M. campechiensis, and their F_1 cross, were planted at Southold, New York and at both St. Croix locations. Only M. campechiensis and F_1 clams were sent to Virginia and Florida, while only F_1 clams and M. mercenaria were sent to Hawaii.

When the clams reached their destinations, a quarantine procedure was followed to insure that no "exotic species" would be introduced to the natural waters. The populations were isolated in trays, without sediment, in flowing sea water and the effluent from the trays was run to waste through a sand filter. For a period up to 20 days, the populations were checked for mortality and infestations of epiphytes and epizooites. This procedure was not followed at Alligator Harbor, Florida. Immediately after the quarantine period, only the clams sent to St. Croix and Hawaii were planted. The delayed planting in the other locations was due to the small size of the clams.

The clams in the artificial upwelling system were reared in three-liter Pyrex baking dishes containing a 4.5-cm thick layer of sand and in eight-liter dishpans containing a 10-cm thick layer of sand. These containers were stacked in a wooden rack to use all the space available in the 750-liter shellfish tanks (2.4 x 0.6 x 0.6 m). In the natural environments, clams were grown in wooden screen trays or Nestier trays filled with sediment and submerged in the bottom. However, in Southold they used polypropylene trays filled with sand and positioned 1.5 cm off the bottom. Protection from predators was accomplished by either covering the planted areas or spreading approximately 2.5-cm gravel over the trays (Table 1).

TABLE 1. SUMMARY OF EXPERIMENTAL CONDITIONS

	RUST-OP-TWIST ST. CROIX	SALT RIVER INLET ST. CROIX	KUPEKE POND HAWAII	ALLIGATOR HARBOR FLORIDA	WACHAPREAGUE VIRGINIA	SOUTHOLD NEW YORK
DATE PLANTED:	5/22/73	6/6/73	3/31/73	9/12/73	10/15/73	SUMMER '73
TRAYS:	3ℓ Pyrex dish (34x21.5x4.5cm) 8ℓ dishpans (32x28x15cm)	Nestier trays (58x58x7cm)		No trays, planted in bottom when >15mm in length	Screen trays (61x30.5x10cm)	Poly-propylene trays (65x55x14cm)
PLACEMENT:	In 2.4x0.6x 0.6m tanks	Submerged in bottom		In bottom (without trays)	Submerged in tidal flat	Stacked trays on bottom
PROTECTION FROM PREDATORS:	None	Trays covered with 1.3cm hardware cloth		Open-bottom plastic covered wire cages (30.5x 30.5x7cm) of 1.2cm mesh covered planted area	2.5cm of gravel spread over trays	Stack of trays wrapped in plastic netting (2.6cm mesh)
WATER TEMPERATURE (°C):	22-30	25-32		9-31	13-18	-1-26
SALINITY (°/oo):	34.75-34.95	33.7-37.6		26-32	28-29.5	24-30
TIDAL RANGE (cm):	None	38		50	Area covered by 60cm on average low tide	120

α

The protocol called for the clam populations to be set out at various densities (3229, 1615, 807 and 323 per m^2) and then observations would be made on survival and growth at the six experimental sites. However, the populations were only planted at these densities in Virginia and at the two locations in St. Croix. In the St. Croix mariculture system, an additional study was undertaken. Clams, designated as "fast growing" populations, were grown at densities that would not over-crowd them or hinder their growth. From time to time the "fast growing" populations were thinned out and/or the slower growing clams discarded.

As the clams increased in size in the mariculture system, they filtered out more than 90% of the food in the water flowing through the tank (flow rate of 8.4 liters/min or a turnover rate of 1.5 h in the tank). When this occurred, the clam population had to be culled before the carrying capacity of the tank was exceeded. The culling was done by discarding clams in the lower 20 percentile of size in a group (as determined by sieving). Only the "fast growing" populations were culled. The factor limiting algal production is volume of nutrient-rich deep water available per day (Table 2); experiments indicate nitrate may be limiting.

In the natural environments, the clams were not disturbed until the experiment was terminated. On several occasions however, spot checks on growth were made. In the mariculture system, all the clams were measured every four weeks.

Initial growth measurements were made at all six locations. The length of 200 randomly selected juveniles from each population was measured with an ocular micrometer. In the St. Croix mariculture system the clams were measured by photocopying (Haines, 1973)

TABLE 2. NUTRIENT COMPOSITION OF ARTIFICIALLY UPWELLED DEEP WATER AT THE ST. CROIX MARICULTURE SYSTEM

(WEEKLY SAMPLES, JANUARY 3 TO MAY 16, 1974)

NUTRIENT	MICRO-EQUIVALENTS PER LITER		
	MEAN	LOW	HIGH
$NO_3 + NO_2$	31.57	29.32	33.37
NO_2	0.23	0.16	0.33
NH_3	0.78	0.30	1.55
PO_4	2.23	1.90	2.40
SiO_4	19.30	16.44	23.20
SALINITY (°/oo)	34.84	34.79	34.92

or with calipers. "Wet" weight of the entire population was recorded initially at all six sites and then at four-week intervals in the mariculture system only. Clams were rinsed and allowed to drip for one minute before weighing.

The experiment was terminated when the majority of the clams in one of the three populations in the density study in the St. Croix mariculture system at Rust-op-Twist reached market size (greater than 25.5 mm in thickness). The collaborators were notified and final growth measurements—length, thickness and "wet" weight—were recorded and survival was determined for all the populations. Since the method of measuring length used in Virginia differed from the other locations, we used the conversion factor described by Haskin (1949) to compare the Virginia data. Final weight measurements were not recorded in Florida or New York and again a conversion factor (Haskin, 1949) was used.

RESULTS AND DISCUSSION

The comparative growth study was terminated after 13 months because the majority of the F_1 clams and M. campechiensis at Rust-op-Twist, St. Croix, had reached market size. These two species of clams began to reach market size as early as 6.5 months after they were planted in the artificial upwelling system. Some F_1 clams in Kupeke Pond, Hawaii, and Alligator Harbor, Florida, were approaching market size after 13 months. In no other locations did any clams from any of the three different populations reach market size during the experiment. Table 3 gives the average thickness of all clams when the experiment was completed.

TABLE 3. AVERAGE THICKNESS (in mm) OF CLAM POPULATIONS AT THE TERMINATION OF THE EXPERIMENT

LOCATION	F_1 CROSS		M. CAMPECHIENSIS		M. MERCENARIA	
	AVERAGE THICKNESS	RANGE	AVERAGE THICKNESS	RANGE	AVERAGE THICKNESS	RANGE
RUST-OP-TWIST, ST. CROIX ("DENSITY STUDY")	28.2	20-32	30.2	20-35.5	6.5[1]	
SALT RIVER INLET, ST. CROIX	10[1]		6.5[1]		x	
KUPEKE POND, HAWAII	17.5[1]		n.p.		x	
ALLIGATOR HARBOR, FLORIDA	19[1]		17.5[1]		n.p.	
WACHAPREAGUE, VIRGINIA	12.4	4.8-20.4	12.8	6.8-16.9	12.6	8.8-17.2
SOUTHOLD, NEW YORK	9[1]		x			
RUST-OP-TWIST, ST. CROIX ("FAST GROWING" POPULATIONS)	27.8	25.5-31	29.9	25.5-34.5	7[1]	

n.p. = not planted

x = no survivors

[1]= values obtained by converting length (Haskin, 1949)

note: market size is a thickness of 25.5 cm or more

It should be noted that the results for the artificial upwelling system have been divided into two groups: the "density study" population and the "fast growing" population. By doing this, one can compare the growth of the "density study" group to the growth of populations in the five natural environments. Culling the "fast growing" population altered its relationship to clam growth in the natural environments.

Table 4 summarizes the survival of the clams planted at all six sites. Wachapreague, Virginia, populations had the best survival but the clams were still too small (Fig. 1) to show any significant differences in survival between the various densities (1615, 807, and 323 per m^2). At Rust-op-Twist, St. Croix, the best survival in the "density study" F_1 clams and M. campechiensis populations was recorded in the 807 and 323/m^2 groups. The F_1 clams at 323/m^2 in Salt River Inlet showed the best survival but this may not be significant since there was such a low (3.7%) survival for the total F_1 clam population. Predation did play an important role in the survival of clams in the natural environments. In Salt River Inlet, hardware cloth was placed over the planted area and at the end of the study the mesh was heavily fouled with seaweed and other debris. Had crushed shells or gravel or open-bottom cages been placed over the planted area, survival may have been higher.

M. mercenaria grew poorly and sustained high mortalities at all locations. The data for the M. mercenaria at Southold, New York are not available because the population was mixed with others during the experiment. In Virginia, the M. mercenaria data are from a VIMS-E laboratory-reared population and not from stock spawned by Paul Chanley. The poor survival and growth of

TABLE 4. SURVIVAL OF THE CLAM POPULATIONS AT THE TERMINATION OF THE EXPERIMENT

LOCATION	% SURVIVAL		
	F_1 CROSS	MERCENARIA CAMPECHIENSIS	MERCENARIA MERCENARIA
RUST-OP-TWIST ST. CROIX ("DENSITY STUDY")	42.8	36.1	<1
SALT RIVER INLET ST. CROIX	3.7	0.7	0
KUPEKE POND HAWAII	22	n.p.	0
ALLIGATOR HARBOR FLORIDA	39	69.5	n.p.
WACHAPREAGUE VIRGINIA	81.9	72.5	89.4
SOUTHOLD NEW YORK	47.7	0	data not available
RUST-OP-TWIST ST. CROIX ("FAST GROWING")	42.7	48.1	<1

n.p. = not planted

Figure 1. Average length (in mm) after 13 months.

F_1 = F_1 cross (*M. mercenaria* ♂ X *M. campechiensis* ♀);

C = *M. campechiensis*;

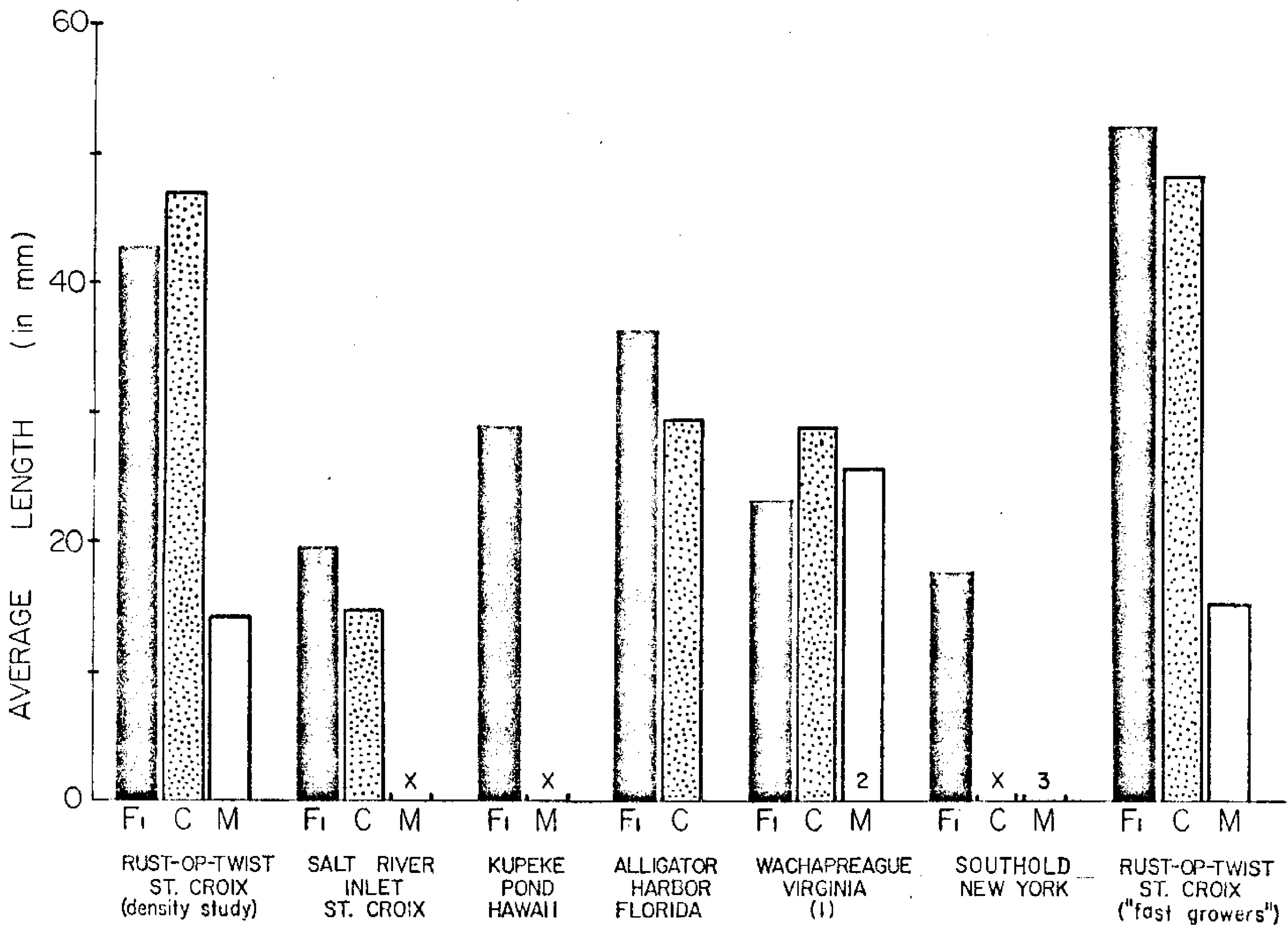
M = *M. mercenaria*;

X = no survivors;

1 = length calculated from thickness data (Haskin, 1949);

2 = VIMS-E laboratory-reared population;

3 = no data available.


AVERAGE LENGTH (in mm)
60
40
20
0
F1 C M
RUST-OP-TWIST
ST. CROIX
(density study)
F1 C M
X
SALT RIVER
INLET
ST. CROIX
F1 M
X
KUPEKE
POND
HAWAII
F1 C
ALLIGATOR
HARBOR
FLORIDA
F1 C M
2
WACHAPREAGUE
VIRGINIA
(1)
F1 C M
X
3
SOUTHOLD
NEW YORK
F1 C M
RUST-OP-TWIST
ST. CROIX
("fast growers")

M. mercenaria in the St. Croix mariculture system may be related to the sustained high temperatures (22°-30°C) and/or to a nutritional deficiency since only three species of diatoms were used as food. In Salt River Inlet and in Kupeke Pond, no northern hard clams survived at the end of the experiment. It is unfortunate that M. mercenaria were not planted in Alligator Harbor for a growth comparison in that environment where temperature reaches 31°C but does go down to 9°C in the winter. Previous observations at Alligator Harbor (Menzel, 1962) showed that the growth rate of M. mercenaria over a 20-month period was 71% of M. campechiensis and 73% of the faster-growing F_1 reciprocal hybrid. The mortality rate was about the same in both species and the reciprocal hybrids. Ansell (1967) reported that considerable deviations in the temperature/growth rate relationships occur, indicating that other factors, possibly food availability, determine the rate of growth of M. mercenaria within the limits set by temperature.

Survival was good for M. campechiensis in three of the five locations. The low survival in Salt River Inlet was undoubtedly due to predation. The total southern hard-clam population in Southold, New York, died in late winter suggesting that M. campechien- was unable to withstand low temperatures (Haven and Andrews, 1957; Chestnut et al., 1957).

Figures 1 and 2 show the average length and average weight of the clam populations at the end of the experiment in May-June, 1974. If the "density study" and the "fast growing" populations at Rust-op-Twist are compared, one finds a significant difference between the two F_1 clam groups as far as increase in length is concerned but this is not the case for M. campechiensis. For the latter

Figure 2. Average weight (in gm) after 13 months.

F_1 = F_1 cross (*M. mercenaria* ♂ X *M. campechiensis* ♀);

C = *M. campechiensis*;

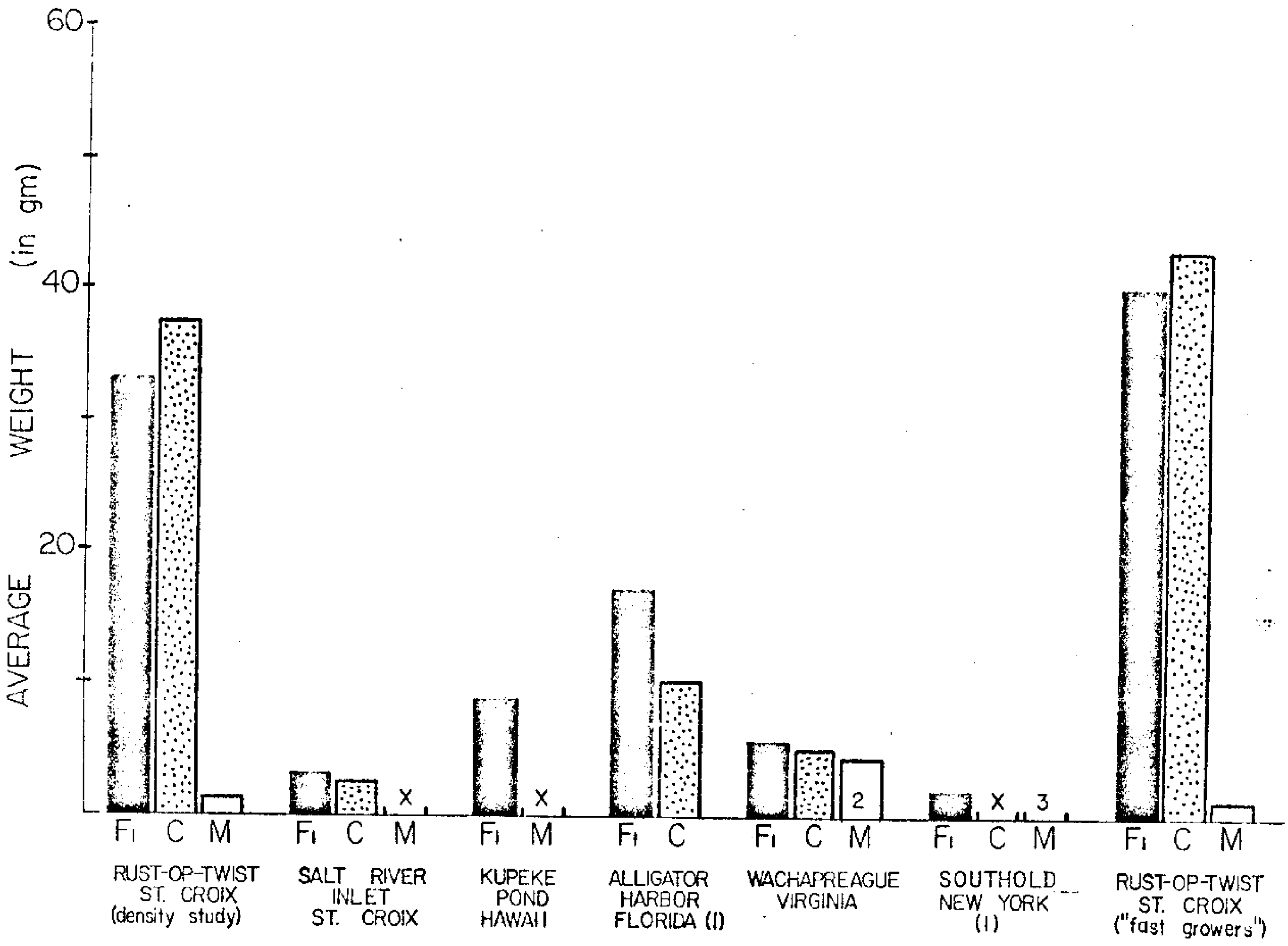
M = *M. mercenaria*;

X = no survivors;

1 = weight calculated from length data (Haskin, 1949);

2 = VIMS-E laboratory-reared population;

3 = no data available.



AVERAGE WEIGHT (in gm)
60
40
20
F1 C M
RUST-OP-TWIST
ST. CROIX
(density study)
F1 C M
X
SALT RIVER
INLET
ST. CROIX
F1 M
X
KUPEKE
POND
HAWAII
F1 C
ALLIGATOR
HARBOR
FLORIDA (I)
F1 C M
2
WACHAPREAGUE
VIRGINIA
F1 C M
X
3
SOUTHOLD
NEW YORK
(I)
F1 C M
RUST-OP-TWIST
ST. CROIX
("fast growers")

species, survival is significantly higher in the "fast growing" population.

The growth of the Rust-op-Twist F_1 clams and M. campechiensis ("density study" populations) surpassed the growth of the populations in the five natural environments. The constant food supply, absence of predators and the extended growing season in the mariculture system are probably responsible for this rapid growth.

LITERATURE CITED

Ansell, A.D. 1967. The rate of growth of the hard clam Mercenaria mercenaria L. throughout the geographical range. Journal du Conseil 31(1):364-409.

Baab, J.S., G.L. Hamm, K.C. Haines, A. Chu and O.A. Roels, 1973. Shellfish mariculture in an artificial upwelling system. Proceedings, National Shellfisheries Association 63:63-67.

Bardach, J.E., J.H. Ryther and W.O. McLarney, 1972. Aquaculture: The farming and husbandry of freshwater and marine organisms. Wiley-Interscience, New York, 868 pp.

Chestnut, A.F., W.E. Fahy and H.J. Porter, 1957. Growth of young Venus mercenaria, Venus campechiensis, and their hybrids. Proceedings, National Shellfisheries Association 47:50-56.

Haines, K.C., 1973. A rapid technique for recording sizes of juvenile pelecypod molluscs. Aquaculture 1:433.

Haskin, H.H., 1949. Growth studies on the quahaug, Venus mercenaria. National Shellfisheries Association, Convention Add. 39:67-75.

Haven, D. and J.D. Andrews, 1957. Survival and growth of Venus mercenaria, Venus campechiensis and their hybrids in suspended trays and on natural bottoms. Proceedings, National Shellfisheries Association 47:43-49.

Menzel, R.W., 1962. Seasonal growth of northern and southern quahogs, *Mercenaria mercenaria* and *M. campechiensis*, and their hybrids in Florida. Proceedings, National Shellfisheries Association 53:111-119.

Menzel, R.W., 1971. Possibilities of molluscan cultivation in the Caribbean. FAO Fisheries Resources Division. Fisheries Report 71.2:183-200. (Presented at November 18-26, 1968 Symposium on Investigations and Resources of the Caribbean Sea and Adjacent Regions, Willemstad, Curacao.)

GROWTH OF THE EUROPEAN OYSTER, *OSTREA EDULIS* LINNÉ, IN THE ST. CROIX "ARTIFICIAL UPWELLING" MARICULTURE SYSTEM AND IN NATURAL WATERS[1]

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ABSTRACT

European oysters, Ostrea edulis Linné, were grown from 3-mm spat to marketable adults in 13 to 16 months in a mariculture operation using "artificial upwelling" on St. Croix, U.S. Virgin Islands. The shellfish were fed three species of diatoms, Bellerochea spinifera Harg. and Guill., Chaetoceros cf. simplex Ostf. and Thalassiosira pseudonana Hasle and Heim. Algal cultures, grown in 45,000-liter pools, were pumped continuously through the shellfish tanks; the salinity was 34.75 to 34.95 °/oo and water temperature varied between 22° and 29°C during the experiments. Larvae produced and released by mature O. edulis were reared through setting, but the spat did not complete metamorphosis.

For comparison, O. edulis were grown in Salt River, a natural inlet on St. Croix. After 82 days, mortality was 100% in the Salt River population. The salinity range was 33.7 to 37.6 °/oo and the temperature fluctuated between 25° and 32°C.

A third experiment, using O. edulis is underway and growth of these oysters indicates that marketable adults may be produced in 12 months.

INTRODUCTION

The European oyster, Ostrea edulis Linné, was grown in a mariculture operation using "artificial upwelling" on St. Croix, U.S. Virgin Islands (Baab, Hamm, Haines, Chu and Roels, 1973). Favorable conditions—constant supply of food,

uniform salinity and absence of predators and suspended sediments—in the managed system produced market-size oysters in 13 to 16 months in the first experiment.

A second experiment, a comparative growth study using *O. edulis*, was begun in Salt River, a natural inlet on St. Croix, and repeated in the "artificial upwelling" mariculture system.

A third experiment with *O. edulis* is underway and preliminary growth results indicate that the mariculture operation may yield marketable adults in 12 months.

MATERIALS AND METHODS

In the "artificial upwelling" mariculture system, sea water was pumped from 870 m depth in the Caribbean Sea through a one-mile-long pipeline into 45,000-liter pools on shore in which unialgal cultures of planktonic diatoms—*Bellerochea spinifera* (clone STX-114), *Chaetoceros simplex* (clone STX-105) or *Thalassiosira pseudonana* (clone 3H)—were grown. The algal cultures (10^4 to 10^6 cells ml^{-1}) were pumped continuously into the shellfish tanks at metered rates. The temperature in the tanks varied between 22° and 29°C. Details of the system are given by Baab *et al.* (1973).

In Salt River, a natural inlet on the North Shore of St. Croix, the oysters were grown in trays (positioned below the low tide mark) suspended from a dock. The water depth at the dock was about 2 m.

Cultchless *O. edulis* used in all experiments were obtained from Pacific Mariculture, Inc., and grown in stacked Nestier trays (Division of Vanguard Industries, Inc., Cincinnati, Ohio). Until the spat were greater than 13 mm in diameter they were held in the trays by liners made from 1/16-inch mesh, plastic-covered fiberglass window screening. At four-week intervals, wet weight and length (measured from umbo to posterior margin) were determined for the populations.

RESULTS AND DISCUSSION

In April 1972, 50,000 3-mm *O. edulis* spat were introduced to the mariculture system. These juveniles were part of an experiment designed to select suitable shellfish species for the "artificial upwelling" mariculture operation. Figure 1 shows the growth of *O. edulis* over a 16-month period. Total mortality, in the first experiment, was 19%. After 13 months, the European oysters reached 75 mm in length and their average weight was 40.7 gm. The oysters were nearly 100 mm in length and averaged 64.1 gm after 16 months. Bardach, Ryther and McLarney (1972) report that *O. edulis* grow to market size of 75 mm in diameter or a total weight of 65 gm in four years in France. These oysters attained "market size" diameter in 13 months and reached "market size" weight in 16 months.

In April 1973, viable larvae (120-140 m in length) were collected with a 62-μ plankton net from the tank containing the *O. edulis*. The larvae were reared in 379-liter polyethylene tanks and fed the same algal diet as the adults. Water

in the tanks was changed daily and no antibiotics were added. When the larvae began to set, cultch (O. edulis shells) was placed in the tanks. Even though the larvae set, they did not complete me amorphosis. Possible causes of this failure to metamorphose may be of nutritional (only diatoms were fed), genetic, environmental (a high temperature of 29-39°C in the afternoon), or infectious origin.

A second experiment, begun in October 1972, was designed to compare the growth of O. edulis (8-mm juveniles) in a controlled environment (the "artificial upwelling" mariculture operation) to growth in an uncontrolled environment (Salt River Inlet)(see Table 1). The O. edulis in the mariculture operation reached 75 mm in length in 10 months; after 82 days no O. edulis survived in the Salt River Inlet population (Fig. 2). Land run-off caused by heavy rains increased the silt content of Salt River Inlet. The oysters were covered with 10-20 mm of silt in their trays; this was believed to be the cause of the 100% mortality. However, fouling by sponges and algae was heavy and predators (crabs and drills, Murex pomum Gmelin and Murex brevifrons Lamarck) were present.

Mortality for the batch of oysters grown in the controlled environment was 81%. This increased mortality (first experiment was 19%) can be attributed to the saturated NaCl-dip given to this batch to remove infestations of the bryozoan, Bowerbankia gracilis Leidy. The shellfish were placed in a saturated salt solution (300 gm NaCl per liter of sea water) immediately after they were removed from the shellfish

tanks. After one minute in the vigorously aerated salt dip, the shellfish were air-dried for one hour. On two occasions however, the oysters were out of water almost an hour before the salt treatment and several days later high mortalities occurred. The total mortality for this experimental batch was 35% if the percent mortality reported is corrected for the deaths caused by the salt treatments.

A third experiment, using 3.2-mm spat, was begun on August 15, 1973, to substantiate the results of previous experiments on St. Croix and to compare growth in other environments—Long Island Sound, Virginia, Florida, and Salt River Inlet. Presently, there are _O. edulis_ in only two of these locations—the St. Croix mariculture system and Greenport, Long Island. Oysters sent to the Virginia Institute of Marine Sciences (Michael Castagna, Eastern Shore Laboratory) arrived in very poor condition and died within a week. From Florida State University, R. Winston Menzel reported that the _O. edulis_ were in satisfactory condition on arrival but died after being suspended in flowing sea water. After 55 days, none of the _O. edulis_ in the Salt River Inlet population survived. Siltation was suspected as the cause of death in the Florida and in the Salt River Inlet populations.

In July 1974, the oysters in Greenport, New York (Paul Chanley, Shelter Island Oyster Company) averaged 44 mm; in June 1974, the _O. edulis_ in the St. Croix mariculture operation averaged 69 mm in length (Fig. 3). Marketable adults are expected after 12 months in the mariculture operation.

Mortality so far in this experiment is 24.3%.

CONCLUSION

Each successive batch of O. *edulis* grown in the St. Croix mariculture operation attained market size in a shorter period of time due to improved handling techniques and due to information gained on requirements for optimization of growth, i.e., densities, food and oxygen requirements (Fig. 4). Densities varied with the size of the oyster—as juveniles, densities greater than $25/ft^5$ were acceptable; at 40-mm length, 15 to $16/ft^2$, and at 75-mm length, 7 to $8/ft^2$. Feeding the oysters three species of diatoms on a rotating schedule appeared to be adequate. Oxygen concentration in the shellfish tanks was kept at 5 ppt or greater.

The excellent growth of O. *edulis* in the "artificial upwelling" mariculture system can be attributed to the constant food supply, the deep water source relatively free of particulate matter, and the extended growing season.

Planned improvements in the supply of food and management of the oysters in the "artificial upwelling" system should further reduce the time they require to reach market size.

LITERATURE CITED

Baab, J.S., G.L. Hamm, K.C. Haines, A. Chu and O.A. Roels, 1973. Shellfish mariculture in an artificial upwelling system. Proc. Natl. Shellfish. Assoc. 63:63-67.

Bardach, J.E., J.H. Ryther and W.O. McLarney, 1972. Aquaculture: The farming and husbandry of freshwater and marine organisms. Wiley-Interscience, New York.

TABLE 1. Comparison of the environmental factors in the "artificial upwelling" mariculture operation and in Salt River Inlet.

ENVIRONMENTAL CONDITIONS	MARICULTURE OPERATION	SALT RIVER INLET
Temperature (°C)	22-29	25-32
Salinity ($^{o}/oo$)	34.8-34.9	33.7-37.6
*Phytoplankton chlorophyll _a_ (mg/m^3)	22.4-54.0	0.56-1.14
*Particulate matter (mg/liter)	negligible (<1)	low during drought; heavy during rainy season
Degree of fouling	light _Bowerbankia gracilis_ Leidy	heavy sponges, algae, bryozoans, tube worms, sea squirts
Predators	absent	crabs and _Murex brevifrons_ Lamarck; _Murex pomum_ Gmelin

*Haines, K.C. (unpublished).

Figure 1. Growth of the first population of *Ostrea edulis* in the "artificial upwelling" mariculture system, St. Croix, U.S. Virgin Islands.

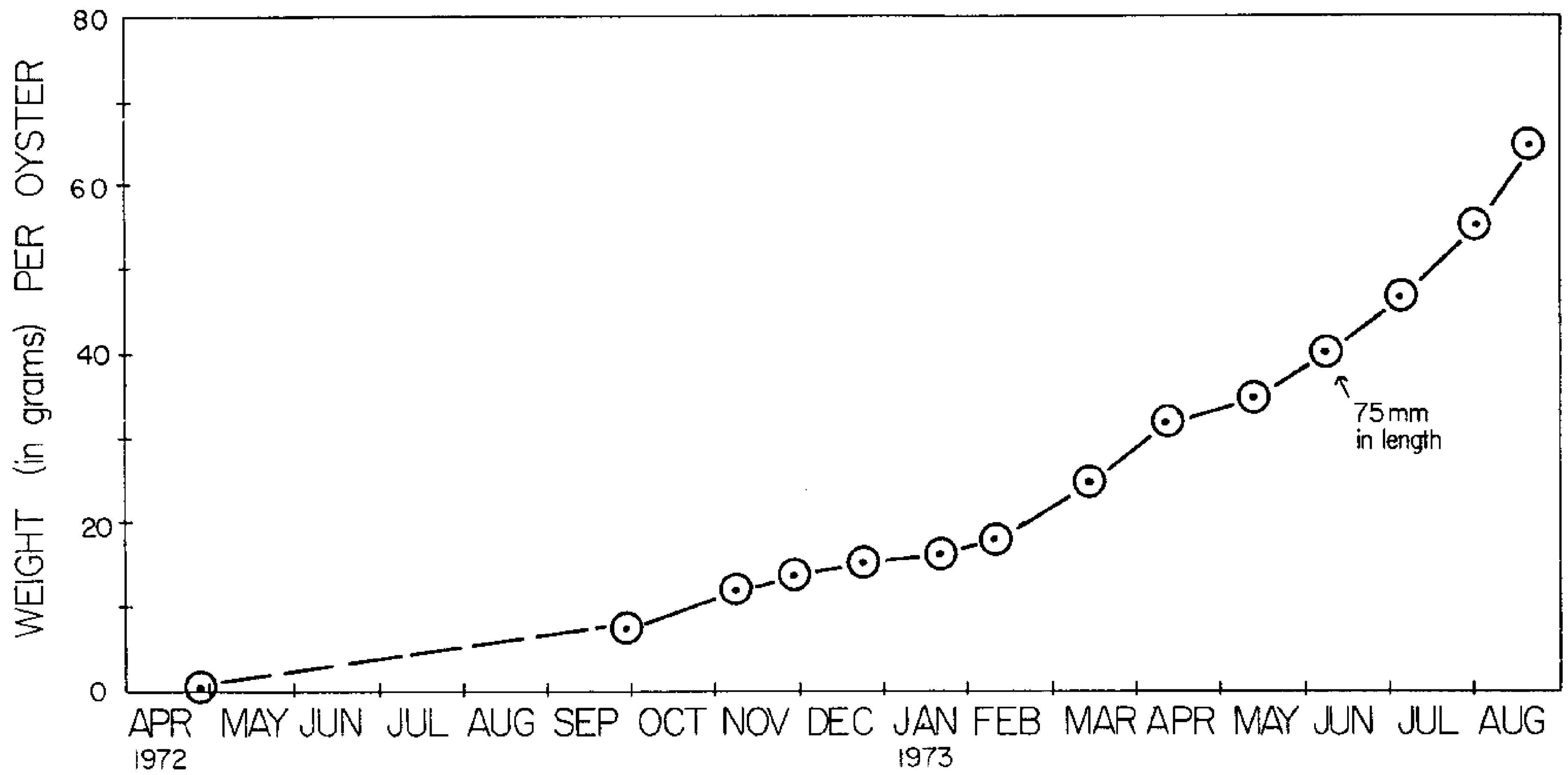

WEIGHT (in grams) PER OYSTER
80
60
40
20
0
APR
1972
MAY
JUN
JUL
AUG
SEP
OCT
NOV
DEC
JAN
1973
FEB
MAR
APR
MAY
JUN
JUL
AUG
75 mm
in length

Figure 2. Growth of the second population of *Ostrea edulis* in the "artificial upwelling" mari-culture system compared to growth in Salt River Inlet, St. Croix.

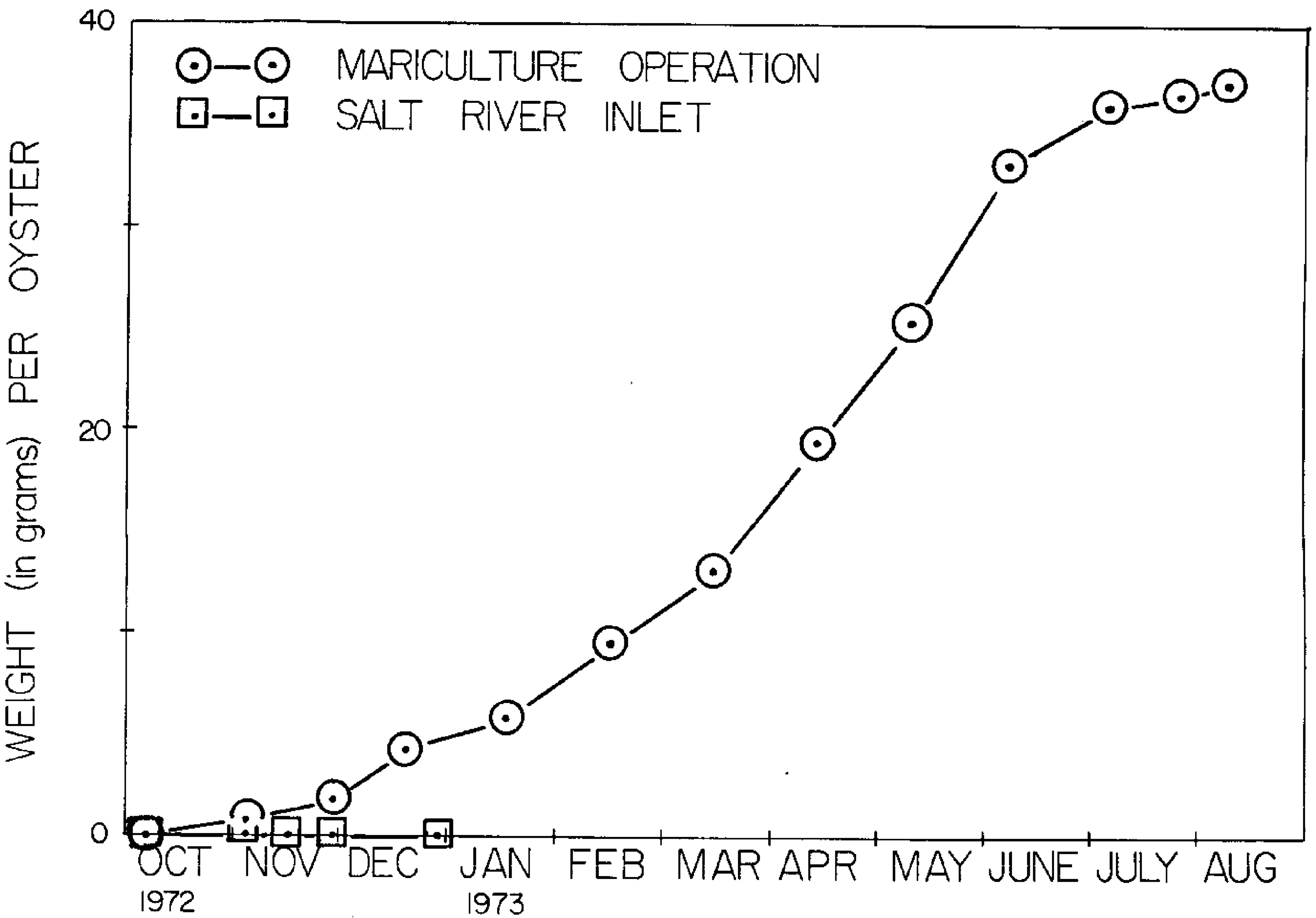

MARICULTURE OPERATION
SALT RIVER INLET
WEIGHT (in grams) PER OYSTER
40
20
0
OCT
1972
NOV
DEC
JAN
1973
FEB
MAR
APR
MAY
JUNE
JULY
AUG

Figure 3. Growth of the third population of *Ostrea edulis* in the "artificial upwelling" mariculture system.

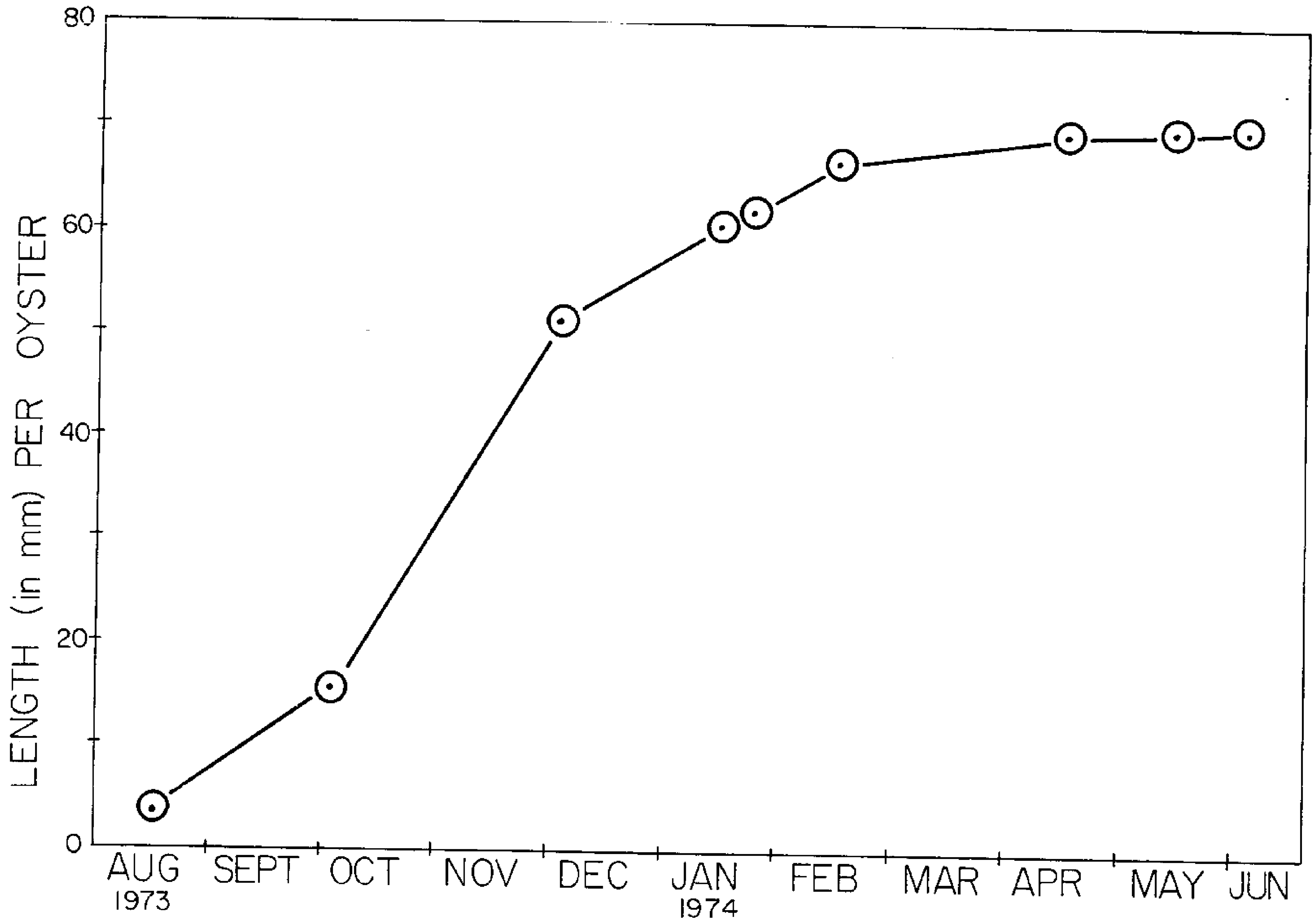
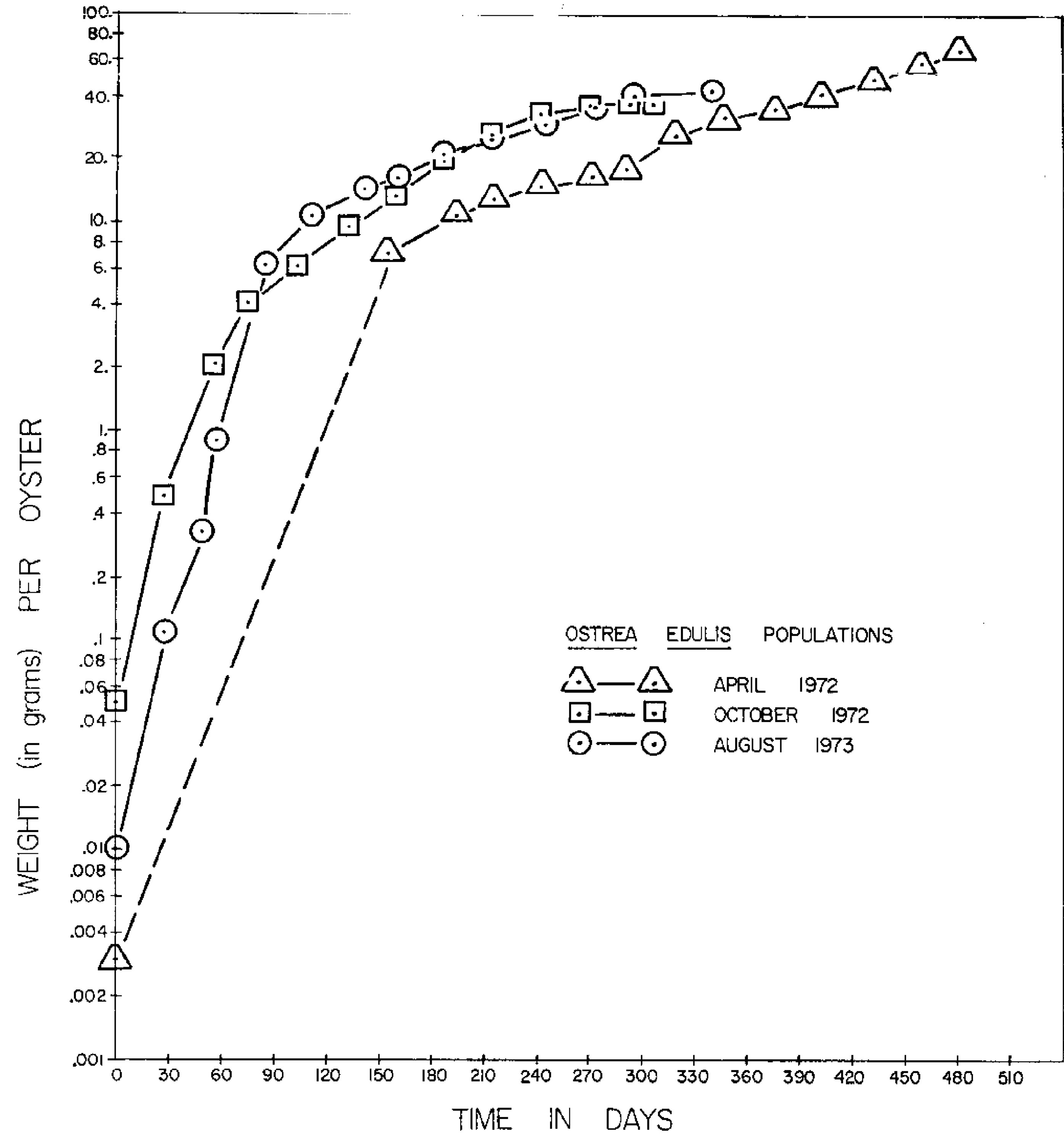

LENGTH (in mm) PER OYSTER
80
60
40
20
0
AUG
1973
SEPT
OCT
NOV
DEC
JAN
1974
FEB
MAR
APR
MAY
JUN

Figure 4. Growth of three populations of *Ostrea edulis* in the "artificial upwelling" mariculture system.



WEIGHT (in grams) PER OYSTER
100.
80.
60.
40.
20.
10.
8.
6.
4.
2.
1.
.8
.6
.4
.2
.1
.08
.06
.04
.02
.01
.008
.006
.004
.002
.001
0
30
60
90
120
150
180
210
240
270
300
330
360
390
420
450
480
510
TIME IN DAYS
OSTREA EDULIS POPULATIONS
APRIL 1972
OCTOBER 1972
AUGUST 1973

THE MARICULTURE POTENTIAL OF *TAPES SEMIDECUSSATA* (REEVE) IN ST. CROIX, U.S. VIRGIN ISLANDS

Judith B. Sunderlin, Mark Brenner and Kenneth M. Rodde

(to be submitted as a
Short Communication to *Aquaculture*)

The life cycle of *Tapes semidecussata* (Reeve), the Japanese "little-neck" clam, was completed in the controlled environment of the artificial upwelling mariculture system in St. Croix, U.S. Virgin Islands. Preliminary growth studies on a *Tapes* population, reared from 5-mm juveniles to adults, and on its progeny are reported. The adult *Tapes* were induced to spawn once and were observed to spawn spontaneously three times in the period April through September 1974. The larvae were reared through setting and metamorphosis; clams from the April spawning averaged more than 20 mm in length in mid-September. This species has excellent mariculture potential in St. Croix. *T. semidecussata* tolerates a salinity of 34.75 to 34.95°/oo and a yearly temperature fluctuation of 22° to 30°C. As juveniles, densities as high as $1600/ft^2$ were tolerated and at market size (>38 mm in length) the clams were maintained at densities of $140\text{-}180/ft^2$. The clams are versatile and can be grown in sand as well as in Nestier trays.

Feeding the juveniles and adults three species of diatoms on a rotating schedule appeared to be adequate. Rapid growth (market size in 7 to 11 months) and good survival (60%) were exhibited by the T. semidecussata population.

A successful molluscan mariculture operation must provide food for a population throughout its life cycle, produce seed reliably and control disease, predators and parasites. The St. Croix laboratory has consistently cultured a variety of algal species that can be used by shellfish in all stages of development. No infectious shellfish diseases have been discovered in the system; however, the bryozoan, Bowerbankia gracilis (Leidy), has been a fouling organism on occasion, but can be controlled by keeping clams in sand and periodically treating oysters with a saturated NaCl solution (Sunderlin et al., 1974).

Initially, research carried out at the St. Croix laboratory concentrated on selection and growth of various algal species in "artificially upwelled," 870-m deep water. Presently, three species of diatoms, Bellerochea spinifera Harg. and Guill., Chaetoceros cf. simplex Ostif, and Thalassiosira pseudonana Hasle and Heim, are cultured as food for shellfish. Baab et al. (1973) list the properties of the deep water and the details of the mariculture system's physical plant.

In conjunction with the algal growth studies, nine species of shellfish have been screened for growth and survival. Seven species grew well and merit more detailed

analysis of nutritional and environmental requirements as well as reproductive capabilities. *Tapes semidecussata*, one of the seven favorable species, was first introduced into the St. Croix system in August 1973. Twenty-seven thousand juveniles, 5 mm in length, were obtained from Pacific Mariculture, Inc. Clams started to attain market size (>38 mm in length) in March 1974 and by July the entire population averaged more than 38 mm in length and 13 g in weight (Fig. 1). Survival for the 11-month growth period was 60%.

During this preliminary growth study, clam densities were also regulated. As juveniles (5 to 20 mm in length), they were grown at 1600/ft^2 in three-inch deep sand. As the clams reached market size, densities in the sand were reduced to 140-180/ft^2. Every four weeks, the *Tapes* were removed from the sand and weighed and measured. Measurements were obtained by using either the photocopying method (Haines, 1973) or calipers. Occasionally, the clams were placed in Nestier trays (Division of Vanguard Industries, Inc., Cincinnati, Ohio) rather than in sand and growth rates did not appear to change.

In April 1974, the population of *T. semidecussata* spawned spontaneously and the larvae from this batch were reared. By mid-September 1974, the juveniles were larger than 20 mm in length.

In May 1974, adult clams were induced to spawn using the following method: approximately 100 *Tapes* were removed

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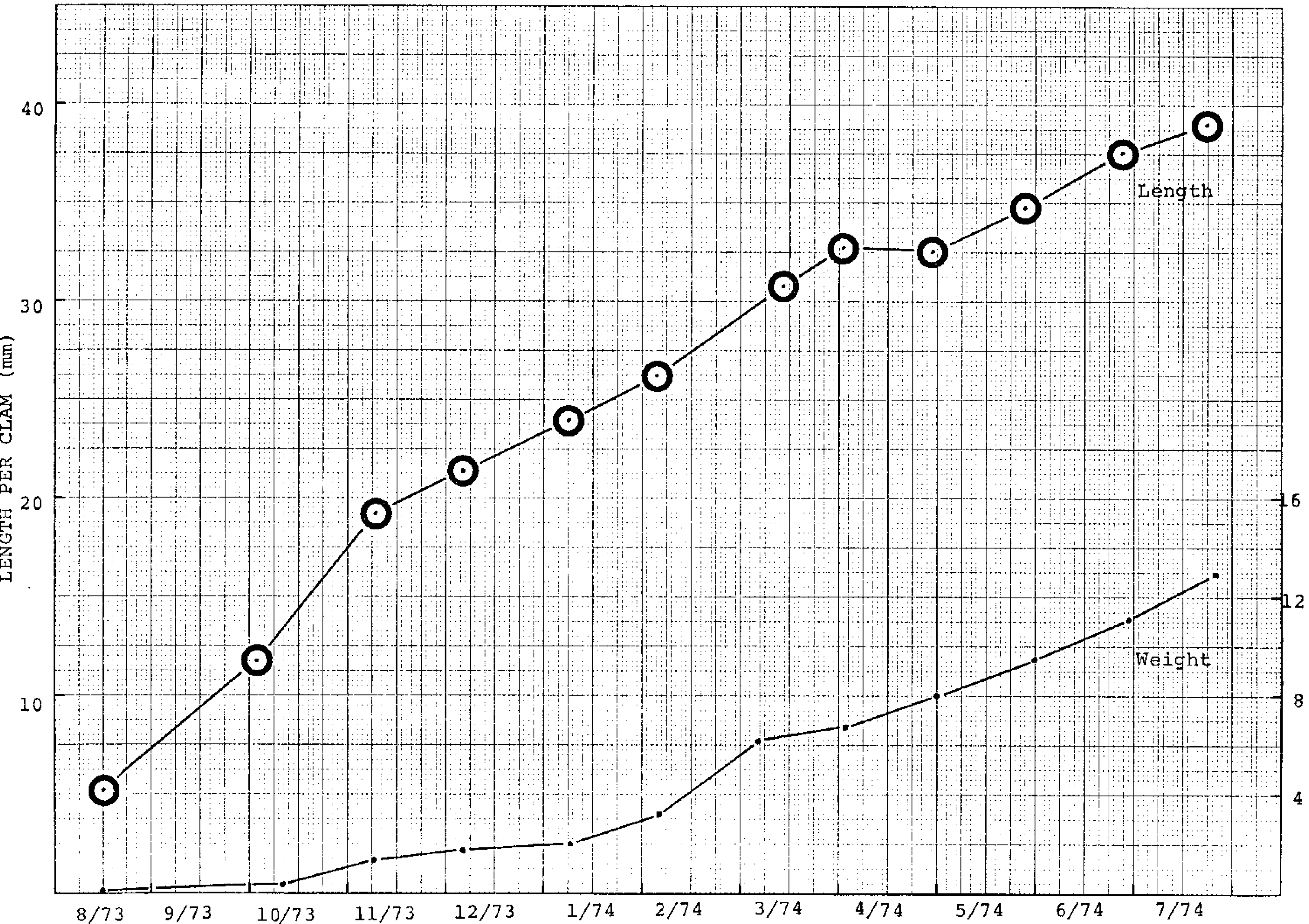


from water of 23°C and placed in a dry bucket and covered with moist paper towels. The bucket was placed in the shade (air temperature, 30°C). After four hours, the clams were placed in two 10-gallon containers of 21.7°C deep water. One hour later, stripped gonad suspension was added and within 40 minutes the clams began to spawn. The larvae were reared in a 100-gallon polyethylene container and fed two species of diatoms, B. spinifera and T. pseudonana, and one undetermined cryptophyto flagellate (clone S-1, isolated by Dr. R.R.L. Guillard from the Sargasso Sea). Vet Strep (25% solution, Merck and Co., Rahway, N.J.) was added to the standing larval culture at a concentration of 0.2 ml/l (M. Castagna, personal communication) to reduce bacterial infection. The water in the larval cultures was filtered every two days and the temperature varied between 22° and 29°C. Setting occurred in 15 to 20 days when the larvae were 250 to 300 μ in size.

In August and September 1974, two additional spontaneous spawnings took place and the larvae were successfully reared in a similar manner. Table 1 compares all four populations of Tapes semidecussata grown in the St. Croix system.

These encouraging results suggest that with continued research on improving algal diets and hatchery techniques, T. semidecussata would be well suited for large-scale mariculture.

References

Baab, J.S., Hamm, G.L., Haines, K.C., Chu, A. and Roels, O.A., 1973. Shellfish mariculture in an artificial upwelling system. Proc. Natl. Shellfish.Assoc. 63: 63-67.

Haines, K.C., 1973. A rapid technique for recording sizes of juvenile pelecypod molluscs. Aquaculture, 1: 433.

Sunderlin, J.B., Tobias, W.J. and Roels, O.A., 1974. Growth of the European oyster, *Ostrea edulis* Linné, in the St. Croix "artificial upwelling" mariculture system and in natural waters. Proc. Natl. Shellfish. Assoc. (submitted).

Figure 1. *Tapes semidecussata*: growth trends (length and weight) in the St. Croix "Artificial Upwelling" mariculture system.

TABLE 1. GROWTH AND SURVIVAL OF FOUR POPULATIONS OF _TAPES SEMIDECUSSATA_ IN ST. CROIX

8-15-73 SEED OBTAINED FROM PACIFIC MARICULTURE				4-2-74 SPAWNING				5-9-74 SPAWNING				9-7-74 SPAWNING			
DATE	DAY	LENGTH (mm)	SUR-VI-VAL (%)*	DATE	DAY	LENGTH (mm)	SUR-VI-VAL (%)*	DATE	DAY	LENGTH (mm)	SUR-VI-VAL (%)	DATE	DAY	LENGTH (mm)	SUR-VI-VAL (%)*
08-15-73	0	5.19		04-02-74	0	0.061		05-09-74	0	-		09-07-74	0	-	
10-02-73	48	11.99		04-04-74	2	0.094		05-11-74	2	0.094		09-09-74	2	0.097	
11-08-73	57	19.13		04-07-74	5	0.109		05-14-74	5	0.112		09-12-74	5	0.126	
12-05-73	112	21.44		04-12-74	10	0.176		05-19-74	10	0.180		09-17-74	10	0.156	
01-09-74	147	23.84		04-18-74	16	-		05-25-74	16	0.210		09-23-74	16	0.194	
02-05-74	174	26.13	64.3	04-22-74	20	-		05-29-74	20	0.290		09-27-74	20	0.205	
03-14-74	211	30.72		05-03-74	31	0.469		06-09-74	31	0.500		10-08-74	31	0.292	
04-02-74	230	32.84		05-08-74	36	0.546		06-14-74	36	0.543		10-13-74	36	0.332	
05-01-74	259	32.27		05-30-74	58	0.905		07-17-74	69	0.5-1.0	0**				
05-29-74	287	34.71	63.6	07-10-74	99	6.77									
06-28-74	317	37.48		08-12-74	132	13.02									
07-23-74	342	38.89	60.4	09-12-74	163	20.39	43								

*after setting and metamorphosis.

**total mortality: suspect high temperature in tank (>33°C)

TEST OF A METHOD FOR ELIMINATING HYDROGEN SULFIDE PRODUCTION IN SAND SUBSTRATE FOR CLAM CULTURE

Kenneth M. Rodde

Summary

Nestier trays lined with a polyvinyl screening/fiberglass layer composite, porous to water movement, containing sand and burrowing clams (*Tapes semidecussata*) were suspended in a continuous flow culture flume along with plastic-lined control trays which would not permit water flow through the liner. After one month of monitoring O_2 and pH in the sand, relative rates of increasing pH values and decreasing O_2 values were similar for both groups of trays, although experimental tray measurements were higher. Visible indications of H_2S production became apparent at the time the screening/fiberglass became clogged by fouling organisms. Thus, while the technique appears to have good potential for practical application, a duplicate experiment, using finer-mesh screening while avoiding clogging of the mesh, is deemed necessary for more definitive data.

H_2S Experiment

In the process of rearing clams, it is sometimes desirable to use sand as a burrowing substrate to reduce the attachment of fouling organisms to the shells as well as to

provide a more "natural" environment for the animals. The latter point is often translated into increased growth rates of the cultured clams (Baab et al., 1972). One disadvantage in using sand, however, is an occasional accumulation of residual algae and fecal material which, when decomposed, produces anoxic sediment conditions and allows sulfur-reducing bacteria to manufacture H_2S. Oxygen-starved sediment is unpleasant to handle during growth studies and, more importantly, seems to be related to increased clam mortality. In addition, a considerable amount of time is required to maintain the substrate in a reasonable state of cleanliness.

In an attempt to reduce or eliminate the H_2S production and better-utilize time devoted to maintenance, a preliminary study of the problem was begun on September 19, 1974. The rationale of the investigation involved a suspended substrate concept, in which two surfaces of sediment per tray are simultaneously exposed to the water flow. Water passing above and beneath the substrate should percolate through the sand in a manner designed to prevent oxygen starvation and subsequent H_2S production.

Three Nestier trays were lined with solid plastic sheeting and three with polyvinyl screen/fiberglass/polyvinyl screen composite layers. Approximately two inches of sand was added to each tray and the trays were placed in a 12-ft flume. Both surfaces of the screened trays were exposed to a water layer of two inches, at a flow rate of 40 ml/sec.

The clams were then put into the trays and allowed to burrow. The system was left to equilibrate for two weeks (i.e., sand shifting from water flow, aeration lines and animal movement), and pH and oxygen measurements began in October. The mean values of screened trays and plastic-bottom control trays are given in Figure 1.

Essentially, the screened trays rose in pH and dropped in O_2 at the same relative rates as the controls, but maintained higher values through the fourth week of measurements. At this point, it is believed that the fiberglass layer, inserted to trap the smaller sand particles, became fouled with organic matter and was impervious to water flow. Oxygen and pH values then were essentially the same since only the surface substrate layer of all trays was subjected to moving water.

An anomaly of the system was noted in measured values from the inflow to outflow ends of the flume. Regardless of tray type, organic debris and diet algae tended to settle in the first two trays at the inflow end. The sand was most discolored in the first tray while the last tray, which was expected to contain an abundance of fecal matter and other debris, was clean by comparison.

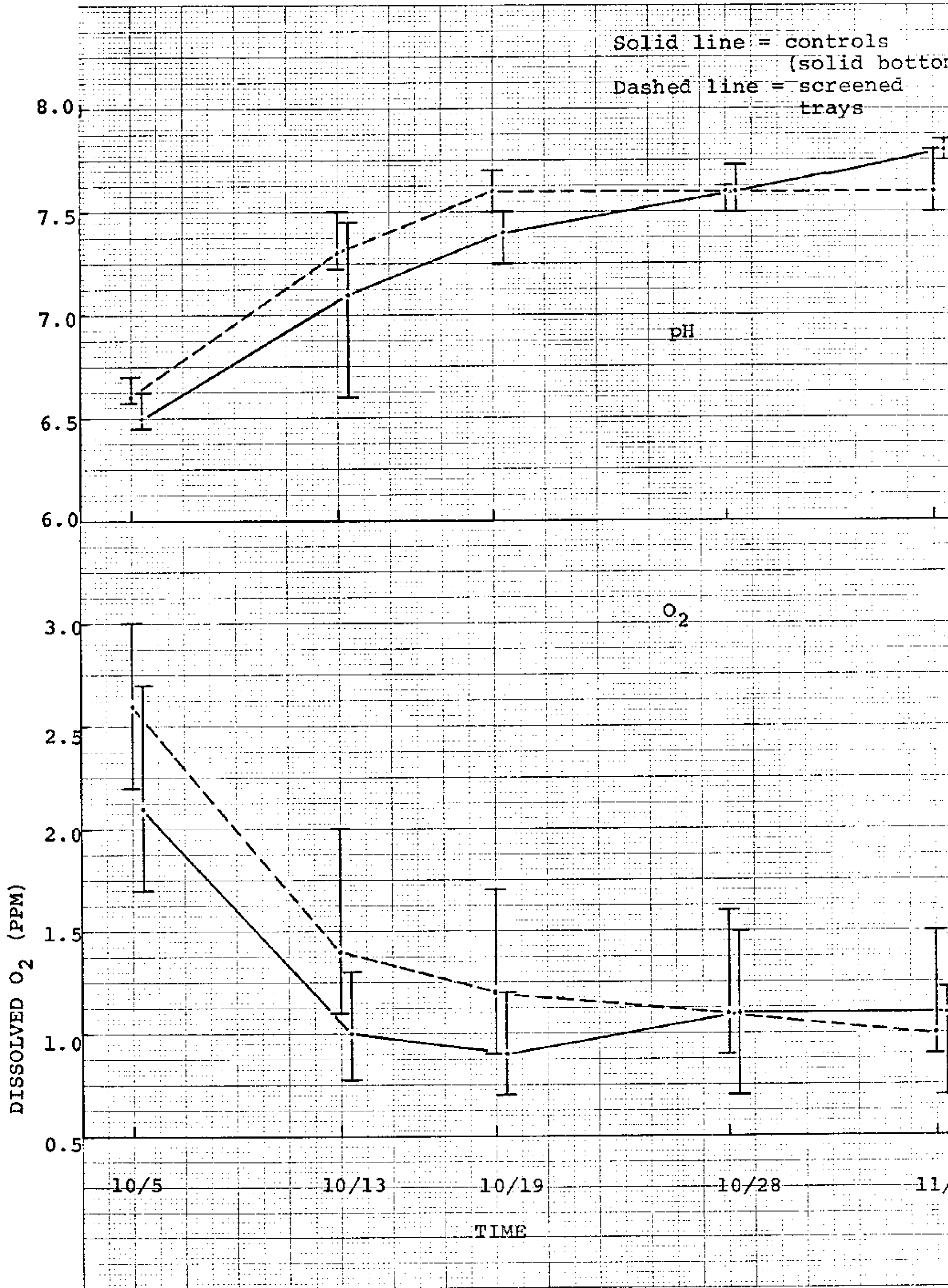
Hydrogen sulfide production was not visible (that is, the sand was not discolored) until the fourth week of measurements, about the time the fiberglass became fouled. Therefore, for more definitive data, a replicate study

using a finer-mesh screen would seem appropriate. The basic principles of this study, however, from the present data, suggest good applicability in juvenile clam culture.

References

Baab, J.S., G.L. Hamm, K.C. Haines, A. Chu and O.A. Roels, 1972. Shellfish mariculture in an artificial upwelling system. Proc. Nat. Shellfish. Assn. 63:63-67.

Figure 1. Change in pH and dissolved oxygen concentration of sediments in trays containing clams in sand. The dashed lines are for the screened-bottom trays; the solid lines for the solid-bottom trays. The plotted values are the means of the samples from three trays; the bars show the range of values obtained.



Solid line = controls
(solid bottom
Dashed line = screened
trays
8.0
7.5
7.0
6.5
6.0
pH
O_2
3.0
2.5
2.0
1.5
1.0
0.5
DISSOLVED O_2 (PPM)
10/5
10/13
10/19
10/28
11/
TIME

COMPARISON OF FRESH AND SALT WATER MUSSEL MEALS AS A REPLACEMENT OF FISH MEAL IN BROILER FEEDS

by

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Centre de Recherches en Nutrition
UNIVERSITÉ LAVAL

November 1974

ABSTRACT

Day-old male broiler chickens were fed isocaloric (3000 kcal M.E./kg) and isonitrogenous rations (22.0% protein = N x 6.25) during a 24-day period to compare the value of meals from fresh and salt water (*Mytilus edulis*) mussels to a control based on fish meal. The overall performance of these groups was also compared to a group fed a chick starter commercial ration containing 22% protein. The diets with fresh or salt water mussel meal were not toxic nor did they cause mortality. However, altough feed intake did not differ appreciably among groups, there were considerable variations in weight gain and feed conversion. The weight gain of the groups fed the commercial diet and the diet based on salt water mussels was markedly higher ($P \leq 0.01$) than that of the groups fed the control diet and the ration based on fresh water mussels. The body weight of birds at 24 days of age was 720 ± 19 g, 713 ± 18 g, 633 ± 16.2 g, and 630 ± 16.2 g for the group fed the commercial, the salt water mussel, the fresh water mussel and the fish meal diets, respectively. Feed efficiency for the 24-day period was also higher for the commercial diet and the ration based on salt water mussels. Feed intake/weight gain ratios were 1.66 and 1.62 with the commercial diet and the salt water mussel diet, respectively, compared to 1.81 with the control ration and 1.90 with the

fresh water mussel ration. The better growth of the chicks on the salt water mussel diet is probably due to its lysine content (12.19% of total amino acids) compared to 8.32% for fresh water mussels. Overall results suggest that salt water mussel meal can advantageously replace a similar amount of fish meal protein. However, the feeding value of fresh water mussel meal appears sligtly inferior to that of fish meal, particularly for chickens of 0-16 days of age.

RESUME

Des poulets à griller mâles, nouvellement éclos ont été alimentés pendant 24 jours de rations isocaloriques (3000 kcal E.M./kg) et isoazotés (22%, N x 6.25) dans le but de comparer la valeur alimentaire de régimes contenant de la farine de moules d'eau douce ou d'eau salée (*Mytilus edulis*) à celle d'un régime témoin contenant de la farine de poisson. Le taux de croissance et l'efficacité alimentaire des poulets recevant ces trois régimes ont également été comparés à ceux d'un groupe recevant un régime commercial de début pour poulet à griller contenant 22% de protéines. Aucune mortalité ni symptôme de toxicité ont été observés chez les poulets alimentés des régimes contenant la farine de moules d'eau douce ou d'eau salée. Cependant, bien que la prise alimentaire ne différait pas d'une façon sensible entre les groupes, des différences appréciables ont été notées dans le poids vif, le gain de poids et l'efficacité alimentaire des divers groupes. Le poids vif et le gain de poids des groupes alimentés du régime commercial et du régime contenant la fairne de moules d'eau salée ont été supérieurs d'une façon très significative ($P \leq 0.01$) à ceux des groupes alimentés du régime témoin ou de la ration contenant la farine de moules d'eau douce. Par exemple, les poids vifs (g) des oiseaux à l'âge de 24 jours étaient respectivement 720 ± 19 g, 713 ± 18 g, 633 ± 16.2 g et 630 ± 16.2 g pour le groupe recevant le régime

commercial, le régime à base de farine de moules d'eau salée, le régime à base de farine de moules d'eau douce et le régime témoin à base de farine de poisson. D'autre part, l'efficacité alimentaire pour la période expérimentale de 24 jours était également supérieure pour le régime commercial et la ration contenant la farine de moules d'eau salée. Les rapports consommation alimentaire/gain de poids étaient respectivement 1.66 et 1.62 avec le régime commercial et le régime à base de moules d'eau salée alors que ce rapport était de 1.81 avec le régime témoin et 1.90 avec le régime à base de moules d'eau douce. Le pourcentage élevé de lysine (12.19%) dans les moules d'eau salée en comparaison avec celui (8.12) des moules d'eau douce pourrait expliquer la meilleure croissance et l'efficacité alimentaire élevée des poulets alimentés du régime à base de moules d'eau salée. L'ensemble des résultats suggère que la farine des moules d'eau salée peut remplacer avantageusement une quantité similaire de protéines provenant de la farine de poisson. Il semble cependant que la valeur alimentaire de la farine de moules d'eau douce soit légèrement inférieure à celle de la farine de poisson, particulièrement chez les poulets âgés de 0 à 16 jours.

INTRODUCTION

Several biological systems to recycle nitrogenous compounds from waste water as a possible source of protein are presently under study. Aquaculture systems using mussels have been developed by Ryther (1968) and Roels (1970) in the U.S.A. to use secondary effluents from waste water treatment plants. However, the economic feasibility of these systems depends on the potential use of molluscs in animal feeds. The nutritive value of salt water mussel was determined earlier in diets for rats (De Siqueira *et al*., 1954; Larralde *et al*., 1965), mink (Juzovicky and Zajcev, 1968) and pigs (Zambriborsc, 1956; Bondarev, 1964). Burlacu *et al* (1968) studied the value of fresh water mussels (*Anodonta Cygnaea* L) in hen feeds. The results of these studies show that fresh water and salt water mussels are a valuable protein supplement.

To establish the economic value of these new products, their nutritive value must be compared to that of the protein concentrates presently used in feed formulae. Furthermore, the toxicity of the shell fish and the accumulation of heavy metals such as cadmium, mercury, copper and zinc, must also be considered since molluscs tend to accumulate metals (Chipman *et al*., 1958; Kopfler and Mayer, 1969; Schuster and Pringle, 1969; Kerfood and Jacobs, 1973).

This paper reports the results of a study comparing the value of meal from fresh water and salt water mussels to that of fishmeal in a diet for broiler chicks. The purpose of this trial was also to check out if mussels picked up from polluted water could be toxic or represent a potential health hazard owing to the accumulation of some metals of any toxic materials.

MATERIALS AND METHODS

A) Sampling sites

The salt water mussels (*Mytilus edulis*) used in the feeding experiment were collected in the spring of 1974 in Powell's cove (in the East River, New York City) within a few yards of the outfall of the Tallmans Island Pollution Control plant.

The fresh water mussels were collected in Quebec in the fall of 1973. The species proportions were as following: *Anodonta cataracta*, 35%, *Lampsilis radiata*, 33%, *Lampsilis ventricosa*, 20% *Elliptio complanata*, 12%. 80% of the mussels were collected from an eutrophic lake, lake StPaul, near Three-Rivers. The rest of the mussels were collected near the shore of the Saint-Lawrence River, at St-Valliers, a small town 30 miles north-east of Quebec city. The river at that point is quite polluted since three big cities, situated upstream, Quebec, Three-Rivers and Montreal, discharge all their sewage raw into the river.

The mussels were frozen at -10°C a few hours after their collection and later freeze-dried. After drying, the shells were removed and the meat was ground with a Wiley Mill using a 40 mesh screen.

b) Composition of the diets

The rations were isonitrogenous (22.0% protein = N x 6.25) and isocaloric (M.E.). Table 1 shows the composition of the control diet based on fishmeal and the two diets in which the fish meal protein was replaced by an equivalent quantity of protein from meal of fresh or salt water mussels. The nutritive value of these three was also compared to that of a commercial chick starter diet from the Ralston-Purina Company, containing 22% protein. Metabolizable energy (M.E.) of the three experimental rations was adjusted to 3000 kcal/kg by varying the proportion of tallow and cellulose. However, the proportion of the other ingredients such as corn, soybean meal, meat meal, feather meal and alfalfa meal remained constant in all diets. The composition of the vitamin and mineral premix used in the three experimental diets is shown in Table 2.

c) Experimental design, management and housing

Day-old male Hubbard broiler chickens used in the present study were obtained from a local hatchery and assigned to 4 different groups in random fashion. A total of 42 birds were used in this trial. There were three groups of 12 chickens each for the control group and the groups fed the commercial and the salt water mussel diets. However, there were only 7 birds in the group fed the fresh water mussel diet since only a small quantity of that meal was available.

During the 24 days of the experiment, the birds were housed in four metallic rearing cages (61 cm x 81 cm x 28 cm) with screened floor mounted on the same framing. The temperature of the cages was thermostatically controlled and kept at 30°C during the first week of the experiment. It was lowered by 3°C per week until a temperature of 22°C was reached. Individual body weight of the birds was recorded on the first day and after 8, 16, and 24 days. Water and feed was provided "ad libitum" but feed intake for each group was recorded for the three experimental periods. This made it possible to determine periodical or cumulative feed conversion for each group of chicks.

c) Chemical analyses

The percentage of protein (N x 6.25) in the fresh and salt water mussels was determined by the micro-kjeldahl method (A.O.A.C., 1970). The amino acid composition of these samples was determined using a Technicon TSM amino acid autoanalyzer equipped with an Autolab integrator. Elution time on the basic column was 22 minutes and 48 minutes on the acidic column. The samples were hydrolyzed before amino acid analysis in 500 ml mason jars, using 200 mg of sample and 100 ml of 6N HCL. After flushing the jars with nitrogen, they were sealed and kept for 22 hours at 105 C. Following hydrolysis, the HCL was evaporated in a rotary vacuum evaporator and the sample was diluted with buffer at *pH* 2.0.

d) Statistical analyses

No statistical analysis was done on feed conversion because feed intake was recorded only for the groups. In the case of body weight and weight gain, the mean, standard error of the mean, the analyses of variance and the comparison among groups were calculated with an IBM computer using Duncan's program. Data were entered by the APL system. Access to the library was given by typing)COPY 307 RAYON 2 DUNCAN.

RESULTS AND DISCUSSION

a) Health of the chicks

The health of the birds fed the fresh or salt water mussels did not differ appreciably from those fed the control and commercial diets. Neither mortality, nor symptoms of toxicity, such as convulsions, ataxy, anorexia, plumage abnormalities, diarrhea, etc, were observed. Rate of growth was also satisfactory with all groups as indicated by the relatively high body weight of chickens at 24 days of age. Body weights, at that age ranged from 633.7 ± 16.2 g to 720.1 ± 19.3 g which compare advantageously with body weight of 4-week-old chickens fed corn and wheat rations (Gardiner, 1973).

b) Body weight and weight gain

There were significant differences in body weight and weight gain (Table 3) between the different groups ($P \leq 0.05$ to $P \leq 0.01$) at the weighing periods. On the 8th day, the chicks fed the commercial and salt water mussel diet showed higher body weight and better weight gain than those fed the fresh water mussel diet. The body weight and the weight gain of the birds fed the control diet did not differ from that obtained with the salt water mussel diet.

On the 16th day, body weight and cumulative weight gain of chickens fed the commercial ration and the salt water mussel ration were similar but higher ($P \leq 0.01$) than that of the birds fed fish meal or fresh water mussel rations. However, although the body weight and the weight gain of the chickens fed the fish meal ration were significantly ($P \leq 0.05$) higher than that of chickens fed the fresh water mussel ration, the difference was not significant. On the 24th day, the body weight and weight gain of the different groups showed the same pattern as that obtained at 8 and 16 days of age. The cumulative weight gain of chicks fed the commercial and the salt water mussel diets was 676.9 ± 19.4 g and 668.7 ± 18.1 g, respectively as compared to 589.3 ± 16.1 g, and 585.8 ± 16.2 g with chicks fed the fresh water mussel and the fish meal diets. It appears therefore that the commercial diet and the salt water mussel diet produced a better overall rate of growth than fish meal and fresh water mussels diets.

The weight gain of the chicks fed the salt water mussel diet was close to that of the chicks fed the commercial diet for each 8-day period, i.e. 0-8, 8-16 and 16-24 days of age. Similarly, the weight gain of the chicks fed the control diet was significantly ($P \leq 0.05$ to $P \leq 0.01$) lower after each of these periods than for those fed the commercial or the salt water diets. It is interesting to note that the rate of gain of the birds fed fresh water mussels increased with

the age of animal. In fact, during the 18-24 day period, the weight gain of the group fed the fresh water mussels was not significantly different from that of chicks fed the commercial or the salt water diet. The weight gain observed during that period was 320.7 ± 9.8 g, 317.6 ± 13.7 g, 309.6 ± 8.4 g and 277.4 ± 10.4 g for the groups fed the salt water mussel, the commercial, the fresh water mussel and the fish meal rations, respectively. Thus, the lower cumulative weight gain of the chicks fed the fresh water mussel or the commercial diets was due to a depressed growth rate during the 0-8 day period.

c) Feed consumption and feed efficiency

Feed consumption for the 24-day period did not vary appreciably among groups (Table 4). However, during the initial period (0-8 days), feed intake tended to be lower for the group fed the fresh water mussel ration than for the groups fed the other diets. In contrast, during the 8-16 days period, the feed intake was the highest for the group fed the fresh water mussels. It is possible taht the taste of the fresh water mussel diet depressed feed consumption during the initial period. The feed intake of the group fed the fresh water mussels improved rapidly, once the birds used to the diet.

The results on cumulative feed conversion (Table 4) indicate that the salt water mussel and the commercial diets had a better feed efficiency than the fresh water mussel and the fishmeal rations. For example, feed conversion for the entire experimental period (0-24 days) was 1.62 and 1.66 for chicks fed the salt water mussel and the commercial rations respectively, compared to 1.81 and 1.90 for those fed the fish meal and the fresh water mussel rations. Feed efficiency also varied within and among periods (Table 4). After only 8 days of trial, the superiority of the feed conversion of the commercial and the salt water mussel diets was obvious. For this period feed consumption / weight gain ratios were only 1.30 and 1.35 for the birds fed the commmercial and the salt water mussel diets, compared to 1.53 and 1.55 for those fed the fresh water mussels and the fish meal rations. During the two following periods (8-16 and 16-24 days) a gradual decrease in feed efficiency was observed for all the rations. Average feed conversion ranged 1.43, 1.58 and 1.91 for the 0-8, 8-16 and 16-24 day periods, respectively. Nevertheless, the feed efficiency of the commercial and the salt water rations remained superior throughout the experiment when compared to fish meal or the fresh water mussel rations. During the 16-24 day period, the feed efficiency of the salt water

mussel diet was markedly higher than that of all the other diets. Furthermore, the feed efficiency of the fresh water mussel diet which was lower than for the fish meal diet became superior during the 16-24 day period. It appears therefore that the lower overall feed conversion (1.90) of the fresh water mussel diet, when compared to the fish meal (1.81), was due to the depressed feed efficiency of the fresh water mussel diet during the 8-16 days period.

Data on the amino acid composition (Table 5) of meals from salt water or fresh water mussels reveal that the protein from salt water mussels contained a smaller proportion of several amino acids such as cystine, phenylalanine, proline, threonine and tyrosine than fresh water mussel but had a greater proportion of arginine, glycine, histidine, lysine and methionine. The high level of lysine (12.19% on the basis of the protein content) in salt water mussel could have been responsible for the overall superior performance of chicks fed this diet. According to the National Research Council (1971), the lysine requirement for broiler chicks is particularly high (1.25% of the diet).

Our work confirms that both salt and fresh water mussel meal can be a valuable protein supplement. Our results show that salt water mussels can advantageously

replace fish meal in broilers chick rations. The fresh water mussel diet was only slightly inferior to the fish meal ration. There was no indication that salt water or fresh water mussels were toxic for the animals.

Table 1. Composition of the control diet and of the diets based on fresh and salt water mussels[1]

Ingredients	Protein (%)	M.E.[2] (Kcal/kg)	Diets-Proportions of ingredients (%)		
			Control (fish-meal)	Salt water mussel	Fresh water mussel
Corn	9.00	3,418	60.00	60.00	60.00
Soybean meal	50.00	2,536	16.78	16.78	16.78
Feather meal	85.00	2,282	2.00	2.00	2.00
Alfalfa meal	17.00	1,654	3.00	3.00	3.00
Meat meal	50.00	1,918	5.00	5.00	5.00
Fish meal	70.00	2,977[2]	5.00	-	-
Salt water mussel meal	67.80	2,977[2]	-	5.16	-
Fresh water mussel meal	43.25	2,977	-	-	8.09
Tallow		7,735	2.38	2.32	1.19
Limestone			0.50	0.50	0.50
Phosphate (Curacao)			0.80	0.80	0.80
Premix			0.50	0.50	0.50
Choline			0.10	0.10	0.10
Cellulose			3.94	3.84	2.04
TOTAL			100.00	100.00	100.00

[1] All the rations contained 22.0% protein (N x 6.25) and 3000 kcal M.E./kg.

[2] It was assumed that the metabolizable energy (M.E.) of fresh and salt water mussel meal was the same as that of fish meal.

Table 2. Composition of the vitamin and mineral premix

Ingredients	Amounts (lbs)
Coban (110 g/ton)	3.0
Vitamin A (60,000 I.U./g)	1.29
Vitamin D_3 (7,500 I.U./g)	1.32
Vitamin E (125,000 I.U./g)	0.36
Menadione (vit. K3) (premix 4%)	0.42
Ascorbic acid	0.30
Vitamin B_{12} (300 mg/lb)	0.21
Calcium pantothenate (80g/lb)	0.42
Folic acid (0.3%)	0.81
Magnesium and potassium sulfate	13.80
Niacin (98%)	0.24
Pyridoxine (1%)	0.60
Riboflavin (18%)	0.96
Santoquin (50%)	1.08
Selenium (0.02%)	3.00
Thiamine (1%)	0.60
Trace mineral premix[1]	3.30
TOTAL:	31.71

[1] Composition: 18.5% Ca (calcium carbonate), 0.30% I (calcium iodate), 17.5% Mn (manganese sulfate and manganese oxide), 12.95% Zn (zinc oxide), 1.25% Cu (copper sulfate).

Table 3. Body weight and periodical and cumulative weight gain of chicks fed the various diets

Parameter	Type of diet	Experimental period (days)			
		0	8	16	24
Body weight (g)	Commercial (chick starter Ralston-Purina)	43.2±0.7[1] *a,A*[2]	170.7±2.8 *a,A*	402.5±7.6 *a,A*	720.1±19.3 *a,A*
	Control (fish meal)	44.7±0.6 *a,A*	153.6±3.8 *b,B*	353.0±7.2 *b,B*	630.4±16.2 *b,B*
	Salt water mussel	44.1±0.9 *a,A*	163.6±4.7 *ab, AB*	392.1±9.6 *a,A*	712.8±18.2 *a,A*
	Fresh water mussel	44.4±1.2[3] *a,A*	127.0±4.0 *c,C*	324.1±9.0 *c,B*	633.7±16.2 *b,B*
Periodical weight gain (g)	Commercial (Chick starter Ralston-Purina)	-	127.5±2.9 *a,A*	231.8±5.4 *a,A*	317.6±13.7 *a,A*
	Control (fish meal)	-	108.9±3.9 *b,B*	199.4±7.1 *b,B*	277.4±10.4 *b,A*
	Salt water mussel (*Mytilus edulis*)	-	119.5±4.3 *a,AB*	228.5±6.6 *a,A*	320.7±9.8 *a,A*
	Fresh water mussel	-	81.7±4.1 *c,C*	198.0±5.2 *b,B*	309.6±8.4 *ab,A*
Cumulative weight gain	Commercial (Chick starter Ralston-Purina)	-	127.5±2.9 *a,A*	359.3±7.6 *a,A*	679.9±19.4 *a,A*
	Control (fish meal)	-	108.9±3.9 *b,B*	308.3±7.1 *b,B*	585.8±16.2 *b,B*
	Salt water mussel	-	119.5±4.3 *a,AB*	348.0±9.4 *a,A*	668.7±18.1 *a,A*
	Fresh water mussel	-	81.7±4.1 *c,C*	279.7±9.0 *c,B*	589.3±16.1 *b,B*

[1] Mean ± standard error of the mean for 12 chicks.

[2] Values for a given parameter in the same column bearing similar lower case letters, are not significantly different at $P \leq 0.05$.
Those bearing similar capital letters are not significantly different at $P \leq 0.01$.

[3] Mean ± standard error of the mean for 7 chicks.

Table 4. Average feed consumption, weight gain and feed efficiency[1] of chickens fed the various diets

Type of diet	Parameter	Experimental period (day intervals)					
		Periodical results			Cumulative results		
		0-8	8-16	16-24	0-8	0-16	0-24
Commercial (Chick starter Ralston-Purina)	Feed consumption (g)	165.3	347.3	607.7	165.3	512.7	1120.3
	Weight gain (g)	127.5	231.8	317.6	127.5	359.3	676.9
	Feed efficiency	1.30	1.50	1.91	1.30	1.43	1.66
Control (fish meal)	Feed consumption (g)	169.0	331.3	557.5	169.0	500.3	1057.8
	Weight gain (g)	108.9	199.4	277.4	108.9	308.3	585.7
	Feed efficiency	1.55	1.66	2.01	1.55	1.62	1.81
Salt water mussel (*Mytilus edulis*).	Feed consumption (g)	160.9	351.6	569.3	160.9	512.5	1081.8
	Weight gain (g)	119.5	228.5	320.7	119.5	348.0	668.7
	Feed efficiency	1.35	1.54	1.78	1.35	1.47	1.62
Fresh water mussel	Feed consumption (g)	125.4	396.7	599.9	125.4	522.1	1122.0
	Weight gain (g)	81.7	198.0	309.6	81.7	279.7	589.3
	Feed efficiency	1.53	2.00	1.94	1.53	1.87	1.90

[1] Feed efficiency = feed consumption/weight gain.

Table 5. Amino acid compostion of fresh and salt water mussels used in the rations

Amino acids	Amino acids (%) 16 gN	
	Fresh water mussel	Salt water mussel (*Mytilus edulis*)
Alanine	4.84	4.52
Arginine	7.29	12.17
Aspartic acid	8.65	8.17
Cystine	1.10	0.0
Glutamic acid	11.02	11.46
Glycine	5.04	7.13
Histidine	2.39	4.01
Isoleucine	3.86	4.32
Leucine	6.66	6.14
Lysine	8.12	12.19
Methionine	2.32	2.77
Phenylalanine	3.67	3.20
Proline	5.37	3.33
Serine	4.31	4.52
Threonine	4.24	3.44
Tyrosine	3.63	2.76
Valine	3.98	3.17
N x 6.25	43.25	67.80

LITERATURE CITED

1. A.O.A.C. (Association of Official Agricultural Chemists). 1970. Methods of Analysis. 11th ed., Washington, D.C.

2. Bondarev, J.E. 1964. A valuable protein and mineral feed. Svinovodstvo, 9: 22-23. Nutrition Abstr. & Revs., 35:533 (1965)

3. Burlacu, G., Nastasescu, G., Marinescu, G., Raduca, C. and Voiculescu, J. 1968. Efficiency of utilisation of the energy of mussels (Anodonta cygnoea L.) in the feed for hens. Studu Cercet. Biol., Ser. Zool., 20: 185-191. Nutrition abstr. & Revs., 39; 687 (1969)

4. Chipman, W.A., Rice, T.R. and Price, T.J. 1958. Uptake and accumulation of radiactive zinc by marine plankton, fish and shell-fish. Fish. Bull. Fish and Wildlife Serv., 58: 279-292.

5. Chuster, C.N. and Pringle B.H. 1969. Trace metals accumulation by the American Earstern oyster, Crassostrea virginica. Proceedings of the National Shellfisheries Ass., Vol. 59.

6. De Sequeira, R., Pechnik, E., Lopes, N. and Guernelli, O. 1954. Studies of Mytilus Mundahuensis. Arg. Bresil, Nutricao, 10: 299-333

7. Gardiner, E.E. 1973. A comparison of corn, Glenlea, Pitic 62, and Neepawa wheats in broiler diets. Can. J. Anim. Sci., 53: 547-550.

8. Kerfoot, W.B. and Jacobs, S.A. 1973. The accumulation of metal by organisms cultured in a combined tertiary treatment aquaculture systems. In: Woods Hole Oceanographic Institute, Progress Report, WHOI-73-2. By Ryther, J.H. Jan. 1973.

9. Kopfler, F.C. and Mayer J. 1969. Studies on trace metals in shellfish. Proceedings, Gulf and South Atlantic Shellfis Sanitation Research Conference, March 1967, Gulf Coast Marine Health Science Laboratory, Dauphin Island, Alabama.

10. Juzovicky, J.A. and Zajcev, A.G. 1968. Mussels for minks. Krolik. Zver., 4:16-17. Nutrition Abstr. & Revs., 39:672 (1969)

11. Larralde, J., Rodriguez, C. and Bello., J. 1965. Coefficier of conversion and protein utilisation index of _Mytilus eduli_ An. Bromatol., 17:239-247.

12. National Research Council. 1971. Nutrient requirements of poultry. 6th Revised Edition. National Academy of Sciences, Washington, D.C.

13. Roels, O.A. 1970. The improvement of the New York estuary through effluent aquaculture and food chain management. Rept. to office of Water Ressources Research, U.S. Dept. Interior, Washington D.C.

14. Ryther, J.H. and Bardach, J.E. 1968. The status and potantial of aquaculture. I. Particularly invertebrate and algal culture; II. Particularly fish culture. Fed. Scientific & Technical Information Bull. BB -177-767, 768, Washington, D.C.

15. Zambriborsc, F.S. 1956. Feeding pigs on mussels (molluscs). Zivotnovodstvo, 12:73-74, Nutrition Abstr. & Revs., 27:9995, (1957).