"ARTIFICIAL UPWELLING"
PROGRESS REPORT / 1975

Report of Progress for the Period
January 1, 1975 to December 31, 1975

for work supported by

Sea Grant Project No. 04-5-158-59

to

NATIONAL SEA GRANT PROGRAM
National Oceanic & Atmospheric Administration
U. S. Department of Commerce
3300 Whitehaven Street N.W.
Washington, D.C. 20235

by

Biological Oceanography
LAMONT-DOHERTY GEOLOGICAL OBSERVATORY
of
Columbia University
Palisades, New York 10964

Oswald A. Roels
Principal Investigator

15 December 1975

C O N T E N T S

PART A—THE ARTIFICIAL UPWELLING PROJECT 2

Summary Progress Report : 1975 2

Papers Published and/or Presented at Meetings in 1975 6

1 ADMINISTRATION 8

1.1 Project Staffing 8

1.2 Consultants and Advisors 10

1.3 Facilities 11

1.4 Allied Projects 12

1.4.1 Open-Bay Mariculture 13

1.4.2 Clam Aquaculture in Wastewater 13

1.4.3 Open-Sea Mariculture 14

1.5 Internships in Mariculture 14

2 ENGINEERING 16

2.1 Deep-Water Pipelines 16

2.2 Surface-Water Pipeline 16

2.3 Pump House 16

2.4 Reactors 17

2.5 Hatchery/Wet Laboratory for Shellfish 17

3. CHEMISTRY 25

3.1 Deep-Water Quality 25

3.2 Protein Measurement 26

3.3 Sources of Error in Silicate Determinations 26

4 MICRO-ALGAE CULTURE 28

4.1 Selection of Algal Species for Feeding Shellfish Larvae and Juveniles 28

4.2 Selection of Clones of Algal Species Capable of Growth in Unsupplemented Deep Water 28

4.3 Determination of Optimum Depth for Cultures of Micro-Algae 29

4.4 Comparison of Techniques for the Estimation of Phytoplankton Culture Density 29

4.5 New Clones of Algae from St. Croix 30

4.6 Interaction of Light and Nutrients on Growth of Chaetoceros curvisetus 31

4.7 Effects of Light and Dark on Growth of Chaetoceros sp. 31

5 SHELLFISH CULTURE : PELECYPODS 33

5.1 Brood Stock Inventory 33

5.2 Hatchery Activities 33

5.2.1 Experimental Spawnings 33

5.2.2 Comparison of Algal Foods for Larvae 35

5.3 Growth of Juvenile Oysters 39

5.3.1 Rearing of the Oysters, Ostrea edulis and Crassostrea gigas Spawned in the Artificial Upwelling Hatchery 39

5.3.2 Growth of the Japanese Pearl Oysters, Pinctada mertensi 44

5.3.3 Growth of the Kumamoto Oysters, Crassostrea gigas 52

5.4 Growth of Tapes semidecussata, the Japanese Littleneck Clam 56

5.4.1 Cultivation of Tapes semidecussata During Long- and Short-Term Feeding Studies 56

5.4.2 Substrates 57

5.4.3 Diet and Light 58.

5.4.4 T. semidecussata growth in Salt River and in the Artificial Upwelling System 64

5.4.5 Maximum Sustained Yield 66

6 SHELLFISH CULTURE : GASTROPODS 68

6.1 Queen Conch, Strombus gigas 68

6.2 West Indian Topshell, Cittarium tica 70

7 CRUSTACEAN CULTURE 71

7.1 Caribbean Lobster, Panulirus argus 71

8 MACRO-ALGAE CULTURE 72

8.1 Growth of Hypnea musciformis, a Tropical Red Seaweed 72

8.1.1 Growth in Continuous-Flow Outdoor Cultures 72

8.1.2 Laboratory Studies of a Unialgal Clone 72

8.2 Growth of Gracilaria verrucosa, Eucheuma isiforme and Agardiella tenera 73

PART B—THE EFFLUENT AQUACULTURE SYSTEM 76

Summary Progress Report : 1975 76

1 ADMINISTRATION 77

1.1 Present Facilities 77

1.2 Staffing 77

2 ENGINEERING 81

2.1 Flow Engineering 81

2.2 Security Precautions 84

3 CHEMISTRY 86

3.1 East River Water and Effluent Chemistry 86

3.1.1 Materials and Methods 87

3.1.2 Results and Discussion 88

3.2 Evaluation of our Clow Secondary Treatment System 91

3.2.1 Visual and Olfactory Inspection 91

3.2.2 Suspended Solids 92

3.2.3 Chemical Oxygen Demand 92

3.2.4 Biochemical Oxygen Demand 92

3.2.5 Summary of STP Operations 93

4 PHYTOPLANKTON 95

4.1 Light-Limited Growth and Cellular Composition of Dunaliella tertiolecta Cultured with Ammonia and Nitrate 95

4.2 Phytoplankton Isolation Studies 96

4.3 Large-Scale Phytoplankton Cultures 97

4.4 Use of a Cream Separator for Concentrating Phytoplankton 99

5 SHELLFISH 101

5.1 Flax Pond Shellfish Hatchery 101

5.2 Shellfish Studies at Tallman Island 101

5.2.1 Routine Shellfish Growth Experiments 101

5.2.2 Dynamics of Protein Production of Mytilis edulis 102

6 SEAWEED 103

6.1 Flax Pond Seaweed Laboratory 103

6.2 Seaweed Tank Design and Operation 103

6.3 Screening for Suitable Seaweed Species 104

6.4 Study of Agardiella verrucosa 107

APPENDICES:

A. Quarterly Progress Report on the Open-Bay Culture of Oysters in the U.S. Virgin Islands (Project supported by the Department of Conservation and Cultural Affairs of the U.S. Virgin Islands Government).

B. Growth of Clam and Oyster Larvae on Different Algal Diets in a Tropical Artificial Upwelling Mariculture System (Sunderlin, Baab, Patry).

C. Nutrients and Salinity Data for Deep-Water Pipelines

D. A Comparison of Techniques for the Estimation of Phytoplankton Density. I: Turbidity, In Vivo Fluorescence and Cell Counts on Selected Unialgal Cultures (Laurence).

E. Interaction of Light and Nutrients on Growth of the Diatom Chaetoceros curvisetus in Outdoor Continuous Culture (Farmer).

F. The Experimental Cultivation of Tapes semidecussata (Reeve) in an Artificial Upwelling Mariculture System (Rodde, Sunderlin, Roels).

G. The Effect of Substrate on the Growth Rate of Tapes semidecussata (Rodde)

H. A Protocol for the Experimental Determination of a Maximum Sustainable Yield of Tapes semidecussata on an Algal Mixture of STX-167 and S-1 (Rodde & Laurence)

I. Growth of the Carrageenan-Producing Tropical Red Seaweed Hypnea musciformis in Surface Water, 870-m Deep Water, Effluent from a Clam Mariculture System, and in Deep Water Enriched with Artificial Fertilizers or Domestic Sewage (Haines).

J. Jeris Method for the Determination of Chemical Oxygen Demand of Wastewaters (Sharfstein).

K. The Potential Yield of Artificial Upwelling Mariculture (Roels, Haines and Sunderlin).

"ARTIFICIAL UPWELLING"
PROGRESS REPORT / 1975

PART A—THE ARTIFICIAL UPWELLING PROJECT

Summary Progress Report: 1975

In May 1975 our deep-water pipelines entered their fourth year of continuous operation, bringing an uninterrupted supply of water from 870-m depth in the sea to our shore-based mariculture facility.

The diatom, Chaetoceros curvisetus (STX-167), which grows very well in deep water alone, has consistently done very well in our pools: the 45,000-liter pool cultures last from 20-30 days, at a turnover rate of 1.1 pool volumes per day. In steady-state continuous cultures of this diatom, up to 99% of the nitrate and nitrite from the deep water are taken up by the diatom. From experiments undertaken in 2000-liter concrete tanks (operating with a water depth of 0.8 m), utilizing different degrees of shading and different turnover rates of the deep water in the tanks during continuous culture, it has been calculated that C. curvisetus (STX-167), in a well-mixed pool of 5-m depth under the St. Croix light conditions, can fix 0.9 g of cellular organic nitrogen per m^2 per day, and 5.3 g of cellular organic carbon per m^2 per day. These numbers will have to be verified in actual deep-tank experiments which are planned for 1976. If this conversion of deep-water nitrate into phytoplankton protein can be maintained the year-round—which we have every reason to believe possible—we would then have achieved a protein production per unit surface area which is 34 times that of the protein yield in soybeans in conventional agriculture.

Four other species of diatoms which can grow in unsupplemented deep water for prolonged periods of time in the laboratory are now ready for testing in the 45,000-liter outdoor pools.

Twenty 2000-liter concrete tanks (called "reactors") have been installed at our beach facility and provided with deep-water supply. These reactors enable us to experiment simultaneously with twenty-two different species of phytoplankton (two species in the two 45,000-liter pools, and twenty in the twenty reactors), so that we can produce a large variety of diets for experimental feeding of shellfish by feeding these twenty-two algal species singly or in any combination.

Our new experimental shellfish laboratory on the beach was completed and put to good use: this building houses our shellfish hatchery, our experimental shellfish area, and has space reserved for the installation of the shellfish pilot plant in 1976.

The hatchery is in operation, and Tapes semidecussata, Crassostrea gigas, and its Kumamoto variety, are being spawned regularly. Nutritional experiments on the larvae of these species are underway, to improve survival and shorten the period of the larval stage. The hatchery supplies us regularly with the necessary animals for experimentation in the experimental shellfish area, in our Open-Bay mariculture project and in our Open-Sea mariculture project. It provides us with the facilities for genetic improvement of our animals.

The experimental shellfish area has been completed and provides facilities for 240 simultaneous quantitative experiments on shellfish. In a first series of experiments, seven different diets were tried in feeding studies with juvenile Tapes semidecussata produced in our hatchery. It was found that a mixture of Chaetoceros curvisetus (STX-167) and S-1 (a phytoflagellate isolated from the Sargasso Sea)

provided the best diet for this clam. In a subsequent experiment, it was found that T. semidecussata fed on the STX-167 + S-1 mixture achieved a conversion of plant protein which is more efficient than any other known conversion efficiency of plant-protein to animal-protein. The efficiency of conversion of phytoplankton-protein to animal-protein by T. semidecussata fed on this diet varied (depending on animal density, flow-rate, etc.) from 30 to 45%. This should be compared with the best known conversion of plant-protein to animal-protein in conventional agriculture which is achieved by the lactating cow and amounts to 31%. We believe that slower flow-rates and higher shellfish densities may further increase this plant-protein to animal-protein conversion efficiency. The efficiency of conversion of deep-water nitrate-nitrogen into algal protein-nitrogen for this particular experiment varied from 60 to 66%.

A carrageenan-producing seaweed, Hypnea musciformis, cultured in the effluent from a shellfish tank, grew about five times faster than in unaltered deep water, and about three times faster than in surface water. The carrageenan yield of H. musciformis grown in this way was excellent and its gelling properties were very good. This seaweed grows very rapidly, thereby competing very successfully for the nutrients in the shellfish tank, effectively outgrowing any other organisms, so that epiphytes did not appear to be a problem in this culture. If the small-scale results obtained in our St. Croix system are applicable to large-scale production, this seaweed could greatly contribute to cleaning-up the effluent from the shellfish mariculture operation and contribute significantly to the economic success of a commercial mariculture operation.

The Japanese pearl oyster, Pinctada mertensi, was introduced in our system in March 1975 and has grown at extraordinarily rapid rates with negligible mortality. If this growth rate continues, the animals will be ready for pearl-seed insertion considerably faster than in their native habitat.

With Crassostrea gigas spat produced in our hatchery, and with the natural set of Crassostrea rhizophorae, excellent growth has been obtained on experimental rafts moored in Salt River, an inlet on the North Shore of St. Croix. We have acquired a new facility there (three-and-one-half acres, including a marina and a trailer house for one of our staff members) which will be used to optimize open-bay mariculture of oysters and clams, using spat produced in our hatchery. The facility will also be used for the training of Virgin Islands' fishermen in raft-culture techniques.

Miss Mary Farmer has completed her experimental work for the doctoral dissertation on the influence of light and turnover rates on the growth of Chaetoceros curvisetus (STX-167) in outdoor continuous cultures. She is writing her doctoral dissertation now, and preparing four publications.

Mr. Gaston Picard has completed his experimental work for the doctoral dissertation on nitrate uptake and assimilation by Chaetoceros simplex (STX-105) and is writing his dissertation.

Papers Published and/or Presented at Meetings in 1975

The following is a list of papers published and/or presented at meetings during 1975. Reprints (where available) 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., K.C. Haines and J.B. Sunderlin (1975). The potential yield of artificial upwelling mariculture. Tenth European Symposium on Marine Biology, September 17-23, Ostend, Belgium.

Malone, T.C., C. Garside, K.C. Haines and O.A. Roels (1975). Nitrogen uptake and growth of Chaetoceros sp. in large outdoor continuous cultures. Limnology and Oceanography 20:9-19.

Farmer, M.W. (1975). Nitrate-limited versus light-limited growth of Chaetoceros curvisetus in outdoor continuous culture. American Society of Limnology and Oceanography, June 22-26, Dalhousie University, Halifax, Nova Scotia.

Anderson, O.R. (1975). The ultrastructure and cytochemistry of resting cell formation in Amphora coffaeformis (Bacillariophyceae). Journal of Phycology 11:272-281.

Sunderlin, J.B., M. Brenner, M. Castagna, J. Hirota, R.W. Menzel and O.A. Roels (1975). Comparative growth of hard shell clams (Mercenaria mercenaria Linné and M. campechiensis Gmelin) and their F_1 cross in temperate, subtropical and tropical natural waters and in a tropical artificial upwelling mariculture system. Proceedings, 6th World Mariculture Society Meeting, January 28-30, Seattle, Washington (in press).

Sunderlin, J.B., W.J. Tobias and O.A. Roels (1975). Growth of the European oyster, Ostrea edulis Linné in the St. Croix artificial upwelling mariculture system and in natural waters. Proceedings, 1974 National Shellfisheries Assoc. (in press).

Tobias, W.J. (1975). A collector trap for lobster larvae. Aquaculture 6:199-201.

Haines, K.C. (1975). Growth of the carrageenan-producing tropical red seaweed Hypnea musciformis in surface water, 870-m deep water, effluent from a clam mariculture system, and in deep water enriched with artificial fertilizers or domestic sewage. Tenth European Symposium on Marine Biology, September 17-23, Ostend, Belgium.

Roels, O.A., A.F. Amos and S. Laurence (1975). Potential mariculture yield of floating sea-thermal power plants. 1975 Fall Meeting American Geophysical Union (Abstract), December 8-12, San Francisco, California.

Roels, O.A. (1975) The economic contribution of artificial upwelling mariculture to sea-thermal power generation. Proceedings, Third Workshop of Ocean Thermal Energy Conversion (OTEC), May 8-10, Houston, Texas.

1 ADMINISTRATION

1.1 Project Staffing*

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

Co-Investigators:
Aust, Leo G. (B. Mech. E.)
St. Croix Station Manager

Haines, Kenneth C. (Ph.D.)
Resident Chief Scientist

Sunderlin, Judith B. (M.S.)
Senior Research Assistant

Van Hemelrijck, Ludo (M.E.E.)
Senior Staff Associate

Supporting Staff: St. Croix:

Angell, Charles (M.S.)
Research Scientist

David, W.
Facilities Upkeep

Dorsey, Thomas E. (Ph.D.)
Research Scientist

Forbes, Milton (Ph.D.)
Faculty Associate

Francis, Oswald A.
Technician

Guyton, Ernest
Technician

Langton, Richard (Ph.D.)
Research Scientist

Laurence, Scott (M.S.)
Research Assistant

Lyon, Richard E. (B.S.)
Laboratory Technician

Monahan, Rosemary (B.S.)
Research Assistant

Muller, Gisele (B.S.)
Research Assistant

Rodde, Cheryl (B.S.)
Laboratory Technician

Rodde, Kenneth (M.S.)
Senior Research Assistant

Williams, Roy G. (B.S.)
Technician

Supporting Staff: New York:

Aikman, Frank (M.S.)
Research Assistant

Amos, Lynn M.
Administrative Assistant

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

Berger, Gail D. (B.S.)
Library and Laboratory Assistant

Farmer, Mary W. (M.S.)
Graduate Student

Hammond, Lynn
Laboratory Assistant

Kostyk, Nadia (M.S.)
Senior Research Assistant

McClain, Irma
Laboratory Assistant

McDonald, Paul (B.S.)
Research Assistant

Pasek, Barbara (B.S.)
Secretary

Peng, Helen (M.S.)
Accountant

Pochapsky, Ruth (B.S.)
Secretary

Trout, Marian E. (B.S.)
Senior Staff Associate; Lab Manager

1.2 Consultants/Advisors

Berg, C. (Ph.D.)
Assistant Professor
The City College of The City University of New York
New York, N.Y. and American Museum of Natural History

Brownell, W. (Ph.D.)
Director, Fundacion Cientifica Los Roques
Caracas, Venezuela

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

Chanley, P.
Shelter Island Oyster Farms, Inc.
Greenport, Long Island, New York

Daemmon, A. (Ph.D.)
Director of Research
Department of Conservation & Cultural Affairs
U.S. Virgin Islands Government
St. Thomas, USVI

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

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

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

Shleser, R. (Ph.D.)
Director, Bodega Bay Project
University of California at Davis
Davis, California

In addition to the above, the following have given us the benefit of their advice and collaboration: Mr. W. Lodge (Consultant, Biological Oceanography at Lamont); Dr. Ken W. Watters and Dr. J. Gonzalez (Puerto Rico Nuclear Center); Dr. Harold J. Humm (University of South Florida); Mr. Harold Norve (Genu Products, Nova Scotia); Dr. Stephen F. Adler, Dr. A.D.F. Toy and Dr. Chifa F. Lin (Stauffer Chemical Co.).

1.3 Facilities

The St. Croix Station occupies all of the buildings (with the exception of the old windmill) on the Estate Rust-op-Twist, located on the North Shore about three miles west of Baron Bluff. We now have seven dwelling units and a cottage for the use of visiting scientists and permanent staff. The station housing is occupied by thirteen people, ten of whom are employees.

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

The shore area (approximately 40,000 sq.ft. of beachfront) 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-gallon 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; (d) deep-water constant head device for constant pressure in the entire deep-water distribution system; (e) two 12,000-gallon capacity concrete pools for algal culture as food for

shellfish; (f) ten 550-gallon elevated tanks ("reactors") mounted on a separate structure to the west of the hatchery/wet lab. Six of these can receive shallow water or deep water; the balance receive deep water only. Both water supply lines have constant-head devices; (g) a shallow-water system consisting of a flexible impeller electric pump and a pipeline of 1-1/2" polyethylene pipe protected by a 2-1/2" galvanized steel pipe clamped to steel bars driven into the bottom; (h) six 200-gallon polyethylene tanks, four of which are used for algal culture in our continuous food-production system; two are used for experimentation; (i) four 200-gallon wood-and-fiberglas tanks in which our prime populations of shellfish are housed; (j) two 150-gallon polyethylene tanks for experimental use; (k) four 200-gallon wood-and-fiberglas tanks for miscellaneous uses; (l) ten additional 550-gallon elevated tanks ("reactors") for comparative algal growth studies; (m) submersible pumps to move the algae-rich water from the large pools to the shellfish tanks; (n) assorted tanks, wood-and-fiberglas or PVC, used in the lobster and seaweed programs; (o) one shipping container for storage of tools and supplies for the beach and pipeline work; (p) 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.

1.4 Allied Projects at the St. Croix Station

The research facilities, equipment and staff at our St. Croix station form a unique center for research in aquaculture. In the past year we have undertaken three other research projects with closely related goals. The first of these (1.4.1) is a project funded by the U.S. Virgin Islands Government from non-federal sources. The objectives of this

project were formally incorporated into those of our Sea Grant-funded Artificial Upwelling project by contract modification in July 1975.

1.4.1 Open-Bay Mariculture

The objectives of this proposed four-year project are to demonstrate the practical and economic feasibility (on a pilot scale) of oyster mariculture in natural open bays in the U.S. Virgin Islands and to expand the project into a viable, self-supporting oyster fishery for local fishermen. During the first year (Phase I) we have concentrated on testing the technical feasibility of open-bay oyster mariculture, using seed oysters and clams produced in our hatchery. If the results of Phase I are favorable, Phase II will be devoted to training local fishermen in successful methods of oyster mariculture, in collaboration with appropriate agencies of the Virgin Islands government. If the results of Phase I are not encouraging, attempts will be made to re-stock open bays in the Virgin Islands with hatchery-reared juvenile shellfish. The implementation of Phases III and IV (provision of seed to local fishermen; technical advice to trained fishermen) are predicated on the success of Phases I and II, and could lead to the development of a new fishery on the three islands, thus providing new employment opportunities and training for Virgin Islanders (see Appendix A for details).

1.4.2 Clam Aquaculture in Wastewater

The Virgin Islands Agricultural Experiment Station of the College of the Virgin Islands funded this project which seeks to establish an aquaculture system based on the nutrients in secondarily treated wastewater from the St. Croix Wastewater Reclamation Plant. The first year was devoted to algal species' isolation and maintenance of continuous outdoor cultures. Preliminary experiments showed that maximum productivity was obtained with a retention time of five days. In

the second year of this project the algae produced will be fed to brackish-water and freshwater clams under various conditions of stocking densities, substrates, and food supply. Optimum harvest time will be determined and the meat produced will be tested as a protein source in feeding studies with young chickens.

1.4.3 Open-Sea Mariculture

In early January 1975 we received a grant from the Energy Research & Development Administration (ERDA) to begin work on a study of the technical and economic feasibility of utilizing sea-thermal power-plant effluent for mariculture purposes in the open sea. Water upwelled by these proposed power plants, or by any other system which draws deep water up to the surface, will be rich in nutrients. We are determining the fate of deep-sea water discharged at the surface, its mixing rate with surface water and vertical and horizontal migration of the resulting mixture, and its nutrient concentration. We are working on the selection of a phytoplankton species best-suited for this "open-sea" mariculture in function of growth rate in different mixtures of deep and surface water, efficiency of nutrient utilization and nutritional value for a second trophic level. Various species of shellfish are being tested for growth in raft and cage cultures suspended in the open sea. Initial, on-shore experiments indicate that a mixture of 70% deep water and 30% surface water is optimal for producing algal blooms which sustain rapid growth of Tapes semidecussata.

1.5 Internships in Mariculture

From June 1 through August 8, 1975, two college juniors participated in the internship-in-mariculture program at the St. Croix station. This program was established in cooperation with the Biology Department of Hood College, Frederick, Maryland. Phyllis Baab and

Elaine Patry worked 40 hours per week for a 10-week period, on shellfish research. While most of their time was spent in the shellfish hatchery conducting larval feeding studies, they also had an opportunity to learn algal culturing techniques, and to participate in experiments on juvenile and adult shellfish.

Three larval feeding studies were completed during their internship; these experiments were written up and have been included in a paper submitted to the World Mariculture Society for presentation at their meeting in San Diego, California in January 1976, and subsequent publication in the Proceedings of that meeting (Appendix B).

The intern program is a valuable experience for students who are able to gain first-hand experience in a given field. We hope to continue this program with Hood College next year, and again have two students working at the station during the summer.

From January to March 1976, a high-school student from Deerfield Academy, Deerfield, Massachusetts, will be working at the laboratory in a similar program. The student will primarily be working in the hatchery, assisting with larval feeding studies, but will also learn the other aspects of the mariculture system.

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 and replacement of the sacrificial anodes. Weekly nutrient and salinity analyses from the individual pipes allow monitoring of the integrity of the pieplines below the depth limits of our divers.

2.2 Surface Water Pipelines

A surface water pipeline has been installed for a separately funded study of phytoplankton growth in mixtures of surface and deep water. The pipeline consists of a 1-1/2" diameter polyethylene pipe threaded through a 2-1/2" galvanized steel pipe which is held on the bottom by steel bars driven into the reef.

2.3 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 (delayed until a lobster biologist can be added to the staff). An integrator in a sealed PVC and acrylic enclosure has been mounted beside the sensor of the recording Eppley pyranometer to facilitate the calculation of the photosynthetically active radiation received during the course of an algal or seaweed growth experiment.

The filters on the discharges of the graphite aeration pumps have been replaced by a different type and relief valves have been added to

protect the pumps from excessive back pressure and resultant high wear. Also, transparent sections of discharge tubing now signal, by graphite deposit, the need to replace filters.

2.4 Algal Reactors

Ten more elevated 550-gallon algal tanks ("reactors") have been added to our beach facility. Five of these are plumbed for deep-water plus surface seawater; the other five are supplied with deep water only. Surface water/deep water mixtures are required for the open-sea mariculture project (section 1.4.3). The addition of these reactors will greatly expand our experimental algal culture capability because we found that the ten reactors associated with the Hatchery /Wet Lab were usually required for routine culture of food for batches of larval or juvenile shellfish, and these were not available for algal culture experiments. These ten new reactors are also elevated, so that shellfish feeding experiments can be conducted under them (see Figure 2.4.1).

2.5 Hatchery/Wet Laboratory

The Hatchery was completed with the addition of a spawning table and a gas-fired hot-water heater for temperature-shocking oysters and clams into spawning.

Ten 550-gallon algal reactors on the west end of the building provide algal culture which is piped throughout the length of the building as is deep water and air. Shellfish spawned in the Hatchery are utilized in feeding and growth studies in the Experimental Shellfish Area of the building. The experimental shellfish area has been equipped with a 9-ft long, 6-ft high test rack for use in studying the growth characteristics of bivalves (see Figures 2.5.1, 2.5.2).

The rack is comprised of five separate horizontal feeding levels

Figure 2.4-1. Ten additional reactors (550-gal. each) installed for outdoor continuous culture studies with phytoplankters. Five of the reactors are provided with surface water as well as deep water; the other five are supplied with deep water only. The vertical pipes are pressure heads for the surface and deep-water systems, to give constant flow rates. The area beneath the reactors is used for juvenile shellfish feeding studies. These studies are conducted in an array of shellfish tanks identical to those in Figure 2.5.2. These ten additional reactors constructed in 1975 bring our total number of reactors to 20.

Figure 2.5-1. Experimental shellfish area of the Hatchery/Wet Lab building. In the foreground are flumes (shallow tanks) used in rearing metamorphosed pelecypod larvae to a size suitable for feeding studies which are conducted in the rack containing 120 small shellfish tanks (in the background).

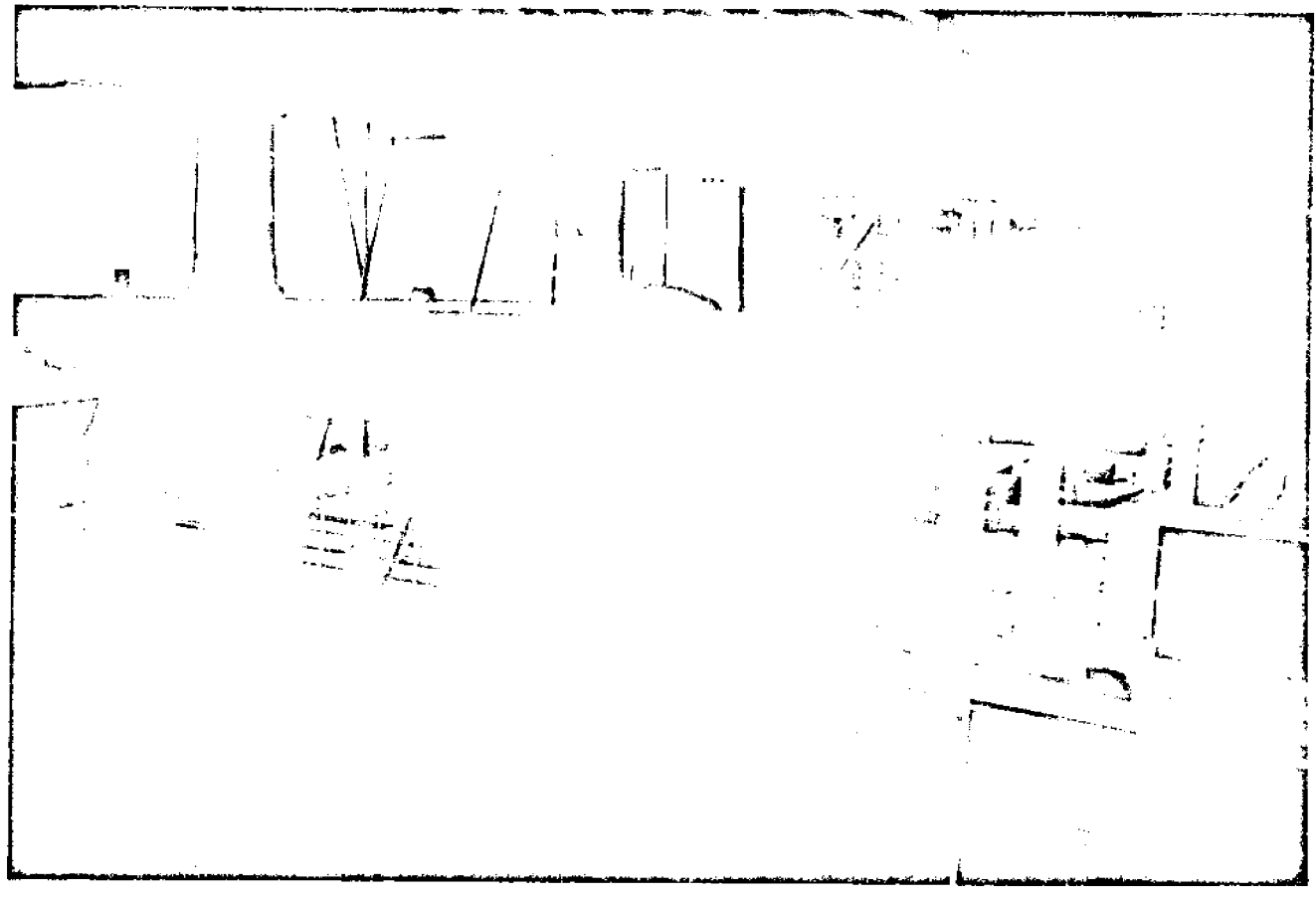
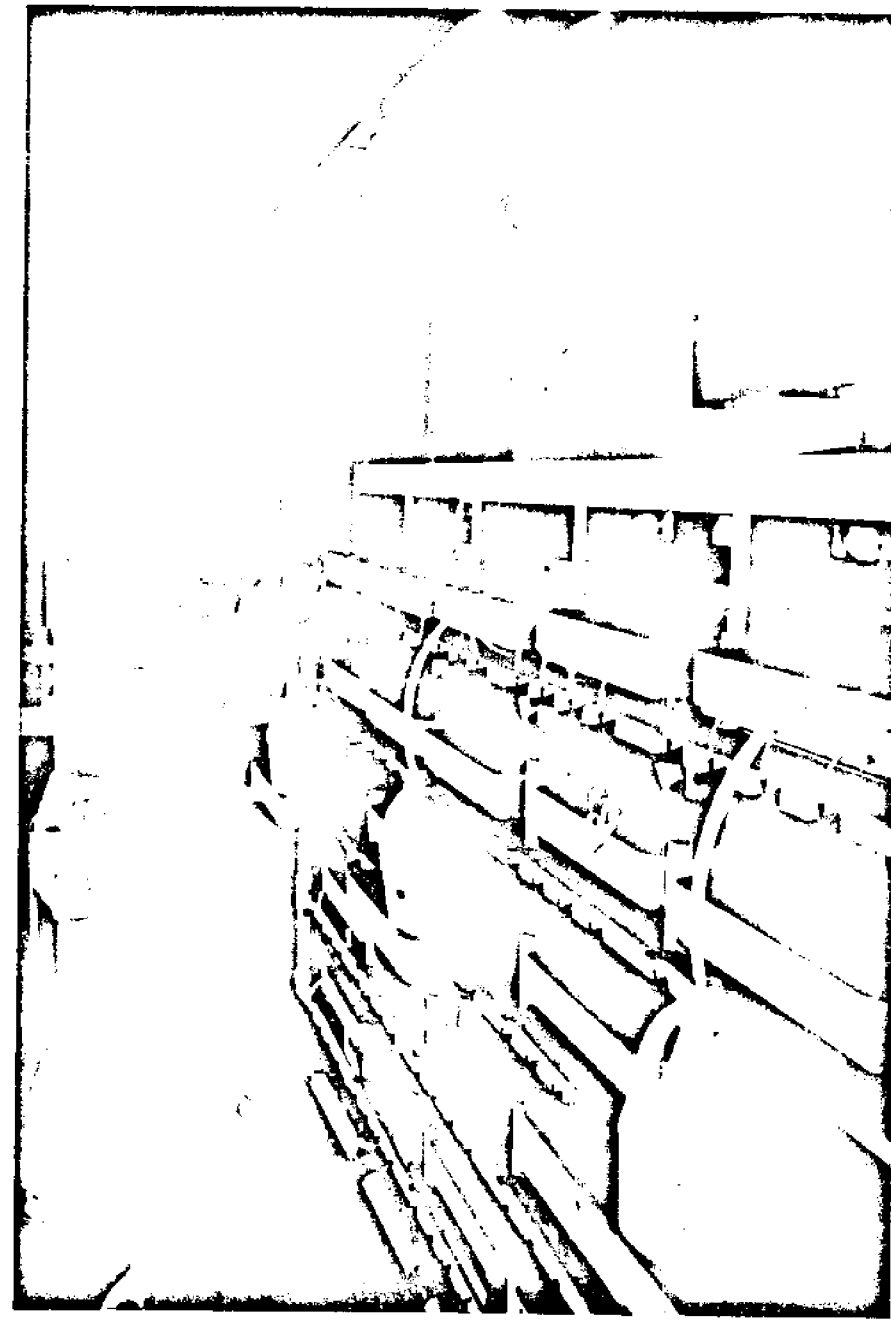


Figure 2.5-2. Rack of shellfish tanks for experimental feeding studies with juvenile pelecypod shellfish. This unit can accommodate up to 120 small batches of oysters or clams for feeding studies at different animal densities, flow rates, and various mixed diets of algae grown in the elevated outdoor tanks ("reactors") and in the large pools. The large tanks at the top of the mixing chambers are constant head devices for feeding different mixed diets to the various shellfish tanks in the rack. Dr. Tom Dorsey is shown collecting three samples of shellfish tank effluent for analysis of nitrogen utilization by the shellfish.



which supply experimental diets to six separate, in-line cells. Each cell contains a drain tray in which up to four animal containers can be placed. An airline accompanies the feed line at each level and provides aeration for the four containers. This arrangement has the capacity for 120 separate test treatments.

The rack is situated under the plumbing of the ten reactors, and any five feed lines can be tapped simultaneously. Algae culture is fed into the shellfish containers through small-diameter capillary tubing (.04" bore). The head is provided by the reactor, or by overhead mixing containers which are also constant-head devices, and flow rates are regulated by adjusting the length of the tubing or its diameter. Each vertical bay of five cells has a common drain to waste.

3 CHEMISTRY

3.1 Weekly Determination of Deep-Water Quality

As in previous years, weekly samples of deep water were taken from the three deep-water pipelines individually. This procedure provides a rapid and relatively inexpensive method of checking pipeline integrity in the event of suspected breakage, because the distribution of nutrients and salinity with depth has been established for the water column. Monitoring of water quality also provides a baseline for nutrient uptake measurements and allows us to evaluate nutrient flux through the system. The parameters investigated weekly include dissolved inorganic nutrients which are determined colorimetrically on a Technicon AutoAnalyser II system, and salinity, which is determined titrimetrically. The data presented below are the mean ($\bar{X}$), standard deviation (S_x), standard error of the mean ($S_{\bar{x}}$), and the range of nutrient values tabulated for the period November 14, 1974 to October 23, 1975. See Appendix C for detailed nutrient and salinity data for the deep-water pipelines.

	SALIN (°/oo)	NO_3+NO_2 (µg-at N/l)	NO_2 (µg-at N/l)	NH_3 (µg-at N/l)	PO_4 (µg-at P/l)	SiO_4 (µg-at Si/l)
$\bar{X}$	34.920	31.27	.18	.82	1.91	19.82
S_x	.2457	1.16	.06	.46	.12	2.59
$S_{\bar{x}}$	.014	.07	.003	.03	.007	.15
Range	34.523-35.151	28.63-33.02	.08-.40	.18-2,43	1.38-2.14	13.95-26.63

3.2 Development of a Direct Method for the Determination of Algal and Shellfish Protein

Since the St. Croix mariculture system is rapidly approaching commercial-feasibility testing, it was necessary to devise a rapid and quantitative method of evaluating the productivity of the system. Continuous monitoring of the fate of nitrogen through the trophic levels was previously limited by chemical methodology, because the particulate nitrogen analyses performed at the Palisades, N.Y. laboratory were extremely time-consuming and therefore costly, due to the large number of samples generated by the system. Dr. Thomas Dorsey has developed a new spectrophotometric method for the determination of protein-nitrogen. The technique is a modification of the Lowry et al. (1951) method which was developed for determining animal-tissue protein. The hard-to-solubilize protein of phytoplankters was a barrier to the successful use of the Lowry technique. Experimentation with various procedures has recently disclosed a rapid method of solubilizing and and measuring algal and shellfish protein in as many as 100 samples per day. A paper on this method is now ready for publication.

3.3 Sources of Error in Silicate Determinations

Samples of 870-m deep water have been found to differ by as much as 30% between replicate samples analyzed for silicate. Silicate values were obtained using a Technicon AutoAnalyzer II (AAII) system on water samples filtered at 8-in. Hg vacuum through Gelman Type A glass-fiber filters, and stored frozen in 1N HCl-washed 125-ml polyethylene sample bottles. All glassware and plastic-ware had been washed in 1N HCl including plastic AAII sample cups. The coefficient of variation at the 99.9% confidence level for ten replicate samples using this procedure is found to be 11.8% for a mean concentration of

22.42 µg-at SiO_4/l. For the same analysis technique, employing ten unfrozen 40 µg-at SiO_4/l standards in distilled water, a coefficient of variation of only .35% was found. Since reproducibility of unfrozen standards was good, sample handling and storage was investigated. Standard deviation was employed as an index of reproducibility since homogeneous samples were used throughout. Preliminary investigation discounted filters and glassware as a source of error, but the following relationships were seen to obtain:

(1) A single homogeneous sample (frozen or unfrozen storage) when placed in HCl-washed AAII plastic sample cups will experience a hyperbolic increase of standard deviation with residence time in the cups during an analysis at room temperature. The same sample placed in chromic acid-washed cups will display negligible deviation over the same residence time in the cups.

(2) Reproducibility is directly related to frozen storage time of samples, i.e., standard deviation among replicate samples increases with increasing frozen storage time, and approximates the linear relation: $Y = .145 (X) + .10$, for Y = standard deviation and X - days of frozen storage.

Samples are now analyzed for silicate immediately after sampling, and chromic acid-washed sample cups are used.

4 MICRO-ALGAE CULTURE

4.1 Selection of Algal Species for Feeding Shellfish Larvae and Juveniles

The experiments on comparisons of foods for shellfish larvae are discussed in section 5.2.2, and the work on test feeding of shellfish juveniles is discussed in sections 5.4.1, 5.4.3 and 5.4.5.

4.2 Selection of Clones of Algal Species Capable of Growth in Unsupplemented Deep Water

Twenty-four clones of micro-algae were tested for their ability to grow in unsupplemented deep water in semi-continuous laboratory cultures. Sixteen of the clones were diatoms, five were green algae, two were cryptophytes, and one was a chrysophyte. Four of the 24 cultures tested grew equally well in deep water with and without the supplement for a period of two weeks or more at reasonably high densities; their identities and origins are:

Clone	Species	Origin
STX-19	*Thalassiosira* sp.	Surface water on St. Croix reef
STX-97	*Thalassiosira pseudonana*	Surface water on St. Croix reef
STX-183	*Thalassiosira* sp.	870-m in St. Croix deep water
581	*Chaetoceros gracilis*	Costa Rica Dome (W.H. Thomas)

The cultures were diluted 50% per day with new medium, prepared daily with fresh deep water, and the cultures were transferred daily to sterile flasks to minimize growth of organisms on the walls. Control cultures received a supplement of chelated iron, trace metals, and vitamins B_1 and B_{12}.

The four clones will next be tested with and without the supplement in 550-gallon continuous-flow deep water reactors outdoors. Four reactors were constructed for this purpose during 1975, to allow replication of culture conditions during an experiment (see section 2.4, Fig. 2.4.1). If these four clones grow as well in deep water continuous-flow cultures outdoors as they did in the semi-continuous laboratory cultures, we will then have five clones suitable for deep-water mariculture. Chaetoceros curvisetus (STX-167) has been grown in 13,000-gallon continuous-flow cultures since mid-1974 at 1.2 turnover/day.

4.3 Determination of Optimum Depth for Deep-Water Cultures of Micro-Algae

Experiments during 1974 in 0.8-m deep tanks with neutral-density screens indicated an optimal depth of 4.1 m for maximum utilization of nutrients. Experiments to repeat this work in real, rather than simulated, depths were not realized because we were unable to acquire a tank of sufficient diameter to avoid excessive shading by the walls at depths greater than 2.5 meters. We have located on St. Croix a scrap tank, 9.1 m deep and 4.6 m in diameter, and we hope to acquire this tank at no cost early in 1976. This tank would give the required depth, and shading of the water column by the walls would be minimized.

4.4 Comparison of Techniques for the Estimation of Micro-Algae Culture Density: Turbidity, In Vivo Fluorescence, and Cell Counts in Unialgal Cultures

Comparisons between cell counting, in vivo fluorescence and turbidity measurements of unialgal culture density were performed on four phytoplankters used in the artificial upwelling mariculture

system (three diatoms and one flagellate). Coefficients of determination between the methods for each species indicated that in vivo fluorescence and turbidity readings provide adequate determinations of culture density, as long as the culture remains unialgal and physiologically active.

However, in cultures of mixed species or containing a significant number of dying cells, direct microscope cell counts are required.

The details of this study are given in Appendix D.

4.5 New Clones of Algae from St. Croix

One new diatom (Thalassiosira sp., STX-183) was isolated as a contaminant from an experimental deep-water culture; it is apparently a new species and is the subject of a taxonomic study in progress. This diatom is also one of four found capable of growing well in unsupplemented deep water (see section 4.2).

Six clones of green algae (Chlorella-like species, Scenedesmus sp., and Dictyosphaerium sp.) were isolated from secondarily treated wastewater cultures in the Clam Culture in Wastewater project (section 1.4.2). These may also be useful in the sewage mariculture project at Tallman Island in New York (see Part B of this report).

Several clones of the red seaweed Hypnea musciformis derived from carpospores were also isolated during the past year. One of these clones is the subject of laboratory studies on the physiology of this carrageenan-producer (see section 8.2).

4.6 Interaction of Light and Nutrients on Growth of the Diatom *Chaetoceros curvisetus* in Outdoor Continuous Culture

Miss Mary Farmer (pre-doctoral student) concluded her culture work with *Chaetoceros curvisetus* on St. Croix early in 1975, and she is in the process of interpreting the mass of data generated in her experiments. A paper (one of three to be published) on the cellular characteristics of the diatom, grown under different combinations of deep water supply rate and light intensity, is presented in manuscript form in Appendix E. An abstract of the paper follows:

ABSTRACT: Farmer, 1976.

The effects of five different light intensities and four different nutrient regimes on the cellular composition of outdoor continuous cultures of *Chaetoceros curvisetus* were examined. Cellular nitrogen and carbon increased with increasing dilution rate at all light intensities. The N:C ratio increased with increasing growth rate in nitrogen-limited but not in light-limited cultures. Chlorophyll *a* per cell, per unit nitrogen, and per unit carbon increased with increasing dilution rate and with decreasing light intensity; these ratios demonstrated the greatest degree of diel periodicity. Interaction of effects of light and nutrients was seen on all ratios involving chlorophyll, but light and nutrients appeared to influence cellular carbon and nitrogen independently of each other.

4.7 Effects of Light and Dark Cycles on the Association of Nitrate Uptake, Assimilation and Cell Growth Rates in *Chaetoceros* sp. (STX-105), a Marine Diatom Grown in Continuous Culture

Mr. Gaston Picard concluded his work with *Chaetoceros* sp.

(STX-105) and has prepared the first of several papers to be published on the results of his work. An abstract of the first of these papers follows:

ABSTRACT: Picard, 1976

The diatom Chaetoceros sp. (STX-105) was grown in a continuous culture in a 12 hr light/12 hr dark illumination cycle, with nitrate as the limiting nutrient. The following parameters were measured for five different growth rates: (1) cell concentration, (2) residual nitrate concentration, (3) cellular content of carbon, nitrogen, chlorophyll a, protein, nitrate, free amino acids and carbohydrate, (4) primary productivity and photosynthetic capacity, nitrate uptake kinetics parameters and the activity of the enzyme nitrate reductase. A diurnal variation was observed for the cellular carbon, nitrogen, protein, nitrate and carbohydrate and for the nitrate reductase activity as well as for photosynthesis. The diel variation was more pronounced at the dilution rate (87%) where the cells had to divide almost daily. The only measured parameters that increased with increasing dilution rate were the cellular chlorophyll a concentration and the nitrate reductase activity; all others decreased. The most stable population characteristics were cell concentration and cellular particulate nitrogen. Results from the rate of carbon fixation indicate that the culture limitation shifted from nutrient to light as the nitrogen limitation decreased (i.e., increasing dilution rate).

5 SHELLFISH CULTURE: PELECYPODS

Completion of the Hatchery/Wet Lab building (see section 2.5) allowed us to shift our emphasis in pelecypod shellfish culture from "species suitability testing" toward spawning clams and oysters to produce batches of juvenile animals of those species that have been found to grow well in the artificial upwelling mariculture system. The juvenile animals are used for (a) studies to determine optimum algal diet (quality and quantity) for growth; (b) quantitative experiments on nitrogen transfer in the phytoplankton-shellfish food chain; (c) raft culture of oysters in Salt River inlet (see section 1.4.1); (d) growth rate studies of filter-feeders on mixtures of deep and surface water (see section 1.4.3).

The results of our many studies to date, from spawning adult animals to growing larvae and juveniles of several species of oysters and clams, are given in the sections which follow.

5.1 Brood Stock Inventory

A list of the species of adult animals maintained as brood stock for the hatchery work is shown in Table 5.1

5.2 Hatchery Studies

5.2.1 Experimental Spawnings

Four species of shellfish were induced to spawn in the hatchery using thermal (23-32°C) and chemical (stripped gonad solution--eggs and/or sperm) stimulation: Tapes semidecussata, Ostrea edulis, and Crassostrea gigas were reared through metamorphosis to produce juveniles. The Kumamoto strain of C. gigas did not metamorphose. We induced male mangrove oysters (Crassostrea rhizophorae)

TABLE 5.1. INVENTORY OF OYSTER AND CLAM BROOD STOCK IN THE ST. CROIX MARICULTURE SYSTEM

SPECIES	NUMBER OF ANIMALS	OBTAINED FROM (DATE)
Tapes semidecussata	890	*Tapes*(3) spawning (9/7/74)
Mercenaria mercenaria	10	VIMS-E as adults (11/73) (P. Chanley)
M. campechiensis	270	Juveniles in comparative study (4/73) (P. Chanley)
F_1 clams (*M. campechiensis* x *M. mercenaria*)	108	-- as above --
Crassostrea gigas	10	Pacific Mariculture Inc. as juveniles (4/72)
Crassostrea gigas	ca. 100	1st year class from P. Chanley (rec'd 10/75)
Ostrea edulis	4	Pacific Mariculture Inc. as juveniles (8/73)
Ostrea edulis	ca. 150	Maine Coast Oyster Co. as adults (6/75)
Kumamoto variety of *C. gigas*	1,400	Bay Center Mariculture Co. as spat (7/74)

to spawn but no female oysters spawned. We also attempted to spawn two clam species, with no success: Mercenaria campechiensis and F_1 clams (M. mercenaria ♂ x M. campechiensis ♀).

Our record for successful and unsuccessful spawnings during 1975 is shown in Figure 5.2. Table 5.2 shows the percent survival of batches of clam and oyster larvae to metamorphosis.

5.2.2 Comparison of Algal Foods for Larvae

Feeding studies on the clam, Tapes semidecussata and the oysters, Ostrea edulis, Crassostrea gigas, and the Kumamoto variety of C. gigas were conducted during 1975. The studies were designed to compare different mixtures of diatoms and flagellates as larval foods. Emphasis was placed on testing algae which can be grown outdoors in the tropical environment.

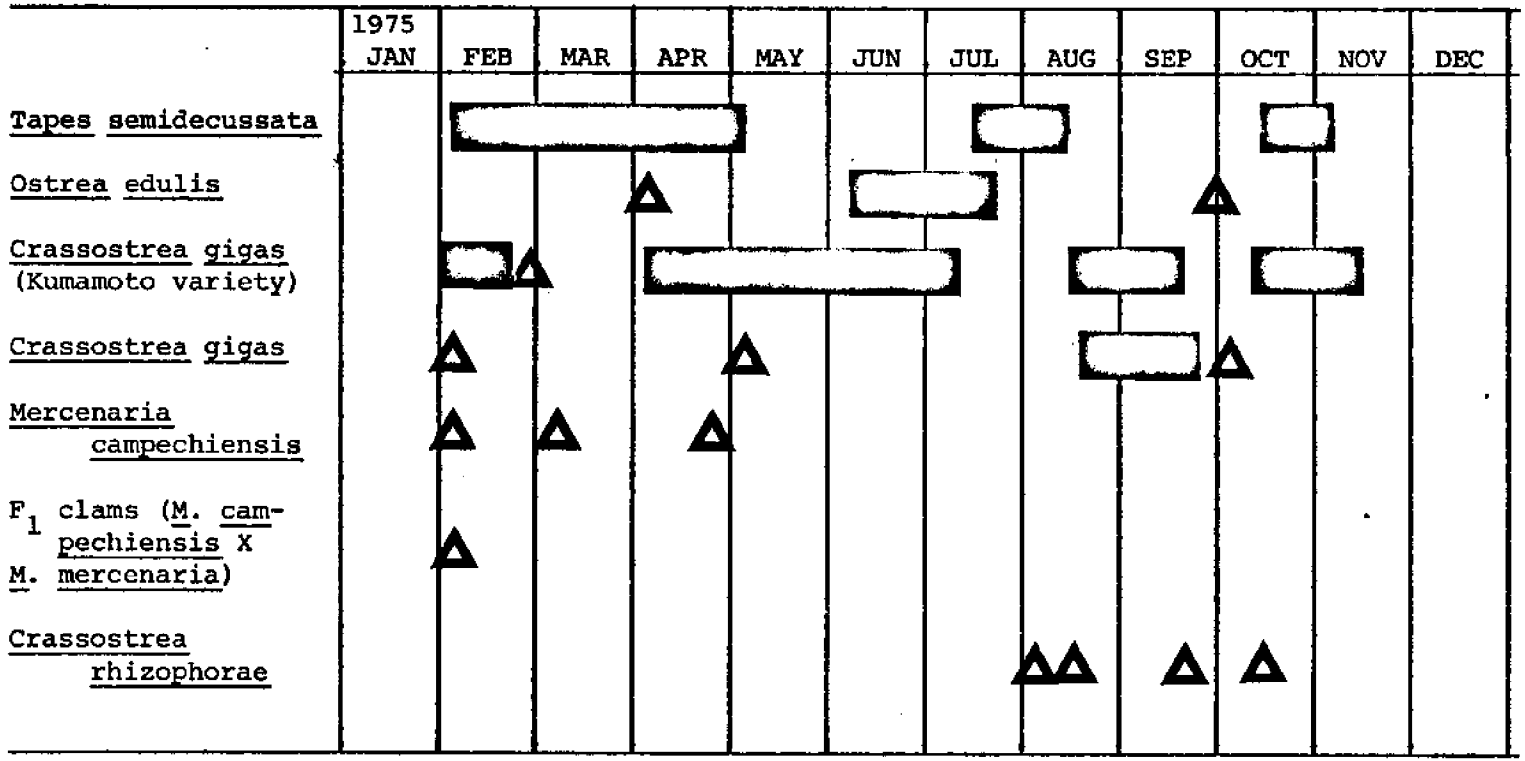
An abstract of a paper on these studies follows; the complete manuscript is given in Appendix B.

ABSTRACT: Sunderlin, Baab and Patry, 1975.

Growth of clam and oyster larvae on different algal diets in a tropical artificial upwelling mariculture system. (Submitted to Proceedings of World Mariculture Soc., January 1976, San Diego)

Feeding studies were conducted on the larvae of Tapes semidecussata Reeve, Kumamoto oysters, Ostrea edulis Linné, and Crassostrea gigas Thunberg, to evaluate different species of algae and their influence on larval growth and survival. The phytoplankton used in the studies were: Isochrysis galbana, Monochrysis lutheri, Bellerochea polymorpha, Thalassiosira pseudonana, and two unknown Cryptophyte flagellates (STX-157 and S-1). Larvae were obtained from the spawning of adult brood stock in the controlled environment of the artificial upwelling

Figure 5.2. Pelecypod shellfish hatchery--milestone chart.



spawning induced and larval rearing experiments underway.

unsuccessful at inducing spawning.

TABLE 5.2. BATCHES OF CLAM AND OYSTER LARVAE REARED IN THE SHELLFISH HATCHERY DURING 1975

SPECIES, NUMBER BATCHES AND SPAWN DATE		% SURVIVAL TO METAMORPHOSIS
Tapes semidecussata -- Five (5) Batches:		
February 4, 1975	Tapes(5)*	41.1%
March 5, 1975	Tapes(6)	44
April 5, 1975	Tapes(7)	62.7
July 16, 1975	Tapes(9)	34
October 16, 1975	Tapes(10)	36.3
Ostrea edulis -- Three (3) Batches:		
June 23-25, 1975	O. edulis(2)*	<1%
June 26, 1975	O. edulis(3)	<1
June 30/July 1/75	O. edulis(4)	<1
Kumamoto oysters -- Six (6) Batches:		
April 4, 1975	Kuma(2)*	No metamorphosis and low survival to setting size
April 22, 1975	Kuma(3)	
May 15, 1975	Kuma(4)	
June 3, 1975	Kuma(5)	
August 18, 1975	Kuma(6)	
October 23, 1975	Kuma(7)	Experiment still underway; larvae not yet reached setting size
Crassostrea gigas -- One (1) Batch:		
August 26, 1975	C. gigas(1)	42%

*Number in parentheses indicates batch number which is assigned chronologically to each batch of animals spawned at the St. Croix artificial upwelling mariculture hatchery.

mariculture system on St. Croix, U.S. Virgin Islands. Salinity was 34.75 to 34.95 °/oo and the water temperature varied between 23 and 28 C during the experiments. Tapes semidecussata larvae could be successfully reared through metamorphosis on algal diets suited for growth in the tropical mariculture system on St. Croix. Mixtures of algal diets were better for growth and survival of Kumamoto larvae than unialgal diets. No significant information could be obtained from the Ostrea edulis study due to poor survival of larval cultures which may have been caused by high temperatures. Crassostrea gigas larvae grew well on a mixed diet and survival was significantly higher than that for the larvae of the Kumamoto variety.

5.3 Growth of Juvenile Oysters

5.3.1 Rearing of the Oysters Ostrea edulis and Crassostrea gigas Spawned in the St. Croix Hatchery

Ostrea edulis Linné adults were given temperature shocks (24-32°C) on June 17, 1975. Six to eight days later, swarming larvae were collected from these brood stock. The larvae began to set 15 days after swarming and metamorphosed on cultch (oyster and scallop shells). Growth (length in mm) of these spat was monitored (Fig. 5.3.1-1) and post-set survival after 18 weeks is 61%. If the spat continue to grow at the present rate, market size oysters should be obtained in another 8 to 9 weeks, for a total of seven months from fertilized eggs to marketable oysters.

Crassostrea gigas Thunberg brood stock were induced to spawn on August 26, 1975. On Day 22, setting began; the C. gigas attached to a variety of substrates--cement-coated plywood boards, mylar drafting film, epoxy-coated boards and oyster, clam and scallop shells. The

Figure 5.3.1-1. Growth of Ostrea edulis(2) spat.

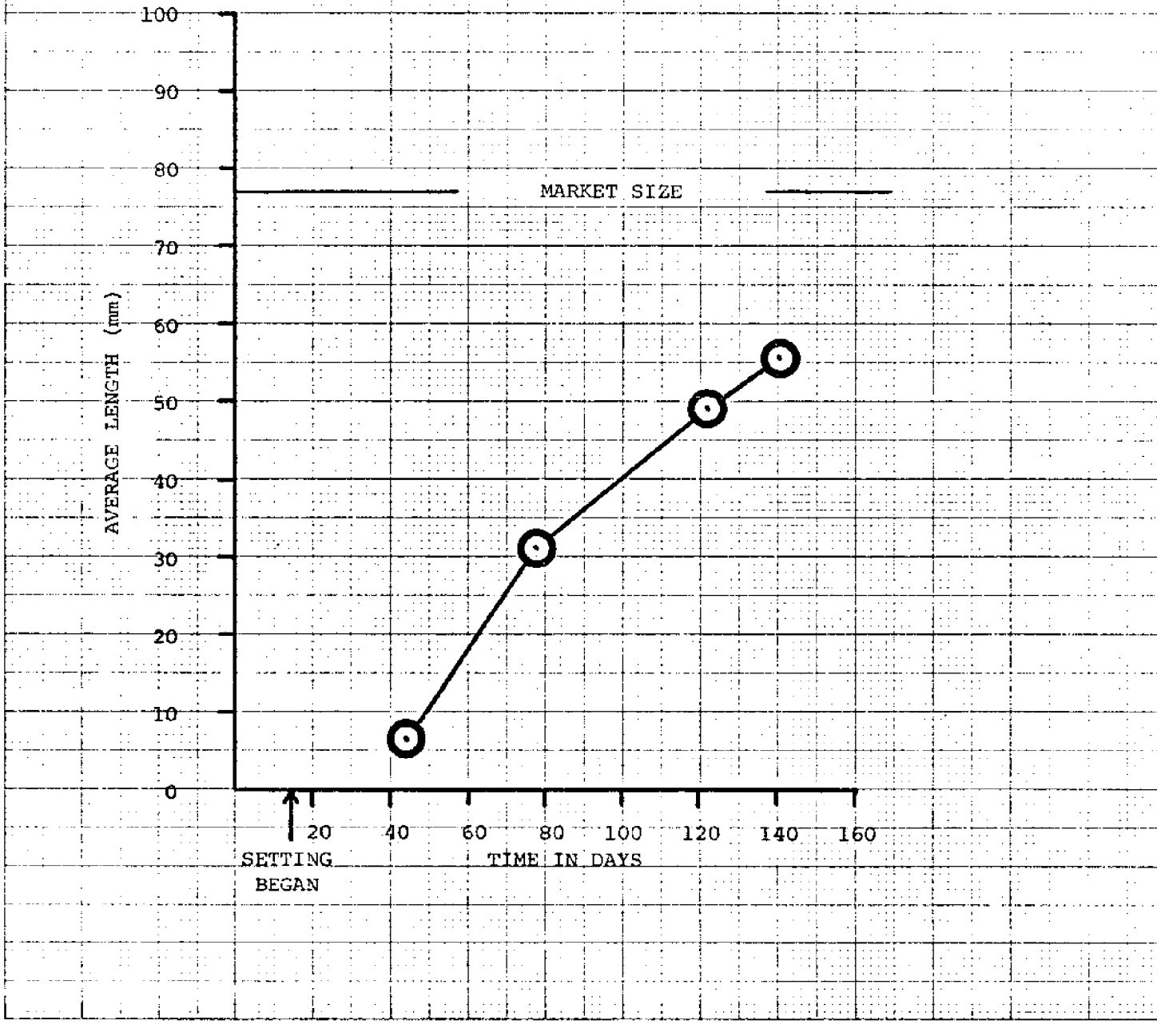
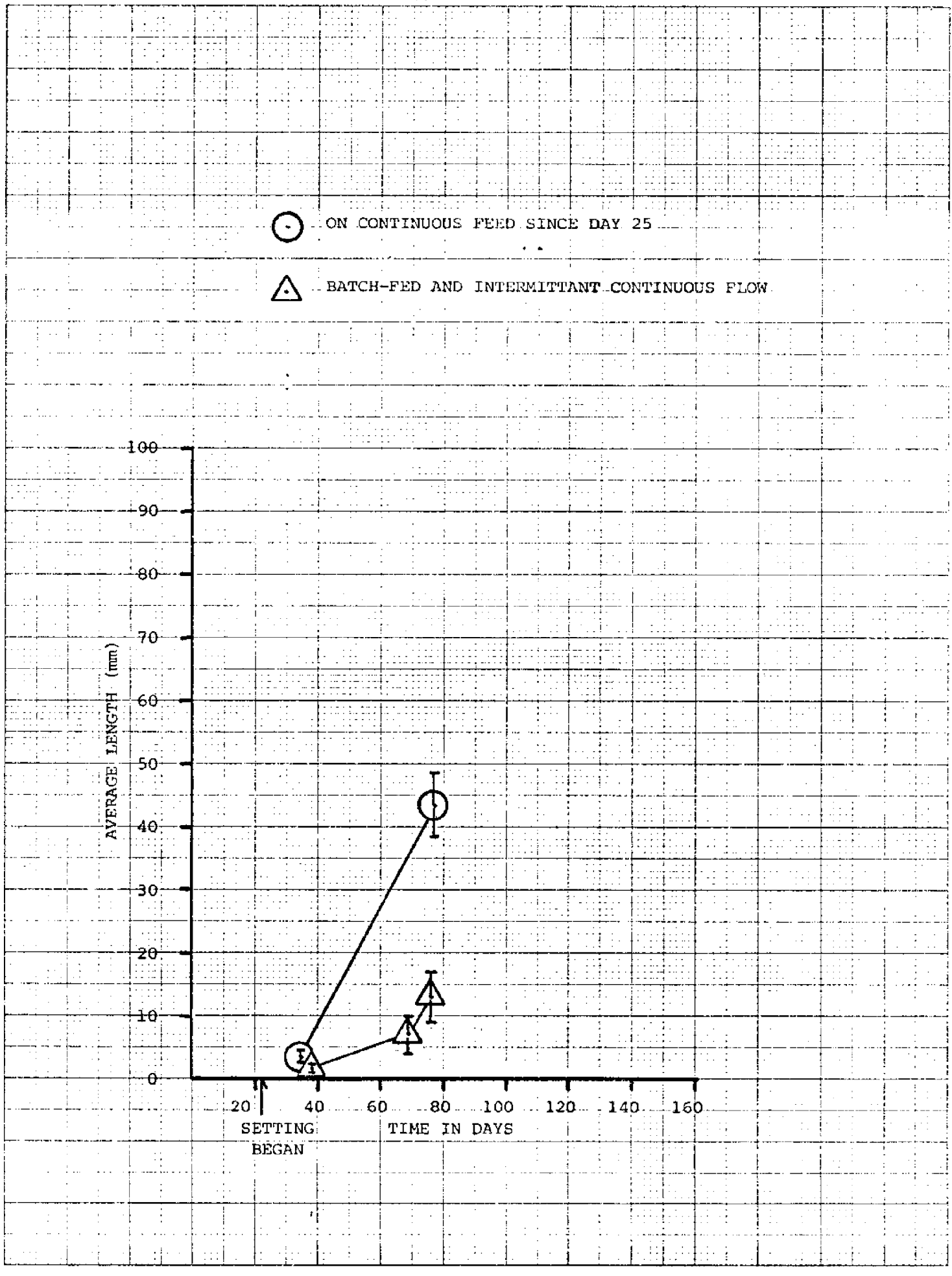

100
90
80
70
60
50
40
30
20
10
0
AVERAGE LENGTH (mm)
MARKET SIZE
20
40
60
80
100
120
140
160
SETTING BEGAN
TIME IN DAYS

Figure 5.3.1-2. Growth of Crassostrea gigas(1) spat.


ON CONTINUOUS FEED SINCE DAY 25
BATCH-FED AND INTERMITTANT CONTINUOUS FLOW
AVERAGE LENGTH (mm)
100
90
80
70
60
50
40
30
20
10
0
20
40
60
80
100
120
140
160
SETTING BEGAN
TIME IN DAYS

heaviest concentration of spat was observed in regions of violent aeration. Some of the spat were placed on continuous feed on Day 25; the others were batch-fed or given intermittant continuous food. The growth of the latter batch was undoubtedly limited from time to time by a low concentration of food in the tank. Figure 5.3.1-2 compares the growth of these two batches of C. gigas spat. Subsequent batches of oyster spat will be involved in strict feeding studies to determine the most efficient way to grow the spat to the 5-10 mm size which can be transplanted to Salt River for raft culture studies.

5.3.2 Successful Growth of the Japanese Pearl Oyster (Pinctada mertensi) Dunker in the St. Croix Mariculture System

The Japanese pearl oyster, Pinctada mertensi, was introduced into the St. Croix system on March 6, 1975 to test the following objectives:

(1) To determine the adaptability of the Japanese pearl oyster in a tropical mariculture environment.

(2) If adaptable, to accelerate natural growth rates using specialized algal diets to decrease the time-frame for insertion of the pearl nucleus and to reduce the overall growing time for marketable pearls.

(3) To compare growth of Pinctada mertensi in St. Croix with its growth in Japan.

The Pinctada mertensi were flown from Tokyo to St. Croix in less than 60 hrs. Damp, cold (ca. 10°C) sawdust was used as the packing medium and the pearl oysters arrived in excellent condition. It is difficult to ship P. mertensi because shortly after being taken out of the water, shells gape and the muscle fails to contract. Taiyo

Fisheries Co. ran several experiments on the best method for shipment and damp, cold sawdust proved to be the best method.

When the P. mertensi arrived in St. Croix, the temperature was 14°C in the sawdust packing. The oysters were removed from the boxes, rinsed and placed in 14°C water containing diatoms for food. The water in the oyster containers warmed up to ambient (ca. 24°C) in only a few hours and only 20 oysters had died during shipment (less than 1% mortality). Initially, 2,141 oysters were received, and as of October 22, 1975 a total of 1,834 oysters were still growing in the system. The cumulative mortality is very low (13.4%). The pearl oysters were divided into two populations -- small size and large size. From March 6, 1975 through September 24, 1975, the pearl oysters were grown in stacked Nestier trays in a 2.4 x 0.6 x 0.6 m shellfish tank. Three stacks of four Nestier trays each were placed in the 750-l shellfish tank. After growth measurements were taken on September 24, the pearl oysters were redistributed into half the number of Nestier trays and placed in mesh baskets (3.4 x 3.4 x 2.0 cm). (Note: these mesh baskets were sent with the pearl oysters from Japan). This redistribution resulted in reduction of the density of the oysters and improvement in the circulation in the tank.

At monthly intervals, growth ("height" in mm, dorsoventral measurement in mm, and weight in grams) and survival were monitored separately for the small-size and large-size populations. Figures 5.3.2-1,2,3 represent the growth of the pearl oysters from March through October 1975.

Taiyo Fisheries Co. informs us that when the Pinctada reach average weights of 28-31 g, they are ready for the nuclear insertion operation. By extrapolating the slope of the line in the graph depic-

Figure 5.3.2-1. Growth of the Japanese pearl oyster, Pinctada mertensi, in the St. Croix artificial upwelling mariculture system. [·] small size population; (·) large size population.

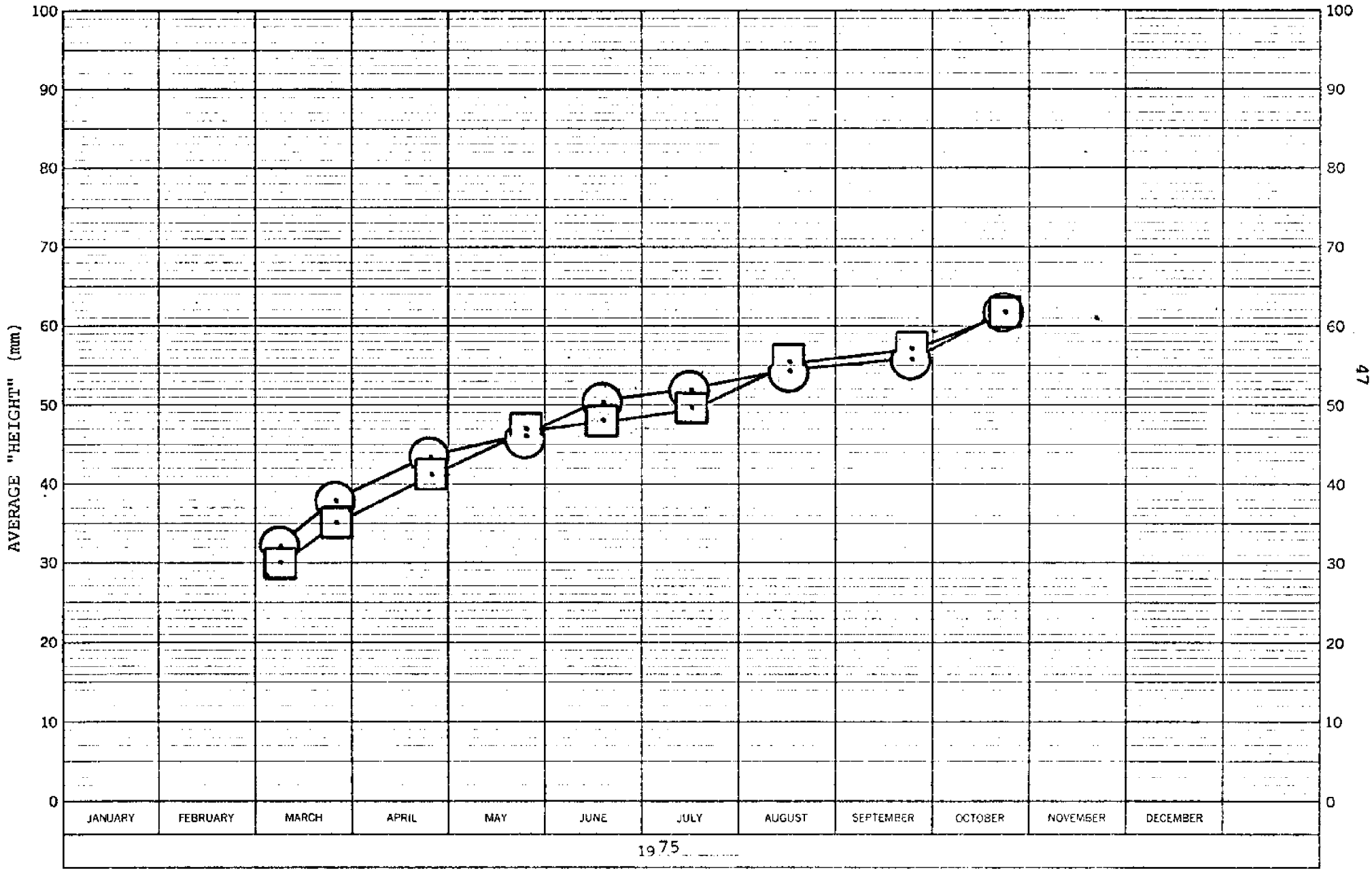
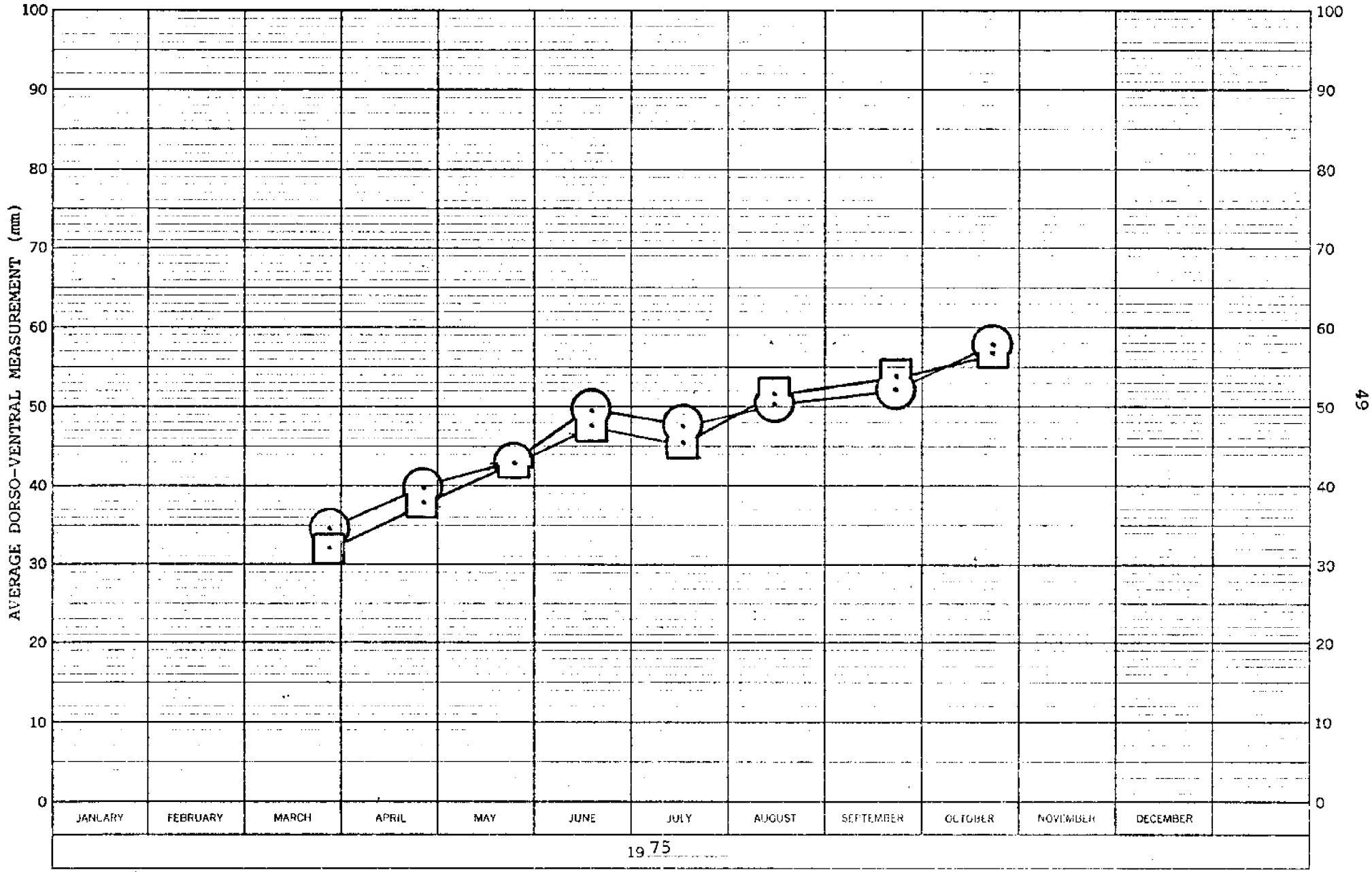

AVERAGE "HEIGHT" (mm)
100
90
80
70
60
50
40
30
20
10
0
JANUARY
FEBRUARY
MARCH
APRIL
MAY
JUNE
JULY
AUGUST
SEPTEMBER
OCTOBER
NOVEMBER
DECEMBER
1975

Figure 5.3.2-2. Growth of the Japanese pearl oyster, Pinctada mertensi, in the St. Croix artificial upwelling mariculture system. ⊡ small size population; ⊙ large size population.


AVERAGE DORSO-VENTRAL MEASUREMENT (mm)
100
90
80
70
60
50
40
30
20
10
0
JANUARY
FEBRUARY
MARCH
APRIL
MAY
JUNE
JULY
AUGUST
SEPTEMBER
OCTOBER
NOVEMBER
DECEMBER
1975

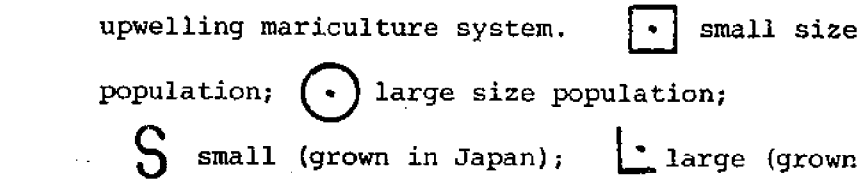
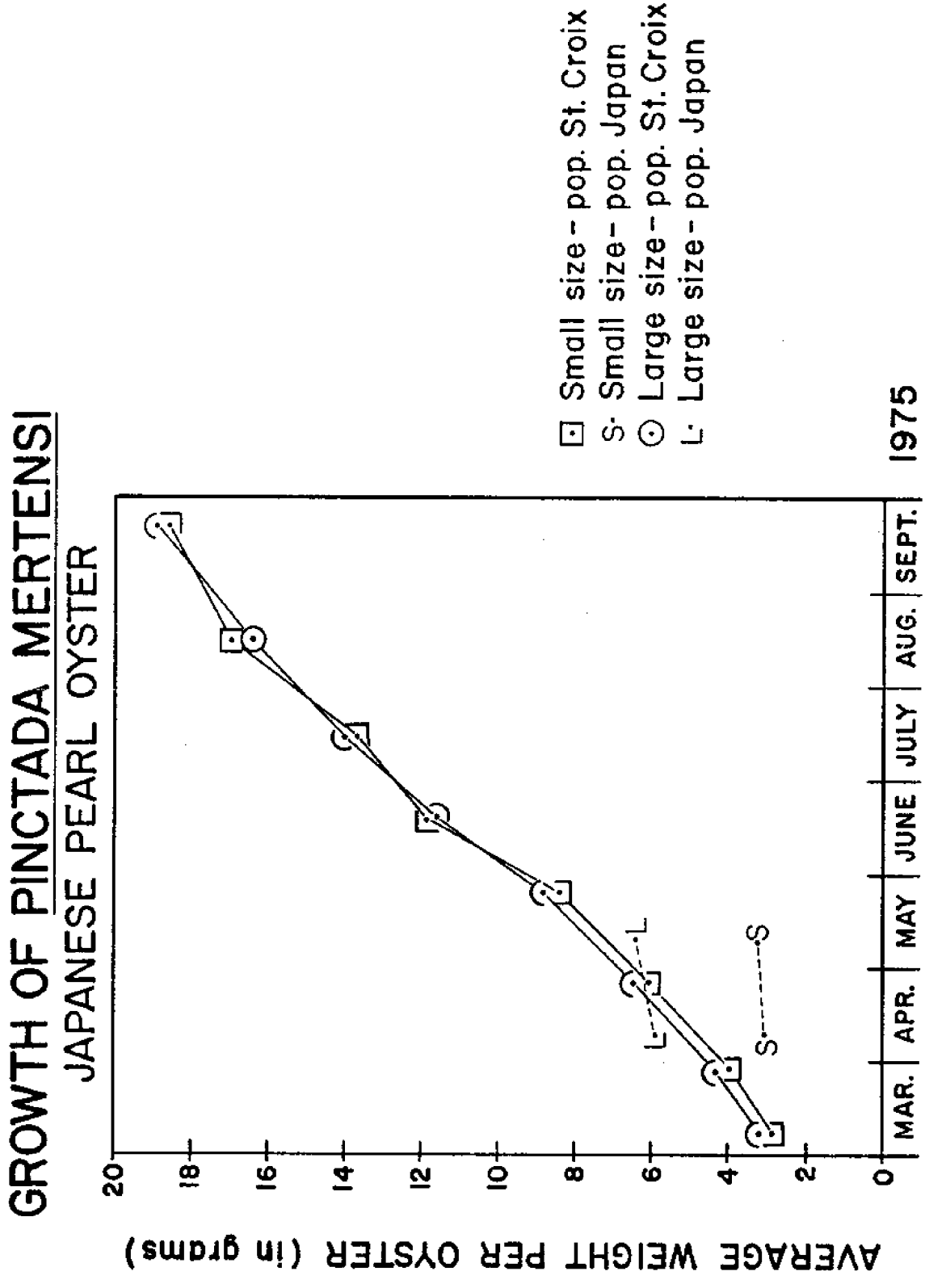


Figure 5.3.2-3. Growth of the Japanese pearl oyster, Pinctada mertensi, in the St. Croix artificial upwelling mariculture system. small size population; large size population; S small (grown in Japan); large (grown in Japan).


GROWTH OF PINCTADA MERTENSI
JAPANESE PEARL OYSTER
AVERAGE WEIGHT PER OYSTER (in grams)
0
2
4
6
8
10
12
14
16
18
20
MAR.
APR.
MAY
JUNE
JULY
AUG.
SEPT.
1975
L
S
S
L
Small size - pop. St. Croix
Small size - pop. Japan
Large size - pop. St. Croix
Large size - pop. Japan

ting average weight per oyster (Fig. 5.3.2-3), the Pinctada are projected to reach 28-31 g around late December 1975, or early January 1976. At this time, Taiyo Fisheries will send an expert from Japan to perform the nuclear insertion.

The excellent growth and survival of Pinctada mertensi in the St. Croix system indicates the favorable adaptation of this species for growth in a tropical mariculture operation. Figure 5.3.2-3 also gives an indication of the comparative growth rate of P. mertensi in Japan.

5.3.3 Growth of Kumamoto Oysters in the St. Croix Mariculture System

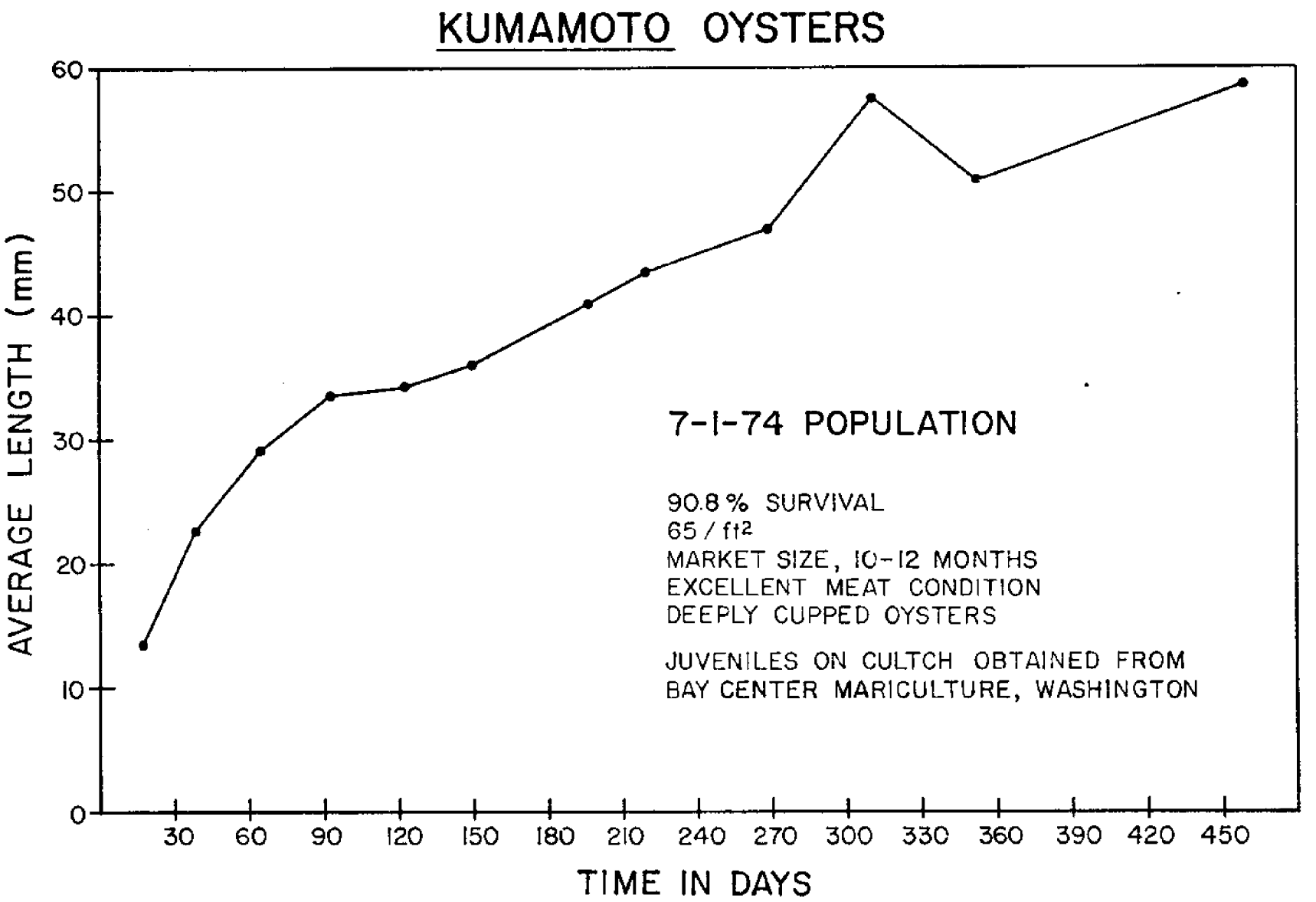
The Kumamoto is a "gigas-type" of oyster but it does not reach the large size of the west coast Japanese oyster, Crassostrea gigas. It is valued for the half-shell trade on the west coast. The growth rate, survival, percentage meat, density per square foot, and taste of the Kumamoto oyster were evaluated in the St. Croix system. When compared with six other shellfish species grown in St. Croix, the Kumamoto is rated as the best (Table 5.3.3). Kumamotos are very plump deeply cupped oysters that seem to be ripe all the time; they spawn readily but to-date larvae have never metamorphosed. Five batches of larvae have reached setting size (ca. 300μ) but have never set (Appendix C). A sixth batch is presently being reared in the hatchery using the same technique--airlift system in a 379-liter polyethylene tank--successfully used for C. gigas larvae.

On July 1, 1974, 2,000 to 3,000 Kumamoto spat (on cultch) were obtained from Bay Center Mariculture Company, Willapa Bay, Washington. At monthly intervals, growth (increase in length and weight) and survival were monitored. Figure 5.3.3 shows average length (mm) from

TABLE 5.3.3. ECONOMIC COMPARISON OF SEVEN PELECYPOD MOLLUSCS FOR ARTIFICAIL UPWELLING MARICULTURE

PELECYPOD MOLLUSCS	TAPES SEMIDECUSSATA (Japanese Little Neck Clam)	MERCENARIA CAMPECHIENSIS (Southern Clam or Quahog)	F_1 CLAMS (cross of M. CAMPECHIENSIS and M. MERCENARIA)	CRASSOSTREA GIGAS (Pacific Oyster)	OSTREA EDULIS (European oyster)	ARGOPECTEN IRRADIANS (Bay Scallop)	KUMAMOTO Oysters (C. gigas variety)
MARKET SIZE	>38 mm in length	>25.5 mm thick	>25.5 mm thick	>30 g in weight	>76.5 mm in length	>50.5 mm in height	50-65 mm length
MONTHS TO REACH MARKET SIZE	7-12	6.5-13	6.5-13	12-16	12-16	7-10	10-12
DENSITY PER SQ.FT. (AT MARKET SIZE)	120	50	50	15	9	25	65
PERCENTAGE SURVIVAL	64	48	42	67	70	73	90.8
UNIFORMITY OF SIZE	+	+	-	+	+	+	+
PERCENTAGE MEAT (SHUCKED WET WEIGHT)	39	28	31	39	22	11 (muscle only)	36
CALCULATED PRODUCTIVITY PER YEAR (LBS. MEAT HARVESTED/SQ.FT)	3.02	2.19	2.66	2.09	1.54	0.44	5.43
RELATIVE GROSS VALUE	56	40	49	39	28	8	100

Figure 5.3.3. Kumamoto oyster growth. Average length in mm from July 1974 through October 1975.


KUMAMOTO OYSTERS
AVERAGE LENGTH (mm)
60
50
40
30
20
10
0
30
60
90
120
150
180
210
240
270
300
330
360
390
420
450
TIME IN DAYS
7-1-74 POPULATION
90.8 % SURVIVAL
65 / ft²
MARKET SIZE, 10-12 MONTHS
EXCELLENT MEAT CONDITION
DEEPLY CUPPED OYSTERS
JUVENILES ON CULTCH OBTAINED FROM
BAY CENTER MARICULTURE, WASHINGTON

July 1974 until October 1975. The oysters reached market size (50-65 mm) in May and June 1975 or 10-12 months after introduction into the St. Croix system. In June, the oysters were removed from their cultch to facilitate measuring and counting procedures. The Kumamoto oysters were grown in Nestier trays in a fiberglas flume (366 x 61 x 16.5 cm) with a turnover period of approximately 25 minutes when both algal pools were active. Market size oysters were grown at densities of 65/ft^2 and survival was 90.8% from introduction until market size was reached.

5.4 Growth of Tapes semidecussata

During 1975 we concentrated on the clam Tapes semidecussata because it appeared to have the greatest economic potential in our system. The following sections give the details on various studies involving this clam.

5.4.1 Experimental Cultivation of Tapes semidecussata During Long- and Short-Term Feeding Studies

The results of our first trial with this species and an intensive study of the value of seven different algal diets are given in a paper prepared for publication. An abstract of the paper follows; the manuscript is given in Appendix F.

ABSTRACT: Rodde, Sunderlin and Roels, 1975

The experimental cultivation of Tapes semidecussata (Reeve) in an artificial upwelling mariculture system.

The growth and reproductive characteristics of Tapes semidecussata were investigated during long- and short-term feeding experiments in an artificial upwelling mariculture system. The long-term study involved the rearing of three successive generations of Tapes on combinations of three planktonic diatoms: Chaetoceros curvisetus, Bellerochea polymorpha and Thalassiosira

pseudonana. Marketable adults (38 mm) were obtained in twelve months from post-set juveniles, and in 10 months from the 5 mm stage. Animal densities at 38 mm were 1775 and 1345 clams/m^2 for first and second generations, with survival rates of 64 and 63 percent, respectively. Ripe gonads found in adult clams during much of the year indicated excellent adaptation to the system by Tapes. Spawning was readily induced in the hatchery and larvae metamorphosed in three weeks. Post-set survival rates of 52 and 84.5 percent were recorded. The short-term feeding study used 6 mm juvenile clams and tested the Chaetoceros and Bellerochea sp. diatoms and an unidentified Cryptophyte flagellate, designated S-1, in seven different mono- and mixed algal diets. The monoculture diets produced the slowest growth in clams after five weeks, yielding a 6.6-fold average increase in wet-weight meat and an average length of 15 mm. The four mixed diets produced similar individual gains in weight, and gave an 11.2-fold average increase in wet-weight meat as a group. The Chaetoceros + S-1 diet showed the best algae-to-meat conversion ratio, however, and was therefore the most effective diet. Clams fed this mixture averaged 16.7 mm in length after five weeks.

5.4.2 The Effect of Substrate on the Growth Rate of Tapes semidecussata

A six-week study was conducted to determine if substrate has an important impact on growth rate of Tapes. Two different size groups of clams, 10 mm and 14 mm, were evenly distributed between both Nestier trays containing sand (1≤3 mm), and trays containing a screen liner. The trays were placed in a 400-liter tank

and received an inflow of diatom culture from pools 1 and 2 at a combined rate of 14.4 liters per minute. This gave a culture residence time of approximately 30 minutes in the tank.

Length and weight measurements were taken every two weeks from each tray in both categories: no significant differences resulted in growth between substrate and non-substrate trays. Therefore, from a growth standpoint alone, there is no advantage in raising Tapes in a substrate.

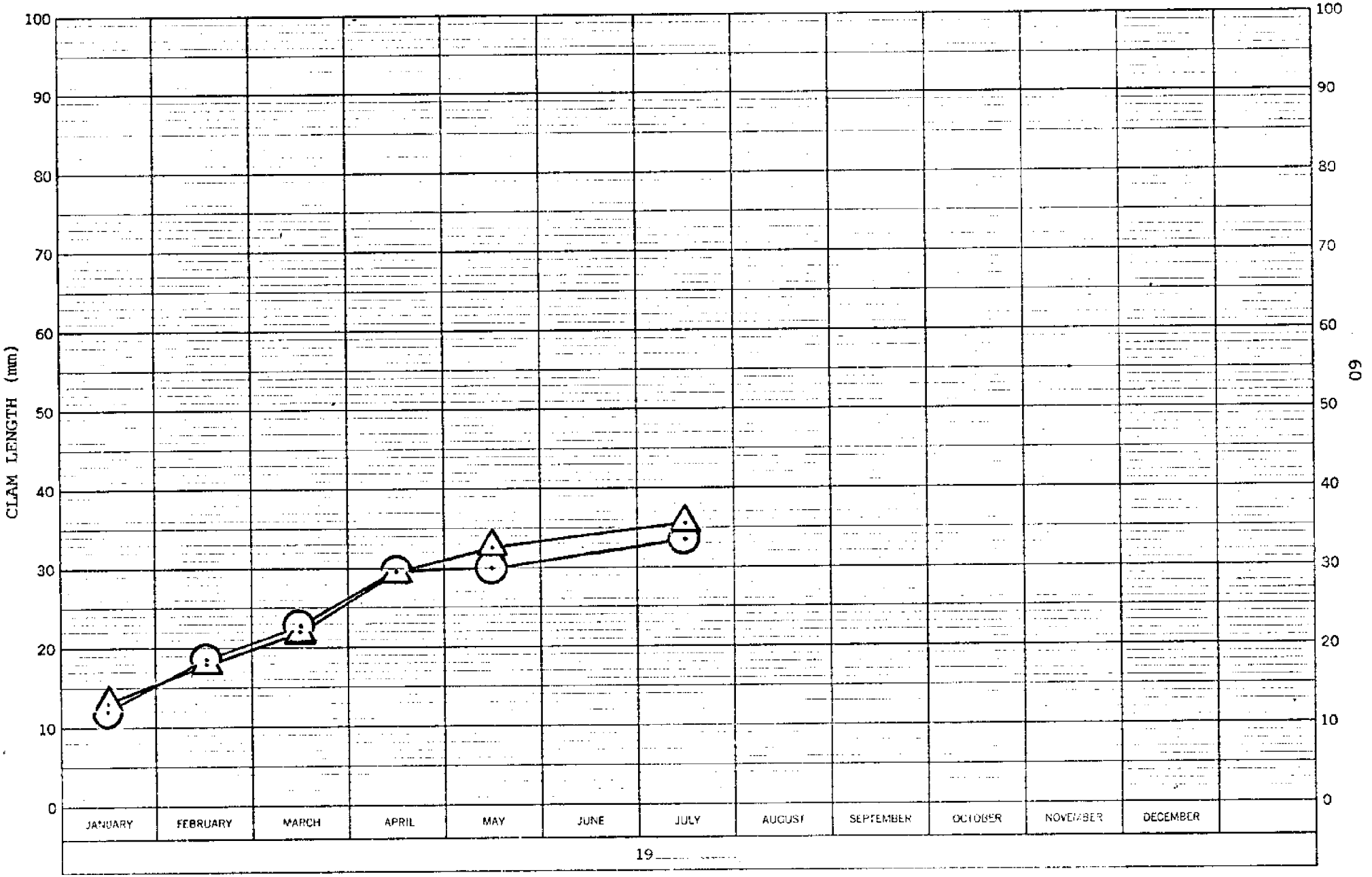
At the end of the experiment, the Tapes were moved to a new location, but the original substrate/non-substrate populations were retained. Since little difference in size between the two groups (10 and 14 mm) was sustained, growth subsequent to March 5, 1975 was averaged; this is shown in Figure 5.4.2. For convenience in presentation, growth in the two size groups is averaged from the beginning of the experiment. The graph shows no significant departures in growth between substrate and non-substrate trays through July 1975.

Details of this experiment are given in Appendix G.

5.4.3 The Effect of Different Algal Diets on the Growth Rate and Gonad Development of Juvenile Tapes semidecussata Under Different Lighting Conditions

This feeding study is the second in a series of growth experiments designed to optimize algal diets for Tapes (see 5.4.1). The purpose of the experiment was to evaluate clone 581 (Chaetoceros sp.) as a potential food (a) as a monoculture to determine its dominant nutritional characteristics as reflected in Tapes growth rates, meat-to-shell ratio, etc., and (b) determine its effect as an ingredient in an algal mixture, i.e., complementary or inhibitory to growth in relation to other diets. In addition, the

Figure 5.4.2 The effect of substrate on the growth rate of Tapes semidecussata. ⊙ substrate; △ screen liner.


CLAM LENGTH (mm)
100
90
80
70
60
50
40
30
20
10
0
JANUARY
FEBRUARY
MARCH
APRIL
MAY
JUNE
JULY
AUGUST
SEPTEMBER
OCTOBER
NOVEMBER
DECEMBER
19

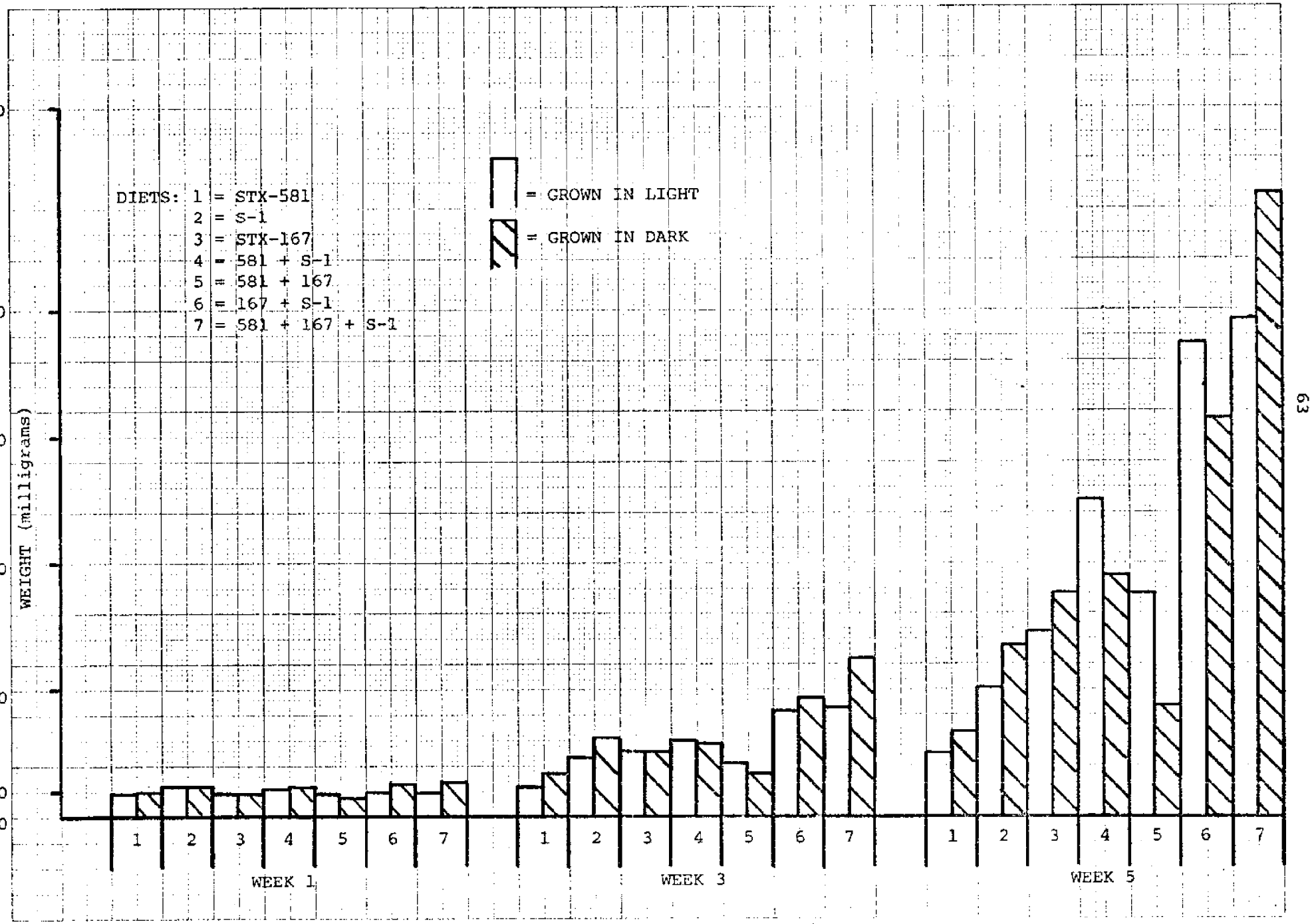
effects of all diets are to be investigated histologically, to determine and/or confirm the suspected impact of a diet on somatic and gonadal development.

Monocultures of three algae, the Cryptophyte flagellate S-1 and two Chaetoceros species, STX-167 (C. curvisetus) and clone 581 (C. gracilis) were cultivated in 550-gallon concrete reactors. Deep water inflow was adjusted for a turnover rate of one volume per day. Each culture was fed into a manifold of five Lucite air-valves adapted for salt-water flow. Supply lines from each manifold fed monocultures at a rate of 2 ml/sec to duplicate 1.5-liter glass trays containing 250 3-mm Tapes, and also to a 2-liter mixing container at a rate of 2.5 ml/sec. The overflow in the mixing containers insured a constant pressure and therefore uniform flow at 2 ml/sec into the animal containers.

Culture outflow and animal tray effluent samples were collected every three days to monitor cell stripping rates and possible preferential selection of algae. The clams were weighed and measured each week and each tray sampled for a histological inspection of the gonad. Particulate nitrogen levels were determined on the clams at the beginning and end of the experiment.

The study has recently terminated but the data have not been analyzed completely, nor has the histological analysis been finished. However, an illustration of the comparative growth rates of Tapes juveniles ($\bar{L}$=3 mm) is presented in Figure 5.4.3. Generally, clone 581 does not appear to be a satisfactory diet ingredient. As a monoculture, it produced extremely slow growth throughout the experiment, and it appears to have had a questionable influence as a component in the three-algae mixture. This can be seen by comparing growth

Figure 5.4.3 Average live weights of Tapes semidecussata grown on different diets in light and dark conditions.


DIETS: 1 = STX-581
2 = S-1
3 = STX-167
4 = 581 + S-1
5 = 581 + 167
6 = 167 + S-1
7 = 581 + 167 + S-1
= GROWN IN LIGHT
= GROWN IN DARK
WEIGHT (milligrams)
300
200
150
100
50
10
0
1 2 3 4 5 6 7
WEEK 1
1 2 3 4 5 6 7
WEEK 3
1 2 3 4 5 6 7
WEEK 5

from the daylight treatments of diets 6 and 7. The growth of Tapes on the three-algae mixture, dark treatment, is clearly superior to all other treatments, but these results are difficult to explain. There is only a 5% difference in growth between daylight treatments of diets 6 and 7, but a 57% difference between dark treatments. Further, the daylight treatment of diet 6 showed better growth than the dark counterpart, while the opposite is the case in diet 7. No firm explanation for this can be given at this time.

5.4.4 Comparative Growth Study of Tapes semidecussata in Salt River and in the Artificial Upwelling Mariculture System

This study was designed to compare growth and survival of Tapes semidecussata in Salt River and in the artificial upwelling mariculture system. Three wire cages containing a total of 500 Tapes were tied to the mangrove roots and suspended 2-3 ft below the surface at three locations in Salt River. Two of the locations appear to be poor sites due to silt accumulation in the cages, but the third had slightly better survival and growth. The 500 Tapes grown as a control in the artificial upwelling mariculture system were reared in a Nestier tray in Tank 3. All Tapes are from the February 1975 batch spawned in the hatchery.

Table 5.4.4 compares the growth (lengh in mm; average weight per clam in grams) of the two populations. Over a three-month period (July 29 - October 30, 1975), 69.8% survival for the Salt River group and 91.2% survival for the Rust-op-Twist group were recorded.

The slow growth of the Salt River Tapes is probably the result of heavy siltation in the cages. Although regular checks are not made on the plankton content of Salt River, one can assume there is a

TABLE 5.4.4. GROWTH OF TAPES SEMIDECUSSATA IN TWO ENVIRONMENTS ON ST. CROIX

DATE	SALT RIVER		RUST-OP-TWIST	
	AV. LENGTH (mm)	AV. WEIGHT (g)	AV. LENGTH (mm)	AV. WEIGHT (g)
07/29/75	22.6	1.7	-	-
07/30/75	-	-	20.6	1.3
08/27/75	22.2	2.3	25.5	2.4
10/16/75	21.6	2.1	-	-
10/30/75	-	-	27.46	-

fair amount of food since oyster spat in Salt River grow very well. However, these oyster spat are located on cultch suspended from a raft anchored in Salt River. Siltation near the raft is much less than where the Tapes cages are located. Therefore, a new study will be started, in conjunction with the present experiment, and Tapes cages will be suspended from the raft.

5.4.5 An Experimental Determination of a Maximum Sustainable Yield of Tapes semidecussata on an Algal Mixture of Chaetoceros (STX-167) and Unidentified Cryptophyte Flagellate (S-1)

This is the third experiment in the series of studies on Tapes growth in the St. Croix system and is scheduled for implementation on November 14, 1975. The two previous experiments have shown that the STX-167 + S-1 diet gives the best, consistent growth of all phytoplankton mixtures tested thus far. This third study will investigate the maximum conversion rate of deep-sea water-NO_3^- to plankton protein and total nitrogen, and its subsequent conversion to protein and total nitrogen in shellfish meat for two separate culture flow-rates.

Five individual population weights of Tapes will be adjusted to their original weights (removing Δw) every nine days for 36 days, to determine the sustainable yield from each population density. The maximum sustainable yield then can be found for a given flow rate of this diet. In addition, an analysis of each nitrogen fraction in the entire experimental system (NO_2, NO_3, NH_3, particulate N) will be conducted for a determination of the total nitrogen budget. The protocol for this experiment is presented in Appendix H.

This feeding experiment (run November-December 1975) has produced

some preliminary results:

(1) Tapes is an efficient filter-feeder—judging by the usually better than 90% drop in particulate protein when comparing inflow and outflow of particulate protein in the shellfish tanks.

(2) Tapes converts vegetable protein (algae) into animal protein more efficiently, on a short-term basis, than any other known grazer (i.e., ruminant). Our efficiency of conversion values range from 30-45% of algae protein nitrogen into shellfish protein nitrogen for the weights studied and the flow-rates employed.

(3) Slower flow-rates or higher shellfish densities might even increase the nitrogen-conversion efficiency.

(4) The conversion of deep-water nitrogen-nitrate into algal protein nitrogen for this particular experiment varied from 60-66%.

6 SHELLFISH CULTURE: GASTROPODS

6.1 Queen Conch (Strombus gigas)

The five Strombus gigas fed on the macroscopic algae growing in the effluent from our other shellfish tanks for two years died of unknown causes in October 1975. The loss was associated with a high mortality rate in a Tapes semidecussata tank feeding the conch tank, and was apparently due to a Clorox spill after the cleaning of one of the pools.

The data from the five animals have been analyzed by Dr. Carl Berg and included in a publication submitted for publication. The following is an abstract of the paper:

ABSTRACT: Berg, 1975

Growth of the queen conch, Strombus gigas with a discussion of the practicality of its mariculture.

Data on the growth of the large marine snail, Strombus gigas were obtained from animals collected as veligers in the plankton and reared through metamorphosis, from larger animals (5.5 cm) reared in a mariculture system, and from field tag-recapture experiments. Using the von Bertalanffy growth equation, it is estimated that 1-, 2-, and 3-year-old juvenile snails are 10.8, 17, and 20.5 cm in maximum shell length, respectively. The animals reach the flaring-lip stage after 3 years and have a mean longevity of another 3 years. Approximately 12% of the total weight of juvenile snail is marketable meat. Measurements of meat weight, shell length, and total weight are highly correlated with one another, thereby providing reliable means of assessing meat yields from living snails. The mariculture of S. gigas is feasible, but because of the snails' slow rate of

growth it may not be economically practical at this time. At least local fisheries in the Caribbean could be re-established and/or maintained by seeding subtidal algal flats with hatchery-reared juvenile snails.

In collaboration with Dr. Carl Berg of The City College of New York and American Museum of Natural History, and with Dr. Willard Brownell of the Fundacion Los Roques in Caracas, Venezuela, a project on conch larva-culture has begun at the Estacion de Investigaciones Biologica on the island of Los Roques, fifty miles off the northern coast of Venezuela. The project is funded by Fundacion Los Roques and the Venezuelan government. Our participation is limited to Dr. Haines' assisting the Fundacion Los Roques to set up a phytoplankton culture system. The goal of the larva-culture project is to restock natural waters with juvenile Strombus and investigate the possibility of raising them to market size in a mariculture system.

Dr. Brownell suceeded in hatching S. gigas eggs and rearing about two dozen larvae through metamorphosis to the snail-like juvenile stage, using only the natural food in surface water.

During September 1975, Dr. Ken Haines of our St. Croix laboratory worked at the Caracas and Los Roques labs to set up a phytoplankton culturing system which would allow better survival and growth of the ca 300,000 larvae hatched from each egg mass produced by a female conch. Axenic one-liter algal cultures produced in the Caracas lab are transported weekly by air from Caracas to Los Roques, where semi-continuous 16-liter cultures are grown to feed the conch larvae. Twelve liters of nearly unialgal cultures of three high-temperature algae are produced daily: Bellerochea polymorpha (STX-114), Chaetoceros sp. (STX-105) and an unknown Cryptophyto flagellate (S-1). In

addition, surface water is enriched to stimulate growth of the natural flora.

Further work on Los Roques will be aided by construction there of a new air-conditioned laboratory powered by a windmill. The present facility produced electrical power for aeration, pumping and other purposes only three hours per day. Our collaboration with this project will continue during 1976; juvenile Strombus gigas hatched and taken through metamorphosis on Los Roques will be reared to market size in the St. Croix mariculture system.

6.2 West Indian Top-Shell (Cittarium tica)

The West Indian top-shell (Cittarium tica), also known as the "whelk" on St. Croix, is an inhabitant of the surf zone, where it feeds on epiphytes growing on rocks, sand, and coral. The animal is greatly prized for its meat, and it is difficult to find large-sized individuals as a result of the heavy fishing pressure (one does not need a boat to harvest them from knee-deep water). In November 1975 several of these animals were introduced into the tank previously occupied by the Strombus gigas (see 6.1 above). Some of the animals have added new shell on the growing lip; if they survive and grow under these artificial conditions, their growth rates will be compared with those of tagged animals in their natural habitat.

7 CRUSTACEAN CULTURE

7.1 Caribbean Lobster (Panulirus argus)

Three Panulirus argus continue to be maintained on culled bivalves from the mariculture system; all three were originally introduced in June, 1972. Another lobster, designated 3A and obtained from Salt River in August 1973 died in June of this year from unknown causes. Table 7.1 shows the initial weights and growth of the surviving animals.

Animal 1A has shown a negligible weight increase since last year, however, the other two continue satisfactory growth. Because of the small numbers of animals being raised, their past diet history and the variability in quantity of food at the present time, this study will be terminated in favor of a more rigid diet experiment with new animals. Consequently, this will be the last report on these animals.

TABLE 7.1. GROWTH OF PANULIRUS ARGUS

DATE	ANIMAL	WEIGHT (grams)	
		INITIAL	PRESENT
06/27/72	1A	110	-
	1B	105	-
	2A	35	-
08/08/73	3A	-	-
10/30/75	1A	-	1095
	1B	-	1280
	2A	-	1030
	3A	-	(final 5/13/75) 210

8 MACRO-ALGAE CULTURE

8.1 Growth of Hypnea musciformis, a Tropical Red Seaweed

8.1.1 Growth in Continuous-Flow Outdoor Cultures

In 1974 we found that H. musciformis cultured in the effluent from a shellfish tank grew about five times faster than in unaltered deep water, and about three times faster than in surface water. During 1975 we determined that the growth stimulation by the shellfish effluent could be achieved by enriching deep water with ammonia (as NH_4Cl) plus a chelated iron/trace metals/vitamins mix, but could not be achieved by enriching the deep water with ammonia alone or with the chelated iron/trace metals/vitamins mix alone. Increasing the ammonia supply from ca 2 to 6 μg-at NH_4-N $liter^{-1}$, while keeping the chelated iron/trace metals/vitamins mix enrichment constant, produced an increased growth rate.

Preliminary experiments, using 4% primarily treated or 35% secondarily treated sewage enrichment of deep water, indicated that this alga could be used in a variety of aquaculture applications, including estuarine systems.

The carrageenan yield of Hypnea musciformis grown in deep water enriched in a variety of ways was 16-27% of dry weight, compared to 20-26% of dry weight for commercially harvested plants from the Mediterranean. The details of this work are given in the manuscript in Appendix I.

8.1.2 Growth Studies in Laboratory Batch Culture

Clonal cultures of Hypnea musciformis were obtained from a carposporic plant collected from nature. A unialgal clone

(STX-HM-14) is being studied in laboratory batch cultures to determine its tolerance limits for such environmental factors as light intensity, temperature, salinity and high levels of nutrients. These studies will indicate the optimum culture conditions and should help in adapting the culture of this seaweed to other aquaculture systems, such as temperate estuarine sewage effluent mariculture like that being carried out at our Tallman Island project in Queens, New York.

To date, we have been developing a medium for prolonged culture of excised tips of Hypnea (to avoid having to change the medium frequently) and testing non-destructive methods for measuring growth. By switching from Guillard's medium (f/4) to medium 2f (=quadruple the nutrient enrichment in f/4), we have increased the growth rate (and decreased the standard deviation for replicates) from 217 ± 161% wet weight per day in medium f/4 to 493 ± 24% wet weight per day in medium 2f over a growth period of three weeks, starting with ca 2 mg (wet weight) Hypnea tips. The "wet weight" method of growth measurement used is very sensitive and is the method of choice "by default"; that is, due to the many-branched pattern of growth, it is impossible to measure increase in area or length as can be done with some seaweeds that form a linear or flat thallus.

8.2 Continuous-Flow Culture of Eucheuma isiforme, Gracilaria verrucosa and Agardhiella tenera

Eucheuma isiforme (obtained through Dr. Clinton Daves), Gracilaria verrucosa (obtained from Dr. Harold Humm) and Agardhiella tenera (from nearby Salt River) were tested for their ability to grow on the nutrients in the effluent from a tank containing Kumamoto

oysters (Crassostrea gigas) fed diatom cultures grown in deep water. Growth of G. verrucosa and E. isiforme in oyster-tank effluent was compared to growth of plants in tanks receiving deep water only. The algae were grown in aerated tanks of 36-liter volume, with 96 volume dilutions per day. Wet weight was measured at four-day intervals. The inoculum plants consisted of three plants in each tank for E. isiforme (total wet weight of 57.2 g in one tank and 65.5 g in the other), and one plant of G. verrucosa divided between the two tanks to give starting weights of 0.87 and 1.3 g. The average temperature in the effluent tank was 25.2°C, and in the deep water tank, 23.6°C.

The Gracilaria verrucosa appeared to grow faster in oyster-tank effluent than in deep water (28 vs. 4.9% per day, respectively) but contamination of the plants by filamentous green algae was so severe after 19 days' growth that at least half of the weight gain may have been due to the contaminants which could not be removed without destroying the Gracilaria.

The Eucheuma isiforme grew at the rate of 8% per day in the shellfish effluent, and 4.5% per day in deep water. These growth rates are underestimated because they were not corrected for losses due to breaking off of small branches of the plants, and they are overestimates because there were some epiphytic filamentous green algae which could not be removed. The contamination with green algae at times was so heavy that the mass of Eucheuma and green algae could not be kept moving by the air circulating system.

The Agardhiella tenera from Salt River was also susceptible to filamentous green algae contamination, and was extremely fragile, so that growth rate could not be measured in the oyster-tank effluent.

From the results with these three species of macro-algae, we

conclude that their growth rates are not fast enough to "outgrow" the contaminating green algae, as is the case with the fast-growing Hypnea musciformis. We will restrict our future seaweed studies almost entirely to Hypnea musciformis.

PART B—THE EFFLUENT AQUACULTURE PROJECT

Summary Progress Report

During 1975 ten phytoplankton tanks and a shellfish rack (containing 120 shellfish trays) were installed in the greenhouse structure at Tallman Island. The present layout now includes baywater and effluent reservoirs, the sewage-treatment plant and all attendant pumps and piping, electrical and air supply, and heating and ventilating equipment.

Since February 1975, two burglaries resulted in losses of equipment totalling $7,000 to $8,000. It therefore became necessary to direct our resources toward the installation of an effective security system. If the measures taken prove ineffective we may be obliged to hire a security guard to ensure the premises are manned round-the-clock.

The Clow secondary-treatment plant was installed in October 1974, but difficulties encountered in developing a reliable system for pumping raw sewage from Manhole #3 to our project site meant that it was not until July 1975 that the treatment plant was operating in a stable, continuous fashion. At this time we began monitoring plant influent and effluent on a weekly basis. Since November 1975, we have been monitoring effluent, river water and phytoplankton-tank phosphate concentrations daily for use in a systematic feedback control program which we are developing.

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 laboratory is located on Powells 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. One trailer is attached to the greenhouse and contains a temperature-controlled phytoplankton culturing room, a chemistry laboratory and a log room. The second trailer contains offices and a conference room, while the third trailer has been converted into a workshop and storage area for laboratory equipment and construction materials.

During 1975, ten phytoplankton tanks and a shellfish rack (containing 120 shellfish trays) were installed in the greenhouse structure (see Fig. 1:1, 1:2). The present layout also includes baywater and effluent reservoirs, the sewage-treatment plant and all attendant pumps and piping, electrical supply, air supply, and heating and ventilating equipment.

1.2 Staffing

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

Supporting Staff:
Sharfstein, Bruce A. (Ph.D.)
Chief Scientist, Tallman Island Station Manager

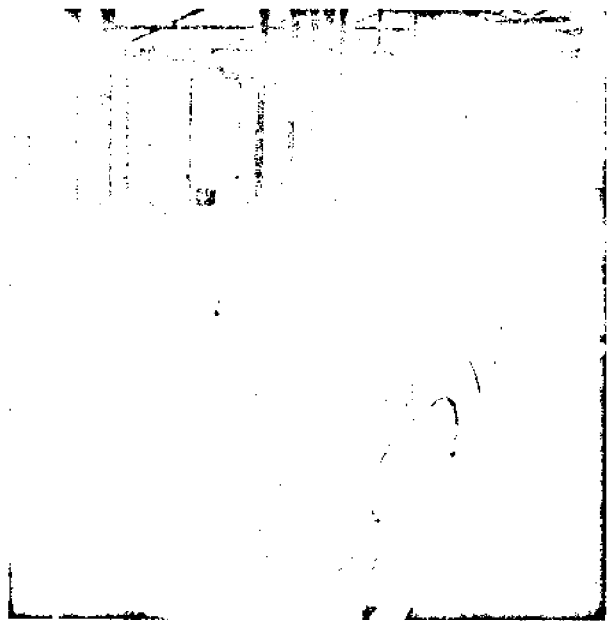


Figure 1:1. Configuration of the new phytoplankton tanks in the greenhouse structure at Tallman Island.

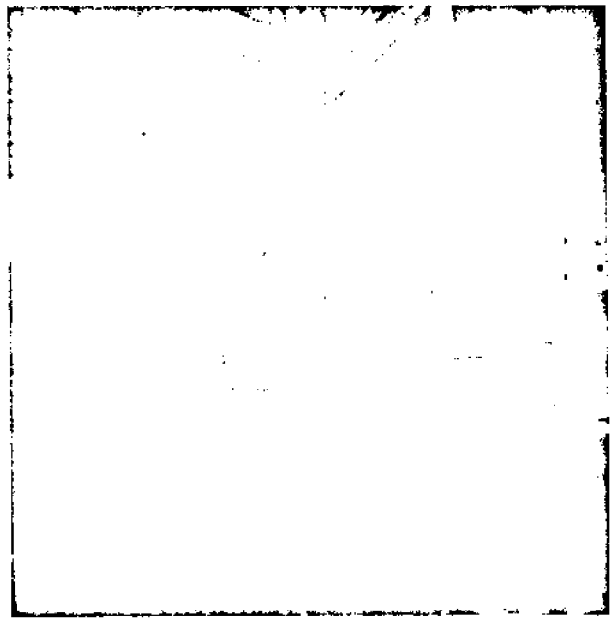


Figure 1:2. A photograph of the new shellfish rack which contains 120 shellfish trays.

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

Dorsey, Thomas E. (Ph.D.)
Postdoctoral Fellow; Water Chemistry

Berg, David (B.S.)
Technician (Shellfish)

Lee, Vicki (B.S.)
Technician (Phytoplankton)

Lewis, Ira
Plant Maintenance

Goldstein, Barry (B.S.)
Doctoral Student; Shellfish

Cavalieri, Rory (B.S.)
Masters Student; Mariculture

KcKeon, Ann-Marie (M.S.)
Volunteer; Phytoplankton Isolations

Chase, Jonathan
Volunteer; Seaweeds

Consultants/Collaborators

Terry, Orville (Ph.D.)
Collaborator on Seaweeds

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

In addition, the New York City Department of Water Resources, which operates the Tallman Island Pollution Control Plant, contributes its support both through consultations concerning aspects of engineering and water analysis and by providing essential services such as trash removal, power, water, fuel-oil delivery, and exterior site maintenance.

2 ENGINEERING

2.1 Flow Engineering

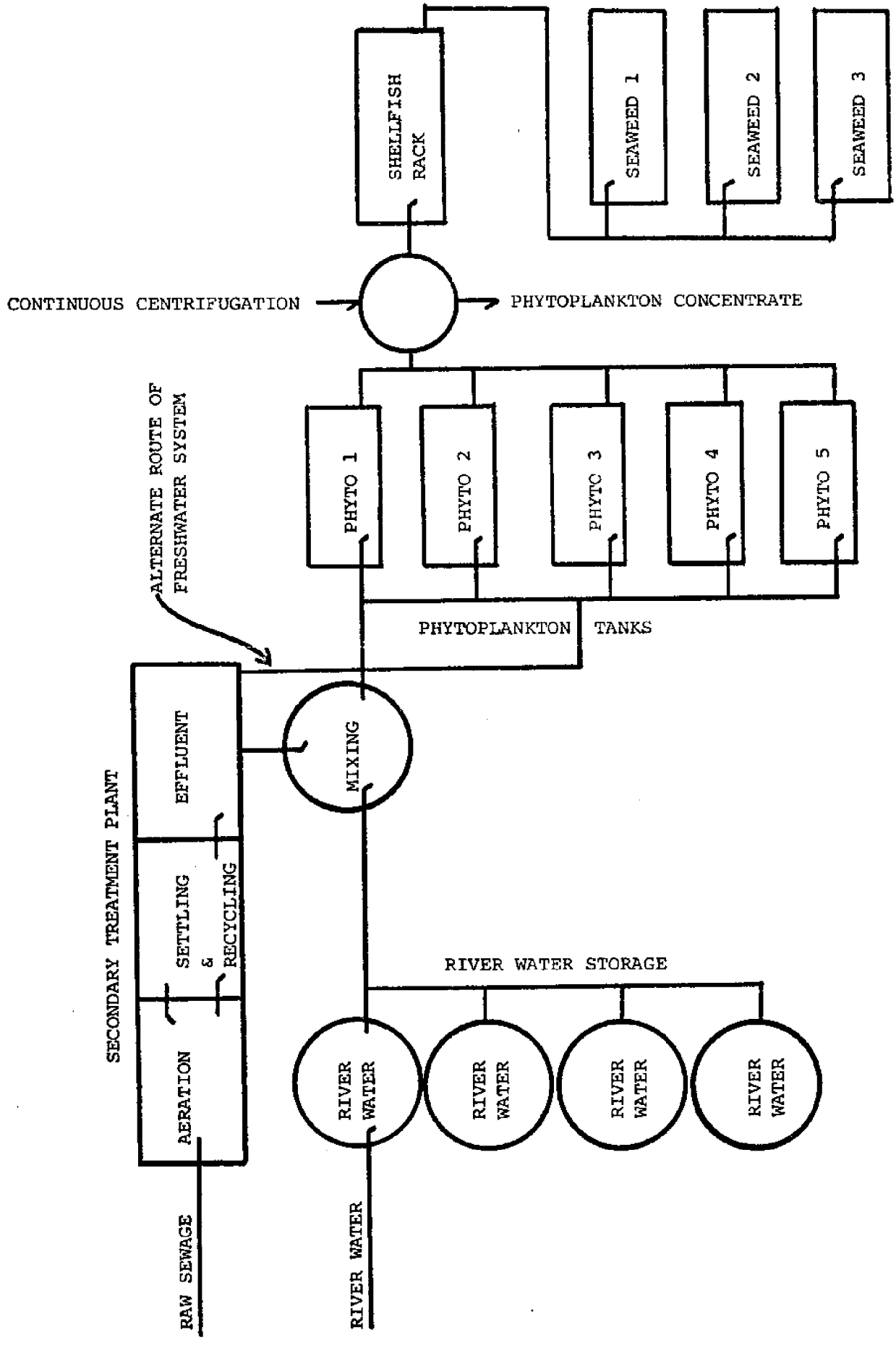
The continuous flow effluent aquaculture system has the capacity to process up to 1,500 gallons per day of sewage. A schematic diagram of the flow within the aquaculture system is shown in Figure 2:1.

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 6"-dia. 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 compartment). The secondarily treated effluent enters the final effluent holding tank from which it is distributed to the aquaculture system.

The secondarily treated effluent is pumped from the effluent holding tank into an overhead constant-level tank from which it is gravity-fed into 500-gallon phytoplankton tanks. The flow rate of effluent entering the phytoplankton tanks 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 pumphouse located beside the Powells Cove

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


SHELLFISH RACK
SEAWEED 1
SEAWEED 2
SEAWEED 3
CONTINUOUS CENTRIFUGATION
PHYTOPLANKTON CONCENTRATE
PHYTO 1
PHYTO 2
PHYTO 3
PHYTO 4
PHYTO 5
PHYTOPLANKTON TANKS
ALTERNATE ROUTE OF FRESHWATER SYSTEM
MIXING
SECONDARY TREATMENT PLANT
EFFLUENT
SETTLING & RECYCLING
AERATION
RIVER WATER STORAGE
RIVER WATER
RIVER WATER
RIVER WATER
RIVER WATER
RAW SEWAGE
RIVER WATER

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 15-micron 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 tanks. The flow rate of river water entering the tanks is controlled by capillary tubing of different diameters.

Mixing of the river water and secondarily treated effluent for our estuarine aquaculture system 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 phytoplankton tanks 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.

2.2 Security Precautions

Since February 1975, we have had two serious burglaries with losses totalling $7,000 to $8,000, as well as several attempted thefts and other incidents of vandalism. As a result, it became necessary to direct our resources toward the installation of an effective security

system. At present, we have installed alarms on all doors and windows and steel-mesh gratings on all windows. We are currently installing double locks and steel cross-bolts on all doors. If these measures prove to be ineffective in foiling thefts, we may be obliged to hire a security guard to ensure that the premises are manned round-the-clock.

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, to optimize their utilization in our aquaculture system. During Phase I of the project weekly measurements were made of the nutrient levels in the secondarily treated effluent from the Tallman Island Pollution Control Plant, and of those levels prevailing in the East River from November 1972 to October 1973.

In April 1973 a massive program of reconstruction and upgrading was begun at the Tallman Island Pollution Control Plant 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.

Reconstruction of the chlorination building at TIPCP, which contained the Phase I effluent aquaculture facilities, was begun in November 1973, and forced relocation of the project's facilities to the present location in three large trailers and a greenhouse structure.

However, we continued to monitor nutrient levels of TIPCP's primary effluent and of East River water on a weekly basis from October 1973 to July 1975.

In October 1974 the Phase II secondary treatment plant was put into operation at our project. Due to difficulties in developing a reliable system for pumping raw sewage from Manhole #3 to our project site, however, it was not until July 1975 that the treatment plant (Clow STP) was operating in a stable, continuous fashion. At this time we began to monitor plant influent and effluent on a weekly basis. The results of this study are reported in section 3.1.2.

Since November 1975, we have been monitoring effluent, river-water, and phytoplankton-tank phosphate concentrations daily for use in a systematic feedback control program we are developing (see section 3.1.2).

3.1.1 Materials and Methods

River water and effluent samples are analyzed for levels of nitrate, nitrite, ammonia, orthophosphate, and silicate. River water samples are filtered through Gelman glass fiber filters (0.3-micron mesh) and the filtrate is stored in both glass and polyethylene bottles. The samples in glass bottles are stored at 5°C; samples in polyethylene bottles are frozen at -10°C. Nitrate, ammonia, and orthophosphate analysis is done on the samples stored in glass; nitrite and silicate analysis is done on the frozen samples. Effluent samples are filtered in the same way and then diluted 1:9 with glas-fiber filtered glass-distilled water before bottling to bring the nutrient concentrations into the range for the analytical techniques used. Water samples are analyzed using an AutoAnalyzer II (Technicon) 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 inorganic nitrate, nitrite, ammonia and orthophosphate in secondary sewage effluent and East River water for the period July 10 to November 11, 1975 are presented in Table 3:1. Mean values and ranges for these same compounds for 1972, the first year of work at Tallman Island, are presented in Table 3:2 for comparative purposes.

Two major changes in effluent composition have occurred since the inception of our system at Tallman Island in 1972. The first of these is an increase in the nitrate fraction of the effluent relative to the ammonia fraction. This is probably related to the improved quality of treatment provided by our small extended aeration plant. Such an interpretation is supported by other treatment parameters as discussed in section 3.2.5. The second change that has been noted is a decrease in the phosphate content of the effluent relative to the total inorganic "nitrogen" load. In 1972, the mean molar N:P ratios of the effluent, and of the 50:50 effluent:river water mixture used as phytoplankton

TABLE 3:1

A: NUTRIENT CONCENTRATIONS (IN µg-at/liter) IN SECONDARILY TREATED EFFLUENT FROM TALLMAN ISLAND POLLUTION CONTROL PLANT, QUEENS (NEW YORK CITY)

NUTRIENT	MEAN[1]	RANGE[2]	PERIOD
Nitrate	236	2 - 1126	July 10 to
Nitrite	215	4 - 779	November 13, 1975
Ammonia	202	10 - 570	
Orthophosphate	30	17 - 53	

B: NUTRIENT CONCENTRATIONS (IN µg-at/liter) IN EAST RIVER WATER TAKEN NEAR THE TALLMAN ISLAND POLLUTION CONTROL PLANT, QUEENS (NEW YORK CITY)

NUTRIENT	MEAN[1]	RANGE[2]	PERIOD
Nitrate	35	7 - 73	July 10 to
Nitrite	6	2 - 23	November 13, 1975
Ammonia	15	5 - 23	
Orthophosphate	5	3 - 6	

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 single lowest value recorded over a particular sampling period.

TABLE 3;2

A: NUTRIENT CONCENTRATIONS (IN µg-at/liter) IN SECONDARILY TREATED EFFLUENT FROM THE TALLMAN ISLAND POLLUTION CONTROL PLANT, QUEENS (NEW YORK CITY)

NUTRIENT	MEAN[1]	RANGE[2]	PERIOD
Nitrate	11	0 - 44	1972
Nitrite	34	11 - 114	
Ammonia	1230	704 - 1670	
Orthophosphate	140	66 - 230	
Silicate	77	33 - 132	

B: NUTRIENT CONCENTRATIONS (IN µg-at/liter) IN EAST RIVER WATER TAKEN NEAR THE TALLMAN ISLAND POLLUTION CONTROL PLANT, QUEENS (NEW YORK CITY)

NUTRIENT	MEAN[1]	RANGE[2]	PERIOD
Nitrate	18	8 - 40	1972
Nitrite	6	4 - 15	
Ammonia	105	30 - 440	
Orthophosphate	17	10 - 70	
Silicate	36	22 - 75	

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 single lowest value recorded over a particular sampling period.

growth media was respectively, 9:1 and 8:1. Presently, the ratios have increased to 22:1 for 100% effluent, and 21:1 for the effluent: river water mixture. These changes may reflect the effects of the current ban on phosphate-containing detergents. Whatever the cause, they strongly affect our ability to accomplish high levels of "nitrogen" removal. This situation is discussed in greater detail in section 4.3.

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 STP has been operating continuously and monitored since July 1975. In addition to the pumping problems reported in section 3.1, during the initial weeks of plant operation it was found that the organic load carried by the fraction of raw sewage we were pumping was not adequate to support a normal bacterial flora. This problem has been solved by a daily addition of raw settled sludge equal to the difference between the required and normally available organic loads. Effluent plant chemistries are corrected for this supplementary organic loading.

3.2.1 Visual and 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 are taken daily from four locations in the STP: influent, aeration compartment, sludge return pipe, and effluent. The samples are individually filtered through pre-dried, pre-weighed glass-fiber filters. The filters are dried again and weighed to determine the weight of suspended solids. A sample of 50 ml is usually sufficient to obtain significant values in milligrams per liter, although occasionally, it is necessary to filter as much as 400 ml, especially when dealing with relatively pure effluent.

For the period July 10, 1975 through November 24, 1975, the mean percent removal of suspended solids was 94.4%, with individual daily values ranging from a low of 21% to a high of 99.9%.

3.2.3 Chemical Oxygen Demand

COD is determined according to the Jeris method, which is a rapid and satisfactory test though not a standard method (see Appendix J for detailed methodology). Average percent removal of COD for the period July 10 - November 24, 1975 was 82.6%.

3.2.4 Biochemical Oxygen Demand

The biochemical oxygen demand is determined according to the standard five-day BOD method outlined in the 13th edition of Standard Methods for the Examination of Water and Wastewater (Amer. Public Health Assn., Washington, D.C.), 1971, pp. 489-495. For the period July 10 - November 24, 1975, the mean percent removal was 92.5%.

3.2.5 Summary of STP Operations

A comparison of the removal values for suspended solids, BOD and COD for our package plant with values supplied by the New York City Department of Water Resources for expected removals in newly upgraded plants (Table 3:3) indicates that our system is producing a considerably higher quality effluent than that with which we will probably be dealing when we enter Phase III. This may result in serious constraints on the predictive validity of our Phase II results. For this reason, planning is now underway for the installation of a piping system to supply Tallman Island secondary sewage effluent to our site as soon as the newly upgraded plant goes on-line (estimated starting date—early spring 1976).

TABLE 3:3. COMPARISON OF CLOW PACKAGE PLANT AND NYC/DWR's UPGRADED TREATMENT PLANT OPERATIONS

PARAMETER	PERCENT REMOVAL	
	AVERAGE CLOW PLANT	OPTIMUM UPGRADED NYC PLANT*
Suspended Solids	94.4%	90%
BOD	92.5%	90%
COD	82.6%	60-70%

*Numbers supplied by Mr. William Pressman of the New York City Department of Water Resources.

4 PHYTOPLANKTON

4.1 Light-Limited Growth and Cellular Composition of *Dunaliella tertiolecta* Cultured with Ammonia and Nitrate

This study which was reported as being in progress as of January 1975, has now been completed. Complete results are reported in the doctoral dissertation of Bruce A. Sharfstein (Light-Limited Growth and Cellular Composition of *Dunaliella tertiolecta* Cultured with Ammonia and Nitrate: The City University of New York, 1975, 173 pages). The thesis abstract is given here:

ABSTRACT: The growth rates, and cellular concentrations of ATP, particulate "carbon", particulate "nitrogen", chlorophyll *a*, protein, carbohydrate and lipid, were measured for *Dunaliella tertiolecta* grown in light-limited continuous culture in nitrate and ammonia medium.

Cells cultured in ammonia medium had consistently higher hourly growth rates at all levels of light intensity, the difference increasing from 22% at the highest intensity to 62% at the lowest intensity. Growth in ammonia medium was shown to be dependent on I_{abs}/unit chlorophyll, the response of cellular chlorophyll *a* to changing light intensity, and the energy demands of division. At the highest intensity studied, nitrate-grown cells showed a similar response to light, while at lower intensities light-limited growth was primarily mediated by the light-dependent component of nitrate assimilation. A theoretical model of light-limited growth was developed based on these results.

Nitrate- and ammonia-grown cells showed similar levels of intracellular chlorophyll a, lipid, and ATP. Nitrate-grown cells showed a tendency to synthesize 40% more protein/division than ammonia-grown cells. It was suggested that it is this additional synthesis, rather than the energy differences in uptake and assimilation of nitrate and ammonia, which account for the observed differences in growth rate at light intensities of 0.018 ly/min, where the light-dependent component of nitrate assimilation is saturated. Further, it was suggested that this additional protein synthesis might be related to the synthetic machinery and enzymes involved in the reduction of nitrate to ammonia by the cell.

A consideration of potential strategies for the operation of an effluent mariculture system was developed, based on the experimental results.

4.2 Phytoplankton Isolation Studies

One criterion used in the selection of phytoplankton for a sewage effluent aquaculture system is that the organism should grow rapidly and thrive in high concentrations of sewage effluent. This increases the efficiency of such an aquaculture system by increasing the volume of sewage which can be processed.

A method for the selection of algal species that meet these criteria was recently developed that enables us to maintain an ongoing selection process within our phytoplankton tanks while they are being used for other culturing purposes. Small vials, fitted with Millipore filters at one end, are filled with a mixed culture that is to be screened for growth in effluent. The vials are allowed to float in the reactors which contain effluent or an effluent:river water mixture.

The Millipore filter functions as a selectively permeable membrane allowing the passage of nutrients into the vial but preventing the exit of algae from the vial. After the vial culture has incubated in the effluent medium for several weeks and increased in density, an aliquot is plated onto a complete algal medium using an atomizer technique. The plated culture is incubated until isolated colonies can be observed and picked. Unialgal colonies of algal species selected for growth in sewage effluent are thus isolated.

This method of algal selection has recently been started as an ongoing selection procedure. We have successfully isolated two species of freshwater algae, Chlorella sp. and Scenedesmus sp. from samples of sewage influent and a mixed phytoplankton-tank culture grown in 100% sewage effluent, respectively.

4.3 Large-Scale Phytoplankton Cultures

Since the completion of our Phase II facilities in July 1975, we have been simultaneously operating two separate effluent aquaculture systems. The first of these is a typical estuarine system utilizing a phytoplankton-culturing medium composed of 50% secondary sewage effluent and 50% East River water. The second is a freshwater system which utilizes undiluted secondary sewage effluent as the growth medium. While the emphasis of our past work has been on the development of a brackish-water system, we are now investigating a freshwater system since we realize that brackish-water systems are limited in applicability to coastal cities, and that even where dilution of the sewage with saline water is possible, the necessity of diluting the secondary sewage effluent would only compound the problems of space limitation characteristic of most urban areas.

As noted previously (section 3.1.2), since the ban on phosphate-

containing detergents, the molar N:P ratio of the secondary effluent has changed markedly. As a result, under current conditions, it is no longer possible to accomplish complete removal of sewage-derived inorganic "nitrogen", although complete stripping of phosphate is routinely attained. The quantity of "nitrogen" that can be removed is dependent on the N:P ratio of the incoming sewage, and on the ratio of utilization of "nitrogen" to "phosphorus" by the phytoplankton. Percent removal of "nitrogen" for the period July 10 to November 24, 1975, has ranged from 17% to 100%, with a mean stripping value of 52%.

Continuous tank cultures are now being regulated by a series of feedback equations so that flow rates and cell densities are adjusted to the prevailing algal growth rate and the phosphate concentration in the incoming sewage effluent (and/or) baywater. This approach has given us runs of up to 11 days with greater than 90% phosphate removal in certain estuarine cultures When the feedback equations are further refined, we hope to be able to increase the length and reliability of these runs through a more precise regulation of culture conditions.

Although we are just beginning to investigate strategies for optimization of the phytoplankton cultures in terms of the relationships that exist between light penetration, growth rate, nutrient uptake rate, depth, and volume throughput, initial area required and volume throughput calculations done by Walrath and Natter (1975), based on our raw data for one-meter deep tanks, are very promising. Their calculations indicate a requirement of 5.5 acres per one million gallons per day (mgd) throughput for a one-meter deep tank under summer conditions of light and temperature, as compared to a value of 26 acres/1 mgd for the Woods Hole effluent aquaculture system under similar seasonal conditions (Walrath, D. and A.S. Natter: A new broom in wastewater

cleanup: aquaculture converts nutrients to food, fuel and fertilizer (in press)).

As noted previously (section 4.2), we are actively evaluating new organisms for use in our system. At present, we are using a total of five estuarine species, and four freshwater species:

Estuarine Organisms

Nannochloris sp.

Isochrysis galbana

Pb_{12} (Jamaica Bay isolate)

X92 (St. Croix isolate)

Dunaliella tertiolecta

Freshwater Organisms

Chlorella sp.

Selenastrun sp.

Chlamydomonas sp.

Scenedesmus quadricata

4.4 Use of a Cream Separator for Concentrating Phytoplankton

We have recently acquired an International Harvester cream separator which we plan to use for producing phytoplankton cell concentrates following techniques suggested by Cornelius Mock (personal communication, 1975).

The ability to mechanically harvest a large percentage of the phytoplankton cells in our tank ("reactor") cultures would have a number of major advantages for an effluent aquaculture system.

The phytoplankton cell densities that must be attained in our reactor cultures to achieve adequate nutrient removal are generally one to two orders of magnitude higher than the cell densities that are suitable for shellfish food. As a result, large volumes of dilution

water are currently needed for the shellfish phase of the system. If a large percentage of the incoming phytoplankton culture could be harvested prior to the shellfish stripping step, an additional usable protein source (the phytoplankton concentrate) could be produced, and the need for dilution water would be eliminated, thereby substantially reducing the area requirements for shellfish culture.

Alternatively, if new phytoplankton growth were constantly harvested from an actively growing continuous culture at a rate equal to the growth rate of the culture, high nutrient removal rates could be accomplished at low standing crop densities. Furthermore, the resultant increase in light penetration might result in higher growth rates, and a greater volume throughput per unit area.

5 SHELLFISH

5.1 Flax Pond Shellfish Hatchery

A shellfish hatchery was operated at the Flax Pond Marine Research Laboratory of the New York State Department of Environmental Conservation from January to August 1975.

Spawning experiments were done with a freshwater species, Rangia cuneata, and an estuarine species, Mytilis edulis. The conditioning and spawning experiments with Mytilis edulis met with limited success and procedures for optimizing conditioning and spawning techniques were begun.

Unfortunately, we have had to temporarily shut down the shellfish hatchery at Flax Pond due to staffing demands at the Tallman Island facility and to the high cost of operating the Flax Pond laboratory. If funding becomes available in the near future, the shellfish hatchery experiments will be re-established.

5.2 Shellfish Studies at Tallman Island

5.2.1 Routine Shellfish Growth Experiments

Shellfish growth studies are currently underway for three estuarine species: Mytilis edulis, Modiolus demissus, and Mya arenaria, and for two freshwater species: Rangia cuneata and Lampsilis sp. (identification unconfirmed). Monthly weight gains and daily stripping efficiences are being monitored with the aim of determining gross food conversion efficiencies for these species. These growth experiments will be used as a guideline in selecting those shellfish which are most suitable for our effluent aquaculture system.

Preliminary results of these growth experiments indicate that of the six shellfish species screened to date, all except Rangia cuneata

are suitable organisms for our system.

We are currently making arrangements to obtain populations of Corbicula manilensis and other potentially suitable freshwater organisms which will be screened for growth in the Tallman system.

5.2.2 Dynamics of Protein Production of Mytilis edulis

Optimization of the effluent aquaculture system in terms of protein production depends in part on the determination of those phytoplankton species and feed densities that result in the maximum protein production for each shellfish species in the system.

A study is beginning with the objectives of generating a series of equations relating protein production of Mytilis edulis to algal species and feeding density, and of describing the transfer of various nitrogen fractions of algal cellular nitrogen through Mytilis edulis in our effluent aquaculture system. During the course of this experiment, the digestibility of algal protein, assimilation of amino and protein nitrogen, and protein conversion efficiences by M. edulis will be determined for different algal species and feeding densities. This information will enable us to select the optimal algal diet and feeding density that will result in maximum protein production.

6 SEAWEED

6.1 Flax Pond Seaweed Laboratory

Six species of seaweed are presently being cultured at the Flax Pond laboratory, including Chondrus crispus, Agardiella verrucosa, Agardiella sp., Hypnea musciformis, Gracilaria sp. and Anfeltia sp. These seaweeds are maintained in continuous culture and provide a readily available supply of stock plants for the effluent aquaculture system at Tallman Island. The first five species of the seaweeds listed here have been introduced and evaluated in the Tallman system. Anfeltia sp. and other potentially suitable seaweeds will be introduced as they are available in sufficient population size (at least 200 g).

Preliminary experiments were done at the Flax Pond laboratory to determine optimum seaweed-tank design and methods of circulation. Circulation systems using aeration and a mechanical paddle were compared. It was concluded that seaweed circulation by aeration alone was excellent for the tank design at Tallman Island.

6.2 Seaweed Tank Design and Operation

The results of the preliminary study of methods of seaweed circulation done at the Flax Pond laboratory suggested that circulation by aeration alone would be adequate for the Tallman Island seaweed tanks. Seaweeds grow best when continuously agitated and exposed to the maximum possible light. For efficient use of the Tallman seaweed "reactors", a circulation pattern was required that provided gentle but continuous movement of the plants, longest possible surface time and largest cycle size combined with fastest cycle speed. A comparison was made of seaweed circulation patterns when agitated by air alone at

three reactor water-levels (0.25, 0.50 and 0.75 levels). Air was supplied by a single air line running the length of the reactor at the mid-line of the bottom of the tank. Observations were made of rate of cycling, cycle size and configuration, plant movement, and surface time. It was concluded that the best circulation pattern occurs with full air supply and a tank water-level of 0.75. Under these conditions, the seaweeds cycle seven times per minute over the entire tank width, with gentle but continuous motion and longest surface time.

6.3 Screening for Suitable Seaweed Species

Initially, the following five species of seaweed were introduced into the Tallman Island system from our Flax Pond laboratory:

Agardiella verrucosa

Agardiella sp.

Gracilaria sp.

Hypnea musciformis

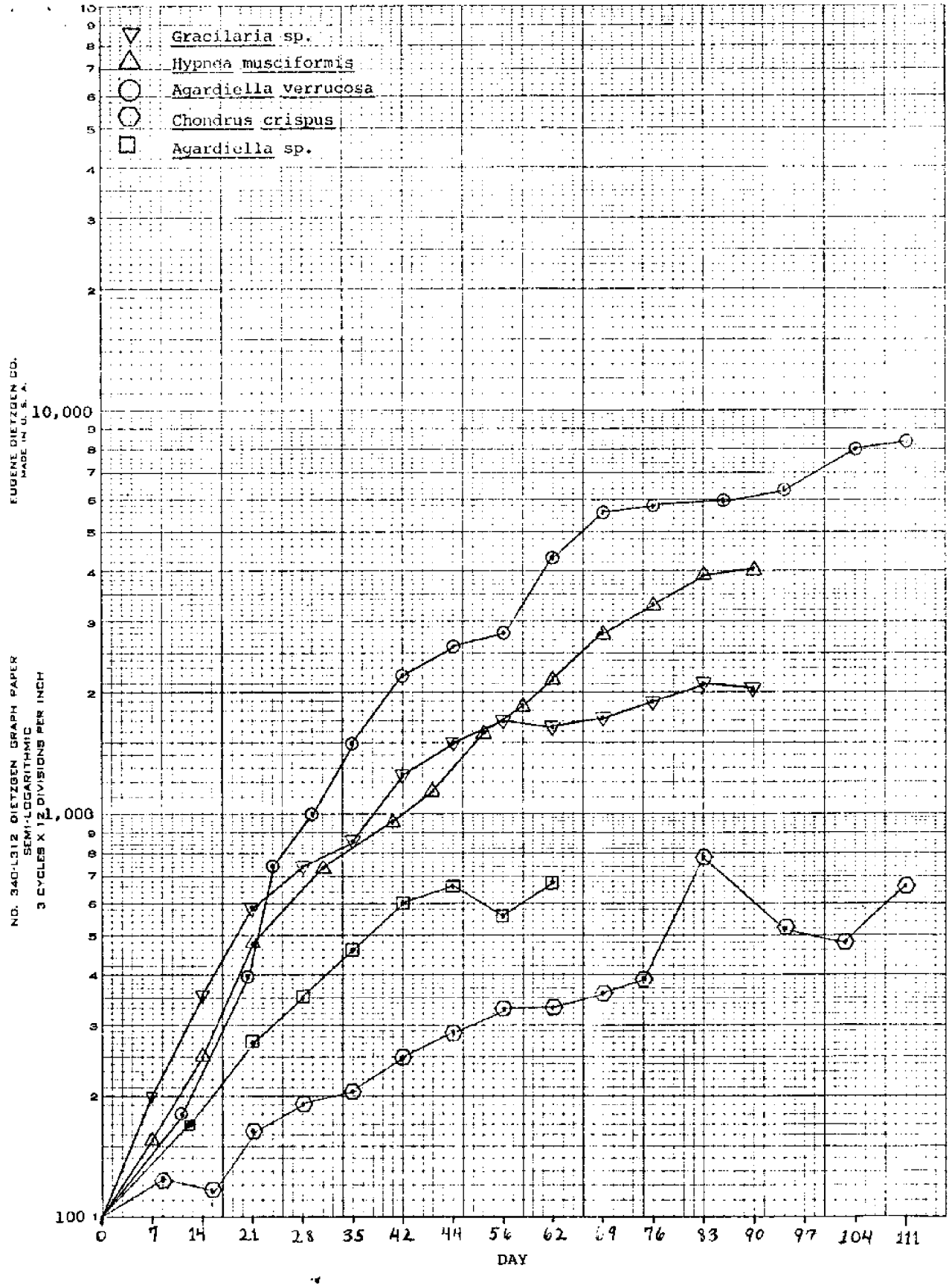
Chondrus crispus

At present, all the seaweeds have been kept in the system for 90-111 days, over the period August-September 1975 to mid-December 1975.

Seaweeds were placed in the reactors, and supplied with a constant flow of a 10% sewage effluent:90% East River water mixture at a rate of one turnover per week. Due to the initially small population sizes, this flow rate was sufficient to maintain adequate nutrient levels. In the case of Agardiella verrucosa and Hypnea musciformis, initial flow rates were increased in response to population gorwth so that the system never became nutrient-limited. Seaweeds were weighed weekly during the study.

Results of this study are summarized in Figure 6:1. The two most

Figure 6:1. Normalized seaweed growth curves for five species of seaweed grown in the Tallman Island effluent aquaculture system.


Gracilaria sp.
Hypnea musciformis
Agardiella verrucosa
Chondrus crispus
Agardiella sp.
10,000
1,000
100
0
7
14
21
28
35
42
44
56
62
69
76
83
90
97
104
111
DAY
EUGENE DIETZGEN CO.
MADE IN U.S.A.
NO. 340-L312 DIETZGEN GRAPH PAPER
SEMI-LOGARITHMIC
3 CYCLES X 12 DIVISIONS PER INCH

successful seaweeds were Agardiella verrucosa, and Hypnea musciformis, although only Agardiella verrucosa has so far attained a population size which permits weekly harvesting. It is notable that neither of these two seaweeds have been attacked by epiphytes.

Chondrus crispus, Agardiella sp. and Gracilaria sp. have all shown relatively slow growth in the system, and have all been attacked by epiphytic alga, Spermothamnion turnerii. At present, these seaweeds have been rejected as suitable choices for the Tallman Island system, although we plan to reintroduce these, along with other new species, at a later date.

6.4 Study of Agardiella verrucosa

Agardiella verrucosa has proven itself to be the most successful seaweed in our aquaculture system (of those tried in the system so far).

At present, we are culturing A. verrucosa in a mixture of 10% secondary sewage effluent:90% East River water, at a turnover rate of 0.36/day. Under these nutrient conditions, and late fall-early winter light conditions, 90% phosphate removal is attained, with a sustainable net production rate of 17 g wet weight/m^2/day for a one-meter deep tank. It is probable that these values are somewhat less than the annual mean production rate, and that productivity can be further optimized. However, using these numbers as a guideline, it can be calculated that a yield of at least 230 tons/acre/year could be attained.

We are currently accumulating a harvest of Agardiella verrucosa that will be sufficient for trace-metals and carrageenan-content analyses. Arrangements for these analyses have already been made with Dr. Sidney Schwartz of the New York State Department of Environmental Conservation and with T.L. Chapman of Marine Colloids, Inc.

QUARTERLY PROGRESS REPORT

Period September 1 to November 30, 1975

on

OPEN-BAY CULTURE OF OYSTERS IN THE U.S. VIRGIN ISLANDS
PHASE I: TECHNICAL FEASIBILITY STUDY

by

LAMONT-DOHERTY GEOLOGICAL OBSERVATORY

OF COLUMBIA UNIVERSITY
Palisades, New York 10964
and
Post Office Box Z, Kingshill
St. Croix, U. S. Virgin Islands 00850

submitted to

DEPARTMENT OF CONSERVATION AND CULTURAL AFFAIRS
U. S. Virgin Islands Government
Post Office Box 578, Charlotte Amalie
St. Thomas, U. S. Virgin Islands 00801

(L-DGO Proposal No. 1186)

Report Due Date: January 1, 1976

0311-439-5639

NOTE

The financial report for this quarter is being prepared by Columbia University and will be forwarded to the Department of Conservation & Cultural Affairs of the U.S. Virgin Islands Government by 1 January 1976.

A prototype raft was anchored in Salt River Inlet on September 11, 1975. The raft measures 12 ft. by 12 ft. and flotation is provided by four 55-gal. drums protected by anti-oxidant paint and liquid tar. The drums are strapped to the raft by stainless steel cables. A single point concrete anchor of approximately 200 lbs. hold the raft in position.

Three locally available materials were chosen for testing as cultch: automobile tire rims, mortar mix coated plywood and Vexar plastic mesh. The tire rims were hung horizontally, each string consisting of 8 rims, 8 inches apart. The mortar mix collectors measure 16 x 32 inches, 2 collectors per string. These strings were hung 8 inches apart on stringers, parallel to the anchor line. Each string carries a 2.2 kilo weight to compensate for the plywood's buoyancy. The Vexar collectors measure approximately 16 x 48 inches and had to be supported by a wooden frame.

We used the natural set of Crassostrea rhizophorae, the mangrove oyster, to test the relative attractiveness of the 3 collector types to oyster spat. Our results clearly showed the superiority of the mortar mix coated plywood as a setting surface. Collectors set out on September 11, 1975, averaged 470 juvenile oysters per collector by October 10. The tire rims and Vexar plastic mesh did not collect enough spat to be of commercial interest. On the basis of these

results, only mortar mix coated plywood or other mortar mix coated material will be used in future experiments. However, mortar mix coated plywood presents some practical problems. Their preparation requires considerable labor and they are difficult to handle in hatchery setting tanks. We plan to try several alternatives including sea scallop shells and corrugated asbestos-cement roofing (mortar mix coated). But these materials must be imported and careful cost comparisons based on oyster production on each material will have to be made.

There were 2 groups of mortar mix collectors hung from the prototype raft. The first was set out on September 11 and the second on September 18, 1975. The growth and estimated numbers of the mangrove oyster are shown in Table I.

TABLE I

Growth of the mangrove oyster as average shell height of a sample of 30 oysters and estimated numbers of oysters per collector. Group A set out in Salt River on Sept. 11, and Group B on Sept. 18, 1975.

	Group A		Group B	
Date	No./collector	Ave. height (mm)	No./collector	Ave. height (mm)
10/10	470 ± 22	4 ± 1		
10/24	656 ± 24	12 ± 2		
11/7	838 ± 10	19 ± 3	232 ± 18	12 ± 2
11/21	768 ± 16	32 ± 2	234 ± 8	17 ± 4
12/4	592 ± 14	39 ± 3	172 ± 2	35 ± 3

Besides individual differences in growth, variability in average shell height was increased by the continuous setting of mangrove oyster spat. Initially the collectors of September 18 had a light set, but improvement was noted in the latter part of November. However, these new oysters were not considered sufficiently developed to count and measure and are not included in Table I.

Preliminary hatchery trials have been inconclusive to date. The Japanese oyster, C. gigas, set lightly on the mortar mix cultch, but was completely absent from the tire rims and Vexar plastic mesh. The Kumamoto variety of C. gigas failed to set.

The Japanese oysters set on or shortly after September 20, 1975, and were fed on phytoplankton species STX S1 and STX 114, according to the availability of these species in reactor and carboy cultures. On November 10, the collectors bearing these hatchery produced Japanese oysters were set out in Salt River Inlet. Their growth rate is shown in Table II.

TABLE II

Growth of juvenile Japanese oysters (C. gigas) set in the hatchery approx. Sept. 20, reared in the hatchery until 11/10 and in Salt River from 11/10 to 11/21.

Date	Ave. shell height (mm)	Sample size
10/3	2	95
11/3	7	245
11/10	13	274
11/21	23	246

Initial growth and survival has been good, with mortality confined to the smaller oysters.

GROWTH OF CLAM AND OYSTER LARVAE ON DIFFERENT ALGAL DIETS IN A TROPICAL ARTIFICIAL UPWELLING MARICULTURE SYSTEM[1]

Judith B. Sunderlin[2], Phyllis T. Baab[3] and Elaine M. Patry[3]

ABSTRACT

Feeding studies were conducted on the larvae of Tapes semidecussata Reeve, Kumamoto oysters, Ostrea edulis Linné and Crassostrea gigas Thunberg to evaluate different species of algae and their influence on larval growth and survival. The phytoplankton used in the studies were: Isochrysis galbana, Monochrysis lutheri, Bellerochea polymorpha, Thalassiosira pseudonana, and two unknown Cryptophyte flagellates (STX-157 and S-1). Larvae were obtained from the spawning of adult brood stock in the controlled environment of the artificial upwelling mariculture system in St. Croix, U.S. Virgin Islands. Salinity was 34.75 to 34.95°/oo and the water temperature varied between 23 and 28 C during the experiments.

Tapes semidecussata larvae could be successfully reared through metamorphosis on algal diets suited for growth in the tropical mariculture system on St. Croix. Mixtures of algal diets were better for growth and survival of Kumamoto larvae than unialgal diets. No significant information could be obtained from the Ostrea edulis study due to poor survival of larval cultures which may have been caused by high temperatures.

---

[1]This work was supported by NOAA Sea Grant 04-5-158-59 from the U.S. Department of Commerce. Lamont-Doherty Geological Observatory Contribution No. 0000.

[2]Lamont-Doherty Geological Observatory of Columbia University, Artificial Upwelling Project, P.O. Box Z, Kingshill, St. Croix, U.S. Virgin Islands 00850.

[3]Hood College, Frederick, Maryland 21701.

Crassostrea gigas larvae grew well on a mixed diet and survival was significantly higher than that for the Kumamoto oyster larvae.

INTRODUCTION

The critical factor in determining the success or failure of a pelecypod shellfish hatchery is water quality. In most hatcheries surface seawater must be filtered or centrifuged to remove undesirable organisms and, in most cases, after filtration, the water should be sterilized. Water quality varies seasonally; when certain dinoflagellate blooms are present, the water may be unfit for bivalve larval culture (Bardach et al., 1972; Chanley, 1975).

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 are grown as food for filter-feeding shellfish in a controlled food chain. Dissolved nitrates, phosphates, silicates, and other nutrients which are essential for algal growth, are abundant in the artificially upwelled water. Nutrient concentrations are stable and do not vary seasonally (Roels et al., 1975). The deep water is free of man-made pollutants, diseases, and predators harmful to shellfish. Therefore, the water quality of the St. Croix system is advantageous for a pelecypod shellfish hatchery.

After water quality, the next important factor is the food supply for the bivalve larvae. Many hatcheries in the temperate region primarily utilize the natural populations of algae but make occasional use of pure algal cultures when light and temperature conditions do not support sufficiently dense cultures during the winter months. The advantage of one method over the other is not clear-cut. Natural algal populations contribute to a wide variety of food organisms (good and bad) in the

larval diet, whereas with pure algal cultures undesirable forms (Chlorella) can be screened out and readily digested forms (Chrysomonads) can be cultured (Bardach et al., 1972).

Literature on larval feeding studies, primarily conducted in temperate areas, reports that Isochrysis galbana, Monochrysis lutheri, Dunaliella euchlora, and Platymonas sp. in unialgal cultures and in mixtures are suitable foods for clam and oyster larvae (Davis and Guillard, 1958; Walne, 1963, 1966; and Loosanoff and Davis, 1963). High temperature is the limiting factor for growing these previously tested food organisms on a large scale in the tropics. In the St. Croix mariculture system the yearly temperature range for algal cultures is 22 to 30 C. Due to these temperatures and intense sunlight, the recommended algae cannot be cultured outdoors. However, in the airconditioned laboratory, small quantities can be grown.

Feeding experiments were designed to evaluate growth of clam and oyster larvae on species of algae that can be grown in large quantities in the St. Croix system. In many of the experiments larvae were fed control diets of Isochrysis galbana and Monochrysis lutheri to compare growth and survival on different diets.

This paper reports the results of controlled feeding studies on one species of clam larvae and three species of oyster larvae: Tapes semidecussata; Kumamoto oyster (a gigas type); Ostrea edulis; and Crassostrea gigas.

MATERIALS AND METHODS

Brood stock from the St. Croix mariculture system were used to produce the Tapes semidecussata, Kumamoto, and Crassostrea gigas larvae. The Tapes and C. gigas were originally purchased as 3-mm juveniles from

Pacific Mariculture, Inc., Pescadero, California. Juvenile Kumamoto oysters were purchased from Bay Center Mariculture Co., Willapa Bay, Washington. All three species were grown to market size in the St. Croix system before they were spawned in the hatchery. Mature Ostrea edulis were imported from Maine (Maine Coast Oyster Corp., Blue Hill, Maine). The following technique was used to induce spawning in all four species.

Adult shellfish were placed in Pyrex spawning dishes (1.4 liter) filled with algal culture, and deep water (22 to 24 C) was circulated around the spawning dishes one-half hour prior to thermal and chemical stimulation. To induce spawning, hot deep water was circulated through the spawning table and within 10 minutes, 32 C was reached in the Pyrex dishes. Immediately after reaching 32 C, cold deep water was circulated through the spawning table and in approximately 45 minutes the spawning dishes reached 22 to 24 C. At the high temperature range a stripped gonad solution (sperm or eggs) was added to each dish and after 45 minutes, if no spawning was observed, this thermal cycle was repeated. Deep water was heated in a glass-lined, gas-fired water heater.

T. semidecussata, Kumamoto oysters, and C. gigas require anywhere from one to three thermal shocks before releasing gametes. Only 5 to 10 ml of the sperm solution were used to fertilize the eggs. After two thermal shocks, small clumps of eggs were observed around several O. edulis and six days later swarming larvae were collected.

Duplicate larval cultures at the initial concentration of 10/ml were used for testing each diet. Either 15, 30 or 50 liter polyethylene containers were used for rearing the larvae. The C. gigas were also grown in a 379-liter polyethylene container. Larval cultures were continuously aerated throughout the experiments.

Every other day the larval cultures were filtered through a graduated series of three 25.4-cm diameter Nytex sieves (nylon-monofilament bolting cloth, Tobler, Ernst and Fraber, Inc., Elmsford, N.Y.). Clumped food and debris were trapped on the top sieve and discarded, while the larvae were collected on the bottom two sieves. The larvae were rinsed from the sieves and combined in a 3 to 4-liter concentrate. Sieve sizes were increased on the next filtration day if a large percentage (>90%) of larvae accumulated on the middle sieve.

A 15-ml sample was taken from each container after the concentrate was thoroughly randomized. After sampling, the proper diets were distributed, each container was filled to its respective volume with deep water, and 0.2 ml of Vet Strep (streptomycin sulfate, Merck and Co., Rahway, N.J.) per liter of larval culture was added. No quantitative measurements (cell counts or turbidities) were made on the algae in the larval cultures prior to filtering, however, a pale green color was observed in all larval containers indicating that food was still present. The initial number of cells/ml added to the larval container was determined either by cell counts or extrapolation from turbidity measurements. The initial food concentration in the larval cultures ranged from 8×10^4 to 2×10^5 cells/ml.

Data on larval growth and survival were obtained from the 15-ml sample taken after filtration. A Sedgwick-Rafter cell was used in determining the number of larvae per ml; duplicate or triplicate 1-ml aliquots of each sample were counted. At least 10 larvae from each sample were measured —length and width in microns (Loosanoff et al., 1966)—using an ocular micrometer.

During these experiments the water temperature in the larval cultures varied between 23 and 28 C and the salinity was 34.75 to 34.95 °/oo. The algae used in these studies were either cultured outdoors in 45,000-liter

ponds or in 2000-liter reactors or cultured in an airconditioned laboratory in 15-liter carboys. Only Isochrysis galbana, Monochrysis lutheri, and STX-157 were grown exclusively in the 15-liter carboys. Bellerochea polymorpha, Thalassiosira pseudonana, and S-1 were grown in large-scale cultures and used regularly for feeding shellfish in the mariculture system. Table 1 summarized the algal diets and the experimental conditions in each feeding study. Additional details of the St. Croix mariculture system are given in Babb et al. (1973).

RESULTS AND DISCUSSION

The feeding studies were terminated when the larvae began to set or when no survivors remained. Tapes semidecussata, Ostrea edulis, and Crassostrea gigas were successfully reared through metamorphosis; the Kumamoto larvae did not metamorphose. To prevent confusion, the results for each species will be discussed separately.

In the Tapes semidecussata feeding study, larvae fed mixtures of diatoms and flagellate(s) began to metamorphose on Days 14 to 16; those fed mixtures of only diatoms and mixtures of only flagellates had not attained setting size by that time. Of the four diets tested (Table 1), Diet I (diatoms only) produced the poorest survival and growth. When the experiment ended, only those larvae fed Diets II and IV had reached setting size of 220 to 280μ (Cahn, 1951; Tamura, 1970). The best survival was shown by those larvae on Diet II, consisting of T. pseudonana, B. polymorpha, and S-1, all algal species that can be grown in large-scale culture for the hatchery in St. Croix. Larvae fed Diet IV were not significantly larger than those fed Diet II. The growth and survival of Tapes semidecussata in this feeding study are given in Figure 1 and Table 3, respectively.

Tapes semidecussata has a high tolerance to temperature with a lower limit of 0 C and an upper limit of 36 C. The optimum temperature for ciliary movement of the gills is 23.4 C (Cahn, 1951; Tamura, 1970). The yearly temperature range in St. Croix (22 to 30 C) is within the tolerance limitations.

Over the past 18 months, seven batches of Tapes have been successfully reared from fertilized eggs to juveniles. Setting size was reached anywhere from Day 12 to 20 when a variety of foods were used throughout the larval period. The best diet found so far is T. pseudonana, B. polymorpha, and S-1, fed as a mixture. When these algal species are fed singly or in combinations of two but not as a mixture of three, the larval growth rate is reduced (Fig. 2).

Five batches of Kumamoto larvae have been spawned and reared in the St. Croix system but none of the larvae have successfully completed metamorphosis. At first, it was believed that food was the major problem, since the first two batches were fed only diatoms (T. pseudonana and B. polymorpha). Two additional experiments were conducted and the larvae were fed these two diatoms and the flagellate S-1, but again, no oyster spat were obtained. The feeding study reported in this paper was designed to test growth and survival of Kumamoto larvae fed unialgal cultures and one mixture (Table 1). All larvae fed unialgal cultures died on or before Day 22; larvae fed the mixture of B. polymorpha and S-1 were still alive on Day 35 but they never metamorphosed (Fig. 3). Even though Diet V (mixture) sustained a small number of larvae over a longer period of time, the survival rate (<1%) was not significantly different from that attained using the other diets. The results of this study indicate that food may be a cause of unsuccessful metamorphosis, but it also indicates that temperature could be the possible cause. The ambient temperature during

this last experiment was 27 to 28 C. Cahn (1950) reports the optimum temperature for development of C. gigas to be between 23 and 25 C.

An additional study on Kumamoto larvae was run to see if there was a difference between growth and survival of larvae cultured at 23 to 24 C and at 26 to 28 C (Table 2). All larvae were fed a mixture of two diatoms and one flagellate. Survival in the "cool" group was not significantly different from that in the "warm" group. The warm group reached setting size 3-5 days sooner than the cool group, but, again, neither group successfully metamorphosed.

Just before setting size was reached, a copper solution was added to obtain a concentration of 100 µg/l in one-half of both the warm and cool larval cultures (Prytherch, 1934). It was hoped that this treatment would help promote setting since deep water has a low concentration of metals. This copper addition appeared to have no effect one way or the other on survival or setting.

Future experiments are planned with Kumamoto larvae and such parameters as food, temperature, aeration, larval culture volume, and larval density will be investigated.

Since temperature was believed to be a problem in rearing Kumamoto oysters, the Ostrea edulis study was designed to include a temperature control. Half of the larvae were grown at ambient temperature (27 to 28 C) and the other half at 23 to 24 C in a deep-water cooled bath. Literature reports that 18 to 20 C is optimal for the setting of O. edulis (Loosanoff and Davis, 1963). The O. edulis larvae began to set on Days 20 to 22. Although less than 1% of the initial larvae set, 95% of the total spat were from the 23 to 24 C cultures.

Due to poor survival in all of the O. edulis cultures (<1%), it is difficult to determine which diet (Table 1) is best for growing this

species in the St. Croix hatchery. The lower temperature in the group fed Diet I may be related to the best larval survival (13.3%) and spat-fall, however, nothing can be concluded from this study. In the spring of 1973 a batch of O. edulis larvae was reared in the mariculture system. The larvae were fed only diatoms and the temperature was 27 to 29 C; these larvae failed to complete metamorphosis.

Further experimentation of O. edulis is planned and temperatures in the larval cultures will be kept at 18 to 20 C. This should reduce the influence of temperature on the feeding study.

Crassostrea gigas larvae, unlike the Kumamoto larvae, did complete metamorphosis in the St. Croix hatchery. Setting began on Day 22 in the 30-liter cultures but was delayed until Day 25 in the 379-liter culture (Fig. 4). The differences in growth rates can be explained by the larval densities. The 30-liter cultures had an initial concentration of 10 larvae/ml, whereas the 379-liter culture had a concentration of 30 larvae/ml. The denser culture sustained a higher survival (47%) than the culture with fewer larvae per ml (37%). The question is why did C. gigas, when reared on the same mixed diet of two diatoms and one flagellate, complete metamorphosis in the St. Croix hatchery and the Kumamoto larvae did not? Future experimentation will be formulated with this question in mind.

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. In: 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.

Cahn, A.R. 1950. Oyster culture in Japan. Department of the Interior, Fish and Wildlife Service. Fishery Leaflet 383, 80 pp.

Cahn, A.R. 1951. Clam culture in Japan. Department of the Interior, Fish and Wildlife Service. Fishery Leaflet FL-399, 103 pp.

Chanley, P. 1975. Laboratory cultivation of assorted bivalve mollusks. In: W.L. Smith and M.H. Chanley (eds.), Culture of marine invertebrate animals, Plenum Press, New York, 297-318.

Davis, H.C. and R.R. Guillard. 1958. Relative value of ten genera of microorganisms as food for oyster and clam larvae. U.S. Department of the Interior, Fishery Bulletin 136, 58:293-304.

Loosanoff, V.L. and H.C. Davis. 1963. Rearing of bivalve mollusks. In: F.S. Russell (ed.), Advances in marine biology, Academic Press, London, 1:1-136.

Loosanoff, V.L., H.C. Davis and P.E. Chanley. 1966. Dimensions and shapes of larvae of some marine bivalve mollusks. Malacologia 4(2):351-435.

Prytherch, H.F. 1934. The role of copper in the setting, metamorphosis, and distribution of the American oyster, Ostrea virginica. Ecological Monograph 4:47-107.

Roels, O.A., K.C. Haines, and J.B. Sunderlin, 1975. The potential yield of artificial upwelling mariculture. 10th European Symposium on Marine Biology, Ostend, Belgium, September 17-23.

Tamura, T. 1970. Marine aquaculture (translated from Japanese). National Sciece Foundation, Washington, D.C. PB 194 05IT.

Walne, P.R. 1963. Observations on the food value of seven species of algae to larvae of Ostrea edulis. I. Feeding experiments. Journal of the Marine Biological Association U.K. 43:767-784.

Walne, P.R. 1966. Experiments in the large-scale culture of the larvae of Ostrea edulis L. Fishery Investigations Series II, 25(4):1-53.

Table 1. Summary of algal diets and experimental conditions for the larval feeding studies

	Tapes semidecussata	Kumamoto oyster	*Ostrea edulis*	*Crassostrea gigas*
Diet I	3H and STX-114	S-1	3H and STX-114	3H, STX-114 and S-1
Diet II	3H, STX-114 and S-1	Iso	Iso and/or Mono, STX-157 and S-1	-
Diet III	Iso and/or Mono, STX-157 and S-1	Mono	3H, STX-114, Iso and/or Mono, STX-157 and S-1	-
Diet IV	3H, STX-114, Iso and/or Mono, STX-157 and S-1	STX-114	-	-
Diet V	-	S-1 and STX-114	-	-
Experimental conditions	Duplicate 15-ℓ cultures for each diet at ambient temperature (26-28 C)	Duplicate 15-ℓ cultures for each diet at ambient temperature (27-28 C)	Duplicate 15-ℓ cultures for each diet at cool temperature (23-24 C); 50-ℓ cultures for each diet at ambient temperature (27-28 C)	30-ℓ cultures with 10 larvae/ml; 379-ℓ culture with 30 larvae/ml at ambient temperature (25-26 C)

3H = *Thalassiosira pseudonana*; STX-114 = *Bellerochea polymorpha*; S-1 and STX-157 = undetermined Cryptophyte flagellates; Iso = *Isochrysis galbana*; Mono = *Monochrysis lutheri*

Table 2. Experimental design of additional Kumamoto feeding study

	Kumamoto oyster
Diet	Mixture of *Thalassiosira pseudonana*, *Bellerochea polymorpha*, and S-1
Experimental conditions	I. 15-ℓ cultures at cool temperature (23-24 C)
	II. 15-ℓ cultures at ambient temperature (26-28 C)
	III. Unfed control (15-ℓ culture)

Table 3. Survival for Tapes semidecussata larval feeding study

Time in days	Percent Survival			
	Diet I	Diet II	Diet III	Diet IV
3	76	79	81	91
6	29	74	69	61
8	14	58	54	47
12	---	40	44	30
14	1	32	34	18
16	1	34	19	18

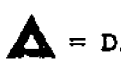


Figure 1. Average growth rate of Tapes semidecussata larvae reared on four different diets. = Diet I; = Diet II; = Diet III; = Diet IV (see Table 1 for key to diets).

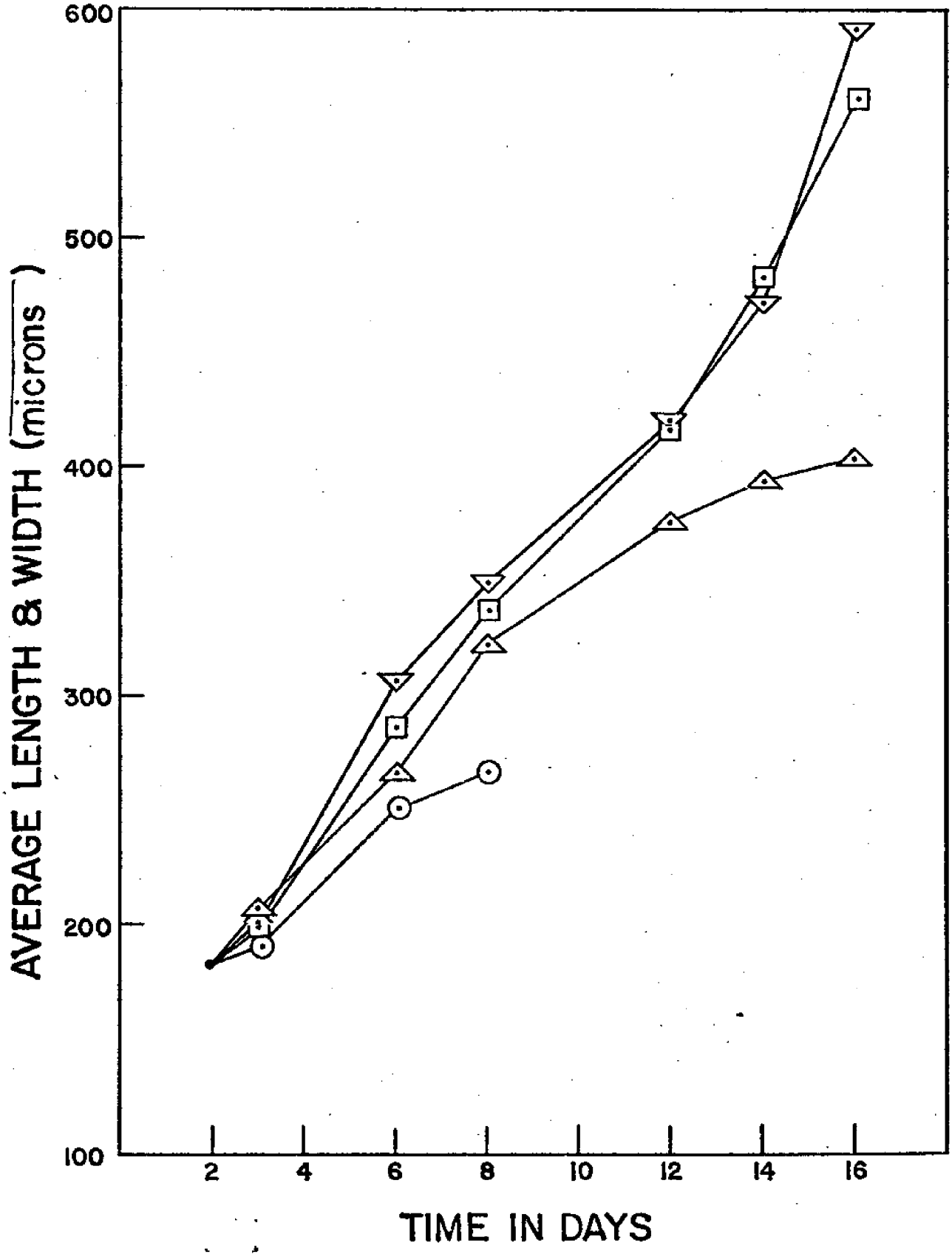

600
500
400
300
200
100
AVERAGE LENGTH & WIDTH (microns)
2
4
6
8
10
12
14
16
TIME IN DAYS

Figure 2. Average growth of Tapes semidecussata larvae.

○ = mixture of three algal species (3H, STX-114 and S-1);

□ = the same algal cultures, fed singly or in combinations of two, but never as a mixture of three.

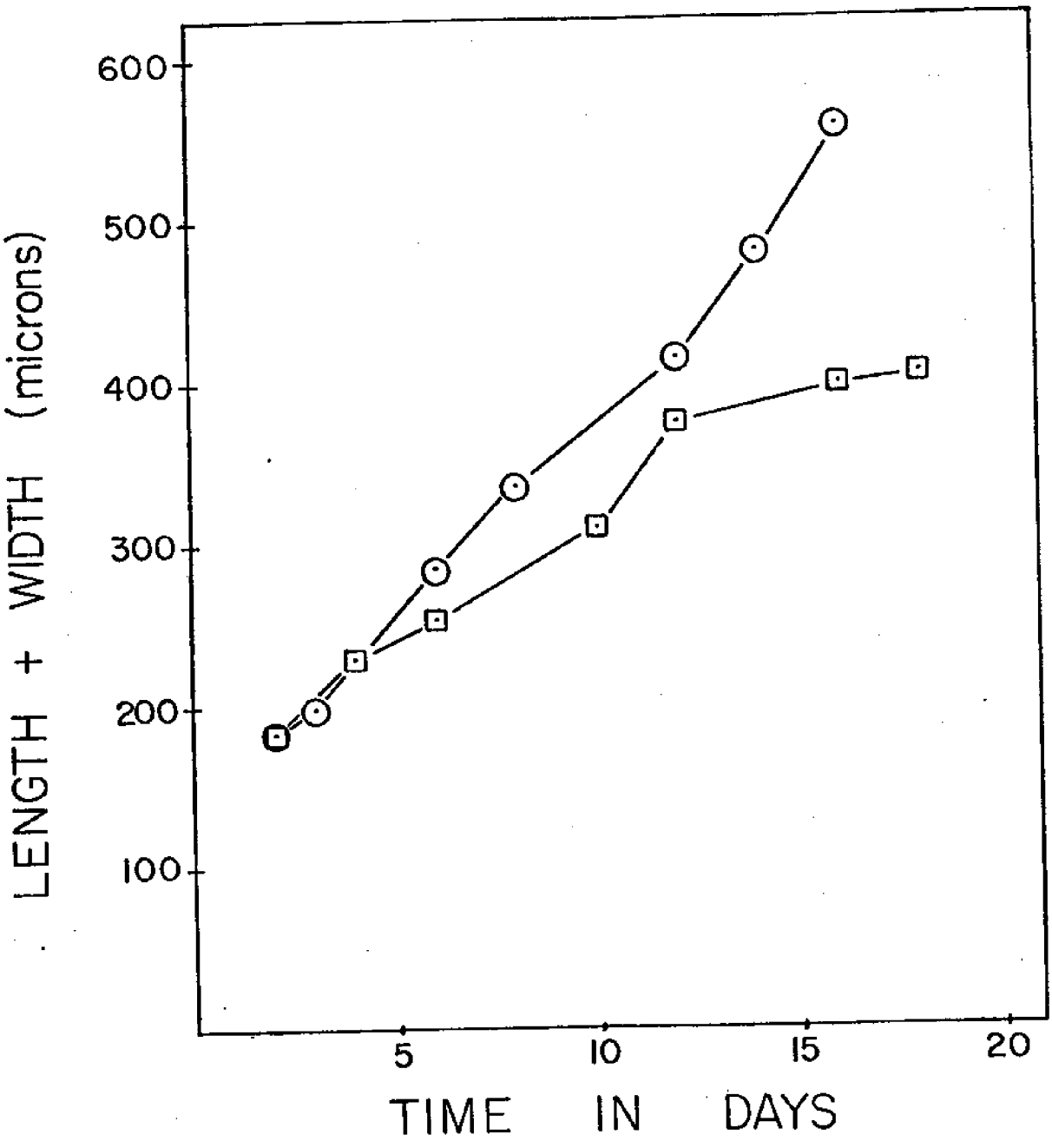
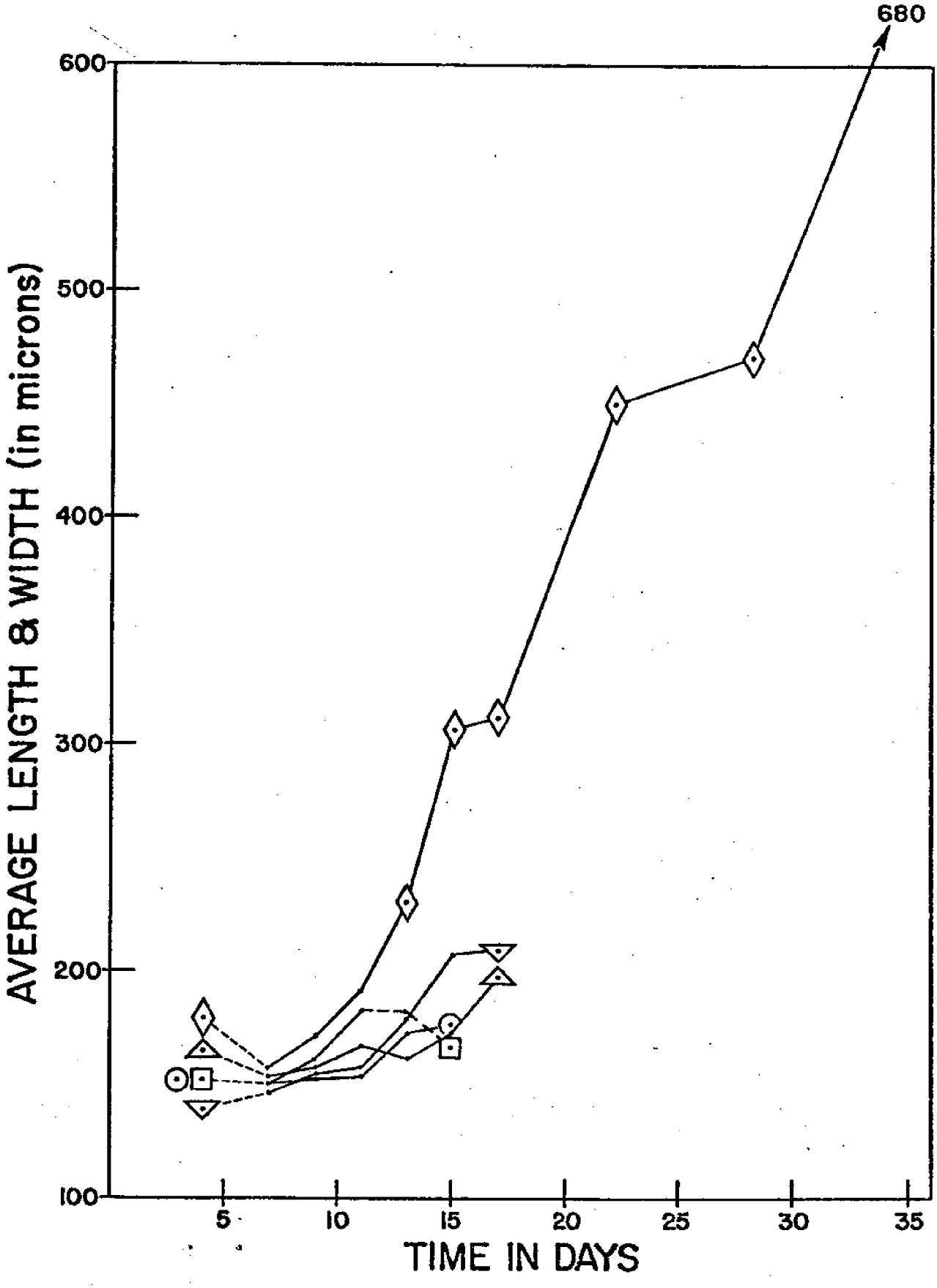

600
500
400
300
200
100
LENGTH + WIDTH (microns)
5
10
15
20
TIME IN DAYS

Figure 3. Average growth rate of Kumamoto oyster larvae reared on five different diets. □ = Diet I; △ = Diet II; ▽ = Diet III; ○ = Diet IV; ◇ = Diet V (see Table 1 for key to diets).


680
600
500
400
300
200
100
AVERAGE LENGTH & WIDTH (in microns)
5
10
15
20
25
30
35
TIME IN DAYS

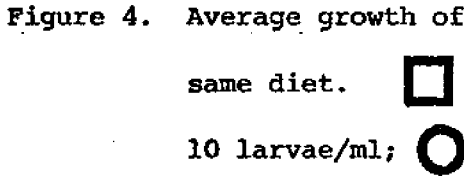
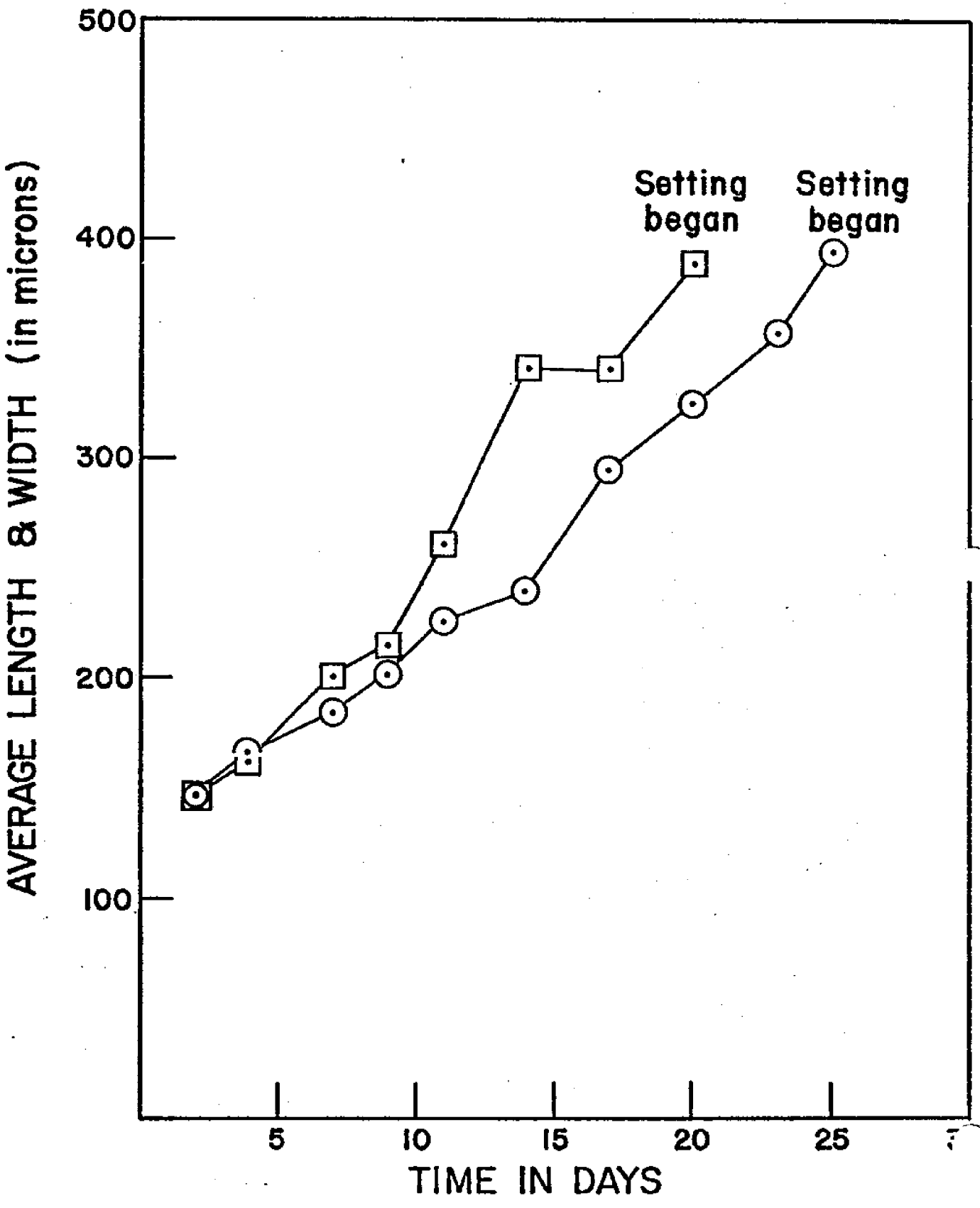


Figure 4. Average growth of Crassostrea gigas larvae reared on the same diet. □ = 30-liter cultures with a density of 10 larvae/ml; ○ = 379-liter culture with a density of 30 larvae/ml.


500
400
300
200
100
AVERAGE LENGTH & WIDTH (in microns)
Setting began
Setting began
5
10
15
20
25
TIME IN DAYS

NUTRIENTS AND SALINITY DATA FOR DEEP-WATER PIPELINES

DATE	SAMPLE STX 1085	SALINITY (°/oo±°/oo)	$NO_3 + NO_2$ (µg-at N/l)	NO_2 (µg-at N/l)	NH_3 (µg-at N/l)	PO_4 (µg-at P/l)	SiO_4 (µg-at Si/l)	PIPE
1974								
12/19	724	34.936	28.63	.17±.01	1.18±.03	1.71	17.51±.134	1
	725	35.049	28.99	.13	.72	1.59	16.44	1
	726	34.826	30.45	.18	.80	1.75	21.17	2
	727	34.933	30.36	.18	.81	1.82	21.03	2
	728	34.920	29.70	.18	.79	1.63	17.77	3
	729	34.891	29.91	.18	.98	1.65	16.94	3
12/26	730	34.999	*25.29±.169	.23±.01	2.43±.03	1.81±.019	17.24±.134	1
	731	34.999	*25.77	.18	.79	1.81	17.47	1
	732	34.970	31.03	.21	1.16	2.02	18.33	2
	733	34.942	31.17	.21	.71	1.96	21.21	2
	734	34.942	30.39	.23	1.43	1.91	15.89	3
	735	34.956	30.98	.23	1.63	1.86	15.62	3
1975								
01/02	NO SAMPLES TAKEN							
01/09	736	34.880	29.23±.169	.23±.04	1.69±.21	1.99±.019	18.6 ±.33	1
	737	34.909	29.89±.21	.28	2.02	1.88±.027	18.0	1
	738	34.794	30.81	.28	1.47	1.98	22.6	2
	739	34.808	31.27	.22	1.38	1.96	22.8	2
	740	34.880	31.79	.31	1.63	2.14	19.9	3
	741	34.851	30.76	.27	1.49	2.00	18.3	3
01/16	742	34.894	31.38±.21	.26±.04	1.50±.21	1.99±.027	19.2 ±.33	1
	743	34.894	31.79	.26	1.78	2.01	19.2	1
	744	*37.794	33.02	.22	1.10	2.09	23.4	2
	745	*37.794	32.81	.29	1.50	2.09	22.7	2
	746	34.880	31.79	.22	1.41	2.03	21.2	3
	747	34.909	29.45	.30	1.54	1.88	20.0	3
01/23	748	34.923	30.62±.21	.26±.04	1.06±.21	2.00±.027	21.6 ±.33	1
	749	34.851	31.43	.33	1.56	2.01	20.5	1
	750	34.794	32.04	.27	1.56	2.09	22.8	2
	751	34.794	32.25	SAMPLE---------	MISSING	2.10	SAMPLE MISSING	2
	752	34.892	31.89	.22	1.43	1.95	21.7	3
	753	34.906	31.33	.20	1.21	1.99	21.7	3

*samples omitted from range.

DATE	SAMPLE STX 1085	SALINITY (°/oo±°/oo)	$NO_3 + NO_2$ (µg-at N/l)	NO_2 (µg-at N/l)	NH_3 (µg-at N/l)	PO_4 (µg-at P/l)	SiO_4 (µg-at Si/l)	PIPE
1975								
01/30	754	*35.151	26.91±.21	.21±.04	1.36±.21	1.55±.027	19.4± .33	1
	755	*35.108	22.75	.37	2.09	1.98	19.8	1
	756	34.806	31.93	.27	1.04	1.99	22.3	2
	757	34.806	32.09	.23	1.57	2.07	23.4	2
	758	*34.921	26.91	.24	1.30	1.96	22.8	3
	759	34.892	31.43	26	1.20	1.97	16.8	3
02/07	760	34.921	31.63±.21	.23±.04	1.59±.21	1.97±.027	20.6± .33	1
	761	34.906	31.93	.22	1.52	2.02	18.8	1
	762	34.835	32.34	.25	.77	2.07	20.9	2
	763	34.835	32.29	.24	1.30	2.01	21.7	2
	764	34.906	31.83	.26	1.64	1.98	13.0	3
	765	34.921	31.93	.23	2.34	1.98	18.6	3
02/14	NO SAMPLES TAKEN							
02/21	766	35.024	29.77±.21	.25±.04	1.27±.21	1.80±.027	18.4±.33	1
	767	35.024	30.58	.29	1.40	1.38	19.2	1
	768	34.880	33.00	.24	1.39	2.03	21.1	2
	769	34.851	32.70	.23	1.97	1.99	22.2	2
	770	34.851	31.84	.26	1.92	1.94	20.8	3
	771	34.880	31.94	.24	1.38	1.96	15.9	3
02/28	772	34.880	32.04±.21	.27±.04	1.56±.21	1.96±.027	20.6± .33	1
	773	34.523	31.79	.26	1.21	1.90	18.7	1
	774	34.851	32.14	.24	1.12	1.97	19.5	2
	775	34.851	32.60	.26	1.49	1.95	22.3	2
	776	34.935	32.26	.23	1.32	1.94	20.0	3
	777	34.909	32.36	.29	1.47	2.02	19.0	3
03/07	778	34.854	31.80±.21	.29±.04	1.64±.21	1.99±.027	21.2± .33	1
	779	34.880	31.90	.27	1.00	1.92	20.4	1
	780	34.794	32.36	.24	1.37	2.01	21.0	2
	781	34.794	32.56	.22	1.13	2.01	25.4	2
	782	34.909	32.31	.24	1.68	1.90	19.3	3
	783	34.935	32.26	.26	1.96	1.85	21.8	3

*sample omitted from range.

DATE	SAMPLE STX 1085	SALINITY (°/oo±°/oo)	$NO_3 + NO_2$ (µg-at N/l)	NO_2 (µg-at N/l)	NH_3 (µg-at N/l)	PO_4 (µg-at P/l)	SiO_4 (µg-at Si/l)	PIPE
1975								
03/14	784	35.000	31.75±.21	.26±.04	1.48±.21	1.83±.027	19.6 ±.33	1
	785	34.966	31.50	.22	.95	1.85	18.8	1
	786	34.851	32.66	.35	1.32	2.01	21.9	2
	787	34.851	32.66	.28	1.41	2.06	21.1	2
	788	34.901	31.80	.26	1.49	2.03	19.7	3
	789	34.880	31.95	.19	.97	2.05	14.3	3
03/21	790	34.880	32.10±.21	.26±.04	1.43±.21	1.94±.027	23.3 ±.33	1
	791	34.880	32.20	.28	1.42	1.88	21.6	1
	792	34.935	29.19	.25	.99	1.97	23.3	2
	793	34.935	32.31	.20	1.23	1.97	22.2	2
	794	34.866	32.71	.40	.87	1.99	22.7	3
	795	34.880	32.81	.34	1.53	1.96	16.2	3
03/28	796	34.876	31.50±.21	.26±.01	1.09±.03	1.96±.019	18.65±.134	1
	797	34.874	31.08±.169	.20	.80	2.02	18.53	1
	798	34.839	32.15	.16	.79	1.86	19.10	2
	799	35.034	29.46	.10	.92	1.99	17.44	2
	800	34.842	31.46	.22±.013	.74±.075	2.01	21.16±.088	3
	801	34.876	31.32	.22	.91	2.07	20.15	3
04/04	802	34.933	32.14±.050	.18±.013	.50±.075	1.87±.013	22.65±.088	1
	803	34.876	32.54	.19	.53	1.96	21.68	1
	804	34.905	32.57	.22	.48	1.96	22.13	2
	805	34.862	32.35	.18	.43	1.92	22.40	2
	806	34.905	32.39	.21	.49	2.09	22.08	3
	807	34.899	32.34	.22	.64	1.91	21.90	3
04/11	808	34.885	31.83±.050	.19±.013	.43±.075	1.84±.013	20.91±.088	1
	809	34.914	31.95	.20	.34	1.85	20.31	1
	810	34.856	32.08	.19	.22	1.88	23.72	2
	811	34.899	32.38	.20	.18	1.85	23.04	2
	812	34.914	32.16	.20	.25	1.84	21.30	3
	813	34.914	32.21	.23	.40	1.87	20.58	3

DATE	SAMPLE STX 1085	SALINITY (°/oo±°/oo)	$NO_3 + NO_2$ (µg-at N/l)	NO_2 (µg-at N/l)	NH_3 (µg-at N/l)	PO_4 (µg-at P/l)	SiO_4 (µg-at Si/l)	PIPE
1975								
04/18	814	35.014	30.74±.050	.24±.013	.42±.076	1.76±.013	18.80±.088	1
	815	35.014	30.74	.22	.27	1.76	18.58	1
	816	34.914	31.98	.24	.32	1.86	23.64	2
	817	34.942	32.20	.23	.30	1.91	22.30	2
	818	34.914	32.03	.25	.22	1.83	20.54	3
	819	34.885	31.68	.23	.26	1.78	20.24	3
04/25	820	34.998	30.78±.050	.19±.013	.47±.075	1.72±.013	18.84±.088	1
	821	34.984	30.51	.15	.21	1.73	19.25	1
	822	34.840	31.26	.21	.54	1.82	24.96	2
	823	34.883	31.35	.20	.42	1.81	22.97	2
	824	34.912	31.13	.21	.26	1.79	20.98	3
	825	34.912	31.44	.21	.29	1.77	20.27	3
05/02	826	34.969	30.21±.169	.10±.01	.47±.03	1.97±.019	16.76±.134	1
	827	34.969	29.96	.15	.55	1.99	14.32	1
	828	34.880	31.19	.10	.63	2.00	20.22	2
	829	34.912	31.34	.10	.70	1.97	20.57	2
	830	34.872	31.24	.16	.99	1.93	12.34	3
	831	34.900	30.95	.15	1.13	1.97	12.45	3
05/09	832	34.929	30.21±.169	.14±.01	.63±.03	1.92±.019	17.70	1
	833	34.929	30.50	.21	.88	2.04	18.40	1
	834	34.828	31.34	.15	.84	1.97	20.66	2
	835	34.900	30.66	.10	.54	1.99	20.53	2
	836	34.900	30.91	.13	.72	1.93	13.95	3
	837	34.857	30.91	.13	1.04	2.00	15.60	3
05/15	838	34.872	29.18±.169	.12±.01	.63±.03	1.93±.019	19.00±.15	1
	839	34.900	31.15	.13	.53	1.97	18.58	1
	840	34.843	31.15	.10	.29	1.92	21.52	2
	841	34.830	31.06	.13	.66	2.00	21.26	2
	842	34.830	31.55	.16	.70	1.97	16.62	3
	843	34.845	31.36	.13	.53	2.02	19.30	3

DATE	SAMPLE STX 1085	SALINITY (°/oo±°/oo)	$NO_3 + NO_2$ (µg-at N/l)	NO_2 (µg-at N/l)	NH_3 (µg-at N/l)	PO_4 (µg-at P/l)	SiO_4 (µg-at Si/l)	PIPE
1975								
05/22	844	34.888	30.00±.169	.15±.01	.97±.03	1.86±.019	16.56±.15	1
	845	34.902	30.15	.13	.85	1.95	18.42	1
	846	34.816	32.05	.15	1.18	2.06	18.94	2
	847	34.830	31.76	.13	.62	2.04	20.12	2
	848	34.902	30.88	.17	.84	1.92	17.77	3
	849	34.931	31.03	.12	.48	2.02	18.29	3
05/29	850	34.859	32.00±.21	.10±.01	1.14±.03	2.10±.019	19.11±.15	1
	851	34.888	30.20	.14	.88	1.89	18.50	1
	852	34.888	29.60	.18	.71	2.04	20.71	2
	853	34.816	31.35	.12	.67	2.04	21.38	2
	854	34.831	31.35	.15	.70	2.06	19.68	3
	855	34.845	31.10	.14	.63	1.96	20.00	3
06/05	856	34.899	31.14±.08	.16±.01	.76±.03	1.86	19.32±.15	1
	857	34.913	31.39	.11	.51	1.84	20.35	1
	858	34.899	32.05	.12	.63	1.95±.02	21.69	2
	859	34.884	32.15	.10	.53	1.95	21.85	2
	860	34.913	32.50	.10	.57	1.98	20.67	3
	861	34.913	32.14	.10	.45	1.92	20.16	3
06/12	862	34.956	31.44±.08	.15±.01	.76±.01	1.91±.02	20.24±.03	1
	863	34.970	31.29	.18	1.56	1.91	21.24	1
	864	34.884	32.04	.18	.91	1.92	26.43	2
	865	34.884	31.55	.17	.87	1.94	23.60	2
	866	34.884	32.05	.15	.84	1.94	24.68	3
	867	34.884	32.10	.17	.62	1.96	22.18	3
06/19	868	34.942	31.40±.08	.13±.01	.90±.01	1.87±.02	22.08±.03	1
	869	34.942	31.44	.18	.74	1.88	21.14	1
	870	34.913	32.14	.23	.87	1.98	26.63	2
	871	34.942	32.34	.22	1.74	1.99	25.60	2
	872	34.913	32.14	.21	.87	1.99	22.92	3
	873	34.942	32.24	.21	.74	1.96	23.06	3

DATE	SAMPLE STX 1085	SALINITY (°/oo±°/oo)	$NO_3 + NO_2$ (µg-at N/l)	NO_2 (µg-at N/l)	NH_3 (µg-at N/l)	PO_4 (µg-at P/l)	SiO_4 (µg-at Si/l)	PIPE
1975								
06/26	874	34.944±.011	31.46±.08	.25±.01	1.39±.01	1.87±.03	19.35±.03	1
	875	34.944	31.46	.20	.83	1.89	19.92	1
	876	34.915	31.99	.17	.79	1.93	19.73	2
	877	34.944	31.99	.18	.91	1.93	17.38	2
	878	35.058	31.46	.16	.66	1.92	20.34	3
	879	34.958	31.41	.16	.81	1.87	22.12	3
07/04	880	34.986±.011	29.61±.16	.20±.01	.81±.01	1.75±.03	18.94±.03	1
	881	34.915	30.07	.20	.97	1.75	20.34	1
	882	35.001	30.12	.21	.97	1.78	21.79	2
	883	34.944	30.46	.17	.62	1.74	23.15	2
	884	34.972	29.83	.21	.89	1.76	20.95	3
	885	34.900	29.83	.21	.89	1.69	20.90	3
07/11	886	34.900±.011	29.73±.16	.16±.01	.50±.01	1.66±.03	21.70±.03	1
	887	34.957	29.83	.15	.62	1.68	21.37	1
	888	34.900	31.00	.15	.58	1.84	23.94	2
	889	34.900	30.81	.19	.77	1.82	22.18	2
	890	34.986	31.05	.15	.52	1.77	18.76	3
	891	35.157	31.05	.13	.46	1.78	22.60	3
07/17	892	34.929±.011	30.81±.16	.13±.01	.50±.01	1.77±.03	21.15±.03	1
	893	34.529	29.14	.18	.73	1.65	19.51	1
	894	34.900	31.15	.17	.78	1.80	21.34	2
	895	34.900	31.44	.16	.65	1.88	24.56	2
	896	34.815	31.54	.15	.63	1.89	21.85	3
	897	34.872	31.39	.15	.56	1.90	21.29	3
07/24	898	34.900±.011	31.30±.16	.11±.01	.50±.01	1.81±.03	20.26±.03	1
	899	34.986	31.10	.14	.59	1.83	18.49	1
	900	34.900	32.47	.15	.57	1.98	19.57	2
	901	34.900	32.27	.11	.42	1.95	22.47	2
	902	34.900	31.88	.12	.65	1.89	19.52	3
	903	34.900	31.78	.10	.73	1.91	19.99	3

DATE	SAMPLE STX 1085	SALINITY (°/oo±°/oo)	$NO_3 + NO_2$ (µg-at N/l)	NO_2 (µg-at N/l)	NH_3 (µg-at N/l)	PO_4 (µg-at P/l)	SiO_4 (µg-at Si/l)	PIPE
1975								
07/31	904	34.929±.011	31.64±.16	.17±.01	.33±.05	1.82±.03	19.35±.10	1
	905	34.929	31.74	.18	.77	1.87	19.02	1
	906	34.900	32.42	.18	.39	1.92	21.50	2
	907	34.900	32.32	.15	.30	1.94	17.10	2
	908	34.900	32.03	.18	.41	1.89	19.35	3
	909	34.929	32.15	.17	.35	1.89	19.21	3
08/07	910	34.843±.011	31.80±.16	.18±.01	.65±.05	1.88±.03	18.27±.10	1
	911	34.843	31.66	.18	.46	1.87	18.08	1
	912	34.986	32.29	.18	.39	1.94	20.15	2
	913	34.957	32.29	.18	.46	1.94	16.40	2
	914	34.929	32.29	.17	.42	1.95	18.52	3
	915	34.957	32.49	.17	.53	1.95	18.34	3
08/15	916	35.014±.00	30.75±.04	.17±.01	.49±.05	1.79±.03	17.12±.10	1
	917	34.957	31.00	.17	.53	1.77	17.03	1
	918	34.943	31.58	.17	.53	1.87	20.82	2
	919	34.957	31.38	.16	.53	1.87	20.72	2
	920	34.900	31.88	.17	.45	1.88	18.48	3
	921	34.900	31.88	.17	.50	1.88	19.27	3
08/21	922	34.900±.011	31.13±.04	.17±.01	.36±.05	1.80±.03	20.07±.10	1
	923	34.900	31.18	.16	.43	1.88	17.40	1
	924	34.929	31.98	.13	.39	1.89	20.22	2
	925	34.929	31.98	.18	.44	1.93	20.17	2
	926	34.929	31.73	.19	.51	1.87	18.21	3
	927	34.943	31.79	.19	.53	1.84	17.33	3
08/28	928	34.872±.011	31.26±.14	.15±.01	1.22±.05	1.98±.02	17.66±.09	1
	929	34.900	30.97	.19	.25	1.91	15.07	1
	930	34.900	31.61	.10	.42	2.08	20.49	2
	931	34.929	31.84	.10	.45	1.95	17.33	2
	932	34.815	31.55	.21	.45	1.67	15.68	3
	933	34.872	31.43	.17	.71	2.01	16.49	3

DATE	SAMPLE STX 1085	SALINITY (°/oo±°/oo)	$NO_3 + NO_2$ (µg-at N/l)	NO_2 (µg-at N/l)	NH_3 (µg-at N/l)	PO_4 (µg-at P/l)	SiO_4 (µg-at Si/l)	PIPE
1975								
09/04	934	34.886±.011	30.80±.14	.19±.01	.31±.05	1.94±.02	16.01±.09	1
	935	34.914	30.80	.24	.35	1.90	16.96	1
	936	34.886	31.84	.21	.31	1.98	20.96	2
	937	34.886	32.53	.20	.35	1.98	22.89	2
	938	34.800	31.84	.12	.39	2.00	24.95	3
	939	34.886	31.55	.15	.43	2.00	23.87	3
09/11	940	34.871±.011	30.80±.14	.21±.01	.39±.05	2.07±.02	21.66±.09	1
	941	34.871	30.97	.26	.35	2.10	21.99	1
	942	34.857	32.13	.23	.35	2.14	21.66	2
	943	34.886	32.13	.16	.39	1.98	26.36	2
	944	34.943	31.72	.17	.32	2.10	22.36	3
	945	34.914	31.38	.18	.32	2.02	22.83	3
09/18	946	34.971±.011	31.26±.14	.13±.01	.32±.05	1.91±.02	21.28±.09	1
	947	34.971	31.26	.21	.32	1.97	18.60	1
	948	34.914	32.37	.18	.39	2.06	17.41	2
	949	34.928	32.13	.15	.53	1.97	24.71	2
	950	34.886	31.85	.13	.60	2.03	17.67	3
	951	34.914	31.56	.23	.67	1.97	18.62	3
09/25	952	34.857±.011	31.38±.14	.11±.003	.34±.05	1.94±.02	20.86±.08	1
	953	34.886	31.56	.10	.31	1.95	18.10	1
	954	34.928	31.85	.10	.30	2.00	22.97	2
	955	34.924	32.13	.11	.48	2.02	23.11	2
	956	34.909	31.85	.11	.51	2.00	17.58	3
	957	34.967	29.55	.11	.49	1.77	18.99	3
10/02	958	34.938±.011	31.29±.14	.12±.003	.55±.05	1.93±.02	20.27±.08	1
	959	34.967	31.29	.13	.58	1.85	23.17	1
	960	34.867	31.87	.11	.49	1.96	24.95	2
	961	34.867	31.75	.10	.33	2.07	28.32	2
	962	34.924	31.87	.13	.36	1.95	20.83	3
	963	34.924	31.98	.11	.58	1.98	20.03	3

DATE	SAMPLE STX 1085	SALINITY (°/oo±°/oo)	$NO_3 + NO_2$ (µg-at N/l)	NO_2 (µg-at N/l)	NH_3 (µg-at N/l)	PO_4 (µg-at P/l)	SiO_4 (µg-at Si/l)	PIPE
1975								
10/09	964	34.915	31.78±.23	.10±.003	.56±.05	1.99±.04	19.02±.05	1
	965	34.858	31.54	.10	.45	2.00	19.51	1
	966	34.829	31.92	.18	.53	2.09	20.98	2
	967	34.801	31.92	.08	.64	2.01	20.98	2
	968	34.829	31.78	.13	.48	2.02	19.27	3
	969	34.943	31.45	.10	.42	2.00	19.27	3
10/16	970	34.886	31.48±.23	.10±.003	.51±.05	2.00±.04	17.16±.08	1
	971	34.858	31.43	.10	.59	2.12	17.39	1
	972	34.743	31.66	.10	.68	2.07	15.66	2
	973	34.829	31.57	.11	.53	2.09	14.49	2
	974	34.858	32.14	.10	.32	2.03	11.59	3
	975	34.801	31.85	.10	1.14	2.06	14.59	3
10/23	976	34.872	32.11±.23	.18±.003	.55±.05	2.07±.04	20.46±.06	1
	977	34.872	32.11	.14	.65	2.06	20.46	1
	978	34.872	32.11	.16	.48	2.08	20.56	2
	979	34.872	32.20	.17	.50	1.99	20.51	2
	980	34.957	31.40	.20	.67	2.00	18.50	3
	981	34.986	31.26	.14	.33	2.10	18.50	3
10/30	982	34.886	32.33±.23			2.10±.04	20.77±.08	1
	983	34.915	32.24			2.10	21.24	1
	984	34.900	31.91			2.04	21.61	2
	985	34.915	32.10			2.09	23.71	2
	986	34.915	32.33			2.05	20.45	3
	987	34.915	31.95			2.04	20.74	3
11/09	988					1.97±.04	19.52±.09	1
	989					2.01	19.47	1
	990					2.07	21.10	2
	991		ANALYSES NOT COMPLETED			2.11	21.73	2
	992					1.96	18.46	3
	993					1.97	18.32	3

DATE	SAMPLE STX 1085	SALINITY (°/oo±°/oo)	$NO_3 + NO_2$ (µg-at N/l)	NO_2 (µg-at N/l)	NH_3 (µg-at N/l)	PO_4 (µg-at P/l)	SiO_4 (µg-at Si/l)	PIPE
1975								
11/13	994						20.93	1
	995						20.84	1
	996						21.30	2
	997			ANALYSES NOT COMPLETED			21.53	2
	998						22.13	3
	999						21.90	3

A COMPARISON OF TECHNIQUES FOR THE ESTIMATION OF PHYTOPLANKTON CULTURE DENSITY. I: TURBIDITY, IN VIVO FLUORESCENCE AND CELL COUNTS ON SELECTED UNIALGAL CULTURES

Scott Laurence

INTRODUCTION

Although the estimation of phytoplankton density can be accurately accomplished through the use of established cell-counting methods, this technique is time-consuming and hence inefficient. In preparation for the increasing number of cultures which must be examined on a daily schedule in our system, and with an eye towards the eventual establishment of a pilot or commercial-scale system in which the semicontinuous monitoring of many cultures will be necessary, a comparison of cell-counting, turbidity and in vivo fluorescent techniques for estimation of culture density was undertaken. Through this study we hoped to establish that turbidity and/or in vivo fluorescence could be used to monitor the density of unialgal cultures grown in our "reactor" system.

METHODS

Unialgal cultures of S-1, an unidentified naked Cryptophyte flagellate used for feeding larvae in our system, 3H (Thalassiosira pseudonana), STX-114 (Bellerochea polymorpha) and STX-167 (Chaetoceros curvisetus) were used for this study. All cultures were started by inoculating 500 liters of deep water contained in 2000-liter concrete "reactors" with two 16-liter carboys of unialgal culture grown in our laboratory.

Following inoculation, cell-counts, turbidity and in vivo

fluorescence were measured at 0800 hrs daily. Once each culture attained "peak" density (a species-specific level determined in prior work), the reactor was successively filled to one-half, full, and finally "activated." Each activated culture was diluted with 230 ml per 10 sec of deep water (one turnover day^{-1}). Flow rates were monitored at 0800 and 1400 hrs daily and adjusted if necessary.

Samples of each culture were collected in 400-ml "turbidity" bottles. Cell counts were performed using our standard procedures on a Speirs-Levy eosinophil counting chamber. All cell species were counted but only the main-species cell count was used for purposes of comparison. Turbidity readings were taken on samples in acetone-washed bottles with a Monitek turbidimeter, Model 250. After turbidity readings were taken, a sample was transferred to an acetone-washed cuvette and in vivo fluorescence was measured on a Turner Model III fluorometer utilizing window 1 (low sensitivity) for all readings.

Once all the data had been collected, linear regressions on the three pairs of data for each culture and for all cultures combined were calculated. Cell counts were calculated from raw data as $X \cdot 10^4$ (= number of cells per ml) so 10^4 was subtracted from each score for computation. Values used for turbidity and in vivo fluorescence were raw data values.

RESULTS

Table 1 illustrates the coefficient of determination (R^2) for each possible set of paired data points (turbidity:in vivo fluorescence, turbidity:cell counts, in vivo fluorescence:cell counts) for each unialgal culture and for all cultures combined.

Figures 1, 2, 3, 4 illustrate the change in culture density over time with the three techniques for the unialgal cultures.

DISCUSSION & CONCLUSIONS

For the purposes of this study, the cell-counting method was used as a "standard," and therefore the degree of linear correlation between turbidity or *in vivo* fluorescence and cell-counting results was used as a measure of the "accuracy" of these two methods.

It is clear that the coefficient of determination for sets of paired data are species-dependent. Very high correlations were obtained on all paired measures for the cultures of 3H and STX-114; coefficients are much lower for cultures of S-1, STX-167 and for all cultures combined. In most cases, turbidity correlates better with cell-count data than does *in vivo* fluorescence, while the coefficients of determination for turbidity:*in vivo* fluorescence measurements are quite high for each unialgal culture.

An examination of the figures and raw data reveals why coefficients are low for all cultures combined; clearly, there is no single relationship between cell counts, turbidity and *in vivo* fluorescence measurements that exists for all species. Note, for example, that *in vivo* fluorescence values are consistently higher than are turbidity or cell-count values for the culture of S-1, while fluorescence scores are consistently lower than the other two measures for the STX-114 culture. For 3H, fluorescense scores consistently occupy a mid-position. Correlations between cell count ratio and turbidity or *in vivo* fluorescence measurements are therefore species-dependent.

The relatively low coefficients of determination for clones S-1 and STX-167 are clearly due in part to the relative amount of algal contamination of these two cultures. The prevalence of dead cells during the final days of the 167-culture also lead to discrepancies between the three measures.

Linear regressions provide us with only a superficial estimate of the relative efficacy of using turbidimeter or *in vivo* fluorescence as an estimation of culture density. The true value of each technique must be examined in the light of the ability of the technique to provide information that is useful in monitoring the physiology and productivity of the culture. Regardless of the degree of linear correlation between the various measures, for example, all three indicate general levels of concentration for each species, and the general increase or decrease in cell concentration is usually followed by similar changes in turbidity or *in vivo* fluorescence measurements. Clearly, the relationships break down when unialgal cultures become contaminated or the number of dead cells increases dramatically. In these cases, there is no substitute for direct microscope inspection of the cultures.

In conclusion, both turbidity and *in vivo* fluorescence provide an adequate estimation of cell concentration and may be used to estimate productivity in unialgal cultures. Visual inspection of cultures is necessary unless species composition is unimportant. In general, turbidity, especially in view of the ease and speed with which this measurement can be taken, appears superior to *in vivo* fluorescence for daily use.

TABLE 1. COEFFICIENTS OF DETERMINATION (R^2)

TURBIDITY, IN VIVO FLUORESCENCE, CELL COUNTS

SPECIES	T:V	T:C	V:C
S-1	.84	.59	.49
(# of data points)	(8)	(8)	(8)
114	.86	.93	.93
(# of data points)	(9)	(9)	(9)
3H	.99	.95	.94
(# of data points)	(6)	(6)	(6)
167	.70	.36	.19
(# of data points)	(11)	(11)	(11)
ALL	.46	.65	.37
(# of data points)	(6)	(6)	(6)

T = turbidity; C = cell counts; V = in vivo fluorescence.

Figure 1. Comparison of techniques for the estimation of culture density: reactor #2, unidentified, naked Cryptophyte flagellate (S-1).

▲----- ▲ *in vivo* fluorescence

O----- O turbidity

●----- ● cell count

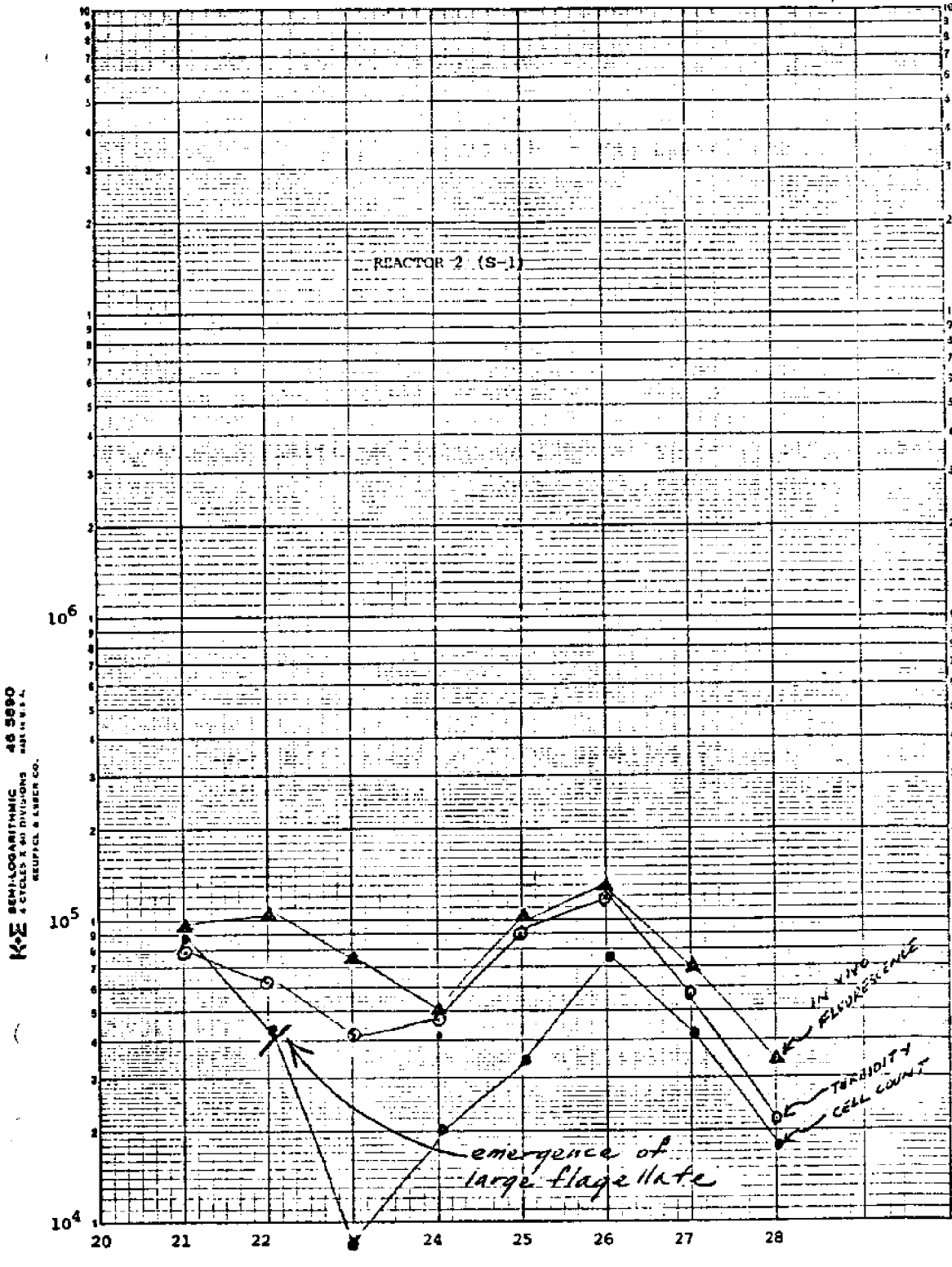

REACTOR 2 (S-1)
10^6
10^5
10^4
20
21
22
24
25
26
27
28
IN VIVO FLUORESCENCE
TURBIDITY
CELL COUNT
emergence of large flagellate

Figure 2. Comparison of techniques for the estimation of culture density: reactor #8, Bellerochea polymorpha (STX-114).

▲-----▲ in vivo fluorescence

O-----O turbidity

●-----● cell count

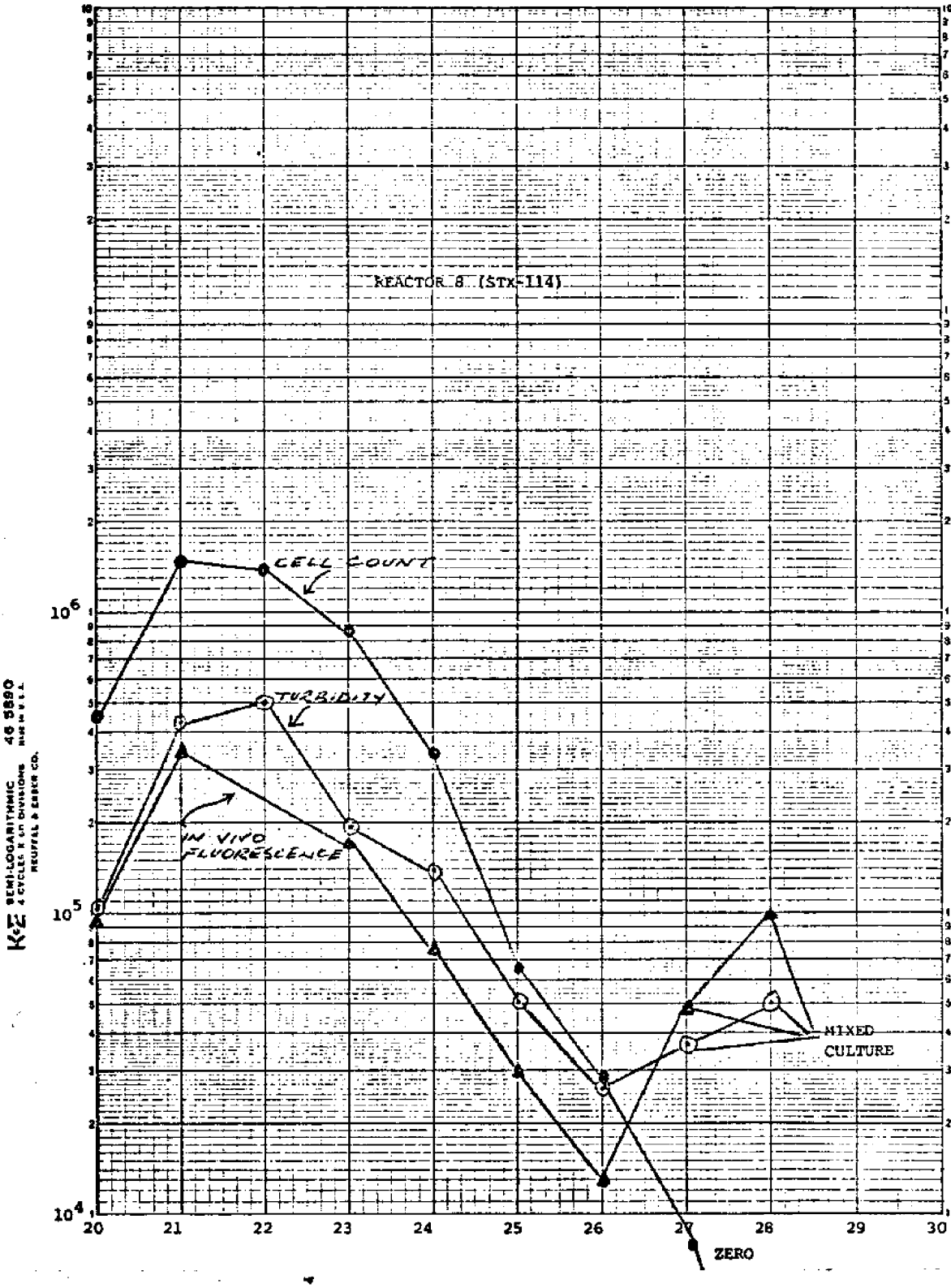

REACTOR 8 (STx-114)
CELL COUNT
TURBIDITY
IN VIVO FLUORESCENCE
MIXED CULTURE
ZERO
10^6
10^5
10^4
20
21
22
23
24
25
26
27
28
29
30
KEUFFEL & ESSER CO.
SEMI-LOGARITHMIC
4 CYCLES X 1 in DIVISIONS
46 5890

Figure 3. Comparison of techniques for the estimation of culture density: reactor #7, Thalassiosira pseudonana (3H).

▲ -----▲ in vivo fluorescence

○ -----○ turbidity

● -----● cell count

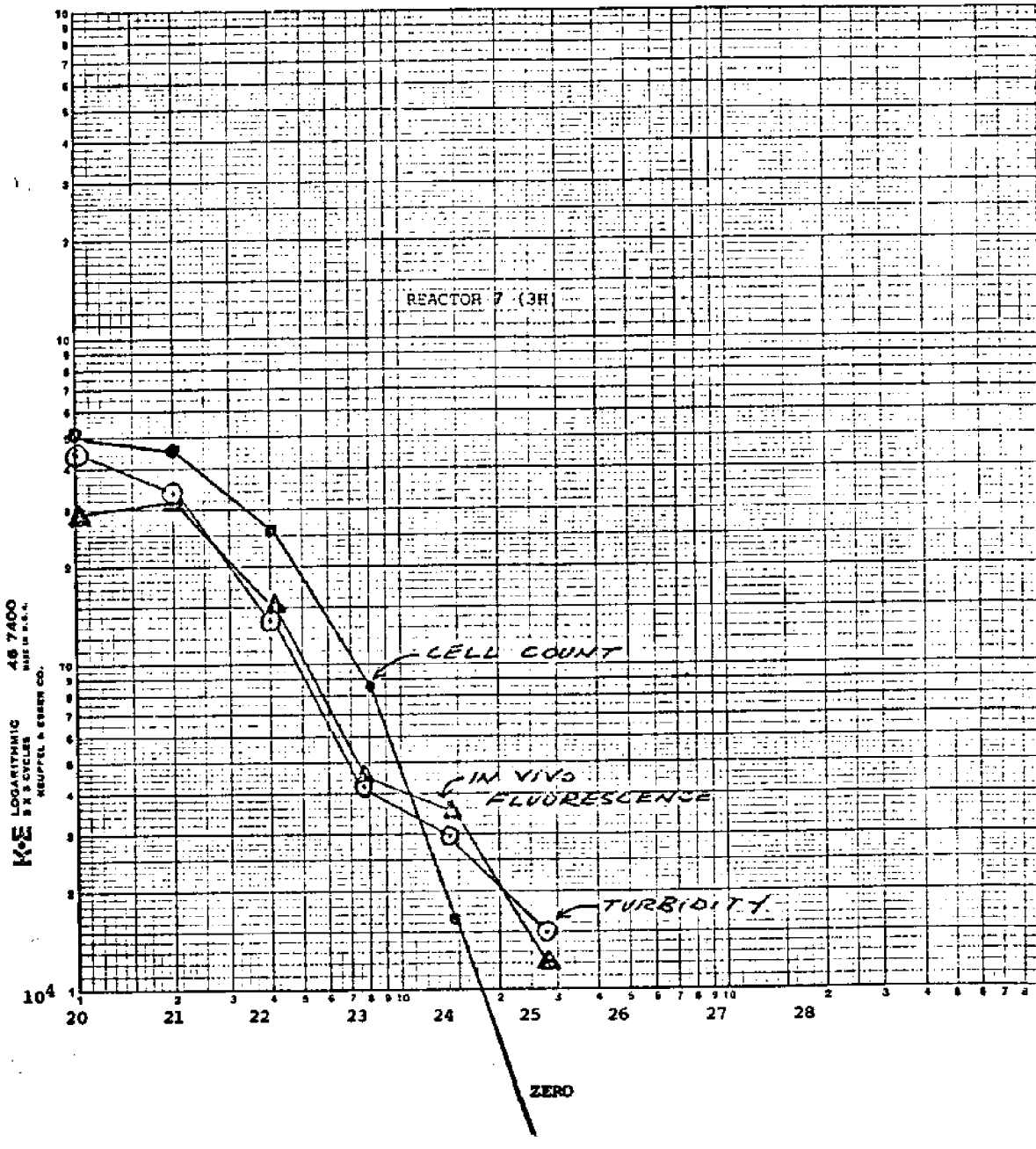
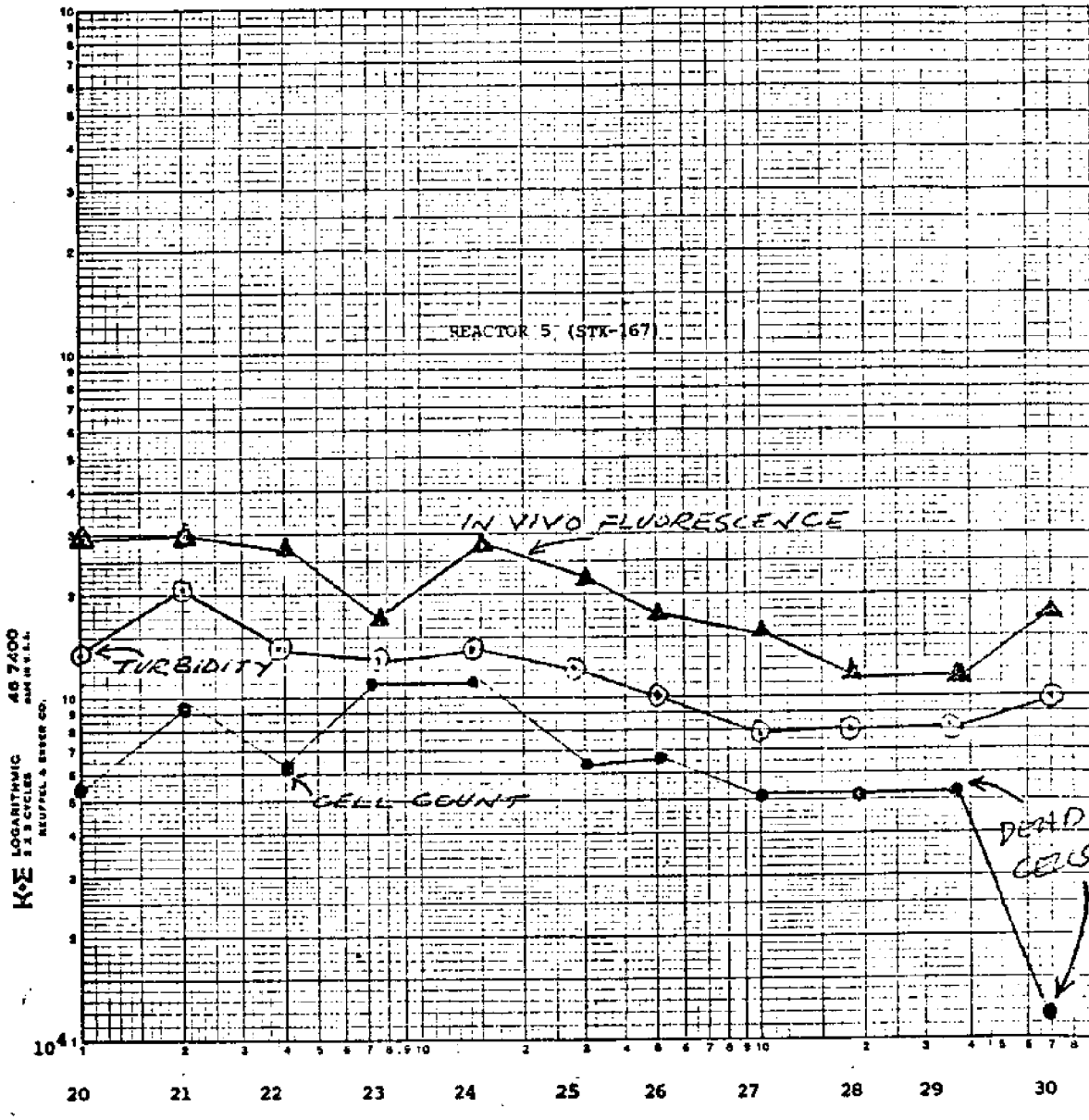

REACTOR 7 (3H)
CELL COUNT
IN VIVO FLUORESCENCE
TURBIDITY
ZERO
20
21
22
23
24
25
26
27
28
10^4
K&E LOGARITHMIC 46 7400
5 X 5 CYCLES
KEUFFEL & ESSER CO.

Figure 4. Comparison of techniques for the estimation of culture density: reactor #5, Chaetoceros curvisetus (STX-167).

▲ ----- ▲ in vivo fluorescence

○ ----- ○ turbidity

● ----- ● cell count


REACTOR 5 (STX-167)
IN VIVO FLUORESCENCE
TURBIDITY
CELL COUNT
DEAD CELLS
20
21
22
23
24
25
26
27
28
29
30

APPENDIX E

INTERACTION OF LIGHT AND NUTRIENTS ON GROWTH OF *CHAETOCEROS CURVISETUS* IN OUTDOOR CONTINUOUS CULTURE: I. CELLULAR CHARACTERISTICS

Mary W. Farmer

ABSTRACT

The effects of five different light intensities and four different nutrient regimes on the cellular composition of outdoor continuous cultures of Chaetoceros curvisetus were examined. Cellular nitrogen and carbon increased with increasing dilution rate at all light intensities. The N:C ratio increased with increasing growth rate in nitrogen-limited but not in light-limited cultures. Chlorophyll a per cell, per unit nitrogen, and per unit carbon increased with increasing dilution rate and with decreasing light intensity; these ratios demonstrated the greatest degree of diel periodicity. Interaction of effects of light and nutrients was seen on all ratios involving chlorophyll, but light and nutrients appeared to influence cellular carbon and nitrogen independently of each other.

INTRODUCTION

Good predictions of primary production in the world oceans can be made on the basis of nutrient concentration alone (Sverdrup, 1955) or of a combination of chlorophyll *a* and light intensity alone (Bannister, 1974). Yet it is well known that both light and the chemical character of available nitrogen affect the rate of uptake of nitrogen (Dugdale and MacIsaac, 1972; Bienfang, 1975), that the rate of uptake of the limiting nutrient affects the rate of uptake of other nutrients (Rhee, 1973; Droop, 1974, 1975), and that the rate of nutrient uptake affects the rate of growth.

When predictions of production need to be more precise than within an order of magnitude or two, such as when estimating effects of pollution or predicting food production within a given system, interactions of environmental factors need to be known.

The purpose of this study is to examine the interaction of effects of light and nutrients on the growth of a marine diatom. The results will be presented in three sections: (1) responses of cellular composition to light and nutrients; (2) rates of uptake and photosynthesis, and (3) diel periodicity and non-steady state conditions such as spring blooms. Paper I (responses of cellular composition to light and nutrients) only is included in this report.

Outdoor continuous cultures were used for this study because they provide an open system in which the cell history is completely known yet are subject to many of the varying conditions found in natural systems.

MATERIALS AND METHODS

Experiments were conducted in five culture vessels ("reactors") of 2-m^3 volume and 2.52-m^2 surface area. Antarctic Intermediate Water was pumped continuously from 870-m depth off the North Shore of St. Croix, U.S. Virgin Islands, for use as culture medium. The nutrient composition of the deep water is shown in Table 1.

The diatom cultured was Chaetoceros curvisetus Cleve, which had been isolated by K.C. Haines from depp water during a preliminary experiment in this study. The diatom was grown from stock for six days, with two transfers, in f/2 medium (Guillard and Ryther, 1962) in the laboratory before inoculation to 200-liter batch cultures of f/4 medium on the beach. After two to three days, when cell density in the f/4 medium was approximately 2 x 10^5 cells/ml, 20 liters of the batch culture were inoculated into 1000 liters of deep water. Inoculation was done at 1600 hr on Day 0 and the cultures were allowed to stand overnight. Deep-water inflow was begun at sunrise on Day 1 at a rate that would equal the dilution rate of the experiment when the reactors reached capacity.

Five cultures in each experiment were run at one dilution rate with each culture shaded to a different light intensity, as shown in Table 2. Shading was provided by neutral density screens that fit over the reactors. The screens were provided with sampling ports (designed by L.G. Aust). Shades were put on the reactors when continuous flow began.

A Weston photocell mounted in watertight polyvinyl-chloride housing was installed in each reactor and could be raised or lowered for surface and bottom light-meter readings (designed by C.L. Carson).

The first samples were taken at sunrise on Day 1, before the deep-water inflow was begun, and were tkane thereafter at sunrise and sunset every day for the duration of the experiment. Three-liter aliquots were taken from mid-depth of the culture for determination of cell number, chlorophyll a, dissolved nutrients (nitrate, nitrite, ammonia, phosphate and silicate), and particulate carbon and nitrogen analyses. Temperatures and light-meter readings were taken at each sampling time. A mechanical pyrheliograph recorded incident light intensity during the first two experiments. An Eppley black and white pyranometer was used for the last two experiments and the pyrheliograph was calibrated against the pyranometer.

Cell numbers were determined manually on samples preserved with Lugol's iodine using a eosinophil counter. Counting error by this technique was ±5%.

Chlorophyll a and phaeopigments were determined on a Turner Model 11 fluorometer by the method described by Strickland and Parsons (1972), but using glass-fiber filters rather than membrane filters. Precision (±2 SE) was 7%.

Analyses for dissolved nutrients were done with standard procedures on the Technicon AutoAnalyzer II and are described in detail in Paper II of this report.

Samples for particulate carbon and nitrogen analyses were filtered onto preheated glass-fiber filters and rinsed with 10 ml of isotonic sodium sulfate to remove salt. Filters were put in individual petri dishes and frozen until time of analysis. A Hewlett-Packard CHN gas-combustion analyzer was used for the determinations.

Growth rates were calculated as increase in biomass per unit biomass per day as follows:

$$\mu_x = \frac{x_1 = (x_0 - wx_0)}{x_0(t_1 - t_0)}$$

$$w = DR(t_1 - t_0)$$

where μ_x = specific growth rate in terms of biomass x (i.e., cell number, particulate carbon or nitroten); w = washout; DR = dilution rate of culture; t_1 and t_0 = time at sampling periods 1 and 0, respectively, expressed in units of days; x_1 and x_0 = biomass present at t_1 and t_0, respectively; and $(x_0 - wx_0)$ = biomass that would be present at t_1 if there were no growth between t_1 and t_0.

RESULTS

Mean temperatures of the cultures and light intensities reaching mid-depth of the cultures are given in Table 3. Extinction coefficients were significantly (r=0.87) correlated to the k calculated using chlorophyll a concentration found by Riley (1956)

$$k = 0.04 + 0.0088C + 0.054C^{2/3}$$

where C = chlorophyll a concentration.

All cultures in all experiments were in exponential growth when continuous flow began and the shades were applied. By Day 3 most cultures attained a mean biomass about which they oscillated, as exemplified by the standard deviations for cell number shown in Table 4. The oscillations were not consistently related to diel periodicity, although most biomass variations showed a diel trend such as was seen with cell number (Fig. 1). Cells growing at the lowest light and at faster dilution rates began to wash out after Day 3 (Fig. 1).

A spike in cell density appeared at sunrise of Day 2 in two experiments. It was caused by the presence of numerous small flagellates, perhaps gametes of the diatom, which persisted to some extent

throughtout the culture growing at the low dilution rate but which washed out of the faster culture. Some wall growth, especially Enteromorphora, appeared by Day 7 of all experiments.

Biomass values and cellular characteristics for each culture were averaged over the samples from the last three days of each experiment. These means, and the variations about these means, were used for statistical tests and all comparisons discussed in this paper (Table 5).

Two-way analyses of variance (ANOVA) demonstrated that both the intensity of light reaching the cultureand the dilution rate of the culture were associated with differences in biomass and that there was an interaction between the effects of light and dilution rate on concentration of chlorophyll a and particulate carbon and nitrogen but not on cell density. That is, the effect of light intensity of cell density was statistically the same at all dilution rates, and the effect of dilution rate on cell number was statistically the same at all light intensities. This observation is demonstrated in Figure 2, where the shapes of the curves for cell number are essentially the same even though the curves fall at different levels. The curves for chlorophyll a, POC, and PN, on the other hand, vary in shape according to the light intensity to which the culture had been exposed.

Cellular composition was also affected by both light intensity and dilution rate, according to the two-way ANOVA test (Fig. 3). There was an interaction of effects on chlorophyll a per cell but not on POC or PN per cell.

Total inorganic nitrogen input to the ractors was calculated; total inorganic nitrogen in the medium was subtracted from the input; the result (a) was compared to the CHN analysis for particulate nitrogen (b) (Table 7). If all inorganic nitrogen was incorporated into the cells,

then a should equal b. The only cultures in which a = b were the ones run at a dilution rate of 0.70. At a dilution rate of 0.25, a was greater than b and at the higher dilution rates, b was greater than a.

Specific growth rate of cultures was statistically the same whether measured by increase in particulate carbon, particulate nitrogen, or cell number (Table 6). Biomass was not related to growth rate, but all measures of cellular composition were related to growth rate (Table 6). Growth rates were approximately equal to dilution rate, as would be expected in chemostat culture, except in cultures growing at 0.25 dilutions per day or at 3% of incident light. Nitrogen, carbon, and chlorophyll a per cell increased with increasing growth rate; nitrogen and carbon per chlorophyll a and the C:N ratio decreased with increasing growth rate.

DISCUSSION

Response of biomass to dilution rate and incident light. One of the characteristics of chemostats containing unicellular algae is that biomass tends to decrease with increasing dilution rate (e.g., Droop, 1966, 1968; Herbert et al., 1956). The only cultures in the present study that demonstrated this trend for all measures of biomass were the cultures grown at 3% of incident light (Fig. 2). These continuous cultures were not chemostats by definition; that is, they were not growth-limited by any nutrient measured in the incoming medium. Specificially, nitrate was present in the reactors in concentrations from 7.29 to 28.3 μg-at liter^{-1}.

All indicators of biomass in the study tended to peak at the 0.70 dilution rate (with the exception of cultures grown at 3% of incident light) and then to follow the expected downward trend. It appears,

therefore, that the biomass was depressed at the 0.25 dilution rate. Some explanation for this depression might be found in connection with the findings shown in Table 7. If the theoretical particulate nitrogen were plotted against dilution rate, a hyperbola would be obtained for each of the three highest light intensities, and the points for the 0.25 dilution rate would fall much lower on the ordinate than the measured values do. The discrepancy might be explained by technical errors in either the CHN or the dissolved nitrogen analyses, but this possibility seems unlikely. Another possibility is that these cells began taking up dissolved organic nitrogen (which was not measured) from the deep water after the dissolved inorganic nitrogen was depleted. If so, then the substitution of organic for inorganic nitrogen could have led to a decrease in cell yield, a phenomenon that has been observed when ammonia is supplemented or replaced by nitrate in other continuous cultures (Sykes, 1975; Bienfang, 1975). To speculate further: a minimum amount of light may be needed for cells to take up organic nitrogen such as urea. Cells growing at low light intensity would not follow the pattern.

Above the 0.70 dilution rate, particulate nitrogen content was less than could be expected from the input and outflow of inorganic nitorgen. Two explanations are possible, other than the unlikely technical difficultires mentioned previously: the cells may have excreted organic nitrogen at the higher dilution rates or there may have been an increase in wall growth at the higher dilution rates. There is no evidence available to decide between these possibilities.

The statistical interaction between light and dilution rate on biomass reflects the fact that for carbon, nitrogen, and chlorophyll a per liter there are no trends with respect to light intensity that

apply at all light intensities. On the other hand, cell density decreased with decreasing light intensity at all dilution rates and, statistically at least, followed the same curve with increasing dilution rate at all light intensities.

Response of cellular characteristics to dilution rate and incident light. Eppley and Sloan (1966) observed that "if growth rate has a physiological basis, its magnitude should be reflected in cell composition." Investigators have, in fact, found considerable variability in cellular characteristics with growth rate (see, for example, Bienfang, 1975; Caperon and Meyer, 1972; Eppley and Renger, 1974; Malone et al., 1975; Thomas and Dodson, 1972; Yentsch and Vaccaro, 1958). The question in this paper is whether these characteristics are different when growth is limited by nutrients than when limited by light. If growth is limited by light, for example, we might expect the C:N ratio to increase with increasing growth rate whereas if it is limited by available nitrogen, we might expect the C:N ratio to decrease with increasing growth rate.

Particulate nitrogen per cell. When growth rate is limited by nitrogen we can expect the cellular nitrogen content to increase with increasing growth rate. Cellular nitrogen did increase with increasing dilution rate at all light intensities in this study, even when growth was probably not limited by nitrogen (i.e., when nitrate was present in the medium). There was a drop in cellular nitrogen at the 1.2 dilution rate, however, that was related to the difference in calculated and measured particulate nitrogen at that dilution rate.

Droop (1968, 1974) has shown that regression of DQ (dilution rate or growth rate (D) times cell quota (Q)) on Q gives a straight line.

Dilution rate and growth rate can be used interchangeably with chemostats but in the continuous cultures in this study they were not interchangeable (Table 5). A plot of DQ on Q when dilution rate was used as D gave a fan of straight lines (Figure 4A) with the slope of the line depending on dilution rate. The relationship was not as clear when specific growth rate (μN) was used for D (Fig. 4B). A straight line drawn through the points in Figure 4B would intercept the abscissa at approximately ten times the value of the intercept in Figure 4A.

The value of treating data in this way is that DQ can be equivalent to rate of uptake in a chemostat steady state (Droop, 1968). If the interpretation can be extended to outdoor ocntinuous cultures, then the curves in Figure 4A suggest that uptake depends on both dilution rate and cell quota, with light intensity determining the interaction. Droop also noted a difference in "fast-adapted" and "slow-adapted" cells in his studies (1974). It may be that the lines in Figure 4A demonstrate an acclimation to the dilution rate of the culture. In Part B of the figure, the separation of curves tends to fall along light intensity values. Perhaps these lines show acclimation to the light intensity of the culture.

Particulate organic carbon per cell. The increase in cellular carbon with growth rate was significant at the 5% level. This positive slope has been found previously in nutrient-limited cultures (Bienfang, 1975; Thomas and Dodson, 1972), although other workers have found negative slopes (Caperon and Meyer, 1972; Malone et al., 1975). The trend may be species-specific.

Nitrogen:carbon ratio. The N:C ratio increased with increasing dilution rate at all light intensities with the following exceptions: at 20%

light, N:C dropped at the highest dilution rate; at 3% light, N:C declined through the three highest dilution rates. These exceptions are the cultures least likely to have been nitrogen-limited and the most likely to have been light-limited. Overall, the N:C ratio was positively correlated to growth rate, a reflection of the fact that in this study growth rate was correlated with dilution rate but not with available light (Table 6).

Chlorophyll a. Chlorophyll a per cell, per unit nitrogen, and per unit carbon increased with increasing growth rate and with decreasing light intensity. Chlorophyll concentration showed an eight-fold variance (ss/df) about the mean in this study and was most sensitive to changes in environmental conditions. Thus, ratios involving chlorophyll demonstrated the greatest degree of diel periodicity (Part III of this report).

Cell volume. Individual cells of Chaetoceros curvisetus vary by as much as 4 to 5 times in length and width. Measurements made under the light microscope and of photographs of cells in this study showed no statistical correlation of cell volume with growth conditions. Samples measured were random and small, however. The increases in cellular carbon and nitrogen with decreasing light as well as with increasing dilution rate suggest changes in cell volume under these conditions.

CONCLUSION

It has been shown that every cellular parameter measured responded to changes in nutrient supply rate and to changes in light intensity. The cells apparently acclimated to the outdoor continuous culture conditions, and this acclimation is reflected in changes in the physiological

state with respect to growth rate.

There was an interaction of effects of light and nutrients on chlorophyll content of the cultures and on all ratios involving chlorophyll. For example, one expects chlorophyll per cell to increase at lower light intensities, but when the dilution rate was high in this study, the chlorophyll per cell decreased at low light intensities. These ratios were therefore difficult to interpret as indicators of the physiological state of the cell.

There was no statistical interaction of effect of light and nutrients on cell density or on carbon or nitrogen content of the cell. This suggests that the changes in cellular carbon and nitrogen with dilution rate at a given light intensity will be similar to the changes with dilution rate at another light intensity. These physiological indicators might be better used in predictive models of phytoplankton productivity than the more variable and sensitive chlorophyll parameters.

REFERENCES

Bienfang, P.K. 1975. Steady state analysis of nitrate-ammonium assimilation by phytoplankton. Limnol. Oceanogr. 20: 402-411.

Caperon, J., and J. Meyer. 1972. Nitrogen-limited growth of marine phytoplankton. I. Changes in population characteristics with steady-state growth rate. Deep Sea Res. 19: 601-618.

Droop, M. R. 1975. The nutrient status of algal cells in batch culture. J. Mar. Biol. Ass. U. K. 55:

---------- 1974. The nutrient status of algal cells in continuous culture. J. Mar. Biol. Ass. U. K. 54: 825-855.

---------- 1968. Vitamin B_{12} and marine ecology. IV. The kinetics of uptake, growth and inhibition in Monochrysis lutheri. J. Mar. Biol. Ass. U. K. 48: 689-733.

Eppley, R. W., and E. H. Renger. 1974. Nitrogen assimilation of an oceanic diatom in nitrogen-limited continuous culture. J. Phycol. 10: 15-23.

Herbert, D., R. Elsworth, and R. C. Telling. 1956. The continuous culture of bacteria; a theoretical and experimental study. J. Gen. Microbiol. 14: 601-622.

MacIsaac, J.J., and R. C. Dugdale. 1972. Interactions of light and inorganic nitrogen in controlling nitrogen uptake in the sea. Deep Sea Res. 19: 209-232.

Malone, T. C., C. Garside, K. C. Haines, and O. A. Roels. 1975. Nitrate uptake and growth of Chaetoceros sp. in large outdoor continuous cultures. Limnol. Oceanogr. 20: 9-19.

Rhee, G-Y. 1974. Phosphate uptake under nitrate limitation by Scenedesmus sp. and its ecological implications. J. Phycol. 10: 470-475.

Riley, G. A. 1956. Oceanography of Long Island Sound 1952-54. II. Physical oceanography. Bull. Bingham. Oceanogr. Coll. 15: 15-46.

Ryther, J. H., and R. R. L. Guillard. 1962. Studies of marine planktonic diatoms. III. Some effects of temperature on respiration of five species. Can. J. Microbiol. 8: 447-453.

Steele, J. H. 1962. Environmental control of photosynthesis in the sea. Limnol. Oceanogr. 7: 137-150.

Sverdrup, H. U. 1953. On conditions for the vernal blooming of phytoplankton. J. Cons. Int. Explor. Mer, 18: 287-295.

References (continued)

Strickland, J. D. H., and T. R. Parsons. 1972. A practical handbook of seawater analysis. 2nd ed. Bull. 167. Fish. Res. Bd. Can. Ottawa. pp. 201-203.

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: 515-523.

Yentsch, C. S., and R. F. Vaccaro. 1958. Phytoplankton nitrogen in the oceans. Limnol. Oceanogr. 3: 443-448.

TABLE 1. INORGANIC NUTRIENT COMPOSITION OF ANTARCTIC INTERMEDIATE WATER, ST. CROIX, U.S. VIRGIN ISLANDS
DEPTH 870 m

NUTRIENT	EXPERIMENT	CONCENTRATION (µM)
Nitrate + Nitrite	1	30.91
	2	31.20
	3	32.42
	4	31.04
Ammonia	1	-
	2	0.34
	3	0.27
	4	0.25
Phosphate	1	2.05
	2	2.10
	3	1.86
	4	3.20
Silicate	1	22.43
	2	25.62
	3	24.60
	4	25.42

TABLE 2. EXPERIMENTAL REGIME OF OUTDOOR CONTINUOUS CULTURES OF *CHAETOCEROS CURVISETUS* GROWN IN ANTARCTIC INTERMEDIATE WATER

EXPERIMENT NUMBER	DILUTIONS PER DAY	REACTOR NUMBER	PERCENTAGE OF INCIDENT LIGHT HITTING SURFACE OF CULTURE
I	0.25	1	100
		2	46
		3	30
		4	20
		5	3
III	0.70	1	100
		2	46
		3	30
		4	20
		5	3
IV	0.95	1	100
		2	46
		3	30
		4	20
		5	3
II	1.20	1	100
		2	46
		3	30
		4	20
		5	3

TABLE 3. MEAN TEMPERATURES OF CULTURES AND LIGHT INTENSITIES REACHING MID-DEPTH OF OUTDOOR CONTINUOUS OF *CHAETOCEROS CURVISETUS*

DILUTION RATE	PERCENT OF INCIDENT LIGHT	MEAN TEMPERATURE (°C)	MEAN AVAILABLE LIGHT (ly/day)
0.25	100	27.4	403
	46	27.0	178
	30	26.9	115
	20	26.9	73
	3	26.5	10
0.70	100	25.9	319
	46	25.3	143
	30	25.3	97
	20	25.2	74
	3	25.0	12
0.95	100	24.5	214
	46	24.2	88
	30	23.8	57
	20	23.7	36
	3	23.7	6
1.20	100	23.4	227
	46	22.9	101
	30	22.9	64
	20	22.8	29
	3	22.7	7

TABLE 4. STANDARD DEVIATIONS ABOUT THE MEAN CELL NUMBER DURING THE LAST THREE DAYS OF GROWTH IN OUTDOOR CONTINUOUS CULTURE

DILUTION RATE	PERCENT OF INCIDENT LIGHT	STANDARD DEVIATION (SD) (10^7 cells liter^{-1})	SD AS PERCENT OF MEAN
0.25	100	2.10	26
	46	3.06	42
	30	2.14	31
	20	0.91	16
	3	0.71	19
0.70	100	1.00	10
	46	1.26	15
	30	1.92	21
	20	1.88	32
	3	0.80	34
0.95	100	0.88	11
	46	1.41	22
	30	2.06	36
	20	1.16	46
	3	0.44	53
1.20	100	1.57	34
	46	2.32	38
	30	1.62	39
	20	0.80	46
	3	0.16	63

TABLE 5. MEAN VALUES OF GROWTH, BIOMASS, AND CELLULAR CHARACTERISTICS OF *CHAETOCEROS CURVISETUS* GROWN IN OUTDOOR CONTINUOUS CULTURES

DILUTION RATE	PERCENT OF INCIDENT LIGHT	SPECIFIC μN	CARBON (mg/liter)	NITROGEN (mg/liter)	ATOMIC C:N	CHLOROPHYLL *a* :PHAEOPIGMENT RATIO	CELL NUMBER (10^7/liter)	CELLULAR CARBON (pg/cell)	CELLULAR NITROGEN (pg/cell)	CELLULAR CHLOROPHYLL (pg/cell)
0.25	100	0.066	1.5515	0.1940	9.33	1.74	7.98	19.44	2.43	0.074
	46	0.044	1.4196	0.1592	10.40	2.10	7.28	19.50	2.19	0.115
	30	0.130	1.8308	0.2154	9.91	1.81	6.89	26.57	2.98	0.162
	20	0.229	1.7800	0.2603	7.97	2.10	5.84	30.48	4.46	0.329
	3	0.378	1.0364	0.1703	7.10	3.04	3.72	27.86	4.58	0.683
0.70	100	0.705	2.3739	0.2944	9.40	1.17	10.09	23.53	2.92	0.226
	46	0.688	2.3421	0.3180	8.59	2.07	8.52	27.49	3.62	0.430
	30	0.888	2.0842	0.3030	8.02	1.35	9.15	22.78	3.31	0.415
	20	0.741	1.6163	0.2714	6.94	2.77	5.88	27.44	4.61	0.776
	3	0.462	0.7176	0.1271	6.58	3.46	2.37	30.28	5.36	0.945
0.95	100	1.02	2.3241	0.3387	8.00	0.76	7.98	29.12	4.24	0.301
	46	0.92	1.7046	0.2711	7.33	1.80	6.29	27.10	4.31	0.539
	30	0.90	1.8381	0.2823	7.59	1.97	5.72	32.13	5.93	0.593
	20	0.86	1.0975	0.1867	6.86	2.22	2.55	43.04	7.32	0.994
	3	0.61	0.3622	0.0485	8.71	2.67	0.83	43.64	5.84	0.745
1.20	100	1.22	1.6026	0.2637	7.09	3.02	4.57	35.07	5.77	0.529
	46	1.24	1.6214	0.2615	7.23	2.42	6.16	26.32	4.24	0.455
	30	1.23	1.0997	0.1954	6.56	2.90	4.18	26.31	4.67	0.555
	20	1.111	0.4873	0.0783	7.26	3.56	1.73	28.17	4.53	0.586
	3	1.09	0.1141	0.0130	10.24	0.46	0.25	45.64	5.20	0.156

TABLE 6. REGRESSIONS OF GROWTH RATE AGAINST BIOMASS AND CELLULAR CHARACTERISTICS OF *CHAETOCEROS CURVISETUS* IN OUTDOOR CONTINUOUS CULTURE

	r	F	p
Growth rates			
μ_n v. μ_c	0.9973	3219	<0.001
μ_c v. μ_{cell}	0.8772	60.103	<0.001
μ_{cell} v. μ_n	0.8758	59.242	<0.001
DR v. μ_n	0.9567	194.40	<0.001
I_d v. μ_n	-0.2353	1.055	NS
Biomass			
μ_n v. cell #	-0.275	1.474	NS
chl *a*	0.283	1.570	NS
POC	-0.118	0.253	NS
PN	0.0703	0.089	NS
Characteristics			
μ_n v. N:C (atomic)	0.4835	5.4907	<0.001
PN/cell	0.5089	6.2908	<0.001
PN/chl *a*	-0.5768	8.4753	<0.001
POC/cell	0.3732	2.9137	<0.05
POC/chl *a*	-0.6399	11.7864	<0.001
chl *a*/cell	0.3856	2.9686	<0.05

TABLE 7. THEORETICAL VERSUS MEASURED PARTICULATE NITROGEN IN OUTDOOR CONTINUOUS CULTURES OF CHAETOCEROS CURVISETUS

DILUTION RATE	PERCENT OF INCIDENT LIGHT INTENSITY	MEASURED PARTICULATE NITROGEN (CHN)	THEORETICAL PARTICULATE NITROGEN*
0.25	100	0.222	0.109
	46	0.200	0.109
	30	0.247	0.109
	20	0.267	0.109
	3	0.188	0.008
0.70	100	0.296	0.309
	46	0.314	0.309
	30	0.302	0.308
	20	0.275	0.294
	3	0.145	0.129
0.95	100	0.318	0.435
	46	0.284	0.409
	30	0.289	0.387
	20	0.200	0.231
	3	0.062	0.061
1.20	100	0.261	0.476
	46	0.256	0.461
	30	0.194	0.398
	20	0.108	0.253
	3	0.014	0.130

*Calculated from $N_{dw} - N_r = N_t$, where N_{dw} = total dissolved inorganic nitrogen in the deep water going into the reactors each day; N_r = total dissolved inorganic nitrogen present in the culture medium; N_t = theoretical amount of particulate nitrogen present.

Figure 1. Cell densities of outddor cultures of Chaetoceros curvisetus grown under four nutrient regimes and five light regimes. The light intensities are given on the ordinate, dilution rates on the abscissa.

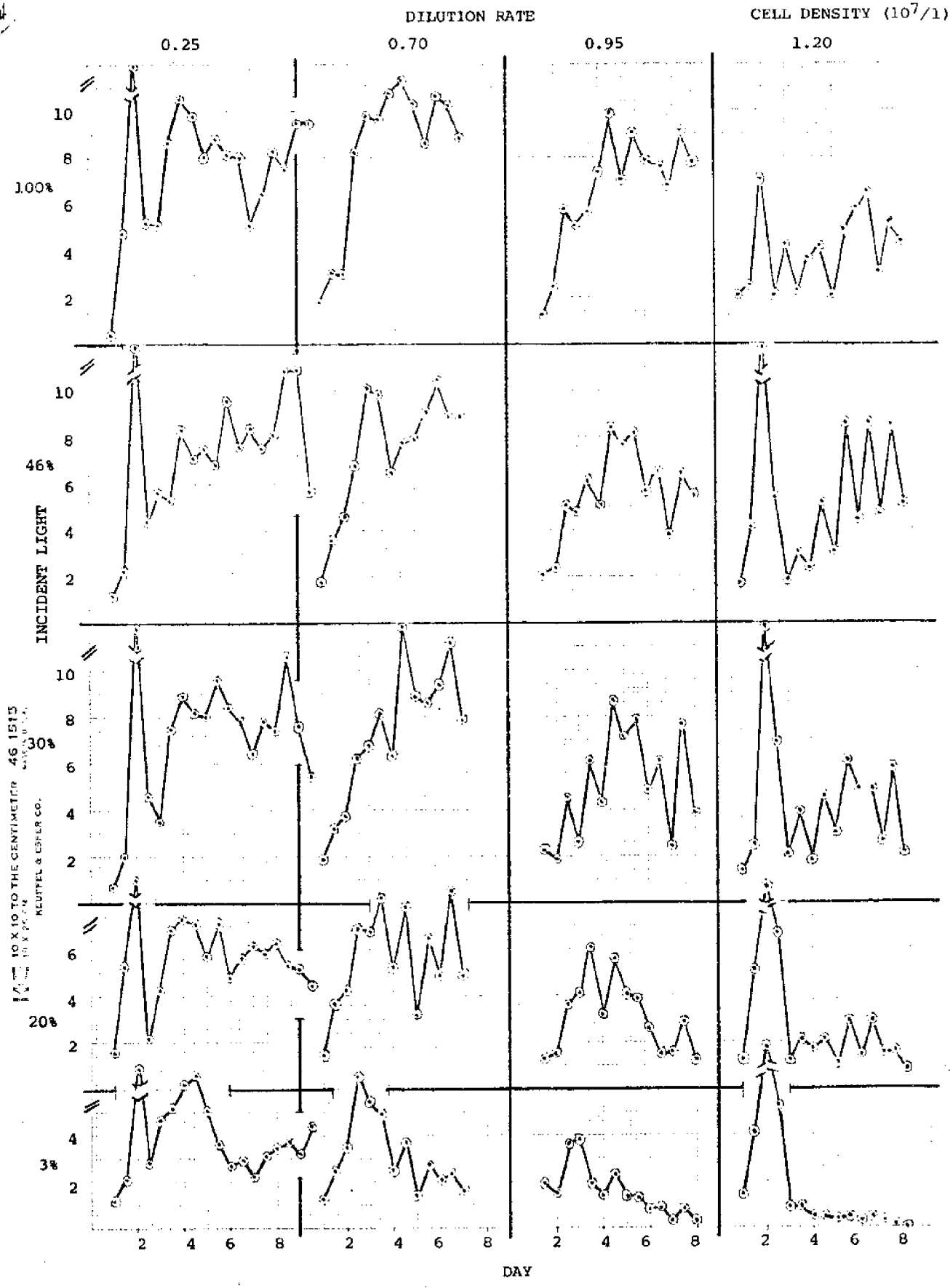

DILUTION RATE
CELL DENSITY (10^7/1)
0.25
0.70
0.95
1.20
100%
46%
30%
20%
3%
INCIDENT LIGHT
DAY
2
4
6
8
10

Figure 2. Effects of light and nutrients on mean biomass of outdoor continuous cultures. Results of analysis of variance are shown for each indicator of biomass. Parameters are shown as a function of dilution rate, with each curve drawn to a single light intensity.

CELL DENSITY
(10^3/LITER)

CHLOROPHYLL A
(μg/LITER)

PVC
(m³/LITER)

PN
(μg/LITER)

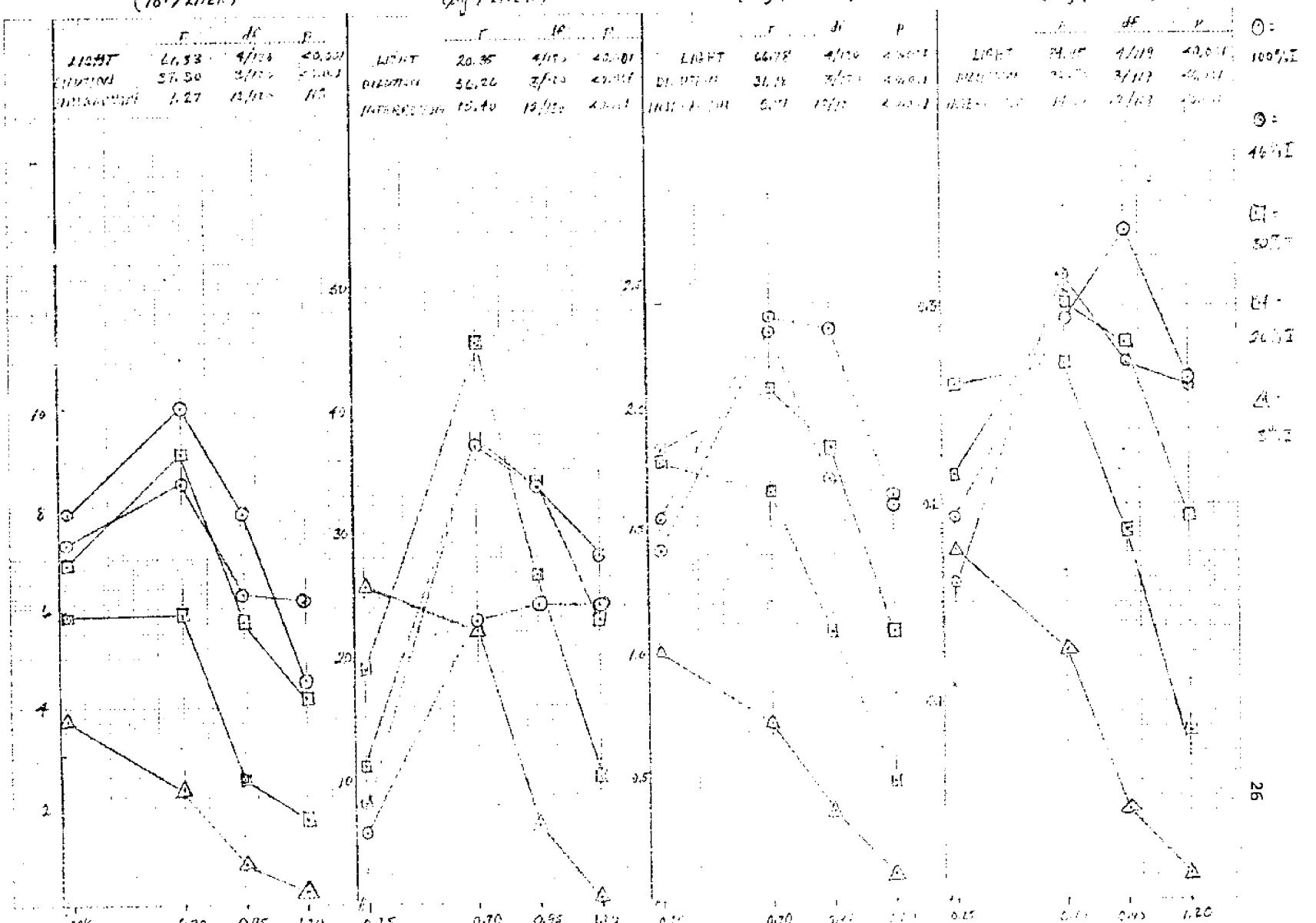
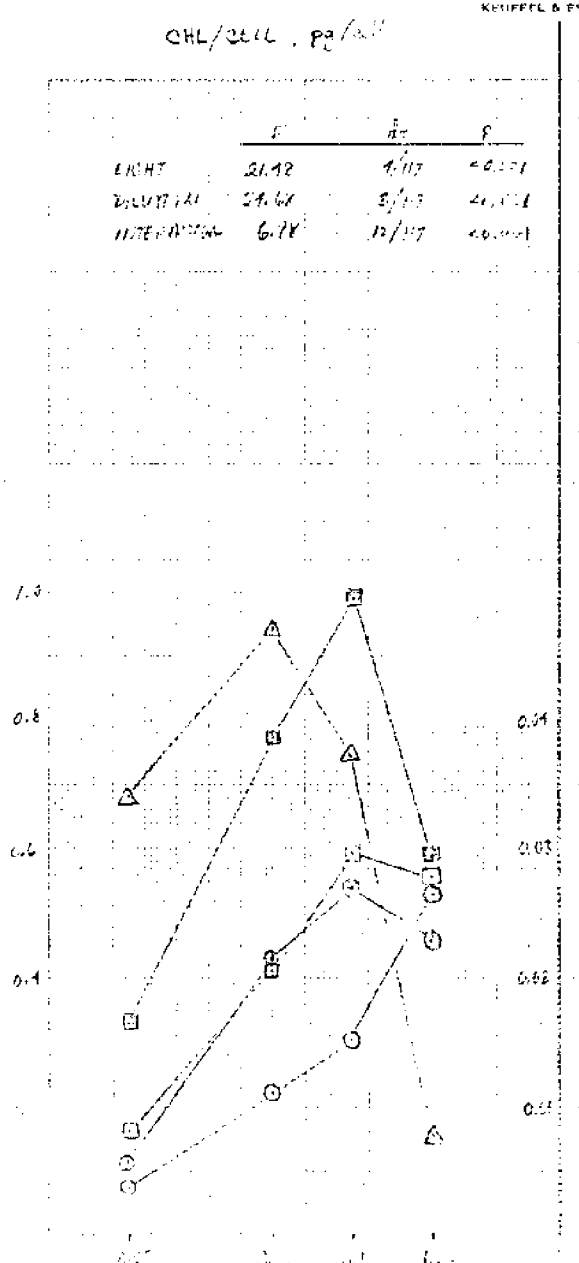
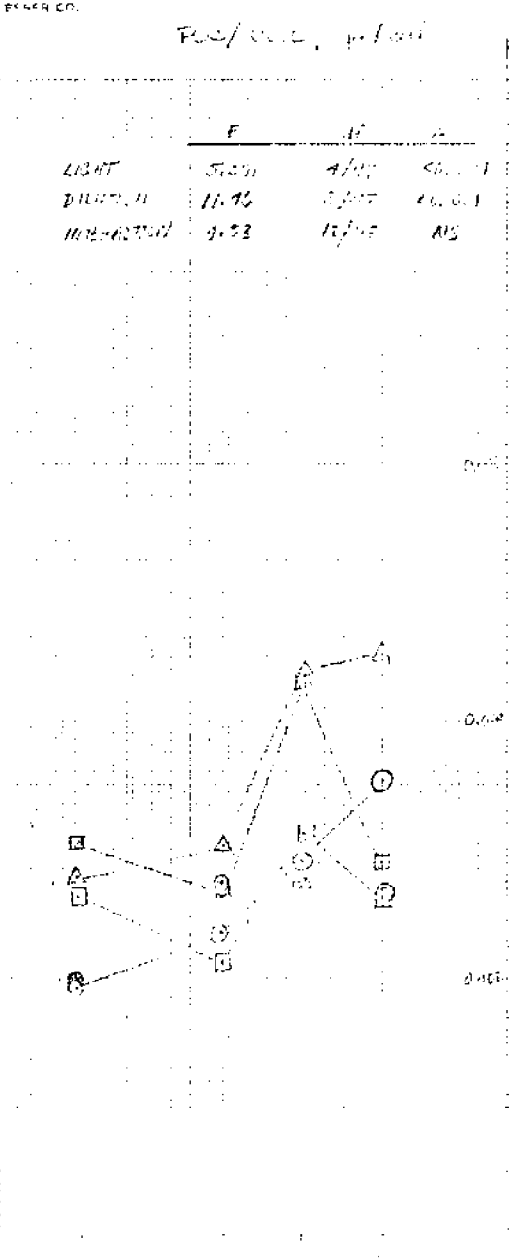


Figure 3. Effects of light and nutrients on mean cellular composition of outdoor continuous cultures. Legend as in figure 2.





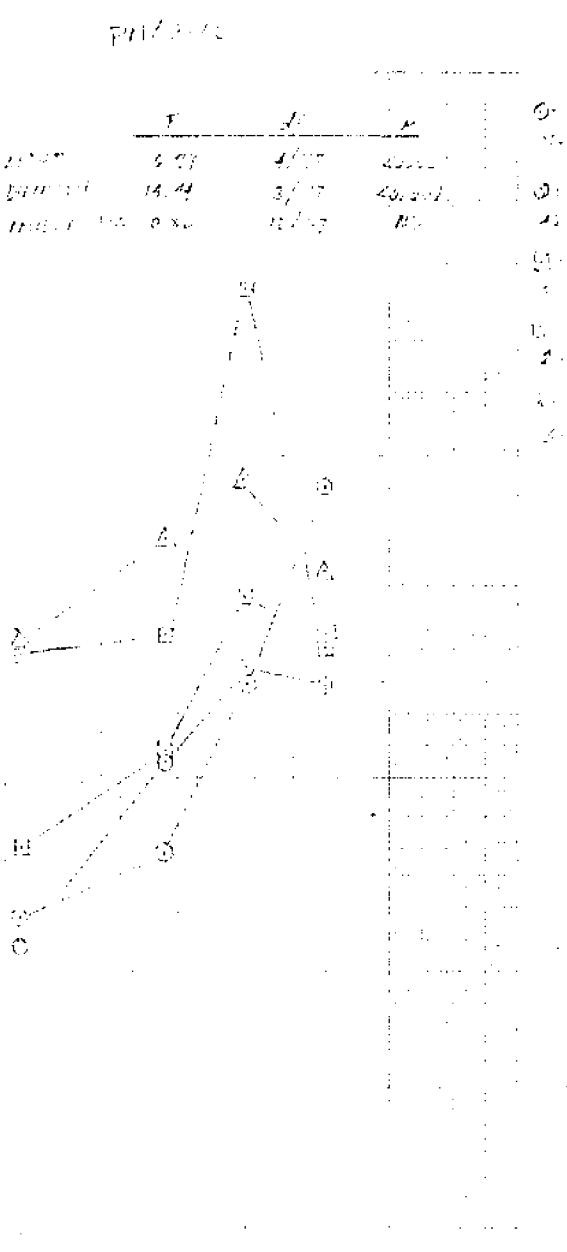
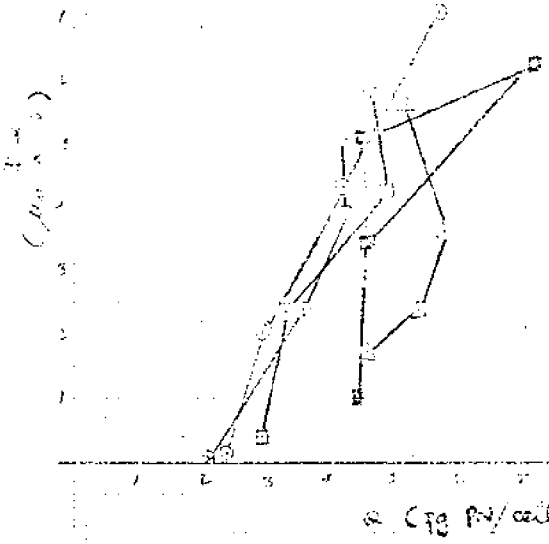
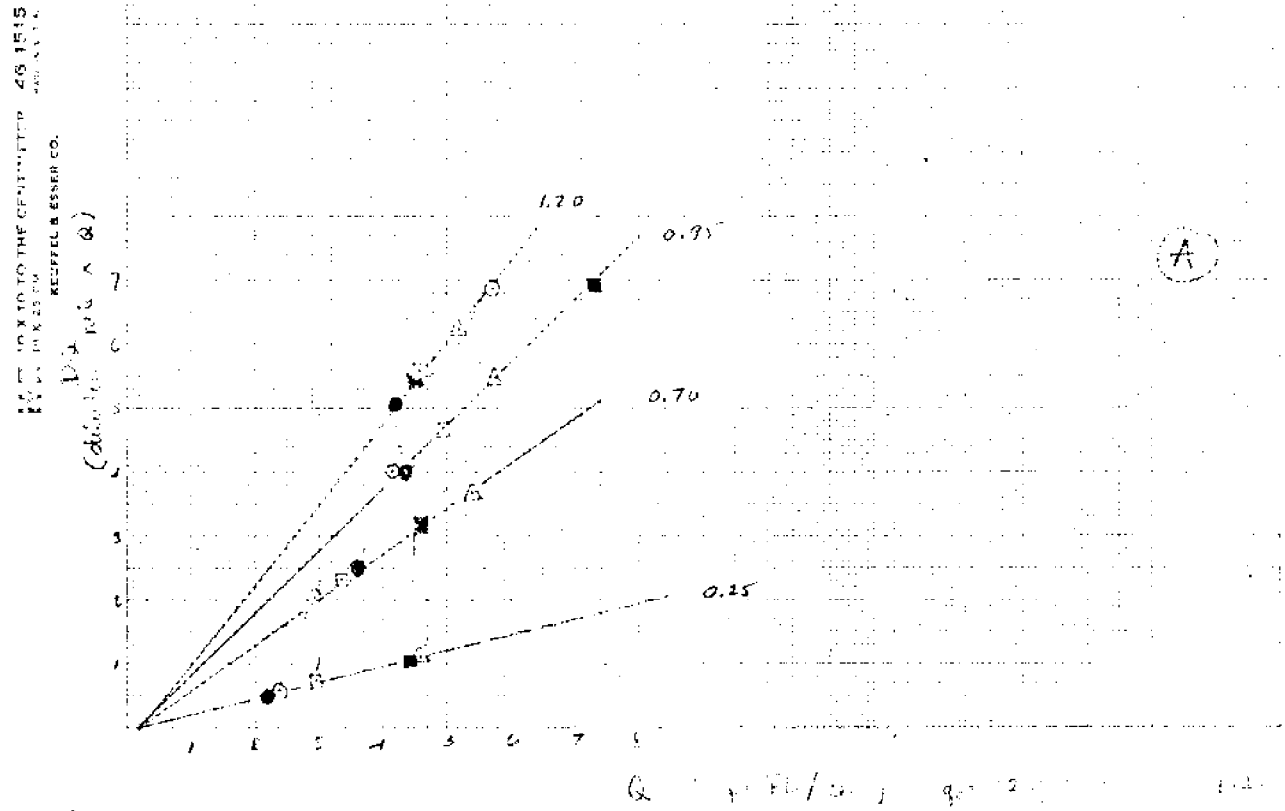


Figure 4. (A) Droop's DQ shown as a function of cellular nitrogen (see text for explanation).

(B) Measured growth rate times Q as a function of cellular nitrogen.



q_o = 2 pg Ar/cell



THE EXPERIMENTAL CULTIVATION OF TAPES SEMIDECUSSATA (REEVE) IN AN ARTIFICIAL UPWELLING MARICULTURE SYSTEM*

Kenneth M.Rodde, Judith B. Sunderlin and Oswald A. Roels

Lamont-Doherty Geological Observatory
of Columbia University
Palisades, New York 10964

*Lamont-Doherty Geological Observatory Contribution #0000.

ABSTRACT

The growth and reproductive characteristics of Tapes semidecussata were investigated during long and short term feeding experiments in an artificial upwelling mariculture system.

The long term study involved the rearing of three successive generations of Tapes on combinations of three planktonic diatoms: Chaetoceros curvisetus, Bellerochea polymorpha and Thalassiosira pseudonana. Marketable adults (38mm) were obtained in twelve months from post set juveniles, and in 10 months from the 5mm stage. Animal densities at 38mm were 1775 and 1345 clams/m^2 for 1st and 2nd generations, with survival rates of 64 and 63%, respectively.

Ripe gonads found in adult clams during much of the year indicated excellent adaptation to the system by Tapes. Spawning was readily induced in the hatchery and larvae metamorphosed in three weeks. Post set survival rates of 52 and 84.5% were recorded.

The short term feeding study used 6mm juvenile clams and tested the Chaetoceros and Bellerochea diatoms and an unidentified, Cryptophyte flagellate designated S-1, in seven different mono- and mixed-algal diets. The monoculture diets produced the slowest growth in clams after five weeks, yielding a 6.6-fold average increase in wet meat weight and an average length of 15mm. The four mixed diets produced similar, individual gains in weight, and gave an

11.2-fold average increase in wet meat weight as a group. The Chaetoceros + S-1 diet showed the best algae-to-meat conversion ratio however, and was therefore the most effective diet. Clams fed this mixture averaged 16.7mm in length after five weeks.

INTRODUCTION

Management of commercial clam stocks often involves the sowing of hatchery-produced seed in preselected, natural growing areas where the animals are left unattended until market size is reached. Along the east coast of the United States, the hard clam Mercenaria mercenaria and the soft shell clam Mya arenaria are cultured in this way (Bardach et al., 1972).

In Japan, the asari, Tapes japonica, is similarly cultivated in bottom sediments and ranks second only to the oyster in annual bivalve production (Tamura, 1970).

The main risk of bottom culture is high mortality due to disease and predation, and is often compounded by fluctuations in temperature, salinity and food availability. Although several techniques are used to minimize this risk, including size-graded gravel and netting devices, control of mortality is better accomplished in a semi-closed, continuous flow culture system which also allows the manipulation of diet for enhanced growth.

The Artificial Upwelling Project on St. Croix employs an experimental system of this kind in which Antarctic Intermediate water, pumped from 870 meters, is used to cultivate

planktonic algae as food for experimental bivalve culture. In addition to containing high concentrations of the inorganic elements needed for algal growth, this deep water is virtually free of disease, pollution and fouling organisms which complicate growth and survival in bottom cultured clam populations.

In the upwelling system, seven species of commercial bivalves have been raised from the juvenile stage to market size at accelerated rates of growth (Roels et al., 1975). This paper describes the growth of the Japanese little neck clam Tapes semidecussata in this system. This species was investigated in two phases: first, a long term study to determine the general growth, survival and propagational characteristics of the animal; secondly, in short term growth experiments, to define the best combination of algae for rapid growth.

METHODS & MATERIALS

A. Long Term Study

Three generations of Tapes were used to determine if adaptation to the system by successive groups of progeny would be attended by consecutive increases in rates of growth.

The first group of clams was obtained from Pacific Mariculture, Inc., in August of 1973 and contained approximately 25,000 clams averaging 5mm in length. These were first quarantined, then distributed on plastic Nestier trays (0.34 m^2/tray) at an initial density of 17216/m^2 and placed in a 750-liter, epoxy-coated plywood tank which measured 2.4 x 0.6 x 0.6

meters. A continuous, regulated flow of food was supplied from two 45,000-liter concrete pools in which three species of planktonic diatoms were alternately cultured: clone STX-114, Bellerochea polymorpha and clone STX-167, Chaetoceros curvisetus which were isolated from deep sea water on St. Croix, and clone 3-H, Thalassiosira pseudonana obtained from R.R.L. Guillard. Deep sea water was fed continuously into the pools at a rate of 40 liters/min, and the addition of vitamins and trace elements provided the necessary nutrients to sustain the algal cultures. The STX-167 diatom was grown in unsupplemented deep water, however.

The flowrate of the pool cultures into the shellfish container was regulated as a function of animal density and algal stripping efficiency. If the animals stripped >90% of the incoming cells for five successive days, an increase in flow and/or a reduction in animal numbers was made to insure adequate food availability (Baab et al., 1973). The initial flow was set at 14.4 liters/min; effluent from the tank was sampled daily to determine the quantity of diatoms removed from the culture by the clams. This was done by comparing influent and effluent cell counts; the density of diatom inflow ranged from 10^4 to 10^6 cells/ml.

Second generation clams were obtained by collecting fertilized eggs from one of many spontaneous spawnings by the parent stock (April, 1974). Larvae were grown in 15-liter buckets for 16 days (Sunderlin et al., 1975), at which time they were transferred to a 370-liter fiberglass flume

measuring 3.66 x 0.61 x 0.2 meters. The larvae were batch-fed mixtures of the three types of diatoms from Day 1 until setting occurred at the end of three weeks. After set, a continuous flow of food was supplied to the animals at an initial rate of 23.2 ml/sec. Four thousand clams were stocked in the flume at an initial density of 2690/m^2 and raised on the same diets until market size was achieved (38mm and/or 10g).

The third generation Tapes used in this study were produced by inducing the F_1 clams to spawn in the hatchery using thermal and chemical (stripped gonad solution) stimulation. Gametes were discharged between 29 and 27C on the 'down side' of the thermal shock, and 4 million fertilized eggs ranging from 61 to 65 μ were collected. Larvae and juveniles of this F_2 group were treated in the same manner as their parents, i.e., batch-fed, set, and raised in the 370-liter flume on the same three types of diatoms. All larvae were filtered through Nytex sieves, then counted and measured under the microscope every two days. After metamorphosis, the growth of each population was monitored on a monthly basis by weighing and measuring the length (antero-postero-axis) from randomly selected individuals of each group.

B. Short Term Study

The short term feeding study tested the monocultures and combinations of three algae: the diatoms Chaetoceros curvisetus and Bellerochea polymorpha used in the long term study, and the unidentified Cryptophyte flagellate designated

S-1 obtained from R.R.L. Guillard. These were grown in 2000-liter containers (reactors), each receiving deep water inflow at a rate of 23.2 ml/sec. Inoculation of duplicate cultures was staggered to insure a constant availability of each culture for the five-week duration of the experiment.

Each reactor culture outflow was fed to a PVC manifold containing five lucite air valves adapted for salt water flow, to which 6.35mm diameter tubing was connected. Two lines of tubing from each manifold supplied mono-algal cultures to duplicate 1.5-liter glass trays (4.5 x 34.5 x 22.2 cm) in which 100 juvenile Tapes averaging 6.5mm in length were placed. The remaining three lines of each manifold fed culture to four plastic 2-liter mixing containers which, in turn, supplied combinations of the three algae to the remaining eight animal trays. The flowrate to the mixing containers was set at 2.5 ml/sec to insure an overflow, thereby providing a constant pressure and uniform supply to the animal trays.

At two-day intervals, both influent and tray effluent samples were collected and analyzed for in vivo chlorophyll using the Turner fluorometer. Initially, serial dilutions of each diet were read for in vivo chlorophyll values and each dilution counted under the microscope for corresponding cell numbers per ml of culture. Plots of each diet were made, and tray effluent fluorometer values were then equated to cell densities. Animal stripping efficiencies were calculated using these data.

RESULTS

A. Long Term Study

The growth rates of the three populations of Tapes are shown in Figure 1. The parent stock curve has been advanced 97 days (estimated time to reach 5mm in the artificial upwelling system) for a better comparison with second generation growth. Including the larval stage, market size was reached in approximately 13 months for both groups. The parent stock grew from 5mm to 38mm in 10 months.

The final population density for the first generation was 1722 clams/m^2, with a survival of 64%. The second generation, raised in a flume, had a final density of 1345 clams/m^2 at market size, and a survival of 63%.

B. Short Term Study

Of the seven experimental diets, those containing mixtures of algae gave better growth in the juvenile clams than the other diets. A summary of the growth data is presented in Table 1.

Figure 2 shows the average shell and meat weights for clams of each test diet. The Bellerochea + S-1 combination produced the greatest increase in shell length, whereas the Chaetoceros + Bellerochea mixture gave the best meat-to-shell ratio. The Chaetoceros + S-1 diet was the most effective combination in producing the best stripped-algae-to-meat-weight conversion ratio (number of cells stripped/gram wet meat weight).

DISCUSSION

The selection of species of bivalves for large-scale cultivation requires careful consideration of many aspects of the animal's biology but at least four points must be evaluated critically: a) the rate of success of inducing spawning in a hatchery, b) the survival rate of larvae through metamorphosis, c) the survival rate at high stocking densities and d) the growth and feeding efficiency on selected diets. These points are now discussed as they apply to Tapes in the upwelling mariculture system.

A. Long Term Study

1. Reproduction. The normal spring and fall spawning cycle of Tapes in nature (Japan) has generally been maintained in the mariculture system, that is, most of the spontaneous discharges were observed during March and April, and September and October. Many animals, however, maintained ripe gonads during much of the year. Evidence for this was seen in the spontaneous spawnings of second generation clams in December and February, and in the induced spawning of parent stock clams in mid-July.

The ripened condition most likely is due to the constant food supply and the uniform water temperature (22-30C) in the system throughout the year. However, other species of bivalves maintained under the same conditions failed to show similar, spontaneous spawning behavior. The fact that Tapes maintains a moderately developed-to-late active gonad without any pre-conditioning further makes this species an attractive

candidate for large-scale mariculture at St. Croix.

The rearing of Tapes larvae in the hatchery was simple and free of problems. Third generation larvae were placed in 15-liter buckets at the straight hinge stage (avg. 90 μ) at a density of 10 larvae/ml, and batch-fed mixtures of Thalassiosira, Bellerochea and Chaetoceros diatoms. Metamorphosis began on Day 21 at an average size of 246 μ, and approximately 624,000 of the 1.2 million larvae, or 52%, survived setting after the 25th day. Although the survival rate dropped to 32% after 68 days in this population, another group was induced to spawn in July and had an 84.4% survival rate on Day 50.

By contrast, survival in nature at this stage of development is substantially lower. For example, a normal December set from a fall spawning of Tapes japonica in areas of the Sea of Ariake is one million larvae per square meter; by February, the survival rate drops to 15%, principally due to winter temperatures and poor food availability (Tamura, 1970).

In addition to the good post-set survival rates obtained in the upwelling system, the total larval time period has recently been reduced; feeding experiments have identified a diet which shortens the larval stage from 21 to 12 days (Sunderlin et al., 1975). This development further enhances Tapes' attractiveness for mass cultivation.

2. Growth. A history of Tapes growth measured as an increase in shell length over time is given in Figure 1 for the three populations which were monitored during the long

term study. Since the parent stock, or first generation, was introduced at a size of 5mm, an adjustment of 97 days (about the time to reach 5mm in the artificial upwelling system) was made to the growth curve to allow a comparison with second generation growth.

From Figure 1, it is readily apparent that the growth rates of the three groups are quite similar. After six months, first and second generations averaged 19.1 and 20.2mm in length and 1.4 and 1.2 grams in live weight. After nine months, these values increased to 26.1 and 28mm, and 3.5 and 3.2 grams, respectively. Including the time for larval development and metamorphosis, market size was reached in 13 months, or, 10 months from the 5mm stage. No increased growth was observed in successive generations reared in the system. Since all three generations grew at the same rate, the next step in the Tapes breeding program will be selection for the fast growing genotype.

As compared to growth in nature, clams cultured in the upwelling system reach market size more quickly. Figure 3 contrasts the growth of Tapes japonica from the Sea of Ariake (Ikematsu, 1957) with second generation T. semidecussata at St. Croix. Since T. japonica spawns primarily in late November and sets in early December, the greatest difference in growth is seen in the first six months when the cultivated clams have a decided advantage over those grown during the winter months in nature: T. japonica averaged 10mm in length after six months as compared to 21mm for T. semidecussata in the artificial upwelling mariculture system. At the end of

one year from post-set, the corresponding measurements are 27mm and 34mm, respectively.

Elsewhere in nature, a greater contrast is seen in the growth rate of Venerupis japonica, a close relative of T. semidecussata and indigenous to British Columbia. In these colder water, 38mm is reached after three or four years; in 1969, less than 200,000 lbs. of V. japonica were harvested (Quale & Bourne, 1972).

Besides rate of growth, the density at which a species can be raised to market size and the survival rate at that point are two other important considerations. The results from the long term study indicate that high stocking densities of T. semidecussata are possible with good survival.

The parent stock and their F_1 were raised at initial densities of 17216 and 2690 clams/m^2 in two types of containers, the 750-liter tank and the 370-liter flume. Both groups were fed the same diet over time at flowrates which were adjusted to compensate for increases in growth; maximum flow to the parent stock was 26.4 liters/min at market size, and 14.4 liters/min for the F_1. Since a number of other shellfish containers were connected to the pool outflow as well, 26.4 liters/min was the maximum flow any one tank could receive from a constant algal culture flow rate of 40 liters/min. With this restriction, final densities at 38mm were 1722 and 1345 clams/m^2 for the two populations, with corresponding survival rates of 64 and 63%.

In comparison with bottom cultured clams, these values are quite satisfactory. Some of the better results from

sowing Venerupis semidecussata in the Sea of Ariake were one year densities of 861 and 1184 clams/m^2 with survival rates of 31 and 42% (Tamura, 1970).

Overall, the long term study has shown that Tapes semidecussata adapts well to a continuous flow mariculture system, spawning readily in the hatchery and showing good post-set survival. Marketable clams can be raised in one year (with a 12-day larval period) at a survival rate of 64%. The minimum limits for an economically feasible culture program, seeding new or existing clam beds and depending on natural food sources for growth, requires a weight increase of 3.2 times and a survival of 65% (Tamura, 1970).

Having met or exceeded these basic requirements, a number of short term feeding experiments are now planned to improve Tapes statistics in both categories by a) determining the optimum algal diet for the different size intervals in the bivalve life cycle, and b) maximizing the efficiency of converting plant protein to shellfish meat. The short term study discussed below is the first experiment in the series.

B. Short Term Study

This phase of the study tested seven different diets, three mono-cultures and four mixtures, to evaluate the effect of each on Tapes growth. The results of the trials are presented in Table 1.

The Chaetoceros and Bellerochea diatoms were selected for the study because they are indigenous to the deep sea

water and gave good growth rates in the long term study. The S-1 Cryptophyte is a naked flagellate similar to Isochrysis and Monochrysis sp., but grows well at higher temperatures (up to 30°C). The juvenile Tapes used for the study were collected from the same population whose previous diet background included the Chaetoceros, Bellerochea and Thalassiosira diatoms.

It can be seen from Table 1 that the monocultures were less effective for rapid growth than the mixed diets. Stripping efficiencies were less than one-third of those for the mixed diets. With the exception of the Chaetoceros-fed clams, the wet meat gain was less than 50% for the clams on the mono-algal diets compared to animals fed the combination diets. The animals fed Chaetoceros only grew well on this monoculture, but the increase in weight above the other monocultures became apparent only after the fourth week. A characteristic of this diatom is that it frequently forms long chains of cells several hundred microns long with large protruding spines. The possibility arises that the clams may have to be a certain size (in this case, 13mm), before these chains can be effectively utilized. Once utilized, however, this diatom gave the best meat-to-shell ratio of the three monocultures (1:1.5) and produced 33% more wet meat than the other species.

Another effect of Chaetoceros on Tapes growth behavior was an early maturation of the gonad. Clams averaging 9.5mm spawned after 18 days on this diet. The next spawning occurred

15 days later by clams ranging from 15 to 16.7mm and fed the S-1 monoculture and the S-1 + Chaetoceros mixture. At the time of the first spawning, the animals weighed 23% less than the S-1 fed clams and only 1% more than those on the Bellerochea diet. This raises a question concerning energy utilization, that is, if gametogenesis was progressing at the expense of somatic tissue growth.

Of the four diets containing algal mixtures, no one combination was clearly superior. However, some mixtures appeared to be more effective in certain areas than others. Figure 2 illustrates the weight fractions of the average whole weight for clams on each diet. The S-1 + Bellerochea mixture produced the largest and heaviest clams, but had the poorest meat-to-shell ratio (Table 1); only 20 grams of meat were obtained from a whole live weight of 82 grams. The influence of S-1 is evident in these results.

The Chaetoceros + Bellerochea diet had the best meat-to-shell ratio, but the three algae combination produced the best wet meat gain of the four mixtures.

The most effective mixture was the Chaetoceros + S-1 diet, based on the ratio of number of algal cells stripped per gram of wet meat gain. This diet also yielded the second highest gain in wet meat. In this sense, it appears to be the best diet of all seven. Further, it is interesting to note that the clams on this diet spawned on the 34th day of the five-week experiment.

The Chaetoceros + S-1 diet may have been the best algal

combination in this feeding trial, but several other species of algae remain to be tested. The optimum diet will include: a) algae which grow well on unenriched deep sea water, b) algae which are the most efficient users of deep water nitrogen, and c) those which contain the necessary combinations of essential amino acids for maximum shellfish growth. The analysis of phytoplankton and shellfish nitrogen conversion efficiencies are projected for subsequent experiments in this series, as are histological investigations of the impact of diets on gametogenesis. The results of research on Tapes to date, however, indicates that this clam is an excellent candidate for large-scale mariculture.

Acknowledgements: We wish to express our thanks to O.A. Francis and M. Brenner for their assistance in the maintenance and data collection on Tapes during the long term study. This work was supported by the U.S. Department of Commerce Sea Grant 04-5-158-59 and by the G. Unger Vetlesen Foundation.

LITERATURE CITED

Baab, J.S., G. Hamm, K.C. Haines, K, Chu, and O.A. Roels. 1973. Shellfish mariculture in an artificial upwelling system. Proc. Nat. Shellfish. Assoc. 63:63-67.

Ikematsu, W. 1957. Ecological studies on the clam, Tapes japonica (Reeve) II. On the setting season and the growth in early young stage. Bull. Jap. Soc. Sci. Fish. 22:736-741.

Quale, D.B. and N. Bourne. 1972. The clam fisheries of British Columbia. Fish. Res. Bd. Can. Bull. No. 179, 70pp.

Roels, O.A., K.C. Haines and J.B. Sunderlin. 1975. The potential yield of artificial upwelling mariculture. 10th European Symposium on Marine Biology, Ostend, Belgium.

Sunderlin, J.B., P.T. Baab and E.M. Patry. 1975. Growth of clam and oyster larvae on different algal diets in a tropical artificial upwelling mariculture system. Submitted to Proceedings of the World Mariculture Society.

Tamura, T. 1970. Marine aquaculture (translated from Japanese). National Science Foundation, Washington, D.C. PB 194 051T.

Figure 1. Growth rates of three generations of Tapes semidecussata raised in the artificial upwelling system. Length in mm.

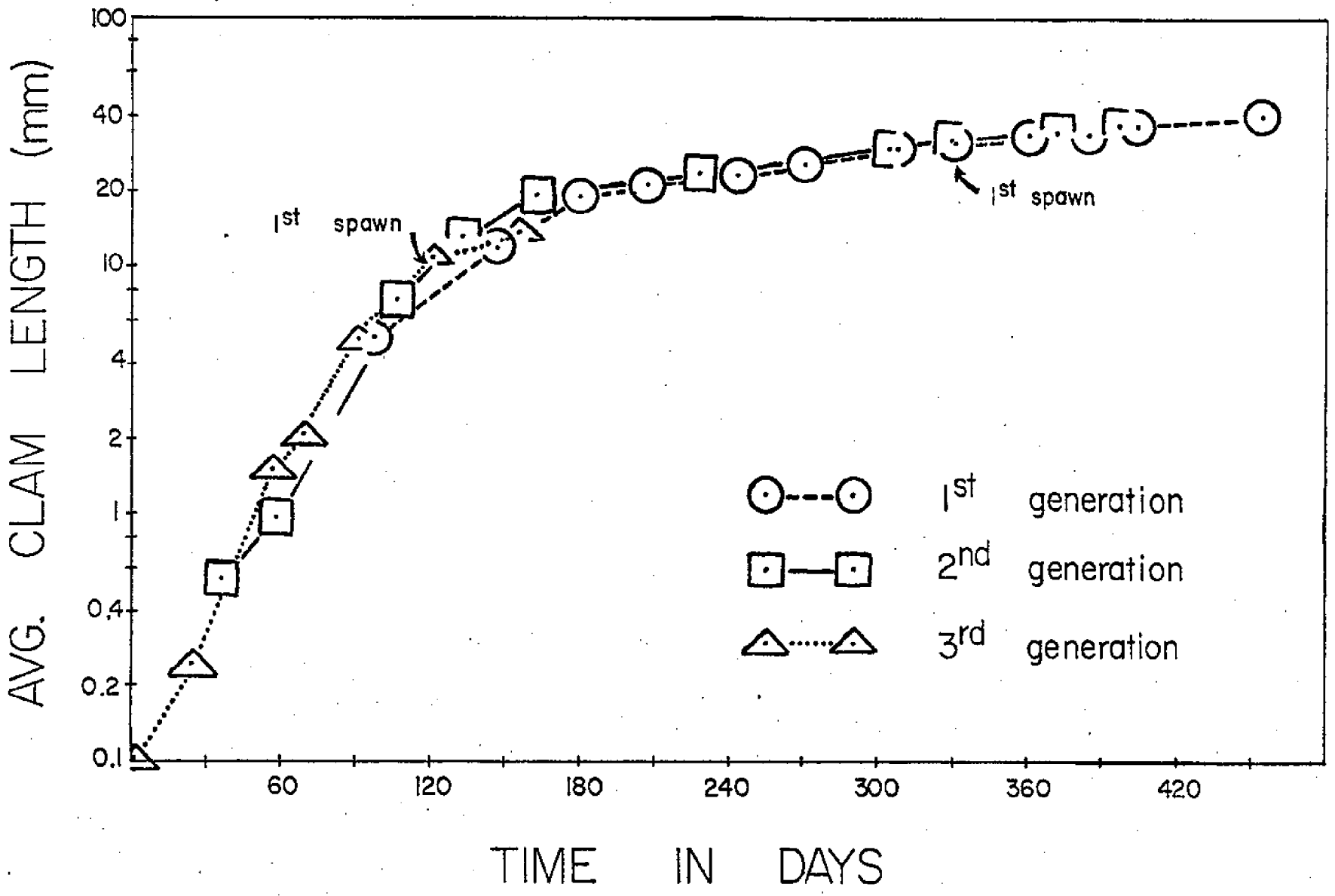

AVG. CLAM LENGTH (mm)
100
40
20
10
4
2
1
0.4
0.2
0.1
1st spawn
1st spawn
60
120
180
240
300
360
420
TIME IN DAYS
1st generation
2nd generation
3rd generation

Figure 2. Average live weight meat and shell fractions for clams of each test diet in the short term study.

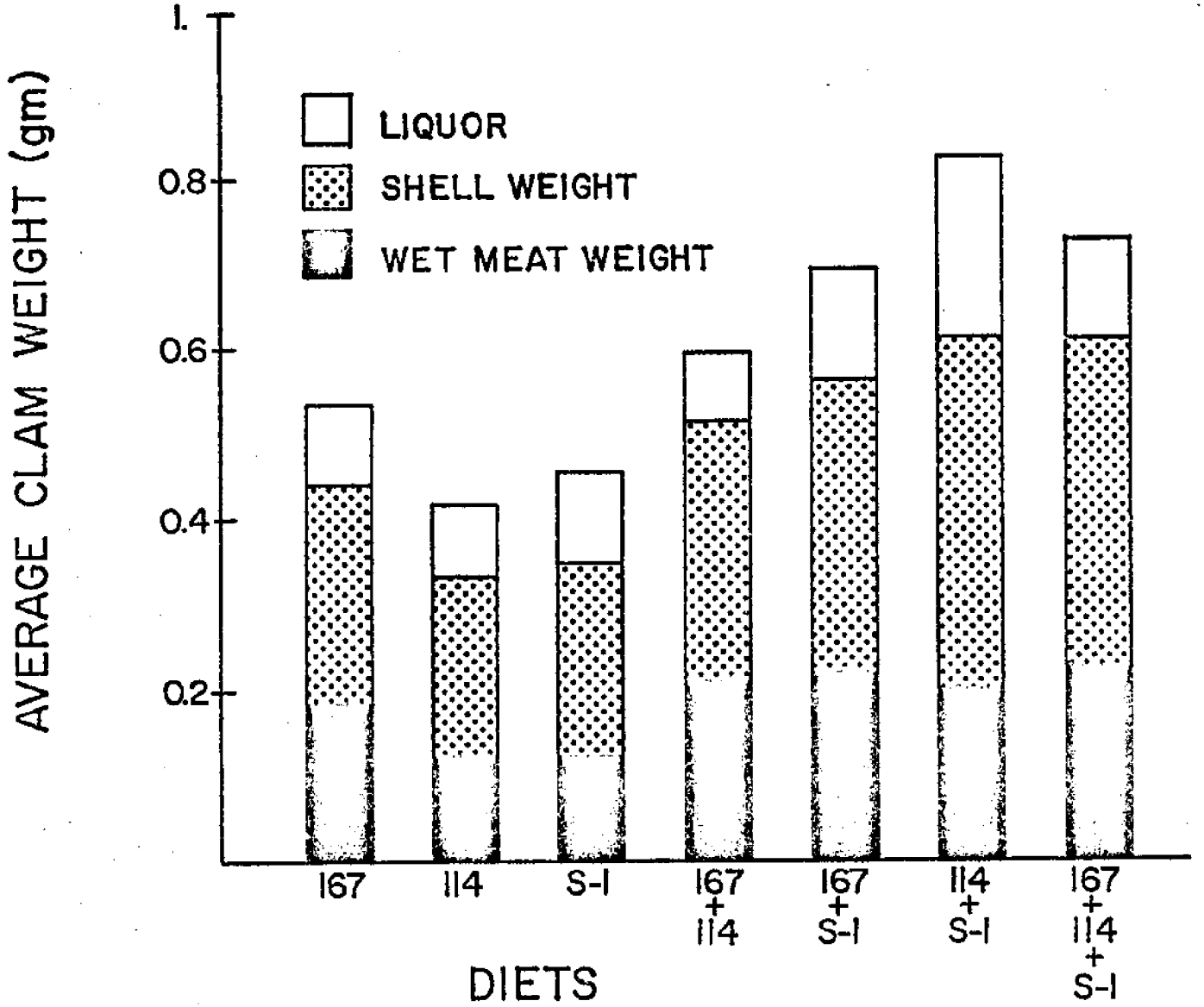

AVERAGE CLAM WEIGHT (gm)
1.
0.8
0.6
0.4
0.2
LIQUOR
SHELL WEIGHT
WET MEAT WEIGHT
167
114
S-1
167 + 114
167 + S-1
114 + S-1
167 + 114 + S-1
DIETS

Figure 3. Comparative growth of St. Croix *Tapes semidecussata* versus natural growth of *Tapes japonica* in the Sea of Ariake, Japan.

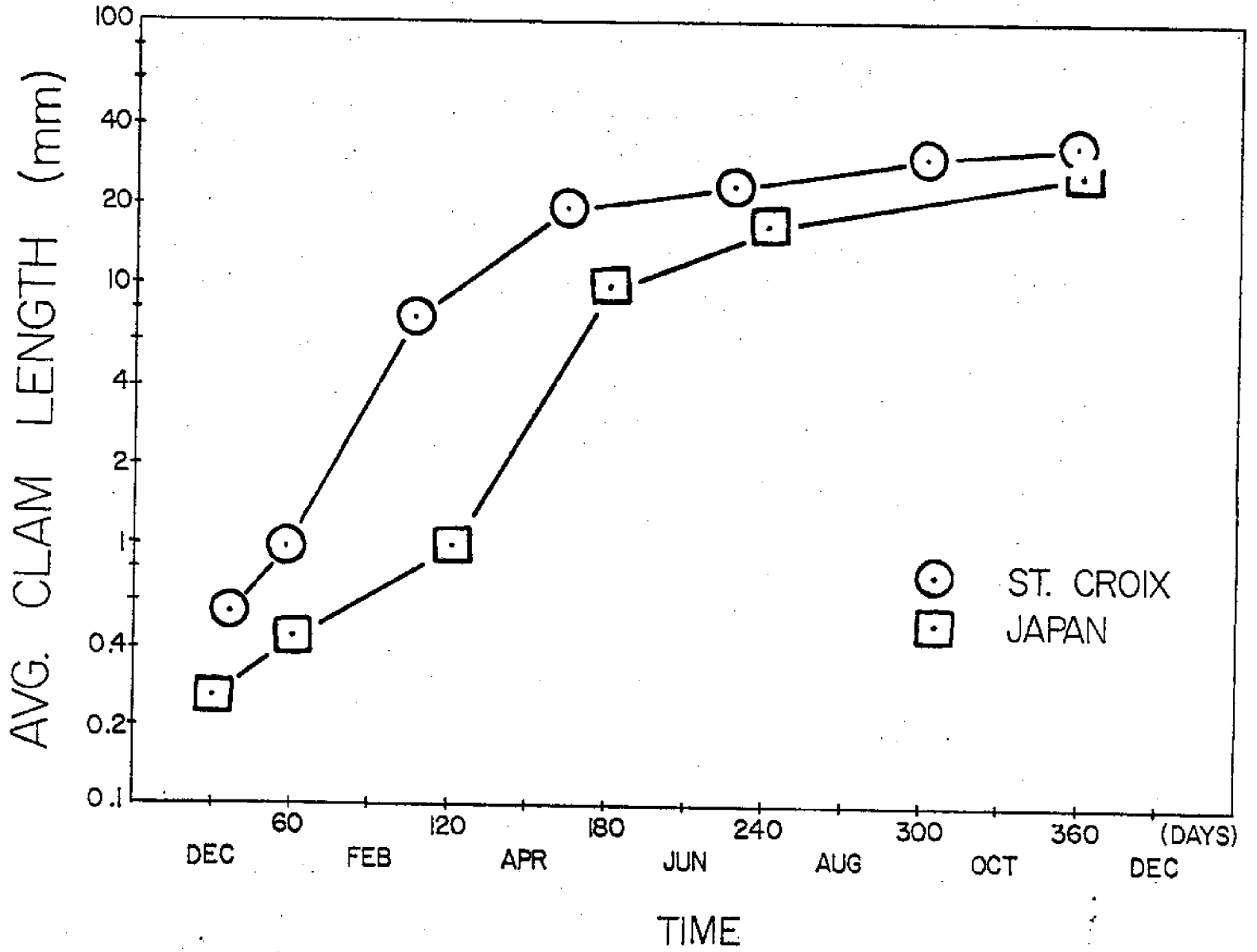

AVG. CLAM LENGTH (mm)
100
40
20
10
4
2
1
0.4
0.2
0.1
60
120
180
240
300
360
(DAYS)
DEC
FEB
APR
JUN
AUG
OCT
DEC
TIME
ST. CROIX
JAPAN

TABLE 1. SUMMARY OF FIVE-WEEK FEEDING STUDY ON JUVENILE *TAPES SEMIDECUSSATA*.

100 CLAMS PER TRAY, FLOWRATE = 2 ml/sec.

AVERAGE MEASUREMENTS	MONOCULTURES			MIXED DIETS			
	Chaetoceros curvisetus	*Bellerochea polymorpha*	S-1	*Chaetoceros* + *Bellerochea*	*Chaetoceros* + S-1	*Bellerochea* + S-1	*Chaetoceros* + *Bellerochea* + S-1
INITIAL							
Length/clam (mm)	6.5	6.5	6.5	6.5	6.5	6.5	6.5
Whole weight/tray(g)	5.10	5.05	5.15	4.90	5.20	5.05	4.95
Wet meat weight/tray(g)	1.75	1.74	1.77	1.69	1.79	1.74	1.71
Shell weight/tray(g)	2.63	2.60	2.65	2.52	2.68	2.60	2.55
FINAL							
Length/clam (mm)	14.9	14.1	15.0	15.4	16.7	17.3	16.6
Whole weight/tray(g)	51.5	40.9	44.1	59.9	68.7	82.3	70.9
Wet meat weight/tray(g)	17.2	11.5	11.4	20.6	22.4	20.0	22.2
Shell weight/tray(g)	25.9	20.9	23.3	30.9	35.3	40.5	37.0
Wet meat weight gain	15.45	9.76	9.63	18.91	19.75	17.4	20.49
Meat:shell ratio	1:1.50	1:1.85	1:2.05	1:1.50	1:1.55	1:2.05	1:1.70
Mortality (%)	.03	.03	.01	.02	0.0	.02	.03
ALGAE							
Cells/ml	6.36×10^4	1.70×10^6	2.20×10^5	1.81×10^6	2.84×10^5	1.96×10^6	2.05×10^6
Cells/day (10^{10})	1.10	29.4	3.80	30.9	4.90	33.40	34.60
5-week cell total (10^{10})	38.50	1029.0	133.0	1081.0	171.5	1169.0	1211.0
% stripping efficiency	11.4	12.3	29.5	87.6	61.5	66.0	84.6
Cells stripped 10^{10})	4.39	126.6	39.2	947.4	105.5	771.5	1024.5
Cells/gram wet meat gain (10^{10})	.284	12.97	4.07	50.10	5.34	44.34	50.0

THE EFFECT OF SUBSTRATE ON THE GROWTH RATE OF *TAPES SEMIDECUSSATA*

Kenneth Rodde

INTRODUCTION

Allowing clams to burrow in substrate (sand) reduces the attachment of fouling organisms and secondarily serves to approximate the natural habitat. This allows the animals to expend less energy in keeping their valves closed, lessens the incidence of gaping, and perhaps contributes to animal survival. One disadvantage of the arrangement is the difficulty in handling the heavy trays during routine inspection or in data collection. From a scientific viewpoint then, rearing clams out of sand is more desirable; from a commercial standpoint, however, a substrate may be advantageous if shellfish can be raised to a marketable size in a shorter period of time.

With both points in mind, this study intends to evaluate the growth of *Tapes semidecussata* under both conditions to determine the effect of the substrate.

METHODS

Two groups of *Tapes* of different sizes were selected to compare growth rates; one group of 2000 individuals averaging 10 mm, and the other, 1600 clams averaging 14 mm. Each group was divided in half, and one-half distributed in a Nestier tray containing two inches of sand of grain size between 1 and 3 mm, and the other half placed in a tray containing a screen liner only.

The trays were stacked (two trays per stack) in a 750-liter shellfish tank with a shortened standpipe to approximate a 400-liter

capacity, and received Pool 1 and 2 diatom cultures (Chaetoceros curvisetus, Bellerochea polymorpha and Thallassiosira pseudonana) at a flow rate of 120 ml/sec each. This rate gave a culture residence time of approximately 30 minutes. Every two weeks the population from each tray was weighed, and 50 individuals randomly selected for length measurements. The positions of the trays within a stack and the stack's position in the tank were rotated every three days for uniformity of exposure to inflow, outflow and center positions in each tank.

RESULTS AND DISCUSSION

The length and weight data of the four experimental populations are presented in Figures 1 and 2, and listed in Table 1.

From these data, it can be seen that very little difference exists in growth between populations in and out of sand during the six-week period. Nor is mortality a significant factor in raising clams under the two conditions. The choice of growing conditions then appears to be one of convenience or necessity, depending upon the amount of handling and the size of the operation, as well as the prevalence of fouling in the system.

Figure 1. A comparison of live weight of Tapes semi-decussata raised with and without a substrate.

O —— O 14 mm population in sand substrate;

O - - - O 14 mm population without sand substrate;

□ —— □ 10 mm population in sand substrate;

□ - - - □ 10 mm population without sand substrate.

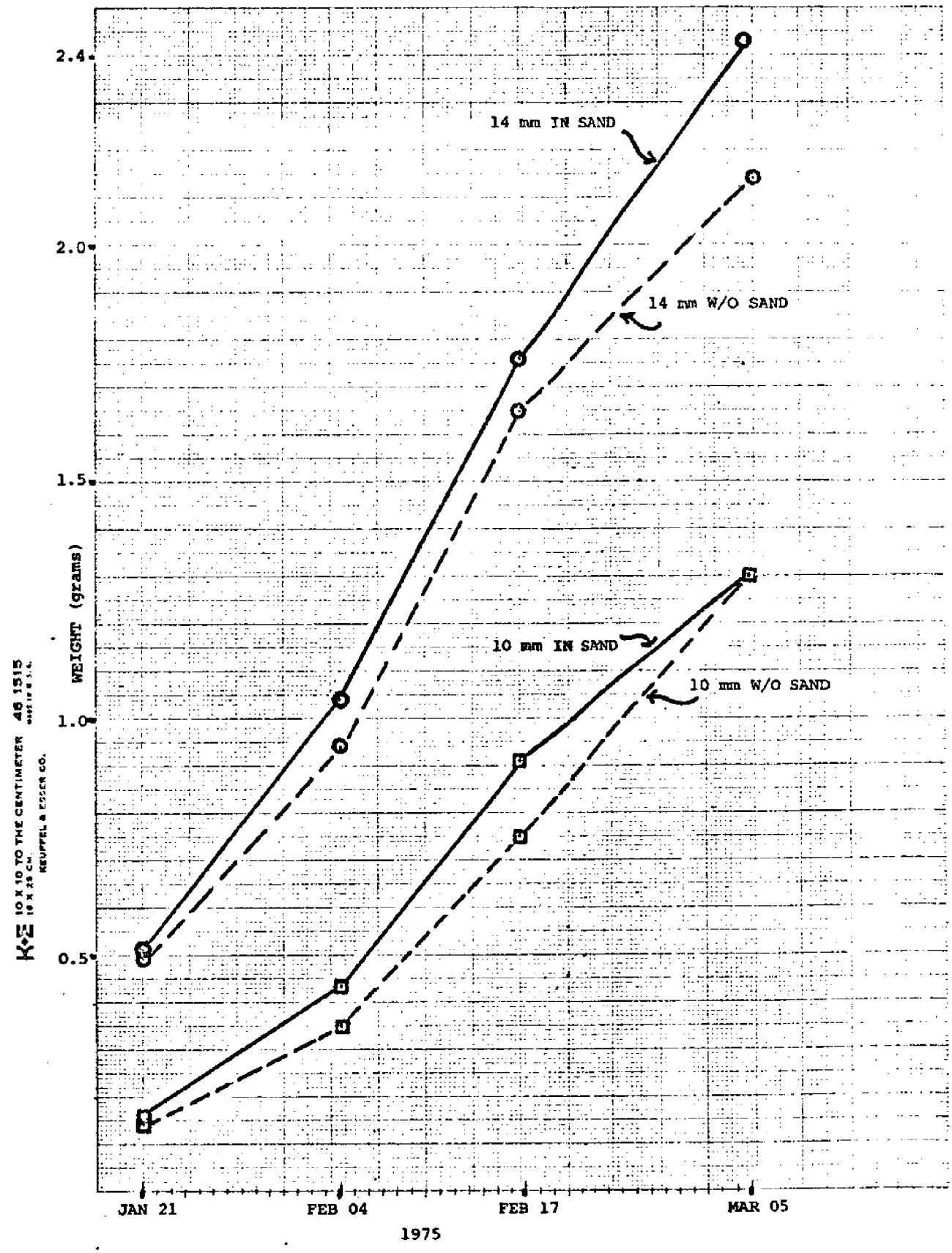

2.4
2.0
1.5
1.0
0.5
WEIGHT (grams)
14 mm IN SAND
14 mm W/O SAND
10 mm IN SAND
10 mm W/O SAND
JAN 21
FEB 04
FEB 17
MAR 05
1975
10 X 10 TO THE CENTIMETER
KEUFFEL & ESSER CO.

Figure 2. A comparison of average length of Tapes semidecussata raised with and without a substrate.

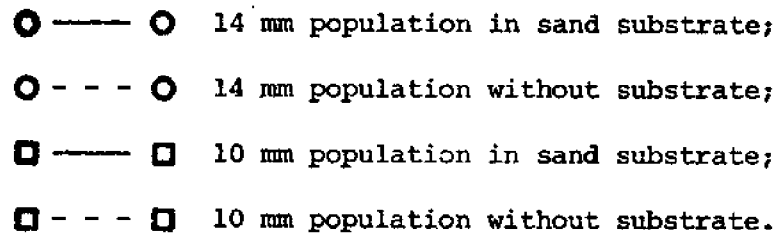
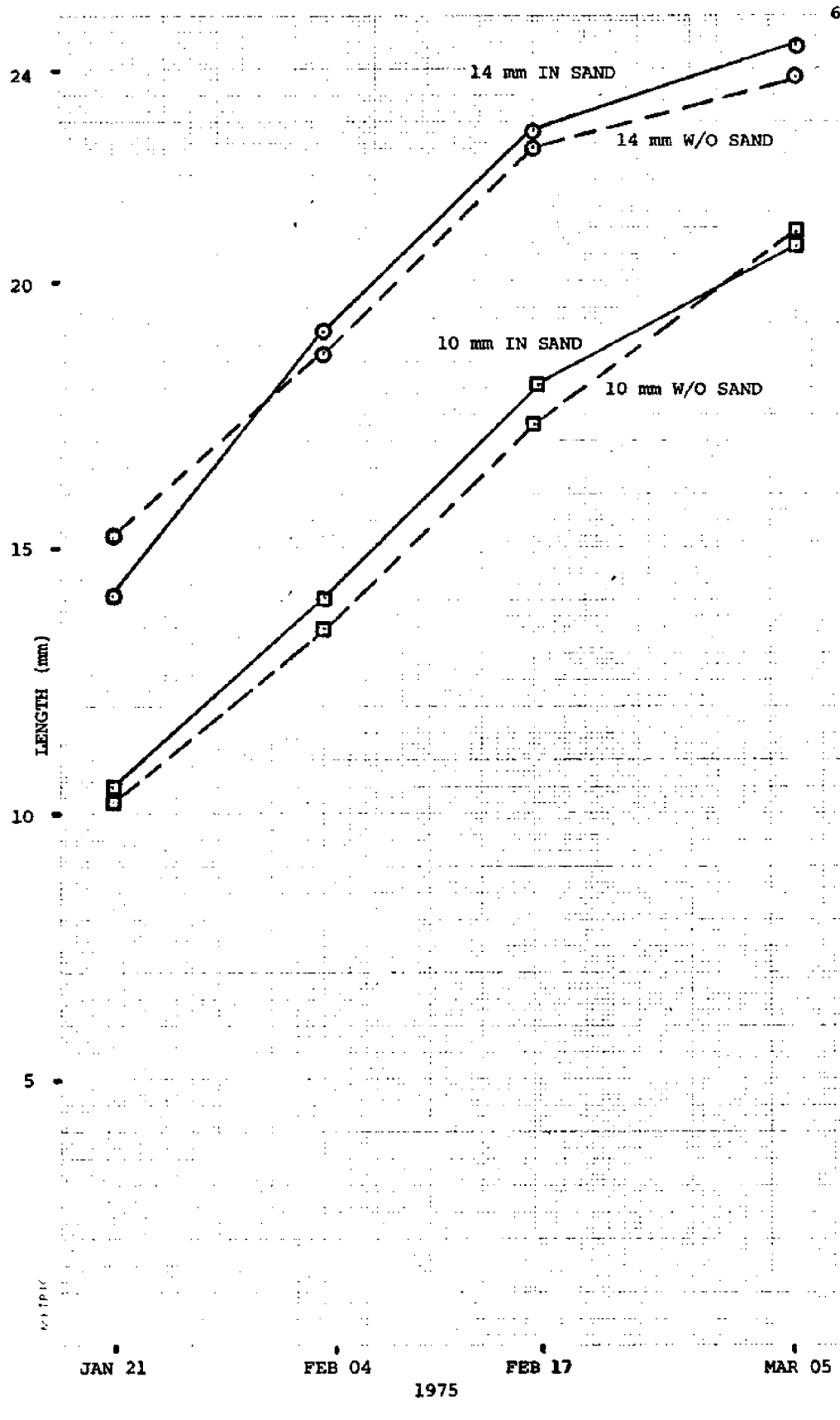


TABLE 1. WEIGHT, LENGTH AND MORTALITY MEASUREMENTS OF TAPES SEMIDECUSSATA WITH AND WITHOUT SUBSTRATE

A = 10 mm POPULATION, B = 14 mm POPULATION

DATE	POP.	SUBSTRATE			SCREEN LINER ONLY		
		AVERAGE LENGTH (mm)	AVERAGE WEIGHT (g)	MORTALITY	AVERAGE LENGTH (mm)	AVERAGE WEIGHT (g)	MORTALITY
JAN 21/75	A	10.50	0.166	0	10.20	0.143	0
	B	14.10	0.512	0	15.20	0.496	0
FEB 04/75	A	14.09	0.435	0	13.47	0.352	0
	B	19.06	1.040	0	18.77	0.944	0
FEB 17/75	A	18.06	0.910	1	17.29	0.75	0
	B	22.90	1.760	0	22.60	1.65	0
MAR 05/75	A	20.71	1.30	0	20.95	1.30	2
	B	24.48	2.43	4	23.87	2.14	0


24
20
15
10
5
LENGTH (mm)
14 mm IN SAND
14 mm W/O SAND
10 mm IN SAND
10 mm W/O SAND
JAN 21
FEB 04
FEB 17
MAR 05
1975

A Protocol

AN EXPERIMENTAL DETERMINATION OF A MAXIMUM SUSTAINABLE YIELD OF *TAPES SEMIDECUSSATA* ON AN ALGAL MIXTURE OF STX-167 AND S-1

Kenneth Rodde and
Scott Laurence

OBJECTIVES

To achieve and maintain the maximum increase in *Tapes semidecussata* meat weight per unit volume of culture media over time, and to determine the nitrogen balance in the experimental system.

RATIONALE

Previous experiments (Protocol #5.1.1, June 14, 1975) investigated the effects of several different diets on the growth rate of *Tapes* over a five-week period. The results showed that the STX-167 + S-1 combination was the most efficient mixture while producing the second greatest increase in wet meat weight.

Using this mixture as the best known diet to date, it is now appropriate to determine the maximum yield of that feed, that is, the maximum conversion rate of deep water nitrate to plankton total nitrogen and protein, converted to total nitrogen and protein of shellfish meat for a given flow rate, turnover time and total weight of an animal population. By removing clams each week to maintain a specified population weight, the yield of the feed can be determined in terms of harvested wet meat weight. By experimenting with a number of initial weight values, the maximum sustainable yield (harvest) will be found for two different flow rates and turnover times.

METHODS AND MATERIALS

Algal culture. The STX-167 (*Chaetoceros curvisetus*) culture for this experiment will be pumped from Pool 2 into a reactor at a rate

of 23 ml/sec. This reactor will have a shortened standpipe (18 inches) to effect a rapid turnover and insure consistency in culture density at all points in the experiment. The S-1 (unidentified, naked Cryptophyte flagellate) will be started in another reactor and will receive deep water inflow at a rate of 23 ml/sec. Back-up cultures of each species will be inoculated on a regular basis in two other reactors in the event the primary cultures collapse. Individual cultures will be monitored daily, both visually and by turbidity measurements.

Experimental shellfish rack. The outflow of the two primary reactors is directed into a cylindrical, 40-liter mixing container positioned above the rack. Vigorous aeration insures a complete mixing of the two cultures. The mixer outflow is connected to the end of a horizontal feeding level of the rack; five cells of this level will hold four separate shellfish containers, one primary and one replicate for each flow rate, and these measure 12 x 7.75 x 5.125 inches and hold 5.3 liters of culture. Teflon capillary tubes of .04 x 1/64 inch will feed the mixed culture to the containers; at 2 ml/sec, this equals a residence time of 44 min, and at 1 ml/sec, 88 min. Each shellfish container is supplied with an 11-inch length of perforated polyethylene tubing attached to one side; air forced through the tube creates a cylindrical-shaped current which homogenates all particulate matter in the water, i.e., algae, feces and pseudofeces.

Animals for the experiment will be selected from the upper 25% size range of a single population of Tapes semidecussata, and five initial weights (one for each of the five cells of a horizontal feed level) will be tested: 35, 50, 70, 100 and 140 grams. These weights were selected on the basis of Tapes growth from the earlier feeding study, the final, best weight of which was approximately 70 grams for the 167 + S-1 diet.

If clams averaging 8 mm in length are used, the number of clams in the min-max weight range will vary from approximately 350 to 1400 clams, respectively.

Sampling. (1) INFLOW: Algal culture will be sampled from the mixing container at 0900 and 1400 hrs daily. Two 400-ml samples will be collected in glass bottles; and cell counts will be performed. The remaining culture will be filtered onto a Gelman glass fiber filter for determination of chlorophyll a, particulate nitrogen, and Lowry protein. The filtrate will be collected for dissolved inorganic nitrogen analyses. Integrated light intensity measurements will be made at 1900 daily from a Li-Cor Quantum Integrator.

(2) TEST ANIMALS: On Day 0 of the experiment, five test population weights of Tapes and their replicates will be selected from the upper 25% (by weight) of a standing population. These are: 35, 50, 70, 100 and 140 grams. A sample of 50 animals will be selected for Lowry protein analysis from the same standing population. The five test populations at each flow rate will be weighed and counted on Days 0, 9, 18, 27, and 36 of the experiment. At each of these samplings the test population weights will be adjusted to maintain the original starting weights at Day 0 (W_0), i.e., the increase in total population weight (ΔW) will be culled ($W_1 - W_0 = \Delta W$). The culled animals comprising ΔW will be counted and weighed for whole wet weight, wet meat and liquor weight, and dry weight. Lowry protein determinations will be made on the wet meat and liquor. Particulate nitrogen will be determined on the dried frozen meat.

(3) OUTFLOW: Shellfish effluent will be sampled alternately between primary and replicate racks at 1400 hrs daily for each experimental weight and flow. Two 400-ml samples will be taken in glass

and filtered onto a Gelman GFF for particulate nitrogen and Lowry protein determiantions on the filters. The filtrate will be analyzed for dissolved inorganic nitrogen species. Dissolved organic nitrogen will be represented as the difference between the total nitrogen entering and incorporated at the shellfish level, and the total determinable nitrogen leaving this level.

Analyses. Shellfish will be blotted and weighed alive for whole wet weight. Wet meat weight will be determined on culled individuals which have been shucked, and the shells dried at 60°C to constant weight. Wet meat weight will be assigned the difference between whole wet weight and dry shell weight. Cell counts will be performed on a minimum of 100 cells/field using a Speirs-Levy Eosinophil Counter. Algal protein will be determined essentially as per Lowry et al. (1951), on cells filtered onto 25 mm Gelman GFF and digested in the alkaline-copper reagent for 60 min at 100°C. Shellfish protein will be determined on a tissue homogenate, diluted to accommodate linearity, as per Lowry et al. (1951). From this tissue homogenate, 25 ml will be pipeted onto aluminum weighing dishes and dried at 60°C to constant weight for dry weight determination and subsequent shipment to LDGO, New York, for particulate nitrogen analysis by combustion gas chromatography (Hewlett-Packard Model 185 CHN analyzer). Filtered algal culture will also be shipped frozen for this analysis. Chlorophyll a will be determined by the fluorometric technique of Strickland and Parsons (1972) on a known volume and density of algal cells filtered at 8 in. Hg vacuum through Gelman GFF.

Dissolved inorganic nitrogen will be determined usinga Technicon AutoAnalyzer II system and standard manifolds.

ANALYSIS OF DATA

(1) Maximum sustainable yield of shellfish meat: $\frac{\Delta W}{W}$, or percen-

tage weight gain per total amount of weight per flow rate.

(2) Nitrogen conversion efficiencies as a function of weight and flow.

(3) Protein conversions: phytoplankton to clam meat for each experimental weight and flow rate.

(4) Daily ratios of various parameter relationships, e.g., sunlight (photometric information)/chlorophyll a, NO_2, NO_3 inflow/outflow, etc.

IMPLEMENTATION

Pending completion of set-up for chemical analyses (Tom Dorsey and Richard Lyon), culture availability: November 10, 1975.

Figure 1. Nitrogen flow in the St. Croix artificial upwelling mariculture system.

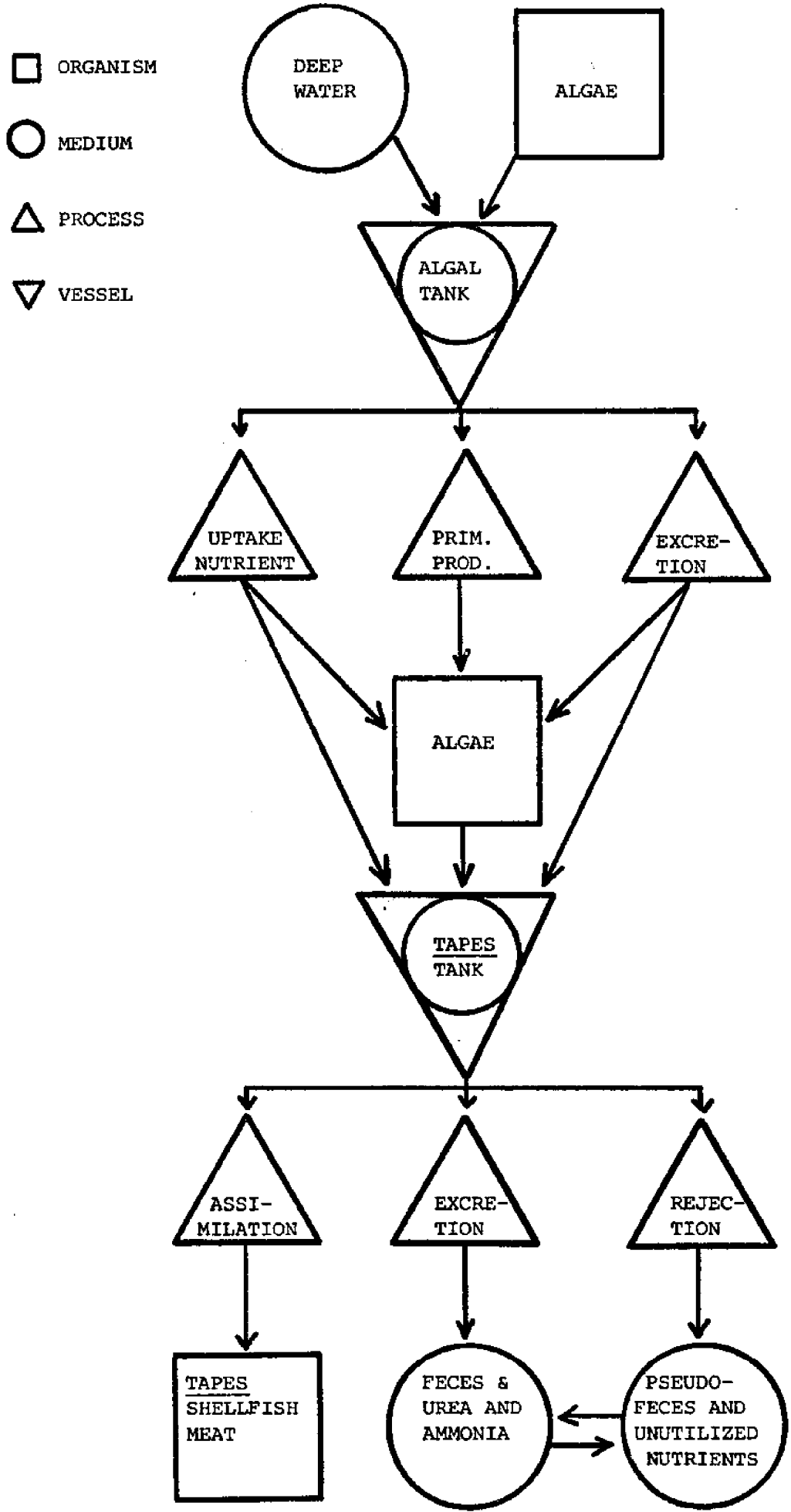
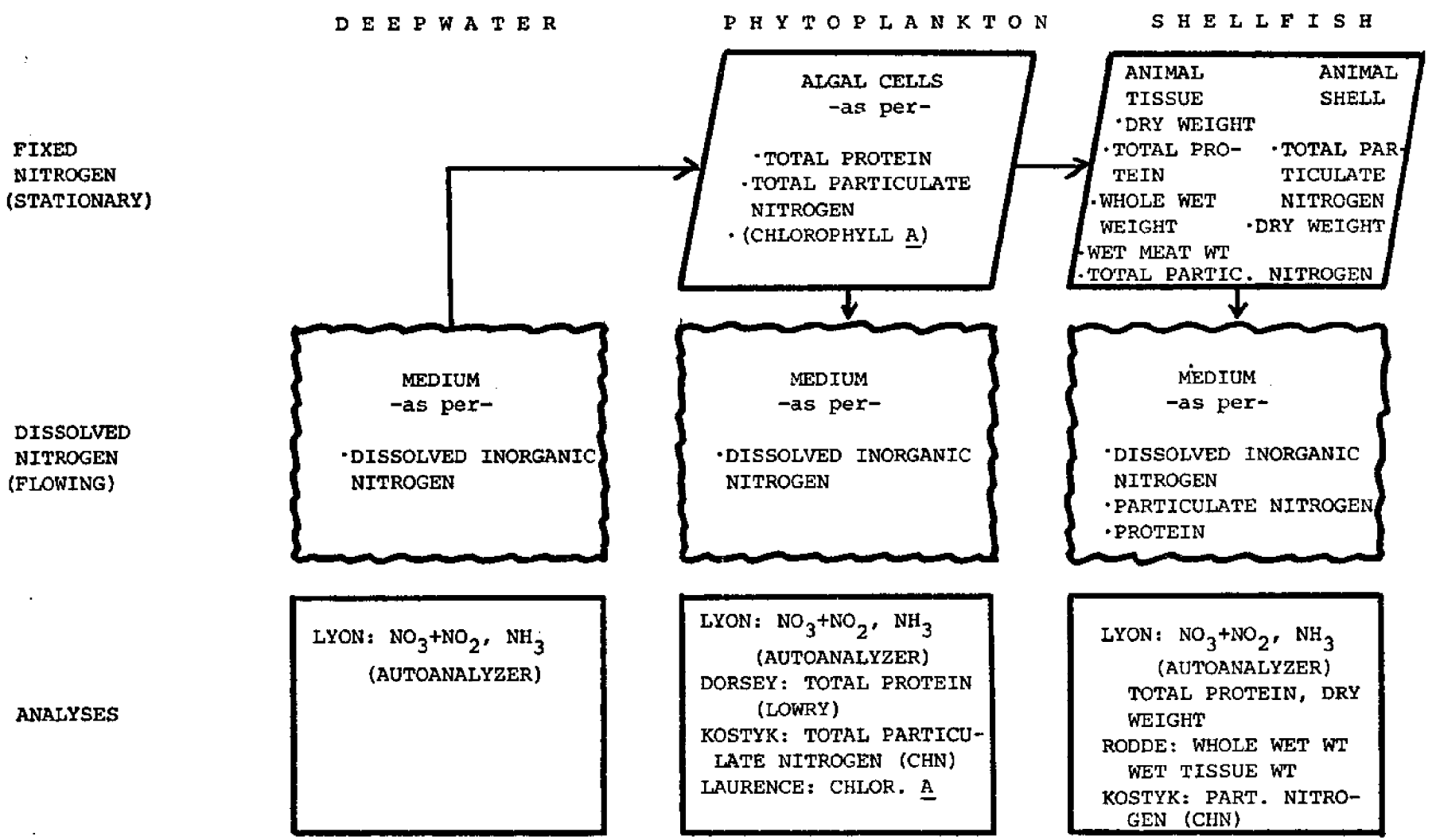

ORGANISM
MEDIUM
PROCESS
VESSEL
DEEP WATER
ALGAE
ALGAL TANK
UPTAKE NUTRIENT
PRIM. PROD.
EXCRE-TION
ALGAE
TAPES TANK
ASSI-MILATION
EXCRE-TION
REJEC-TION
TAPES SHELLFISH MEAT
FECES & UREA AND AMMONIA
PSEUDO-FECES AND UNUTILIZED NUTRIENTS

Figure 2. Trophic level breakdown of nitrogen balance experiment and attendant analyses.


DEEPWATER
PHYTOPLANKTON
SHELLFISH
FIXED NITROGEN (STATIONARY)
DISSOLVED NITROGEN (FLOWING)
ANALYSES
ALGAL CELLS -as per-
·TOTAL PROTEIN
·TOTAL PARTICULATE NITROGEN
·(CHLOROPHYLL A)
ANIMAL TISSUE
·DRY WEIGHT
·TOTAL PROTEIN
·WHOLE WET WEIGHT
·WET MEAT WT
·TOTAL PARTIC. NITROGEN
ANIMAL SHELL
·TOTAL PARTICULATE NITROGEN
·DRY WEIGHT
MEDIUM -as per-
·DISSOLVED INORGANIC NITROGEN
MEDIUM -as per-
·DISSOLVED INORGANIC NITROGEN
MEDIUM -as per-
·DISSOLVED INORGANIC NITROGEN
·PARTICULATE NITROGEN
·PROTEIN
LYON: NO3+NO2, NH3 (AUTOANALYZER)
LYON: NO3+NO2, NH3 (AUTOANALYZER)
DORSEY: TOTAL PROTEIN (LOWRY)
KOSTYK: TOTAL PARTICULATE NITROGEN (CHN)
LAURENCE: CHLOR. A
LYON: NO3+NO2, NH3 (AUTOANALYZER)
TOTAL PROTEIN, DRY WEIGHT
RODDE: WHOLE WET WT WET TISSUE WT
KOSTYK: PART. NITROGEN (CHN)

GROWTH OF THE CARRAGEENAN-PRODUCING TROPICAL RED SEAWEED HYPNEA MUSCIFORMIS IN SURFACE WATER, 870-M DEEP WATER, EFFLUENT FROM A CLAM MARICULTURE SYSTEM, AND IN DEEP WATER ENRICHED WITH ARTIFICIAL FERTILIZERS OR DOMESTIC SEWAGE[1]

by

Kenneth C. Haines[2]

Lamont-Doherty Geological Observatory

of Columbia University

Palisades, New York 10964

Running head: "Growth of Hypnea musciformis"

to be presented at the
Tenth European Symposium on Marine Biology
Ostend (Belgium) September 17-23, 1975

[1] Supported by the National Sea Grant Program, NOAA, U. S. Department of Commerce (04-5-158-59) and the G. Unger Vetlesen Foundation; Lamont-Doherty Geological Observatory Contribution No. 0000.

[2] Present address: Artificial Upwelling Project, P.O. Box Z, Kingshill, St. Croix, U.S. Virgin Islands 00850

GROWTH OF THE CARRAGEENAN-PRODUCING TROPICAL RED SEAWEED HYPNEA MUSCIFORMIS IN SURFACE WATER, 870-M DEEP WATER, EFFLUENT FROM A CLAM MARICULTURE SYSTEM, AND IN DEEP WATER ENRICHED WITH ARTIFICIAL FERTILIZERS OR DOMESTIC SEWAGE

Kenneth C. Haines
Lamont-Doherty Geological Observatory
of Columbia University

A B S T R A C T

Growth of Hypnea musciformis (Wulf.) Lamaroux in the effluent from an artificial upwelling mariculture system on St. Croix, U.S. Virgin Islands, in which 870-m deep water is used to grow planktonic algae which are then fed to bivalve shellfish, was about five times faster than growth in unaltered deep water, and about three times faster than in surface seawater. Growth was positively correlated with ammonia concentration in the water supplied to the seaweed, but there was no correlation with nitrate or phosphate concentration.

The growth stimulation by the effluent from the shellfish mariculture system could be achieved by enriching deep water with ammonia plus a chelated iron/trace metals/vitamins mix, but could not be achieved by enriching the deep water with ammonia alone or with the chelated iron/trace metals/vitamins mix alone. Increasing the ammonia supply, while keeping the chelated iron/trace metals/vitamins mix enrichment constant, produced an increased growth rate.

Enrichment of deep water with 4% primary-treated sewage and 35% secondary-treated sewage also increased

growth of H. musciformis relative to its growth in deep water alone. Carbon, nitrogen, and carrageenan contents of the seaweed grown under various nutritional conditions are presented.

INTRODUCTION

Hypnea musciformis (Wulfen) Lamaroux is a common red alga of tropical seas. It is valued for its content of kappa-carrageenan, a cell-wall polysaccharide, which is important as a gelling agent in a variety of commercial applications (Levring et al., 1969). Humm and Kreuzer (1974) found that H. musciformis on the north shore of St. Croix, U. S. Virgin Islands, doubled its weight every two to four days in its natural nutrient-poor environment, and concluded that it may be economically feasible to rear the alga in tanks into which surface seawater would be pumped.

This paper presents results of studies undertaken to determine whether the growth rate of Hypnea musciformis could be increased by cultivating the alga in seawater containing higher concentrations of dissolved nutrients than normally found in surface seawater. It was hoped that H. musciformis could be utilized to remove residual nutrients and animal-waste products from the effluent from a tropical "artificial upwelling" mariculture system (Roels et al., 1975), or in a sewage-enriched seawater mariculture system such as that proposed by Prince (1974) for temperate seaweeds.

MATERIALS AND METHODS

Hypnea musciformis was collected between October 1974 and May 1975 by wading in shallow waters at one of the following locations on the north shore of St. Croix: Tague Bay,

adjacent to the Fairleigh-Dickinson University's West Indies Laboratory pier; Boiler Bay; Rust-op-Twist; or Sugar Bay in Salt River Inlet. All of these locations normally have salinities of ca 35 °/oo. Inoculum plants for experiments were picked free of epiphytic seaweeds and care was taken to divide algal material equally between experimental tanks both by weight and by apparent physiological state (relative pigmentation of the thalli); this was sometimes accomplished by dividing plants. Sexually reproductive thalli were seldom seen and were not used for growth experiments. In some cases, inocula for experiments were aliquants of algal material harvested from the previous experiment.

The experimental tanks were 30.2 cm x 45.7 cm x 30.2 cm deep opaque polyvinylchloride (PVC) tanks fitted with inlet and outlet ports for the water supply near the corners on opposite ends of the long sides of the tanks. The outlet port was shielded by a vertical perforated PVC panel to prevent the algal mass from clogging the outlet port. The water level was maintained by means of an external standpipe so that the working volume was 36 liters. An aeration manifold (a 2.0 cm inside diameter PVC pipe with small holes drilled along its length) was located next to one side of the long axis of the tank. Aeration was adjusted so that the algal mass was kept constantly revolving around the horizontal long axis of the tank. The tanks were kept outdoors in full sun. Temperatures and seawater flow rates were measured twice daily; flow rates were set by means of garden hose ball valves.

Where applicable, nutrient or sewage enrichments were metered to the tanks by means of a peristaltic pump.

Wet weights of the masses of thalli were determined after slinging them through a 180-degree arc at arms' length in a plastic mesh bag to remove free water. On days when the Hypnea was weighed, the tanks were scrubbed with a sodium hypochlorite solution to control colonization of the tank walls by pennate diatoms. Dry weights of harvested thalli were obtained by drying at 60°C to constant weight. Dissolved nitrate, nitrite, ammonia and orthophosphate concentrations in the seawater supplies and seaweed-tank effluents were determined in duplicate on glass fiber-filtered samples by methods given by Parsons and Strickland (1972) on an Auto-analyzer II (Technicon Corp.), using standard manifolds. The 95% confidence limits (±2 σ) for the analyses were (in μg-at l^{-1}): NO_3-N, ±0.41; NO_2-N, ±0.07; NH_4-N, ±0.20; PO_4-P, ±0.13. Particulate carbon and nitrogen contents of the harvested algae were determined on a Hewlett-Packard CHN Analyzer, Model 185. Carrageenan analyses were done on oven-dried samples of Hypnea by the Stauffer Chemical Company, Eastern Research Laboratory, Dobbs Ferry, N.Y.

The growth studies were carried out at the Artificial Upwelling Project at Rust-op-Twist, St. Croix, except that the Tague Bay surface-water tank was maintained at Fairleigh-Dickinson University's West Indies Laboratory, approximately 20 km east of Rust-op-Twist.

Growth in Surface Water, Deep Water and Effluent from a Bivalve Shellfish Culture Tank

Hypnea from Tague Bay was grown in three tanks receiving equal continuous flows of seawater from (1) Tague Bay surface water, pumped from inside the reef; (2) water pumped from 870-m depth, approximately 1.8 km offshore (hereafter called "deep water"); or (3) the effluent from a tank containing juvenile Japanese little-neck clams, Tapes semidecussata (Reeve). The seawater supply to the clam tank was from continuous cultures of three species of diatoms grown either in unsupplemented deep water or in deep water supplemented with a chelated iron/trace metals/vitamins mix to yield the following levels of enrichment: disodium ethylenediamine tetraacetate (EDTA), 1.5 µM; ferric chloride, 1.3 µM; Cu, 0.005 µM; Co, 0.0006 µM; Mn, 0.1 µM; Mo, 0.003 µM; Zn, 0.009 µM; cyanocobalamin (vitamin B_{12}, 0.04 µg l^{-1}, thiamine HCl (vitamin B_1), 2.0 µg l^{-1}. The clams were fed from cultures of three species of diatoms grown in supplemented deep water about 85% of the time, and from cultures grown in unsupplemented deep water about 15% of the time. Consequently, the effluent from the clam tank fluctuated widely in its content of NO_3-N and NH_4-N, due to variations in the food supply (species and cell concentrations of diatoms) to the clams, varying degrees of nitrogen uptake by the diatom species, and different efficiencies of the clams in removing diatom species from suspension. By contrast, the seawater supplies from deep water and Tague Bay were relatively constant in nutrient content (Table I).

Table I

The flow rates in all three tanks were set to give 96 volume changes per day; this prevented the algal mass from completely stripping the nutrients from the water and permitted calculations of nutrient uptake by the alga. (When the Hypnea was removed from the tank there was no measurable nutrient uptake by diatoms remaining in the clam tank effluent during their 15-minute passage through the Hypnea tank [one observation].) A repeat experiment in the three tanks was made using inoculum from tanks receiving clam tank effluent for the deep water and clam effluent tanks, and from Tague Bay for the tank supplied with Tague Bay water. Measurements of nutrient uptake were made for the Tague Bay tank during the repeat experiment, and for the effluent and deep water tanks during the first experiment.

Growth in Deep Water with Artificial Enrichments

In these experiments deep water was supplemented with (1) the chelated iron/trace metals/vitamins mix used in the diatom cultures to yield the concentrations of the components given above, or (2) ammonium chloride to yield concentrations of ca 4 and 12 µg-at NH_4-N l^{-1} ("low" and "high" levels, respectively), or (3) both the chelated iron/trace metals/vitamins mix and ammonium chloride. Control cultures of the alga received unsupplemented deep water. Flow rates to the tanks were set to give 96 volume changes per day. The inoculum for the first experiment in this series was obtained from Boiler Bay on January 5, 1975.

Growth in Deep Water Enriched with Domestic Sewage

Hypnea collected March 18, 1975 from Sugar Bay and April 17, 1975 from Rust-op-Twist was grown in deep water supplemented with ca 4% primary-treated and ca 35% secondary-treated sewage, respectively. Control cultures were grown in unsupplemented deep water. The total flow rates to the tanks was set to give 3-4 volume changes per day, to reduce the amount of sewage required. Flow rates were more variable in these experiments than in the previous ones with faster flow rates. Failure of the peristaltic pump used for metering the sewage necessitated the use of a gravity flow system; this led to fluctuations in flows of the enrichments due to changes in pressure head. The sewage was collected in 80-liter batches from the Advanced Wastewater Treatment Plant on St. Croix, sealed airtight in 20-liter glass carboys to prevent oxidation of ammonia to nitrate, and stored in the dark to inhibit algal growth.

RESULTS

Growth in Surface Water, Deep Water and Effluent from a Bivalve Shellfish Culture Tank

Growth of Hypnea in the effluent from the shellfish tank was much more rapid than growth in deep water or in Tague Bay water (Figure 1).

Figure 1

Maximum growth rates during the two experiments in each tank were 64.5 and 62.5 (wet weight) g day^{-1} in the shellfish effluent, 16.1 and 16.2 g day^{-1} in Tague Bay water and 12.1 and 15.4 g day^{-1} in deep water. Growth ceased when a maximum wet weight of about 200 g was reached in the Tague Bay and deep water tanks; this suggests that one or more nutrients may have been limiting. (It was not feasible to continue growing the Hypnea in the shellfish effluent tank until growth stopped there also, because the large mass of alga could no longer be kept in motion by the aerating system.)

There was a marked depression in growth rate of the Hypnea in the effluent tank which may have been caused by an eight-day period of overcast weather (days 31-39). There did not appear to be any direct influence of temperature on growth rates in the tanks; the temperature ranges and averages (in parentheses) for the tanks were (°C): effluent, 23.2-31.2 (26.1); Tague Bay, 27.3-30.0 (29); deep water, 22.6-26.5 (24.3).

When the maximum growth rates of the Hypnea from the appropriate growth curves in Figure 1 are plotted vs the mean concentrations of nutrients measured in the seawater supplied, a direct correlation between growth rate and nutrient concentration is observed for NH_4-N, but not for PO_4-P or NO_3+NO_2-N (Figure 2).

Figure 2

Uptake of NH_4-N per unit weight of Hypnea in the effluent tank was directly related to the ambient concentration of the nutrient, defined here as the total amount of nutrient in the

tank (the residue after uptake) divided by the weight of Hypnea in the tank (Figure 3).

Figure 3

The maximum uptake efficiency for NH_4-N (about 50%) was observed at an NH_4-N residue concentration of 0.4-0.8 µg-at NH_4-N g^{-1} Hypnea. The decreased uptake at the highest residue concentration may have been caused by the turnover rate being too fast for maximum uptake (Figure 4).

Figure 4

Similar uptake rates for NO_3-N were observed in the deep water tank (0.3-0.7 µg-at g^{-1} Hypnea), but uptake efficiency was less than 10% and the growth rate in deep water was about one-fifth that observed in the effluent tank.

Growth in Deep Water with Artificial Enrichments

The addition of NH_4-N alone (ca 4 µg-at l^{-1}) to deep water did not produce a growth rate any faster than that obtained with deep water alone, nor did the addition of the chelated iron/trace metals/vitamins mix alone (Figure 5). The addition of NH_4-N plus the chelated iron/trace metals/vitamins mix prevented the Hypnea from disintegrating as badly as did the plants in the unenriched deep water, and growth was eventually resumed in this combined enrichment (Figure 5).

Figure 5

When a new experiment was started with and without the NH_4-N plus chelated iron/trace metals/vitamins mix (using inoculum Hypnea harvested from the enriched water at the end

of the experiment shown in Figure 5, growth with the enrichment (21.7 g wet wt day^{-1}) was four times faster than in deep water alone, but about one-third as fast as the growth rate in the shellfish effluent (62.5-64.5 g wet wt day^{-1}) (Figure 6).

Figure 6

Increasing the NH_4-N enrichment from ca 4 to ca 12 µg-at l^{-1}, while using the same level of enrichment with the chelated iron/trace metals/vitamins mix, produced a further increase in growth rate: the growth rates with the "high" and "low" NH_4-N enrichment levels were 35.7 and 23.6 g wet wt day^{-1}, respectively (Figure 7).

Figure 7

The average temperatures for this series of enrichment experiments were 22.3 to 22.7°C. The minimum temperatures were 21.1-21.8°C, and the maximum temperatures were 23.3-24.6°C, except that 29.9°C was observed once when flow was interrupted to one of the tanks.

Growth in Deep Water Enriched with Domestic Sewage

Figure 8 gives the results of single growth experiments with Hypnea in deep water enriched with 4% primary-treated sewage and 35% secondary-treated sewage. In both cases there was an initial lag in growth rate in the sewage-enriched tanks. Enrichment with 4% primary sewage gave only slightly better growth (7.6 g wet wt day^{-1}) than deep water alone. Secondary sewage stimulated Hypnea growth about 22% over that in deep water alone: the growth rates were 15 g and 11.8 g wet wt day^{-1}

with and without secondary sewage, respectively. Temperature was highly variable in these experiments due to the low flow rates used (three volumes per day) and difficulties in regulating the flows. The average temperatures in the tanks were 26.3-26.8°C, and maximum temperatures of 30-33°C in the afternoon were common. The minimum temperatures were 21.8-23.0°C.

Figure 8

Carbon, Nitrogen and Carrageenan Content of Hypnea musciformis

The carbon and nitrogen contents of Hypnea grown in shellfish effluent, deep water and Tague Bay water are given in Table II. The carbon and nitrogen contents were inversely related to the growth rates measured during the most active periods of growth prior to harvest (compare Table II with Figure 1). However, the carbon and nitrogen contents reported may not accurately reflect the composition during active growth. The C:N ratio was also inversely related to growth rate for the Hypnea grown in effluent and Tague Bay water. The deep water Hypnea did not fit this pattern, possibly due to uptake of NO_3-N from deep water in excess of the requirements for growth.

Table II

The carrageenan yields and the kappa:lambda ratios are given in Table III for Hypnea grown under a variety of conditions in this study, as well as collected from nature. The carrageenan yields for Hypnea grown during this study were 16-29% of the dry weight, compared to 20-26% for Hypnea

collected from nature. Correlations between carrageenan yield and growth rate, and between kappa:lambda ratio and growth rate are difficult to make, because--as for the carbon and nitrogen contents--for many samples the growth rates given were measured during the period of maximum growth rate, while the samples for carrageenan analysis were often taken after growth had slowed or stopped. This applies to samples of Hypnea grown in deep water and in Tague Bay water; similarly, there is no way of knowing the rates of growth of populations harvested from nature. The carrageenan contents of Hypnea harvested during the period of maximum growth rate (samples 5 and 8 in Table III, grown in deep water at two levels of NH_4-N, with addition of chelated iron/trace metals/vitamins mix) had carrageenan contents and kappa:lambda ratios comparable to those of Hypnea commercially-harvested from nature in the Mediterranean.

Table III

DISCUSSION

This study has shown that Hypnea musciformis can be grown in seawater much richer in dissolved nutrients than its normal habitat, and can be used as a means of reducing the loss of nutrients in the effluent from mariculture systems, while producing commercially-valuable carrageenan. Roels et al. (1975) have calculated that Hypnea grown in the effluent from a tropical mariculture system would have a gross harvest value of $ 107,250 per hectare annually.

It is interesting to note that Hypnea musciformis grown in effluent from the St. Croix mariculture system has a higher productivity than has been observed for some marine plants or communities, micro-algae mass culture, and some conventional agricultural crops (Table IV).

Table IV

Additional study is needed to determine the optimal conditions for growing Hypnea, expecially with respect to achieving maximum carrageenan production. The use of wild populations of plants for experiments, as was the case in this study, has made it difficult to make valid comparisons between experiments. The alga has recently been brought into unialgal culture from individual tetraspores, so that genetically-uniform plants can be utilized for future experiments.

ACKNOWLEDGEMENTS

I thank D. Berg, M. Farmer, O. Francis, N. Kostyk, S. Laurence, R. Ryons, R. Monahan, N. Ogden, S. Richardson, and C. Rodde for their technical assistance, and Dr. O.A. Roels for his support and encouragement of this work and for reading the manuscript. Dr. S. Adler (Stauffer Chemical Co.) kindly supplied the carrageenan contents of Hypnea samples.

LITERATURE CITED

Goldman, J.C., J.H. Ryther, L.D. Williams and B.E. Lapointe, 1975. The mass culturing of macroscopic marine algae. Presented at the 38th Annual Meeting, Amer. Soc. Limnol. Oceanog., Dalhousie Univ., Halifax, Nova Scotia, June 22-26.

Humm, H.J. and J. Kreuzer, 1974. On the growth rate of the red alga, Hypnea musciformis, in the Caribbean Sea. Unpublished manuscript.

Levring, T., H.A. Hoppe and O.J. Schmid, 1969. Marine algae: A survey of research and utilization. Bot. Mar. Handbook No. 1. Cram De Gruyter und Co., Hamburg, Germany, 42[illegible] pp.

Morris, I., 1974. The limits to the productivity of the sea. Sci. Prog., Oxf., Vol. 61, pp. 99-122.

Prince, J.S., 1974. Nutrient assimilation and growth of some seaweeds in mixtures of sea water and secondary sewage treatment effluents. Aquaculture, Vol. 4, pp. 69-79.

Ryther, J.H., 1960. Organic production by plankton algae, and its environmental control. In, The ecology of algae, edited by C.A. Tryon, Jr. and R.T. Hartman, Special Publ. No. 2, Pymatuning Laboraotyr of Field Biology, Univ. Pittsburgh, pp. 72-83.

Roels, O.A., K.C. Haines and J.B. Sunderlin, 1975. The potential yield of artificial upwelling mariculture. Presented at the 10th European Symposium on Marine

Biology, Ostend (Belgium), September 17-23.

Strickland, J.D.H. and T.R. Parsons, 1972. A practical handbook of seawater analysis. Fish. Res. Bd. Can. Bull. 167, 2nd edition, 310 pp.

TABLE I. RANGES OF DISSOLVED NUTRIENT CONCENTRATIONS (μG-AT L^{-1}) IN THE THREE TYPES OF SEAWATER USED FOR THE *HYPNEA* TANKS

NUTRIENT	870 M DEEP WATER[1]	TAGUE BAY WATER[2]	SHELLFISH TANK EFFLUENT[1]
NO_3+NO_2-N	30.2-30.8[3]	2.5-3.4	0.7-15.4
NO_2-N	0.2	0.5-0.8	0.4-1.0
NH_4-N	0.8-1.2	2.6	3.0-28.8
PO_4-P	1.8-2.3	0.7	1.1-1.9

[1]SEVEN SAMPLES AT 1-3 DAY INTERVALS

[2]TWO SAMPLES EIGHT DAYS APART

[3]ALL CONCENTRATIONS ROUNDED TO NEAREST 0.1 μG-AT L^{-1}

TABLE II. CARBON AND NITROGEN CONTENT OF *HYPNEA MUSCIFORMIS* ($\pm 2\sigma$)

GROWING CONDITIONS	PER CENT OF DRY WEIGHT		C:N
	CARBON	NITROGEN	
SHELLFISH TANK EFFLUENT	18.32 (±0.33)	2.57 (±0.11)	7.1
DEEP WATER	25.12 (±0.56)	3.28 (±0.07)	7.7
TAGUE BAY WATER	24.63 (±2.21)	2.63 (±0.04)	9.4

TABLE III. CARRAGEENAN CONTENT OF *HYPNEA MUSCIFORMIS*

SAMPLE	GROWTH CONDITIONS	YIELD (% DRY WT)	K:λ	GROWTH RATE (G WET WT/DAY)
1	DEEP WATER	29	2.7	12.1
2	DEEP WATER + LOW NH_4-N + EDTA/TM/VITAMINS	27	3.8	21.7
3	TAGUE BAY (*IN SITU*)	26	11.5	N/A
4	MEDITERRANEAN (*IN SITU*, STAUFFER #372)	26	N/A[2]	N/A
5	DEEP WATER + HIGH NH_4-N + EDTA/TM/VITAMINS	25	11.5	35.7
6	TAGUE BAY WATER	22	5.7	16.2
7	MEDITERRANEAN (*IN SITU*, STAUFFER #1974)	20	13.3	N/A
8	DEEP WATER + LOW NH_4-N + EDTA/TM/VITAMINS	19	11.5	23.6
9	DEEP WATER + 4% PRIMARY-TREATED SEWAGE	19	5.2	7.6
10	SHELLFISH TANK EFFLUENT	16[1]	N/A	64.5

[1]DATA DOUBTFUL (INSUFFICIENT MATERIAL FOR ACCURATE MEASUREMENT)

[2]NOT AVAILABLE

TABLE IV. PRODUCTIVITY OF *HYPNEA MUSCIFORMIS* COMPARED WITH OTHER PLANTS, COMMUNITIES OR LAND-BASED AGRICULTURE.

COMMUNITY OR CROP	DRY WEIGHT GRAMS/M^2/DAY[1]	CARBON GRAMS/M^2/DAY[2]
HYPNEA MUSCIFORMIS	45.1	8.3
SHELLFISH-TANK EFFLUENT (THIS STUDY)	43.7	8.0
HYPNEA MUSCIFORMIS[3]	6-17.6	-
GRACILARIA SP.[3]	9.8-16.9	-
CHLORELLA MASS CULTURE[4]	28	-
SUGAR CANE[4]	18.4	-
TURTLE GRASS FLAT[4]	11.3	-
INTENSIVE AGRICULTURE[5]	-	10-25
MOST AGRICULTURE[5]	-	3-10
LITTORAL SEAWEED[5]	-	2.1-2.8

(1) BASED ON THE OBSERVATION THAT DRY WEIGHT IS 10% WET WEIGHT

(2) BASED ON DATA IN TABLE II

(3) GOLDMAN, RYTHER, WILLIAMS AND LAPOINTE, 1975

(4) RYTHER, 1960

(5) MORRIS, 1974

Figure 1. Growth of Hypnea musciformis in surface water from Tague Bay, deep water from 870 m and in the effluent from a culture tank in which juvenile clams were reared on diatoms grown in 870-m water. The inoculum labelled Batch 4 came from another effluent tank whose growth curve is not shown.

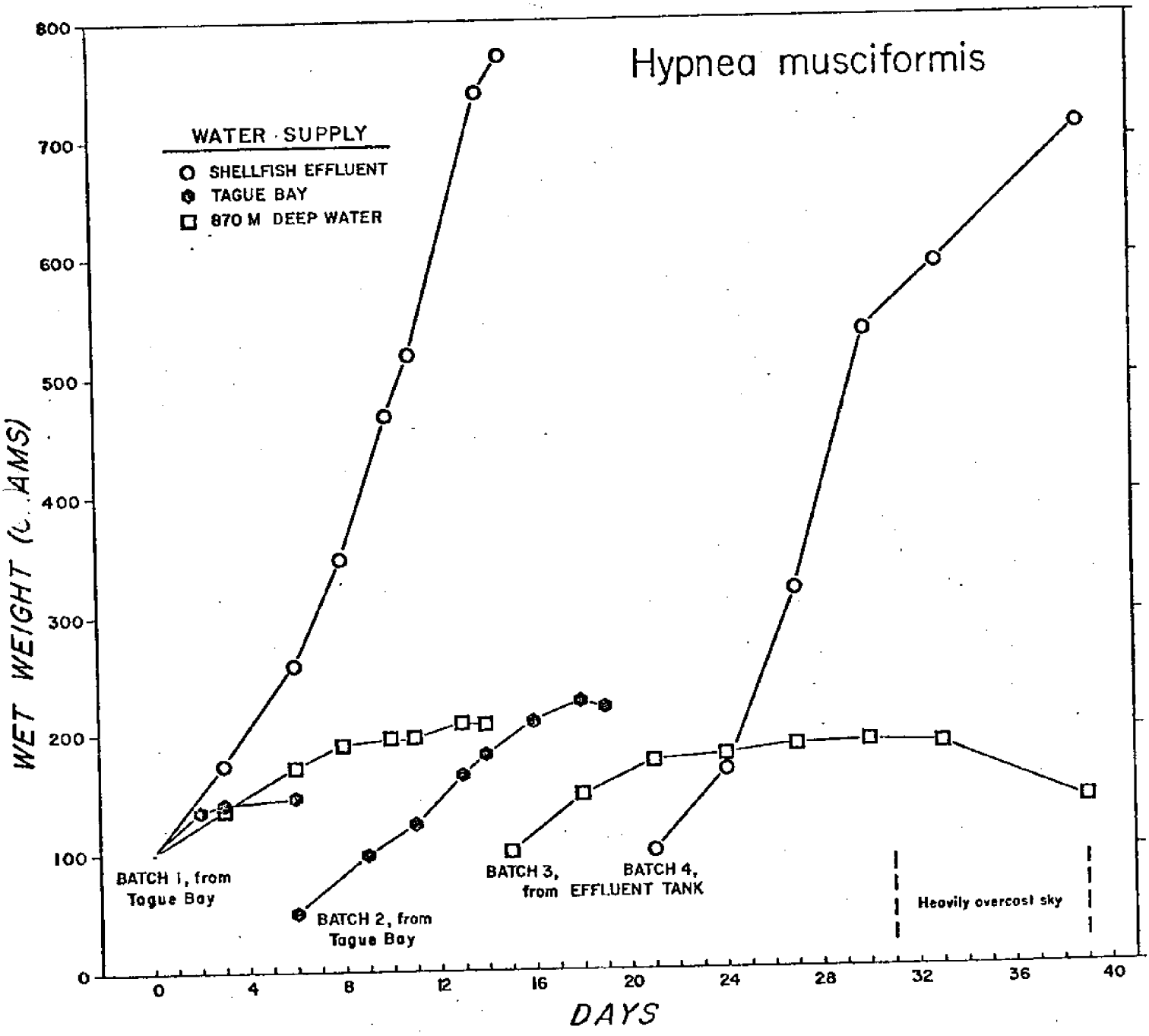

Hypnea musciformis
WATER SUPPLY
SHELLFISH EFFLUENT
TAGUE BAY
870 M DEEP WATER
WET WEIGHT (c. GMS)
DAYS
BATCH 1, from Tague Bay
BATCH 2, from Tague Bay
BATCH 3, from EFFLUENT TANK
BATCH 4,
Heavily overcast sky
0
100
200
300
400
500
600
700
800
0
4
8
12
16
20
24
28
32
36
40

Figure 2. The relationship between maximum growth rate of Hypnea musciformis and mean nutrient concentration in the seawater supply. Seawater supply:  , 870-m deep water;  , Tague Bay water;  , shellfish tank effluent. The ranges for nutrient concentrations are indicated wherever they do not fall within the area of the symbol.

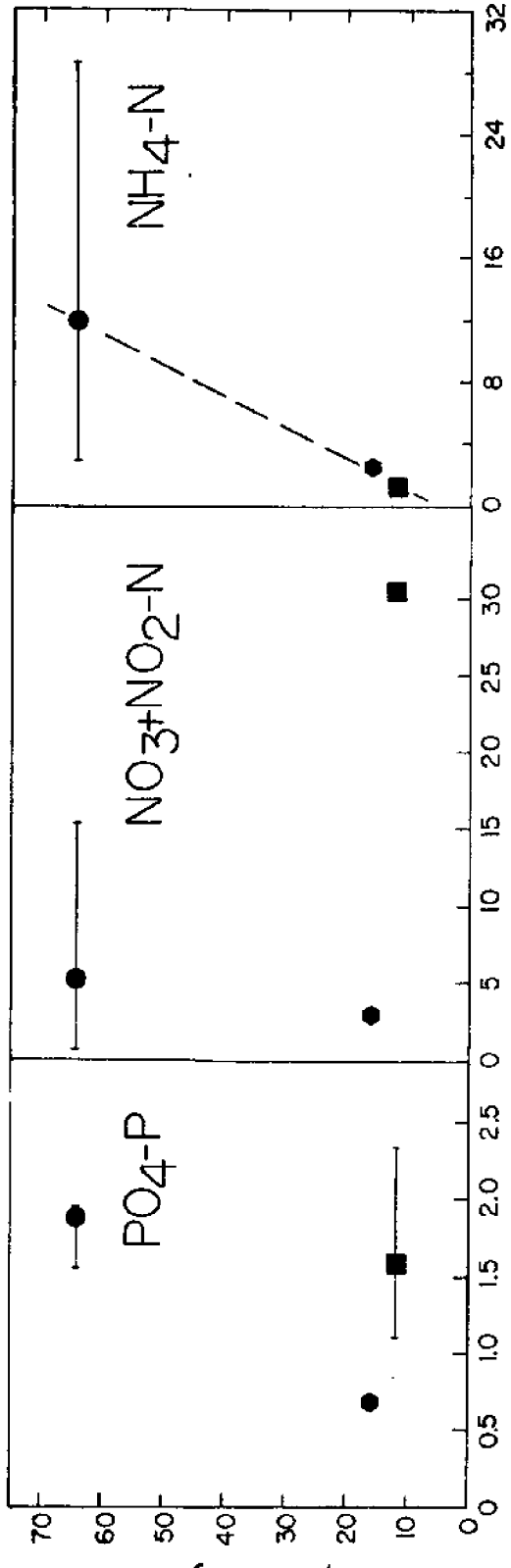

GROWTH RATE
(grams wet weight
per day)
70
60
50
40
30
20
10
0
PO4-P
0
0.5
1.0
1.5
2.0
2.5
NO3+NO2-N
0
5
10
15
20
25
30
NH4-N
0
8
16
24
32
NUTRIENT IN SUPPLY (µg-at l-1)

Figure 3. The relationship between NH_4-N uptake by Hypnea musciformis and the ambient NH_4-N concentrations (residue after uptake) in the shellfish effluent tank. The values plotted are means; bars indicate the ranges for duplicates. The ranges for NH_4-N residue fall within the line symbols. The numbers next to the plotted values indicate the sequence in which the values were obtained during the growth of the alga.

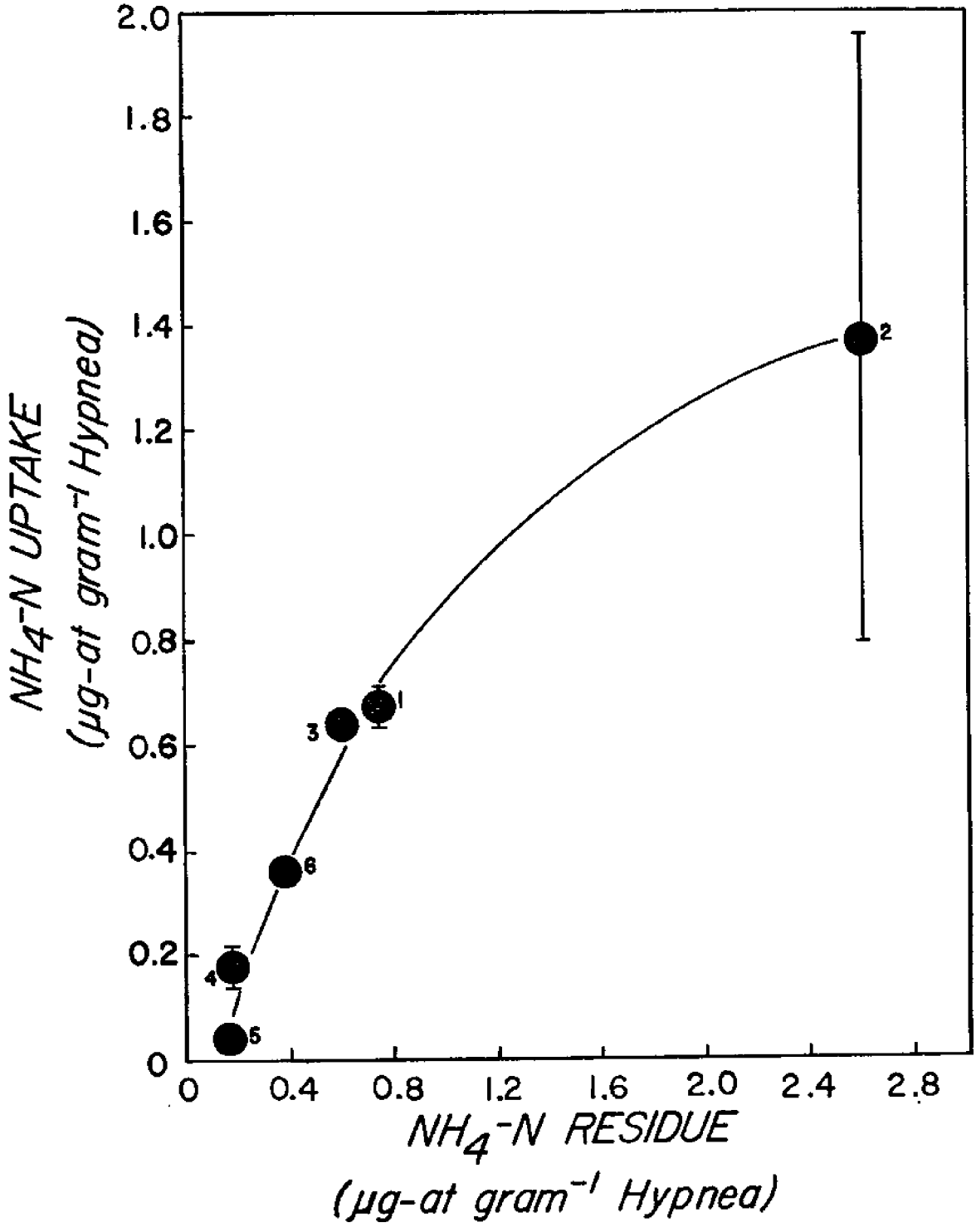

2.0
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0
0
0.4
0.8
1.2
1.6
2.0
2.4
2.8
NH4-N UPTAKE
(µg-at gram-1 Hypnea)
NH4-N RESIDUE
(µg-at gram-1 Hypnea)
1
2
3
4
5
6

Figure 4. Uptake efficiency for NH_4-N by Hypnea musciformis as a function of ambient NH_4-N concentration (residue after uptake) in the effluent tank. Values plotted as described for Figure 3.

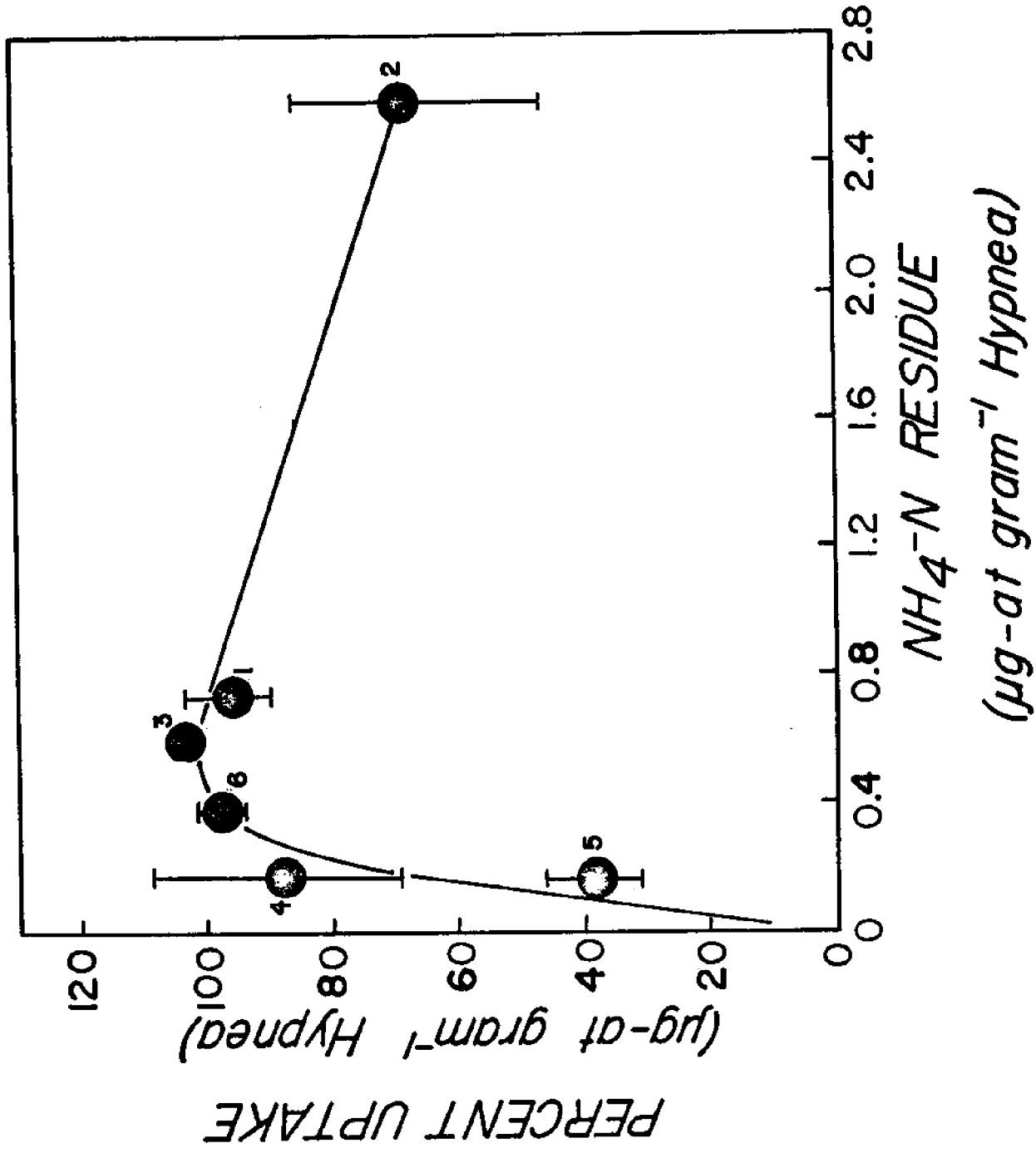

PERCENT UPTAKE
(µg-at gram⁻¹ Hypnea)
120
100
80
60
40
20
0
0.4
0.8
1.2
1.6
2.0
2.4
2.8
NH4-N RESIDUE
(µg-at gram⁻¹ Hypnea)
1
2
3
4
5
6

Figure 5. Effects of enrichment of deep water with NH_4-N, with a chelated iron/trace metals/vitamins mix, or with NH_4-N plus a chelated iron/trace metals/vitamins mix, on the growth of Hypnea musciformis. Open symbols, unenriched deep water; closed symbols, deep water enriched as indicated between the vertical dashed lines. Growth during the last six days was underestimated because about one-third of the algal mass was lost when the tank accidently overflowed.

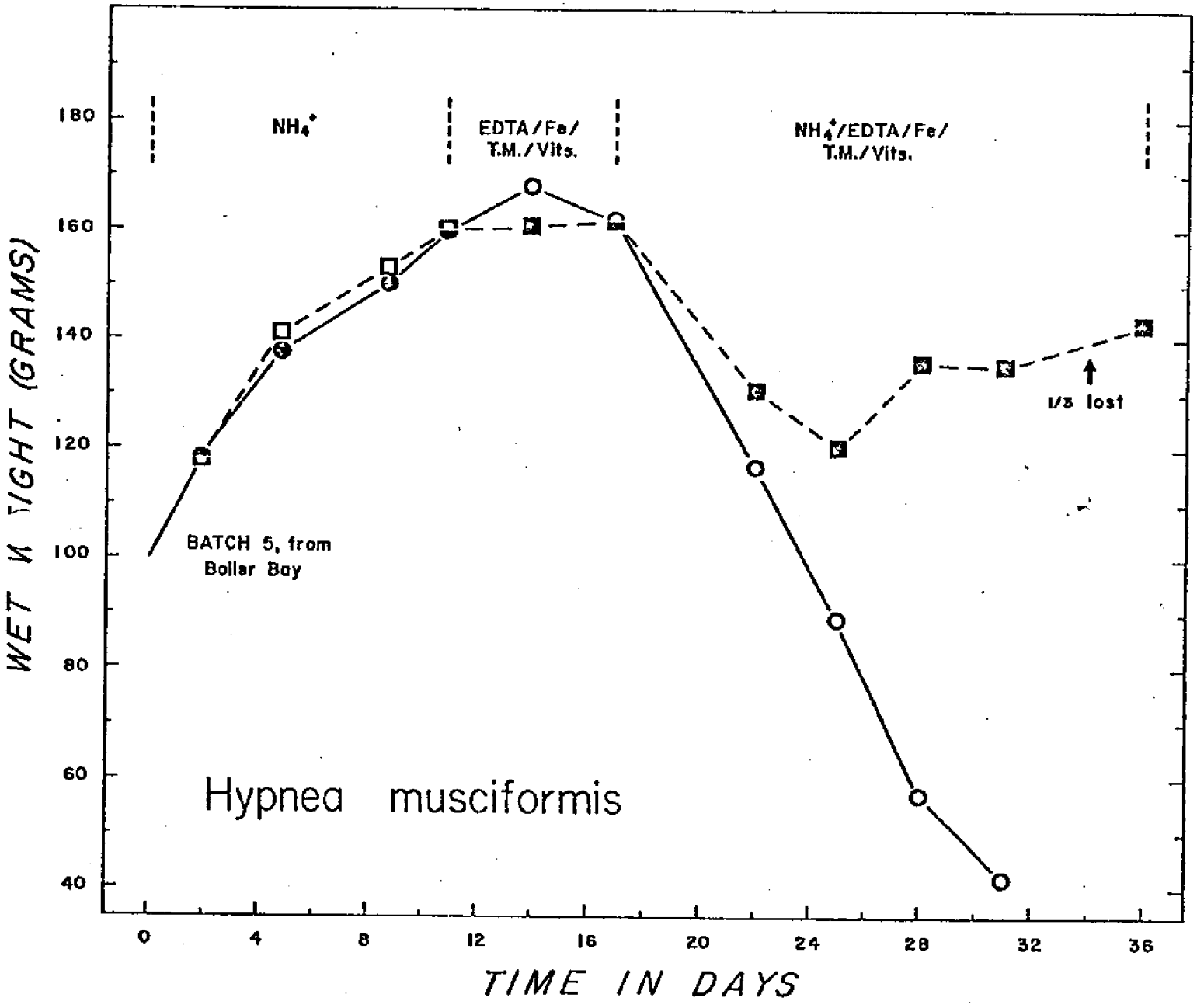

NH4+
EDTA/Fe/ T.M./Vits.
NH4+/EDTA/Fe/ T.M./Vits.
1/3 lost
BATCH 5, from Boiler Bay
Hypnea musciformis
WET WEIGHT (GRAMS)
TIME IN DAYS
180
160
140
120
100
80
60
40
0
4
8
12
16
20
24
28
32
36

Figure 6. Effect of enrichment of deep water with the combination of NH_4-N plus a chelated iron/trace metals/vitamins mix. The inoculum was taken from the experiment shown in Figure 5.

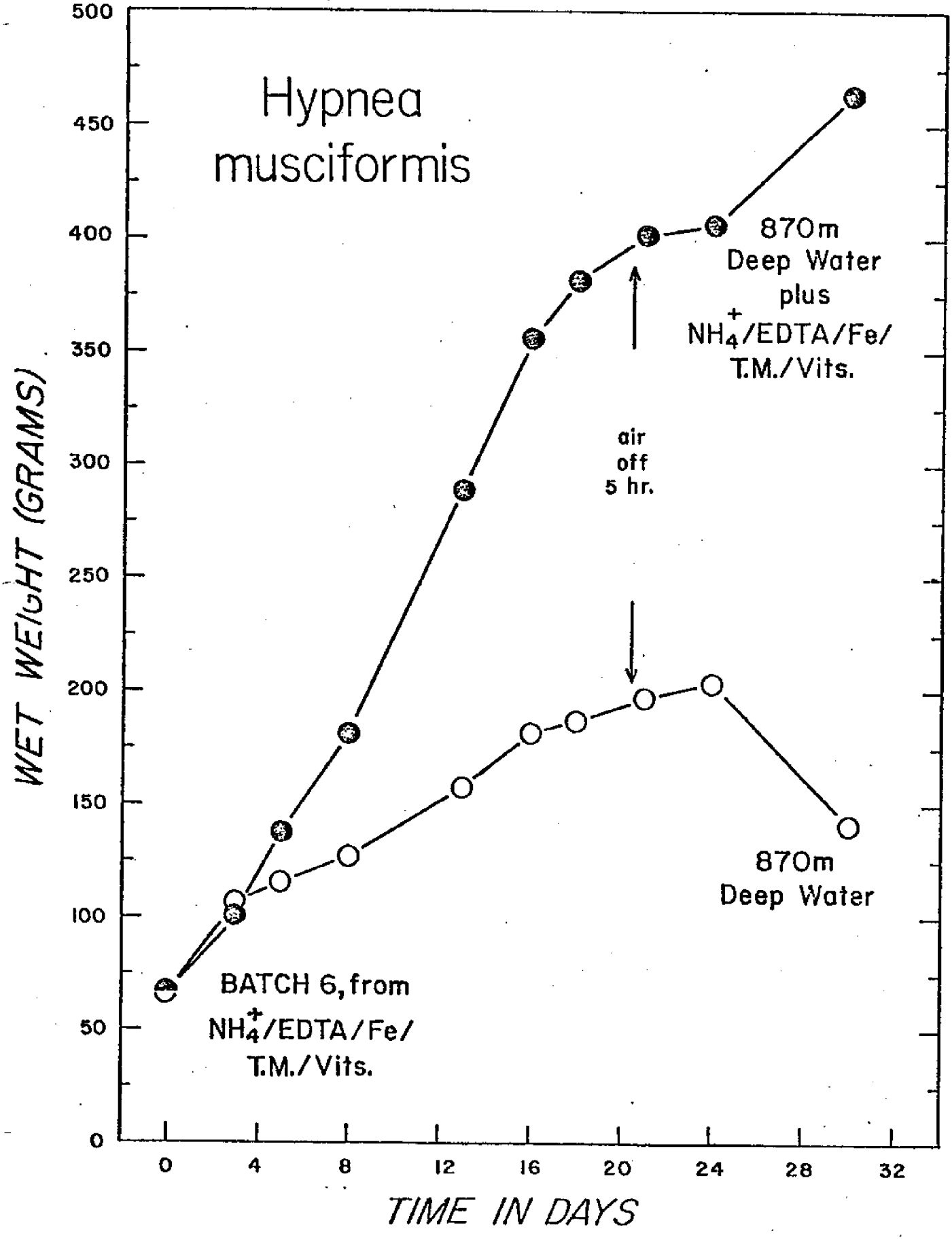

Hypnea
musciformis
870m
Deep Water
plus
NH4+/EDTA/Fe/
T.M./Vits.
air
off
5 hr.
870m
Deep Water
BATCH 6, from
NH4+/EDTA/Fe/
T.M./Vits.
WET WEIGHT (GRAMS)
TIME IN DAYS
0
50
100
150
200
250
300
350
400
450
500
0
4
8
12
16
20
24
28
32

Figure 7. Growth of Hypnea musciformis in deep water enriched at two levels of NH_4-N but with the same enrichment level with the chelated iron/ trace metals/vitamins mix. The inoculum came from similarly-enriched deep water from the previous experiment (see Figure 6).

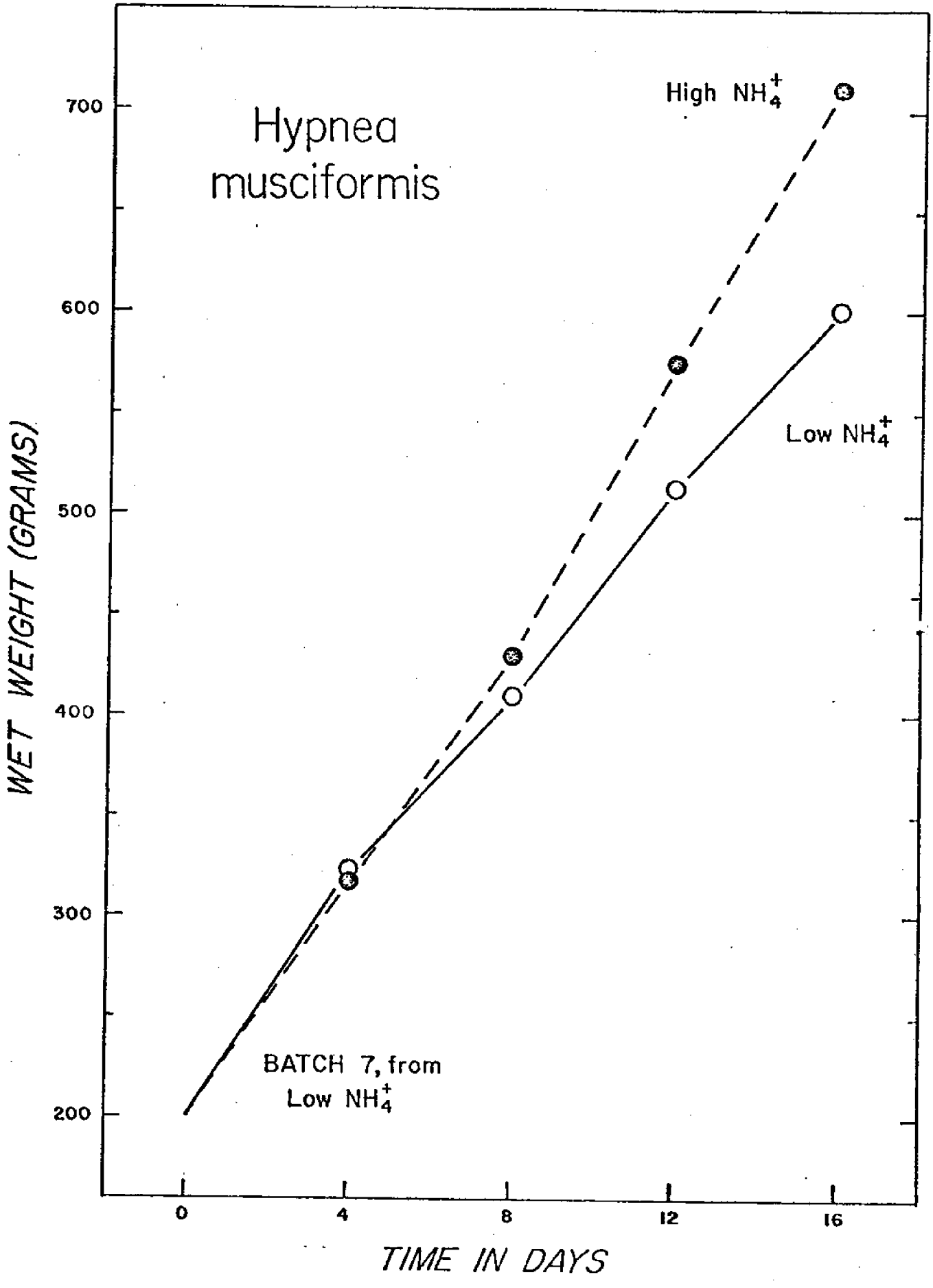
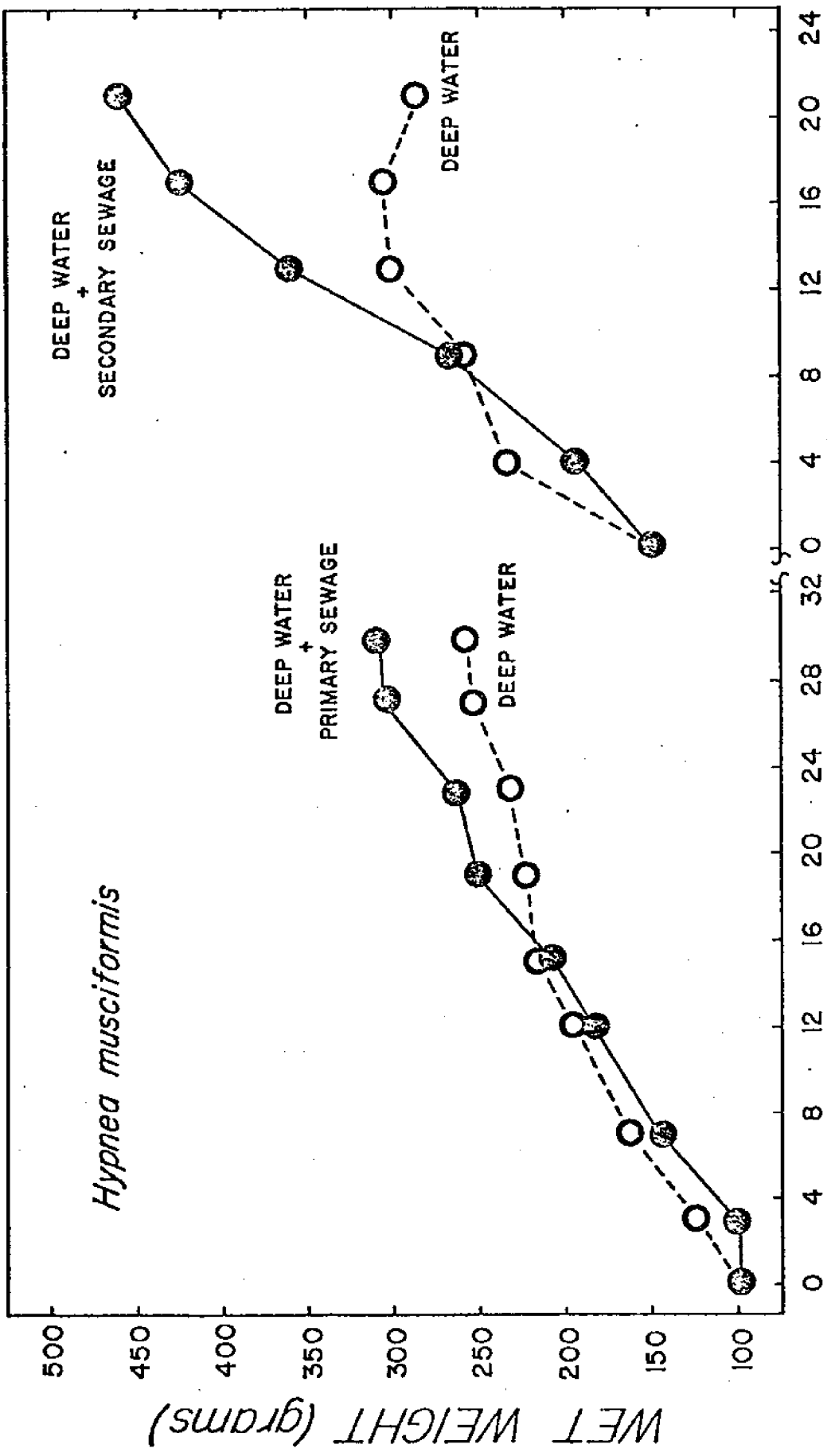

Hypnea
musciformis
High NH_4^+
Low NH_4^+
BATCH 7, from
Low NH_4^+
700
600
500
400
300
200
0
4
8
12
16
WET WEIGHT (GRAMS)
TIME IN DAYS

Figure 8. Effect of enrichment of deep water with 4% primary-treated sewage and with 35% secondary sewage on growth of Hypnea musciformis.


Hypnea musciformis
DEEP WATER + SECONDARY SEWAGE
DEEP WATER
DEEP WATER + PRIMARY SEWAGE
DEEP WATER
WET WEIGHT (grams)
500
450
400
350
300
250
200
150
100
0
4
8
12
16
20
24
28
32
0
4
8
12
16
20
24
DAYS

JERIS METHOD FOR THE DETERMINATION OF CHEMICAL OXYGEN DEMAND OF WASTEWATERS

(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 a water bath to cool to ambient temperature.

(7) After cooling, add five (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 concentrated H_2SO_4

1 liter concentrated H_3PO_4

(b) Ferrous ammonium sulfate, 0.05N

20 grams $Fe(NH_4)_2(SO_4)_2.6H_2O$

5 milliliters concentrated H_2SO_4

} dissolve in distilled 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)

THE POTENTIAL YIELD OF ARTIFICIAL UPWELLING MARICULTURE*

by

Oswald A. Roels, Ph.D.
Lamont-Doherty Geological Observatory
of Columbia University
Palisades, New York 10964 and
Professor, The City College of N.Y.

Kenneth C. Haines, Ph.D.
Lamont-Doherty Geological Observatory
of Columbia University
Palisades, New York 10964

Judith Baab Sunderlin, M.S.
Lamont-Doherty Geological Observatory
of Columbia University
Palisades, New York 10964

to be presented at the
Tenth European Symposium on Marine Biology
Ostend (Belgium) September 17-23, 1975

Running head: "Artificial Upwelling Mariculture"

*Supported by the National Sea Grant Program, NOAA, U.S. Department of Commerce (04-5-158-59) and the G. Unger Vetlesen Foundation; Lamont-Doherty Geological Observatory Contribution No. 0000; City University Institute of Marine & Atmospheric Sciences Contribution No. 00.

ABSTRACT

The Potential Yield of Artificial Upwelling Mariculture

Oswald A. Roels, Kenneth C. Haines and Judith B. Sunderlin

Lamont-Doherty Geological Observatory and City College of New York

In our Artificial Upwelling project in St. Croix, Antarctic Intermediate water is pumped continuously from 870 meters depth in the sea into 45,000-liter concrete pools on shore in which unialgal cultures of planktonic diatoms are grown. The pool cultures are started by inoculating them with cultures grown in 800-liter tanks. The growth rate of the algae is regulated by the rate at which nutrients are supplied by the incoming deep water, thus assuring nearly complete utilization of the nutrients in the deep water.

This system produces 113,550 liters of nearly unialgal diatom culture per day (10^4 - 10^6 cells per ml) which is pumped continuously into shellfish tanks at metered rates based on the feeding activity of the animals. The total flow pumped to the shellfish matches the flow of deep water into the algal pools, so that the pool volume remains constant. The filter-feeding shellfish remove up to 90% of the algae pumped from the pools. The yearly temperature range in the shellfish tanks is 22-29°C.

The diatom clones used in the system are: Thalassiosira pseudonana (3H), from New York estuarine waters; Chaetoceros simplex (STX-105) from 200 m depth local waters, and Bellerochea polymorpha (STX-114), from 870 m deep water, both high-temperature tolerant clones, and Chaetoceros curvisetus (STX-167), from 870 m water.

Ten species of shellfish have been screened for growth and survival in the St. Croix system. Seven species grew well and reached market size quickly. They are: Ostrea edulis (European oyster); Crassostrea gigas (Pacific oyster); C. gigas, Kumomoto variety; Tapes semidecussata (Japanese) little-neck clams); Mercenaria campechiensis (Southern clam or quahog); F_1 Clam (a cross between M. Campechiensis x M. mercenaria); Argopecten irradians (Bay scallop). Pinctada mertensi (the pearl oyster) is also growing very rapidly in the system.

Spiny lobsters, Queen conch and carrageenin-producing seaweeds are grown in the effluent of the shellfish tanks.

In large scale operations, it is envisaged that the deep sea water would be utilized first for its cold temperature (5-7°C) before entering the mariculture system. Power generation by the Claude process, desalination and air conditioning are some of the potential applications of this.

A 100-megawatt sea-thermal power plant would require a flow of 4.5×10^7 liters of deep sea water per minute. Such a flow of deep water might yield 125,000 tons of shellfish meat and 59,000 tons (dry weight) of carrageenin-producing seaweed per year.

INTRODUCTION

Natural upwelling occurs in several areas of the world's oceans where deep water is brought to the surface by natural forces. When the nutrient-rich deep water is mixed with nutrient-poor surface water, blooms of phytoplankton are stimulated by the nutrients (nitrate, phosphate, and silicate) in the deep water. The algae form the base of marine food chains which yield valuable animal protein. The upwelling associated with the Peru Coastal Current gives rise to the world's most productive fishery.

The cold temperature of deep ocean water can be used for a wide variety of cooling applications and for sea thermal power production by the "Claude process" in areas where the temperature differential between the surface and the deep water is great enough. Some of the possible cooling applications are airconditioning, ice-making, cooling for electrical power generating plants (avoiding thermal pollution) and fresh water production by desalination. The discharged deep water from these cooling systems is a valuable resource for mariculture since its nutrient content, essential for algal growth, is much higher than that of surface water.

We have started an Artificial Upwelling Project on the island of St. Croix (U.S. Virgin Islands) in the Caribbean Sea (17°47'N, 64°48'W), to determine the feasibility of producing phytoplankton as the primary food source for a

mariculture system, using deep-sea water and sunshine as raw materials.

In our Artificial Upwelling Project, we pump deep water into ponds onshore, where diatoms are grown as food for filter-feeding shellfish (oysters, clams and scallops) in a controlled food chain. The productivity of our system is much higher than that of natural upwelling systems because we do not dilute the deep water with nutrient-poor surface water. Another advantage of using deep water is that it is free of man-made pollutants, diseases and predators that are harmful to shellfish. The St. Croix site was chosen because the ocean reaches a depth of 1,000 m, approximately 1.8 km offshore.

The system has been in continuous operation, utilizing deep-sea water, since May 1972.

This paper describes results obtained in the Artificial Upwelling system and forecasts the potential of large-scale applications of this concept.

DESCRIPTION OF THE ARTIFICIAL UPWELLING MARICULTURE SYSTEM

Three 1,830-m long, 7.5-cm diameter, polyethylene pipelines were installed from the shore into the sea, to a depth of 870 m. A shallow reef extends approximately 400 m offshore at our beach. In this reef zone, the three polyethylene pipelines are threaded through a 35-cm (internal diameter) fiberglass pipeline. This fiberglass pipeline is attached to the reef by anchored concrete saddles and serves to protect the polyethylene pipes from abrasion by surf action.

At the seaward end of the fiberglass pipeline the water depth is approximately 20 m. From that point on the sea floor slopes down at a 33° angle. On this slope, the three polyethylene pipelines are lying on the sea floor, held down by lead ballast weights. The seaward intake of the pipes, at 870-m depth, is held 30 m above the sea floor by a system of anchors and floats.

A glass-lined centrifugal pump on shore, driven by a 2-hp motor, yields a combined flow of 170 liters per minute through the three pipelines. The composition of the surface water 1,800 m offshore, of the reef water inshore and of the water from 870-m depth pumped up through our pipelines is given in Table I.

Table I

TABLE I. ST. CROIX WATER PROPERTIES

	NUTRIENTS ($\mu eq.\ell^{-1}$)					SALINITY	IN-SITU TEMPERATURE
	NO_3+NO_2-N	NO_2-N	NH_3-N	PO_4-P	SiO_4-Si	(°/oo)	(°C)
OFFSHORE SURFACE WATER[1]	0.2	0.2	0.9	0.2	4.9	35.539	26-29
REEF SURFACE WATER[2]	0.2	0.2	0.8	0.3	2.1	35.596	26-29
870-M DEEP WATER[3]	31.3 (±0.31)	0.2 (±0.05)	0.7 (±0.13)	2.1 (±0.10)	20.6 (±0.37)	34.852 (±0.008)	7

[1]Mean value from samples taken June 11 and 20 (1975), 1,800 m offshore from our beach.

[2]Mean value from samples taken June 3 and 20 (1975), 5 m offshore from our beach.

[3]Mean values, $\pm 2\sigma$, for weekly samples taken in the period January 1973 to October 1974.

A constant head device maintains constant pressure of the deep-water flow throughout the system onshore. The deep water is pumped continuously into 5 m x 10 m concrete pools of 1-m depth. Nearly unialgal diatom cultures have been grown continuously in these pools since May 1972, producing 111,000 liters of culture per day, containing 10^4 to 10^6 cells per milliliter. The cell concentration depends on cell size and species (see Table 2). This diatom culture is pumped continuously into shellfish tanks at metered rates based on the feeding activity of the animals. The total flow pumped to the shellfish matches the flow of deep water into the algal pools, so that the pool volume remains constant. The filter-feeding shellfish remove up to 90% of the algae pumped from the pools. The yearly temperature range in the shellfish tanks is 22-29°C.

In 1974, ten 2,000-liter concrete tanks, provided with deep-water flow, were added to the system to expand the number of species of algae which can be grown and experimented with simultaneously. Another ten 2,000-liter concrete tanks with provision for deep-water flow are under construction at the time of writing (August 1975).

The phytoplankton produced in the pools and in the 2,000-liter concrete tanks is fed continuously to a shellfish hatchery, a shellfish larvae and juveniles rearing area, an experimental shellfish area and a pilot shellfish area. In the larvae-setting and juvenile-rearing shellfish area the young animals produced in the hatchery are kept

until they are 2-5 mm in size, in fiberglass flumes of 366 x 61 x 16-cm deep.

The experimental shellfish area has provision for up to 240 individual shellfish trays for experimentation. Each one of these trays can be provided with any combination of the different unialgal cultures grown in the smaller concrete tanks and in the two large pools.

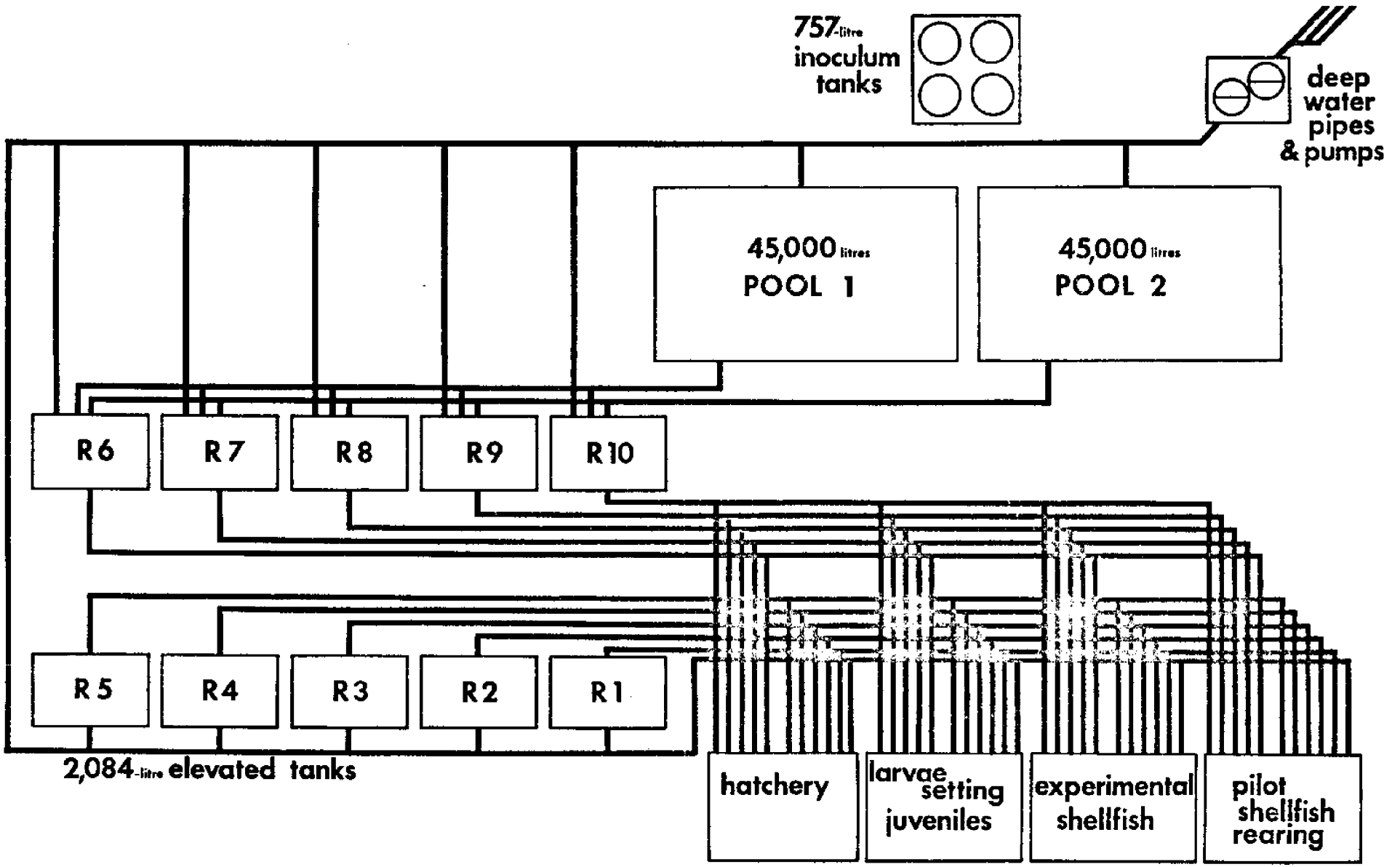
In the pilot shellfish area, new species of shellfish are screened for growth and survival in the system, and design parameters for shellfish-growing in large-scale mariculture systems are tested. Figure 1 gives a schematic representation of the flow of deep water and phytoplankton suspension through the mariculture system.

Figure 1

Small-scale experimentation is underway with Strombus gigas (the queen conch) which graze on the epiphytic algae on the walls of the pools and tanks. Panulirus argus (the spiny lobster) is being reared successfully on the slow-growing shellfish, which do not meet our standard growth requirements and are culled systematically from the system.

The carrageenan-producing seaweed, Hypnea musciformis, has been grown very successfully in the effluent from the shellfish tanks, thereby utilizing some of the nitrogen-containing compounds excreted by the shellfish which would otherwise be a net loss from the system (1).

Figure 1. Schematic representation of the flow of deep water and phytoplankton suspension through the St. Croix "Artificial Upwelling" mariculture system.


757-litre inoculum tanks
deep water pipes & pumps
45,000 litres POOL 1
45,000 litres POOL 2
R6
R7
R8
R9
R10
R5
R4
R3
R2
R1
2,084-litre elevated tanks
hatchery
larvae setting juveniles
experimental shellfish
pilot shellfish rearing

PHYTOPLANKTON PRODUCTION

The diatom clones used in the system are listed in Table II.

Table II

These diatoms are maintained in the laboratory in axenic culture, in F/2 medium (2). Laboratory inoculations are arranged to produce 32 liters of axenic culture of the different species of diatoms on a regular schedule. These 32 liters of axenic inoculum are then transferred to 757-liter open polyethylene tanks on the beach, containing deep-sea water enriched to F/2 strength. These polyethylene tanks provide starter cultures for the 45,000-liter pools. Figure 2 gives the growth curve of Chaetoceros sp. (STX-105) in this system.

Figure 2

Thalassiosira pseudonana (3H), Chaetoceros sp. (STX-105) and Bellerochea polymorpha remain unialgal for one to two weeks when cultured in the deep-sea water pools supplemented with chelated iron (1 μM Fe-EDTA), trace metals and vitamins; unsupplemented cultures of these three species do not remain unialgal as long as those receiving the supplement. For the continuous culture of these three diatoms in our pool system, a 1.5-times daily turnover is used, i.e., 67,500 liters of deep water are pumped through a 45,000-liter pool per 24-hour period, at the same rate during both day and

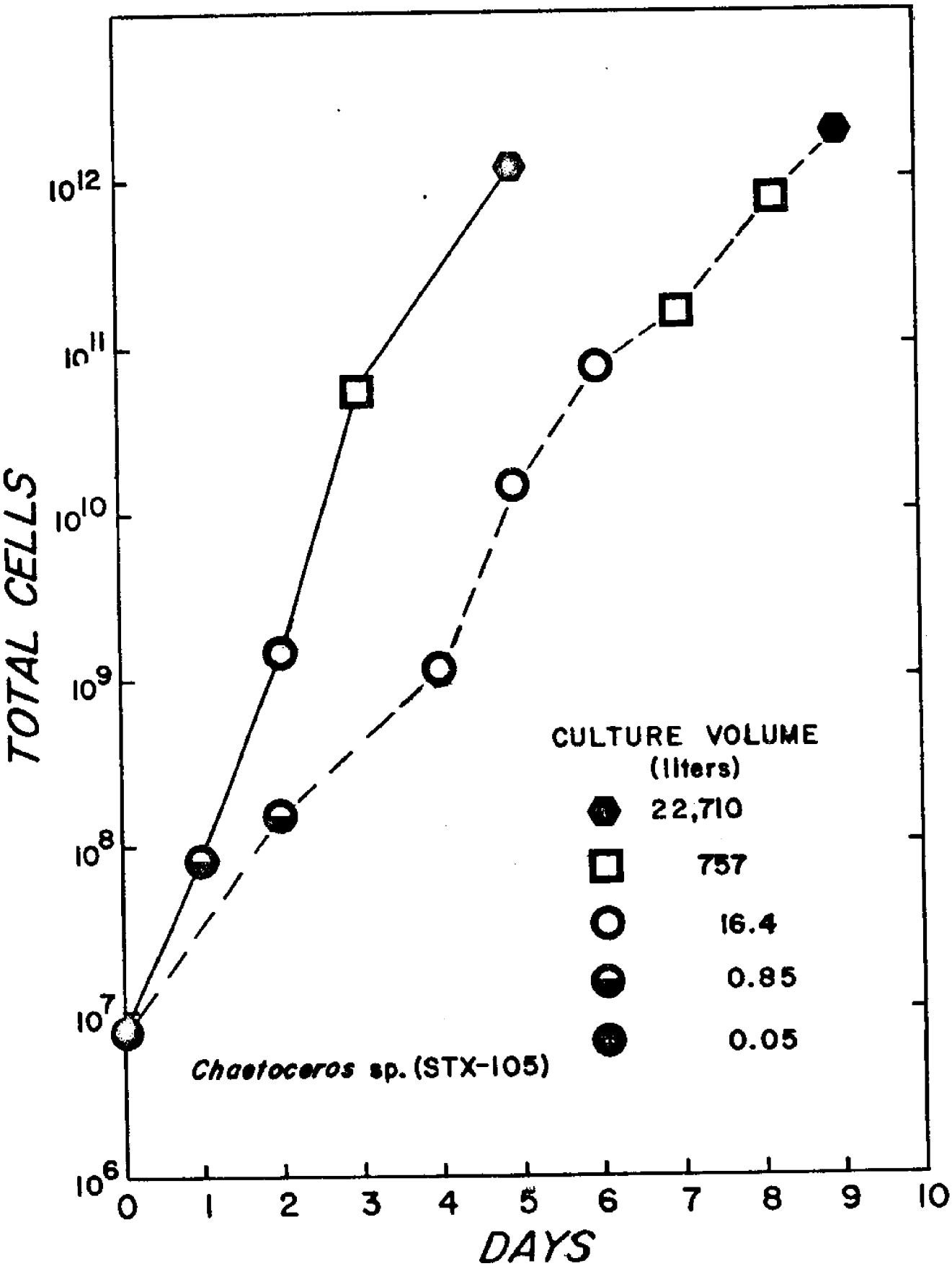
TABLE II. DIATOM SPECIES USED IN ST. CROIX ARTIFICIAL UPWELLING MARICULTURE

SPECIES	ORIGIN	SIZE (μ^3)
Thalassiosira pseudonana (3H)	New York Estuary (Guillard)	203 (3)
Chaetoceros sp. (STX-105)	St. Croix, 200 m (Haines)	57-650
Bellerochea polymorpha (STX-114)	St. Croix, 870 m (Haines)	1.9-9.6
Chaetoceros curvisetus (STX-167)	St. Croix, 870 m (Haines)	116-5325
Unidentified cryptophyte (S_1)	Sargasso Sea (Guillard)	3.5-17.4

Figure 2. Growth curve of Chaetoceros sp. (STX-105) in the St. Croix mariculture system from a 50-ml laboratory inoculum to 22,710-l. (half our pool volume).

_____ one-day inoculum schedule

_ _ _ _ two-day inoculum schedule


TOTAL CELLS
10^{12}
10^{11}
10^{10}
10^{9}
10^{8}
10^{7}
10^{6}
0
1
2
3
4
5
6
7
8
9
10
DAYS
CULTURE VOLUME
(liters)
22,710
757
16.4
0.85
0.05
Chaetoceros sp. (STX-105)

night. Chaetoceros curvisetus (STX-167) can be maintained in unsupplemented deep water for more than four weeks at a turnover rate of 1.1 pool volumes per day. In steady-state continuous cultures of this diatom, up to 99% of the nitrate and nitrite from the deep water are taken up by the diatom. The silicate uptake is approximately 90% of that present in the incoming deep water, and the phosphate uptake is 77% (4).

From experiments undertaken in 2,084-liter concrete tanks (operating with a water depth of 0.8 m) utilizing different degrees of shading and different turnover rates of the deep water in the tank during continuous culture, it has been calculated that Chaetoceros curvisetus (STX-167), in a well-mixed pool of 5-m depth under the St. Croix light conditions, can fix 0.9 grams of cellular organic nitrogen/m^2/day and 5.3 grams of cellular organic carbon/m^2/day (4). These numbers will have to be verified in actual deep-tank experiments.

Several other clones of phytoplankters which can grow in unsupplemented deep water for prolonged periods of time in continuous culture are ready for testing in the 45,000-liter pools.

SHELLFISH PRODUCTION

Several populations of different species of pelecypod mollusks have been grown from spat to market size in our St. Croix system. Mercenaria mercenaria (the northern hard clam or quahog) and Crassostrea virginica (the North American oyster) did not grow well in the system: C. virginica had high mortality whereas M. mercenaria survived but grew very slowly (5).

Ostrea edulis (the European oyster) was grown from 3 mm spat to market size adults in 12 months (6).

A comparative growth study of hardshell clams (Mercenaria mercenaria and Mercenaria campechiensis, and their F_1 cross) in temperate, subtropical and tropical natural waters and in our tropical Artificial Upwelling mariculture system revealed that M. campechiensis and the F_1 cross grow from 1 mm spat to market size in 6.5 to 13 months in the Artificial Upwelling system, while no animals had reached market size in any of the other environments after 13 months (7).

Tapes semidecussata (the Japanese clam) has been grown from egg to market size in 13 months and from set to market size in 12 months (8).

Argopecten irradians (the bay scallop) grew from 1-2 mm to market size in 7 to 10 months.

Table III lists the shellfish which have been grown successfully from seed to market size in our Artificial Upwelling system.

Table III

Two more species of shellfish have been introduced in the Artificial Upwelling mariculture system and are growing very rapidly: they are the Kumomoto variety of Crassostrea gigas and Pinctada mertensi.

To avoid the introduction of parasites and diseases, we have started our own hatchery, utilizing deep-sea water exclusively. Tapes semidecussata has already been reared through several generations in our system. Post-set survival of the larvae has been as high as 85%.

As a result of improved handling techniques and better and more reliable food supply, each successive batch of a given species of shellfish has reached market size more rapidly in our system.

The taste of the shellfish grown in our system was found to be superior to that of the same species from several other conventional natural environments by a New York panel of seafood experts.

TABLE III. PELECYPOD MOLLUSKS GROWN SUCCESSFULLY FROM SEED TO MARKET SIZE IN THE ARTIFICIAL UPWELLING MARICULTURE SYSTEM

	TAPES SEMI-DECUSSATA (Japanese Little Neck Clam)	*MERCENARIA CAMPECH-IENSIS* (Southern Clam or Quahog)	F_1 CLAMS (cross of *M. CAMPECH-IENSIS and M. MERCEN-ARIA)*	*CRASSOSTREA GIGAS* (Pacific Oyster)	*OSTREA EDULIS* (European Oyster)	*ARGOPECTEN IRRADIANS* (Bay Scallop)
MARKET SIZE	>38-mm length	>25.5-mm thick	>25.5-mm thick	>30-gm weight	>76.5-mm length	>50.5-mm height
MONTHS TO ATTAIN 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
PERCENTAGE SURVIVAL	64	48	42	67	70	73
PERCENTAGE MEAT (SHUCKED WET WEIGHT)	39	28	31	39	22	11 (muscle only)

SEAWEED PRODUCTION

To recover some of the nitrogen losses from the system and to reduce a potential environmental hazard, the carrageenan-producing seaweed Hypnea musciformis has been grown very successfully in the effluent from the shellfish tanks.

Hypnea musciformis grew five times faster in the shellfish tank effluent than in unaltered deep-sea water, and about three times faster than in surface seawater. The growth rate was positively correlated with the ammonia concentration in the growth medium.

The seaweed grown in the effluent doubled its weight every 60 hours (1). The carrageenan content of the seaweed grown in the effluent was 16% (on a dry weight basis) and the gelling properties of the carrageenan in food applications were excellent.

At the high turnover rates used in the study on which these calculations are based (96 turnovers of the volume of the tanks used per day), the maximum ammonia-nitrogen utilization by the seaweed was 50% and averaged 41%. The efficiency of ammonia-nitrogen utilization would no doubt be higher at slower turnover rates, but this would also lower the growth rate.

THE POTENTIAL YIELD OF LARGE-SCALE ARTIFICIAL UPWELLING MARICULTURE

In large-scale systems, pumping massive volumes of deep-sea water to the surface, the temperature differential between the cold deep-sea water (5-7°C) and the warm surface water (26-29°C) which has a potential energy of 120 kilogram-meters per kilogram of deep water brought to the surface, will be utilized for sea-thermal power generation and/or desalination. We have described the potential of these applications in an earlier publication (9).

However, despite the rapid increase in energy costs caused by the rapid rise of oil prices during the last two years, the potential mariculture yield of cold deep-sea water in tropical and subtropical areas remains far more economically valuable than the potential energy yield from the temperature differential. It would, therefore, obviously be advantageous to utilize all the economically valuable properties of deep-sea water rather than only one of them. This would also distribute the cost of the deep-sea water pumping system over different products: power, fresh water, seafood and carrageenan.

According to our most conservative estimates, based on experimental results in our system, the potential yield of shellfish meat from an Artificial Upwelling mariculture system would be 25.8 tons of fresh shellfish meat/hectare/year. At $2.50/lb of shellfish meat, this would represent

$113,520/year/hectare.

We are reasonably confident that it will be possible to increase this yield several times, in part by improved management of the system, but mainly by a better choice of diet provided by the algal mixture fed to the shellfish.

By extrapolating our small-scale seaweed data, we calculated that the potential yield of Hypnea musciformis would be 165 metric tons (dry weight)/hectare/year. At $650/ton, this would represent a yield of $107,250/hectare/year.

In view of these results, we recommend at this time the construction of a pilot plant, pumping 100 m^3 deep-sea water per minute.

Its purpose would be to test the economic feasibility of Artificial Upwelling mariculture, and, to make efficient use of the deep water in this plant, it should be used in technical feasibility studies of sea-thermal power generation and desalination prior to entering the mariculture section of the plant.

REFERENCES

(1) Haines, K.C. (1975) Growth of the carrageenan-producing tropical red seaweed, Hypnea musciformis, in surface water, 870-m deep water, effluent from a clam mariculture system, and in deep water enriched with artificial fertilizers or domestic sewage. 10th European Symposium on Marine Biology, Ostend (Belgium), September 17-23, 1975.

(2) Guillard, R.R.L. and J.H. Ryther (1962) Studies on marine planktonic diatoms. I: Cyclotella nana Hustedt and Detunula confervacea Cleve (Gran.). Canadian J. Microbiol. 8:229-239.

(3) Guillard, R.R.L. and U. Cessie (1963) Minimum cyanocobalamin requirements of some marine centric diatoms. Limnol. Oceanography., 8:161-165.

(4) Farmer, M.W. (1975) Nitrate-limited versus light-limited growth of Chaetoceros curvisetus in outdoor continuous culture. 38th Annual Mtg., American Soc. Limnol. Oceanogr., Dalhousie Univ., Halifax, N.S., June 22-26, 1975.

(5) Baab, J.S., G.L. Hamm, K.C. Haines, A. Chu and O.A. Roels (1972) Shellfish mariculture in an artificial upwelling system. Proc. National Shellfish. Assn., 63:63-67.

(6) Sunderlin, J.B., W.J. Tobias and O.A. Roels (1974) Growth of the European oyster, Ostrea edulis Linné, in the St. Croix Artificial Upwelling mariculture system and in natural waters. Proc. 66th Joint SINA/NSA Ann. Mtg. & Conf., Phila., Pa., June 16-20, 1974 (in press).

(7) Sunderlin, J.B., M. Brenner, M. Castagna, J. Hirota, R.W. Menzel and O.A. Roels (1974) Comparative growth of hardshell 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. Proc. World Mariculture Soc. (in press).

(8) Rodde, K.M., J.B. Sunderlin and O.A. Roels (1975) The mariculture potential of Tapes semidecussata (Reeve) in an artificial upwelling system. 67th Joint SINA/NSA Mtg. & Conf., Charleston, S.C., June 22-26, 1975.

(9) Othmer, D.F. and O.A. Roels (1973) Power, fresh water and food from cold, deep-sea water. Science, 182: 121-125.