ARTIFICIAL UPWELLING
PROGRESS—1976-1977

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C O N T E N T S

G: Ammonium and Nitrate Uptake Rates of the Seaweeds Hypnea musciformis (Rhodophyta) and Macrocystis pyrifera (Phaeophyta)

H: Ammonia-Nitrogen Production by the Bivalve Mollusc Tapes japonica and its Recovery by the Red Seaweed Hypnea musciformis in a Tropical Mariculture System

I: Report of ^{14}C Studies on Individual Cells in Populations of Chaetoceros curvisetus on St. Croix

J: Organic Production Potential of Artificial Upwelling Mariculture

ABSTRACT

A pilot-stage, two-trophic-level, deep-sea water mariculture system was operated on St. Croix, U.S. Virgin Islands over a nine-month period. Antarctic Intermediate Water from about 870 m depth was pumped continuously into two 50,000-liter onshore pools. The pools were inoculated with laboratory grown cultures of the diatom Chaetoceros curvisetus (STX-167) and operated at a turnover rate of 1.15 day^{-1}. Twenty-nine (29) such cultures were started and lasted an average of 17.0 days each. Mean down-time required for deactivation and restarting of cultures was 54 hours.

Protein-nitrogen production in each pool was measured three times weekly and averaged 23.61 µg-at $liter^{-1}$. This production represented a conversion of available deep-water 'nitrogen' (32.16 µg-at $liter^{-1}$) into algal-protein nitrogen of over 73%. Extrapolated yearly production of algal protein was about 101% of expected levels, based upon previous experience.

Of the total deep-water 'nitrogen', 93.5% was accounted for at the first trophic level, in the forms of dissolved inorganic nitrate, nitrite, ammonia and algal-protein nitrogen. Unaccounted-for nitrogen increased towards the end of culture life and was probably in the form of dissolved organics and wall growth (attached algae).

The production was 6.4 metric tons of algal protein/ha/yr. This compares to a production of 0.71 tons/ha/yr for alfalfa, currently the greatest terrestrial producer of plant protein. Based on earlier work using 2000-liter vessels under various light and flow-rate regimes, a model was derived which predicts a production of >23 tons/ha/yr at a pool depth of 4.88 m and a turnover rate of .81 day^{-1}. Further analysis using a computerized economic sensitivity model has projected an economically optimum pool depth of about 3 m, with a turnover rate of .84 day^{-1} and an algal protein production of 16.5 tons/ha/yr.

Representative environmental observations including air, deep water and pool temperatures are included, as are ambient light readings. The pools, which were not light-limited, produced about 2 g of algal protein per kWh of measured light.

The algae were pumped continuously to three populations of the clam Tapes japonica. Over a nine-month period, mean "stripping" (inflow less outflow ÷ inflow) of algal-protein nitrogen was about 48%, or about 55% of expected levels. All three populations demonstrated a decreasing ability to convert stripped algal-protein nitrogen to Tapes meat-protein nitrogen over time; near the end of the study about 15% conversion was measured. This low conversion (about 45% of that expected) was

traced to the tank configuration, which led to a build-up of fecal material, ammonia and bacteria, with a corresponding increase in mortality. Adjustments in the shellfish technical description may be necessary to reduce discrepancies between measured and expected stripping rates; this deviation was greatest for larger animals.

Over the nine-month period studied, about 1.5×10^7 liters of water flowed through the second trophic level, with a total deep-water 'nitrogen' content of about 6.78 kg. About 3.7 kg (or 55%) was accounted for in the shellfish-tank effluent as particulate protein and dissolved inorganic nitrate, nitrite, and ammonia. About 0.53 kg (or about 8%) of the total 'nitrogen' in the deep water was recovered in the form of live Tapes meat-protein nitrogen. An estimated loss of 0.114 kg occurred through mortality (about 2%) and another 0.024 kg was estimated as lost through spawning (<1%). In sum, about 65% of the total deep-water 'nitrogen' available was accounted for at the second trophic level; tank deposit particulate matter and dissolved organic nitrogen probably constituted most of the unaccounted-for nitrogen.

The second trophic level demonstrated a net uptake of dissolved nitrate plus nitrite of about 0.4 kg, probably due to the presence of epiphytic algae and attached pennate diatoms in the tanks. A new production of about 0.46 kg of ammonia was also measured, due to excretion by the shellfish.

The biological value of the diet was calculated at 22.

Preliminary analysis of the results indicated that previously expected levels of production (about 23% conversion of total available deep-water inorganic nitrogen to live Tapes meat-protein nitrogen) could be obtained with appropriate changes in shellfish-tank configuration and with implementation of appropriate adjustments to the shellfish technical description.

PREFACE

The following report describes what was accomplished from 1 July 1976 to 30 June 1977, with Sea Grant support, on the "Artificial Upwelling" mariculture project, St. Croix, U.S. Virgin Islands.

—During the year a pilot demonstration plant was operated on the basis of the best information collected previously.

—The inputs on which the pilot plant operation is based are summarized in a technical description.

—The pilot operation was subjected to a set of observations to determine the validity of the methods of operation.

—The procedures which were applied in all phases of the effort have been identified.

—A series of developmental research studies was undertaken to increase understanding or to improve the productivity of the processes involved.

—The data collected, both as a result of observations and of research studies, have been subjected to an initial analysis.

—The final output of this program is the substantiation of the aquaculture budget generator program, which provides guidance as to the economically critical aspects of the project, and will ultimately provide a rationale for further development.

1 INFRASTRUCTURE

1.1 Facilities

The St. Croix Marine Station occupies all of the buildings (except the old windmill) on the Estate Rust-op-Twist, located on the North Shore about a half mile west of Baron Bluff, St. Croix, U.S. Virgin Islands. Station housing provides accommodation in seven apartments for visiting scientists and permanent staff of the project.

The laboratory area (approximately half an acre) is about 1400 ft back from the shoreline. On the lower floor of the renovated building is the laboratory, including a dust-free culture transfer room, business office, comprising approximately 1,600 sq.ft. A 582 sq.ft. section of the warehouse houses two diesel-powered emergency generators, a workshop, tool and spare-parts storage space.

The shore area (approximately 63,000 sq.ft. of beachfront) contains the mariculture complex. This complex includes: (a) the onshore terminus of the three 3-inch deep-water pipelines; (b) pumphouse with two 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 (integrating radiometer) for continuous readout of solar radiation; (c) deep-water constant head device for constant pressure in the entire deep-water distribution system, (d) two 50,000-liter concrete pools for algal culture; (e) the shellfish hatchery and wet lab for shellfish

feeding and growth experiments, with associated set of ten 2,000-liter elevated tanks ("reactors"); (f) ten 2,000-liter elevated tanks ("reactors") mounted on a separate structure west of the hatchery/wet lab. These reactors can receive shallow or deep water. Both water supply lines have constant-head devices; (g) a shallow-water system consisting of a self-priming centrifugal pump and a pipeline of 1-1/2" polyethylene pipe protected by 2-1/2" galvanized steep pipe clamped to steel bars driven into the bottom; (h) six 800-liter polyethylene tanks, four of which are used for algal culture, two for experimentation; (i) eight 800-liter wood-fiberglass tanks for shellfish and miscellaneous uses; (j) two 600-liter polyethylene tanks for experimental use; (k) twelve rectangular (86 x 62 x 22 cm) tanks constituting the Model 1 shellfish pilot plant; (l) fourteen round (66 cm diameter x 15 cm deep) tanks constituting the Model 2 shellfish pilot plant; (m) submersible pumps routing algal suspension to shellfish tanks; (n) storage container for tools and supplies for beach and pipeline work.

1.2 Staffing

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

Research and Supporting Staff:

In St. Croix:
Aust, Leo G. (B. Mech. E.)
Station Manager
Research Program Manager

Laurence, Scott (Ph.D.)
Project Coordinator
Research Scientist Associate II

Haines, Kenneth C. (Ph.D.)
Research Scientist

Sunderlin, Judith B. (M.S.)
Research Scientist Associate II

Tobias, William J. (M.S.)
Research Scientist Assistant II

Widdowson, Robert R. (B.A.)
Research Scientist Assistant II

Robichaux, David (B.A.)
Research Scientist Assistant I

Railey, Melinda A. (B.A.)
Research Scientist Assistant I

Francis, Oswald A.
Technical Staff Assistant IV

Tucker, Paul A.
Technical Staff Assistant III

Stapleton, Charles
Maintenance Worker

Powell, Albert
Maintenance Helper

David, Winston J.
Groundskeeper

Boatswain, Gladys
Building Attendant

In Port Aransas:

Van Hemelryck, Ludo (M.E.E.)
Chief Engineer
Research Engineer

McDonald, Paul W. (B.S.)
Research Scientist Assistant III

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

Amos, Lynn M.
Executive Assistant

3 PROCEDURES

3.1 Pilot Plant Operations

3.1.1 Phytoplankton

The deep (Antarctic Intermediate) water being pumped on St. Croix is an excellent medium in which to grow photosynthetic plants, but it contains very few of them. Dense "starter" cultures for the inoculation of deep water are required, to accelerate the establishment of a steady-state continuous-flowthrough culture in the pools. At present, this is accomplished by producing axenic algal cultures in the laboratory in highly enriched seawater medium under controlled environmental conditions, and using these cultures to inoculate still larger cultures on the beach, where temperature, light, and other factors cannot be easily manipulated. The phytoplankter used is Chaetoceros curvisetus (clone STX-167). A schematic diagram of the flow through the algal culture system is given in Figure 1.

The algal cultures are produced daily by transfer of small cultures at intervals into larger growth vessels containing new media. The last stage in the algal culture system is a large-volume culture in 50,000-liter concrete pools, from which the algae are pumped continuously to the shellfish-rearing tanks. The laboratory and beach culture methods are further detailed.

3.1.1.1 "Starter" Cultures

Inocula for the outdoor pools are reared as axenic laboratory cultures (16-liter carboys) which are used to inoculate 760-liter unialgal polytank (PT) cultures which, in

Figure 1. Schematic diagram of the flow through the algal culture system.

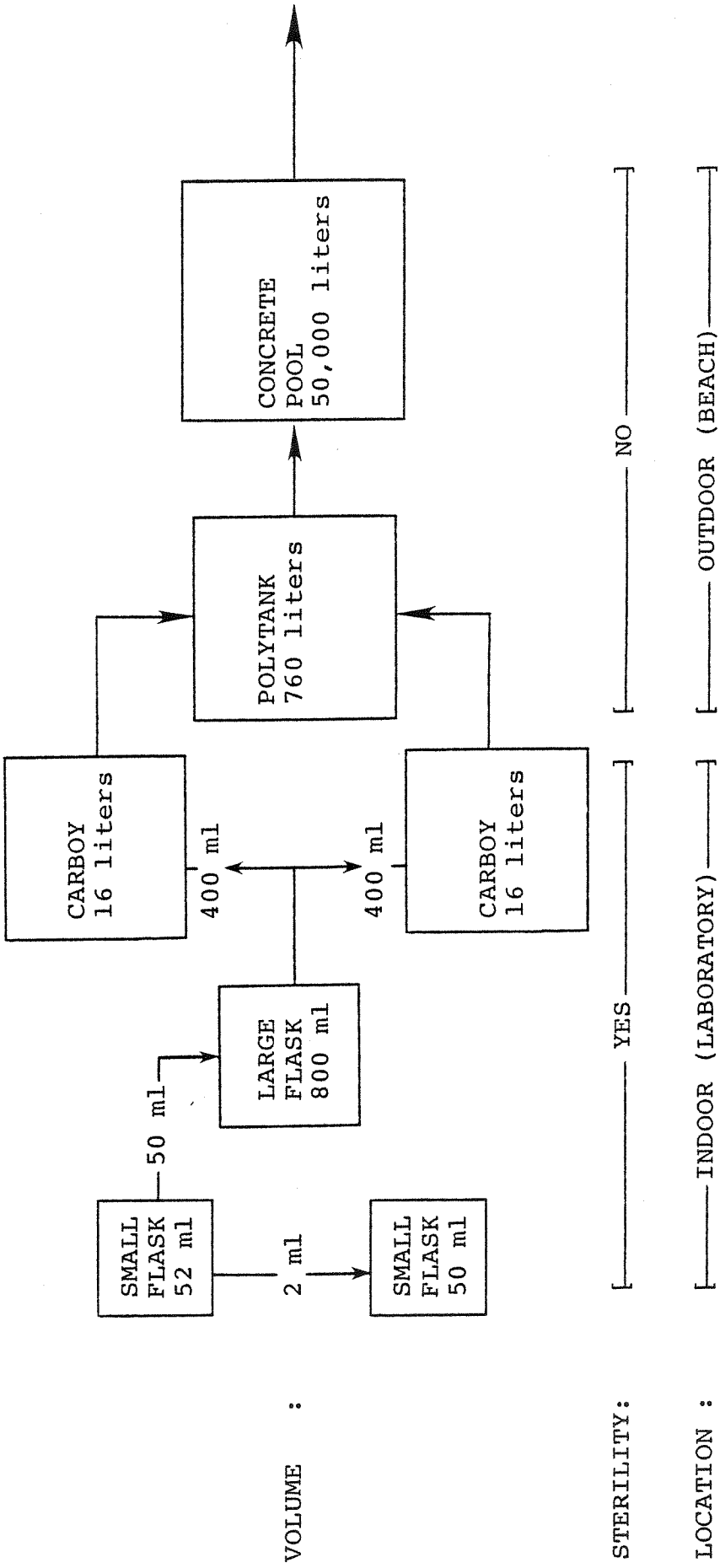
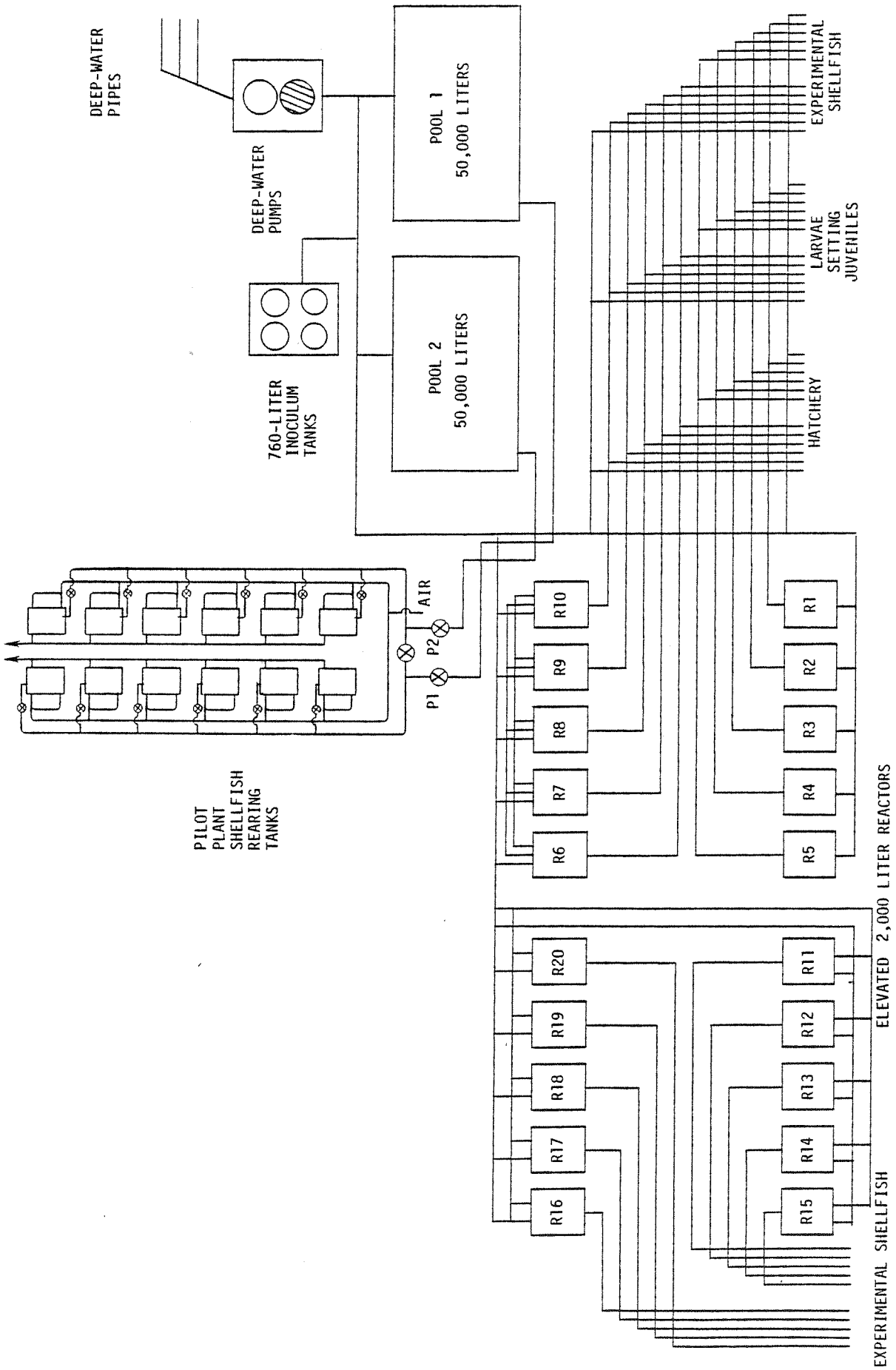

CONCRETE POOL 50,000 liters
POLYTANK 760 liters
CARBOY 16 liters
CARBOY 16 liters
400 ml
400 ml
LARGE FLASK 800 ml
SMALL FLASK 52 ml
50 ml
2 ml
SMALL FLASK 50 ml
VOLUME :
STERILITY:
YES
NO
LOCATION :
INDOOR (LABORATORY)
OUTDOOR (BEACH)

Figure 2. Schematic diagram of the St. Croix "Artificial Upwelling" mariculture system.


DEEP-WATER PIPES
DEEP-WATER PUMPS
760-LITER INOCULUM TANKS
POOL 1 50,000 LITERS
POOL 2 50,000 LITERS
HATCHERY
LARVAE SETTING JUVENILES
EXPERIMENTAL SHELLFISH
AIR
P1
P2
PILOT PLANT SHELLFISH REARING TANKS
R1
R2
R3
R4
R5
R6
R7
R8
R9
R10
R11
R12
R13
R14
R15
R16
R17
R18
R19
R20
ELEVATED 2,000 LITER REACTORS
EXPERIMENTAL SHELLFISH

turn, are used to inoculate the 50,000-liter pools. Preparation of the laboratory cultures includes growing the diatom in a 50-ml small flask (SF) for two days and using it to inoculate an 800-ml large flask (LF). The LF, after two days' growth, is used as inoculum for two 16-liter carboys which are incubated in the laboratory for two days prior to being used as inoculum for a polytank (PT). The SF is incubated in a 12-hr light/12-hr dark cycle; the LFs and the carboys are incubated under continuous illumination, provided by fluorescent light bulbs; temperature is normal room temperature (ca 25°C). The medium used in laboratory cultures is Guillard's medium F/2, except that silica is omitted from the SFs and LFs. The medium used in the outdoor PTs is F/4 (except for the F-level silica). The PTs are inoculated with two 16-liter carboy cultures from the laboratory. Aeration is provided by a polyvinylchloride (PVC) manifold which rims the bottom of the PT. The PT is prepared for inoculation by scrubbing with a solution of household bleach (sodium hypochlorite) and a swimming-pool chloride (sodium dichloroisocyanurate), filling with deep water and adding the enrichment nutrients. Carbon dioxide is metered into the PT cultures when the pH goes above 8.7; this rarely occurs.

3.1.1.2 Beach Cultures

The two 50,000-liter concrete pools are ~1 m deep and are provided with a continuous flow of deep water at a turnover (dilution) rate of 1.15 volumes/day. Culture is pumped out continuously, using submersible pumps. During 1976-77, pool culture produced in excess of the needs of the

pilot plant requirement were used for maintenance of brood stock and other animals, or overflowed to waste. The pools are scrubbed (as for the PTs) and reinoculated on a 28-day cycle, so that a different pool is inoculated every two weeks. Following scrubbing of the pool and airlines, and flushing of the lines which distribute the culture to the pilot plant and other animal tanks, the pool is filled to half-volume, inoculated with a PT culture and allowed to set overnight. The following day, the pool is filled to 50,000-liters and is activated that or the following day by starting continuous flow of deep water in and culture out. Two to three inoculum cultures are produced every week so that there is a backup PT culture available in the event that a pool culture "collapses" below a useful level (ca 10^4 cells/ml). The decision to scrub a pool on a day other than the scheduled day is made by the scientist in charge (SIC), based on visual estimation of pool density and/or on quality of culture as revealed by microscopic examination.

3.1.2 Shellfish

3.1.2.1 Design and Operation

The shellfish pilot plant, located on the beach west of the phytoplankton pools and perpendicular to the northeast corner of the hatchery building, consists of two parallel rows of six rectangular tanks (approx. 86 x 62 x 22 cm), which incorporate an airlift recirculation system (Figs. 2,3,4). Tanks 1, 3, 5, 7, 9 and 11 receive flow from Pool 2, while Tanks

Figure 3. Diagram of the shellfish pilot demonstration plant, consisting of two parallel rows of six rectangular tanks (approx. 86 x 62 x 22 cm) and incorporating an airlift recirculation system.

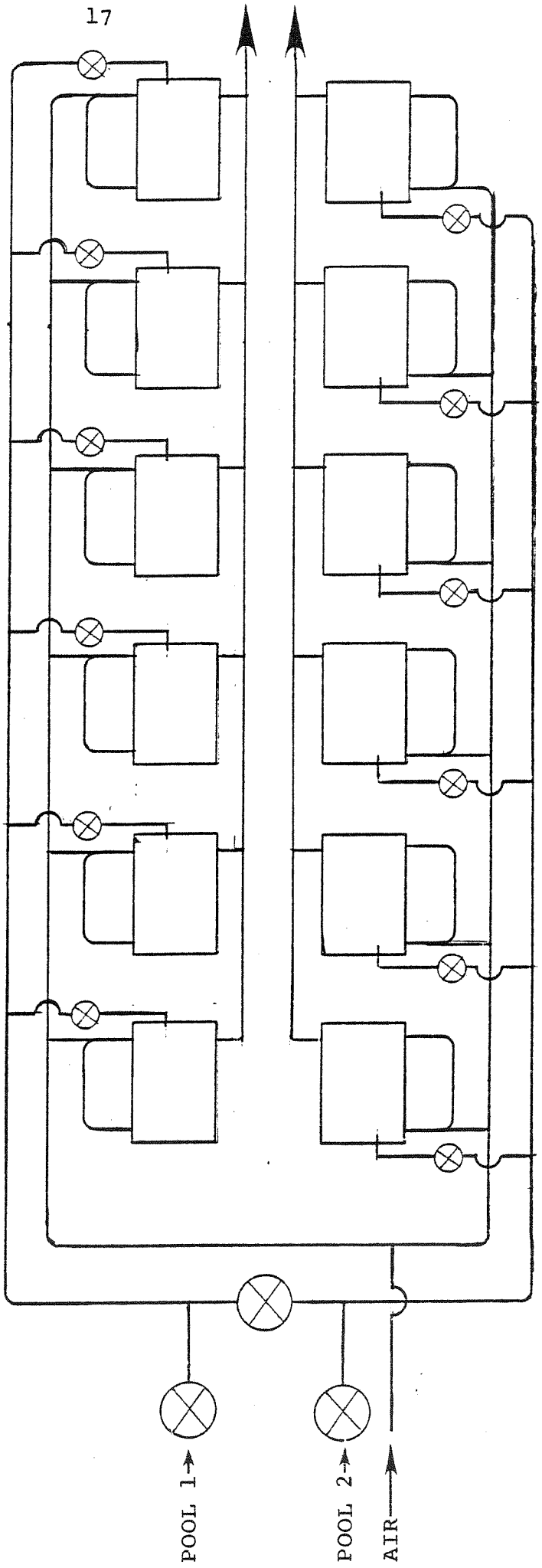
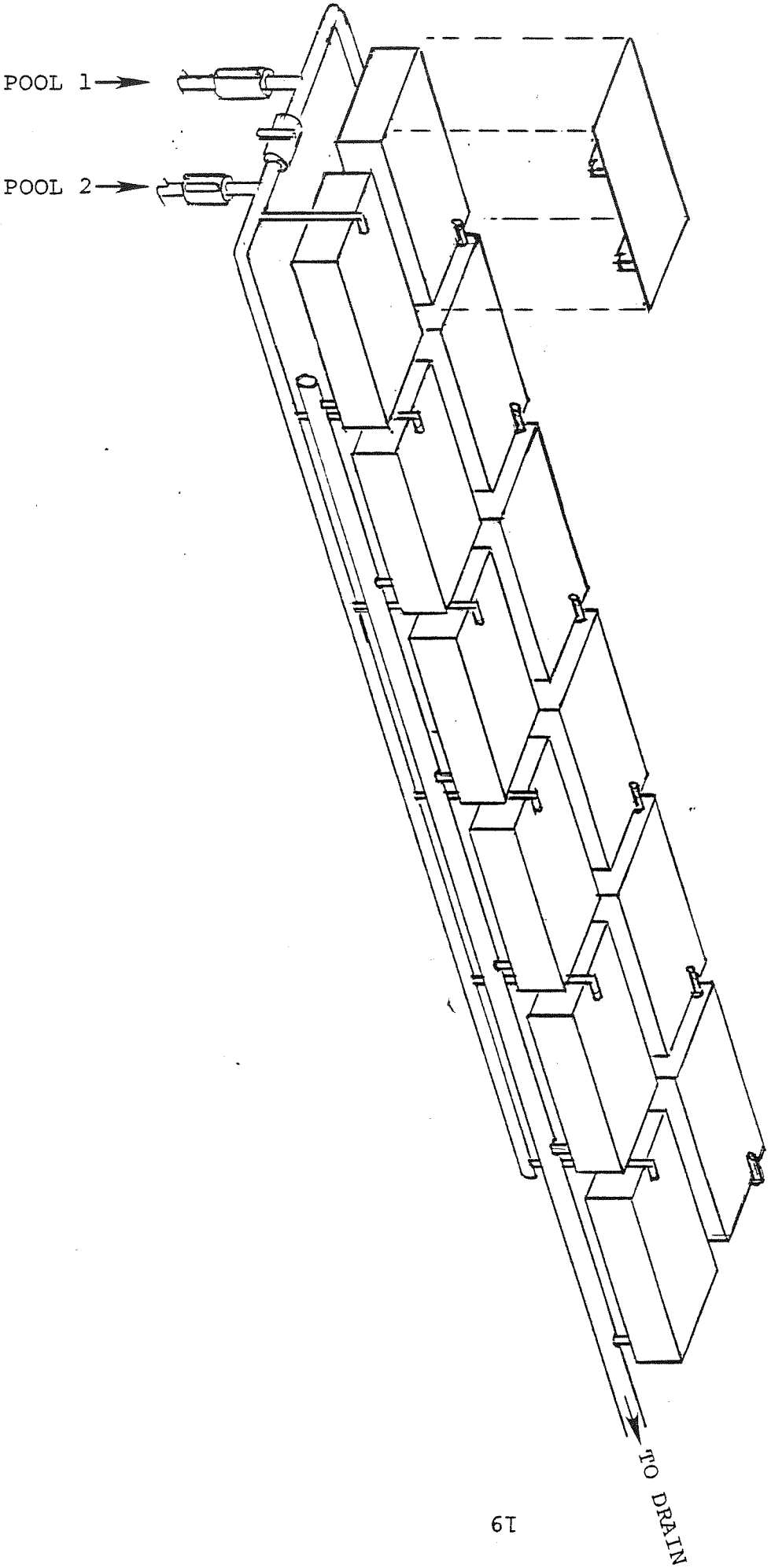

POOL 1
POOL 2
AIR

Figure 4. Another view of the shellfish pilot plant, consisting of 12 covered rectangular tanks.


POOL 1
POOL 2
TO DRAIN

2, 4, 6, 8, 10 and 12 receive flow from Pool 1. All tanks can receive the flow from one pool when only one is on-line.

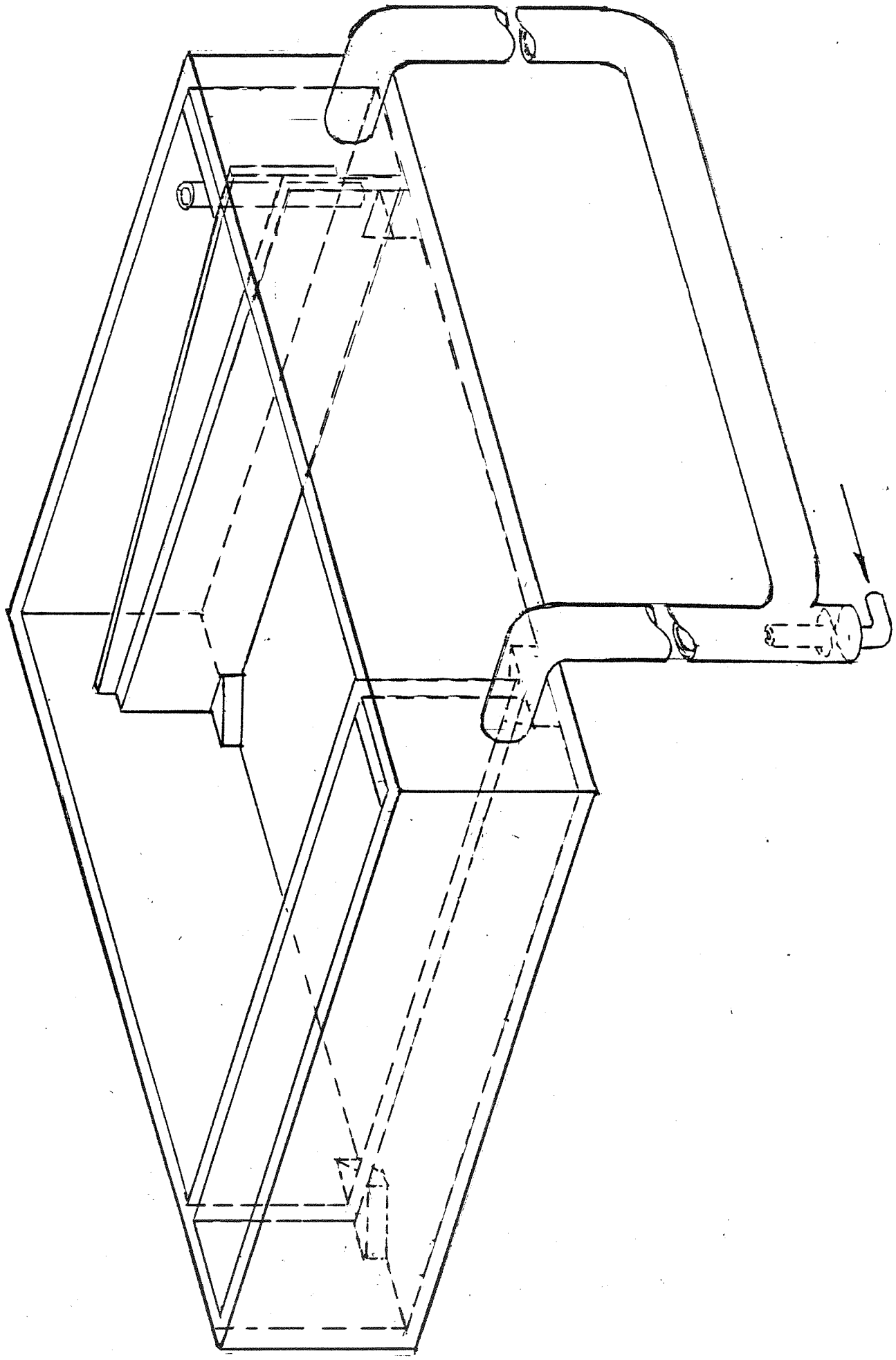
The tanks are constructed of 5/8" exterior plywood with the main shellfish compartment separated from the inflow and outflow chambers by 3/4" plywood partitions (Fig. 5). The tanks are made watertight by trim-fitting corners and applying exterior spackling compound to fill the seams, then covered with polyester resin. The tanks are painted with a non-toxic, non-yellowing, white enamel paint. Covers for the tanks are made from corrugated aluminum roofing material.

Low-pressure air, supplied by a Siemens compressor (Model #2CH4-001-1U), is injected through capillary tubing at the base of the left-vertical section of the external recirculation system (Fig. 5), creating a recirculation flow through the tank. Pool culture entering from the left side of the tank flows under the inflow partition, up through a Nestier tray via the main compartment containing shellfish, and over the effluent chamber partition. A portion of the flow then exits via the overflow standpipe and the remainder is recirculated back through the tank.

Tank capacity is 113 liters and tank turnover time is 17 min at a flow of 110 ml/sec. The pilot plant requires the total flow from both pools ($\sim$ 1.32 liters/sec) when operating at full capacity.

Tapes japonica spat (approx. 10,000 clams) are introduced into the pilot plant 56 days after spawning at an expected shell length of 4.3 mm (0.01g whole wet weight). The clams are placed in a 1/16" mesh bag in one-fourth of the Nestier tray and a weighted

Figure 5. Detail of an individual shellfish tank.



wood plate is used to cover the remaining 3/4ths of the tray. This directs the flow of pool culture up through the clams when the entire tray is not being used. As the clams increase in size, a larger open-mesh Vexar screening replaces the mesh bag.

The subsequent feeding plan is based on a projected growth, from this size on, as derived from a 36-day feeding experiment (Roels et al., 1977; Appendix B) in November-December 1975, which used animals averaging 12.7 mm in length at the start.

On Day 70 the wood partition covering 3/4ths of the Nestier tray is removed and the shellfish are spread out on a tray liner over the entire tray. At Day 126, the shellfish are divided equally into quarters and spread out into four trays of the pilot plant. One batch occupies Tanks 1-4, the second occupies Tanks 5-8, and the third occupies Tanks 9-12. Tray liners are removed when the clams are large enough to be retained in the Nestier tray.

The expected growth of a population of 10,000 clams is shown in Figure 6. The predicted feeding flow required is shown in Figure 7. Actually, the flow is adjusted stepwise, as shown in Table 1.

After 140 days, three batches of 10,000 clams are expected to need a flow rate which exceeds our present pool capacity. Accordingly, after that date the shellfish population is reduced every 28 days, by culling the excess over a predetermined weight. The weight to which each population (four trays) has to be reduced each time, and the amount expected to be culled is shown in Table 2.

3.1.2.2 Maintenance

At 14 and 35 days after introduction, and after 35 days on a regular weekly schedule, the shellfish are

Figure 6. The expected growth of a population of 10,000 clams (*Tapes japonica*).

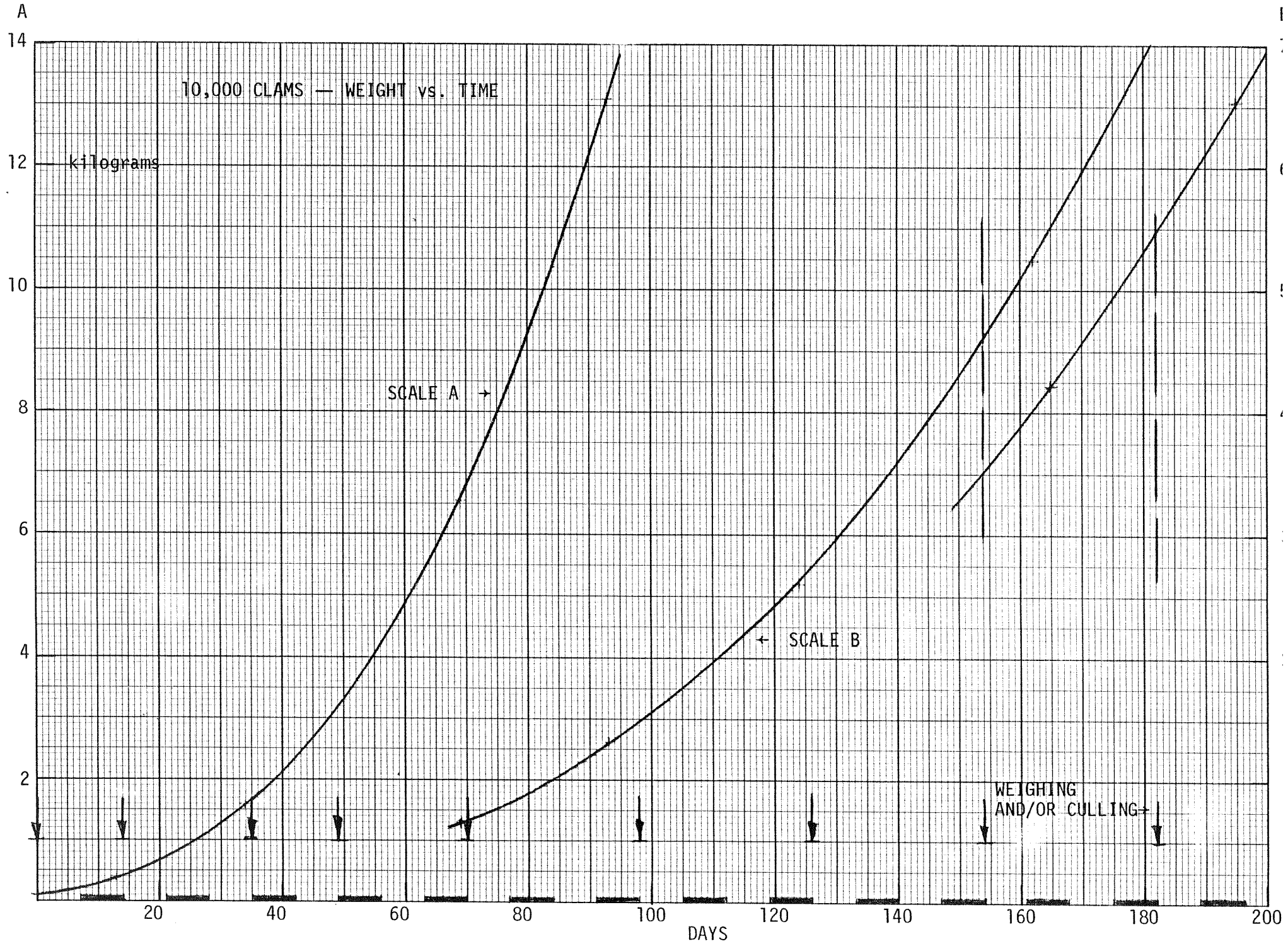


Figure 7. The predicted feeding flow required to feed a population of 10,000 clams (Tapes japonica). (In practice, the flow is adjusted stepwise.)

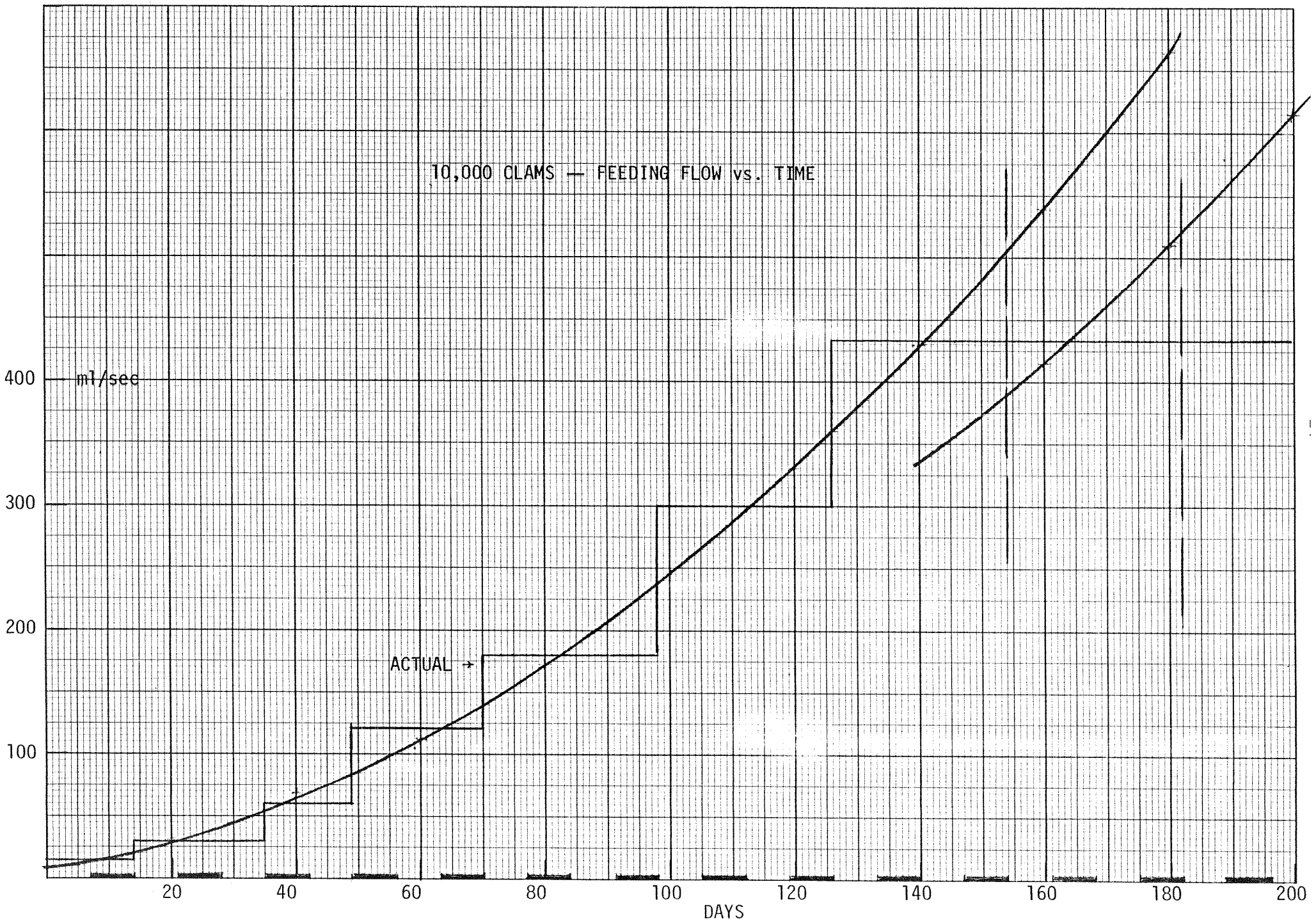


TABLE 1. INTENDED FLOW RATE CHANGES

DAY	FLOW ($ml\ sec^{-1}$)	SHELLFISH DIVISION
0	15	1/4 tray
14	30	1/4 tray
35	60	1/4 tray
49	120	1/4 tray
70	180	1 tray
98	300	1 tray
126 and on	110	4 trays

TABLE 2. INTENDED CULLING OF EXCESS OVER PREDETERMINED WEIGHT OF SHELLFISH SUPPORTABLE BY PRESENT FOOD FLOW RATE FOR EACH OF THREE POPULATIONS

DAY	WEIGHTS (kg)		
	BEFORE CULLING	AFTER	CULLED
154	45.858	33.734	12.124
182	52.258	39.812	12.446
210	58.340	45.914	12.426
238	64.444	52.030	12.414
266	70.565	58.159	12.406
294	76.697	64.298	12.399
322	82.839	70.443	12.396
350	88.987	-	88.987

cleaned. During some portions of the 1976-77 year, half of the trays were on a biweekly cleaning schedule.

Prior to the initiation of shellfish maintenance, the shellfish tank covers are removed, the tank feedline switched off, and the accumulation of fecal material, water circulation, and general clam condition noted. The Nestier tray containing the shellfish is then removed and placed in a shaded area while the tank is cleaned. Fecal accumulation and bacteria, if present, are noted.

The following procedure for shellfish maintenance is then implemented: the effluent standpipe and main compartment chain plug are removed and the tank drained. The aeration for the individual tank airlift recirculation system is shut off and intake fitting disconnected to drain the external recirculation system. With a hose connected to the hatchery deep-water system, the tank is rinsed and scrubbed with a stiff nylon hand-brush. The vertical sections of the external airlift recirculation system are scrubbed with a long-handled wire brush and rinsed. The silicone stopper in the airlift system is removed and the capillary tubing rinsed free of deposits. The stopper in the pool culture feed-lines is removed and the flow rate tubing cleaned by passing a smaller diameter section of tubing through it. The airlift system is then reassembled and the pool flow valve opened to allow the tank to fill while the clams are rinsed.

The clams, tray, and tray liner (if present) are rinsed with deep water to remove fecal material and the dead shellfish (if any) are removed and counted. The tray is placed back into the

tank and recovered. Fouled Nestier trays are replaced periodically.

The individual tank/shellfish cleaning time is approximately 15 mins.

If spawning occurs in the pilot plant tanks, the tanks and recirculating systems are drained after spawning is completed and the shellfish rinsed to avoid accumulation and decomposition of organic material. Individual tray and population spawning records are maintained.

3.1.3 Hatchery

The procedures followed in our hatchery operation are described in the hatchery operating manual (Appendix C).

3.2 Pilot Plant Observations

3.2.1 Phytoplankton

Cell counts (algae) are made twice daily on samples taken at 0800 and 1400 hrs from the pools and PT cultures. Cell counts from the individual pilot shellfish tanks are performed every Monday, Wednesday and Friday at 1400 hrs. The purposes of the cell-counting exercise are:

(a) To follow the growth and "state-of-health" of the pools and the PTs. This information is used to verify that visual monitoring of the pools is indeed adequate. Cell counts from the pools and the PTs are plotted on semilogarithmic graph paper to obtain a graph showing the growth rates and/or yields of the algal cultures.

(b) To calculate the efficiency of the shellfish in stripping the algal cells from the pool water pumped to their tanks.

Turbidities are taken twice daily on samples taken at 0800 and 1400 hrs of the pools to indicate the cell densities of the cultures. A Monitek, Model 250, laboratory turbidimeter is used.

3.2.2 Shellfish

Flow rates are measured with a graduated cylinder and a stopwatch and adjusted according to instructions given by the scientist-in-charge (SIC).

3.2.2.1 Population Growth Measurements

Growth data consisting of mean individual size (length and width in mm), mean individual weight (g), total population weight (g), and estimated population number are taken when periodic transfers are made.

The individual populations are passed through a series of sieves to divide them according to size. The clams are then blotted dry and each fraction is weighed to the nearest .1 g on a Mettler balance (Model PW-1210). A random sample of 25 clams from each fraction is also removed, weighed, and measured, and this data used to determine mean clam weight, length, width, population weight, and population number per group.

As the clams become larger, the sievings are discontinued.

3.2.2.2 Allometric Growth Measurements

A subsample of 25 clams is removed from each population whenever the population is weighed (at intervals never exceeding 28 days) and the following procedure implemented :

(a) Number aluminum weighing dishes and record tare weights.

(b) Measure length, width, and depth of clams, to nearest .1 mm.

(c) Weigh clams in groups of 5 to nearest 0.1 mg.

(d) Shuck meat from clams and weigh meat and shell separately to the nearest 0.1 mg.

(e) Place weighing dishes containing meat and shell in oven at 60°C for 24 hr or until constant dry weight is reached.

(f) Remove aluminum dishes with dried clam and shell from oven and cool in dessicator for 1 hr.

(g) Reweigh dried clam meat and shell.

(h) Retain dried clam meat for protein analysis.

This information is used to provide a detailed analysis of shellfish growth, wet weight-dry weight relationship (% wet meat weight of whole clam wet weight and % dry meat of wet meat), and algal protein-nitrogen to shellfish meat protein-nitrogen conversion.

3.2.3 Chemistry

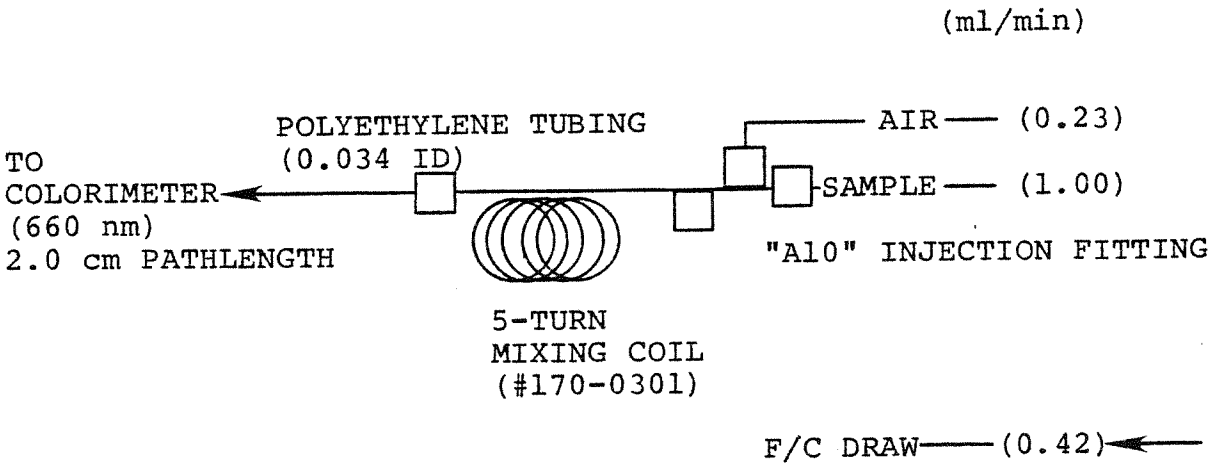
Chemical observations performed on the pilot plant monitor the nutrient balance at different stages in the process. This involves the determination of the concentration of:

NO_2^-	PO_4^{---}
NO_3^-	SiO_4^{---}
NH_4^+	Particulate protein

Standard Technicon Autoanalyzer methods are used for the first five determinations. The particulate protein determination follows a semiautomated procedure outlined by Dorsey et al. (1977) for a heated Biuret-Folin protein assay. Samples are first digested in alkaline-copper solution (Biuret reagent), the copper-protein complex reduced by Folin-Ciocalteau phenol reagent and the molybdate blue color stabilized by cooling:

Method: Samples may be either particulate and filtered onto 25-mm, 0.45µ glass fiber filters, or dissolved in a volume of 0.1-0.4 ml. To the sample in a 17 x 126 mm test tube, add 5.0 ml of alkaline copper solution (reagent A) with an automatic pipette and heat in a 100°C water bath for 100 mins. Remove sample from hot water bath, add 0.5 ml Folin-Ciocalteau reagent, mix immediately on a Vortex mixer, and quickly place into a 10°C cold water bath for 10-15 mins. Centrifuge at 2500 rpm for 2 mins to remove filter fibers and cell debris. Transfer the supernatant to AA-II sample cups by careful decantation or pipeting.

Protein Manifold:



Brij-35 (1 ml/liter) may be used in the sample wash to improve flow characteristics. A 40 hr^{-1}, 1:1 (wash to sample ratio) sampler cam, 15 x 2.0 cm flow cells, 660 nm interference filters at a standard calibration setting of 2.00 gives 50% full-scale deflection of recorder pen at the 20 µg protein-nitrogen level.

3.2.4 Environment

Temperatures in the polytanks are taken approximately six to eight inches from the side of the tank, just inside the

air-bubble stream; in the pools, temperatures are taken from the catwalk in the area of the standpipe; in the shellfish tanks, temperatures are taken from either side of the tank about half-way down the length of the tank. Temperatures are taken @ 0800 and 1400 hrs. daily.

The deep-water temperature is taken from the over-flow hose which runs continuously (0800 and 1400 hrs. daily).

Once a day, at 0800, a reading is taken from the LI-500 integrator on the beach. These numbers are then converted to kW-hr m^{-2} and to Langleys, using a pre-programmed HP-97 calculator.

Weather, sea state, and wind speed are estimated.

All these environmental observations are recorded on a daily beach data sheet (Fig. 8).

Figure 8. All environmental observations are recorded on a beach data sheet, daily.

BEACH DATA SHEET

OBSERVER_____ WEEKDAY_____ DATE_____

WEATHER_____ SEAS_____ AIR TEMP_____ DW TEMP_____

WIND_____ RAINFALL_____

POOL LEVEL: P_1_____ REACTOR ENRICHMENT (202 ml) DW PUMP : [VACUUM_____ / FLOWRATE_____

P_2_____ R-_____ AIR PUMPS: 1)_____ 2)_____

0800

	temp	flow rate ml/10 sec	pool		temp	flow rate ml/10 sec	pool		temp
T1				T7				P1	
T2				T8				P2	
T3				T9				PT1	
T4				T10				PT2	
T5				T11				PT3	
T6				T12				PT4	

WEATHER_____ LIGHT READING

SEAS_____ 0800_____

POOL LEVEL: P_1_____ DW TEMP_____

P_2_____ DW PUMP : [VACUUM _____ / FLOWRATE_____

AIR TEMP_____

WIND_____ mph AIR PUMPS: 1)_____ 2)_____

HYPNEA TANKS:	A	B	C
0800 TEMP			
# OF CLAM TANKS			

HYPNEA TANKS:	A	B	C
1400 TEMP			
# OF CLAM TANKS			

1400

	temp	flow rate ml/10 sec	pool		temp	flow rate ml/10 sec	pool		temp
T1				T7				P1	
T2				T8				P2	
T3				T9				PT1	
T4				T10				PT2	
T5				T11				PT3	
T6				T12				PT4	

Scientist-In-Charge_____

SIC Approval_____

4 RESULTS—PILOT PLANT AND RELATED DATA

4.1 Deep Water Inorganic Nutrients and Deep Water 'Nitrogen' Budget

The major goal of the Artificial Upwelling mariculture program is to initiate and control growth of the primary and secondary trophic levels using only sunlight and deep water as raw materials. Observations on deep water inorganic nutrients are performed on replicate samples from each of the three pipelines once weekly.

These observations, while not comprising a part of the regular pilot plant operations, serve the following purposes:

(1) Through comparison of weekly NO_3+NO_2 values to baseline, a check on the structural integrity of each of the three pipelines is maintained.

(2) By comparing dissolved inorganic 'nitrogen' values (NO_3+NO_2 and NH_4) to values for SiO_3 and PO_4, confirmation that the system is 'nitrogen' limited is maintained.

(3) Most importantly, the concentration of inorganic 'nitrogen' in the deep water provides the basis for a nitrogen budget for the system as a whole. This budget is used to trace the efficiency with which this nutrient is converted to usable nitrogen at the various trophic levels in the system, and also serves as a check on the accuracy, scope and consistency of our chemical and biological observations.

Table 3 represents a portion of 'raw' (transformed into concentrations (μg-at liter^{-1}) data collected from three pipelines over the period July 1, 1976 through June 30, 1977. (All raw data

TABLE 3. DEEP WATER DISSOLVED INORGANIC NUTRIENTS RAW DATA

DATE	PIPE	$NO_3^-+NO_2^-$	NO_2^-	NH_4^+	$PO_4^{\equiv}$	$SiO_4^{\equiv}$
(representative data):						
.	.	.	.	.	.	.
.	.	.	.	.	.	.
.	.	.	.	.	.	.
06/15/77	1	32.90	-	0.54	2.64	27.70*
	1	27.44	-	0.08	1.87	21.32
	2	29.86	-	0.22	2.11	22.25
	2	27.40	-	0.00	2.21	22.60
	3	29.62	-	0.99	2.15	19.89
	3	27.17	-	0.80	2.26	19.75
06/22/77	1	26.94	-	0.29	2.74	19.80
	1	29.62	-	1.20	2.83	19.40
	2	27.13	-	0.54	2.72	20.34
	2	34.11	-	0.66	2.62	19.01
	3	33.72	-	0.38	2.73	20.78
	3	33.91	-	1.00	2.69	19.21
06/29/77	1	34.30	-	0.09	2.40	22.45*
	1	33.95	-	1.01	2.38	23.43*
	2	22.37*	-	1.34	2.18	32.17*
	2	29.00	-	0.59	2.24	-
	3	33.09	-	0.28	1.98	-
	3	30.56	-	0.80	2.22	39.44*
.	.	.	.	.	.	.
.	.	.	.	.	.	.
.	.	.	.	.	.	.

For N = 39 (weeks)

$\bar{x}$ NO_3+NO_2= 30.64

$\bar{x}$ NO_2 = 00.04

$\bar{x}$ % NO_2 of NO_3+NO_2= 0.1

*Suspected contamination; value not included in summary tables.

is on file at both the St. Croix and Port Aransas laboratories.) For samples (N=39) for which NO_3 and NO_2 were determined separately, NO_2 comprised approximately 0.1% of the total NO_3+NO_2. For this reason, the remainder of the tables use the NO_3+NO_2 values only; NO_2 values are not listed separately as virtually all of the NO_3+NO_2 is comprised of NO_3-N.

Table 4 summarizes the mean weekly values for deep water inorganic NO_3+NO_2, NH_4, SiO_4 and PO_4. This data is summarized in Table 7, where total mean dissolved inorganic 'N' present in the deep water is shown as 31.62 μg-at liter^{-1}, with a standard deviation between weekly means of ±0.54 μg-at liter^{-1}. NH_4 values average to 0.98 μg-at liter^{-1}, indicating that the great majority of deep water inorganic 'nitrogen' is present in the form of nitrate (>99.9%). The N:P and N:SiO_4 ratios of 13.72:1 and 1.40:1, respectively, indicate that, insofar as the major inorganic nutrients are concerned, the system is probably nitrate-limited (also see pool data, below).

Table 5 summarizes four ANOVAS performed on inorganic nutrients for the three pipelines. The F ratios indicate that values obtained for NO_3+NO_2, NH_4 and PO_4 from each of the lines are from the same population. Although the F value for SiO_4 does not reach the 5% level of significance, the low N (10, 12 and 11) for pipelines 1, 2 and 3, respectively, indicates that differences between the lines in SiO_4 concentration may be small, but significant. It is known, from a visual inspection by "ALVIN" in March 1976, that one of the lines has its intake on the sea bed, and may be bringing up SiO_4 leached into the surrounding waters.

Not all of the nitrogen present in the deep water is in inorganic forms. Dissolved organic nitrogen has not been measured.

TABLE 4. DEEP-WATER INORGANIC NUTRIENTS: WEEKLY VALUES
ALL VALUES IN μg-at liter^{-1}

DATE	NO_3+NO_2	NH_4	TOTAL "N" INORGANIC	SiO_4	PO_4
	($\bar{x}$ ± S.D. between pipeline means)				
7-1-76	33.94±2.25	-	33.94±2.25	-	-
7-8-76	32.23±0.32	-	32.23±0.32	-	-
7-15-76	33.34±0.13	-	33.34±0.13	-	-
7-22-76	33.19±0.39	-	33.19±0.39	-	-
7-30-76	33.71±0.89	-	33.71±0.89	-	2.80±0.06
8-6-76	33.21±0.61	-	33.21±0.61	-	2.69±0.14
8-13-76	28.99±1.68	-	28.99±1.68	-	2.45±0.19
8-28-76	33.22±0.41	-	33.22±0.41	-	-
9-10-76	33.78±0.30	-	33.78±0.30	-	-
9-16-76	33.58±0.12	-	33.58±0.12	-	-
9-23-76	33.73±0.16	-	33.73±0.16	-	-
10-1-76	28.97 ±1.01	-	28.97±1.01	-	2.02±0.03
10-7-76	29.40±0.41	0.28±0.14	29.68±0.52	-	1.95±0.05
10-15-76	28.03±1.81	0.89±0.84	28.92±1.50	-	1.92±0.04
10-22-76	29.10±0.44	0.05±0.00	29.15±0.48	-	1.96±0.04
10-28-76	DATA ELIMINATED				
11-4-76	27.80±2.62	1.72±0.52	29.52±0.89	18.90±1.27	1.94±0.06
11-11-76	27.29±2.94	1.08±0.35	28.37±2.00	-	1.99±0.01
11-18-76	29.03±0.79	1.08±0.35	30.11±1.01	-	-
11-25-76	29.44±0.29	1.45±0.49	30.89±1.77	20.38±0.18	2.21±0.05
12-2-76	29.97±0.48	0.22±0.15	30.19±0.60	-	-
12-10-76	30.15±0.35	1.36±1.37	31.51±1.14	-	-
12-17-76	30.44±0.50	1.79±0.66	32.23±1.04	-	-
12-22-76	30.45±0.36	1.08±0.12	31.53±0.39	19.18±0.49	2.07±0.04
12-31-76	29.70±0.56	1.42±0.93	31.12±1.26	-	-
1-5-77	29.71±0.54	0.12±0.04	29.83±0.61	-	-
1-12-77	28.46±1.04	0.32±0.11	28.78±0.93	-	-
1-19-77	30.58±0.61	-	30.58±0.61	-	-
1-26-77	30.29±0.60	0.87±0.88	31.16±1.36	20.51±1.25	1.84±0.19
2-2-77	29.85±0.52	0.23±0.03	30.08±0.57	-	-
2-9-77	29.85±0.55	0.31±0.02	30.16±0.61	-	-
2-16-77	29.57±0.58	0.27±0.05	29.84±0.66	-	-
2-23-77	31.07±0.31	0.55±0.15	31.62±1.61	-	-
3-2-77	31.05±0.77	0.90±0.30	31.95±0.61	-	-
3-9-77	29.51±0.54	1.24±0.45	30.75±0.64	29.57±2.49	2.84±0.15
3-16-77	33.05±0.45	1.24±0.71	34.29±1.06	-	-
3-23-77	29.93±0.71	0.82±0.14	30.75±0.65	-	-
3-30-77	31.33±0.39	0.92±0.11	32.25±0.43	-	-
4-6-77	31.49±0.38	1.83±0.93	33.32±0.90	-	-
4-13-77	31.64±0.28	0.97±0.24	32.61±0.50	-	-
4-20-77	33.06±0.83	0.85±0.09	33.91±0.76	-	-
4-27-77	30.05±0.32	1.32±0.18	31.37±0.69	-	-
5-4-77	29.23±0.77	1.34±0.41	30.57±0.78	23.56±5.32	2.58±0.78
5-11-77	29.19±0.30	1.80±0.77	30.99±0.93	-	-
5-18-77	29.44±0.21	1.68±0.78	31.12±0.82	25.67±1.74	2.43±0.97
5-25-77	29.27±0.49	1.66±0.35	30.93±0.82	25.67±1.74	2.43±0.97
6-1-77	29.34±0.36	1.79±1.03	31.13±0.75	23.90±1.20	2.65±0.20
6-8-77	30.40±0.00	1.05±0.65	31.45±0.65	23.19±1.04	2.46±0.09
6-15-77	29.07±2.22	0.39±0.37	29.46±2.36	21.16±1.31	2.21±0.25
6-22-77	30.91±3.43	0.68±0.35	31.59±3.51	19.76±0.69	2.72±0.07
6-29-77	32.18±2.30	0.58±0.50	32.76±2.34	-	2.24±0.17

TABLE 5. SUMMARY TABLES: PIPELINE DEEP WATER INORGANIC NUTRIENT ANOVAS (one-way)

$NO_3^- + NO_2^-$			
Pipe #1	N = 84	Total SS	841.52
Pipe #2	N = 82	Treatment SS	8.33
Pipe #3	N = 83	Error SS	833.19
		df_1	2.00
		df_2	246.00
		F	1.23
		F for 5% level of significance	3.04
NH_4^+			
Pipe #1	N = 60	Total SS	95.35
Pipe #2	N = 61	Treatment SS	0.79
Pipe #3	N = 61	Error SS	94.57
		df_1	2.00
		df_2	179.00
		F	0.74
		F for 5% level of significance	3.05
PO_4			
Pipe #1	N = 24	Total SS	18.73
Pipe #2	N = 24	Treatment SS	0.09
Pipe #3	N = 22	Error SS	18.64
		df_1	2.00
		df_2	67.00
		F	0.16
		F for 5% level of significance	3.14
SiO_4			
Pipe #1	N = 10	Total SS	93.81
Pipe #2	N = 12	Treatment SS	16.27
Pipe #3	N = 11	Error SS	77.53
		df_1	2.00
		df_2	30.00
		F	3.15
		F for 5% level of significance	3.32

Table 6, however, illustrates the raw data collected on protein-nitrogen content of the deep water over the period 6/6/77-7/23/77. The data indicate infrequent and aperiodic "peaks" of protein-nitrogen* of up to 2.65 µg-at liter^{-1} and averaging 0.54 µg-at liter^{-1}. The presence of various diatoms (including Bellerochea sp., Chaetoceros sp. and Nitzschia sp., and various flagellate species) has been confirmed through microscopic examination of the deep water, through the growth of these organisms in deep water to which the medium F/2 has been added, and by the infrequent takeover of "reactor" and "polytank" cultures by these organisms. Bellerochea sp. and various Nitzschia and flagellates have been observed in pool cultures as well. The aperiodic appearance of protein-nitrogen in the deep water may account for the infrequent measurement of total dissolved inorganic nitrogen "+" particulate protein-nitrogen values in the pools exceeding 32 µg-at liter^{-1}. Assuming a mean protein-nitrogen content of 0.54 µg-at liter^{-1} in the deep water, total deep water 'nitrogen' is 32.16 µg-at liter^{-1}.

4.2 Pilot Plant Pool Cultures—9/21/76-7/5/77

The rate of production of algae in the present mariculture system is limited by a combination of various factors, including the volume of deep water pumped, the concentration of nitrate in the deep water, the volume of the phytoplankton pools, and the maximum turnover rate sustainable in these pools. The latter is

*Data for the deep water protein-nitrogen content may not always correspond to pool protein-nitrogen at low levels (<3.00 µg-at liter^{-1}); 75 ml of pool sample is taken regardless of turbidity, while deep water proteins are determined on one liter of water.

TABLE 6. DEEP WATER: PROTEIN-NITROGEN, JUNE 6, 1977 TO JULY 23, 1977

Date	Pro-N µg-at l^{-1}	Mean Pro-N	Date	Pro-N µg-at l^{-1}	Mean Pro-N	Date	Pro-N µg-at l^{-1}	Mean Pro-N
06/06/77	0.00	0	06/23/77	-	-	07/10/77	0.24	0.34
	0			-			0.43	
06/07/77	0.73	0.45	06/24/77	0.05	0.23	07/11/77	0.29	0.64
	0.16			0.40			0.99	
06/08/77	0	0	06/25/77	0	0	07/12/77	0.59	0.50
	0			0			0.40	
06/09/77	1.05	1.44	06/26/77	0	0	07/13/77	0.04	0.11
	3.82			0			0.17	
06/10/77	2.26	1.13	06/27/77	1.41	1.44	07/14/77	0	0.04
	0			1.46			0.08	
06/11/77	0	0.21	06/28/77	1.41	1.92	07/15/77	0.50	0.75
	0.42			2.43			1.00	
06/12/77	0	0	06/29/77	3.59	2.62	07/16/77	0.30	0.95
	0			1.64			1.59	
06/13/77	0	0	06/30/77	-	-	07/17/77	1.14	1.12
	0			-			1.09	
06/14/77	0	0	07/01/77	-	-	07/18/77	0	0
	0			-			0	
06/15/77	0	0	07/02/77	2.60	2.34	07/19/77	0	0
	0			2.09			0	
06/16/77	-	-	07/03/77	0	0	07/20/77	0	0
	-			0			0	
06/17/77	-	-	07/04/77	-	-	07/21/77	0	0
	-			-			0	
06/18/77	0	0	07/05/77	0	0	07/22/77	0	0
	0			0			0	
06/19/77	0	0	07/06/77	0	0.84	07/23/77	0	0
	0			1.68			0	
06/20/77	1.21	0.61	07/07/77	0	0.07			
	0			0.13				
06/21/77	-	-	07/08/77	1.32	1.32			
	-			1.32				
06/22/77	-	-	07/09/77	3.26	2.65			
	-			2.03				

SUMMARY OF VALUES:

w = 40
x = 0.54
δ = 0.77

0 = none detected

TABLE 7. SUMMARY DATA—DEEP WATER INORGANIC NUTRIENTS AND PARTICULATE PROTEIN-NITROGEN (all values in μg-at liter^{-1})

	N (=number)	$\bar{x}$ ± S.D.
NO_3+NO_2*	50	30.64 ± 1.79
NH_4	37	0.98 ± 0.43
Total dissolved inorganic 'N'	50	31,62 ± 1.67
Particulate protein-N	40	0.54 ± 0.77
Total 'nitrogen'		32.16 ± -
SiO_4	12	22.62 ± 3.24
Dissolved 'N':SiO_4		1.40:1
PO_4	21	2.30 ± 0.33
Dissolved		13.72:1

*Approx. 99.9% NO_3-N (Table 3)

limited by the maximum division rate of the diatom, Chaetoceros curvisetus (STX-167), used in the system since mid-1974 and the sole algae grown in the pools during the 1976-77 year. To date, this diatom remains unique in its ability to grow on unsupplemented deep water.

The present pools are relatively shallow (about 1.0 m deep) and do not represent the "optimum" or "maximum" dimensions for pools as calculated by Roels et al., 1976 (Appendix J), who determined that a 4.88 m deep pool with a turnover rate of .81 day^{-1} would provide an optimized relation between pool depth and algal protein production per m^2 of surface area and per unit volume (m^3) of deep water. Van Hemelryck (Appendix D) investigated optimal pool depth in the context of the aquaculture budget generator and concluded that a depth of approximately 3 meters would be optimal. The present pools thus do not make maximum use of available light (see below).

An example of worksheets for pool cultures and polytank cultures is given in Tables 8 and 9. Data include dissolved inorganic 'nitrogen' (NO_3+NO_2, NO_2, NH_4) and particulate protein values on sampled dates, in replicate. Table 10 shows representative pool temperature data at 0800 and 1400 hr daily. Table 11 is a sample of mean daily light readings (in kWh/m^2 and Langleys) recorded for each culture.

Some of this data is summarized in Table 12, which provides information on 29 pilot plant pool cultures over the period 9/21/76-7/5/77. The dates and total time each pool culture was on continuous flow, the mean ($\bar{x}$) and standard deviation (s) of the

TABLE 8. SAMPLE OF WORKSHEETS FOR DATA COLLECTION FROM POOLS

DATA LOG - ARTIFICIAL UPWELLING PROJECT, ST. CROIX (NOV. 5, 1976 - NOV. 25, 1976)

POOL 1

Mo-DAY-YR	Time	Algal Species	10^3 cells per ml	pool status	pool turbidity ppm	pool protein mg/L	pool protein µg-at N/L	pool NO_3+NO_2 µg-at N/L	pool NH_4-N µg-at N/L	pilot plant
11-5-76	0800	167	48	ON	5.6					+
	1400	167	35	ON	8.5					+
11-6-76	0800	167	24	ON	4.6					+
	1400	167	33	ON	7.2					
11-7-76	0800	167	6	ON	2.7					
	1400	167	0	ON	1.7		9.08			
11-8-76	0800	167	—	ON	—					
	1400	167	—	ON	—					
11-9-76	0800	167	—	SCRUB	—					
	1400	167	—	INOC	—					
11-10-76	0800	167	12	OFF	3.0					
	1400	167	13	OFF	3.6		13.64			
11-11-76	0800	167	29	OFF	6.2					
	1400	167	42	ON	18.0					
11-12-76	0800	167	59	ON	7.6					+
	1400	167	65	ON	10.5	2390	26.20			+
11-13-76	0800	167	56	ON	9.2					+
	1400	167	79	ON	14.9					+
11-14-76	0800	167	70	ON	9.2					+
	1400	167	55	ON	11.5					+
11-15-76	0800	167	75	ON	9.6	2010	22.92			+
	1400	167	77	ON	10.1					+
11-16-76	0800	167	55	ON	7.5					+
	1400	167	58	ON	11.2					+
11-17-76	0800	167	50	ON	6.2					+
	1400	167	54	ON	8.4	2250	25.74			+
11-18-76	0800	167	55	ON	12.1					+
	1400	167	62	ON	7.9					+
11-19-76	0800	167	52	ON	6.0	1640	18.77	$3.96 \pm .05$	$1.76 \pm .16$	+
	1400	167	56	ON	8.3					+
11-20-76	0800	167	90	ON	4.1					+
	1400	167	37	ON	8.4					+
11-21-76	0800	167	26	ON	3.8					+
	1400	167	37	ON	5.2					+
11-22-76	0800	167	23	ON	4.2					+
	1400	167	28	ON	5.4	1380	15.81	* ND	$0.19 \pm .05$	+
11-23-76	0800	167	21	ON	2.6					+
	1400	167	51	ON	6.3					+
11-24-76	0800	167	35	ON	4.6	920	10.52	$0.75 \pm .06$	2.2 ± 0.11	+
	1400	167	45	ON	5.4					+
11-25-76	0800	167	24	ON	3.7					+
	1400	167	35	ON	4.6					+

* ND = NOT DETECTABLE

POOL 2

Algal Species	10^3 cells per ml	pool status	pool turbidity ppm	pool protein µg/L	pool protein µg-at N/L	pool NO_3+NO_2 µg-at N/L	pool NH_4-N µg-at N/L	pilot plant
167	49	ON	10.8					
167	44	ON	20.0					
167	70	ON	12.5					
167	63	ON	15.5					+
167	59	ON	9.0					+
167	68	ON	10.5		22.61			+
167	58	ON	7.7					+
167	37	ON	10.5					+
167	41	ON	6.6					+
167	29	ON	11.9					+
167	32	ON	5.6					+
167	42	ON	6.6		22.49			+
167	34	ON	4.5					+
167	38	ON	6.4					+
167	27	ON	9.1					
167	24	ON	7.2	1590	18.20			
167	20	ON	3.9					
167	35	ON	5.8					
167	21	ON	4.0					
167	26	ON	5.7					
167	24	ON	3.8	1120	12.76			
167	21	ON	5.7					
167	18	ON	5.8					
167	18	ON	6.6					
167	14	ON	5.0					
167	36	ON	5.8	1870	21.39			
167	34	ON	4.6					
167	29	ON	6.4					
167	25	ON	4.4	1280	14.57	$7.45 \pm .05$	2.4 ± 0.16	
167	24	ON	6.6					
167	21	ON	4.6					
167	44	ON	8.6					
167	20	ON	4.6					
167	37	ON	5.2					
167	19	ON	4.2					
167	28	ON	6.0	1380	15.81	$0.97 \pm .05$	$2.98 \pm .05$	
167	22	ON	3.9					
167	35	ON	6.1					
167	18	ON	3.7	900	10.29	$10.71 \pm .06$	$2.71 \pm .11$	
167	45	ON	6.4					
167	19	ON	3.0					
167	33	ON	4.4					

DATA LOGS:
CELL COUNTS - STX 1020 BOOK 21 (SEPT. 2, 1976 - DEC. 4, 1976)
TURBIDITY - STX 1320 (FEB. 16, 1974 - JAN. 30, 1977)
CHEM. NUTRIENTS PROTEIN DATA - STX 1349
PILOT PLANT PROTEIN SAMPLING LOG - STX 1292

TABLE 10. REPRESENTATIVE POOL TEMPERATURE VALUES (°C)

DATE	POOL 1 0800	POOL 1 1400	POOL 2 0800	POOL 2 1400	DATE	POOL 1 0800	POOL 1 1400	POOL 2 0800	POOL 2 1400
07/01/76	24.0	-	24.0	26.9	08/20/76	23.0	26.5	22.5	-
02	25.0	27.9	24.5	27.9	21	23.0	26.5	24.0	26.5
03	24.0	26.5	24.0	26.5	22	22.5	27.0	25.0	27.0
04	24.9	26.5	24.9	26.5	23	23.5	26.0	24.0	26.5
05	24.5	26.5	24.5	-	24	23.9	26.0	23.5	25.5
06	24.5	24.5	26.5	-	25	23.5	26.1	24.0	25.9
07	24.9	27.9	25.1	28.0	26	23.5	23.0	23.9	23.0
08	24.5	27.5	24.9	27.0	27	22.0	25.0	21.0	25.0
09	25.0	-	25.0	-	28	23.9	25.9	23.9	-
10	25.5	27.5	25.0	27.5	29	23.0	26.2	24.5	26.2
11	25.5	27.1	25.5	27.1	30	23.0	26.0	25.0	27.5
12	24.5	27.1	24.9	27.1	31	23.0	25.0	23.9	25.5
13	24.5	27.5	24.5	27.5					
14	24.5	27.5	25.0	27.5	09/01/76	23.0	24.5	23.1	24.5
15	24.9	27.9	25.0	27.9	02	23.0	26.0	23.0	26.0
16	24.5	27.0	24.9	27.0	03	23.5	25.5	24.0	26.0
17	25.5	27.9	24.5	27.5	04	23.0	23.9	25.0	25.0
18	24.9	27.0	24.9	27.0	05	22.9	-	23.0	24.9
19	24.0	26.9	24.5	27.0	06	25.0	30.0	24.0	27.0
20	25.0	27.9	24.9	27.5	07	24.5	28.0	23.5	26.0
21	26.1	29.0	25.1	27.5	08	25.0	23.5	25.0	24.0
22	25.0	27.0	24.9	27.0	09	23.0	26.0	23.5	26.9
23	24.9	-	24.9	-	10	23.0	26.0	23.9	26.9
24	24.1	27.9	24.5	28.0	11	24.3	26.5	24.5	27.0
25	24.9	-	24.9	-	12	28.0	26.2	23.5	25.9
26	24.1	24.5	27.9	28.0	13	23.0	27.0	23.0	27.0
27	25.0	26.5	28.0	29.5	14	23.0	27.0	23.0	27.0
28	25.1	29.5	28.5	27.0	15	23.0	27.5	23.0	27.5
29	24.9	25.1	27.1	28.0	16	24.0	27.0	24.0	27.0
30	24.9	25.0	28.0	-	17	24.0	27.0	24.0	27.0
31	25.1	25.1	28.1	28.5	18	23.9	26.0	23.9	26.0
					19	23.9	25.9	23.9	25.9
08/01/76	24.1	25.1	25.1	26.9	20	24.0	26.5	24.0	-
02	25.0	27.0	26.0	28.0	21	23.5	26.0	24.0	26.5
03	24.0	27.0	24.0	27.5	22	24.0	26.0	24.5	27.0
04	24.5	27.0	24.0	27.0	23	24.0	27.0	24.0	26.5
05	24.0	28.0	24.0	27.9	24	23.1	25.5	23.0	24.5
06	26.1	-	24.1	-	25	24.0	-	24.0	25.0
07	24.5	26.5	24.5	26.5	26	24.0	24.0	24.5	24.5
08	24.1	27.0	24.1	27.0	27	24.5	24.5	23.0	24.5
09	24.1	27.0	24.0	27.0	28	24.0	27.0	23.5	26.5
10	24.5	26.0	24.5	26.0	29	24.0	24.0	24.0	24.0
11	23.9	25.5	23.5	25.5					
12	23.0	25.9	22.5	25.9	10/01/76	24.0	27.0	27.0	27.0
13	22.5	25.5	24.5	25.5	02	23.9	26.0	23.9	26.0
14	23.0	25.5	23.1	25.5	03	24.0	26.9	23.5	26.5
15	22.9	25.9	22.9	25.5	04	24.0	-	24.0	-
16	22.5	26.0	22.5	25.5	05	25.0	26.5	26.5	28.0
17	23.9	26.9	23.5	26.5	06	24.9	27.0	27.5	28.5
18	23.0	23.9	23.0	23.9	07	25.0	-	25.0	-
19	22.9	26.0	22.5	25.0	08	24.5	24.5	25.5	24.5

TABLE 11. REPRESENTATIVE AMBIENT LIGHT VALUES. SIMILAR DATA FOR CULTURES 1-29 AVAILABLE

Culture #1	ON OFF (1400-0800)	kWh/m^2	Langley's
9-21-76		4.20	103.30
9-22-76		4.35	377.24
9-23-76		5.28	454.21
9-24-76		3.99	343.31
9-25-76		4.10	352.43
9-26-76		3.44	295.71
9-27-76		5.05	434.46
9-28-76		3.20	274.95
9-29-76		2.81	241.53
9-30-76		3.55	304.83
10-1-76		4.29	368.63
10-2-76		4.64	399.01
10-3-76		4.49	386.35
10-4-76		4.38	376.23
10-5-76		3.94	338.76
10-6-76	(0800)	4.59	394.45

N=16	N=16
$\bar{x}$=3.96	$\bar{x}$ 340.34
s=0.98	s=84.62

TABLE 12. PILOT PLANT POOL CULTURES (9/21/76 - 7/5/77): INOCULATION CHARACTERISTICS, DURATION OF CULTURES, TIME REQUIRED FOR INOCULATION AND ACTIVATION, ENVIRONMENTAL DATA

CULTURE #	POOL #	DATE ON 1400 HR	DATE OFF 0800 HR	HOURS ON ACTUAL	[1]% OF EXPECTED	[2]TEMPERATURE (°C) MEAN	S.D.	[3]DAILY LIGHT (kWh/m^2) MEAN	S.D.	[4]TOTAL LIGHT/ CULTURE	POLYTANK INOCULUM (760 LITERS) [5]TEMPERATURE (°C)	[5]CELL CONC. x10^7 LITERS	[6]K(DIV. PER DAY	[8]HOURS INOCULATION +ACTIVATION
1	2	09/21/76	10/07/76	378	56.8	25.40	±2.23	3.96	±0.98	62.37	30.0	7.3	3.05	24
2	1	09/26/76	10/12/76	378	56.8	25.10	±1.50	3.58	±1.07	56.39	30.0	8.9	3.21	24
3	2	10/08/76	10/20/76	282	42.3	25.76	±1.39	1.87	±0.45	21.97	30.1	6.8	7.67	24
4	1	10/13/76	10/26/76	306	45.9	26.27	±1.40	2.84	±0.96	36.21	30.0	6.5	3.25	24
5	2	10/26/76	11/02/76	234	35.1	25.44	±1.55	1.82	±1.95	17.75	-	10.2	1.33	48
6	1	10/28/76	11/09/76	282	42.3	25.45	±1.37	3.20	±1.23	37.60	31.0	7.5	0.92	42
7	2	11/04/76	11/26/76	522	78.4	24.41	±1.22	3.46	±0.89	75.26	30.0	19.7	3.21	48
8	1	11/11/76	11/30/76	450	67.6	24.25	±1.18	3.34	±0.94	62.23	28.5	3.0	1.53	48
9	2	11/29/76	12/16/76	402	60.4	23.15	±1.08	2.73	±0.76	45.73	28.0	12.0	-0.33	48
10	1	12/02/76	12/23/76	498	74.8	23.12	±1.01	2.92	±0.85	60.59	28.0	18.0	1.05	48
11	2	12/18/76	01/06/77	450	67.6	22.83	±0.95	3.18	±0.81	59.63	27.5	15.4	-	48
12	1	12/26/76	01/11/77	378	56.8	22.94	±1.09	3.22	±0.89	50.72	26.0	6.3	0.48	72
13	2	01/08/77	01/25/77	402	60.4	22.73	±1.08	3.22	±0.79	53.94	27.0	7.4	0.32	48
14	1	01/13/77	02/08/77	618	92.8	22.55	±1.00	3.24	±0.77	83.43	26.5	13.0	16.09	48
15	2	01/27/77	02/22/77	618	92.8	22.76	±1.11	3.50	±0.95	90.12	26.2	19.1	-0.82	48
16	1	02/10/77	03/10/77	666	100.0	23.18	±1.29	4.17	±1.01	115.72	26.1	9.1	1.60	48
17	2	02/24/77	03/14/77	426	64.0	25.53	±1.23	4.31	±1.22	76.50	27.9	12.5	2.80	48
18	1	[7]03/13/77	03/29/77	383	57.5	23.87	±1.48	4.46	±0.99	71.17	28.0	15.1	2.49	66
19	2	03/19/77	03/27/77	192	28.8	24.73	±1.49	4.89	±0.35	39.12	25.5	10.7	-	90
20	1	[7]03/31/77	04/24/77	600	90.1	24.28	±1.51	5.10	±0.80	127.50	26.0	9.7	2.49	36
21	2	03/29/77	04/15/77	402	60.4	24.00	±1.38	5.12	±1.13	85.76	26.0	36.3	1.38	48
22	1	04/28/77	05/16/77	426	64.0	24.69	±1.34	5.28	±1.02	93.72	27.0	13.5	2.60	48
23	2	04/17/77	05/03/77	378	56.8	24.32	±1.56	5.49	±0.97	86.47	29.0	13.3	0.94	48
24	1	05/19/77	06/01/77	306	45.9	24.90	±1.34	4.24	±1.17	54.06	29.9	-	-	48
25	2	05/05/77	05/22/77	402	60.4	24.74	±1.20	4.54	±1.32	76.05	28.5	-	-	48
26	1	06/03/77	06/18/77	354	53.2	25.18	±1.46	5.32	±0.97	78.47	30.0	-	-	36
27	2	05/26/77	06/09/77	330	49.5	25.80	±1.41	4.79	±1.11	65.86	29.0	12.9	2.69	72
28	1	06/20/77	07/05/77	354	53.2	25.54	±1.55	5.71	±1.69	84.22	29.9	-	-	48
29	2	06/11/77	06/28/77	402	60.4	25.48	±1.37	5.37	±0.20	89.95	29.8	10.4	4.00	48

[1]Expected hours ON=666 (27.75 days). [2]$\bar{x}$ and s of culture temperatures taken at 0800 and 1400 hr daily. [3]Based on daily recording (0800-0800) from integrating radiometer; raw data and daily values in Langleys available. [4]Mean daily value ÷ 24 x total hours culture on continuous flow. [5]Prior to inoculation of pool. [6]Based on change in cell density from 0800-1400 hr on day of inoculation. [7]Culture activated at 0800 hr. [8]For total "down-time" add 6 hr to each value for cleaning and refilling to 23,000 liters; expected hours required for inoculation + activation is 48 hr, or 54 hr total "down-time".

pool culture temperature (^{o}C), the mean and standard deviation of ambient light (daily and culture totals) in kWh m^{-2} is shown, as are selected characteristics of the polytank inoculum used for the culture (temperature, cell concentration and division rate per day on day of inoculation). The time required from inoculation to activation of each pool is also shown. Six more hours are required for draining, cleaning, and refilling to 23,000 liters.

This data is further summarized in Table 13. On the average, cultures were on continuous flow for 17 days, or 11 days less than had been expected. (As is shown below, however, total algal protein produced was actually greater than had been expected, because of the greater mean protein-n production.

The difference in mean culture duration between Pools 1 and 2 (Table 13) may be of significance, but has not been subjected to a statistical analysis.

Since the cultures are not light-limited, we would not anticipate any close correlation between light availability and algal protein or cell production in culture duration. This seems supported from casual inspection of the data, but these data have not been subjected to any statistical analyses.

Table 14 illustrates dissolved inorganic NO_3+NO_2 and NH_4 values, cell density, and protein-nitrogen concentration, as well as protein-nitrogen/cell ratios and total 'nitrogen' accounted for. Data is for twenty-nine (29) pilot plant pool cultures (Tables 12 and 13). The values represent the means of replicate samples taken on Monday, Wednesday and Friday of each week at 1400 hr.

Total nitrogen values in Table 14 are computed for those days on which all 'nitrogen' values are available (no measurements

TABLE 13. MEAN OF VALUES PRESENTED IN TABLE 12
PILOT PLANT POOL CULTURES, 1976-77

		POOL 1	POOL 2	POOLS 1&2
(1) Number of cultures	29			
(2) Mean hours (days) cultures on continuous flow				
N		14	15	29
$\bar{x}$ (to nearest hr)		428(17.8)	388(16.2)	408(17.0)
S (of culture means)		123.0(5.1)	105.0(4.4)	113.8(4.7)
Mean % of expected (666 hr)				61.3
(3) Culture temperature (°C)				
$\bar{x}$	24.43			
S	01.11			
(4) Ambient light per day (0800-0800) (kWh/m^2)				
$\bar{x}$	03.96			
S	01.08			
(5) Total ambient light (kWh/m^2) per culture				
$\bar{x}$	67.53			
S	25.24			
(6) Total ambient light (kWh/m^2) all cultures	1958.51			
(7) Mean temperature (°C) of 760-liter inoculum				
N	28			
$\bar{x}$	28.26			
S	01.66			
(8) Mean inoculum cell concentration (x 10^7 liters)				
N	25			
$\bar{x}$	12.18			
S	06.54			
(9) Mean K (divisions/day) on day of inoculum (0800-1400 hr)				
N	23			
$\bar{x}$	00.67			
S	00.85			
(10) Hours (days) inoculation → activation				
N	29			
$\bar{x}$	47.38(1.97)			
S	14.46(0.60)			

Note: Add 6 hr for total "down-time" (emptying, cleaning, refilling, inoculation → activation)

for dissolved inorganic 'nitrogen' were taken on pool cultures prior to 12/10/76). Thus, a full 'nitrogen' balance is available for 21 cultures.

The data shown in Table 14 indicate a sharp decline in NO_3+ NO_2 concentration from deep water values. Concentration of NO_3+NO_2 may fall below 0.1 µg-at l^{-1} while the culture is on continuous flow. Concentrations are often much higher after a pool has "crashed" and still on continuous flow. NH_4 concentration fluctuates around low values but may peak occasionally to as high as 4-8 µg-at l^{-1}. Mean pool culture NH_4 values are higher than the mean NH_4 concentration in the deep water (1.53 vs 0.98 µg-at l^{-1}, respectively), but this difference has not been subjected to statistical analysis to determine if it is significant. Occasional peaks in pool NH_4 values indicate possible excretion by the algae and/or their competitors/predators. It is unlikely that these levels cause any significant suppression of nitrate (NO_3) uptake.

As shown in Table 14, particulate protein often reaches high levels of concentration (> 27 µg-at l^{-1}). Mean protein-nitrogen prediction for the 29 cultures was 23.61 µg-at l^{-1} (Table 15).

The total dissolved inorganic 'nitrogen' and particulate protein-nitrogen often account for a high percentage of the total deep water nitrogen (32.16 µg-at l^{-1}). In general, failure to account for over 60% of the deep water 'N' in the form of pool culture dissolved and protein 'N' occurs when a "crashed" pool is sampled during the final days of a culture's lifetime. This probably indicates the presence of significant amounts of dissolved organic nitrogen, not accounted for with the methods used. The

TABLE 14. POOL CULTURE CELL PRODUCTION AND 'NITROGEN' BUDGET

Culture No.	Pool No.	Date Sampled	NO_3+NO_2 (µg-at L^{-1})	NH_4 (µg-at L^{-1})	Total Dissolved Inorganic 'N' (µg-at L^{-1})	Algal Protein-Nitrogen (µg-at L^{-1})	Total 'Nitrogen' (µg-at L^{-1})	Cells x 10^7 liter	Protein-N/cell x 10^7 (µg-at L^{-1})
1	2	9/29/76	--	--	--	27.77	--	7.7	3.61
		10/02/76	--	--	--	23.54	--	3.7	6.36
		10/05/76	--	--	--	10.46	--	0.4	26.15
	$\bar{x}$					20.59		03.93	
	S					9.02		03.66	
2	1	9/29/76	--	--	--	28.46		6.2	04.59
		10/02/76	--	--	--	26.86	--	5.7	04.71
		10/05/76	--	--	--	--	--	7.2	--
		10/08/76	--	--	--	13.31	--	1.1	12.10
	$\bar{x}$					22.88		05.05	
	S					08.32		02.71	
3	2	10/08/76	--	--	--	04.74	--	0.2	23.70
		10/11/76	--	--	--	20.34	--	4.4	04.62
		10/14/76	--	--	--	24.46	--	3.9	06.27
	$\bar{x}$					16.51		02.83	
	S					10.40		02.29	
4	1	10/14/76	--	--	--	17.26		0.3	57.53
		10/20/76	--	--	--	23.02		5.8	03.97
		10/23/76	--	--	--	05.13	--	--	--
	$\bar{x}$					15.14		3.05	
	S					09.13		3.89	
5	2	10/23/76	--	--	--	23.21	--	1.0	05.13
		10/26/76	--	--	--	26.45	--	3.8	06.96
		10/29/76	--	--	--	17.90	--	3.1	05.80
		11/01/76	--	--	--	02.04	--	0.0	>>>0
	$\bar{x}$					17.42		01.98	
	S					10.83		01.77	
6	1	10/29/76	--	--	--	24.55	--	5.9	04.16
		11/01/76	--	--	--	22.97	--	7.2	--
		11/04/76	--	--	--	21.90	--	5.7	03.19
		11/07/76	--	--	--	09.08	--	0.0	03.84
	$\bar{x}$					19.63		04.70	
	S					07.11		03.20	
7	2	11/04/76	--	--	--	28.71	--	5.0	05.74
		11/04/76	--	--	--	22.61	--	6.8	03.33
		11/10/76	--	--	--	22.49	--	4.2	05.35
		11/12/76	--	--	--	18.20	--	2.4	07.58
		11/15/76	--	--	--	12.76	--	2.1	06.08
		11/17/76	--	--	--	21.39	--	3.6	05.94
		11/19/76	--	--	--	14.57	--	2.4	06.07
		11/22/76	--	--	--	15.81	--	2.8	05.65
		11/24/76	--	--	--	10.29	--	4.5	02.28
	$\bar{x}$					18.54		03.76	
	S					05.79		01.54	
8	1	11/12/76	--	--	--	26.20	--	6.5	4.03
		11/15/76	--	--	--	22.92	--	7.7	2.98
		11/17/76	--	--	--	25.74	--	5.4	4.77
		11/19/76	--	--	--	18.77	--	5.6	3.35
		11/22/76	--	--	--	15.81	--	2.8	5.65
		11/24/76	--	--	--	10.52	--	4.5	2.34
		11/26/76	--	--	--	8.96	--	0.5	17.92
		11/29/76	--	--	--	6.56	--	0.0	>>0
	$\bar{x}$					16.94		04.13	
	S					7.71		02.78	
9	2	11/29/76	--	--	--	18.82	--	2.8	6.72
		12/01/76	--	--	--	26.98	--	7.3	3.70
		12/03/76	--	--	--	15.67	--	6.4	2.45
		12/06/76	--	--	--	23.68	--	5.8	4.08
		12/08/76	--	--	--	19.07	--	8.3	2.29
		12/10/76	03.07	0.50	03.57	24.14	27.71	6.9	3.50
		12/13/76	01.85	0.89	02.74	27.40	30.14	3.6	7.61
		12/15/76	00.19	0.75	00.94	15.14	16.08	1.3	11.65
	$\bar{x}$		01.70	0.71	02.42	21.36	24.64	05.30	
	S		01.45	0.20	01.34	04.84	07.51	02.45	
10	1	12/03/76	--	--	--	19.93	--	3.7	5.39
		12/06/76	--	--	--	28.12	--	9.4	2.99
		12/08/76	--	--	--	25.10	--	5.4	4.65
		12/10/76	00.05	01.59	01.64	30.65	32.29	6.0	5.11
		12/13/76	01.85	01.30	03.15	29.41	32.56	5.9	4.98
		12/15/76	00.19	0.75	00.94	29.04	29.98	6.2	4.68
		12/17/76	08.19	--	8.19	15.81	--	7.3	2.17
		12/20/76	--	--	--	17.82	--	2.2	8.10
		12/22/76	16.36	01.10	17.46	08.75	26.21	0.9	9.72
	$\bar{x}$		05.33	01.19	05.80	22.74	30.26	05.22	
	S		07.00	00.35	07.83	07.56	02.94	02.60	

TABLE 14 (CONTINUED)

11	2	12/20/76	0.14	02.06	02.20	30.14	32.34	8.6	3.50
		12/22/76	0.10	00.50	00.60	27.22	27.82	8.2	3.32
		12/24/76	2.05	01.92	03.97	21.70	25.67	6.5	3.34
		12/27/76	--	02.53	--	23.67	--	6.1	3.88
		12/29/76	6.37	10.62	16.99	20.69	37.68	5.4	3.83
		12/31/76	1.14	03.14	04.28	22.64	26.92	6.5	3.48
		1/03/77	4.28	01.66	05.94	09.90	15.84	1.0	9.90
		1/05/77	17.19	00.36	17.55	04.34	21.89	0.0	>>0
	$\bar{x}$		04.47	02.85	07.36	20.04	26.88	05.29	
	S		06.06	03.28	06.97	08.67	07.02	03.15	
12	1	12/27/76	0.83	01.64	02.47	21.31	23.78	2.1	10.15
		12/29/76	7.81	03.28	11.09	18.26	29.35	4.3	04.25
		12/31/76	--	02.25	--	28.48	--	8.6	3.31
		1/03/77	4.28	01.66	5.94	24.02	29.96	6.2	3.87
		1/05/77	0.03	0.29	0.32	28.17	28.49	7.9	3.57
		1/07/77	8.67	0.13	8.80	21.02	29.82	8.8	2.39
		1/10/77	4.84	2.64	7.48	22.72	30.20	2.5	9.09
	$\bar{x}$		4.41	1.70	6.02	23.43	28.60	5.77	
	S		3.52	1.16	4.02	3.78	2.49	2.84	
13	2	1/10/77	0.13	0.68	0.81	30.56	31.37	4.3	7.17
		1/12/77	--	0.23	--	30.96	--	5.8	5.34
		1/14/77	3.51	1.08	4.59	26.59	31.18	4.2	6.33
		1/17/77	0.19	0.35	0.54	29.80	30.34	5.0	5.96
		1/19/77	4.29	1.36	5.65	23.98	29.29	4.7	5.03
		1/21/77	4.43	0.38	4.81	23.31	28.12	1.8	12.95
		1/24/77	19.48	0.52	20.00	03.43	23.03	--	>>0
	$\bar{x}$		5.34	0.66	7.12	24.09	28.96	4.30	
	S		7.20	0.42	7.47	9.63	2.97	1.35	
14	1	1/14/77	0.35	0.71	1.06	26.47	27.53	3.0	8.82
		1/17/77	0.23	0.18	0.41	29.30	29.71	2.8	10.46
		1/19/77	4.29	1.36	5.65	23.64	29.29	3.8	6.22
		1/21/77	0.59	0.30	0.89	31.55	32.44	3.2	9.86
		1/24/77	0.07	0.60	0.67	26.58	27.25	3.9	6.82
		1/26/77	0.54	0.73	1.27	29.82	31.09	3.4	8.77
		1/28/77	0.16	--	--	29.06	--	7.0	4.15
		1/31/77	0.27	0.21	0.48	30.63	31.11	4.5	6.81
		2/02/77	0.14	0.81	0.95	28.73	29.68	4.3	6.68
		2/04/77	0.13	0.09	0.22	29.24	29.46	5.2	5.62
		2/07/77	0.35	0.21	0.56	31.06	31.62	6.8	4.57
	$\bar{x}$		0.65	.52	1.22	28.73	29.92	4.35	
	S		1.22	.40	1.59	2.33	1.69	1.44	
15	2	1/28/77	0.57	0.32	0.89	27.75	28.64	4.8	5.78
		1/31/77	0.47	0.28	0.75	30.54	31.29	7.7	3.97
		2/02/77	0.18	0.19	0.37	29.93	30.30	4.1	7.30
		2/04/77	0.04	0.13	0.17	28.72	28.89	4.7	6.11
		2/07/77	0.35	0.21	0.56	33.40	31.62	5.5	5.64
		2/09/77	0.09	0.39	0.48	32.19	32.67	6.2	5.19
		2/11/77	0.30	0.24	0.54	23.96	24.50	7.5	3.19
		2/14/77	0.31	--	--	32.03	--	11.0	2.91
	$\bar{x}$,		0.29	0.25	0.54	29.82	29.70	6.44	5.01
	S		0.18	0.09	0.24	3.02	2.71	2.26	1.53
16	1	2/11/77	0.18	0.08	0.26	29.82	30.08	5.5	5.42
		2/14/77	0.49	0.11	0.60	24.39	24.99	7.3	3.34
		2/16/77	0.07	0.25	0.32	27.69	28.01	6.7	4.13
		2/18/77	0.43	0.15	0.58	27.77	28.35	8.0	3.47
		2/21/77	0.71	0.77	1.48	26.29	27.76	5.5	4.78
		2/23/77	2.29	1.28	3.57	21.19	24.76	5.8	3.65
		2/25/77	0.94	0.76	1.70	22.20	23.89	4.2	5.29
		2/28/77	2.43	0.79	3.22	30.08	33.30	8.9	3.38
		3/02/77	3.04	0.82	3.86	32.12	35.98	8.1	3.97
		3/04/77	4.24	0.65	4.89	29.04	33.93	10.6	2.74
		3/07/77	5.25	0.78	6.03	24.51	30.54	8.5	2.88
		3/09/77	5.19	1.13	6.31	19.94	26.25	6.0	3.32
		3/10/77	20.70	3.49	24.19	13.46	37.65	--	7.70
	$\bar{x}$		<3.54	<0.85	<4.39	<25.27	29.65	7.09	
	S		<5.48	<0.88	<6.32	< 5.11	4.43	1.81	
17	2	2/25/77	2.02	1.47	3.49	28.57	32.05	3.8	7.52
		2/28/77	2.38	1.18	3.56	31.31	34.86	9.6	3.26
		3/02/77	2.07	0.96	3.03	28.70	31.72	8.4	3.42
		3/04/77	2.21	0.78	2.99	25.93	28.92	4.7	5.52
		3/07/77	2.74	1.04	3.78	25.87	29.65	9.7	2.67
		3/09/77	4.55	1.00	5.55	25.22	30.77	10.0	2.52
		3/10/77	20.70	3.49	24.14	13.46	37.65	0.1	134.60
		3/14/77	7.77	1.84	9.61	14.16	23.77	--	<<0
	$\bar{x}$		5.56	1.47	7.03	24.15	31.17	6.6	
	S		6.43	0.88	7.28	6.69	4.12	3.8	

TABLE 14 (CONTINUED)

18	1	3/14/77	4.89	1.72	6.61	31.74	38.34	7.4	4.29
		3/16/77	4.52	0.75	5.27	27.71	32.98	8.7	3.19
		3/18/77	0.31	--	--	29.78	--	9.2	3.23
		3/21/77	5.41	1.20	6.60	26.58	33.18	5.4	4.92
		3/23/77	0.5	1.17	1.67	24.12	25.79	5.4	4.46
		3/25/77	2.24	0.91	3.15	21.48	24.63	3.5	6.14
	x̄		2.98	1.15	4.66	26.90	30.98	6.60	
	S		2.27	0.37	2.19	3.73	5.71	2.20	
19	2	3/21/77	0.33	1.06	1.39	25.70	27.08	5.7	4.5
		3/23/77	0.35	0.88	1.23	24.83	26.06	6.1	4.07
	x̄		0.34	0.97	1.31	25.27	26.57	5.90	
	S		0.01	0.13	0.11	00.62	00.72	0.28	
20	1	4/01/77	0.27	0.84	1.11	28.42	29.53	3.0	9.47
		4/04/77	3.48	0.86	4.33	30.41	34.80	6.5	4.68
		4/06/77	0.67	1.02	1.69	30.67	32.36	6.2	4.95
		4/08/77	0.68	1.60	2.28	26.71	28.98	5.6	4.77
		4/11/77	0.47	1.07	1.54	27.21	28.75	6.6	4.12
		4/13/77	0.67	1.30	1.97	29.36	31.33	6.2	4.74
		4/15/77	--	2.06	--	32.36	--	8.4	--
		4/18/77	3.01	0.72	3.75	27.98	31.73	10.9	2.57
		4/20/77	3.56	0.94	4.51	27.33	31.84	1.6	1.71
		4/22/77	10.30	1.18	11.72	10.31	22.03	0.2	51.55
	x̄		2.57	1.14	3.66	27.08	30.15	5.52	
	S		3.21	0.41	3.27	06.16	03.59	3.17	
21	2	3/30/77	0.57	1.23	1.94	28.01	29.95	3.8	7.37
		4/01/77	0.32	0.80	1.12	28.10	29.22	7.2	3.90
		4/04/77	2.48	0.94	3.42	32.6	35.58	7.5	4.29
		4/06/77	0.38	1.43	1.81	31.62	33.43	8.7	3.63
		4/08/77	0.89	0.90	1.75	23.92	25.67	5.3	4.51
		4/11/77	0.42	0.82	1.24	27.50	28.74	10.5	2.62
		4/13/77	0.67	1.30	1.97	29.36	31.33	9.1	3.23
		4/15/77	13.88	3.41	17.29	12.98	30.27	--	>> 0
	x̄		2.32	1.35	4.69	26.71	30.52	07.44	
	S		4.68	0.86	5.35	06.11	03.01	02.29	
22	1	4/29/77	0.91	2.09	3.00	29.01	32.01	3.7	7.84
		5/02/77	0.75	1.15	1.93	28.79	30.72	3.4	8.47
		5/04/77	0.46	1.08	1.54	27.51	29.05	6.3	4.37
		5/06/77	1.10	1.00	2.10	28.99	31.09	7.9	3.67
		5/09/77	0.43	1.49	1.92	27.77	29.69	6.8	4.08
		5/11/77	0.41	2.70	3.11	29.55	32.66	6.9	4.28
		5/17/77	0.58	2.29	2.87	29.49	32.36	6.9	4.27
	x̄		0.66	1.69	2.35	28.73	31.08	5.99	
	S		0.27	.67	.63	.80	1.37	1.73	
23	2	4/18/77	0.48	1.05	1.53	30.41	31.94	5.2	5.80
		4/20/77	0.64	1.28	1.92	32.19	34.11	3.5	9.20
		4/22/77	0.65	0.68	1.33	29.87	31.20	6.6	4.73
		4/25/77	1.43	0.98	2.41	28.60	31.01	6.3	4.54
		4/27/77	0.87	1.53	2.40	27.00	29.40	6.1	4.43
		4/29/77	0.45	1.50	1.95	23.62	25.57	4.7	5.03
		5/02/77	5.41	1.76	7.17	14.24	21.41	0.8	17.80
	x̄		01.42	01.25	02.67	26.56	29.23	04.74	
	S		01.79	00.37	02.02	06.09	04.34	02.04	
24	1	5/20/77	0.75	1.25	2.00	--	--	3.6	--
		5/23/77	0.32	0.92	1.24	21.53	22.77	6.9	3.12
		5/25/77	0.53	3.53	4.06	27.57	31.63	7.6	3.63
		5/27/77	0.35	3.63	3.97	21.15	25.12	5.8	3.65
		5/30/77	0.92	1.47	2.38	23.24	25.62	5.1	4.56
	x̄		0.57	02.16	02.73	23.37	26.29	05.80	
	S		0.26	01.31	01.24	02.94	03.77	01.56	
25	2	5/06/77	0.46	1.30	1.76	30.50	32.26	5.5	5.55
		5/09/77	0.73	1.81	2.54	26.13	26.67	7.1	3.68
		5/11/77	--	--	--	31.50	--	7.0	4.50
		5/13/77	0.55	1.74	2.28	31.35	33.63	7.0	4.48
		5/16/77	0.17	8.05	8.22	23.80	32.02	6.4	3.72
		5/18/77	0.42	2.16	2.58	21.01	23.59	5.3	3.96
		5/20/77	12.53	2.13	14.66	07.17	21.83	--	<<0
	x̄		02.48	02.87	05.34	24.49	28.33	06.38	
	S		04.93	02.56	05.15	08.64	04.99	00.80	
26	1	6/03/77	--	--	--	29.40	--	7.4	3.97
		6/06/77	0.61	2.58	3.19	28.88	32.07	7.1	4.06
		6/08/77	0.59	1.75	2.34	27.28	29.62	5.5	4.96
		6/10/77	0.52	1.11	1.63	30.29	31.92	6.2	4.89
		6/13/77	0.68	1.84	2.52	14.17	16.69	4.7	3.01
		6/15/77	0.55	1.12	1.67	22.60	24.26	3.4	6.65
		6/17/77	15.71	2.28	17.98	04.56	22.57	--	<<0
	x̄		3.11	1.78	4.89	22.45	26.19	05.72	
	S		6.17	0.60	6.44	9.69	06.10	01.51	

TABLE 14 (CONTINUED)

18	1	3/14/77	4.89	1.72	6.61	31.74	38.34	7.4	4.29
		3/16/77	4.52	0.75	5.27	27.71	32.98	8.7	3.19
		3/18/77	0.31	--	--	29.78	--	9.2	3.23
		3/21/77	5.41	1.20	6.60	26.58	33.18	5.4	4.92
		3/23/77	0.5	1.17	1.67	24.12	25.79	5.4	4.46
		3/25/77	2.24	0.91	3.15	21.48	24.63	3.5	6.14
	x̄		2.98	1.15	4.66	26.90	30.98	6.60	
	S		2.27	0.37	2.19	3.73	5.71	2.20	
19	2	3/21/77	0.33	1.06	1.39	25.70	27.08	5.7	4.5
		3/23/77	0.35	0.88	1.23	24.83	26.06	6.1	4.07
	x̄		0.34	0.97	1.31	25.27	26.57	5.90	
	S		0.01	0.13	0.11	00.62	00.72	0.28	
20	1	4/01/77	0.27	0.84	1.11	28.42	29.53	3.0	9.47
		4/04/77	3.48	0.86	4.33	30.41	34.80	6.5	4.68
		4/06/77	0.67	1.02	1.69	30.67	32.36	6.2	4.95
		4/08/77	0.68	1.60	2.28	26.71	28.98	5.6	4.77
		4/11/77	0.47	1.07	1.54	27.21	28.75	6.6	4.12
		4/13/77	0.67	1.30	1.97	29.36	31.33	6.2	4.74
		4/15/77	--	2.06	--	32.36	--	8.4	--
		4/18/77	3.01	0.72	3.75	27.98	31.73	10.9	2.57
		4/20/77	3.56	0.94	4.51	27.33	31.84	1.6	1.71
		4/22/77	10.30	1.18	11.72	10.31	22.03	0.2	51.55
	x̄		2.57	1.14	3.66	27.08	30.15	5.52	
	S		3.21	0.41	3.27	06.16	03.59	3.17	
21	2	3/30/77	0.57	1.23	1.94	28.01	29.95	3.8	7.37
		4/01/77	0.32	0.80	1.12	28.10	29.22	7.2	3.90
		4/04/77	2.48	0.94	3.42	32.6	35.58	7.5	4.29
		4/06/77	0.38	1.43	1.81	31.62	33.43	8.7	3.63
		4/08/77	0.89	0.90	1.75	23.92	25.67	5.3	4.51
		4/11/77	0.42	0.82	1.24	27.50	28.74	10.5	2.62
		4/13/77	0.67	1.30	1.97	29.36	31.33	9.1	3.23
		4/15/77	13.88	3.41	17.29	12.98	30.27	--	>> 0
	x̄		2.32	1.35	4.69	26.71	30.52	07.44	
	S		4.68	0.86	5.35	06.11	03.01	02.29	
22	1	4/29/77	0.91	2.09	3.00	29.01	32.01	3.7	7.84
		5/02/77	0.75	1.15	1.93	28.79	30.72	3.4	8.47
		5/04/77	0.46	1.08	1.54	27.51	29.05	6.3	4.37
		5/06/77	1.10	1.00	2.10	28.99	31.09	7.9	3.67
		5/09/77	0.43	1.49	1.92	27.77	29.69	6.8	4.08
		5/11/77	0.41	2.70	3.11	29.55	32.66	6.9	4.28
		5/17/77	0.58	2.29	2.87	29.49	32.36	6.9	4.27
	x̄		0.66	1.69	2.35	28.73	31.08	5.99	
	S		0.27	.67	.63	.80	1.37	1.73	
23	2	4/18/77	0.48	1.05	1.53	30.41	31.94	5.2	5.80
		4/20/77	0.64	1.28	1.92	32.19	34.11	3.5	9.20
		4/22/77	0.65	0.68	1.33	29.87	31.20	6.6	4.73
		4/25/77	1.43	0.98	2.41	28.60	31.01	6.3	4.54
		4/27/77	0.87	1.53	2.40	27.00	29.40	6.1	4.43
		4/29/77	0.45	1.50	1.95	23.62	25.57	4.7	5.03
		5/02/77	5.41	1.76	7.17	14.24	21.41	0.8	17.80
	x̄		01.42	01.25	02.67	26.56	29.23	04.74	
	S		01.79	00.37	02.02	06.09	04.34	02.04	
24	1	5/20/77	0.75	1.25	2.00	--	--	3.6	--
		5/23/77	0.32	0.92	1.24	21.53	22.77	6.9	3.12
		5/25/77	0.53	3.53	4.06	27.57	31.63	7.6	3.63
		5/27/77	0.35	3.63	3.97	21.15	25.12	5.8	3.65
		5/30/77	0.92	1.47	2.38	23.24	25.62	5.1	4.56
	x̄		0.57	02.16	02.73	23.37	26.29	05.80	
	S		0.26	01.31	01.24	02.94	03.77	01.56	
25	2	5/06/77	0.46	1.30	1.76	30.50	32.26	5.5	5.55
		5/09/77	0.73	1.81	2.54	26.13	26.67	7.1	3.68
		5/11/77	--	--	--	31.50	--	7.0	4.50
		5/13/77	0.55	1.74	2.28	31.35	33.63	7.0	4.48
		5/16/77	0.17	8.05	8.22	23.80	32.02	6.4	3.72
		5/18/77	0.42	2.16	2.58	21.01	23.59	5.3	3.96
		5/20/77	12.53	2.13	14.66	07.17	21.83	--	<<0
	x̄		02.48	02.87	05.34	24.49	28.33	06.38	
	S		04.93	02.56	05.15	08.64	04.99	00.80	
26	1	6/03/77	--	--	--	29.40	--	7.4	3.97
		6/06/77	0.61	2.58	3.19	28.88	32.07	7.1	4.06
		6/08/77	0.59	1.75	2.34	27.28	29.62	5.5	4.96
		6/10/77	0.52	1.11	1.63	30.29	31.92	6.2	4.89
		6/13/77	0.68	1.84	2.52	14.17	16.69	4.7	3.01
		6/15/77	0.55	1.12	1.67	22.60	24.26	3.4	6.65
		6/17/77	15.71	2.28	17.98	04.56	22.57	--	<<0
	x̄		3.11	1.78	4.89	22.45	26.19	05.72	
	S		6.17	0.60	6.44	9.69	06.10	01.51	
27	2	5/30/77	0.74	1.57	2.31	28.52	30.83	9.9	2.88
		6/01/77	0.44	2.32	2.76	25.28	28.04	6.9	3.66
		6/03/77	--	--	--	24.33	--	5.8	4.19
		6/06/77	0.61	2.58	3.19	28.88	32.07	7.1	4.07
		6/08/77	14.11	1.84	15.95	06.10	22.05	--	>>0
	x̄		03.98	02.08	06.05	22.62	28.25	07.43	
	S		06.76	00.46	06.61	09.45	04.46	01.75	
28	1	6/20/77	15.71	02.28	17.98	04.57	22.57	05.5	0.83
		6/22/77	0.68	05.47	06.14	22.98	29.12	07.5	3.06
		6/24/77	0.47	2.11	02.58	27.28	29.86	5.2	5.25
		6/27/77	0.23	1.40	01.63	23.73	25.36	2.2	10.79
		6/29/77	0.74	2.32	03.06	--	--	0.4	--
		7/01/77	1.39	5.01	06.40	26.58	32.98	--	>>0
		7/04/77	21.58	0.71	22.29	04.00	26.29	--	>>0
	x̄		5.83	2.76	8.58	18.19	27.70	04.16	
	S		8.92	1.79	8.18	10.90	03.70	02.83	
29	2	6/13/77	--	--	--	16.12	--	4.7	3.43
		6/15/77	0.42	1.44	1.85	25.68	27.53	3.4	7.55
		6/17/77	0.40	0.62	1.02	27.32	28.34	--	--
		6/20/77	0.25	4.60	4.84	26.12	30.96	3.3	7.92
		6/22/77	0.28	4.97	5.25	12.69	17.94	3.4	3.73
		6/24/77	0.30	1.86	2.16	26.58	28.74	4.7	5.66
		6/27/77	1.63	2.73	4.26	14.16	18.52	6.3	2.25
		6/29/77	29.77	2.25	32.02	--	22.07	6.6	0.00
	x̄		4.72	2.64	7.36	18.58	26.29	4.63	
	S		11.06	1.61	11.00	9.66	5.72	1.38	

All samples taken at 1400 hrs. "--" indicates no sample taken.

TABLE 14 (CONTINUED)

NITROGEN BUDGET—SUMMARY TABLE, 29 SEPTEMBER 1976

Culture No.	Pool No.	NO_3+NO_2 ($\mu g\text{-at } L^{-1}$)	NH_4 ($\mu g\text{-at } L^{-1}$)	Total Dissolved 'Nitrogen' ($\mu g\text{-at } L^{-1}$)	Algal Protein-N ($\mu g\text{-at } L^{-1}$)	Total 'Nitrogen' ($\mu g\text{-at } L^{-1}$)
1	2	-	-	-	20.59	-
2	1	-	-	-	22.88	-
3	2	-	-	-	16.51	-
4	1	-	-	-	15.14	-
5	2	-	-	-	17.42	-
6	1	-	-	-	19.63	-
7	2	-	-	-	18.54	-
8	1	-	-	-	16.94	-
9	2	1.70	0.71	2.41	21.36	23.77
10	1	5.33	1.19	6.52	22.74	29.26
11	2	4.47	2.85	7.32	20.04	27.36
12	1	4.41	1.70	6.11	23.43	29.54
13	2	5.34	0.66	6.00	24.09	30.09
14	1	0.65	0.52	1.17	28.73	29.90
15	2	0.29	0.32	0.54	29.82	30.36
16	1	3.54	0.85	4.39	25.27	29.66
17	2	5.56	1.47	7.03	24.15	31.18
18	1	2.98	1.15	4.13	26.90	31.03
19	2	0.34	0.97	1.31	25.27	26.58
20	1	2.57	1.16	3.73	27.08	30.81
21	2	2.32	1.35	3.67	26.71	30.38
22	1	0.66	1.69	2.35	28.73	31.08
23	2	1.42	1.25	2.67	26.56	29.23
24	1	0.57	2.16	2.73	23.37	26.10
25	2	2.48	2.87	5.35	24.49	29.84
26	1	3.11	1.78	4.89	26.19	31.08
27	2	3.98	2.08	6.06	28.25	34.31
28	1	5.83	2.76	8.59	27.70	36.29
29	2	4.72	2.64	7.36	26.29	33.65
	$\overline{N}$ =	21	21	21	21 29	21
	$\overline{X}$ =	02.97	01.53	04.49	25.58 23.61	30.07
	S =	01.87	00.78	02.29	02.53 04.08	02.75

Notes:

For 21 cultures (#s 9-29)(all values in $\mu g\text{-at liter}^{-1}$):

1) Mean total 'nitrogen' accounted for : 30.07 ± 2.75

2) Mean total deep-water 'nitrogen' (Table 5) : 32.16 (includes 0.59 $\mu g\text{-at liter}^{-1}$ protein nitrogen)

3) Mean % deep-water 'nitrogen' accounted for in pools : 93.5

4) Mean algal-protein nitrogen : 25.58 ± 2.53
   % of total pool 'nitrogen' : 85.1

5) Mean dissolved inorganic 'nitrogen' : 4.49 ± 2.29
   % of total pool 'nitrogen' : 14.9

6) Mean NO_3+NO_2-N : 2.97 ± 1.87
   % of total pool 'nitrogen' : 9.9

7) Mean NH_4-N : 1.53 ± 0.78
   % of total pool 'nitrogen' : 5.0

8) Efficiency of conversion, total deep-water 'nitrogen' ÷ phytoplankton pool protein 'nitrogen' (29 cultures) : 23.61 ÷ 32.16 = 73.4%

growth of filamentous algae on the walls of the pools also accounts for the "disappearance" of deep-water nitrogen.

Since the cell count at 1400 hr represents a count of live C. curvisetus (STX-167) cells only, the increased protein-nitrogen cell count ratio during the last few days of culture life indicates the presence of dead cells and contaminants in the pool. This conclusion is often confirmed by visual (microscopic) examination of the pools before "crashing" in which contaminants in the form of other diatoms, flagellates, amoebas and/or bacteria may be seen. (The presence of such contaminants, however, has not been positively correlated with the onset of pool collapse.)

In general, the methods used for measurement of 'nitrogen' in the pools accounted for about 93.5% of total deep water 'nitrogen' (Table 15). Of the total 'nitrogen' measured in the pools (21 cultures), 85.1% was in the form of particulate protein 'N', and 14.9% was in the form of dissolved inorganic 'N' (9.9% NO_3 and 5.0% NH_4).

Table 15 summarizes the data collected on algal protein production over the period 9/21/76-7/5/77 in the artificial upwelling system.

As shown, the two pools, while on continuous flow, produced a mean of 0.234 kg algal protein per day (~50% that amount per pool). This translates to a production of 1.98 g/m^2/day of algal protein.

The extrapolated yearly production for the two pools is 75.4 kg algal protein yr^{-1} or 6.4 tons protein/ha/yr. These values for the 1976-77 cultures correspond to the 6.99 g/m^2/day predicted

TABLE 15 (CONTINUED)

2) Extrapolated Yearly Algal Protein Production

a) Twenty-nine (29) cultures were ON continuous flow for a mean of

408 hr (Table 13)

and OFF continuous flow (total down-time) for a mean of

~53.4 hr (Table 13)

Therefore, mean total time for each culture was

461.4 hr

b) For a 365-day year, each pool would be ON continuous flow for

(408)÷(461.4) ((365))
= 322.7568 days

c) Yearly algal protein production for the two pools is therefore:

(322.7568)(.233798)
= 75.45990 kg yr^{-1}
OR 37.72995 kg yr^{-1} per pool

d) With a combined surface area of 117.8 m^2, this translates to a total yearly algal protein production of

0.6405764 kg/m^2/yr
OR 6.4 tons protein/ha/yr

Note: This compares to a figure of 5.2 tons/ha/yr of algal protein, computed for the pools by Roels et al. 1976, p. 5; Appendix J)

and 0.710 tons protein/ha/yr for alfalfa (highest terrestrial plant protein producer) (see Roels et al. 1976, p. 9)

and 23.063 tons/protein/ha/yr for an optimized pool depth and turnover rate as computed by Roels et al. 1976, p. 9.

by Roels et al. (Appendix J) for a 4.88 m deep pool at a turnover rate of .81 day^{-1} and the 23.063 tons or protein/ha/yr predicted by Roels et al. (1976) for the same pool depth and turnover rate (4.88 m deep and .81 day^{-1}). The value of 6.4 tons/ha/yr is 1.2 tons/ha/yr greater than was estimated by Roels et al., 1976 (Appendix J) for the actual pools. This is entirely due to the fact that mean concentration of particulate protein, especially for the last 21 cultures, was significantly greater than that assumed by Roels et al. (23.61 µg-at l^{-1} vs 21.4 µg-at l^{-1}). This value for protein production is nine times greater than that of the .71 tons/ha/yr of plant protein produced by alfalfa, which yields the highest amount of protein/ha in conventional land-based agriculture (see Roels et al., 1976; Appendix J).

4.3 Hatchery

The primary function of the hatchery is to provide the pilot plant shellfish area with batches of juvenile Tapes japonica. The hatchery also provides shellfish for experimental feeding studies.

During the period 7/1/76-6/30/77, sixteen (16) batches of Tapes were reared in the hatchery and all but two of these batches successfully completed metamorphosis (Table 16). Batches #20, 21 and 22 were used in the pilot plant.

One striking difference between batches of Tapes #21 through Tapes #32 was the percent survival to metamorphosis: the mean percent survival for these 13 batches is 9%. Survival decreased from an average of 40% in batches #5-13, with

TABLE 16. *Tapes japonica* REARED IN THE HATCHERY FROM AUGUST 1976 THROUGH JUNE 1977

SPAWNING DATE	BATCH NUMBER	INDUCED OR SPONTANEOUS	PERCENTAGE SURVIVAL TO METAMORPHOSIS	MEAN SIZE (LENGTHxWIDTH) WHEN METAMORPHOSIS BEGAN
08/03/76	20	induced	33	207 x 198
08/16/76	21	induced	14	212 x 202
08/24/76	22	induced	10	194 x 179
08/31/76	23	induced	11	171 x 156
01/03/77	24	spontaneous	6	199 x 184
01/24/77	25	spontaneous	4.5	202 x 193
01/31/77	26	spontaneous	0.4	148 x 114
02/08/77	27	spontaneous	15	198 x 187
02/28/77	28	induced	2	162 x 144
03/15/77	29	spontaneous	0	not measured
03/16/77	30	spontaneous	0	not measured
03/30/77	31	sponteneous	6	197 x 188
04/01/77	32	spontaneous	13	205 x 193
04/25/77	33	induced	64	207 x 197 ("Vet Strep")
			71	195 x 187 ("Neomycin")
05/23/77	34	induced	68	195 x 181 ("Vet Strep")
			58	169 x 158 ("Neomycin")
			35	192 x 176 (no antibiotics)
06/17/77	35	induced	54	205 x 190 ("Vet Strep")
			57	207 x 197 (no antibiotics)

successive batches. The mean survival to metamorphosis for Tapes #16, 17 and 18 was 51%. A dramatic increase in survival for Tapes batches #33, 34 and 35 (58%) was observed. There are several factors which have been identified as responsible for the decreased survival rate of batches spawned from August 1976 through April 1977:

(1) The deep water pipeline in the hatchery has never been cleaned since operation began in December 1974. At times, H_2S has been noticed in the hatchery plumbing when the deep water is first turned on. To reduce the H_2S, a valve has been left partially open, since April 1977, to allow deep water to flow through the pipes at all times.

(2) Since the hatchery operation began, the antibiotic Streptomycin sulfate ("Vet Strep") has been used in the larval cultures at 50 mg/liter. Bacteria do build up a resistance to this antibiotic, therefore others are being tested.

(3) A change in hatchery personnel for the period August 1976 to April 1977 may have had an influence on larval survival.

The increase in percent survival for Tapes batches #33, 34 and 35 is very encouraging and helped institute a few changes and proposed changes in the hatchery routine. These three batches were reared under slightly different conditions than the previous 32 batches.

(a) Deep water was allowed to flow slowly through the hatchery plumbing at all times;

(b) larvae were reared in large 379-liter cultures rather than in 15-liter cultures, thus a different surface to volume ratio

was established in the larval cultures;

(c) these larvae (batches #33, 34, 35) were from induced spawnings rather than spontaneous spawnings and survival, on the average, appears to be better for induced batches (see Tables 16-18).

Since the first batch of Tapes japonica larvae was reared in the artificial upwelling mariculture system in April 1974, Tapes have been induced to spawn, by thermal and chemical (stripped gonads) stimulation, ten months of the year (Table 17), spontaneous spawnings have been observed seven months of the year. Generally, Tapes larvae have been obtained whenever a batch was required.

After rearing 33 batches of Tapes in the hatchery system, it is apparent that the feasibility of a hatchery in the tropics has been demonstrated. Of course, refinements and improvements in the system are necessary. In this coming year, procedures for increasing the efficiency and decreasing labor and equipment costs on the present hatchery operation will be implemented.

4.4 Pilot Plant Shellfish

In this section, the general manner in which the pilot plant shellfish area was managed is described, important data is illustrated, and the data is subjected to a preliminary analysis. Refer to Section 3 (Procedures) for a description of intended operations.

Three populations of juvenile T. japonica clams were introduced into the pilot shellfish area: H20, spawned on 8/3/76; H21, spawned on 8/16/76, and H22, spawned on 8/24/76. These three populations were designated as pilot plant populations

TABLE 17. *Tapes japonica* LARVAE BATCHES FROM FEBRUARY 1975 to JANUARY 1976

SPAWNING DATE	BATCH NUMBER	PERCENTAGE SURVIVAL TO METAMORPHOSIS
02/04/75	5	41
03/05/75	6	44
04/05/75	7	63
07/16/75	9	34
10/16/75	10	36
12/30/75	11	4
01/02/76	12	51
01/27/76	13	46

TABLE 18. PERCENTAGE SURVIVAL TO METAMORPHOSIS FOR BATCHES OF *Tapes* GROWN WITH OR WITHOUT ANTIBIOTICS

BATCH NUMBER	WITH ANTIBIOTICS				WITHOUT ANTIBIOTICS
	Streptomycin	Neomycin			
	50 (mg/l)	50 (mg/l)	25 (mg/l)	12.7 (mg/l)	
31	6%	-	-	-	-
32	-	13%	-	-	-
33	64%	-	71%	-	-
34	68%	-	-	58%	35%
35	54%	-	-	-	57%

1, 5, and 9 (PP1, PP5, PP9) in reference to the tank number into which each population was first placed.

For various reasons, the pilot plant shellfish area was operated in a highly structured manner, in which distribution of animals and control of flow rate were based on projected shellfish growth rates as described in the technical description. That is, manipulations of shellfish and flow rates were based on a pre-determined schedule of expected population weights based on the number of days the population was in the pilot plant and actual population weights were not used as feedback for either distribution or flow-rate control. This point is of importance in comparing "expected" and "actual" growth rates of the three populations.

The shellfish were introduced into the pilot plant according to the schedule in Table 19.

From Days 0-35 (through 10/26/76), PP1 was placed in a single tray at an intended flow rate of 30 ml/sec. From 10/26/76-11/30/76, the intended flow rate was 60 ml/sec. This was changed to 180 ml/sec on 11/30/76 and to 300 ml/sec on 12/28/76. On 1/25/77, the population was redistributed to 4 trays, each at a flow rate of 110 ml/sec, for a population flow of 440 ml/sec. A highly similar sequence was used for PP5 and PP9: the intended flow rates are summarized in Table 20. Note deviations from the original flow-rate schedule (Table 1). Table 20 also illustrates the total intended flow in liters through each population over the various weighing periods, and the "actual" flow for the identical periods of time. This "actual" flow was calculated on the basis of a pilot plant interruption data log on which all increases or decreases

TABLE 19. INTRODUCTION OF SHELLFISH INTO THE PILOT PLANT

BATCH #	TRAY #	DATE OF INTRODUCTION SCHEDULED	ACTUAL	CORRECTED*
20	1	09/01/76	10/13/76	09/22/76
21	5	09/21/76	10/15/76	10/12/76
22	9	10/11/76	10/19/76	10/19/76

*Note: According to our plan of work as described in the section 2.1.2 (page 20) of our proposal for 1976-77, we intended to introduce the shellfish into the pilot plant when they had reached an average length of 4.3 mm and a weight of .01 g. Corrected date equals estimated time this size was actually reached.

TABLE 20. TOTAL FLOW THROUGH EACH PILOT PLANT POPULATION FOR EACH WEIGHING PERIOD (1976-77)

DAYS	DATES	INTENDED FLOW RATE (ml/sec)	TOTAL THEORETICAL FLOW (LITERS) (ml/sec x ml/day x days)	A 30 MINUTES OFF FOR WEIGHING & CLEANING	B LOSS FOR INTERRUPTIONS	TOTAL SUBTRACTED (A+B)	ESTIMATED FLOW (LITERS) PER POPULATION
	PP1						
(13)	10/13-10/26	30	33,696	54	0	54	33,642
(35)	10/26-11/30	60	181,440	108	0	108	181,332
(28)	11/30-12/28	180	435,456	324	0	324	435,132
(28)	12/28-01/25	300	725,760	540	0	540	725,220
(28)	01/25-02/22	440	1,064,448	792	119,315	120,107	944,341
(28)	02/22-03/22	440	1,064,448	792	224,697	225,489	838,959
(28)	03/22-04/19	440	1,064,448	792	231,330	232,122	832,326
(28)	04/19-05/17	440	1,064,448	792	239,879	240,669	823,779
(28)	05/17-06/14	440	1,064,448	792	170,478	171,270	893,178
							5.7079032 x 10^6 liters
	PP5						
(11)	10/15-10/26	30	28,512	54	0	54	28,458
(21)	10/26-11/16	30	54,432	54	0	54	54,378
(14)	11/16-11/30	30	36,288	54	0	54	36,234
(21)	11/30-12/21	60	108,864	108	0	108	108,756
(28)	12/21-01/18	180	435,456	324	0	324	435,132
(28)	01/18-02/15	300	725,760	540	95,022	95,562	630,198
(28)	02/15-03/15	440	1,064,448	792	280,302	281,094	783,354
(28)	03/15-04/12	440	1,064,448	792	245,256	246,048	818,400
(28)	04/12-05/10	440	1,064,448	792	244,035	244,827	819,621
(28)	05/10-06/07	440	1,064,448	792	172,656	173,448	891,000
							4.6105531 x 10^6 liters
	PP9						
(14)	10/19-11/02	30	35,288	54	0	54	36,234
(21)	11/02-11/23	30	54,432	54	0	54	54,378
(35)	11/23-12/28	60	181,440	108	0	108	181,332
(28)	12/28-01/25	180	435,456	324	0	324	435,132
(28)	01/25-02/22	300	725,760	540	81,351	81,891	643,869
(28)	02/22-03/22	440	1,064,448	792	224,631	225,423	839,025
(28)	03/22-04/19	440	1,064,448	792	231,330	232,120	832,236
(28)	04/19-05/17	440	1,064,448	792	239,877	240,669	823,779
(28)	05/17-06/14	440	1,064,448	792	170,478	171,270	893,178
							4.7391630 x 10^6 liters

in flow due to pool scrubbing operations and removal of trays for cleaning and weighing were recorded. These interruptions affected total flow only once the entire pilot plant area was filled; prior to this time, one pool was sufficient to feed all trays at the intended rate. It will be noticed that the actual total flow to all populations (once the pilot shellfish area was filled) was about 80% of expected. It should be stressed that these "actual" flow rates account for interruptions only and that the accuracy of these values depends on the actual precision of flow-rate control. Periodic checks on flow rates indicated that significant deviations from the intended rates were uncommon. At any rate, the total flow into all three populations was limited by pool flow capacity. Thus, real mean flow rates may have been somewhat lower, if decreases in flow rates during p.m. hours occurred, but mean values above the intended flow rate should not have occurred for any significant length of time (this would have had the practical effect of draining the pools). Since the real flow rates were more likely to be somewhat below rather than above the intended, the estimated conversion efficiencies, calculated below, may be somewhat conservative.

At the time of introduction, batches H20, H21 and H22 had exceeded the size planned for introduction. The corrected date shown in Table 19 corresponds to the day on which the planned introduction size is considered to have been reached.

It should be noted that these dates are at 50, 57 and 56 days after spawning. A 45-day span had been anticipated based on extrapolation of the 1975 "constant weight" study. The observed discrepancy can be attributed entirely to a different growth rate

before the larvae "set".

The pilot plant shellfish weight increases since corrected introduction date are given in Table 21.

These "expected" growth rates were based on a predicted growth rate for an assumed 10,000 individuals per population on corrected date of introduction fed at the intended flow rate and with an assumed concentration of algal protein-nitrogen in the inflow of 21.4 μg-at liter^{-1}. Pool 'down-time' was not accounted for (assumes continuous flow from both pools). Thus, this expected growth rate assumes precise control over shellfish numbers on date of introduction, and does not account for losses due to mortality, or to possible deviations from expected flow rates, and/or to deviations from expected concentration of food in the inflow. In short, it is derived strictly from operation and predictions based on the shellfish technical description.

The actual number of animals in each population was measured on 5/10/77 and 5/17/77. From these values and from mortality records kept beginning on 1/11/77 (previous to this date very little mortality was noticed and an assumed mortality rate of 1% per weight period prior to 1/11/77 was used in the following calculations) and accounting for the 25 animals removed from each population at the end of a weighing period for the allometric study, the actual number of animals in each population over each weighing period was calculated.

Based on the actual total weight of each population, a mean individual weight was also computed. Values for PP1 are illustrated in Table 22, where the number (N), whole weight (W) and mean individual weight (w) for the population at the beginning (N_0, W_0, w_0)

TABLE 21. EXPECTED AND MEASURED PILOT PLANT SHELLFISH WHOLE WET WEIGHT PRODUCTION DAYS 14-322

Days in system	[1]Expected weight per population, after culling (kg)	Expected culled weight per population (kg)	Expected total weight production per population (kg)	Measured weight				Mean % of total expected population
				PP1 (H2O)	PP5 (H21)	PP9 (H22)	Mean (PP1,5,9)	
14	0.400	0	0.400	-	.333	.935	.634	159
35	1.600	0	1.600	1.340	1.339	2.100	1.593	100
70	6.400	0	6.400	3.500	3.500	4.700	3.900	61
98	14.827	0	14.827	7.348	8.376	9.414	8.379	57
126	27.480	0	27.480	10.705	13.270	13.490	12.488	45
154	33.734	12.124	45.858	15.660	19.650	19.530	18.280	40
182	39.812	12.446	64.382	19.720	21.100	23.430	21.413	33
210	45.914	12.426	82.910	22.760	24.800	27.000	24.853	30
238	52.030	12.414	101.440	26.200	28.450	31.520	28.723	28
266	58.159	12.406	119.975	29.430	31.250	33.630	31.437	26
294	64.298	12.399	138.513	30.520	32.570	36.040	33.043	26
322	70.443	12.396	157.054	30.790	34.930	38.620	34.780	22

[1]Expected weights are based on projections from the Nov-Dec 1975 "Constant Weight" study. Expected values assume a constant inflow of 21.4 $\mu gat\ L^{-1}$ of algal protein-nitrogen; a conversion efficiency (to shellfish meat protein nitrogen) of 33% and a meat protein to whole wet weight factor of 33.125 (see Appendix B) (Pool "down-time" is not accounted for)

TABLE 22. PILOT PLANT POPULATION #1 (H20) DATA FOR "INTENDED", "ACTUAL", "THEORETICAL", AND "THEORETICALLY ADJUSTED" FLOW RATES 10/13/76-6/14/77

Dates	N_o[1]	N_n[1]	$\bar{N}$[1]	W_o[2] (g)	W_n[2] (g)	W_o[3] (g)	W_n[3] (g)	$\bar{W}$[3] (g)
10/13-10/25	9156	9066	9111	.720	1339.33	.079	.148	.114
10/26-11/30	9041	8951	8996	1335.64	3436.03	.148	.384	.266
11/30-12/28	8926	8876	8901	3426.43	7234.16	.384	.815	.600
12/28-01/25	8851	8628	8740	7213.78	10704.59	.815	1.241	1.028
01/25-2/22	8603	8157	8380	10673.57	15660.0	1.241	1.920	1.581
02/22-3/22	8132	7686	7909	15612.0	19720.0	1.920	2.566	2.210
03/22-4/19	7661	7215	7438	19655.9	22760.0	2.566	3.154	2.860
04/19-5/17	7190	6744	6967	22681.14	26210.0	3.154	3.886	3.572
05/17-6/14	6719	6563	6641	26112.8	29430.0	3.886	4.484	4.185

[1] N = live animals only. N was computed by taking an actual count performed on 5/17/77 (at end of period 4/19-5/17). N_o = number at end of period plus a mean mortality figure per day X number of days in each weighing period. For N_n of earlier period, another 25 animals was added since 25 were removed at end of each period for an allometric study. Mortality figures were taken from actual counts over periods which did not exactly correspond to weighing periods above. See Table 25 for mortality data. $\bar{N}$ = mean number $((N_n+N_o)=2)$. For periods before 1/11/77 (first date on which actual mortality was measured), a death rate of 1% per period was assumed.

[2] W_o = whole wet weight of population on day 0 of the weighing period. Slight differences exist between W_n of previous period and W_o as latter accounts for 25 animals removed for allometric study. W_n = actual whole wet weight measured at end of period.

[3] W_o = (W_n) (from previous weight period) X (N_o) for that period. W_n = $W_n \div N_n$. $\bar{W}$ = $(W_o+W_n) \div 2$. Mean individual weights for each period may not precisely correspond when computed on the basis of the allometric study.

and end (N_n, W_n, w_n) of each weighing period are shown, as are mean values ($\bar{N}$ and $\bar{W}$).

For PP1, these values were used to calculate a new expected population weight; the only difference between these values and the expected weights as shown in Table 21 are in the number of animals assumed to exist in the population. Table 22 illustrates the original and revised population weight for PP1 for Days 14-238 (assuming no culling for Days 154, 182, 210 and 238). The data indicate that revised expected values are about 80% of original expected values. This indicates the need for (1) more precise control over numbers at time of introduction, and (2) the elimination or decrease of mortality in the pilot plant, or the inclusion of a larger expected mortality rate into the shellfish technical description.

Since the actual number of animals introduced into the pilot plant was about 9% less than expected, since significant mortalities occurred in each population, and since the individual weight (and therefore area) of the surviving animals within those populations did not grow as fast as had been predicted, it is clear that the operation of flow rates at the intended values represents considerable "over-feeding", in comparison to flow rate based on the actual numbers and individual whole wet weight in the population.

This difference between intended operating value and a theoretically optimum value based on the feeding criterion ($F = .0185\ N(w)^{\frac{2}{3}}$ = ml/sec) is shown (for PP1 only) in Table 23. Also shown are corrected flow rate values taking into consideration not only the actual population characteristics but the actual

TABLE 23. "INTENDED", "ACTUAL", THEORETICAL" AND "THEORETICALLY ADJUSTED" MEAN FLOW RATES FOR PP1 10/13/76-6/14/77

Dates	All values in ml/sec. I	A	F	FA	% Change I-A	% Change I-F	% Change I-FA	% Change A-FA
10/13-10/26	30	30	40.21	39.76	0	34.03	32.53	32.53
10/26-11/30	60	60	69.45	73.58	0	15.75	22.63	22.63
11/30-12/28	180	180	117.54	100.65	0	-34.70	-44.08	-44.08
12/28-01/25	300	300	164.66	136.89	0	-45.11	-59.37	-54.31
01/25-02/22	440	390.4	209.75	163.68	-11.27	-52.33	-62.80	-58.07
02/22-03/22	440	346.8	246.94	220.73	-21.18	-43.88	-49.83	-36.53
03/22-04/19	440	344.0	275.31	235.83	-21.82	-37.43	-46.40	-31.44
04/19-05/17	440	340.5	295.87	247.70	-22.61	-32.76	-43.70	-27.25
05/17-06/14	440	369.2	316.03	320.98	-16.09	-28.18	-27.05	-13.06

1) I = Intended = Set flow rate, based upon the equation

$$F = .0185\ N\ (w)^{2/3} = \text{ml/sec, where}$$

N and w = expected (median) number and individual whole wet weight, respectively.

2) A = Actual = mean flow rate as shown in Table 20; accounts for interruptions of flow to population due to pool down-time and shellfish weighing time requirements.

3) F = Theoretical = $.0185\ N\ (w)^{2/3}$ = ml/sec, based upon computed N and w as shown in Table 20.

4) FA = Theoretically adjusted. F assumes a concentration in inflow of 21.39 µg-at L^{-1}. FA adjusts flow to deliver the same amount of algal protein-nitrogen per unit time, taking into account the mean algal protein-n constant as measured over the various weight periods. FA = (F)(21.39)÷(x conc.). x conc. is from Table 20.

constant weight study, the 35 g, 2 ml/sec group grew significantly faster than did the 35 g, 1 ml/sec group (72.2 g whole wet weight increase over the 36-day study period as opposed to 62.4g). Thus, since the animals were fed at a greater food flow rate than is indicated by the requirements of the feeding criterion, and since smaller animals grow at a faster rate (see technical description), we would expect the animals to "catch up" to the expected weight gains. In fact, they continued to fall behind throughout their residence in the pilot plant.

These values imply that flow rates adjusted to actual, as opposed to expected, numbers (N) and individual weights (w), would have resulted in less "wasted" food. However, this conclusion assumes that the animals would have removed a much higher proportion of incoming food at the lower flow rates. This is compatible with the results obtained and with known relationships between food inflow concentration and shellfish stripping, but it is possible that difficulties in "presentation" level dynamics (such as tank configuration) may have introduced constants into feeding activity. That is, it is possible, although unlikely, that only a fraction of the food would have been consumed, even at lower flow rates, and that the conversion of total algal protein-nitrogen into clam meat protein-nitrogen could have been even less.

4.5 Spawning Record

"Spontaneous" (natural) spawnings were observed in the pilot plant beginning 1/10/77.

Table 24 shows the spawning statistics for individual pilot plant tanks from 1/20/77 to 7/15/77. The total number of spawnings

TABLE 24. SUMMARY SPAWNING RECORD

20 JANUARY TO 15 JULY 1977*

PILOT PLANT TANKS

	H20				H21				H22			
	1	2	3	4	5	6	7	8	9	10	11	12
Number of spawnings	10	10	9	5	3	7	8	6	10	11	8	7
Total of population				34				24				36
Total												94

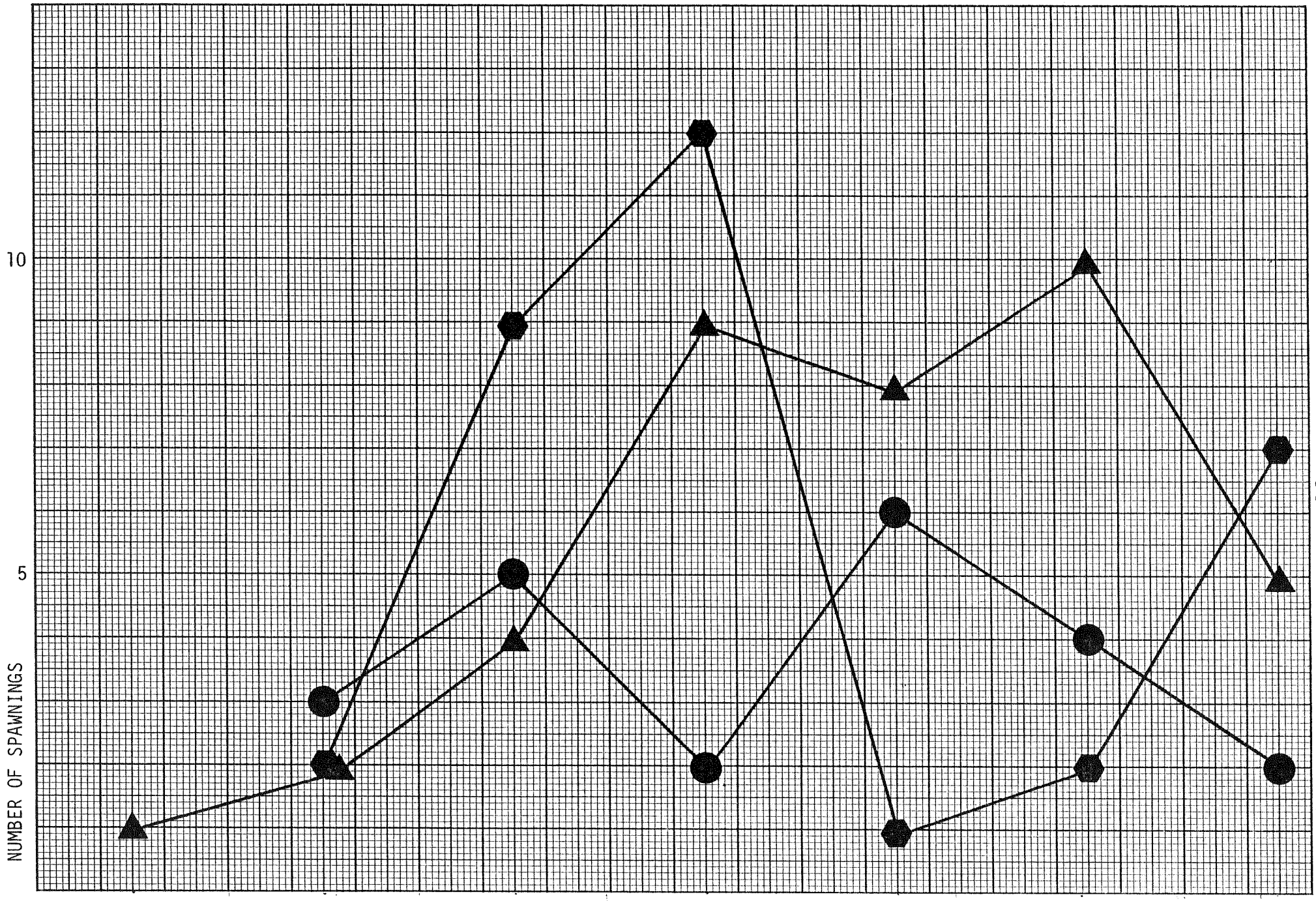
*Note: First tank spawning observed on 20 January 1977. Table represents summary of daily observations. Data on spawnings for each tank over each weighing period are available.

Figure 9. Pilot plant shellfish spawnings:

flow changes

manipulations

undetermined



changed to a biweekly cleaning schedule with no changes in water flow. Eleven spawnings occurred in the tanks cleaned weekly, compared to six spawnings in those cleaned biweekly. From 4/12/77 to 7/19/77, half of the pilot plant tanks were converted to a once-through flow; half of which were cleaned weekly and half biweekly. A total of 17 spawnings occurred in the once-through/ weekly cleaned tanks, 13 in the recirculation/weekly cleaned tanks, 17 in the once-through/biweekly cleaned tanks, and 17 in the recirculation/biweekly cleaned tanks. Thus, these differential treatments had little effect on spawning rates.

The high amount of spawning activity is indicative of significant energy loss through gonadal production. Further, the gonadal material, which often settled in areas of poor water movement in the tanks, degraded rapidly and provided a good medium (in combination with accumulated fecal and pseudo fecal material) for bacterial growth.

An estimate of protein-nitrogen lost to the system through spawning activity (to June 30, 1977) was made and is included in the nitrogen budget section, below.

4.6 Mortality Record

Table 25 illustrates mortality data collected on all three populations. As shown, mortality was quite high, with a total number dead of 2,714; 2,323 and 2,218 animals from populations H20, H21 and H22, respectively. Assuming that each population consisted of 10,000 animals initially. This represents a mean mortality per population of about 24%. This data covers the period 1/11/77 (the first date mortalities were evident and recorded) and

TABLE 25. MORTALITY DATA, 11 JANUARY TO 28 JUNE 1977

Population #	H20				H21				H22			
Tank #	1	2	3	4	5	6	7	8	9	10	11	12
Date												
1/11/77	-	-	-	-	-	-	-	-	-	-	-	-
1/18/77	-	-	-	-	-	-	-	-	-	-	-	-
1/25/77	38	-	-	-	15	-	-		57	-	-	-
2/ 1/77	46	14	40	22	14	-	-		50	-	-	-
2/ 8/77	30	14	21	14	26	-	-		35	-	-	-
2/15/77	9	13	18	20	23	-	-		63	-	-	-
2/22/77	17	28	114*	19	3	4	6	4	39	-	-	-
2/24/77	9	13	9	6	-	-	-	-	-	-	-	-
3/ 1/77	7	6	23	18	6	12	1	5	4	2	12	3
3/ 8/77	31	28	26	35	12	10	-	-	16	8	9	18
3/15/77	17	25	-	-	132	14	27	11	25	16	-	-
3/22/77	36	33	67	40	18	35	-	-	28	25	37	41
3/29/77	37	34	-	-	231	25	38	23	48	33	-	-
3/31/77	-	-	-	-	430**	-	-	-	-	-	-	-
4/ 5/77	52	31	89	70	295	22	-	-	53	25	108	123
4/12/77	50	29	-	-	9	9	70	26	47	19	-	-
4/19/77	53	30	61	78	5	29	-	-	60	23	69	82
4/26/77	52	50	-	-	6	22	96	41	63	18	-	-
5/ 3/77	16	34	40	55	5	18	-	-	20	19	75	51
5/10/77	22	20	-	-	1	28	53	53	30	13	-	-
5/17/77	41	26	58	88	5	20	-	-	41	22	68	78
5/24/77	27	25	-	-	4	23	39	32	21	20	-	-
5/31/77	21	38	55	63	3	21	-	-	15	16	38	49
6/ 7/77	16	32	-	-	5	22	49	21	18	11	-	-
6/14/77	30	30	53	43	9	21	-	-	18	20	57	43
6/21/77	42	14	-	-	8	15	43	38	32	29	-	69
6/28/77	33	27	60	57	6	26	-	-	39	14	38	-
Σ Tank	758	594	734	628	1271	376	422	254	822	328	511	557
Σ Population		2714				2323				2218		

NOTES: - = no observation made *tray accidentally dropped **antibiotic treatment (Streptomycin sulfate)

6/28/77, when the animals were about 4.7 grams (individual whole wet weight) each.

After Day 140, mortality in all populations increased markedly. The primary peak in mortality occurred on Day 168 for batch #5, Day 182 for batch #9, and Day 196 for batch #1. Mean clam lengths for batches #1, 5, and 9 during these periods were 25mm, 24mm, and 22mm, respectively. The production of fecal material and pseudofeces was extremely heavy during this period. Organic material settled out on the Nestier tray edges, underneath the tray, in the re-circulation system plumbing, and clogged the tray liners, which retained the clams within the tray. In general, an unfavorable environment for clam growth existed, reducing water flow through the tanks, creating "dead" areas of poor circulation due to the entrapment of air underneath the trays and liners, and providing suitable quantities of organic material which accumulate and decompose. Pink Pseudomonas bacteria often heavily covered the bottom and sides of the tanks.

Upon removal of individual tray liners on Days 182 and 196, mortality levels decreased; however, individual tank mortality rates are still very high. It is evident that good water circulation and the removal of fecal material are essential to maintain good shellfish growth and reduce mortality.

On the other hand, it is not known to what extent the mortality experienced in the pilot plant is abnormal . In the Nov-Dec 1975 'constant weight' study from which the technical description was derived, no mortalities occurred, but the 35g-1ml group was only 1.29 in weight at the end of the 36-day study. Laurence and Roels

(1976), who used animals from the same population as that used in the constant-weight study and measured growth over a 100-day period, found a great increase in mortality past the 25mm (∿ 3.5 g) size: 17% of the animals past this size died over a 34-day period, and tank conditions were described as optimal. The high mortalities measured in the 76-77 plant were probably the result of interaction between normal die-off of older (~20mm) animals and an "unfavorable" environment.

4.7 Allometric Data

As described in section 3.2.2.2 above, 25 animals from each population were sampled at the end of each weighing period, and measured for whole wet weight, length, width and depth, whole (wet) meat weight, shell weight, dry meat weight, and for particulate protein-nitrogen content. Mean allometric values for the 25 animals subsampled between 10/26/77 - 6/14/77 for population H20 are illustrated in Table 26. (Results from all three popularions were very similar; data on individual animals and for populations H21 and H22 are available).

The allometric data was also used to compute mean percent wet meat of whole wet weight, mean percent dry meat of wet meat weight, mean percent protein and protein-nitrogen content of dry meat for each population over each weighing period. These values are illustrated in Table 27. These percentages were then used, in combination with recorded whole wet weight gains, to calculate the total wet meat, dry meat, meat protein and meat protein-nitrogen produced in the pilot plant.

TABLE 26. REPRESENTATIVE ALLOMETRIC DATA, PILOT PLANT POPULATION #1 (H20)
26 OCTOBER 1976 - 14 JUNE 1977 (SPAWNED ON 3 AUGUST 1977)

Date	Day (Past Spawning)	Length (mm)	Width (mm)	Depth (mm)	Whole Wet Weight (g)	Wet Meat Weight (g)	Dry Meat (g)
10/26/76	84	9.7	6.4	3.4	0.132	0.056	0.009
11/30/76	119	13.4	8.8	5.0	0.421	0.179	0.032
12/68/76	147	17.5	11.8	7.0	1.04	0.48	0.10
01/25/77	175	19.2	12.9	8.0	1.38	0.60	0.14
02/22/77	203	22.7	15.2	9.9	2.17	1.01	0.21
03/22/77	231	23.2	15.5	10.1	2.39	1.10	0.22
04/19/77	259	24.6	16.5	11.3	3.14	1.44	0.28
05/17/77	287	26.2	18.0	12.3	3.75	1.69	0.35
06/14/77	315	27.8	18.3	13.3	4.70	2.19	0.44

Note: Values represent means for 25 animals selected at the end of each weighing period. Data on individual animals for all three populations are available.

TABLE 27. WHOLE WET WEIGHT GAIN, MEAN PERCENT WET MEAT OF WHOLE WET WEIGHT, MEAN PERCENT DRY MEAT OF WET MEAT, MEAN PERCENT PROTEIN OF DRY MEAT, AND EXTRAPOLATED WET MEAT, DRY MEAT, PROTEIN AND PROTEIN-NITROGEN WEIGHT GAIN FOR PILOT PLANT POPULATIONS #1, 5 AND 9 (H20, H21 AND H22), 10/13/76 - 6/14/77. WEIGHT VALUES IN GRAMS.

Dates	Population Whole Wet Wt Gain(s)	Mean % Wet of Whole	Mean % Dry of Wet	% Protein of Dry	(Extrapolated) Total Wt Meat Gained	(Extrapolated) Total Dry Meat Gained	(Extrapolated) Total Protein-Gained	(Extrapolated) Protein-N Gained
Population # 1								
10/13/76-10/26/76	619.33	42.02	15.62	45.60	260.24	40.65	18.54	2.835
10/26/76-11/30/76	2096.70	42.42	17.19	50.90	889.42	152.89	77.82	11.899
11/30/76-12/28/76	3912.30	46.15	20.83	49.20	1805.53	376.09	185.04	28.294
12/28/76- 1/25/77	3356.26	43.48	22.84	46.40	1459.30	333.30	154.65	23.647
1/25/77- 2/22/77	4955.41	46.38	21.23	43.10	2298.32	487.93	210.30	32.156
2/22/77- 3/22/77	4060.00	45.34	19.92	41.10	1840.80	366.69	150.71	23.044
3/22/77- 4/19/77	3040.00	45.82	19.16	41.70	1392.93	266.89	111.29	17.017
4/19/77- 5/17/77	3450.00	45.19	20.47	42.30	1559.06	319.14	135.00	20.642
5/17/77- 6/14/77	3220.00	46.67	19.90	40.40	1502.77	299.05	120.82	18.474
Population # 5								
10/15/76-10/26/76	271.90	43.48	19.20	49.00	118.22	22.70	11.12	01.700
Pop. culled back to 10,000								
10/26/76-11/16/76	1005.90	42.97	16.73	51.30	432.24	72.31	37.10	05.673
11/16/76-12/21/76	2138.17	45.62	19.77	48.50	975.43	192.84	93.53	14.301
12/21/76- 1/18/77	4898.96	43.36	22.85	44.50	2124.19	485.38	215.99	33.026
1/18/77- 2/15/77	4894.35	43.84	22.53	43.90	2145.68	483.42	212.22	32.450
2/15/77- 3/15/77	6380.00	37.59	24.27	39.60	2398.24	582.05	230.49	35.243
3/15/77- 4/12/77	1450.00	39.41	24.01	39.20	571.45	137.20	53.78	8.223
4/12/77- 5/10/77	3730.00	41.32	22.03	37.00	1541.20	339.53	125.63	19.209
5/10/77- 6/ 7/77	3620.00	44.01	20.70	39.60	1593.16	329.78	130.59	19.968
Population # 9								
10/19/76-11/ 2/76	734.20	43.34	15.23	48.40	318.20	48.46	23.46	3.587
11/ 2/76-11/23/76	1165.15	42.82	13.78	51.80	498.92	68.75	35.61	5.445
11/23/76-12/28/76	2546.72	43.87	21.07	46.90	1117.25	235.40	110.40	16.880
12/28/76- 1/25/77	4768.37	42.91	22.06	46.40	2046.11	451.37	209.44	32.024
1/25/77- 2/22/77	4075.56	41.13	21.66	44.20	1676.28	363.08	160.48	24.538
2/22/77- 3/22/77	6040.00	41.27	23.10	39.50	2492.71	575.82	227.45	34.778
3/22/77- 4/19/77	3890.00	41.54	21.67	38.70	1615.91	350.17	135.51	20.720
4/19/77- 5/17/77	3590.00	44.03	22.18	35.50	1580.68	350.59	124.46	19.031
5/17/77- 6/14/77	4510.00	39.88	20.78	37.60	1798.59	373.75	140.53	21.488

4.8 'Nitrogen' Budget and Conversion Efficiencies

Table 28 is an example of worksheets used for the construction of a 'nitrogen' budget for the second trophic level. As shown, these sheets represent values for incoming and effluent 'nitrogen' content, in the forms of NH_4, NO_3+NO_2, and algal particulate protein-nitrogen (cell concentration and turbidity values, also shown, have not been subjected to analysis.) The values illustrated in Table 28 represent the computed mean of replicate samples taken on Monday, Wednesday and Friday of each week at 1400 hr. Effluent data was collected (on file at St. Croix and Port Aransas labs) on individual pilot plant shellfish tanks, but only population means are used in the following.

For each population, over each weighing period, the mean NH_4, NO_3+NO_2, and algal protein-nitrogen content in the inflow and outflow was calculated, as were the mean "removed" values (inflow less outflow). Total 'nitrogen' accounted for in the inflow and outflow was also computed.

The results are shown in Tables 29, 30 and 31 for populations PP1, PP5 and PP9, respectively. Percent "stripping" of protein-nitrogen (mean inflow less mean outflow ÷ mean inflow) for each weighing period if also shown. It should be noted that no measurements on tank deposit, epiphytes, etc. were taken, and that the "removed" values here are thus not strictly comparable to those obtained in the 1975 constant-weight study, where effluent and tank deposit values were combined to provide a "stripped" protein ratio. Further, failure to account for tank deposit is probably one of the major reasons why the total incoming 'nitrogen' is not accounted for in terms of effluent protein-nitrogen and

TABLE 29. 'NITROGEN' BUDGET, POPULATION #1, H20, 10/13/76 - 6/19/77

DATES	IN (INFLUENT) NH_4	NO_3+NO_2	ALGAL PROTEIN-N	TOTAL NITROGEN	OUT (EFFLUENT) NH	NO_3+NO_2	ALGAL PROTEIN-N	TOTAL NITROGEN
10/13-10/26	0.63	00.01	21.63	22.27	1.09	01.20	04.22	06.51
10/26-11/30	1.31	10.39	20.19	31.89	5.39	05.48	08.55	19.42
11/30-12/28	1.46	01.94	24.98	28.38	2.56	00.23	13.36	16.15
12/28-01/25	1.75	03.12	25.73	30.60	3.69	01.14	16.12	20.95
01/25-02/22	0.47	01.14	27.41	29.02	2.76	01.27	17.19	21.22
02/22-03/22	1.19	07.28	23.93	32.40	3.95	01.12	13.26	18.33
03/22-04/19	1.28	03.88	24.97	30.13	4.14	03.00	13.51	17.65
04/19-05/17	1.73	02.98	25.55	30.26	3.37	01.45	13.18	18.00
05/17-06/14	2.10	04.47	21.06	27.63	4.41	01.65	11.47	17.53
N = 9	9	9	9	9	9	9	9	9
$\bar{x}$ =	01.32	03.91	23.94	29.18	03.48	01.84	12.32	17.31
S =	00.52	03.20	02.44	03.01	01.24	01.55	03.92	04.31

DATES	I N - O U T NH_4	NO_3+NO_2	PROTEIN-N	% STRIPPING PROTEIN-N
10/13-10/26	-0.46	-1.19	17.41	80.49
10/26-11/30	-4.08	4.91	11.64	57.65
11/30-12/28	-1.10	1.71	11.62	46.52
12/28-01/25	-1.94	1.98	09.61	37.35
01/25-02/22	-2.29	-0.13	10.22	37.29
02/22-03/12	-2.76	6.16	10.67	44.59
03/22-04/19	-2.86	0.88	11.46	45.90
04/19-05/17	-1.64	1.53	12.37	48.42
05/17-06/14	-2.31	2.82	09.59	45.54
N =	9	9	9	9
$\bar{x}$ =	-2.16	2.07	11.62	49.31
S =	-1.06	2.31	02.38	13.16

TABLE 30. 'NITROGEN' BUDGET, POPULATION #5, H21, 10/15/76 - 6/7/77

DATES	IN				OUT			
	NH_4	NO_3+NO_2	ALGAL PROTEIN-N	TOTAL NITROGEN	NH_4	NO_3+NO_2	ALGAL PROTEIN-N	TOTAL NITROGEN
10/15-10/26	0.63	0.01	20.53	21.17	0.55	nd	16.09	16.64
10/26-11/16	-	-	22.87	nd	-	-	8.84	nd
11/16-12/21	2.75	4.58	20.76	28.09	2.53	1.88	14.39	18.80
12/21-01/18	1.87	2.82	24.93	29.62	2.25	0.68	17.17	20.10
01/18-02/15	0.29	0.63	28.92	29.84	4.50	0.70	17.86	23.06
02/15-03/15	1.23	6.05	23.22	30.50	3.44	0.95	13.82	18.21
03/15-04/12	1.06	5.23	23.81	30.10	4.45	2.49	11.52	18.46
04/12-05/10	1.44	4.02	25.56	31.02	4.02	1.81	12.70	18.53
05/10-06/09	2.48	2.64	23.35	28.47	4.73	1.25	12.07	18.05
N =	8	8	9	8	8	7	9	8
$\bar{x}$ =	01.47	03.25	23.77	28.60	03.31	01.39	13.83	18.98
S =	00.86	02.14	02.55	03.16	01.44	00.69	02.90	01.90

(nd=not done)

DATES	IN - OUT			
	NH_4	NO_3+NO_2	PROTEIN-N	% STRIPPING PROTEIN-N
10/15-10/26	0.08	-	04.53	21.40
10/26-11/16	-	-	14.03	61.35
11/26-12/21	0.22	2.70	06.37	30.68
12/21-01/18	-0.38	2.14	07.76	31.13
01/18-02/15	-4.21	-0.07	11.06	38.24
02/15-03/15	-2.21	5.10	09.40	40.48
03/15-04/12	-3.39	2.74	12.29	51.62
04/12-05/10	-2.58	2.21	12.86	50.31
05/10-06/07	-2.25	1.39	11.28	48.31
N =	7	7	9	9
$\bar{x}$ =	-2.11	2.32	09.95	41.50
S =	1.57	1.56	3.18	12.55

TABLE 31. 'NITROGEN' BUDGET, POPULATION #9, H22, 10/19/76 - 6/14/77

DATES	IN				OUT			
	NH_4	NO_3+NO_2	ALGAL PROTEIN-N	TOTAL NITROGEN	NH_4	NO_3+NO_2	ALGAL PROTEIN-N	TOTAL NITROGEN
10/19-11/02	-	-	24.04	-	0.90	nd	11.07	-
11/02-11/23	2.37	2.47	22.06	26.90	7.85	1.27	4.41	13.53
11/23-12/28	1.49	4.50	21.97	27.96	2.77	1.61	12.92	17.30
12/28-01/25	1.75	2.68	25.71	30.14	4.16	1.11	11.84	17.11
01/25-02/22	0.47	1.14	27.41	29.02	5.11	0.37	14.05	19.53
02/22-03/22	1.19	7.28	23.93	32.40	3.61	0.97	11.11	15.69
03/22-04/19	1.28	3.33	24.97	29.58	4.04	3.06	12.77	19.87
04/19-05/17	1.73	2.98	25.55	30.26	4.12	1.42	11.76	17.30
05/17-06/14	2.10	4.47	21.06	27.63	4.60	1.72	10.43	16.75
N =	8	8	9	8	9	8	9	8
$\bar{x}$ =	01.55	03.61	24.08	29.19	04.13	01.44	11.15	17.14
S =	00.59	01.84	02.07	01.67	01.86	00.78	02.76	02.02

(nd=not done)

DATES	IN - OUT			
	NH_4	NO_3+NO_2	PROTEIN-N	% STRIPPING PROTEIN-N
10/19-11/02	-	-	12.97	53.95
11/02-11/23	-5.48	1.20	17.65	80.01
11/23-12/28	-1.28	2.89	09.05	41.19
11/28-01/25	-2.41	1.57	13.87	53.95
01/25-02/22	-4.64	0.77	13.36	48.74
02/22-03/22	-2.42	6.31	12.82	53.57
03/22-04/19	-2.76	0.27	12.20	48.86
04/19-05/17	-2.39	1.56	13.79	53.97
05/17-06/14	-2.50	2.72	10.63	50.47
N =	7	8	9	9
$\bar{x}$ =	-2.88	2.16	12.93	53.86
S =	1.32	1.90	2.37	10.65

dissolved inorganic nitrogen forms and meat-protein nitrogen "gained."

The values shown in Tables 29, 30, 31 were used in combination with the calculated meat-protein nitrogen "gained" values (see Tables 27, 28) as the basis for the 'nitrogen' budget. However, since a total of 6,670 animals died over the period 1/11/77 to 6/14/77 in the three populations (see Table 25), an estimate of the loss which occurred as a result of death was also made and included in the budget. Similarly, the large number (72) of spawnings observed during this same period indicated that a significant portion of 'nitrogen' may have been lost through this pathway, and gross estimates of this loss were also made.

Methods and values obtained for 'nitrogen' lost through mortality for population H20 (PP1) are illustrated in Table 32. The same methods were used for H21 (PP5) and H22 (PP9). For each population, actual mortality figures for the period 1/11/77 to 6/14/77 (for PP1 and PP9) and 1/11/77 to 6/7/77 (for PP5) were noted. These numbers are derived from actual counts taken of dead animals in each population; mortality figures were not always recorded on the same days as total weight gains were taken. Data collected via the allometric study for mean individual whole wet weight, wet and dry meat, protein, and protein-nitrogen content for each weighing period beginning 1/18/77 (PP5) and 1/25/77 (PP1 and PP9) were then averaged and used as an estimate for total whole wet weight, wet and dry meat, protein and protein-nitrogen lost over this period. Mean protein-N lost per day from 1/11/77 onwards was also calculated. Estimated mortalities which occurred prior to 1/11/77 were not included in the analysis since mortality

TABLE 32. ESTIMATED MEAT PROTEIN-NITROGEN LOST FROM SECOND TROPHIC LEVEL THROUGH MORTALITY PILOT PLANT POPULATION #1 (H20) JANUARY 11, 1977 — JUNE 14, 1977

DATES[a]	MEAN INDIV. WET WT[b] (g)	MEAN % WET MEAT OF WHOLE	MEAN % DRY MEAT OF WET	MEAN % PROTEIN OF DRY	MEAN % PROTEIN-N OF PROTEIN (BSA STANDARD)
1/25-2/22	2.17	46.38	21.23	43.1	15.3
2/22-3/22	2.40	45.34	19.92	41.1	15.3
3/22-4/19	3.14	45.82	19.16	41.7	15.3
4/19-5/17	3.75	45.19	20.47	42.3	15.3
5/17-6/14	4.70	46.67	19.90	40.4	15.3
$\bar{x}$ =	3.23	45.88	20.14	41.72	15.3
S =	1.03	0.64	0.77	1.04	-

Total Mortality, 1/1/77-6/14/77 = 2,481

Estimated Total (Whole Wet) Weight Lost
= (2,481)(3.23)
= 8,013.63 grams

Estimated Wet Meat Lost
= (8,013.63)(.4588)
= 3,660.63 grams

Estimated Dry Meat Lost
= (3,676.65)(.2014)
= 740.48 grams

Estimated Meat Protein Lost
= (740.48)(.4172)
308.93 grams

Estimated Meat Protein-Nitrogen Lost
= (308.93)(.153)
= 47.27
= 47 grams

Notes: [a] Dates used are those over which allometric data were collected.
[b] Values are for data collected at end of respective weighing periods.

rates were very low prior to this date and the animals small. The estimated protein-nitrogen loss through mortality was calculated at 47, 40, and 27 grams for populations H20, 21 and 27, respectively, for a total estimated loss of about 114 grams.

For estimates of particulate protein-nitrogen lost through spawning, a much less precise method was used (not illustrated). Here, the great barrier to precision was the lack of quantitative data on the size of the spawning. Spawning records only indicated whether or not spawning occurred and if it was "light", "medium" or "heavy". On 3/16/77 a spawning in a pilot plant tank, characterized as "heavy", was completely collected, and the total number of gametes estimated at 4.4×10^7 (this was accomplished using standard light microscopy and established larval counting procedures employing a counting chamber).

For the following estimates, it was assumed that the average spawning resulted in the loss of 1.0×10^7 gametes. Protein-nitrogen content of 3.0×10^6 eggs from the spawning on 3/16/77 was performed in replicate, and indicated that 1.0×10^6 eggs = 2407 ± 240 μg-at of particulate protein-nitrogen. Since 72 spawnings occurred over the period 10/13/76 through 6/19/77, the estimated protein-nitrogen lost through this pathway is:

$$(2407 \pm 240)\ (72)\ (10)\ (14)\ 10^{-6}) = 24.26 \pm 2.4 \text{ g}$$

Thus, total estimated protein-nitrogen lost to the second trophic level through mortality and spawning over the period 1/11/77 - 6/14/77 was about 138 grams. This represents a significant fraction, since total meat protein-nitrogen gained over this period was about 526 grams (Table 36).

Before the nitrogen budget is discussed in more detail, conversion efficiency data is presented. Tables 33, 34 and 35 illustrate conversion efficiencies based on total "available" (total presented) and "removed" (total presented less total measured in the effluent) phytoplankton protein-nitrogen. Concentration values are from Tables 29, 30 and 31 and total liters through each population are from the "actual" flow rates as illustrated in Table 20 and discussed above.

The protein-nitrogen gains for each weighing period are based on values illustrated in Table 27.

It will be noticed that except for apparently minor exceptions, the data for inflow and outflow protein-nitrogen values, and for efficiencies of conversion, appear highly consistent across the three populations, and display a strong and consistent trend. These data, however, have as yet to be subjected to any statistical analyses. Some anomalous data appear in conversion efficiencies for "removed" protein-nitrogen to Tapes meat 'nitrogen' "gained." These values, which are all high, are for PP5, 10/15 to 10/26 and 11/16 to 12/21, and 12/21 to 1/18; and for PP9 over the period 11/23 to 12/28, 1976. These abnormally high conversions are attributable to low values obtained for "removed" protein-nitrogen which is, in turn, attributable to high effluent values. Probably, tank deposit particulate protein, spawning, mortalities, or a combination of these factors contributed to incorrectly high effluent values. Possible measurement and/or computational errors, however, have not as yet been investigated and may be partially responsible. These values do not affect "available" conversions

TABLE 34. CONVERSION EFFICIENCIES, PILOT PLANT POPULATION #5 (H21), 10/15/76 - 6/7/77

DATES	PROTEIN-N IN (µg-at L^{-1})	PROTEIN-N REMOVED (µg-at L^{-1})	TOTAL LITERS IN	TOTAL PROTEIN-N[a] IN (g)	TOTAL PROTEIN-N REMOVED (g)	TOTAL PROTEIN-N GAINED (g)	% C "A"	% C "R"
10/15/76-10/26/76	20.53	04.53	28,458	08.18	01.80	01.700	20.78	94.44[b]
10/26/76-11/16/76	22.87	14.03	54,378	17.41	10.68	05.673	32.58	53.12
11/16/76-12/21/76	20.76	06.37	144,990	42.14	12.93	14.301	33.94	110.60[b]
12/21/76-01/18/77	24.93	07.77	435,132	151.87	47.33	33.026	21.75	69.78[b]
01/18/77-02/15/77	28.92	11.06	630,198	255.15	97.58	32.450	12.72	33.25
02/15/77-03/15/77	23.22	09.40	783,354	254.65	103.09	35.243	13.84	34.19
03/15/77-04/12/77	30.10	12.29	818,400	344.87	140.81	08.223	02.38	05.84
04/12/77-05/10/77	31.02	12.86	819,621	355.95	147.56	19.209	05.40	13.02
05/10/77-06/07/77	28.47	11.28	891,000	355.13	140.71	19.968	05.62	14.19

[a] $(\bar{x}$ protein-N)(total liters)(14)(10^{-6})=(g)

[b] see text

% C "A" = Conversion of total presented (available) algal protein-nitrogen to Tapes meat protein-nitrogen

% C "R" = Conversion of removed algal protein-nitrogen to Tapes meat protein-nitrogen

TABLE 35. CONVERSION EFFICIENCIES, PILOT PLANT POPULATION #9 (H22), 10/19/76 - 6/14/77

DATES	PROTEIN-N IN (μg-at L^{-1})	PROTEIN-N REMOVED (μg-at L^{-1})	TOTAL LITERS IN	TOTAL PROTEIN-N[a] IN (g)	TOTAL PROTEIN-N REMOVED (g)	TOTAL PROTEIN-N GAINED (g)	% C "A"	% C "R"
10/19/76-11/02/76	24.04	12.97	36,234	12.19	06.58	03.587	29.43	54.51
11/02/76-11/23/76	22.06	17.65	54,378	16.79	13.44	05.445	32.43	40.51
11/23/76-12/28/76	21.97	09.05	181,332	55.77	22.97	16.880	30.27	73.49[b]
12/28/76-01/25/77	25.71	13.87	435,132	156.62	84.49	32.024	20.45	37.90
01/25/77-02/22/77	27.41	13.36	643,869	247.08	120.43	24.538	09.93	20.38
02/22/77-03/22/77	23.93	12.82	839,025	281.09	150.59	34.778	12.37	23.09
03/22/77-04/19/77	24.97	12.20	832,236	290.93	142.15	20.720	07.12	14.58
04/19/77-05/17/77	25.55	13.79	823,779	294.67	159.04	19.031	06.46	11.97
05/17/77-06/14/77	21.06	10.63	893,178	263.34	132.92	21.488	08.16	16.17

[a] $(\bar{x}$ protein-N)(total liters)(14)(10^{-6})=(g)

[b] see text

% C "A" = Conversion of total presented (available) algal protein-nitrogen to Tapes meat protein-nitrogen

% C "R" = Conversion of removed algal protein-nitrogen to Tapes meat protein-nitrogen

TABLE 36. SUMMARY 'NITROGEN' BUDGET AND CONVERSION DATA (AS WEIGHT), PILOT PLANT POPULATIONS #1 (H20), #5 (H21) AND #9 (H22), 10/13/76 - 6/14/77

POPULATION NUMBER	DATES	TOTAL LITERS	NH_4 (g)	NO_3+NO_2 (g)	TOTAL INORGANIC 'N' (g)	ALGAL PROTEIN 'N' (g)	TOTAL 'N' (g)	TOTAL DEEPWATER 'NITROGEN' @ 32.16 µg liter^{-1}
	"IN"							
1	10/13/06/14	5.70790920×10^6	105.48	312.45	417.93	1913.06	2330.99	
5	10/15-06/07	4.61055310×10^6	94.89	209.78	304.67	1534.29	1838.96	
9	10/19-06/14	4.73916300×10^6	102.84	239.52	342.36	1597.67	1940.03	
		1.50576250×10^7	303.21	761.75	1064.96	5045.02	6109.98	6779.55
	"OUT" (EFFLUENT)							
1			278.08	147.04	425.12	984.50	1409.62	
5			213.65	89.72	303.37	892.70	1196.07	
9			274.02	95.54	369.56	739.78	1109.34	
			765.75	332.30	1098.05	2616.98	3715.03	
	"REMOVED" (IN-OUT)							
1			-172.60	165.41	- 7.19	928.56	921.37	
5			-118.76	120.06	1.30	641.59	642.89	
9			-171.18	143.98	-27.20	857.89	830.69	
			-462.54	429.45	-33.09	2428.04	2394.95	
	"GAINED" (MEAT PROTEIN-N)							
1			178.0					
5			169.8					
9			179.5					
			526.3					
	"LOST" (THROUGH MORTALITY; MEAT PROTEIN-N)				—ESTIMATED (SEE TEXT)			
1			47					
5			40					
9			27					
			114					
	"LOST" THROUGH SPAWNING							
1,5,9			24		—ESTIMATED (SEE TEXT)			

and, even if grossly incorrect, have a negligible impact on mean computed "removed" conversions and on the nitrogen budget as a whole, since total clam meat protein-N produced during these periods represents only 6% of the total meat protein-N produced over the entire period of the pilot plant operation.

These conversions, of course, account for live weight gains only. For the three populations as a whole, conversion of available algal protein-N to Tapes meat protein-N increases from about 7.3% to about 9.7% if we add protein-N "lost" through mortality to protein-N "gained." Similarly, "removed" efficiencies increase from 16.4 to 21.8%. Although, as mentioned, no statistical analyses have as yet been performed on data collected at the shellfish level, a careful inspection of the data reveals that the three populations behaved in a highly similar fashion.

A strong trend in percentage conversion over time is evident in all three populations, both in terms of "available" and "removed" efficiencies. "Available" conversion, for example, approximates 26% during the first weighing period to less than 7% over the last weighing period. Similarly, mean percent "removed" conversions drop from approximately 60% to approximately 15%. The large and consistent difference between "available" vs "removed" conversion ($CR \cong 2CA$) can be attributed to the low mean stripping rate (approximately 48%), and in consequence to total food available to the animals vs the amount they actually consumed. (Also see discussion on "intended," "actual," "theoretical" and "theoretically adjusted" flow rates.)

There was a consistent decrease in both types of conversion, however, it is not possible to conclude whether age, size or residence

time in the system is more strongly correlated with decreases in conversion. Mortality and spawning undoubtedly contributed to declines in conversion, but the precise degree to which they are responsible cannot be ascertained. Even accounting for possible losses due to mortality and spawning, the decrease is still evident.

Table 36 summarizes the collected 'nitrogen' data, expressed in terms of weight. These figures were obtained by multiplying mean concentration values of the various parameters by the total volume of water pumped through each; this latter figure is from Table 27 and represents an estimated "actual" flow. Summary figures for the three populations as a whole are also included. A number of points related to these data will be discussed.

First, it will be noted that approximately .3 kg of NH_4 was presented to the shellfish, while approximately .77 kg was measured in the effluent, for a net production of ammonia of approximately .46 kg. For a two-trophic-level system, this production is a loss of nitrogen, and must also be considered as a possible pollutant. If a third trophic level such as macrophytic algae were added to the system, it is clear that the amount of total nitrogen produced or recovered could be increased by a significant amount. (Note that total meat protein-nitrogen "gained" is only approximately .6 kg more than was lost through ammonia production.)

Values for dissolved inorganic NO_3+NO_2 indicate uptake at the shellfish level. Inspection strongly indicates that this removed

NO_3+NO_2 of approximately .4 kg will be significant. It is highly likely that this represents uptake of these inorganic nutrients by epiphytic algae (Enteromorpha sp.), phytoplankton residing in the shellfish tank (especially pennate diatoms clinging to the sides), and by other, unidentified organisms. Probably a cleaner system would result in less uptake of this type; at any rate, the contribution of the shellfish themselves to the possible production of NO_3+NO_2 is entirely masked by the activities of contaminating organisms.

It will be observed that of the total estimated deep water 'nitrogen' which flowed through the system (including NO_3+NO_2, NH_4, and algal protein-N, see Table 7, above) of approximately 6.8 kg, about 6.1 kg was accounted for in the shellfish inflow (approx. 90%), which is about three percentage points less than was accounted for in the 21 pool cultures discussed earlier (see pp. 56). About 5.0 kg of this 6.1 kg total was in the form of algal protein-nitrogen (approx. 82%). Over 2.6 kg of this algal protein-nitrogen was in the shellfish tank effluent and was not utilized as food for the shellfish (approx. 50% of incoming food). About 2.4 kg of algal protein-nitrogen was "removed" by the shellfish (including tank deposit, which was not measured), of which about .53 kg was recovered in the form of live shellfish meat protein-nitrogen. An estimated .11 kg was probably gained in meat and subsequently lost through mortality, and about .02 kg may have been lost through spawning activity by the shellfish.

Thus, of the total or approx. 6780 g of 'nitrogen' "available", in the deep water about 526 g, or about 7.8%, was recovered in the

form of live shellfish meat protein-nitrogen. This live meat protein-nitrogen represents about (526.3 ÷ 5045) x 100 $\tilde{=}$ 22% of removed algal protein-nitrogen. If we add the estimated loss of nitrogen through mortality to the live weight gain, this conversion becomes (526.3 + 111.86) ÷(2428) x 100 $\tilde{=}$ 26%, and if possible loss through spawning is added, the conversion is (526.3 ÷ 111.86 + 24.26) ÷ 2428 x 100 $\tilde{=}$ 27%. Thus, we may assume that a reasonable biological value for the diet is approximately 27, indicating that it is, at least potentially, an excellent diet. (It should be noted here that a strictly unialgal diet of STX-167 (Chaetoceros curvisetus) should not be assumed as the only source of food for the shellfish, since contaminants were periodically observed in the pools). Of course, a biological value of 27 assumes that mortality and spawning were not closely linked to diet; but even if they are, a biological value of 22 appears easily obtainable with this diet at pilot-level production.

5 DEVELOPMENTAL RESEARCH

5.1 Phytoplankton: Stability of Outdoor Pool Cultures

Pool collapses are typified by a decline in cell density much faster than the dilution rate, indicating that cessation of growth cannot be the only cause for the collapses. The diatom cells are being killed by some agent, or are being removed from the population (as by grazing), or both; cessation of growth of existing cells may also be occurring.

Possible causes have been classified into the following four categories:

(a) Toxicity of dissolved compounds in the pools.

(b) Interaction with other organisms.

(c) Changes in our laboratory stock cultures of Chaetoceros curvisetus, STX-167.

(d) Environmental factors.

A multi-pronged plan of work has been drawn up and is partly implemented. The work undertaken or planned is summarized as follows:

(a) Interaction between lab cultures and pool samples taken from a "collapsed" pool. This type of experiment is performed with filtered or unfiltered pool samples.

(b) Nutrient supplementation of pool samples, with or without filtration and/or reinoculation.

(c) Qualification and quantification of biological contaminants in pool cultures.

(d) Observation of cell size evolution.

(e) Use of other clones of Chaetoceros curvisetus.

(f) Evaluation of the correlation between environment and "collapse."

Two types of laboratory experiments were performed to see if the agent(s) causing pool collapse could be removed by filtration and whether reinoculation or nutrient supplements (and various combinations of filtration, reinoculation, and nutrient supplements) would result in growth of Chaetoceros. There were "long-term" experiments, where single observations after 5-10 days of incubation evaluated the effects of the culture manipulations, and "short-term" experiments, where observations were made at least daily during a 3-4 day period beginning the day the manipulations were made. The results from these two types of experiments are given in the two following sections.

5.1.1 Long-Term Laboratory Culture Collapse Experiments (Nos. 1-5)

The results of five "long-term" experiments undertaken at the time of five different pool culture collapses are given in Table 37. There was much variability in the results, both between experiments and between replicates within individual experiments. It is likely that this is largely due to the 5-10 day incubation period being too long, so that many of the cultures had entered the "stationary" or "decline" phase of batch culture growth characterized by death and cell lysis. This was evident in some cases, where dead Chaetoceros cells outnumbered live ones. Variations in size and physiological state of inoculum between experiments could have an effect similar to that of too-long an

TABLE 37. EFFECTS OF COMBINATIONS OF FILTRATION, NUTRIENT-ENRICHMENT AND REINOCULATION ON REGROWTH OF *Chaetoceros curvisetus* IN WATER FROM COLLAPSED OUTDOOR DEEP-WATER CULTURES OF THE SAME CLONE

TREATMENT	EXPERIMENT #:	1	2	3	4	5	5
	POOL #:	P2	P1	P2	P2	P2	P1**
	DATE :	12/16	12/22	01/04	01/24	02/21	02/21
Unfiltered Pool Water, Not Reinoculated							
1A No additions		1.5	<0.25	<0.25	<0.25	<0.25	<0.25[b]
B		<0.25	<0.25	<0.25	<0.25	<0.25	<0.25[b]
2A Plus nutrients		<0.25	<0.25	<0.25	<0.25	<0.25	<0.25
B		<0.25	<0.25	<0.25	<0.25	<0.25	<0.25
Unfiltered Pool Water, Reinoculated							
3A No additions		<0.25	<0.25	<0.25	<0.25	0.75[b]	<0.25
B		<0.25	<0.25	<0.25	<0.25	0.75[b]	<0.25
4A Plus nutrients		<0.25	<0.25	<0.25	<0.25	<0.25	<0.25
B		<0.25	<0.25	<0.25	<0.25	<0.25	<0.25[b]
Filtered Pool Water, Reinoculated							
5A No additions		143	27	1	89	<0.25[b]	2.8
B		136	33	1	32[b]	<0.25[b]	3.8
6A Plus nutrients		347	4	<0.25	34	32	<0.25[b]
B		376	2	25	28[b]	<0.25[b]	<0.25[b]
Sterile Media, Inoculated							
7A Deep water		177	18*	52	42	78	-[a]
B		126	41	-[a]	40	<0.25[b]	-[a]
8A Deep water + 2 ml unfiltered		4	50*	5	134	<0.25[b]	<0.25[b]
B pool water		7	88	34	78	9.30[b]	<0.25[b]
9A Deep water + 2 ml filtered		78	3*	11	149	<0.25[b]	8.3
B pool water		131	29	-[a]	32	<0.25[b]	2.4
10A f/2		-[a]	145	-[a]	344	475	-[a]
B		-[a]	147	-[a]	327	315	-[a]
11A f/2 + 2 ml unfiltered pool water		0.5	14	0.25	0.25	0.25	0.25[b]
B		0.5	17	-[a]	0.25	0.25[b]	0.25
12A f/2 + 2 ml filtered pool water		178	7	18	0.25[b]	520	0.25[b]
B		153	7	-[a]	2.50[b]	574	510
Inoculum Age (Days) :		8	7	8	6	5	
Medium + Number Cells in Inoculum :		DW=3.3x10^5	f/2=1.1x10^4 DW=1.8x10^4	DW=1.7x10^5	DW=1.9x10^4	DW=1.57x10^5	
Incubation Time (Days) of Experiment :		5	5	7	10	9	

Notes:
*One-month-old deep water
**Healthy pool
[a]Treatment omitted
[b]Almost as many (or more) dead cells also present

In each experiment, 50 ml of collapsed pool culture was incubated in duplicate, non-sterile, polyethylene foam-plugged 125-ml Pyrex Erlenmeyer flasks. If the pool water was filtered, 2 Gelman Type A-E glass fiber filters were used. Nutrients were added to f/2 strength. Sterile media had been autoclaved 15 min at 15 psi. Inocula were 1 ml aliquots from 50 ml, 5- to 8-day-old axenic cultures of clone STX-167. Incubation was at 24-27°C with a 12/12, light/dark cycle under "daylight" fluorescent light; at the end of the incubation period the cultures were preserved with Lugol's iodine and duplicate samples were counted in a Speirs-Levy eosinophil counting slide. Observations of numbers of dead *Chaetoceros* cells were made only in Experiments 4 and 5. Values reported are thousands of cells per milliliter; densities of <0.25 x 10^3 ml^{-1} reported indicate no cells were seen in the samples. Pennate diatoms, flagellates, amoebas and many unidentifiable spherical cells were observed in unfiltered pool water flasks, but are not reported here.

incubation period. Also, because the cultures in a single experiment were counted all at the same time, rather than when they reached their peak density, it is difficult to attach any significance to the relative densities observed. Nonetheless, it is possible to draw some general conclusions from the data.

(1) Reinoculation alone, addition of nutrients alone and reinoculation plus addition of nutrients did not result in good growth of Chaetoceros in unfiltered pool water (treatments 1-4 in Table 37. (But note that addition of a small amount of unfiltered pool water sometimes allowed good growth.) Note, also, that in experiment 5 the treatment of a healthy pool culture in the same way as a collapsed pool culture produced similar results.

(2) Filtration of collapsed pool cultures with a glass fiber filter to achieve "99.9% retention of particles 0.3 μm and larger" (according to the A. H. Thomas Co. catalog) allowed growth of reinoculated Chaetoceros in 78% of the cases (36 out of 46 tests in treatments 5, 6, 9, 12).

These conclusions do not tell us what the mechanism is for Chaetoceros growth inhibition in unfiltered collapsed pool culture, or the cause for the original culture collapse. However, the fact that the inhibitory agent can usually be removed by filtration indicates that it is either particulate—and probably a microorganism—or it is soluble and loses its inhibitory activity rapidly in seawater.

5.1.2 Short-Term Laboratory Culture Collapse Experiments (Nos. 6-11)

The short-term experiments were designed to minimize

the pitfalls encountered when samples of continuous-flow outdoor cultures are brought into the laboratory, and to concentrate on the events occurring in the first few days of an experiment.

Experiments 6 & 7: Effects of filtration of collapsed pool culture on growth of reinoculated Chaetoceros: Experiments 6 and 7 show conflicting results of filtration of collapsed pool culture on growth of C. curvisetus. In experiment 6, filtration had no significant effect on growth, with good growth being obtained with and without filtration (Fig. 10). Perhaps the vigorous aeration used inhibited the toxic agent in the vessel containing unfiltered Pool 1 culture. In experiment 7A, a mixture of collapsed Pool 2 culture and sterile carboy culture similar to that in in experiment 6 showed a favorable effect of filtration (Fig. 11). Filtering the pool culture also had a favorable effect where the collapsed Pool 1 was mixed with a healthy Pool 2 culture (experiment 7B; see Fig. 11).

Experiment 8: Effect of collapsed Chaetoceros curvisetus cultures on axenic cultures, separated by a sub-micron porosity filter: The purpose of this experiment was to see if the agent causing pool collapse in Pool 2 would pass through an 0.4 μm pore-size filter. If it would, this would be good evidence that the active agent is a poison excreted by a microorganism that is too large to pass through the pores, so that physical contact between the Chaetoceros and the attacking organism would not be required, or it could mean that the agent is smaller than 0.4 μm in size. The culture vessels consisted of two 125-ml polycarbonate Erlenmeyer flasks with a portion of the wall and bottom of each vessel cut

Figure 10. Experiment 6—Effect of glass-fiber filtration of collapsed pool culture on increase in Chaetoceros curvisetus density. Growth was measured as in vivo fluorescence of chlorophyll a on a Turner Model III fluorometer equipped with a high sensitivity door, as described by Parsons and Strickland (1972)*. The culture vessels were aerated one-liter aspirator bottles containing 800 ml mixtures (1:1) of 2-day-old Chaetoceros curvisetus carboy culture (f/2 medium) and filtered or unfiltered collapsed pool (Pool 1) culture. Incubation was as described for previous experiments.

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*Strickland, J.D.H. and T.R. Parsons, 1972. A practical manual of seawater analysis. Bulletin 167 (2nd ed.), Fish. Res. Bd. Canada.

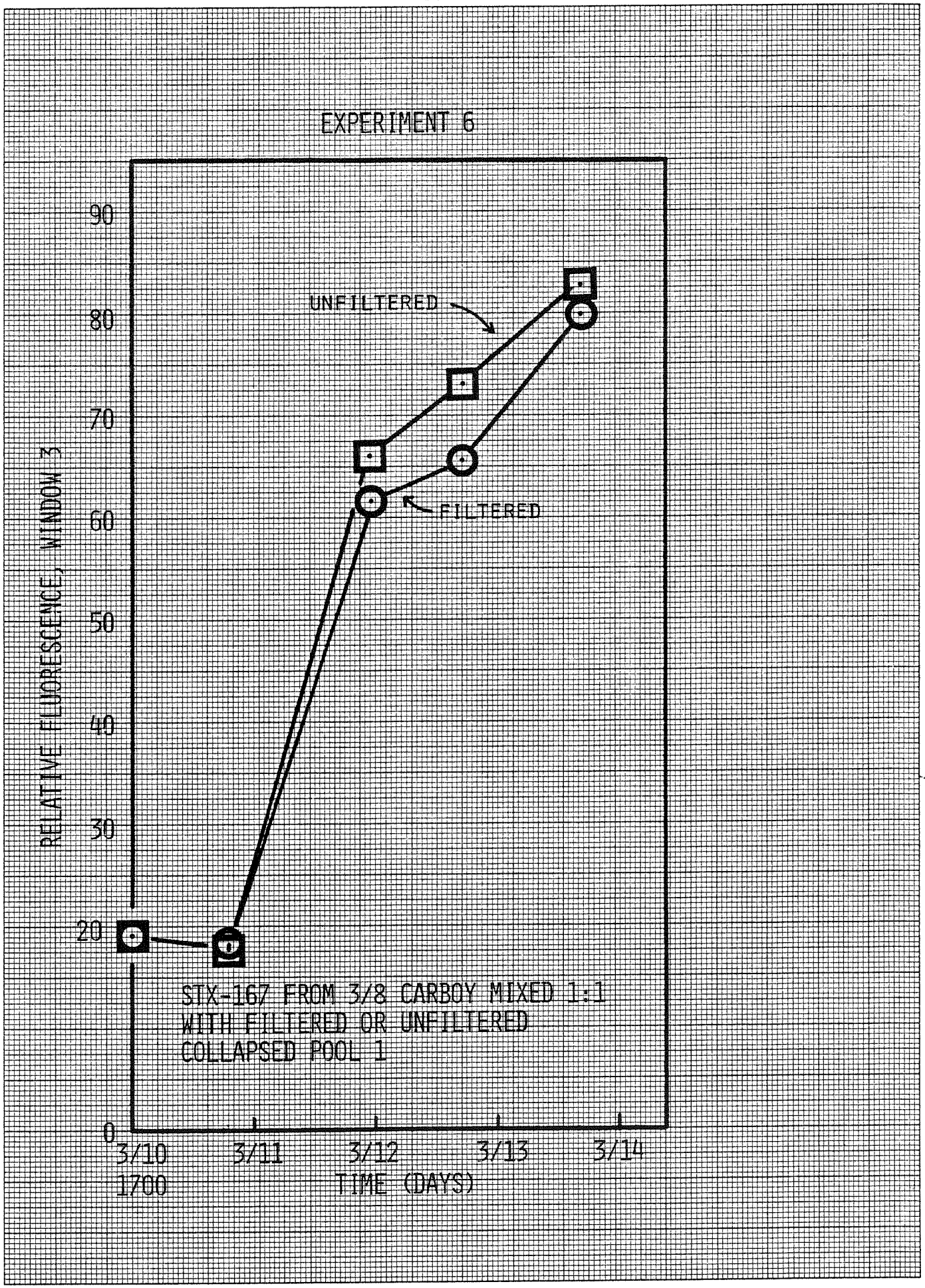
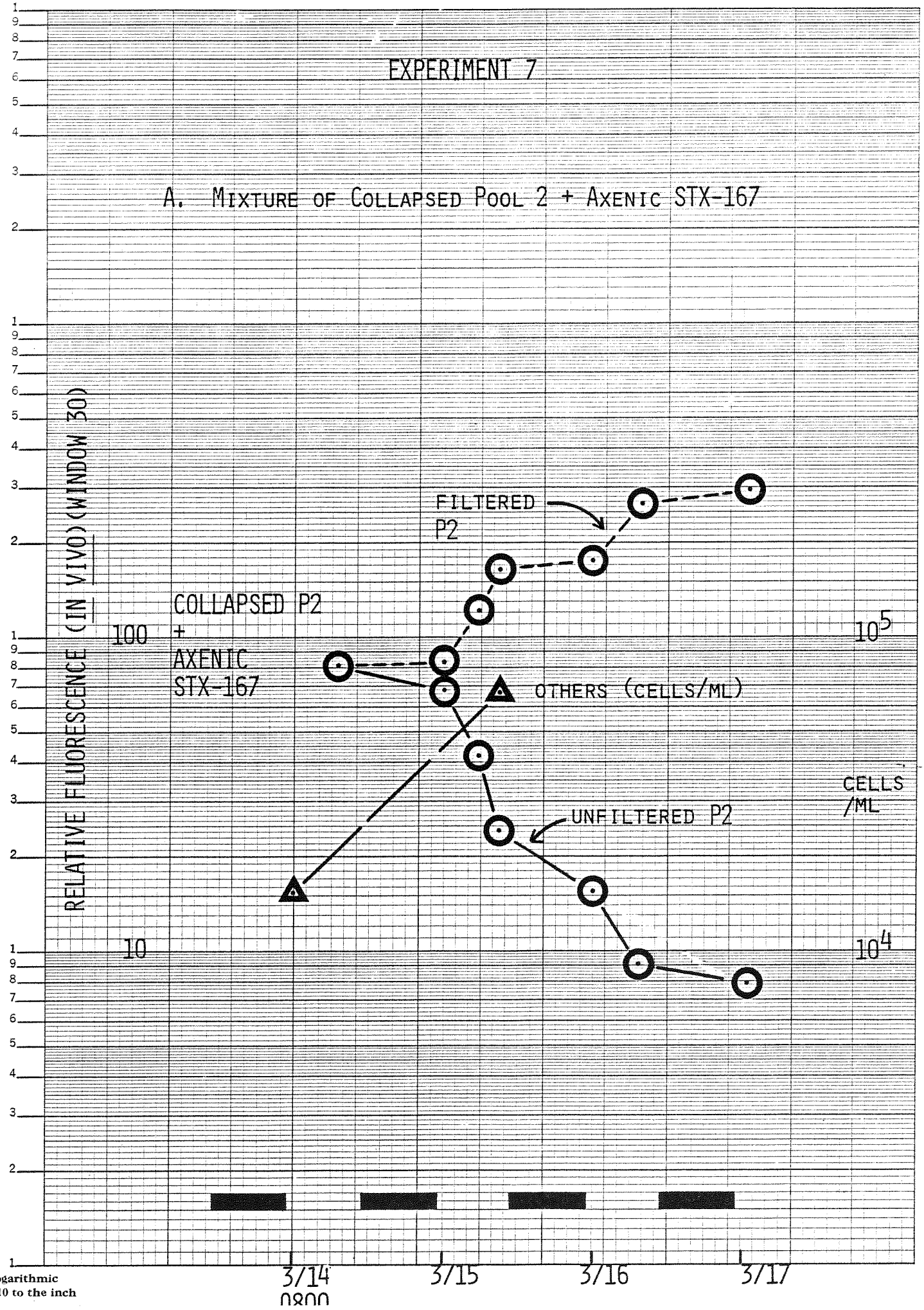


Figure 11. Experiment 7—Effect of glass-fiber filtration of collapsed pool culture on increase in Chaetoceros curvisetus density. Growth measured as in vivo fluorescence, as in Experiment 6. A—1:1 mixture of an f/2 culture of C. curvisetus and filtered or unfiltered collapsed Pool 2 culture. B—1:1 mixture of a healthy Pool 1 culture and filtered or unfiltered collapsed Pool 2 culture. The experimental vessels were cotton-plugged, non-sterile, 3000-ml Fernbach flasks containing 2000 ml of culture; the flasks were incubated as in previous experiments and were hand-shaken 3 to 4 times daily. Note the increase in density of contaminants ("other") in the flask containing unfiltered Pool 2 culture, while total chlorophyll a decreased.

2-184

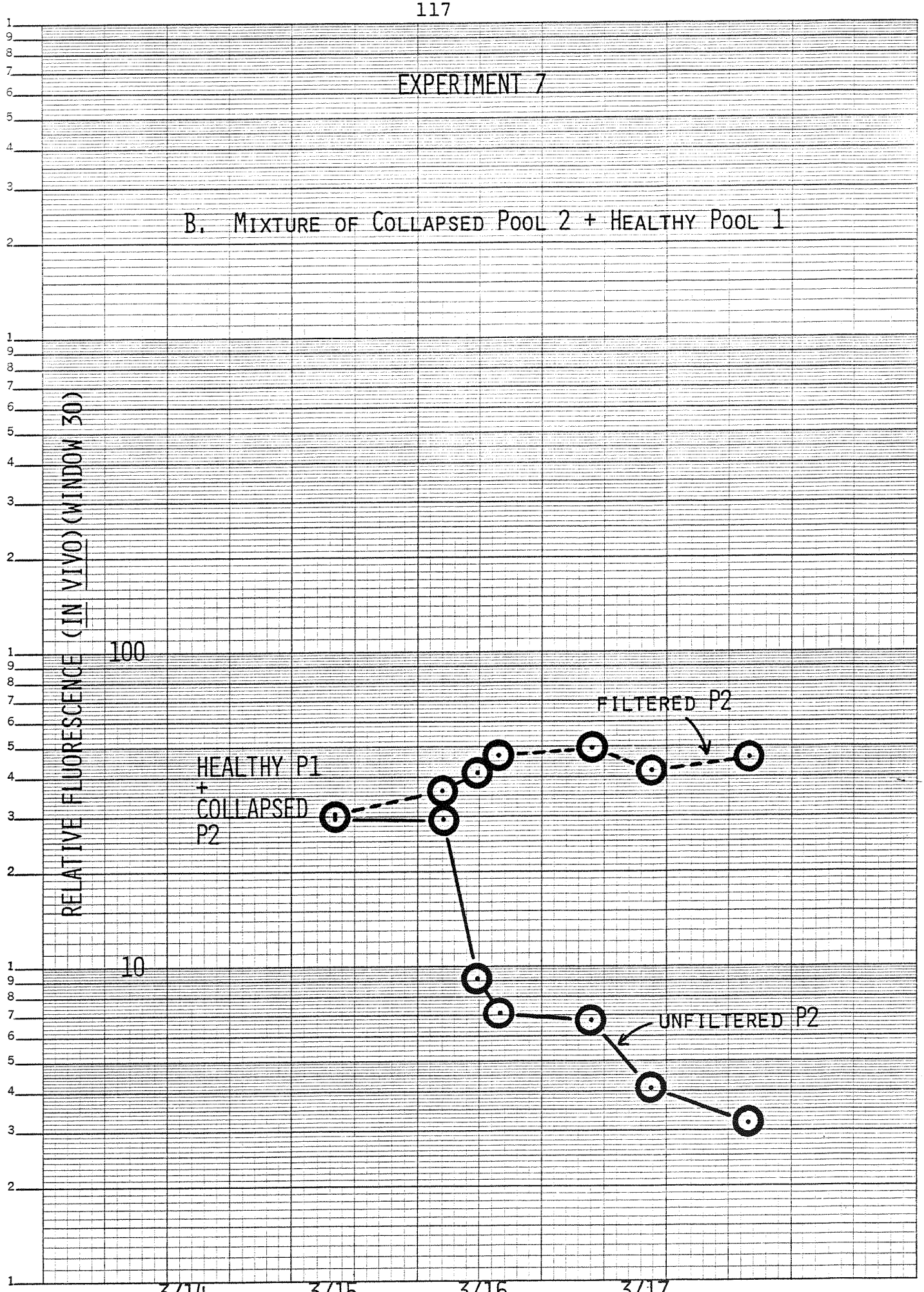


Semi-Logarithmic
Cycles x 10 to the inch

12-184

EXPERIMENT 7

B. MIXTURE OF COLLAPSED POOL 2 + HEALTHY POOL 1



Semi-Logarithmic
4 Cycles x 10 to the inch

off in a vertical plane; the flasks were glued together with silicone cement, with a 47 mm diameter 0.4 µm pore size polycarbonate Nucleopore membrane filter separating the interiors of the two flasks. An epoxy putty bridge between the necks of the two flasks made the unit rigid, and the unit could be autoclaved repeatedly and re-used without replacing the membrane. For this experiment on 4/15/77, two pairs of flasks were set up with the contents as follows:

	Flask A	Flask B
Pair 1	50 ml sterile f/2	25 ml sterile f/2 + 25 ml glass fiber filtered collapsed Pool 2 culture
Pair 2	50 ml sterile f/2	25 ml sterile f/2 + 25 ml unfiltered collapsed Pool 2 culture

Each side in each pair was inoculated with 5 ml of 5-day-old Chaetoceros to give 21.4 x 10^3 cells ml^{-1}; incubation was as described for other laboratory experiments.

The changes in cell densities over a four-day period in the flasks are shown in Table 38. Filtering the pool culture to remove cells allowed growth of the added Chaetoceros in the same flask (1B), while growth of Chaetoceros was inhibited in the flask where the pool culture was not filtered (2B). Growth of Chaetoceros in the attached axenic cultures (in communication with the non-axenic cultures via the 0.4 µm membrane) was not inhibited, indicating that either direct contact with the agent was necessary or possibly that the toxic factor produced in the unfiltered pool culture did not diffuse through in sufficient

TABLE 38. EXPERIMENT 8—EFFECT OF COLLAPSED Chaetoceros curvisetus CULTURES ON AXENIC CULTURES SEPARATED BY A SUBMICRON POROSITY FILTER. VALUES ARE 10^3 CELLS/ML. DETAILS ARE GIVEN IN THE TEXT

PAIR:		1				2			
SIDE:		A		B		A		B	
CONTENTS OF FLASKS; SEPARATED BY 0.4 μM MEMBRANE:		AXENIC 167		FILTERED P2 + 167		AXENIC 167		UNFILTERED P2 + 167	
DATE	TIME	167	others	167	others	167	others	167	others
4/15	1300	21.4	-	21.4	-	21.4	-	21.4	8.6
4/16	0800	45	0.5	36.8	1	44	0.5	28	13
4/16	1830	81	0.8	71	1.8	111	0	23	64.5
4/18	1400	234	12	228	20	202	0	1.8	12.5
4/19	1600	310	24	363	9	434	0	3	3.2

quantities or was degraded too rapidly to be effective. However, the density of "other" organisms (flagellated cells—most likely gametes of Chaetoceros) was much lower in Flask A of Pair 2 than it was in Flask A of Pair 1, indicating that something in unfiltered pool water was passing through the membrane and affecting gamete production but not vegetative reproduction. The 0.4 μm pore membranes are not completely effective in preventing passage of bacteria, so they may have selectively let some bacteria through and not others. Carlucci et al. (Can. J. Microbiol. 22:1667-1671, 1976) showed that the majority of the deep-sea bacteria they examined were 0.2-0.4 μm in diameter.

The antibiotics penicillin and streptomycin (effective against gram-negative and against both gram-negative and gram-positive bacteria, respectively, were used in the following experiments (numbers 9, 10, 11) to pinpoint whether or not bacterial contaminants were responsible for failure of Chaetoceros to grow when reinoculated into collapsed pool culture.

Experiment 9: Effect of antibiotics on growth of Chaetoceros: This batch culture experiment was carried out in conjunction with the experiments made in two outdoor reactor cultures (see 5.1.6). The experiment showed a promotional effect of the antibiotics penicillin and streptomycin on in vivo fluorescence during the first day or two, but all the cultures declined simultaneously and at the same rate on the second or third day (due to nutrient depletion) before a predicted culture collapse occurred. The data are not reported, because they shed no light on the culture collapse problem.

Experiment 10: Effects of antibiotics on growth of Chaetoceros inoculated into filtered collapsed reactor culture: This experiment repeated a test of the effect of the antibiotics on C. curvisetus in 1:1 mixtures of filtered collapsed reactor 6 or 8 cultures and deep water from 6/21 (see section 5.1.6 for history of the Chaetoceros culture collapses in reactors 6 and 8), to verify the promotional effects of the antibiotics observed in experiment 9. The results are given in Table 39. Note that in this experiment, in contrast to experiment 9, the antibiotics allowed less growth than where antibiotics were not used. No explanation for the difference between the two experiments is obvious.

Experiment 11: The effect of antibiotics and nutrients on growth of Chaetoceros inoculated into filtered and unfiltered collapsed pool culture: In this experiment, the effects of the antibiotics were again investigated, and some observations were made of the population composition in the unfiltered treatments. The results, given in Table 40 show that simple reinoculation of unfiltered collapsed pool culture produced growth of other organisms but not of Chaetoceros, while addition of antibiotics allowed some growth of Chaetoceros. The best growth of Chaetoceros in unfiltered water was with the addition of nutrients alone. The growth of Chaetoceros in filtered water was best with no additions or only nutrients added; antibiotics were strongly inhibitory to Chaetoceros growth. These results indicate that the reason for the failure of Chaetoceros to grow after reinoculation into unfiltered pool water was some sort of inter-

TABLE 39. EXPERIMENT 10—EFFECTS OF ANTIBIOTICS ON GROWTH OF Chaetoceros curvisetus INOCULATED INTO FILTERED COLLAPSED REACTOR CULTURE

DATE	TIME	REACTOR:	WITH ANTIBIOTICS			WITHOUT ANTIBIOTICS		
			6	8	8 +Nutrients	6	8	8 +Nutrients
6/21	1730		5.1	5.2	5.1	5.0	5.3	5.1
6/22	1700		6.6	6.1	6.1	13.3	13.5	14.1
6/23	1630		11.1	10.0	9.5	23.0	56.4	60.0
6/24	1730		14.2	14.2	10.8	42.7	68.1	101

Note: Filtered collapsed C. curvisetus culture from reactors 6 or 8 which had been maintained at 1.0 dilutions per day after dilution 1:1 with collapsed pool culture (or with deep water on 6/18) was mixed 1:1 with deep water and incubated under laboratory conditions in 125 ml Erlenmeyer flasks as described earlier. The flasks were all inoculated from a 5-day-old f/2 C. curvisetus culture to give 3.6×10^3 cells ml^{-1}. Growth was measured as in vivo fluorescence of chlorophyll a as described for Experiment 6. Nutrients, if added, were at f/2 strength. Antibiotics added were: Penicillin G, 100 mg $liter^{-1}$, Dihydrostreptomycin, 50 mg $liter^{-1}$.

TABLE 40. EXPERIMENT 11—EFFECT OF ANTIBIOTICS AND NUTRIENTS ON GROWTH OF *Chaetoceros curvisetus* INOCULATED INTO FILTERED AND UNFILTERED COLLAPSED POOL CULTURE

		Unfiltered				Filtered			
Additions:		None	Nutrients	Antibiotics	Antibiotics + Nutrients	None	Nutrients	Antibiotics	Antibiotics + Nutrients
Date	Time								
6/28	1815	14	13.2	14.6	13.2	10.6	11.1	10.2	11.0
6/29	1730	12.6	18.7	10.4	13.8	17.5	15.8	9.9	9.2
6/30	1750	29.8	75.4	16.0	29.1	66.2	51.3	13.2	9.8
7/01	1600	90.6	352.0	25.5	62.2	182.5	152.5	14.9	8.9
C. curvisetus on 7/1?		No	Many	Few	Few				
Flagellates (f) or pennate diatoms (p) present?		f,p	f,p	f,p	p				

Note: Pool 2 water was inoculated after filtered through a glass-fiber filter, or without filtering, to give 2.6 x 10^3 cells ml^{-1} (5-day-old f/2 culture). Growth measurement, incubation and antibiotic additions as described for Experiment 10.

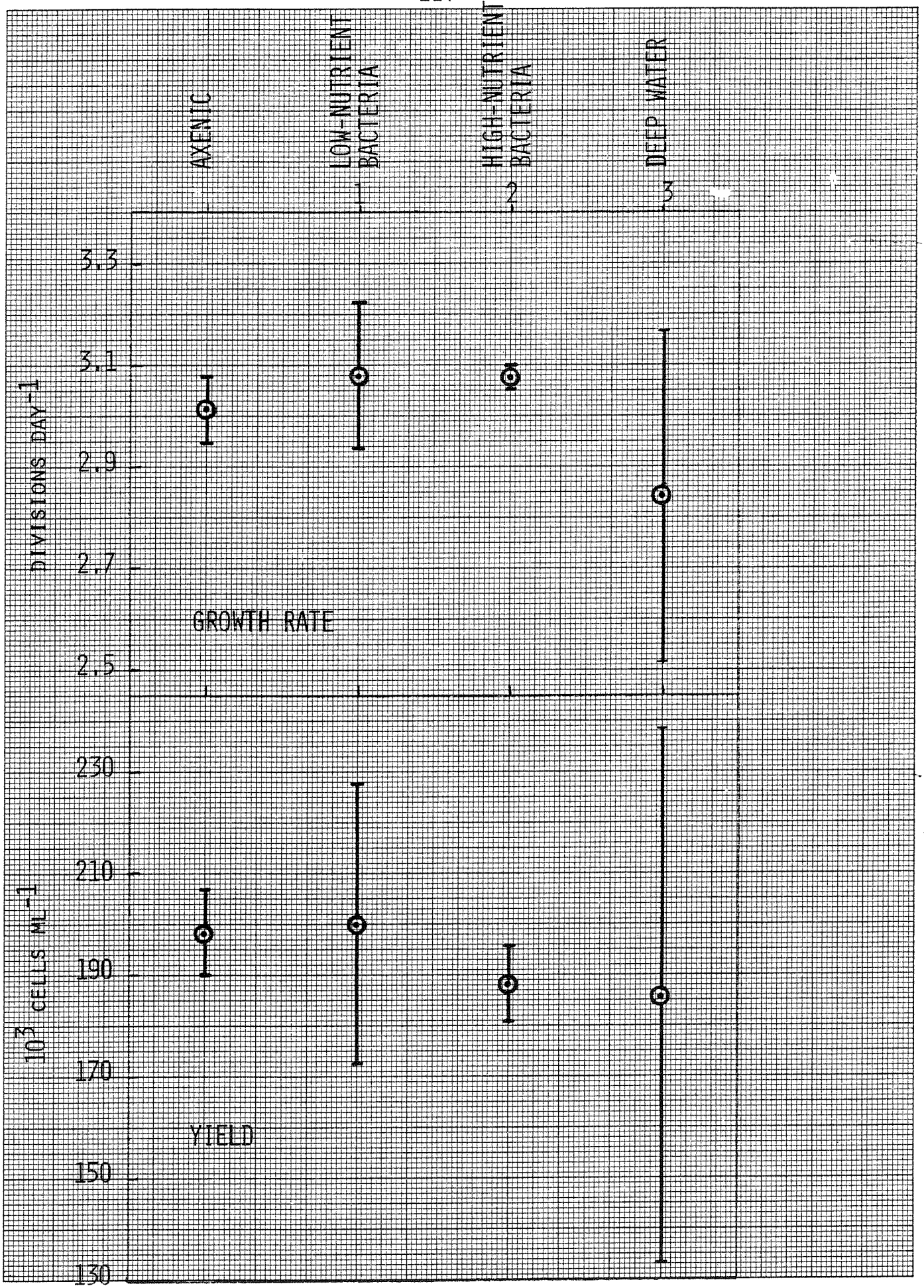
curvisetus in batch culture, where metabolic products of the diatom can accumulate and provide a nutrient source for bacteria. Dense bacteria cultures were obtained by inoculating a heat-killed C. curvisetus culture and a rich synthetic medium (seawater with added glucose, peptone and yeast extract, medium GPYS) with foam from a pool culture and incubating overnight. Growth rate and yield of Chaetoceros were determined over a three-day period in duplicate cultures inoculated from the above two sources of bacteria and from deep water. The results are shown in Figure 12. Neither growth rate nor yield were significantly affected by the additions of bacteria, as shown by the overlapping standard deviations about the means. None of the cultures collapsed when incubated for several more days. One can conclude that the normal bacterial flora in an outdoor continuous culture of Chaetoceros curvisetus that is growing well is not pathogenic for batch laboratory cultures of C. curvisetus.

5.1.4 Scanning Electron Microscopy of Collapsed Outdoor Chaetoceros curvisetus Cultures

Laboratory experiments showing that the agent causing culture collapses was usually removable by filtration suggested that it might be possible to identify the agent by means of scanning electron microscopy (SEM). This study was undertaken in collaboration with Dr. Garry Cole, Department of Botany, The University of Texas at Austin.

A sample collected from a collapsed Pool 2 Chaetoceros culture of 1/24/77 was concentrated 232x by reverse filtration, fixed in 4% buffered (pH 7.4) glutaraldehyde, and shipped

Figure 12. Growth rates and yields of 3-day-old laboratory cultures of Chaetoceros curvisetus (STX-167) under axenic and bacterized conditions. Bacteria were added from three sources: bacteria from foam on an outdoor 13,000-gallon deep-water culture of C. curvisetus grown in (1) a heat-killed C. curvisetus culture (= low-nutrient bacteria) or in (2) a glucose/peptone/yeast extract medium (= high-nutrient bacteria); or (3) bacteria in fresh, untreated deep water. Values plotted are means of two cultures ±1 standard deviation.



refrigerated (with ice packs) in buffered $35^{o}/oo$ NaCl with 1mM mercuric chloride added as a preservative, to Austin for SEM study. The sample had large numbers (1.7×10^{4} ml^{-1}) of spherical organisms possessing fine pseudopodia (a heliozoan), and some algal flagellates. The SEM pictures showed diatoms (C. curvisetus and pennate diatoms) and unidentified subspherical pitted particles; the heliozoans were not seen, and would not be expected to be preserved with the techniques we used, because heliozoans lack cell walls. There were also filamentous bacteria (actinomycetes?) attached to some of the pennate diatoms; these might have been responsible for the culture collapse, but previous work (see section 5.1.3) indicates that bacteria are probably not the cause of culture collapse.

A second sample for SEM study was collected from a collapsed Pool 2 culture on 2/21/77 and prepared for SEM in a manner reputed to allow for storage of samples for two weeks (Paerl, H. W. and S. L. Shimp, Limnol. Oceanog. 18:802-805, 1973). However, the method did not work for us, so we abandoned the method of shipping fixed samples in liquid to Austin.

A portable critical drying point apparatus, on loan from Dr. Cole, was used to prepare a Chaetoceros and deep-water particulate sample for SEM on 6/3/77. This method has the advantage of allowing storage and shipping in a dry state. We have not yet used the method on a collapsed pool culture.

Dr. Patricia Johansen at UT Port Aransas Marine Laboratory saw some of the preserved (in Lugol's iodine) heliozoans and suggested that they were in that taxonomic group; she doubted

that they would be able to feed on Chaetoceros, which is generally larger than the heliozoans.

5.1.5 Transmission Electron Microscopy of Collapsed Outdoor Chaetoceros Cultures

The possibility exists that viruses may be responsible for the collapse of our outdoor Chaetoceros cultures. Viruses would not be seen in the course of daily light microscope observations of the cultures, and would require different culture and isolation techniques than those we have used for bacteria, so we cannot eliminate viruses as a possible cause of culture collapse until we have looked for them with the appropriate techniques during a pool collapse. Three samples from a Pool 2 culture before, during and after collapse, on 6/28/77, were processed for transmission electron microscopy (TEM). The samples were shipped to Dr. Garry Cole in Port Aransas for TEM observation but they were delayed in the mail and arrived too late for observation this summer. The samples were embedded, sectioned and observed during September, October 1977 at the University of Texas, Austin. No virus particles were found in the post-crash sample; perhaps the samples deteriorated too much during shipment. The search for viruses should be repeated on other collapsing pool cultures, in case viral contamination is only one of several causes (we may have sampled a pool collapse that was not caused by a virus). In future studies we will seek collaboration with a virologist, and hand-carry samples to the mainland for TEM, or embed them in St. Croix.

5.1.6 Changes in Cell Density, Population Composition, Photosynthesis, Respiration and Carbon-14 Fixation in Outdoor Continuous *Chaetoceros curvisetus* Cultures

In June 1977, Dr. Bassett Maguire and his graduate student, Jim Davenport, from the Zoology Department at the University of Texas, Austin, measured photosynthesis and respiration using carbon-14 and the light-and-dark bottle method. The carbon-14 method they used measures carbon fixation by individual cells in the population as well as by the population as a whole. The method involves incubation of cells which have incorporated carbon-14 with photographic emulsion for several months, and then counting exposed silver grains in the vicinity of cells. The method would indicate whether photosynthesis of only some cells is affected by the agent causing culture collapse, or whether the whole population is affected simultaneously. A predicted culture pool collapse did not occur during a period of closely-spaced observations, but a few observations were made during a culture collapse in a reactor where we mixed a collapsed Pool 1 culture with a healthy reactor 8 culture, 1:1. Table 41 shows the time course of change in densities of live and dead *Chaetoceros curvisetus* and of all other organisms (amoebas, flagellates) in the experimental reactor 8, and in a "control" reactor where we mixed the culture with fresh deep water, 1:1. Note that the control reactor collapsed a day earlier than the experimental reactor. We had expected the experimental reactor culture to collapse no later than the day after mixing the collapsed

TABLE 41. CHANGES IN DENSITIES OF LIVE AND DEAD *Chaetoceros curvisetus* AND OTHER ORGANISMS IN STEADY-STATE REACTOR CULTURES

		Experimental Reactor 8			Control Reactor 6		
		Chaetoceros			Chaetoceros		
Date	Time	Live	Dead	Others	Live	Dead	Others
6/18	1000	42.2	--[a]	3.2	36.2	--[a]	1.4
	1300	53.8	--	3.5	38.7	--	0.3
	1500	41.8	--	4.9	41.0	--	0.9
6/19	0700	55.5	8.0	5.0	80.5	1.8	1.8
	1930	67.5	4.8	5.8	64.5	0.8	0.8
6/20	0800	53.8	16.2	4.2	62.2	1.5	3.5
	1900	48.3	10.7	10.0	74.5	5.2	5.5
	0800	33.0	21.5	4.8	40.8	27.8	12.0
	1630	37.3	8.8	5.0	5.5	24.2	14.5
6/22	0800	3.5	17.0	4.5	<0.3[b]	2.0	7.0

[a] no data collected
[b] < 0.3 = no cells seen

Note: Reactor cultures mixed 1:1 with collapsed Pool 1 culture ("control reactor 6") and put on continuous flow immediately at 1.0 dilutions per day. The control reactor had been put on continuous flow on 6/13, the experimental reactor on 6/15. Densities given are thousands of cells per milliliter.

pool culture with the reactor culture. The collapse of the experimental culture was not associated with a rapid increase of "other" organisms, as in the control culture, nor was there a rapid increase in the number of dead Chaetoceros prior to the collapse as was observed in the control culture. This suggests that two different agents or mechanisms may have been responsible for culture collapse in the two reactors. Protozoans (oligotrichs, family Strobililidiidae) were observed in the experimental reactor on the last day; these may have been responsible for the slow decline in live Chaetoceros densities over the period 6/19/77 to 6/21/77, prior to the collapse, but they were probably not the cause of the final collapse. Efforts to culture the tintinnids to test this assertion were fruitless.

The data for carbon fixation are given in Appendix I and will be summarized here.

The oxygen production technique showed a general decline in photosynthetic activity during the collapse of the experimental reactor on June 18, 21 and 22 (400, 222, and 19 mg C fixed per m^3 per 2 hrs, respectively). Production per cell during the same period decreased from 8.7 to 6.3 to 5.7 mg C fixed per m^3 per 10^{12} cells. The ^{14}C technique showed that during the same period of time, there was a shift from high production per cell for the majority of cells in the culture before the collapse to low production per cell after the collapse. These results are consistent with the hypothesis that the population is affected by interaction with another organism, such as a virus or bacterium, with "infection" spreading to more and more cells in the population,

but inconsistent with predation (by large filter-feeders) as a cause of culture collapse.

5.1.7 Recommendations for Future Studies of the Problem of Collapse of *Chaetoceros curvisetus* Cultures

During this past year we undertook experiments which would investigate the most likely causes of pool collapses. As a result of our studies we believe that contaminants are the causes, but the contaminants and their mechanism(s) of causing culture collapse have yet to be identified. The information we have to date does not suggest an immediate solution to the problem of increasing the longevity of the *Chaetoceros* cultures, but it is possible to attack the problem in a number of ways.

It is easy to see similarities between the problems we are having with our *Chaetoceros* cultures and the problems faced by land agriculturists when they attempted to grow mono-specific plant crops, such as corn. Predictable high yields of corn and other crops are only possible if pesticides and special strains of plants developed over the years are routinely used to *prevent* infestations by insects, fungi and viruses. We do not have such an armament of chemicals and strains and a great deal more time and effort will have to be spent before we first understand what is happening in our collapsing cultures and then develop methods for preventing culture collapses.

We have not yet investigated the effects of light intensity (by increasing depth, or by shading) or dilution rate on the onset of culture collapse, for example. Perhaps making the

cultures light-limited rather than nitrate-limited (as they now are) will make the cultures less susceptible to interference by contaminant organisms. Perhaps we are pushing the Chaetoceros too close to the limits of one or more of its physiological processes, so that it is highly susceptible to perturbation by changes in the environment or interactions with other organisms. Future work should involve a biochemical approach: What changes occur in cell constituents, such as synthetic and respiratory enzymes, and metabolic pools during a culture collapse? What is the normal pattern of these constituents in a steady-state culture? An understanding of how cells in a normal steady-state culture function will have to precede recognition of why cells die in a collapsing pool.

5.2 Selection of Algal Species for Growth in Unsupplemented Deep Water

5.2.1 New Clones of Algae from Deep Water

A new clone of Chaetoceros curvisetus (STX-199) was isolated into unialgal culture from a June 6 sample of deep water, one of the daily samples collected since December 4, 1976. Live C. curvisetus cells were seen after 10 days or more incubation of deep water samples in only 1.49 of the 208 samples collected. This clone will be made bacteria-free and tested in the outdoor continuous culture system and in batch laboratory cultures to compare its growth characteristics with those of clone STX-167, which has been failing in outdoor cultures more often than usual during the past year.

Two other clones of centric diatoms (STX-197 and STX-198,

a Thalassiosira (?) sp. and a small chain-forming Chaetoceros sp., respectively) were also isolated from deep water, but have not been tested for growth in deep water.

An unidentified cryptomonad alga (clones STX-195 and STX-196) was isolated from deep water collected March 3, 1977. Clone STX-195 grows well in unsupplemented deep water; the growth data are given in the following section. This uniflagellated alga is about 2-3 µm x 4-6 µm in size.

5.2.2 Testing of Algal Species for Growth in Unsupplemented Deep Water

During the first quarter of this year, five algal species were selected for testing in continuous culture after demonstrated ability to grow in batch culture of unenriched deep water. Of these clones, three (diatoms) were isolated at the St. Croix station: STX-19, STX-183 and STX-97. These clones, along with clone-581 (Chaetoceros sp., from W. H. Thomas) and Tetraselmis tetrathele (from Tahiti), were all tested in reactor cultures at a dilution rate of 1.0 turnovers per day. STX-19 and STX-183 washed out at a rate greater than the dilution rate, indicating that not only did they not divide fast enough but were dying off as well. STX-97, also tested in the same conditions, washed out at a slower-than-dilution rate, indicating that it may grow successfully at a lower dilution rate. It was later grown in reactor cultures at .25, .50, .75 and 1.0 dilutions/day. Results using these dilution rates were erratic; growth did occur, but was unpredictable. Clone-581 was abandoned because preliminary tests indicated that it is not a

good food for juvenile shellfish.

T. tetrathele, a flagellate clone received 9/5/76 from Tahiti, was tested in 2000-liter outdoor containers and was found to grow equally well in enriched and unenriched deep water. It could be maintained at a steady state over a range of .25-1.0 dilutions/day in unsupplemented deep water. However, the cultures could not be maintained unialgally for more than one week. Unidentified diatoms took over in all cases. This alga may be useful sometime in the future for hatchery work where larval food is needed for a short period of time.

A high-temperature Isochrysis sp., clone T. Iso, also received from Tahiti (CNEXO) and reisolated from a mixed culture on St. Croix, was started in a one-month outdoor continuous flow experiment at .50 dilutions/day. Identical tanks were employed, one enriched with chelated trace metals and vitamins, the other completely unsupplemented deep water. Cell production averaged three times higher in the enriched cultures than in the cultures without enrichment (see Table 42). Washout did not occur in any of these cultures, nor were any other organisms observed in routine counting.

We tested the ability of this alga to grow in unsupplemented deep water in two separate experiments at dilution rate of 0.25, 0.5, 0.75 and 1.0 reactor volumes per day. The results are given in Table 43, cell density was highly variable as in the previous "plus enrichment/no enrichment experiment, both between treatments and, in this experiment, between replicate cultures at the same flow rates, especially at the lower flow rates. The

TABLE 43. EFFECT OF ENRICHMENT OF DEEP WATER ON GROWTH OF CLONE T. Iso IN CONTINUOUS FLOW OUTDOOR REACTOR CULTURES AT .50 DILUTIONS/DAY

	Cell Density*	
	10^3 cells/ml	(± 1 S.D.)
Plus Enrichment*		
Reactor 17	217	(±191)
Reactor 20	98	(± 96)
Average:	157	
No Enrichment		
Reactor 18	46	(± 40)
Reactor 19	44	(± 43)
Average:	45	

*Enrichment includes the trace metals Fe, Cu, Ca, Mn, Mo, and Zn, and vitamins B_{12} and B_1.

**Cell densities given are averages of 26 daily measurements made in duplicate for each culture at 0800 hr.

TABLE 43. EFFECT OF DILUTION RATE ON CELL DENSITY AND DAILY CELL PRODUCTION OF CLONE T. Iso

Dilution Rate (volumes day^{-1})	Mean Cell Density (10^3 cells ml^{-1} ± S.D.)	Cell Production (10^9 cells day^{-1} ± S.D.)	Days to end of experiment or until washout
1.0	25± 31	50± 61	16* (washout?)
	29± 53	58±105	35
0.75	33± 32	50± 42	16* (washout?)
	27± 34	40± 51	34 (washout)
0.5	44± 42	44± 41	32
	99±110	98±109	33 (washout)
0.25	44± 41	22± 21	32
	206± 78	103± 39	35

*Cultures were terminated when cell densities decreased to less than 10^3 ml^{-1}.

Note: Grown in unsupplemented deep water in 2000-liter (528 gal.) outdoor continuous cultures; the data are from two separate experiments started 12/25/76 and 1/31/77.

TABLE 44. EFFECT OF INOCULUM SIZE ON GROWTH OF CLONE T. Iso

Inoculum (liters)	Cell Density (10^3 cells/ml) At Start of Continuous Flow	21-Day Mean (± S.D.)	On Final Day of Experiment
30	37	64(± 60)	40
60	120	44(± 39)	58
120	220	131(±116)	48
240	450	176(±169)	50

Note: Grown in unsupplemented deep water in 2000-liter (528-gal.) outdoor continuous cultures. The cultures were inoculated at one-half volume from a 29-day-old f/2 polytank batch culture. Continuous flow was started (0.25 dilutions day^{-1}) 24 hr later, after filling the reactors. The experiment was terminated after 21 days; none of the cultures had washed out.

maintaining constant supplies of this clone as a food organism, because inocula cultures could be held for long periods of time at high density, and larger cultures could be inoculated and continuous flow started immediately, without having to wait for growth to occur or worry about "overshooting" the carrying capacity of the medium. However, further studies of dilution rate and utilization of nutrients (nitrogen) by this clone are needed, as well as quantitative testing of it as a food source, before considering using it as a replacement for Chaetoceros curvisetus in our system.

A single test was made to grow the flagellate clone STX-195 (isolated from deep water) in unsupplemented deep water, using dilution rates of 1.0, 0.75, 0.50 and 0.25 per day. Although cell density and production per day were both higher at the lowest flow rates, both cultures grown at the low dilution rates (0.50 and 0.25 per day) collapsed after 26 days (i.e., cell density decline was much faster than washout alone). Thus, this initial test indicates that STX-195 does not have the apparent resistance to culture collapse that clone T. Iso has. The data are presented in Table 45.

5.3 Possible Antibiotic Production by an Algal Flagellate

Outdoor 200-gallon batch cultures of T. Iso have been maintained constantly since November 19, 1976, in enriched seawater medium f/2 minus Si. These cultures have remained virtually free of contaminating organisms (bacteria, other algae and protozoans), non-bacterial contaminants averaging less than 0.02% of cell counts. Normally, batch cultures of diatoms and

TABLE 45. EFFECT OF DILUTION RATE ON CELL DENSITY AND DAILY CELL PRODUCTION OF CLONE STX-195

Dilution Rate (volumes day^{-1})	Mean Cell Density (10^3 cells ml^{-1} ± S.D.)	Cell Production (10^9 cells day^{-1} ± S.D.)
1.0	29 ± 35	58 ± 69
0.75	29 ± 27	44 ± 41
0.5	72 ± 71	71 ± 71*
0.25	127 ± 99	64 ± 49*

*Culture collapse (not washout) occurred on day 26.

Note: Clone STX-195 is an unidentified flagellate isolated from deep water. Grown in unsupplemented deep water in 2000-liter (528-gal) outdoor continuous cultures. Experiment was terminated 27 days after continuous flow was started.

other flagellates become heavily contaminated following exponential growth phase (usually after 3-4 days), making the cultures useless as innocula for pool or reactor cultures. In contrast, batches of T. Iso have repeatedly grown without any manipulation for over two months, maintaining a cell density of Ca 4 x 10^6 cells/ml (Figure 13).

A possible explanation for the apparent resistance of cultures of clone T. Iso to invasion by other organisms is that it excretes a substance which is toxic to other organisms (an antibiotic). This was tested by culturing marine bacteria and three other clones of microalgae in the 0.5 µm-filtered supernatant taken from an outdoor culture when it was two weeks old. Growth of the mixed marine bacteria population, and of two of the algae tested (a flagellate and a diatom) was inhibited; growth of a green algal flagellate and of the producer (T. Iso) was not inhibited (Table 46).

Recently, a first test of cell extracts of lab-grown T. Iso, in collaboration with Mrs. Helen Gjessing of the College of the Virgin Islands, St. Croix, showed no antibiotic activity with pathogenic bacteria. Similar experiments with marine bacteria gave similarly negative results. Although these results are somewhat disappointing from a pharmacological view, the importance of T. Iso to shellfish mariculture is not diminished when considering the 49% inhibition of marine bacteria from cell-free supernatant and its impressive stability in beach experiments.

We reasoned that the apparent antibiotic proprieties of

Figure 13. Growth of clone T. Iso (Isochrysis sp.?) in four outdoor 757 l. (200 gal) polytank batch cultures in medium f/2 minus Si. Circles, inoculated 11/18/76; squares, inoculated 12/17/76; triangles; inoculated 2/2/77; x's, inoculated 3/15/77. The arrow at day 29 indicates when the 2/2/77 culture was diluted 75% with deep water and enriched back to f/2 level of nutrients. The cultures were maintained in full sunlight, with continuous aeration.

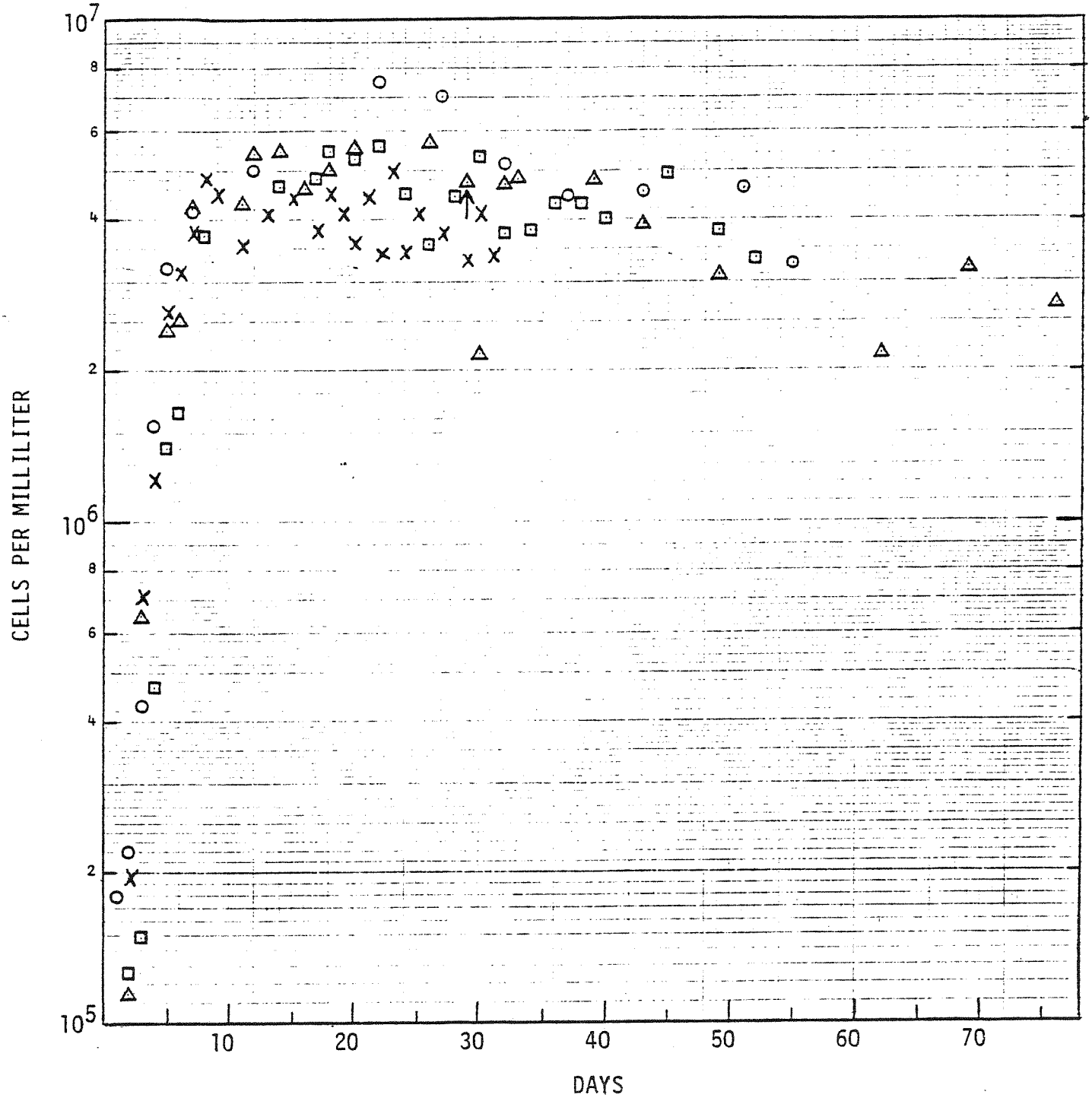

CELLS PER MILLILITER
10^7
8
6
4
2
10^6
8
6
4
2
10^5
10
20
30
40
50
60
70
DAYS

TABLE 46. GROWTH OF TEST BACTERIA AND ALGAE IN MEDIUM f/2 IN THE SUPERNATANT FROM A TWO-WEEK-OLD T. Iso CULTURE

TEST ORGANISM	GROWTH IN T. Iso SUPERNATANT AS % OF GROWTH IN DEEP-WATER CONTROLS
Mixed marine bacteria	50.7 (±1.7)
S-1 (flagellate)	53.0 (±4.3)
STX-114 (diatom)	85.6 (±2.3)
STX-92 (green flagellate)	105.8 (±4.1)
T. Iso	126.9 (±2.3)

Note: Values are average yields of duplicate cultures in T. Iso supernatant, expressed as a percentage of yields observed in duplicate control cultures in f/2 medium made from deep water. Values in parentheses are ±1 standard deviation. Growth of bacteria was measured as turbidity; growth of algae by means of cell counts.

clone T. Iso might be taken advantage of in solving the problem of Chaetoceros curvisetus culture collapses (see section 3.2.2), perhaps by adding enough T. Iso to a collapsing pool culture to reverse the decline in the diatom population, or in combination with a reinoculation with the diatom. This was tested in the lab in a preliminary way using a collapsed Pool 2 culture: wherever T. Iso was inoculated in combination with Chaetoceros curvisetus (with or without filtration and/or nutrient additions), growth of C. curvisetus was poorer than that of T. Iso, and poorer or no better than where C. curvisetus was inoculated alone. The test was not repeated.

5.4 Macroalgae

The first half of the period covered by this report dealt with the parameters of nitrate and ammonia uptake by Hypnea musciformis on the beach and in the laboratory. Laboratory experiments were run using the Technicon AutoAnalyzer-II to determine nitrate and ammonia uptake rates for H. musciformis and for Macrocystis pyrifera, in collaboration with Dr. Patricia Wheeler (from Dr. Wheeler North's group at California Institute of Technology). These studies showed that ammonia is taken up much more rapidly than nitrate, supporting earlier observations of higher growth rate and ammonia uptake in clam tank effluent (rich in ammonia) than in nitrate-rich deep water. At the concentrations studied (less than 30 μM) nitrate- and ammonia-nitrogen uptake of nitrate by H. musciformis followed saturable kinetics, but ammonia did not. The results for M. pyrifera and the details of the work on H. musciformis are given

in the draft manuscript (submitted to J. Phycol.) in Appendix G. In a beach experiment in July, uptake of ammonia excreted by Tapes japonica was studied over four 24-hr cycles. Fragmentation of H. musciformis was a problem in several of the tanks, and was related to high ammonia concentrations in the clam tank effluents. Where fragmentation was not a problem, ammonia uptake was highly correlated with influent ammonia concentrations both day and night. Details of this work are given in the published paper (Appendix H).

The second half of the present period was devoted to continuous culture growth experiments with H. musciformis in 200-gal tanks on the beach. The problem of fragmentation mentioned above was also encountered in these large mass cultures. The growth rates obtained with tetrasporophytic and gametophytic clones were abnormally low for the months of February and March, 3.3-3.6% increase in fresh weight per day, as compared to 8.4, 10.3 and 15.8% per day for the same period last year. In previous years' studies, the micro-algae used to feed the shellfish were grown 50-85% of the time in deep water supplemented with chelated trace metals and vitamins, whereas during this past year only unsupplemented deep water micro-algae cultures were used as food for the shellfish (see the period II/20-III/29/1977 in Table 47).

During the period April 15-May 19 we tested the hypothesis that the absence of enrichment to the deep water was the cause for the poor growth rates. Equal flows from the two sides of the Tapes pilot plant were diverted to duplicate 200-gal tanks

TABLE 47. GROWTH RATE OF *Hypnea musciformis* IN EFFLUENT FROM BIVALVE SHELLFISH UNDER VARIOUS CULTURE CONDITIONS

EFFLUENT SOURCE	PERCENT SUPPLEMENTED DEEP WATER	DATES	PLANT SOURCE	PLANT DENSITY (g wet wt liter^{-1})	DILUTION RATE (tank vol. day^{-1})	TANK DEPTH (m)	WATER TEMPERATURE (range, °C)	SOLAR RADIATION (Langleys day^{-1})	GROWTH RATE (% day^{-1})
Tapes japonica clams	ca 85	10/02/74-10/17/74	Tague Bay tetrasporophyte (?)	4.8 -21.4	96	0.3	23.2-31.2	na[1]	20.0
		10/23/74-11/10/74		2.7 -14.9	96	0.3			27.3
Crassostrea gigas (Kumomoto var.) and *Pinctada martensii* oysters	ca 50	01/21/76-01/31/76	Clone STX-14-HM tetrasporophyte	2.8 - 8.5	25-72	0.6	21.1-23.0	na	16.6
		01/31/76-02/10/76		2.5 - 6.1				na	18.0
		02/10/76-02/20/76		2.5 - 4.4				na	8.4
		02/20/76-03/12/76		2.5 -11.0				na	10.3
		03/12/76-03/27/76		2.5 -10.4				434± 74[2]	15.8
		03/27/76-04/10/76		2.5 - 5.9				436± 80	12.5
		04/10/76-04/23/76		2.5 - 6.6				410±124	11.0
Tapes japonica pilot plant	0	02/11/77-03/29/77	Clone STX-14-HM gametophyte	0.57- 1.9	31-63	0.6	21.0-26.5	372± 77	3.6
Tapes japonica pilot plant	0	03/20/77-03/29/77	Clone STX-14-HM tetrasporophyte	0.15- 0.19	47	0.6	22.0-27.9	421± 67	3.3
Tapes japonica pilot plant	0	04/15/77-05/19/77	Rust-op-Twist beach tetrasporophyte	0.13- 2.0	47	0.6	na	na	1.7
Tapes japonica pilot plant	100 (½ rate used in 1974-76)	04/15/77-05/19/77	Rust-op-Twist beach tetrasporophyte	0.13-13.2	47	0.6	na	na	9.7

[1]Data not available. [2]Standard deviation (±); 400-700 nm waveband.

containing 1 kg each of wild H. musciformis collected from the Rust-op-Twist beach. One tank received a supplement equal to one-half that used in previous years (to simulate some of the enrichment being unavailable, incorporated into microalgae and clam feces). The experiments showed that the trace nutrients in the supplement were indeed limiting growth in the unsupplemented deep water: the growth rate in the supplemented tank was 9.7% per day, whereas in the unsupplemented tank it was only 1.7 per day. During the last two weeks the H. musciformis in the unsupplemented tank (only) began to fragment and wash out of the system (see Table 47). Experiments have not been made to determine which component(s) of the supplement allows growth in deep water clam tank effluent.

When the beach H. musciformis cultures were growing very slowly, infestation by epiphytic chlorophytes was a problem. However, when good growth of H. musciformis was obtained in the supplemented clam tank effluent, the epiphytes grew poorly and were not a problem. This may have been due to shading by the rapidly growing H. musciformis.

Experiments to compare carrageenan production of tetrasporophytic and gametophytic phases of H. musciformis were abandoned because we had insufficient laboratory control of the factor(s) (probably temperature) controlling spore production. Unwanted sporulation, resulting in mixtures of gametophytic and tetrasporophytic plants made cultures useless

for the planned experiment.

The responses of Hypnea to elevated temperatures in laboratory cultures were investigated, to determine if high temperatures (>28°C) inhibited growth, as suggested by Dr. John Ryther's group working on Hypnea in sewage mariculture in Florida.

The growth rates of the gametophytic (clone STX-14-HMG) and tetrasporophytic (clone STX-14-HM) phases of H. musciformis were calculated from increases in fresh weight of duplicate 2-cm tip cuttings over a seven-day period. The Hypnea was grown in 200 ml of medium 2f (= f/2 with 4 times the nutrient levels, except f/2 level P) at 28°C, and in medium f at 30 and 32°C, in 10 cm diameter x 7 cm deep Pyrex glass dishes with glass lids, without aeration. The same waterbath and light system were used simultaneously for the Hypnea and for the microalgae experiments reported elsewhere. The results are given in Table 48. The results show that growth rates of both clones of Hypnea are very similar and are very little affected by temperatures as high as 32°C. However, in outdoor cultures where nutrients are sometimes growth-rate limiting, it is possible that high temperature in combination with low nutrient stress may inhibit growth even further. This possibility would have to be tested for in another experiment.

5.5 Shellfish Diet Testing

A preliminary diet test was conducted with Isochrysis

TABLE 48. GROWTH RATES OF Hypnea musciformis AT 28, 30 AND 32°C.

TEMPERATURE	CLONE STX-14-HM (tetrasporophyte)	CLONE STX-14-HMG (gametophyte)
28	0.37 ± 0.01	0.42 ± 0.02
30	0.43 ± 0.04	0.43 ± 0.02
32	0.44 ± 0.11	0.36 ± 0.02

Note: Values are mean doublings in fresh weight per day (mean ± SD).

sp., a flagellate clone isolated from Tahiti (T. Iso), on Tapes japonica larvae (batch #24). The survival and growth of larvae fed a monoculture diet of T. Iso was compared to identical concentration of Tapes batch #24 larvae fed Bellerochea polymorpha (STX-114) and Thalassiosira pseudonana (3H). Through metamorphosis to Day 25, the larvae were maintained in 15-liter buckets, filtered and fed every other day (antibiotic Streptomycin sulfate added), and transferred to 40-liter flumes on Day 15 to set.

Results from the preliminary test are shown in Tables 49 a and b.

Initial growth of the larvae fed T. Iso was more rapid than the larvae fed STX-114 and 3H; however, little growth occurred after Day 8. The larvae fed T. Iso failed to reach setting size by Day 30.

Growth and survival of larvae fed the diet of STX-114 and 3H was average through setting (up to Day 15), but decreased markedly thereafter. The survival of both groups to Day 30 was poor.

A second diet test was conducted to determine the survival and growth of replicate larval concentrations fed T. Iso and 3H or STX-114 (depending on which was available), and larvae fed S-1 (another, unidentified naked flagellate) and 3H or STX-114. Food concentrations were proportioned, as determined by culture turbidity samples, to provide a 1×10^5 ml^{-1} cell concentration. The same larval culturing techniques were followed as in the first diet test.

The results of the second diet test are shown in Table 50a and b.

TABLE 49. GROWTH AND SURVIVAL OF SHELLFISH LARVAE (*Tapes japonica*) FED TWO DIFFERENT DIETS

(a) DIET: STX-114 and 3H

DAY	LARVAE LENGTH AND WIDTH (μ)	% SURVIVAL
4	114 x 91	60
8	155 x 135	43
10	171 x 151	43
15	215 x 198	40
25	229 x 223	39
30	233 x 221	13

(b) DIET: T-Iso (Tahiti *Isochrysis*)

DAY	LARVAE LENGTH AND WIDTH (μ)	% SURVIVAL
4	130 x 108	75
8	165 x 145	63
10	169 x 149	63
15	169 x 156	23
25	186 x 170	23
30	186 x 170	13

TABLE 50. GROWTH AND SURVIVAL OF SHELLFISH LARVAE (Tapes japonica) FED TWO DIFFERENT DIETS

(a) DIET: S-1 and 3H or STX-114

DAY	LARVAE LENGTH AND WIDTH (μ)	% SURVIVAL
2	106 x 85	73
4	110 x 90	31
6	115 x 93	18
8	112 x 93	7

(b) DIET: T-Iso and 3H or STX-144

DAY	LARVAE LENGTH AND WIDTH (μ)	% SURVIVAL
2	98 x 77	66
4	106 x 83	22
6	112 x 92	12
8	114 x 94	6

Growth and survival of both groups of larvae were extremely poor and the experiment was terminated on Day 8.

No valid conclusions may be drawn concerning the quality (as food for shellfish) of T. Iso. The poor survival of larvae in both experiments may be attributed to bacterial immunity to the present antibiotics, or to increased bacterial concentration in the hatchery plumbing.

5.6 Comparative Shellfish Growth Study: Pilot Plant vs. Flume

The present pilot plant operation incorporates a new tank design with an airlift recirculation system in which the culture mixture flows underneath and up through the clams, rather than over and across them, as in previous shellfish tank designs. The comparative growth study of the pilot plant Tapes vs. an identical population reared in trays in a hatchery flume was designed to determine if there were gross differences in shellfish growth rates due to tank design.

Due to food constraints, the Tapes flume populations (initially size and weight groups identical to those in the pilot plant) were reduced by 75%, with matched populations in the pilot plant. Flume populations received a total STX-167 reactor culture flow of 46 ml/10 sec (at approximately 10^5

cells/ml) and the populations were rotated in the flume weekly. After distribution into four trays, the pilot plant populations received 110 ml/sec/tray of STX-167 pool cultures (approximately 1×10^5 cells/ml). The flume populations are cleaned weekly, weighed when their corresponding pilot plant populations are weighed, and mortality recorded.

Due to shellfish priorities and food constraints, the pilot plant-flume comparative study was terminated on 6/7/77. A comparison of individual clam size and weight and total population weight is represented in Table 51.

All pilot plant populations are larger (mean individual length) and weigh approximately twice as much as the flume population clams.

Significant data were available to determine the volume of food (ml) available per second per clam in the flume and pilot plant populations. The results are shown in Table 52. The pilot plant populations are receiving an average of .07 ml/sec/clam or seven times more food than the flume population's .01 ml/sec/clam.

Cumulative mortality, from 12/21/76 to 6/7/77 for the flume and pilot plant populations are given in Table 53. Mortality is significantly greater in all pilot plant tanks than in the corresponding flume populations. Percent mortality figures for the pilot plant and flume populations batches #20, 21, and 22, were 44%, 42%, 27% in the pilot plant and 20%, 5%, 13% in the flume.

At no time in the flume were there large amounts of fecal material on the shellfish, tray, or tray liner. Fecal material

TABLE 51. Tapes COMPARATIVE GROWTH STUDY—PILOT PLANT vs. FLUME—INDIVIDUAL CLAM SIZE AND WEIGHT AND TOTAL POPULATION WEIGHT

	Start (12/21/76)			Finish (06/07/77)					
	Flume & Pilot Plant			Flume			Pilot Plant		
Batch Number:	20	21	22	20	21	22	20	21	22
Size Clam (length mm)	12.6	11.8	9.7	22.7	24.3	20.4	28.6	27.3	25.0
Size Clam (weight g)	.4	.3	.3	2.2	2.9	1.7	5.2	4.9	3.5
Total Clam Population (weight kg)	4.4*	2.3*	3.7*	3.7*	4.9	4.3	29.4	28.5	31.5

*Flume populations culled back by 75% on 12/27/76.

TABLE 52. Tapes COMPARATIVE GROWTH STUDY—PILOT PLANT vs. FLUME—VOLUME OF FOOD AVAILABLE PER SECOND PER CLAM AS OF 06/07/77

	Flume			Pilot Plant		
Batch Number:	20	21	22	20	21	22
Total Clam Population (weight g)	3900	3900	4300	29400	28500	31500
Number Clams	2027	2158	2540	6467	6436	7979
Flow (ml/sec)	18	18	18	440	440	440
Flow (ml/sec/clam)	.01	.01	.01	.07	.07	.06

TABLE 53. Tapes COMPARATIVE GROWTH STUDY—PILOT PLANT vs. FLUME—CUMULATIVE MORTALITY (12/21/76 - 6/7/77)

BATCH NUMBER	FLUME			PILOT PLANT		
	20	21	22	20	21	22
Initial Number	2523	2271	2964	10000	10000	10000
Final Number	2027	2158	2580	6467	6436	7979
Total Mortality	496	113	384	4368	4153	2735
% Mortality	20	5	13	44	42	27

did collect below the Nestier tray on the bottom of the flume; however, no areas of decomposing organic material were found. Tray liners remained in the trays until the experiment was terminated. No spawning was observed in the flume populations.

5.7 Shellfish Feeding Rate Experiment—Experiment No. 5.1.14

This preliminary study was designed to determine if stacking densities in the pilot plant might have been partially responsible for the observed discrepancy between measured and expected weight gains.

Four different weight groups of clams were used, calculated on the basis of individual clam weight and flow rate. The clams used in this experiment were selected from Tapes batch #24's hatchery population.

The experiment was conducted in clear acrylic tubes held vertically in a rack. Individual tube flow rates were set at an initial rate of 4 ml/sec, controlled by metering pumps. The "Tapes tube" study (tube/tray comparison) was run over two one-week periods, with consistent results. Table 54 illustrates the results obtained.

A "standard weight" of 150 g fed at 4 ml/sec was placed in tubes 3 and 4, and identical weights and flow rates were used for trays 7 and 8; the latter constituted a partial replication of the November-December (1975) "constant weight" study (although no aeration was provided for trays 7 and 8;

TABLE 54. Tapes TUBE STUDIES—EXPERIMENT 5.1.14

#1: 5/20/77 - 5/26/77 (F = 4.0 ml/sec):

Tube No.	W_0 (g)	W_6	ΔW 0-6	w_0	w_6*	Δw 0-6	N_{0-6} (Estimated)	Mortality D_{0-6}
1	49.97	62.88	12.91	0.430	0.542	0.112	116	0
2	130.06	153.57	23.51	0.447	0.527	0.080	291	2
3	150.13	169.30	19.17	0.392	0.442	0.050	383	2
4	149.89	171.52	21.65	0.4326	0.496	0.063	346	8
5	259.86	266.18	06.32	0.4309	0.441	0.011	603	44
6	449.95	429.69	-20.26	0.4210	0.402	-0.019	1,069	147
7†	150.06	194.77	44.71	0.4013	0.521	0.119	374	0
8†	150.00	191.83	41.83	0.4552	0.581	0.126	330	0

*Based on a subsample weight of 100 animals. †Trays; no air.

#2: 5/27/77 - 6/3/77 (F = 4.0, Tubes 1, 2, Trays (7+8); F = 8.0 for Tube 3):

Tube No.	W_0 (g)	W_7	ΔW 0-7	w_0	w_7	Δw 0-7	N_{0-7}	Mortality D_{0-7}
1	175.0	207.77	32.77	0.5352	0.6354	0.1002	327	2
2	175.0	198.72	23.72	0.5224	0.5932	0.0708	335	16
3	350.0	401.58	51.58	0.5087	0.5837	0.1144	688	11
7†	175.0	238.24	63.24	0.5401	0.7353	0.1952	324	0
8†	175.0	231.75	56.75	0.5255	0.6959	0.1704	333	1

†Trays.

this difference was explored in more detail in Experiment 5.1.16, undertaken during August 1977 and not discussed in this report.) Other tubes had weights related by a factor of 3 to the standard weights (see Table 54). For Week 2, highly similar conditions were employed, except that only standard weights were used, and Tube 3 (see Table 57) had twice this weight fed at twice the flow rate (8.0 ml/sec).

Unfortunately, protein-nitrogen values for incoming algae to the two experimental configurations are invalid, due to the contaminating presence of a blue-green alga in the feed lines (values obtained exceeded 60 µg-at liter^{-1} in some cases). This problem was corrected during later studies. For expected weight gains, a factor of (1.75) (F) was used to estimate total wet weight gain per day (see Table 57). This assumes a protein-nitrogen content of the inflow of 23.2 µg-at liter^{-1}. Expected whole wet weight increases were calculated for the tube and tray treatments (Table 57) and demonstrate that (1) predicted whole wet weight gains can be achieved in trays, and (2) much less growth (about half) occurs when the animals are stacked in tubes. These results were replicated during the second week. Perhaps the most potentially useful result of these studies was that expected weight gains were achieved under monoculture (STX-167) conditions; the earlier constant weight study had used STX-167 and S-1 as food, while the 1976-77 pilot shellfish tanks were supplied STX-167 only; it had, therefore, been hypothesized that the discrepancy between (E) and (O) might be due to the monoculture diet. Because the above results indicated that high

TABLE 55. FLOW RATES—Tapes TUBE STUDIES (EXP. 5.1.14)—VALUES IN ML/MIN

#1: 5/20/77 - 5/26/77 (E=240 (=4.0 ml/sec); a.m./p.m. readings):

Date	1	2	3	4	5	6	7	8
05/21/77	240	240	240	240	240	240	240	240
	238	240	240	242	240	238	240	240
05/22/77	235	238	240	240	242	240	230	234
	237	239	240	240	240	240	230	236
05/23/77	250	234	237	236	238	240	233	233.5
	240	238	240	240	240	240	238	240
05/24/77	242	242	240	244	240	240	238	242
	-	-	-	-	-	-	-	-
05/25/77	242	230	240	238	240	238	240	230
	232	238	250	240	238	240	248	232
05/26/77	232	234	248	250	248	242	240	230
$\bar{x}$ =	238.80	237.30	241.50	241.00	240.60	239.80	237.70	235.75
s =	5.37	3.59	4.09	3.80	2.84	1.14	5.46	4.49
$\bar{x}$ ml/sec	3.98	3.96	4.03	4.02	4.01	4.00	3.96	3.93

TABLE 56. Tapes TUBE STUDIES (EXP. 5.1.14)—VALUES IN ML/MIN

#2: 5/27/77 - 6/3/77 (EF=4.0 m/sec. 1,2,7,8; 8.0 ml/sec. 3; Tank 3 = 30 sec. sample):

Date	1	2	3	7	8
05/27/77	240	238	240	240	241
	240	240	238	240	240
05/28/77	242	242	240	240	240
	240	242	242	240	240
05/29/77	238	240	242	240	240
	240	238	260	240	238
05/30/77	240	242	270	240	240
	240	239	240	242	241
05/31/77	242	240	250	245	245
	240	240	250	242	246
06/01/77	245	240	250	242	246
	242	242	250	242	246
06/02/77	248	242	249	242	250
	240	242	240	244	248
06/03/77	238	244	240	242	234
	244	240	277.5	244	246
$\bar{x}$ =	241.19	240.69	248.66	241.56	242.56
s =	2.61	1.66	11.55	1.67	4.24
$\bar{x}$ ml/sec	4.02	4.01	8.29	4.03	4.04

TABLE 57. EXPECTED (E) AND OBSERVED (O) WEIGHT GAINS
EXPERIMENT 5.1.14

#1: 5/20/77 - 5/26/77:

Tube No.	"O" 0-6	"E"* 0-6	% "O" of "E"	$\bar{x}$ % "O" of "E"
1	12.91	-	-	
2	23.51	-	-	
3	19.17	42	45.6	48.6 (tubes)
4	21.65	42	51.5	
5	06.32	42	15.0	
6	-20.26	42	-	
7†	44.71	42	106.5	103.0 (trays)
8†	41.83	42	99.6	

†Trays. *$E\Delta w/day = (1.75)(F) = (1.75)(4) = 7.0$; $E\Delta w/6$ days = 42 g whole wet weight

#2: 5/27/77 - 6/3/77:

Tube No.	"O" 0-7	"E" 0-7	% "O" of "E"	$\bar{x}$ % "O" of "E"
1	32.77	49	66.9	57.7 (tubes)
2	23.72	49	48.4	
3	51.58	98	52.6	
7†	63.24	49	129.1	122.5 (trays)
8†	56.75	49	115.8	

†Trays. $E_{0-7} = (1.75(4.0)(7) = 49$ g.

TABLE 58. Tapes TRAY STUDY—6/4/77-6/11/77
Tapes 24; FLOW = 46.0 ML/SEC

W_0 (g)	W_7	ΔW_{0-7}	w_0^*	w_7^*	Δw_{0-7}
1,600.0	2,270.62	620.62	0.6487	0.8140	0.16530

*Based on mean of two (2) subsamples of 50 animals each

Δw_{0-7} = 620.62

$E\Delta w_{0-7}$ = (1.75(46)(7) = 563.50. 0 = 110% E

of 3,200 animals with a mean wet weight of 1.0 g/clam and a mean shell length of ~20 mm/clam.

This represents the upper boundary of animals covered by the November-December 1975 "constant weight" study. The populations were housed in trays with outside dimensions of 30.5 x 20 x 13 cm. The position of an outflow tube controlled tank volume; for all but the tank volume experimental group (see below) which was located 9 cm above the tank floor and will maintain volume at 4 liters. Flow rates were identical for all groups at 4.5 ml sec^{-1}. All groups were fed from 2000-liter reactor cultures inoculated from 16-liter axenic laboratory-grown cultures of STX-167. Reactor cultures were mixed in a PVC chamber affixed to the shellfish rack. With the exception of the "vertical feed" group (see below), which were fed using a tandem bellows pump, all flow rates were controlled by 1/8" i.d. capillary tubing. The western experimental shellfish feeding rack and reactors 14-20 were used for these studies.

Trays	Group Designation	Description
1, 2	MST	Control group; monolayer with screen; 3.8 cm from bottom of tank
3, 4	MSB	As above, with screen on bottom of tank
5, 6	MSN	As above, with no screen (animals on floor of tray)
7, 8	S4	Animals stacked to a depth of 4" in 3" I.D. PVC pipe affixed to bottom of tray
9, 10	S1	As above, stacked to 1" in 6" I.D. PVC pipe
11, 12	SF	As for S4, with feeding from below (vertical feed)

Trays	Group Designation	Description
13, 14	SV4	As above, animals in 3" Vexar mesh
15, 16	SV1	As for S1, stacked to 1" in Vexar mesh
17, 18	MV5	Final layer of animals on bottom of tank with screen; tank volume 500 ml
19, 20	MV14	As above, with tank volume of 1400 ml

Sampling: Samples for protein-N content were taken on inflow at 1400 hrs daily. Approximately 4 liters were sampled, shaken, and 75 ml filtered in replicate for later analysis by the modified Lowry (Folin-Biuret) method. Protein-N samples were taken on the effluent of Tanks 1 and 2 at 1400 daily. Flow rates were measured and recorded at 0800 and 1400 daily and readjusted if deviation exceeded ±5%. On Days 0, 7, 14 and 21, the entire population of each tray was weighed and culled back to 150 g. Culled samples were frozen, and later measured for wet meat weight, dry meat weight and protein content. Shell length to nearest .5 mm was taken for 25 randomly selected individuals from each tray on Days 0, 7, 14 and 21. Total number of animals in each tray was recorded and mean individual wet weight computed.

Tables 59, 60 and 61 illustrate the whole wet weights, mean shell lengths, mean individual weights and mortalities for each tray over the periods Days 0-7, 7-14 and 14-21, respectively. These data are summarized in Table 62. Table 63 illustrates the daily and mean flow rates for all trays, and Table 64 represents a conversion of mean tray flow rates to

TABLE 59. WEIGHT, SHELL LENGTH AND MORTALITY DATA, D_{0-7}. EXPERIMENT 5.1.15.

Tank No.	Tank Designation	W_0 (7/8/77)	N_0	w_0	SL_0	W_7	N_7	w_7	SL_7	ΔW_{0-7}	Δw_{0-7}	ΔSL_{0-7}	Mortality (M)
1	MST	150.4	112	01.34	19.82	172.3	112	01.54	20.61	21.90	0.20	0.79	0
2		150.3	115	01.31	19.89	167.9	115	01.46	19.96	17.60	0.15	0.21	0
3	MSB	150.0	108	01.39	19.36	170.5	108	01.58	20.92	20.50	0.19	1.56	4
4		150.0	110	01.36	20.38	178.6	110	01.62	21.14	28.60	0.26	0.76	0
5	MSN	150.2	119	01.26	19.54	182.5	119	01.53	20.96	32.30	0.27	1.42	0
6		150.7	111	01.36	19.17	181.5	111	01.64	20.88	30.80	0.28	1.71	1
7	S4	150.2	103	01.46	20.24	161.6	103	01.57	21.08	11.40	0.11	0.84	4
8		150.2	125	01.20	19.64	163.5	125	01.31	20.54	13.30	0.11	0.90	3
9	S1	150.5	118	01.28	19.94	179.1	118	01.52	20.09	28.60	0.24	0.15	2
10		150.0	117	01.28	20.28	117.6	117	01.52	21.28	27.60	0.24	1.00	0
11	SF	150.2	110	01.37	19.96	175.1	110	01.59	19.09	24.90	0.22	-0.87	1
12		150.0	112	01.34	18.82	170.4	112	01.52	20.47	20.40	0.18	1.64	3
13	SV4	150.1	123	01.22	18.85	170.5	123	01.39	19.81	20.40	0.17	0.96	3
14		150.3	118	01.27	19.94	173.0	118	01.47	19.64	22.70	0.20	-0.30	3
15	SV1	150.3	124	01.21	20.10	178.0	124	01.44	20.83	27.70	0.23	0.73	0
16		150.6	117	01.29	20.03	179.6	117	01.54	20.48	29.00	0.25	0.45	0
17	MV14	150.0	111	01.35	19.72	176.2	111	01.59	20.78	26.20	0.24	1.06	2
18		150.7	119	01.27	19.08	180.6	119	01.52	21.04	29.90	0.25	1.96	1
19	MV5	150.4	107	01.41	19.29	173.8	107	01.62	21.46	23.40	0.21	2.17	2
20		150.2	115	01.31	19.82	171.8	115	01.49	21.12	21.60	0.18	1.30	2

TABLE 60. WEIGHT, SHELL LENGTH AND MORTALITY DATA, D_{7-14}. EXPERIMENT 5.1.15.

Tank No.	Tank Designation	W_7 (7/15/77)	N_7	w_7	SL_7	W_{14}	N_{14}	w_{14}	SL_{14}	ΔW_{7-14}	Δw_{7-14}	ΔSL_{7-14}	Mortality (M)
1	MST	150.35	96	1.57	20.61	160.58	96	1.67	20.90	10.23	0.10	0.29	9
2		150.33	100	1.50	19.96	131.05	100	1.31	21.83	-19.28	-0.19	1.87	18
3	MSB	150.40	93	1.62	20.92	153.50	93	1.65	21.28	03.10	0.03	0.36	8
4		150.35	91	1.65	21.14	159.77	91	1.87	21.88	19.42	0.22	0.74	5
5	MSN	150.10	96	1.56	20.96	173.65	96	1.81	20.59	23.55	0.25	-0.37	0
6		150.95	89	1.70	20.88	174.27	89	1.96	21.32	23.32	0.26	0.44	0
7	S4	151.05	94	1.61	21.08	164.15	94	1.75	20.80	13.10	0.14	-0.28	3
8		151.05	113	1.34	20.54	169.42	113	1.50	20.80	18.37	0.16	0.26	3
9	S1	150.30	99	1.52	20.09	177.74	99	1.80	20.79	27.44	0.28	0.70	1
10		150.00	95	1.58	21.28	171.04	95	1.80	20.35	21.04	0.22	-0.24	0
11	SF	150.00	92	1.63	19.09	176.18	92	1.92	21.29	26.18	0.29	2.20	0
12		150.10	96	1.56	20.47	171.40	96	1.79	21.03	21.30	0.23	0.56	1
13	SV4	150.11	106	1.42	19.81	169.85	106	1.60	20.75	19.74	0.18	0.94	2
14		150.45	100	1.50	19.64	171.45	100	1.71	21.52	21.00	0.21	1.88	2
15	SV1	150.75	100	1.51	20.83	173.68	100	1.74	21.73	22.93	0.23	0.90	0
16		150.15	97	1.55	20.48	172.65	97	1.78	20.89	22.50	0.23	0.41	0
17	MV14	150.45	90	1.67	20.78	165.10	90	1.83	21.26	14.65	0.16	0.48	5
18		150.80	94	1.60	21.04	170.62	94	1.82	21.20	19.82	0.22	0.16	1
19	MV5	150.98	88	1.72	21.46	160.40	88	1.82	21.53	09.42	0.10	0.07	9
20		150.54	99	1.52	21.12	162.75	99	1.64	21.28	12.21	0.12	0.16	6

TABLE 61. WEIGHT, SHELL LENGTH AND MORTALITY DATA, D_{14-21}. EXPERIMENT 5.1.15.

Tank No.	Tank Designation	W_{14} (7/22/77)	N_{14}	w_{14}	SL_{14}	W_{21} (7/29/77)	N_{21}	w_{31}	SL_{21}	ΔW_{14-21}	Δw_{14-21}	ΔSL_{14-21}	Mortality (M)
1	MST	149.70	93		20.90	165.70	93		21.30	16.00		0.60	4
2		150.20	93		21.83	163.00	93		21.09	12.80		-0.76	5
3	MSB	150.68	87		21.28	159.45	87		21.42	08.77		0.14	5
4		150.00	75		21.88	168.15	75		22.56	18.15		0.68	3
5	MSN	150.41	81		20.59	171.50	81		22.13	21.09		1.54	4
6		150.66	76		21.32	172.90	76		22.31	22.24		0.99	2
7	S4	150.13	84		20.80	162.75	84		21.60	12.62		0.80	3
8		150.32	94		20.80	168.05	94		21.95	17.73		1.15	1
9	S1	150.77	83		20.79	175.65	83		22.69	24.88		1.90	0
10		150.55	80		20.35	172.35	80		22.45	21.80		2.10	0
11	SF	150.95	77		21.29	175.52	77		22.59	24.57		1.30	0
12		150.00	81		21.03	169.78	81		22.61	19.78		1.58	0
13	SV4	150.66	91		20.75	173.65	91		21.76	22.99		1.01	0
14		150.85	84		21.52	175.90	84		22.44	25.05		0.92	0
15	SV1	150.55	85		21.73	168.85	85		21.78	18.30		0.05	4
16		150.85	85		20.89	177.60	85		22.56	26.75		1.67	1
17	MV14	150.72	78		21.26	171.30	78		22.27	20.58		1.01	5
18		150.67	80		21.20	160.15	80		21.52	09.48		0.32	12
19	MV5	150.95	77		21.53	165.05	77		22.78	14.10		1.25	8
20		150.12	89		21.28	173.81	89		22.33	23.69		1.05	0

TABLE 62. SUMMARY—GROWTH DATA, SHELLFISH EXPERIMENT 5.1.15: 7/8/77-7/29/77

Tank No.	Tank Designation	ΔW 0-7	$\bar{T}_{\bar{x}}$	7-14	$\bar{T}_{\bar{x}}$	14-21	$\bar{T}_{\bar{x}}$	0-21	$\bar{T}_{\bar{x}}$	ΔSL 0-7	$\bar{T}_{\bar{x}}$	7-14	$\bar{T}_{\bar{x}}$	14-21	$\bar{T}_{\bar{x}}$	0-21	$\bar{T}_{\bar{x}}$	MORTALITY 0-7	$\bar{T}_{\bar{x}}$	7-14	$\bar{T}_{\bar{x}}$	14-21	$\bar{T}_{\bar{x}}$	0-21	$\bar{T}_{\bar{x}}$	$\bar{T}_{\bar{x}} / \frac{\tilde{m}}{N_0}$
1	MST	21.90	19.75	10.23	-04.53	16.00	14.40	48.13	29.63	0.79		0.29		0.60				0	0	9	13.50	4	4.5	13		
2		17.60		-19.28		12.80		11.12		0.21		1.87		-0.76				0		18		5		23		18 / .16
3	MSB	20.50	24.55	03.10	11.26	08.77	13.46	32.37	49.27	1.56		0.36		0.14				4	2.0	8	6.50	5	4.0	17		
4		28.60		19.42		18.15		66.17		0.76		0.74		0.68				0		5		3		8		12.5/ .11
5	MSN	32.30	31.55	23.55	23.44	21.09	21.67	76.91	76.64	1.42		-0.37		1.54				0	0.5	0	0	4	3.0	4		
6		30.80		23.32		22.24		76.36		1.71		0.44		0.99				1		0		2		2		3.0/ .03
7	S4	11.40	12.35	13.10	15.74	12.62	15.18	37.12	43.26	0.84		-0.28		0.80				4	3.5	3	3.0	3	2.0	10		
8		13.30		18.37		17.73		49.40		0.90		0.26		1.15				3		3		1		7		8.5/ .07
9	S1	28.60	28.10	27.44	24.24	24.88	23.34	80.92	75.68	0.15		0.70		1.90				2	1.0	1	0.5	0	0	3		
10		27.60		21.04		21.80		70.44		1.00		-0.24		2.10				0		0		0		0		1.5/ .01
11	SF	24.90	22.65	26.18	23.74	24.57	22.18	75.65	68.57	-0.87		2.20		1.30				1	2.0	0	0.5	0	0	1		
12		20.40		21.30		19.78		61.48		1.65		0.56		1.58				3		1		0		4		2.5/ .02
13	SV4	20.40	21.55	19.74	20.37	22.99	24.02	63.13	65.94	0.96		0.94		1.01				3	3.0	2	2.0	0	0	5		
14		22.70		21.00		25.05		68.75		-0.30		1.88		0.92				3		2		0		5		5 / .04
15	SV1	27.70	28.35	22.93	22.72	18.30	22.53	68.93	73.59	0.73		0.90		0.05				0	0	0	0	4	2.5	4		
16		29.00		22.50		26.75		78.25		0.45		0.41		1.67				0		0		1		1		2.5/ .02
17	MV14	26.20	28.05	14.65	17.24	20.58	15.03	61.43	56.82	1.06		0.48		1.01				2	1.5	5	3.0	5	8.5	12		
18		29.90		19.82		09.48		52.20		1.96		0.16		0.32				1		1		12		14		13 / .11
19	MV5	23.40	22.50	09.42	10.82	14.10	18.90	46.92	52.21	2.17		0.07		1.25				2	2.0	9	7.5	8	3	19		
20		21.60		12.21		23.69		57.50		1.30		0.16		1.05				2		6		0		8		13.5/ .12

Note: No tanks provided aeration

Key: MST = screen, near top; MSB = screen, bottom; MSN = no screen; S4 = 4" PVC; S1 = 1" PVC; SF = 4" vertical (↑) flow; SV4 = 4" Vexar; SV1 = 1" Vexar; MV14 = 1400 ml volume, screen on bottom; MV5 = 500 ml volume, screen on bottom.

0-7 = Day 0 (7/15) - Day 7 (7/2

$\bar{T}_{\bar{x}}$ = Treatment mean

SL = Shellfish length

$\frac{\tilde{m}}{N_0}$ = Mean (treatment) mortalit mean number on Day 0

TABLE 63. FLOW RATES—SHELLFISH EXPERIMENT 5.1.15—7/8/77-7/29/77
F = ML/MM; EF = 150 (2.5 ML/SEC)

Date	T1	T2	T3	T4	T5	T6	T7	T8	T9	T10
07/08	150	150	150	150	150	150	150	150	150	150
07/09	148	152	156	140	148	162	138	150	142	150
07/10	147.50	150	144	156	149	142	135	144	138	150
07/11	146	146	150	152	150	152	148	150	153	154
07/12	150	150	144	146	130	112	144	132	134	150
07/13	150	150	148	150	150	148	148	148	150	154
07/14	-	-	-	-	-	-	-	-	-	-
07/15	144	153	146	144	146	150	147	138	130	140
07/16	148	146	146	150	150	148	146	142	150	151
07/17	-	-	-	-	-	-	-	-	-	-
07/18	150	158	150	152	150	155.33	154	156	146	152
07/19	150	156	140	146	146	150	150	146	154	144
07/20	150	160	138	150	150	156	144	152	152	150
07/21	140	140	150	156	160	150	144	156	152	152
07/22	154	148	152	144	150	146	142	140	156	150
07/23	150	155	152	130	150	148	155	150	155	155
07/24	147	150	153	145	150	150	145	150	150	150
07/25	152	150	142	146	142	155	160	154	156	150
07/26	150	155	160	150	150	145	150	150	160	150
07/27	147	150	145	148	149	153	148	142	150	146
07/28	150	140	136.50	139	150	150	150	140	150	150
07/29	140	155	155	150	150	150	150	150	155	142
$\bar{x}$	148.18	151.20	147.88	147.20	148.50	148.62	147.40	147.00	149.15	149.50
s	3.54	4.56	6.12	6.04	5.41	9.65	5.66	6.34	7.68	3.82

Date	T11	T12	T13	T14	T15	T16	T17	T18	T19	T20
07/08	150	150	150	150	150	150	150	150	150	150
07/09	158	146	152	150	164	148	140	134	146	144
07/10	153	148	144	149	152	146	146	146	147	146
07/11	154	147	148	150	148	146	147	148	148	148
07/12	150	150	150	150	150	156	146	144	148	150
07/13	154	148	150	148	148	150	148	150	150	146
07/14	-	-	-	-	-	-	-	-	-	-
07/15	150	134	110	144	146	120	144	146	144	150
07/16	150	145	155	147	148	139	146	147	146	147
07/17	-	-	-	-	-	-	-	-	-	-
07/18	152	160	144.67	150	157.33	155.33	146	150	148	150
07/19	152	159	140	150	145	142	144	148	148	144
07/20	154	154	130	152	150	150	150	150	150	150
07/21	148	154	142	156	160	150	160	160	150	148
07/22	150	152	139	144	141	137	150	150	140	146
07/23	150	150	150	140	150	150	145	145	150	150
07/24	150	150	155	145	150	150	145	145	145	145
07/25	158	146	146	161	152	150	146	154	150	140
07/26	160	150	140	145	140	140	145	140	140	145
07/27	158	150	150	150	137.50	140	140	147.50	150	150
07/28	110	150	137	145	110	127.50	140	137.50	137.50	127.50
07/29	150	150	142	150	120	100	140	130	140	140
$\bar{x}$	150.55	149.65	143.73	148.80	145.94	142.34	146.40	146.10	146.38	145.08
x	10.14	5.35	10.19	4.55	12.39	13.35	5.13	6.75	4.05	5.44

TABLE 64. FLOW RATES—SHELLFISH EXPERIMENT 5.1.15—7/8/77-7/29/77. MEAN FLOW, ML/SEC

	T 1/T 2	T 3/T 4	T 5/T 6	T 7/T 8	T 9/T10	T11/T12	T13/T14	T15/T16	T17/T18	T19/T20
	2.47/2.52	2.46/2.45	2.48/2.48	2.46/2.45	2.49/2.49	2.51/2.49	2.40/2.48	2.43/2.37	2.44/2.44	2.44/2.42
$\bar{x}$	2.49	2.46	2.48	2.45	2.49	2.50	2.44	2.40	2.44	2.43

$\bar{x}$ of treatment means (N=10) = 2.46

x = 0.03

treatment mean flow rates (E = 2.50 ml/sec). Flow rate control, while not perfect, appears to have been adequate. Table 65 shows the mean daily inflow concentration of protein-nitrogen, NO_3+NO_2, and NH_4. Outflow values for tanks 1 and 2 are also shown. The dissolved inorganic data were not collected after July 22, 1977.

Table 66 summarizes the experiment by comparing expected to observed weight gains. The treatment groups display a wide variation (observed ranges from 31.4 to 81.1% of expected for the three-week study). None of these differences have been subjected to statistical analysis, and it is very likely that differences between replicates in some conditions invalidate the observed treatment differences. Lowest weight gains occurred in tanks 1 and 2 (epoxy-coated hardware cloth screen about 1/2" from top of water in tank, animals in monolayer), tanks 3 and 4 (screen on bottom), tanks 19 and 20 (screen on bottom, 500 ml volume), tanks 17 and 18 (screen on bottom, 1400 ml volume) and in tanks 7 and 8 (animals stacked to 4" in a PVC cylinder). Greatest weight gains occurred in tanks 5 and 6 (animals on bottom, no screen), tanks 9 and 10 (stacked on bottom to 1" in PVC cylinder), tanks 15 and 16 (as in 9 and 10, with a Vexar cylinder), tanks 11 and 12 (stacked to 4", fed from the bottom), and tanks 13 and 14 (stacked to 4" in Vexar cylinder).

The conclusions of the study (which may be altered in the wake of more complete analysis) are:

(1) The presence of a screen does not improve weight gain, nor does it decrease mortality. In fact, most conditions with

TABLE 65. PROTEIN-NITROGEN AND DISSOLVED INORGANIC 'NITROGEN' VALUES
SHELLFISH EXPERIMENT 5.1.15—7/11/77-7/29/77

	Date	Pro-N IN	Pro-N OUT T1	Pro-N OUT T2	NH_4 IN	NH_4 OUT T1	NH_4 OUT T2	NO_3+NO_2 IN	NO_3+NO_2 OUT T1	NO_3+NO_2 OUT T2
	07/11	30.44	06.11	06.51	01.07	02.61	02.35	00.51	00.73	00.59
WK	07/12	30.42	10.67	07.67	01.21	02.86	02.70	00.37	00.52	00.29
1	07/13	21.98	07.69	10.13	01.09	02.81	02.67	01.34	01.77	01.71
	07/14	21.50	05.23	07.80	00.33	03.07	03.05	26.25	22.60	22.71
	07/15	34.85	07.48	10.73	00.43	02.97	03.26	00.82	02.16	02.07
	07/16	24.81	16.11	21.99	00.17	00.25	00.17	00.33	00.38	00.48
	07/17	27.56	17.45	23.68	-	00.70	01.53	00.84	03.11	01.65
WK	07/18	22.43	15.49	19.75	00.18	00.69	00.84	00.80	00.66	00.66
2	07/19	22.69	12.60	14.04	00.31	03.03	03.44	00.53	05.23	05.02
	07/20	19.83	09.48	22.39	00.11	01.64	06.04	03.19	05.77	04.87
	07/21	15.56	04.89	09.63	00.22	06.52	06.63	01.54	03.01	03.06
	07/22	19.67	02.96	07.02	00.38	03.62	04.07	11.58	12.81	13.10
	07/23	19.16	02.56	02.64	discontinued----------					
	07/24	21.14	04.76	01.85	----------					
WK	07/25	23.22	07.91	03.46	----------					
3	07/26	22.70	06.98	06.59	----------					
	07/27	24.10	15.82	07.43	----------					
	07/28	23.81	20.81	10.99	----------					
	07/29	----------								
Days	$\bar{x}$	26.09	07.43	08.03	00.93	02.84	02.69	07.12	06.41	06.33
0-7	s	05.02	02.39	01.52	00.40	00.19	00.29	12.76	10.81	10.94
Days	$\bar{x}$	23.96	11.93	17.46	00.24	02.26	03.13	01.15	02.90	02.54
7-14	s	06.11	04.77	05.87	00.21	02.18	02.50	00.97	02.07	01.85
Days	$\bar{x}$	21.97	08.83	05.71	----------					
14-21	s	02.00	06.92	03.23	----------					
Days	$\bar{x}$	23.66	09.72	10.79	00.50	02.56	03.06	04.01	04.90	04.68
0-21	s	04.65	05.43	06.86	00.41	01.67	01.89	07.67	06.58	06.70

Notes: 7/8-7/10 eliminated due to blue-green algae contamination; all values are means of replicates and are in µg-at liter^{-1}; all samples taken at 1400 hr.

TABLE 66. EXPECTED (E) AND OBSERVED (O) WHOLE WET WEIGHT GAINS—TANK AND TREATMENT MEANS SHELLFISH EXPERIMENT 5.1.15—7/8/77-7/29/77

Note: For $E\Delta W$,

$$\Delta w/day = (.306)(\beta x 2.8x10^{-6})(24x3600xF)(33.125)$$

where $\beta = \bar{x}$ protein-nitrogen inflow ÷ deep-water nitrate
$= 23.66 \div 32$
$= .74$

$$\Delta w/day = (.306)(.74x2.8x10^{-6})(24x3600xF)(33.125)$$

$$= 1.81$$

	F	(21x)(1.81)(F)	EΔW	OΔW	O%E	O%ET
T 1	2.47		94.12	48.13	51.1	31.4
T 2	2.52		96.03	11.12	11.6	
T 3	2.46		93.74	32.37	34.5	52.6
T 4	2.45		93.36	66.17	70.7	
T 5	2.48		94.50	76.91	81.4	81.1
T 6	2.48		94.50	76.36	80.8	
T 7	2.46		93.74	37.12	36.4	44.7
T 8	2.45		93.36	49.40	52.9	
T 9	2.49		94.89	80.92	85.3	79.8
T10	2.49		94.89	70.44	74.2	
T11	2.51		95.65	75.65	79.1	72.0
T12	2.49		94.89	61.48	64.8	
T13	2.40		91.46	63.13	69.0	70.9
T14	2.48		94.50	68.75	72.8	
T15	2.43		92.60	68.93	74.4	80.5
T16	2.37		90.31	78.25	86.6	
T17	2.44		92.98	61.43	66.1	61.1
T18	2.44		92.98	52.20	56.1	
T19	2.44		92.98	46.92	50.5	56.5
T20	2.42		92.22	57.50	62.4	

the hardware cloth screen did poorly. Metal ion contamination cannot be ruled out as a cause, but it is unlikely to be the single or major factor since some groups with these screens did fairly well.

(2) Stacking to 1" does not appear to have a significantly detrimental effect on growth or mortality, while stacking to 4", at least under some configurations, does have a detrimental effect.

(3) Feeding from the bottom apparently does not, by itself, significantly interfere with feeding and growth.

(4) A low (500 ml) volume at the flow rate (2.5 ml/sec) does appear to have a deleterious effect on feeding and/or growth, while increasing the volume to 1400 ml appears to reduce or eliminate the effect.

(5) Animals of this size (about 20 mm) apparently do much better on the bottom of the tanks; elevating the animals had a clear, negative effect. (This effect was confirmed in a later experiment, 5.1.16, completed during August, 1977 and not presented in this report.)

(6) Most significant, at least from the long-range point of view, was the fact that the "constant weight" controls did poorly, and that factors not controlled in the study (e.g., "fecal accumulation") may be required to explain the results post hoc.

Although this study definitely requires more complete analysis,

it did serve to confirm earlier results of the negative effect of high-density stacking, and it aided in the design of new pilot plant shellfish tanks by pointing out that elevation of animals and/or the presence of a screen is not advantageous and may, in fact, have a significant detrimental effect.

A number of other points also require emphasis:

—The study was relatively short-term and certainly small-scale, with significant mortalities in some replicates, but not others. Data collected during the third week indicate a diminishing treatment effect with time, which may indicate some adaptation of the animals to a "new" environment.

—The results were obtained on 20-mm animals and may not be applicable to animals of widely varying sizes (or ages) and/or with widely varying prehistories. For example, stacking may be more detrimental to older or larger animals, or they may be more susceptible to bacterial contamination resulting from fecal degradation.

6. PUBLICATIONS AND PAPERS PRESENTED AT MEETINGS

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7. ACKNOWLEDGEMENTS

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APPENDIX A

ARTIFICIAL UPWELLING MARICULTURE:

TECHNICAL DESCRIPTION

May 1977

ARTIFICIAL UPWELLING MARICULTURE

TECHNICAL DESCRIPTION

Introduction

— Organic material is synthesized in the ocean from inorganic compounds. This transformation is performed by autotrophic organisms utilizing sunlight as a source of energy. The most important photo-autotrophs, quantitatively, are the algae (1; p. 58).

— Both energy and inorganic material (nutrients) are required to sustain the production of high-energy organic compounds. In tropical oceans, the "primary productivity" is limited by the rate of nutrient renewal. Much of these nutrients is derived from the decomposition of organic matter. Whenever this decomposition occurs below the euphotic zone, the nutrients remain unused and, except in areas of natural upwelling, return to the surface by slow diffusion. High nutrient concentrations are encountered at depths of 500 fm. (900 m)(2; p. I-19). This nutrient-rich deep-sea water can be brought to the surface with a minimal expenditure of energy.

— Direct harvesting of planktonic algae (phytoplankton) is impractical because of the small size and low concentration at which these organisms occur. The phytoplankton can sustain larger animals, which constitute usable sources of human food.

— A set of conditions under which a two trophic-level phytoplankton-shellfish "artificial upwelling" mariculture can operate has been established (3). Extrapolation of the collected data to large installations, and the economic optimization of such plants, requires a definition of the laws governing total productivity.

Objective

Our objective is to establish an adequate quantitative relationship (transfer function) between the mariculture's outputs, its inputs, and its environment.

The expressions are to be based on our understanding, or best interpretation, of the mechanisms or reactions involved. They will be deemed adequate if their application yields predictions within 10% of actual observations, or within observational error, if larger.

In areas where no logical mechanism can be introduced, empirical expressions satisfying experimental evidence may be used. These sections are identified in the margin and are subject to a continuing analysis.

Initial Hypothesis

I. A spectrum of elements is involved in the exchanges taking place within our system. "Nitrogen" is considered the limiting chemical component in the food stream.

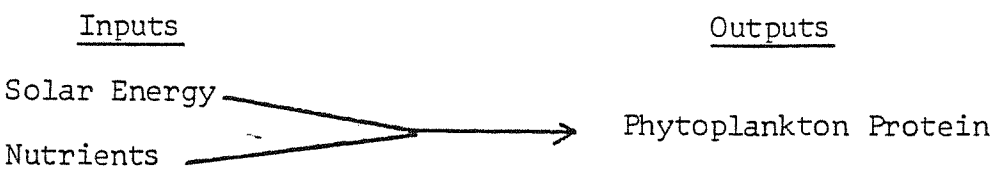
II. Each trophic level is considered an independent entity, related to the other level only through the output/input link, as defined.

III. Some operating conditions can be controlled and/or

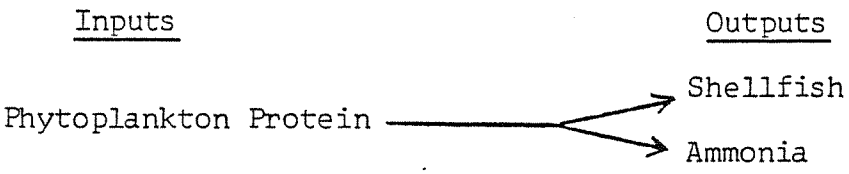
maintained constant. Others have time-dependent fluctuations. It is assumed that storage mechanisms within the organisms will absorb these fluctuations and allow time-variant inputs to be replaced by constant averages.

Definition of Trophic Levels

- First Level : Phytoplankton



- Second Level : Shellfish



First Trophic Level : Phytoplankton

Phytoplankton absorbs nutrients and transforms them into protein with the help of solar energy, according to:

$$P = c \alpha \eta_1 v$$

where:

P =	phytoplankton protein produced	(g)
c =	nutrient concentration	(g-at. N cm^{-3})
α =	equiv. N to protein ratio (14 x 6.25) = 87.5	-
η_1 =	conversion efficiency of NO_3-N to phytoplankton protein-N	-
V =	volume of deep-sea water handled	(cm^3)

η_1, for a specific phytoplankter, is a function of the exposure to sunlight of the culture. This exposure is, in turn, a function of the pool turnover rate and of the pool depth.

At a compensation depth (D_c) the light intensity equals the respiration needs of the phytoplankton (1; p. 83).

If this depth is exceeded, a net decrease in total production results.

The compensation depth corresponds approximately to the depth at which incident light is attenuated by a factor of 0.01. At that depth:

$$e^{-\mu D_c} = 0.01 \quad \therefore$$

$$-\mu D_c = \ln 0.01 = -4.61$$

where μ = extinction coefficient (cm^{-1})

The average light attenuation, in a column of depth D_c is:

$$\frac{I_{av}}{I_o} = \frac{1}{D_c} \int_o^{D_c} e^{-\mu z} dz = \frac{1}{\mu D_c} (1-e^{-\mu Dc}) = .215$$

where z is the vertical axis.

From experimental data collected in St. Croix by Mary W. Farmer (4), a reactor shaded to 20% of incident light could be operated at a turnover rate of .7 day^{-1}. The measured value of μ = .01158 cm^{-1}, and:

$$D_c = \frac{\ln 0.01}{-\mu} \approx 400 \text{ cm}$$

In reactors approximately 80 to 100 cm deep, a turnover rate of 1.2 day^{-1} can be maintained.

A linear expression for turnover rate versus depth can be derived to fit both data points. For:

$$\text{turnover rate} = f(z) = k_i + az = \frac{\dot{V}}{Az} \qquad (sec^{-1})$$

where: $\dot{V}$ = deep-sea water flow rate $(cm^3\ sec^{-1})$

A = pool area (cm^2)

$$f(100) = 1.2/(24 \cdot 3600) = 13.9 \times 10^{-6}$$

$$f(400) = 0.7/(24 \cdot 3600) = 8.10 \times 10^{-6}$$

$$a = \frac{(8.10-13.9)\times 10^{-6}}{400-100} = -1.93 \times 10^{-8}$$

$$k_i = (13.9\times 10^{-6}) - 100\ a = 13.5 \times 10^{-6} \qquad (sec^{-1})$$

The turnover rate expression can be normalized into:

$$\frac{\dot{V}}{Az} = k_i\left[1 + \left(\frac{aD_c}{k_i}\right)\frac{z}{D_c}\right]$$

and rounded:

$$\approx k_i\left(1-\frac{z}{2D_c}\right)$$

Note: The value of D_c, as determined from MWF's experiment, may be different for different phytoplankters. If a higher concentration of nutrients is used (c), the cultures are expected to have a higher density and extinction coefficient. The product μD_c should remain constant, which will be approximated by maintaining $c \cdot D_c$ constant (neglecting the contribution to μ of clear seawater).

In the expression which relates the turnover to the pool depth, $1/k$ represents the exposure factor.

η_1 can now be defined as a function of k. In our present pool system, with a 1-m depth and a turnover rate of 1.15 day^{-1}

$$\frac{\dot{V}}{Az} = 1.33 \times 10^{-5} = k\left(1-\frac{z}{2D_c}\right)$$

and: $k = 1.52 \times 10^{-5}$

Measured conversion efficiency (5), averaged over a 36-day period, under these operating conditions, shows that:

$$\eta_1 = .69$$

No other measurements allowing us to relate η_1 to k are available at this time.

It is known, however, that high turnover rates lead to a "washout" of the culture, resulting in a drastic drop in conversion. It is also impossible for η_1 to exceed 1.0 even at high values of the exposure factor.

Evaluating the coefficients of:

$$\eta_1 = a_2k^2 + a_1k + a_0$$

for:

$$\begin{cases} \eta_1\big|_{k=0} = 1 \\ \dfrac{d\eta_1}{dk}\Big|_{k=0} = 0 \\ \eta_1\big|_{k=1.52\times10^{-5}} = .69 \end{cases}$$

yields:

$$a_0 = 1$$

$$a_1 = 0$$

$$a_2 = -1.34 \times 10^9$$

$$\eta_1 = 0 \text{ for } k \sqrt{\frac{1}{1.34\times10^9}} = 2.73 \times 10^{-5}$$

This value of k corresponds to a turnover rate of 2.06 day^{-1} in a 1-m pool, or 1.18 day^{-1} in a 4-m pool (or .215 shaded reactor). At these turnover rates, complete "washout" can be expected.

Second Trophic Level : Shellfish

Shellfish assimilates phytoplankton, and in the process increases its wet weight, according to:

$$S = \beta \eta_2 P$$

where:

S = shellfish wet weight increase (g)

β = protein to wet weight ratio (33.125) -

η_2 = conversion efficiency of plant protein to animal protein -

η_2, for a specific phytoplankton/shellfish combination, is a function of the rate at which phytoplankton is presented to the shellfish. If this rate is low, only the vital needs will be

satisfied, and no net weight increase will result ($\eta_2 = 0$). If the rate is too high, all available food cannot be assimilated.

Growth rates, in terms of $\frac{\dot{w}}{w}$, are not constant, over the life span of the shellfish. Studies have shown that over a wide range of sizes, the filtration rate of shellfish is proportional to $w^{0.73}$, where w represents the individual weight of each animal (6). A further decrease of this rate, for large (or old?) animals was noticed, but not evaluated.

The filtration rate, and the criterion by which to characterize feeding rate, seems more closely related to the animal's area $\left[l^2=(\frac{w}{\rho})^{\frac{2}{3}}\right]$ than to its weight, or volume ($l^3= \frac{w}{\rho}$).

In our analysis we have adopted:

$$F = \frac{\dot{P}}{N(w)^{\frac{2}{3}}} \qquad (g^{\frac{1}{3}}\ sec^{-1})$$

as our feeding criterion.

F = protein feeding rate per (individual weight)$^{\frac{2}{3}}$

$\dot{P}$ = phytoplankton protein inflow rate ($g\ sec^{-1}$)

N = number of animals -

w individual weight (g)

Applying this criterion to data collected in St. Croix during a constant-weight study in November-December 1975, has provided the following quadratic least-square fit expression for $\eta_2 = f(F)$:

$$\eta_2 = a_2(\ln F)^2 + a_1(\ln F) + a_0$$

$a_0 = -16.89144976$

$a_1 = -\ 1.891173337$

$a_2 = -\ 0.0518$

within the following boundary:

$$7 \times 10^{-9} \leq F \leq 9 \times 10^{-8}$$

Notes & References

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APPENDIX B

NITROGEN BALANCE AND CLAM GROWTH IN AN ARTIFICIAL UPWELLING MARICULTURE SYSTEM AT DIFFERENT FOOD FLOW RATES AND SHELLFISH DENSITIES

by

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Abstract

The conversion of deep-sea water nitrate to algal protein and further to clam-meat protein was studied at the St. Croix Artificial Upwelling Project (U.S. Virgin Islands), where nutrient-rich water, pumped from 870-m depth in the sea, is used as the raw material for a mariculture system. Unialgal cultures of Chaetoceros curvisetus (STX-167) and S-1, an unidentified naked flagellate, were grown individually and continuously in onshore pools provided with deep-sea water. The cultures were combined 1:1 in a mixing tank and fed continuously to several batches of Tapes japonica for 36 days. Thirty-five, 70 and 140 g batches of clams, in 4-liter containers, received a continuous food flow-rate of 1 ml/sec. Thirty-five, 50, 70, 100, and 140 g batches of clams, in 4-liter containers, received a 2 ml/sec food flow-rate. The particulate protein nitrogen and dissolved NH_4^+ and (NO_3^- + NO_2^-) entering and leaving each shellfish tank were measured daily. Every nine days all of the clams were weighed and measured, enough clams were harvested from each batch to bring the total weight back to its starting level, and the tank deposit was separated, measured and analyzed for each group.

Sixty-nine percent of the deep-water nitrate-nitrogen (concentration 31 µg-at/l) was converted into algal protein-nitrogen over the 36-day period. From 31% to 35% of the algal protein entering the Tapes feeding tanks was converted into clam meat protein by the 1 ml/sec flow groups, and between 24% and 33% of the algal protein was converted into clam meat protein by the 2 ml/sec flow groups.

Maximal individual clam growth was obtained in the 35 g, 2 ml/sec group, with a 1.42 mm/week shell-length increase and a 41.1% weekly increase in fresh weight per clam. This fastest individual clam growth was obtained at the lowest percent stripping of algal protein. The greatest clam population growth occurred for the 100 g, 2 ml/sec group, with a total weight gain of 134 g in 36 days.

Ammonium ion concentration increase in the shellfish tank was highest at the slowest individual clam growth.

The Protein Efficiency Ratios in this experiment varied between 8 and 14, indicating that the algal food source used is a good one for Tapes japonica.

Introduction

In our Artificial Upwelling Project in St. Croix, Antarctic Intermediate Water is pumped from 870-m depth in the sea into 45,000-liter concrete pools on shore in which unialgal cultures of planktonic diatoms are grown continuously. The pool cultures are started by inoculating them with cultures grown in 757-liter polyethylene tanks. The growth rate of the algae is regulated by the rate at which nutrients are supplied by the incoming deep water; the deep water flow is regulated to assure nearly complete utilization of the nutrients in the deep water.

The system produces 114,000 l of nearly unialgal diatom culture per day (10^4-10^6 cells/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.

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*, Kumamoto variety; *Tapes japonica*, japanese little-neck clam; *Mercenaria campechiensis*, southern clam or quahog; F_1 clam, a cross between *M. campechiensis* x *M. mercenaria*; *Argopecten irradians*, bay scallop. *Pinctada martensii*, the pearl oyster, is also growing very rapidly in the system.

Panulirus argus, the spiny lobster, Strombus gigas, the queen conch and carrageenin-producing seaweeds are grown in the effluent of the shellfish tanks (Othmer and Roels, 1973; Roels et al., 1976).

The purpose of the present experiment was to determine

1. optimum shellfish densities and food flow rates to achieve maximum shellfish growth
2. the efficiency of "nitrogen"-transfer in this food chain, from deepwater nitrate to phytoplankton protein, to clam meat protein.

In the present experiment, unialgal cultures of the diatom Chaetoceros curvisetus (St. Croix 167) and of S_1, an unidentified naked flagellate were grown in deep water as food for the clam, Tapes japonica.

Tapes japonica was chosen as a test animal because it grows well in the system and can easily be spawned in our St. Croix hatchery. We have demonstrated earlier that a mixture of Chaetoceros curvisetus (St. Croix 167) and S_1 is a good food for Tapes japonica in our system (Rodde et al., 1976).

Materials and Methods

Animals

The clams used in this nitrogen balance and growth experiment were Tapes japonica (Deshayes). The average length of the clams was 12.7 mm at the beginning of the study. Only the fastest growing clams comprising the top 25%, less the top 1%, of the original population were used for our work. These were second

generation clams grown at the Artificial Upwelling Mariculture Station on St. Croix from brood stock originally obtained from Pacific Mariculture in California. Standard spawning procedures outlined by Loosanoff and Davis (1963) were used.

Phytoplankton

The clams were fed a mixed diet of Chaetoceros curvisetus (STX 167) and S-1, an unidentified naked flagellate. The mixed algal diet contained on the average 21.5 µg-at PPN (particulate protein nitrogen) per liter. Previous work has shown the PPN of these phytoplankton to be 90% of the total nitrogen (Dorsey et al., 1977). Assuming a chemical composition equal to that given for Chaetoceros sp. by Parsons et al. (1961), the dry weight of this algal suspension would be approximately 5.7 mg/l. At the two flow rates employed in this experiment, 938 or 469 µg-at PPN day^{-1}, equivalent to 246 or 123 mg dry weight of algal cells, were fed per day to each batch of Tapes in a 4-liter tank. A cell suspension with 22 µg-at PPN contains 5.3×10^4 cells/ml $\pm$ 15% of STX 167 or 1.2×10^5 cells/ml $\pm$ 15% of S_1. Chaetoceros curvisetus (STX 167) was isolated by Dr. Kenneth Haines at the Artificial Upwelling Station and grows well at temperatures up to 30°C. The S-1 flagellate was obtained from Guillard and isolated by him from the Sargasso Sea.

Chemicals

All chemicals for the protein and inorganic assays were obtained from Fisher Scientific, Freehold, New Jersey, or from J. T. Baker Chemical Co., Phillipsburg, New Jersey. The crystallized and lyophilized bovine serum albumin protein

standard came from Sigma Chemical Co., St. Louis, Mo.

Experimental Design

Nutrient-rich deep sea water containing 31 µg-at liter^{-1} NO_3^- was pumped continuously through three 3" diameter polyethylene pipes from 870 meters below the sea surface into a 12,000 gallon concrete pool used for growing Chaetoceros curvisetus. S_1-was grown in continuous culture in several 2,000 liter concrete containers. The turnover rate for both cultures was 1.1/day. Backup cultures of the phytoplankton were always ready in case of mishap or contamination to maintain an average of 21.5 µg-at PPN/l for the mixed culture.

The cultures of Chaetoceros curvisetus and S-1 flowed at 23 ml/sec each into a 40-liter cylindrical polyethylene mixing container. The culture mix was fed to sixteen shellfish tank feeding lines at constant pressure. Figure 1 gives a schematic diagram of the experimental layout.

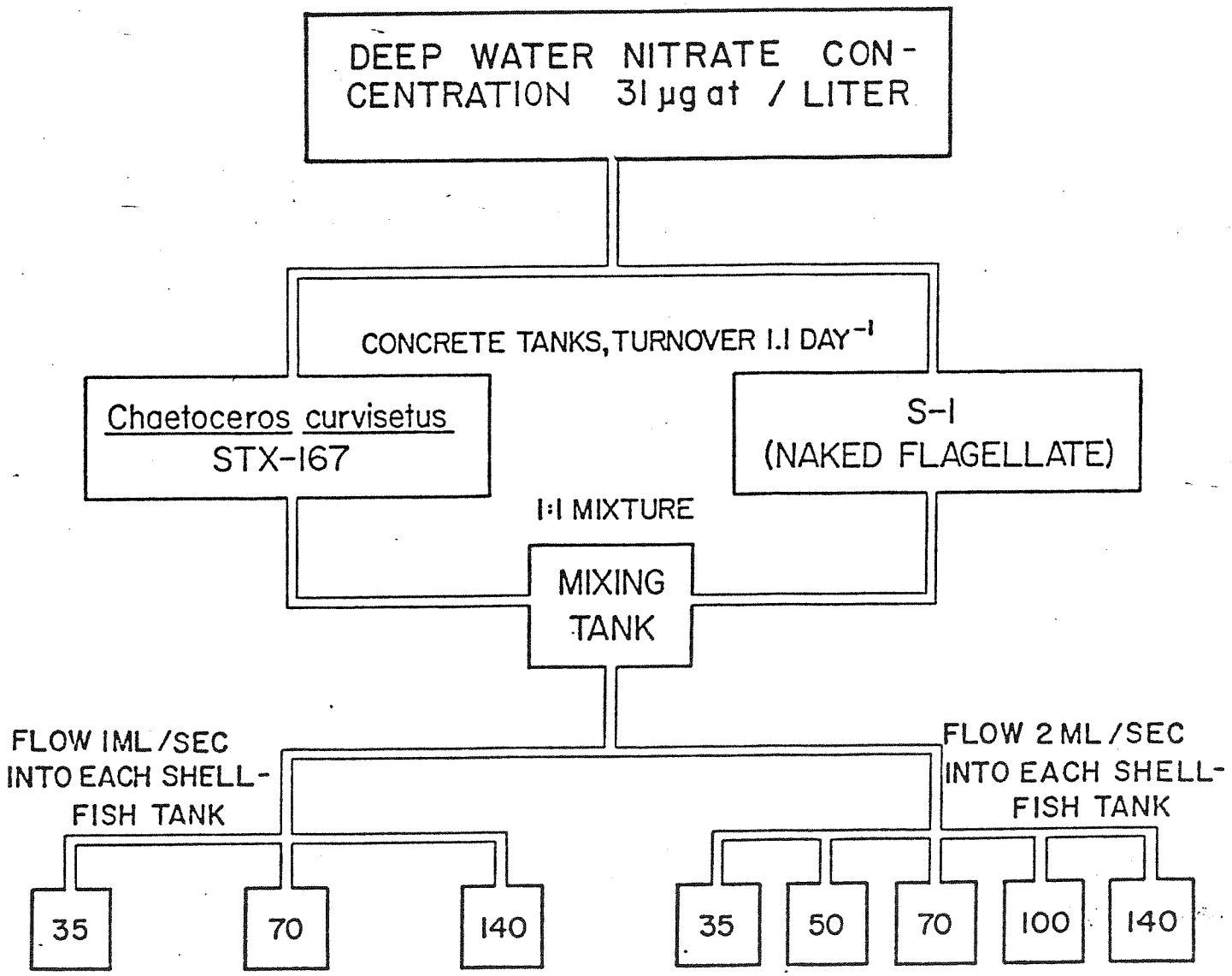
Figure 1

The mixing chamber was vigorously stirred by an air stream. The outflow from the mixing tank flowed to 16 shellfish tanks with a capacity of 4 l each.

The flow to the 4-liter shellfish tanks (12" x 7.75" x 5.13") was regulated to provide either 2 ml/sec or 1 ml/sec. The flow to each shellfish tank was controlled by a length of 1/16" teflon tubing coming from the feed level pipe.

The shellfish feeding tanks had a perforated air tube at each end to mix and aerate the water. In addition 1/4" chicken screening,

DIAGRAM OF NITROGEN BALANCE STUDY



coated with polyester resin to avoid metal ion contamination, was laid horizontally 1.5" off the tank bottom. This screening kept the clams off the bottom and prevented them from coming in contact with the tank deposit. No shellfish died during this experiment.

The shellfish tanks had an overflow tube keeping their volume at 4 liters. One set of shellfish tanks received an algal culture flow of 2 ml/sec and another set received 1 ml/sec resulting in turnover times of 33 or 66 minutes respectively. The 2 ml/sec flowrate provided 173 l/day and the 1 ml/sec flowrate provided 86.5 l/day or algal culture to the shellfish.

The clam densities used were 35, 70 and 140 g per 4.0 liter tank for the 1.0 ml/sec flow and 35, 50, 70, 100 and 140 g per 4.0 liter tank for the 2.0 ml/sec flow. All experiments were run in duplicate, i.e. there were 16 experimental shellfish tanks in operation simultaneously for 36 days.

The salinity of the system was 34.75-34.95% and the temperature was $25^{o}C \pm 3^{o}C$.

The flow rates into the algal culture tanks and out of the shellfish feeding tanks were measured and adjusted, if necessary, at 8 o'clock in the morning and again at 2 o'clock in the afternoon each day of the experiment. Flow rates were adjusted if they deviated by 2% or more from the planned rate.

Sampling

Duplicate one-liter samples of the mixing-tank outflow were taken each day at 2 o'clock for the determination of the particulate protein nitrogen and dissolved NO_3^-, NO_2^- and NH_4^+.

Duplicate two-liter samples of the shellfish-tank outflow were taken each day at 2 o'clock for the determination of particulate protein nitrogen and dissolved inorganic nitrogen leaving the shellfish feeding tanks for each of the different experimental treatments.

The test populations of Tapes japonica were weighed, counted, measured and culled back to the original clam weight on days 9, 18, 27 and 36 of the experiment. After all measurements were taken, the culled animals were frozen until we were ready to determine wet and dry meat weight and meat protein nitrogen.

For simplicity, the experimental treatment in which 35 g of Tapes were kept in a 4-liter tank receiving a flow rate of 1 ml/sec is referred to as the 35 g, 1 ml/sec group.

Analytical Methods

Clam growth. The clam population growth rate of Tapes japonica was measured by the increase in the mass of each batch of whole clams. The individual clam growth rate was determined by measuring the average increase in length of 25 culled clams at the end of the 36-day experiment, using a micrometer.

Whole clam weight and wet meat weight. Tapes japonica were blotted dry with paper towels and weighed alive for whole clam weight. To get the wet meat weight, the shellfish were first frozen and then shucked by using a spatula to open the clams and to scoop out the frozen meat as a single mass. The wet meat was then placed in a tared glass petri dish and weighed. This method of shucking was necessary because of the small size and the large number of clams used in this experiment. The frozen meat could

be quickly and more easily removed in this manner. While shucking a batch of clams, the remaining clams in that same batch were kept from melting by keeping them on a surface kept at low temperature.

Dry meat weight. Each batch of clam meat was freeze-dried at 60°C for 24-48 hr and then placed in a desiccator for several days. After the clam meat had reached constant weight, the dry meat was scraped from the glass petri dish completely with a single-edged razor blade and weighed on a glassine paper. The clam shells were dried separately in a similar manner.

Shell weight and shell protein. The wet and dry weights of the shells were determined. After the dry weights were taken, the shells were ground to a fine powder with a mortar and pestle. Ten mg of ground shell gave an appropriate final color absorbance with the heated biuret-Folin assay for protein-nitrogen measurement (Dorsey et al., 1977).

Clam protein nitrogen. The dry clam meats were pulverized to a fine powder with an all-glass mortar and pestle. A portion was weighed and then homogenized at 1000 rpm for 2 min in a Potter-Elvehehm homogenizer with biuret-reagent before measuring protein by the heated biuret-Folin method (Dorsey et al., 1977).

Particulate protein nitrogen. Particulate protein in the algal pools, the clam tank deposits, and the clam-tank effluents were also measured by the heated biuret-Folin method with some modifications. The particulate algal protein samples were filtered onto Gelman 25-mm diameter, 0.45 micron pore-size, glass fiber filters. The entire filter was then heated in biuret reagent for 100 min at 100°C. After this, Folin reagent was

added immediately. After the final color development was complete, the heavy-walled test tubes were centrifuged at 100 x g for 5 min to remove the glass fiber filter before comparing the color with bovine serum albumin standards which were also heated for the same length of time (Dorsey et al., 1977). The absorbance was read at 660 nm in a Gilford model 240 spectrophotometer with 1.0 cm cuvettes.

Inorganic "nitrogen" determinations. The ammonia, nitrite and nitrate concentrations were all determined with a Technicon AutoAnalyzer II, running at 30 samples per hr. The colorimetric assays were done as described in the standard Technicon AutoAnalyzer methodology handbook (methods which are based on procedures given by Strickland and Parsons, 1972). A computerized peak integrator was used to determine the concentrations of these nitrogen-containing compounds.

Results

Deep-Water Nitrate Conversion into Algal Protein

At the 1.1 day^{-1} turnover rates used for Chaetoceros curvisetus (STX-167) and S-1 culture tanks, 69% of the deep-water nitrate nitrogen was converted into phytoplankton protein nitrogen, determined on the mixture of the cultures of the two species taken from the mixing-tank outflow. Sixteen percent of the nitrate remained unaltered, leaving 15% of the incoming nitrate unaccounted for. A good portion of this is probably in the nucleic acids of the phytoplankton cells, some may be present as intracellular nitrate and some organic nitrogen may have been released to the medium. The percentages given in Table 1 are derived from the

particulate protein and nitrate concentrations determined daily and averaged over the entire 36-day period of the experiment.

Table 1

Clam Feeding and Growth

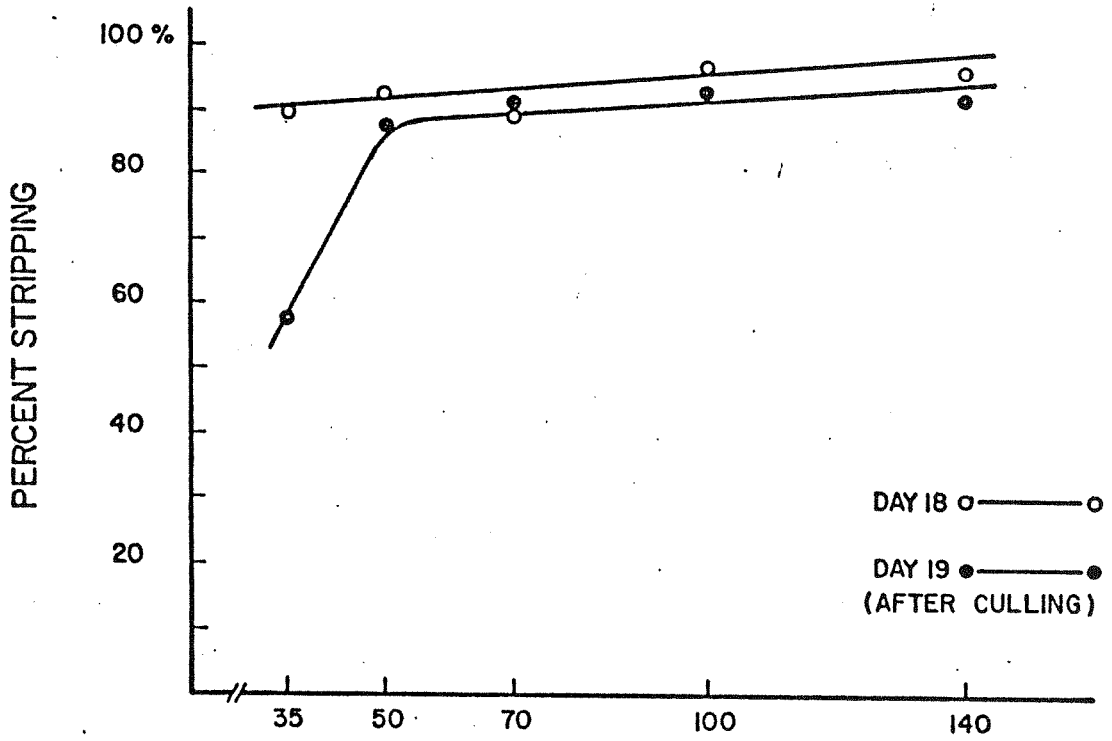
"Percent stripping" is defined as the fraction of the PPN removed in the clam tank from the food inflow. This includes uptake by the clams as well as loss of PPN to the tank deposit. The advantage of using PPN as a measurement, is that algal cells of vastly different sizes or shapes can be quantitatively compared.

The "percent stripping" was constant for most experimental clam groups. For the 35 g, 2 ml/sec group, the individual clam growth rate was the greatest, and for this group, culling on day 18 resulted in reduced percent stripping, as shown in Figure 2.

Figure 2

On days 18 and 19 of the experiment, the food concentration flowing into the Tapes feeding tanks was 24.1 and 21.9 µg-at PPN/l, respectively. On day 18, the clam weights were reduced to the original starting weight by culling. Thus, for the 35 g, 2 ml/sec group, culling on day 18 removed half the weight of clams in the tank. Figure 2 shows that the percent stripping was almost the same for all groups, except for the 35 g, 2 ml/sec group on day 19. The percent stripping for this group was lower on the average during the entire experiment: 74% compared to 87-93% stripping for all other groups.

CHANGE IN PERCENT STRIPPING AFTER Tapes CULLING



The increase in length of the different groups of clams over the 36-day experimental period is given in Figure 3.

Figure 3

The 35 g, 2 ml/sec group had the lowest percent stripping and the greatest increase in length, as shown in Figure 3. This figure gives the average increase above the starting length of the clams remaining in the experiment on day 36 of the experiment. The 35 g, 2 ml/sec group grew from 12.7 mm to 19.8 mm, an increase of 7.1 mm, in 36 days. This was the highest average individual growth rate achieved: 1.42 mm/week.

Figure 4 gives the weight gain (g) for each experimental group of Tapes japonica.

Figure 4

The total weight gain is greatest for the 100 g, 2 ml/sec group, which showed an increase in wet weight of 134 g over the 36-day period. Although this population had the greatest weight increase as a group, the growth rate of the individual clams averaged .268 g/week/g whole clam, compared to the 35 g, 2 ml/sec group which had an average growth rate of .411 g/week/g whole clam.

The composition of the clams varied slightly, but on the average, 47% of the whole wet weight was wet meat, 16% of the wet meat was dry meat, and 42% of the dry meat was protein.

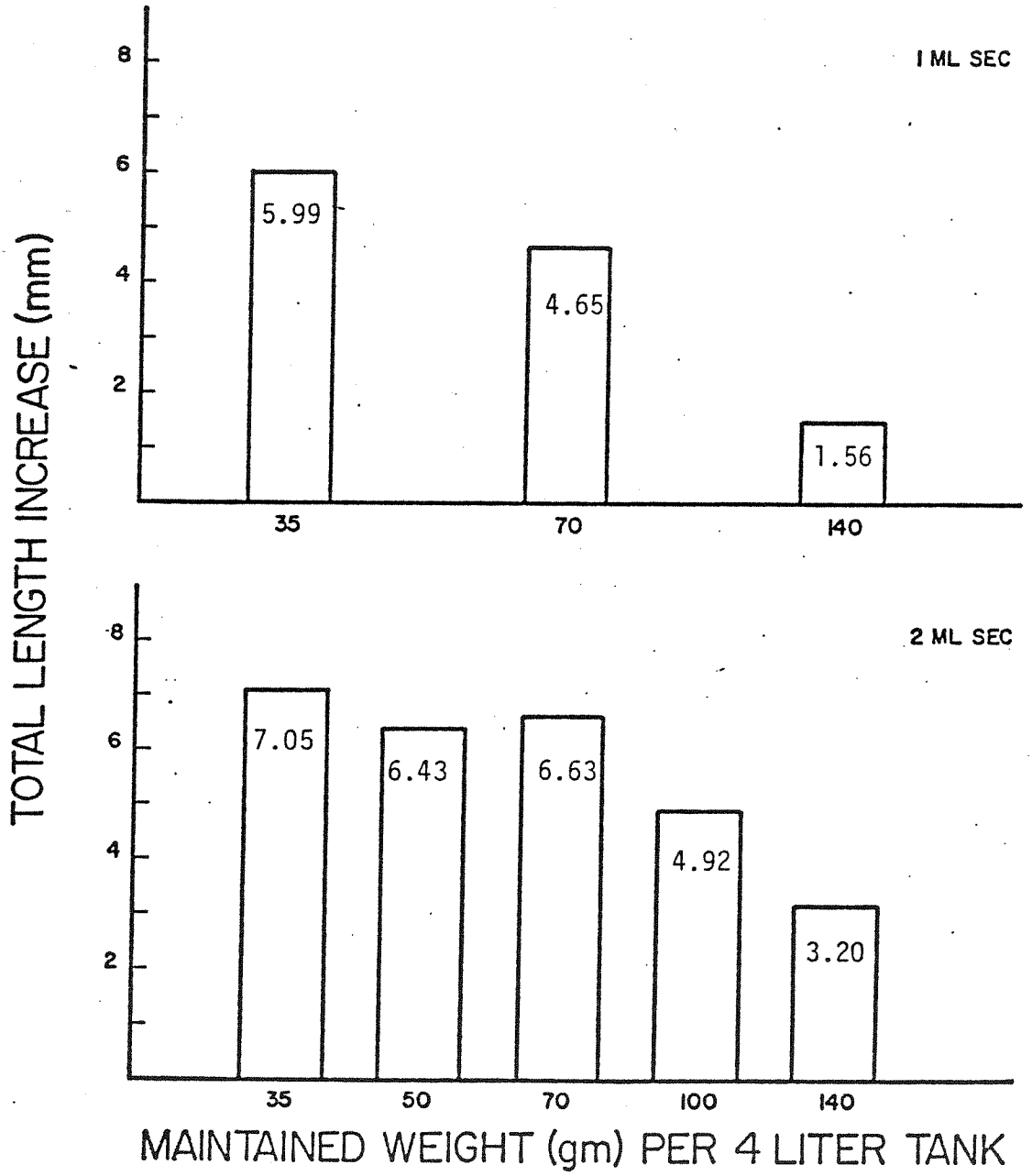
Nitrogen Balance

The nitrogen recovery is shown in Table 2.

Table 2

TOTAL LENGTH INCREASE (mm) OF *Tapes japonica* IN 36 DAYS

STARTING LENGTH 12.7 mm



TOTAL WEIGHT GAIN (gm) IN 36 DAYS

Tapes japonica — FED S-I AND STX-167

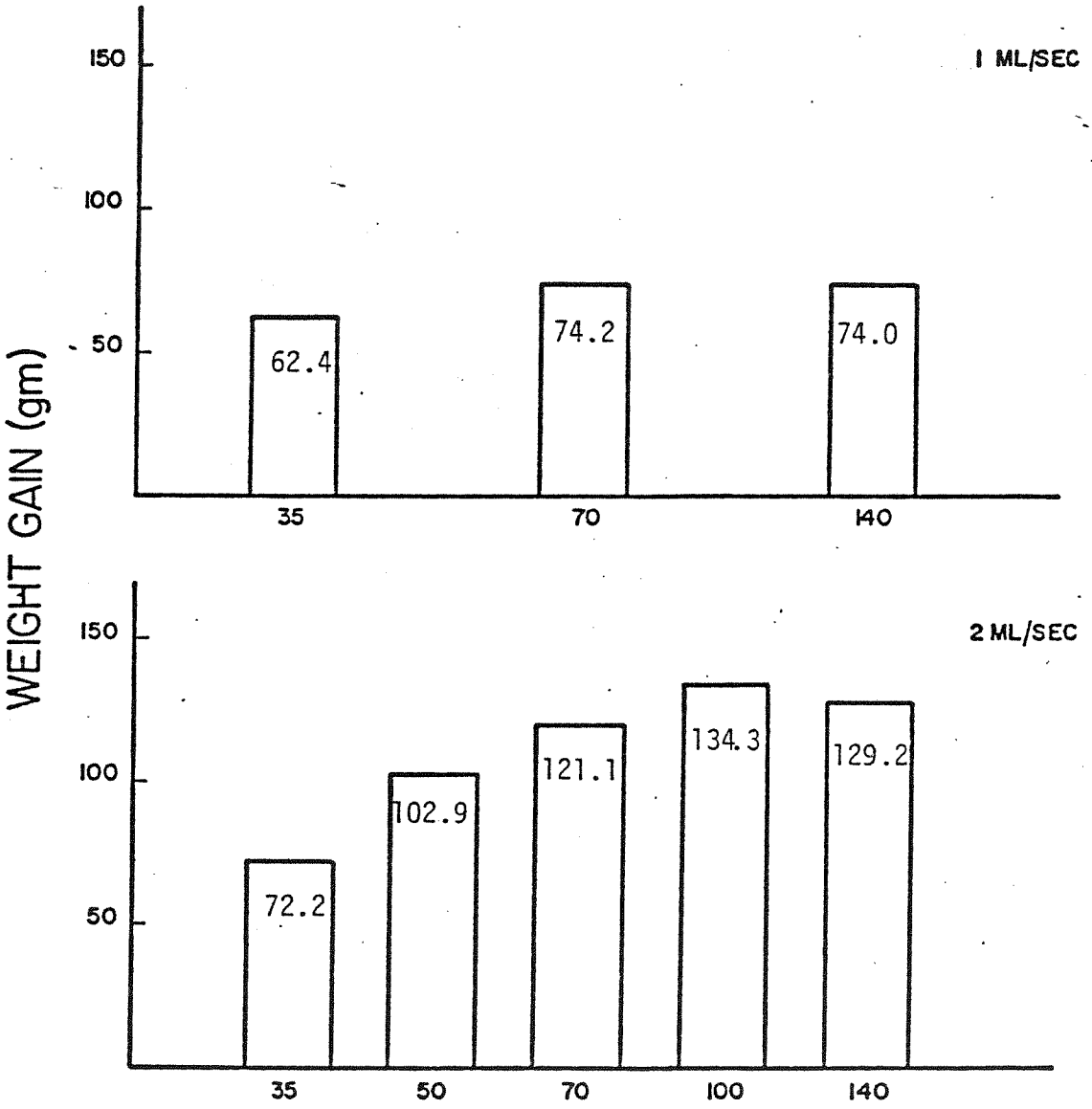


Table 2. The total nitrogen recovered in the shellfish feeding tanks and their effluent as a percentage of the inflowing algal protein nitrogen.

Plankton Suspension Flow Rate	Starting Clam Weight (g)	"N" Recovered as % of Algal Protein-N Inflow
	Total Nitrogen Recovered During 36-Day Period	
1 ml/sec	35	73.9
	70	76.6
	140	88.2
2 ml/sec	35	70.6
	50	72.4
	70	73.1
	100	70.2
	140	71.9

"N" recovered = meat protein-N + shell protein-N + tank deposit protein-N + tank effluent (protein-N + NO_3^--N + NO_2^--N + NH_4^+-N)

The utilization of the algal protein by the clams is shown in Table 3.

Table 3

To trace the fate of algal protein nitrogen (PPN) through the system, the PPN flowing into the shellfish feeding tanks was assigned a value of 100%. The incorporation of this PPN into clam meat protein, tank deposit particulate protein, tank effluent particulate protein, effluent ammonia and effluent (nitrate + nitrite), were then expressed as a percentage of algal PPN in the food flow coming into the shellfish tanks. This percentage value for each fraction was averaged, based on the daily values obtained, over the entire duration of the 36-day experiment. Three to four hours' time was subtracted from each nine-day period while weighing, measuring and culling was taking place.

Figure 5 shows the percentage incorporation of PPN into clam meat protein.

Figure 5

The greatest total amount of protein was incorporated into the shellfish meat by the 100 g, 2 ml/sec group; this group also had the greatest fresh weight gain. Thirty-two percent of inflowing PPN was incorporated into clam meat protein by this group. The rate of protein-nitrogen incorporation was equal to 12.0 µg-at protein N/day per g whole clam for this group. The 35 g, 2 ml/sec group had the least efficient food utilization and the fastest growth of individual clams. This group converted 24% of inflowing algal protein into clam-meat protein, corresponding

Table 3. Protein utilization by *Tapes japonica* of a mixture of *Chaetoceros curvisetus* (STX-167) and S-1.

Weight Density (g/4-l)	Flow Rate (ml/sec)	Total Whole Weight Gain of Clams (g)	Algal Protein in the Inflow (g)	Algal Protein Stripped (g)	Algal Protein Absorbed (g)	Algal Protein Retained (g)	Protein Efficiency Ratio[1]	"Stripped" Protein Efficiency Ratio[2]	Bio-logical Value[3]
35	1	62.4	5.72	5.01	4.58	1.86	10.9	12.5	40.6
70	1	74.2	5.72	5.33	4.97	1.97	13.0	13.9	39.6
140	1	74.0	5.72	5.34	4.96	1.78	12.9	13.9	35.9
35	2	72.2	11.5	8.52	7.75	2.71	6.30	8.47	35.0
50	2	102.0	11.5	10.0	9.24	3.42	8.99	10.2	37.0
70	2	121.0	11.5	10.3	9.56	3.71	10.6	11.6	38.8
100	2	134.0	11.5	10.6	9.84	3.75	11.7	12.7	38.1
140	2	129.0	11.5	10.6	9.95	3.49	11.3	12.1	35.0

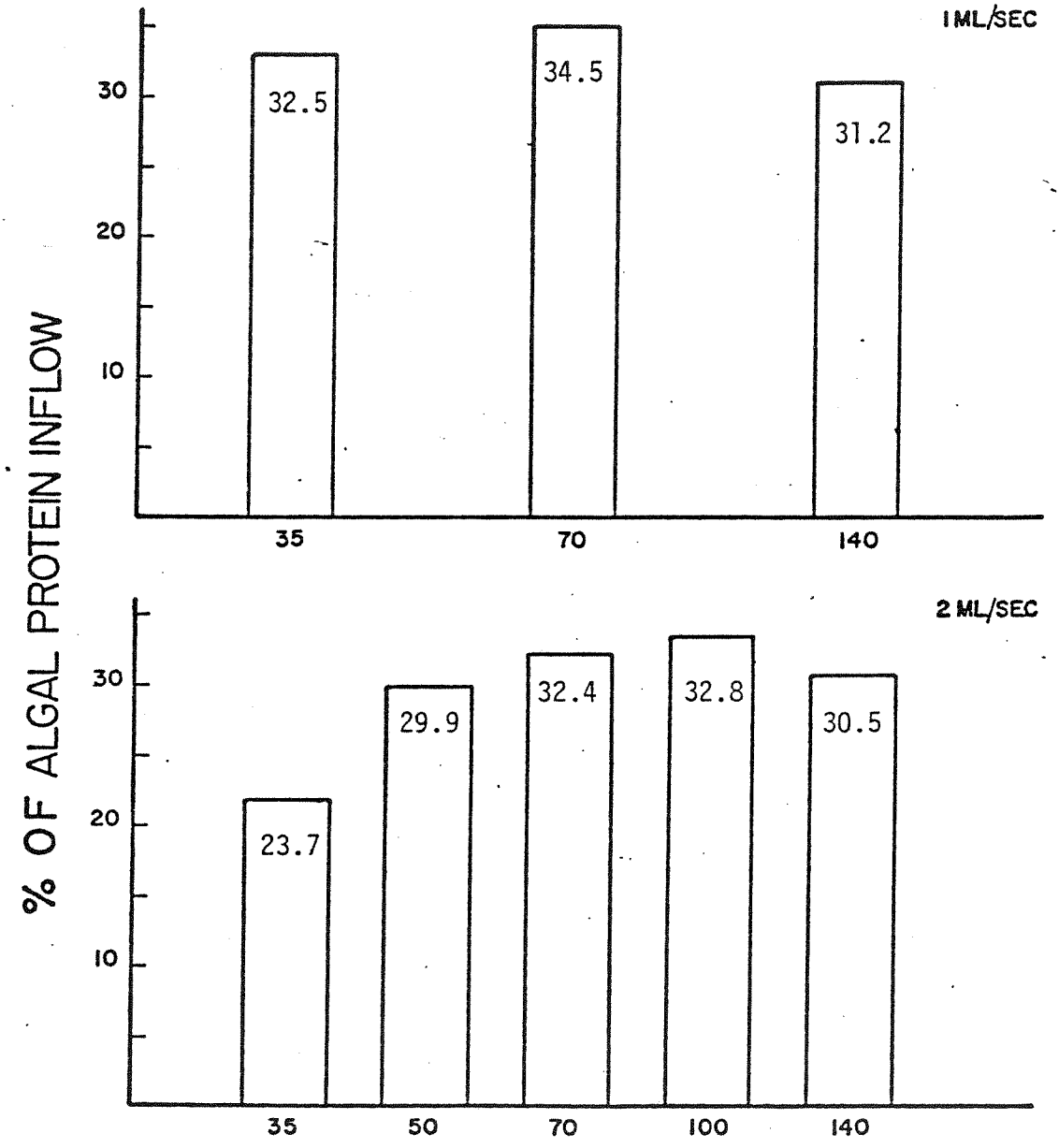
[1]Protein Efficiency Ratio = total weight gain/weight protein presented.

[2]"Stripped" protein efficiency ratio = total weight gain/weight protein stripped.

[3]Biological Value =(retained protein/absorbed protein) x 100.

PROTEIN CONVERSION EFFICIENCY

SHELLFISH MEAT PROTEIN PRODUCED AS % OF ALGAL PROTEIN INFLOW OVER A 36 DAY PERIOD



to an incorporation rate of 24.7 µg-at protein N/day/g whole clam. The 70 g, 1 ml/sec group had the highest efficiency of protein conversion: 34.5%, the highest percent of phytoplankton protein incorporated into shellfish meat protein.

The percentage of inflowing PPN appearing as tank deposit particulate protein and as effluent PPN is shown in Figures 6 and 7.

Figure 6

Figure 7

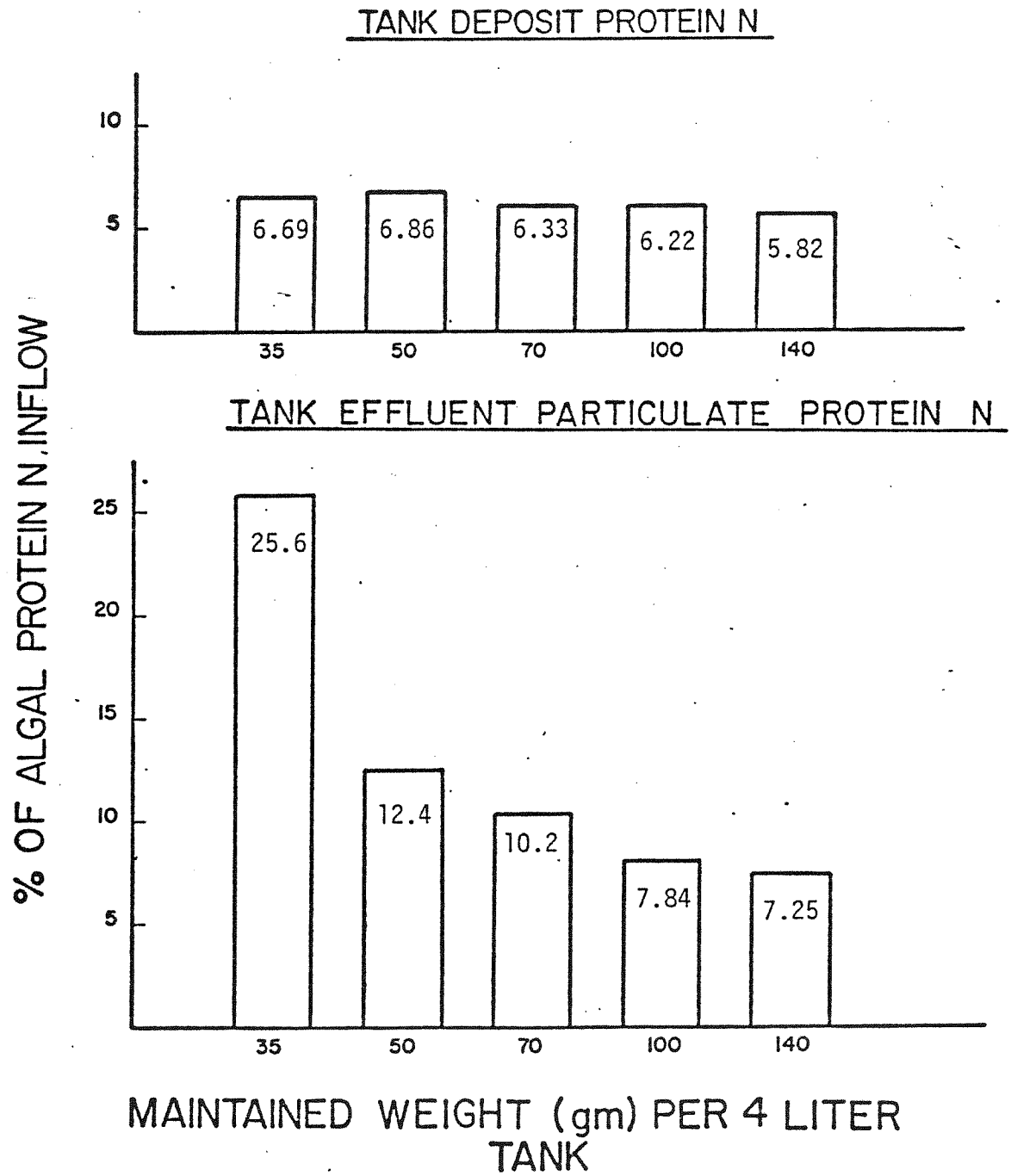
Tank deposit PPN varied little with the clam density or with flow rate and averaged 5-7%.

The amount of particulate protein nitrogen in the effluent varied a great deal among the different experimental groups. Figures 6 and 7 show that a major portion (26%) of the entering protein leaves in the effluent in the least efficient 35 g, 2 ml/sec group. The percentages in Figures 6 and 7 are really equal to 100 minus the percent stripping averaged over the entire period of the experiment. For the entire experimental period, the concentration of PPN in the effluent in µg-at/l was 2.69, 1.48 and 1.45 for the 35, 70 and 140 g, 1 ml/sec groups, and 5.50, 2.67, 2.19, 1.69 and 1.59 for the 35, 50, 70, 100 and 140 g, 2 ml/sec groups, respectively. Only 7.9% of the inflowing PPN remained in the effluent of the group with maximum population growth: 100 g, 2 ml/sec.

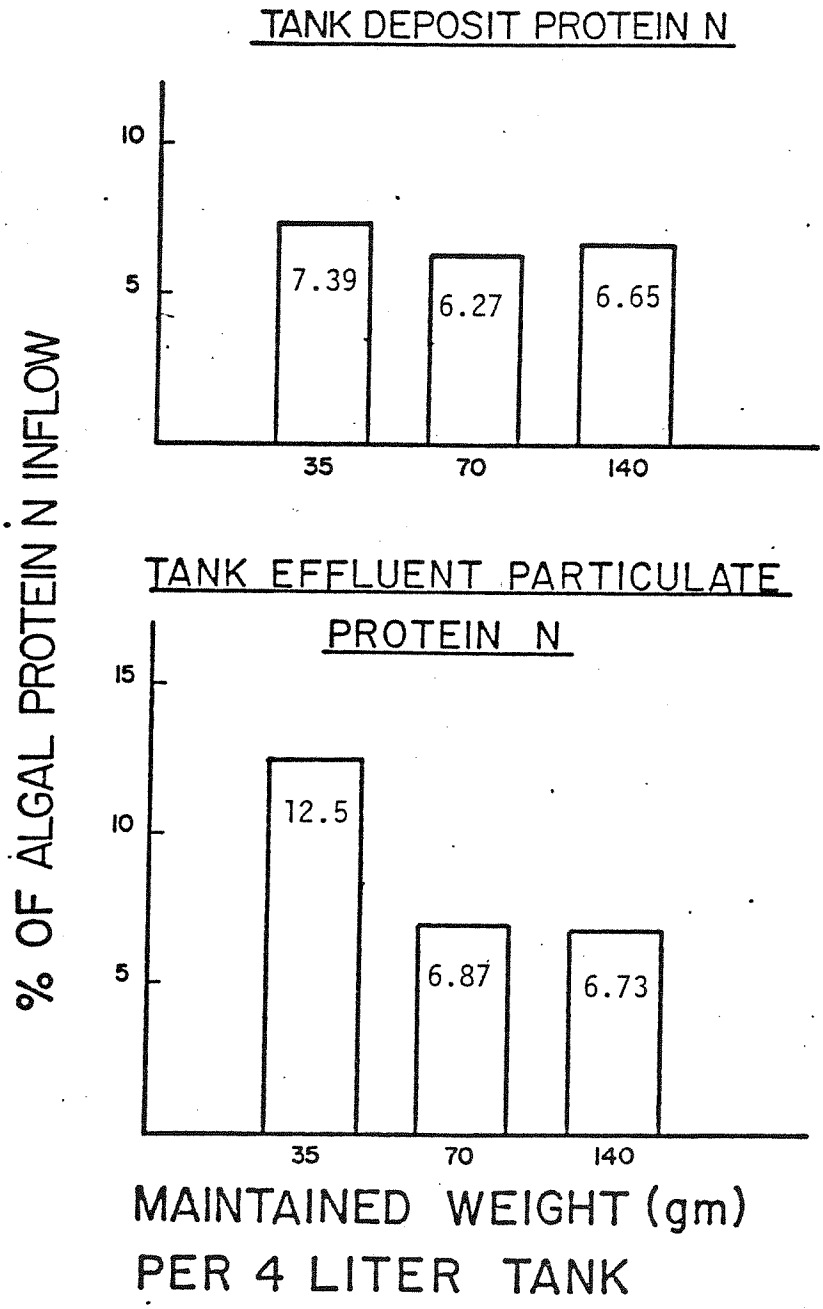
The ammonia-N-concentration in the shellfish tank effluent expressed as a percent of PPN inflow is given in Figure 8.

Figure 8

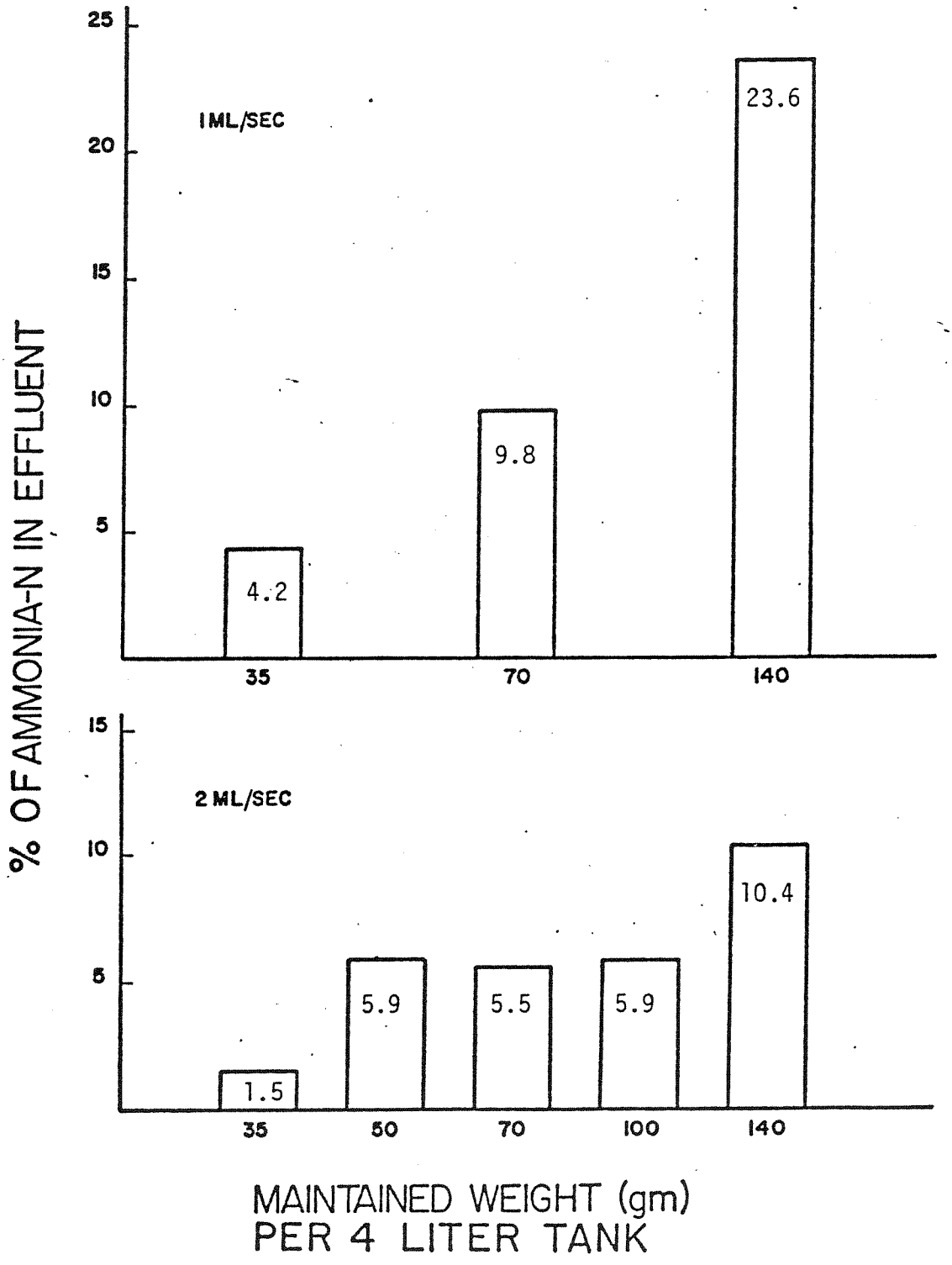
COMPARISON OF TANK DEPOSIT PROTEIN N AND TANK EFFLUENT PROTEIN N AS % OF ALGAL PROTEIN N INFLOW (2 ML/SEC)



COMPARISON OF TANK DEPOSIT PROTEIN N AND TANK EFFLUENT PROTEIN N AS % OF ALGAL PROTEIN N IN INFLOW (IML/SEC)



AMMONIA PRODUCTION AS % OF INFLOWING ALGAL PROTEIN NITROGEN



The absolute NH_4^+ concentrations are 0.91, 2.12 and 5.07 µg-at NH_4^+-N/l for the 35, 70 and 100 g, 1 ml/sec groups, respectively and 0.33, 1.21, 1.20, 1.26 and 2.20 µg-at NH_4^+-N/l for the 35, 50, 70, 100 and 140 g, 2 ml/sec groups generated above the 0.89 µg-at NH_4^+-N/l entering the Tapes feeding tanks. The 140 g, 1 ml/sec group had 23% of its inflowing PPN in the form of ammonia nitrogen in the shellfish-tank effluent, and, although they were actively growing, the growth of the inidividual shellfish was far from maximal for this species.

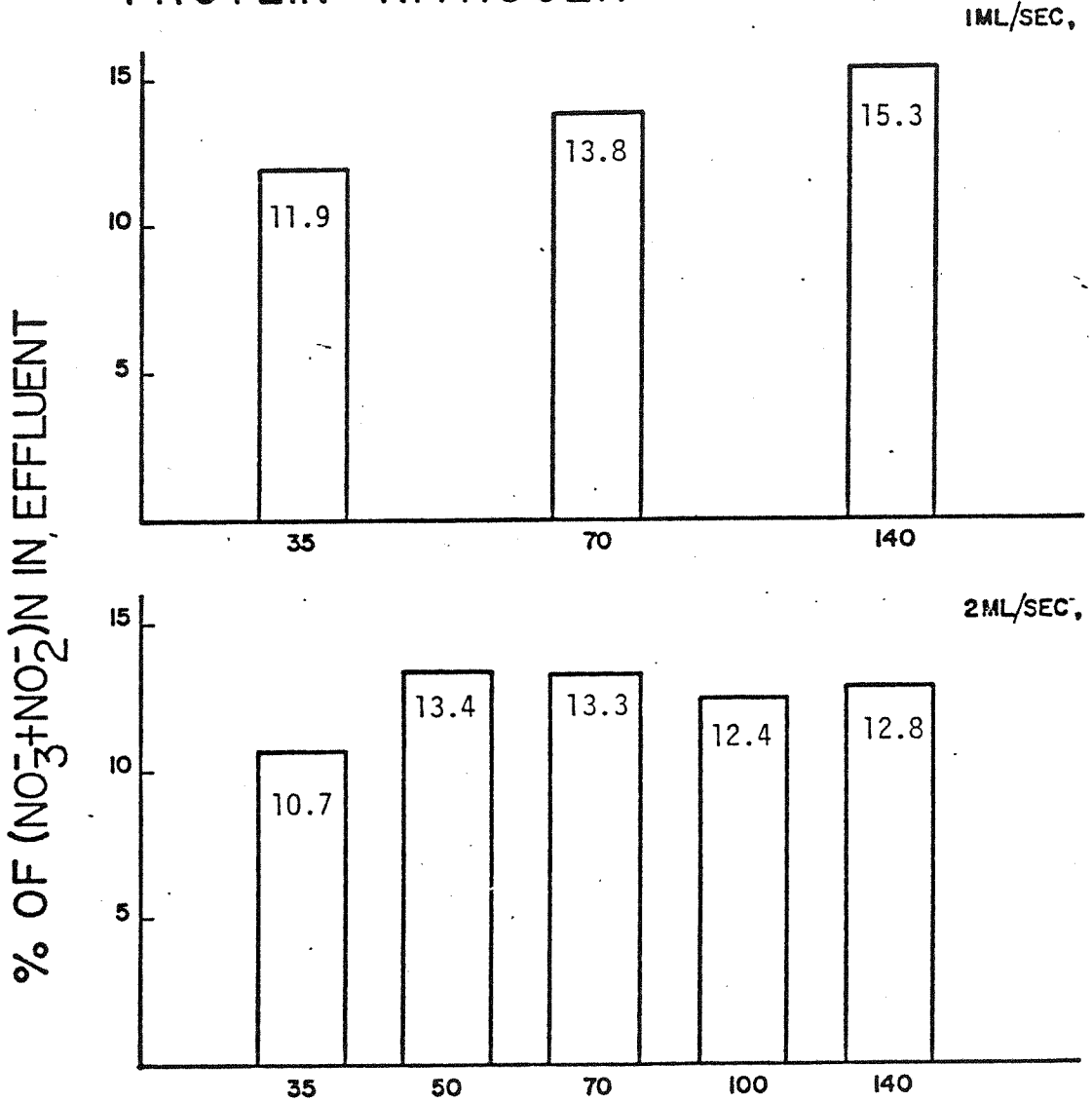
One unexpected result was the amount of NO_3^- produced in the shellfish tanks. As indicated in Figure 9, an amount of NO_3^-

Figure 9

equal to 12-14% of the PPN entering the shellfish tanks was generated in the tanks over and above the original unreacted NO_3^- present in the outflow from the phytoplankton tanks entering the shellfish tanks. The amount of NO_3^- and NO_2^- also did not vary greatly with the weight of the shellfish populations. The most likely explanation for this finding is that nitrifying bacteria are breaking down the tank deposit and converting it into nitrate. Control experiments on tank-deposit protein-nitrogen also show large losses if accumulated tank deposit is allowed to remain in the tanks for several weeks with aeration and no culture flow.

The increase of (NO_2^- + NO_3^-) in the Tapes feeding tanks lies between 2.30 and 3.27 µg-at of combined (NO_2^- + NO_3^-) N/l.

$NO_3^- + NO_2^-$ FORMATION IN THE SHELLFISH TANKS AS % OF INFLOWING ALGAL PROTEIN NITROGEN



The fate of the algal protein nitrogen entering the shellfish tanks for three of the experimental populations is given in Figures 10, 11 and 12.

Figure 10

Figure 11

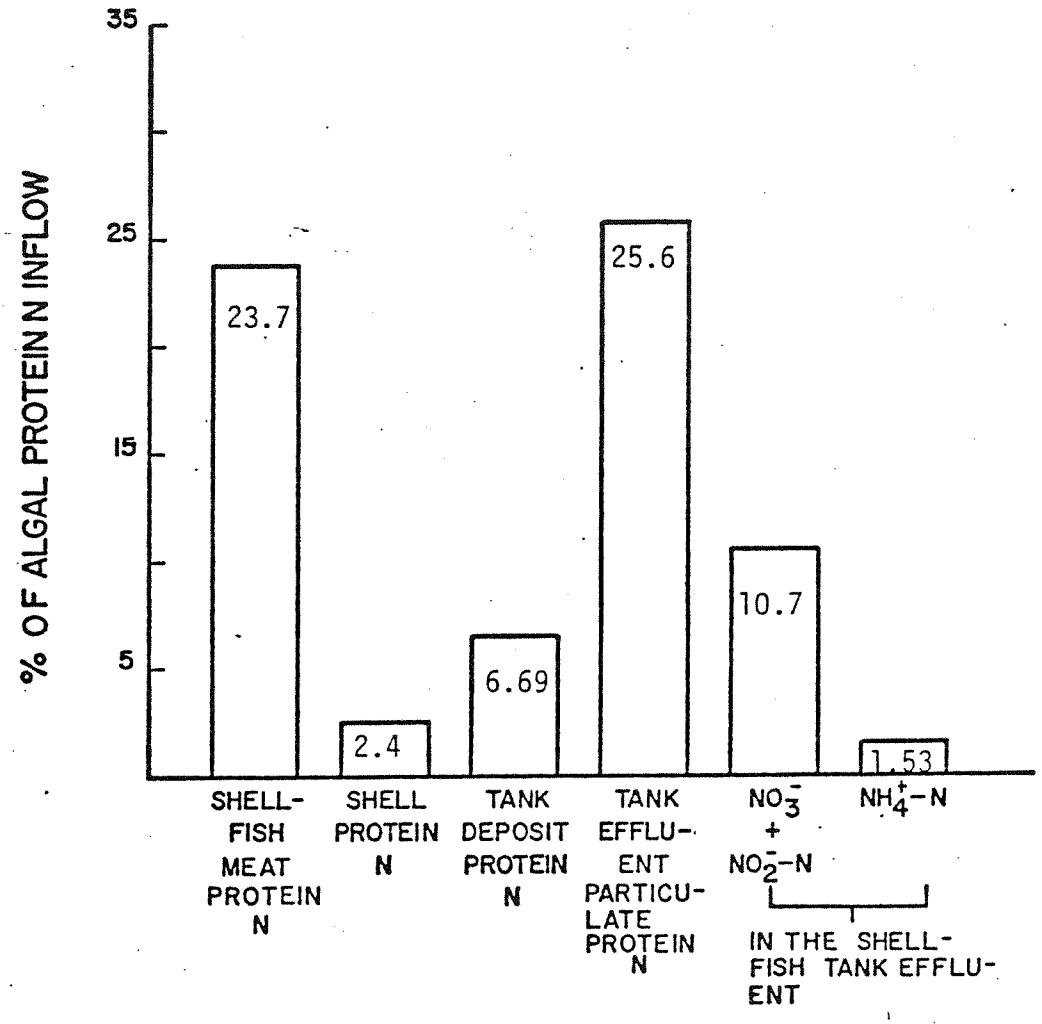
Figure 12

The largest differences appear in the PPN and the NH_4^+-N in the Tapes tank effluent. The highest effluent PPN is found in the group with the fastest individual growth. The highest NH_4+-N is generated by the group with the slowest individual growth.

Discussion

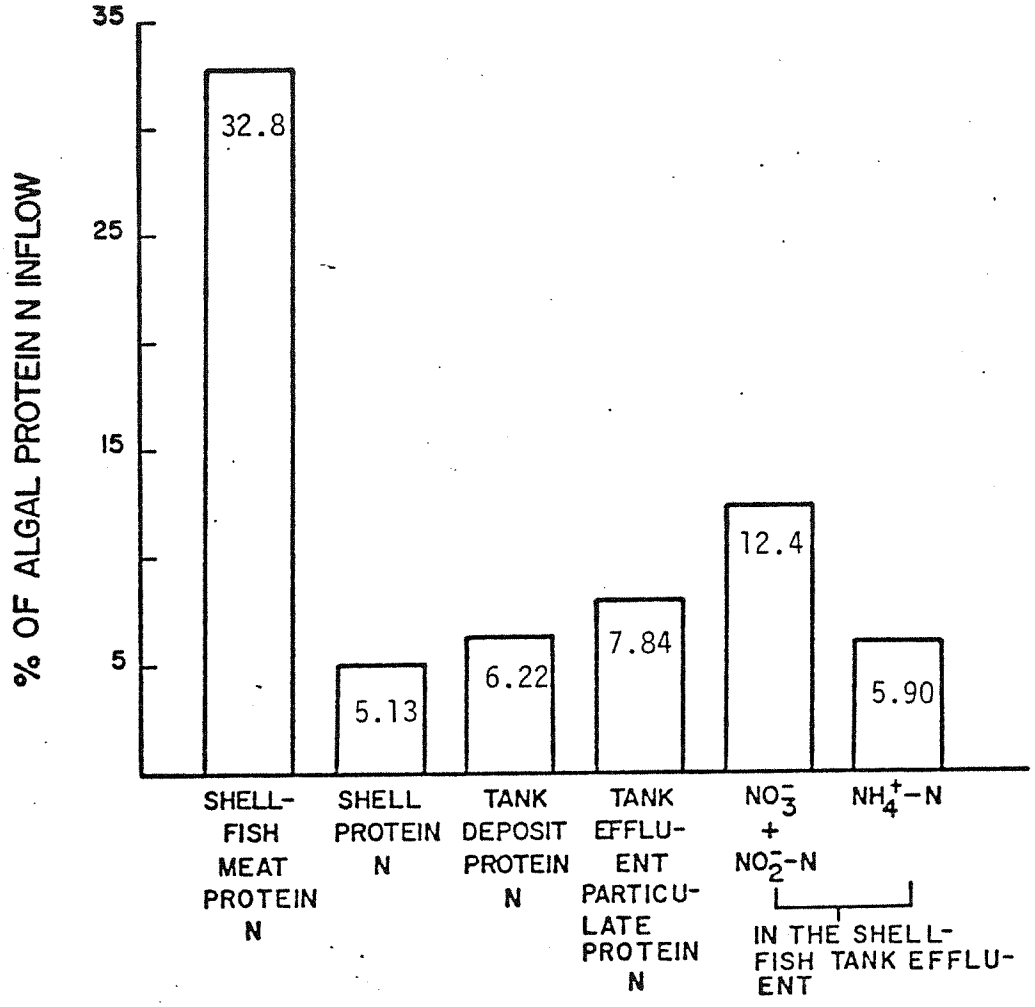
The utilization of deep-water nitrate by the phytoplankton used in this work, Chaetoceros curvisetus (STX-167) and the flagellate S-1, was somewhat lower than normally obtained in the larger-scale mariculture system in St. Croix, where only C. curvisetus (STX-167) is grown. The average conversion of deep-water nitrate-nitrogen into phytoplankton protein-nitrogen by C. curvisetus (STX-167) in the 45,000-liter pools is 78%, corresponding to better than 90% utilization of incoming deep-water nitrate, since the deep-water nitrate is naturally also utilized for the synthesis of nitrogen-containing compounds other than protein. Assuming 70% efficiency of nitrate-nitrogen to phytoplankton protein-nitrogen conversion in our system, the protein production per square meter per year in the St. Croix experimental system, for 330 days' operation of the pools per year, would be 0.52 kg, corresponding to 5.2 tons protein per hectare per year (Roels et al., 1975). By comparison, the best

THE FATE OF ALGAL PROTEIN N FLOWING INTO SHELLFISH TANKS CONTAINING *Tapes japonica*



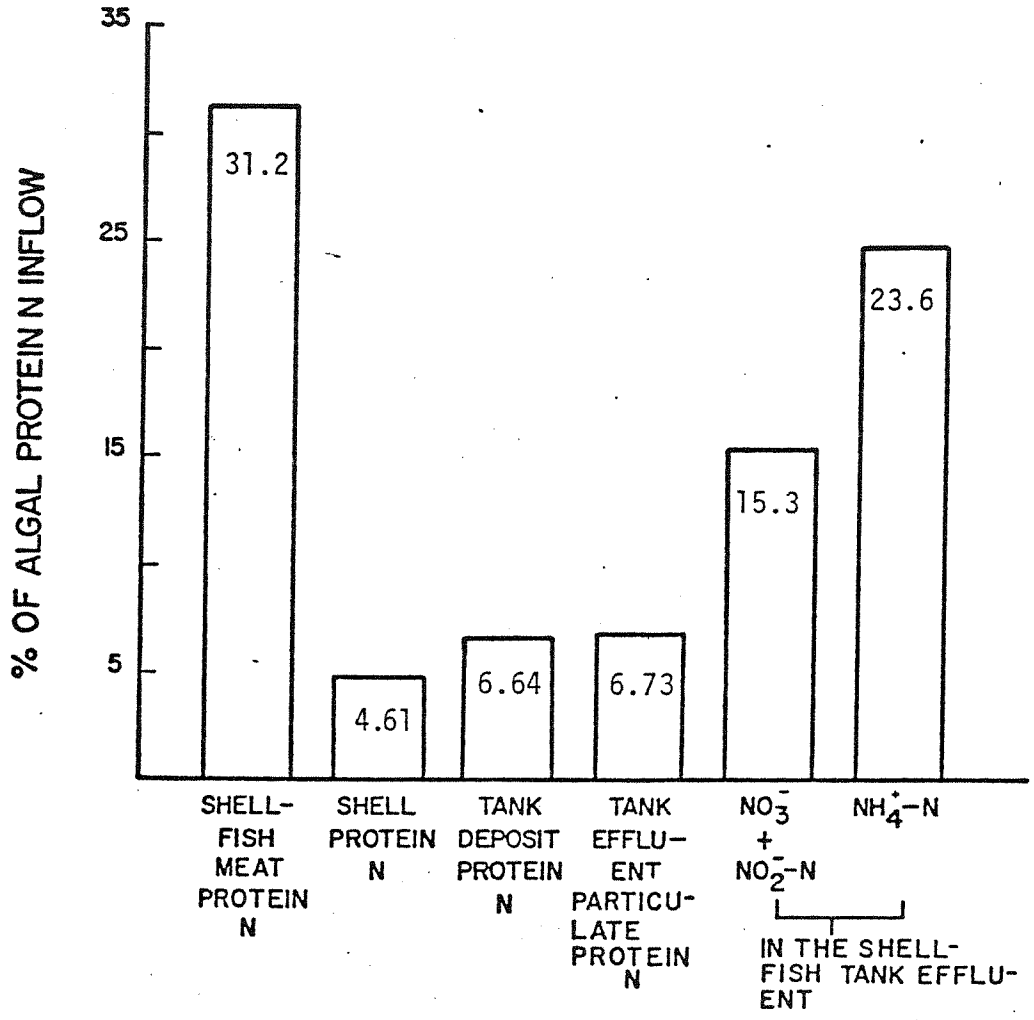
35 GM, 2 ML/SEC

THE FATE OF ALGAL PROTEIN N FLOWING INTO SHELLFISH TANKS CONTAINING *Tapes japonica*



100 GM, 2 ML/SEC

THE FATE OF ALGAL PROTEIN N FLOWING INTO SHELLFISH TANKS CONTAINING *Tapes japonica*



140 GM, 1ML/SEC

protein output in land-based agriculture is that of alfalfa crops, yielding 0.71 tons protein/ha/yr (Pimentel et al., 1975). The 5.2 tons phytoplankton-protein/ha/yr quoted here applies for a 0.8-m deep pool, as presently used in St. Croix. Extrapolation of experimental results indicates that far higher phytoplankton-protein productivities per unit area could be achieved by using deeper pools (Roels et al., 1976).

The "percent stripping" of incoming phytoplankton in the shellfish tanks was uniformly high for the groups, varying between 87-94%, except for a brief period immediately following each culling, when the percent stripping for the 35 g, 2 ml/sec group was lower (60% on day 19 of the experiment). The 35 g, 2 ml/sec group averaged 74% stripping for the entire experimental period. This group was obviously wasting food. There are several reports in the literature describing the filtration rate of shellfish. These generally show a maximum or optimal filtration rate for different species. Ali (1970), using Hiatella arctica, a suspension filter-feeder, and Winter (1973), using Modiolus modiolus, show a decreasing filtration rate with increasing cell densities. Work with Mytilus edulis by Thompson and Bayne (1972, 1974) and by Tenore and Dunstan (1973), as well as by Forster-Smith (1975) and Schulte (1975), shows that the filtration rate of the mussel reaches a maximum. Forster-Smith (1975) shows the same for Cerastoderma edule and Venerupis pullastra; Tenore and Dunstan (1973) demonstrate it for Crassostrea virginica and Mercenaria mercenaria. This optimum filtration rate must have been reached by the 35 g, 2 ml/sec group for which a marked decrease in stripping was observed. This decrease in percent stripping

might be due to the saturation of the feeding sites on the gills of the clams. There is no indication of ill effects and the 35 g, 2 ml/sec group is growing somewhat faster than the 35 g, 1 ml/sec group. Loosanoff and Engle (1974) have shown that Crassostrea virginica can adapt to cell concentration by pumping at different rates but this condition should have an upper limit.

The 35 g, 2 ml/sec group had the lowest "percent stripping" and the maximal individual growth rate, as shown by the average increase in shell length in Figure 3 and the weight increase per gram of clam. We should stress here that in our experimental design, there is a marked difference in the individual growth of each animal and the mass population growth: Figure 4 clearly demonstrates that the 100 g, 2 ml/sec group of clams showed the greatest increase in total weight over the experimental period. However, the clams in the 35 g, 2 ml/sec group had the greatest individual weight gain and thus would reach market size more quickly. We have therefore determined that optimum shellfish densities differ depending upon whether the greatest growth is desired for the mass population of clams or for the individual clams, or whether maximum food utilization is the goal.

The reason for these differences in growth rate and food utilization is probably the availability of feeding acceptor sites: the clam feeding acceptor sites for the 35 g, 1 ml/sec group are nearly saturated as shown by the fact that doubling the amount of food given to the clams resulted in only moderate increase in growth (Fig. 4). The 35 g, 2 ml/sec group must certainly have all its food-acceptor sites saturated (indicated by reduced percent stripping). This

group had the maximum individual growth. By increasing the clam density in the tanks, i.e., by going from 35 g, 2 ml/sec group to the 140 g, 2 ml/sec group, we are increasing the number of food-acceptor sites while keeping the food constant. The food-acceptor sites at a higher clam density (such as the 100 g, 2 ml/sec group) are not all filled, but a greater number of the sites are accepting food so that the total mass growth is greater but the rate of individual growth is slower.

The results on maximal individual and mass population growth show that if clams are grown in a confined space, small change in clam density affects overall individual and mass population growth.

High clam densities will require high feeding rates for maximum individual growth. If the food concentration (PPN/l) cannot be increased by fertilization because of too high an ammonium ion buildup in the shellfish feeding tanks, higher culture flow rates will be required to maintain maximal individual growth rate. Because water current surrounding the clams affects feeding (Walne, 1972) the current that a clam species can tolerate might well be one of the limiting factors for very high clam densities in an industrial-type mariculture system.

Another purpose of this feeding experiment was to obtain a nitrogen balance on the particulate protein nitrogen entering the shellfish tanks. A complete nitrogen balance for clam feeding is very difficult, since the food and the feces are not separable. However, the fate of the entering PPN was determined, and, on the average, 75% of the entering PPN could be accounted for. At the

clam densities used in this experiment, the efficiency of conversion of phytoplankton protein to shellfish meat protein was equal to 33%, 35% and 31% for the 35, 70, and 140 g, 1 ml/sec flow groups and 24%, 30%, 32%, 33% and 30% for the 35, 50, 70, 100 and 140 g, 2 ml/sec flow groups. The nitrate buildup in the shellfish feeding tanks was constant (11-15%) as was the tank deposit protein (5-7%) for all experimental groups. Only the NH_4^+-N and the PPN in the effluent varied considerably. In fact these variations might become valuable indicators of the growth rate of the clams in a mariculture system. The slowest growing group studied, the 140 g, 1 ml/sec group had high NH_4^+-N concentration in the shellfish tank effluent, equal to 24% of the entering PPN whereas the PPN leaving the shellfish tank was the lowest, i.e., 6.7% of that entering. Conversely, the group with the fastest individual growth had the lowest NH_4^+-N concentration buildup in the effluent, 1.5% of the entering PPN concentration, and the highest PPN remaining in shellfish tank effluent, 26%.

Table 3 shows the protein value of this mixed algal diet for Tapes japonica. This type of information will be important to future nutritional studies. Two classical indicators of protein food value are employed, the Protein Efficiency Ratio and the Biological Value of the diet. Protein absorbed or consumed is the total PPN inflow minus the total PPN in the tank deposit and tank effluent for the duration of the experiment, which is the same as the PPN stripped minus the PPN in the tank deposit. The retained protein-N is the protein nitrogen incorporated into the Tapes meat protein. The definition of Protein Efficiency Ratio and Biological Value is given by the following equations:

$$\text{Protein Efficiency Ratio (P.E.R.)} = \frac{\text{weight gain (g)}}{\text{protein consumed (g)}}$$ (Osborn et al. 1919)

$$\text{The Biological Value (B.V.)} = \frac{\text{Retained protein N} \times 100}{\text{Absorbed protein N}}$$ (Mitchell 1924)

Problems inherent in making comparisons of the biological value of protein in food such as the level of protein and caloric intake are given by Forbes et al. (1956), Rosenthal and Allison (1951), Hegsted and Worcester (1947), and Sherwood and Weldon (1953).

The Protein Efficiency Ratios obtained in our feeding studies (6.30 - 13.0) are higher than those obtained in studies with land animals for which values between 2.0 and 4.0 were found by Morrison and Campbell, by Derse, and by Rosenberg as reported by Campbell (1961). Higher P.E.R. values might be expected in clam feeding studies because of the weight of the shells which are low in protein and also because of the higher water content of clam meat compared to the meat of most land animals.

Our P.E.R. values however do compare well with those obtained on mice fed egg protein, peanut flour protein and wheat gluten as is described by Barnes and Bosshardt, who obtained values between 5 and 12, with a diet containing approximately 2% nitrogen.

An advantage of determining Protein Efficiency Ratios over Biological Value figures is that the P.E.R.s do not require killing the animals to determine the amount of retained protein as do the B.V. data. The Protein Efficiency ratio does require determination of the tank deposit protein nitrogen for the entire experimental period. Since this determination does have some unpleasant drawbacks, a "Stripped" Protein Efficiency Ratio as given in Table 3

might well have as much meaning without necessitating the tedious measurements of protein nitrogen on the tank deposit. We certainly recommend the use of the stripped Protein Efficiency Ratio for more routine work.

The information on the nutritional value of the phytoplankton obtained in this experiment with Tapes japonica is a consequence of using protein-N to quantify the efficiency of food transfer in this marine food chain and represents significant progress over dry weight used in the past to describe the efficiency of phytoplankton conversion to shellfish.

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APPENDIX C

HATCHERY OPERATING MANUAL

FOR THE "ARTIFICIAL UPWELLING" MARICULTURE PROJECT

AT THE

ST. CROIX MARINE STATION

THE UNIVERSITY OF TEXAS MARINE SCIENCE INSTITUTE

JUDITH B. SUNDERLIN

PHYLLIS T. BAAB

C O N T E N T S

I. BROOD STOCK

Brood stock shellfish, usually those introduced as juveniles and grown to market size in the system, are maintained in the "Artificial Upwelling" mariculture system at ambient temperature. These shellfish are continuously fed algal cultures from the 2000-liter reactors. Turnover periods in the rearing tanks range from 13 to 20 minutes. The yearly temperature range in the brood stock tanks is 22°C to 27°C.

New Tapes japonica brood stock, from either California or Washington State, are introduced into the system every six to nine months. This introduction should improve the genetic strain and help avoid inbreeding in this species.

Several days before spawning is attempted, two to three brood stock animals are sacrificed and the gonads examined for ripeness. Selected numbers of the following populations: Tapes japonica, Crassostrea gigas, and the C. gigas Kumamoto variety—are usually ripe throughout the year in the "Artificial Upwelling" system.

II. SPAWNING

Equipment

The following is a list of the equipment used in spawning procedures at the St. Croix "Artificial Upwelling" mariculture project.

a) Shellfish shucking knife

b) Glass-lined, gas-fired water heater

c) Spawning table, 2 ft x 4 ft (0.6 m x 1.2 m) —minimum size

d) Vexar screening, 3/4" (19 mm) mesh, two layers on bottom of spawning table to allow for water circulation under the spawning dishes

e) Pyrex loaf pans, 1.4-liter capacity. For a 2 ft x 4 ft (0.6 m x 1.2 m) table, 14 pans are required

f) Thermometers (4)

g) Beakers, 50 ml (3)

h) Pasteur pipettes and bulbs

i) Sieves, 25.4 cm in diameter, made from Nitex screening ranging in mesh size from 35 to 350 μ, stretched and glued to acrylic tubing

j) 1,2 Dichloroethane (when mixed with acrylic dust, is an excellent glue for the sieves)

k) Polyethylene buckets: 12-, 17-, and 21-liter capacity (a total of 10)

l) Disposable pipettes, 1 ml, for larval counts

m) Alcohol: 10 drops to each 1 ml larval sample

n) Sedgwick-Rafter cells (2 or more)

o) Microscope (100X magnification)

Spawning Procedure

Shellfish brood stock animals are subjected to thermal and chemical stimulation to induce spawning. The following technique has been used to induce spawning in four species of shellfish (Tapes japonica, Crassostrea gigas, C. gigas Kumamoto, and Pinctada martensii).

Adult shellfish are placed in Pyrex spawning dishes (1.4 liter) filled with algal culture, and deep water (22°C to 24°C) is circulated around the spawning dishes one-half hour prior to thermal and chemical stimulation (Fig. 1). To induce spawning, hot deep water is circulated through the spawning table and within 10 mins 31-32°C is reached in the Pyrex dishes. Immediately after reaching 31-32°C cold deep water is circulated through the spawning table and in approximately 45 mins the spawning dishes reach 22-24°C. At the high temperature range, a stripped gonad solution (sperm or eggs) is added to each dish and after 45 mins, if no spawning is observed, this thermal cycle is repeated. Deep water is heated in a glass-lined, gas-fired water heater (Fig. 2).

Figure 1. Spawning table (2 ft x 4 ft; 0.6 m x 1.2 m) containing 14 Pyrex spawning dishes. Four thermometers are used to check for uniform water temperature in the spawning dishes.

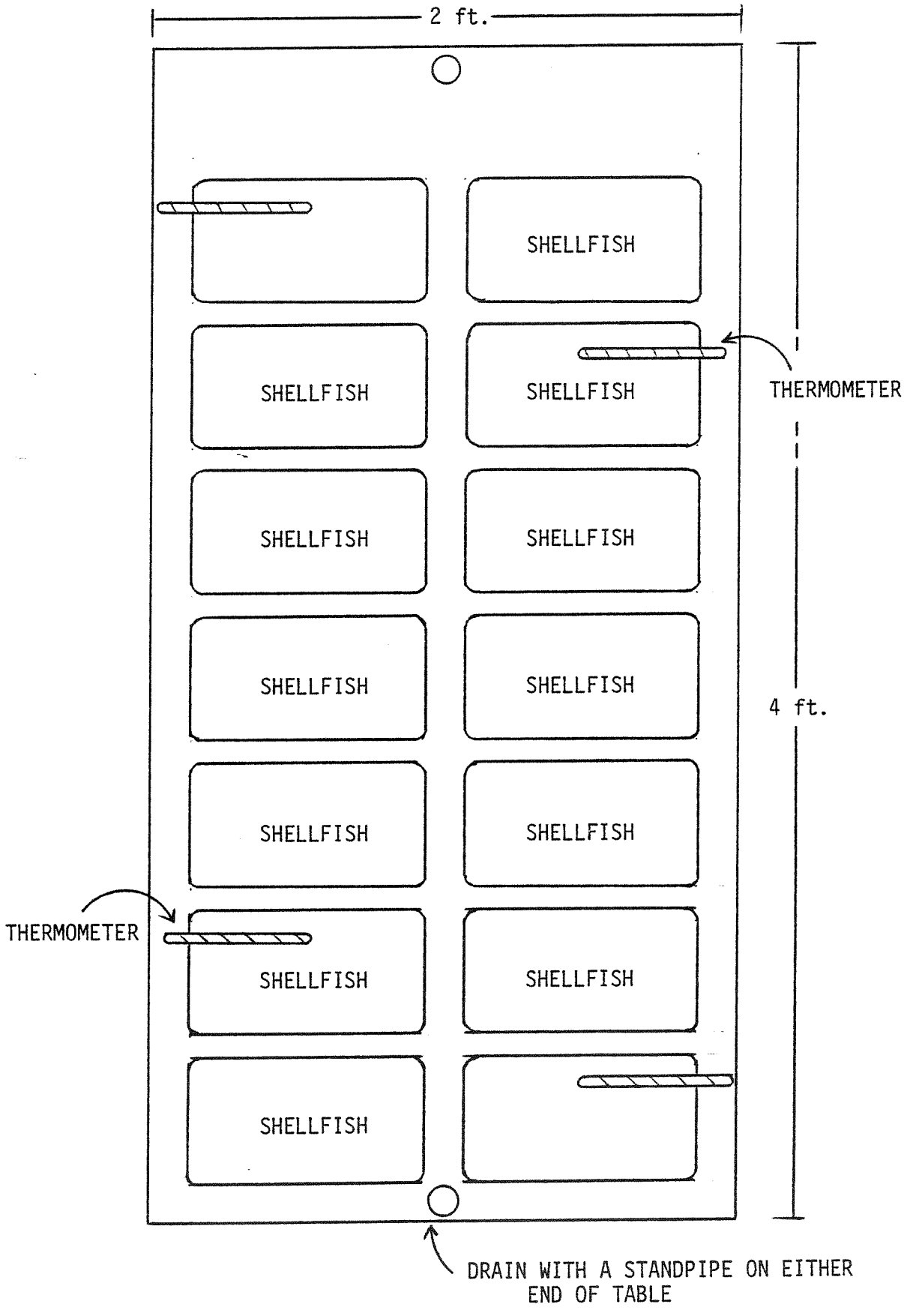
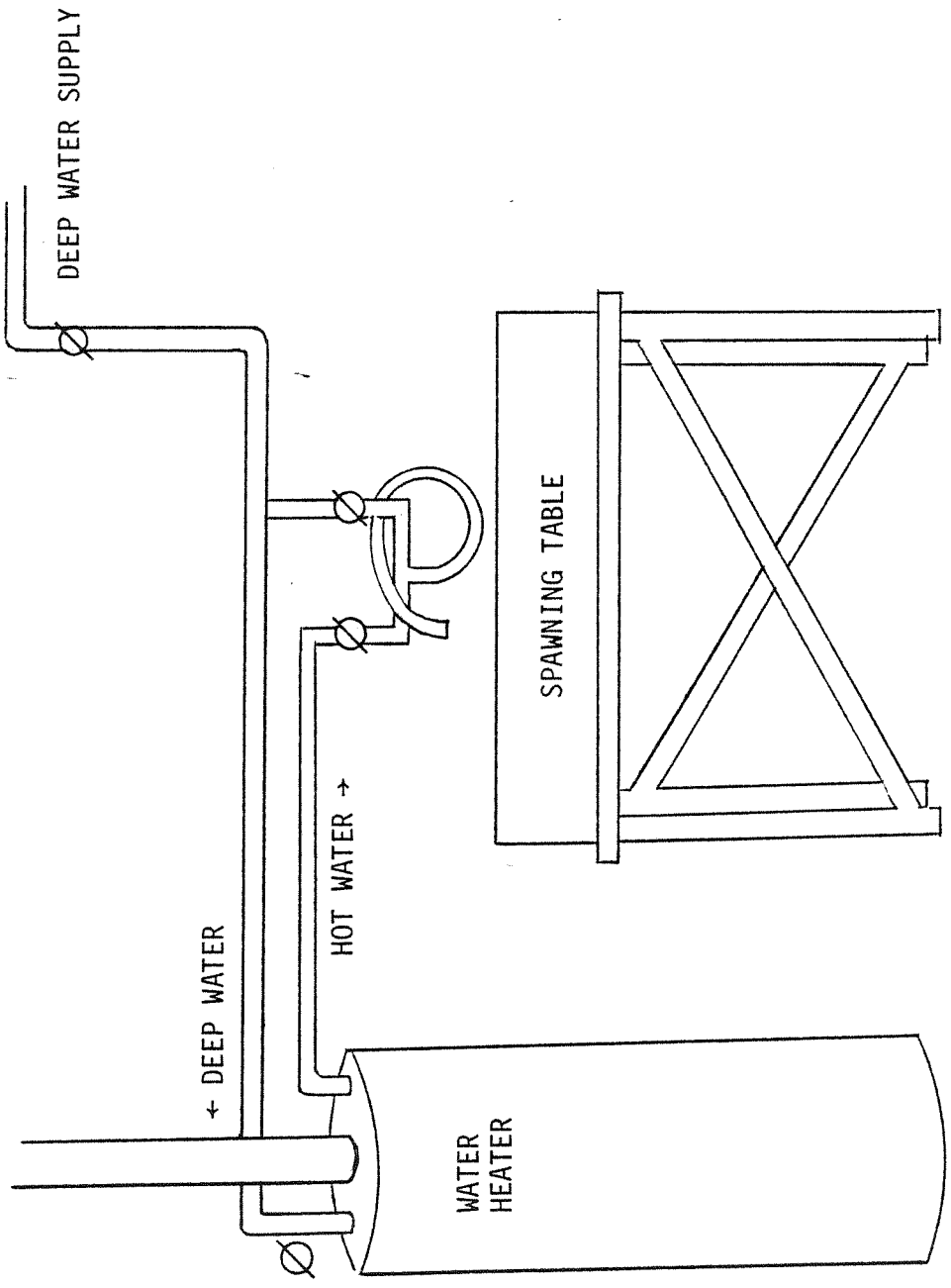

2 ft.
SHELLFISH
SHELLFISH
SHELLFISH
SHELLFISH
SHELLFISH
SHELLFISH
SHELLFISH
SHELLFISH
SHELLFISH
SHELLFISH
SHELLFISH
SHELLFISH
THERMOMETER
THERMOMETER
4 ft.
DRAIN WITH A STANDPIPE ON EITHER END OF TABLE

Figure 2. Thermal stimulation to induce spawning in shellfish. Deep water is heated in a glass-lined, gas-fired water heater to a temperature of 31-32°C.


DEEP WATER SUPPLY
SPAWNING TABLE
HOT WATER →
← DEEP WATER
WATER HEATER

After two or three thermal cycles, if no spawning occurs, all the water is siphoned out of the dishes and they are refilled with algal culture. Then a heat shock is given; the shellfish usually spawn after this treatment.

If spawning is induced, it is important to isolate any spawning male from dishes that may contain females. Therefore, two Pyrex dishes containing algal culture are devoid of shellfish during spawning attempts and are ready to accommodate spawning males. We have found that too much excess sperm in a dish or bucket of fertilized eggs may interfere with cleavage and irregular larvae are produced.

The sex of the clam or oyster can be determined only at time of spawning or only after the shellfish has been sacrificed. Male oysters (C. gigas and Kumamoto) release sperm in a thin, erratic stream from the area of the excurrent siphon. Female oysters release a cloud of eggs by opening and closing the valves almost with a clapping motion (Fig. 3).

Tapes japonica release sex products through the excurrent siphon (Fig. 4). Sperm released into the water gives a milky appearance to the spawning dish; eggs released in the water are observed as tiny, individual specks that will eventually settle out (sometimes in clumps) on the bottom of the dish. The observations of sperm and eggs in the water are similar for both oysters and clams.

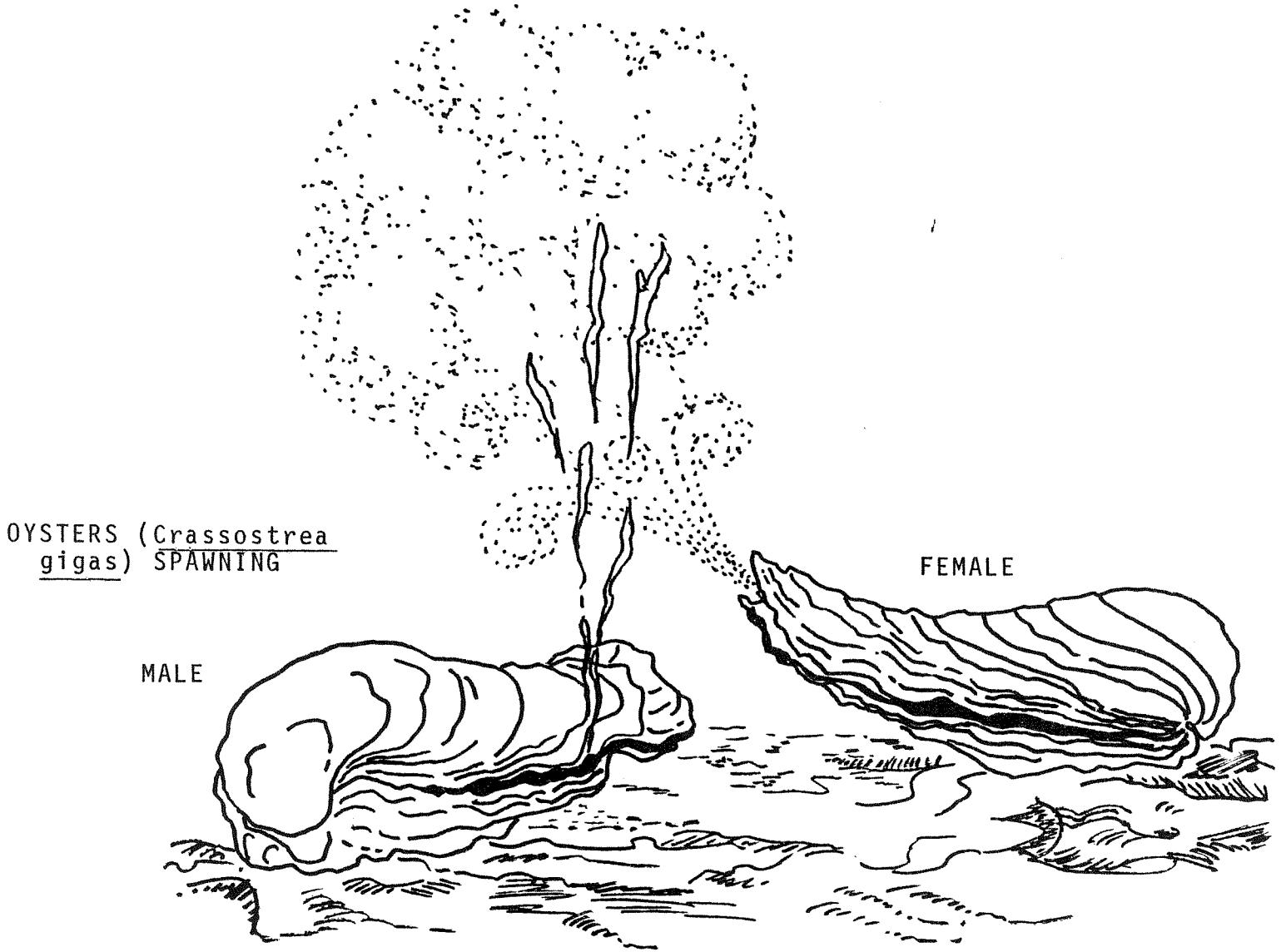
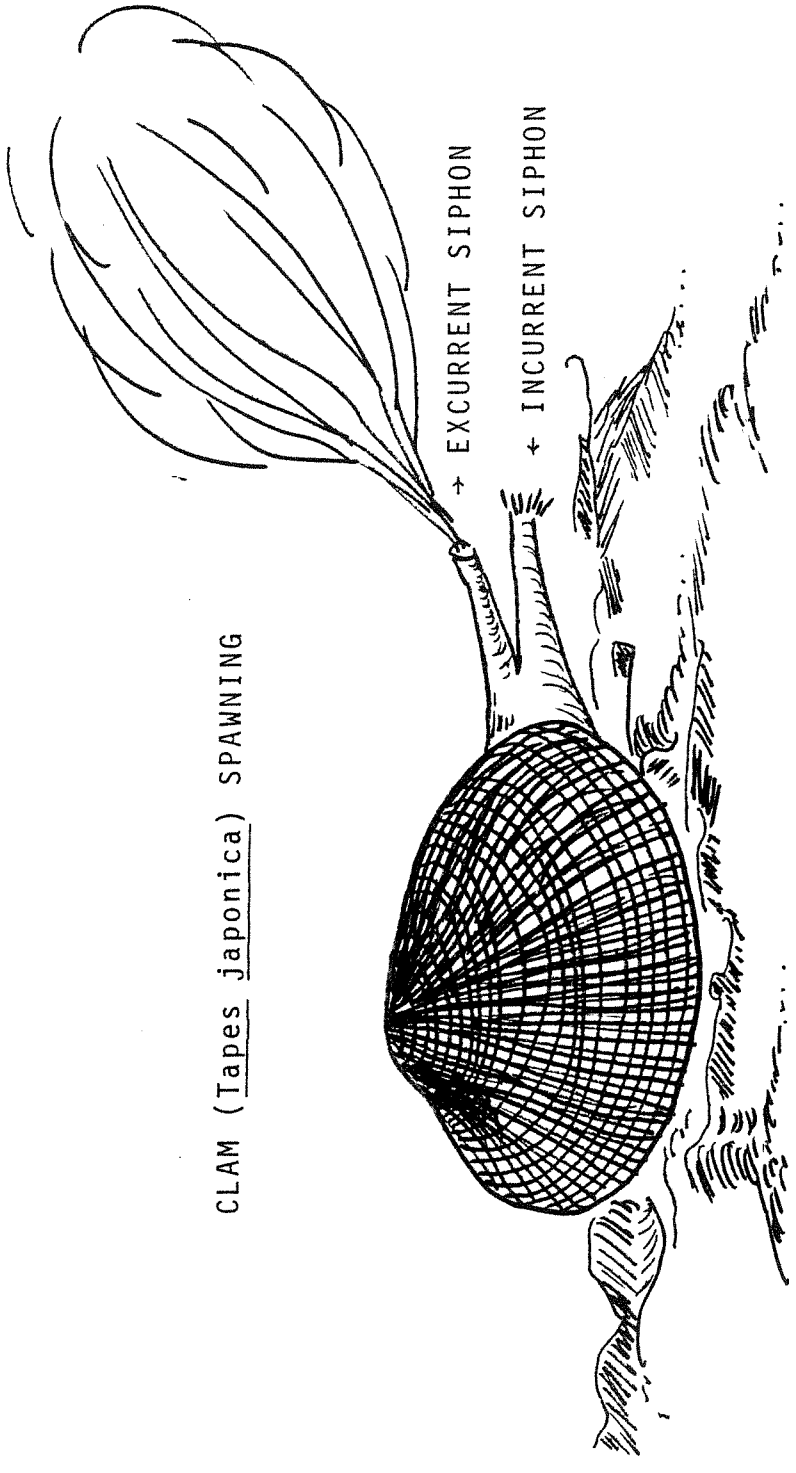

OYSTERS (Crassostrea gigas) SPAWNING
FEMALE
MALE

Figure 4. Tapes japonica clams release sex products through the excurrent siphon. Sperm released into the water gives a milky appearance; eggs appear as tiny, individual specks.


CLAM (Tapes japonica) SPAWNING
EXCURRENT SIPHON
INCURRENT SIPHON

After female shellfish have been induced to spawn, the eggs are siphoned out of the spawning dishes into a bucket containing approximately 3-4 liters of deep water. Please note—when siphoning out the eggs, care should be taken to avoid draining all the water out of the dish and exposing the spawning female. After siphoning, refill the dish with water of comparable temperature. As long as the female continues to spawn, repeat the siphoning and refilling steps to collect as many eggs as possible.

Fertilize the eggs with approximately 10-15 ml of sperm solution (color of watered-down milk). If sperm is less dense, use 5-10 ml more for fertilization. After the sperm solution has been added, allow the bucket of eggs (with volume of 10-12 liters) to stand, with one air-line bubbling slowly, for 30 mins. Then filter the contents of the bucket through a 130μ sieve. This sieve will collect any debris or clumped gonad material and allow the clean, fertilized egg solution to pass through. Tapes eggs are approximately 61μ in diameter and C. gigas and Kumamoto oyster eggs about 48μ.

Note—If Tapes spawn spontaneously in a tank, the eggs can be filtered through a 130μ sieve (to remove debris) and onto a 35μ sieve; the eggs will be caught on the 35μ sieve but the extra sperm will pass through. Several batches of Tapes have been reared after collecting cleaving eggs on a 35μ sieve and growth was normal.

After filtering through a 130μ sieve, the fertilized

eggs are randomized and sampled (a 15 ml sample is taken) for counting and observation. A Sedgwick-Rafter cell is used to determine the number of fertilized eggs (or larvae) per milliliter; duplicate or triplicate 1-ml aliquots of the sample are counted.

The total number of fertilized eggs can be calculated if the volume of the fertilized egg concentrate is known.

Example of a calculation:

16-liter concentrate of fertilized eggs

Counts: 1. 212 eggs/ml

2. 193 eggs/ml

Average # eggs/ml = 203

or $\frac{(212+193)}{2} = 203$

Total # eggs in concentrate = 3,248,000

or (16,000 ml x 203/ml = 3,248,000)

The eggs are then dispersed into either 15-, 30-, 50-, or 379-liter polyethylene or fiberglass containers at 15-20/ml for oysters and 10-15/ml for Tapes. These containers are slowly aerated at all times.

Algal culture, previously filtered through a 35μ sieve, is added on Day 0 to the polyethylene containers to achieve a concentration of approximately 5×10^4 cells/ml since straight-hinge, veliger larvae develop within the first 24 hr. The larvae begin to feed at this point in their cycle and the first filtration and regular feeding are not scheduled until 36-48 hr after spawning. The reason for the delay in regular filtering is to ensure complete development

of the larval shell. If larvae are filtered too early, the shell may be injured and deformities and eventually mortality occurs (Loosanoff and Davis, 1963).

Word of caution! After spawning has been attempted using thermal and chemical stimulation, the brood stock animals should NEVER be placed back into the tank containing the other brood stock. This rule should be followed even if attempts at spawning are not successful. There is always a chance that the brood stock you were working with will spawn late in the day. Thus, if they were put back with the others all your brood stock may spawn and put you out of business for a while. Therefore, brood stock used in the hatchery should be placed in a separate pan with a continuous food supply for 4-5 days to ensure that no spawning will occur.

III. LARVAL REARING AND FEEDING

Rearing of Larvae

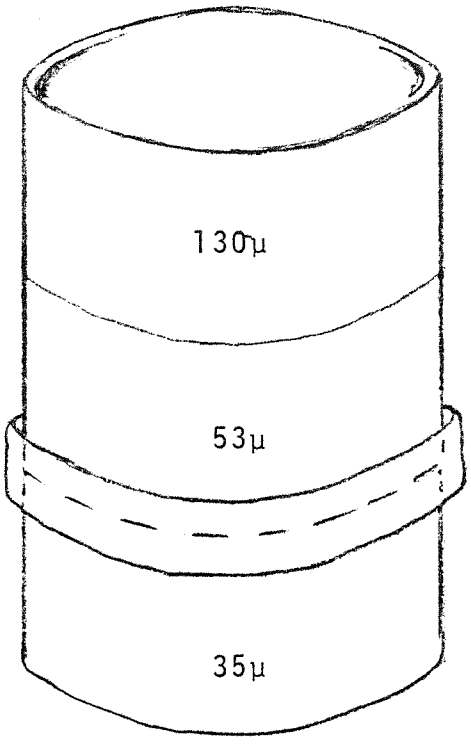
The following will be a basic description for rearing all bivalve larvae in the "Artificial Upwelling" mariculture system. If there is an exception for a particular species, it will be mentioned.

Every other day, the larval cultures are filtered through a graduated series of three 25.4-cm diameter Nitex sieves (made with nylon-monofilament bolting cloth; Tobler Ernst, Fraber Inc., Elmsford, New York)(see Fig. 5). In the top sieve, clumped food and debris are trapped and discarded while larvae are collected on the bottom two sieves. The larvae are rinsed from the sieves and combined in a concentrate of 3 liters or more, depending on the number of larvae present; the concentrate should have at least 30 larvae/ml.

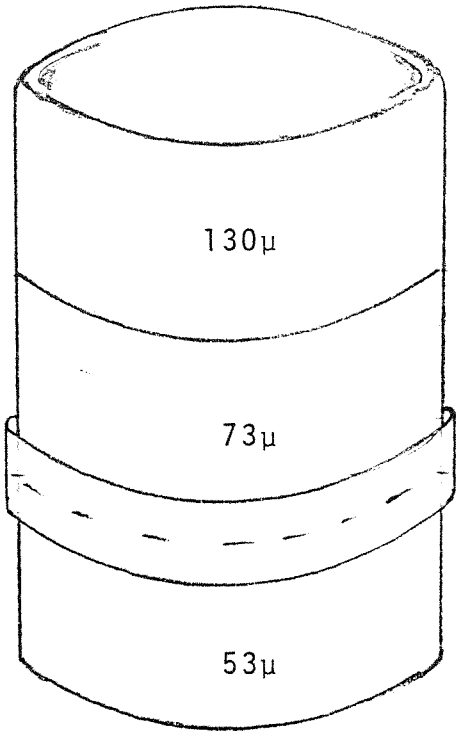
Sieve sizes are increased on the next filtration day if a large percentage (90% or more) of the larvae accumulate on the middle sieve. When selecting sieve sizes, be sure to check the "diagonal" of the specific mesh size being used. In all cases, the diagonal is considerably larger than the mesh size recorded on the sieves and unless care is taken in selecting sieves, many larvae may be lost. Table 1 lists sieve mesh size and the corresponding diagonal in microns.

A 15-ml sample of the larval concentrate is taken after

Figure 5. Series of sieves used to separate different size fractions of oyster larvae and of clam larvae. The 130μ sieve fits loosely on top of the middle sieve to allow air to pass, but the bottom two sieves are aligned with each other using a plastic collar.



FIRST SERIES OF SIEVES
FOR OYSTER LARVAE
(C. gigas & Kumamoto)



FIRST SERIES OF SIEVES
FOR CLAM LARVAE
(Tapes japonica)

TABLE 1. HATCHERY SIEVE SIZES

MESH SIZE IN MICRONS (MARKED ON SIEVES)	CORRESPONDING DIAGONAL IN MICRONS
350	495
300	424
253	358
211	293
163	231
130	184
102	144
86	122
73	103
53	75
35	49
20	28

thorough randomization (Fig. 6). Procedure for randomizing the larval concentrate is to gently plunge the "randomizer" up and down in a bucket at least 10 times, taking care not to touch the "randomizer" to the bottom of the bucket and not to splash the contents of the bucket.

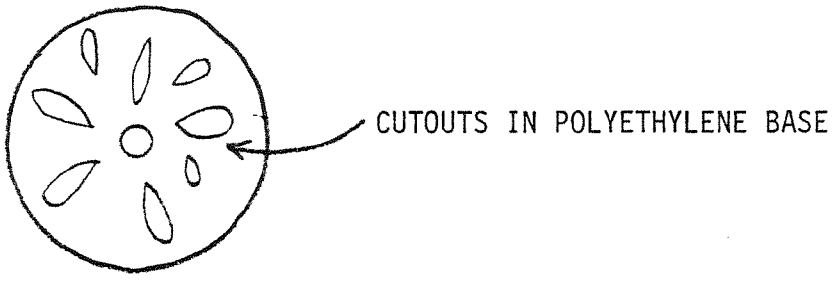
Data on larval growth and survival are obtained from the 15-ml sample. A Sedgwick-Rafter cell is used to determine the number of larvae per ml and either duplicate or triplicate 1-ml aliquots of each sample are counted on Days 2 and 10. At least 10 larvae from each sample are measured (each time the larvae are filtered)—length and width in microns (Loosanoff et al., 1966)—using an ocular micrometer (Fig. 7). Ten drops of alcohol are placed in the Sedgwick-Rafter slide, before counting, to make the larvae settle on the bottom of the slide.

Feeding of Larvae

All algal culture should be filtered through a 35μ sieve before introducing it into the larval cultures. The source of the algal cultures (i.e., Pool #, Reactor #, Polytank #, or Carboy date) and the quantity used should be recorded in the notebook each time the larval culture is filtered. The initial number of cells/ml added to the larval containers is determined either by cell counts or extrapolation from turbidity measurements. The initial food concentration in the larval cultures ranges from 8×10^4 to 2×10^5 cells/ml. Larvae fed a mixture of algal species reach setting size faster than those fed diets of monocultures. For Tapes, the mixture of 3H, STX-114, and S-1 (two diatoms and a naked flagellate: Thalassiosira

Figure 6. A randomized sample of larvae is taken by gently plunging the "randomizer" up and down in the bucket at least 10 times, taking care not to touch the "randomizer" to the bottom of the bucket and not to splash the contents of the bucket.

THE "RANDOMIZER"



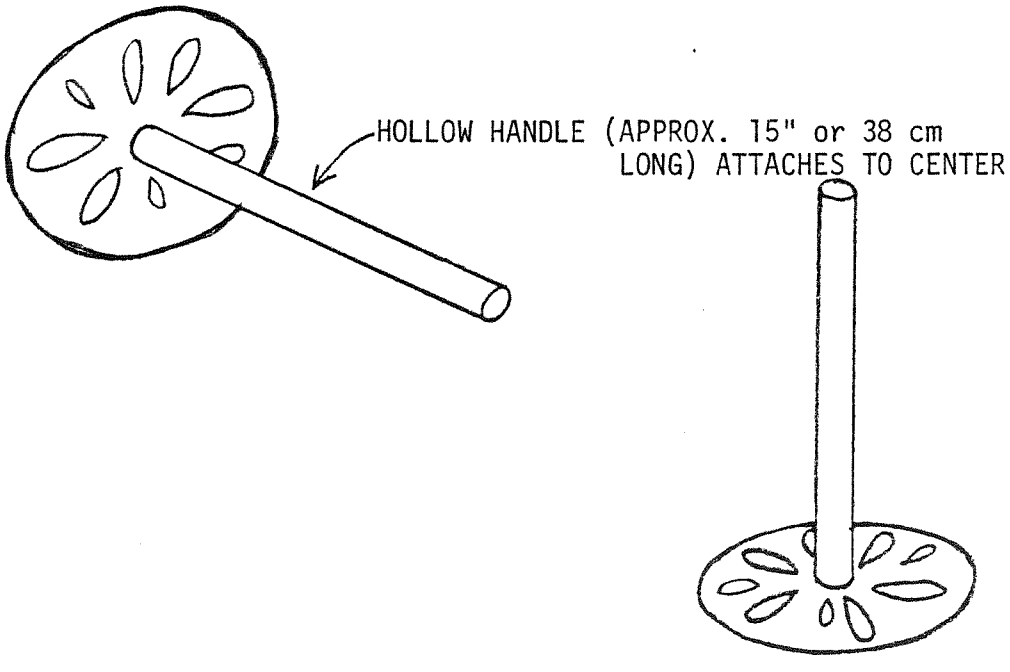
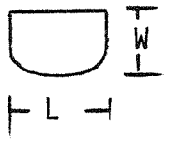


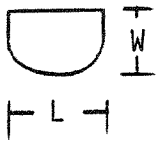
Figure 7. Sample of a page of larval measurements for oysters and clams.

OYSTER LARVAE

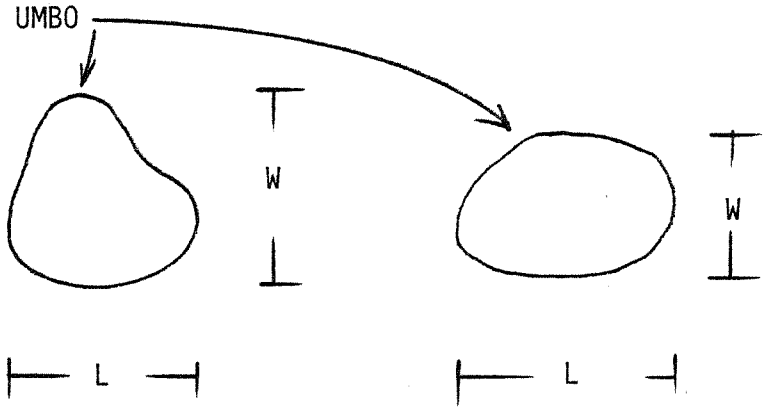
CLAM (Tapes) LARVAE

STRAIGHT HINGE or
"D"-SHAPED LARVAE





LARVAE WITH AN UMBO



(1) Microscope used and magnification with ocular micrometer (e.g., Wild 125X)

(2) State size of smallest unit in micrometer as calibrated on microscope (e.g., @ unit = 9.52μ)

(3) Record length and width in units (to nearest 1/10)

e.g.:	L	W
	9.8	8.7
	10.0	8.9
	9.4	8.7
	9.8	8.8
	10.0	9.0
	9.2	8.5
	9.5	8.6
	9.8	8.8
	9.7	8.7
	9.6	8.5
Average:	9.7	8.7

(4) Calculate average size in microns (i.e., multiply average # units by # microns in @ unit:

92μ x 83μ
(length x width)

pseudonana, Bellerochea polymorpha, and an unidentified Cryptophyte flagellate) gives the best results, but a mixture of two of those cultures gives satisfactory results (Sunderlin et al., 1976).

Prior to filtering the larval cultures, no quantitative measurements (cell counts or turbidities) are made on the algae remaining in the larval cultures. However, a pale green color is observed in the cultures, indicating that food is still present.

After the food is added to the larval culture vessel, the larvae from the larval concentrate are added. The larvae are distributed equally among the culture vessels and the final volume in each vessel is reached by adding deep water.

The 15-, 30- and 50-liter larval cultures are aerated with one air-line each—using a black rubber #12 stopper (or bung) as a weight on the air-line. Only slow, gentle aeration is used. In the 379-liter cultures, two air-lines (with stoppers as weights) are used to keep the larvae in suspension.

Both techniques of keeping larvae in suspension work well when an initial stocking density of 10 larvae/ml is used. On each filtration day, the larvae are returned to the same size and number of containers. Therefore, when metamorphosis is reached, there are usually 5-6 Tapes larvae per ml. For oysters, the concentration is closer to 1/ml when setting size is reached.

Cleaning of Culture Vessels

It is preferable but not essential to have twice as many containers as one uses for any given experiment. This way, the extra clean containers can air dry (upside-down) for the days they are not being used. Air drying in a hatchery is one of the best ways to control contamination since most chemicals (Chlorox) can be harmful to larvae if misused. The 15-, 30-, and 50-liter containers are best scrubbed with one's clean hands and a thorough rinsing with deep water followed by draining (and preferably air drying) inverted or upside-down on a slatted surface to allow air circulation inside the buckets. A stiff brush—clean and only to be used for hatchery culture containers—may be used for scrubbing culture vessels, especially the 379-liter vessels.

A good rule of thumb for hatchery vessels/containers is: if found upside-down, vessel is clean; if found right side-up, assume vessel to be dirty and scrub, rinse and drain before using.

IV. SETTING AND METAMORPHOSIS

Tapes japonica larvae are transferred into fiberglass setting flumes, 10 x 1.3 x 0.5 ft (3.05 x 0.38 x 0.15 m) when the average length is 200-225μ, usually Day 14 (1×10^6 larvae/flume). The larvae in the flumes are filtered every other day and batch-fed algal cultures until Day 20 or until the larvae have set and have completed metamorphosis. Three to four air-lines are placed in each flume. After the larvae have completed metamorphosis (usually Day 20) the flumes are placed on continuous flow, receiving food from 1 or more 2000-liter reactors.

After Day 35, when the juvenile population is evaluated for uniformity of size and survival, it is advisable to thin the population down to 50,000 per flume; at this point, the flume receives food from 2 or more 2000-liter reactors.

TABLE 2. TAPES CLAM SPAT DATA

DATE_____ BATCH #___ TECHNICIAN_____

TOTAL POPULATION WEIGHT_____grams

SAMPLE	WEIGHT	NUMBER
1	_____	_____
2	_____	_____
3	_____	_____
4	_____	_____
5	_____	_____
6	_____	_____
7	_____	_____
8	_____	_____
9	_____	_____
10	_____	_____
TOTALS	W = _____	N = _____ W/N = _____

NUMBER	LENGTH	WIDTH	NUMBER	LENGTH	WIDTH
1	_____	_____	9	_____	_____
2	_____	_____	10	_____	_____
3	_____	_____	11	_____	_____
4	_____	_____	12	_____	_____
5	_____	_____	13	_____	_____
6	_____	_____	14	_____	_____
7	_____	_____	15	_____	_____
8	_____	_____	16	_____	_____

VI. LITERATURE CITED

Loosanoff, V.L. and H.C. Davis, 1963. Rearing of bivalve mollusks. In: F.S. Russel (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.

Sunderlin, J.B., P.T. Baab and E.M. Patry, 1976. Growth of clam and oyster larvae on different algal diets in a tropical "artificial upwelling" mariculture system. Proc., World Mariculture Soc. 7:215-228.

ARTIFICIAL UPWELLING MARICULTURE:

AQUACULTURE BUDGET GENERATOR

PROGRAM LISTINGS

May 1977

(a) AQUA2B

(b) INDATA

(c) INDISK

(d) SUMDATA

```
GET ACP/AQUA23
#WORKFILE ACP/AQUA23: ALGOL, 658 RECORDS, SAVED
LIST
1000 BEGIN
2000 COMMENT
3000
4000              AQUACULTURE BUDGET GENERATOR PROGRAM
5000                        METRIC
5100
5200      VERSION 23, REVISED 5.27.76 BY G ALLEN
5300        ST CROIX ARTIFICIAL UPWELLING PROJECT APPLICATION
6000
7000 ;
8000 FILE      REMIN(KIND=REMOTE),
9000           REMOUT(KIND=REMOTE,MAXRECSIZE=14),
10000           LINEOUT(KIND=PRINTER);
10500 FILE DISKIN2(KIND=DISK,FILETYPE=7,TITLE="ACP/SUMDATA1.");
11000 FILE      DISKIN(KIND=DISK,FILETYPE=7,TITLE="ACP/BNAR.");
11500 FILE  DISKIN3(KIND=DISK,FILETYPE=7,TITLE="ACP/BNAR2.");
12000       ARRAY      VAR[0:960],A[0:10],C902[0:900],A903[0:900],A910[0:900];
12500      ARRAY SUMTIT[0:28,0:5],A911[0:900];
13000       ARRAY WT[0:900],TMP[0:5];
14000      INTEGER    I,TIME,IWANT,IREC,INDX,J,IPICK,LOW,HIGH,CAPITAL,II,
14050                  LENGTH,GRWRP,IJ,K;
15000      REAL       LAG906,LAG907,LAG909,DUM,VAL,VALU;
15050 BOOLEAN TEST;
16000      LABEL      CALC925,NOWRITE,ENDOFPROG,START,CHANGES,LISTIT,INSERT,
17000                 PICKONE,MOREBNGES,C204,C207,C402,SECTION7,LIST1,ERR1,
17500                  LIST2,LIST3,TABLES,FX903,FX910,LSST,LAST;
18000 FORMAT  FMTOUT1 ("IF YOU WANT TO: (1) CHANGE AN ASSUMPTION"
19000                  ,"(2) LIST A VARIABLE"/T5,"(3) RUN THE "
20000                  ,"BUDGET PROGRAM;(4) CHANGE A TABLE:"
21000                 ,"(5) STOP THE PROGRAM."/T5,
21500                "TYPE 1,2,3,4 OR 5"),
22000          BRIEF1("PICKONE. FOR HELP TYPE 9"),
24000         FMTOUT2    ("TYPE ASSUMPTION NUMBER, COMMA, AND NEW"
25000                    ," VALUE",/,"WHEN DONE TYPE '999,0'"),
27210          FMTOUT5("IF YOU WANT TO:"/T5,"(1) SEE A SECTION"/
27220                  T5,"(2) SEE AN ASSUMPTION",/,T5,
27230                  "(3) SEE A TABLE",/,T5,
27240                  "(4) RETURN TO THE PROGRAM",/,
27250                  "TYPE 1,2,3, OR 4"),
27260          FMTOUT6("TYPE THE RANGE OF THE SECTION YOU WISH TO SEE",/,
27270                  "(E.G. 1,47)"),
27280         FMTOUT7("IF YOU WANT TO:",/,T10,"(1) SEE THE"
27290                  " MORTALITY ADJ. FACTOR TABLE"
27300                 ,/,T10,"(2) SEE THE COMPARTMENT SIZE TABLE",/,
27310                 "TYPE 1 OR 2"),
27320        FMTOUT8(5("T",J3,"=",F7.4,X1,)),
27340        FMTOUT9("TO SPECIFY THE TABLE:",/,
27350                T10,"(1) A903",/,T10,"(2) A910",/,
27360                T10,"(3) RETURN TO THE PROGRAM",/,
27370                "TYPE 1,2 OR 3"),
27380       FMTOUT10("TYPE THE RANGE, COMMA, NEW VALUE"
27390                ,/,"(E.G.5,12,0.02). WHEN DONE TYPE '999,999,999'"),
28000          FMTERR1    (I3,"  IS NOT A VALID NUMBER FOR "
29000                      "AN ASSUMPTION***TRY AGAIN"),
29500       FMTOUT4   ("TYPE THE ASSUMT. NUMBER YOU WISH TO SEE",/,
29510                  "IF YOU WISH TO SEE ANOTHER ASSUMT. - TYPE ",/,
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29520                  "IN THER NEXT ASSUMT. YOU WISH TO SEE. WHEN",/,
29530                  "DONE TYPE '-999'"),
29535          FMTOUT11(T22,"PHYSICAL PLANT REQUIREMENTS",/,T12,
29540                   "TOP LINE FOR SINGLE BATCH BOTTOM LINE FOR",
29545                   " ONE BATCH PER MONTH",/,"MNTH",T7,"NUMBER",
29550                   T17,"SPACE",T24,"FLOWRATE",T35,"HEATING",
29555                   T44,"PUMPING",T54,"AERATION",T64,"FEED COST"),
30000            FMTOUT3  (I3,X1,E16.8,X2,8A6),
30100         FMTOUT12("TYPE TRANSFER NUMBER, AREA (M2), WEIGHT(G)",'
30150                  "(E.G. 3,0.02,150.0) WHEN DONE TYPE 999");
31000 SWITCH   SWLAB:=C204,C207,C402;
31500 SWITCH SWLAB1:=LIST1,LIST2,LIST3,PICKONE;
34000     VALUE ARRAY ASST(1,2,4,5,8,10,11,13,14,15,16,17,18,19,20,
35000                      22,24,26,27,29,31,34,35,36,47,101,102,
36000                      103,104,105,106,107,108,109,110,201,202,
37000                      203,204,205,206,207,208,209,210,211,212,213,
38000                      214,216,222,301,302,303,304,305,312,401,402,403,404,
39000                      405,406,407,408,409,410,411,412,413,414,415,416,
40000                   417,418,419,420,426,431,441,442,443,445,501,502,503,
41000                      504,505,506,507,509,601,602,603,604,605,
41025                      606,608,610,611,618,620,641,642,643,644,
42000                      701,703,704,705,801,802,803,
43000                      804,805,806,808,809,901,920,921,927,928);
44000     VALUE ARRAY CALC(204,207,402);
44100     VALUE ARRAY RITE(931,936,932,937,933,938,934,939,935,
44200                        940,941,807,947,615,950,949,944,946,
44300                        945,414,948,948);
44400   DEFINE TEMP=FOR I:=1 STEP 1 UNTIL 950 DO
44420          IF VAR[I] NEQ 0.0 THEN
44440          WRITE(REMOUT,<I3,E16.8>,I,VAR[I])#;
45000     DEFINE RITEOUT=A[0]:=VAL;
45500            VAR[IREC]:=VAL;
46000                     WRITE(DISKIN[IREC],11,A[*])#,
47000             REED=    READ(DISKIN[IREC+1],11,A[*])#;
47100   READ(DISKIN3[1],301,A903[*]);
47200   READ(DISKIN3[2],301,A910[*]);
47300    READ(DISKIN[3],301,A911[*]);
47400    FOR I:=0 STEP 1 UNTIL 300 DO BEGIN
47500       A903[I+300]:=A910[I];
47600       A903[I+600]:=A911[I];
47700    END;
48000     FOR I:=0 STEP 1 UNTIL 122 DO
49000          READ(DISKIN[ASST[I]], * ,VAR[ASST[I]]);
49100    FOR I:=0 STEP 1 UNTIL 25 DO
49110    READ(DISKIN2,<4A6>,FOR J:=0 STEP 1 UNTIL 3 DO SUMTIT[I,J]);
50000F'ICKONE:       WRITE(RE
51000                READ(REMIN, / ,IWANT);
51500                CAPITAL:=0;
52000                IF IWANT EQL 1 THEN GO TO CHANGES ELSE
53000                IF IWANT EQL 2 THEN GO TO LISTIT ELSE
54000                IF IWANT EQL 3 THEN GO TO START ELSE
54500                 IF IWANT EQL 4 THEN GO TO TABLES ELSE
54510                 IF IWANT EQL 5 THEN GO TO ENDOFPROG ELSE
54517               IF IWANT EQL 9 THEN
54519               BEGIN
54521                 WRITE(REMOUT,F
54523                 GO TO PICKONE;
54525               END ELSE
54527               GO TO PICKONE;
```

```
54530 TABLES:   WRITE(REMOUT,FMTOUT9);
54540           READ(REMIN,/,IPICK);
54550           IF IPICK EQL 1 THEN
54560           BEGIN
54570                WRITE(REMOUT,FMTOUT10);
54580           FX903:  READ(REMIN,/,LOW,HIGH,VALU);
54584                 IF LOW EQL 999 THEN GO TO PICKONE;
54592                 FOR I:=LW STEP 1 UNTIL HIGH DO
54596                 BEGIN
54600                   A903[I]:=VALU;
54608                 END;
54610                   WRITE(DISKIN3[1],301,A903[*]);
54612                 GO TO FX903;
54620           END ELSE
54630           IF IPICK EQL 2 THEN
54640           BEGIN
54650                WRITE(REMOUT,FMTOUT12);
54660            FX910:READ(REMIN,/,TIME);
54670                  IF TIME EQL 999 THEN GO TO PICKONE;
54680                  READ(REMIN,/,VALU,VAL);
54950                END ELSE
54960                   IF IPICK EQL 3 THEN GO TO PICKONE;
55000 CHANGES:        WRITE(REMOUT,FMTOUT2);
56000 MORECHANGES:    READ(REMI, / ,IREC,VALU);
57000                 IF IREC EQL 999 THEN GO TO PICKONE;
57050            IF IREC EQL 808 THEN
57100            BEGIN
57150               VAL:=-LN(VALU)/180.0;
57200               FOR I:=1 STEP 1 UNTIL 901 DO
57250               A903[I-1]:=EX(-VAL*I);
57300            END;
57350            WRITE(DISKIN[3],901,A903[*]);
57500                READ(DISKIN[IREC],11,A[*]);
58000                IF MASKSEARC(IREC,4"FFF",ASST) NEQ -1 THEN
59000                BEGIN
60000                IF INDX:=MASKSEARCH(IREC,4"FFF",CALC) NEQ -1 THEN GO TO
61000                SWLAB[INDX+1];
61500                GO TO INSERT;
62000                C204: VALU:=1800*VALU**0.5;
63000                      GO TO INSERT;
64000                C207: VALU:=28000.0*VALU**.548;
65000                      GO TO INSERT;
67000                C402: VAL:=95000.0*VALU**.565;
68000                      RITEOUT;  REED;
69000                      VAL:=140000.0*VALU**.554;
70000                      RITEOUT;  REED;
71000                      VAL:=230000.0*VALU**.538;
72000                      RITEOUT;  REED;
73000                      VAL:=510000.0*VALU**.50;
74000                      RITEOUT;  REED;
75000                      VAL:=42000.0*VALU**.438;
76000                      RITEOUT;  REED;
77000                      VAL:=14.0*VALU**(-.169);
78000                      RITEOUT;  REED;
79000                      VAL:=86.0*VALU**(-.346);
80000                      RITEOUT;  REED;
81000                      VAL:=130.0*VALU**(-.262);
82000                      RITEOUT;  REED;
83000                      VALU:=150.0*VALU**(-.354);
```

```
84000                     READ(DISKIN[IREC],11,A[*]);
85000      INSERT:        A[0]:=VALU;
85500                     VARI[IREC]:=VALU;
86000                     WRITE(DISKIN[IREC],11,A[*]);
87000                     WRITE(REMOUT,<"##">);
88000                     GO TO MORECHANGES;
89000                END;
90000                 WRITE(REMOUT,FMTERR1,IREC);
91000                 GO TO MORECHANGES;
91010 LISTIT:   WRITE(REMOUT,FMTOUT5);
91020           READ(REMIN,/,IPICK);
91030           GO TO SWLAB1[IPICK];
91040           GO TO LISTIT;
91050 LIST1:    WRITE(REMOUT,FMTOUT6);
91060           READ(REMIN,/,LOW,HIGH);
91070           FOR I:=LOW STEP 1 UNTIL HIGH DO
91072           BEGIN
91074             IF HIGH GTR 950 THEN HIGH:=950;
91076             IF VARI[I] NEQ 0 THEN
91080             WRITE(REMOUT,<I3,F30.10>,I,VARI[I]);
91084           END;
91090           GO TO LISTIT;
91500 LIST2:          WRITE(REMOUT,FMTOUT4);
92000  LSST: READ(REMIN,/,IREC);
93000                 BEGIN
93500                       IF IREC EQL -999 THEN GO TO LISTIT;
93600                       IF MASKSEARCH(IREC,4"FFF",ASST) EQL -1 THEN
93700                       BEGIN
93800                             WRITE(REMOUT,<I3," IS NOT A VALID ASST."
93850                                   " **** TRY AGAIN">,IREC);
93870                             GO TO LSST;
93880                       END;
94000                       READ(DISKIN[IREC],11,A[*]);
95000                       WRITE(REMOUT,FMTOUT3,IREC, FOR I:=0 STEP 1
96000                       UNTIL 7 DO A[I]);
97000                 END;
97500                 GO TO LSST;
97510 LIST3:   WRITE(REMOUT,FMTOUT7);
97520          READ(REMIN,/,IPICK);
97530          IF IPICK EQL 1 THEN
97540          BEGIN
97550               WRITE(REMOUT,<"MORTALITY ADJ. FACTOR TABLE (A903)"
97555                     " ,%, IN 10 DAY INTERVALS"/>);
97560               FOR I:=1 STEP 5 UNTIL 150 DO
97570               WRITE(REMOUT,FMTOUT8,I,A903[I],I+1,A903[I+1],
97580                     I+2,A903[I+2],I+3,A903[I+3],I+4,A903[I+4]);
97590          END ELSE
97600          IF IPICK EQL 2 THEN
97610          BEGIN
97620               WRITE(REMOUT,<"AREA (M2)-WEIGHT (G) RELATIONS ",
97630                     "(A910,A911) FOR CONTRAINER SIZE TRANSFER"/>);
97640               FOR I:=0 STEP 1 UNTIL LENGTH - 1 DO
97650               WRITE(REMOUT,<X10,I3,2F12.4>,I+1,A910[I],A911[I]);
97660           END;
97670          GO TO LISTIT;
97700 START:
97705  TEST:=FALSE;
98100 % TIME OR WEIGHT DEPENDENT GROWTH LIMIT
98150  HIGH:=899;
```

```
98200 IF VAR[2] GTR 0 THEN HIGH:=MIN(VAR[2]-1.0,899);
98250 LOW:=0;
98300 %INITIALIZE VARIABLES
98305  FOR I:=457,458,459,460 DO VAR[I] := 0.0;
98310 FOR I:=311,435,437,452,453,454,455,456,463,514,612,924,925,926
98315   DO VAR[I] := 0.0;
98325  II:=0;
98350 WT[LOW]:=VAR[805];
98400 C902[LOW]:=VAR[901];
98450 VAR[922]:=HIGH+1;
98500 %  BIOLOGICAL SUBSYSTEM
98550 %    SET STARTING DAY (1-360)
98560   IJ:=VAR[10];
98565 %  STARTING WEIGHT,NUMBER OF TRAYS,SHARE OF TRANSFER
98570   VAR[98]:=VAR[805]*VAR[11];
98575   VAR[99]:=VAR[19]*1000.0/VAR[98];
98580   VAR[900]:=1.0;
98585   VAR[706]:=1.0;
98600 FOR I:=LOW STEP 1 UNTIL HIGH DO
98650 BEGIN
98660     IF I EQL HIGH THEN TEST:=TRUE;
98700 %  SET TEMPERATURE, AMBIENT AND OPERATING
98750    VAR[229]:=VAR[201];
98800    IF VAR[202] GTR 0 THEN VAR[229]:=VAR[202];
99050 %  OXYGEN CONSUMPTION (MG/HR)
99070    VAR[520]:=VAR[503]+VAR[620]*VAR[229];
99100    VAR[513]:=(VAR[520]*WT[I]**VAR[501])*C902[I];
99110 %  PHYTOPLANKTON PRODUCTION
99120 %  DEPTH FACTOR
99130   IF VAR[18] GTR VAR[22] THEN
99131     BEGIN
99133       WRITE(REMOUT,<"ACTUAL POOL DEPTH EXCEEDS COMPENSATIN~ DEPTH",
99135             2(X2,F8.1)>,VAR[18],VAR[22]);
99137       VAR[51]:=VAR[22];
99140      END ELSE VAR[51]:=VAR[18];
99150    VAR[51]:=VAR[51]-(VAR[51]**2)/(2.0*VAR[22]);
99160 %  EXPOSURE FACTOR
99170   VAR[52]:=VAR[24]/VAR[51];
99180 %  CONVERSION EFFICIENCY
99200    VAR[53]:=1.0+VAR[26]*VAR[52]+VAR[27]*VAR[52]**2.0;
99202   IF VAR[53] LSS 0.0 THEN BEGIN
99204   WRITE(REMOUT,<"CALCULATED PHYTOPLANKTON CONVERSION EFFICIENCY IS",
99206         " NEGATIVE,",/,"IMPLYING EXCESSIVE FLOWRATE. COMPUTATION ",
99208         "TERMINATED. REDUCE VARIABLE 24">);
99210   GO TO PICKNE;
99212   END;
99214 %  RATE OF PROTEIN PRODUCTION (G/CM2/DAY)
99220   VAR[54]:=6.25*(VAR[20]+VAR[644]);
99230   VAR[55]:=VAR[54]*VAR[53]*VAR[24];
99300 %  GROWTH INCREMENT
99350    WT[I+1]:=WT[I];
99380 %  ANIMAL GROWTH FUNCTION
99400    BOBIN:=1 STEP 1 UNTIL 2 DO
99500 %  FEEDING CRITERION
99650 % LIMITED BY FEED QUANTITY AND TYPE
99655   VAR[56]:=VAR[29]*VAR[31];
99661 % CONVERSION EFFICIENCY
99667   VAR[57]:=VAR[34]+VAR[35]*LN(VAR[56])+VAR[36]*LN(VAR[56])**2;
```

```
99670 %  CONVERSION FACTOR
99673    VAR[58]:=VAR[14]*VAR[15]*VAR[17];
99676 %  RATE OF FEEDING PHYTO (G/SEC/ANIMAL IN)
99685    VAR[59]:=VAR[56]*WT[I]**VAR[802];
99691 %  GROWTH INCREMENT PER DAY
99694    VAR[807]:=VAR[58]*VAR[57]*VAR[59]*86400.0;
99700 % LIMITED BY METABOLITE/OXYGEN CONC/PH NOT OP
99750 % LIMITED BY SPACE, NOT OP
99900          WT[I+1]:=WT[I+1]+VAR[807];
99950      END;
99975        VAR[807] := (WT[I+1] - WT[I])/2.0;
100000    WT[I+1]:=(WT[I+1]+WT[I])/2.0;
100010 %  FOOD CONSUMPTION, CORRECTED FOR MORTALIT  G/SEC/ANIMAL IN
100020    VAR[621]:=VAR[59]*C902[I];
100031 %  METABOLITE PRODUCTION (G/DAY) AMMONIUM-NH3 (TH MAX)
100032     VAR[432]:=(1.0-VAR[57])*VAR[621]/6.25;
100034 % NH3 DISSOCIATION BASED ON HENDERSON-HASSELBACH EQUATION AND
100035 % LOG KA=.2804+2717.(1/ABCTEMP) PKA=VAL
100036     VAL:=.2804+2717.0/(VAR[229]+273.0);
100037 % PROPORTION OF NH3 (VALU) THEN ACTUAL NH3
100038      VALU:=1.0/(10.0**(VAL-VAR[445])+1);
100039     VAR[436]:=VAR[432]*VALU;
100040      VAR[463]:=VAR[621]*VAR[420]*VAR[603];
100050 % TARGET WEIGHT REACHED?
100100     IF WT[I+1] LSS VAR[920] THEN
100150     BEGIN
100200        IF I EQL 899 THEN
100250        BEGIN
100300          WRITE(REMOUT,("TARGET WEIGHT CANNOT BE REACHED IN ",
100350               "900 DAYS",/,"RESET ASSUMPTIONS JUST CHANGED"));
100400          GO TO PICKONE;
100450        END;
100500     END ELSE
100550     BEGIN
100600        VAR[922]:=I+1;
100650          TEST:=TRUE;
100700     END;
100850 % SURVIVAL
100900      C902[I+1]:=A903[I+1]*VAR[901];
100950 % SURVIVAL MODIFIED BY O2/METAB CONC, PH, SPACE, FEED, NOT OP
100960 % WEIGHT & NUMBER, SINGLE BATCH
100970      ` VAR[915]:=WT[I+1]*C902[I+1];
100985 VAR[916]:=C902[I+1];
100990 %  TRANSFER CRITERION
100992     IF VAR[915] GEQ VAR[98] THEN
100994     BEGIN
100996 %  SHARE OF TRANSFER & NUMBER OF TRAYS (/TRAY INPUT)
100999        IJ:=IJ-1;
101000        IF IJ GTR 0 THEN BEGIN
101001          VAR[900]:=VAR[900]*VAR[11];
101002          VAR[706]:=VAR[706]+VAR[900];
101003          VAR[98]:=VAR[98]*VAR[11];
101006        END ELSE
101008       BEGIN
101010         VAR[922]:=I+1;
101012         TEST:=TRUE;
101014         I:=HIGH;
101016       END;
101018     END;
```

```
102808     IF VAR[416] GTR 0.0 THEN
102811     BEGIN
102817       VAR[494]:=1.0-VAR[493]/VAR[416];
102820       IF VAR[494] LSS 0.0 THEN
102823       BEGIN
ABBS26ESS THANWRITE(REMOUT,<"SPECIFIED FLOWRATE IN ANI
102829            " INFLOW. ",/,"RATE OF ",E16.8," (CMS/SEC/G OF ANIMAL ",
102832            "IS ASSUMED.">,VAR[493]);
102835         VAR[494]:=0.0;
102838       END;
102841     END ELSE VAR[494]:=VAR[414];
102844 %  CALCULATED FLOWRATE REQUIRED (CMS/SEC)
102850 VAL:=VAR[440]-VAR[4]/10.0**6.0;
102875 VALU:=(VAR[643]/100.0)*VAR[440]/VAL;
102900 VAR[438]:=(VAR[436]/VAL)/(1.0-VAR[494]+VAR[494]*VALU);
102950     VAR[438]:=VAR[438]*VAR[926]/VAR[915];
102955 TEST:=FALSE;
102965 IF VAR[416] GTR 0.0 THEN BEGIN
102975     IF VAR[416] LSS VAR[438] THEN TEST:=TRUE;
102985     VAR[438]:=VAR[416]*(1.0-VAR[494]);
102990  END ELSE IF VAR[495]:=VAR[493]*(1.0-VAR[494]) LSS VAR[438] THEN BEGIN
102995      TEST:=TRUE;
102998      VAR[438]:=VAR[495];
103000  END;
103005  IF TEST THEN
103010 WRITE(REMOUT,<"WARNING - FLOWRATE IN ANIMAL TANKS INSUFFICIENT TO ",
103020     "MEET DESIGN ",/,"METABOLITE LOADING. MODIFY ONE OF VAR 4,414,",
103030     "416,441,442,443,603.">);
103040 VAR[438]:=VAR[438]*VAR[915]*86.4;
103100 % AERATION REQUIREMENT FLOWRATE
103150 % TOTAL OXYGEN CONSUMPTION
103200 VAR[517]:=VAR[513]*VAR[926];
103250 % OXYGEN INTAKE CONC (OMEGAF)  MG/L
103300 VAR[515]:=11.52*EXP((-0.0207)*VAR[201]);
103350 % CALCULATED FLOWRATE  L/DAY
103400 VAL:=VAR[502]-VAR[515];
103500 VAR[515]:=11.52*EXP((-0.0207)*VAR[229]);
103550 VALU:=((-VAR[5]))*(VAR[515]-VAR[502])/VAL;
103600 VAR[439]:=VAR[517]+VAL*VAR[438]*(1.0-VAR[494]
103620             +VAR[494]*VALU)/24.0;
103650 % DIRECT OXYGENATION, IF NECESSARY
103700 IF VAR[439] GTR 0 THEN
103750 VAR[519]:=VAR[439]/(1000000.0*VAR[504])
103800 ELSE VAR[519]:=0.0;
105300 COMMENT   HEATING REQUIREMENTS, HEAT RECIRCULATING WATER
105400           THE AMOUNT OF TEMP LOSS (VAR[214]). HEAT INTAKE
105500           WATER TO OPERATE TEMPERATURE + 50% OF TEMP LSS
105600 ;
105700           VAR[200]:=((1.0-VAR[494])*(VAR[229]+0.5*VAR[214]-
105800                VAR[201]) + VAR[494]*VAR[214]);
105900           VAR[215]:=VAR[200]*VAR[438];
106100 %  NUMBER OF TRAYS
106200      VAR[950]:=VAR[900]*VAR[926];
106300      VAR[916]:=VAR[916]*VAR[926];
106400
106550  % PUMP CAPACITY, INTAKE AND RECIRC (KW) - 100% EFFIC
106600    VAR[326]:=0.00001*VAR[663]*VAR[302];
106650   VAR[327]:=0.00001*VAR[443]*VAR[302]*VAR[915];
```

```
106700  VAR[306]:=VAR[312]*(VAR[19]*1000.0)**2.0;
106720  VAR[306]:=VAR[306]*VAR[950]/1000.0;
106810 %  FEEDING EQUIPMENT REQUIREMENT
106815  IF VAR[618] GTR 0.0 THEN BEGIN
106820           VAR[629]:=VAR[950]*VAR[605];
106840           VAR[627]:=VAR[618]*(INTEGER(VAR[900]/VAR[606])+1)*
106841                        (1.0+VAR[604])*VAR[401]/2000.0;
106850  END;
107000          FOR II:=438,215,438,235 DO VAR[II]:=VAR[II]/1000.0;
107100            IF CAPITAL EQL 1 THEN WRITE(REMOUT,<I2,7F10.1,/,X2,
107200            7F10.1>,J,VAR[916],VAR[950],VAR[438],VAR[215],VAR[326],
107300            VAR[518],VAR[627],VAR[926],VAR[924],VAR[438],VAR[235],
107400            VAR[306],VAR[519],VAR[607]);
107450          FOR II:=438,215,438,235 DO VAR[II]:=VAR[II]*1000.0;
117000 COM    (SECTION 2)
118000                        COST OF SPACE
119000 ;
119500 %  COST OF LAND
120000                VAR[111]:= VAR[101]/VAR[102];
121000                VAR[112]:= VAR[111]*VAR[928];
121100 % COST OF PHYTO TANKS
121200   VAR[124]:=(3.0*VAR[803]+1.0)*SQRT(VAR[662])/100.0;
121300   VAR[125]:=4.0*VAR[18]/100.0+2.0*(VAR[18]/100.0)**2.0;
121400   VAR[126]:=VAR[804]*VAR[124]*VAR[125]*10000.0/VAR[662];
121500   VAR[127]:=VAR[126]*VAR[928];
122000                VAR[113]:=VAR[103]*(VAR[927]/((VAR[927]+1.0)
122500                          **VAR[104]-1.0)+VAR[928]);
123000 %  COST OF ANIMAL TANKS
124000                VAR[115]:=VAR[105]*(VAR[927]/((VAR[927]+1.0)
124500                          **VAR[106]-1.0)+VAR[928]);
125000                VAR[116]:= VAR[641]*VAR[107];
126000          VAR[117]:=VAR[116]*(VAR[927]/((VAR[927]+1.0)
126500                     **VAR[642]-1.0)+VAR[928]);
127000                VAR[118]:=VAR[108]*(VAR[927]/((VAR[927]+1.0)
127500                          **VAR[109]-1.0)+VAR[928]);
128000              VAR[119]:= VAR[111]+VAR[103]+VAR[105]+VAR[116]+VAR[126];
129000                VAR[120]:= VAR[112]+VAR[113]+VAR[115]+VAR[117]+VAR[127];
130000          VAR[121]:=(VAR[111]+VAR[105]+VAR[116])/VAR[110]+VAR[108];
130500          VAR[122]:=(VAR[112]+VAR[115]+VAR[117])/VAR[110]+VAR[118];
131000 COMMENT             END OF COST SPACE                          ;
161000                COMMENT                                          (SECTION 9)
162000                                                                 COST OF FEED
163000                ;
175000  IF VAR[602] GTR 0.0 THEN BEGIN
175050                VAR[615]:=VAR[621]*30.0/(WT[VAR[922]]
175060                          *C902[VAR[922]]);
175090              VAR[609]:=VAR[607]*(VAR[927]/((VAR[927]+1.0)
175095                        **VAR[608]-1.0)+VAR[928]);
175100  VAR[613]:=VAR[629]*30.0*VAR[611]/(VAR[921]*VAR[610]);
175110              VAR[614]:= VAR[609]/(12.0*VAR[921])+VAR[613];
175120              VAR[616]:= VAR[615]*VAR[602];
175130                VAR[617]:=VAR[616]+VAR[614]*1000.0/WT[VAR[922]];
175500  END;
176000 COMMENT             END OF SECTION 4&5                          ;
177000 COMMENT             (SECTION 6)
178000                     COST OF WASTE TREATMENT
179000 ;
179050 % DISCHARGE WASTE TREATMENT
179100    VAR[444]:=VAR[621]*VAR[926]*VAR[603]*86400;
```

```
179200 VAR[433]:=VAR[419]*VAR[444];
179300     IF VAR[432]:=VAR[431]*VAR[915]
179400      GTR VAR[433] THEN VAR[433]:=VAR[432];
179800 VAR[432]:=VAR[444]*VAR[420];
179900 VAR[422]:=VAR[494]*VAR[438];
180000   VAR[421]:=VAR[432]*VAR[417]/((VAR[438]-VAR[422])*100.0);
180020   VAR[423]:=(VAR[432]-VAR[433])/VAR[421];
180040   IF VAR[423] GTR VAR[438]-VAR[422] THEN
180060   BEGIN
180080     VAR[423]:=VAR[438]-VAR[422];
180100     WRITE(REMOUT,<"WARNING - DISCHARGE WASTE TREATMENT TOO ",
180120        "INEFFICIENT TO MEET",/,"EPA REGULATIONS IN SINGLE PASS">);
180140   END;
180210  IF VAR[423] GTR 0 THEN
180220     BEGIN
180300 VAR[452]:=(18.243*VAR[423]**0.565)*VAR[402];
180400 VAR[453]:=(31.758*VAR[423]**0.554)*VAR[403];
180800 VAR[457]:=(181.063*VAR[423]**(-0.169))*VAR[402];
180900 VAR[458]:=(16237.723*VAR[423]**(-0.346))*VAR[403];
180910     END;
181200   VAR[424]:=VAR[452]+VAR[453];
185000     VAR[425]:=(VAR[424]*VAR[401])/2000.0;
186000     VAR[427]:=VAR[425]*(VAR[927]/((VAR[927]+1.0)
186500              **VAR[426]-1.0)+VAR[928]);
187000     VAR[428]:=VAR[427]*1000.0/(365.0*(VAR[438]-VAR[422]));
188000 VAR[429]:=(VAR[457]+VAR[458]+VAR[411]*
188100          VAR[412]*VAR[413]*VAR[422]/1000000.0)/VAR[438]*1000.0;
190000     VAR[430]:=VAR[428]+VAR[429];
191000 COMMENT             END OF SECTION 6                          ;
192000 COMMENT             (SECTION 7)
193000                     COST OF AERATION
194000 ;
194500  SECTION7:
195000     VAR[508]:=VAR[506]*(VAR[519]*VAR[401])/2000.0;
196000     VAR[510]:=VAR[508]*(VAR[927]/((VAR[927]+1.0)
196500              **VAR[509]-1.0)+VAR[928]);
197000     VAR[511]:=(VAR[519]*VAR[413]*24.0*365.0+VAR[507]*VAR[508])/100.0);
199000     VAR[512]:=VAR[510]+VAR[511];
200000 COMMENT             END OF SECTION 7                          ;
201000 COMMENT             (SECTION 8)
202000                     COST OF HEAT
203000 ;
205000   IF VAR[202] GTR 0.0 THEN BEGIN
206000 VAR[217]:=(VAR[229]-VAR[201]-0.5*VAR[214])*(VAR[438]-VAR[422])*
206010           (1.0-EXP(VAR[203]*VAR[216]));
207000     VAR[218]:=5900.0*VAR[216]**0.5;
208000     VR[219]:=VAR[218]*VAR[401]/2000.0;
209000     VAR[220]:=VAR[219]*((VAR[206]/100.0)+(VAR[927]/((VAR[927]
209500              +1.0)**VAR[205]-1.0)**VAR[205]-1.0)+VAR[928]);
210000     VAR[221]:=VAR[215]-VAR[217];
211000     VAR[223]:=(1.0+VAR[222]/100.0)*(VAR[221]/24.0)*(1.0/VAR[212]);
212000     VAR[224]:=30.7*VAR[223]**0.548;
213000     VAR[225]:=VAR[224]*VAR[401]/2000.0;
214000     VAR[226]:=VAR[225]*(VAR[927]/((VAR[927]+1.0)
214500              **VAR[208]-1.0)+VAR[928]);
215000     VAR[227]:=VAR[225]*VAR[209]/100.0+VAR[210]*VAR[211]*365.0+VAR[223]
216000              *24.0*365.0*VAR[213]/(10000000.0);
217000     VAR[228]:=VAR[220]+VAR[226]+VAR[227];
218000   END;
```

```
220000 COMMENT             END OF SECTION 8                          ;
220010 COMMENT                    SECTION 10
220020                          COST OF PUMPING
220030 ;
220032   VAR[330]:=0.0;VAR[331]:=0.0;
220035   VAR[328]:=VAR[306]/VAR[8];
220040  FOR I:=326 STEP 1 UNTIL 329 DO BEGIN
220045    VAR[I]:=VAR[I]/VAR[301];
220050 VAR[307]:=(560.0*VAR[I]**0.6)*(1.0+VAR[303]/100.0)
220055           *VAR[401]/2000.0;
220060 VAR[308]:=VAR[307]*(VAR[927]/((VAR[927]+1.0)**VAR[304]
220065           -1.0) + VAR[928]);
220070 VAR[309]:=VAR[307]*VAR[305]/100.0 + VAR[I]*8760.0*
220080              VAR[413];
220090   VAR[I-6]:=VAR[308]+VAR[309];
220092   VAR[330]:=*+VAR[307];
220094   VAR[3#1]:=*+VR[#08];
220098 END;
220100     VAR[311]:=VAR[320]+VAR[321];
220105    VAR[310]:=VAR[322]*VAR[8];
220110 COMMENT               END OF SECTION 10
220120 ;
220130 COMMENT                  (SECTION 11)
220140                  COST OF LABOR - HARVEST,TRANSFER, AND CLEANING
220150 ;
220240           VAR[706]:=(VAR[706]*VAR[611])/(VAR[703]);
220500  VAR[708]:=VAR[661]*VAR[611]*VAR[704]*12.0/(VAR[705]*10000.0);
221000 COMMENT              (SECTION 9)
222000                     COST SUMMARY
223000 ;
224000   VAL:=VAR[921]*365.0/VAR[806];
225000   VAR[931]:=VAR[122]*VAR[950]/VAL;
226000   VAR[932]:=VAR[120]*VAR[662]/(10000.0*VAL);
227000   VAR[933]:=VAR[228]/VAL;
228000   VAR[934]:=VAR[323]/VAL;
229000   VAR[935]:=VAR[512]/VAL;
230000   VAR[936]:=VAR[708]/VAL;
231000   VAR[937]:=(VAR[311]+VAR[663]*0.864*365.0*VAR[13]+VAR[663]*86.4
231100              *365.0*VAR[664]*VAR[610])/VAL;
232000   VAR[938]:=VAR[662]/10000.0;
233000   VAR[939]:=VAR[310]/VAL;
234000   VAR[940]:=VAR[18]/100.0;
235000   VAR[941]:=VAR[616];
236000   VAR[942]:=VAR[663]/10.0**6.0;
237000   VAR[943]:=VAR[932]+VAR[934]+VAR[936];
238000   VAR[944]:=VAR[53];
239000   VAR[945]:=VAR[430]*(VAR[438]-VAR[422])*0.365/VAL;
240000   VAR[946]:=WT[VAR[922]];
241000   VAR[947]:=VAR[706]*VAR[926]*365.0/(VAR[806]*VAL);
242000   VAR[948]:=VAR[921];
243000   VAR[949]:=VAR[701];
245000   VAR[951]:=VAR[47]*1000.0/(WT[VAR[922]]*C902[VAR[922]]);
246000   VAR[952]:=VAR[57];
247000   VAR[953]:=0.0;
248000   FOR I:=931 STEP 2 UNTIL 951 DO
249000     VAR[953]:=*+VAR[I];
250000   VAR[954]:=VAR[922];
250100   VAR[955]:=(VAR[119]*VAR[662]/10000.0+VAR[121]*VAR[950]+VAR[219]
250200              +VAR[225]+VAR[330]+VAR[425]+VAR[508]+VAR[607])/1000.0;
```

```
250300    VAR[956]:=(VAR[932]*VAL+VAR[122]*VAR[950]+VAR[220]+VAR[226]
250400                +VAR[331]+VAR[427]+VAR[510]+VAR[609])/1000.0;
251000    WRITE(REMOUT,<T30,"OUTPUT SUMMARY">);
252000    WRITE(REMOUT,<T2,"ANIMAL PRODUCTION COST($/KG OUTPUT)",
E5BEB8URES">);T45,"PERFOR
252300           FOR I:=931 STEP 2 UNTIL 955 DO BEGIN
252350    IJ:=I-931;
252400     WRITE(REMOUT,<2(F12.4,X1,4A6)>,VAR[I],
252500       FOR J:=0 STEP 1 UNTIL 3 DO SUMTIT[IJ,J],VAR[I+1],
252600       FOR J:=0 STEP 1 UNTIL 3 DO SUMTIT[IJ+1,J]);
252700    END;
258000 GO TO PICKONE;
259000 ENDOFPROG:
260000 END.
#
REMOVE
#
```

```
GET ACP/INDATA
#WORKFILE ACP/INDATA: DATA, 123 RECORDS, SAVED
LIST
100    1              1.00000 FOR SINGLE BATCH, 1 FOR REPEATED
200    2            900.0    TIME LIMIT ON GROWTH (DAYS) MAX 900
300    4              0.0000INLET NH3 CONC (MG-N/L)
400    5              0.90000XYGENATION EFFIC IN RECIRC LOOP (PROPN)
500    8              1.0000NUMBER OF RECIRCULATION PUMPS IN ANIM TANKS
600   10             5.0     NUMBER  OF TRANSFER CYCLES
700   11             4.0     TRANSFER DECISION: FINAL WT/START WT
800   13              0.00   COST OF DEEP SEA WATER ($/M3)
900   14             2.65    TOTAL ANIM WEIGHT/WET MEAT WT (#)
1000   15              2.50  TOTAL MEAT/MEAT PROTEIN (#,D.M. BASIS)
1100   16              0.00  RESERVE PHYTO TANK CAPY (PROPN)
1200   17              5.00  WET WEIGHT/DRY WEIGHT (#)
1300   18            100.0   PHYTO POOL DEPTH (CM)
1400   19            20.0    MAXIMUM WT OF ANIMALS  (KG/TRAY)
1500   20    0.000000448     NUTRIENT CONCENTRATION DSW (G-N/CM3)
1600   22            400.0   COMPENSATION DEPTH FOR PHYTO (CM)
2700C)24            0.00106  FLOWRATE TO PHYTO TANK (C
1800   26               0.0  COEFF B1 IN PHYTO EFFIC FN (#)
1900   27   -1750000000.0    COEFF B2 IN PHYTO EFFIC FN (#)
2000   29              3.0   FEED RATE/BIOL EFFIC FD RATE (#)
2100   31   0.000000011808   BIOL EFFIC SPECIFIC FD CRITERION (G/SEC/1G AN)
2200   34  -16.89145         COEFF A0 IN ANIMAL EFFIC FN (#)
2300   35    -1.891173        COEFF A1 IN ANIM EFFIC FN   (#)
2400   36    -0.0518          COEFF A2 IN ANIMAL EFFIC FN (#)
2500   47             0.0010COST OF "SPAT" ($/HEAD)
2600  101           10000.0000COST OF LAND ($/HA)
2700  102           8000.0000AREA UTILIZABLE BY BUILDING OR STRUCTURE (M2/HA)
2800  103             10.0000CAPITAL COST OF PHYTO TANKS INCL  ($/M2)
2900  104              5.0000LIFE OF PHYTOPLANKTO TANKS (YR)
3000  105              0.0000COST OF COVER (PLASTIC WITH STEEL OR WOOD FRAME) ($/

3100  106              5.0000LIFE OF COVER (YR)
3200  107              0.0063OFFICE & STOR AGE REQUIRED AS PROPORTION OF TANK
3300  108             20.0000CAPITAL COST OF ANIMAL TANKS ($/TRAY)
3400  109              5.0000LIFE OF TANK COMPARTMENTS (YR)
3500  110              3.0   STACKING FACTOR PER AREA STRUCTURE (TRAYS/M2)
3600  201             20.0000MEAN AMBIENT SEAWATER TEMPERATURE ('C)
3700  202              0.0000OPERATING TEMPERATURE OF TANKS ('C)
3800  203              0.5000EFFICIENCY OF HEAT EXCHANGER (PROPN)
3900  204              0.0000CAPITAL COST FUNCTION=HEAT EXCHANGER WHERE C IS CAPI

4000  205             10.0000LIFE OF HEAT EXCHANGER (YR)
4100  206              2.0000ANNUAL MAINTENANCE CHARGE (% OF CAP)
4200  207              0.0000CAPITAL COST FUNCTION=GAS OR OIL FIRED HEATERS
4300  208             10.0000LIFE OF HEATER (YR)
4400  209              3.0000ANNUAL MAINTENANCE (% OF CAP)
4500  210              4.0000LABR REQUIREMENT FOR HEATER (HR/DAY)
4600  211              8.0000MAINTENANCE ENGINEER WAGE RATE ($/HR)
4700  212              0.9000EFFICIENCY OF HEATER (PROPN)
4800  213             11.0000COST OF FUEL ($/MIL BTU)
4900  214              0.0000LOSS OF HEAT WITHIN SYSTEM ('C)
5000  216              0.0000AREA OF HEAT EXCHANGER (M2)
5100  222             50.0000RESERVE CAPACITY OF HEATER REQUIRED  (%)
5200  301              0.8500EFFICIENC OF PUMP (PROPN)
5300  302             10.0000PUMP LIFT -INTKE (M)
5400  303            100.0000STANDBY REQUIREMENT (PERCENT)
```

```
 5500 304            10.0000LIFE OF PUMP (YEARS)
 5600 305             3.0000PUMP MAINTENANCE (PERCENT)
 5700 312         0.00000025RECIRC PUMPING POWER COEFF (#)
 5800 401         2400.0000ENGINEERING NEWS RECORD COST OF CONSTRUCTION INDEX(E

 5900 402             1.0000CAPITAL COST FUNCTION FOR SCREENING
 6000 403             1.0000CAPITAL COST FUNCTION FOR FILTRATION
 6100 404             0.0000CAPITAL COST FUNCTION FOR NITRIFICATION
 6200 405             0.0000CAPITAL COST FUNCTION FOR CARBON ABSORPTION
 6300 406             0.0000CAPITAL COST FUNCTION FOR OZONATION
 6400 407             0.00000 & M COST OF SCREENING
 6500 408             0.00000 & M COST OF FILTRATION
 6600 409             0.00000 & M COST OF NITRIFICATION
 6700 410             0.00000 & M COST OF CARBON ABSORPTION
 6800 411             5.00000ZONE DOSAGE
 6900 412            22.0000ELECTRICAL CONCUMPTION IN OZONE PRODUCTION (KWH/KG)
 7000 413             0.0400ELECTRICAL POWER COST ($/KWH)
 7100 414             0.9000PROP OF FLOW IN ANIMAL TANKS RECIRC (#)
 7200 415             0.1000RROPORTION OF RECIRCULATION TREATED WITH CARBON
 7300 416             0.0000FLOWRATE THROUGH TANKS (CMS/SEC/G)
 7400 417            90.0000EFFIC OF WASTE TRT SYSTEM IN REM SUSP SOL(%)
 7500 418            90.0000EFFIC OF WASTE TRT SYSTEM IN REM NITRATE(%)
 7600 419             0.1200EPA DISCHARGE REQUIREMENT (G SS/G FD/DAY)
 7700 420             0.8000WASTE PRODUCTION (G SS/G FD(DM BASIS))
 7800 426            10.0000LIFE OF WASTE TREATMENT PLANT (YR)
 7900 431             0.0030ALTERN EPA DISCHARGE REQ (G SUSP SOL/G ANIM)
 8000 441            10.0000DESIGN AMMONIACONCENTRATION (% OF 96 HR LC50)
 8100 442             1.2000ACUTE TOXIC AMMONIA CONC. (MG/L)
 8200 443             0.0000DIRECT SW FLOWRATE TO ANIM TANKS (CMS/SEC/G
 8300 445             8.0   OPERATING PH
 8400 501             0.8800XYGEN CONSUMPTION RATE (DELTA)
 8500 502             5.0000MINIMUM TOLERABLE 02 CONCENTRATION IN TANK (MG/L)
 8600 503        -0.013908  COEFF 30 IN SPECIFIC 02 CONSN FN (#)
 8700 504             0.6000AERATION EFFICIENCY, SURFACE AERATOR (PROPN)
 8800 505             1.0000ELECTRIC MOTOREFFICIENCY (PROPN)
 8900 506           500.0000CAPITAL COST OF AIR LIFT PUMP ($/INST KW)
 9000 507             1.0000MAINTENANCE COT OF AERATOR (% OF CAP)
 9100 509             5.0000LIFE OF AERATOR (YR)
 9200 601             0.0025PHYTO POOL RECIRC POWER REQ (KW/MS)
 9300 602             0.0000COST OF ARTIFIC FEED (0 IF NONE) ($/KG-PROT)
 9400 603             2.5000PHYTO COMPOSITION(G-DM/G-PROT)
 9500 604             0.0000FEEDING UNITS STANDBY CAPACITY (PROP)
 9600 605             0.0000FREQ OF FEEDING ARTIF FOOD (TIMES/DAY)
 9700 606             0.0000CAPACITY OF FEEDING EQUIP (M2/DAY/UNIT)
 9800 608             0.0000LIFE OF FEEDING EQUIP  (YEAR)
 9900 610             0.0000COST  OF ADDED NUTRIENT ($/KG-N)
10000 611              4.00  WAGE RATE ($/HR)
10100 618             0.0000CAPITAL COST OF FEEDING EQUIP ($/UNIT)
10200 620          0.002413 COEFF 31 IN SPECIFIC 02 CONSN FN (#)
10300 641           200.00  CAPITAL COST OF OFFICE,STORAGE,&C ($/M2)
10400 642            15.00  LIFE OF OFFICE,STORAGE,&C (YEAR)
10500 643             0.0000EFFIC OF RECIRC WASTE TRT IN REM NH3 (%)
10600 644             0.0   NUTRIENT ADDED TO INTAKE WATER(G-N/CM3)
10700 701             0.1000COST OF LABOR=HARVEST SUPERVISORY ($/KG OUTPUT)
10800 703           20.0000 RATE OF TRANSFER AND HARVEST (TRAYS/HR)
10900 704             1.0000FREQUENCY OF HAND CLEANING PHYTO TANK (TIMES/MONTH)
```

```
 11000 705              50.0000RATE OF HAND CLEANING PHYTO TANKS (M2/HR)
 11100 801               7.2000"BIOLOGICAL ZERO" TEMPERATURE (°C)
 11200 802               0.6700COEFFICIENT   ALPHA (METABOLIC BODY SIZE)
 11300 803               3.0000NUMBER OF PHYTOPLANKTON TANKS
 11400 8 4               1.0000COST OF EXCAVATION,PHYTO TANK ($/M3)
 11500 805             0.01025 INITIAL WEIGHT (G)
 11600 806               5.00  BATCH INTERVAL (DAYS)
 11700 808               0.80  PROP ANIMALS SURVIVING AFTER 180 DAYS (=)
 11800 809               0.0000NOT OP
 11900 901               1.0000STARTING POPU LATION, 4TH STAGE, T=0
 12000 920              10.0000MAX WEIGHT OF ANIMAL AT HARVEST (G)
ALBI00K62IATCH)       5000.0000TARGET OUTPUT FRO
 12200 927               0.1000INTEREST RATE ON SINKING FUND (PROPORTION)
 12300 928               0.1000RETURN ON     CAPITAL (PROPORTION)
 #
 REMOVE
 #
```

```
GET ACP/INDISK
#WORKFILE ACP/INDISK; ALGOL, 83 RECORDS, SAVED
LIST
1000 BEGIN
2000 FILE      DISKIN    (KIND=DISK,FILETYPE=7,TITLE="ACP/INDATA."),
2500           REMOUT(KIND=REMOTE),
5000           DISKOUT   (KIND=DISK,MAXRECSIZE=14,BLOCKSIZE=420,
6000                      AREASIZE=30,AREAS=35,SAVEFACTOR=999,
7000                      TITLE="ACP/3NAR."),
7100         DISKOUT2(KIND=DISK,MAXRECSIZE=301,BLOCKSIZE=903,
7200                  AREASIZE=9,AREAS=2,SAVEFACTOR=999,
7300                  TITLE="ACP/3NAR2.");
8000 ARRAY     A[0:13],A903[0:900],A910[0:300],A911[0:300];
8100      VALUE ARRAY ASST(1,2,4,5,8,10,11,13,14,15,16,17,18,19,20,
8105                       22,24,26,27,29,31,34,35,36,47,101,102,
8110                       103,104,105,106,107,108,109,110,201,202,
8115                       203,204,205,206,207,208,209,210,211,212,213,214,
8120                       216,222,301,302,303,304,305,312,401,402,403,404,
8125                       405,406,407,408,409,410,411,412,413,414,415,
8130                       416,417,418,419,420,426,431,441,442,443,445,501,502,
8135                       503,504,505,506,507,509,601,602,603,604,
8140                       605,606,608,610,611,618,
8142                       620,641,642,643,644,701,703,801,802,803,
8145                       804,805,806,808,809,901,920,921,927,928);
8150     VALUE ARRAY CALC 204,207,402);
9000 INTEGER   I,IREC,ISET;
10000 LABEL     DBEWARE,INSERT,C204,C207,C402;
12000 SWITCH      SWLAB:=C204,C207,C402;
12100 DEFINE RITEOUT=  A[0]:=VAL;
12110                  WRITE(DISKIN[IREC],11,A[*])#,
12120         REED    =  READ(DISKIN[IREC],11,A[*])#;
12130      FILL A910[*] WITH 150(0);
12134 %  SURVIVAL FUNCTION, S=VAR[808] WHEN I = 180
12136      VALU:=-(LN(0.8)/180.0);
12140    FOR I:=1 STEP 1 UNTIL 301 DO BEGIN
12141             A903[I-1]:=EXP(-VALU*I);
12142      A903[I-1]:=EXP(-VALU*I);
12144      A910[I-1]:=EXP(-VALU*(I+299));
12146      A911[I-1]:=EXP(-VALU*(I+599));
12148    END;
12150      WRITE(DISOUT2[1],301,A903[*]);
12160      WRITE(DISKOUT2[2],301,A910[*]);
12170      WRITE(DISKOUT[3],301,A911[*]);
13000 AGAIN:    READ(DISKIN,<I3,F20.2,9A6>,IREC,FOR I:=0
14000           STEP 1 UNTIL 9 DO A[I])[EOF];
15000           VALU:=A[0];
15500          GO TO INSERT;
16000           IF ISET:=MASKSEARCH(IREC,4"FFF",ASST) NEQ -1 THEN
17000           BEGIN
18000                IF ISET:=MASKSEARCH(IREC,4"FFF",CALC) NEQ -1 THEN
18500                BEGIN
21000                GO TO SWLAB[ISET+1];
22000                C204: VALU:=1800*VALU**0.5;
23000                      GO TO INSERT;
24000                C207: VALU:=28000.0*VALU**.548;
24500                      RITEOUT;  REED;
25000                C402: VAL:=95000.0*VALU**.565;
26000                      RITEOUT;  REED;
```

```
27000                       VAL:=140000.0*VALU**.554;
28000                       RITEOUT;  REED;
29000                       VAL:=230000.0*VALU**.538;
30000                       RITEOUT;  REED;
31000                       VAL:=510000.0*VALU**.50;
32000                       RITEOUT;  REED;
33000                       VAL:=42000.0*VALU**.438;
34000                       RITEOUT;  REED;
35000                       VAL:=14.0*VALU**(-.169);
36000                       RITEOUT;  REED;
37000                       VAL:=86.0*VALU**(-.346);
38000                       RITEOUT;  REED;
39000                       VAL:=130.0*VALU**(-.262);
40000                       RITEOUT;  REED;
41000                       VALU:=150.0*VALU**(-.354);
42000                 INSERT:A[0]:=VALU;
43000                       WRITE(DISKOUT[IREC], * ,FOR I:=0
44000                       STEP 1 UNTIL 10 DO A[I]);
45000                 GO TO AGAIN;
46000                 END;
47000                 WRITE(DISKOUT[IREC],*,FOR I:=0 STEP 1 UNTIL 10 DO A[I]);
48000                 GO TO AGAIN;
49000            END;
49500             WRITE(REMOUT,<I3,"IS NOT A VALID ASSUMPTION #">);
49550             GO TO AGAIN;
50000 EF:      LOCK(DISKIN); LOCK(DISKOUT); LOCK(DISKOUT2);
51000 END.
#
REMOVE
#
```

```
GET ACP/SUMDATA
#NO FILE:ACP/SUMDATA.
GET ACP/SUMDATA1
#WORKFILE ACP/SUMDATA1: DATA, 26 RECORDS, SAVED
LIST
100 SPACE
200 PHYTO SPACE COST ($/KG)
300 HEAT
400       RECIRC COST ($/KG)
500 AERATION
600       LABOR COST ($/KG)
700 INTAKE WATER (PUMPED)
800 PHYTO TANKS AREA (M2)
900 RECIRCULATION PUMPING
1000  -"-  TANKS DEPT@ (M)
1100 FEED:ARTIFICIAL
1200  -"-  TANKS FLOW(M3/SEC)
1300     :PHYTOPLANKTON
1400  -"-  CONVERSION EFFIC
1500 WASTE TREATMENT
1600 ANIMAL INDIVID WEIGHT(G)
1700 LABOR,REGULAR
1800  -"-   BATCH WEIGHT (KG)
1900  -"- ,SUPERVISORY,&C
2000  -"-   NUMBER OF TRAYS
2100 LARVAE
2200  -"-   CONVERSION EFFIC
2300   TOTAL
2400  -"-   DAYS TO HARVEST
2500  TOTAL CAPITAL (000)
2600  ANN'L TOTAL CAP (000)
#
REMOVE
#
```

ARTIFICIAL UPWELLING MARICULTURE:
SAMPLE OUTPUT FROM BUDGET GENERATOR PROGRAM
(INFLUENCE OF POOL DEPTH
AND TURNOVER RATE ON PRODUCTION COST)

```
TYPE ASSUMPTION NUMBER, COMMA, AND NEW VALUE
WHEN DONE TYPE '999,0'
27,-1340000000.0
***
24,0.00133333
***
18,200
***
24,0.0016
***
999,0
```

OUTPUT SUMMARY

ANIMAL PRODUCTION COST ($/KG OUTPUT)		PERFORMANCE MEASURES		
0.0841	SPACE	0.2748	PHYTO	SPACE COST ($/KG)
0.0000	HEAT	0.2046		RECIRC COST ($/KG)
0.0032	AERATION	0.0874		LABOR COST ($/KG)
0.0686	INTAKE WATER (PUMPED)	33247.0172	PHYTO	TANKS AREA (M2)
0.5472	RECIRCULATION PUMPING	2.0000	-"-	TANKS DEPTH (M)
0.0000	FEED:ARTIFICIAL	0.5320	-"-	TANKS FLOW (M3/SEC)
0.5668	:PHYTOPLANKTON	0.8475	-"-	CONVERSION EFFIC
0.0524	WASTE TREATMENT	10.0292	ANIMAL	INDIVID WEIGHT (G)
0.2462	LABOR,REGULAR	5000.0000	-"-	BATCH WEIGHT (KG)
0.1000	-"- ,SUPERVISORY,&C	5714.4768	-"-	NUMBER OF TRAYS
0.1262	LARVAE	0.3073	-"-	CONVERSION EFFIC
1.7946	TOTAL	189.0000	-"-	DAYS TO HARVEST
699.9000	TOTAL CAPITAL (000)	153.2176	ANN'L	TOTAL CAP (000)

```
TYPE ASSUMPTION NUMBER, COMMA, AND NEW VALUE
WHEN DONE TYPE '999,0'
24,0.00187
***
999,0
```

OUTPUT SUMMARY

ANIMAL PRODUCTION COST ($/KG OUTPUT)		PERFORMANCE MEASURES		
0.0841	SPACE	0.2520	PHYTO	SPACE COST ($/KG)
0.0000	HEAT	0.1879		RECIRC COST ($/KG)
0.0032	AERATION	0.0801		LABOR COST ($/KG)
0.0732	INTAKE WATER (PUMPED)	30451.4127	PHYTO	TANKS AREA (M2)
0.5472	RECIRCULATION PUMPING	2.0000	-"-	TANKS DEPTH (M)
0.0000	FEED:ARTIFICIAL	0.5694	-"-	TANKS FLOW (M3/SEC)
0.5200	:PHYTOPLANKTON	0.7917	-"-	CONVERSION EFFIC
0.0524	WASTE TREATMENT	10.0292	ANIMAL	INDIVID WEIGHT (G)
0.2462	LABOR,REGULAR	5000.0000	-"-	BATCH WEIGHT (KG)
0.1000	-"- ,SUPERVISORY,&C	5714.4768	-"-	NUMBER OF TRAYS
0.1262	LARVAE	0.3073	-"-	CONVERSION EFFIC
1.7525	TOTAL	189.0000	-"-	DAYS TO HARVEST
662.7118	TOTAL CAPITAL (000)	144.7479	ANN'L	TOTAL CAP (000)

```
TYPE ASSUMPTION NUMBER, COMMA, AND NEW VALUE
WHEN DONE TYPE '999,0'
24,0.00213
**
999,0
```

OUTPUT SUMMARY

ANIMAL PRODUCTION COST ($/KG OUTPUT)		PERFORMANCE MEASURES		
0.0841	SPACE	0.2402	PHYTO	SPACE COST ($/KG)
0.0000	HEAT	0.1793		RECIRC COST ($/KG)
0.0032	AERATION	0.0763		LABOR COST ($/KG)
0.0791	INTAKE WATER (PUMPED)	29003.2620	PHYTO	TANKS AREA (M2)
0.5472	RECIRCULATION PUMPING	2.0000	-"-	TANKS DEPTH (M)
0.0000	FEED:ARTIFICIAL	0.6178	-"-	TANKS FLOW (M3/SEC)
0.4957	:PHYTOPLANKTON	0.7298	-"-	CONVERSION EFFIC
0.0524	WASTE TREATMENT	10.0292	ANIMAL	INDIVID WEIGHT (G)
0.2462	LABOR,REGULAR	5000.0000	-"-	BATCH WEIGHT (KG)
0.1000	-"- ,SUPERVISORY,&C	5714.4768	-"-	NUMBER OF TRAYS
0.1262	LARVAE	0.3073	-"-	CONVERSION EFFIC
1.7341	TOTAL	189.0000	-"-	DAYS TO HARVEST
643.8918	TOTAL CAPITAL (000)	140.4345	ANN'L	TOTAL CAP (000)

```
TYPE ASSUMPTION NUMBER, COMMA, AND NEW VALUE
WHEN DONE TYPE '999,0'
24,0.0024
**
999,0
```

OUTPUT SUMMARY

ANIMAL PRODUCTION COST ($/KG OUTPUT)		PERFORMANCE MEASURES		
0.0841	SPACE	0.2368	PHYTO	SPACE COST ($/KG)
0.0000	HEAT	0.1768		RECIRC COST ($/KG)
0.0032	AERATION	0.0752		LABOR COST ($/KG)
0.0874	INTAKE WATER (PUMPED)	28594.4381	PHYTO	TANKS AREA (M2)
0.5472	RECIRCULATION PUMPING	2.0000	-"-	TANKS DEPTH (M)
0.0000	FEED:ARTIFICIAL	0.6863	-"-	TANKS FLOW (M3/SEC)
0.4889	:PHYTOPLANKTON	0.6570	-"-	CONVERSION EFFIC
0.0524	WASTE TREATMENT	10.0292	ANIMAL	INDIVID WEIGHT (G)
0.2462	LABOR,REGULAR	5000.0000	-"-	BATCH WEIGHT (KG)
0.1000	-"- ,SUPERVISORY,&C	5714.4768	-"-	NUMBER OF TRAYS
0.1262	LARVAE	0.3073	-"-	CONVERSION EFFIC
1.7356	TOTAL	189.0000	-"-	DAYS TO HARVEST
639.4812	TOTAL CAPITAL (000)	139.3638	ANN'L	TOTAL CAP (000)

```
TYPE ASSUMPTION NUMBER, COMMA, AND NEW VALUE
WHEN DONE TYPE '999,0'
18,300
**
999,0
```

OUTPUT SUMMARY

ANIMAL PRODUCTION COST ($/KG OUTPUT)		PERFORMANCE MEASURES		
0.0841	SPACE	0.2059	PHYTO	SPACE COST ($/KG)
0.0000	HEAT	0.2216		RECIRC COST ($/KG)
0.0032	AERATION	0.0633		LABOR COST ($/KG)
0.0742	INTAKE WATER (PUMPED)	24069.8266	PHYTO	TANKS AREA (M2)
0.5472	RECIRCULATION PUMPING	3.0000	-"-	TANKS DEPTH (M)
0.0000	FEED:ARTIFICIAL	0.5777	-"-	TANKS FLOW (M3/SEC)
0.4907	:PHYTOPLANKTON	0.7805	-"-	CONVERSION EFFIC
0.0524	WASTE TREATMENT	10.0292	ANIMAL	INDIVID WEIGHT (G)
0.2462	LABOR,REGULAR	5000.0000	-"-	BATCH WEIGHT (KG)
0.1000	-"- ,SUPERVISORY,&C	5714.4768	-"-	NUMBER OF TRAYS
0.1262	LARVAE	0.3073	-"-	CONVERSION EFFIC
1.7242	TOTAL	189.0000	-"-	DAYS TO HARVEST
604.8969	TOTAL CAPITAL (000)	128.4736	ANN'L	TOTAL CAP (000)

```
TYPE ASSUMPTION NUMBER, COMMA, AND NEW VALUE
WHEN DONE TYPE '999,0'
24,0.00267
**
999,0
```

OUTPUT SUMMARY

ANIMAL PRODUCTION COST ($/KG OUTPUT)		PERFORMANCE MEASURES		
0.0841	SPACE	0.1986	PHYTO	SPACE COST ($/KG)
0.0000	HEAT	0.2137		RECIRC COST ($/KG)
0.0032	AERATION	0.0610		LABOR COST ($/KG)
0.0792	INTAKE WATER (PUMPED)	23185.8635	PHYTO	TANKS AREA (M2)
0.5472	RECIRCULATION PUMPING	3.0000	-"-	TANKS DEPTH (M)
0.0000	FEED:ARTIFICIAL	0.6191	-"-	TANKS FLOW (M3/SEC)
0.4732	:PHYTOPLANKTON	0.7283	-"-	CONVERSION EFFIC
0.0524	WASTE TREATMENT	10.0292	ANIMAL	INDIVID WEIGHT (G)
0.2462	LABOR,REGULAR	5000.0000	-"-	BATCH WEIGHT (KG)
0.1000	-"- ,SUPERVISORY,&C	5714.4768	-"-	NUMBER OF TRAYS
0.1262	LARVAE	0.3073	-"-	CONVERSION EFFIC
1.7117	TOTAL	189.0000	-"-	DAYS TO HARVEST
592.9490	TOTAL CAPITAL (000)	125.7941	ANN'L	TOTAL CAP (000)

TYPE ASSUMPTION NUMBER, COMMA, AND NEW VALUE
WHEN DONE TYPE '999,0'
24,0.00293
***
999,0

OUTPUT SUMMARY

ANIMAL PRODUCTION COST ($/KG OUTPUT)		PERFORMANCE MEASURES		
0.0841	SPACE	0.1959	PHYTO	SPACE COST ($/KG)
0.0000	HEAT	0.2109		RECIRC COST ($/KG)
0.0032	AERATION	0.0602		LABOR COST ($/KG)
0.0854	INTAKE WATER (PUMPED)	22871.2501	PHYTO	TANKS AREA (M2)
0.5472	RECIRCULATION PUMPING	3.0000	-"-	TANKS DEPTH (M)
0.0000	FEED:ARTIFICIAL	0.6701	-"-	TANKS FLOW (M3/SEC)
0.4670	:PHYTOPLANKTON	0.6728	-"-	CONVERSION EFFIC
0.0524	WASTE TREATMENT	10.0292	ANIMAL	INDIVID WEIGHT (G)
0.2462	LABOR,REGULAR	5000.0000	-"-	BATCH WEIGHT (KG)
0.1000	-"- ,SUPERVISORY,&C	5714.4768	-"-	NUMBER OF TRAYS
0.1262	LARVAE	0.3073	-"-	CONVERSION EFFIC
1.7117	TOTAL	189.0000	-"-	DAYS TO HARVEST
589.2928	TOTAL CAPITAL (000)	124.9378	ANN'L	TOTAL CAP (000)

TYPE ASSUMPTION NUMBER, COMMA, AND NEW VALUE
WHEN DONE TYPE '999,0'
24,0.00213
***
999,0

OUTPUT SUMMARY

ANIMAL PRODUCTION COST ($/KG OUTPUT)		PERFORMANCE MEASURES		
0.0841	SPACE	0.2185	PHYTO	SPACE COST ($/KG)
0.0000	HEAT	0.2351		RECIRC COST ($/KG)
0.0032	AERATION	0.0673		LABOR COST ($/KG)
0.0702	INTAKE WATER (PUMPED)	25592.2228	PHYTO	TANKS AREA (M2)
0.5472	RECIRCULATION PUMPING	3.0000	-"-	TANKS DEPTH (M)
0.0000	FEED:ARTIFICIAL	0.5451	-"-	TANKS FLOW (M3/SEC)
0.5209	:PHYTOPLANKTON	0.8271	-"-	CONVERSION EFFIC
0.0524	WASTE TREATMENT	10.0292	ANIMAL	INDIVID WEIGHT (G)
0.2462	LABOR,REGULAR	5000.0000	-"-	BATCH WEIGHT (KG)
0.1000	-"- ,SUPERVISORY,&C	5714.4768	-"-	NUMBER OF TRAYS
0.1262	LARVAE	0.3073	-"-	CONVERSION EFFIC
1.7504	TOTAL	189.0000	-"-	DAYS TO HARVEST
626.0680	TOTAL CAPITAL (000)	133.1872	ANN'L	TOTAL CAP (000)

```
TYPE ASSUMPTION NUMBER, COMMA, AND NEW VALUE
WHEN DONE TYPE '999,0'
18,350
***
999,0
```

OUTPUT SUMMARY

ANIMAL PRODUCTION COST ($/KG OUTPUT)		PERFORMANCE MEASURES		
0.0841	SPACE	0.2181	PHYTO	SPACE COST ($/KG)
0.0000	HEAT	0.2680		RECIRC COST ($/KG)
0.0032	AERATION	0.0660		LABOR COST ($/KG)
0.0689	INTAKE WATER (PUMPED)	25104.2343	PHYTO	TANKS AREA (M2)
0.5472	RECIRCULATION PUMPING	3.5000	-"-	TANKS DEPTH (M)
0.0000	FEED:ARTIFICIAL	0.5347	-"-	TANKS FLOW (M3/SEC)
0.5521	:PHYTOPLANKTON	0.8432	-"-	CONVERSION EFFIC
0.0524	WASTE TREATMENT	10.0292	ANIMAL	INDIVID WEIGHT (G)
0.2462	LABOR,REGULAR	5000.0000	-"-	BATCH WEIGHT (KG)
0.1000	-"- ,SUPERVISORY,&C	5714.4768	-"-	NUMBER OF TRAYS
0.1262	LARVAE	0.3073	-"-	CONVERSION EFFIC
1.7804	TOTAL	189.0000	-"-	DAYS TO HARVEST
635.7113	TOTAL CAPITAL (000)	133.5048	ANN'L	TOTAL CAP (000)

```
TYPE ASSUMPTION NUMBER, COMMA, AND NEW VALUE
WHEN DONE TYPE '999,0'
24,0.0024
***
999,0
```

```
TYPE ASSUMPTION NUMBER, COMMA, AND NEW VALUE
WHEN DONE TYPE '999,0'
24,0.0024
***
999,0
```

OUTPUT SUMMARY

ANIMAL PRODUCTION COST ($/KG OUTPUT)		PERFORMANCE MEASURES		
0.0841	SPACE	0.2044	PHYTO	SPACE COST ($/KG)
0.0000	HEAT	0.2509		RECIRC COST ($/KG)
0.0032	AERATION	0.0617		LABOR COST ($/KG)
0.0724	INTAKE WATER (PUMPED)	23456.3707	PHYTO	TANKS AREA (M2)
0.5472	RECIRCULATION PUMPING	3.5000	-"-	TANKS DEPTH (M)
0.0000	FEED:ARTIFICIAL	0.5630	-"-	TANKS FLOW (M3/SEC)
0.5169	:PHYTOPLANKTON	0.8009	-"-	CONVERSION EFFIC
0.0524	WASTE TREATMENT	10.0292	ANIMAL	INDIVID WEIGHT (G)
0.2462	LABOR,REGULAR	5000.0000	-"-	BATCH WEIGHT (KG)
0.1000	-"- ,SUPERVISORY,&C	5714.4768	-"-	NUMBER OF TRAYS
0.1262	LARVAE	0.3073	-"-	CONVERSION EFFIC
1.7486	TOTAL	189.0000	-"-	DAYS TO HARVEST
612.0632	TOTAL CAPITAL (000)	128.3128	ANN'L	TOTAL CAP (000)

```
TYPE ASSUMPTION NUMBER, COMMA, AND NEW VALUE
WHEN DONE TYPE '999,0'
24,0.00267
***
999,0
```

OUTPUT SUMMARY

ANIMAL PRODUCTION COST ($/KG OUTPUT)		PERFORMANCE MEASURES		
0.0841	SPACE	0.1956	PHYTO	SPACE COST ($/KG)
0.0000	HEAT	0.2400		RECIRC COST ($/KG)
0.0032	AERATION	0.0589		LABOR COST ($/KG)
0.0767	INTAKE WATER (PUMPED)	22438.5666	PHYTO	TANKS AREA (M2)
0.5472	RECIRCULATION PUMPING	3.5000	-"-	TANKS DEPTH (M)
0.0000	FEED:ARTIFICIAL	0.5983	-"-	TANKS FLOW (M3/SEC)
0.4945	:PHYTOPLANKTON	0.7535	-"-	CONVERSION EFFIC
0.0524	WASTE TREATMENT	10.0292	ANIMAL	INDIVID WEIGHT (G)
0.2462	LABOR,REGULAR	5000.0000	-"-	BATCH WEIGHT (KG)
0.1000	-"- ,SUPERVISORY,&C	5714.4768	-"-	NUMBER OF TRAYS
0.1262	LARVAE	0.3073	-"-	CONVERSION EFFIC
1.7305	TOTAL	189.0000	-"-	DAYS TO HARVEST
597.2646	TOTAL CAPITAL (000)	125.0527	ANN'L	TOTAL CAP (000)

```
TYPE ASSUMPTION NUMBER, COMMA, AND NEW VALUE
WHEN DONE TYPE '999,0'
24,0.00293
***
999,0
```

OUTPUT SUMMARY

ANIMAL PRODUCTION COST ($/KG OUTPUT)		PERFORMANCE MEASURES		
0.0841	SPACE	0.1912	PHYTO	SPACE COST ($/KG)
0.0000	HEAT	0.2346		RECIRC COST ($/KG)
0.0032	AERATION	0.0576		LABOR COST ($/KG)
0.0819	INTAKE WATER (PUMPED)	21881.8050	PHYTO	TANKS AREA (M2)
0.5472	RECIRCULATION PUMPING	3.5000	-"-	TANKS DEPTH (M)
0.0000	FEED:ARTIFICIAL	0.6411	-"-	TANKS FLOW (M3/SEC)
0.4833	:PHYTOPLANKTON	0.7032	-"-	CONVERSION EFFIC
0.0524	WASTE TREATMENT	10.0292	ANIMAL	INDIVID WEIGHT (G)
0.2462	LABOR,REGULAR	5000.0000	-"-	BATCH WEIGHT (KG)
0.1000	-"- ,SUPERVISORY,&C	5714.4768	-"-	NUMBER OF TRAYS
0.1262	LARVAE	0.3073	-"-	CONVERSION EFFIC
1.7245	TOTAL	189.0000	-"-	DAYS TO HARVEST
590.2257	TOTAL CAPITAL (000)	123.4796	ANN'L	TOTAL CAP (000)

```
TYPE ASSUMPTION NUMBER, COMMA, AND NEW VALUE
WHEN DONE TYPE '999,0'
24,0.0032
***
999,0
```

OUTPUT SUMMARY

ANIMAL PRODUCTION COST ($/KG OUTPUT)		PERFORMANCE MEASURES		
0.0841	SPACE	0.1906	PHYTO	SPACE COST ($/KG)
0.0000	HEAT	0.2338		RECIRC COST ($/KG)
0.0032	AERATION	0.0574		LABOR COST ($/KG)
0.0888	INTAKE WATER (PUMPED)	21810.2374	PHYTO	TANKS AREA (M2)
0.5472	RECIRCULATION PUMPING	3.5000	-"-	TANKS DEPTH (M)
0.0000	FEED:ARTIFICIAL	0.6979	-"-	TANKS FLOW (M3/SEC)
0.4817	:PHYTOPLANKTON	0.6460	-"-	CONVERSION EFFIC
0.0524	WASTE TREATMENT	10.0292	ANIMAL	INDIVID WEIGHT (G)
0.2462	LABOR,REGULAR	5000.0000	-"-	BATCH WEIGHT (KG)
0.1000	-"- ,SUPERVISORY,&C	5714.4768	-"-	NUMBER OF TRAYS
0.1262	LARVAE	0.3073	-"-	CONVERSION EFFIC
1.7299	TOTAL	189.0000	-"-	DAYS TO HARVEST
590.1087	TOTAL CAPITAL (000)	123.4026	ANN'L	TOTAL CAP (000)

```
TYPE ASSUMPTION NUMBER, COMMA, AND NEW VALUE
WHEN DONE TYPE '999,0'
18,100
***
24,0.00133
***
999,0
```

OUTPUT SUMMARY

ANIMAL PRODUCTION COST ($/KG OUTPUT)		PERFORMANCE MEASURES		
0.0841	SPACE	0.3976	PHYTO	SPACE COST ($/KG)
0.0000	HEAT	0.1527		RECIRC COST ($/KG)
0.0032	AERATION	0.1291		LABOR COST ($/KG)
0.0834	INTAKE WATER (PUMPED)	49099.2984	PHYTO	TANKS AREA (M2)
0.5472	RECIRCULATION PUMPING	1.0000	-"-	TANKS DEPTH (M)
0.0000	FEED:ARTIFICIAL	0.6530	-"-	TANKS FLOW(M3/SEC)
0.6794	:PHYTOPLANKTON	0.6904	-"-	CONVERSION EFFIC
0.0524	WASTE TREATMENT	10.0292	ANIMAL	INDIVID WEIGHT(G)
0.2462	LABOR,REGULAR	5000.0000	-"-	BATCH WEIGHT (KG)
0.1000	-"- ,SUPERVISORY,&C	5714.4768	-"-	NUMBER OF TRAYS
0.1262	LARVAE	0.3073	-"-	CONVERSION EFFIC
1.9220	TOTAL	189.0000	-"-	DAYS TO HARVEST
879.1415	TOTAL CAPITAL (000)	197.5353	ANN'L	TOTAL CAP (000)

ARTIFICIAL UPWELLING MARICULTURE: RECOMMENDED REVISIONS TO BE MADE TO THE AQUACULTURE BUDGET GENERATOR COMPUTER PROGRAM

by

DR. GEOFFREY P. ALLEN

March 26, 1977

Introduction

This report consists of three sections.

I. Improvements to the program and notes for conversion to FØRTRAN. Several redundant statements have been deleted. Removal of the option to evaluate single batch or continuous production has permitted further simplification. (Single batch production can still be evaluated if the interval between batches is set equal to or greater than time to harvest.) Statements which made reference to a compartment size table, not used in this version of the program, have also been deleted.

II. Modifications to the program. Five sets of modifications are described and the required changes in the program noted.

III. Revised definitions of variables. The list of variables and their definitions which was provided in an earlier report is updated to reflect changes in parts I and II. In addition, sources are given for baseline values of variables listed in INDATA.

Future work

Some additional documentation may be sought for the baseline values of variables in the input data file. Part of the intent of the program layout is that the degree of confidence in the values assumed for the variables should be exposed. Concern here can lead in two directions:

(1) an investigation of the sensitivity of the calculated costs to changes in assumed values of variables where justification of those values is weak; and

(2) a search for more reliable or empirically validated values relating to the species and site under consideration.

It is important to remember that the cost of production estimates can only be fully validated when commercial, or large scale pilot plant operations occur. We are measuring changes in cost assuming that the model is correct. It would therefore seem appropriate to work through the cost components of greatest magnitude in detail to ensure that the best representation of the real-world situation is being modeled. An investigation of the impact of modifications to the existing technology can be carried out at the same time. These steps should provide firmer delineation of areas of ignorance concerning biological responses, feasible technologies and costs, and the importance of better information in reducing, or in giving more precision to, estimated cost of production.

lines	comments
*27340-27370	Omit. Only one table.
*30100-30150	Omit. Refers to compartment size table, not used.
*31000	Omit.
31500	Omit in FTN. Use assigned GØTØ statement on program.
34000-44300	These are data tables in FTN.
*44000	Omit.
*44400-44440	Omit. Debugging aid.
*45000-47000	Omit. Procedure refers to section no longer used.
47000	This is the last line of declaration statements. In FTN the format statements (lines 18000-30150) could be placed anywhere after this line.
*47100	Change to 47100 READ(DISKIN3, 901, A903[*]); A change in INDISK must also be made. See page I-4 . If Note 1 followed, read statement in DØ loop. Add error branch. See line 54511 below.
*47200-47700	Delete
49000	If Note 1 is followed, add error branch. See line 54511 below.
49100-49110	If Note 1 followed, omit.
*51500	Omit. Not used.
52000-54517	Can use assigned GØTØ in FTN provided default for incorrect value of IWANT transfers control to line 54527.
54511	If Note 1 followed insert branch statement here. At the statement branched to (i) Read from INDATA (ii) Write to CURRDATA (iii) Insert lines to calculate number surviving, that is ACP/INDISK lines 12134-12141(except line 12140 would then read 12140 FØR I:=1 STEP 1 UNTIL 901 DØ) (iv) Write values for number surviving on disk file (BNAR2). The error branches on lines 47100 and 49000 should transfer control to this group of statements. If either disk file of current data cannot be read (e.g. if it has been removed) then original values of parameters will be placed in these files.
*54530	Change to 54530 TABLES:

lines	comments
*54540-54550	Omit. Only one table.
*54610	Change to 54610 WRITE (DISKIN3, 901, A903 [*]);
*54620	Change to 54620 END;
*54630-54950	Omit. Only one table.
*54960	Change to 54960 GØ TØ PICKØNE;
*6000-84000	Omit. No longer used in this form.
91030	In FTN use assigned GØTØ statement.
*97510-97560	Replace by the following statements which provide for printing out sections of the mortality adjustment table.
97510 LIST 3:	WRITE (REMØUT, FMTØUT6);
97520	READ (REMIN,/,LØW, HIGH);
97530	IF LØW LEQ 0 THEN LØW:=1;
97540	IF HIGH GTR 900 THEN HIGH: = 900;
97550	BEGIN
97560	WRITE (REMØUT,< "MØRTALITY ADJ. FACTØR TABLE"/>):
97565	FØR I: = LØW STEP 5 UNTIL HIGH DØ
*97590-97650	Omit. Table no longer used.
99122-99220	Changes are described in section IIb.
*98310-102650	A number of changes are required in this section of the program to facilitate removal of the single batch option. They are described in detail below.
98310	Change to 98310 FØR I:=311, 432, 436, 452, 453, 454, 455, 456, 463, 513, 621, 915, 916, 924
99100	Change [513] to [514]
100020	Change [621] to [612]
100032	Change [432] to[435] and [621] to [612]
100039	Change [436] to [437] and [432] to [435]
100040	Change [463] to [462] and [621] to [612]

line	comments
100970	Change [915] to [925]
100935	Delete
100992	Change [915] to [925]
101400	Change to 101400 VAR[621]:= *+VAR[612];
101500	Change to 101500 VAR[513]:= *+VAR[514];
101600-101670	Change to 101600 VAR[432]: = *+ VAR[435]; 101650 VAR[436]: = *+ VAR[462]; 101670 VAR[463]: = *+ VAR[462];
101850	Change [925] to [915]
101900	Change [926] to [916]
101910-101920	Delete. The logic appears at fault here. Number of transfers should not be cummulated for continuous production. The output will need the same number of transfers regardless of whether it is single batch or continuous production.
102130	Delete.
102150	Change to 102150 VAR[900]:= VAR[924]/VAR[99];
102200-102650	Delete
*106300	Delete. not used.
*107000-107450	Delete. Alternative listing of capital cost is provided in the program.
212000	Change 30.7 to 0.012. Error made in translating units to metric. Influences capital cost of heating equipment. No change in results from present program.
*223500	Delete. Single batch option eliminated
*224000	Change to 224000 VAL: = VAR[921]* 365.0/VAL;
INDISK 8000	ARRAY A[0:13], A903[0:900]; Eliminates use of arrays A910, A911 not required. Lines 12130-12146 will then require the following changes.
*12130	Delete
*12140	Change 301 to 901

lines	comments
INDISK	
*12142-12146	Delete
*12150	WRITE (DISKØUT2, 901, A903,[*]);
*12160 12170	DELETE

II Modifications to program

a.) Addition of artificial nutrients and calculated compensation depth dependent on nutrient level.

Order of calculations

1. Nutrient concentration at inlet is sum of nutrient concentration in deep sea water and nutrients added artifically.

$$N_t = N_d + N_a$$

where

N_t is total nutrient concentration in inlet water G-N/CM^3.

N_d is nutrient concentration in deep sea water G-N/CM^3.

N_a is nutrient concentration added artifically G-N/CM^3.

2. Compensation depth is a function of nutrient concentration

$$D_k = a + \frac{b}{N_t} \cdot D_c(448 \times 10^{-9})$$

where

D_k is the calculated compensation depth (CM).

D_c is the standard compensation depth (CM).

$D_c = 400$ when $N_d = 448 \times 10^{-9}$ is assumed.

Comment: D_k has been expressed in this way to make use of information available in D_c. In the function the values a=0.0, b=1.0 have been used. The function has the properties that as $N_t \to 0$, $D_k \to \infty$, as $N_t \to \infty$, $D_k \to 0$, and when $N_t = N_d$, $D_k = D_c$.

Modifications to the program are described in section IIb.)

b.) Actual depth not compensation depth of phytoplankton tank used in calculation.

Order of calculations

1. Depth factor D´ is calculated according to the formula

$$D' = D - D^2/2D_k$$ where D´ is the apparent pool depth (cm)

D is the actual pool depth (cm)

2. Exposure factor, K

$K = \frac{\dot{V}}{D'}$ where $\dot{V}$ is the flow into the phytoplankton tank ($CM^3CM^{-2}sec^{-1}$)

$\frac{1}{K}$ is the apparent detention time (sec)

$\frac{1}{K}$ measures the apparent length of time taken for a volume of water to be totally replaced. It is less than the actual detention time e.g. if $D' = 200$ and $\dot{V} = 0.1$ then $\frac{1}{K} = 20000$ seconds or about 5½ hours. The actual detention time if D=400 would be 40000 seconds or about 11 hours.

3. Efficiency of conversion, η_1

$$\eta_1 = 1.0 + b_1 k = b_2 k^2$$

Note: a functional form which avoids the decreasing value of apparent depth that occurs at higher values of actual depth is

$D' = a(1-e^{-bD})$ where a and b are parameters

If a=200, b=0.01 the function gives similar values to the existing one.

e.g.

D	D´ using original function	D´using suggested replacement
0	0	0
10	9.9	19.0
100	87.5	126.4
200	150.0	172.9
400	200.0	196.3
600	150.0	199.5
800	0	199.9

A better fit to the data could be found for this suggested non-linear function. The above values are solely for illustration.

Modifications to the program

AQUA2B

Lines 99122-99220 should appear as follows:

```
99122          VAR[60]:= VAR[644]+ VAR[20];
99124          VAR[61]:= 0.0 + 1.0*(VAR[20]* VAR[22]/VAR[60]);
99130          IF VAR[18]GTR VAR[61]THEN
```

```
99131 }
99133 }          unchanged

99135            2(x2, F8.1)>, VAR[18], VAR[61]

99137 }
99140 }          omit

99150            VAR[51]:=VAR[18]-(VAR[18]**2.0)/(2.0*VAR[61]);

99155            IF VAR[51]LEQ 0.0 THEN VAR[51]:=0.0001;

99160 }
99214 }          unchanged

99220            VAR[54]:=6.25* VAR[60];
```

Line 99155 is added since the functional form permits negative values of VAR[51], which would be meaningless and would interfere with subsequent calculations.

c.) Cost of artificial nutrients included in total cost.

Order of calculations

1. Cost of artificial nutrients

$$C_n = F \cdot R \cdot C_n \ (86.4) \ 365.0/Q$$

where

C_n is cost of nutrients $/kg output

F is flow rate into phytoplankton tanks cm^3/sec

R is rate of addition of nutrients g-N/cm^3

C_u is unit cost of nutrients $/kg-N

Q is output from the facility kg/year

2. Cost of feed (phytoplankton)

$$C_p = C_{ps} + C_{pr} + C_{pl} + C_n$$

where

C_p is cost of feed $/kg output

C_{ps} is cost of space for growing phytoplankton $/kg output

C_{pr} is cost of recirculation pumping in phytoplankton tanks $/kg output

Modifications to the program

AQUA2B
line

224500 VAR [930]:= VAR[663]*VAR[664]*VAR[610]*86.4*365.0 /VAL;

237000 Change to 237000 VAR[943]:=VAR[930]+VAR[932] +VAR[934]+VAR[936];

Comment: The cost of artifical nutrients does not appear separately in the printed output but can be calculated as the difference between the cost of feed (phytoplankton) and its major components (space, recirculation pumping and labor).

d.) Inclusion of manpower requirements.

Order of calculations

1. Cost of labor in animal production facility

$$C_L = C_a \cdot A/B$$

where C_L is the cost of labor in the animal production facility ($/day)

A is the number of animals started per batch

B is the interval between batches (days)

C_a is the share of cost of transfer and harvest

2. Total regular labor requirement

$$C_{TL} = (7C_L + C_{PL}/52)/W$$

where C_{TL} is the regular labor requirement for transfer, harvest and cleaning (hours/week)

C_{PL} is the total cost of cleaning phytoplankton tank ($/year)

W is the regular labor wage rate ($/hr)

3. Number of supervisory and technical staff

$$S_T = C_b \cdot V/S_a$$

where S_T is the total number of full time equivalent supervisory and technical staff required (#)

C_b is the overhead cost assigned to output ($/kg)

V is the output per year (kg/year)

S_a is the annual salary for supervisory and technical staff

Modifications to the program

AQUA2B line		
49100		Change 25 to 27
102130		(This line can be deleted in present program. Replace by VAR[706]:=VAR[706]+ VAR[900]; This treats harvest as another transfer operation, carried out at the same rate and with same labor.)
220125		Move line 224000 to this location
220300		VAR[707]:=VAR[706]* VAR[926]/VAR[806];
220600		VAR[957]:= (VAR[707]* 7.0+VAR[708]/52.0)/VAR[611];
220700		VAR[958]:= VAR[701]* VAL/VAR[809];
224000		Delete after moving to line 220125.
		Change to 241000 VAR[947]:=VAR[707]*365.0/VAL;
252300		Change 955 to 957
INDATA		
11800	809	16.000.0 ANNUAL SALARY, TECH & SUPER LABØR ($) (these must line up correctly for format I3, F20.2,9A6)
SUMDATA		
2700		REG LABØR (HRS/WK)
2800		SUPER & TECH LABØR (#) (these must line up correctly for format 4A6)

e.) Changes in baseline assumptions

1. Var[27], coefficient b_2 in phytoplankton conversion efficiency function

   Source Memo of 2/25/77 LVH. Original value did not include light attenuation at depth.

2. Var [24], unit flowrate into phytoplankton tanks

   Source Memo of 2/25/77 LVH. Corresponds to turnover rate of 1.15 times per day in 100cm deep pool rather than 1.5 times per day assumed previously.

Modifications to the program

INDATA

line 1700 24 0.001331 FLØWRATE TØ PHYTØ TANK (CM3/CM2/SEC)

1900 27 -134000000.0 CØEFF B2 IN PHYTØ EFFIC FN (#)

(these must line up correctly for format I3,F20.2, 9A6)

III. Definitions of Variables and Sources for Assumed Values

Notes: key to abbreviations in sources.

EPA report	Blecker, H.C. and T.M. Nichols. Capital and Operating Costs of Pollution Control Equipment Modules, Vol II- Data Manual. Environmental Protection Agency Report EPA R5-73-0236 July 1973.
Gravitz	Gravitz, N., L. Gleye, G. Tchobanoglous and R. Shleser "Preliminary Acute Toxicity Studies of Some Inorganic Compounds in Homarus americanus" unpublished manuscript.
Lightburn & Roels	Lightburn K.D. and O.A. Roels "Economic Analysis of a Mariculture Plant" unpublished manuscript, December 1971.
Liao and Mayo	Liao, P.B. and R.D. Mayo "Salmonid Hatchery Water Reuse Systems" Aquaculture 1:317-35. 1972.
LVH	Estimate by Mr. L. van Hemelryck.
T/S	Tchobanoglous, G. and R. Shleser. "Waste Treatment Costs for Saltwater Aquaculture Facilities" unpublished manuscript. [may have been published in Aquaculture.]

III. Definitions of Variables (Revised 3/26/77)

Variable Number	Variable Value	Description	Units
1	0.0	Not operational	
2	900.0	Time limit on growth. Purpose to permit examination of production cost over selected intervals. Overridden if either maximum harvest weight [920] or maximum number of transfer cycles [10] is reached. Source Limit on array storage of 900 in this program.	day
4	0.0	Ammonia concentration in intake water Source None. Zero value assumed.	mg-N/l
5	0.9	Efficiency of oxygenation in animal tank recirculation pumping unit Source Assumption.	propn.
8	1.0	Number of recirculation pumps in animal tanks. Source Assumption: Increasing the number of pumps will increase capital costs but permits isolation of each section of the production unit, a protection against epidemic losses.	(#)
10	5.0	Number of transfer cycles in the animal system. In conjunction with [11] it will determine harvest day, which will occur when a transfer is required but none is available. Overridden if maximum harvest weight [920] or maximum growth time [2] is reached. Source Assumption.	#
11	4.0	Ratio of final weight per tray to initial weight per tray. When exceeded one tray is transferred to several others, the number being the value in [11]. Source Assumption. In combination with variable 19 it determines the limits in which the load of a tray will lie.	#
13	0.0	Cost of deep sea water delivered to site excluding in-site pumping costs. Source No cost figure available. Will depend on whether water supplied as by-product from industrial process or pumped specifically for aquaculture facility.	$/m^3
14	2.65	Ratio of total animal weight to wet meat weight Source Experimental observation-LVH	g total /g wet meat

Variable Number	Variable Value	Description	Units
15	2.50	Ratio of total meat weight to meat protein weight (dry matter basis) Source Experimental observations-LVH.	g dry meat /g dry prot.
16	0.0	Reserve phytoplankton tank capacity as proportion of utilized capacity. Source Assumption. No reserve capacity to protect against accidental loss of the use of a tank.	propn.
17	5.0	Ratio of clam wet meat weight to dry meat weight Source Experimental observation-LVH.	g total /g dry
18	100.0	Depth of phytoplankton tanks Source Depth of experimental pools, St. Croix-LVH.	cm
19	20.0	Maximum weight of animals in a tray. Source Design criterion, greatest weight which can be conveniently manipulated without mechanical aids-LVH.	kg
20	0.000000448	Concentration of nutrients in deep sea water Source Experimental observation, St. Croix-LVH.	$g\text{-}N/cm^3$
22	400	Specific compensation depth for phytoplankton production Source Assumed value for seawater with 448×10^{-9} $g\text{-}N/cm^3$ of nutrients-LVH.	cm
24	0.001331	Unit flowrate into phytoplankton tanks Source Calculated value based on a turnover rate of 1.15 times per day in tanks of 100 cm depth-LVH.	$cm^3/cm^2/sec$
26	0.0	Coefficient b, in phytoplankton conversion efficiency function Source see variable 27.	sec
27	-1340000000.0	Coefficient b_2 in phytoplankton conversion efficiency function Source Based on calculations by LVH. Uses experimental observation that efficiency of conversion is 0.69 when flowrate into 100 cm deep tank is 0.001331 $cm^3/cm^2/sec$ and efficiency is zero when flowrate is 0.002778 $cm^3/cm^2/sec$. See memos 5/26/76 and 2/25/77.	sec^2
29	3.0	Animal feed rate decision ratio of actual feeding rate to biologically efficient (maximum conversion rate) feeding rate for animal Source Assumption recognizing the trade-off between biologically efficient production of an animal and the cost of providing it with a suitable environment by LVH.	#

Variable Number	Variable Value	Description	Units
31	0.000000011808	Biologically efficient specific feed supply rate Source Calculation based on experimental observation-LVH.	g-prot/sec /lg animal
34	-16.89145	Coefficient a_0 in animal conversion efficiency function	#
35	-1.891173	Coefficient a_1 in animal conversion efficiency function	#
36	-0.0518	Coefficient a_2 in animal conversion efficiency function Source Animal conversion efficiency function fitted to limited experimental and theoretically required values-LVH.	#
47	0.001	Cost of larva on intering the production system Source Assumption. Reflects unknown hatchery costs. No known market exists from which values could be obtained.	$/head
51		Phytoplankton tank apparant depth	cm
52		Phytoplankton tank exposure factor (its reciprocal is the exposure time)	/sec
53		Phytoplankton conversion efficiency	g out/g in (Nitrogen or protein basis)
54		Equivalent protein concentration (nitrogen x 6.25) in deep sea water	g-prot/cm^3
55		Rate of protein production in phytoplankton tanks	g-prot/cm^2/sec
56		Actual specific feeding rate of animals	g-prot/sec/1 g animal
57		Specific animal conversion efficiency	g-prot out/g-prot in/1 g animal
58		Coefficient to convert growth measured as gain in protein to growth measured as total wet weight	g-total/g-prot
59		Rate of feeding an individual animal based on the average weight in a batch on that day	g-prot/sec/ animal

Variable Number	Variable Value	Description	Units
60		Nutrient concentration in intake water to phytoplankton tanks	g-N/cm^3
61		Calculated compensation depth, dependent on nutrient concentration	cm
98		Weight of individual animal, adjusted for mortality, at which a transfer to more trays must be made	g/animal in
99		Number of animals started on one tray at the start of the growth calculations	$ animals in/tray
101	10000.0	Cost of land Source Highly location specific. Reflects a premium of about 100% over prime agricultural land prices in the United States.	$/ha
102	8000.0	Area of facility utilizable by building or tank structures Source Assumed that four-fifths of the land area can be actively used and one-fifth will be waste, access roads, etc.	m^2/ha
103	10.0	Capital cost of phytoplankton tanks excluding excavation Source Lightburn and Roels (1971) provide calculations which indicate a capital cost for phytoplankton tanks 12 feet deep excluding excavation costs of between $13.50 and $15.00 per square meter. Approximately three-fourths of the cost is for piping and ancillary equipment. If this part of the cost is cut to one-third for a one meter tank but costs of lining remains the same then capital costs range between $7.10 and $8.15 in 1971 dollars. The baseline figure is therefore a high cost, which might be reduced by less substantial engineering standards.	$/m^2
104	5.0	Life of phytoplankton tanks Source Assumption. This value is lower than the lifetimes typically applied to equivalent structures but is intended to recognize the likelihood of obsolescence in an activity where the technology is relatively undeveloped. The class life system (ADR) used by the U.S. Treasury has the following asset depreciation ranges (in years)	year

Variable Number	Variable Value	Description	Lower Limit	Guideline Period	Upper Limit	Units
		Agricultural machinery & equipment	8	10	12	
		Farm buildings	20	25	30	
		Light trucks	3	4	5	
105	0.0	Cost of cover Source Assumed that no shading or other protection would be required for animal tanks				$/m^2
106	5.0	Life of cover Source See variable 104.				year
107	0.0063	Ratio of office laboratory and storage space area to phytoplankton tank area Source Assumption based on providing approximately 250m^2 of office, laboratory and storage area for an operation using 40,000m^2 of phytoplankton tank and producing 1 metric ton per day.				#
108	20.0	Capital cost of animal tanks, complete Source Estimate by LVH.				$/tray
109	5.0	Life of animal tanks and equipment Source See variable 104.				year
110	3.0	Stacking factor (Number of trays per square meter of structure) Source Assumption.				#
111		Effective capital cost of utilized land				$/m^2
112		Rental cost of raw land				$/m^2/yr
113		Annual cost of phytoplankton tanks (excluding excavation)				$/m^2/yr
115		Annual cost of cover for phytoplankton or animal tanks				$/m^2/yr
116		Capital cost of office and storage space expressed per area of tank				$/m^2 - tank

Variable Number	Variable Value	Description	Units
117		Annual cost of office and storage space	$/m^2 - tank/yr
118		Annual cost of animal tanks, complete	$/tray/year
119		Total capital cost of phytoplankton tanks	$/m^2
120		Total annual cost of phytoplankton tanks	$/m^2/yr
121		Total capital cost of animal tanks	$/tray
122		Total annual cost of animal tanks	$/tray/yr
123		Total cost of excavating phytoplankton tanks	$/m^2
124		Length of embankments, phytoplankton tanks	m
125		Cross-sectional area of phytoplankton tank embankments	m^2
126		Total cost of excavation work, phytoplankton tanks	$/m^2 tank
127		Annualized cost of excavation work, phytoplankton tanks	$/m^2/year
200		Heat input required to maintain operating temperature	Kcal/l
201	20.0	Mean ambient sea-water temperature as it enters the system Source Approximates average value observed in phytoplankton tanks, St. Croix.	^oC
202	0.0	Operating temperature of tanks Source Assumes no external temperature control.	^oC
203	0.5	Efficiency of heat exchanger, proportion of available heat recovered.	#
204	0.0	Not operational	
205	10.0	Life of heat exchanger Source see variable 104.	year
206	2.0	Annual maintenance cost for heat exchanger Source EPA report.	% of capital cost/year

Variable Number	Variable Value	Description	Units
207	0.0	Not operational	
208	10.0	Life of conventional oil or gas fired boiler Source Assumption.	years
209	3.0	Annual maintenance cost, excluding regular labor, conventional fired boilers Source EPA report.	% of capital cost/year
210	4.0	Labor requirement for conventional fired boiler Source EPA report.	hrs/day
211	8.0	Maintenance engineer wage rate Source typical skilled labor rate, United States.	$/hr
212	0.9	Efficiency of conventional fired boiler Source Assumption	proportion
213	11.00	Cost of fuel oil Source Assumes 34500 kcal/gal and 38 cents/gal for #2 fuel oil	$/mil Kcal
214	0.0	Loss of heat within system Source Assumed zero since no external heating employed.	^{o}C per pass
215		Heat input required to maintain system at operating temperature	Kcal/day
216	0.0	Area of heat exchanger Source Assumed zero. No external heat source employed.	m^2
217		Heat input provided by recovery through heat exchanger	Kcal/day
218		Standard capital cost, heat exchanger	$
219		Capital cost of heat exchanger, adjusted for changes in cost of construction index	$
220		Annual cost of heat exchanger, including capital recovery charge and maintenance cost	$/year
221		Heat input required from conventional source	Kcal/day
222	50.0	Reserve capacity provided for conventional heater Source Assumption. Value will depend on extent to which animals can tolerate acute temperature drop to ambient water temperature.	% of utilized capacity

Variable Number	Variable Value	Description	Units
223		Total conventional heater capacity required	Kcal/hr
224		Standard capital cost of conventional heater	$
225		Capital cost of conventional heater, adjusted for changes in cost of construction index	$
226		Annualized capital cost for conventional heater	$/year
227		Annual operating cost for conventional heater	$/year
228		Total annual cost for conventional heater	$/year
229		Operating temperature in animal tanks. If [202] greater than zero; [229]=[202], otherwise [229]=[201], ambient temperature	^{o}C
301	0.85	Efficiency of pump Source EPA report	propn
302	3.0	Pump lift-intake Source Assumes water is delivered to site and represents head loss within the plant only.	m
303	100.0	Intake pump standby capacity Source Assumes single intake pump and need for continuous operation.	% of utilized capacity
304	10.0	Life of pump Source see variable 104.	year
305	3.00	Pump annual maintenance cost Source EPA report.	% of capital cost
306		Animal tank recirculation pumping power total requirements	Kw
307		Capital cost of individual pump (successively: phytoplankton recirculation, animal recirculation)	$
308		Annual cost of pump equipment (successively, same order as [307]).	$/yr
309		Operating cost of pumps (successively, same order as [307]).	$/yr
310		Total cost of recirculation pumping in animal tanks	$/yr

Variable Number	Variable Value	Description	Units
311		Total cost of intake pumping	$/yr
312	0.00000025	Animal tank recirculation pumping power coefficient Source LVH. Function assumes that resistance of shellfish to water passage increases with their depth in tray, represented by the square of grams of animal per tray. Equivalent to assumption that to drive 2.5 l/sec of water through a tray containing the maximum of 20 kg of shellfish requires 0.1 kw of pump power	watts/(g of animals in tray)^2
320		Total cost of intake pumping into phytoplankton tank	$/yr
321		Total cost of intake pumping direct to animal tanks	$/yr
322		Total cost of recirculation pumping in animal tanks	$/yr
323		Total cost of recirculation pumping in phytoplankton tanks	$/yr
326		Pumping capacity, intake to phytoplankton tanks	Kw
327		Pumping capacity, intake direct to animal tanks	Kw
328		Pump capacity required for each isolated animal tank recirculation circuit	Kw
329		Pump capacity required for recirculation in phytoplankton tanks	Kw
330		Total capital cost of pumping equipment (Sum of steps described under [307]).	$
331		Annual cost of pumping equipment (Sum of steps described under [308]).	$/year
401	2400.0	Cost of construction index for inflating those cost functions constructed on the basis ENRCC=2000 Source Engineering News Record.	#
402	1.0	Screening	
402	1.0	Filtration	

Variable Number	Variable Value	Description	Units
404	0.0	Nitrification	
405	0.0	Carbon absorption	
406	0.0	Ozonation	
407-410	0.0	Not operational	

The variables [402] to [406] are used for both capital and operating costs. They take the value 1 if the process is included and 0 if the process in not included. It is assumed that if the equipment is installed it will be used, hence variables [407] to [410] which formerly acted to include operating and maintenance costs, when desired, no longer serve any purpose. The functions used are described at the end of this section.

411	5.0	Ozone dosage required for sterilization Source T/S	mg/l
412	22.0	Electricity consumption in ozone production Source T/S	kwh/kg
413	0.04	Electrical power cost Source Typical cost for medium size users, United States.	$/kwh
414	0.9	Proportion of flow in animal tanks recirculated Source Assumption.	propn.
415	0.1	Proportion of recirculated water treated with carbon absorption Source T/S. Only operates if var[405]=1.	propn.
416	0.0	User specified flowrate through animal tanks. If set to zero then var [414] determines flowrate.	cm^3/sec/ g animal
417	90.0	Efficiency of waste treatment system in removing suspended solids Source Assumption. Not supported by data.	%
418	90.0	Efficiency of waste treatment system in removing nitrates Source Assumption. Not supported by data.	%
419	0.12	Environmental Protection Agency proposed discharge guideline Source T/S	g suspended solids/g feed
420	0.8	Rate of solid waste production Source T/S. Based on experimental observation on lobster.	g suspended solids/g food fed, dry matt basis

Variable Number	Variable Value	Description	Units
421		Rate of removal of suspended solids in single pass through discharge waste treatment	g-ss/l
422		Rate of flow in recirculation circuits in animal tanks	l/day
423		Required flowrate through discharge waste treatment plant to meet Environmental Protection Agency regulations	l/day
424		Total capital costs of waste treatment plant based on ENRCC index of 2000	$
425		Current capital cost of waste treatment plant based on current ENRCC index	$
426	10.0	Life of waste treatment plant Source See variable 104.	year
427		Annual cost of waste treatment plant	$/yr
428		Capital cost of waste treatment plant per 1000 liters treated	$/Kl
429		Operating cost of waste treatment plant per 1000 liters treated	$/Kl
430		Total cost of waste treatment per 1000 liters treated	$/Kl
431	0.003	Alternative form of Environmental Protection Agency proposed discharge guidelines Source T/S.	g suspended solids/g animal weight/day
432		Cumulated rate of metabolite production, theoretical maximum, continuous batch	g-N sec/ animal in
	(179300)	Suspended solids discharge allowance under Alternative Environmental Protection Agency regulations (see [431])	g-ss/day
	(179800)	Weight of suspended solids actually produced	g-ss/day
433		Suspended solids discharge allowance under Environmental Protection Agency regulations (see [419])	g-ss/day
	(179400)	The least limiting discharge allowance	g-ss/day

Variable Number	Variable Value	Description	Units
435		Rate of metabolite production, theoretical maximum based on conversion efficiency and expressed as nitrogen, mortality adjusted.	g-N/sec/animal in
436		Cumulated rate of un-ionized ammonia production in animal tanks	g-N/sec/animal in
437		Rate of un-ionized ammonia production in animal tanks (based on dissociation proportion of total theoretical ammonia production)	g-N/sec/animal in
438	(102950)	Calculated flowrate required in animal tanks to meet safe metabolite loads	cm^3/sec/g
	(103040)	Actual flowrate in animal tanks	l/day
439		Rate of deficit in oxygen supply. If less than zero, excess oxygen supplied, no additional aeration. If greater than zero, additional aeration supplied.	mg/hr
440		Maximum ammonia concentration allowed in animal tanks	mg/l
441	10.0	Design ammonia concentration (% of concentration causing 50% mortality in 96 hours-96 hours LC_{50}) Source Assumed suitable safety margin.	%
442	1.2	Acute toxic ammonia concentration Source Gravitz et al. Refers to observations with 1g and 3g lobsters. No data for clams.	mg/l
443	0.0	Rate of flow of (surface) seawater direct to animal tanks Source Assumed zero under present system.	cm^3/sec/g animal
444		Weight of feed entering the animal tanks (dry matter basis)	g/day
445	8.0	Operating pH Source Assumed same as seawater (7.8-8.1)	#
452		Capital cost of screening equipment in waste treatment plant	$
453		Capital cost of mechanical filtration equipment in waste treatment plant	$
457		Operating cost of screening equipment in waste treatment plant	$/day

Variable Number	Variable Value	Description	Units
458		Operating cost of mechanical filtration equipment in waste treatment plant	$/day
462		Rate of production of suspended solids in animal tanks, adjusted for mortality	g-ss/sec/ animal in
463		Cumulated rate of production of suspended solids in animal tanks, continuous production	g-ss/sec/ animal in
493		Rate of flow of water from phytoplankton tanks and direct from sea into animal tanks	cm^3/sec/g
494		Proportion of animal tank flow recirculated. If [416] is equal to zero, [494] equals [414]. If insufficient flow to maintain safe metabolite load, warning message is issued.	#
501	0.88	"Metabolic body size" factor in oxygen consumption equation Source Unpublished experiments on lobster (Schuur) No data for clams.	#
502	5.0	Minimum tolerable oxygen concentration in animal tanks Source Assumed value for lobster. Adult animals would tolerate much greater acute deficits (below 3mg/ℓ). No data for clams.	mg/ℓ
503	-0.013908	Coefficient b_0 in specific oxygen consumption function Source Calculated from unpublished experimental data on lobster by Schuur. No data for clams.	mg/hr /lg animal
504	0.6	Aeration efficiency; surface aerator Source Based on Liao & Mayo, figure 5, average oxygen deficit of 2 mg/ℓ.	kgO_2/kwh
505	1.0	Not operational. Was used as conversion factor for kwh to HP including efficiency loss.	
506	500.0	Capital cost of aerator Source Subjective estimate by Tchobanoglous.	$/installed K
507	1.0	Maintenance cost of aerator Source EPA report.	% of capital cost/year
509	5.0	Life of aerator Source See variable 104.	year

Variable Number	Variable Value	Description	Units
513		Oxygen consumption rate, continuous production, adjusted for mortality	mg/hr/ animal in
514		Oxygen consumption rate in animal tanks, adjusted for mortality	mg/hr/ animal in
515		Saturation oxygen concentration in intake water (103500). Saturation oxygen concentration in recirculation water	mg/hr
517		Rate of oxygen consumption in animal tanks	mg/hr
519		Direct aeration capacity required	Kg/hr
520		Temperature corrected specific oxygen consumption rate	mg/hr/lg animal
601	0.0025	Phytoplankton tank recirculation pumping power requirement Source Extrapolation of existing recirculation pumping capacity in phytoplankton tanks at St. Croix.	kw/m^3
602	0.0	Cost of artificial feed Source Zero value indicates no artificial food fed.	$/kg
603	2.5	Composition of phytoplankton Source Experimental observations-LVH.	g-dry matter /g protein
604	0.0	Reserve capacity of feeding equipment Source Zero indicates none used.	proportion of utilized capac
605	0.0	Frequency of feeding artificial food Source Zero indicates none used.	#/day
606	0.0	Capacity of feeding equipment Source None used.	m^2/day/unit
608	0.0	Life of feeding equipment Source None used.	year
610	0.0	Cost of added nutrients Source Not investigated, none used.	$/kg-N
611	4.0	Wage rate, regular labor Source Assumption. A high value for U.S. conditions. U.S. average wage for hourly paid workers in agriculture, Dec. 1976 was $2.84 per hour (USDA-Economic Research Service-Farm Labor) Fringe benefits typically at 10% to employer's cost.	$/hour

Variable Number	Variable Value	Description	Units
612		Rate of feeding animals of given weight, adjusted for mortality	g-prot/sec /animal in
618	0.0	Capital cost of feeding equipment Source None used.	$/unit
620	0.002413	Coefficient b_1 in temperature corrected specific oxygen consumption function Source See variable 503.	mg/hr/^{o}C /lg animal
621		Rate of feeding animals, continuous production, adjusted for mortality	g-prot/sec /animal in
640		Rate of feeding phytoplankton to animals, total plant	g-prot/sec
641	200.0	Capital cost of office, laboratory and storage space Source	$/m^2
642	15.0	Life of office, laboratory and storage space Source See variable 104.	year
643	0.0	Efficiency of animal tank recirculation loop waste treatment system in removing ammonia Source None used.	%
644	0.0	Rate of addition of artificial nutrients to phytoplankton tank intake water (Note: where these nutrients are added in dilute form e.g. sewage sludge, the volume is the total volume of deep sea water and diluted nutrient and the quantity is related to this volume) Source Assumes no addition.	g-N/cm^3
661		Utilized area of phytoplankton tanks in the plant	cm^2
662		Total area of phytoplankton tanks in the plant, including reserve	cm^2
663		Total flowrate of deep sea water into phyto-plankton tanks	cm^3/sec
664		Utilized volume of phytoplankton tanks	m^3

Variable Number	Variable Value	Description	Units
701	0.1	Cost of supervisory and technical labor Source Assumption. No data available.	$/kg output
703	20.0	Rate of transfer and harvest of animal trays (Represents number transferred into) Source Assumption.	trays/hour
704	1.0	Frequency of hand cleaning of phytoplankton tanks Source Assumption. Supported by typical cleaning frequency at St. Croix.	#/month
705	50.0	Rate of hand cleaning phytoplankton tanks Source Based on subjective estimate, LVH.	m^2/hour
706	(101002)	Number of transfers per tray at end of growth calculations	# transfers /tray in
	(102140)	Share of number of transfers per animal at end of growth calculations	# transfers /animal in
	(220240)	Cost of transfer and harvest	$/animal in
707		Total cost of labor associated with animal production facility	$/day
708		Cost of hand cleaning phytoplankton tanks	$/year
801	7.2	"Biological zero" temperature Source Not used in current program. Estimated for lobster from experimental observations. Apparent origin of temperature dependent growth function.	^oC
802	0.67	Metabolic body size coefficient used to determine feeding rate of animals Source Not used in current program. Estimated for lobster from experimental observations.	
803	3.0	Number of phytoplankton tanks. The reserve capacity of phytoplankton tanks, [16] should be made consistent with [803] which includes reserve area Source Assumption.	#
804	1.0	Unit cost of excavation work, phytoplankton tanks Source Lightburn and Roels (1971).	$/$m^3$ of embankment
805	0.01025	Initial weight of animals entering the system Source Experimental observations-LVH.	g

Variable Number	Variable Value	Description	Units
806	5.0	Interval between batches in continuous production Source Assumption.	day
807		Daily rate of weight gain for an individual animal for the given average weight	g/day/animal
808	0.8	Proportion of animals surviving after 180 days Source Subjective estimate-LVH.	propn.
809		Annual salary for supervisory and technical labor Source	$/year
900	(101000)	Number of trays in use per tray at start	#trays/tray in
	(102150)	Share of cumulated number of trays per animal started	#trays/animal in
	(106200)	Total number of animal trays in plant	#
901	1.0	Proportion of animals surviving at start of initial growth period. (Normally equal to one, but can be set to lower values in program is to be started from middle of a growth sequence, as when transferring from one system to another	animals surviving / animals started
915		Cumulated weight of animals in system, adjusted for mortality	g/animal in
916		Number of animals in the system, continuous production	#/animal in
920	10.0	Maximum weight of individual animal at harvest. Overridden if either maximum time [2] or maximum number of transfers [10] reached. Source Estimated average meat weight of marketed wild clams.	g
921	5000.0	Target output from a batch Source Assumption	kg/batch
922		Number of days to harvest of animals	day
924		Cumulated number of trays in use per tray at start of growth	#trays/tray
925	(100970)	Weight of individual animal, at end of ith day, adjusted for mortality	g
	(102705)	Weight of animals in the system	g

Variable Number	Variable Value	Description	Units
927	0.1	Interest rate on sinking fund Source Represents a rate of borrowing for high risk ventures. Current rate on low grade corporate bonds ranges between 9½% and 11½%.	proportion
928	0.1	Return on capital, a rate charged the fixed investments of the firm Source See variable [927]. May be set at higher levels to calculate market price sought in high risk situation.	proportion
930		Cost of artifical nutrients added to phyto-plankton tanks	$/kg output
931		Cost of space for animals including land, buildings, tanks, trays	$/kg output
932		Cost of space for phytoplankton production including land and tanks	$/kg output
933		Cost of heating	$/kg output
934		Cost of recirculation pumping in phyto-plankton tanks	$/kg output
935		Cost of aeration in animal tanks	$/kg output
936		Cost of labor to maintain phytoplankton production	$/kg output
937		Cost of pumping intake water	$/kg output
938		Total area of phytoplankton tanks	m^2
939		Cost of recirculation pumping in animal tanks	$/kg output
940		Depth of phytoplankton tanks	m
941		Cost of feed supplement fed directly to animals	$/kg output
942		Flowrate into phytoplankton tanks	m^3/sec
943		Cost of feed (phytoplankton). This is the sum of [932], [934] and [936] plus the cost of artifical nutrients, if any.	$/kg output
944		Phytoplankton. Conversion efficiency	g out/g in (Nitrogen or protein basis)

Variable Number	Variable Value	Description	Units
945		Cost of waste treatment	$/kg output
946		Weight of individual animal at harvest	g
947		Cost of regular labor employed in animal production section	$/kg output
948		Weight of a single batch of animals at harvest	kg
949		Cost of supervisory labor	$/kg output
950		Number of trays of animals in the system with continuous production	#
951		Cost of larvae	$/kg output
952		Specific animal conversion efficiency	g protein out /g protein in /lg animal
953		Total cost of production	$/kg output
954		Number of days to harvest	#
955		Total capital cost	thousand $
956		Annualized capital cost	thousand $/year
957		Amount of regular labor for cleaning tanks and transferring animals	hrs/week
958		Supervisory and technical staff required	# full time equivalent

The following cost functions are fixed within the program:

Heat exchanger capital cost — $

$C = 5900\ Q^{0.5}$ where Q is the exchanger area — m^2

Source EPA report, converted to metric units

Conventional boiler capital cost — $

$C = 0.012\ Q^{0.548}$ where Q is heat output required — kcal/hr

Source EPA report, converted to metric units

Pump capital cost — $

$C = 560\ Q^{0.6}$ where Q is pumping power — kw

Source EPA report

Waste Treatment-screening, capital cost — $

$C - 18.243\ Q^{0.565}$ where Q is flowrate — ℓ/day

Source T/S

Waste Treatment-filtration, capital cost — $

$C = 31.758\ Q^{0.554}$ where Q is flowrate — ℓ/day

Source T/S

Waste Treatment-screening, operating and maintenance cost — $/day

$C = 181.063\ Q^{-0.169}$ where Q is flowrate — ℓ/day

Source T/S

Waste Treatment-filtration, operation and maintenance cost — $/day

$C = 16237.7\ Q^{-.346}$ where Q is flowrate — ℓ/day

Source T/S

The waste treatment functions apply to flowrates exceeding 50,000 ℓ/day.

AMMONIUM AND NITRATE UPTAKE RATES OF THE SEAWEEDS *HYPNEA MUSCIFORMIS* (RHODOPHYTA) AND *MACROCYSTIS PYRIFERA* (PHAEOPHYTA)[1]

by

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Condensed running title: N uptake by *Hypnea* and *Macrocystis*

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ABSTRACT

Ammonium and nitrate uptake were measured by continuous sampling with an autoanalyzer. For Hypnea musciformis (Wulfen) Lamouroux, nitrate uptake followed saturable kinetics (K_s = 4.9 μg-at $N \cdot l^{-1}$, V_{max} = 2.85 μg-at $N \cdot g(wet)^{-1} \cdot h^{-1}$. The ammonium uptake data fit a truncated hyperbola, i.e., saturation was not reached at the concentrations used. Nitrate uptake was reduced one-half in the presence of ammonium, but presence of nitrate had no effect on ammonium uptake. Darkness reduced both nitrate and ammonium uptake by one-third to one-half.

For Macrocystis pyrifera (Linnaeus), nitrate uptake followed saturable kinetics; K_s = 13.1 μg-at $N \cdot l^{-1}$, V_{max} = 3.05 μg-at $N \cdot g(wet)^{-1} \cdot h^{-1}$. Ammonium uptake showed saturable kinetics at concentrations below 22 μg-at $N \cdot l^{-1}$ (K_s = 5.3 μg-at $N \cdot l^{-1}$, V_{max} =2.38 μg-at $N \cdot g(wet)^{-1} \cdot h^{-1}$; at higher concentrations uptake increased linearly with concentration. Nitrate and ammonium were taken up simultaneously; presence of one form did not affect uptake of the other. Previous growth on a restricted nitrogen supply stimulated nitrate uptake rates.

Key index words:

ammonium
Hypnea
Macrocystis
nitrate
uptake kinetics

INTRODUCTION

There is evidence that uptake and storage of nitrogen by seaweeds can be affected by the concentration of dissolved inorganic nitrogen in seawater, and that environmental fluctuations in inorganic nitrogen can result in corresponding fluctuations in tissue nitrogen and in growth rate. *Laminaria longicruris* accumulated nitrate during the winter when concentrations were high, then grew rapidly using the internal nitrogen reserve when external nitrate was depleted (5). Fertilization of a natural *Laminaria* bed with nitrate increased internal nitrate and growth rate compared to unfertilized control beds (5). Fertilization with nitrate and phosphate increased growth rate in *Alaria esculenta* (2) and in *Chondrus crispus* (18). Rapid growth periods of tropical *Eucheuma isiforme* (6) and temperate *Macrocystis pyrifera* (15) coincided with high environmental concentrations of nitrate.

Both nitrate and ammonium can be utilized as a nitrogen source for growth. For example, *Fucus spiralis* grew similarly on either ammonium or nitrate (24). *Gelidium nudifrons* took up nitrate and ammonium equally over a period of several days when they were present singly or in combination (1). *Gracilaria foliifera* and *Neogardhiella baileyi* grew faster in ammonium-enriched cultures than in nitrate- or sewage-enriched cultures (7). *Hypnea musciformis* grew better in the ammonia-rich effluent from a clam mariculture system then in nitrate-rich 870 m deep water, but trace nutrients may have been limiting in the deep water (13). However, ammonium can be toxic at high concentrations in some sewage effluent-seawater mixtures

or in enriched seawater media (21 and authors cited therein).

A knowledge of kinetics of nitrogen uptake was needed for maricultural experimentation using commercially important seaweed species in the California Institute of Technology's Marine Farm project (19) and in the University of Texas' Artificial Upwelling project on St. Croix (16,20). It should be theoretically possible to specify optimal cultural conditions for nitrogen supply if the relation between uptake rates, external nitrogen concentration, and other environmental factors are known. Uptake rates in phytoplankton often vary hyperbolically with external nutrient concentration and are characterized by the kinetic parameters V_{max} (the maximal uptake rate) and K_s (the half-saturation constant, i.e. the nutrient concentration at which uptake is one-half maximal) (9). Thus, V_{max} is an indicator of uptake ability at high nutrient concentrations, whereas K_s is an indicator of uptake ability at low nutrient concentrations compared to that at high concentrations. The predictive value of K_s for ammonium and nitrate uptake has been shown for several phytoplankters (10).

Less is known about nitrogen uptake by macrophytes. Kinetic parameters have been reported recently for three species: Fucus spiralis (23), Gracilaria foliifera, and Neogardhiella baileyi (7,8).

Here, we report relations between external ammonium and nitrate concentrations and uptake rates for the tropical red alga Hypnea musciformis and the temperate brown alga Macrocystis pyrifera. Uptake kinetics were determined by a continuous sampling method which eliminated problems associated with

Antarctic Intermediate deep water (pumped continuously from 870 m depth to which 10^{-6}M disodium ethylene tetraacetic acid (EDTA) was added, or in flowing surface water. The deep water contained (in $\mu g\text{-at} \cdot l^{-1}$): NO_3-N, 27.3; NH_4-N, 1.1; PO_4-P, 2.1 (20). Surface water contained approximately 0.2 μg-at $NO_3\text{-}N \cdot l^{-1}$ and 0.8 μg-at $NH_4\text{-}N \cdot l^{-1}$. The experimental plants were visibly free of epiphytic growth.

Experimental Apparatus. A two-channel Technicon Auto-Analyzer II was used to analyze continuously for nitrate and ammonium in 1-liter Pyrex flasks containing the experimental plants. The analytical methods were standard Technicon methods, based on those described by Strickland and Parsons (1972). Additions of nitrate (KNO_3) or ammonium (NH_4Cl) to yield 1-40 μg-at $N \cdot l^{-1}$ were made by volumetric pipet from 10 mM N stocks. Uptake was measured as loss of added nutrient from the seawater. We added successively larger doses in some experiments, or simply added a single large dose in other experiments. Both methods yielded similar results. The uptake flasks containing 4-6 g(wet) of alga and one liter of glass fiber-filtered surface seawater were held in a water bath at 26 ± 1 C for Hypnea and 16 ± 1 C for Macrocystis. Light was provided by two 20-watt fluorescent light bulbs (one "cool" white and one "daylight") at the flask surface (to give a spectral illuminance of 1.6 $\mu W\ cm^{-2} nm^{-1}$ measured as above with the light probe inside an empty uptake flask. Seawater in the flasks was uniformly mixed by aeration, to give a current speed of ca 6-7 cm sec^{-1}. No loss of ammonium-N due to stripping by the air stream was observed over a one-hour period in control experiments (without

algae) run at 24 C (pH 8.2 and 9.3) with 20 ug-at $NH_4N \cdot l^{-1}$ added.

Calculation of Uptake Rates. Nitrate and ammonium concentrations were calculated for selected points on the recorded nutrient disappearance curves at 5 or 10 min intervals, using baseline-corrected peak heights of calibration standards. After correcting for volume changes due to sample removal and nutrient additions (about 10% of the initial volume), we calculated the amount of nutrient taken up per unit time by the alga during each time interval. Mean nutrient concentration for each interval was used for determination of uptake kinetics. The kinetic parameters K_s and V_{max} for nutrient uptake were determined from the weighted linear regression of S/V on V as described by Wilkinson (1961).

RESULTS

Hypnea musciformis. The steeper slopes of the ammonium disappearance curves, compared to the nitrate curves, reflect more rapid uptake of ammonium than of nitrate (Figure 1). Uptake of nitrate and ammonium at low nutrient concentrations (< 5 ug-at $N \cdot l^{-1}$) increased rapidly with increasing concentrations (Figures 2 and 3). Increases in concentration at higher levels had less effect on uptake rates. The nitrate uptake data fit a hyperbolic curve (Figure 2) yielding estimates for the kinetic parameters K_s and V_{max} Table 1). The curve for ammonium uptake was a truncated hyperbola; i.e., saturation was not attained at the ammonium concentration used. At all nutrient concentrations ammonium uptake was much faster than nitrate uptake. High nitrate levels had no effect on ammonium uptake (Figure 2), but high levels of ammonium reduced nitrate uptake by about one-half (Figure 3). Uptake of both nitrate and ammonium were reduced by one-third to one-half in the dark. Uptake recovered considerably in both cases after only 10 minutes exposure to light. Ammonium-grown Hypnea showed reduced rates of nitrate uptake

but uptake rates approached those of nitrate-grown plants after 50 minutes exposure to nitrate in the absence of ammonium (Figure 3).

Macrocystis pyrifera. Nitrate uptake followed saturation kinetics (Figure 4), yielding values for K_s and V_{max} (Table 1). Ammonium uptake followed saturation kinetics below 22 μg-at $N \cdot l^{-1}$; but at higher concentrations uptake increased linearly with increasing concentration (Figure 5). This suggested that ammonium uptake may occur by more than one process. We calculated the kinetic parameters given in Table 2 only from those points below 22 μg-at $N \cdot l^{-1}$. Nitrate and ammonium were taken up simultaneously (Figure 6). Presence of one form had no effect on uptake of the other (Figures 4 and 5).

Kinetics of nitrate uptake by Macrocystis were affected by the concentration of nitrate in the growth medium (Table 3). The V_{max} for the deep water-grown plant was higher than for the surface water-grown plant on a wet weight basis; this relation was reversed, however, when nitrogen-specific uptake rate was considered (Table 3). Furthermore, K_s for the plant grown in surface water was significantly lower than K_s for the deep water plant. Thus growth on a restricted nitrogen supply led to an increased ability to take up nitrate.

DISCUSSION

Ammonium was taken up more rapidly than nitrate by both seaweeds, although both nitrogen sources were taken up simultaneously. Simultaneous uptake has been reported for other seaweeds, Fucus (23) and Gelidium (1), but it does not occur generally in planktonic algae (10). High ammonium concentrations partially inhibited nitrate uptake by Hypnea but not by Macrocystis. Macrocystis in this respect is similar to Fucus (25). When each nitrogen source was supplied singly, uptake by Hypnea was faster than

by Macrocystis; this may reflect the difference in the experimental temperatures (25 C and 16 C respectively), differences in degree of nitrogen starvation of the plants, in the case of ammonia (7,24), surface:volume ratio differences, or a genetic difference between these two algae. Ammonium and nitrate uptake by Fucus increased with temperature (23); similar temperature dependence of uptake has been observed in marine phytoplankters (10, 25). The faster uptake by Hypnea compared to Macrocystis, may be an adaptation to a nitrogen-poor environment. Upwelling of nutrient-rich water occurs in the California waters where Macrocystis grows (15), but does not occur in St. Croix waters where Hypnea grows. Marine phytoplankters from nitrogen-poor environments generally have lower K_s values for nitrogen uptake than those from nitrogen-rich environments; this has been shown at the population level (17), the species level (4,10,11), and when clones of the same species were compared (4,25).

Nitrate uptake followed saturable kinetics for both seaweeds, and the calculated K_s's and V_{max}'s give an indication of uptake abilities at low and high concentrations respectively. The half-saturation constants, K_s's are close within the confidence limits given and are similar to those reported for Fucus, but somewhat higher than those reported for Gracilaria and Neoagardhiella (Table 1). The V_{max}'s are more difficult to compare since the rates have been reported in different units. Maximal nitrate uptake rates for Macrocystis and Hypnea are similar on a wet weight basis. However, maximal nitrate uptake rates for Macrocystis and Hypnea are considerably higher than those reported for Gracilaria and Neoagardhiella on a dry weight basis, and are considerably lower for Macrocystis than those reported for Fucus on an areal basis. The K_s's for all the seaweeds is about one order of magnitude higher than K_s's reported for marine phytoplankton (4,10, 11, 17, 25).

Ammonium uptake kinetics were more complex. Although the ammonium uptake data for Hypnea fit a truncated hyperbola (Figure 3); saturation of ammonium uptake was not achieved at the external concentrations used, and it is quite possible that ammonium uptake by Hypnea does not follow saturation kinetics. For Macrocystis, the relation between ammonium uptake rates and external ammonium concentration shows a sharp discontinuity above 20 μg-at N·l^{-1}. A saturable uptake system seems to be operating at low concentrations, but another system becomes manifest at higher external ammonium concentrations. The latter system could be either simple diffusion resulting in a linear relation between external concentration and uptake rate or another uptake system with a higher V_{max} and K_s than the system operating at lower concentrations.

Deviation of uptake kinetics from simple hyperbolic relations over wide concentrations have been reported previously, and in fact seem prevalent for ammonium uptake at high external concentrations. Topinka (23) found that uptake rates for nitrate and ammonium deviated from hyperbolas at higher nutrient concentrations. Ammonium uptake by Gracilaria and Neoagardhiella was not saturated at a concentration as high as 40 μg-at l^{-1}, and D'Elia et al. (8) suggest that a strong diffusive component predominates ammonium uptake at high external ammonium concentrations.

Although ammonium uptake does not follow saturation kinetics over the whole range of external concentrations used, it is still possible to estimate a K_s for uptake at relatively low substrate concentrations when the two processes can be separated (for Macrocystis, Neoagardhiella, and Gracilaria) or when deviation from saturable kinetics is not extreme (for Fucus). The K_s's for ammonium uptake by seaweeds in these cases (Table 2) are relatively close within the given confidence limits, but are about an order of magnitude higher than values obtained for phytoplankton (10, 17). Any

comparisons of nitrogen uptake kinetics between species are necessarily uncertain because of our lack of knowledge of how such factors as growth history, internal nutrient reserves, and environmental parameters influence uptake rates. The effect of these factors needs to be examined in future studies.

The original purpose for determining the kinetics of nitrogen was to provide guidelines for optimizing the nitrogen supply for Hypnea and Macrocytis culture. Since nitrate uptake follows saturation kinetics, the uptake curve provides information on minimum external concentrations required to supply the seaweeds with a given amount of nitrogen and also indicates the external concentration beyond which no further increase in uptake rate occurs. The ammonium uptake curves also provide minimum external concentrations required to supply the algae with a given amount of nitrogen, but indicate that uptake is not saturated at high external concentrations. The results presented here also indicate that both seaweeds take up ammonium more rapidly than nitrate at all concentrations; this could influence the choice of chemical form of nitrogen fertilizer if artificial enrichment of seawater is used.

Because the K's for ammonium and nitrate uptake by Hypnea and Macrocystis are greater than the levels of ammonium and nitrate commonly found in the natural habitats of these plants, accumulation of nitrogen from the environment may often be restricted by the ambient concentration. Thus, there is also the potential for a nitrogen limitation on growth.

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Legends for Figures

Figure 1. A smoothed tracing of a portion of a chart record showing nutrient disappearance (uptake) in simultaneous nitrate (heavy line) and ammonium (light line) uptake experiments with Hypnea musciformis. The arrows indicate addition of nutrients (μg-at $N \cdot l^{-1}$) to the flasks. The circles at five or ten minute intervals indicate points for which nutrient concentrations were calculated; uptake rates were calculated for the intervals between these points (see text for details). The Hypnea had been grown to N-depletion prior to the experiment, which began with 1 μg-at $N \cdot l^{-1}$ additions (not shown here).

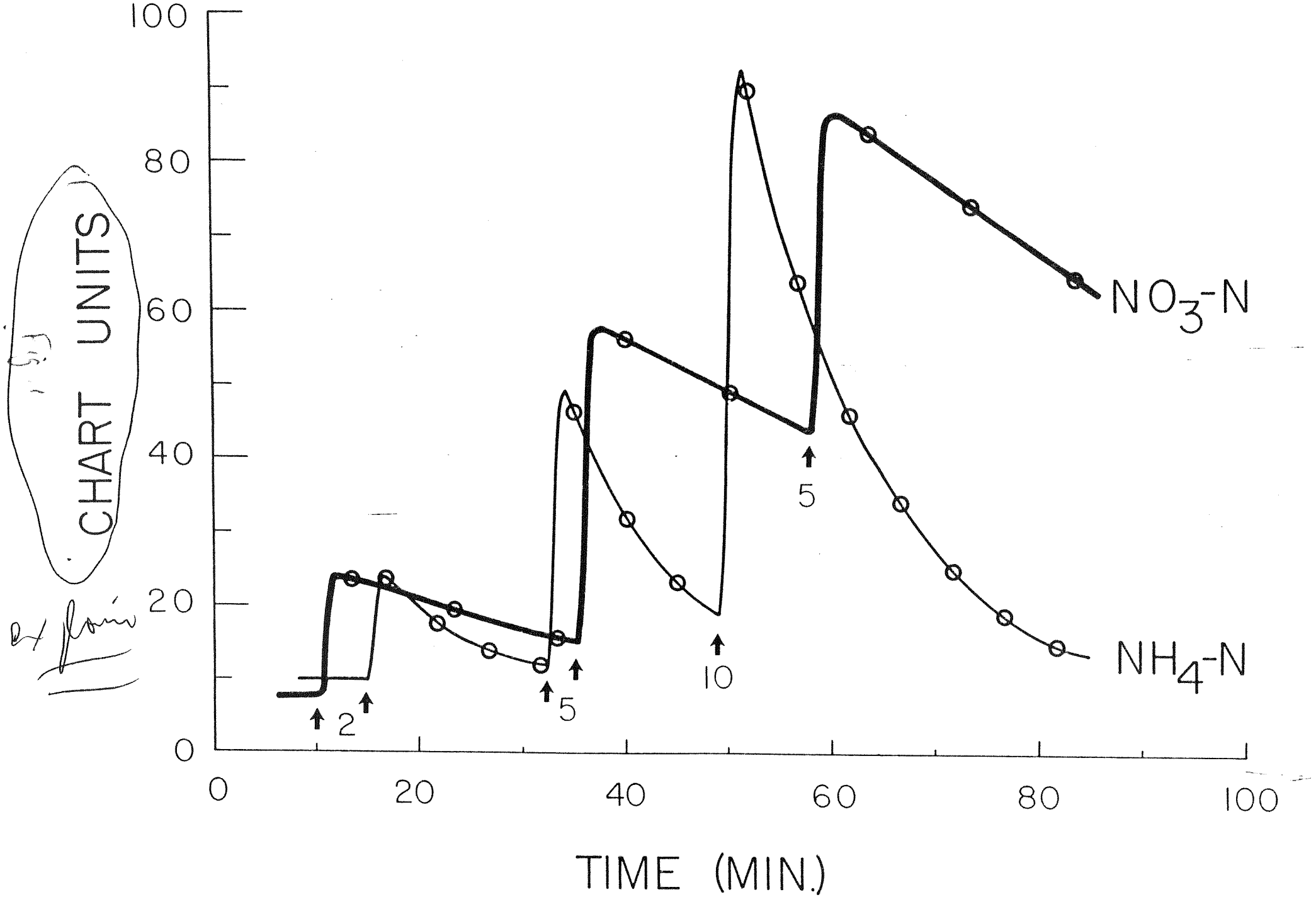
Figure 2. Nitrate uptake by Hypnea. Nitrate-grown Hypnea: (x), 12.5 hr in light; (O), 14.5 hr in dark; ($\triangle$), after 10 min exposure to light; (·), 14.5 hr in light, in presence of 18 μg-at ammonium-$N \cdot l^{-1}$. Ammonium-grown Hypnea: ($\odot$), 13.2 hr in light; the six points, reading from right to left, are in the order of observation at 10 min intervals and thus indicate an increase in uptake rate as the concentration decreased.

Figure 3. Ammonium uptake by Hypnea. (x), 13 hr in light; (··), 14.5 hr in light, in presence of 19 μg-at nitrate-$N \cdot l^{-1}$; (O), 14.5 hr in dark; ($\triangle$), after 10 min exposure to light.

Figure 4. Nitrate uptake by Macrocystis. (x), nitrate only; (·), nitrate in the presence of 30 μg-at ammonium-N·l^{-1}; (○), uptake by nitrogen-deprived algae.

Figure 5. Ammonium uptake by Macrocystis. (x), ammonium only; (·), ammonium in the presence of 30 μg-at nitrate-N·l^{-1}.

Figure 6. Simultaneous uptake of nitrate and ammonium by Macrocystis.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60
40
20
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100
TIME (MIN.)
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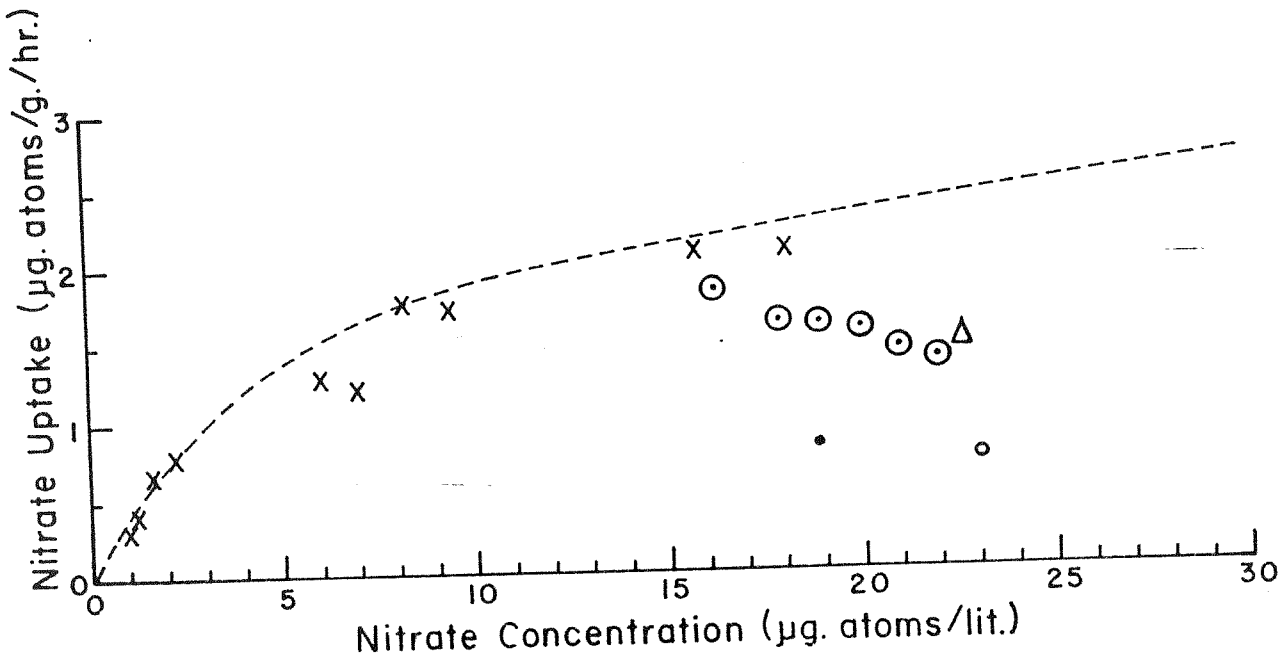
NH_4-N
2
5
10
5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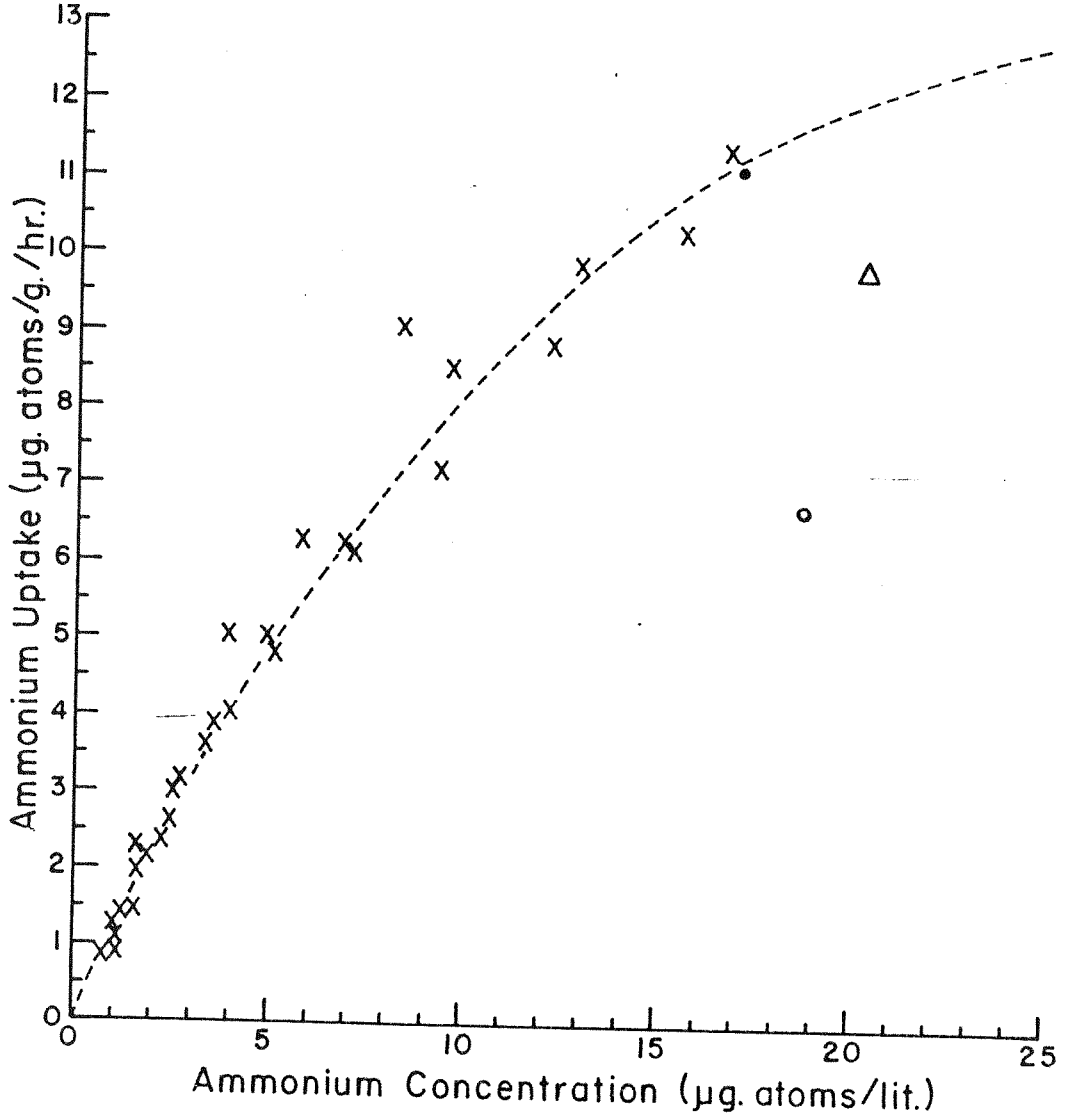
10
15
20
25
30
Nitrate Concentration (µg. atoms/lit.)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2
3
4
5
6
7
8
9
10
11
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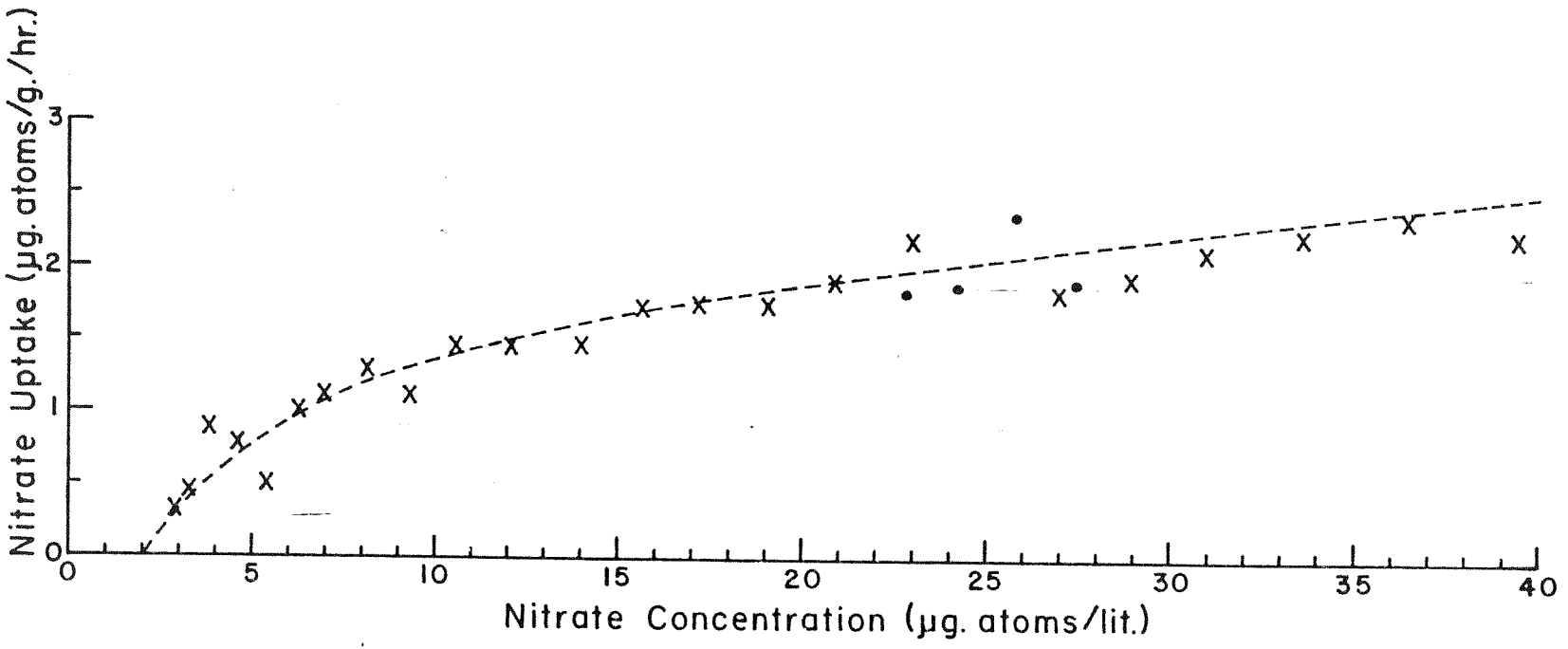
5
10
15
20
25
Ammonium Concentration (µg. atoms/lit.)

Fig. 3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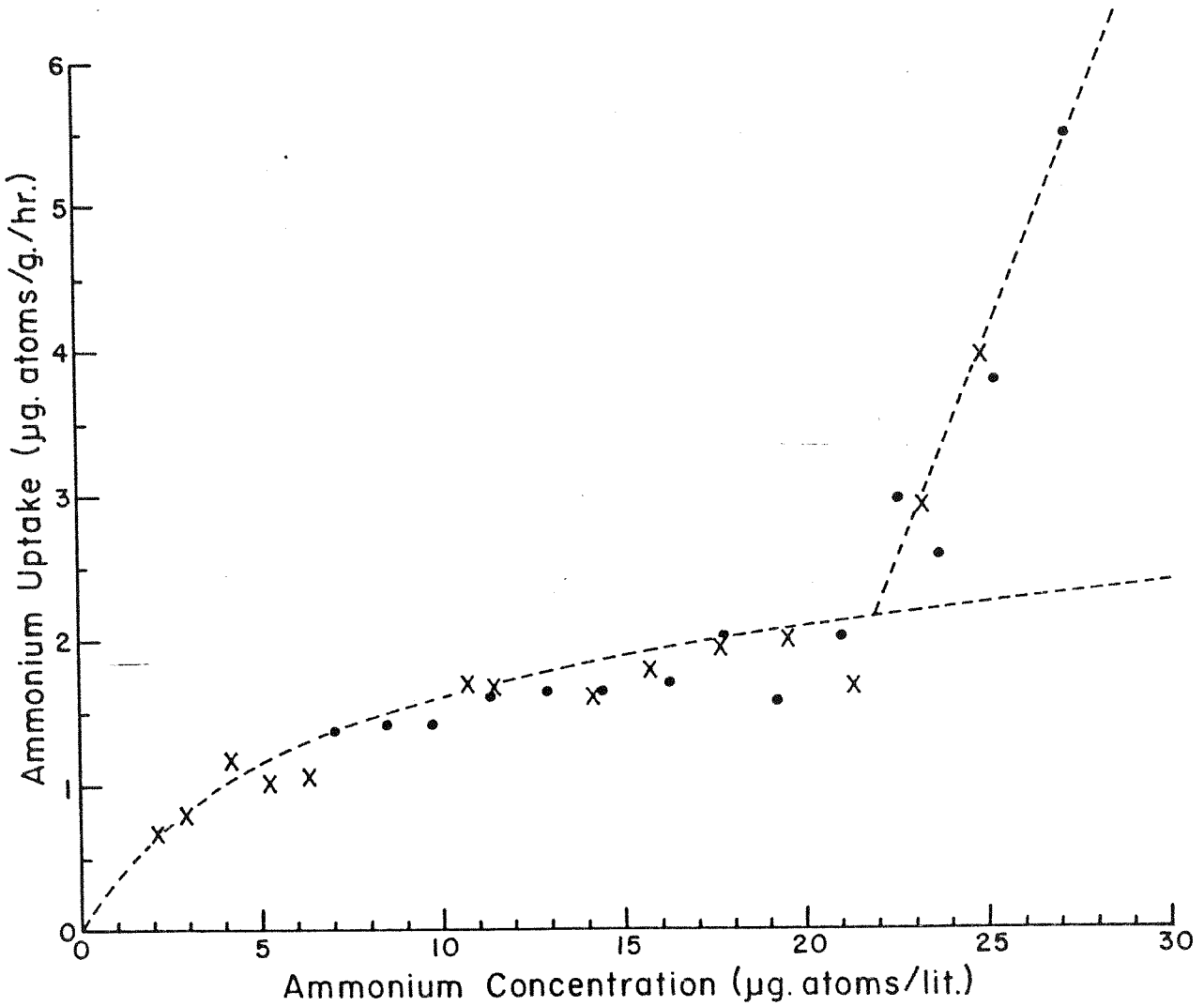
Nitrate Concentration (µg. atoms/lit.)

Fig. 4

Fig. 5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Ammonium Concentration (µg. atoms/lit.)

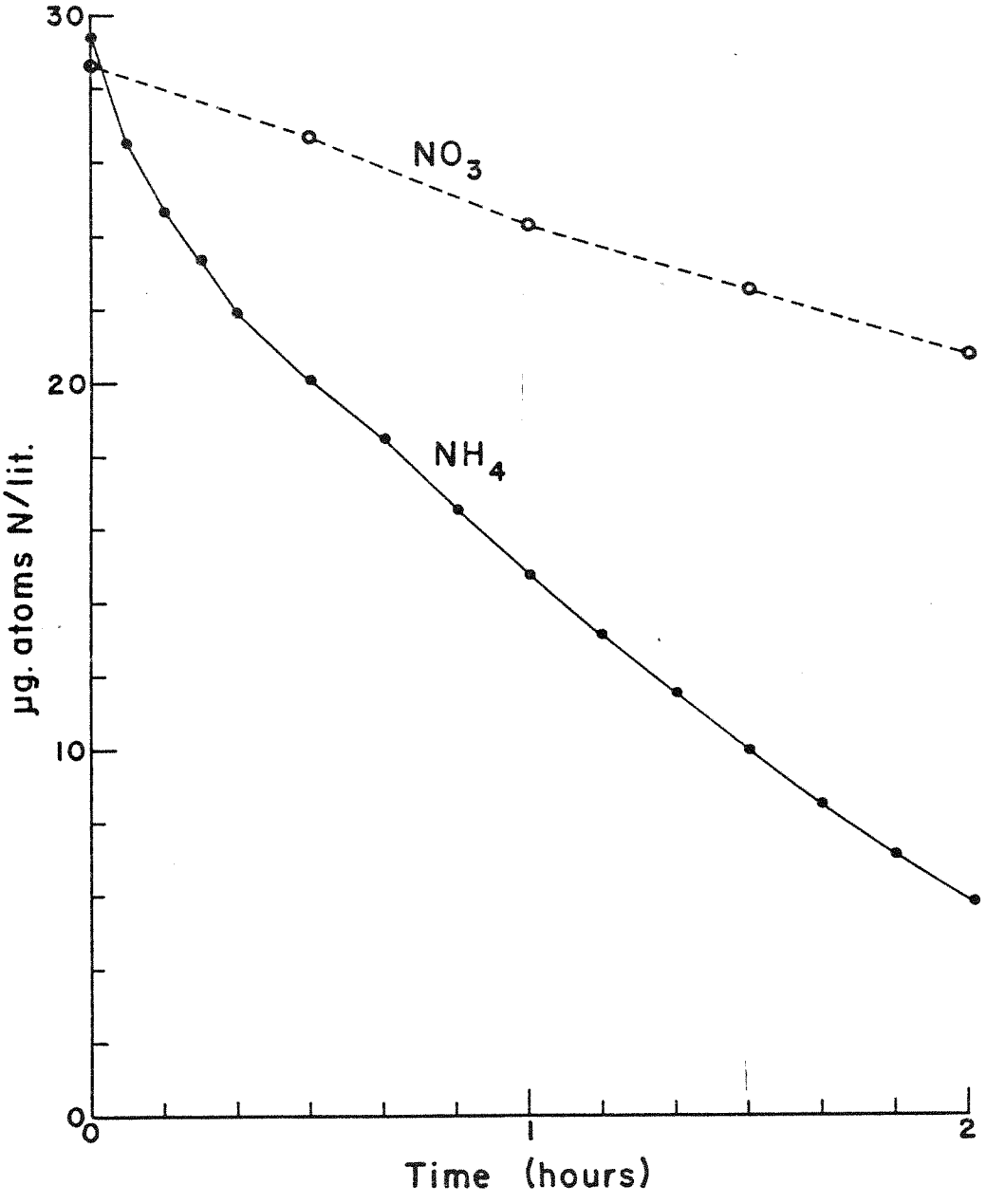

Fig. 6

Table 1. Kinetic parameters for nitrate uptake.

Alga	Temperature C	K_s ($\pm$ S.E.) $\mu g\text{-at.}l^{-1}$	V_{max} ($\pm$ S.E.) $\mu g\text{-at.}g(wet)^{-1}.h^{-1}$	V_{max} ($\pm$ S.E.) $ng\text{-at.}g(dry)^{-1}.min^{-1}$	V_{max} ($\pm$ S.E.) $\mu g\text{-at.}cm^{-2}.h^{-1}$	Reference
Hypnea	26	4.9 $\pm$ 3.9	2.85 $\pm$ 0.79	475 $\pm$ 132[a]		this study
Macrocystis	16	13.1 $\pm$ 1.6	3.05 $\pm$ 0.17	508 $\pm$ 28[a]	0.095 $\pm$ 0.005[b]	this study
	16	8.7 $\pm$ 1.6	2.24 $\pm$ 0.16	373 $\pm$ 27[a]	0.069 $\pm$ 0.005[b]	this study
Fucus	5	5.6 $\pm$ 0.9			0.160 $\pm$ 0.008	(25)
	10	6.7 $\pm$ 0.8			0.202 $\pm$ 0.009	(25)
	15	7.8 $\pm$ 1.4			0.228 $\pm$ 0.018	(25)
Gracilaria	20	2.5 $\pm$ 0.5		162 $\pm$ 19		(8)
Neoagardhiella	20	2.4 $\pm$ 0.3		194 $\pm$ 15		(8)

[a] Calculated assuming dry weight is 10% of wet weight

[b] Calculated using everage relation of 32.2 $cm^2.g(wet)^{-1}$

Table 2. Kinetic parameters for ammonium uptake at low external ammonium concentrations (< 22 µg-at NH_4-N . l^{-1}).

Alga	Temperature C	K_s ($\pm$ S.E.) µg-at . l^{-1}	Reference
Hypnea	26	16.64 $\pm$ 1.78	this study
Macrocystis	16	5.31 $\pm$ 1.00	this study
Fucus	5	6.4 $\pm$ 2.0	(25)
	10	6.4 $\pm$ 4.9	(25)
	15	9.6 $\pm$ 2.6	(25)
Gracilaria	20	1.59	(8)
Neoagardhiella	20	4.94	(8)
	20	4.52	(8)
	20	2.27	(8)

Table 3. Comparison of nitrate uptake by Macrocystis sporophytes grown in deep water or in surface water.

	Growth Medium	
	Deep Water	Surface Water
Nitrate Concentration ($\mu g\text{-at } NO_3\text{-N}.l^{-1}$)	27.34	0.23
Tissue Nitrogen (% dry weight)	2.94	1.85
V_{max} ($\mu g\text{-at } NO_3\text{-N}.g^{-1}.h^{-1}$)	3.05 ± 0.17	2.24 ± 0.16
(h^{-1})a	0.0146 ± 0.0008	0.0171 ± 0.0012
K_s ($\mu g\text{-at } NO_3\text{-N } .l^{-1}$)	13.06 ± 1.62	8.66 ± 1.56

[a] Calculated assuming that dry wright is 10% of wet weight and using the value given above for tissue nitrogen.

Sym. 100

Running title: Ammonia-nitrogen production

Helgoländer wiss. Meeresunters. 30, ●–● (1977)

Ammonia-nitrogen production by the bivalve mollusc *Tapes japonica* and its recovery by the red seaweed *Hypnea musciformis* in a tropical mariculture system

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ABSTRACT: Production and recovery of ammonia-N was studied in the second and third trophic levels of a mariculture system on St. Croix, US Virgin Islands. The diatom *Chaetoceros curvisetus*, grown on nutrients in artificially upwelled deep water from 870 m depth, was the food source for *Tapes japonica*. Consumption of the phytoplankton by *T. japonica* increased throughout the day and decreased at night, and was related to corresponding changes in algal culture density. Feeding efficiency was highest at night. Ammonia-N production by the clams fluctuated over a typical 24 h period; dropping during the day and increasing at night. The ammonia-N concentration in the shellfish tank effluent was inversely related to the quantity of phytoplankton consumed by the clams. At all ration levels the small clams produced ammonia-N at a greater rate than large clams. *H. musciformis* fragmented and washed out of some tanks; the fragmentation was related to high ammonia-N concentrations in the inflowing water. High light intensity and temperature alone do not appear to cause fragmentation, but may have induced a trace nutrient deficiency in *H. musciformis* grown in the ammonia-rich seawater. Where fragmentation was not obvious, ammonia-N uptake per g *H. musciformis* was highly correlated with the average ammonia-N concentration of the inflowing seawater both day and night. Percent uptake of ammonia-N increased with increasing concentration of the nutrient in the inflowing seawater, reaching a plateau of about 70 % uptake of the available ammonia-N at concentrations above 4 µg-at/l.

INTRODUCTION

In the past few years the interest in controlled marine ecosystems has increased markedly due to the concern over recycling of waste nutrients and developing new food and energy sources (Othmer & Roels, 1973; Goldman et al., 1974; Ryther et al., 1975; Roels et al., 1976). In these systems there are a limited number of trophic levels, each of which is interrelated in such a manner that, starting with a dissolved nutrient source, each level utilizes one or more of the nutrients that is not available to, or has been regenerated by, the previous level. At the artificial upwelling project on St. Croix (US Virgin Islands) the nutrient source is seawater that is pumped from a depth of 870 meters and has high concentrations of nitrate, phosphate and silicate (Othmer & Roels, 1973; Roels et al., 1976). These nutrients are used for the production of phytoplankton which is then fed to bivalve molluscs. Recovery of the soluble waste produced by the shellfish is accomplished by growing seaweed in the effluent from the shellfish tanks.

This paper deals with the generation of ammonia-N by the bivalve mollusc *Tapes japonica* Dehayes feeding on the diatom *Chaetoceros curvisetus* Cleve and the recovery of the ammonia-N by the red seaweed *Hypnea musciformis* (Wulfen) Lamouroux.

MATERIALS AND METHODS

Experimental design

The experimental system consisted of three trophic levels connected in series so that seawater flowed continuously from one trophic level to the next, as follows: phytoplankton – bivalve molluscs – seaweed. The methods employed with each trophic level are given in the following sections.

Phytoplankton

The diatom *Chaetoceros curvisetus* (clone STX-167, isolated from St. Croix deep water by KCH) was grown in an outdoor 2000 liter epoxy-coated concrete tank (reactor) (Malone et al., 1975). The growth medium used was Antarctic Intermediate Water, pumped from a depth of 870 m, hereafter referred to as deep water. Deep water was continuously pumped into the reactor to give a dilution rate of one reactor volume per day. The overflow from the reactor was fed by gravity to the tanks containing the shellfish.

Shellfish

Four different populations, comprised of two different size classes, of the bivalve mollusc *Tapes japonica* were maintained in the flow through system. Either 70 or 140 gms, total wet weight, of clams of a particular size class were placed in 4.6 l tanks (28 cm × 16.4 cm × 10 cm deep) and the algal culture was metered into each tank at the rate of 1 ml sec^{-1}. The inflow into the tanks was adjusted by restricting the flow with an appropriate length of capillary tubing. The water in the shellfish tanks was continuously mixed by aeration from a horizontal air manifold on one short side of each tank, and the tanks were shaded from direct sunlight. The clams were suspended off the bottom of the tank on an epoxycoated wire mesh. A single population of clams consisted of two tanks containing an equal biomass of the same size clams. The choice of flow rate and clam biomass was based on unpublished data which indicated that these flow rate/biomass combinations approached the maximum conversion rate of phytoplankton protein to shellfish meat protein.

Every fourth day, at the conclusion of a 24 h experiment, the clams were removed from each tank, blotted dry with a paper towel and the total wet weight determined. Enough clams were removed to return the weight to the the initial 70 or 140 g. Samples of clams were taken from both the large (mean shell length = 18.9 mm, mean dry meat weight = 84.0 mg) and small (mean shell length = 10.1 mm; mean dry meat weight = 9.7 mg) size classes at the beginning and end of the experiment and the dry meat weight was found to be 6.02 ∓ 0.91 % of the total wet weight. This factor (.0602) was used to convert total wet weight to dry meat weight for the entire experiment.

A number of taxonomic synoyms exist in the English and Japanese literature for the Japanese little neck clam, *Tapes japonica*. The more common names being *Venerupis semidecussata*, *Tapes semidecussata*, *T. japonica* and *Paphia philippinarum*. Throughout this paper we refer to the clam as *Tapes japonica* Dehayes, 1853 (see Cahn, 1951; Ohba, 1959).

Seaweed

The effluent from each of the four shellfish populations flowed into a 4.6 l tank containing the red seaweed *Hypnea musciformis*. The inflow was maintained at the rate of 2 ml sec^{-1}, or 37.6 tank volumes per day. A second *Hypnea* tank received the effluent from the first in an attempt to maximize nutrient removal. The tanks had the same dimensions as the clam tanks, and the seaweed was kept constantly revolving by aeration from a manifold located along the long axis on one side of the tank. The tanks were exposed to full sunlight throughout the day. The *Hypnea musciformis* used for these experiments was collected from a single population on a local reef (0–0.5 in depth) and none of the seaweed was sexually reproductive. The plants were picked free of epiphytic seaweeds and only actively growing branches were used in the experiment. An initial wet weight of 20 g of seaweed was placed in each tank. Every four days, at the conclusion of a 24 h experiment, the seaweed was removed from each tank, blotted dry on a paper towel and the wet weight determined. 20 g of *H. musciformis* was replaced in the appropriate tank. The total wet weight at the end of each experiment was used to calculate the ammonia-N uptake per gram of seaweed for the entire 24 h experiment. In some of the shellfish-seaweed combinations the seaweed fragmented and washed out of the system, resulting in a decrease in biomass. Data collected from these tanks was not used for calculating ammonia-N uptake. When fragmentation did occur *H. musciformis* was replaced with newly collected plants. Control *Hypnea* tanks were set up as described above, except that instead of receiving the effluent from the clam tanks they received the phytoplankton culture directly at the rate of 2 ml sec^{-1}. In a separate experiment an additional control was run to test for ammonia-N uptake by residual phytoplankton in the effluent from the shellfish tanks by omitting the *H. musciformis* from the system.

Sampling program

A series of five experiments, each covering a 24 h period were carried out on July 9, 13, 17, 21 and 26, 1976. Samples of the phytoplankton culture flowing into the shellfish tanks, the effluent from each of the shellfish populations and the effluent from the seaweed tanks were collected at four hour intervals beginning at 0600 h (except on July 26, when sampling began at 0200 h). The water temperature in the banks from one of the shellfish-seaweed combinations was measured at each sampling time. The means and ranges of temperature are given in Table I.

Table 1

Mean and range of temperatures (°C) in one serial combination of a *Tapes japonica* population and two *Hypnea musciformis* tanks. Times of high and low temperatures are given in parentheses

Temperature range	*Tapes japonica*	*Hypnea musciformis* 1st Tank	2nd Tank
High	30.0° (18:00 h)	30.5° (14:00 h)	31.0° (14:00 h)
Low	25.0° (06:00 h)	25.0° (06:00 h)	24.8° (02:00 h)
Mean ± S.D. (n = 24)	27.1 ± 1.4°	27.2 ± 1.8°	27.1 ± 2.0°

The turbidity of the influent and effluent samples from the shellfish tanks was measured using a model 250 Monitek laboratory turbidimeter (Monitor Technology, Inc., 303 Convention Way, Redwood City, Calif. 94063). All samples were filtered through Gelman Type A–E glass fiber filters and the filtrate was analysed for ammonia-N and nitrate + nitrite-N concentrations using a Technicon Auto-Analyser II. The analytical methods used are described in the standard Technicon Auto-Analyser methodology handbook and are based on the procedures given by Strickland & Parsons (1972). For the influent samples, into the shellfish tanks, a known sample volume was filtered and a protein determination carried out on the filter according to the method of Dorsey et al. (1976).

RESULTS

Feeding activity of *Tapes japonica*

Figure 1 shows the diel periodicity in the average phytoplankton consumption, over five 24 h experiments, for the four populations of *Tapes japonica*. The quantity of *Chaetoceros curvisetus* consumed by *T. japonica* was based on the difference in the turbidity between the influent and effluent water from the shellfish tanks. Although there were some differences in the amount of phytoplankton consumed by the four clam populations the diel periodicity in the rate of consumption was similar for all groups. The actual amount of phytoplankton consumed increased throughout the day, reached a peak around sunset, and then decreased throughout the night.

The periodicity in the amount of phytoplankton consumed by the clams may be related to the periodicity in the algal cell density, measured as turbidity, of the influent phytoplankton culture (Fig. 1). It can be seen that the turbidity of the influent water increased throughout the day, peaking near sunset, and then dropped during the night. The change in the rate of consumption by the clams reflects this change in algal cell density.

If the average amount of phytoplankton consumed by the four clam populations is expressed as a percentage of the available phytoplankton it is clear that a higher percentage of the phytoplankton was removed at night (Fig. 1). This suggests that the clams increased their pumping activity or altered their filtration efficiency; this resulted in a greater percentage of the available phytoplankton being consumed.

Ammonia-N production by *Tapes japonica*

Figure 2 shows the temporal change in the average ammonia-N production for the four populations of *Tapes japonica* over five 24 h experiments. The differences in ammonia-N production (Fig. 2) between the four clam populations were greater than the differences in the amount of phytoplankton consumed (Fig. 1). The highest con-

centration of ammonia-N was produced by the 140 (× 2) gm population of small clams. Despite the differences in the quantity of ammonia-N produced, the periodicity in the observed ammonia-N concentrations was similar for all four populations (Fig. 2). The ammonia-N concentration in the shellfish tank effluent gradually decreased throughout the day with the lowest levels observed between 14:00 and 18:00 h; this was followed by an increase in the average ammonia-N concentration during the night.

For comparison the average turbidity of the phytoplankton culture being metered into the shellfish tanks is also shown in Figure 2. The turbidity increased and reached its peak during the day while the ammonia-N concentration in the shellfish tank effluent was dropping to its lowest level. This suggests that the ammonia-N production by *T. japonica* in our experimental mariculture system is inversely related to the phytoplankton concentration flowing into the shellfish tanks.

In Figure 3 the ammonia-N produced by *T. japonica* per g dry meat per day is plotted against protein-N consumed per gram dry meat per day for the two different size classes of clams. The quantity of phytoplankton consumed was measured as turbidity and converted to protein-N using the regression equation protein-N = 31.86 (turbidity) ($r = 0.97$; d.f. = 29) in order to present the system as protein-nitrogen consumed versus ammonia-N produced. At virtually every level of food consumed the smaller clams, as represented by the upper line, produced ammonia at a greater rate than the larger clams. It is also clear that ammonia-N production and the quantity of phytoplankton, or protein-N, consumed are inversely related for both size classes of clams.

Fragmentation of *Hypnea musciformis*

Hypnea musciformis in a number of tanks fragmented and many of the pieces washed out of the system. This resulted in a decrease in biomass and a decrease in the percent uptake of ammonia-N in these tanks. The process of fragmentation did not appear to be reversible once fragmentation commenced. This suggested that fragmentation might be related to the nutrient concentration, especially since the second *Hypnea* tank in a series had a lower inflowing ammonia-N concentration due to the uptake of the nutrients by the first *Hypnea* tank, and fragmented less often. Figure 4 shows the relationship between the average ammonia-N concentration of the seawater flowing into the *Hypnea* tanks and resistance of the seaweed to fragmentation. At the low ammonia-N concentrations *H. musciformis* could be maintained for longer periods than at the higher ammonia-N concentrations before fragmentations occurred.

The fragmentation did not appear to be specifically related to either light or temperature alone. The seaweed tanks in series had a nearly identical temperature regime (Table 1) but the *Hypnea* in the first tank in each pair usually fragmented more often than, and earlier than, the *Hypnea* in the second tank. The seaweed in the control tanks experienced the same lighting regime as the experimental tanks but the control did not fragment during the 21 days of the experiment.

Growth of *Hypnea musciformis*

Excluding all of the *Hypnea* tanks where fragmentation obviously occurred, growth (increase in wet weight) during the 4 day periods between harvest was greater in the tanks receiving the shellfish tank effluent directly than it was in those tanks receiving effluent from seaweed tanks. The percent growth of the weed grown directly in the shellfish tank effluent was 37.4 % ($n = 10$) and in the second seaweed tank it was 22.0 % ($n = 15$). In some of these tanks there was actually a slight loss of weight which was possibly due to the onset of fragmentation.

Ammonia-N uptake by *Hypnea musciformis*

The uptake of ammonia-N per gram wet weight of *Hypnea musciformis* was found to be highly correlated with the average ammonia-N concentration of the seawater flowing into the tanks (Fig. 5). The values plotted in Figure 5 are for both day and night samples. The diel variation in ammonia-N uptake followed the diel variation in the ammonia-N concentration flowing into the *Hypnea* tanks. Consequently, any direct influence of light on ammonia-N uptake was probably masked by the diel fluctuations in ammonia-N production by *Tapes japonica* (Fig. 2). Support for this is provided by uptake studies with an ammonia-N starved laboratory culture of *H. musciformis* where dark uptake of ammoniak-N was about 90 % of the uptake rate in the light, over the range of 12–18 μg-at/l (Haines, unpublished data).

At inflow ammonia-N concentration of 1.1 μg-at/l, or less, excretion (shown as negative uptake in Fig. 5) was observed in six out of eight tanks. Perhaps *H. musciformis* in these tanks was fragmenting but not to the extent that it was readily visible, or the seaweed may have been excreting intracellular nitrogen prior to the onset of fragmentation.

On a percentage basis, uptake of ammonia-N increased with increasing concentration of nutrient in the inflowing water, reaching a plateau of about 70 % uptake of the available ammonia-N above an inflowing concentration of 4 μg-at/l (Fig. 6).

The pigmentation of *Hypnea* changed when it was maintained in our system. The natural light brown color changed to a yellow-brown color in tanks receiving low levels of ammonia-N, and to a dark red-brown in the tanks receiving the highest ammonia-N concentrations. Lapointe et al. (1976) observed similar changes in pigmentation in *Hypnea* and *Gracilaria* species in their mariculture system utilizing sewage-enriched seawater.

DISCUSSION

Feeding activity of *Tapes japonica*

The quantity of phytoplankton consumed by the four populations of clams in our flow through mariculture system fluctuated over a 24 h period (Fig. 1). The pattern of change, which reflected a change in the algal cell density of our phytoplankton culture was similar for all four populations except that a biomass of small clams consumed slightly more of the available phytoplankton than an equal biomass of large clams. This might be expected since it is well known that filtration rate is dependant upon body size, being relatively higher for smaller animals (Winter, 1973). The fact that there was not a greater difference in the quantity of phytoplankton consumed by an equal biomass of different size clams may indicate that our system was food limited. The phytoplankton culture was metered into the shellfish tanks at a constant

rate and this rate probably resulted in an algal cell density immediately surrounding the animals which was below the level where filtration rate is reduced, and yet high enough to stimulate a near-maximum feeding efficiency for both size classes and weight groups of *Tapes japonica* (Winter, 1969; Winter & Langton, 1976; see also Hildreth & Crisp, 1976). Therefore the differences in the quantity of phytoplankton consumed by equal biomasses of clams may reflect the absolute difference in filtration rate for the animals of different sizes.

In a mariculture system such as ours the percentage of the available phytoplankton consumed by the shellfish might be expected to remain constant. This was not the case; a greater percentage of the available phytoplankton was consumed at night (Fig. 1). The reasons behind this are not yet fully understood but it may be that the increasing phytoplankton concentration flowing into the tank during the day stimulated an increased feeding efficiency which was maintained for a number of hours after the algal cell concentration had decreased during the night (Winter & Langton, 1976).

Ammonia production by *Tapes japonica*

The ammonia-N production by *Tapes japonica* in our experimental system showed a similar daily pattern of change for all four populations (Fig. 2). The decrease in ammonia-N concentration form the shellfish tank effluent during the day contrasts with the algal cell density of the inflowing phytoplankton culture, which increased during the day. It therefore appeared as if the ammonia-N concentration in the shellfish tank effluent was inversely related to the influent algal cell concentration. Since the quantity of phytoplankton consumed by the clams reflected the phytoplankton concentration coming into the tanks (Fig. 1) and, the ammonia-N concentration was inversely related to the inflowing phytoplankton concentration (Fig. 2) it follows that the ammonia-N production was inversely related to the quantity of food consumed, as shown in Figure 3. Little data exists on the influence of nutritional levels on the rate of ammonia excretion for bivalve molluscs, except for starved animals (Bayne, 1973, 1976; Bayne et al., 1976). However, the effects of nutrition have been more thoroughly investigated for zooplankton where it has usually been found that nitrogen excretion increases with an increased ration (Corner et al., 1965; Butler et al., 1970; Takahaski & Ikeda, 1975). In contrast to this more general pattern, Martin (1968) found an inverse relationship between ammonia excretion and the quantity of phytoplankton ingested by natural zooplankton populations. However, in a more recent publication Takahaski & Ikeda (1975) have demonstrated that the excretion rates, measured using a method similar to Martin's, have to be corrected for the uptake of ammonia-N by the phytoplankton culture which is serving as a food source for the zooplankton. They divided the zooplankton excretion rate into an apparent excretion rate and the uptake rate by the phytoplankton and have shown that the apparent excretion rate was inversely related to the phytoplankton concentration in the experimental flask. This situation is very similar to what takes place in our mariculture system. It would appear that our ammonia-N production data for *Tapes japonica* only approximates an actual excretion rate and would need to be corrected for ammonia-N uptake by the phytoplankton being continuously metered into the shellfish tanks. Nevertheless, our ammonia-N production data accurately reflects the levels of ammonia which are available for culturing commercially valuable seaweeds such as *Hypnea musciformis*. One other possibility has to be considered before we may conclude that nutritional levels are or are not inversely related to ammonia-N excretion for *T. japonica*. Bivalves will normally satisfy their metabolic energy requirements by using carbohydrates, then lipids and then protein (Gabbott & Bayne, 1973; Bayne, 1976). So, it is possible that at the lower food levels the protein in the phytoplankton would be utilized for the metabolic requirements and that the resulting deamination of the protein would increase the rate of ammonia excretion at the lower food levels.

Growth and ammonia-N uptake by *Hypnea musciformis*

The growth rates of *Hynaea musciformis* and uptake of ammonia-N per gram of seaweed observed in this experiment were lower than in a previous study (Haines, 1976), but the maximum percent uptake was higher. This may be explained by the use here of lower dilution rates, lower ammonia-N concentrations, and a higher biomass of *H. musciformis* per unit volume of the tanks. In this experiment we are trying to maximize nutrient removal, whereas in the previous study the emphasis was on maintaining a maximum growth rate and avoiding nutrient limited growth.

Fragmentation of *Hypnea musciformis*

The fragmentation observed in this experiment appeared to be related to high levels of ammonia-N, but it does not necessarily follow that high concentrations of ammonia-N alone caused the fragmentation. In the previous study fragmentation did not occur in *Hypnea musciformis* cultures receiving either *Tapes japonica* effluent or deepwater when supplemented with 4–12 µg-at ammonia-N/l and a chelated trace metals + vitamins mix (Haines, 1976). The *Tapes japonica* individuals in that study were fed algal cultures grown in deep water supplemented with the chelated trace metals + vitamins mix 85 % of the time, and with cultures grown in unsupplemented deepwater alone the remaining 15 % of the time. In the more recent experiments, no supplement was used in the algal culture. There were also differences in light intensity and temperature between the two experiments. Previously the temperature was lower in the *Hypnea* tanks receiving supplemented deep water, where the average temperature was 22–23° C (maximum 24.6° C), and in the tanks receiving shellfish effluent where the average temperature was 26.1° C (compare with Table 1). Light intensity was also undoubtedly higher than in the previous study because the tanks used in the present experiment were shallower (0.1 m versus 0.3 m depth) and translucent rather than opaque. Furthermore this study was completed during the summer months, rather than in the winter when light intensity is lower. Our experimental conditions may have induced, in *Hypnea musciformis* that fragmented, an increased requirement for a trace metal or vitamins caused by using an ammonia rich, high light intensity, and high temperature environment. Lapointe et al. (1976) observed fragmentation of *H. musciformis* in their tanks at the Harbor Branch Foundation (Florida, USA). They suggested that fragmentation was caused by high light intensity, rather than the high temperatures (above 28° C) in their non-nutrient limited cultures. The total dissolved-N concentration in their tanks was at least 10–20 times higher than those in our experiments. It therefore seems unlikely that the trace nutrient deficiency at high light

intensities and high temperature that we suggested as a possible cause of fragmentation would occur in their system. From these studies of *H. musciformis* it is difficult to identify a specific factor(s) which causes fragmentation especially since our control *H. musciformis* did not fragment. A comprehensive study on nutrient, light, and temperature interaction is necessary before the mechanism causing fragmentation will be understood.

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Fig. 1: Diel perodicity in the average phytoplankton consumption by four populations of *Tapes japonica* over five 24 h experiments. The 06:00 h values are repeated on the right hand side of the graph. ○ = 70 g (total wet weight) large clams; □ = 140 g large clams; ● = 70 g small clams; ■ = 140 g small clams; = turbidity of water entering clam tanks; = average percent consumption of phytoplankton for all four clam populations

Fig. 2. Temporal changes in the average ammonia-N production by four populations of *Tapes japonica* over five 24 h experiments. Symbols as in Figure 1, except average ammonia-N production for the four clam populations

Fig. 3: Ammonia-N production versus protein-N consumed for four populations of *Tapes japonica* comprised of two different size classes of clams. The upper regression line is for the small animals where $Y = -0.18 \times + 118.25$ ($r = 0.78$; d.f. = 8) and the lower regression line is for the larger clams where $Y = -0.11 \times + 45.64$ ($r = 0.82$; d.f. = 8). Symbols are as in Figure 1

Fig. 4: Relationship between the average ammonia-N concentration of the seawater flowing into the *Hypnea musciformis* tank and the resistance of the seaweed to fragmentation. Data points are included here if fragmentation of *H. musciformis* was readily visible during the experiment or if fragmentation did not occur during the 21 day experimental period. Ammonia-N concentrations are the means calculated from values determined at 4 h intervals over a 24 h period every four days. ○ = 0.80 — 1.16 µg-at ammonia-N/l; ● = 1.32 — 7.51 µg-at ammonia-N/l

Fig. 5: Uptake of ammonia-N per gram wet weight of *Hypnea musciformis* as a function of the ammonia-N concentration of the seawater flowing into the seaweed tank. A straight line fit to the data follows the form where: Uptake = —0.77 (inflowing ammonia-N concentration) + 0.74; ($r = 0.95$; d.f. = 25). Data were excluded for tanks in which fragmentation of the seaweed was obvious. Control *Hypnea musciformis* tanks receiving phytoplankton culture only are also included in the graph

Fig. 6: Percent uptake of ammonia-N by *Hypnea musciformis* as a function of the ammonia-N concentration of the seawater flowing into the seaweed tank. The values are derived from the same data presented in Figure 5 expect that the negative uptake (excretion) data were omitted

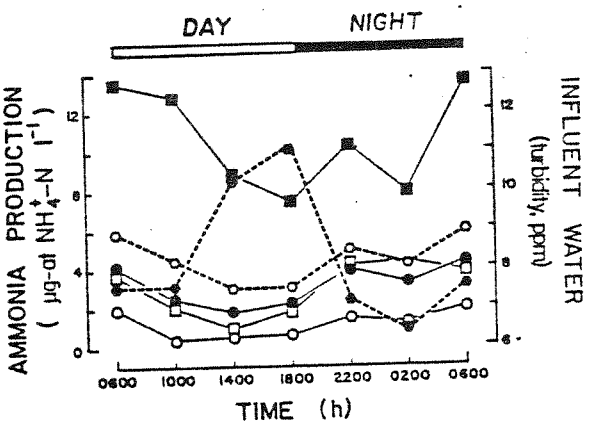


Fig 1 Langton et al.

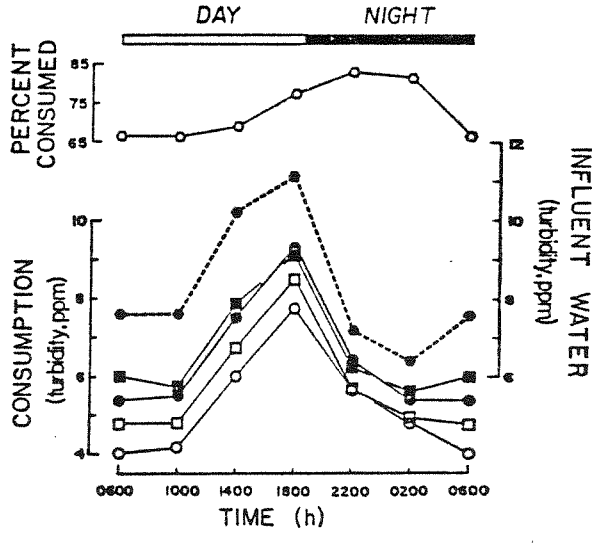
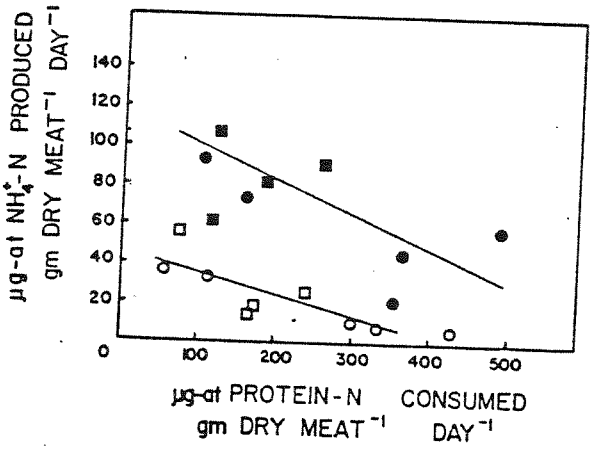


Fig 2 Langton



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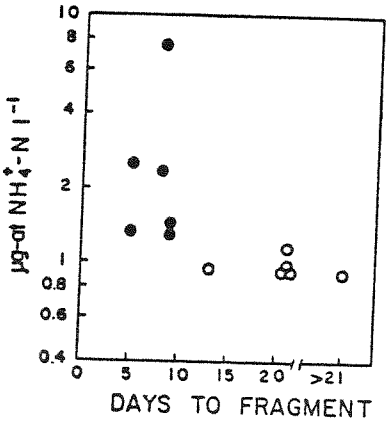

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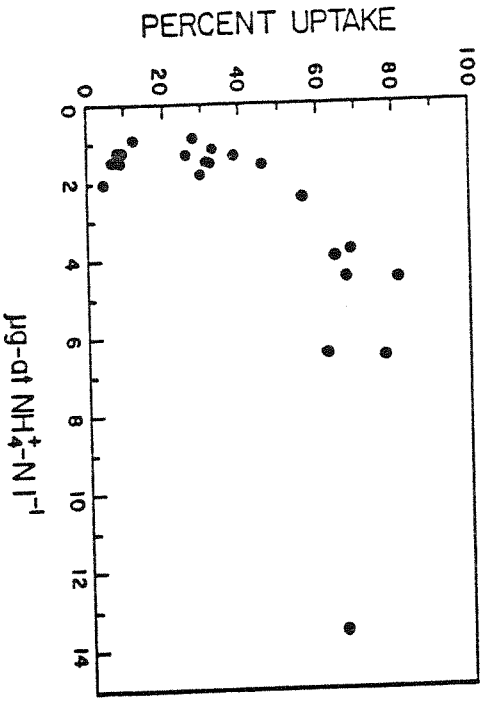

Fig. 6 hanson et al.

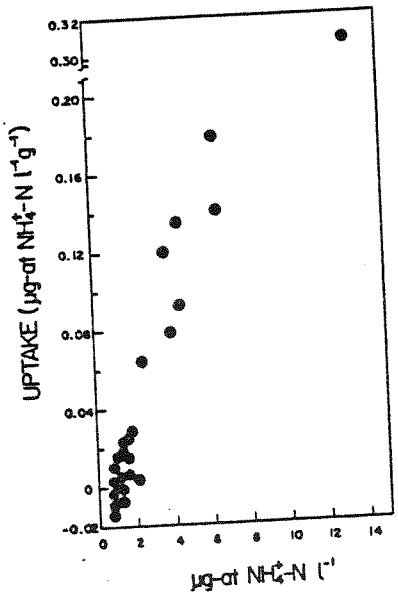

Fig 5 hanson et al.

Report on ^{14}C Studies on Individual Cells in Populations of Chaetoceros curvisetus on St. Croix

by

Bassett Maguire, Jr. & James B. Davenport

The ^{14}C autoradiographic method (Maguire and Neill, 1971, Ecology) was used to examine the photosynthetic rate of individual cells within Chaetocerous populations in Pool 1 and in experimental reactor 8 and its "control" (reactor 6) in June 1977.

The decline in Pool 1 which occurred between June 13th was especially long and slow and was so highly unusual that the beach people, who apparently are usually quite accurate in their predictions, missed in their estimates of when the crash would come. Because of this we did not get the before (and after) measures we wished on the pool. Upon the crash of Pool 1, however, some of its contents were used to innoculate Reactor 8, and Reactor 6 was established as a control.

Light and dark bottle oxygen and ^{14}C experiments were run at .33m in these reactors from 1100 June 18 through 1700 on June 19, twice on June 21, and once on June 22. The most pertinent and interesting results are summarized on Figure 1.

The histograms illustrate the number of grains of silver, each resulting from the radioactive disintegration of a ^{14}C atom, for a number of cells. On the 18th the mode was 15-17 silver grains/cell; the mode was the same on the 21st, as can be seen by the dotted histogram, but there was a slight decrease in the mean. However, because of our knowledge of the decrease in productivity of the cells (from the oxygen data which will be discussed below), the incubation of the cells with the ^{14}C was lengthened from 2 to 3 hr. The solid lines of the middle histogram give the number of grains/cell/2 hr. of incubation, and show clearly that there was a decrease in photosynthetic pro-

ductivity on the part of a large fraction and the Chaetoceros cells on the 21st. For the reason given above, the ^{14}C incubation of June 22nd was further lengthened to 4 hr. The mean and mode, even with the 4 instead of 2 or 3 hr. incubations, had moved strikingly to the left, as shown by the lower dotted line histogram, and when productivity distribution for the Chaetoceros cells was plotted on a 2 hr. incubation basis (the lower, solid histogram), the reduction in productivity of essentially all of the cells remaining alive in the population was dramatic. Reduction in the number of living cells also was very great---only 51 cells were seen on one slide as a result (usually numbers are much greater), and the histogram of the 22nd was therefore normalized to 102 to give size and shapes easily comparable with these above. The caret on the X axis of each histogram gives the background radiation level for the slide preparation, and, can roughly be considered to be the zero point on that axis.

These data strongly suggest that the entire population was affected by some agent or condition which reduced productivity of each member of the population (note that the skew to the right of the 18th is followed by a shift towards a normal distribution on the 21st and to a skew towards the left on the 22nd).

Good supporting evidence comes from the light/dark oxygen bottle results which are summarized to the right of each histogram. In these, photosynthesis is estimated from the difference in oxygen change in the light and dark bottles, and expressed here in terms of carbon fixed in terms of $mg/m^3/hr$. On June 18, the Chaetocerous was producing at a rate of about 8.7×10^{-9} gm $C/10^3$ cells. This productivity dropped to 6.3×10^{-9} $g/10^3$ living cells on the 21st and then to 5.7×10^{-9} $g/10^3$ living cells on the 22nd. The decrease was striking, though not as great in these data as with the ^{14}C data, a situation which may well be the result of interference with oxygen bottle results as density of living cells decreased, and as amount of dead cell material, bacteria, and predators all increased.

Light levels (raw meter counts) during these 3 runs were 103, 78, and 93 respectively. This means, if one assumes a photosynthetic rate directly proportional to light level, that the middle histogram should be moved to the left by about 30%, and the bottom histogram moved to the left by somewhat less than 10%. Such corrections for light levels would make the results even more dramatic, especially for the 21st, and reinforces our conclusion that an increasingly large decrease occurred, at the level of the individual cell productivity, within the experimental time interval.

Reactor 6, which was supposed to be the control, apparently also became affected by the same agent as did Reactor 8, as its pattern through time was much the same as that of Reactor 8.

In summation, from these results, it is our judgement that the Chaetocerous population was affected by some agent, possibly a virus (or metabolite?) which progressively reduced the photosynthetic capacity of the algal cells and resulted in their death (washout could explain it, at most, partly). In addition, however, large populations of a good-sized ciliate (Oligotrichina) developed in one reactor, and a very large population of small amoebae (Hartmannellidae or Vahlkampfiidae) developed in the other. These and other predators could also, on occasion, be the cause of great and rapid reduction of algal populations.

Organic Production Potential of
Artificial Upwelling Marine Culture

O.A. Roels, S. Laurence, M.W. Farmer and L. Van Hemelryck

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ORGANIC PRODUCTION POTENTIAL OF ARTIFICIAL UPWELLING MARINE CULTURE

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Abstract: Increased population and rising expectations have put an enormous strain on the world's energy and food supplies. Petroleum reserves and land-resource limitations severely limit the expansion of conventional agriculture and animal husbandry.

In tropical and subtropical areas of the oceans, the warm surface waters constitute the world's largest storage of solar energy. The underlying cold deep water, less than 1,000 meters below the ocean's surface, constitutes a cold sink, making it possible to generate mechanical energy by inserting a suitable heat engine between the warm and cold waters. The energy required for pumping the deep water to the surface is typically 6.5% of the total energy produced by the plant.

The nitrate, phosphate and other nutrients dissolved in the deep sea water constitute the raw materials for plant growth when brought into the light at the surface. Extrapolation of results from small-scale experiments conducted at the St. Croix (United States Virgin Islands) "Artificial Upwelling" station, indicate that this system could produce twenty times more algal protein per hectare than alfalfa, the highest protein-producer per hectare in land-based agriculture. The algal protein can be converted into clam protein with better than 30% efficiency.

It is recommended that a commercial feasibility test of a combined sea-thermal power plant and mariculture operation utilizing deep-sea water and sunshine as major raw materials be undertaken.

Introduction: Increased population and rising expectations have put an enormous strain on the world's energy and food supplies. Humanity is expected to increase from the present four billion to between six and seven billion by the year 2000. Already now, five hundred million people are suffering from protein-deficiency. Pimentel and his collaborators (1975) have demonstrated that both petroleum and land-resource limitations make it impossible to feed the present population a United States diet (in which 69% of the total protein intake is of animal origin) based on United States agricultural technology.

Very high levels of agricultural productivity requiring few man-hours have been achieved in highly developed nations through the intensive use of petroleum-dependent machinery and fertilizers. Pimentel et al. (1975) have estimated that even without an increase in world population, the world-wide use of these techniques would exhaust

presently known petroleum reserves in 13 years. Another factor limiting the expansion of agricultural production is the availability of arable land. Most of the available land is in use, and although the arable land area can be increased through irrigation, this is a capital and energy-intensive practice. Because of these pressures on arable land and petroleum reserves, it is imperative that we explore alternative methods for protein production.

The constraint on arable land naturally points to the vast area of the oceans—particularly in the tropical and subtropical latitudes—as the world's largest collector and storage of solar energy.

However, the animal protein production derived from the sea is disappointingly small. The sea presently accounts for only 5-10% of world protein consumed. The world fish catch has apparently stabilized at 1970 levels (Mayer, 1976) and overfishing of many species has already occurred (e.g., sardines, whales, herring). The total fish production in the oceans, which is obviously closely related tc total biomass production, varies greatly with the different oceanic areas: thus, the "open ocean" with 90% of the surface area of the world's oceans produces 0.7% of its fish, the coastal zones with 9.9% of the area produce 54% of the fish and the upwelling regions with only 0.1% of the surface area of the world's oceans produce 44% of the fish (Crisp, 1975).

This natural phenomenon of high biological productivity in upwelling areas has stimulated our research in "Artificial Upwelling." "Artificial Upwelling" utilizes sunshine and deep-ocean water as raw materials to produce energy and high-quality protein.

Its protein production potential per unit area exceeds that of most agricultural systems, and it is not dependent on petroleum for fertilizer and energy.

"Artificial Upwelling" derives mechanical energy from the temperature differential between the warm surface water and the cold deep water in the tropical and subtropical oceans. Only a small proportion of the total energy generated is required to drive the pumps to bring the deep water to the surface. The deep water is also rich in nutrients (nitrate, phosphate, etc.) compared to surface water. These nutrients can be used as fertilizer to produce plant biomass for marine food chains. In a small land-based pilot plant on the North Shore of St. Croix (U.S. Virgin Islands), the authors have demonstrated the technical feasibility of the biomass production based on deep-sea water and numerous paper studies have analyzed the engineering and economic feasibility of power generation from the sea's temperature differential.

The Energy Resource: The ocean's waters are horizontally stratified and the deep-ocean water is uniformly cold. In tropical areas the temperature differential between the sun-warmed surface layer and the deep cold water is 20°C; this differential varies but little throughout the year. This temperature difference can be utilized to create mechanical energy by inserting a suitable heat engine between the warm and cold layers; such an engine would have a low Carnot efficiency because of the small temperature difference, but the resource is practically inexhaustible and renewable because it is powered by the sun. As demands for fossil fuels increase and they become more difficult to mine, the net energy gains (gross energy less the energy cost of extraction and delivery) resulting from their recovery decreases: we have seen examples of this in the current expensive exploitation of Alaskan and North Sea oil.

The concept of utilizing this temperature differential to run a heat engine is credited to d'Arsonval (1881). Claude (1930) constructed and operated such a plant on the North Shore of Cuba; the plant's operation was short-lived because of trouble with the cold-water pipeline, but he did demonstrate that the process was technically feasible. Since that time, numerous paper studies (Anderson and Anderson, 1966; Lockheed,

1975; TRW, 1975) have demonstrated that such a plant could be constructed utilizing present-day technology. There are wide variations in the projections of the cost of the power produced by such "sea-thermal power plants." These plants could use the "open" or Claude-cycle process, in which the warm water is evaporated under low pressure to drive a turbine, or the "closed" cycle process, in which an intermediary fluid such as ammonia, freon or propane, is evaporated by the warm surface water and condensed by the cold deep water. The "closed" cycle is advantageous because the working pressures are greater and therefore allow for conventional turbine design. However, this system requires very large and expensive heat-exchangers between the working fluid and the warm and cold water. The "open" cycle avoids this problem, but requires a large turbine capable of efficient work at low pressures, and may involve problems with dissolved gases in sea water. Other problems with ocean-thermal energy conversion (OTEC) plants include corrosion and biofouling, but the general consensus is that the difficulties can be solved.

The energy available from the resource is best expressed in terms of cubic meters of deep water, since long and large-diameter pipelines must be used to obtain the deep water, and because pumping costs must be considered. For a cold source at 280°K and a warm source at 300°K, the maximum theoretical efficiency for full utilization of the resource (Van Hemelryck, 1975) is:

$$\eta_{max} = \frac{1}{2}\left(\frac{300}{280} - 1\right) = .0357$$

and the maximum available energy is:

$$W_{max}/m^3 = k\Delta\eta_{max} = 2.91 \times 10^6 \text{ J/m}^3$$

where k = specific heat of water = 4.187×10^6 J/(°K m^3)

Van Hemelryck (1975) has discussed a Rankine-cycle plant which makes optimal use of this resource. At the limit for an optimal plant, the discharge temperature of the cold water (T'_c), and that of the warm water (T'_h), should be equal ($T'_c = T'_h$). Further, assuming a three-stage (multiple evaporation) plant and a surface water:deep water ratio of 3:1, he has shown that with a ΔT of 20°K the theoretical output would be 1.642×10^6 J/m^3, neglecting irreversible losses associated with the operating equipment. Because net yield will depend upon various economic factors and the actual design of turbines, pumps, and (for the "closed" cycle) heat-exchangers, a precise estimate of usable energy from this process cannot be made. For a proposed "open"-cycle plant at Abidjan, Ivory Coast, Salle and Capestan (1957) estimated a gross energy production of about 1.0×10^6 J/m^3 of deep water. A 100 MW plant would thus require approximately 3.15×10^9 m^3/yr. In contrast, a recent "closed"-cycle design commissioned by the U.S. Energy Research and Development Administration (Lockheed, 1975) would require pumping almost 18 times the volume of deep water, 5.7×10^{10} m^3/yr, to achieve a gross output of 250 MW. Anderson and Anderson (1965, 1966) estimated that, for a 33 MW plant producing 302 kW/m^3/sec the pumping costs would require 6.5% of the gross power production. For a plant of this size, pumping costs would therefore be 1.96×10^4 J/m^3. This cost would undoubtedly go down for larger plants.

The Organic Production Potential in Artificial Upwelling Marine Culture: After its utilization in the condenser of a sea-thermal power generating plant, the deep water is unaltered except for its temperature, and can be utilized as a source of nutrients for mariculture.

The technical feasibility of "Artificial Upwelling" mariculture has been demonstrated in a small plant on the North Shore of St. Croix,

U.S. Virgin Islands (17°47'N, 64°48'W) in the Caribbean Sea. The site on St. Croix was chosen because the ocean reaches a depth there of 850 m, approximately 1.6 km offshore. Three polyethylene pipelines, each 1830 m long and 7.5 cm in diameter, were installed from shore into the sea to a depth of 870 m. The pipelines were installed in 1972 and have brought deep water to shore continuously since that time; the present deep-water flow is 250 liters/min. As shown in Figure 1, the deep water is pumped into two 45,000-liter (12,000 gallon) pools. Diatoms grown in these pools are started from laboratory cultures, then cultured in 757-liter (200-gallon) tanks which are used to inoculate the pools. One diatom, Chaetoceros curvisetus Cleve (STX-167) can be grown in continuous culture in unsupplemented deep water, frequently for up to 40 days, at a turnover rate of one pool volume per day. The pool cultures are pumped continuously into shellfish tanks at metered rates, depending upon the feeding activity of the shellfish: an algal stripping rate of about 90% is maintained. The system also contains a hatchery, where the clam Tapes japonica (Deshayes) is regularly produced, a larvae-setting area for juveniles, an experimental shellfish area used to determine optimum feeding ratios, animal density, etc., and a pilot shellfish-rearing area used to test results of small-scale studies and for preliminary economic determinations. Food to these areas can be supplied from the pools or from a wide range of algae grown in elevated 2000-liter culturing vessels ("reactors"). In addition, a separate set of ten 2000-liter reactors is used to study the possibility of maintaining continuous cultures using a surface-water inoculum.

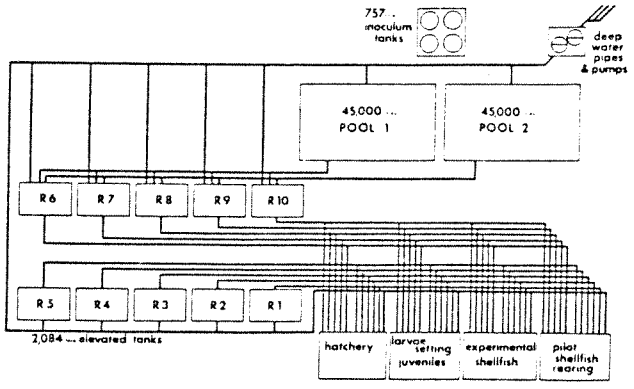

Figure 1. Outline of the experimental deep-sea water mariculture facility on the North Shore of St. Croix, U.S. Virgin Islands.

Table 1 gives the concentration of the major nutrients necessary for algal growth in deep and surface water at St. Croix.

Table I. Nutrient Concentration in the Deep (870-m) and Surface Waters North of St. Croix, U.S. Virgin Islands

	Nutrients (µg-at/liter)				
	(NO_3+NO_2)-N	NO_2-N	NH_3-N	PO_4-P	SiO_4-Si
Surface Water (3 km offshore)	.2	.2	.9	.2	4.9
870-m Deep Water	31.3	.2	.7	2.1	20.6

The yearly temperature range in the shellfish tanks is 22-29°C. Ten species of shellfish have been screened for growth and survival in the

St. Croix system. Eight species grew well and reached market size quickly. They are the European oyster (Ostrea edulis), the Pacific oyster (Crassostrea gigas), the Pacific oyster for the half-shell trade (C. gigas, Kumomoto variety), the Japanese little-neck clam (Tapes japonica), the Southern clam or quahog (Mercenaria campechiensis), F_1 clam (a cross M. campechiensis X M. mercenaria), the bay scallop (Argopecten irradians), and the Japanese pearl oyster (Pinctada martensii).

The Japanese little-neck clam reproduced in the system and a third generation has been produced.

Spiny lobsters, Queen conch and carrageenan-producing seaweeds are grown in the effluent of the shellfish tanks. Studies to test the feasibility of rearing the Queen conch (Strombus gigas) on algae growing on the sides and bottoms of ponds are underway. The spiny lobster, Panulirus argus, is being reared on culled shellfish. Hypnea musciformis, a carrageenan-producing red seaweed, doubles its weight every three days by stripping ammonia (an animal excretory product) from the shellfish tank effluent.

Primary production: One of our main goals at St. Croix is maximal algal productivity. Since inorganic nitrogen (nitrate, nitrite and ammonia) is the limiting nutrient for algal growth in deep-sea water, we have expressed our algal production in terms of protein per hectare, based on the efficiency of inorganic-nitrogen to algal-protein conversion. The latter is dependent upon internal (or species-specific) variables as well as upon external variables such as temperature, nutrient concentration, dilution rate, and pool depth.

In the pools at St. Croix, Chaetoceros curvisetus regularly attains a concentration of 25 µg-at of protein-nitrogen/liter. Since the inorganic nitrogen concentration in the incoming deep-sea water is 32 µg-at per liter, this represents a conversion of deep water dissolved inorganic nitrogen to phytoplankton protein-nitrogen of over 78%. Assuming only 70% efficiency of inorganic nitrogen to protein-nitrogen conversion, at one turnover per day, and with a pool depth of 1.0 m, the protein production/m^2/yr in the St. Croix experimental system for 330 days operation of the pools per year (35 days down-time for cleaning and restarting of the pools) would be:

$$\frac{32}{10^9} \times 14 \times \frac{70}{100} \times 6.25 \times 800 \times 330 = 0.52 \text{ kg}$$

or 5.2 tons protein/hectare/year.

To maximize the phytoplankton-protein which can be produced per unit surface area and per m^3 of deep water, the optimal pool depth and turnover (dilution) rate of the pools should be determined.

Farmer (M.W. Farmer, 1976, Doctoral dissertation, Biology Department of The City College, New York; in preparation) studied productivity of Chaetoceros curvisetus (STX-167) in outdoor cultures in 80-cm deep, 2000-liter vessels as a function of the culture turnover rate and light intensity. Light intensity was controlled through the use of neutral density screens which regulated the surface light intensity of the cultures at 3%, 20%, 30%, 46%, or 100% of the natural sunlight intensity (I_o) on the beach in St. Croix. Light attenuation in each culture was determined at sunset and sunrise each day by measuring subsurface and bottom light intensities. Four different deep-water flow rates were used for each light condition: .25; .70, .95 and 1.20 turnovers/day. For simplicity, we discuss below the results of those cultures in which the surface light intensity was 0.3 x I_o only; or $\alpha = 0.3$. From these data, pool depth, light attenuation, turnover rates, and hence productivity values, for an optimized algal system were constructed. It must be emphasized that an "optimum" set of algal pool parameters (depth vs length and width) must take into account economic factors such as cost of excavation, maintenance, etc., and therefore that depth which pro-

vides the maximum production per unit surface area may not be the best in terms of capital or maintenance costs. For this reason, we have chosen to base our productivity estimates upon what at present appears to provide the optimum cost/productivity ratio in addition to those estimates providing greatest absolute productivity.

To determine the optimized productivity estimates, differences between the light intensity at the top and bottom of the reactors at different dilution rates were used to calculate the light attenuation coefficient, k. From these absorbance values, a least-squares parabola regression was constructed to extrapolate to other dilution rates. This parabola is shown in Figure 2. The peak absorbance value is

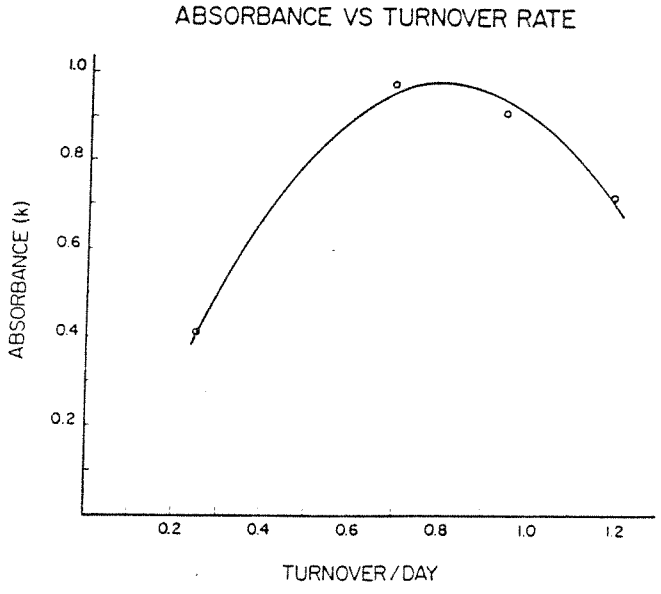

Figure 2. Absorbance vs turnover rate.

obtained for .81 turnover/day, under these experimental conditions. Next, the corresponding pool depths were calculated. The depth for a pool with 100% incident light was defined as that depth at which the average light intensity (I_{av}) in the culture is the same as the average light intensity in the screened experiment at the same turnover rate. Pool depths were calculated by first defining the average light for each culture according to the expression:

$$I_{av} = \alpha I_o \left(\frac{1-e^{-kz}}{kz} \right)$$

where α = proportion of incident light penetrating a neutral density screen and striking the surface of the culture;
I_o = illumination immediately below the surface in the absence of a screen;
z = depth.

For the selected data, obtained for $\alpha = 0.30$, I_{av} was very close to the theoretical 0.215 I_o average light for a 100% incident light culture with depth equal to the compensation depth. The compensation depth is the depth at which energy lost through respiration is equal

to energy gained through photosynthesis. Light attenuation at that depth is 0.01.

In a second step, the depth of cultures with the same absorbtivity (k), for each turnover rate, which would also "see" the same average illumination, when subjected to unattenuated (α=1.0) sunlight, was determined. Figure 3 illustrates the relationship between turnover rate and equivalent depth.

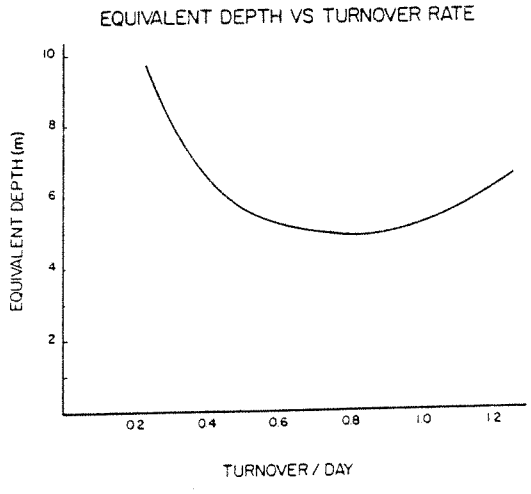

Figure 3. Equivalent depth vs turnover rate.

In these studies, direct measurements were made of cell density and particulate nitrogen: 10^8 cells contained 0.388 mg particulate nitrogen. From these data protein concentration vs turnover rate could be estimated: the relationship is illustrated in Figure 4. Protein concentration decreases with increasing turnover rate.

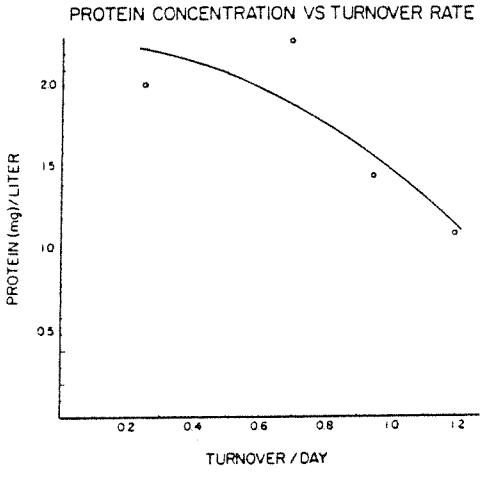

Figure 4. Protein/liter vs turnover rate.

Figure 5 illustrates the relationship between the volume of deep-sea water (m^3) handled per m^2/day and the protein produced in g/day. Since they are not linearly related there will undoubtedly be a trade-off between increased productivity, the cost of constructing a deep pool, and the cost of pumping large volumes of water.

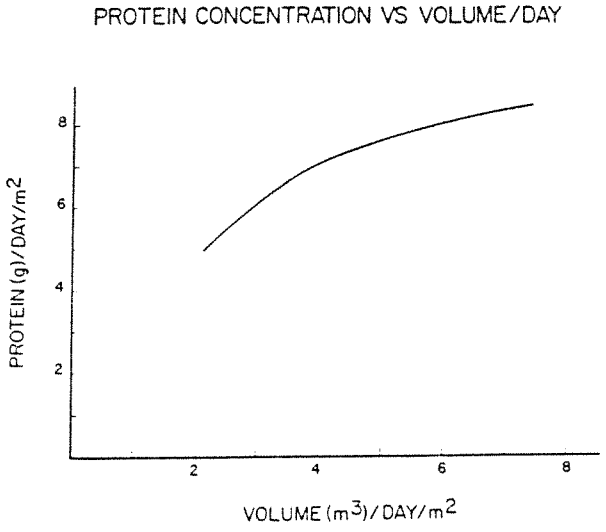

Figure 5. Protein vs volume.

Table 2 summarizes the data discussed above. Tentatively, we chose our "optimum" pool depth as 4.87 m; this represents peak light attenuation and a good compromise between depth and productivity (6.99 grams

Table II. Turnover Rates, Attenuation Coefficient (k), Average Light Intensity, Depth, Volume Pumped per Unit Surface Area per Day, Cell Density and Cell Production, and Protein Production Values Based on Data Collected on a 0.8-m Deep Culture, with α=0.3

Turnover/ Day	k	I_{av}	Depth (m)	Volume/Day /m^2 (m^3)	Cells/ Liter (x 10^7)	Cells/ Day/m^2 (x 10^{10})	Protein/m^2/ Day (g)
.25	.4110	.2557	9.30	2.325	9.20	21.4412	5.20
.50	.8021	.2214	5.57	2.785	8.60	23.9844	5.81
.70	.9533	.2099	4.95	3.465	7.80	27.1344	6.58
.81	.9754	.2083	4.88	3.953	7.30	28.8159	6.99
.95	.9408	.2108	5.00	4.750	6.45	30.8180	7.48
1.00	.9114	.2130	5.10	5.10	6.20	31.4721	7.63
1.20	.7044	.2293	6.11	7.332	4.75	34.7463	8.42

protein/m^2/day). The maximum productivity within the explored range is obtained with a 6.10-m pool depth at a turnover rate of 1.2/day and yields 8.42 grams protein/m^2/day. These extrapolations are based on many assumptions and have to be verified experimentally.

The energy cost of producing phytoplankton protein is another important consideration. As indicated earlier, the deep-water pumping costs are typically 1.96 x 10^4 J/m^3. For a plant of given size this is a constant, but the costs per hectare of ocean or land surface, and therefore the energy input vs food energy output ratios for a given surface area, will depend upon the depth and turnover rates of the pools.

Table 3 illustrates these relationships for the deep-water effluent from the condenser of a hypothetical 100 MW OTEC plant. Clearly, the

Table III. Hectares Required for Full Utilization of 100 MW OTEC Plant Deep-Water Discharge, Pumping Energy Required, Food Energy Produced, and Energy Input/Output Ratio for Pools Varying in Depth and Turnover Rate

Turnover/ Day	Depth (m)	Hectares Required*	Pumping Energy Required (kg-cal/ha/day) ($\times 10^5$)**	Food Energy Produced (kg-cal/ha/day) ($\times 10^5$)***	Energy Input/ Energy Output
.25	9.30	2787	1.09	4.48	.24
.50	5.57	2328	1.30	5.01	.26
.70	4.95	1870	1.62	5.67	.29
.81	4.88	1639	1.85	6.02	.31
.95	5.00	1364	2.22	6.44	.34
1.00	5.10	1270	2.39	6.58	.36
1.20	6.11	883	3.44	7.25	.47

*Lockheed Design (1975) = 6.48×10^7 m^3/day.
**Deep-water pumping costs = 1.96×10^4 J/m^3. 10^5 kg-cal=4.185×10^8 J.
***Algal protein = .58 ash-free dry wt. 1.0 g ash-free dry wt=5 kg-cal.

area required decreases with increasing pool depth and turnover rate. On the other hand, the cost of energy per hectare and hence the ratio of the energy input/food energy output increases. Perhaps the most relevant way of judging the importance of these figures is to compare the potential productivity of an "Artificial Upwelling" system to various terrestrial crops.

Table 4 illustrates the primary (algal) productivity which could be obtained with a deep-sea water mariculture system with the primary productivity of selected land products. These products which provide the best protein output (alfalfa), highest output in terms of weight (corn silage) and require the lowest fuel energy input (cassava) were chosen for comparison. All comparative data are from Pimentel et al. (1975). The "minimum" figures assume a 9.3-m deep pool with a .25/day turnover rate. "Optimum" and maximum figures assume a 4.87 or 6.1-m deep pool

Table IV. Primary Production per Hectare per Year for Chosen Terrestrial Crops and for Phytoplankton Grown in 100% Deep Water. For "Artificial Upwelling", 1 Year = 330 Days' Production

Type of Crop	Crop Yield in Protein (kg)	Crop Yield (kg)	Crop Yield Food Energy ($\times 10^6$ kg-cal)	Energy Input** ($\times 10^6$ kg-cal)	Energy Input/ Energy Output	Energy Input (kg-cal)/ Protein Output (grams)
Terrestrial*						
A) Alfalfa (highest protein production)	710	6,451†	11.4	2.694	.24	3.79
B) Corn Silage (highest crop yield in weight)	393	30,200	24.1	5.493	.23	13.97
C) Cassava (lowest fossil energy input)	58	5,824†	19.2	0.016	.0008	.27
Marine (Artificial Upwelling; Phytoplankton)						
Minimum***	17,160	28,586†	147.9	35.93	.24	2.10
"Optimum"***	23,063	39,764†	198.8	61.10	.31	2.65
Maximum***	27,793	47,719†	239.6	113.32	.47	4.08

Footnotes to Table IV:
*Comparative data are from Pimentel et al. (1975).
**Energy input = fossil-fuel energy for terrestrial sources and deep-water pumping costs for "Artificial Upwelling".
***See text for explanation of terms.
†Dry weight (kg).

with turnover rates of .81 and 1.2/day, respectively. In terms of its production of protein, dry weight of crop and food energy, the mariculture system compares very favorably. The energy input required for each hectare/year is, of course, greater than for the terrestrial products, but for the terrestrial products this is fossil-fuel energy which we again stress is becoming increasingly scarce and expensive. In terms of energy input vs food energy output, the various systems are very close, except for cassava. The reason for this is that labor is substituted for fossil-fuel-derived energy. Pimentel et al. (1975) estimate that in excess of 1,200 man-hours/year are required for each hectare of cassava production; this compares with about 25 man-hours for corn silage. Labor costs are an important element of comparative data, but they are not yet available for the mariculture system. Considering that a source of energy will be easily available from the OTEC facility, mariculture is likely to be a highly mechanized, energy-intensive business with low man-hour requirements. Our calculations do not include energy costs other than the costs of pumping the deep-ocean water. In any case, it will be noticed that in terms of kg-cal input vs protein output, the mariculture system compares very favorably with corn silage and encompasses the figure for alfalfa.

Secondary production: The algae produced in the St. Croix "Artificial Upwelling" system have been used as food for filter-feeding shellfish: clams, oysters and scallops. The conversion of deep-sea water nitrate to algal protein and further to clam-meat protein was studied at the St. Croix Station. A mixture of unialgal cultures of Chaetoceros curvisetus (STX-167) and S-1 (an unidentified naked flagellate) was used. The cultures were grown individually and continuously in onshore pools, combined in a mixing tank and fed continuously to several batches of Tapes japonica for 36 days. The clams in each batch were culled every 9 days to bring them back to the original weights. Thirty-five, 70 and 140-gram batches of clams in a 4-liter container received a continuous food flow-rate of 1.0 ml/sec. Thirty-five, 50, 70, 100, and 140-g batches of clams in 4-liter containers received a 2.0 ml/sec food flow rate. The particulate protein and dissolved NH_4^+, NO_3^- plus NO_2^-, entering and leaving each shellfish tank were measured daily. Every 9 days, all the clams were weighed and measured; enough clams were harvested to bring the total population weight back to its starting level, and the tank deposit was determined for each group. Sixty-nine percent of the 31 µg-at/liter nitrate-nitrogen in the deep water was converted into algal protein nitrogen over the 36-day period. From 31% to 35% of the algal protein entering the Tapes feeding tanks was converted into clam-meat protein by the 1 ml/sec flow groups and between 24% and 33% of the algal protein was converted into clam-meat protein by the 2 ml/sec flow groups. The fastest individual clam growth was obtained in the 35-g, 2 ml/sec group, with a 1.42 mm/week shell-length increase and a .411 g/week/g whole clam weight increase. The greatest clam population growth occurred for the 100-g, 2 ml/sec group with a total weight gain of 134 g in 36 days.

The fastest individual clam growth was obtained at the lowest percent stripping of algal protein nitrogen. Ammonium ion concentration in the shellfish tank was highest at the slowest individual clam growth rate. The Protein Efficiency Ratios in this experiment varied between

8 and 14, indicating that the algal food source is a good one for Tapes japonica. (Roels et al., 1977. To be presented at World Mariculture Soc. Mtg., January 9-12, Costa Rica.) These results are summarized in Figure 6.

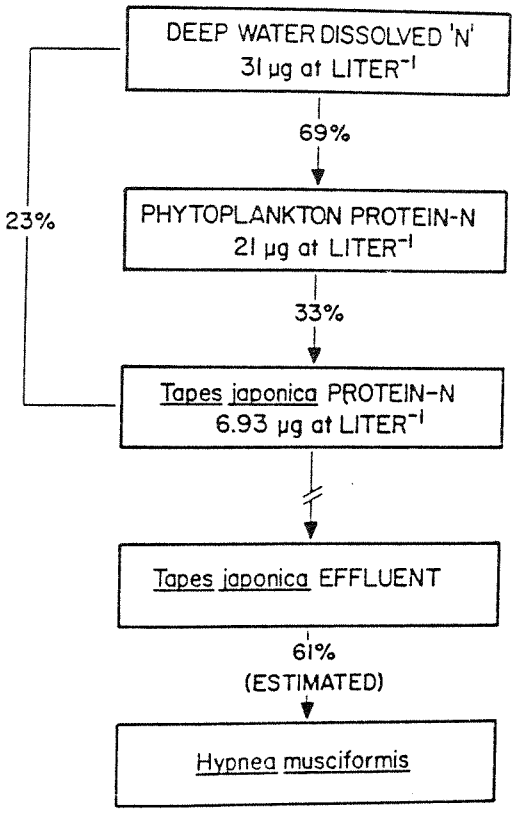

Figure 6. A summary of a recent (December 1975) food conversion study carried out at the "Artificial Upwelling" site at St. Croix. Total conversion efficiency for deep-water nutrients to Tapes japonica meat protein was 23%, considerably higher than achieved in conventional agriculture and animal husbandry. The red seaweed Hypnea musciformis has been grown in the Tapes tank effluent with a 61% efficiency of conversion of the ammonia in this tank effluent.

We have also grown the carrageenan-containing seaweed in the Tapes tank effluent. In the experiment described above, this effluent contained about 2 µg-at NH_3-N per liter, and preliminary studies indicate that about 61% of this available nitrogen can be incorporated by Hypnea. The use of another such primary producer could further benefit the overall biological and economic productivity of deep-sea water OTEC plants and mariculture systems.

Comparison between "Artificial Upwelling" marine culture and agriculture and animal husbandry: Table 5 compares the protein conversion efficiency obtained in "Artificial Upwelling" mariculture experiments with those of various other secondary producers. In small-scale experiments, the St. Croix mariculture system achieved a better plant to animal protein conversion than is achieved in cow's milk production, which is the most efficient animal protein production system known in conventional agriculture and animal husbandry.

In recent studies, surface water was used as an inoculum for deep water and the resultant phytoplankton was fed to the clam Tapes japonica (S. Laurence and O.A. Roels, 1976, "Plant and animal protein production in a mariculture system utilizing deep (870-m) and surface water mixtures," in preparation). Such inoculations would be economically

Table V. A Comparison of the Plant-to-Animal Protein Conversion Efficiency Obtained in the "Artificial Upwelling" System with Other Efficient Secondary Producers

Animal Product	Animal Protein Output / Vegetable Protein Input
Milk*	31.4
Eggs*	27.1
Beef (feedlot)*	6.5
Catfish*	10.5
Shellfish (Tapes japonica)**	33.0

*The comparative data for these products are from Pimentel et al. (1975).

**Shellfish data from the St. Croix "Artificial Upwelling" mariculture system.

advantageous since the system would not require extensive laboratory culturing facilities. A mixture of 80% deep and 20% surface water was used. Cultures obtained peak density within five days and were usually dominated by Chaetoceros curvisetus. These cultures were maintained for up to 40 days at 1 turnover/day on a 2,000-liter scale. Shellfish fed phytoplankton grown under these conditions converted the plant protein into animal protein with an efficiency of 35%. The results are summarized in Figure 7.

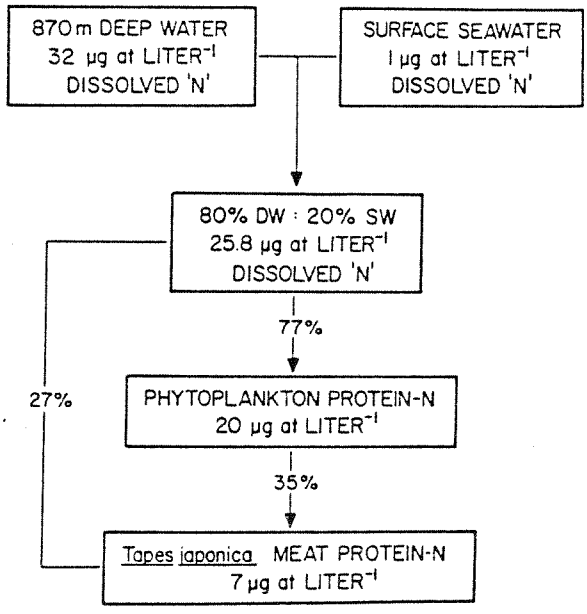

Figure 7. Summary of a food conversion study using surface water to inoculate deep water. Cultures were dominated by the diatom Chaetoceros curvisetus and were maintained at one turnover per day for up to 40 days with a constant ratio of 80% deep and 20% surface water.

Conclusion: Available experimental evidence indicates that "Artificial Upwelling" marine culture can produce substantial quantities of high-quality plant and animal protein. While conventional agriculture and animal husbandry are faced with increasing cost and scarcity of arable land and petroleum, "Artificial Upwelling" could generate more electrical power than the food production requires and would not utilize artificial fertilizers.

In view of the present population pressure on food, energy, water and land resources, the outlook for improving humankind's lot is grim.

If successful, "Artificial Upwelling" would generate power from the sun and could produce high-quality animal protein in large quantities. Its commercial feasibility should be tested now.

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