# ARTIFICIAL UPWELLING Final Report on the Shellfish Model II Pilot Plant Operation

1977 - 1978

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## ARTIFICIAL UPWELLING

Final Report on the Shellfish Model II Pilot Plant Operation 1977 - 1978

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### 1 SUMMARY

Although the upwelling areas cover only 0.1% of the total surface area of the world's oceans, they produce 44% of the fish (Crisp, 1975). In these upwelling areas, the deep water rising to the surface as a result of natural forces, supplies the nutrients necessary for phytoplankton blooms, which in turn can sustain rich animal life, which forms the basis of the abundant fishery in these areas.

In the tropical oceans, the deep water is considerably colder than the surface mixed layer. Surface temperatures range from 24-28°C over the year and the deep ocean water is approximately 20°C colder. The deep water also contains more dissolved nutrients (nitrate, phosphate and silicate) than the surface waters. As early as 1881, d'Arsonval recognized that it would be possible to generate electrical power based on this temperature differential.

The authors and their colleagues have demonstrated the technical feasibility of utilizing the deep water as an ideal medium for mariculture. It would thus be possible to utilize both the cold temperatures and the nutrients of the deep water. Certain species of algae grow well in the deep ocean water, once it is exposed to the sunlight, because of its relatively high nutrient content (nitrate, phosphate, silicate). The algae grown in this way can be used as a food source for filter-feeding shell-fish or other phytoplankton consuming organisms. Moreover, the deep water is free of parasites, pollutants, predators, epizoites, epiphytes and disease-bearing organisms which plague many mariculture operations.

The St. Croix site was chosen for the Artificial Upwelling experiment because 1000 m deep water is only 1.5 kilometers from the shore at the laboratory site. Three 7.5 cm diameter polyethylene pipelines were installed to a depth of 870 m in the sea. A glass lined pump on shore pumps deep water into two concrete lined pools of 50 m $^3$  each (5 m x 10 m by 1 m deep). The deep water in the pools is inoculated with phytoplankton culture. Once the required cell density is reached, usually in less than 24 hours, the pools are put on continuous flow. Deep water is pumped into the pools at the same rate as phytoplankton suspension is pumped to tanks containing filter feeding bivalve molluscs. The system was completed in 1972.

Between 1972 and 1976, standard procedures for phytoplankton culturing in this system were established and ten species of molluscan bivalves were tested for survival and growth. Eight species grew well: they were Mercenaria campechiensis, an  $F_1$  hybrid of M. mercenaria  $\times$  M. campechiensis, Tapes japonica, Ostrea edulis, Crassostrea gigas, C. gigas Kumamoto variety, Argopecten irradians and Pinctada martensii. Crassostrea virginica and Mercenaria mercenaria grew slowly and suffered high mortalities.

From October 1976 until October 1978, the St. Croix Artificial Up-welling Mariculture system was operated in pilot plant fashion to determine yields and provide a basis for cost estimates of the system. Tapes japonica used in the pilot plant were produced in the hatchery of the Artificial Upwelling system in St. Croix. The diatom Chaetoceros curvisetus

(STX 167) was grown in the pools as the sole food source for <u>Tapes</u> in the pilot plant, although other algal species were grown for larval and juvenile <u>Tapes</u> in the hatchery.

During 1976-1977, a Model I pilot plant was operated which gave shellfish growth below expectations. A full report on the operation of this Model I pilot plant was submitted to the Sea Grant Office in January 1978 (Artificial Upwelling - Progress 1976-1977).

The shellfish tank design was modified, based on the results of 1976-1977 experience, and the Model II pilot plant started operating in October 1977.

This report describes the results of 12 months' operation (October 1977 - October 1978) of this Model II pilot plant.

Over a twelve month period, the phytoplankton pools (100 m<sup>2</sup>, 1 m deep) averaged a phytoplankton protein production of 2.2 g/m<sup>2</sup>/day. This corresponds to a plant protein production of 8.1 Tons/Ha/year and should be compared to maximum plant protein production for alfalfa of 0.7 T/Ha/year. Alfalfa is the best protein producer in conventional agriculture.

Since the 1 m deep pools were operated in nutrient-limited rather than in light-limited fashion, it is estimated that 3 m deep pools could produce 19.3 T protein/Ha/year.

The Model II pilot plant produced 423 kg of <u>Tapes japonica</u> in 12 months, for an overall conversion efficiency of 15.8% from phytoplankton protein to shellfish meat protein. With improved techniques, based on the experience gained in the Model II pilot plant, it is expected that

an overall conversion efficiency of 24.2% of phytoplankton protein to shellfish meat protein can be achieved.

An Aquaculture Budget Generator, which provides cost of production estimates was developed and is described in detail. Excluding the cost of the deep sea water (in case an OTEC plant or other plant utilizing the cold temperature would provide its effluent free to the mariculture system), the production of the clam <u>Tapes japonica</u> would cost \$1.57 per kilogram whole fresh weight in a system comparable in all respects to the way the St. Croix system pilot plant Model II was operated. In a system with as small a deep sea water flow as used in the St. Croix pilot plant (1.3 l/sec) the deep sea water cost would be exorbitantly high (\$48.54 per kg shellfish produced).

Based on reasonable improvements suggested by the Model II pilot plant, the Aquaculture Budget Generator predicts a production cost of \$1.06 per kg whole fresh weight <u>Tapes japonica</u> for a plant producing 365 T shellfish per year. This price includes a deep sea water cost of \$0.3854 per kg of clams produced. Excluding the deep sea water cost, the price would be \$0.67 per kg clams. Such a plant would have a deep sea water flow of 624.7 l/sec. It would utilize 6 pools with a depth of 3 m and a total surface area of 2.4 Ha. The animals would be grown from 1 mg spat to 11.5 g market size in 252 days.

For a mariculture plant coupled to a 10 Megawatt OTEC plant, with a deep sea water flow of 37.5 m<sup>3</sup>/sec, the cost per Kg. whole clams would be \$0.7722 if the mariculture plant pays for the Deep Sea Water pipeline installation and pumping costs. If the deep water pipeline and pumping cost was borne by the OTEC plant, the clam production cost would be \$0.6703/Kg. Such a plant would produce 21,900 Tons clams/year. The description of such a plant is shown in Table 1.1.

PICKONE. FOR HELP TYPE 9.

	OUTPUT		
ANIMAL	PRODUCION COST(\$/KG OUTPUT:	PE	RFORMANCE MEASURES
	DEEP-SEA WATER DSW DISTRIBUTION		DSW FLOW (M3/SEC) # OF PIPELINES
.1933 .1279	PHYTO SPACE " LABOR		PIPELINE DIAM (M) PIPELINE LENGTH (M)
	" TOTAL SFSH SPACE	6.0000 * .432E+07	# OF POOLS POOL VOLUME (M3)
.0625 .1188	" LABOR " LARVAE		POOL AREA (M2) POOL DEPTH (M)
	" TOTAL SUPERVISION		TRAY AREA (M2) INDIVIDUAL WEIGHT (G)
.7722 3722.5848	TOTAL PROD. COSTS ANNUAL CAP. COST(000)	252.0000 * .219E+08	DAYS TO HARVEST OUTPUT (KG/YEAR)
35332.1308 PICKONE.	TOTAL CAPITAL (000) FOR HELP TYPE 9.	0.0000	से से के के के

PROGRAM EXECUTION TERMINATED BY USER REQUEST GOOD BYE FROM AQUASA

CC:

ACCOUNT-RUN LN-MIN LN-COST TM-SEC TM-COST MSAU313-279 13 \$0.06 2.349 \$0.15

### 2 INTRODUCTION

Although the upwelling areas cover only 0.1% of the total surface area of the world's oceans, they produce 44% of the fish. In these upwelling areas, the deep water rising to the surface as a result of natural forces, supplies the nutrients necessary for phytoplankton blooms, which in turn can sustain rich animal life, which forms the basis of the abundant fishery in these areas.

In the tropical oceans, the deep water is considerably colder than the surface mixed layer. Surface temperatures range from 24-28°C over the year and the deep ocean water is approximately 20°C colder. The deep water also contains more dissolved nutrients (nitrate, phosphate and silicate) than the surface waters. As early as 1881, d'Arsonval recognized that it would be possible to generate electrical power based on this temperature differential. This is best done in these areas of the oceans between 20°N and 20°S.

The St. Croix Artificial Upwelling Project has demonstrated the technical feasibility of utilizing the deep water as an ideal medium for mariculture. Certain species of algae grow well in the deep ocean water, once it is exposed to the sunlight, because of its relatively high nutrient content (nitrate, phosphate, silicate). The algae grown in this way can be used as a food source for filter-feeding shellfish or other phytoplankton consuming organisms. Moreover, the deep water is free of parasites, pollutants, predators, epizoites, epiphytes and disease-bearing organisms which plague many mariculture operations.

The St. Croix site was chosen for the Artificial Upwelling experiment because 1000 m deep water is only 1.5 kilometers from the shore at the laboratory site and St. Croix is situated in the tropical region at  $17^{\circ}N$ . Three 7.5 cm diameter polyethylene pipelines were installed to a depth of 870 m in the sea. A glass lined pump on shore pumps deep water into two concrete lined pools of 50 m<sup>3</sup> each (5m x 10m x 1m deep). The deep water in the pools is inoculated with phytoplankton culture. Once the required cell density is reached, usually in less than 24 hours, the pools are put on continuous flow. Deep water is pumped into the pools at the same rate as phytoplankton suspension is pumped to tanks containing filter-feeding bivalve molluscs. The system was completed in 1972.

Between 1972 and 1976, standard procedures for phytoplankton culturing in this system were established and ten species of molluscan bivalves were tested for survival and growth. Eight species grew well: they were Mercenaria campechiensis, an  $F_1$  hybrid of M. mercenaria x M. campechiensis, Tapes japonica, Ostrea edulis, Crassostrea gigas, C. gigas Kumamoto variety, Argopecten irradians and Pinctada martensii. Crassostrea virginica and Mercenaria mercenaria grew slowly and suffered high mortalities.

From October 1976 until October 1978, the St. Croix Artificial Upwelling Mariculture system was operated in pilot plant fashion to determine yields and provide a basis for cost estimates of the system. <u>Tapes japonica</u> used in the pilot plant were produced in the hatchery of the Artificial Upwelling system in St. Croix.

The following report is a summary and analysis of the "Artificial Upwelling" project operated in pilot plant fashion on St. Croix, U.S. Virgin Islands. The report focuses on the technical and economic feasibility of artificial upwelling mariculture, based on data collected on a two-trophic level marine food chain operated at the St. Croix site over the period October 11, 1977 to October 10, 1978.

The pilot stage, two-trophic level deep seawater mariculture system was operated on St. Croix, U.S. Virgin Islands, over a twelve month period. Antarctic Intermediate Water from 870 meter depth was pumped continuously into two 50,000 liter on-shore 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<sup>-1</sup>. The algae were pumped continuously to 28 tanks containing the clam Tapes japonica. A prior pilot experiment (Pilot Plant Model I) had been run over a nine month period during 1976-1977 and has been reported in full in the "Artifical Upwelling - Progress Report 1976 - 1977" submitted to the National Sea Grant Office in January 1978. During this earlier pilot run, the shellfish tank design was found to be inappropriate resulting in poor shellfish growth. This report provides the results obtained in the Model II Pilot Plant operated for a twelve month period, during which a greatly improved design of shellfish tank was utilized, resulting in considerably better growth.

Quarterly reports on this work for the period October 1, 1977 - December 31, 1977, January 1, 1978 - March 31, 1978, and April 1, 1978 - June 30,

1978 have been submitted to the Sea Grant Office respectively on January 27, 1978, April 15, 1978 and August 10, 1978 and should be consulted in conjunction with the present report.

The report also refers to data collected over the years 1974 - 1977 on the first trophic level. A complete description of the data base used for this report can be found in Appendix A.

Because the amount of data collected over this period of time is very large (analysis utilized several million individual data points) and because a full appreciation of the upwelling process requires consideration of a multitude of complex and interacting factors, the body of the report is a distillation of our main conclusions only. In some sections it has been necessary to refer the reader to appendices, to published papers and manuscripts submitted for publication and to data files far too voluminous for reproduction in this report. Nevertheless, the report stands by itself and is intended to serve as the single most cogent assessment of the Artificial Upwelling process to date.

The report is divided into seven main sections. First, the <u>guiding</u> <u>concepts</u> ("Conceptual Approach") underlying our approach to the process are described. This involves a coherent set of assumptions brought to bear in designing, operating and analyzing the Artificial Upwelling process in St. Croix. Next is a brief <u>history of the project</u>; this provides context for operations and designs which have an historical as opposed to a strictly logical basis, and serves to highlight the accomplishments of the station since its inception to the present day.

A description of the Artificial Upwelling system at St. Croix (as of

10-10-78) is then provided.

The <u>methodology</u> used in operating the two trophic-level food chain is then described, including brief descriptions of chemical measurement and data analysis techniques. The results of the phytoplankton and shell-fish production for the year 10/77 - 10/78 is then given. This is followed by the analysis and discussion of the <u>economic feasibility</u> of Artificial Upwelling. This section incorporates the technical description of the mariculture system as operated on St. Croix over the 1977-78 year. A revised aquaculture budget generator, based on the St. Croix results and on an extrapolation of these results with cost data per Kg of shellfish produced is presented.

The report then summarizes the conclusions.

### 3 CONCEPTUAL APPROACH

The actual operations of the Artificial Upwelling project have been the result of a number of biological and practical constraints, but since its inception, the project has been guided by a few underlying principles. In this section some of the underlying philosophy of operation is qualitatively described, followed by a more specific outline of our basic approach. The descriptions and analyses which comprise the remainder of the report should be evaluated within the context of these general principles.

The primary goal of the project has been that of operating a controlled multi-level food chain <u>using deep</u> (Antarctic Intermediate) <u>water</u> and <u>sunlight only as raw materials</u>. Since its inception, the project has emphasized the production of <u>plant</u> and <u>animal protein</u> in such a system.

Although it has been understood that various technical and especially, economic factors may require that extraneous energy or nutrient resources be incorporated at one or more trophic levels in the process, every attempt has been made to evaluate its biological and technical feasibility in the light of this restriction. As will be seen below, the emphasis on protein production using deep water and sunlight alone has guided the choice of such diverse elements as the particular species of alga used, to means employed for rearing shellfish.

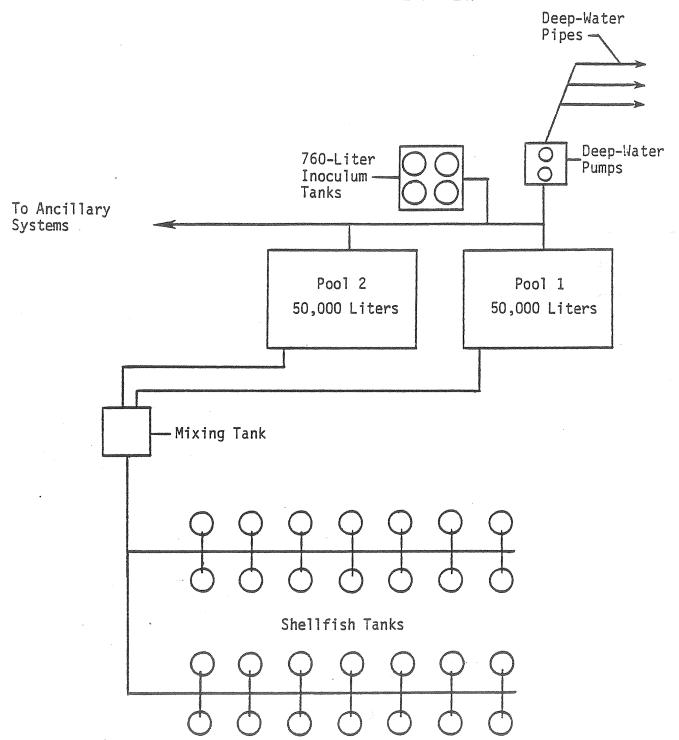
The emphasis on protein production stems from the fact that the limiting macronutrient in the St. Croix Artificial Upwelling system is nitratenitrogen ( $NO_3^-$  - N). By tracing the transfer of nitrogen from its inorganic

form in deep water to its presence as plant and animal particulate protein-nitrogen, a simple accounting of the productivity and efficiency of the system can be made.

Employing deep water and sunshine alone as raw materials reflects a more general emphasis on simplicity in design, operation and analysis. This emphasis stems from an understanding of the extreme complexity and exceptionally large number of possible biological relations and types of measurements which can be utilized, and the need to establish an invariant and easily-monitored system as opposed to one that may be thought of as more biologically "realistic" or "interesting". We have attempted to increase the complexity or scope of the guiding quantitative equations or engineering only if a significant discrepancy (based on well-defined analyses) develops between predicted and actual outputs. The approach has therefore allowed for a relatively simple evaluation of the process as well, since the number of potential inter-variable relations has been kept to a minimum, and it has a positive influence on the technical and economic feasibility of the process by reducing the technical sophistication and cost of operation. As will be seen below, emphasis on simplicity has led to significant trade-offs in the level of control exercised over the process and in the level of output at both trophic levels. This conceptual approach is presented schematically in Figure 3.1.

The procedures reflect an awareness that the actual accomplishment of these goals, at least at commercial scale, will require an unequivocal economic evaluation of the process based on actual data supplemented by understanding generated from a variety of sources.

FIGURE 3.1 SCHEMATIC REPRESENTATION OF THE ST. CROIX ARTIFICIAL UPWELLING PILOT PLANT II MARICULTURE SYSTEM.



The core of this approach is the <u>technical description</u>, a set of simple, quanitative equations which are used (1) to generate a set of procedures for operating the process and (2) as inputs for the economic analysis. Because the process involves a two trophic-level food chain, the technical description involves equations which describe outputs (under a well-defined set of boundary conditions) at both trophic levels. The technical description allows for the generation of <u>predicted outputs</u> (in the present case, from the pilot-level project under consideration), given that the operations are consonant within the boundary conditions of the equations. The technical descriptions which served to guide the pilot plant Model II operations over 1977-78 may be found in Appendix B. This is the same technical description as was used for the operation of the Model I pilot plant during 1976-77. This report contains a revision of the phytoplankton technical description in section 7.

The technical description serves as the core model for the economic analysis, produced by a computer program, the "Aquaculture Budget Generator" or A.B.G.

The A.B.G. is the basis of the economic sensitivity analysis of the system: the budget generator clearly indicates the major costs inherent in the different phases of the system to produce the end-product:shellfish. The sensitivity analysis guides the manipulation of selected variables (pool depth, shellfish transfer schedules, etc.) and determines the influence of changes on other variables, the most important of which is the cost of production (\$ per Kg of live shellfish). Thus, the model allows for an evaluation of these variables which have the most important impact on production costs.

This type of manipulation is the basis for the economic evaluation and data analysis presented in the present report. First, a priority listing of the most cost-sensitive items (variables) was generated. Since their impact on production costs depends on the validity of the technical description, a comparison was made between the predicted outputs and the measured results obtained from the pilot plant. An initial and qualitative determination was made on the degree of actual or possible discrepancy. In those instances where discrepancy was indicated, a further and more rigorous analysis of the data was made to determine the extent and cause of the discrepancy. Once it was determined that the measured or calculated results were obtained under conditions analagous to those dictated by the technical description (and therefore not due to chance, accident, or operating procedures beyond the scope of the expressions), an evaluation of the (1) need to alter the technical description and (2) ability to supply the information necessary for change was accomplished. If a determination of need for change was made but insufficient data or understanding was available, the item was placed on a priority list for further research and development. Otherwise, the change was incorporated, and a re-evaluation of cost-sensitive items through manipulation of the A.B.G. was done.

4	DESC	RIPTION OF THE ST. CROIX ARTIFICIAL UPWELLING MARICULTURE
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		DESCRIPTION OF ELECTRICAL SYSTEM
		GENERAL

4 DESCRIPTION OF THE ST. CROIX ARTIFICIAL UPWELLING MARICULTURE
PLANT

### 4.1 SEA WATER COLLECTION

The sea water enters three divergent pipelines, each 6400 ft (1920 m) in length whose lower ends are at a depth of  $\overline{\text{ca}}$  2900 ft (870 m). Their position on the sea floor is shown in Figure 4.1.

The lines are made of 3" nominal (i.e. 3.5"  $0.D. \times .396$ " wall or  $8.89 \text{ cm} \times 1.01 \text{ cm}$ ) polyethylene pipe which was supplied in lengths of 1000' (305 m) in coils of approximately 8 ft (2.44 m) in diameter. The polyethylene pipes were heat butt-welded and these butt-welds were reinforced by a Kellems grip as shown in Figure 4.2.

Each line was made up in three sections for deployment purposes.

The two joints which were made after deployment consist of monel assemblies heat swaged over stubs of the pipe by the pipe manufacturer. These stubs in turn were heat welded to the pipe lengths.

Over a length of approximately 1031 ft (309 m) in the area where the lines might be exposed to abrasion from coral and rock due to water currents, the pipe was covered by reinforced rubber hose nominal 4" I.D.  $\times$  1/4" wall (10.2 cm  $\times$  .63 cm).

Over the entire exposed lengths, cylindrical split 5 lbs (2.27 kg) lead weights were strapped to the pipe on centers ranging from 8 ft (2.44 m) to 32 ft (9.76 m) predicated on the predicted currents acting on the emplaced lines. These lead weights are shown in Figure 4.3. Near the intake, the lines are anchored with lead blocks (450 lbs) to which a

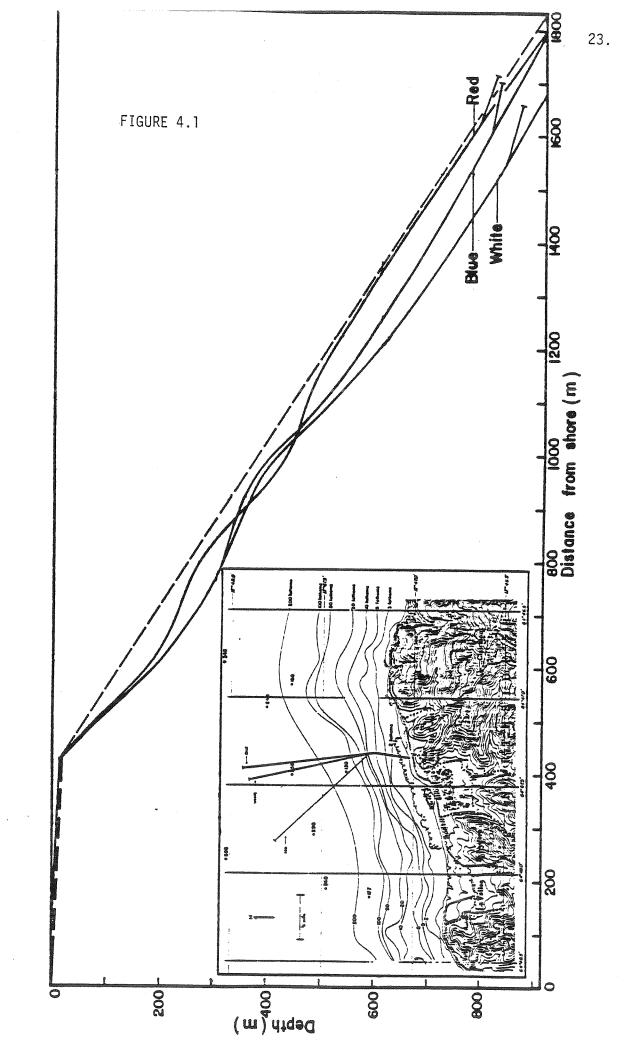
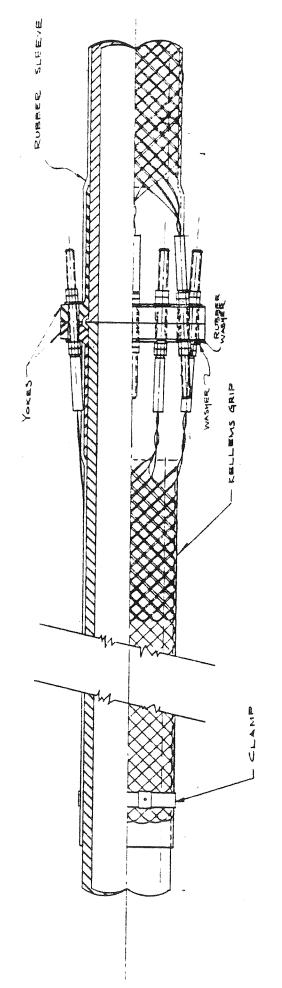


Figure 4.2. Polyethylene pipe butt-weld protection assembly with Kellem grips.





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stainless steel cable 100 ft long is attached. The other end of this cable is connected to the intake of the pipeline, which floats 100 ft above the bottom, supported by a plastic encased glass float for bouyancy (Figure 4.4).

The lines are placed in a diverging pattern and converge at a point approximately 1300 ft from shore and are restrained by a system of Kellem grips, steel cables and steel stakes, grouted into holes drilled into the bottom. From this point the pipes are threaded through a 1130 feet long 14" diameter (35 cm) bell and spigot pipe made of fiberglass and epoxy resin ("Techite" pipe).

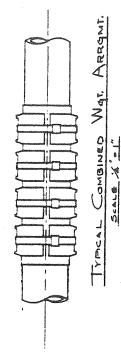
This protective conduit in 10' or 20' sections is fastened in place by concrete saddles (refer to Figure 4.5) staked down with (2) epoxied steel bars -- #8 or 1" (2.54 cm) in diameter and approximately 30" (76 cm) in length. These bars are grouted into holes drilled 18" (46 cm) into the bottom using perforated sleeves and grout fills the clearance between the bars and the oversized holes precast through the saddles. A schematic diagram of this Techite pipe system is shown in Figure 4.6.

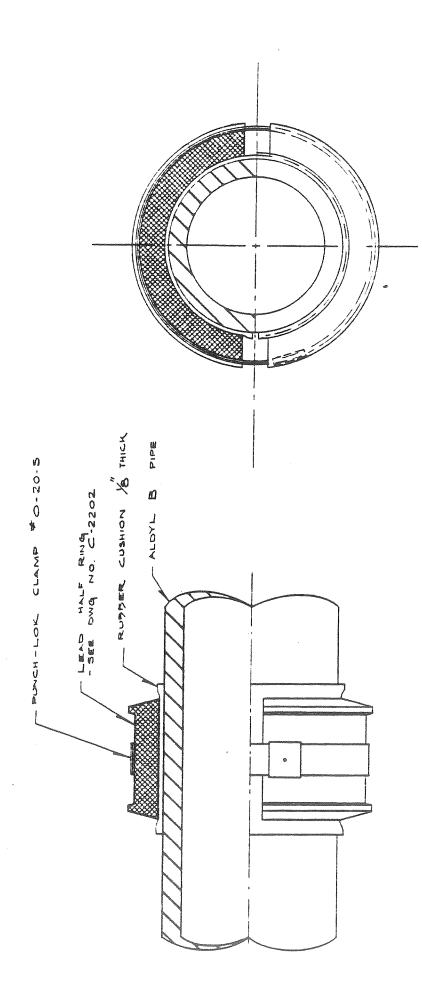
Upon reaching the water's edge the pipes leave the conduit and are led through (3) 5" schedule 40 PVC (14 cm x .65 cm wall) pipes which are covered by a 40 ft (12.2 m) long prestressed double tee concrete beam, the space between the tees encompassing the 5 inch pipes was filled with concrete. The emerging pipes, which are at water's edge, are fitted with a monel flange and stud as described above.

Figure 4.3. Lead weight assembly.



LETTER





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Figure 4.4. Anchor connection and float assembly.

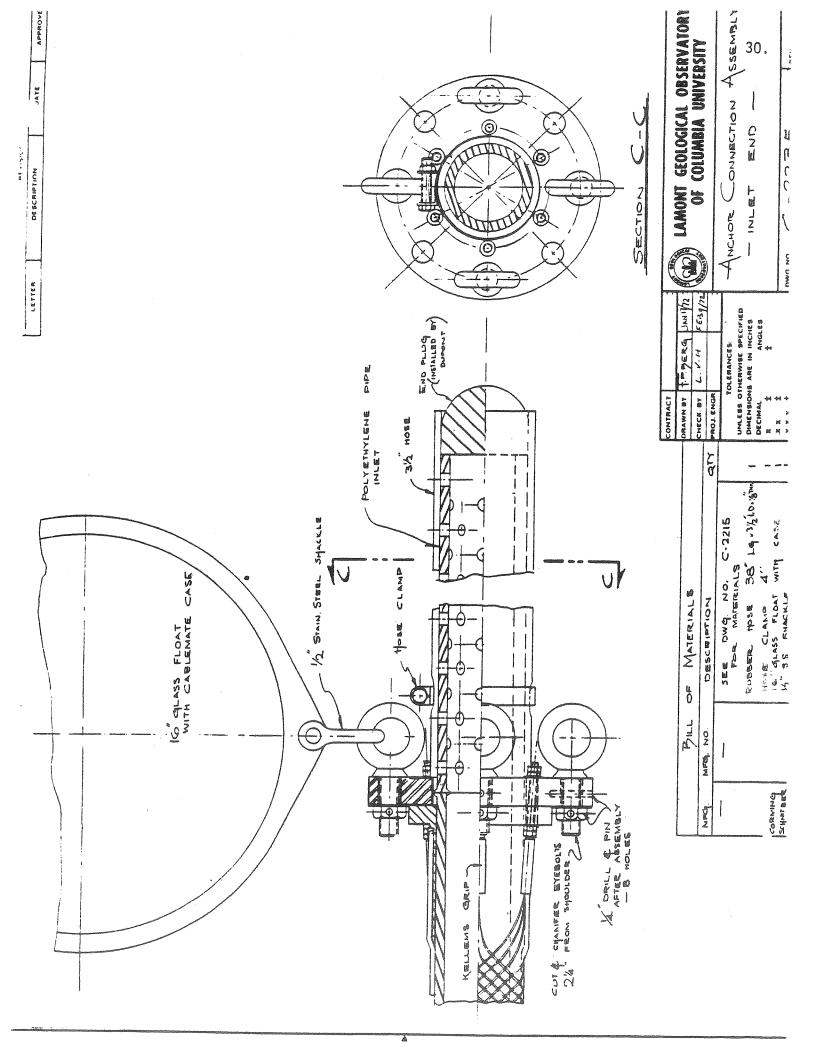


Figure 4.5. Concrete saddles for the Techite pipe anchoring system.

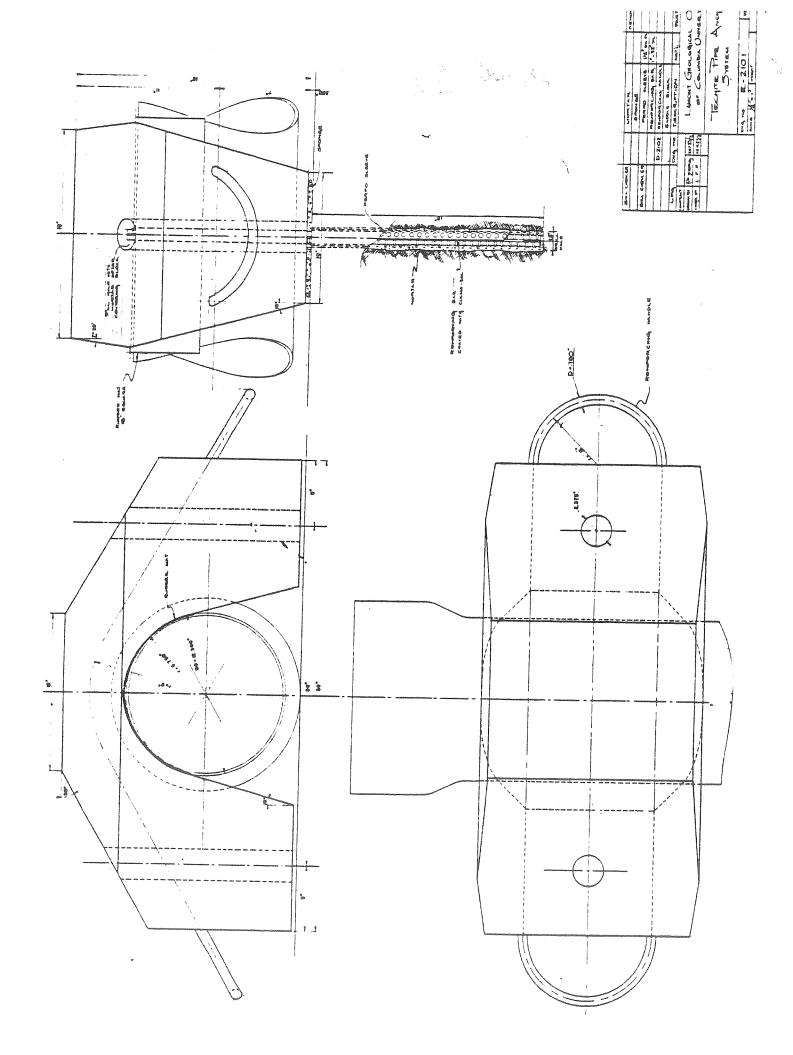
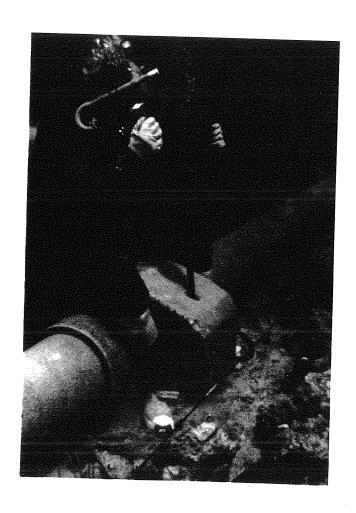
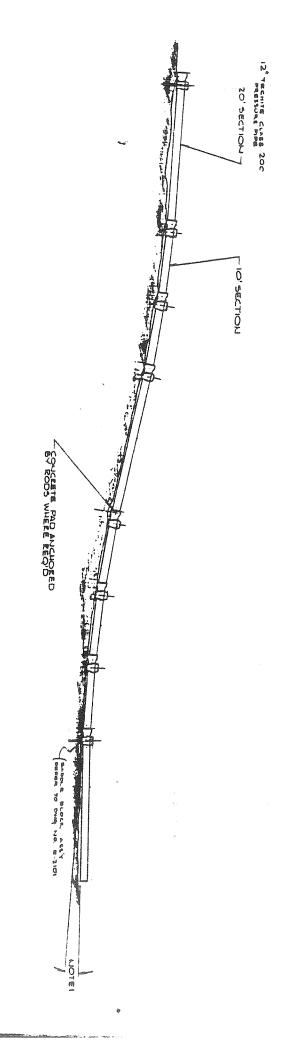


Figure 4.6. Techite fiberglass pipe. Drawing on next page.



Diver installing concrete saddle to anchor Techite pipe.



INSTALATION DRAWING

NOTES

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From this point on, all pipe and fittings are made of PVC. Each pipe has a 3" ball shut valve before connection to the manifold. The valves are normally open; they are closed only when samples of water are taken from each line for chemical analysis to monitor the integrity of the lines and also to observe the minor if any seasonal fluctuations of the nutrient content.

An overall schematic drawing of the pipeline is shown in Figure 4.7.

The lines converge at the shoreline and, each with a ball shutoff valve, are manifolded to a single header leading to the inlet of one of two glass lined centrifugal pumps which are at an elevation of 38" (96.5 cm) above normal sea level. The annual sea level variation in this area is 12" (30.5 cm) or less.

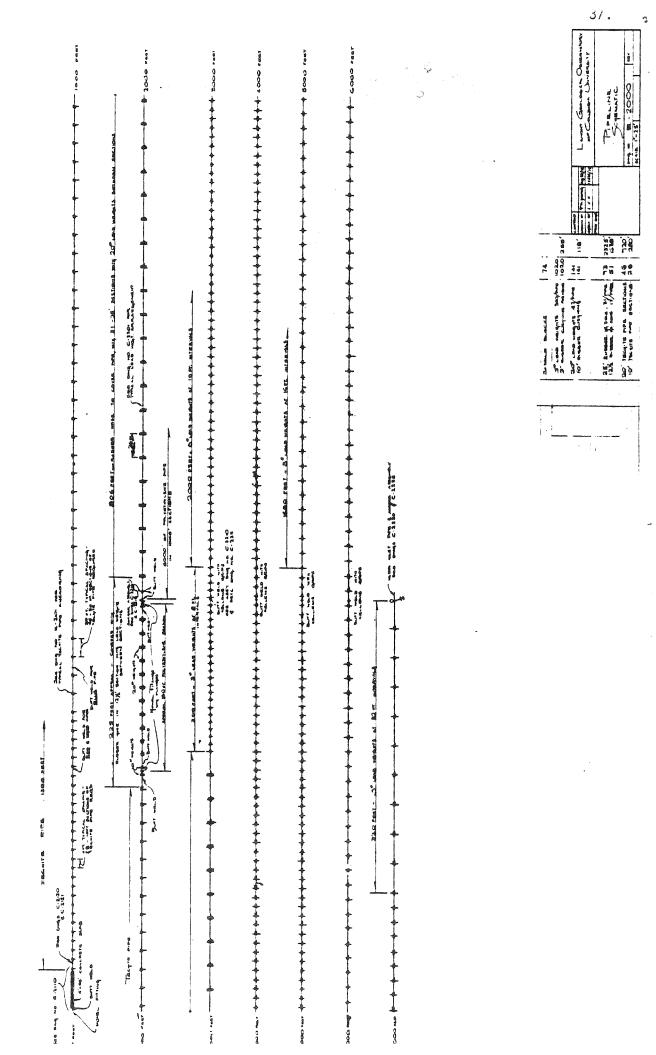
The system is equipped with two pumps (Goulds Pump Co., Model 3708) arranged in parallel and valved on both the suction and discharge sides for rapid switch to the standby pump in case of emergency and for routine preventive maintenance. A 300 gal (1135 1) polyethylene priming tank is mounted on an elevated platform outside of the pump house.

The pump house also contains the aeration pumps, the electrical panels and switches.

The shallow section of the deep water pipelines are inspected each month by a team of divers to a depth of  $20 \, \text{m}$ .

This system has given extremely reliable operation from its installation in May 1972 until the end of the Model II pilot shellfish plant operation.

Figure 4.7. Deepwater pipeline schematic.



at the shore terminal of the three pipelines to the deep sea water pump showed a number of hairline cracks due to exposure of the PVC pipeline for a period of six and one-half years to sunlight. Replacing this 4" PVC line caused the first significant interruption in the deep sea water supply since the installation of the pipelines in May 1972. After installation of the new 4" line, (above sea level) it was shielded from direct sunlight to avoid repetition of this problem.

The deep sea water supply coming up through each of the pipelines was sampled weekly and analyzed for nitrate, nitrite, phosphate and silicate. The salinity was also determined. The results of these analyses indicated that the pipelines had remained intact throughout the period.

## 4.2 SEA WATER DISTRIBUTION

### 4.2.1 CONSTANT HEAD DEVICE

From either of the two pumps (the other being a standby) a 3" Schedule 40 PVC line leads to a 6" PVC tee into whose vertical branch is fixed a 6" PVC stand pipe holding a concentrical 3" PVC internal drain whose upper end is at an elevation of 29 feet ASL (8.7 m). Since the pump provides ca 15% more flow than is required, a constant head pressure is provided. The drain pipe exits through the side wall of the stand pipe at a height to permit filling of an elevated tank having a capacity of 528 gallons (2000 1) thus providing a source of "secondary" deep water for filling inoculum tanks, cleaning and other intermittent uses without disturbing the constant head of the "primary" deep water which permits accurate continuous controlled flow through fixed diameter orifices.

The secondary water storage tank is fitted with an over flow stand pipe and excess flow is conducted through a filter bed before returning to sea. The tank is tightly covered to eliminate sunlight and hence unwanted algae growth. The stand pipe is capped with a 6" PVC cap and a 11/2" double elbowed vent line turned downward for the same purpose. The standpipe is shown in Figure 4.8.

# 4.2.2 POOL SUPPLY FLOW REGULATION

From the discharge run of the 6" tee at the lower end of the stand pipe a 3" PVC header leads to the two algae pools. From this header, 1 1/2" PVC lines, with ball shutoff valves bring the water to the pools through an orifice plate which is fixed in place by half of a 1 1/2" PVC union. This system is detailed in Figure 4.9. Figure 4.10 details the orifice sizes used. Table 4.1 shows the flow control data through the orifices.

## 4.3 DESCRIPTION OF THE ALGAL POOLS

The two pools, of reinforced concrete are rectangular and have sloping sides of 45°. At the rim, they measure 35'-6 (10.8 m) by 19'-7 (5.97 m) the depth is 4' (1.22 m) providing a working capacity of 50,000 l. A l m PVC stand pipe extends to the working level and any overflow drop in level indicates an imbalance in the overall system. The stand pipe is pulled for draining for periodic cleaning, the drainage lines lead to an underground filter bed.

Figure 4.8. "Constant-head" standpipe.

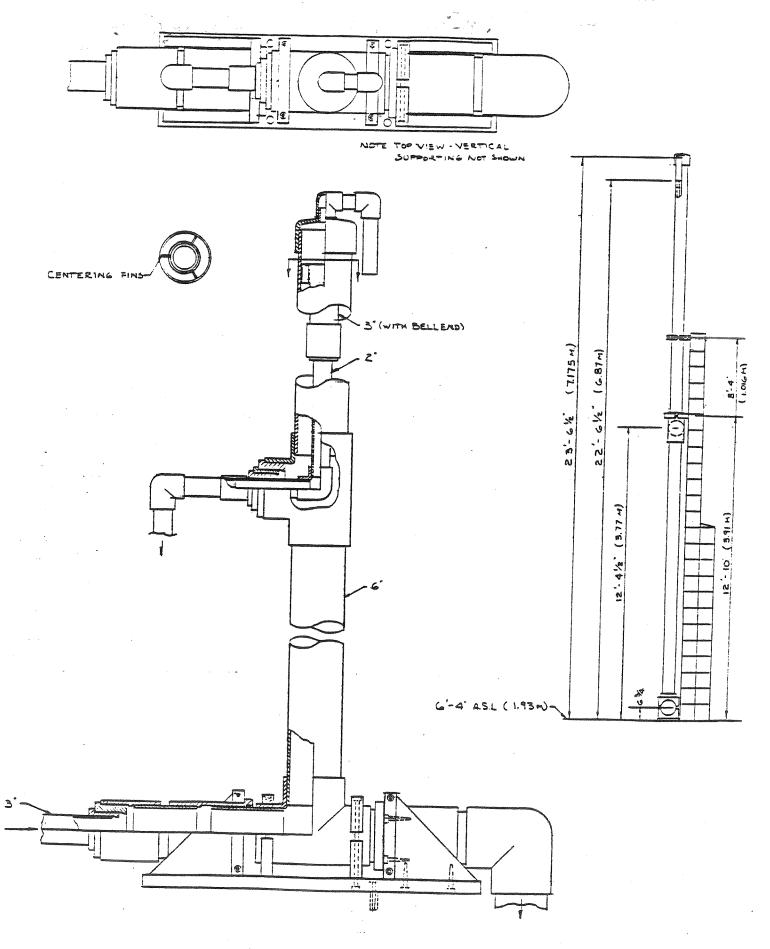
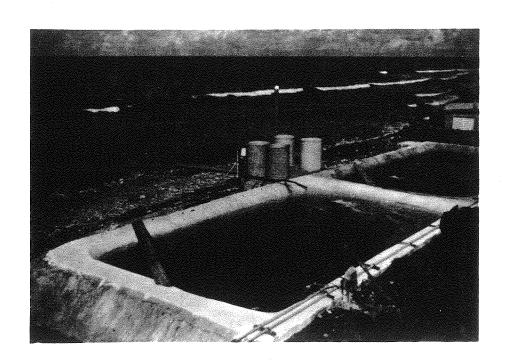


Figure 4.9. Diagram of the deep seawater flow-through the mariculture system. Drawing on next page.



Phytoplankton Pools

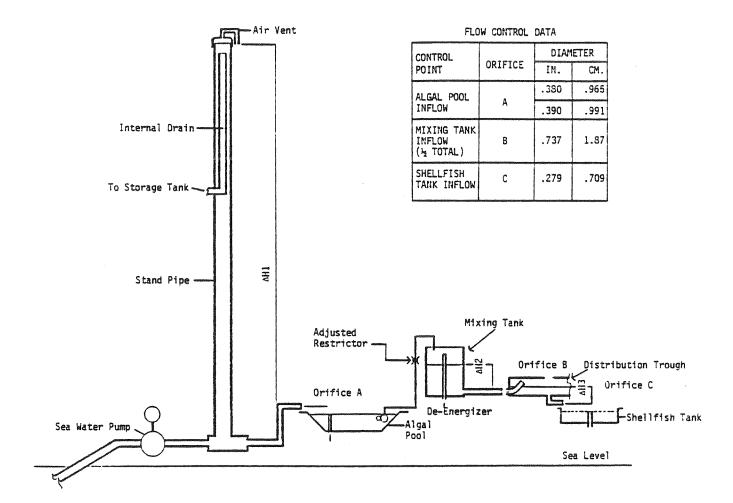


Figure 4.10. Orifice detail.

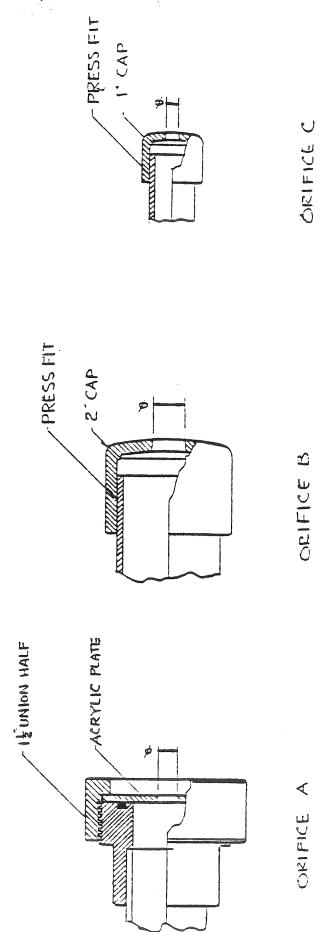


TABLE 4.1 FLOW CONTROL DATA

CONTROL POINT IN SYSTEM	ORIF DIAN INCHES	FICE METER CM	G.P.M.	LOW RATI L/m	ℓ/sec	
Deep water through orifice to pool	. 380	. 965	10.54	39.9	.665	
Mixing tank through orifice to trough (1/2 total)	.737	1.87	10.54	39.9		
Trough through orifice to shellfish tank	.279	. 709	. 79	3.0	.050	

è

4.4 DISTRIBUTION OF THE ALGAL POOL CULTURES AND SHELLFISH AREA

Submerged pumps are used to pump the pool cultures from the pools to the shellfish area. A total of three pumps are used in each pool: Two in series to obtain the required pressure and the third to increase the flow. The rate of flow is governed by the positioning of ball shut-off valves placed in each of the 1 1/2" PVC lines. The pumps have magnetically coupled impellors. Downstream of the control valves, the pool water enters the covered shellfish area where it is directed to an elevated mixing tank and from there enters a 2" PVC header with two branches, each with a fixed diameter discharge orifice, which directs the water to an "open" 6" PVC distribution trough.

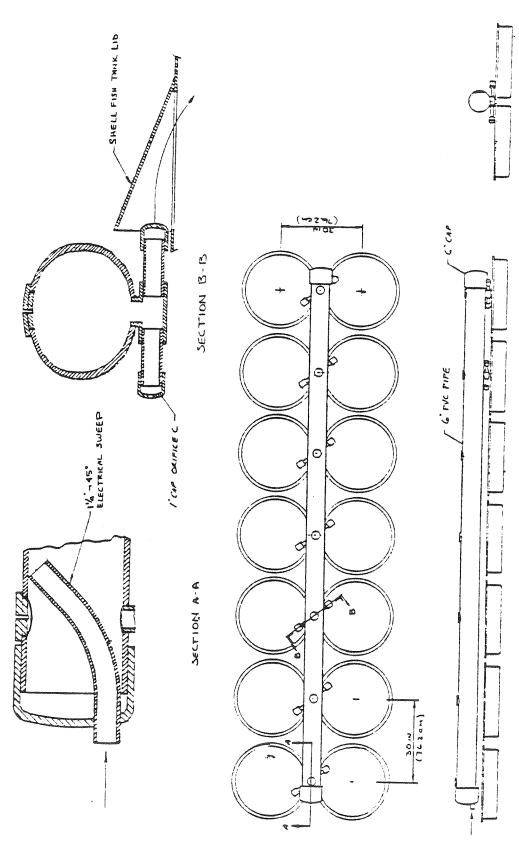
Each trough services 14 shellfish tanks arranged in two rows of seven each. A detail is represented in Figure 4.11. The troughs are elevated and positioned between the two rows. Seven 1" tees on 30" center are cemented into holes in the underside of the trough of a size to accept the branch of the tees, the tees are placed at an angle. In the runs of the tees are cemented a length of 1" pipe. A 1" cap, drilled through the end to provide a fixed orifice, is pressed over the 1" pipe to overcome the difficulty of producing 28 identically sized orifices, the caps are drilled off center to allow "fine tuning" by rotating the cap and then marking the proper position.

The angle at which the tees are cemented into the trough plus the length of the horizontal 1" pipes cause the jet of water to impinge at

Figure 4.11. Details of pilot-plant shellfish area.

Drawing on next page.





the proper distance from the center of the circular shellfish tanks to impart rotation of the water and promote the flow of floating matter toward the center. The shellfish tanks, (Figure 4.12) made of fiberglass are 26" (66 cm) inside diameter, 6" (15.2 cm) inside depth and have a top flange making the overall diameter 28 3/8" (72 cm), wall thickness is 3/16" (.47 cm). A 1" PVC coupling is epoxied into the bottom to provide a socket for a 1" standpipe (which maintains a 3.54" (9 cm) water depth) and a drain line. The tank working volume is 9.24 gal (35 1).

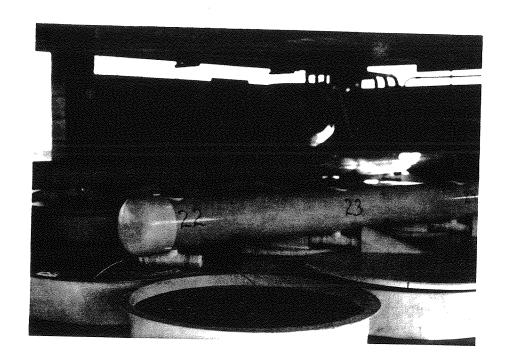
A fiberglass cover, 29 7/8" (75.9 cm) outside diameter, with a 1/2" (1.27 cm) turned down lip, also 3/16" thick is fitted with three riser blocks to center it over the tank and provide ventilation but reduce the entry of light to reduce unwanted algae growth. The cover also has a scooped opening which covers the incoming water jet and the orifice for the same purpose. The tank drain pipes consist of a 1" PVC electrical 45° sweep which can be rotated for sampling. Normally the overflow is conducted to a system of open gutters leading to drainage lines to underground sand bed before returning to sea.

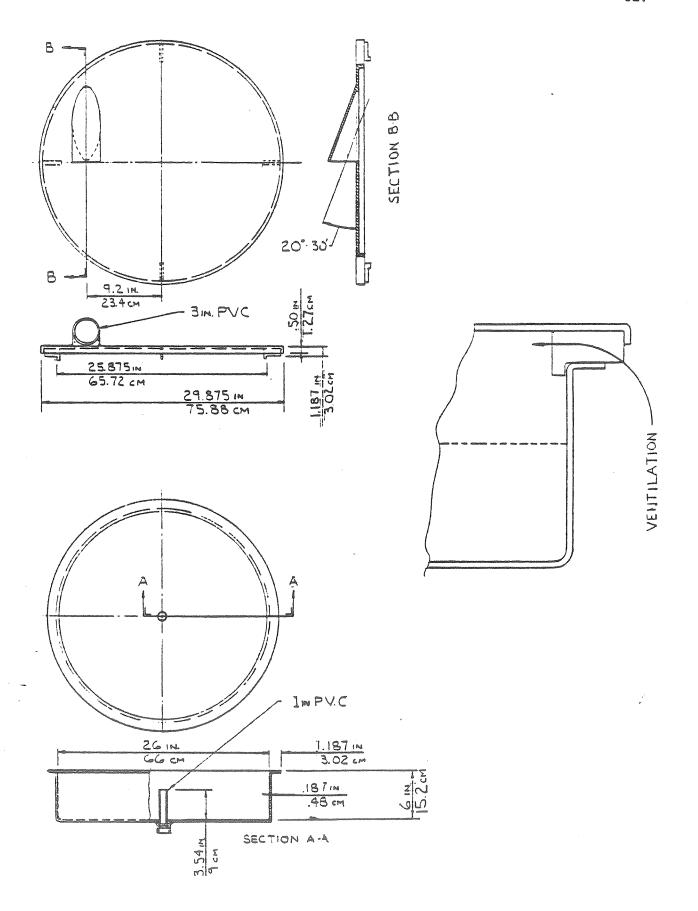
#### 4.5 CLEANING

Since the primary deep water system is enclosed and the deep water itself is clean, the only cleaning required is that of the pool filling orifice plate which is easily removed by unscrewing the union nut.

The pools are periodically drained and scrubbed down with a chlorine solution and rinsed with deep water. The submersible pumps are immersed in a chlorine solution and rinsed during the downtime.

Figure 4.12. Fiberglass shellfish tank. Drawing on next page.





The pool algal distribution system, however, which is exposed after reaching the mixing tank, requires periodic cleaning. For this purpose, a second mixing tank and header are on hand. The distribution troughs, while described as "open", are made of 6" PVC pipe with access holes with fitted caps over each of the seven discharge points and also with loose caps at each end. The cap at the entry end has a 1 1/4" PVC 45° electrical sweep cemented off center to just clear the inside wall of the 6" pipe and directed upward to deenergize the water received from the nozzle and thus provide a uniform water level over the length of the trough (Figure 4.11, Section A-A). All of these features permit cleaning of the interior surfaces with bottle brushes.

#### 4.6 DESCRIPTION OF THE AERATION SYSTEM

Two graphite vane pumps (Conde Milking Machine Company No. 3) in parallel supply air to the algal pools and to the inoculum tanks.

The introduction of air to the pools is through a 1" PVC pipe restrained by 6" concrete column blocks at a height of approximately 4 1/2" (11.4 cm) off the bottom. This pipe is placed along the long centerline of the pool and has holes drilled at regular intervals through the bottom element. The inlet end of the pipe is elbowed up 45° for the attachment of a rubber hose. The blind end has a stabilizing cross bar made up of a tee and two short capped off pipes.

The air to the inoculum tanks is introduced through an assembly made of 1/2" PVC pipe and consists of a vertical leg teed into an

octagonal ring and a 45° reinforcing bar teed into the ring and elbowed and teed into the vertical leg. An epoxied concrete cylinder placed on the vertical leg provides negative bouyancy. Forty holes 0.041" (0.104 cm) diameter are drilled into the bottom element of the pipes making up the octagon. The aeration system is shown in Figure 4.13.

#### 4.7 DESCRIPTION OF ELECTRICAL SYSTEM

The standard power supply is from the Virgin Islands Water & Power Authority and comes to the station in the form of two separate single phase supplies providing an "open  $\Delta$ " three phase network.

In the case of failure of this power, an automatic transfer system starts one of two standby emergency diesel powered generators. These are 15 kw units, Fermont Model MB-16.

The distribution of the 3 phase current is through a 4-wire system.

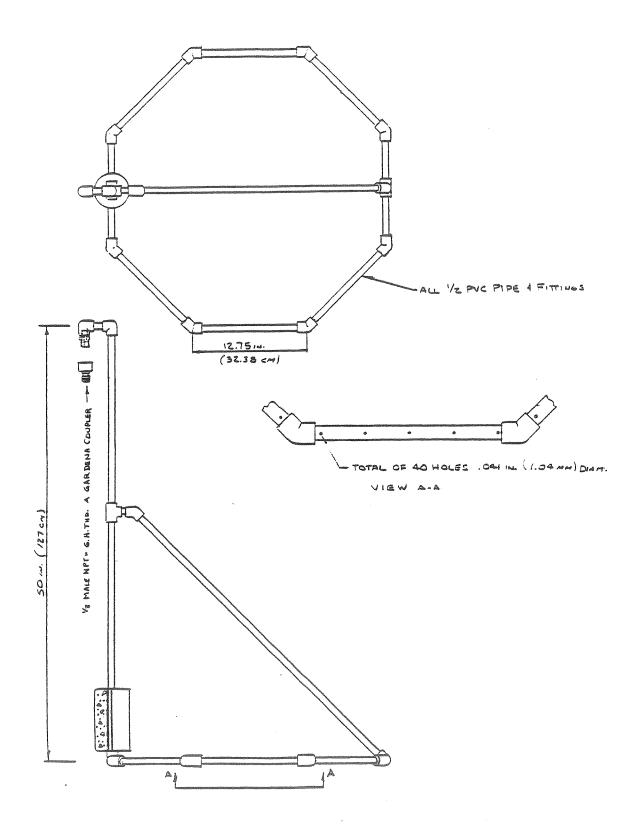
Three phase power is supplied to the deep water pumps and to the aeration pumps. The submersible pumps are on single phase and this system includes automatic ground fault switches to prevent injury to personnel.

A time delay automatic contactor in the circuit feeding the deep water pumps disconnects the pump motors should there be a failure of power supply for a period of more than 30 seconds. This prevents the pumps restarting unprimed upon resumption of power.

#### 4.8 GENERAL

Table 4.2 lists the conversion of pipe sizes in inches to centimeters.

Figure 4.13. Inoculum tank aeration system.



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TABLE 4.2 CONVERSION OF PIPE SIZES TO METRIC

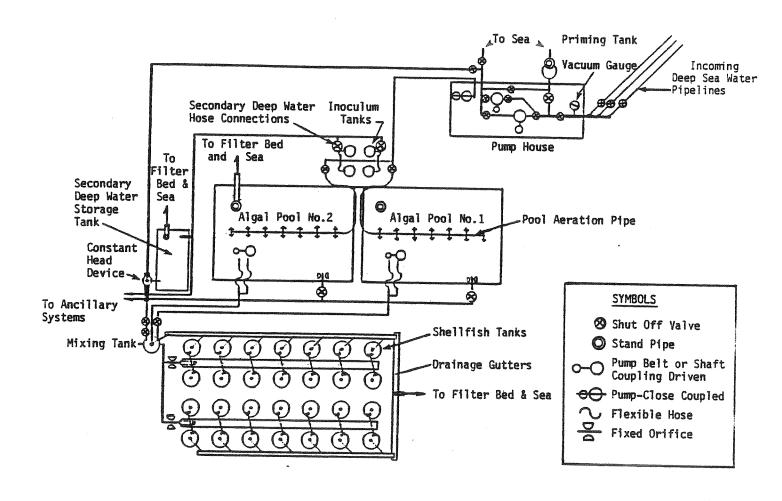
NOMINAL DIAMETER	SCHEDULE No	OUTSIDE Inches	DIAMETER Cm	WALL THICKNESS Inches	(Nom.) Cm
1/2	40	.840	2.13	. 109	.277
3/4	40	1.050	2.67	.113	.287
1	40	1.315	3.34	.113	.338
1 1/4	40	1.660	4.22	. 140	. 356
1 1/2	40	1.900	4.83	. 145	.368
2	40	2.375	6.03	. 154	.391
3	40	3.5	8.89	.216	.549
4	80	3.5	8.89	.300	.762
4	40	4.5	11.43	.237	.602
6	40	6.625	16.83	.280	.711

Table 4.3 gives a listing of the major components used in the system and their sources. A schematic representation of the overall system is shown in Figure 4.14.

TABLE 4.3. LISTING OF MAJOR COMPONENTS AND SOURCES

ITEM	SOURCE	MODEL NO.
Sea Water Pumps	Goulds Pump Co. Seneca Falls, N.Y. 13148	No. 3703 Glass lined
Aeration Pump	Conde Milking Mach. Co. Sherrill, N.Y. 13461	No. 3 assembled for use as a blower
Submersible Pump	Little Giant Pump Co. (Distributor - Harry Alter, Chicago, Ill.	No. 4 Si1D
PVC Valves, Fittings & Pipe	Plastic Piping Systems 169 Freling Huysen Ave. Newark, N.J. 07114	
Inoculum Mixing & Priming Tanks	Nalgene.	Fisher Scientific
Shellfish Tanks	H&M Systems St. Thomas, U.S.V.I.	Special
Sea Water Pipelines	Dupont	3" Schedule 30
Abrasion Resistant Hose	Gates Rubber Co.	3 1/2" x 1/8" wall
Surf Zone Conduit	Techite Division United Aircraft Products Co. California	14" "Techite"
Anchoring Bars & Grounding Sleeves	Perfo Division SIKA Chemical Corp. Lyndhurst, N.J. 07071	1 1/4" (for 1" bars in 1 1/2" hole)
Grout Additive	SIKA Chemical Corp. Lyndhurst, N.J. 07071	SIKA set
Anchoring hardware & chain	Schnitzer	
Kellems grips	Kellems	No. 020-30-1311
Hose & weight clamps	Punch-Lok	No. 0-30-S
Weld protection yokes		Special (DWG C-2216)
Bouyancy float	Corning Glass Corning, N.Y.	16" diameter

Figure 4.14. Schematic representation of the mariculture facility.



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	5.2	INCOMI	NG DEEP S	SEA (	VATE	R	» (		6	۰	٥		٠	۰	•	٠	9	۰	•	•	٠	65
		5.2.1	INTRODUC	CTIO	٧.	0	• •					٠	6		0	•	٠	٠		۰	6	65
		5.2.2	METHODS	AND	MAT	ER1	ALS		٠		٠	•	٠	٠		8	۰	۰		•		67
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		5.2.3	RESULTS	ø •	9 9	٠				٠	٠	•	é		8	٠	۰	۰	۰		٠	69
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		5.3.2	RESULTS	o e	• •	٠		•	•		٠	•	٠	•	•	٠	۰	٠	٠		6	79
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			5.3.2.2	EN	/IRO	NME	NTA	L (	BS	ER	VA	TI	ON.	S	•	•	s	٠	6	•	•	90
			5.3.2.3	P00	L C	HEM	IST	RI	ES	٠	•	•	•	•	•	•	٠	•		٠	•	93
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#### 5 PHYTOPLANKTON PRODUCTION

## 5.1 INTRODUCTION

In this section, the results of one-hundred (100) phytoplankton pool cultures operated over the period 7/3/76 -- 10/10/78 are described and analyzed. Separate treatment of cultures activated over the period encompassing the Model II shellfish idemonstration project (10/8/77 -- 10/10/78) is also included. In addition, results of deep-sea water sampling over the period 1972-1973 are described.

In general, it is the purpose of this section to describe how these algal cultures were initiated and maintained, and to report the results obtained. Emphasis is placed on the production of algal protein and the efficiency with which available deep water inorganic 'nitrogen' was converted to particulate protein-nitrogen. The conversion of available (incident) solar radiation to usable biomass is also estimated.

These data do not represent a means for direct extrapolation to large (commercial)-scale operations. Since the two pools used for these studies are only 1 m. deep, production is nutrient--rather than light-limited, and the technical description predicts much greater production per unit area at large scale in deeper pools than was obtained with the 1 m. deep pools in St. Croix. Thus the pools are not a model or mimic of larger-scale operations and are thus not, strictly speaking, a pilot-level operation. Actual production to be expected at commercial scale can be derived from the technical description assuming the latter is sufficiently precise for proposed conditions at large scale.

The two pools were used to provide food to the second trophic level, and were not operated with the intention of providing experimental support or verification of the phytoplankton technical description. The number of treatments possible with the two pools is generally inadequate for the systematic variation necessary for such evaluation, and the necessity of providing food often required non-systematic changes in procedures. However, all procedures were systematized over the period 10/8/77 -- 10/10/78, and significant variation in food supply to the shellfish resulted. Finally, it should be noted that culture 'crash' or mass cell death, necessitating pool scrubbing and reinoculation constitutes a problem so that both pools were "active" (supplying food to the shellfish) for 83% of the time for the period from 10/8/77 until 10/10/78. Seventeen percent (17%) of the time was taken by draining, scrubbing, reinoculation and start-up of the pool cultures. Neither the present technical description nor any of those models surveyed in the literature provide a prediction or understanding of these culture crashes.

In summary, the pool data reported provide a means for determining the relationship between various monitored parameters. Further, we may determine if the results are in substantial agreement or disagreement with the basic assumptions of the model. The data may also be used to determine the relative degree of stability or invariancy in output obtainable under outdoor conditions with the use of the systematic set of procedures described below. Another use of the data is to provide a tight budgeting of food availability to the second trophic level (shellfish), thus providing the basis of an evaluation of its conversion efficiency. Finally, the data

can be used to determine which parameters are most effective for monitoring the system and to point to those areas related to phytoplankton production requiring immediate or long-term research efforts. These issues are treated in the concluding parts to this section.

#### 5.2 INCOMING DEEP SEA WATER

## 5.2.1 INTRODUCTION

In this section, selected physical, chemical and biological properties of the Antarctic Intermediate ("Deep") Water Mass, used as the nutrient source for the microalgae, are reviewed. A knowledge of these characteristics is crucial for an adequate evaluation of the study described in this report. The absolute temperature of the deep water and its thermal stability over time are of central importance for an evaluation of the utilizable thermal energy to be gained through use of an Ocean Thermal Energy (OTEC) plant. Temperature also influences biological activity at all trophic levels. Both salinity and temperature play a significant role in determining the fate of deep sea water discharged into open bays or into the open sea (see Laurence and Roels, 1976, Marine Pastures, ERDA). The biological composition of deep water (the species and concentration of organisms present in the water) will affect a combined OTEC/mariculture operation in various ways. It is important for an understanding of OTEC heat exchanger biofouling, for species succession and possible contamination in the microalgae ponds, and for a consideration of potential disease and fouling problems at all subsequent (higher) trophic levels.

The nutrient concentration of the deep sea water, its constant composition and the absence of pollutants, predators and disease-bearing organisms make it a uniquely suitable medium for Artifical Upwelling mariculture. At the St. Croix Marine Station, the incoming deep water is sampled for dissolved inorganic nutrients on a routine basis. This permits verification of pipeline structural integrity (through comparison to baseline, or expected values) and provides a quantitative basis for estimating the absolute limits to nutrient availability and thus, to the amount of cytoplasm which can be produced by the microalgae grown in it, per unit volume of deep water pumped.

Nutrient-limitation of batch cultures of the diatom Chaetoceros curvisetus, STX-167, grown in Antarctic Intermediate seawater, was investigated. A factorial nutrient enrichment experiment indicated simultaneous nutrient-limitation by phosphate and nitrate with synergistic involvement of various vitamins and trace metals. A copy of the paper describing this study is available in Appendix C. Since one of the primary aims of the upwelling process is the production of high-quality plant and animal protein, and since most (over 90%) of the incorporated inorganic 'nitrogen' is transformed into protein, an analysis of the amount of available deep sea water inorganic 'nitrogen' and the efficiency with which it is transformed into plant and subsequently into animal, protein represents the chief means by which the over-all productivity and efficiency of the mariculture system is evaluated. For these reasons complete data on the nutrient concentration of the deep water are important.

## 5.2.2 METHODS AND MATERIALS

The St. Croix Marine Station is located ca. 3 km due north of 17°14'N, 64°67'W. Three polyethylene pipelines, each with an internal diameter of 3 in., and a length of 1.9 km, were installed on May 29, September 3 and September 29, 1972, respectively. The three intakes are situated approximately 1.6 km offshore at a depth of ca. 370 meters. The intakes are known to be separated both vertically and horizontally in the water mass. The three lines have brought up a continuous flow of deep water since 1972; total flow is at present 250 liters/minute. The two 50,000 liter pools require less than 100 liters/minute, while the twenty 2,000 liter 'reactors' require less than 50 liters/minute. The remaining water is used to maintain an adequate head and is returned to sea. At periodic intervals, this water is also used for cleaning.

A manifold arrangement constructed of PVC and located near the shoreline allows for the sampling or use of water from any combination of 1, 2, or 3 pipelines.

# 5.2.2.1 SAMPLING, 1972-1978

In the years immediately following pipeline installation (1973-1975), sampling for salinity and for macronutrient concentration was both intensive and extensive. In addition, variations in biological parameters such as Chlorophyll <u>a</u> were occasionally determined over short (24 hour) intervals. As the mariculture goals of the laboratory were successively accomplished and the stability of the deep water supply was made manifest, sampling was reduced in scope and was carried out with longer intervals between sampling.

Generally, samples for macronutrient composition were taken in replicate from each pipeline at weekly intervals over the period 1973-1978. Sampling for phosphate and silicate were taken routinely and aperiodically during the 1976-1977 year (see results below), but were resumed on a weekly basis for the following year. For these analyses a 4 liter sample from each line was first obtained. Aliquots (ca. 250 ml) were filtered through Gelman type A/E glass fiber filters (47 mm., 0.45µ) at 8" Hg immediately after sampling. Samples for nitrate, nitrite and ammonia were stored in glass bottles and samples for silicate and phosphate were placed in plastic bottles. All weekly samples were frozen and removed at monthly intervals for chemical determinations. For analysis, a Technicon Autoanalyzer II with standard manifolds was used. Standard Technicon chemical methodology was used but a modification of the phosphate method, according to Berhardt and Wilhelms (1967), was introduced in 1974.

Samples for salinity determinations were taken from the 4 liter sample used for chemical determinations over the period 1972-1975, but were discontinued thereafter. Aperiodic checks on the deep water were made since that time but results were not recorded; virtually no changes were found. Salinity was determined either by titration according to a modified version of the method described by Strickland and Parsons (1972), or by using a Hytech bench-type inductive salinometer.

Deep sea water (DSW) temperature as delivered by the pump at the surface was recorded at 0800 and 1400 hours daily, using a hand-held centigrade thermometer. Data for the period 1976-1978 were recorded in machine readable format and are reported here.

Turbidity measurements, were done daily on a combined DSW sample over the period 2/2/77 -- 8/1/77, using an 800 ml sample and a Monitek model 250 turbidimeter. Samples were calibrated against a diatomaceous earth standard.

The presence of microorganisms in the deep water was studied most extensively in 1977 (coincident with turbidity readings) and determined at various intervals over the period 1972-1978. Generally, samples of DSW were treated with Lugol's solution and settled in a plexiglass chamber. Following settling and removal of the top 90% of the sample, the remainder was sampled for microscopic review. Another technique used was to add unfiltered DSW to a 50 ml Erlenmeyer flask containing Guillard's "F/2" enrichment medium. The flasks were then kept at low light at ca. 25°C for various periods and sampled aseptically to determine microalgae and zooplankton present. These studies generally identified genus only. Because of the very low concentration of organisms in the DSW, no attempts were made to quantify their relative or absolute abundance.

## 5.2.3 RESULTS

Table 5.1 illustrates summary physical and chemical measurements on the DSW over the period 1973-1978.

The 678 salinity measurements made over the period 1973-1975 indicate a very high degree of stability in regard to this parameter. Since salinity often varies by large amounts in estuaries and other off-shore environments commonly used as sources of water for mariculture (daily fluc-

(1976-1978) n=1,582

SELECTED PROPERTIES OF DEEP SEA WATER PUMPED TO THE SURFACE FROM 🛨 870 m DEPTH ON THE NORTH SHORE OF ST. CROIX (17047'N, 64047'W) 1003+ 11TER-1 TABLE 5.1

SHORE		(1/4/'N, 64'47	SHOKE OF SI. CKUIA (1/4/'N, 64'47'W) µgat LITER''.	RESULTS FOR ALL THREE	L THREE PIPELINES.
	1973	1974	1974-1975	1976-1977	1977-1978
$(N0_3^++N0_2^-)-N$	31.42 <sup>±</sup> 0.16	31.28 <sup>+</sup> 0.14	31.27-1.16	30.53-1.93	30.57+1.48
	n=233	n=144	n=372	n=242	n=352
NO2-N	0.21+0.03	$0.20^{+}_{-}0.02$	0.18+0.06	í	
	n=239	n=144	n=365		
(NH3+NH4)-N	0.75-0.07	0.68+0.06	$0.82^{+}_{-}0.46$	0.84+0.52	1.09+0.54
	n=207	n=144	n=365	n=163	n=339
P03 -p	2,12±0,05	2 14+0 03	1 91+0 12	2 28 0 37	+0 +20 0
*				75.0-03.3	******
	n=239	n=144	n=377	n=60	n=320
Si04 -3 -Si	21.29+0.19	19.91+0.10	19.82 <sup>+</sup> 2.59	20.90 <sup>+</sup> 2.05	21.83 <sup>+</sup> 2.28
	n=239	n=144	n=377	n=32	n=311
Salinity ( <sup>0</sup> /oo)	34.84+0.00	34.86+0.01	34.89+0.05	I	1
	II 8	n=144	n=350		
Temperature ( <sup>0</sup> C)			(1976-1978)	21.1 <sup>±</sup> .97	

tuations of 5  $^{\rm O}$ /oo and seasonal fluctuations of 20  $^{\rm O}$ /oo or more are common), this extreme stability provides a virtually unmatched water quality. Routine salinity analyses were discontinued in 1975, but periodic checks since that time have indicated no detectable variation over mean values obtained during 1973-1975.

The measured DSW temperature delivered at the surface is the result of significant heat-transfer from the water surrounding the small diameter pipelines and is ca.  $15^{\circ}\text{C}$  warmer than is the <u>in situ</u> temperature (6.7°C). Nevertheless, the measured DSW temperature of  $21.1 \pm 0.97^{\circ}\text{C}$  indicates a high degree of stability in regard to this parameter.

Macronutrient measurements also indicate a very high degree of stability over the 6 year period.

Separate nitrite  $(NO_2^-N)$  measurements were discontinued in 1976 as over 99.9% of the total  $(NO_3^- + NO_2^-)$  - N is comprised of  $NO_3^-$  - N.

Note that for all measurements some variation over the years is noticeable, and that this coincides with an increased intra-year standard deviation. Because a single technician performed the measurements during 1973-1974 while four (4) technicians performed the later analyses, this increased variation does probably not reflect changes in deep water inorganic nutrients. Rather, measurement error is probably the main source of variation. In the absence of more information, the very low deviation around values obtained over the 1973-1974 period, indicates that these numbers should be accepted as "standard" values. We doubt that any significant variation in macronutrient concentration exists.

The nutrient content of water from the individual pipelines is tabulated for the periods 1976-1977 and 1977-1978 in Table 5.2. The three pipelines represent three different sampling areas and the high consistency between mean values for each of the lines, for each of the four macronutrients, reflects the stability of the Antarctic Intermediate water mass with respect to these chemical parameters.

In contrast to the very consistent physical and chemical features of the deep water, all biological measurements to date indicate a quantitatively low but highly variable composition.

Organisms identified in deep sea water samples to date include

Amphora coffaeiformis (see Anderson, 1975), various Bellerochea sp. including Bellerochea polymorpha, a diverse population of Chaetoceros sp. including Chaetoceros curvisetus, Navicula sp., Nitszchia closterium,

Nitszchia pacifica and various other Nitszchia sp., and various unidentified microflagellates. Various types of bacteria have also been identified in deep water samples.

There has been no systematic attempt to determine the full range of organisms present in the deep water, nor is a precise knowledge of this concentration known. Because the actual number of species is probably great (and may include as yet unidentified sexual forms of known species), because their concentration is very low (total particulate matter in DSW is invariably below 0.2 ppm), and because all evidence to date indicates great variability in both species composition and concentration, a full understanding of DSW biological composition would require an intensive and extensive series of studies.

NUTRIENT CONCENTRATION OF DEEP SEA WATER PUMPED TO THE SURFACE FROM ± 870 m DEPTH ON THE NORTH SHORE OF ST. CROIX DURING 1976-1978. VALUES ARE EXPRESSED IN µgat LITER<sup>-1</sup> AND ARE TABULATED FOR EACH OF THE THREE PIPELINES INDIVIDUALLY (#1,2,3). TABLE 5.2

		1976-1977			1977-1978	
	_	2	8	_	2	e
$(N0_3^2 + N0_2^2) - N$	30.70 <sup>+</sup> 1.89 n=81	30.65 <sup>+</sup> 1.88 n=81	30.23 <sup>‡</sup> 2.02 n=81	30.51 <sup>±</sup> 1.58 n=116	30.75 <sup>±</sup> 1.35 n=117	30.45 <sup>+</sup> 1.51
(NH <sub>3</sub> +NH <sup>+</sup> <sub>4</sub> )-N	0.85 <sup>+</sup> 0.55	0.82 <sup>+</sup> 0.50	0.85 <sup>+</sup> 0.51	1.07 <sup>±</sup> 0.54	1.13 <sup>±</sup> 0.55	1.07 <sup>+</sup> 0.53
	n=54	n=55	n=54	n=110	n=117	n=112
P04-3-p	2.29 <sup>‡</sup> 0.39	2.28 <sup>‡</sup> 0.39	2.25 <sup>+</sup> 0.33	2,48 <sup>+</sup> 0,37	2.45 <sup>+</sup> 0.56	2.46 <sup>+</sup> 0.37
	n=20	n=21	n=19	n=102	n=107	n=111
Si0 <sub>4</sub> -3-Si	21.58 <sup>+</sup> 2.55	21.19 <sup>±</sup> 1.74	19.81 <sup>+</sup> 1.23	21.65 <sup>+</sup> 2.29	21.75 <sup>+</sup> 2.30	22.09 <sup>+</sup> 2.25
	n=12	n=10	n=10	n=100	n=104	n=107

#### 5.2.4 CONCLUSIONS

This brief summary of data collected on Antarctic Intermediate water pumped to the surface on the north shore of St. Croix over the years 1972-1978 leads to the following conclusions:

- (1) Structural integrity of all three (3) polyethylene lines to a depth of 870 m. has been maintained over a six year period.
- (2) Physical and chemical measurements indicate a very high degree of stability over time. The thermal and chemical composition of the water mass may be assumed to be constant.
- (3) Biological composition is generally unexplored, but the concentration of living material is low, with a high degree of variability. Implications of DSW biology on mariculture remain to be investigated.
  - 5.3 THE CONVERSION OF DEEP WATER INORGANIC NITROGEN ALGAL PROTEIN-NITROGEN

### 5.3.1 METHODS AND MATERIALS

Appendix D is a detailed set of operating procedures for the initiation of large (50,000 liter) outdoor mass algal cultures at the St. Croix Marine Station.

This is a generalized set of procedures and a number of minor modifications have been employed during the period of time covered by this report (7/76-10/78). Generally, many attempts have been made to introduce time, labor and energy savings at one or more steps in the process. Over the period 10/11/77--10/11/78, during which the operation was run in a fully documented and systematic fashion, such modifications were introduced

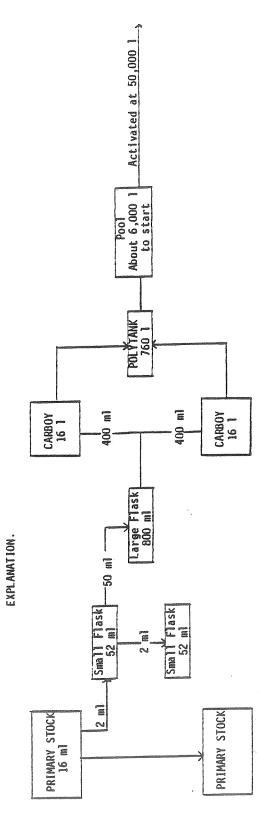
when it appeared that major cost savings could result if these changes were employed at large scale. These changes were documented in the quarterly reports to the agency for the 1977-78 year, and will not be described in this report. However, it should be noted that the procedures outlined in Appendix D are a full and highly reliable methodology for initiating outdoor cultures but that they do not stem from an explicit theoretical treatment of the subject. As such, they represent a summary of one, but by no means the only, series of steps which can be used, and are based generally on practical experience.

The revised phytoplankton technical description has been employed as a means of testing various inoculation procedures notably, through manipulation of volume of water nutrient concentration, algal (protein) - inoculum concentration, and duration between transfers. Results of more investigations are not sufficiently complete for inclusion here but it has been shown that the model will be valuable for determining efficient and cost-effective methods for inoculation procedures at large scale. It is important to realize that the predictions based on the theoretical model are used as inputs to the economic analysis, and not the actual procedures used in St. Croix and described in Appendix D.

Because the methodology used for initiating and sustaining the outdoor mass algal cultures is described in detail in Appendix D and may be found in several previous reports to the agency, only selected principal features of these methods will be described here.

The general technique is described in Figure 5.1.

GENERALIZED PROCEDURE FOR TRANSFER OF CHAETOCEROS CURVISETUS CLEVE (STX-167) THROUGH SUCCESSIVE STAGES FOR INOCULATION OF OUTDOOR POOLS. SEE TEXT FOR FIGURE 5.1:



ı	1	1	1	1	1		
*	Wed or Sat 1530	Beach	NO	ins/m²/sec		76. Kluo MSO	
qualiq	Tues & Fri 1530	Beach	2	incident light (0-3000 $\mu$ Einsteins/m <sup>2</sup> /sec	(with no control)	DSW only	
2	Sun & Wed 1530	Beach	ON			F/4; S104 @ "F"	
2%	Fri & Mon 0800	Lab	Axenic (autoclaving)			F/2	
2	Wed & Sat 0800	Lab	Axenic (autoclaving)	/m²/sec		F/2	
N	Mon & Thurs 0800	Lab	Axenic (autoclaving)		25 ‡ 2	F/2	
2	Med 0800	A G	Axenic (autoclaving)	051		2	
RESIDENCE TIME (days)	TRANSFER DAY	LOCA LOCA LOCA LOCA LOCA LOCA LOCA LOCA	>	Inc.	TEMPERATURE (°C)	MEDIA	The same of the sa

The following points concerning this technique should be noted:

- (1) The residence time in each stage is determined by the initial size of the inoculum (cellular protein), the volume of the vessel (volume of media), media concentration, and the growth rate of the organism ( $K_s$ ). As noted above, the actual schedules used to date are based on experience, but the revised technical description allows for a quantitative prediction of the increase in cellular protein in time, given any set of inoculum size, vessel size, organism growth rate, and concentration of limiting nutrient in the media (for continuous flow, inflow rate, and outflow rate can be manipulated).
- (2) The transfer schedule used is dictated by practical considerations such as personnel availability and by the reliability of the cultures. This latter factor dictates the need for back-up cultures. At the scale used in St. Croix, multiple cultures are prepared easily at low cost. At commercial scale, a much more precise analysis of reliability would be required to keep redundancy to a minimum. This is of great importance as economic analysis indicates that inoculation costs are a large portion of total production costs.
- (3) The location of the vessels is subject to change and may be different depending on the layout of the facility and on other practical considerations. For example, there is no a priori reason for the smaller vessels to be within a laboratory environment. (During small scale experiments carboys often have been kept outdoors with shading during full sunlight.) Further, some of the stages may be eliminated entirely (especially the large flask stage).

- (4) The need for sterility is also somewhat dependent on practical considerations. At the St. Croix lab, all vessels of 16 liter size and less are autoclaved. Because the deep water does contain various types of phytoplankton and bacteria, research goals are best accomplished with a highly controlled, axenic culture system. Less expensive methods (heat probes and/or use of chemicals such as hypochlorite) have been used successfully. At large scale, sterility is entirely impracticable.
- (5) Light conditions may be varied considerably. The light cycle used in the laboratory in St. Croix is based on practical experience and convenience. Laboratory light cycles of 12 hours on/12 hours off have been successfully employed and may preadapt the cultures to outdoor conditions. The revised technical description does not provide any input for the optimization of light variables. The time of inoculation for polytank and pool cultures (1530 hours) is dictated by the fact that morning and early afternoon sunlight may be too intense for the alga, and might result in bleaching of the chlorophyll.
- (6) The temperatures used are species-specific. STX-167 grows well between 20 and  $27^{\circ}$ C, while growth slows outside this range. Temperature variables are a simple extension of the revised technical description.
- (7) The concentration of nutrients in the media is also subject to variation. Since one of the prime goals of the Artificial Upwelling process is the use of deep water as the only nutrient source, the use of enriched sea water in the laboratory is not necessarily desirable. In the past, a number of pools have been inoculated following a series of transfers in deep water only; the residence time in each stage is, of course,

increased. The addition of  ${\rm CO}_2$  to highly enriched cultures ("F" level and above) has also been accomplished and results in very rapid growth and a very dense inoculum for the succeeding stage (71.0 x  $10^8$  cells/liter), with a consequent reduction in residence time and/or vessel size. In general, the case of nutrient supplementation may be analyzed quantitatively through the use of the revised technical description and its use at commercial scale is dependent upon economic and practical consideration.

The system used to maintain the phytoplankton cultures, including the flow-regulation system, pools, air system is described in Appendix D. It is important to re-emphasize the point that the St. Croix system is not a scaled-down version, model or mimic of any proposed system. Thus a commercial scale system would require engineering appropriate to the change in scale and to changes in techniques dictated by practical and economic constraints.

### 5.3.2 RESULTS

This section summarizes the results obtained when the mass algal outdoor cultures in St. Croix are operated according to the procedures described above.

Since production in the pilot system is nitrogen limited and since the final product of both trophic levels is protein, the production of algal protein and the efficiency with which deep water inorganic nitrogen was converted to protein-nitrogen is emphasized in this section. Essential features of the cultures are described and significant problems

with the process are reviewed.

Over the period from July 1976 through October 1978, one hundred (100) monocultures were activated. Inoculations which failed to result in an activated culture (operation on invariant, continuous flow) were not given a culture number. Thus, the extra time required to initiate the following culture increased the mean down-time between cultures.

## 5.3.2.1 REPRESENTATIVE CULTURES

Figures 5.2 A, B, and C illustrate examples respectively of a rapidly collapsing culture, a typical or average culture, and a highly stable, long-lasting culture. These cultures were chosen for illustrative purposes only.

Similar graphs for each of the 100 cultures are on file and are available. The graphs display a curvilinear fit (spline function) to measurements taken on dissolved inorganic ammonia, dissolved inorganic nitrate and nitrite, particulate protein-nitrogen, cell density and temperature of the culture. Ambient (air) temperature and integrated light values also are shown.

The following general points should be noted:

- (1) For all three cultures, ammonia-nitrogen values are consistently low (generally below  $2\mu$ gat  $1^{-1}$ ), but rarely approach zero. In general, ammonia values in the pool cultures did not deviate significantly from deep water concentrations. As discussed below, this supports the observation that STX-167 did not make use of ammonia as a nitrogen source.
  - (2) Particulate protein-nitrogen and dissolved inorganic (nitrate +

Figure 5.2 A Representative unstable culture

Legend: Solid green line: Particulate Protein-N μg atoms 1<sup>-1</sup>

Solid red line:  $(NO_3^- + NO_2^-) - N \mu g$  atoms  $1^{-1}$ 

Solid black line: Number of cells ml<sup>-1</sup>

Solid blue line:  $(NH_3 + NH_4^+) - N \mu g$  atoms  $1^{-1}$ 

Dashed red line: Air temperature <sup>O</sup>C

Dashed blue line: Water temperature in the pools OC

Green vertical bars: Irradiance (watts/hr/m<sup>2</sup>)

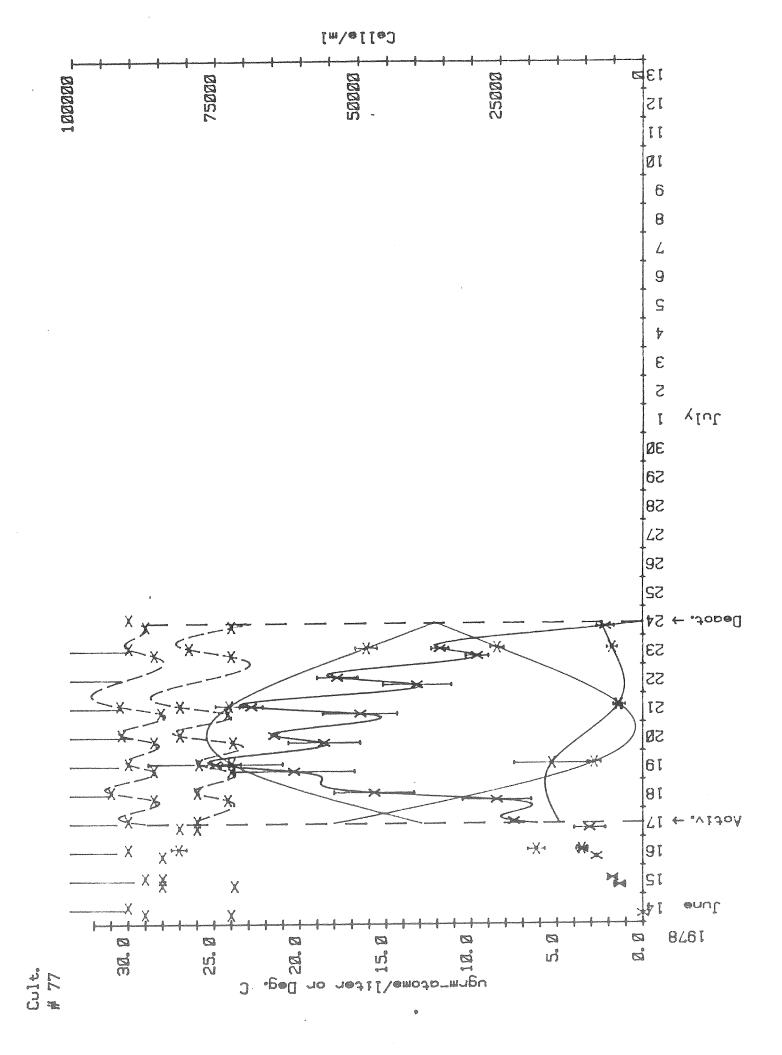


Figure 5.2 B Representative typical culture.

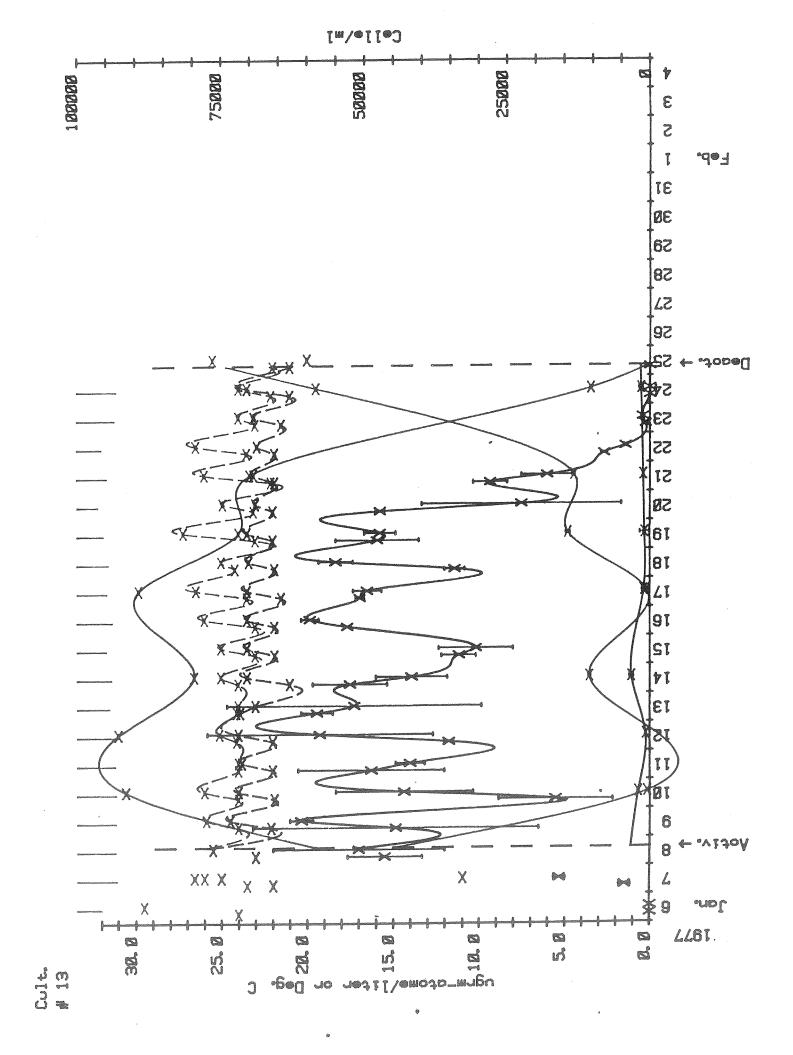
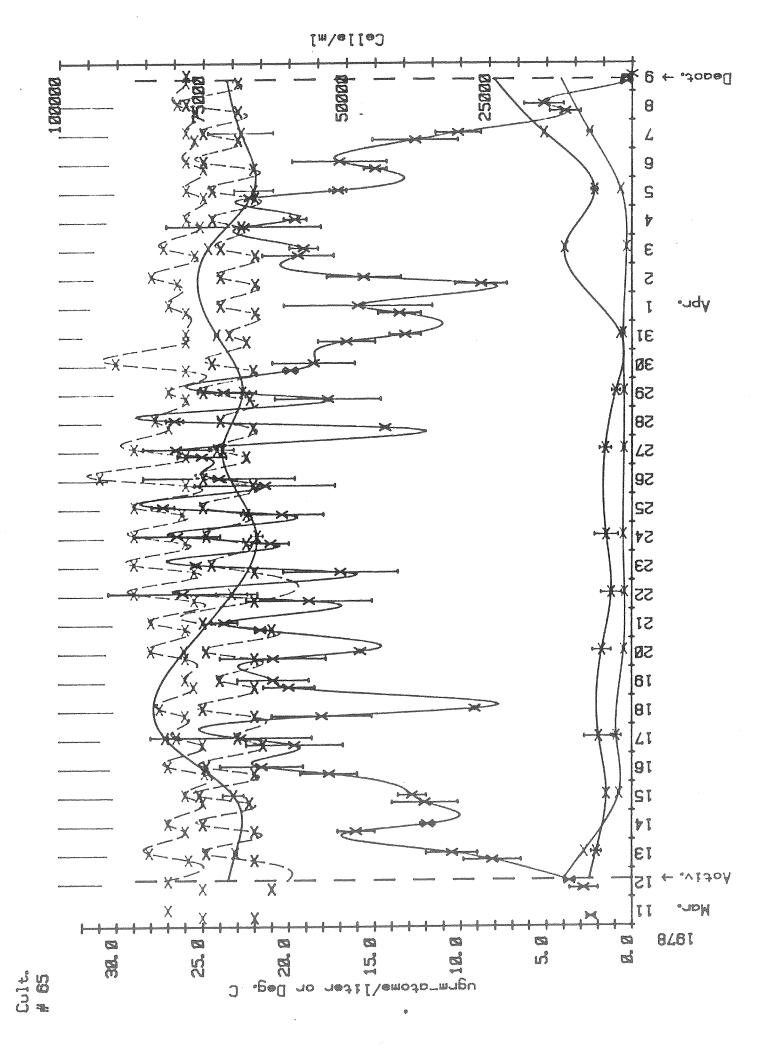


Figure 5.2 C Representative stable culture.



nitrite) demonstrate a high degree of within and between culture variation.

- (3) Concentrations of PPN and total DIN appear to be strongly and inversely correlated. As supported by more complete and quantitative evidence below, this indicates a marked preference of STX-167 for nitrate over ammonia as the nitrogen source, at least in the St. Croix system.
- (4) Cell-density generally correlates positively with PPN concentrations, but displays more variation. Potential implications are discussed below.
- (5) Culture temperature is a smoothly varying function with a significant increase in P.M. (1400) vs. A.M. (0800) readings. The potential influence of temperature on culture stability is also discussed below.
- (6) There appears to be no simple or obvious correlations between the environmental observations and either instantaneous or total culture protein production.

From both the technical and economic point of view, the culture characteristics of most concern focus on maximum sustainable protein production. This, in turn, depends upon the maximum instantaneous yield and the longevity of the culture.

Figure A is an example of a highly unstable and short-lived culture. Although the culture was on a constant dilution rate, protein-nitrogen concentration increased steadily for the first 4 days, reached a peak, and declined thereafter. The peak in protein-nitrogen production is accompanied by a reduction of inorganic nitrate + nitrite to negligible levels.

This culture was inactivated after 7 days because of the need to maintain sufficient food to the shellfish. However, other, similar cultures which have remained activated showed a continued decline in protein concentration, accompanied by an increase in the number of dead cells detected, levels of (nitrate + nitrite) to near deep sea water values and contamination with unwanted species, generally pennate diatoms and protozoans, and by an increase in observable bacterial levels. No recovery of production in such cultures has been observed but a resurgence of production by <a href="Chaetoceros">Chaetoceros</a> may occur given sufficient time or adequate control measure.

Figure B demonstrates a similar but more typical pattern. Protein production increases gradually, then declines after reaching a peak. The nearly complete conversation of available deep water 'nitrogen' to protein-nitrogen at the peak of production is a common if short-lived phenomenon and speaks well for the potential productivity of the system.

The gradual increase in PPN concentration as illustrated by cultures 77 and 13 (Figures A & B) is noteworthy for two reasons.

First, this implies that at the flow-rates employed (1.15 dilutions/day<sup>-1</sup>), the organism can grow at significantly faster rates for periods of days. Increasing protein concentration has been observed in cultures with dilution rates exceeding 1.5 day<sup>-1</sup>, so the maximum sustainable division rate of the diatom may exceed this value. A general increase in dilution rate is therefore possible and, if no detriment to culture longevity results, an increase in protein production would occur.

The phenomenon also implies that steady-state, if obtainable, may take a period of days to occur. This is very important, especially if greater control over cultures (by manipulation of dilution rate, nutrient supply, etc.) is considered. In fact, preliminary computer runs using the revised phytoplankton technical description indicate that steady-state is reached only after a period of days, indicating that feedback control using short time intervals may be counter-productive.

Figure C, displays the results of culture #65, representing a good culture in terms of stability and longevity.

Since economic analysis to date has indicated that inoculation costs are a significant part of overall production costs, it is most desirable to initiate cultures which last as long as possible. Cultures such as #65 indicate that steady-state can be maintained for periods approaching one month and longer. The ability to maintain a high quality monoculture for such periods of time under constantly changing environmental conditions without changes in operating procedures is remarkable. Very few systems have achieved the ability to maintain a healthy monoculture under outdoor conditions. The factors responsible for producing long-lasting cultures have not yet been identified. No significant relation between environmental parameters and culture longevity or total culture protein production have been identified to date.

The more general problem of culture collapse will be discussed following a summary presentation of environmental and culture data obtained over the period 1976-1978.

## 5.3.2.2 ENVIRONMENTAL OBSERVATIONS

Table 5.3 illustrates the mean and standard deviation of selected environmental observations on one hundred pool cultures. For these summary data, all data points available in machine readable format were employed.

As expected, there was a significant degree of correlation between light, air temperature and culture temperature.

An initial linear analysis on these variables indicated that combined a.m. and p.m. air and culture temperature readings (n=3030) had a coefficient of determination ( $R^2$ ) of 0.47. Light, which was integrated over 24-hour periods only, demonstrated an  $R^2$  with culture temperature of 0.15 (n=1383) and with air temperature (n=1386) of 0.10.

A partial correlation analysis indicated that low concentrations between temperature and light readings was due to significant variation in culture density, as measured by PPN content. Controlling for PPN raised the  $R^2$  value between light and culture temperature to 0.52; additional control over other culture variables had no significant effect on  $R^2$ .

Incoming deep sea water temperature will be determined by the efficiency of the ocean thermal energy conversion process, the residence time in the pools, air temperature, wind and relative humidity. This is important as STX-167, isolated from the deep water, has a temperature optimum of  $21 \pm 2^{\circ}$ C. In fact, laboratory experiments have indicated a significant slowing of division with this diatom at temperatures exceeding

TABLE 5.3. SUMMARY ENVIRONMENTAL OBSERVATIONS 7/76 -- 10/78.

	Mean ± s.d.	N of Data Points
Air Temperature ( <sup>O</sup> C)	27.9 + 2.5	3190
Culture Temperature	24.5 + 1.7	3106
Light (kw/hr/m <sup>2</sup> /day)	4.42 ± 1.39	708

- 1. Based on readings of a hand-held thermometer taken twice daily @ 0800 and 1400 hours. Data for A.M. vs. P.M. values and for individual cultures are available in machine-readable format.
- 2. Based on readings .5 m below surface of pool cultures at 0800 and 1400 hours daily. A.M. vs. P.M. and individual culture data available in machine-readable format.
- 3. Based on 24-hour (0800-0800) readings taken from integrating radiometer; 169.8 units≡1.0 kw/hr/m²/day. More detailed information available in machine-readable format.

27°C. The actual temperature reached in the St. Croix pools often approached this value, and mean pool temperatures were above optimum for the organism.

The slight but statistically significant and deleterious effect of elevated pool temperatures on the cultures was demonstrated in a zero-order partial correlation analysis. Culture temperature was inversely correlated with PPN ( $R^2 = -.1123$ )<sub>n</sub> and with the  $\log_{10}$  of the cell concentration ( $R^2 = -.2640$ ), while it was positively correlated with ( $NO_3 + NO_2$ ) concentration ( $R^2 = 0.1274$ ). (No significant effect with ammonia was found which, as discussed above, was generally unutilized by the cells). Controlling for variations in light intensity in the partial correlations analysis increased the inverse  $R^2$  value between pool temperature and  $\log_{10}$  cell concentration to -.3224, while that between pool temperature and PPN was raised only slightly to -.1390.

Considering the non-systematic nature of the changes in variables measured and the low variation in pool temperatures in general, these results present good preliminary evidence for the notion that lower pool temperatures resulting from deeper pools would improve the growth and possibly the longevity of STX-167 in outdoor culture. Of further statistical interest would be an analysis of culture longevity and/or steady-state conditions and fluctuations in pool temperature, especially at the higher end of the scale and as a function of light variations. Certainly, the possible role of elevated temperatures in the phenomenon of culture collapse warrants more detailed experimental investigation.

### 5.3.2.3 POOL CHEMISTRIES

Table 5.4 is a summary of pool chemistry and cell density data collected over the period of July 1976 -- October 1978.

The mean concentration of PPN produced in the pools was 21.83  $\mu$ gat 1. $^{-1}$ . This is equivalent to a conversion efficiency of total deep water inorganic nitrogen (31.5  $\mu$ gat 1. $^{-1}$  is assumed) to PPN of 69%. This conversion efficiency is identical to that predicted by the original (1976) phytoplankton technical description for 1.0 meter deep pools at a turnover rate of 1.15  $day^{-1}$ . This is more a reflection of the long-term consistency of the results obtained than it is a reflection of the adequacy of the technical description. However, the reason for this is that the conversion efficiencies predicted by the original description were based on empirical measurements made during a November-December 1975 study using 2000 liter vessels ('reactors'), and not on a quantitative or mechanistic understanding of algal nutrient uptake and growth. However, the long-term predictability of conversion is important and well established; similar summary measurements of conversion efficiency performed on pools over various lengths of time ranging from 1 month to 1 year during the years 1975 - 1978 have indicated little deviation from this mean value.

Despite the fact that mean PPN values do not vary over the long-term, considerable within-culture variation is a consistent observation (see Figures 5.2 A, B, and C above). The standard deviation of 7.55  $\mu$ gat<sup>-1</sup> is almost 35% of the mean value.

Of course, dissolved inorganic ( $NO_3^- + NO_2^-$ )-N concentration over

TABLE 5.4. SUMMARY OF POOL CHEMISTRY AND CELL COUNT DATA JULY 1978 -- OCTOBER 1978<sup>1</sup>

	×	std dev.
$NO_3^- + NO_2^-$ -N (µgat L <sup>-1</sup> )	4.80	7.42
$NH_4^+ + NH_3$ -N (µgat L <sup>-1</sup> )	1,18	0.83
CELL DENSITY $(\times 10^7 L^{-1})$	4.00	1.56
PARTICULATE PROTEIN NITROGEN $(\mu gat L^{-1})$	21.83	7.55
TOTAL NITROGEN ( $\mu gat L^{-1}$ )	27.81	

For all data, n = 390. Only days on which all data was available, including environmental observations, were used for this summary. This data base was used for all partial correlation analyses. (see text).

this long period of time also reflects this variation ( $\bar{x}$  = 4.8  $\mu$ gat 1. $^{-1}$ ; s.d. = 7.42  $\mu$ gat 1. $^{-1}$ ). As expected, a strong inverse linear relationship exists between PPN and ( $NO_3^- + NO_2^-$ )-N concentration. A fourth order partial correlation analysis (controlling for cell concentration, pool temperature, light intensity and ammonia) indicated a linear correlation between PPN and ( $NO_3^- + NO_2^-$ )-N of -.3010.

Figures 5.3 A and B illustrate simple linear functions of PPN versus  $(NH_4^+ + NH_3)$ -N and  $(NO_3^- + NO_2^-)$ -N, respectively. The high degree of variation in PPN concentration with virtually no concomitant change in ammonia content is clearly evident. The lack of a perfect inverse correlation between PPN and  $(NO_3^- + NO_2^-)$ -N concentration can be deduced from Figure 5.3 B. Most of the variation around the line exists at the higher end of the PPN scale, where  $(NO_3^- + NO_2^-)$ -N concentration was very low. Much of the random variation is probably due to  $(NO_3^- + NO_2^-)$ -N determinations which are close to the limits of detection at high PPN concentrations.

Another, probably more significant reason for this type of variation in the correlation between the PPN values and the  $(NO_3^- + NO_2^-)$ -N concentration is that following uptake of the nitrate, and subsequent conversion to protein, the cell may release dissolved organic nitrogen. Thus, simply measuring PPN and  $(NO_3^- + NO_2^-)$ -N content does not account for the total nitrogen available and no perfect correlation between low  $(NO_3^- + NO_2^-)$ -N and high PPN values can be expected.

The mean total nitrogen accounted for in the pools was 27. 81  $\mu$ gat L $^{-1}$ , or about 88% of total inorganic deep sea water 'N' available. In fact, the

Figure 5.3 A. Particulate protein nitrogen plotted as a function of  $(NH_4^+ + NH_3)-N$  concentration.

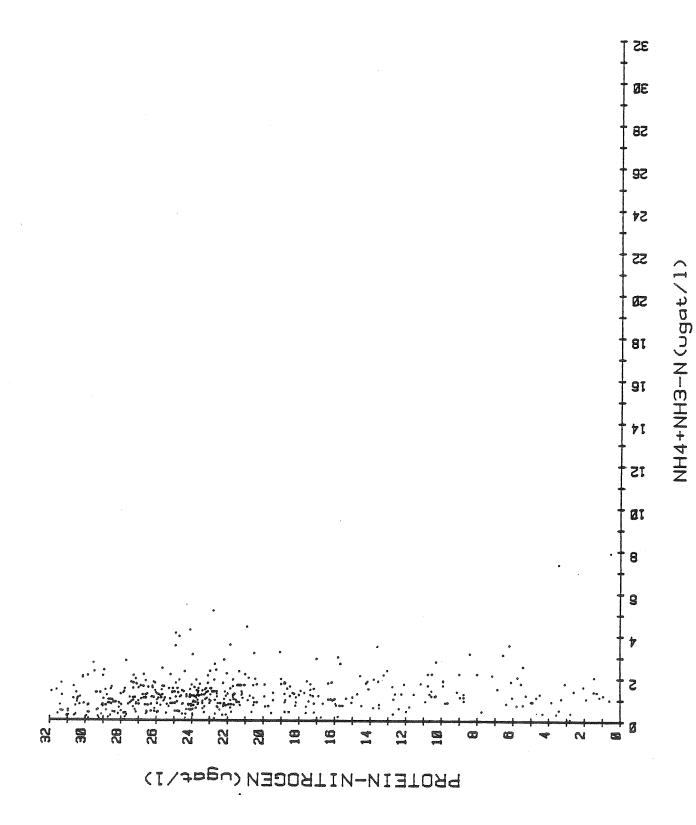
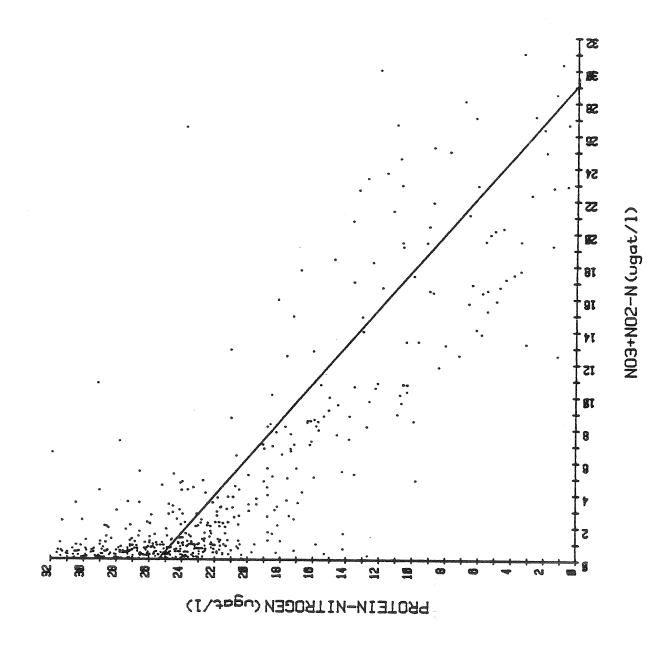


Figure 5.3 B. Particulate protein nitrogen plotted as a function of  $(NO_3^- + NO_2^-)-N$  concentration.



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addition of a total dissolved organic nitrogen analysis to the spectrum of measurements employed has increased this accountability to 96%. This provides a crude indication that total measurement errors are above 4% stemming probably from the lack of precision or sensitivity in the methods used.

In any event, the preferential uptake of nitrate over ammonia by STX-167 under these conditions—probably due to the very low concentration of ammonia present—apears well established. Further, the high degree of inverse correlation between PPN and  $(NO_3^2 + NO_2^2)$ —N content indicates that the latter may be very useful for a continuous monitoring of culture conditions, perhaps supplemented by PPN and total dissolved organic nitrogen analyses at aperiodic intervals. Data to date indicate that these supplemental measurements would be most useful to verify and/or diagnosis of culture health during the latter phase of a culture's lifetime.

Table 5.5 summarizes the data obtained and time required for reinoculation between cultures ("down-time") and of culture longevity ("activation-time").

These data indicate clearly that outdoor continuous-flow cultures of Chaetoceros curvisetus (Cleve) can be initiated and sustained using deep-sea water only as the culturing medium. However, the relatively short mean active life of the cultures (12.4 days) and the high standard deviation (6.2 days) points to the problem of maintaining a constant

100.					
uly useful ironmental t. Data		tion			
lysis tures used		Standard Deviation 41.58	148.39 (6.2 days)		
the year 1977-		Mean 59.92	.74 day)	25	
s different in		Me 59	297.74 (12.4 day)	83.25	
<pre>d be operated. ield at the</pre>			2		
ls in a fashion					
trient limited					
rated.	- 1				
roduced per unit					
oth and turn-	1	Down-Time¹ (Hours)	Active-Time <sup>2</sup> (Hours)		
iology .		<u> </u>	ле <sup>2</sup> (	ime ime	
ity of	1	Ë	Ë	% Active-Time	
cm deep,	•	own-	ctive	Acti	
ate and light		i)	Ř	26	
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l Time required between deactivation of one culture and activation (placing on continuous flow of Succeeding culture.

2 Total time each culture was on continuous flow. See text for discussion.

TABLE 5.6 SUMMARY PRODUCTION OF ALGAL PLANT PROTEIN IN THE ARTIFICIAL UPWELLING SYSTEM JULY 1977 -- JUNE 1978.

NUMBER OF CULTURES	50 cultures
DEPTH OF POOLS <sup>1</sup>	1 m
EFFECTIVE AREA (two pools)	$100 \text{ m}^2$
MEAN TURNOVER RATE <sup>2</sup>	1.23
MEAN PPN PRODUCTION	21.58 μgat L <sup>-1</sup>
MEAN % ACTIVATION TIME	82.86%
MEAN PROTEIN PRODUCTION	2.22 $g/m^2 day = 8.1 Tons/Ha/year$

Note that pools are very shallow and thus are not light-limited. Areal production would increase considerably at large scale under light-limited conditions.

<sup>2</sup> Turnover rates used varied from  $1.15 - 1.56 \, \mathrm{day}^{-1}$ .

intensity. Light intensity was controlled through the use of neutral density screen which regulated the surface light intensity of the cultures at 3%, 20%, 30%, 46%, or 100% of the natural sunlight intensity ( $\mathbb{I}_{0}$ ) 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: 0.25, 0.70, 0.95 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  $_{0}$  only; or  $\alpha$  = 0.3. From these data, pool depth, light attenuation, turnover rates, and hence productivity values, for an optimised 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 provides 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 optimised productivity estimates, differences between the light intensity at the top and bottom of the reactors at difference 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. The

peak absorbance value is 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:

$$I_{av} = \alpha I_0 \left( \frac{I - e^{-kz}}{kz} \right)$$

where  $\alpha$  = proportion of incident light penetrating a neutral density screen and striking the surface of the culture;

 $I_0$  = 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  $_{0}$  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 absorptivity (k), for each turnover rate, which also "see" the same average illumination, when subjected to unattenuated ( $\alpha = 1.0$ ) sunlight, was determined.

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. Protein concentration decreases with increasing turnover rate.

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.

The actual St. Croix pilot plant operation utilized 1 m deep pools with a turnover rate of 1.15/day. This resulted in actual phytoplankton protein production equivalent to 8.1 tons/Ha/year.

It was estimated that a 3 m deep pool would provide an economic optimization between good utilization of the solar energy incident on the surface area and cost of excavation: as pool depth increases beyond 3 m, relatively little further increase in productivity per unit surface area is gained. A 3 m deep pool would have a turnover rate of 0.75 per day and would produce 19.3 T of phytoplankton protein/Ha/year, allowing for a duty cycle of 0.93 (14 days on, 1 day off).

It might be possible, of course, to achieve better utilization of the incident solar radiation in shallow pools by increasing the nutrient content of the incoming deep sea water through the addition of fertilizer. This in turn would increase the density of the phytoplankton cultures and might require dilution of the phytoplankton produced prior to feeding the culture to the shellfish. Experimentation along these lines was started during 1978 and will be reported on separately.

The major problem with the phytoplankton cultures remains the unpredictable collapse of the cultures after varying periods of time. A basic investigation of this phenomenon is underway. A brief summary of the results is enclosed as Appendix E.

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# 6 THE PRODUCTION OF CLAMS, TAPES JAPONICA

### 6.1 INTRODUCTION

In this section, we turn to a discussion of the focus of work on "Artificial Upwelling" over the 1977-1978 year. During this year emphasis was given to a feasibility test of growing clams <a href="Tapes">Tapes</a> japonica. Technical feasibility was evaluated through a series of systematic observations on growth and food conversion data. These data were also used to determine the validity of the technical description, which was used to perform an economic evaluation of the process.

The test can be characterized as a practical demonstration project. While the major focus of work was on the shellfish, parallel work on economic factors indicated that major cost items centered around primary production.

It is important to realize that the second trophic level was not intended to provide specific inputs for the technical description, which serves as the quantitative, theoretical basis for economic evaluation. Rather, the shellfish were manipulated and fed according to a pre-existing set of equations based on a generalized understanding of shellfish growth functions. Since the description predicted specific weight gains as a function of animal population parameters and food availability, it was intended to compare these predictions with actual weight gains. This comparison would serve to demonstrate the adequacy of the description or to point to areas requiring revision and/or more empirical work.

Since the shellfish technical description is based on experimental

work carried out at the laboratory between 1970 and 1975 and on a literature review of similar work done on various species of bivalves, the work described here is best seen as a test of scale. That is, it was assumed that the existing equations were adequate extrapolations from earlier, experimental-sized studies and that the process most required practical demonstration at the largest scale possible. This decision had important implications. Attempting to grow the maximum amount of shellfish required, for example, that both pools be employed. Since the cultures sometimes "crash", and because the limits to efficient phytoplankton growth were being pushed to the maximum, fluctuations in the food supply to the shellfish occurred. On one hand, this dynamic condition made systematic monitoring difficult, and introduced variables not necessarily accounted for in the original equations. On the other, the production system was pushed to its limits. Since procedures were entirely scheduled and non-routine intervention was not permitted, any difficulties in growing the animals were assured of being demonstrated. In short, some precision in data was sacrificed to assure that the techniques employed were sound, and could be duplicated at any scale without fear that similar growth could not be maintained.

In general, a highly conservative approach was taken, and the data that were generated support the claim that the actual production obtained is below that which might be expected at commercial scale. This is not to say that the means for increasing such production are entirely at hand, for our understanding of shellfish feeding and growth remains inadequate.

Before moving to a description of the system, it should be noted that these points can be carried specifically to certain design features of the system, such as the shellfish tanks. Other designs are fully compatible with the technical description, and may prove both technically and economically superior to the design employed for this test. An economic analysis which incorporates these design features, however, is dealing with fewer unknowns since an actual, adequate test has been performed. Those aspects of the actual system used in St. Croix which have and have not been used as the basis for economic evaluation represent the most crucial and difficult decisions required for an adequate evaluation of commercial feasibility.

In this section, we will discuss a comparison of the results obtained in Model I and Model II pilot plants, generated respectively during 7/76-- 7/77 and 10/77--10/78.

The experience gained from the problems encountered in the Model I pilot plant led to a vastly improved design for the Model II plant.

This section is followed by a description of the Methods and Materials in the operation of a Model II pilot plant.

The following sections describe the results obtained and the chapter concludes with a brief discussion.

6.2 COMPARISON OF RESULTS OBTAINED IN MODEL I vs. MODEL II PILOT SHELLFISH PLANTS

The design and operation of the Model II (1977-1978) shellfish area was based in part on the results of the Model I (1976-1977) pilot demonstration. Full results of that earlier test, were described in our

earlier report to Sea Grant for 1976-1977. The problems experienced using Model I were:

#### 6.2.1 CONFIGURATION

Significant problems were encountered in cleaning the shell-fish trays (Nestier) in the Model I pilot plant. There was a significant build-up of fecal material. Bacterial plaques ( $\underline{Pseudomonas\ sp}$ ) were frequently noted. A significant amount of dissolved  $\underline{NO_3}$  and  $\underline{NO_2}$  was taken up at the second trophic level, probably by the filamentous algae ( $\underline{Enteromorpha}$ ) which accumulated on the insides of the tanks. Cleaning of tanks was difficult and time-consuming. Flow rate control was somewhat difficult and time consuming.

Although food chain conversion efficiency was quite good (generally ranging from a high of 30% to a low of 5% and strongly and inversely correlated with age and size), the actual growth rates and conversion efficiencies in the Model I pilot plant were generally 25-50% of that expected, based on the original shellfish technical description.

We may summarize the results of analysis of these problems in the context of changes which were incorporated into the new plant design.

Some of these conclusions were based in part on experiments done during the 1976-1977 year (see our Sea Grant Progress Report 1976-1977, pp. 160 ff). Various theoretical and practical factors were also incorporated into the final design stage of our Model II pilot plant.

Because of the compounding of variables in the plant (since it was not an experimental design), it was determined that the significant drop-off

in conversion efficiency with age, and the significant deviation between predicted and actual results (this deviation also widened with age), provided insufficient evidence to indicate if or how the original description might be changed. Of primary importance was the need to improve the environment of the animals, and to disentangle the age/size-dependent variables from the "configurational" (environmental) variables. Although it is well-known that mortality increases exponentially with age once adulthood is reached, it was assumed that the fouling of the tanks was responsible at least in part for the abnormally high death rates, especially in instances involving very heavy die-off in individual tanks. Fouling was attributed in part to low food ingestion rates ( $\bar{u} = 50\%$ ) on the part of the shellfish, poor circulation and drainage in the tanks (due mainly to the presence of the plastic Nestier tray liner), and to the seepage of sunlight into the tank, thus allowing for the growth of Enteromorpha.

The low food conversion rates in our Model I pilot plant were traced in part to low ingestion rates, especially for larger (older) animals, and possibly to fouling, gametogenesis and spawning variables, to age (size) dependent changes in assimilation and to the stocking density (loading factors) of the animals (again, the older/larger animals were more densely stacked in the trays).

Since it was known that variables related to maturation were at least in part responsible for the drop-off in food conversion with age, and that the original technical description did not include such variables, it was expected that the Model II plant could provide this information by eliminating the deleterious influence of as many environmental variables as possible. Therefore, the following changes were incorporated into the plant.

- (1) The trays were placed under a roof and covered with fiberglass tops. This was done to prevent the growth of algae in the tanks and to lessen the amount of sunlight and heat exposure to the animals.
- (2) Shallow (9 cm deep), circular trays were used. This improved drainage and circulation, greatly simplified cleaning procedures, reduced costs, and provided less surface for the growth of algae, bacteria, and the accumulation of metabolic by-products.
- (3) The animals were spread in a loose monolayer thus reducing the stocking density or "loading factor".
- (4) By adding deep sea water to the inflow when one or both pools were off-line, the amount of flow to each tray was kept constant, thus reducing the amount of stimulation thought to contribute to the release of gametes.

Before presenting the results of the Model II plant in detail, these results which have a direct bearing on attempts made to eliminate problems encountered with the earlier, Model I (1976-1977) plant will be presented.

#### 6.2.2 MORTALITY

A significant amount of protein was lost through the Model I plant because of high mortality rates and frequent spawning. A very significant problem for the analysis of the Model I plant was that

the variables of shellfish age, size (as indicated by all shell and weight measurement variables), spawning frequency, food availability and consumption, food flow variability and tank fouling all covaried to some extent. Therefore, the causes of high mortality rates could not be determined based on a strictly quantitative evaluation of these data. The ultimately successful solutions to reduce mortality and spawning in the Model II pilot plant are discussed below.

Table 6.1 is a summary of mortality in the Model I and Model II plants. Note the vastly improved survival rates for the Model II plant. Frequent and regular inspections of the Model II trays revealed no significant fecal and detrital accumulations.

The mortality for populations of <u>Tapes</u> in Pilot Plant Model I and Pilot Plant Model II is listed in Tables 6.1A and 6.1B. Direct comparison between <u>Tapes</u> survival in the two pilot plant models is difficult: there were differences in population numbers, population sizes, time the populations spent in the pilot plants, method of culling and harvesting, etc.

Pilot Plant Model I (Table 6.1A) reared three populations of shellfish for a six month period. During this time 2714, 2323, and 2218 individuals died from <u>Tapes</u> populations 20, 21 and 22 respectively. Since approximately 10,000 juvenile clams were introduced into the system for each population a gross mortality of 24% was observed.

TABLE 6.1A MORTALITY DATA FOR PILOT PLANT I, JANUARY 11 TO JUNE 28, 1977.

DATE	POPULATION #20	POPULATION #21	POPULATION #22
1/11/77 1/18/77 1/18/77 1/25/77 2/1/77 2/8/77 2/15/77 2/22/77 2/22/77 3/1/77 3/15/77 3/22/77 3/22/77 3/29/77 3/31/77 4/5/77 4/12/77 4/19/77 4/19/77 5/10/77 5/10/77 5/17/77 6/14/77 6/14/77 6/21/77 6/28/77	- 38 122 79 60 178 37 54 120 42 176 71 - 242 79 222 102 145 42 213 52 177 48 156 56 177	- 15 14 26 23 17 - 24 22 184 53 317 430 317 114 24 165 23 135 25 98 24 97 30 104 32	57 50 35 63 39 - 21 51 41 131 81 - 309 66 234 81 165 43 209 41 118 29 138 130 91
Σ POPULATION	2714	2323	2218

TOTAL: 7255

MORTALITY DATA FOR PILOT PLANT II, JANUARY 3, 1978 TO OCTOBER 10, 1978. TABLE 6.1B

POPULATION #	20	21	22	34	35	37	39	40	41	42	43	44	45	46	47.	48	49	50
DATE																		
1/3/78 1/17/78 1/31/78 2/28/78 2/28/78 3/14/78 3/28/78 4/11/78 4/25/78 5/23/78 6/6/78 6/6/78 6/20/78 7/4/78 8/1/78 8/19/78 9/12/78			211111111111111111111111	+111111111005133	50 81 881 198 814 1126	66 101 101 103 80 80 80 103 114 117 117 117 117 117 117 117 117 117	10 13 13 13 14 14 12 12 13 14 17 17 17 17 17 17 17 17 17 17 17 17 17	0 0 0 24 33 117 118 119 32 32 37 119	00000000000000000000000000000000000000	26 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 10 10 1	111100000111111111111111111111111111111	00 00 00 00 00 00 00 00 00 00 00 00 00	1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1	111111111111111111111111111111111111111	1111175300111111		111111111111110000	
E POPULATION TOTAL: 4144	-		12	55	1390	599	294	265	317	271	153	595	74	65	45	6	0	0

Pilot Plant Model II (Table 6.1B) grew 16 shellfish populations for time periods ranging from one week to eight months. Total mortality from January 3, 1978 to October 10, 1978 was 4144. Table 6.3 lists approximate number of individuals introduced into the pilot plant for each population and from these figures a mortality of less than 1% was achieved.

These data and observations support the conclusions based on 1976-1977 data and indicate that the corrective measures taken to improve the environment of the animals was successful and contributed to a greatly improved survival rate with age.

### 6.2.3 SPAWNING

The frequent tank spawnings observed in the Model I pilot plant were attributed in part to the methods used in handling of the animals during cleaning and weighing. It was judged that the animals were out of the water too long, exposed to sunlight, and subject to excessive mechanical stimulation. Finally, it was determined that spawnings which occurred between cleaning periods were due to changes in flow-rates to the animals; this was due to the deactivation and reactivation of pool cultures: in pilot plant Model I, the flow rate of

sea water to the animals was reduced by half when one pool was off line.

Spawning data for the two configurations (Models I and II) are not strictly comparable because of the different number of trays (12 and 28, respectively) and other differences. From 01/20/77 to 07/15/77 in the Model I plant, a total of 94 tank spawnings were observed. Total tank spawnings recorded for Model II over the one-year 10/11/77 to 10/10/78 was only 28. Thus, greater consistency of flow rate, reduced handling, reduced sunlight and other improvements helped to reduce the release of gametes. Note that since the hatchery could obtain ripe individuals for spawning purposes almost at any time in the pilot plant, gametogenesis was probably not interfered with.

#### 6.3 METHODS AND MATERIALS

Figure 6.1 illustrates schematically the system used for producting shellfish. The primary production system has been described in detail in Section 4. For purposes of analysis, the primary and secondary production systems were considered as wholly separate. The shellfish production system consisted of (1) a larval and setting area, (2) a juvenile grow-out area and (3) the shellfish demonstration facility. Each of these three areas had a distinct food supply system. In addition, brood stock were maintained for the purpose of providing a constant supply of animals.

## 6.3.1 BROOD STOCK

Note: A shellfish hatchery operation manual describes in detail the methods outlined below (see Sunderlin and Baab, 1977 in

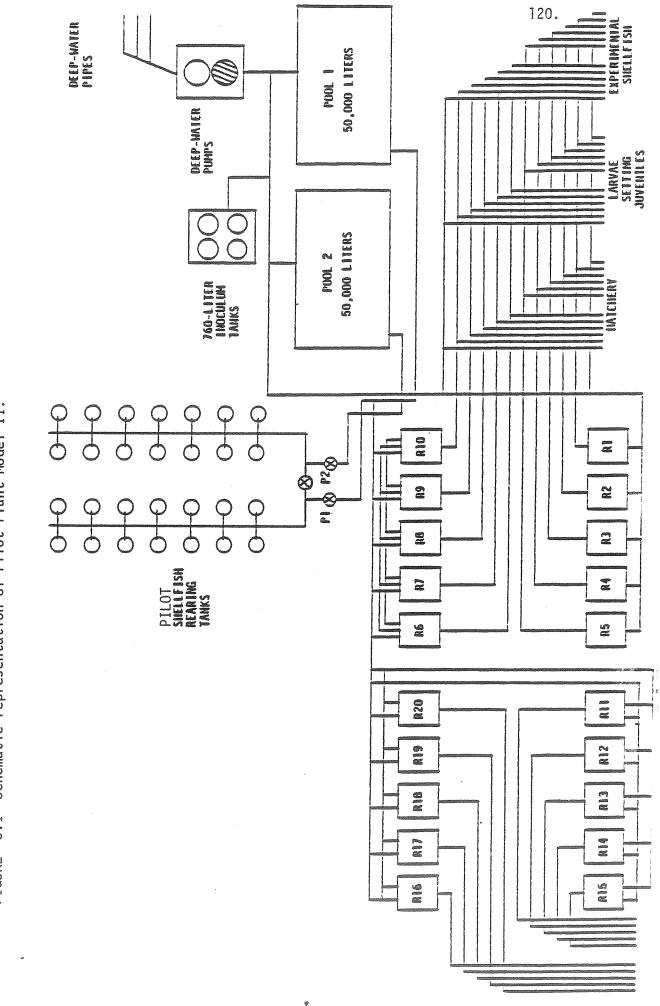


FIGURE 6.1 Schematic representation of Pilot Plant Model II.

Laurence, et al. 1978; also see Rodde et al., 1977). Brood stock animals consisted of three (3) original populations of the clam <a href="Tapes japonica">Tapes japonica</a> (DesHayes) obtained from California and Washington State (Rodde et al., 1977), as well as a number of populations produced from these animals on St. Croix. (Full records of all populations used for the test and of their spawning history is available; the latter data is stored on disk file and magnetic tape).

It is important to note that none of the hatchery procedures were intended to serve as inputs for economic analysis. Although procedures for brood stock and for animals less than 56 days old were standardized, little control was maintained over such parameters as food supply. Aperiodic manipulation of brood stock, larvae and juveniles was permitted to maintain healthy stock. Thus, hatchery procedures were not considered as part of the demonstration test per se. Rather, maintenance of brood stock and production of spat was undertaken for practical considerations and demonstration of the feasibility of hatchery procedures in the subtropical climate was valuable in its own right. This is especially important to bear in mind since growth in the shellfish demonstration plant was clearly affected by the quality and quantity of animals produced by the hatchery. For purposes of later economic analysis, a more dependable and vigorous supply of animals to the grow-out facility was assumed, such as would be obtained if the spat were purchased from outside sources or if the existing hatchery would produce more animals per batch.

The brood stock were housed in fiberglass flumes and fed a mixed

diet of algae grown in continuous culture in 2,000 liter concrete tanks provided with deep sea water. Chaetoceros curvisetus (STX-167), Bellerochea polymorpha (STX-114), Thallasiosira pseudonana (3H) and an unidentified flagellate designated S-1 were used to feed the brood stock. During interruptions in food supply to these animals (these interruptions were not documented), brood stock were either left unfed with flowing sea-water or were supplied the effluent from several tanks in the shellfish demonstration plant.

A particular demand, which shaped a large number of parameters at the second trophic level, was that of introducing populations on a regular, scheduled basis. This was in contrast to the previous years' work in which three (3) populations only of <u>Tapes</u> were grown, introduced simultaneously and this growth monitored for about one full year.

To test the ability of the system to go on a production-line basis, populations were spawned at 28-day intervals, by subjecting selected brood stock animals to thermal shock using a close variant of the Milford method. This procedure did not always result in an adequate number  $(1 \times 10^7)$  of fertilized eggs, in which case, larvae were obtained by subjecting selected pilot plant shellfish to an identical treatment, and/or by obtaining larvae which resulted from spawnings following manipulation of the shellfish tanks (see results, below).

## 6.3.2 LARVAL CULTURING AREA

Larvae were reared at an initial concentration of 10/ml. The animals were housed in 379 liter conical fiberglass tanks and supplied

moderate aeration. The larvae were fed a mixed diet consisting of sterile, laboratory grown cultures of S-1, 3H and STX-114 at an initial concentration of 8 x  $10^4$  to 2 x  $10^5$  cells/ml.

It is important to note that this diet, based on a long series of studies undertaken on <u>Tapes</u> larvae at the St. Croix station, does not include <u>Chaetoceros curvisetus</u> (STX-167). This is important for the following reasons. First, STX-167 was not used because it is apparently too large (probably because of its chain forming tendencies and extensive spine formations) to be ingested efficiently by larvae. However, no precise determination of filtration efficiency with size in <u>Tapes</u> has been accomplished. In St. Croix, STX-167 was fed to the animals beginning on day 56, and the animals were expected to be about 4.3 mm in shell length. The influence of this size, based on the original shellfish technical description (Van Hemelryck, 1978), upon subsequent growth rates is unknown. It should be realized, however, that some of the populations consisting of unusually small animals on day 56 may have continued to grow poorly in part because of this switch to an organism too large for efficient ingestion.

The question of diet for larvae and juvenile animals assumes great importance when considering that only STX-167 grows well on unsupplemented deep water. Thus, a mixed diet requirement for the younger animals also demands a totally different algal culturing system, to which an "enrichment" solution must be added. This point is especially important to consider since there is generally technical and economic pressure to produce the smallest (youngest) animals acceptable by the market place, because of

their high food conversion efficiencies, lower maintenance costs, etc.

The following manipulations of <u>Tapes</u> larvae were carried out as a routine part of hatchery procedure. The algal culture was renewed three times weekly by filtering the larval culture through a graduated series of 3 Nitex sieves. Clumped food and debris were trapped on the top sieve and discarded, while larvae were collected in a 10-12 liter concentrate. Sieve sizes were increased for the subsequent filtration of over 90% of larvae accumulated on the middle sieve.

A random 15 ml sample was taken from the 10-12 liter concentrate and used to monitor growth and survival after each filtration. Ten (10) or more larvae were measured for length and width using an ocular micrometer (Loosanoff et al., 1966). On days 2, 10 and 14, a Sedgwick-Rafter cell was used to determine the concentration of larvae; duplicate or triplicate 1 ml aliquots were counted. Results were used to estimate food requirements for the population as well as to determine survival. Following filtration, the tanks were refilled with deep sea water and streptomycin sulfate ("Vetstrep") was added to  $50 \text{ mg.L}^{-1}$ .

On day 14, the larvae usually began to metamorphose, and were transferred to the juvenile grow-out area.

Larval tank temperatures were not controlled and were monitored frequently. Temperatures ranged from 23 to 25°C. Salinity, also monitored periodically, ranged from 34.75 to 34.95 ppt (also see results below).

# 6.3.3 JUVENILE GROW-OUT AREA

Larvae and juveniles were transferred to the juvenile grow-out area on day 14, regardless of size or condition. This area

consisted of two (2) fiberglass flumes, each measuring 30.5 x 3.1 x 14 cm. From  $1.0 - 1.5 \times 10^6$  larvae were placed in each setting flume and streptomycin and food were added. Up to the age of 21 days, the larvae/juveniles were filtered three times weekly and batch fed the mixed diet of laboratory-reared algae. By day 21, most of the larvae had metamorphosed, and the flume was placed on a continuous flow of algal culture from two or three of the outdoor, 2000-1 culture vessels containing STX-114, S-1 and 3-H. (All unmetamorphosed animals were therefore washed down the drain). Food densities in the reactors were sampled infrequently and fluctuated considerably. A range of  $7.1 \times 10^4$  to above  $1.0 \times 10^6$  cells per ml was recorded. Prior to 02/18/78, total flow to each flume from the reactors was 1.38 liters/minute for animals 21-35 days old, and 2.76 - 4.14 liters per/minute (depending on population size and growth rate, and concentration of cells in the food supply), for animals 35 - 56 days old.

Because of observations which indicated that population growth rates at this age were food-limited, the flow rates were increased on 02/18/78 to 2.76  $\ell$ /min and 5.52  $\ell$ /min over days 21-35 and 35-56 respectively.

Three air lines were placed in each flume beginning on day 14 to aid in mixing, and the flumes were cleaned three times weekly. Populations were weighed on days 35 and 56, to the nearest .1 gram. Animals were patted dry and weighed on a Mettler balance.

## 6.3.4 PILOT SHELLFISH AREA

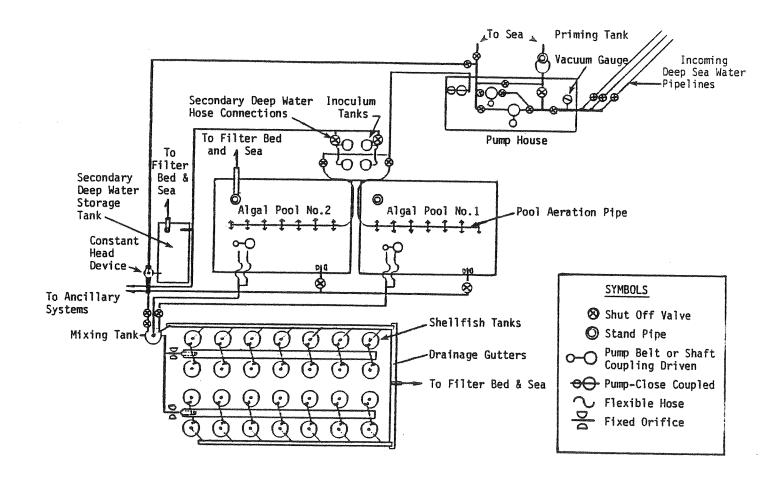
Because of its central importance, the pilot shellfish area will be described in some detail.

Figures 6.2, 6.3 and 6.4 illustrate the system used. As indicated in Figure 6.5, algal culture from the two pools was pumped into a 100-liter capacity polyethylene tank. This mixing tank could also accept deep sea water directly, when one or both pools was offline, to maintain a constant flow of water to the shellfish. The mixing tank was located in the shellfish wet lab and was elevated above the shellfish tanks. The mixing tank was covered with opaque polyethylene film to prevent the entry of sunlight and thus of the growth of algae. The tank was cleaned once weekly (Tuesdays).

The algal culture from the mixing container was fed into a PVC header and divided equally into two (2) parts. One-half of the flow went into each one of two troughs. Flow into the trough was regulated by a fixed diameter orifice. Each of the two troughs (designated north and south) delivered water to fourteen (14) tanks, consisting of seven (7) pairs of tanks situated opposite one another. Water from the trough flowed down into a PVC "tee" and out two fixed-diameter orifices and into the shellfish tanks. As shown in Figure 6.3, the water entered the tank at an angle, thus imparting a circular flow to water in the tank and aiding in circulation and cleaning. The headers and trough were cleaned once weekly (Tuesdays) with a stiff plastic brush. At no time were any chemicals used to clean components in the shellfish pilot plant.

The twenty-eight (28) shellfish tanks were each circular in shape (diameter = 26" = 66.04 cm) with a flat bottom and area of 530.9 inches<sup>2</sup> or 3425.3 cm<sup>2</sup>. A removable standpipe of 1/2" PVC was placed in the center of the tank and maintained water level at 9 cm depth. The water flowed

FIGURE 6.2 SCHEMATIC OF THE DEEP WATER DELIVERY SYSTEM TO THE MARICULTURE FACILITY



-SHELL FISH THINK LID 6.000 SECTION B-13 10 6 PUC PIPE I'G' -45° ELECTRICAL SWEEP 0 SECTION A-A

FIGURE 6.3 DETAILS OF THE PILOT-PLANT SHELLFISH AREA.

FIGURE 6.4 FIBERGLASS SHELLFISH TANK

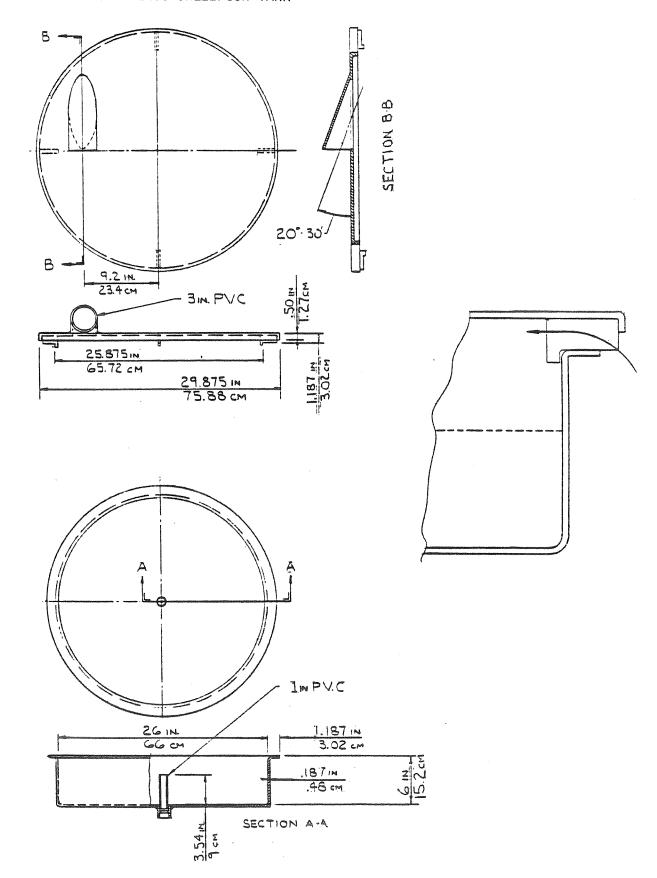
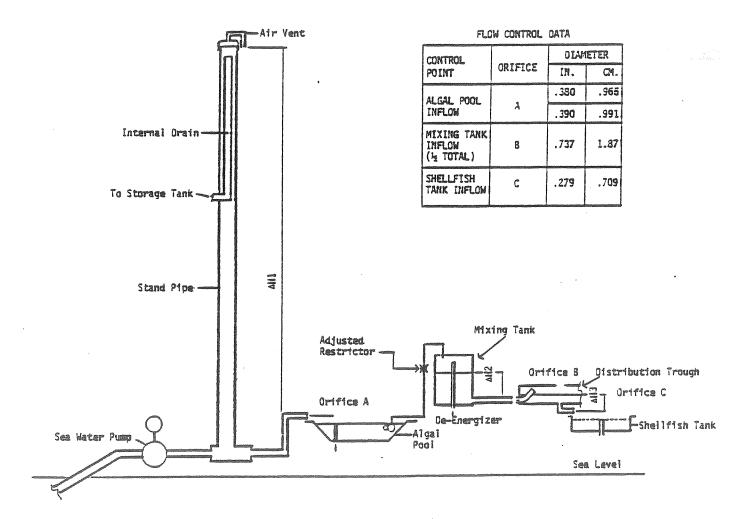


FIGURE 6.5 DIAGRAM OF PUMPING SYSTEM

# NOT TO SCALE



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out the standpipe to a fiberglass trough and then out to sea. The tanks were not aerated at any time. This elimination of air supply to the shellfish was a product of the economic evaluation of the Model I shellfish pilot plant. That analysis indicated that air costs were a significant portion of the operating costs. Tanks were scrubbed and rinsed clean every two (2) weeks only (Tuesdays). If bacteria were observed in the tank (this occurred rarely), the tank was placed in full sunshine for about one hour and re-rinsed. No other cleaning was done. Cleaning was only done on regular, scheduled days regardless of the condition of the population.

Probably the most notable feature of the shellfish systems was the use of a low-head constant-flow food delivery system. The low-head employed (11 cm. between top of water in the trough and exit point in the PVC "tee") allowed for a very even distribution of water to all of the tanks. Slight variations in flow to one or more tanks was quickly compensated for. Flow-rates into each tank were measured three times weekly by collecting tank effluent in a graduate cylinder for a 30-second period. Flow-rates were adjusted by rotating the PVC cap at the end of each "tee"; since the orifice was off-center, this had the effect of changing the head and therefore the flow-rate. With all 28 tanks operating, nominal flow to each was 47.14 mls/sec.

## 6.3.4.1 SHELLFISH TRANSFER PROCEDURES

The goal of introducing a new population of <a href="Tapes">Tapes</a> juveniles into the plant every 28 days combined with the limited

amount of culture available (an absolute maximum of 140,000 liters/day) required that each population be moved successively through the plant, and be harvested after 266 or 280 days in the plant.

Maintaining a constant and identical flow rate to all tanks permitted a simple means of maintaining flow rate control, but required periodic culling of the tank populations to maintain an optimum feeding rate to the shellfish. The populations were manipulated according to predictions of feeding and growth as described in the shellfish Technical Description (see Van Hemelryck, Laurence, et al., 1978). This quantitative description was based in part on the results of earlier feeding studies undertaken in St. Croix (see Roels, et al., 1978). The original technical description assumed that growth was a function of food concentration (protein/ml/sec) and the area of the animal ( $W^{0.66}$ ), where W = whole wet weight (g).

Assuming that the amount of food available remained constant at  $21.4~\mu gat~L^{-1}$  of protein-nitrogen, this equation permitted a constant relation between water flow and tray area. Based on the technical description, the ration allowed for each  $2550~cm^2$  tray was supplied at the nominal flow rate of 47.14~ml/sec. This assumes that the animals in the tray have a combined area of  $2550~cm^2$ , and are distributed in a monolayer. To provide some flexibility in the number of animals which could be placed in each tray, the tray area was increased to  $3380~cm^2$ . Thus, each population covered only about 2/3 of the bottom of the tray.

Since it was assumed that the animals fed according to an area ( $W^{0.66}$ ) function, the growing populations were culled back periodically to a constant area of 2550 cm<sup>2</sup>. The actual procedures used were as follows:

On day 56, when the animals were expected to be .01 g in whole wet weight and 4.3 mm in shell length each, 200 g (20,000 animals) were placed in tray #1, where they remained for 14 days. Because the animals were very small and fast growing at this stage, this was intended to ensure that the animals were over fed (otherwise, they would soon outstrip the food supply). About 50% of the food supplied to tray #1 was assumed to be in excess and went down the drain.

On day 70, the population was transferred to Tank #2, where it was also over fed for the following 14 days.

On day 84, the animals were considered sufficiently large to obtain accurate numbers and individual whole wet weights. Ten (10) subsamples of 100 animals each were weighed to the nearest .0001 g (Mettler balance). The entire population was then patted dry and weighed to the nearest .1 g; the subsample mean individual whole wet weight was divided into the population wet weight to obtain a population number.

The population was then divided into two (2) portions and placed into trays #3 and #4 for 14 days. The proper whole wet weight of the population assigned to each tray was determined on the basis of an

equation derived from the technical description in which  $F = .0185 \text{No.(wo)}^{2/3}$ 

where F = flow rate in ml/sec. (assumes protein-nitrogen in the food supply of 21.14  $\mu$ gat L<sup>-1</sup>); N<sub>O</sub> = number of animals on day 0 of each 14 day period and w<sub>O</sub> = mean individual whole wet weight, day 0.

Since F = 47.14 ml/sec and the mean individual whole wet weight  $(w_0)$  of the population was known, the number  $(N_0)$  and therefore, the whole wet weight of the population  $(W_0 = N_0(w_0))$  could be calculated.

The constant in the equation (K = .0185) was derived empirically during November-December 1975 feeding trials (Roels, et al., 1977). The feeding criterion used was determined on the basis of some computer runs using the Aquaculture Budget Generator (ABG) which indicated that a compromise between high stripping efficiency (and thus of gross growth efficiency) and individual animal size would be economically optimum. The value used for the feeding criterion (K = .0185) predicted a stripping efficiency of 88% and a gross growth efficiency of 27%. Thus, the animals were not fed to maximize either conversion efficiency or individual growth rate.

On day 84, prior to being transferred to two (2) trays, the population was sieved and the smaller animals discarded if the total weight available exceeded the pre-determined loading capacity of the trays. (If this occurred, a new individual weight and number for the population was determined.) On all subsequent transfer days, however, a randomly selected weight of animals were culled, and no more selective

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## sieving was performed

When 98 days of age, the animals were divided into three (3) trays, on day 112 into four (4) trays. From then on, the population remained in four (4) trays. Instead of allowing the population to expand to more trays, the animals were culled to maintain a constant area.

Full harvesting of each population began 210 days after introduction to the plant (266 days old) when two (2) of the four tank populations were removed. The remaining two tanks of the population were harvested 14 days later (280 days old).

Fluctuations in the concentration of algal protein-nitrogen in the food supply were not used to alter the weight of animals per tray. Thus, changes in protein-N concentration due to pool crash or diurnal fluctuations in algal concentration represented an unstable input within the context of relatively invariant water flow to population area (F = .0185  $N_O(w)^{0.66}$ ) ratio.

Since the area of each population in the pilot plant was held constant (except for populations 56-84 days of age which were over fed), the number of animals per tray decreased in inverse proportion to the age (Ag) of the animals and the total weight per tray (W); Ag and W covaried.

These standard procedures allowed for invariancy in control of pilot plant flow rate, manipulation of populations through the plant, and systematic observations (as described more fully below). Thus, the

demonstration feasibility test could operate with a minimum of labor and with no disturbance of the animals except at 14-day intervals. At all times, it was considered more important to let actual failures in control disrupt the ingestion of food and of subsequent growth rather than provide emergency support. This was done to eliminate unforeseen labor and maintenance costs in scaled-up operations.

Conversely, the facility could not provide systematic variation among all relevant variables for a complete test of the technical description. Discrepancies between predicted and actual weight gains as a function of area were used to generate elaborations upon the original technical description, but could not test differential predictions of the influence of say, age and mean whole wet weight, since these variables co-varied in the pilot plant. Food function variables were also purposefully compounded as all populations regardless of age, weight or number were fed an identical amount of food.

Other points relevant to the analysis of data collected in the pilot plant are discussed more fully below.

Each tray population was weighed at 14 day intervals. Original and final weights for each 14-day period were recorded, as were the number of dead and their weight, and whether or not spawning occurred. This latter observation was actually made on transfer days but was sometimes observed during the daily visual inspection of the plant.

## 6.3.4.2 SHELLFISH "NITROGEN" BUDGET

Understanding and accounting of the two-trophic level food chain were expressed through predictions and analyses of nitrogen

transfer through the algae and shellfish.

At the shellfish stage, inflow nitrogen was measured on Mondays, Wednesdays and Fridays at 1400 hours. Replicate samples for particulate protein-nitrogen (PPN) analyses were carried out on 75 ml of culture. The culture was filtered at low (<8" Hg) vacuum and the algae collected on a 25 mm Gelman A-E filter. The filters were placed in test tubes and frozen for later analysis using a modified Lowry technique, (see Dorsey, et al. 1977). Filtered culture water was frozen in glass and plastic bottles for later analysis of dissolved inorganic nitrate and nitrite ( $NO_3^- + NO_2^-$ )-N and for ammonia ( $NH_4^+ + NH_3$ )-N, using a Technicon Autoanalyzer II system and standard Technicon manifolds.

The effluent from each shellfish population in the plant (each population occupied 1-4 trays, and some of the earlier populations from the Model I plant occupied up to 8 trays each) was collected on Monday, Wednesday and Friday at 14:00 hours and were analyzed in replicate for particulate PPN,  $(NO_3^- + NO_2^-)-N$  and for  $(NH_4^+ + NH_3)-N$ .

A subsample of twenty-five (25) animals from each population was taken every 14 days (culled animals at transfer day were used). The animals were patted dry, and measured individually for shell length, width and depth (mm) (Loosanoff, et al., 1966). Dry shell weights, wet meat and dry meat weights were obtained (see Laurence and Roels, Sea Grant Progress Report 1976 Appendix B for details). The dried meat

was pulverized and its protein content was determined using the modified Lowry technique of Dorsey et al., 1977.

The amount of PPN remaining in each tank at the end of the 14-day period was estimated by determining PPN in randomly selected, diluted aliquots. Generally, this PPN fraction, assumed to be unassimilated algal protein-nitrogen, was very low, and often below levels of detection.

Tank temperatures were taken daily with a hand held thermometer.

In addition to these routine observations, a large number of aperiodic measurements were taken. Salinity, pH, and  $0_2$  concentration were measured at various locations in the plant. Measurements of Chl <u>a</u> concentration and of dissolved organic nitrogen (D.O.N.), and of  $P0_4^{3-}$  and  $Si0_4^{4-}$  concentration were also made occasionally. The inflow and outflow of each population in the plant were sampled over 24-hour intervals twice during the year. Randomly selected animals from various populations in the plant were also subjected to separate study of their  $0_2$  consumption, food stripping rates as a function of food concentration, and ammonia tolerance. These data are not included in this report.

### . 6.3.4.3 DATA MANAGEMENT

All routine observations were catalogued and numerical equivalents placed on appropriate data collection sheets (see Appendix A). Manual transformation of these data were used to generate Quarterly Reports and staff updates.

Following verification of all data points, data were entered into tape and disk file on a Hewlett-Packard HP9825A minicomputer system. The computerized data file is shown in Appendix A. All data entries were verified against original data sheets. Data files and access programs were tabulated and all routine observations on the one-year (10/11/77 - 10/10/78) operation of the plant are immediately available in machine readable format.

# 6.4 RESULTS OBTAINED IN THE MODEL II SHELLFISH PILOT PLANT 6.4.1 HATCHERY

The results obtained in the shellfish hatchery are written up for publication. A copy of the manuscript is enclosed as Appendix D.

# 6.4.2 SPAT SUPPLY TO PILOT PLANT MODEL II

The hatchery was operated for practical purposes only and was not included in the analysis of pilot shellfish data, nor were any of the results incorporated within the shellfish technical description or the computer based Aquaculture Budget Generator. Generally, the reason for this omission is that, due to the small numbers of animals required, labor intensive hatchery techniques were used on St. Croix. The size of a hatchery required for Artificial Upwelling mariculture at commercial scale would require mechanization of procedures, or the spat could be purchased from outside sources. Here, only general results of the hatchery operations are provided. A complete treatment of hatchery operations may be found in Sunderlin, Laurence and Roels, "Spawning and Growth of the clam Tapes japonica in a Subtropical Hatchery", in Appendix D.

Some basic characteristics of populations spawned and introduced into the pilot plant are shown in Table 6.2

A total of eighteen (18) populations spawned in the hatchery between 7/76 and 7/78 were introduced into the Model II pilot plant.

The larval period for these populations averaged 14 days, and mean survival to metamorphosis was 51%.

The growth rates of successive populations were highly consistent as indicated by shell length and width measurements taken on day 14 shown in Table 6.2. Length/width ratios were also highly consistent for larval animals as shown in Figure 6.6. These data are virtually identical to measurements taken by Loosanoff, et al. 1966 on Tapes.

Tables 6.3 through 6.23 supply some basic growth rate information (shell length plus width measurements) for the eighteen populations.

Referring again to Table 6.2 for populations <u>Tapes</u> 34 through <u>Tapes</u> 43, the weights produced on Day 35 and Day 56 were erratic. The disappointing weights for populations #42 and #43 were specifically believed to be related to food (poor quality reactor cultures, primarily S-1) and lower temperatures in the winter (23°C versus 25-27°C in summer) contributing to slower growth.

Consequently, on February 18, 1978, starting with <u>Tapes</u> 44, several changes were made in the hatchery procedures:

(1) At least  $6 - 8 \times 10^6$  fertilized eggs were obtained on spawning days.

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OT PLANT	AVERAGE	WEIGHT PER CLAM		0.0786	0.0238	0.0222	!!	1 1	0.0118	0.0010	0.0100	0.0036	0.0063	0.0050	0.0055	0.0066	0.0114	0.0373	0.0244	0 0404	
INTRODUCED TO PILOT PLANT	A MILITAN L'D	CLAMS		9,156	9,000	9,000	1	1	12,000	142,000	25,000	47,500	13,500	5,312	29,167	19,375	21,493	9.722	22.6	9 886	2000
INTRODU	HEIVIT	(m5)		720	215	200	!	!	142	142	250	175	98	27	162	128	246.6	363	200	400	2 !
DAY 56	THEIGHT			-	1 1	1 1	4	166.4	642.3**	142.0***	1434.79	327.5	86.48	27.0	162.33	128.16	752.25	1325.4	2722.43	2994 45	5026.48
DAY 35	UFICUT	(gm)	-	o.	n. a	sutwitte	WC02000		-	<20.0		-	TO MENT OF THE	No.	KKOIOW	on the same of		-	Carry State	Constant or the last of the la	93.84
	(microns)	RANGE		٠	+ 228.5	+ 238.0	→ 219.0	→ 209.4	+ 223.7	+ 199.9	<b>+ 236.1</b>	→ 214.2	÷	٠	+	٠	٠	٠	٠	+	152.3 + 209.4
	MIDTH (m)	SD		16.66	24.09	30.08	11.61	7.52	8.94	9.71	13.23	9.04	14.56	15.89	18.65	7.47	10.34	11.97	16.94	15.99	17.99
14		×	(	198.4	201.6	178.5	206.7	197.1	202.7	184.7	214.5	200.9	217.2	211.3	206.5	198.1	207.7	222.9	187.1	211.7	196.3
DAY 14	i(crons)	RANGE		٠	٠	٠	٠	٠	*	+	٠	+	+	195.2 + 247.5	٠	٠	†	ተ	÷	÷	161.8 + 219.0
	LENGTH (micro	SD	f	17.52	28.27	33.42	10.66	8.09	6.56	8.09	7.23	8.75	17.42	13.99	18.84	6.80	9.77	10.34	15.99	16.66	17.80
		×	ř	207.4	212.5	194.1	213.3	206.4	210.6	195.2	215.8	206.4	224.2	221.7	219.0	8.80	215.6	232.0	206.1	219.0	206.1
% SHRVIVAL	TO MFTA.		ć	2) 2)	7	10	63	57	71	59	26	06	28	38	46	(M)	,	99	40	23	~
	3	(105)	S	36	5.52	13.84	29.84	16.40	12.78	18.56	7.62	30.96	4.32	96.9	13.76	25.40	27.40	31.90	19.20	15.80	27.40
		GENERATION	-	ي ت ت	250	3rd	- S	St	- S.	mix (1&3)	400	St	4	2nd	2nd	mix (18.4)	Sud	Sta	mix (1&3)	چ د د	mix (183)
	TAPES	POPULATION	c	200		22	Ť	32	37	36	40		2	(L)	44	ه د د	46		\$	9	20

\* Culled to 34.0 \*\* (D57) \*\*\* (D55) Tapes 20, 21 and 22 were spawned on 8/3/76, 8/10/76 and 8/24/76 respectively and were reared in the 1976-77 (Model I) pilot plant and transferred to the Model II configuration 10/04/77.

Iapes 34 and Iapes 35 were spawned on 5/23/77 and 6/17/77 and introduced into the Model I plant and transferred to Model II on 10/04/77. Tapes 36 was a poor population and was not introduced to the pilot plant. Tapes 37 was the first population which spent days 56 ff entirely in the Model II pilot plant. Excess animals from Tapes 37 were used to occupy the tray positions intended for Tapes 36. NOTE:

Populations 20, 21 and 22 were not introduced on day 56 into pilot plant Model I, whereas populations 37-50 were introduced on day 56 into pilot plant Model II.

FIGURE 6.6 LENGTH: WIDTH RATIOS TAKEN ON DAY 14 OF ALL TAPES JAPONICA
POPULATIONS INTRODUCED IN PILOT PLANT MODEL II TAKEN
ON DAY 14.

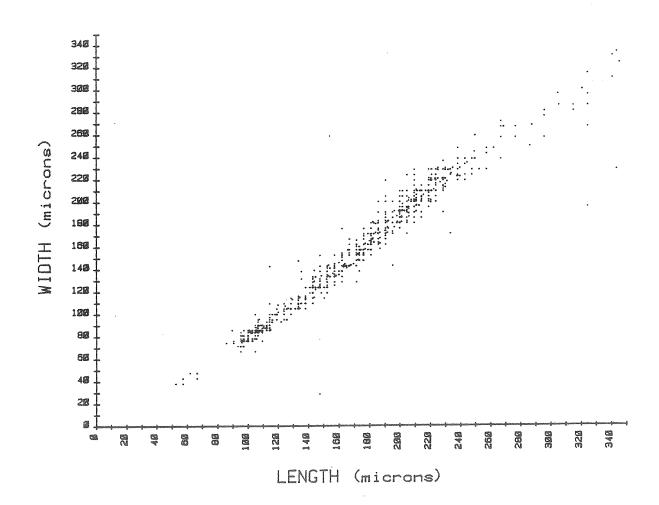


TABLE 6.3 LENGTH AND WIDTH OF CLAM LARVAE AND SPAT

Date Spawned Aus. 3,1976 Population # 20 Maximum values: for Hatchery data N=10

# HATCHERY

H∋e(da 1	r) 1(um) 97.10	
, mary Sampa	109.48	90.44
3	121.86	99.96
6	163.74	144.70
l'	209.44	190.40
10	223.72	209.44
13	228.48	217.06
15	238.00	228.48
17	261.80	247.52
20	323.68	295.12
22	437.92	437.92
27	720.00	660.00
29	740.00	680.00
31	890.00	780.00
34	920.00	840.00
36	1320.00	1140.00
38	1240.00	1060.00
41	2100.00	1700.00
43	1400.00	1180.00
49	3100.00	2360.00
57	2400.00	1860.00

TABLE 6.4 LENGTH AND WIDTH OF CLAM LARVAE AND SPAT

Date Spawned Aus. 16,1976 Population # 21 Maximum values: for Hatchery data N=10

# HATCHERY

Ase(day)	1(um) 99.96	w(um) 80.92
ente Comme	109.48	37.53
i.	133.28	112.34
· ·	178,98	161.84
1	199.92	190.40
11	220.86	214.20
14	257.04	228.48
16	285.60	271.32
18	399.84	371.28
	504.56	466.48
	540.00	500.00
28	820.00	700.00
30	940.00	820.00
32	940.00	830.00
37	920.00	860.00
45	1820.00	1460.00

TABLE 6.5 LENGTH AND WIDTH OF CLAM LARVAE AND SPAT

Date Spawned Aug. 24,1976 Population # 22 Maximum values: for Hatchery data N=10

# HATCHERY

Ase(day)	l(um) 99.96	w(um) 85.68
C) None	109.48	90.44
6	152.32	131.38
3	180.88	171.36
10	233.24	171.36
13	199.92	190.40
15	266.56	238.00
17	295.12	276.08
20	440.00	400.00
22	560.00	500.00
22	720.00	640.00
37	1720.00	1440.00

Date Spawned Aus. 31,1976
Population # 23
Maximum Values: for Hatchery data N=10

## HATCHERY

Age(day) 1	l(um) 102.82	w(um) 78.06
, con , co bosse	114.24	90.44
Ö	147.56	123.76
8	154.22	133.28
10	166.60	152.32
13	190.40	180.88
15	209.44	195.16

# TABLE 6.7 LENGTH AND WIDTH OF CLAM LARVAE AND SPAT

Date Spawned May 23,1977

Population # 34

Maximum values: for Hatchery data N=10

Ase(day)	l(um) 114.24	w(um) 90.44
7	130.88	157.08
11	209.44	195.16
13	223.72	209.44
1.5	229.48	218.96

# TABLE 6.8 LENGTH AND WIDTH OF CLAM LARVAE AND SPAT

Date Spawned June 17,1977 Population # 35 Maximum values: for Hatchery data N=10

## HATCHERY

Нав(qax)	l(um) 109.48	⊍(um) 90.44
10	204.68	190.40
12	214.20	199.92
14	218.96	209.44

# TABLE 6.9 LENGTH AND WIDTH OF CLAM LARVAE AND SPAT

Date Spawned July 18,1977 Population # 36 Maximum values: for Hatchery data N=10

ਜਿਵਦ(day) 2	l(um) 112.34	w(um) 93.30
4	138.04	114.24
7	171.36	142.80
9	180.88	164.70
11	176.12	161.84
14	180.88	171.36
15	195.16	180.88
13	204.68	204.68

## TABLE 6.10 | LENGTH AND WIDTH OF CLAM LARVAE AND SPAT

Date Spawned Aus. 15,1977 Population # 37 Maximum values: for Hatchery data N=10

## HATCHERY

(Yab)sefA 2	l(um) 106.62	w(um) 85.68
4	133.28	196.62
Э	204.68	195.16
11	204.68	204.68
14	213.36	214.20
16	238.00	223.72
23	514.08	456.96

# TABLE 6.11 LENGTH AND WIDTH OF CLAM LARVAE AND SPAT

Date Spawned Sept. 14,1977 Population # 39 Maximum values: for Hatchery data N=10

Ase(day)	l(um) 112.34	w(um) 93.30
5	157.08	138.04
, i	195.16	173.26
9	199.92	190.40
14	223.72	209.44
31	228.48	223.72

# TABLE 6.12 LENGTH AND WIDTH OF CLAM LARVAE AND SPAT

Date Spawned Oct. 11,1977 Population # 40 Maximum values: for Hatchery data N=10

# HATCHERY

Нэе(day) З	l(um) 114.24	w(um) 109.48
6	180.88	171.36
3	199.92	199.92
13	228.48	236.10

# TABLE 6.13 LENGTH AND WIDTH OF CLAM LARVAE AND SPAT

Date Spawned Nov. 7,1977 Population # 41 Maximum values: for Hatchery data N=10

(Yab)seft S	l(um) 112.34	w(um) 90.44
4	142.80	109.48
7	185.64	171.36
12	218.96	214.20

## TABLE 6.14 LENGTH AND WIDTH OF CLAM LARVAE AND SPAT

Date Spawned Dec. 6,1977
Population # 42
Maximum values: for Hatchery data N=10

## HATCHERY

Age(day) 4	l(um) 128.52	w(um) 104.72
Ë	152.32	133.28
9	180.88	176.12
15	247.52	238.00

# TABLE 6.15 LENGTH AND WIDTH OF CLAM LARVAE AND SPAT

Date Spawned Jan. 2,1978

Population # 43

Maximum values: for Hatchery data N=10

Aae(day) 2	l(um) 114.24	w(um) 90.44
9	195.16	130.88
	204.58	199.92
16	242.76	233.24

TABLE - 6.16 LENGTH AND WIDTH OF CLAM LARVAE AND SPAT

Date Spawned Jan. 31,1978
Population # 44
Maximum values: for Hatchery data N=10

## HATCHERY

Aas(day) 2	1(um) 104.72	w(um) 85.68
4	133.28	109.48
7	190.40	176.12
11	218.96	209.44
13	214.20	209.44
15	238.00	228.48

# TABLE 6.17 LENGTH AND WIDTH OF CLAM LARVAE AND SPAT

Date Spawned Feb. 27,1978

Population # 45

Maximum values: for Hatchery data N=10

Ase(day) 2	l(um) 114.96	w(um) 95.30
9	196.39	180.10
12	210.76	191.50
16	210.76	205.97

# TABLE 6.18 - LENGTH AND WIDTH OF CLAM LARVAE AND SPAT

Date Spawned March 27,1978

Population # 46

Maximum values: for Hatchery data N=10

## HATCHERY

Ase(day) 5	l(um) 162.86	w(um) 141.78
	220.34	199.26
1:3		220.34
,	244.29	234.71

# TABLE 6.19 LENGTH AND WIDTH OF CLAM LARVAE AND SPAT

Date Spawned April 25,1978

Population # 47

Maximum values: for Hatchery data N=10

Hae (day)	l(um)	w(um)
1	105.38	38.14
5	136.81	167.65
4 4	229.92	229.92
16	249.08	244.29

# TABLE 6.20 LENGTH AND WIDTH OF CLAM LARVAE AND SPAT

Date Spawned May 23,1978 Population # 48 Maximum values: for Hatchery data N=10

# HATCHERY

Aae(day) 3	l(um) 56.64	ω(um) 47.60
10	180.88	180.88
15	223.72	204.68
20	223.72	223.72

# TABLE 6.21 LENGTH AND WIDTH OF CLAM LARVAE AND SPAT

Date Spawned July 17,1978 Population # 50 Maximum values: for Hatchery data N=10

Age(day) 2	l(um) 112.34	w(um) 90.44
.5	157.08	140.90
9	209.44	192.30
12	218.96	209.44
19	257.04	247.52

# TABLE 6.22 LENGTH AND WIDTH OF CLAM LARVAE AND SPAT

Date Spawned June 19,1978

Population # 49

Maximum values: for Hatchery data N=10

## HATCHERY

Age(day) 2	l(um) 114.24	w(um) 95.20
5	166.60	142.80
ġ	214.20	199.92
12	238.00	238.00
16	247.52	238.00

# TABLE 6.23 LENGTH AND WIDTH OF CLAM LARVAE AND SPAT

Date Spawned July 17,1978

Population # 50

Maximum values: for Hatchery data N=10

Hae(day)	l(um) 112.34	w(um) 90.44
ente len 'uni	157.08	140.90
9	209.44	192.30
12	218.96	209.44
13	257.04	247.52

- (2) On Day 14, the population was culled to 2 3 x  $10^6$  and 1  $1.5 \times 10^6$  larvae were placed in each of two setting flumes.
- (3) On Day 21, the two flumes were combined when placed on continuous flow and the food flow to the flume was increased by a factor of 2.
  - (4) On Day 35, the population was culled to 25 gm.
- (5) On Day 56, was culled by sieving and 200 gm of the largest clams were placed in the pilot plant.

It was expected that on Day 56 the hatchery would provide a homogenously distribued population of 20,000 - 25,000 animals, each 4.3 mm in shell length and 0.01 gm in individual whole wet weight. Ten (10) populations of 18 were composed of individuals weighing 0.01 gm or more on day 56. The last four populations (<u>Tapes</u> 47 through <u>Tapes</u> 50) exceeded this weight two or three times and averaged over 7 mm in length (Table 6.2).

One disappointment was the number of induced spawnings (11 out of 18) versus the number of spontaneous spawnings required for the 18 populations destined for the pilot plant. Brood stock were not always ripe, primarily due to inadequate holding tank facilities and lack of constant food supply. We did learn over the last two years that Tapes grown in the pilot plant will undergo gametogenesis and can be easily spawned by manipulating temperature, flow rate or a variety of stress-inducing variables. Clams can be kept in a ripe condition at all times if sufficient care and over feeding is maintained. No artificial lowering of temperature

below the 21 - 23<sup>o</sup>C range supplied by the deep water is necessary.

Regular cleaning of the brood stock facilities is very important.

It is clear that a separate brood stock facility is not required and enough animals of a pre-selected size, condition and genetic history can be removed from the pilot plant and induced to spawn. In the present pilot plant, such a procedure has provided spat more reliably than has the use of a separate brood stock operation.

# 6.4.3 SHELLFISH ALLOMETRY

Extensive data were collected on shell length, width and depth, and on mean whole wet weight, wet mean weight, dry shell and dry meat weight, and protein-nitrogen content of the meat of populations 34 through 50 (for similar data collected on populations 20, 21 & 22, see our 1976 - 1977 Sea Grant Progress Report). The data displayed in Tables 6.24 - 6.39 reflect mean values for 25 animals selected from each population at biweekly intervals. Allometric relations were very consistent, indicative of reliable sampling and measurement procedures.

Figure 6.7 illustrates interrelationships among the three shell dimension measurements. For these plots, raw data (n > 4000) were used. Note the high degree of consistency in the values and the generally linear relationship between length and width ( $r^2 = 0.98$ ). Depth, however, displays a non-linear relationship with length and width; in general the organism's thickness grows at an accelerated rate in comparison with length and width as a function of age. Variation in these depth/length

# TABLE 6.24

Date Spawned May 23,1977 Population # 34 All values=mean,+-S.D.

Age (in days)	1(mm)	w(mm)	d(mm)	Н	MM(a)	(e)MW	DM(a)	DS(a)	PRT.(9)	PPN(a)
141	16.34 2.73	-1.00 -1.00	-1.00 -1.00	25	0.7644	0.3617	0.0594	0.4027	0.02139	0.00342
155	17.67 3.02	-1.00 -1.00	-1.00 -1.00	29	9.9581	0.4570	0.0760	0.5012	0.02643	0.00423
169	17.72	-1.00 -1.00	-1.00 -1.00	25	1.0962	-1.0000	-1.0000	-1.0000	-1.00000-	-1.00000
183	18.31	-1.00 -1.00	-1.00 -1.00	50	0.6027	-1.0000	-1.0000-	-1.0000	-1.00000-	-1.00000
197	19.68	13.54	8.19 1.87	25	1.6521	0.7597	0.1202	0.3924	0.05481	0.00877
211	21.59	14.75 2.24	9.18 1.94	25	2.0981	0.8714	0.1766	1.2268	0.07175	0.01148
225	21.74	15.26 2.36	8.90 1.56	25	1.9960	0.8828	0.1616	1.1132	0.07033	0.01125
239	20.96	14.16	3.56 2.24	25	1.8968	0.9598	0.1524	0.9370	0.07237	0.01158
253	22.40	15.67 2.28	9.86 2.05	25	2.4491	1.1112	0.1458	1.3380	0.07638	0.01222
267	25.59 3.82	17.51 2.77	11.12	23	3.6902	1.6900	0.2744	2.0002	0.12849	0.02056

TABLE 6.25

Date Spawned June 17,1977 Population # 35 All values=mean,++3.B.

Hee (in days)	1(mm)	w(mm)	d(mm)	Н	(e)WW	WM(∍)	DM(a)	DS(a)	PRT.(3)	PPN(#)
130	15.10	10.40	5.30	54	0.5245	0.2558	0.0409	0.2687	0.01690	0.00270
144	2.97 18.97	2.05 12.57	1.38 7.48	100	1.0727	0.5362	0.0816	0.5365	0.03308	0.00529
158	3.31 19.75	1.83 13.45	1.69 7.92	100	0.9635	0.4733	0.0783	0.4902	0.03621	0.00579
172	2.51 19.29	1.65 13.19	1.24 7.94	25	1.4465	0.6933	0.1107	0.7533	0.04642	0.00743
186	3.60 19.73	2.32 13.28	1.63 7.82	25	1.4584	0.7337	0.1211	0.7247	0.06092	0.00975
200	3.13 19.82	2.07 13.90	1.63 8.19	25	1.6722	0.8164	0.1529	0.8558	0.07085	0.01134
214	3.90 19.63	2.70 12.96	2.30 7.46	25	1.3170	0.7147	0.1024	0.6023	0.04520	0.00723
228	3.27	2.19 15.22	1.49 9.55	25	2.2611	1.0832	0.1695	1.1779	0.07363	0.01178
	2.68	2.01	1.46 10.97			1.6827		1.5852	0.13538	0.02166
242	2.62	2.14	1.42						0.08134	0.01302
25 <del>6</del>	24.54	16.29 2.38	2.15						0.07872	
270	23.18 3.59	15.50 2.56	9.65			1.2328			0.08245	
284	22.43 4.07	15.12 2.64	9.65 2.61	25	2.5096	1.2121	0.1742	1.47(5	9.90240	0.01017

# TABLE 6.26

Date Spawned July 18,1977 Population # 36 All values=mean,+-S.B.

Age (in days)	I(mm)	w(mm)	d(mm)	Н	MM(a)	(e)MW	DM(⊕)	DS(9)	PRT.(9)	PPN(∌)
àà	17.32	11.75 2.05	6.99 1.55	32	0.9114	0.4489	0.0698	0.4625	0.02938	0.00470
113	13.10 2.36	8.43 1.21	4.66 0.98	143	0.1877	0.1055	0.0120	0.0821	0.00517	0.00083
169	16.80 3.42	11.18	6.36 1.47	25	0.8839	-1.0000-	-1.0000-	-1.0000	-1.00000-	1.00000

TABLE 6.27

Date Spawned Aus. 15,1977 Population # 37 All values=mean,++8.D.

Age (in days)	1 (mm)	w(mm)	d(mm)	Н	MM(a)	(E)MW	(e)MU	DS(a)	PRT.(4)	PPM(3)
57	3.82	-1.60	-1.00	25	1.1606	0.6839	0.0881	0.4767	0.02611	0.00418
71	1.49	0.00 5.74	-1.00 3.04 0.50	323	0.0702	0.0390	0.0052	0.0312	0.00228	0.00036
35	1.52 13.72 1.59	0.80 8.68 0.93	9.35 4.77 9.61	107	0.2497	0.1379	0.0176	0.1118	0.00698	0.00112
99	19.70	13.25	7.94 1.44	50	1.1895	0.5759	0.0883	0.6136	0.04211	0.00674
113	15.58	10.23 1.36	5.55 0.39	25	0.5289	0.2598	0.0443	0.2691	0.01813	0.00290
127	17.44 2.65	11.13	6.17 1.08	50	0.6099	0.3151		0.2948	0.02368	0.00379
141	17.97 2.60	11.63	6.54 1.21	25	0.9586		0.0838	0.4344	0.03379	0.00541
155	17.75 3.32	11.89 1.97	6.69 1.43	24	1.0117	0.5450	• • • • • • • • • • • • • • • • • • • •	0.4668	0.03822	0.00611
169	20.60 3.25	13.36 2.31	7.89 1.68	25	1.5555	0.3487	0.1352		0.05674 0.05516	0.00883
183	20.70 3.55	13.50	7.87 1.85	25	1.5278	0.8082	0.1358 0.1340	0.7196 0.9352	0.07397	
197	22.48 3.21	2.13	8.30 1.68	25	2.0451	1.1099	0.2652		0.10246	
211	26.18	17.54	10.66	20 15	2.9488	1.5478		1.4010	0.11732	
225	25.19	16.50	10.17 2.23 10.16	ತ್ತವ ಇ	2.3164	1.4217		1.3947	0.09931	0.01589
239	24.74 4.07 25.74	16.50 2.76 17.17	2.31	25	3.2590	1.7625		1.4965		0.02061
253 267	3.85 26.52	2.67 17.79	2.16	26	3.5889	1.8207	0.2794			0.01937
281	3.82 26.27	2.60	1.93		3.3114		0.2361			0.01591
201	2.95	2.00	1.69	(m. cm)	- water 6					

TABLE 6.28

Date Spawned Sept. 14:1977 Population # 39 All values=mean;+-S.B.

Age (in days)	1 (mm)	พ(คค)	d(mm)	Н	ИМ(а)	ЫМ( <b>э</b> )	DM(=)	DS(q)	PRT.(9)	PPN(9)
55	1.30 0.52	-1.00 -1.00	-1.00 -1.00	25	0.0667	0.0438	0.0061	0.0230	0.00159	0.00025
69	3.99 2.21	-1.00 -1.00	-1.00 -1.00	252	0.0069	0.0035	0.0006	0.0034	0.00020	0.00003
83	7.30 1.69	4.99	2.52 0.61	38	0.0456	0.0212	0.0040	0.0243	0.00146	0.00023
97	9.12 2.85	6.10 1.71	3.13	14	0.1277	0.0661	0.0123	0.0616	0.00366	0.00059
111	13.21	3.95 2.28	4.83 1.66	25	0.4614	0.2451	0.0384	0.2162	0.01712	0.00274
125	3.35 14.94 3.32	9.93 2.03	5.39 1.41	25	0.5852	0.3087	0.0493	0.2765	0.02197	0.00351
139	16.65	10.32	6.06 1.63	25	0.7967	0.4183	0.0706	0.3783	0.03110	0.00498
153	20.85 3.33	13.47	7.96 1.55	25	1.4551	0.7491	0.1383	0.7060	0.06297	0.01007
167	21.48 2.93	14.07	8.32 1.56	25	1.6526	0.8562	0.1506	0.7964	0.06486	0.01039
181	22.35 3.42	1.94 14.78 2.37	9.00	25	2.0957	1.0630	0.1674	1.0277	0.07531	0.01205
195	3.42 24.06 3.82	15.85 2.52	9.56	25	2.6023	1.3408	0.2049	1.2514	0.09984	0.01598
209	3.62 24.07 3.66	15.88	9.81 1.95	25	2.6244	1.3411	0.2503	1.2833	0.10636	0.01710
223	25.64	2.30 16.84 3.07	10.72	25	3.3135	1.6709	0.2581	1.6426	0.11687	0.01870
237	4.66 27.18 3.22	17.88 2.19	11.36	25	3.3836	1.5111	0.3207	1.8725	0.12841	0.02055
251	3.22 26.99 3.87	17.84 2.66	11.19	25	3.6180	1.8042	0.3912	1.8138	0.15534	0.02485
265	3.36 23.43	18.39	12.16	25	4.3382	2.1770	0.3794	2.1612	0.16174	0.02588
279	30.39	19.82	13,26	25	5.7186	2.9888	0.5624	2.7297	0.20351	0.03256
389	33.82 4.25	22.27 22.38	15.49 2.62	25	8.5133	4.3619	0.7359	4.1514	0.41546	0.06647
420	33.82 4.25	22.27 22.88	15.49 2.62	25	7.7133-	-1.0000-	-1.0000-	-1.0000	-1.00000-	1.00000

TABLE 6.29

Date Spawned Oct. 11:1977 Population # 40 All values=mean:+-S.D.

Ase (in days)	l(mm)	w(mm)	d(mm)	Н	MM(a)	MM(a)	DM(a)	DS(a)	PRT.(a)	PPN(∌)
55	5.09	3.58	1.75	102	0.0161	0.0037	0.0014	0.0074	0.00044	0.00007
70	1.06 8.07	0.72 5.40	0.38 2.74	24	0.0774	0.0418	0.0069	0.0356	0.00236	0.00038
34	1.75 11.38	1.08 7.73	0.60 3.92	25	0.2211	0.1205	0.0173	0.1007	0.00734	0.00117
98	1.81 12.72	1.16 3.44	0.90 4.38	25	0.2993	0.1544	0.0254	0.1449	0.01054	0.00169
112	2.33 15.69	1.46 10.28	1.01 5.48	25	0.6214	0.3338	0.0512	0.2876	0.02286	0.00366
126	3.36 17.36	2.20 11.38	1.42 6.34	25	0.3605	0.4707	0.0799	0.3899	0.03143	0.00503
140	3.27 19.86	2.09 13.18	1.53 7.42	25	1.3274	0.7319	0.1231	0.5955	0.05191	0.00831
154	3.15 22.02	2.17 14.57	1.48 8.50	25	1.3848	1.0310	0.1686	0.8538	0.06333	0.01013
168	4.24 22.68	2.83 15.16	2.07 8.97	25	2.1146	1.1448	0.1736	0.9698	0.07901	0.01264
182	3.62 24.10	2.57 16.06	1.90 9.58	25	2.4909	1.3294	0.2107	1.1615	0.09366	0.01499
196	3.69 26.25	2.42 17.33	1.87 10.78	25	3.1798	1.7494	0.2446	1.4304	0.10735	0.01716
210	2.70 25.51	1.90 17.00	1.53 10.29	25	2.8131	1.4227	0.2132	1.3954	0.09658	0.01545
224	2.89 25.36	2.26 17.07	1.70 10.29	25	3.2581	1.7454	0.2677	1.5127	0.10940	0.01750
238	5.08 25.16	3.60 16.60	2.70 10.14	25	3.0813	1.6108	0.2329	1.4705	0.09883	0.01581
252	4.26 29.41	2.89 19.39	2.24 12.55	25	4.5676	2.3374	0.4032	2.2302	0.14911	0.02386
266	4.17 29.21	2.83 19.46	2.10 12.50	25	4.7874	2.5307	0.4147	2.2567	0.16297	0.02508
280	4.90 28.46 3.01	3.37 19.32 2.11	2.52 12.10 1.66	25	3.8406	1.7419	0.3565	2.0986	0.13059	0.02089

TABLE 6.30

Date Spawned Nov. 7,1977 Population # 41 All values=mean,++S.D.

Age (in days)	1(mm)	⊌(ฅฅ)	d(mm)	Н	(E)MM	WM(=)	DM(a)	DS(a)	PRT.(#)	PPN(a)
57	4.16 1.06	-1.00 -1.00	-1.00 -1.00	358	0.0135	0.0075	0.0013	9.8060	0.00047	0.99993
35	6.91 1.33	4.82 0.88	2.40 0.48	208	0.0378	0.0183	0.0029	0.0195	0.00106	0.00017
99	7.34 1.38	5.16 0.94	2.58 0.59	25	0.0729	0.0299	0.0073	0.0430	0.00249	9.99949
113	10.43	7.05 1.78	3.73 1.09	25	0.2302	0.1022	0.0203	0.1279	0.00522	9.00084
127	11.93	3.0ĭ 1.67	4.39 1.16	25	0.3340	0.1516	0.0313	0.1823	0.01114	0.00178
141	14.86	10.02	5.79 1.23	25	0.5443	0.3031	0.0599	0.3412	0.02574	0.00412
155	16.08 2.25	10.73	6.36 1.15	25	0.7776	0.3432	0.0662	0.4344	0.03021	0.00463
169	17.47 2.90	11.65	7.09 1.52	25	1.0571	0.5008	0.0821	0.5563	0.03548	0.00568
183	18.20	12.08 1.59	7.27 1.19	25	1.1206	0.5089	0.0613	0.6117	0.94450	0.00712
197	18.80	12.54 2.30	7.63 1.88	25	1.3981	0.6784	0.1151	0.7197	0.05013	0.00802
211	19.53	13.07 1.67	8.00 1.54	25	1.4905	0.7216	0.1537	0.7689	0.05413	0.00886
225	22.36 3.14	14.91 2.04	9.62 1.75	25	2.2644	1.0539	0.2044	1.2104	0.08081	0.01293
239	22.83	15.20	9.90 1.74	25	2.5251	1.2368	0.1381	1.2883	0.07767	0.01243
253	23.10	15.42 1.88	10.07	25	2.5989	1.2151	0.2285	1.3838	0.08746	0.01399
267	25.75 2.26	17.62 2.38	11.72	25	3.6834	1.7690	0.3118	1.9145	0.10664	0.01706
281	25.79 2.60	17.31 1.69	11.72	25	3.8238	1.7897	0.3254	2.0341	0.08216	0.01315

TABLE 6.31.

Date Spawned Dec. 6,1977 Population # 42 All values=mean,+-S.D.

Age (in days)	1(mm)	w(mm)	d(mm)	N	MM(€)	(e)MW	DM(a)	DS(a)	PRT.(9)	PPN(∌)
70	2,94	-1.00	-1.00	507	0.0048	0.0027	0.0004	0.0020	0.00017	0.00003
34	1.66 5.39	-1.00 3.65	-1.00	25	0.0302	0.0150	0.0038	0.0153	0.00729	0.00117
98	1.96 9.59	1.28 6.51	0.60 3.32	25	0.1511	0.0839	0.0156	0.0672	0.00660	0.00106
112	2.55 11.62	1.68 7.94	1.00 4.03	25	0.2533	0.1368	0.0247	0.1165	0.00858	0.00137
126	2.47 12.82	1.37 8.57	0.83 4.34	25	0.3412	0.1838	0.0280	0.1573	0.01278	a.00204
140	2.76 15.54	1.66 10.02	1.10 5.52	25	0.5857	0.3205	0.0459	0.2652	0.01996	0.00319
154	2.64 16.92	1.51 11.12	1.12 6.06	25	0.7583	0.3927	0.0538	0.3657	0.02393	0.00383
163	2.87 17.45	1.39 11.45	1.32 6.32	25	0.9300	0.4984	0.0819	0.4316	0.02791	0.00447
182.	3.65 20.70	2.27 13.50	1.65 7.60	25	1.4531	0.7954	0.1236	0.6577	0.04910	0.00786
196	2.69	1.73 15.38	1.23 3.93	25	2.1630	1.1503	0.1942	1.0127	0.07400	0.01184
210	2.79 25.99	1.93	1.63 10.20	25	3.0074	1.6458	0.2748	1.3615	0.10282	0.01645
224	2.51	1.59	1.15	25		1.6040		1.4336	0.12257	0.01961
	3,23	2.14	1.45	25	3.6850	1.8910		1.7939	0.11382	0.01821
233	4.21	3.20 18.80	1.99	25	4.5180	2.3051		2.2129	0.12724	0.02036
252	3.70	2.77	2.10	25	4.0799	1.9885		2.0914	0.12785	9.92946
256	27.78	18.78	2.34	25	4.5276		0.3731		0.12796	0.02047
280	29.22 4.35	19.10 2.86	11.74 2.16	40	₩.J&(©	in a TUW!	<i>-</i>			

TABLE 6.32

Date Spawned Jan. 2,1978 Population # 43 All values=mean,+-S.D.

SHELLFISH ALLOMETRY										
Age (in days)	1(mm)	พ(คค)	d(mm)	Н	MM(±)	(e)MW	DM(9)	DS(a)	PR7.(9)	PPN(a)
57	1.00	-1.00 -1.00	-1.00 -1.00	456	0.0008	0.0007	0.0001	0.0002	0.90002	9.00000
71	0.00 3.20	-1.00 -1.00	-1.001 -1.00	8008	0.0001	0.0000	0.0000	0.0000	0.00000	9.20000
35	1.25 5.38	3.93 1.27	2.02	138	0.0202	0.0110	0.0019	0.0092	0.00073	0.90612
99	1.94 8.34	5.87	2.93 0.89	25	0.1074	0.0596	0.0135	0.0478	0.00474	0.00076
113	2.40 11.10	1.52 7.64 1.15	3.93 0.78	25	0.2204	0.1236	0.0193	0.0969	0.00745	0.00119
127	1.30	9.32 1.60	5.02 1.20	25	0.4230	0.2153	0.0305	0.2077	0.01338	0.00214
141	2.39 13.55	9.17	4.86 1.25	24	0.4440	0.2350	0.0372	0.2090	0.01676	0.00258
155	2.90 17.45	1.89	6.92 2.25	25	0.9230	0.4738	0.0613	0.4442	0.02910	0.00456
169	2.32	1.49 12.85 2.21	7.72 1.30	25	1.3093	0.6897	0.1182	0.6196	0.04253	0.00682
183	2.69	13.92	7.99	25	1.6088	0.3467	0.1470	0.7620	0.35854	0.00937
197	4.68 23.06	15.68 2.10	9.50 1.98	25	2.2579	1.0817	0.1766	1.1762	0.11358	0.01817
211	2.88 25.48	17.25	10.78	25	2.9631	1.3634	0.2630	1.5997	0.08782	0.01405
225	3.20 25.31	17.85 2.85	10.97	25	3.4618	1.7227	0.3151	1.7391	0.09794	9.01567
239	3.13 25.24	17.19 3.05	10.58	25	2.9639	1.2537	0.2796	1.7102	0.08145	0.01303
253	4.44	18.65 2.99	11.91	25	3.7911	1.6022	0.2993	2.1389	0.12353	0.01976
267	4.08 28.69 3.35	19.23	12.45	25	4.7000	2.2928	0.3973	2.4072	0.24958	0.03993

TABLE 6.33

Date Spawned Jan. 31,1978 Population # 44 All values=mean,+-5.D.

Age (in days)	1(mm)	w(mm).	d(mm)	H	MM(a)	WM(a)	DM(q)	DS(a)	FRT.(∍)	PPN(3)
56	1.57 0.32	-1.00 -1.00	-1.00 -1.00	651	0.0008	0.0006	0.0001	0.0002	-1.00000	-1.00000
79	2.74	-1.00 -1.00	-1.00 -1.00	320	0.0053	0.0036	0.0004	0.0017	0.00014	0.00002
84	5.64 1.83	4.12	1.93	25	0.0337	0.0178	0.0026	0.0159	0.00097	0.00016
98	7.26 2.69	5.01 1.73	2.43 0.94	25	0.0772	0.0412	0.0051	0.0361	ō.00194	0.00031
112	9.68 2.58	6.56 1.62	3.30 0.96	23	0.1587	0.0887	0.0160	0.0699	0.00564	0.00090
126	11.36	7.94 2.24	4.11 1.45	25	0.3193	0.1750	0.0269	0.1443	0.01049	0.00168
140	15.16 2.50	10.12	5.48 1.26	25	0.5675	0.2981	0.0469	0.2694	0.01759	0.00281
154	17.39 1.82	11.61	6.36 0.99	25	0.8497	0.4525	0.0752	0.3972	0.02936	0.00470
168	20.53	13.58 1.76	3.00 1.51	25	1.5131	0.3254	0.1303	0.6927	0.07992	0.01279
132	20.52 3.01	13.72 1.80	8.03 1.28	25	1.4957	0.3026	0.1394	0.6931	0.04870	0.00779
196	22.54 2.28	14.74	9.12 1.34	25	2.0116	1.0554	0.1487	0.9562	0.05792	0.00927
210	22.40	15.12 1.52	8.90 1.11	25	1.9653	0.9878	0.1742	0.9774	0.06350	0.01016
224	23.61	16.01 1.74	9.76 1.48	25	2.2516	1.0691	0.1306	1.1825	0.06653	0.01064
238	24.37	16.25 2.08	10.40	25	2.8383	1.4924	0.2508	1.3459	0.11491	0.01839
250	24.68 3.70		10.20	25	3.0366	1.3501	0.2365	1.1865	0.10673	0.01708

TABLE 6.34

Date Spawned Feb. 27,1978 Population # 45 All values=mean,+-8.D.

Ase (in days)	1(mm)	w(mm)	d(mm)	Н	ИМ(э)	WM(a)	DM(a)	DS(#)	PRT.(a)	PPN(9)
57	1.58 0.50	-1.00 -1.00	-1.00 -1.00	644	0.0010	0.0007	0.9001	0.0003	0.00003	9.99999
71	3.55 1.03	-1.00 -1.00	-1.00 -1.00	243	0.0077	0.0050	0.0005	0.0027	0.00016	0.00003
35	6.61 1.73	4.54 1.13	2.22 0.54	25	0.0433	0.0247	0.0015	0.0240	0.00224	0.00036
99	10.25 2.08	6.88 1.28	3.46 0.33	25	0.1694	0.0948	0.0121	0.0745	0.80548	0.00088
113	12.68 2.69	8.40 1.63	4.50 1.14	25	0.3332	-1.0000-	-1.0000-	-1.0000	-1.00000-	-1.00000
127	15.42 3.16	10.03 2.03	5.51 1.39	25	0.5889	-1.0000-	-1.0000-	-1.0000	-1.80000-	-1.00000
141	17.95 2.65	11.67	6.65 1.28	25	0.9330	0.4987	0.0742	0.4343	0.04561	0.00730
155	21.68 2.59	14.29 1.90	3.68 1.43	25	1.6795	0.9095	0.1527	0.7690	0.05665	0.00906
169	21.10	13.82 1.61	8.28 1.43			0.8347			0.05380	0.00861
133	22.64 2.56	14.76 1.72	8.76 1.81	25	1.9426	1.0648	0.1647	0.3779		
197	23.17	15.21 2.14	9.13 1.61	25	1.9098	0.3980	0.1501	1.0118	0.04952	0.00792
211	24.87 3.25	16.36 2.20	10.03 1.80	25	2.7446	1.5047	0.2158	1.2400	0.13401	0.02144
223	26.18 2.76	17.10 1.82	10.72 1.59	25	3.1324	1.5440	0.3044	1.4884	0.17102	0.02736

TABLE 6.35

Bate Spawned March 27:1978 Population # 46 All values=mean:+-S.B.

Ade (in days)	1(mm)	w(mm)	d(mm)	Н	(e)WW	(e)MW	DM(s)	DS(4)	PRT.(a)	PPN(a)
71	7.31 1.20	4.92 0.81	2.62 0.41	•	0.0669			0.0304		0.00038
85	9.30 1.93	6.78 1.40	3.47 0.81						-1.00000	
99	12.65	11.85 18.27	4.36 1.08	25	0.3089	-1.0000	-1.0000-	-1.0000	-1.00000	-1.00000
113	15.45 2.56	10.62 1.68	6.00 1.12	25	0.6902	0.3779	0.0503	0.3123	0.03451	0.00552
127	13.26	11.38	6.88 1.14	25	1.0383	0.5340	0.0798	0.4544	0.03035	0.00486
141	20.28	13.08 1.68	7.64 1.17	25	1.3448	0.7317	0.0948	0.6130	0.86660	0.01066
155	19.54	12.85	7.24 1.20	25	1.1233	0.5364	0.0913	0.5869	0.04086	0.00654
169	21.52	14.17	8.30 1.20	25	1.5317	0.7534	0.1271	0.7783	0.04368	0.00699
183	23.48	15.30 2.06	9.16 1.60	25	2.2013	1.2162	0.2021	0.9856	0.08919	0.01427
195	24.70 2.65	16.62 3.01	9.77 1.24	24	2.6065	1.4269	0.2236	1.1796	0.10540	0.01686

TABLE 6.36

Bate Spawned April 25,1978 Population # 47 All values=mean,+-S.D.

Age (in days)	1(mm)	w(mm)	d(mm)	N	(€)MM	(e)MW	DM(a)	DS(9)	PRT.(⊕)	PPN(a)
84	14.39 1.78	9.88 2.22	5.27 0.71	25	0.3626	0.1365	0.0339	0.1761	9.01005	0.00161
98	16.16	10.93 0.90	6.07 0.73	24	0.6713	0.3780	0.0559	0.2938	5.02011	0.00322
112	18.59	12.21	7.06 0.86	25	1.0098	0.5633	0.0797	0.4465	3.03036	0.00486
126	19.39	12.87	7.22 0.81	25	1.1583	0.6298	0.0922	0.5285	0.03815	0.00610
140	20.82	13.64	7.92 0.98	25	1.3698	0.6965	0.1089	0.6734	3.03941	0.00631
154	21.94	14.49	8.46 0.99	25	1.7705	0.9876	0.1603	0.7829	3.09405	0.01505
166	23.05 3.06	15.28 2.13	9.24 1.52	25	2.1509	1.0760	0.1956	1,0749	0.08748	0.01400

TABLE 6.37

Date Spawned May 23,1978 Population # 48 All values=mean,+-3.D.

Ase (in days)	1(mm)	พ(พพ)	d(mm)	Н	(e)WW	(E) MM	DM(⇒)	DS(a)	⊃RT.(⊕)	PPH(a)
79	10.28	6.82 1.23	3.95 0.98	24	0.1737	0.0972	0.0159	0.0815	0.00728	0.00116
34	14.13	9.37 0.89	5.46 0.59	25	0.4449	0.2499	0.0377	0.1949	3.01275	0,00204
98	16.05	10.59 0.93	7.02 3.01	25	0.6530	0.3595	0.0573	0.2935	3.01979	0.00317
112	13.48	12.12 1.41	7.19 1.07	25	0.9467	0.4583	0.0739	0.4884	3.02779	0.00445
126	19.96	13.03	7.80 0.93	25	1.3411	0.7098	0.1162	0.6313	3.05493	0.00879
138	22.55 2.19	14.86 1.43	3.85 0.96	25	1.9726	1.0531	0.1724	0.9145	3.09601	0.01536

# TABLE 6.38

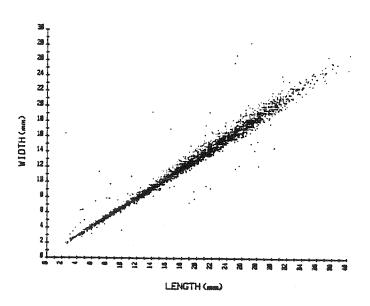
Date Spawned June 19,1978 Population # 49 All values=mean,+-S.D.

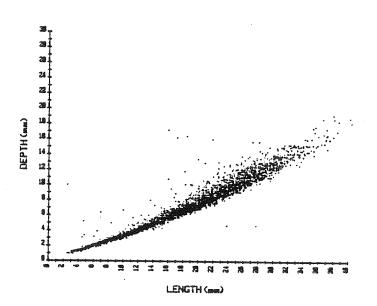
Ase (in days)	1(mm)	W(mm)	d(mm)	Н	MM(a)	WM(a)	DM(a)	DS(a)	PRT.(9)	PPN(a)
71	8.30 1.09	6.01 0.72	3.05 0.44	25	0.1058	0.0556	0.0104	0.0502	3.00403	0.00064
35	12.05	8.14 1.17	4.14 0.73	25	0.2528	0.1277	0.0222	0.1250	a.00702	0.00112
99	15.56 2.39	10.33	5.67 1.01	25	0.5981	0.3224	0.0525	0.2757	3.02893	0.00463
111	17.10 2.75	11.28	6.35 1.33	25	0.3248	0.4523	0.0693	0.3726	ð.0411i	0.0 <b>0</b> 658

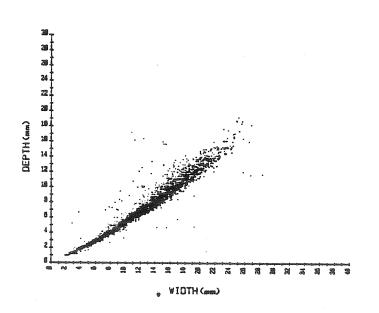
# -- TABLE - 6.39

Date Spawned July 17,1978 Population # 50 All values=mean,++S.D.

Hae (in days)	1(mm)	w(mm)	d(mm)	Н	MM(a)	(e)MW	(E)MC	DS(a)	PRT.(⊕)	PPM(a)
71			3.89	25	0.2113	0.1160	0.0240	0.0953	a.01214	0.00194
83	13.33			25	0.3806	0.2071	0.0310	0.1735	9.01692	0.00271







and depth/width relations also increases with size. Deviation from linearity is especially noticeable once the animals are over 15 mm in shell length (10 mm in width). These allometric trends may have important implications for a quantitative description of feeding and growth and are discussed in more detail below.

Consistency in shell length, width and depth measurements versus whole wet weight, wet and dry meat, dry shell and dry meat weights is also evident, as illustrated in Figures 6.8 and 6.9.

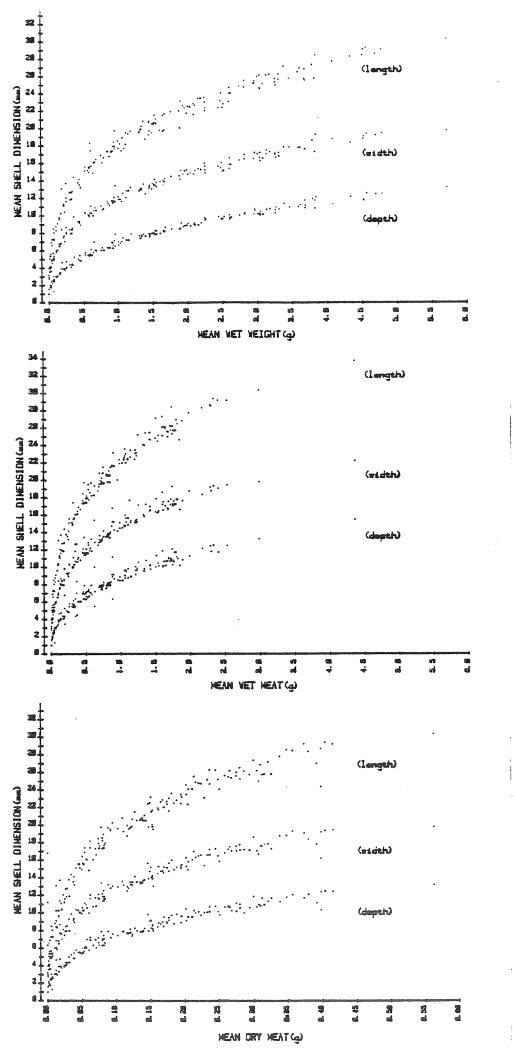
Data from Tables 6.24 - 6.39 were used to generate Figure 6.10 which shows percent protein as a function of mean whole wet weight because the <u>Tapes</u> protein content was used as a basis for determining protein conversion rates (algae-<u>Tapes</u>). The shellfish technical description assumes that protein is 3% of whole wet weight; data collected from the Model II plant indicates that this is a reasonable assumption. Variation in this value has been related to fluctuations in food supply (slope = -0.07).

#### 6.4.4 FODD SUPPLY TO THE SHELLFISH

#### 6.4.4.1 FLOW RATES TO THE SHELLFISH TRAYS

As shown in Table 6.40, flow rates were virtually identical for all trays in the system. The north (trays 1-14) and south (trays 15-28) were fed from separate orifices but received nearly identical mean flow rates. For purposes of analysis, it was assumed that flow to all trays was identical and equal to 50 mls/second (1500 mls/30 seconds).

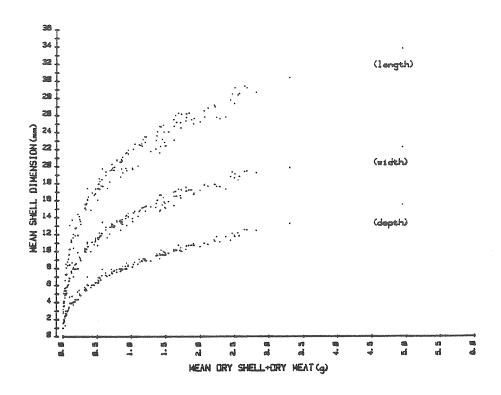
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RATIO OF SHELL DIMENSIONS TO WEIGHT FOR ALL SHELLFISH POPULATIONS 6.8 FIGURE

FIGURE 6.9 RATIO OF SHELL DIMENSIONS TO WEIGHT FOR ALL <u>TAPES</u>

GROWN IN PILOT PLANT MODEL II.



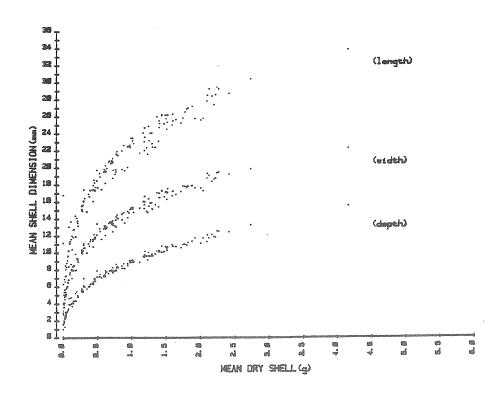


FIGURE 6.10 PERCENT PROTEIN VERSUS WHOLE WET WEIGHT OF TAPES

GROWN IN SHELLFISH PILOT PLANT MODEL II.

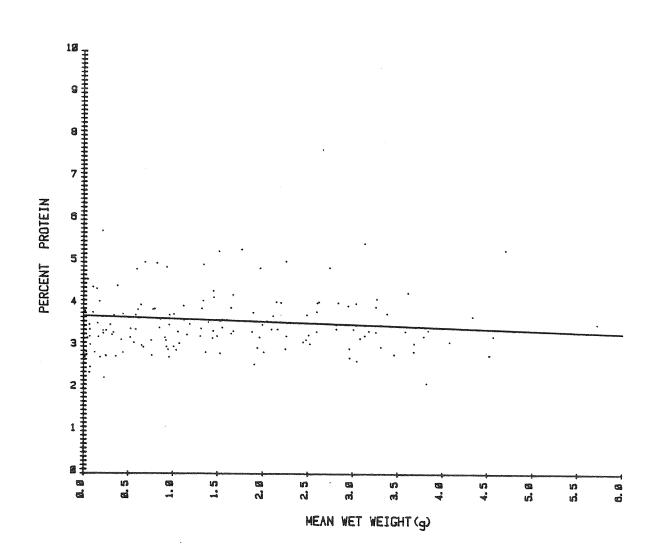


TABLE 6.40

# SUMMARY SHELLFISH EFFLUENT FLOW RATES 10/11/77-10/10/78

# INDIVIDUAL TRAY DATA

(1.)flow-rates in mls/30secs.

TRAY# 1 2 3 4 5 6 7 8 9 10 11 12 13 14	NORTH 133 134 139 139 139 139 139 139 139 133 134	MEAN +- S.D.  1487+-149 1513+- 89 1511+-103 1514+- 88 1523+-106 1537+-103 1506+-147 1518+-125 1549+-161 1532+-121 1523+-152 1497+-147 1514+-100 1466+-153	TRAY# 15 16 17 18 90 122345678	SOUTH 133 139 139 139 139 139 139 139 139 139	SIDE  MEAN +- S.1  1425+-175  1501+-100  1491+- 83  1480+- 99  1534+-122  1492+- 89  1498+-149  1462+-112  1510+- 88  1552+- 83  1535+- 95  1513+- 89  1429+-142
MEAN STD.DEV.		1514 21			1496 38

In general, flow-rates were consistent, and were indicative of great improvement of design over the Model I plant.

6.4.4.2 NITROGEN BALANCE IN THE SHELLFISH PILOT PLANT
Against the background of highly consistent
shellfish allometry and flow rates, considerable variation was observed
in influent and effluent chemistry values, as well as in individual
population and biweekly weight gains. We turn first to the flow-through
chemistry values.

Table 6.41 illustrates mean and standard deviation values for  $(NH_4^+ + NH_3) - N$ ,  $(NO_3^- + NO_2^-) - N$  and Particulate Protein Nitrogen (PPN) and for total 'N'  $(NH_4^+ + NH_3 + NO_3^- + NO_2^- + PPN) - N$  for each biweekly test period. For each test period, the inflow was sampled six times in replicate. Table 6.42 gives the same data for effluent values. For these data, the values are the mean  $\frac{+}{}$  standard deviation for the 5 - 8 shellfish populations which were sampled for effluent chemistries in the plant over each biweekly period. (Effluent data for individual populations are discussed below). Mean inflow values are also displayed in Figure 6.11.

Total ammonia levels are consistently low (grand mean  $^+$  standard deviation = 1.5  $^+$  0.7 µgat L $^{-1}$ ). The values are very close to those measured in the pools (see Table 5.4 in the Phytoplankton section).

Because of changes in nutrient uptake by the algal cultures and due to culture collapse, the  $(NO_3^- + NO_2^-)-N$  values vary considerably. Figure 6.11 shows that the  $(NO_3^- + NO_2^-)-N$  and PPN values are inversely related. This is also consistent with the pool data (see Table 5.4

# SUMMARY SHELLFISH INFLOW CHEMISTRIES 10/11/77-10/10/78

(1.)a11 values in usat/l (2.)Mean+-

(2.)Mean+-Std.dev.

T	EST PERIOD	NH4+NH3-N	N03+N02-N	PPN	TOTAL'N'
1 .	10/11/77-	1.4	11.8	15.4	28.5
	10/25/77	0.7	8.5	9.4	3.2
in it	10/25/77-	1.0	7.4	19.4	27.9
	11/08/77	0.4	6.6	7.1	2.8
	11/08/77-	1.5	8.5	18.2	28.1
	11/22/77	1.0	7.4	5.1	4.3
4.	11/22/77-	1.0	18.9	10.6	30.4
	12/06/77	0.4	8.7	7.8	8.7
5.	12/06/77-	0.6	1.7	19.7	22.1
	12/20/77	0.4	2.0	3.4	2.4
5.	12/20/77-	1.0	4.2	19.5	24.7
	01/03/78	0.4	4.3	3.2	2.6
i a	01/03/78-	0.8	9.1	15.3	25.2
	01/17/78	0.4	6.4	4.6	2.3
3.	01/17/78-	1.2	2.3	20.2	23.7
	01/31/78	0.4	0.9	3.7	1.9
7.	01/31/78-	1.6	7.9	17.6	27.1
	02/14/78	1.9	6.4	4.7	3.9
10.	02/14/78-	0.8	5.3	19.6	25.7
	02/28/78	0.3	4.6	3.7	2.2
4-4	02/28/78-	1.4	5.5	17.7	24.6
	03/14/78	1.2	5.0	4.3	1.9
12.	03/14/78-	1.2	2.3	21.5	25.0
	03/28/78	0.5	2.2	2.4	1.6
13.	03/28/78-	3.0	4.9	20.1	28.1
	04/11/78	1.7	6.5	5.4	4.2

# SUMMARY SHELLFISH INFLOW CHEMISTRIES 10/11/77-10/10/78 (Continued)

14.	04/11/78-	0.9	12.7	14.2	27.8
	04/25/78	0.3	3.7	4.1	2.2
15.	04/25/78-	2.3	18.0	6.8	26.9
	05/09/78	0.8	6.3	4.7	2.0
16.	05/09/78-	3.0	10.6	16.7	30.3
	05/23/78	3.6	9.6	8.6	3.9
17.	05/23/78-	1.4	5.8	19.9	27.1
	06/06/78	1.1	6.1	6.0	4.7
18.	06/06/78-	2.5	8.9	17.1	28.5
	06/20/78	1.4	7.8	5.3	3.9
19.	06/20/78-	1.6	3.5	21.2	27.1
	07/04/78	0.6	3.0	2.8	0.8
20.	07/04/78-	1.1	8.8	18.2	28.1
	07/18/78	0.6	12.3	3.0	9.8
21.	07/18/78-	1.6	8.5	16.3	26.8
	08/01/78	2.1	6.0	4.4	4.5
22.	08/01/78-	1.6	13.3	12.3	26.2
	08/15/78	1.0	4.4	3.1	3.2
23.	08/15/78-	0.9	11.6	18.0	28.9
	08/29/78	0.5	11.7	12.3	4.9
24.	08/29/78-	1.6	13.1	12.4	23.8
	09/12/78	1.2	10.5	8.8	1.5
25.	09/12/78-	1.1	4.3	19.1	24.5
	09/26/78	0.5	5.5	4.4	2.7
26.	09/26/78-	^3.0	10.1	14.8	27.7
	10/10/78	3.2	7.9	6.2	1.7
	MEAN	1.5	3.4	17.0	26.7
	STD.DEV.	0.7	4.5	3.5	2.1

# SUMMARY SHELLFISH OUTFLOW CHEMISTRIES 10/11/77-10/10/78

(1.)all values in usat/l

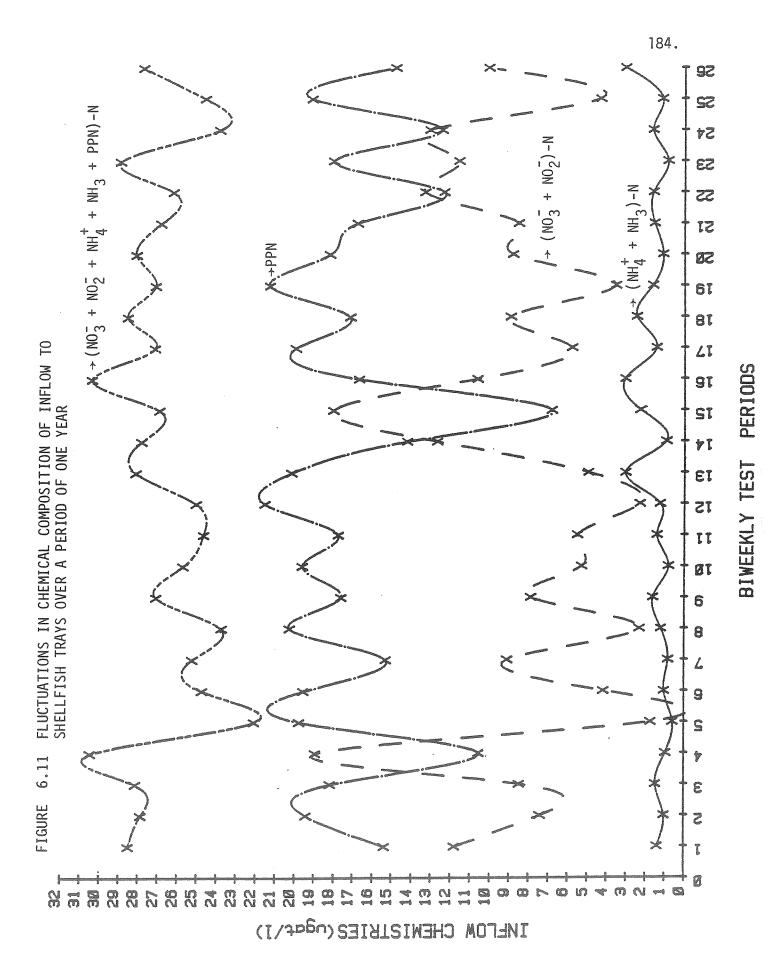
(2.)Summed data for 5-8 shellfish populations for each biweekly test period

(3.)Mean+-Std.dev.

	EST PERIOD	NH4+NH3-N	N03+N02-N	PPH	TOTAL'H'
1 .	10/11/77-	2.5	11.7	7.6	21.7
	10/25/77	1.1	8.7	4.8	7.3
ines &	10/25/77-	3.7	7.7	9.1	20.5
	11/08/77	1.5	5.7	5.0	6.3
3.	11/08/77-	3.7	8.5	9.2	21.3
	11/22/77	1.8	7.3	5.6	5.9
4.	11/22/77-	3.7	19.4	4.7	27.8
	12/06/77	2.9	8.0	3.4	4.7
long	12/06/77-	2.1	2.1	14.8	19.0
- esp 55	12/20/77	0.9	2.2	6.6	5.3
Ö a	12/20/77-	3.0	4.7	11.4	19.1
	01/03/78	0.9	4.8	5.4	6.5
energy	01/03/78-	3.6	9.2	7.4	20.2
1 m	01/17/78	1.4	6.2	4.6	6.7
3.	01/17/78-	5.2	2.7	11.7	19.6
	01/31/78	1.0	1.0	5.8	5.1
2.	01/31/78-	3.8	8.3	8.7	20.8
	02/14/78	1.2	6.4	4.8	5.2
10.	02/14/78- 02/28/78	5.1	4.7	12.1 4.8	22.9 4.4
11.	02/28/78-	5.2	6.1	10.5	21.9
	03/14/78	2.8	5.0	6.2	4.3
12.	03/14/78-	5.0	2.6	14.2	21.8
	03/28/78	1.9	2.0	6.1	5.2

## SUMMARY SHELLFISH OUTFLOW CHEMISTRIES 10/11/77-10/10/78 (Continued)

13.	03/28/78-	6.8	5.1	15.0	26.9
	04/11/78	4.5	6.2	7.2	5.7
14.	04/11/78-	4.1	12.9	5.2	22.2
	04/25/78	1.5	3.8	3.9	4.1
15.	04/25/78-	5.2	18.8	3.5	24.6
	05/09/78	1.1	6.3	3.3	4.5
16.	05/09/78-	4.4	10.9	7.5	22.5
	05/23/78	1.2	9.8	6.9	5.8
17.	05/23/78-	4.3	5.0	9.8	19.1
	06/06/78	1.2	5.6	6.9	6.2
18.	06/06/78-	5.7	9.1	8.1	22.7
	06/20/78	1.9	7.7	6.9	6.6
19.	06/20/78-	4.6	4.6	14.3	23.0
	07/04/78	1.5	4.4	7.5	7.2
20.	07/04/78-	4.3	4.6	7.2	16.6
	07/18/78	1.6	5.2	4.6	5.5
21.	07/18/78-	3.9	8.4	8.5	21.0
	08/01/78	1.4	5.1	5.2	6.3
22.	08/01/78-	4.7	13.6	3.9	22.2
	08/15/78	1.2	4.7	2.1	5.4
23.	08/15/78-	4.0	12.2	7.3	23.3
	08/29/78	1.9	11.4	5.8	8.7
24.	08/29/78-	5.8	13.3	8.5	25.5
	09/12/78	5.4	10.4	7.2	10.8
	MEAN	4.4	8.5	9.0	21.9
	STD.DEV.	1.0	4.6	3.2	2.8



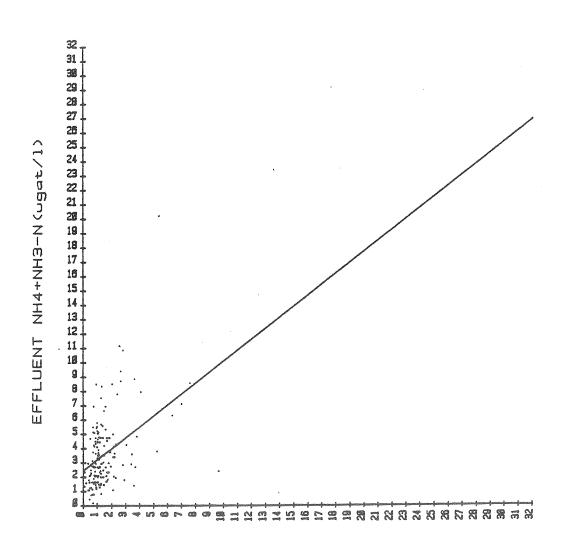
and Figures 5.2.A, B and C in the Phytoplankton Section #5).

Figures 6.12 and 6.13 illustrate relations between inflow and outflow ammonia (NH $_4^+$  + NH $_3$ )-N and nitrate and nitrite (NO $_3^-$  + NO $_2^-$ )-N respectively. Note that effluent ammonia values are generally higher than the inflow values (grand mean = 4.4  $_2^+$  1.5  $_{\mu}$ gat L $_3^{-1}$ ), and that the effluent values appear to fluctuate randomly in relation to inflow values. Clearly, this reflects ammonia excretion by the shellfish, and this excretion was correlated to concentration of ammonia in the inflow.

In contrast, there is a nearly perfect linear relationship between inflow and outflow  $(NO_3^- + NO_2^-)$ -N values  $(r^2 = 0.97)$ . This is significant for three reasons. First, the data represent a good internal check on the  $(NO_3^- + NO_2^-)$ -N measurements and indicate high accuracy. Second, no uptake of  $(NO_3^- + NO_2^-)$ -N was observed in the shellfish tanks as was the case in the Model I plant. This provides a much more accurate and complete 'nitrogen' budget than was available the previous year. Third, no  $(NO_3^- + NO_2^-)$ -N production was observed either, as was observed with the 1975 constant weight study (used as the basis for the shellfish technical description). In that study, effluent  $(NO_3^- + NO_2^-)$ -N values exceeded inflow values.

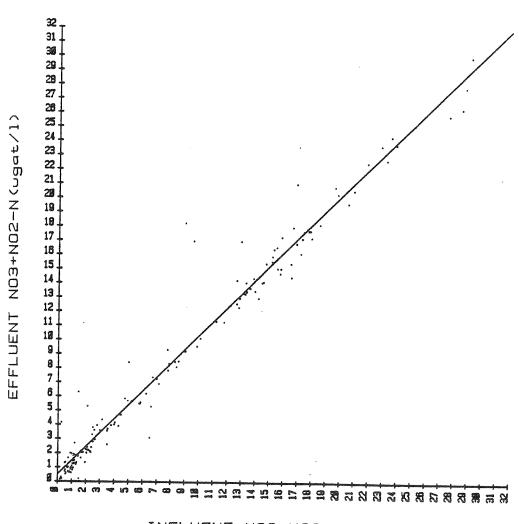
The mean  $\stackrel{+}{}$  standard deviation values for inflow PPN is shown in Figure 6.14. Individual values obtained for each of the 156 sampling days (3 times weekly x 52 weeks) are shown in Figure 6.15. The high

FIGURE 6.12

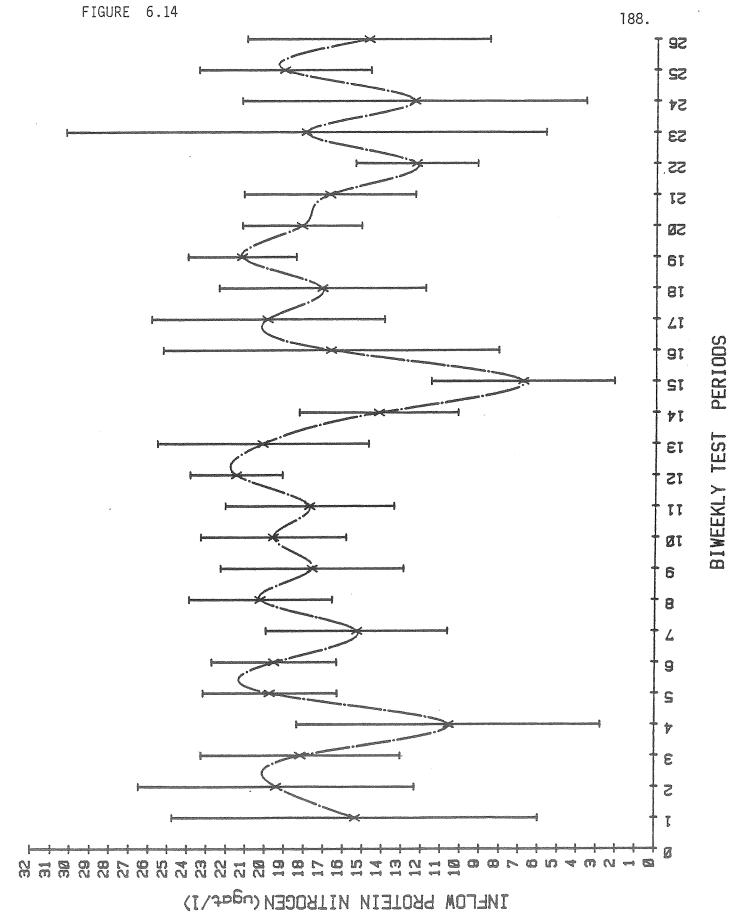


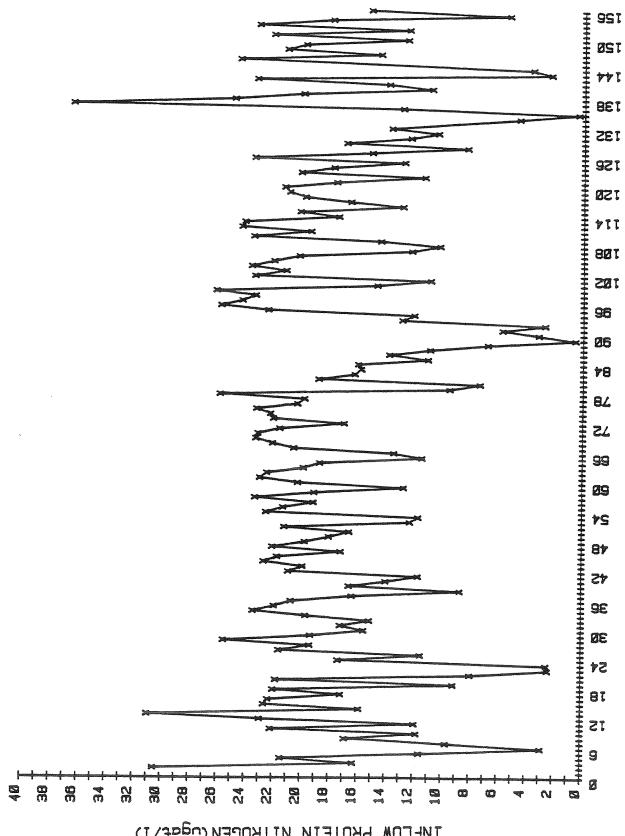
INFLUENT NH4+NH3-N (ugat/1)

FIGURE 6.13



INFLUENT NO3+NO2-N(ugat/1)





INFLOW PROTEIN NITROGEN (Ugat/I)

degree of variability in the PPN values is very significant and will be reviewed below, especially in relation to pilot plant shellfish weight gains.

The  $8.4 \pm 4.5 \, \mu gat \, L^{-1} \, (NO_3^- + NO_2^-) - N$  in the shellfish inflow represented about 26% of the available inorganic nitrogen in the incoming deep sea water which is about 32  $\mu gat \, L^{-1}$ . As indicated earlier, effluent  $(NO_3^- + NO_2^-) - N$  values are virtually identical. Ammonia  $(NH_4^+ + NH_3) - N$  inflow values  $(1.5 \pm 0.7 \, \mu gat \, L^{-1})$  represent a mean of 4.7% of available incoming inorganic nitrogen. In the effluent, the ammonia - N corresponds to 13.8% of the inorganic nitrogen in the Deep Sea Water.

PPN values in the inflow (17.0  $\pm$  3.5  $\mu$ gat L $^{-1}$ ) represent a mean of 53% of available Deep Sea Water 'nitrogen'.

Of great importance is the fact that the effluent contained a mean of 9.0  $\mu$ gat L<sup>-1</sup> of PPN, or still 28.1% of deep sea water 'nitrogen'. Thus only 47% of the incoming PPN was assimilated by the shellfish.

The total 'N' measured in the inflow (26.7  $\pm$  2.1  $\mu$ gat L $^{-1}$ ) and outflow (21.9  $\pm$  2.8  $\mu$ gat L $^{-1}$ ) represent 83.4% and 68.4% of total available deep sea water 'nitrogen'.

Inflow values for total 'N' are in agreement with the pool culture measurements given in Section 5 of this report and show that a significant quantity of dissolved organic nitrogen was not measured. The difference between inflow and outflow total 'N' values is due to the uptake of algal PPN and its subsequent conversion to shellfish meat protein-nitrogen. On the basis of the flow-through chemistry values

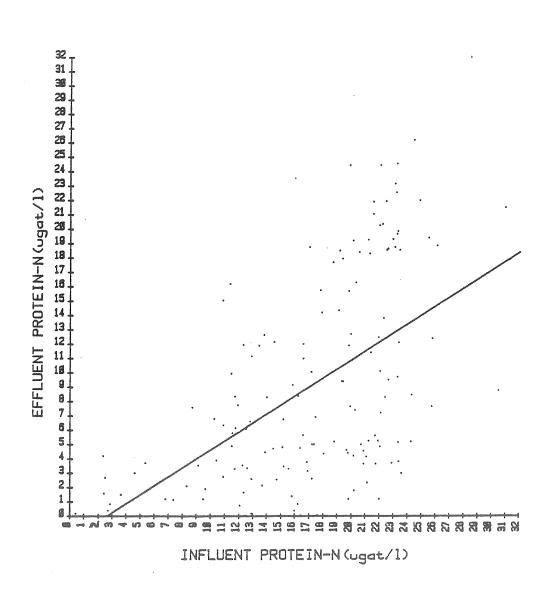
 $4.8~\mu gat~L^{-1}$  or 15% of deep sea water 'nitrogen' was converted to Tapes meat nitrogen or about 28% of PPN in the inflow and 60% of the PPN removed in the tanks was converted to meat protein-N. This assumes that all of the meat protein-nitrogen is accounted for in the total 'N' outflow less inflow values, which may not be true since there is a measurement error, for example. However, it provides a good internal check for conversions based on total whole wet weight gains and percent PPN of whole wet weight.

This discussion of flow-through chemistry results will be completed by a brief reference to selected inflow versus outflow relations.

One of the first questions which arises from the observation that the removal of PPN by the shellfish (47%) was quite low, pertains to inflow versus outflow PPN results. Since shellfish filtration rates are to some extent dependent on the concentration of the food supply (cells/ml, PPN  $\mu$ gat L<sup>-1</sup>, etc) (Winter, 1973; 1978), one might suppose some relation between inflow and outflow PPN concentration.

This relationship for 148 complete sampling days is shown in Figure 6.16. A least squares linear fit of the data is superimposed over the data points. It must be stressed that the effluent values represent the mean effluent values for from 5-8 populations; individual population data are explored in more depth below. Despite this intentional homogenization of data, a weakly positive, linear trend can be observed ( $r^2 = 0.31$ ). This trend is important, since it does not support

FIGURE 6.16 INFLUENT VERSUS EFFLUENT PPN FOR 148 SAMPLING
DAYS IN THE MODEL II PILOT SHELLFISH PLANT



the hypothesis that the fairly low concentration of PPN in the food supply which was 17.0  $\mu$ gat L<sup>-1</sup>, as opposed to the 21.4  $\mu$ gat L<sup>-1</sup> assumed by the technical description and used as the basis for the number of animals in each tray, was limiting uptake efficiency. The data here must be considered as suggestive only, but effluent values rarely go down with an increase in inflow values. Rather, they generally increase, indicating no increase in feeding efficiency where there is an increase in the food concentration.

Figures 6.17 and 6.18 are scattergrams with a superimposed least-squares linear fit to the data, of ammonia production (outflow-inflow) versus PPN presented and ammonia production versus PPN absorbed (PPN presented-PPN in effluent). Once again, data (N = 151 for 6.17 and N = 140 for 6.18) are for the combined effluent values for all populations in the plant, and are therefore relatively crude. Clearly, some general relation between PPN presented and/or absorbed and ammonia production exists. However, there is a great deal of scatter evident in both plots.

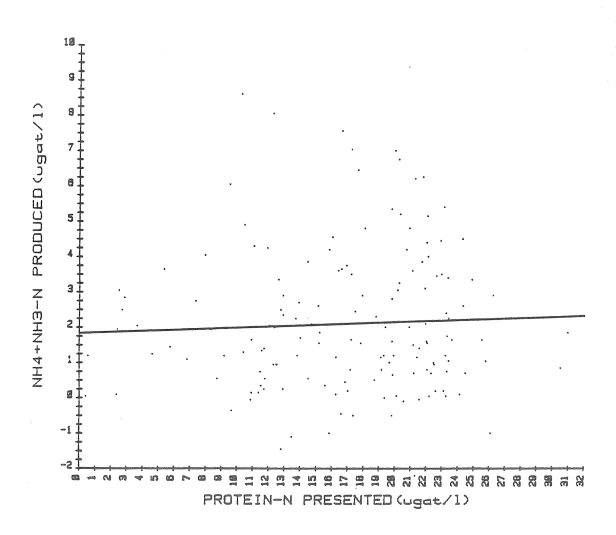
These figures provide some support for the need to include some type of tank population parameter, expressed as a function of individual shellfish age in size variables when analyzing food conversion. By averaging tank populations which vary considerably in age and size, few clear relationships in 'nitrogen' conversion can be established.

#### 6.4.5 SHELLFISH GROWTH

Fable 6.43 illustrates live whole wet weight gains for the entire year (10/11/77 - 10/10/78). Live weight gains for each of

FIGURE 6.17 AMMONIA PRODUCTION AS A FUNCTION OF PPN PRESENTED

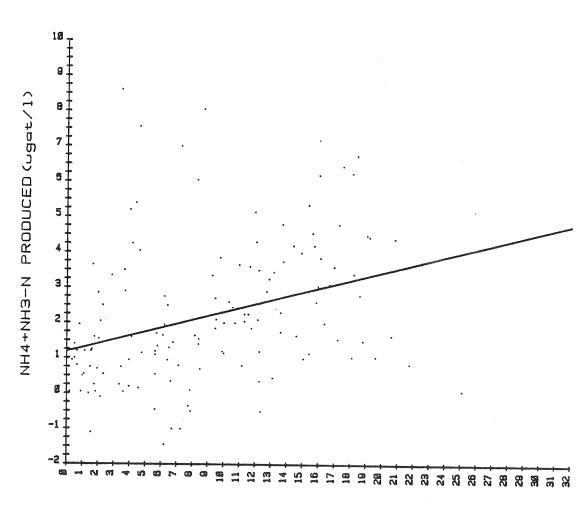
TO THE SHELLFISH



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FIGURE 6.18 AMMONIA PRODUCED AS A FUNCTION OF PPN ABSORBED IN THE SHELLFISH TANKS



PROTEIN-N ABSORBED (ugat/1)

TABLE 6.43

# SUMMARY SHELLFISH WHOLE WET WEIGHT GAINS 10/11/77-10/10/78

## (1.)all weights in grams

TEST PERIOD	MEAN +- S.D.	TOTAL WT.GAIN
3. 11/08/77-11/22/77 4. 11/22/77-12/06/77 5. 12/06/77-12/20/77 6. 12/20/77-01/03/78 7. 01/03/78-01/17/78 8. 01/17/78-01/31/78 9. 01/31/78-02/14/78 10. 02/14/78-02/28/78 11. 02/28/78-03/14/78 12. 03/14/78-03/28/78 13. 03/28/78-04/11/78 14. 04/11/78-04/25/78 15. 04/25/78-05/29/78 16. 05/09/78-05/23/78 17. 05/23/78-06/06/78 18. 06/06/78-06/20/78 19. 06/20/78-07/04/78 20. 07/04/78-07/18/78 21. 07/18/78-08/29/78 23. 08/15/78-08/29/78	30.8+-606.4 263.7+-674.1 256.5+-627.7 300.2+-667.9 295.9+-685.1 338.9+-710.6 385.6+-560.2 193.6+-320.0 293.6+-320.9 157.2+-620.9 157.2+-890.9 223.8+-893.0 347.8+-893.0 347.8+-890.1 330.8+-770.9 347.8+-475.9 160.9+-432.1	16391.0 17730.3 15879.9 15879.0 169875.0 18975.0 19185.0 19185.0 19459.0 17359.0 17360.0 17360.0 184275.0 18600.0 12100.0 12100.0 12290.0
TOTAL WEIGHT GAIN MEAN STD.DEV.	423619 16293 4879	

the 26 two-week test periods are shown, as is the mean  $\frac{1}{n}$  standard deviation weight gain per tray (n = 28).

A total of 423.619 kg. of live whole wet weight of <u>Tapes</u> was produced over the 12-month period of the study. The mean weight gain per two-week test period was 16.293 kg, with a standard deviation of 4.879 kg. Weight gains per test period ranged from a low of 4.75 kg (11/22/77 - 12/06/77) to a high of 25.003 kg (5/23/78 - 6/06/73). Thus considerable variation in weight gains between individual test periods was observed. Since considerable control was exerted over the weight and area of animals per tray and since the tray environment was healthy (see above) this variation was due to fluctuations in food supply and to differences between individual populations.

The mean  $^+$  standard deviation data for individual trays is important and illuminating. The original shellfish technical description predicted equal weight gains for shellfish populations with equal animal area fed equal quantities of food. All trays in the pilot plant were culled to maintain this constant area (ca. 2550 cm²), and all trays were supplied identical flow rates. However, variation in weight gain between trays was very large--in all cases the standard deviation exceeded the mean value by a wide margin. A highly similar set of data was obtained from the Model I pilot plant, in which three populations were studied longitudinally for a one-year period. As indicated above, those results were clouded by high mortality and spawning rates which reflected a poor tank environment, and no substantial conclusions con-

cerning the validity of the technical description could be drawn.

Data collected on the Model II pilot plant (a configuration strikingly different from the Model I design) support the Model I results. Further, the data indicate that although the rearing of shellfish can be accomplished routinely and with little technical problems, the qualitative feed model requires fundamental change. It is very difficult to determine at this stage if problems encountered with the shellfish are attributable to the type of food provided, to the manner in which it is fed to the animals (tank design, etc.) or some basic physiological process in the animals themselves. The shellfish growth data indicate that the fluctuations in weight gains of the animals in the pilot plant are traceable to problems with the algae, i.e. their food supply.

Commercial exploitation requires a consistent and <u>predictable</u> output (weight gain, in this case). For this reason, the first issue to be addressed in relation to technical feasibility is the considerable deviation observed between the twenty-six (26) two-week test periods, as illustrated in Table 6.43.

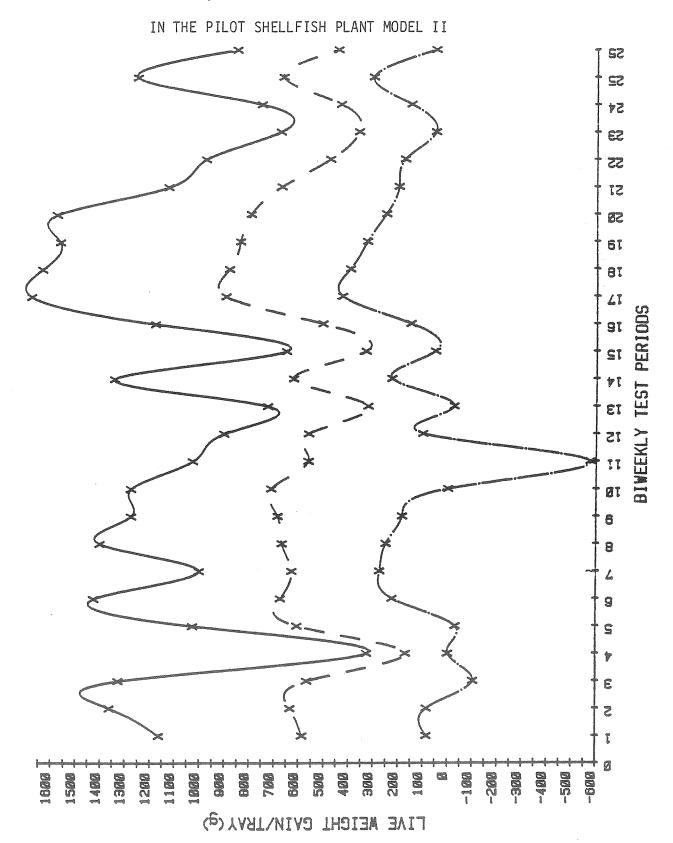
First, it should be pointed out that the total weight gains for each two-week period reflect highly consistent patterns within each test period (between the individual test populations and trays). That is, when total weight gains were low, the weight gains from all individual trays were generally low, and conversely, when total weight gains

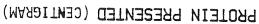
were high so were all individual tray weight gains: the fluctuations in biweekly weight gains did not reflect random combinations of high and low tray weight gains. Figure 6.19, illustrates the maximum, average and minimum weight gains per tray over each test period; note the high degree of covariance.

These data imply that for any particular test period, the weight gains of the fastest and slowest-growing animals were limited by a common variable.

Naturally, this implies further that total weight gains for each test period were limited by food supply. Indeed, this appears to be the case. Figure 6.20 illustrates total live weight gain and the total protein presented to the pilot plant for each test period. Total protein presented was calculated by multiplying the mean concentration of PPN in the food supply by the total flow of water through the plant and is, therefore, a relatively crude measure. Nevertheless, there is a very high correlation between weight gain and food supply. As shown in Figure 6.21, a least squares linear fit of the data indicates a  $r^2$  of 0.31; this should be considered as quite strong given the nature of the data. It is especially important to note that the total protein presented was calculated from mean values and does not take into account fluctuations in the food supply within each test period. For example, the relatively high total amount of food presented during test period 23 (8/15 - 8/29/78) coincides with a relatively low weight gain, but this may be attributable to the high standard deviation of inflow PPN values during this period (see Tables 6.41 and 6.42 above).

FIGURE 6.19 MAXIMUM, AVERAGE AND MINIMUM WEIGHT GAIN PER TRAY





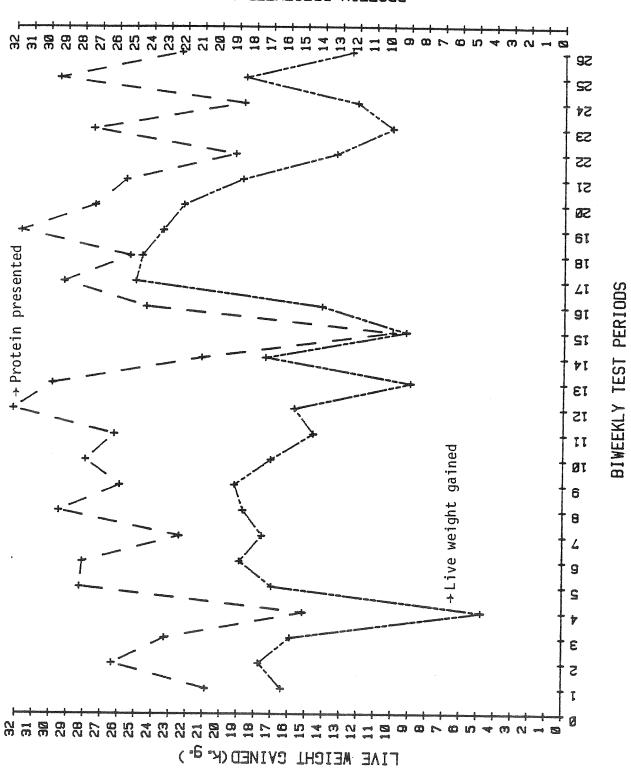
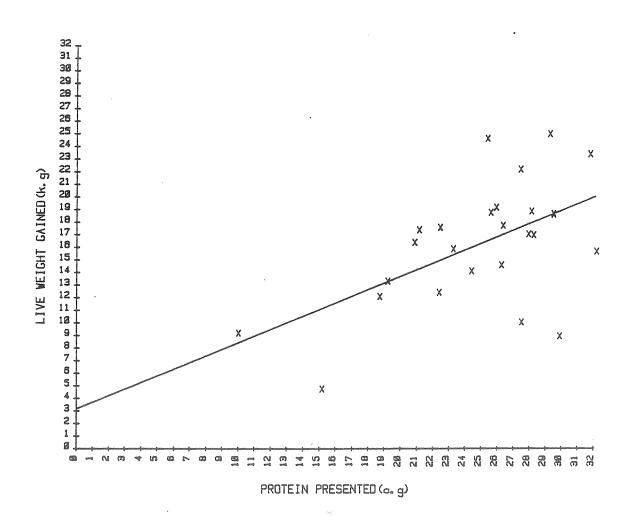


FIGURE 6.21 LEAST SQUARES FIT OF PROTEIN PRESENTED

(CENTIGRAMS) vs. LIVE WET WEIGHT GAINED (k.g.).



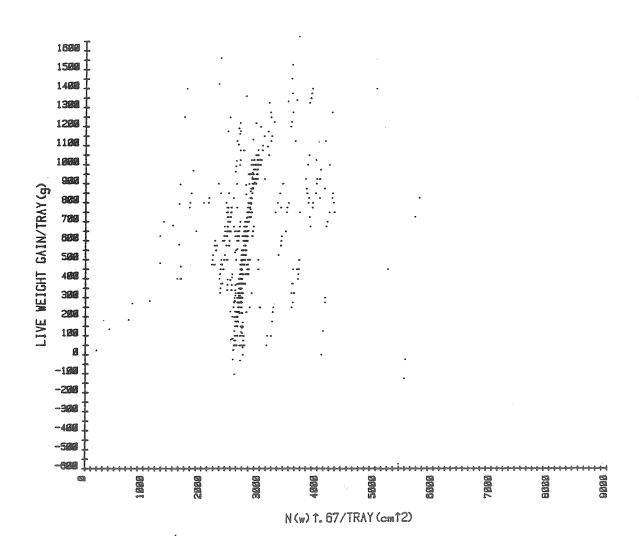
Of course, weight gain results from the complex interplay of many factors. From the viewpoint of technical feasibility and the need to obtain high and constant weight gains, however, it is evident that the periods of very low weight gain (particularly during periods 4, 13 and 15) are attributable to a low and possibly fluctuating food supply. This qualitative examination of data implies strongly that an improvement in the first trophic level would dramatically improve the overall weight gains recorded. Below, we discuss potential weight gains in more detail by examining the growth of superior populations during periods of high and consistent PPN concentration in food supply.

Tentatively, we may conclude that these fluctuations in weight gain do not invalidate the technical approach taken to rear <u>Tapes</u> in the pilot plant and that a relatively simple experimental program could iron out most of the problems to be encountered at commercial scale. Most of the problems are apt to be economic and will reflect problems of scale; there is nothing to suggest in the data collected in the Model II pilot plant that shellfish (particularly <u>Tapes</u>) are inherently difficult to rear.

Figure 6.22 is a scattergram of live weight gain per tray (all trays, all test periods) versus the animal population area per tray on the first day of each test period (population area =  $N(w)^{0.66}$  were N = number of animals and w = mean whole wet weight per animal; see Methods and Materials above). The high concentration of points (many are overlapped and thus indistinguishable on this plot) where  $X \cong 2550 \text{ cm}^2$  reflects the intended animal area per tray as determined in the shell-

FIGURE 6.22 LIVE WEIGHT GAIN PER TRAY VERSUS SURFACE

AREA OF THE CLAMS

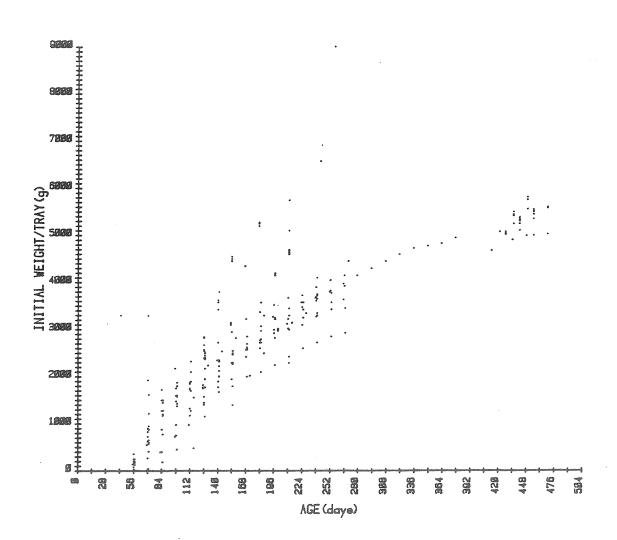


in

fish technical description. Points to the left of this line do not reflect poor control over population area; rather, they represent animals less than 84 days of age which intentionally occupied only a portion of the tray area. These points increase positively and linearly as they approach the ideal area equation. The points to the right of this intended value represent those trays for which the "loading factor" or area/tray was intentionally increased by a factor of two (2) during test periods 10 and 11. This was done to test the hypothesis that total weight and/or area per tray and not age was responsible for the drop-off in weight gain per tray. Deviations in weight gain per tray among those trays with a similar area contradict predictions based on the original technical description.

In Figure 6.23 the whole wet weight per tray on the first day of each test period is plotted against the age of the animals. Since area was held constant, the total weight of animals in each tray increased with individual size (weight) and age. Some notes on the more obvious deviations from the general trend of the data will be instructive. The cluster of points above the general function represent those trays in which the loading factor was increased during test periods 10 and 11. The cluster of points where age was more than 420 days reflect populations 20, 21 and 22 which were transferred from the Model I pilot plant. Deviations in initial weight/tray for a given age, of course, reflect variations between populations in individual weight as a function of age. This reflects variations in growth rate between populations.

FIGURE 6.23 INITIAL WEIGHT OF SHELLFISH PER TRAY AS A FUNCTION OF AGE



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This variation in individual wet weight per animal as a function of age is illustrated in Figure 6.24. Figures 6.25 - 6.39 illustrate mean individual weights as a function of age for those populations for which data are available. The mean individual wet weight is a calculated figure based on a known population weight and an assumed population number.

As indicated in (Table 6.43), there is considerable deviation in weight gain/tray within each test period. Was this random déviation or does it reflect a consistent trend?

In Figure 6.40, live weight gains per tray are plotted against the age of the animals per tray. First, the tendency to see a parabolic relationship in the data with a peak at x=84 days should be resisted since, as indicated above, all populations spent the first two test periods while in the plant (56-84 days of age on the initial day of each test period) in an "overfed" condition. During this period, the animals did not cover the bottom of the tray and the total animal area was below that dictated by the shellfish technical description. Rather, the drop-off in weight gain appears to be logarithmic. Of course, this linear scattergram does not take food supply into account, which probably explains most of the variations in weight gain per tray for a given age. Genotypic variations probably also account for some of the variation, but this cannot be determined precisely from the data.

Since the initial weight/tray increased with age (Figure 6.24) we

FIGURE 6.24 INDIVIDUAL WET CLAM WEIGHTS AS A FUNCTION OF AGE

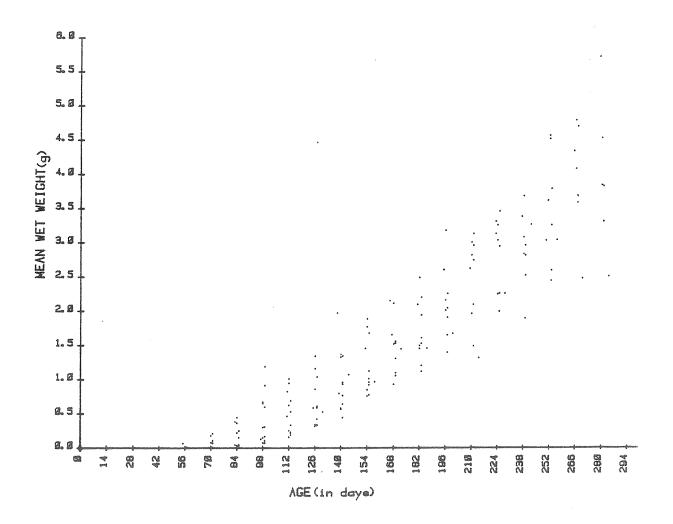
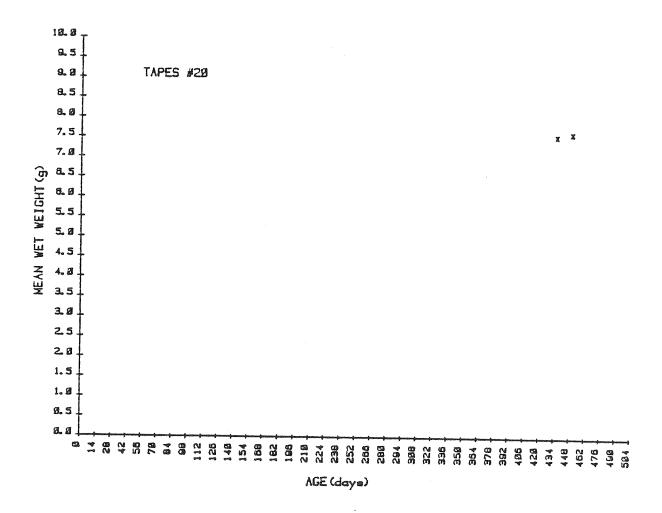


FIGURE 6.25 INDIVIDUAL WET CLAM WEIGHTS AS A FUNCTION OF AGE



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FIGURE 6.26 INDIVIDUAL WET CLAM WEIGHTS AS A FUNCTION OF AGE

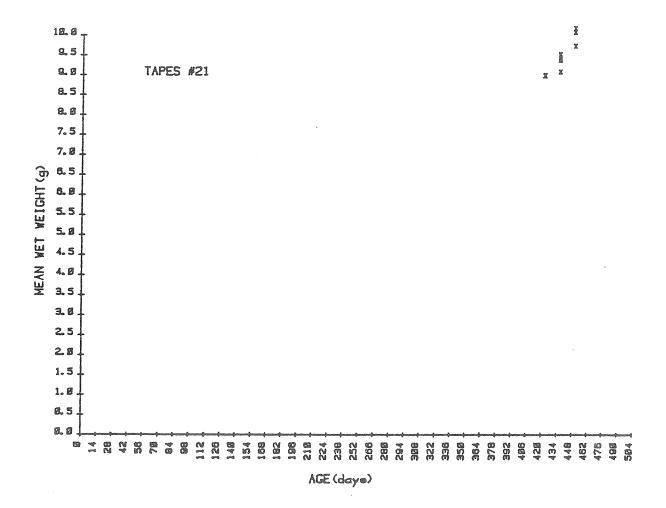


FIGURE 6.27 INDIVIDUAL WET CLAM WEIGHTS AS A FUNCTION OF AGE

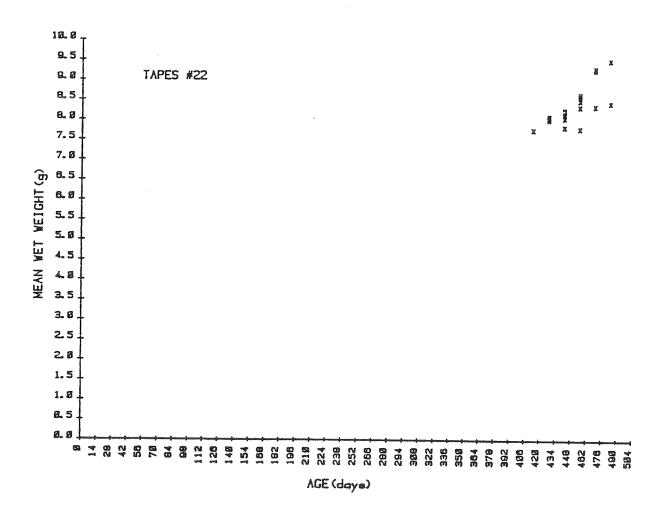
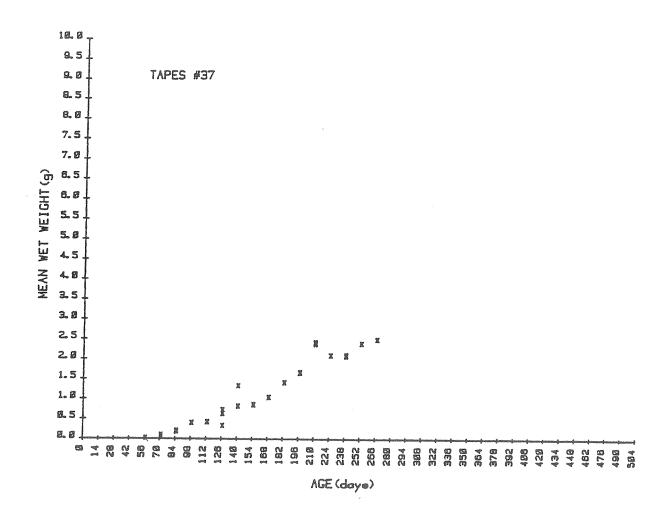


FIGURE 6.28 INDIVIDUAL WET CLAM WEIGHTS AS A FUNCTION OF AGE



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FIGURE 6.29 INDIVIDUAL WET CLAM WEIGHTS AS A FUNCTION OF AGE

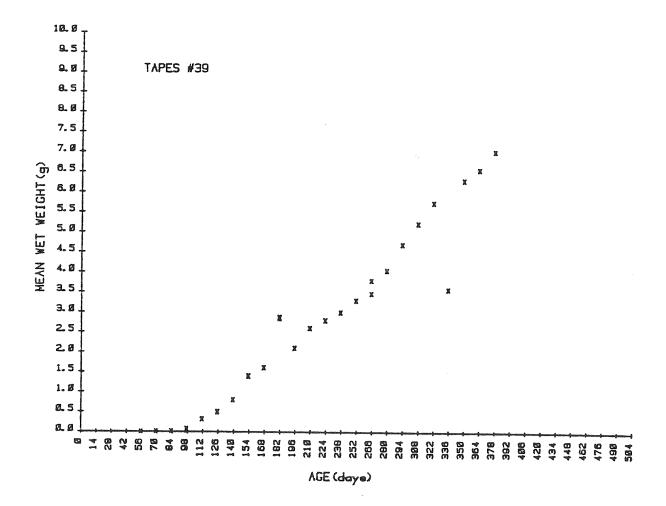


FIGURE 6.30 INDIVIDUAL WET CLAM WEIGHTS AS A FUNCTION OF AGE

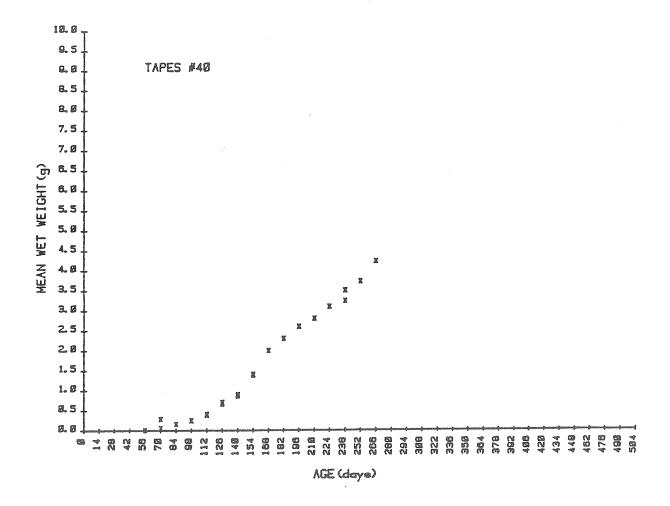


FIGURE 6.31 INDIVIDUAL WET CLAM WEIGHTS AS A FUNCTION OF AGE

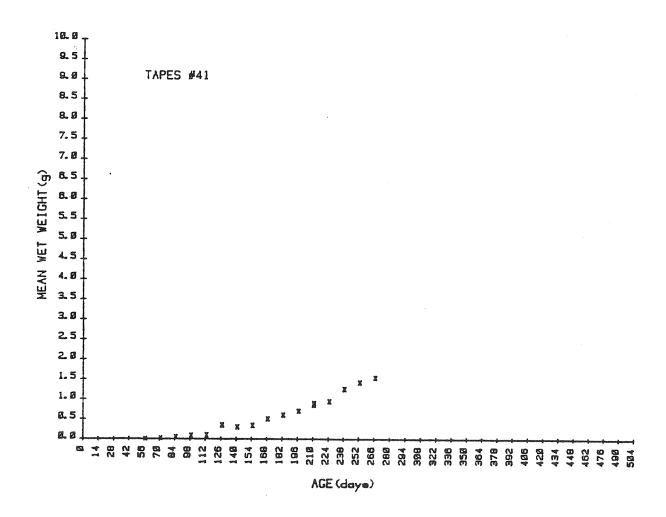


FIGURE 6.32 INDIVIDUAL WET CLAM WEIGHTS AS A FUNCTION OF AGE

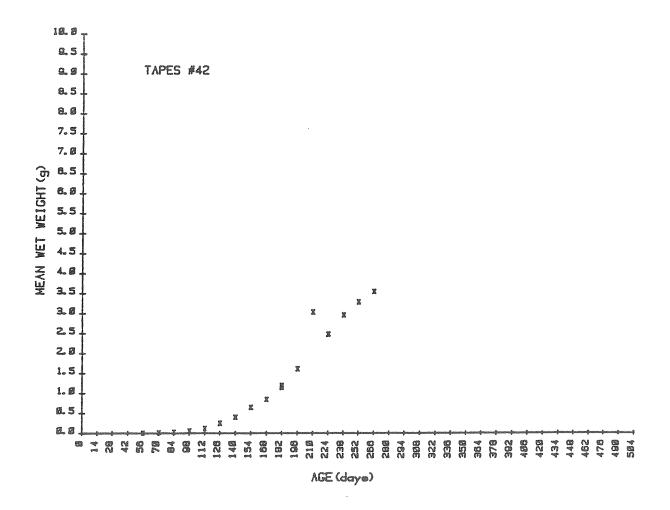


FIGURE 6.33 INDIVIDUAL WET CLAM WEIGHTS AS A FUNCTION OF AGE

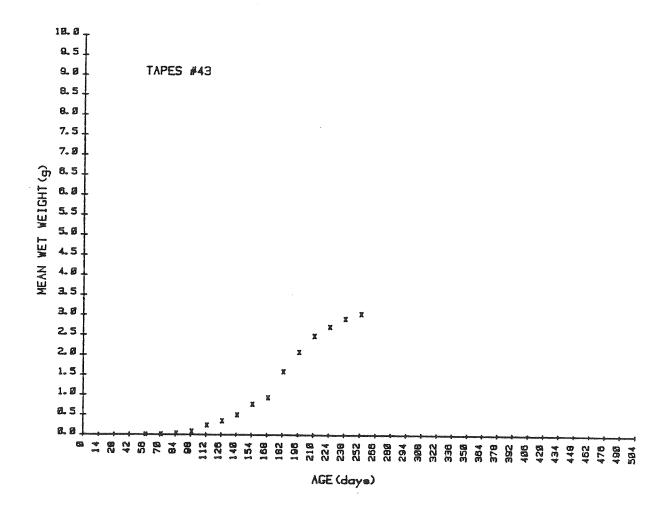


FIGURE 6.34 INDIVIDUAL WET CLAM WEIGHTS AS A FUNCTION OF AGE

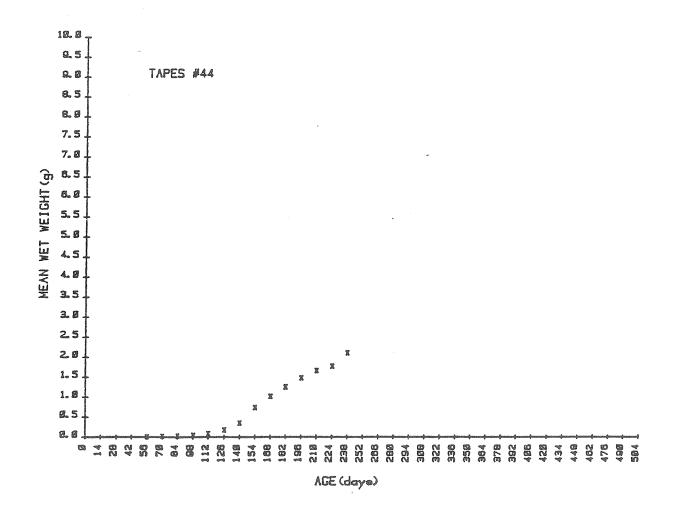


FIGURE 6.35 INDIVIDUAL WET CLAM WEIGHTS AS A FUNCTION OF AGE

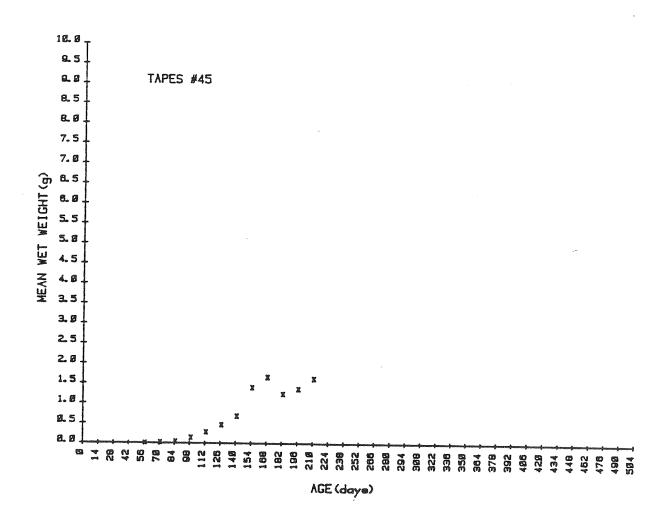
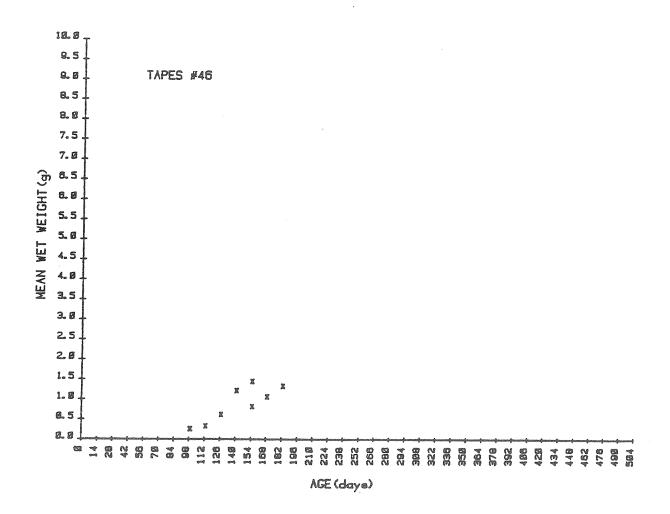


FIGURE 6.36 INDIVIDUAL WET CLAM WEIGHTS AS A FUNCTION OF AGE



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FIGURE 6.37 INDIVIDUAL WET CLAM WEIGHTS AS A FUNCTION OF AGE

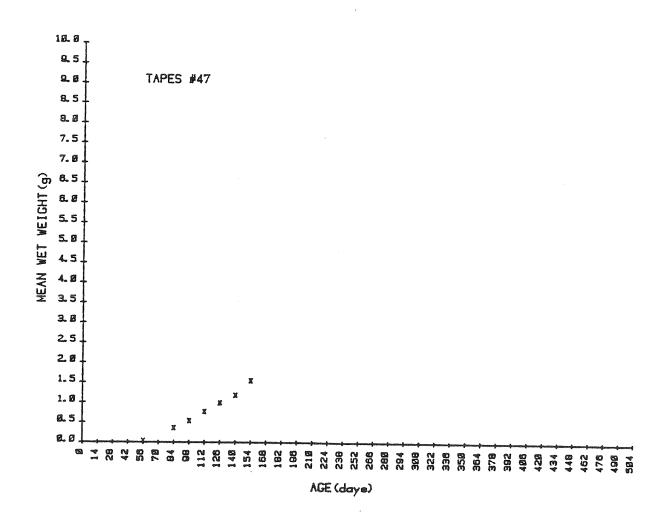


FIGURE 6.38 INDIVIDUAL WET CLAM WEIGHTS AS A FUNCTION OF AGE

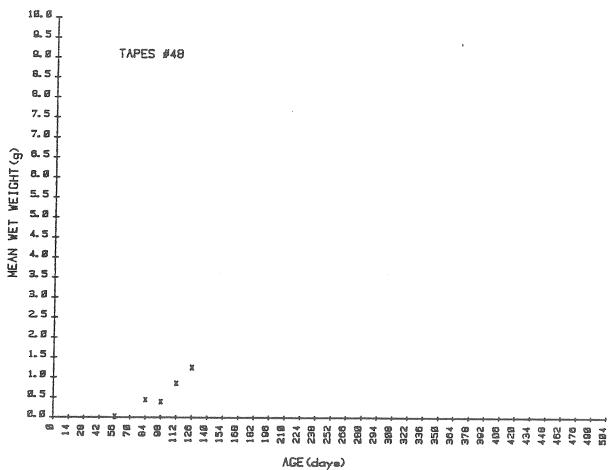


FIGURE 6.39 INDIVIDUAL WET CLAM WEIGHTS AS A FUNCTION OF AGE

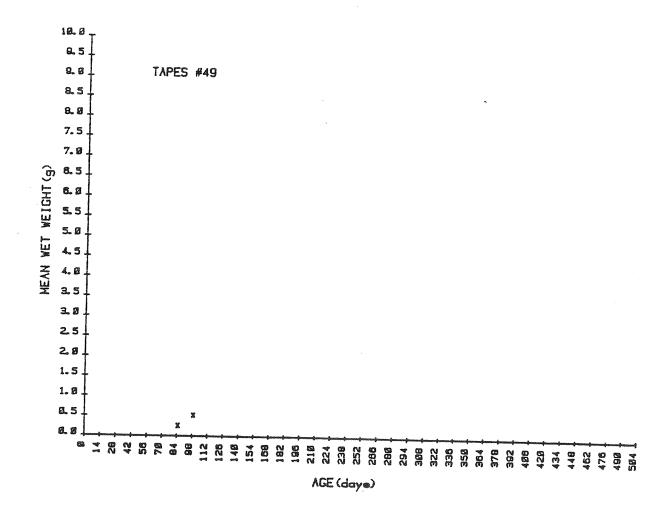
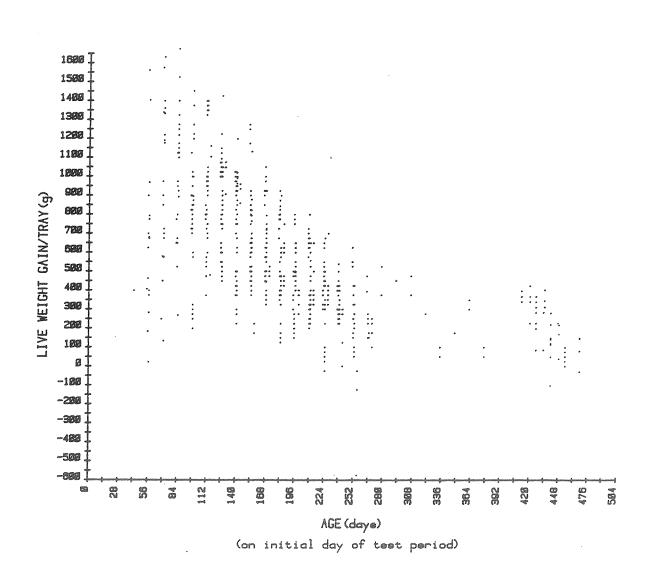


FIGURE 6.40 LIVE WEIGHT GAIN/TRAY (g) vs. AGE OF ANIMALS/TRAY (DAYS) ON THE INITIAL DAY OF EACH TEST PERIOD.



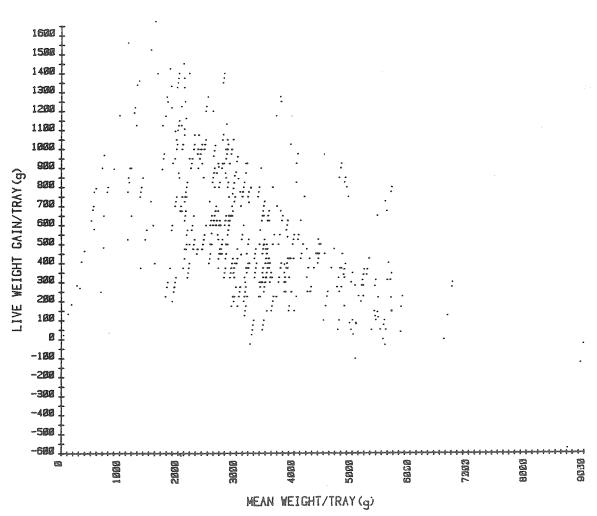
would also expect a drop-off in weight gain per tray as a function of the population weight/tray. This is confirmed in Figure 6.41. Here, the drop-off is more dramatic than is apparent in the weight gain versus age plot (Figure 6.40) because the loading factor was increased during test periods 10 and 11. The results of that test demonstrated dramatically that an increase in total weight/tray, independent of age and without a corresponding increase in food supply led to a dramatic drop in weight gains. In fact, the only losses in tray weight were recorded during these periods (Figure 6.41) and occurred in trays with the greatest initial whole wet weight.

Of course, age and individual weight per animal may be treated as separate variables (as they are above), but generally we expect them to covary in a controlled situation, where food supply and genotypic variation would be minimized. In any event, there is no direct correlation between age and growth of clams. Other developmental/physiological variables which generally covary with age (size, various physiological parameters such as metabolic rate, etc.) are more important for growth.

For a given size clam, maximum weight gains per unit tray area can be obtained by increasing food supply up to an optimum level, beyond which the population will be unable to remove it efficiently. This is shown in Figure 6.42, where weight gain per tray is plotted against the ratio of protein presented over initial weight per tray. Clearly, we expect that low food levels and low population weights will lead to low

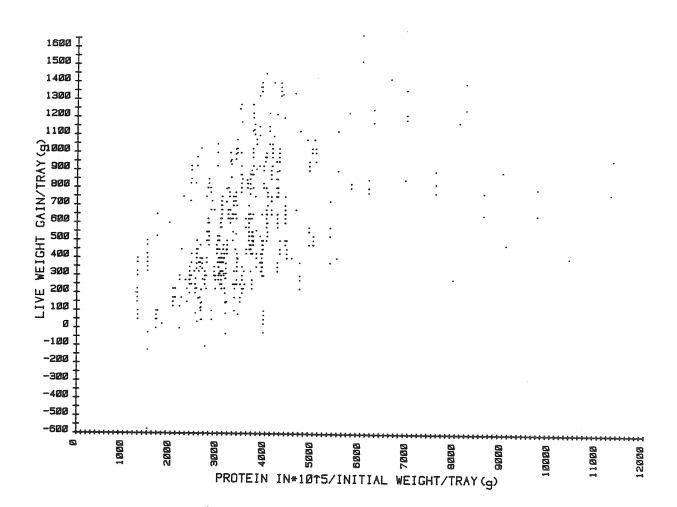
9

FIGURE 6.41 THE INFLUENCE OF MEAN WEIGHT OF <u>TAPES</u>/TRAY (g)
ON LIVE WEIGHT GAIN/TRAY (g).



(for first day of each test period)

FIGURE 6.42 PROTEIN INPUT/INITIAL WEIGHT/TRAY (g) vs. LIVE WEIGHT GAIN/TRAY (g).



weight gain, even if food is efficiently assimilated. The greatest weight gains should occur in those trays containing sufficient clams fed optimal amounts of food and containing the most efficient growers (e.g. the youngest animals). This is the case as shown in Figures 6.40 and 6.41.

If maximum weight gains per tray (i.e., per unit pilot plant area) is the main goal, then animals should be harvested at the earliest possible age. Weight gains can be kept high as animals grow by feeding an increasingly large amount of clams a greater amount of food. Weight gain per unit pilot plant area is limited only by maximum animal stacking densities and limits of animal tolerance to water flow. A trade-off exists between maximum harvestable animal size and food conversion efficiency.

These are only economic and engineering problems, however, and the data presented herein indicate no special technical problems.

However, economic projections must rest on a firm theoretical and empirical base and the data indicate clearly that a drop-off in weight gain occurs as a function of animal size and/or age and/or total population (tray) weight at a far greater rate than that predicted by the shellfish technical description, independent of animal population area. Thus, the technical description requires change to reflect the drop-off in food conversion as a function of maturation.

#### 6.5 DISCUSSION

The operation of the pilot plant to produce <u>Tapes japonica</u> by Artificial Upwelling in St. Croix during the year 10/77 - 10/78 was different in many ways from the manner in which a commercial plant would be operated. Indeed, we have learned from both Model I and Model II Pilot Plants how to improve the yields of the Artificial Upwelling mariculture system.

The major differences which would lead to increased yields at the shellfish stage would be the following:

- 1) Food supply to the animals.
- <u>a</u>. Since the St. Croix system has only two pools, the food supply was diluted with straight deep-sea water whenever one pool was shut down due to its scheduled draining, cleaning, re-filling and reinoculation. This down-time accounted for 17% of the total time during a one year period. If more pools were available, a system could be devised whereby pools producing high density cultures would be "on line" providing a constant volume of flow containing a constant concentration of phytoplankton protein to the shellfish. This would require an increase in the surface area of the pools for phytoplankton production and/or an improvement in the management of the pools. This would increase the food supply to the shellfish by 20% ( $\frac{100 \times 17}{83}$ ), and an increase of 20% in shellfish production might be expected.
  - b. As is apparent from Figure 5.2B, describing a representative

typical phytoplankton pool culture, the pools were activated before maximum levels of particulate protein nitrogen were reached and were de-activated after collapse was well advanced. If pools at high production level (e.g.  $27.5~\mu gat~PPN.L^{-1}$ ) were kept active, it is estimated that a further increase of 30% of food supply to the shellfish could be achieved. Since the mean PPN production in the pools was  $21.58~\mu gat~L^{-1}$  (see Table 5.6) a PPN concentration of  $27.5~\mu gat.L^{-1}$  would represent 90.1% conversion of incoming  $30.57~\mu gat~(NO_3^- + NO_2^-) - N.L^{-1}$  to PPN.  $\sqrt{\frac{27.5~x~100}{30.57}} = 90~$ 

If these % increases in food supply (20 + 30 = 50%) would give rise to similar % increases in whole shellfish weight produced in the pilot plant, an output of 423.6 x 1.5 = 635 kg might be expected (423 kg of shellfish were actually produced over the 12 month operation of the Model II shellfish pilot plant).

The projection that 635 kg of whole shellfish weight could be produced in one year in the shellfish plant by improved food supply does not appear unreasonable: Table 6.43 indicates that the best increase in total weight gain of the <u>Tapes</u> in pilot plant Model II for a two week period was 25 kg, achieved between 5-23-78 and 6-6-78. This would correspond to 25 kg x 26 = 650 kg production in one year if this growth rate could be sustained, and would correspond to a conversion efficiency of 24.2% of phytoplankton protein-N to shellfish meat protein-N, compared to the conversion efficiency of 15.8% actually achieved during 12 month's operation of the St. Croix Model II pilot plant.

- 2) Improved management of the shellfish.
- <u>a.</u> Since it was scheduled by the granting agency that the pilot plant would be operated for 12 months only (10/77 10/79), and we were attempting to produce as much shellfish as possible during that period, the <u>Tapes</u> populations #20, 21 and 22 which had been in Model I pilot plant since 8/3/76, 8/10/76 and 8/24/76 respectively, were transferred from the Model I pilot plant to the Model II pilot plant on 10/4/77, (see Table 6.2). These populations were then approximately 16 months old. They were larger animals, with slower growth and a history of relatively poor growth and high mortality in the Model I pilot plant.

The weight of these populations when introduced in the pilot plant Model II was

#20 14.714 kg in 3 trays

#21 25.373 kg in 5 trays

#22 37.422 kg in 8 trays

Populations #34 and #35 (see Table 6.2) had also been in the Model I pilot plant since July and August 1977 respectively and were transferred to Model II on 10/4/77.

These populations were introduced in the Model II pilot plant because insufficient 56 day old animals were available to stock up the full pilot plant (28 trays) on day one.

These larger animals with their previous unsatisfactory history in Pilot Plant Model I could be expected to have poorer growth and food

conversion efficiency than the populations specifically produced in the hatchery for Pilot Plant II. Their presence would therefore, depress the amount of shellfish which could be produced in the pilot plant if only young 56 day old populations had been introduced into the pilot plant.

These older populations were moved through the pilot plant and harvested as new populations were introduced. Only their weight gains were counted as Pilot Plant Model II shellfish production.

<u>b.</u> The other <u>Tapes</u> populations introduced in the Model II Pilot Plant when they were 56 days old (#37, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49 and 50 -- see Table 6.2) varied greatly in total and individual weight and size. Individual average weights ranged from 1 mg for <u>Tapes</u> 39 to 40.4 mg for <u>Tapes</u> 50. The total weight introduced in the pilot plant varied from 27 g for <u>Tapes</u> 43 to 400 g for <u>Tapes</u> 50.

It is obvious from Table 6.2 that the drastic action taken to improve the hatchery management (see section 6.3.3) in February 1978 greatly improved the growth of the spat between setting and day 56 when the animals were introduced in the pilot plant. This is demonstrated by the average weight of the clams of populations 47, 48 and 49 (see Table 6.2) which were 37.3, 24.4 and 40.4 mg on day 56 compared to mean weights ranging from 1 to 11.4 mg per clam for populations 37 through 47 on day 56 when they were moved from the hatchery into the pilot plant. The later influence of this improved starting weight of the shellfish in the pilot

plant is best shown in Figures 6.28 through 6.39 giving the growth of  $\underline{\text{Tapes}}$  populations #37 through #49 in the pilot plant.

On day 98, six weeks after their introduction in the pilot plant (when they were 56 days old), populations 37 through 46 had an average individual weight per clam of 180 mg; populations 47, 48 and 49 averaged 500 mg on that day.

Similarly, on day 126, populations 37 through 46 had an average individual weight per clam of 440 mg; the corresponding weight for population #47 was 1,000 mg and for population #48 was 2,300 mg.

The most important influence of improving the size and condition of the animals introduced from the hatchery into the plant is likely to be on the length of time the animals will have to be kept in the plant to reach market size.

7	TECHI	VICAL I	DESCF	RIPTI	ON	۰	٠	٥	6	۰		•	•	6	٠	•	•	۰	۰	۰	235
	7.1	INTRO	DUCT 1	ON		٠	٠	9	•	•	•	٠		*	٠	•	٠	•	٠	•	235
	7.2	GENERA	AL AF	PROA	СН		٠	٠	ь	٠	•	•		٠	•	٠	•		6		235
	7.3	INITIA	AL HY	POTH	ESI:	S	٠	•	٠	•	•		٥	•	۰	۰	9	۰	•		237
	7.4	DEFIN	ITION	OF	TROT	PHI	C	LE	VE	LS		•	•	•	•	•		٠	۰	ø	237
		7.4.1	FIR	ST T	RO PH	HIC	L	ΕV	EL		P	НУ	ΤΟ	PL	AN	KT	ON	Í	۰	٠	237
		7.4.2	SEC	OND	TROI	PHI	С	LE	VE	L		SH	EL	LF	IS	Н	6	٥	٠	٠	240
			7.4	.2.1	SI	PAC	Ε	RE	QU	IR	EMi	EN	TS		•		ø	6	۰	•	241
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#### 7 TECHNICAL DESCRIPTION

#### 7.1 INTRODUCTION

Autotrophic organisms synthesize organic material in the ocean from inorganic compounds, utilizing sunlight as a source of energy and constitute the first trophic level in the ocean's food chain. The most important photoautotrophs, quantitatively, are the unicellular algae.

The nutrients required to produce organic compounds are derived from the decomposition of organic matter. In the tropical oceans, the surface waters are nutrient-depleted, whereas comparatively high nutrient concentrations occur in deep ocean water.

Energy is introduced only at the first trophic level in the marine food chain. The phytoplankton biomass produced is dilute and individual size is small. At higher trophic levels, the individual size generally increases and individuals congregate, making harvesting practical.

Natural upwelling of deep-ocean water causes and sustains the most fertile fisheries in the ocean. "Artificial Upwelling" projects to domesticate this natural process.

#### 7.2 GENERAL APPROACH

The efficiency of utilization of solar energy for protein synthesis can be maximized by management.

The process can be directed toward the end product most desirable to man.

We have initially selected a two-trophic level system consisting of

a phytoplankton stage, followed by shellfish cultivation. This approach satisfies the requirements of a minimum number of trophic levels to preserve efficiency and provide a usable food.

In the St. Croix "Artificial Upwelling" project, the required chemical input is derived from nutrient rich deep-ocean water. It is recognized that a spectrum of nutrients is involved in the development of phytoplankton. The transformation of "nitrogen," generally the "limiting" nutrient, is used to characterize successive trophic levels.

Finally, each trophic level is maximized individually, neglecting as yet undefined trade-offs in each level, which may improve the efficiency of the total system.

Our objective, within this description, 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 rational mechanism has been established, empirical expressions satisfying experimental evidence may be used. These sections are identified and are subject to a continuing analysis.

#### 7.3 INITIAL HYPOTHESIS

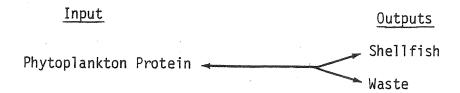
- I. A spectrum of elements is involved in the exchanges taking place within our system. "Nitrogen" is considered the limiting chemical component in the food stream.
- II. Each trophic level is considered an independent entity, related to the other level only through the output/input link, as defined.
- 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.

## 7.4 DEFINITION OF TROPHIC LEVELS

FIRST LEVEL: PHYTOPLANKTON



SECOND LEVEL: SHELLFISH



### 7.4.1 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<sup>-3</sup>)

 $\alpha$  = equiv. N to protein ratio

 $(14 \times 6.25) = 87.5$ 

 $\eta_1$  = conversion efficiency

v = volume of deep-sea water handled (cm<sup>3</sup>)

 $\eta_1$ , 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  $(D_{\rm C})$  the light intensity equals the respiration needs of the phytoplankton. 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:

$$e^{-\mu D_C} = 0.01$$

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

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

$$\frac{I_{av}}{I_{c}} = \frac{1}{D_{c}} \qquad \int_{0}^{D_{c}} e^{-\mu Z} dz = \frac{1}{\mu D_{c}} (1 - e^{-\mu D_{c}}) = .215$$

From experimental data collected in St. Croix by Mary W. Farmer, a reactor shaded to 20% could be operated at a turnover rate of .7  $\rm day^{-1}$ .

The measured value of  $\mu = .01158 \text{ cm}^{-1}$ , and:

$$D_{c} = \frac{\ln 0.01}{-u} \approx 400 \text{ cm}$$

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

An attempt has been made to develop expressions, compatible with these data, which would provide a continuous relationship between pool depth turn-over rate and conversion efficiency.

An empirical relationship, proposed in our original "Technical Description", is considered unsatisfactory. A better understanding of the phytoplankton growth mechanism (MODEL) is required, before a rational expression can be introduced. Some progress has been made, in the development of a "Phytoplankton Model", but not to the extent that a set of continuous expressions can be derived.

Based on the analysis of the data collected we estimate that:

 $1^{\rm O}$ ) in pools 1 m deep, at a turnover rate of 1.15/day under the climatic conditions prevalent on St. Croix, the "nitrogen" to protein-"nitrogen" conversion is .9.

On the basis of the data collected on shaded reactors, we estimate that:

2°) the same conversion can be achieved, in an extrapolation of our present experiment, in pools 3 m deep at a turnover rate of .75/day.

No estimate of the effect of different operating conditions, on the conversion efficiency, is available.

# 7.4.2 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)

 $\beta$  = protein to wet weight ratio (33.125) -

 $n_2$  = conversion efficiency feeding rate

 $\eta_2$ , 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  $\frac{w}{w}$ , 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  $w^{0.73}$  is the individual weight of each animal (Jurgen E. Winter, "The filtration rate of Mytilus edulis", Marine Biology, 22:317-328, 1973). 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  $\ell^2 = (\frac{w}{\rho})^{\frac{3}{3}}$  than to its weight, or volume  $(\ell^3 = \frac{w}{\rho})$ .

as our feeding criterion.

F = protein feeding rate per (individual weight)<sup>3</sup>

 $\dot{P}$  = phytoplankton protein inflow rate (g sec<sup>-1</sup>)

N = number of animals

w = individual weight (g)

The empirical relationship, proposed in our original "Technical Description", is considered unsatisfactory. A better understanding of the shellfish growth mechanism (Model) is required, before a rational expression can be introduced. Some progress has been made, in the development of a "Shellfish Model", but not to the extent that a set of continuous expressions can be derived.

According to data collected in St. Croix during a constant-weight study in November-December 1975, the optimum conversion was achieved for a feeding rate; per 1 g-equivalent animal of:

$$F = 1.18 e^{-8}$$
 (q-prot/sec)

Maximum conversion is the slowest feeding rate, which should be considered. In practice, a feeding rate, three times higher than optimum conversion as determined by this experiment was suggested. The feeding factor aimed for in the pilot plant is:

$$F = 3 \times 1.18 e^{-8} = 3.54 e^{-8}$$
 (g-prot/sec)

### 7.4.2.1 SPACE REQUIREMENTS

To the extent that the feeding rate has an influence on both the growth and food conversion efficiency, it is

important that the distribution of food is uniform.

To achieve uniform access to the food stream, for all shellfish in a given compartment,

- 10) a circulation is imparted to the medium, considerably stronger than the fresh food flow. This is easily achieved in a "closed loop" container, such as a circular tray, or a "raceway" configuration.
- 20) Space is to be provided so that no shellfish interferes with the access to food of other shellfish.

This last requirement translates into a distribution of the shellfish into a limited number of layers, possibly a "monolayer", on the bottom of a tray. The space requirements are defined in terms of the individual animals area, rather than it's volume or weight.

The highest packing density which can still be considered a monolayer is of the order of:

$$S \times (w)^{1/3}$$
 g/cm<sup>2</sup>

where:

$$S = apparent density \gtrsim 1$$
  $g/cm^3$ 

this corresponds to:

$$N = \frac{(w)^{1/3}}{w} = (w)^{-2/3}$$
 #/cm<sup>2</sup>

where:

 $N = number of animals/cm^2$ 

The food flow required, for a tightly packed tray is of the order of:

$$F/cm^2 = 3.54 e^{-A}$$
 (gm-prot/sec/cm<sup>2</sup>)

For a conversion efficiency of .9 and a nutrient content of 32  $\mu$ gat-N/l, in the phytoplankton stage, this food flow is achieved with a culture flow of:

$$\frac{3.54 \text{ e}^{-8}}{32 \text{ e}^{-9} \times 87.5 \times .9} = .01405 \quad (\text{cm}^3/\text{sec/cm}^2)$$

For every  ${\rm cm}^3/{\rm sec}$  of deep sea water phytoplankton culture flow, a tray area of:

$$1/.01405 \approx 70$$
  $(cm^2/cm^3/sec)$ 

has to be provided.

## 7.4.2.2 CONVERSION EFFICIENCY

The actual productivity of phytoplankton achieved in the St. Croix pilot plant is equivalent to 8.1T/Ha/year. The productivity, in terms of shellfish whole wet weight is 42.3T/Ha/year. On the basis of a protein to wet weight ratio of

$$\beta = 33.125$$

the achieved conversion efficiency, for an entire year:

$$\eta_2 = \frac{42.3}{8.1 \times 33.125} = .158$$

over a bi-weekly period (period 17), in the pilot operation, a total weight gain of 25 kg was achieved, as opposed to an average gain of 16.3 kg, per two week period over the entire year.

For an extrapolation of our present experiment, assuming perfected techniques, a conversion efficiency of

$$\eta_2 = .159 \times \frac{25}{16.3} = .242$$

can be expected.

No estimate of the effect of different operating conditions, on the conversion efficiency, is available.

8	AQUAC	CULTURE	BUDGET	GENE	RAT	OR	•		•	٠	•	•	•	٠	•	٠	9	٠	٠	٠	246
	8.1	FILE F	UNCTIONS				• (			•						•	•	•	•	•	246
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# 8 AQUACULTURE BUDGET GENERATOR

The Aquaculture Budget Generator package consists of an interactive computer program (AQUA3A) with supporting data files (DISK3\$ and SUMD3A). The main program also calls a subroutine (CNSTRCT). The programs are written in Fortran (MNF compiler), for use on the University of Texas at Austin CDC CYBER 170/750 computer.

Appendix B contains a listing of

- -- the main program "AQUA3A"
- -- the subroutine "CNSTRCT"
- -- the files "DISK30"

"DISK31"

"SUMD3A"

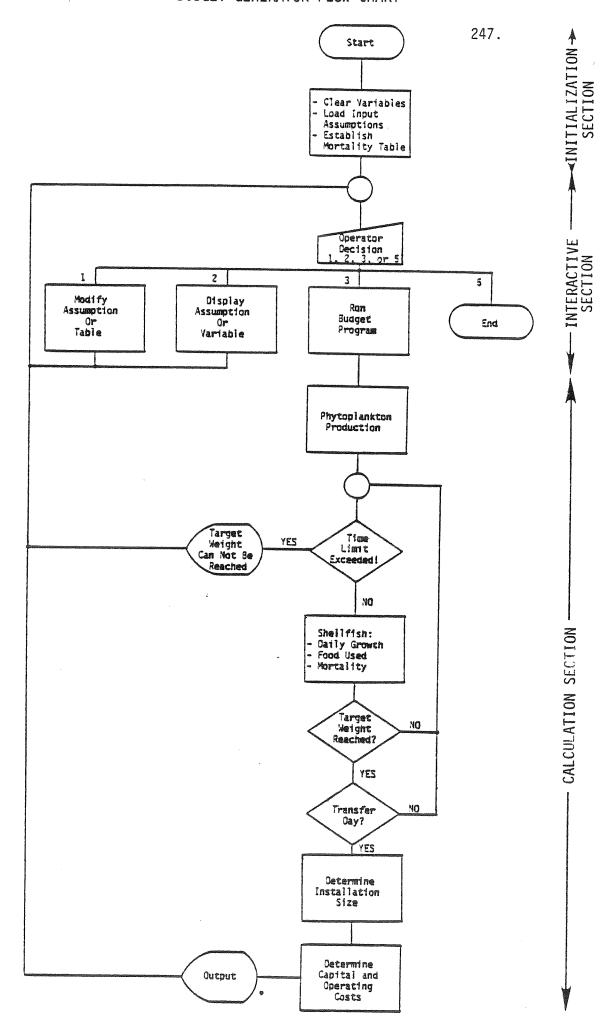
--a listing of variables used in the program

### 8.1 FILE FUNCTIONS

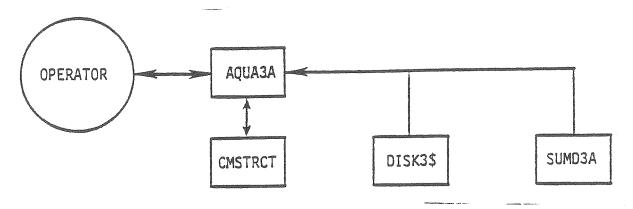
- a. AQUA3A—the main program, provides cost of production estimates. The values of input parameters and variables can be examined or modified interactively.
- b. DISK3\$--(The \$ sign stands for individual versions, numbered  $\emptyset$  to 9).

Source of default values for the input assumptions.

- c. SUMD3A--Source of the titles for AQUA3A, when producing a tabulated list of production cost estimates.
- d. CNSTRCT--Subroutine which interprets operator commands, to display or modify variables.



### 8.2 FILE RELATIONSHIPS



### 8.3 PROGRAM AQUA3A--PRINCIPAL PARTS

The program consists of an initialization section, an interactive section and a calculation section.

# 8.3.1 INITIALIZATION SECTION (up to statement #120)

Within the initialization section, the array of variables (VAR) is set to Ø. The default values of the input assumptions (DISK3\$) and the output table titles (SUMD3A) are read from their respective files.

A mortality table is generated (A9Ø3).

## 8.3.2 INTERACTIVE SECTION (statements #150 to #480)

The interactive section controls the display of requested variables and modification of input assumptions. It allows the operator to:

- 1) Change an assumption
- 2) Examine a variable
- 3) Run the budget program
- 4) (N.A.)
- 5) Exit from the program

- 8.3.3 CALCULATION SECTION (from statement #500 to end)

  There are three parts to the calculation section.

  These are:
- a) A biological subsection, in which plant and animal growth and survival are computed on a daily basis, and total requirements are calculated.
- b) A physical subsection, in which are determined the facilities needed to satisfy the requirements specified in section a) to produce the desired output; and
- c) A cost subsection, in which the physical and biological requirements to produce a unit output are costed, following standard accounting practices. The cost components and selected performance measures for the system are then printed out.
  - 8.3.3.1 BIOLOGICAL SUBSECTION (from statement #500 to 800.)

Within the biological subsection, the conversion of "nitrogen" into phytoplankton protein, then into shellfish, is evaluated in accordance with the "Technical Description" of the Artificial Upwelling process.  $1^{0}$ ) a constant phytoplankton conversion efficiency (VAR(27) = .9) is assumed, unless the compensation depth is exceeded: the volume beyond this depth is considered unproductive.

The compensation depth, for deep sea water (VAR(23)) is accepted to be 400 cm. This compensation depth is corrected, for nutrient enriched DSW, by making it inversely proportional to the nutrient concen-

tration (VAR(22)).

In a pool, whose depth does not exceed the compensation depth, each cm<sup>3</sup> of DSW, with a "nitrogen" content of:

$$= 14 \times 32e-\emptyset9$$

= 
$$4.48e-\emptyset7$$
 g-N/cm<sup>3</sup> (VAR(21))

yields:

$$0.9 \times 4.48e-07 \times 6.95$$

$$= 2.52e - 06 g - Prot/cm^3$$

How this yield would vary with, for example, the duration of exposure to sunlight is not defined at present, in the "Technical Description". The conversion yield, as defined above, is assumed to occur, for the St. Croix environment in:

- a) A pool 1 m deep at a turnover rate of 1.15
- b) A pool, 3 m deep, at a turnover rate of .75.

Pool depth (VAR(70)) and turnover rate (VAR(72)) are introduced from the assumption initialization files. Set (a) represents the actual operating conditions contained in the file "DISK3Ø" and set (b) is part of set of assumptions, contained in file "DISK31". This second set of operating conditions is deemed representative of what would be achieved in a large scale operation.

20) The shellfish conversion efficiency is also assumed constant.

An animal of a total wet weight size of 1 g, is fed at a rate of:

$$3 \times 1.18 e - 08 g - Prot/sec (VAR(57))$$

Animals of different sizes are fed at rates proportional to an

exponential (VAR(803) = 2/3) function of total wet weight. The required food flow rate (VAR(60)), and the resultant weight increases (VAR(808)), is evaluated on a daily basis from the introduction of the spat, at a size provided by VAR(806) = .001 g, until the desired market size (VAR(921) = 10 g) is reached. Actual harvesting occurs at the next transfer operation, whose intervals are determined by VAR(61).

Daily evaluations are corrected for mortality, according to the mortality table A903. This mortality table provides the daily mortality to be expected, on the basis of an estimate of the fraction of animals surviving after 180 days (VAR8Ø9).

8.3.3.2 PHYSICAL SUBSECTION (from statement 820 to Section 1)

From the output of the biological subsection, the following parameters are evaluated:

- -- Days to harvest (VAR(923))
- --"Standard" harvest weight/animal (VAR(916))
- --Mortality correction (VAR(917))
- --Protein Consumed/animal introduced (VAR(622))
- --Phytoplankton production rate per unit area (VAR(55))

From these parameters, the area, volume, flow and number of animals introduced per batch, to achieve the desired output is computed. The desired output is defined in terms of the size of successive batches, in kg (VAR(922)), and of the interval between batches, in days (VAR(807)).

For the selected subdivision of the total pool area a ratio of the

number of walls per pool is determined, since several pools share common walls.

The area required by the shellfish and the total area involved in transfers, over one year, are also calculated.

### SECTION 1

This section evaluates the capital and operating costs, related to the seawater intake system. This evaluation requires a selection of the size (diamter of the intake line(s), in order to minimize the sum of capital and operating costs.

The capital cost of a set of intake pipelines is obtained according to:

$$C = F + GD + KNLD^{1.8}$$

where:

An interpolation between published cost data, provided the following values for the coefficients:

$$F = 140,000$$
 (VAR(247))  
 $G = 370,000$  (VAR(248))  
 $K = 740$  (VAR(249))

A capital annualization factor is applied to the capital investment cost, in order to determine annual capital costs. This factor:

$$A_{f} = \frac{I_{sf}}{(I_{sf} + 1)^{Am} - 1} + Ret. + Maint.$$

where:

$$A_f$$
 = Annualization Factor (VAR(251)) (#/year)  $I_{Sf}$  = Interest rate on sinking fund (VAR(928)) (#/year)  $A_m$  = Life of pipeline (VAR(242)) (year) Ret. = Return on capital (VAR(929)) (#/year) Maint. = Annual fraction of capital cost, required for mainenance (VAR(244)) (#/year)

The pumping power required to overcome pipeline friction losses, is determined as follows: from our internal pipe friction evaluation program it is derived that the unit friction head is closely approximated by:

$$UFH = \frac{U(\frac{FLOW}{N})^{1.8}}{D^{4.8}}$$

FLOW = DSW Flow (VAR(664))

where:

 $(m^3/sec)$ 

The power loss; per m of pipeline length:

$$W/L = 1000. \times (\frac{FLOW}{N}) \times UFH$$
 (kgm/sec)  
= 9.807 \times (\frac{FLOW}{N}) \times UFH (kW)

and the power cost, per year, per pipeline.

$$\frac{Y_{\text{op}}}{N}$$
 = (Duty factor) x 365. x 24. x w x W/(Pumpn)

where:

Combining the above expressions, the power operating costs, per year, can be expressed as:

$$Y_{OD} = NQL(\frac{FLOW}{N})^{2.8} \times D^{-4.8}$$

where:

Q = (Duty Factor) x 365. x 24. x w x 
$$U/(pump \eta)$$
  
= (VAR(252)

The yearly capital costs are:

$$Y_{cap} = A_f(F + GD + KNLD^{1.8})$$

and the total yearly costs:

$$\gamma_{tot} = \gamma_{cap} + \gamma_{op}$$

The optimum pipeline diameter is obtained by solving the first derivative, with respect to D, for this yearly cost equation.

$$\frac{\partial Y_{\text{tot}}}{\partial D} = A_f G + 1.8 A_f KNLD^{.8} - 4.8 NQL \left(\frac{FLOW}{N}\right)^{2.8} \times D^{-5.8} = \emptyset$$

This implicit expression is evaluated by iteration, to within .1%. For the selected diameter (VAR(250)), the required capital investment (VAR(258)) and yearly capital and operating costs (VAR(259)) are then evaluated.

### SECTION 2

This section concerns itself with components of the system, whose costs-are directly proportional to their area. These components include:

- --The phytoplankton pools, required excavation, liners, embankments;
- --The shellfish area;
- --Ancillary office/storage facilities

The cost of phytoplankton pools consists of three components, the first two non-recurring, the last recurring.

(1) Cost of Land. This is the cost of raw land divided by the number of usable square meters in a hectare (making allowance for roadways, support equipment, wasted space, etc.).

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(2) Cost of excavation. This depends on the number of pools, total area and depth. With more than one pool there are some embankments which are common boundaries for two pools. Assuming approximately square pools, the length of each side will be:

$$L = A^{\frac{1}{2}}$$
 where A is the pool area in m<sup>2</sup>

A correction to the total length of embankments, for contiguous as opposed to isolated single pools, is introduced by the equivalent wall/cell number (VAR(67 $\emptyset$ )) calculated earlier. The total length of embankment:

$$L_T = (wall/cell) \times A^{\frac{1}{2}}$$
 (m)

Assuming a 4 meter roadway on top of each embankment and sloped sides to the pools, let the cross-sectional area of the embankment be:

$$A_E = 4\left(\frac{D}{100}\right) + 2\left(\frac{D}{100}\right)^2$$
 (m<sup>2</sup>)

where D is the actual pool depth in cm.

Thus, the volume of excavation work is:

$$V_E = A_E L_T \tag{m3}$$

Let the cost of excavation by  $K_{\rm E}$  \$/m<sup>3</sup> then cost of excavation,  $C_{\rm E}$ , is given by:

$$C_E = K_E V_E = K_F A_E L_T \tag{$}$$

(3) Cost of lining. If the pool lining is concrete its life may be quite long, although for accounting and investment purposes a relatively short period may be selected. Non-rigid liners such as butyl

rubber may have a much shorter actual life.

If the pool sides have a gradual slope, a reasonable approximation to the quantity of liner is given by the pool area.

### SECTION 3

This section sizes and costs the required DSW distribution channels and lift.

A single hypothetical channel, carrying the total flow and having a length (VAR(154)) equal to the square root of the total pool area, is substituted for the distribution network.

The flow velocity (VAR(76) = 1 m/sec) is provided as an input assumption.

The channel cross-section (VAR(151)) is obtained by dividing the total flow (VAR(664)) by this flow velocity. The product of the cross-section by the length of the channel provides the excavated volume.

To achieve the assumed flow velocity in a given channel, requires a slope (VAR(153)) which is computed according to:

SLOPE = 
$$\frac{n^2 v^2}{m^{4/3}}$$

where:

n = roughness factor (VAR(77) = .
$$\emptyset$$
2) (#)  
V = flow velocity (VAR(76)) (m/sec)  
m = Hydraulic radius (VAR(152)) (m)  
= ( $\sqrt{2/3}$  A) /2

where:

A = channel cross-section (VAR(151)) 
$$(m^2)$$

The total lift required:

The Regulation factor (VAR(75) accounts for additional head losses, required to control the distribution of the deep sea water between a number of pools.

### SECTIONS 4-9

These sections, in previous versions of the Aquaculture Budget Generator, were handling heating, aeration and effluent treatment aspects. They are deleted from the present "AQUA3A" version.

### SECTION 10

Within this section, the circulation pumping costs are determined.

These costs include capital investment in pumping equipment, maintenance and energy costs.

The program provides for four separate pumping operations.

- (1) Deep sea water distribution to the pools.
- (2) Deep sea water distribution to the shellfish
- (3) Recirculation of water in the shellfish trays
- (4) Recirculation of water in the phytoplankton pools

Only the first of these operations is active in "AQUA3A". The other operations are set to  $\emptyset$ .

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VAR(328) = Pumping capacity, DSW to Shellfish =  $\emptyset$ .

VAR(313) = Shellfish tray recirculation power =  $\emptyset$ .

 $VAR(6\emptyset2)$  = Phytoplankton pool recirculation power =  $\emptyset$ .

The deep sea intake pumping power requirements are accounted for in Section 1.

The capital cost of pumping ( $C_p$ ) is given by:  $C_p = 560.W^{0.6}$ 

where W is the calculated power requirement in KW.

The operating cost is the power cost, for continuous operation, and the maintenance cost is a fixed percentage of the capital cost.

### SECTION 11

Labor costs, for handling of shellfish, cleaning of shellfish and phytoplankton areas, are evaluated in this section.

The tasks are defined in terms of the areas to be handled, and of the frequency of these operations. These tasks are transformed into a time requirement, to which an hourly rate is then applied.

Supervisory and laboratory technician labor is specified as an input assumption (VAR(702) in terms of a direct component of the unit output cost, in \$/kg total wet weight.

Specialized labor required to maintain the plant and equipment is costed separately for each component in the relevant section of the program.

### SECTION 12

In this section the results of cost evaluations are summarized for insertion into the output display.

#### 8.4 PROGRAM OPERATION

Operation of this program on the UT computer, is described in the introductory comment of the "AQUA3A" program. After the prompting response:

"PICK ONE. FOR HELP TYPE 9."

If the user types "9", the response is:

"IF YOU WANT TO:

- "1) CHANGE AN ASSUMPTION
- "2) EXAMINE A VARIABLE
- "3) RUN THE BUDGET PROGRAM
- "5) EXIT FROM THE PROGRAM

"TYPE 1, 2, 3, or 5

If the user types '1', the computer responds:

"TYPE ASSUMPTION NUMBER, COMMA, AND NEW VALUE

"WHEN DONE TYPE 999,0

NOTE: No acknowledgement of the assumption change is provided. Space successive requests adequately and verify that the desired value has been accepted, by examining the modified assumption (see below). If the user types "2", the computer responds:

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"IF YOU WANT TO:

- "1) SEE A SECTION
- "2) SEE AN ASSUMPTION
- "3) SEE A TABLE
- "4) RETURN TO THE PROGRAM

"TYPE 1, 2, 3 or 4

If the user transmits '1', the computer returns:

"TYPE THE RANGE OF THE SECTION YOU WISH TO SEE

" e.g. 1, 47

Only a single range can be examined, at which time the previous message appears. If the user transmits '2', the computer responds:

"TYPE THE ASSUMPTION NUMBER YOU WISH TO SEE

"IF YOU WISH TO SEE ANOTHER ASSUMPTION, TYPE IN THE

NEXT NUMBER

"ASSUMPTION YOU WISH TO SEE. WHEN DONE TYPE -999
Transmitting "-999", returns the display prompt.

If the user now types '3', the message displayed is:

"IF YOU WANT TO:

"1) SEE THE MORTALITY ADJUSTMENT FACTOR

"TYPE 1

If the user types '1', a 900 day survival table is printed out, 5 days per line. Any other response is considered negative.

In both cases, the system returns to the display prompt.

If the user responds to the display prompt, by typing '4' the program returns to the initial prompt:

"PICK ONE. FOR HELP TYPE 9

If the user types '3', the calculation part of the program is entered, using the latest values of assumptions, and the "Output Summary" is printed out, before returning to the start of the program.

If the user types '5', the execution of the job is terminated.

#### 8.5 PROGRAM OUTPUT

A number of the variables (VAR), manipulated by the program, constitute input assumptions and can be defined interactively in the absence of specific requests, these input variables have to be provided with a set of "default" values. These default values are provided by the "DISK3\$" files.

Two sets of default values for the assumptions are available at this time:

"DISK3Ø" contains a set of conditions, representative of the actual St. Croix operation;

"DISK31" contains a set of conditions, representing an extrapolated realistically improved operation.

The main differences between the two sets are summarized as follows:

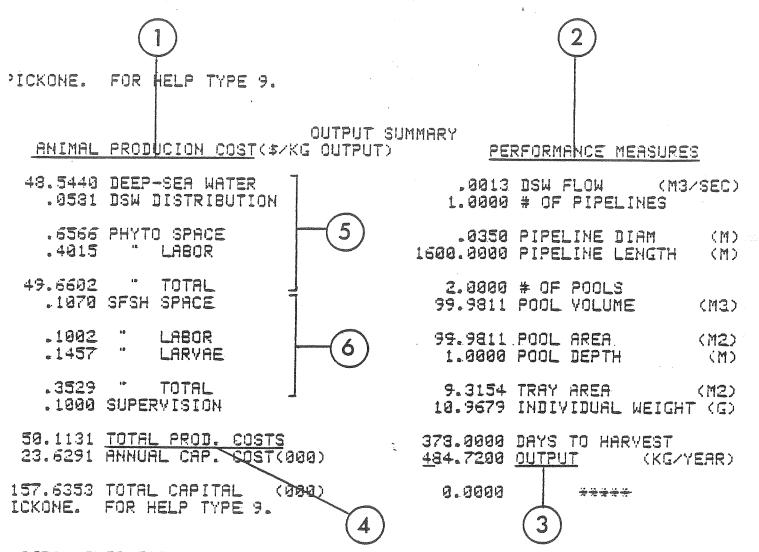
--The actual St. Croix operation utilized a deep sea water flow of  $\approx 1.3$  l/sec, exposed to sunlight in two pools with a combined volume of  $\approx 100$  m<sup>3</sup>, a depth of  $\approx 1$  m and at a turnover rate of  $\approx 1.15$ /day. The shellfish phytoplankton protein conversion efficiency is set at .158. The total output, neglecting interruptions under these conditions, would have been  $\approx 485$  kg/year of shellfish (whole wet weight).

--The extrapolated operation analyzes a plant producing 1000 kg/day of shellfish. The phytoplankton is being grown in six pools, 3 m deep, at a turnover of .75/day. The shellfish conversion efficiency is assumed to be .242.

When either set of initial assumptions is loaded by the "AQUA3A" program, and execution is requested, an "Output Summary" is produced which is a baseline output, for either the St. Croix or the extrapolated operating conditions. Baseline summarizes for both the "DISK3Ø" and "DISK31" set of default assumptions are shown in Table 8.1 and 8.2.

As detailed on Table 8.1, the "Output Summary" contains two columns:

- 1) Lists a number of components of the total shellfish production costs. These costs are expressed in \$/kg of whole wet weight shellfish output. (The last two items in this column, relate to the annual capital costs and to the required capital investment. These amounts, expressed in k\$, concern the entire plant).
- 2) Lists a number of characteristics of the plant, which are determined within the program.
- 3) Represents the plant output, in kg/year. The desired output is one of the inputs provided to the program. It is formulated in terms of the size of each batch (VAR(922)), in kg, and of the interval between batches (VAR(8 $\emptyset$ 7)) in days.
- 4) Represents the summation of all costs, divided by the plant's output. It also corresponds to the sum of the identified cost components, listed in Column 1.
- 5) This subsection relates to the phytoplankton production. (Note that it is expressed in terms of \$ per quantity required to produce 1 kg of shellfish).



ROGRAM EXECUTION TERMINATED BY USER REQUEST OOD BYE FROM AQUASA

# TABLE 8.2

# PICKONE. FOR HELP TYPE 9.

SMIND	DUT PRODUCTON COOK AND ON	PUT SUMMARY	
IIIILI	PRODUCION COST(\$/KG OL	JTPUT) PEI	RFORMANCE MEASURES
.3854 .003 <b>0</b>	DEEP-SEA WATER DSW DISTRIBUTION	.6247 1.0000	DSW FLOW (M3/SEC) # OF PIPELINES
.1987 .1279	PHYTO SPACE " LABOR	.5558 1600.0000	PIPELINE DIAM (M) PIPELINE LENGTH (M)
	" TOTAL SFSH SPACE	6.0000 71970.7129	# OF POOLS POOL VOLUME (M3)
.0625	" LABOR " LARYAE	23990.2376 3.0000	POOL BEPTH (M2)
	" TOTAL SUPERVISION	4373.2204 11.5074	TRAY AREA (M2) INDIVIDUAL WEIGHT (G)
1.0630 166.4604	TOTAL PROD. COSTS ANNUAL CAP. COST(000)	252.0000 * .365E+06	DAYS TO HARYEST OUTPUT (KG/YEAR)
1180.3442 PICKONE.	TOTAL CAPITAL (000) FOR HELP TYPE 9.	9.9999	

PROGRAM EXECUTION TERMINATED BY USER REQUEST GOOD BYE FROM AQUASA

6) This subsection represents the costs, specifically related to the shellfish section.

Under the "Performance Measures", column 2, are listed:

- --The required deep-sea water flow, to achieve the desired output, and the characteristics of the intake pipeline system, as optimized within the program;
  - --The phytoplankton pool size (Total) and configuration assumed;
  - -- The shellfish area requirement;
- --Listed next are the shellfish mean harvesting size, in grams, and the number of days required for the introduced larvae, to attain that size.

A comparison of both baseline tables shows the major influence exerted by the size of the plant, on unit output production costs, particularly the deep sea water component.

At the size of the St. Croix operation, the deep sea water costs represent an exorbitant \$48.54/kg. In a plant handling about 500 times the St. Croix water flow, the contribution of the deep sea water costs, to the total production costs, shrinks to % 36%.

REMARK: The pipeline characteristics listed in the "Performance Measures" column of the St. Croix baseline "Output Summary" (Table 8,1) do not represent our actual installation. The program suggests, and utilizes, a pipeline which is optimized for the utilized deep sea water flow. Our St. Croix installation provides more water than was utilized by the demonstration plants. The deep sea water intake installation was constructed as a verification of feasibility, and was not optimized.

9	REFERENCES.															26	Ω
-		•	•	۰	۰	•	*		۰	•	•	•	٠			40	0

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# APPENDIX A

Artificial Upwelling Pilot Plant Model II

Computerized Data File

# STX COMPUTERIZED DATA FILE

# PURPOSE:

Improve accessibility of the data collected through scheduled observations.

# LIST OF MEASUREMENTS:

atom who had also	Committee of the commit		
I)	DEEP SEAWATER CHEMISTRY		l/week
			- Thursday-a.m.
	•		2 x 3 samples
	NH <sub>4</sub>	D001	
	NO3+NO2	D001	
	PO <sub>4</sub>	D001	
	SiO <sub>4</sub>	- D001	
II)	POOL HISTORY		
	a) filling rate		as required
٠	Dilutions/Day	D002	
	Operating volume	D002	
	b) status change		as required
	Inoc. source/Pool #	D007	
•	Inoc. date/time	D007	
	Activ. date/time	D007	
	Deactiv. date/time	D007	
III)	POOL CHEMISTRY	···.	3/week
			Mon/Wed/Fri-p.m.
			2 x 2 samples
	NH <sub>4</sub>	D003	
	NO3+NO2	D003	
	Partic. ProtN	D003	

	•		
IV)	POOL CELL COUNTS		273. 2/day
			a.m./p.m.
			2 x 2 samples
	Count (167)/Contam. (+)	D004	
V)	ENVIRONMENT		2/day (except *)
			a.m./p.m.
	Level, Pool I	D005	
	Level, Pool II	D005	
	Temp., Pool I	D005	
	Temp., Pool II	D005	
	Temp., DSW	D005	
	Temp., Air	D005	
	Light*/Rain*	D005	
VI)	POLYTANK HISTORY		
	(To be defined)		
VII)	POLYTANK OBSERVATIONS		2/day
			a.m./p.m.
		,	2 x 4 samples
	a) cell counts		
	count (167)/contam.	( <u>+</u> ) D004	
	b) environment		4 readings
	Temp.	D005	
	рН	D008	·
VIII)	HATCHERY SPAWNINGS		1/4 week
			variable
	Parents, sperm	_ D009	
	Parents, eggs	D009	
	Spawnings Date	D009	
	Number of fert. eggs	D009	
		•	

IX)	HATCHERY OBSERVATIONS		% 3/week
			variable
			Sample of 10
	Date/weight	D009	
	Number/fraction removed	D009	
	Length/width (10x)	D009	
X)	PILOT PLANT SHELLFISH HISTORY		2 weeks
	•		?
			28 trays
	Batch # introduced	D010	
	Weight introduced	D010	
	Batch # removed '	D010	
	Weight removed	D010	
XI)	SHELLFISH FLOWTHROUGH & CHEMIS	TRY	3/week
			Mon/Wed/Fri-p.m.
			2 on inflow
		***	2 x 8 outflow
	NH <sub>4</sub>	D006	
	NO <sub>3</sub> +NO <sub>2</sub>	D006	
	Partic. ProtN	D006	
		,	
XII)	SHELLFISH PLANT CONTROL		
	a) environment		2/day
			a.m./p.m.
	Mixing tank level	D005	

D005

Av. tray temp.

400 - 36 468 1872 8 56 728 2912 14 98 1274 5096 (to be defined) 16 112 1456 5824 104											
Cay         Week         Trim.         Year           -         24         312         1248           -         -         -         126           -         -         -         426           -         36         468         1872           8         56         728         2912           (ta be defined)         16         112         1456         5824           -         -         -         104           -         -         -         7488           -         (56)         728         2912           -         162         2126         8424           -         162         2126         8424           4         28         364         1456           -         168         2184         8736           -         168         2184         8736           -         168         2184         8736           -         168         2184         8736           -         168         2184         8736	-	CATA FILE SIZE									
- 24 312 1248 100 36 488 1872 8 56 728 2912 14 98 1274 5096 (to be defined) 16 112 1456 5824 16 112 1456 5824 104 7488 - (56) 728 2912 - 162 2106 8424 4 28 364 1456 - 168 2184 8736 - (320) 4160 16640			Pe	er:							
400 - 36 468 1872 8 56 728 2912 14 98 1274 5096 (to be defined) 16 112 1456 5824 16 112 1456 5824 104 7488 - (56) 728 2912 - 162 2106 8424 4 28 364 1456 - 168 2184 8736 - (320) 4160 15640	* † * †	Cey	Heek	Trim.	Yecr						
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- 162 2106 8424 4 28 364 1456 - 168 2184 8736 - (320) 4160 18640		-	-		7488						
4 28 364 1456 - 168 2184 8736 - (320) 4160 16640		-	(56)	728	2912						
- 168 2184 8736 - (320) 4160 18640	Management	-	162	5106	8424						
- (320) 4160 16640	encus,	4	28	364	1456						
			168	2184	8736						
- (16) 208 832	(Alakanega		(320)	4160	16642						
	Name of the last o	-	(16)	208	832						

3/week

Sun/Tues/Thurs-p.m.

2 x 28 readings

D005

D011

2 weeks

?

8 batches, 25 samples

	, <b>#</b>	D011
	pled	D011
	weight	D011
	weight	D011
. •		
		D011
	5x	D011
		D011
	dry weight	D012
е	dryshell	D012
	meat N weight	D012

ate

- 2. The data input is obtained from a number of datacollecting sheets (Appx. A). As individual files will usually be
  established one at a time, it is of interest to group all
  measurements, recorded on a specific data sheet, into the same
  file.
- 3. All data will be stored into a "Master File," tape cassette library. Only a fraction of the "Master File" is directly accessible, in the system's memory at any time, due to memory size limitations. The individual files will be subdivided into sequential "pages," each of which can be accommodated by the available memory. The location of such pages becomes part of the primary access parameter.

Measurements have been tabulated in function of shared major access parameter, data source and file size, in order to select a convenient page organization (See table 1).

The sequence of pages, constituting each individual file for a calendar year, are grouped into books.

The adopted organization distributed the measurements over 10 books, identified by the letters A to J.

Measurement	Book
I	E
II a)	G
II b)	G
III	C
IV	A
V -	В
VI	(To be defined)

(a II (d II	00 <u>S</u> 00T 000	0SZ 0S 00Z	7	Year	Ð
	- 4	240 ()		e de la	
I	1248	375	ħ	Quarter	E
(q IIX	9878	89T	25	Меек	D
	96701	86T			_
IX	8424	762			
III	787	9 E	25	Меек	S
	9 Z E Z T	238			
(s IIX	95†T	82			
(d IIV	78S	112			
Λ	9605	86	25	Меек	В
	9878	89T			
(b IIV	5824	ZTT			
ΛI	2912	99	25	Меек	A
. sasM	Data/Year	Data/Page	Pages/Year	Page Unit	Воок
		cy pook tollow			J
	r		•	IIIX	
	r		(1)	IIIX	
	D		( c	IIX	
	B - B	•	ਭ)	IIX	
	ວ			IX	
	I		,	X	
	H			XI	
	· H			IIIA	
	В		(q	IIV	
	A			IIA	
	Воок			meaurem	

Book	Page Unit	Pages/Year	Data/Page	Data/Year	Meas.
Н	1/2 Batch	26	(4)	104	VIII
			288	7488	IX
			292	7592	
I	4 Trays	14	260	3640	X
J	4 Batches	52	320	16640	XIII a)
			16	832	XIII b)
			- 336	17472	

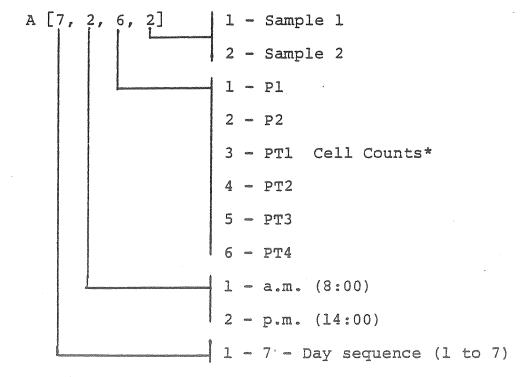
More detailed shellfish tray temperature measurements made during 1976-77 are filed under:

B' week 52 168 8736 XII a)

The data location within each page follows:

# A - PAGE IV & VII a)

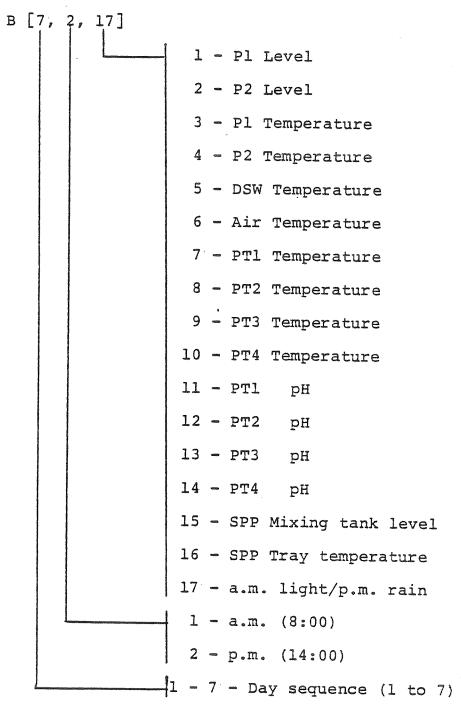
(cell counts)



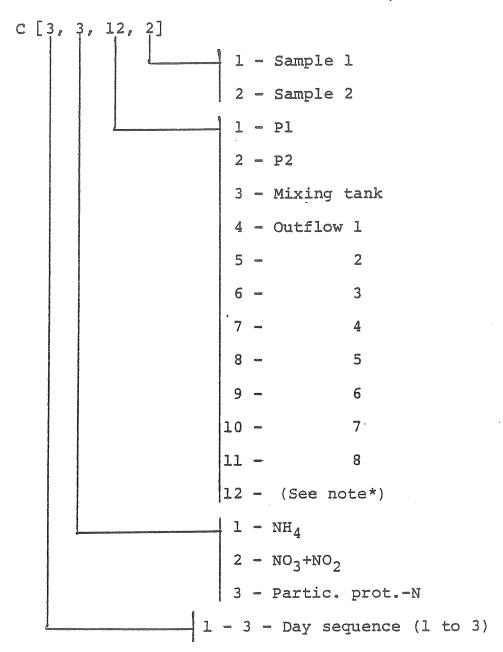
\* Note: If contaminants are detected, use (-)

V + VII. b) + XII a)

(beach observations)



(culture chem.)



283.

\*Note: Frames C [P, Q, 12, S] are used to

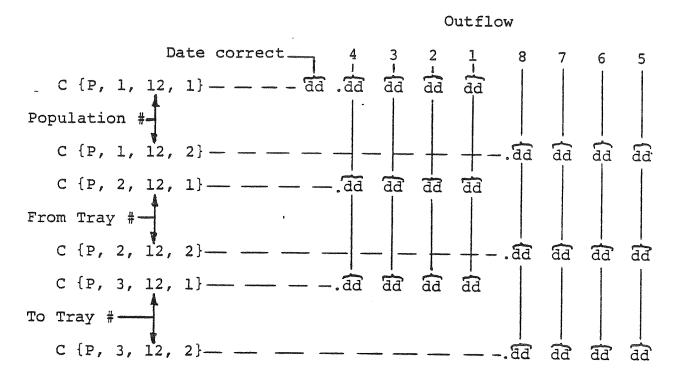
Store:

Date corrections

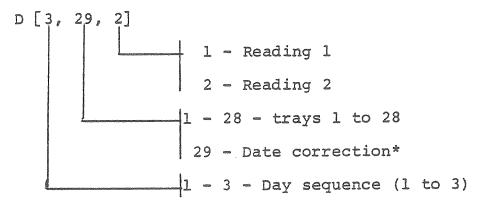
Population #

Tray #

in 2 digit blocks, as follows:



(tray flow rates)



\* Note: "Date Correction" only in frames:

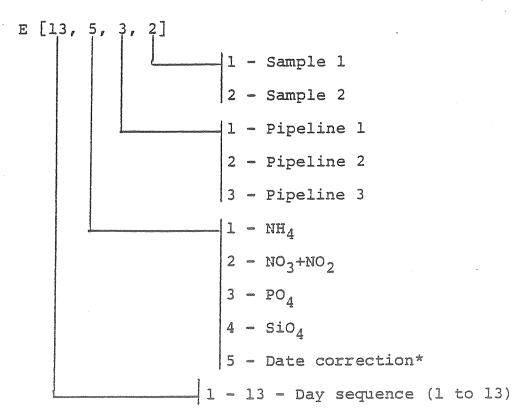
Frames:

are blank.

### E - PAGE

T

(DSW chemistry)



\* Note: "Date Correction" only in frames:

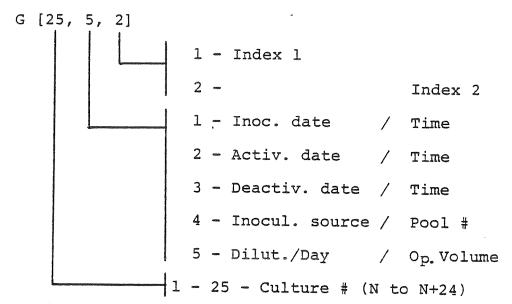
Frames:

are blank.

# G - PAGE

II a) + II b

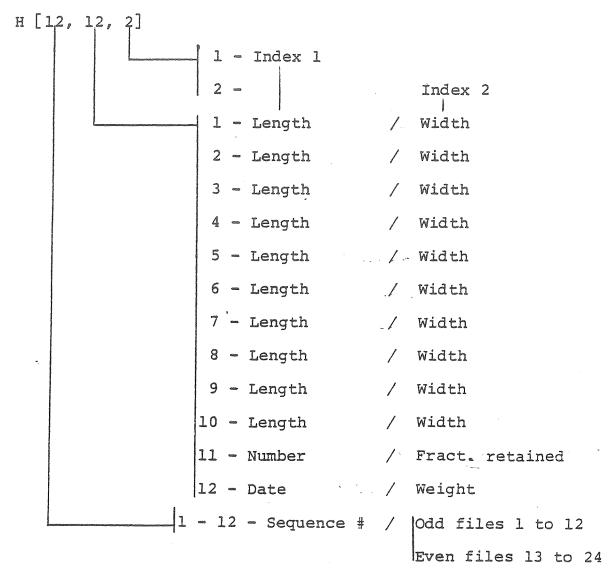
(pool history)



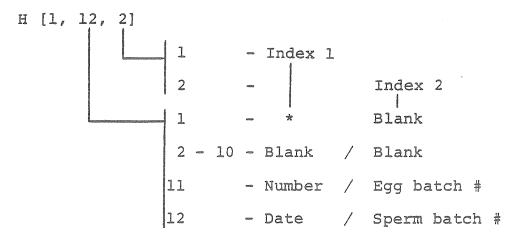
286.

VIII + IX

(hatchery)



Note: For sequence #1 only (spawning):



<sup>\*(</sup>Next available sequence #)

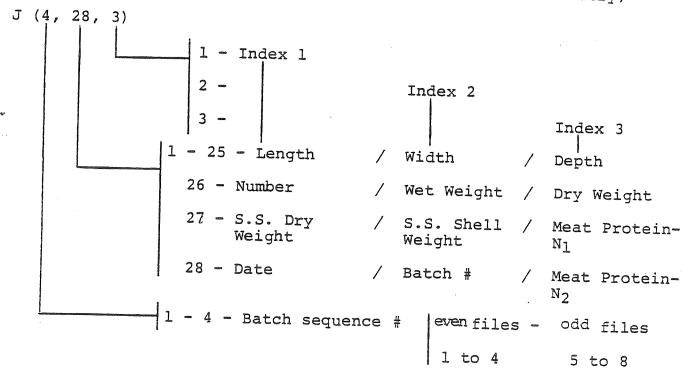
X

(p.p. shellfish)

### J - PAGE

XIII a) + XIII b)

(shellfish allometry)

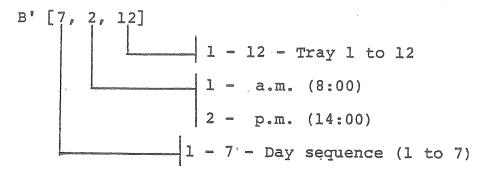


# FOR 1977 ONLY

# B'- PAGE

XII a)

(shellfish tray temp.)



For a 52-week year (364 days), the total data library will consist of:

Book	Page Format	Slots	Pages		Size*
		/Page	/Year	(Words)	(Bytes)
A	[7,2,6,2]	168	52	8736	69888
В	[7,2,17]	238	52	12376	99008
С	[3,3,12,2]	216	52	11232	89856
D	[3,29,2]	174 '	52	9048	72384
E	[13,5,3,2]	390	4	1560	12480
G	[25,5,2]	250	2	500	4000
H	[12,12,2]	288	26	7488	59904
I	[4,13,5]	260	14	3640	29120
J	[4,28,3]	336	52	17472	139776
				Total:	576416
For	1976-77 only:			- On white could be to be a proper of the country o	and the second s
B	[7,2,12]	168	52	8736	69888
				Total 1977:	646304

<sup>\*</sup>Note: Because of unused blank slots, some file sizes are slightly larger than the corresponding data set.

A 9825-A tape cassette has a nominal capacity of 250000 bytes (two tracks). A total of three cassettes/year will be used

as follows:		For l	76	For 19	77	For 19	78
Cas. #1: Books:	A	1/3 to	7/2	1/1 to	7/1	1/0 to	6/30
	В	78	88	90		10	99
	B'	t g	88	86	99	10	86
	С	80	98	<b>20</b>	98	69	<b>5</b> §
	D	90	<b>98</b>	48	6.9	10	99
	E	68	46	- 66	80	68	70
	G	68	99	80	88	60	10
Cas. #2: Books:	A	7/3 to	12/31	7/2 to	12/30	7/1 to	12/29
	В	10	\$\$ •	\$ <b>8</b> ,	99	89	00
	В'	88	88	<b>66</b>	<b>†</b> 8	48 .	88
	С	18	68	40	99	99	99
	D	99	88	10	89	8 <b>8</b>	9 <b>0</b>
	E	98	10	15	18	18	16
	G	78	88	88	90	40	\$ <del>6</del>
Cas. #3: Books:	H	1/3 to	12/31	1/1 to	12/30	1/0 to	12/29
	I	#8	78	99	99	10	10
	J	£8	4.6	10	68	99	ep

File location and sizes, within each tape, will be as follows:

Cas. #1, Trk Ø		File Size (Bytes)
File Ø =	Loading Programs	5000
Files 1-26 =	A[168]	26 x 1344
File No. =	$1+Integer (\frac{Date-1}{7})$	
Files 27-52 =	B[238]	26 x 1904
	$\frac{27+Integer}{7} \left(\frac{Date-1}{7}\right)$	**ANTHQUARA REQUIRE OFFICE THE ANTHONY OF THE ANTHO
Total	Number of Bytes:	89448

	a		
For 1976-77 Only:		291	
<del>-</del>	- ni licol	26 7244	
	= B' {168}	26 x 1344	
File No.	= 53+Integer $(\frac{\text{Date-1}}{7})$		
Total Number of Bytes:		124392	
Cas. #1, Trk 1			
File Ø	= Display Programs (A,B)	5000	
Files 1-26	$= C \{216\}$	26 x 1728	
File No.	= 1+Integer $(\frac{\text{Date-1}}{7})$		
Files 27-52	$= D\{174\}$	26 x 1392	
File No.	= $27+Integer \left(\frac{Date-1}{7}\right)$		
Files 53-54	•	2 x 3120	
File No.	= 53+Integer $(\frac{\text{Date-l}}{91})$		
File 55	and the same of th	2000	
File 56	= Display Programs (C-G)	5000	
Total Number of Bytes:		99360	
Cas. #2, Trk Ø			
File Ø	= Loading Programs	5000	
Files 1-26	$= A\{168\}$	26 x 1344	
File No.	= 1+Integer ( $\frac{\text{Date}-183}{7}$ )		
Files 27-52	= B{238}	26 x 1904	
File No.	$= 27 + Integer(\frac{Date-183}{7})$	·	
Tota	l Number of Bytes:	89448	
For 1976-77 Only:			

Files  $53-78 = B'\{168\}$ 26 x 1344

File No. =  $53+Integer \frac{Date-183}{7}$ 

Total Number of Bytes: 124392

Cas. #2, Trk 1		292.
File Ø	= Display Programs (A,B)	5000
Files 1-26	$= C{216}$	26 x 1728
File No.	= l+Integer(Date-183) 7	
Files 27-52	$= D\{174\}$	26 x 1392
File No.	= 27+Integer(\frac{Date-183}{7})	
Files 53-54	$= E{390}$	2 x 3120
File No.	= 53+Integer(\frac{Date-183}{91})	
File 55	$= G{250}$	2000
File 56	= Display Programs (C,G)	5000
Tota	l Number of Bytes:	99360
Cas. #3, Trk Ø		
File Ø	= Loading Programs	5000
File l	= (Empty)	3200
Files 2-27	= H {288}	26 x 2304
File No.	= 2(Population#-P) +0 +1	
Files 28-41	= I{260}	14 x 2080
File No.	= $2xInteger(\frac{Tray \#=1}{4}) + 28 + 0 + 1$	
File 42	= Display Programs	5200
Tota	l Number of Bytes:	102424
Cas. #3, Trk 1		
Files 0-51	= J {336}	52 x 2688
File No.	$= 2xInteger(\frac{Date-1}{14}) +0 +1$	with the second
Tota	l Number of Bytes:	139776

```
A HP-9885 disk has a capacity of 468480 bytes (1830 records).
total of 2 disks/year will be used as follows:
1976 Disks: Disk Ø: Book A Jan. 3 to Dec. 31
                           B
                           B' "
                           H
                           I
             Disk 1: Book C Jan. 3 to Dec. 31
                           D
                           E
                           G
                           J
1977 Disks: Disk Ø: Book A Jan. 1 to Dec. 30
                           В
                          B'"
                           H
                           I
             Disk 1: Book C Jan. 1 to Dec. 30
                           D
                          G
                          J
1978 Disks: Disk Ø: Book A Jan. 0 to Dec. 29
                          B
                          H
                          I
             Disk 1: Book C Jan. 0 to Dec. 29
                          D
```

E

G

File location and sizes, within each disk, will be as follows:

Disk Ø	Data Files	Fil (Re	e 9	Siz	e
Files "A-1" to "A-52"		6	X	52	
1 + Integer ( $\frac{\text{Date}}{7}$	<u>l</u> ) = File Seq. #				
Files "B-1" to "B-52"		8	X	52	
$1 + Integer (\frac{Date-7}{7})$	<u>l</u> ) = File Seq. #				
Files "H-1" to "H-26"			10	x	26
[2 ( Population #-	-l - P )] +0 = File Seq.	#			
Files "I-1" to "I-14"			9	X	14
[2xInteger ( $\frac{\text{Tray}\#-1}{4}$	$\frac{+1}{+2}$ = File Seq.	#			
Total	Number of Records		1.	114	
For 1976-77 Only:					
Files "b-1" to "b-52" (	B')		6	X	52
$1 + Integer (\frac{Date-1}{7})$	= File Seq. #				
Total	Number of Records	***************************************		312	angalanian meneranj i

	Data Files	File Size
( · ·		7 x 52
<u>L</u> )	= File Seq. #	C = 50
,	= File Seq. #	6 x 52
4 .	-	13 x 4
=)	= File Seq. #	8 x 2
<b>1)</b>	File Seq. #	11 x 52
'e*	- <u>l</u> )]	11 A J2
. ta	al Number of Records	1316

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Date	Lab Technician
Time	Chemist

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	Plastic						
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	Plastic	2					
	Glass	3					
	Plastic	3					

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x 10	<u>/liter</u>				
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T19

T20

T21

SHELLFISH TANK PT1 PT2 PT3 PT4

LEVELS: POOL 1 POOL 2 MIXING TANK

T24

T23

T22

LIGHT READING

T 5

T 6

T 7

1400 HOURS

T10

T 9

T 8

temp temp

TEMPERATURE: AIR \_\_\_\_\_ DW\_\_\_\_\_

# PILOT PLANT

	IN	FLOW	
SAMPLE #	NH <sub>4</sub>	NO3 +NO2	PROTEIN N
		Garanteen talle and the second se	
		AZENIA DE LA CALLA DE LA C	

DATE
ACTIVATED—POOL 1POOL 2
BEACH TECHNICIAN
LAB TECHNICIAN
All values in µg-at liter-1 All proteins based on 75ml filtered

OUTFLOW						on rome possesses
POPULATION #	TANK #	SAMPLE #	LT	NH <sub>4</sub>	NO3 +NO2	PROTEIN N
	÷.					
÷**						
						Name of the second
			************************			
						Official and the second and the seco
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	CULT. # POOL #	INOCULATION	a	*	ACTIVATION	a l	DEACTIVATION	a a a a a a a a a a a a a a a a a a a	FINAL DEACTIV.	ACTIV.
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COMMENTS			
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# D009A

Day 0
Date
Population #
# Fertilized Eggs
Eggs from <u>Tapes</u> population #
Sperm from Tapes population #

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# D009B

Date				
Popul	ation #	Million of Market Million and Contract of Million and		
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308.

DATE DAY All weights in grav | Wd/TANK | Wd/POPN POP # | Wd-14/TANK Wd-14/POPN ∆W/TANK ΔW/POPN | MORTALI TOTALS

Date	_
Population #	
Tare wt.	
<pre># clams/sample</pre>	
Whole wet wt.	
Whole dry wt.	
Dry shell wt.	
Dry meat wt.	
Wet meat wt.	

<u>Depth</u>

<u>Length</u>

Width

Depth

Length 
$$\begin{array}{c} \overline{x} \\ S_x \\ \overline{x} \\ S_x \\ \end{array}$$
 Width  $\begin{array}{c} \overline{x} \\ S_x \\ \overline{x} \\ S_x \\ \end{array}$ 

Length

Width

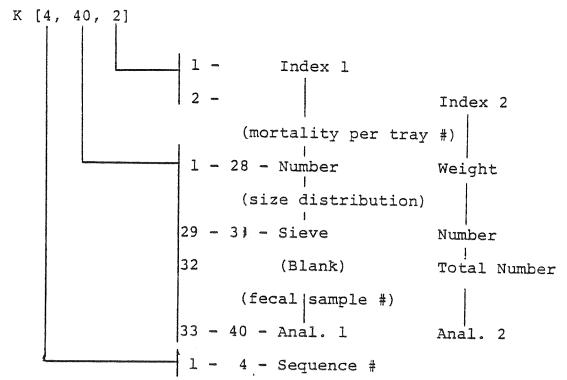
# PROTEIN-"N" ASSAY .

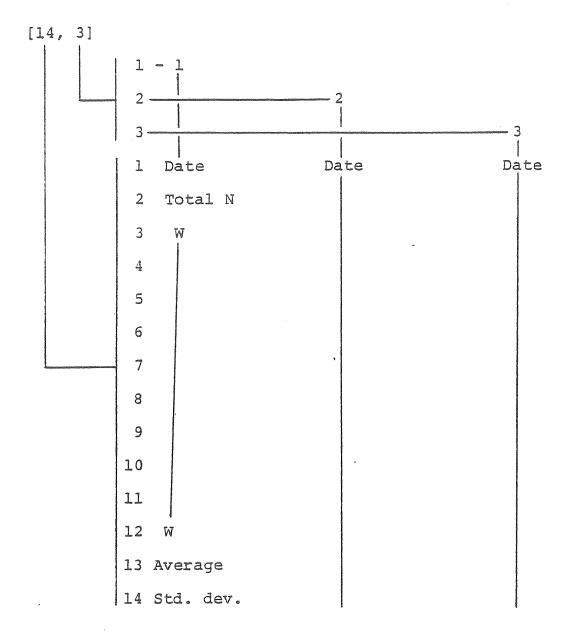
THE FOLLOWIN	IG DATA MAS OBTAINED OSTING A SOSSAMEET THE	, , , , , , , , , , , , , , , , , , ,
	NOTEBOOK #	and the same
	PAGE #	
POPULATION #		
DATE SAMPLED		
	WHOLE DRY WEIGHT (gross)	_grams
	TARE	grams
	WHOLE DRY WEIGHT (net)	
	DRY SHELL WEIGHT (gross)	_grams
	TARE	grams
	DRY SHELL WEIGHT (net)	_grams
	NCE BETWEEN THE ABOVE TWO PARAMETERS (DRY ED, DILUTED, AND SUBSAMPLED AGAIN:	MEAT WEIGHT)
	DILUTION	
	SUBSAMPLE RATIO	
	PROTEIN-"N" ASSAY-1	micrograms

# ADDITIONAL MEASUREMENTS

XIV)	НАІ	RVEST SIZE DISTRIBUTION AND M	ORTALITY RECORD	2 weeks (as for X) 28 trays
	a)	# of dead	D013	
		weight of dead	D013	
	b)	sieve size	D013	(3 sieves
		# retained	D013	+ total)
XV)	FEC	CAL PROTEIN-N		2 weeks
	a)	protein-N	DO14	(as for X)
	b)	# of trays	DO14	
XVI)	SHE	LLFISH INDIVIDUAL WEIGHT		(variable) (as for X) 15 populations
	a)	date	DO15	
	b)	up to 10 weights	D015	
	c)	total N	DO15	
	d)	average and std. dev.	(calc.)	
FILE	ORGA	NIZATION		
XIV)	a)	tray #/sequence #		
J.	b)	sieve size		
XV)	a)	origin/sample #		
	b)	origin/sample #		
XVI)	dat	e/batch #		
MEASU	REME	NT .	воок	
XIV)	a)		K	
	b)		K	
XV)	a)		К	
	b)		K	

XVI)	a)		L		
	b)		L		
	c)		L		
	d)		L		
BOOK	PAGE UNIT	PAGES/YEAR	DATA/PAGE	DATA/YEAR	MEAS.
K	8 weeks	7	224	1568	XIV a)
			32	224	XIV b)
			6.4	448	XV
			320	2240	
K-P	age		X	IVa) + XIVb)	+ XV
L	Population	15	3	45	XVI a)
		,	30	450	b)
			3	45	c)
			6	90	d)
			42	630	
L-P	age		X	VI	
воок	Page Format	Slots/Page	Pages/Ye		e Size s Bytes
K	[4, 40, 2]	320	7	2240	17920
L	[14, 3]	42	16	672	5376
1978,	Cas. #3, Trk	Ø			
	Files	43-49 = K [320]	1	7 ×	2560
	File	s No. = Unit (	$\frac{\text{Date-l}}{56}) + 43$		
	File 5	0 Loading prog	ram for K [*]		1200
	File 5	l Display prog	ram for morta	ality	1200
	File 5	2 Display prog	ram size dist	tribution	1200
	File 5	3 Display prog	ram for tray	deposits	1250





.

•

Files 54-69 = L[42]	16x336
File No. = Population #+20	
File 70 Loading program (L)	646
File 71 Display program (L)	1772

L Nov. 8, 1977 to Oct. 10, 1978

File location and sizes, within the disk, will be as follows:

Disk Ø Data File File Size (Records)

Files "K-1" to "K-7"

Unit  $(\frac{\text{Date-l}}{56})$  = File Seg. # 7x10

Files "L-1" to "L-16"

Population # - 33 = File Seq. # 16x2

Total Number of Records 102

### PROGRAM FILES

# "ADDIT" Loading "Addit" Programs 'Disk 1 "ADD1" "ADD2" "ADD3" "ADD3" Programs "Add"

Transfer	<sup>-</sup> Day		Date
ta	anks of Tapes # harve	ested today	•
Та	ank #		
Ta	ank #		
	To	otal wt. wt	•
Clams we	ere sieved and counted.		
Re	etained on 12.5 mm sieve:	i Versende Solich for State of All Production and All Solice of State of St	clams
Re	etained on 9.5 mm sieve	*econology of Starmet Education and State Education	clams
Pa	ssed thru 9.5 mm sieve		clams
			Final number
	pawnings were observed a	fter trans	fers
Tanks	,, and represe	nting <u>Tape</u>	<u>s</u> , and
Many gap	ers observed in the last	tani	ks.
Mortalit	ies:		
Tank #	# De	<u>ad</u>	Weight of Dead

FECAL PROTEIN-NITROGEN

B.T.:	Protein N accumulated mg population <sup>-</sup> 1		6 °		_ u			
anks	Calculations	x Protein N (μg-at.L <sup>-1</sup> ) X 10 (10:100 dil.)	X 32.64 L. (Tank Volume, calc.)	% 14 (atomic wt. N) ÷ 1000 μg mg <sup>-1</sup>	X # of tanks population <sup>-1</sup> = mq protein N popula-	tion-1		
Jant Ta	# of Tanks							
ion: Pilot Plant Tanks	Protein N µg-at.Ll							
Accumulation:	L.T. 0.K.			-				
Faecal Acc	Sample No.							
	Tank No.							
Date:	Population No.							

POPULATION NUMBER DETERMINATION
Day 28 (84 days past spawning)

DATE	
POPULATION	West Organization of the Control of
TECHNICIAN	

# 1. Individual whole wet weight

	N	W	W
1.	100		
2.	100		
3.	100		
4.	100		
5.	100		
6.	100		
7.	100		
8.	100		
9.	100		
10.	100		
mean			
std. d	lev.		

# 2. Population weight

	+ tare	less tare	
1.			
2.			
3.			
4.			
5.			
6.		mennes formande hand connecticio de escondricio principio de significante de la financia de la referencia (segui	
7.			

Population	weight/mean	individual	whole we	et weig	ht			
				emile trade		X	104	animals

# APPENDIX B

В.	AQU.	ACULTURE BUDGET GENERATOR COMPUTER FILE
	LIS	TINGS
	1.	MAIN PROGRAM "AQUA3A"
	2.	SUBROUTINE "CNSTRCT"
	3.	"DISK30"
	4.	"DISK31"
	5.	"SUMD3A"
	6.	LISTING OF THE VARIABLES USED IN THE PROGRAM

А ПОТОТИТЕТИЕ ПОТОТИТЕТИЕ ПОТОТИТЕТИЕ В В В В В В В В В В В В В В В В В В В	AAAAAAAA	9000	0.00000000	110	nn	AAAAAAAAA	ABAAA	53333333	AAAAA	AAAAAAAA	
40         40         40         AA         AA         43         43           40         40         40         AA         AA         33 </th <th>ANAANAAAA</th> <th>70000</th> <th>nonnnonr</th> <th>93</th> <th>nn</th> <th>AAAAA</th> <th>AAAAAA</th> <th>535333333333</th> <th>AAAAA</th> <th>AAAAA</th> <th></th>	ANAANAAAA	70000	nonnnonr	93	nn	AAAAA	AAAAAA	535333333333	AAAAA	AAAAA	
U	AA	27	20.00	3	99	AA	AA	22	AA	4	
GG         GG<	AA	99	() PI	nn	nn	AA	AA	**	AA	AA	
BU         UU         AA         AB         53           UU         UU         AA         AA         33           UU         UU         AA         AA         3333           UU         UU         UU         AAAAAAAAAAAA         3533           UU         UU         AAAAAAAAAAAAAAAAAA         33           UU         UU         AA         AA         35           UU         UU         AA         AA         AA         33           UU         UU         UU         AA         AA         AA         33           UU         UU         UU         AA         AA         AA         33         33           UU         UU         UU         UU         AA         AA         AA         33         33           UU         UU         UU         UU         AA         AA         AA         33         33           UU         UU         UU         AA         AA         AA         33         33         33         33           UU         UU         UU         AA         AA         AA         AA         33         33         33         33 <td>AA</td> <td>OF</td> <td>70</td> <td>nn</td> <td>nn</td> <td>AA</td> <td>AA</td> <td>~</td> <td>AA</td> <td>AA</td> <td></td>	AA	OF	70	nn	nn	AA	AA	~	AA	AA	
HG         UR         UR         AA         AA         33           BB         UR         UU         UU         AAAAAAAAAAAAA         3333           BB         UR         UU         UU         AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA	AA	0.0	ÖN	m	nn n	AA	A	ক <u>া</u>	AA	8	
BIG         DIG         UU         UU         AAAAAAAAAA         \$333           BIG         BIG         UU         UU         AAAAAAAAAAA         333           BIG         BIG         UU         UU         AA         AA         AA         33           BIG         BIG <td>AA</td> <td>00</td> <td>O C</td> <td>nn</td> <td>nn</td> <td>AA</td> <td>AA</td> <td>***</td> <td>AA</td> <td>4 4</td> <td></td>	AA	00	O C	nn	nn	AA	AA	***	AA	4 4	
U4         U1         U4         AAAAAAAAAAA         3333           U0         UU         UU         AAAAAAAAAAA         35           U0         UU         AA         AA         35           U0         UU         AA         AA         53           U0         UU         AA         AA         53           U0         UU         AA         AA         AA         33           U0         UU         UU         AA         AA         33         33           U0         UU         UU         AA         AA         AA         33         3333333           U0         U0         AA         AA         AA         333333333         33333333	NA	88	1361	nn	nn	AA	AA	~~~	44	A	
AAAAAAAAAA         UU         UU         AAAAAAAAAAA         33           AA         Wu         UU         UU         AA         AA         AA         S3           AA         Wu         UU         UU         AA         AA         AA         S3           AA         WU         UU         UU         AA         AA         AA         AA         AA           AA         WU         UU         UU         AA	AAAAAAAAAAA	0.0	73	nn	nn N	AAAAA	AAAAA	N N N N N N N N N N N N N N N N N N N	BAAAA	AAAAA	
OUR         OUR         AA         AA         33           OUR         OUR         OUR         AA         AA         53           OUR         OUR         OUR         AA         AA         53           OUR         OUR         OUR         AA         AA         53         33           OUR         OUR         OUR         AA         AA         53	ANAAAAAAA	1917	00	20	111	AAAAAA	AAAAA	₹.	AAAAA	AAAAA	
BB	AA	44	0.0	35	nn	AA	AA	per n	AA	AA	
QQ         QQ         QQ         AA         AA         S3         S3         S3         S3         B3         B3<	AA	00		750	ſΩ	AA	AA	~J ~J	AA	AA	
QQ         GQ/10         UU         AA         AA         33         33           GQ/GQ/QQQ/QQQ         GQ/QQQQQQQQ         AA         AA         33335555555333	A4	35	(11)	35	nn	AA	AA	N.	AA	4	
งติงและสินเลยายน บบบบบบบบบบบบ AA AA 3 เมลินใช้เมลินเลย บบบบบบบบบบบ AA AA	AA	00	0000	nn	m	AA	AA	1. N.	AA	A	
GGGGGGGGGG AA AA AA	AA	2000	noannan	nnnnnnn	nnnn	AA	AA	5535555555	AA	44	
	AA	ากถูก	SHOOMS	OHIO OHIO	nnn	AA	44	3333333333	АА	AA	

```
PROGRAM ANUAZA(TAPE1, TAPE3, TAPE4, OUTPUT=TAPE1)
    C
    C
                AQUACULTURE BUDGET GENERATUR PROGRAM
    C
                               METRIC
    Û
    C
           VERSION 3A, REVISED 10.1.79 BY L. VAN HEMELRYCK.
           THIS VERSION EVOLVED FROM VERSION 28, AS ADAPTED TO THE ST.
    C
          CROIX ARTIFICIAL UPABLLING SYSTEM BY G. ALLEN, IN MAY, 1976,
    C
           AND THANSLATED FROM ALGOL INTO FURTRAN BY S. DAVIS, IN FEB-
    C
    C
           HUARY, 1978.
    C
    C
              THIS VERSION IS DOCUMENTED INDEPENDENTLY FROM THE PREVI-
          UUS VERSIUNS. IT RETAINS THE INTERACTIVE STRUCTURE, PERMIT-
    C
           TING INDIVIDUAL INPUT PARAMETERS TO BE MODIFIED, BEFORE THE
    C
    Ü
           OUTPUT IS EVALUATED. THE INITIAL VALUES OF THE SET OF ASSUM-
           TIONS CAN BE INTRUDUCED FROM A NUMBER OF INITIAL VALUE FI-
    C
           LES, NAMED DISK38, WHERE $ = 0,1,2,...,9.
    Ü
    C
              TO KUN THIS PROGRAM, ON THE UNIV. OF TEXAS UT-2D SYSTEM
    L
    C
          THE FULLOWING SEQUENCE IS FOLLOWED. (SYSTEM RESPONSES ARE IN
à
    Ü
          BUUTES). AFTER LUGIN:
    C
    C
             ≡CC≡
    C
              READPF, 1748, BAQUA3A, DISK35, SUMD3A
    C
             ≡((() ≡
    C
            ■COPIED FILE 54QUA3A=
    C
            =CUPIEU FILE DISK35≡
    C
            ≡COPIED FILE SUMD3A

■
    C
            ≡CC≡
    C
             LUAD BAQUAZA, MNFLIB/
    Ü
             EXECUTE,, TTY, DISK35, SUMD3A
    C
            ≅GÚ≡
    Û
            EFL USED .... =
    C
            =LOAD TIME .... TM SEC=
    C
            EPICKUNE. FUR HELP TYPE 9.5
    C
    C
          YOU ARE NUW IN THE BUDGET GENERATOR PROGRAM.
          LIMENSION VAR(961), A(11), C902(900), A903(900),
              SUMTIT(26,4), NT(901)
          INTEGER I, TIME, INANT, IREC, INDX, J, IPICK, LOW, HIGH, LENGTH,
              IJ, K, ERR
          KEAL DUM, VAL, VALI
          LUGICAL TEST
    C
          DIMENSION ASST (96)
          INTEGER ASST, PIC (80), SIGN, CEL, ML
          DATA (ASST(I), I = 1,90,1)/2,3,5,14,15,10,17,18,21,23,27,30,
             32,35,30,48,61,52,03,64,65,60,67,68,69,70,71,72,73,74,75;
             70,77,78,79,80,162,103,104,105,106,107,108,109,110,111,
             202,203,212,240,241,242,243,244,245,246,247,248,249,
         Ĺ
             302,305,304,305,300,313,402,414,444,446,
             500,507,500,510,602,603,604,611,612,642,643,645,702,704,
             705,706,803,804,805,806,807,809,902,921,922,928,929/
          DU = 1,901,1
       50 vak(1) = 0.
    Ū
    C
```

READ IN INITIAL VAR VALUES FROM DISK35; IE INITIALIZE ASSUMPTIONS

Ü

```
UO 112 1 = 1, 90, 1
                                                                 324.
      READ (5,105) VAR(ASST(1))
  195 FURMATIOX, E19.121
  114 CONTINUE
      KENINU 3
C
      VALU = ALUG(VAR(889))/180.
      Du 112 l = 1,900,1
      \Delta903(1) = EXP(VALU*1)
  112 CUNTINUE
C
   READ IN OUPUT HEADINGS FROM SUMD34
C
      UU 120 I = 1, 26, 1
         READ(4,115) (SUMTIT(I,J), J = 1, 4, 1)
         FURMAT (4A7)
  126 CUNTINUE
C
      PICKONE:
C
  150 CUNTINUE
      WRITE (1,155)
  155 FURMAT (* PICKONE. FOR HELP TYPE 9.*)
C
      READ(1,160) (PIC(I), I = 1, 60, 1)
  168 FURMAT (68A1)
      CALL CHSTRCT (PIC, X, Y, Z, ERR)
      IF ( ERR .GT. 0 ) GO T O 150
      INANT = X + .5
C
C
      IF ( IWANT .EQ. 1 ) GO TO 300
      IF ( IMANT .EW. 2 ) GO TO 400
      IF ( IWANT .EG. 3 ) GO TO 500
      IF ( IWANT .EQ. 5 ) GO TO 990
      IF ( IWANT .NE. 9 ) GU TO 150
C
      VIRITE ( 1, 170)
  170 FURMAT (* IF YUU WANT TO- *
         /18x, *1) CHANGE AN ASSUMPTION*/18X, *2) EXAMINE A VARIABLE*
     B
          /182, *3) RUN THE BUDGET PROGRAM*/18X, *5) EXIT FROM
          THE PROGRAM*//* TYPE 1,2,3 OR 5 *)
      GU TO 150
C
C
   CHANGES:
  340 WRITE (1, 345)
  305 FORMAT ( * TYPE ASSUMPTION NUMBER, COMMA, AND NEW VALUE*/
          * WHEN DONE TYPE **999, 6 ** *)
  314 READ (1,320) (PIC(I), I = 1, 00, 1)
  320 FORMAT (69AI)
C
      CALL CHSTRCT (PIC, X, Y, Z, ERR)
       IF ( ERR .GT. 4) GO TO 300
      IREC = x + 0.5
      VALU = Y
       IF ( IREC .EQ. 999 ) GO TO 150
C
       IF ( IREC . WE. 534 ) GU 10 340
       VAL = ALUG(VALU) / 188.8
      DO 330 1 = 1, 903,1
          4903(I) = EXP(VAL*I)
  350 CONTINUE
```

```
325.
```

```
C
      00 350 I = 1, 90, 1
          INUX = 1
          IF ( IREC .EN. ASST(INDX) ) GO TO 370
  352 CHATINUE
  355 WRITE (1,364) TREC
  360 FORMAT (13, * 15 NOT A VALID NUMBER FOR AN ASSUMPTION --- TRY AGAIN*)
      GU TO 310
C
  370 CONTINUE
       VAR (IKEC)
                 = VALU
      60 TO 31J
C
    LISTIT:
  464 HRITE (1,410)
  410 FURMAT (* IF YOU WANT TO: */
     T5, *1) SEE A SECTION*/T5, *2) SEE AN ASSUMPTION*/
        15, *3) SEE A TABLE*/T5, *4) RETURN TO THE PROGRAM*/
        * TYPE 1,2,3 UR 4*)
      READ (1,420) 1910K
  420 FURMAT (11)
Ü
      IF ( IPICK .EQ. 1 ) GO TO 425
      IF ( IPICA .Eu. 2 ) GO TO 445 .
      IF ( IPICK .EQ. 3 ) GO JO 470
      IF ( IPICK .EG. 4 ) GO TO 150
      GO TO 499
C
    LISTI:
  425 MRITE (1,430)
  430 FORMAT (* TYPE THE RANGE OF THE SECTION YOU WISH TO SEE*
        /* (E.G. 1,47)*)
      READ (1,435) ( PIC(1), I = 1, 60, 1)
  435 FURMAT ( 6KA1 )
C
      CALL CNSTRCT (PIC, X, Y, Z, ERR)
      IF ( ERR .GT. 0 ) GO TO 425
      LUW = X + 0.5
      H1GH = Y + U.5
      IF ( HIGH .GT . 961 ) HIGH = 961
      DG 440 1 = LON, mIGH, 1
         IF ( VAR(I) .NE. 0.) WRITE (1,437) I, VAR(I)
         FORMAT (13, F30.10)
  440 CUNTINUE
      GU TO 400
C
    LISTZ:
  445 NRITE (1,450)
  450 FORMAT (* TYPE THE ASSUMPT. NUMBER YOU WISH TO SEE.*/
        * IF YOU WISH TO SEE ANOTHER ASSUMPT. TYPE IN THE NEXT*/
         * ASSUMPT. YOU WISH TO SEE. WHEN DONE TYPE **=999** *)
  455 READ (1,456) (PIC(1),1= 1, 60, 1)
  450 FURMAT (50A1)
C
      CALL CHSTRCT(PIC, X, Y, Z, ERR)
      IF ( ERR .EN. 1) GO TO 445
      IREC = X + 0.5
      IF ( IKEC .LT. =900) GO TO 400
C
      00 457 1 = 1, 95, 1
```

1

1

```
451 CUMITANUÉ
        ARITE (1,300) IREC
                                                                   326.
        60 TU 455
  Ü
    400 CUNTINUE
        00 463 1 = 1,86,1
        KEAD (3,404) J_{r}(A(K), K = 1,11,1)
        IF (J .EU. IREC) GO TO 465
    403 CUNTINUE
    404 FURMAT (14,11A7)
   405 WHITE (1,464) J_{r}(A(K),K = 1,11,1)
        KENIND 3
 C
        GO TO 455
 C
     LIST3:
   470 AKITE (1,475)
   475 FURMAT (* 1F YOU WANT TO:*/
          TID, *1) SEE THE MURTALITY ADJUSTMENT FACTOR*/
           * TYPE 1*)
       READ (1,476) IPICK
   476 FURMAT (II)
 C
       IF ( IPICK .E.G. 1 ) GO 10 480
       WRITE (1,477) IPICK
   477 FORMAT (* ENTERED CHARACTER:** *, II, * **IS NOT A VALID CHOICE.*)
       GO TO 470
 C
   480 WRITE (1,483) (I, 4903(1) ,I=1,900,1)
   463 FORMAT L* MORTALITY ADJUSTMENT FACTOR TABLE (A903) */
      6160(5( *T* ,I3, *=*, F7.4,1X)/))
       GU TO 420
 C
 C
C
     START:
         WHEN COMPARING THE FORTRAN VERSION TO THE ALGOL
(
    REMEMBER THAT I HAS BEEN ADDED TO EVERY VAR SUBSCRIPT.
C
C
  SUB CONTINUE
C
C
     INITIALIZE VARIABLES
       VAR (613) = M.
       VAR(918) = 0.
       VAR(919) = 0.
       VAR(22) = (VAR(21) + VAR(645)) / VAR(21)
      VAR(23) = VAR(23)/VAR(22)
      VAR(74) = VAR(74)/VAR(22)
      VAR(19)=VAR(70)
      VAR(25)=VAR(72)
                       *VAR(19)/86460.
C
C
    PHYTOPLANKTON PRODUCTION
       DEPTH FACTUR
         IF ( VAR(19) .GT. VAR(23)) WRITE (1,505) VAR(19), VAR(23)
         FORMAT (* ACTUAL PUUL DEPTH FACTUR EXCEEDS COMPENSATION DEPTH*
  505
         ,2(2x,Fd.1))
         VAR(52) = AMINI (VAR(23), VAR(19))
C
C
       CONVERSION EFFICIENCY
      VAR (54)=VAR (27) *VAR (19)/VAR (52)
Ĉ
```

11 1 4331(1) . EN. 1KEL 1 60 TO 460

```
RAIL OF PROTEIN PRODUCTION G/CM2/SEC
                                                                  327.
  525
          VAR(55) = 0.25 * (VAR(21) + VAR(645))
          VAR(SO) = VAR(SS) * VAR(S4) * VAR(25)
C
C
   SHELLFISH PRUDUCTION
C
   TIME DEPENDENT GRUNTH LIMIT
      ULIM = 980 - VAR(01)
      mlGm = ULIM
      IF ( VAR(3) .GT. v.) HIGH = MIN1 (VAR(3), ULIM)
      LUM = 1
C
   INITIALIZE
         MT (LUM) = VAR(BMB)
         I = L \cup N = 1
C
C
   FEEDING CRITERION
      VAR(57) = VAR(30) \times VAR(32)
C
   DAILY INCREMENT LOOP
C
         DU 800 IZ = LOW, HIGH, 1
         l = I+1
C
C
         ANIMAL GRUNTH FUNCTION
C
   RATE OF FEEDING G-PRUT/SEC/ANIMAL IN
      VAR(60)=VAR(57)*"T(1)**VAR(803)
C
C
    CONVERSION EFFICIENCY
      VAR (50) = VAR (35)
C
C
          TUTAL MEIGHT/PROTEIN RATIO
               VAR(59) = VAR(15) * VAR(16) * VAR(18)
C
         GROWTH INCREMENT PER DAY
C
               VAR(808) = VAR(59) * VAR(58) * VAR(60) * 86400.
               AT(I+1) = AT(I) + VAR(808)
C
C
      SURVIVAL
         C902(I)
                      = 4903(1)
                                    * VAR(902)
   CUMULATED FOUD FLUNS, CORRECTED FOR SURVIVAL (G/SEC/ANIMAL IN)
C
      VAR(613)=VAR(613)+VAR(60)*C902(I)
C
   CUMULATED WEIGHT AND NUMBER, CORRECTED FOR SURVIVAL
      VAR(918)=VAR(918)+WT(I+1)*C902(I)
      VAR(919)=VAR(919)+C902(I)
C
   TARGET WEIGHT REACHED
      IF (NT(1+1) .GE. VAR(921)) 700,800
  700 K = 100*((I)/VAR(61)=INT((I)/VAR(61)))
      IF (K .NE. 0) 710,820
  710 I2 = I2-1
  BUR CONTINUE
      MRITE (1,810) HIGH
  818 FORMAT (* TARGET WEIGHT CANNOT BE REACHED IN *, 13, * DAYS. *)
      GO TO 150
C
  FINAL WEIGHT AND NUMBER, PER ANIMAL IN
C
  620 VAR(925) = I+.1
      VAR(916) = NI(I+1)
      VAR(917) = C982(I)
```

```
INTERCEDIAL LOOP LEGIS GLUDINGFOLWITUME TIM
                  VAR (022) = VAR (613) / VAR (007)
                                                                                                                                                                                   328.
  L
          WEIGHT AND NUMBER OF ANIMALS SIMULTANEOUSLY IN THE SYSTEM
 C
                  VAR (918) = VAR (918) / VAR (837)
                  VAR (919)=VAR (919)/VAR (807)
 C
         TUTAL NUMBER OF ANIMALS STARTED/BATCH
 C
                  VAR(927) = 1000. * VAR(922) / (VAR(916) * VAR(917))
 C
         TUTAL FEED RATE, AREA UTILIZED, FLOW, VOLUME
                  VAR(041) = VAR(927) * VAR(622)
                  VAK(602) = VAR(641) / VAR(56)
                  VAR(603) = VAR(662) * (1. + VAR(17))
                  VAK(064) = VAR(602) * VAR(25) / 1000000.
                  VAR(665) = VAR(663) * VAR(19) / 1000000.
 C
         PUOL WALL TO CELL RATIO
                 CEL = 1
                 WL = 1
                 N. = 1
       880 00 890 L = 1,2,1
                  VIL = NL + 1
                 DO 890 N = 1, M, 1
                 CEL = CEL + 1
                 ML = ML + 2
                 IF (CEL.EU. VAR(H04)) 960
      090 CUNTINUE
                 14 = M+1
                 IF (CEL.LT. VAR (804)) 880
      900 VAR(670) = NL/CEL
C
C
         THAY AREA (M2), TRANSFERS
                 VAR(949)=VAR(662) *VAR(25) *VAR(74)/10000.
                 VAR(767)=VAR(949) *365./VAR(61)
C
                                                                                                                                      (SECTION 1)
C
C
         INTAKE PIPELINE COSTS
                 IF (VAR(2)) 902,902,912
      982 VAR(251) = VAR(928)/((VAR(928)+1.)**VAR(242)=1.)+VAR(929)+VAR(244)
                 VAR(252) = VAR(243) * 8760 * VAR(414) * 9.807 * VAR(245) / VAR(302)
                AH = VAR(251) * VAR(248)
                A1 = VAR(246) * VAR(251) * VAR(249) * VAR(240) * VAR(241)
                A2 = -(VAR(246) + 3.) * VAR(252) * VAR(240) * VAR(241) *
              B (VAR(664) / (VAR(240) * VAR(243))) ** (VAR(246) + 1.)
               01AM = (-A2/A1)**(1/(2.*VAR(246)*3.))
      965 DD = Av + A1*DIAM**(VAR(246) - 1.) + A2*DIAM**(-VAR(246) - 4.)
                D\dot{U} = UD/((VAR(246) - 1.) * A1*DIAM**(VAR(246) - 2.) -
              5 (VAR(246) + 4.) * A2*D[A***(-VAR(246) - 5.))
                DIAM = DIAM -UD
                IF ( AbS(DD / DIAM) = .001) 910,910,905
      412 VAR(250) = DIAM
                VAR(258) = VAR(247) + VAR(248) * VAR(250) +
             U VAR(240) * VAR(241) * VAR(249) * VAR(250)**VAR(246)
                VAR(259) = VAR(251) \times VAR(253) + VAR(240) \times VAR(241) \times VAR(252) \times VAR(251) 
             o(VAR(004)/VAR(240))**(VAR(240)+1.)/(VAR(250)**(VAR(240)+3.))
C
C
                                                                                                                                     (SECTION 2)
C
          COST OF SPACE
C
          CUST OF LAND
     912 VAR(112) = VAR(102) / VAR(103)
```

```
VAKILLO) = VAKILLS * VAKIACA)
                                                                   329.
C
   SM / STRON LATINAN CHARLIFACHA
      VAR(125) = VAR(804) * VAR(670) * SQRT(VAR(663)/VAR(804)) / 100.
      VAR(126) = 4. * VAR(19)/100. + 2. * (VAR(19)/100.) ** 2.
      VAR(127) = VAR(805) * VAR(125) * VAR(126) * 10000. / VAR(663)
      VAR(12b) = VAR(127) * VAR(929)
      VAR(114) = VAR(184) * (VAR(928) / ((VAR(928) + 1.) ** VAR(125) =
         1.) + VAR(929)
      VAR(116) = VAR(106) * (VAR(928) / ((VAR(928) + 1.) ** VAR(107) =
          1_a) + VAR(929))
      VAR(117) = VAR(642) * VAR(168)
      VAR(118) = VAR(117) * (VAR(928) / ((VAR(928) + 1.) ** VAR(643) =
         1.) + VAR(929)
      VAR(119) = VAR(139) * (VAR(928) / ((VAR(928) + 1.) ** VAR(110) =
         1.) + VAR(929))
      VAR(120) = VAR(112) + VAR(104) + VAR(106) + VAR(117) + VAR(127)
      VAR(121) = VAR(113) + VAR(114) + VAR(116) + VAR(118) + VAR(128)
      VAR(122) = VAR(112) + VAR(106) + VAR(117) + VAR(109)
      VAR(123) = VAR(113) + VAR(116) + VAR(118) + VAR(119)
C
C
    END OF SECTION 2
C
                                                   (SECTION 3)
C
C
    DISTRIBUTION CHANNELS
      VAR(150) = SURT(VAR(662))/200.
      VAR(151) = VAR(664)/VAR(76)
      VAR(152) = SQRT(2.*VAR(151)/3.)/2.
      VAR(153) = (VAR(76) * VAR(77)) * * 2. / VAR(152) * * (4./3.)
      VAR(303) = VAR(150) * VAR(153) * VAR(75)
C
C
    AMORTIZATION AND CAPITAL COSTS
      VAR(155) = VAR(150) * VAR(151) * VAR(805)
      VAR(156) = VAR(155) * VAR(929)
      VAR(160) = VAR(154)*(VAR(112)+VAR(104)+VAR(117))+VAR(155)
      VAR(161) = VAR(154)*(VAR(113)+VAR(114)+VAR(118))+VAR(156)
C
Ç
    END OF SECTION 3
C
                                                   (SECTION 10)
    COST OF PLUMBING
      VAR(331) = 0.
      VAR(332) = 0.
C
Ľ
    PUMP CAPACITY, INTAKE AND RECIRCULATION(KW) - 1001 EFFIC.
      VAR(327) = 9.807 \times VAR(664) \times VAR(303)
      VAR(329) = VAR(313) * VAR(949)
      VAR(330) = VAR(602) * VAR(665)
C
      00 948 I = 327, 338, 1
         VAR(I) = VAR(I) / VAR(302)
         VAR(306) = (560. * VAR(I) ** 0.6) * (1. + VAR(304) / 100.) *
              VAR(492) / 2000.
     C
         VAR(309) = VAR(308) * (VAR(928) / ((VAR(928) + 1.) ** VAR(305)
               -1.) + VAK(929)
     D
                                  )
         VAR(310) = VAR(308) * VAR(306) / 100. + VAR(1) * 8760. *
              VAR (414)
         VAR(I - 6) = VAR(309) + VAR(310)
         VAR(331) = VAR(331) + VAR(308)
         VAR(332) = VAR(332) + VAR(389)
  946 CUNTINUE
C
```

C

```
C
          END OF SECTION 10
                                                                      330.
 Ü
                                                     (SECTION 11)
     COST OF LABOR - MARVEST, TRANSFER, AND CLEANING
 Ù
       VAR(707) = (VAR(707) * VAR(612)) / VAR(704)
       VAR(789) = VAR(662) * VAR(612) * 365.
         / (VAR(785) * VAR(786) * 10000 )
C
C
          END OF SECTION 11
(SECTION 12)
C
     CUST SUMMARY
       VAL = VAR(922) * 305. / VAR(807)
       VAR(932) = (VAR(259)+VAR(14)*VAR(664)*VAR(243)*31536000.)/VAL
       VAR(933) = VAR(664)
       VAR (934) = VAR (321) / VAL
       VAR (935) = VAR (240)
       VAR(930) = VAR(121) x VAR(663)/(10000 = xVAL)
       VAR(937) = VAR(250)
       VAR(938) = VAR(729)/VAL
       VAR(939) = VAR(241)
       VAR(940) = VAR(932) + VAR(934) + VAR(936) + VAR(938)
       VAK (941) = VAR (804)
       VAR(942) = VAR(123) * VAR(949) / VAL
       VAR(943) = VAR(665)
       VAR (944) = VAR (727)/VAL
       VAR(945) = VAR(663)/10000.
       VAR(946) = VAR(46) * 1000_ / (VAR(916) * VAR(917))
      VAR(947) = VAR(19)/100
      VAR(948) = VAR(942) + VAR(944) + VAR(946)
       VAR(950) = VAR(702)
       VAR(951) = VAR(916)
      VAR(952) = VAR(940) + VAR(948) + VAR(950)
      VAR(953) = AINT(VAR(923))
      VAR(954) = (VAR(934) * VAL + VAR(123) * VAR(949)
     b +VAR(161)+VAR(332)+VAR(259))/1000.
      VAR(955) = VAL
      VAR(956) = (VAR(120) * VAR(663) / 10000. + VAR(122) * VAR(949)
     в +VAR(160)+VAR(331)+VAR(258))/1000.
C
C
C
                           OUTPUT RESULTS
      WRITE (1,960)
  960 FURMAT (T30, * OUTPUT SUMMARY*)
      WHITE (1,961)
  961 FORMAT (T5,*ANIMAL PRODUCION COST(S/KG OUTPUT)*,
        T48, *PERFURMANCE MEASURES*)
C
      UN 980 I = 932, 956, 2
         IJ = I - 931
      RRITE (1,970) VAR(1), (SUMTIT(1J,J), J=1,4,1),
         VAR(I+1), (SU = TIT(IJ+1,J), J=1,4,1)
         FURMAT (2(F10.4,1X,4A7))
  980 CONTINUE
      GO TO 150
C
C
    ENDOFPRUG
C
  990 ARITE (1,995)
  995 FURMAT (* PROGRAM EXECUTION TERMINATED BY USER REQUEST*/
         * GUOD BYE FROM AGUAZA *)
      STUP
```

```
<u>⊊</u> 19 ₽
                                                                    331.
C
C
C
                            SUBROUTINES
C
       SUBROUTINE CHSTRCT (PIC, LOW, HIGH, VALU, ERR)
Ĺ
       THIS SUBROUTINE SIMULATES A FREE FORMAT ALGOL READ.
  IS USED ANY PLACE WHERE:
            1) FORMAT IS UNDETERMINED OR FREE
            2) MULTIPLE ENTRIES SEPARATED BY CUMMAS ARE ANTICIPATED
C
       UP TO 3 REAL NO.S ARE CONSTRUCTED FROM THE ENTRIES ( EMBEDDED
Ç
  BLANKS ARE IGNORED).
       ERHORS DETECTED IN THE SUBROUTINE ARE COMUNICATED TO THE DRIVER
C BY PLACING A 1 IN ERR.
       INTEGER PIC(80)
       INTEGER ERR, SIGN
       INTEGER CUMMA, DASH, BLNK, HO, HI, HZ, H3, H4, H5, H6, H7, H8, H9
       INTEGER DEC
      REAL LUN, NIGH, VALU
      LUGICAL FOUND
      DATA COMMA, DASH, BLNK, H0, H1, H2, H3, H4, H5, H6, H7, H8, H9 /
         1H,, 1H=, 1H,, 1H0, 1H1, 1H2, 1H3, 1H4, 1H5, 1H6, 1H7, 1H8, 1H9/
      DATA DECILAR
C
C
      1411TALIZE VARIABLES
C
      ERK = 0
       INDX = 0
      LON = U.
      mIGH = n.
      VALU = 4.
C
      00 \ 250 \ 1 = 1, 3, 1
      ACC = v.
      IEXCNT = -1
      SIGN = 1
      FOUND = .FALSE.
C
C
      AWALYZE INPUT STRING 1 CHARACTER AT A TIME. J CHECK FOR INCORRECT
C
   CHARACTERS
Ü
      00 \ 245 \ J = 1, \ 20, \ 1
      IMDX = IMDX + I
      IF ( PIC(INDX) .EQ. COMMA ) GO TO 243
      IF ( PIC(INDX) .EW. BLNK ) GO TO 248
      FOUND = . TRUE.
C
C
      CHECK SIGN - UNLY ONE SIGN CHARACTER ALLOWED PER NO.
C
      IF ( P(C(INDX) .NE. DASH ) GU TO 235
      1F ( $16m .EQ. 1 ) GO TO 22W
      WRITE (1,214)
  216 FURMAT (* MORE THAN ONE MINUS SIGN FOUND IN ONE OF ENTRIES*)
      ERK = 1
      KETURA
\mathbb{C}
```

220 SIGM = -1

C

```
CHECK FUR DECIMAL POINT
                                                                332.
C
  235 IF ( PIC(INDX) .NE. DEC ) GU TO 237
      IF ( 1EXCOT .GT. =1 ) WRITE (1,236)
  236 FORMAT (* SECOND DEC. POINT FOUND IN SAME NO. -- FIRST ONE IGNORED*)
      IEXCUI = V
      GU TU 240
  237 CONTINUE
C
      ASSUME IT IS SOME DIGIT 4-9 AND NOT CHAR.
C
      ACC2 = -1
      IF ( PIC(INOX) .EW.
                            HO ) ACC2 = 0.
      IF ( PIC(INDX) .EQ.
                            H1) ACC2 = 1.
                            m2) ACC2 = 2.
      IF ( PIC(INDX) .EG.
                            H3) ACC2 = 3.
      1F ( PIC(INDX) .EG.
      IF ( PIC(INDX) .EW.
                            H4 ) ACC2 = 4.
      IF ( PIC(INDX) .EW.
                            H5 ) ACC2 = 5.
                     .EQ.
      IF ( PIC(INDX)
                            mo ) ACC2 = 6.
                            H7 ) ACC2 = 7.
      IF ( PIC(INDX) .EQ.
      IF ( PIC(INDX) .EU.
                            H8) ACC2 = 8.
                            H9) ACC2 = 9.
      IF ( PIC(INDX) .EG.
C
C
      IF ACC2 = -1 THEN NO LEGAL CHAR. FOUND
C
      1F ( ACC2 .GT. -1 ) GU TO 239
      WRITE (1,238) PIC(INDX)
  238 FORMAT (* 1LLEGAL CHARACTER FOUND IN NO. FIELD. CHAR: *,A1)
      ERR = 1
      RETURN
C
  239 CONTINUE
C
C
      MUST BE A LEGAL CHARACTER. *STACK UP* NO.
C
      ACC = ACC * 10.
      IF ( IEXCNT .GE. 0 ) IEXCNT = IEXCNT + 1
      ACC = ACC + ACC2
  240 CUNTINUE
      IF (.NUT. FOUND ) GO TO 200
  243 CONTINUE
  247 IF ( lexcol .Eu. -1 ) IEXCOT = 0
      ACC = SIGN * ACC/ (10**IEXCNT )
C
      IF ( I \cdotEU\cdot 1) LOV = ACC
      IF ( I .Eu. 2 ) HIGH = ACC
      IF ( I.EU. 3) VALU = ACC
  254 CONTINUE
  260 CONTINUE
      RETURN
      END
```

MSAU313-277

10.00.40 EDIT AGUASA 10.13.14 PRINT AGUASA/33,HD

0000000000	troop troop troop seed seed seed troop troop troop	888888888	¥	×	3333333333		880000
anaaaaaaaaa	touch trough tro	88888888888	×	×	3333333333333		SOUGHANAN
00 00	gand general	88 88	×	×	33	20	3
00 00	posed posed	SS	¥	X	33	2	22
00 00	Toxic (Cook)	88	×	×	5.5	S	3
00 00	pood prose	88	×	×	.×2.	25	មាល
00 00	Scent    wood	88	¥	××	33	3	88
00 00	Brook Brook	8888888888	X X X X X X	×	3333	25	200
00 00	posmi) motod	88888888888	X X X X X X X	×	3333	20	3
00 00	event event	88	×	** **	3.3	53	20
00 00	general Species	88	×	×	~	200	NO
00 00	pand	SS	×	天天	3.5	848	3
00 00	biso) tempj	SS	×	X X	33	HH	9
00 00	Street,	88 88	¥	×	53 33	2 2	99
000000000000	journal jurnal jurnal jurnal jurnal jurnal jurnal jurnal jurnal jurnal jurnal jurnal	888888888888888888888888888888888888888	¥	X	3333333333333		BUNNUBUN
0000000000	poord poord poord poord poord poord poord poord poord	8888888888	X	X	33333333333		NUMBUN

```
2
              SET TU 1. TO REMOVE DSW COSTS
                                                                        335.
                                                                  ( m)
        3
              9.6946946946946616162
                                  TIME LIMIT ON GROWTH (900MAX)
                                                                  (UAY)
        5
              INLET NH3 CONC
                                                                  (UG-N/L)
       14
              ୬.ଜନ୍ଧ୍ରମ୍ୟର୍ଷ୍ଟ୍ର୍ୟ୍ର୍ୟ୍ୟ କ୍ଷ୍ୟ
                                  COS1 OF
                                          DEEP SEA WATER
                                                                  (\$/M3)
       15
              2.65ของชุมเบินคนะ+ชุม
                                  TOTAL WET
                                            NEIGHT/WET MEAT WI
                                                                  ( r>)
              BO+30000000000000E+00
       10
                                        MEAT/MEAT PROTEIN
                                  DRY
                                                                  ( rp )
       17
              RESERVE PHYTO POOL CAPY
                                                                  ( 00)
       18
              5.ชเลดเยยตบทุพพ¥+พิพ
                                  NET/DRY MEAT WEIGHT
                                                                  ( rè)
              4.4800000000000000000007
       21
                                  NUTRIENT CONCENTRATION DSA
                                                                  (G-N/CM3)
       23
              COMP DEPTH FOR PHYTO POOLS
                                                                  (CM)
       27
              9.60000000000000000001
                                  PHYTO CONVERSION EFFICIENCY
                                                                  ( r)
       30
              FEED RATE/BASELINE FD RATE
                                                                  ( eq )
      32
              1.183100000000000000000
                                  BASELINE FEED CRITERION
                                                                 (G/SEC/1G-ANIM.
      35
              1.5888888888888888
                                  SHELLFISH CONV EFF
                                                                 ( rd)
      30
                                  (SPARE)
      48
              1. ตาเดยผลผลผลสลย-63
                                  COST UF SPAT
                                                                 ($/HEAD)
              1.400000000000000E+01
      01
                                  INTERVAL BETWEEN TRANSFERS
                                                                 (DAY)
      02
                                  (SPARE)
      .03
                                  (SPARE)
      04
                                  (SPARE)
      05
                                  (SPARE)
      06
                                  (SPARE)
      07
                                  (SPAKE)
      00
                                  (SPARE)
      09
                                  (SPARE)
      10
             POOL DEPTH
                                                                 (CM)
      71
                                  (SPARE)
      72
             1.15ยองยดงอังยังE+ชัย
                                  TURNUVER RATE
                                                                 (1/DAY)
      73
                                  (SPARE)
      74
             7.000000000000000E+01
                                  SHFSH TRAY A/FLOW
                                                                 (CM2/CM3/SEC)
      75
             FLOW REG. HEAD MULT.
                                                                 ( p>)
3
      70
             1. ผู้ยือพิทยุปยิงหมันE+พูน
                                 CHANNEL FLOW VELOCITY
                                                                 (M/SEC)
      77
             2. EQUADAMARAMARE-02
                                 ROUGHNESS FACTOR
                                                                 ( p)
      78
                                 (SPARE)
Þ
      79
                                 (SPARE)
      83
                                 (SPARE)
     102
             COST OF LAND
                                                                 (5/HA)
     163
             AREA USABLE BY BLDG OR STRUCT
                                                                 (AH\SM)
     104
             CAP CUST PHYTO POULS
                                                                 (3/M2)
     1175
             LIFE OF PHYTOPLANKTON POULS
                                                                 (YEAR)
     140
             O. DUDGGGGGGGGGGGGGG
                                 COST OF COVER
                                                                 ($/M2)
     1.17
             5. พยยทยทิยทยพลพัสธ+พย
                                 LIFE UF COVER
                                                                 (YEAR)
     108
             5.300000000000000<del>0</del>E-83
                                 OFF A STO AREA REGIPPOOL AREA
                                                                 ( r>)
à
     109
             2.ชชชชทยังภิยยย E+ช1
                                 CAPITAL COST OF ANIMAL TRAYS
                                                                 (S/M2)
     110
             5. อนอบทดทดทดของอย+อง
                                 LIFE OF TRAYS
                                                                 (YEAR)
             111
                                 SHELLFISH STACKING FACTOR
                                                                 ( 14)
     505
             2.ชติปทองคทุพพลซูE+ติ1
                                 MEAN AMBIENT SEA WTR TEMP
                                                                 (()
     263
             2. BURRABBORRABBE+91
                                 OPERATING TEMP OF POOLS
                                                                 (C)
     212
             8. อหาอทอดออกดอย+บอ
                                 MAINTENANCE ENGI WAGE RT
                                                                 (-$/HR)
     244
             1. Kadarakanangétan
                                 NUMBER OF PIPELINES
                                                                 ( ⋈)
     241
             1.60HUNNUNUNUNE+03
                                 PIPELINE LENGTH
                                                                  (M)
     242
             2.00000000000000000001
                                 PIPELINE LIFE
                                                                 (YEAR)
     243
             1. พยะพยยยยยยยยยยองE+ษย
                                 PIPELINE DUTY CYCLE
                                                                 (m)
     244
             PIPELINE MAINTENANCE FACTOR
                                                                 ( A)
     245
             FRICTIONHEAD 1M3/SEC-IM
                                                                ( p)
    240
             FLOW EXPONENT
                                                                 ( P)
    247
             F COEF., PIPELINE COST
                                                                ($)
    245
             S COEF., PIPELINE COST
                                                                (mm)
    244
             7.4996630000000E+02
                                 K COEF., PIPELINE COST
                                                                ($0)
    342
             8.5ชพทยบทุศยุยิลพ์E-พิ1
                                 EFFICIENCY OF PUMP
                                                                (r)
    303
                                 PUMP LIFT (INTAKE)
                                                                (M)
```

305 305 306 513 402 414 444	<ul> <li>1. พอกอกของคพพพพพธ + พธ</li> <li>1. ชอบของปีกไม่ตุ้งของธิ + พธ</li> <li>3. ผลของของปีกไม่ตุ้งของธิ + พพ</li> <li>3. ผลของปีของปีของปักษะ E + พพ</li> <li>3. และสังผลของพพพพพธ E + พ</li> <li>4. และสังผลหลังผลของพพพพ E + พ</li> <li>4. และสังผลหลังผลของพพพพ E + พ</li> <li>4. และสังผลพพพพพพพพพพพพพพพพพพพพพพพพพพพพพพพพพพพพ</li></ul>	STANDBY REMUTRED LIFE OF PUMP PUMP MAINTENANCE AN TRAYS RECIRC POWER ENG. NEWS RECORD COST INDEX ELECTRICAL POWER COST (SPARE) (SPARE)	(+) 336. (YEAR) (+ CAP) (kw/M2) (r) (\$/KNH)
500 557 500 510	1 ∵ କଥ୍ୟର୍ଷ୍ଟ୍ରମ୍ୟର୍ଷ୍ଟ୍ରି + ଅନ୍	ELECTRIC MOTOR EFFIC (SPARE) (SPARE) (SPARE)	(e)
002	ช่.มชทุสหกฎทฤหลัง€+ตับ	PHYTO PL RECIRC PAR REG (SPAKE) (SPARE)	(KW/M3)
011	d. HedodadyyonanE+ou	CUST OF ADDED NUTR	(3/KG=N)
012	4.ปที่ปัจยทั้งถึงปัจจัย+ยิย	WAGE RT	(\$/HR)
045	Sa+BannanganangE+e2	CAP COST OF OFF, STRG, AC	(\$/MZ)
043	1.5ยปิงหันพิสัยกันพิษัธิ+ัก1	LIFE OF OFF, STRG, AC	(YR)
045		NUTR ADDED TO INTK WTR	(G-N/CM3)
762	1 . ପର୍ମ୍ୟନ୍ତ୍ରମ୍ୟନ୍ତ୍ରମ୍କ୍	CST OF LBK=HRVST SUPER	(\$/KG OUT)
104	2.00000000000000000001	RT OF TRASFR AND HRVST	(M2/HR)
7:15	1.50 ୨ହିନ୍ନମହନ୍ତ୍ର ବଳ 🕶 1	PHYTO POOL CYCLE	(DAY)
740	5.6000000000000000001	RATE OF HAND CLEANING	(M2/HR)
803	6.50000000000007E-81	METABOLIC COEFFICIENT	( r)
<b>6</b> 84	2.UNENDABENNEƏE+RU	NO OF PHYTO POOLS	( p> )
b 215	1.୭୯୯୭୯/ମର୍ମ୍ୟର୍ମ୍ୟ 🛨 🗜 🤄	COST OF EXCUTN PHYTO POOL	(\$/M3)
800	1.4254466066666-62	INITIAL WEIGHT	(G)
8×17	5. Jajabanana6+86	BATCH INTERVAL	(DAY)
044	5. /ไปทัพทย์พยาพยย์ = พไ	FRACI SURVIVING AFTER 180 DAYS	(r)
9192	1.2000000000000000000000000000000000000	START POP SURVIVAL	(4)
921	1.พะยุพบพัพษ์ยุษัพE+พ1	MAX WEIGHT OF AN AT HRVST	(G)
925	6.0464666804089E+66	TARGET OUPPT FRM PLNT	(KG/BATCH)
928	1 . ମୟଷ୍ଟ୍ରପ୍ରପ୍ରସ୍ଥିଲେମ୍	INT RATE ON SINKING FND	( p> )
929	1.6503000000000000000	RETURN ON CAPITAL	(r))

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MSAUS13-274

14.14.56 AGUASA PRINTED. 14.15.22 PRINT DISK30/33,HD

```
53
53535333333
53535333333
      33333
ирововрава
вотворова
            DEEDEEDEE
             nanananana
```

. 6

**0 0**.

). O. (

```
339.
        2
               d. nowodddddde+ab
                                    SET TO 1. TO REMOVE DSW CUSTS
                                                                      ( 10)
        3
               4. Juoningnanane+62
                                    TIME LIMIT ON GROWTH (900MAX)
                                                                      (DAY)
        5
                                    INLET NH3 CONC
               S. DOGGERNAGANANAE+NO
                                                                      (UG-N/L)
       14
               COST UF
                                             DEEP SEA WATER
                                                                      ($/M3)
       15
               2.6599999999996+99
                                    TOTAL MET
                                                WEIGHT/WET MEAT WT
                                                                      ( m)
               2.5ยอทย์ทย์สหมอย์ช£+ยย
       10
                                    DRY
                                           MEAT/MEAT PROTEIN
                                                                      ( rè)
       17
               ป. ยอยองยอยอยอยอยอย<del>ะ+</del>อย
                                    RESERVE PHYTO POOL CAPY
                                                                      (r)
       15
               5.60000003830006E+66
                                    NET/DRY MEAT WEIGHT
þ
                                                                      ( m)
       <1
              4.45ยที่ยัยติดติดติดติดติ=ต7
                                    NUTRIENT CONCENTRATION DSA
                                                                      (G=N/CM3)
       23
              COMP DEPTH FOR PHYTO POULS
                                                                      (CM)
       27
               9. ทมตทยยทมยทมยท9E-พ1
                                    PHYTO CONVERSION EFFICIENCY
                                                                      (m)
       30
               3.800000000000000000000000
                                    FEED RATE/BASELINE FD RATE
                                                                      ( ↔ )
       32
               1.150000000000003JE-08
                                    BASELINE FEED CRITERION
                                                                      (G/SEC/1G-ANIMA
       35
              2.4266666666666666
                                    SHELLFISH CONV EFF
                                                                      ( ↔ )
       30
                                    (SPARE)
              1.062000000000000000000
       48
                                    COST OF SPAT
                                                                      (D/HEAD)
       01
              1.4ยปปัจของของสิทธิ+ป1
                                    INTERVAL BETWEEN TRANSFERS
                                                                     (DAY)
       02
                                    (SPARE)
       03
                                    (SPARE)
       04
                                    (SPARE)
       65
                                    (SPARE)
       06
                                    (SPAKE)
       07
                                    (SPAKE)
       68
                                    (SPARE)
       69
                                    (SPARE)
       70
              POOL DEPTH
                                                                     (CM)
       71
                                    (SPAKE)
       72
              7.5000000000000000000001
                                    TURNOVER RATE
                                                                     (1/DAY)
       73
                                    (SPARE)
       74
              7.6000000000000000000001
                                    SHFSH TRAY A/FLUW
                                                                     (CM2/CM3/SEC)
       75
              FLOW REG. HEAD MULT.
                                                                     (m)
       70
Ì
              1.00000000000000E+00
                                    CHANNEL FLOW VELOCITY
                                                                     (M/SEC)
       17
              2.000000000000000E-02
                                    KOUGHNESS FACTOR
                                                                     (4)
       7 B
                                    (SPARE)
       79
                                    (SPARE)
      80
                                    (SPARE)
      102
              1.0000000000000E+04
                                    COST OF LAND
                                                                     ($/HA)
      103
              8. KOEGUGGGGGGGGE+03
                                    AREA USABLE BY BLDG OR STRUCT
                                                                     (M2/HA)
      104
              1. WUSARBANBRABUE+61
                                    CAP COST PHYTO PUOLS
                                                                     (SM/8)
      105
              5. DUNBUUNNNNNNE+BU
                                    LIFE OF PHYTOPLANKTON POOLS
                                                                     (YEAR)
      140
              N. NONNONOBBANNE+20
                                    COST OF COVER
                                                                     ($/M2)
      1417
              5. ย่อทอดิตดิยคลดดE+ดิต
                                    LIFE OF COVER
                                                                     (YEAR)
              6.3000000000000E-03
      100
                                   OFF A STO AREA REGIPPOOL AREA
                                                                     ( r>)
      1,19
                                    CAPITAL COST OF ANIMAL TRAYS
              2. VYDEGORIGINARE + WI
                                                                     ($/M2)
     111
              5. ยยอยหมายหยายหลาย + คน
                                   LIFE OF TRAYS
                                                                     (YEAR)
     111
              1. ยอยออยิงหมุทอยิยะ+ยิย
                                    SHELLFISH STACKING FACTOR
                                                                     ( r>)
     202
              2. EUROUNUNGUNUE+K1
                                   MEAN AMBIENT SEA WIR TEMP
                                                                     (C)
     2.13
              2.0002000000000000001
                                   UPERATING TEMP OF POOLS
                                                                     (C)
     215
              MAINTENANCE ENGI WAGE RT
                                                                     (5/HR)
     240
              1.00000000000000000000
                                   NUMBER OF PIPELINES
                                                                     ( r)
     241
              1.00000000000000E+03
                                   PIPELINE LENGTH
                                                                     (M)
     242
              PIPELINE LIFE
                                                                     (YEAR)
     243
              PIPELINE DUTY CYCLE
                                                                     ( p)
     244
              3.46666666666666666
                                   PIPELINE MAINTENANCE FACTUR
                                                                     ( ↔ )
     245
                                   FRICTIONHEAD 1M3/SEC-1M DIAM.
              ( m)
     240
              1.8สหอังที่ถ้นชายอินE+ยัง
                                   FLOW EXPONENT
                                                                     (m)
     247
              F COEF., PIPELINE COST
                                                                     ($)
     248
              3.7000000000000000E+k5
                                   G COEF., PIPELINE COST
                                                                     (何克)
     249
              K CUEF., PIPELINE CUST
                                                                     ($#)
     302
              5.50000000000000E-31
                                   EFFICIENCY OF PUMP
                                                                     ( +)
     3.3
                                   PUMP LIFT (INTAKE)
                                                                     ( ivi )
```

305 305 313 402 414 445 507 508 510 602	1.000000000000000000000000000000000000	LIFE OF PUMP PUMP MAINTENANCE AN THAYS RECIRC POWER FIG. NEWS DECORPORATE	340. (
003 014 011 012 043 043 0445 7045 0445 7045 0445 0445 0445 0445	1.0200000000000000000000000000000000000	PHYTO PL RECIRC PWR REG (SPARE) (SPARE) COST OF ADDED NUTR WAGE RT CAP COST OF OFF, STRG, AC LIFE OF OFF, STRG, AC NUTR ADDED TO INTK WTR CST OF LBR=HRVST SUPER RT OF TRNSFR AND HRVST PHYTO POUL CYCLE RATE OF HAND CLEANING METABOLIC COEFFICIENT NO OF PHYTO POOLS COST OF EXCVTN PHYTO POOL INITIAL WEIGHT BATCH INTERVAL FRACT SURVIVING AFTER 180 DAYS START POP SURVIVAL MAX WEIGHT OF AN AT HRVST TARGET OUPPT FRM PLNT INT RATE ON SINKING FND RETURN ON CAPITAL	(KW/M3)  (\$/KG=N) (\$/HR) (\$/M2) (YR) (G=N/CM3) (\$/KG OUT) (M2/HR) (DAY) (M2/HR) (P) (P) (\$/M3) (G) (DAY) (P) (P) (H) (H) (H) (H) (H) (H) (H) (H) (H) (H

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USM FLU (M3/SEC)
DSN DISTRIBUTION
# UF PIPELINES
PHYTO SPACE
PIPELINE DIAM
                (%)
  = LAGOR
PIPELINE LENGTH
                (≅)
≡ TUTAL
" UF PUULS
SESH SPACE
FOUL VOLUME
               (13)
≡ LABUR
PUUL AHEA
                (82)
= LARVAE
PUUL DEPTH
                (M)
≡ TUTAL
THAY AREA
                (M2)
SUPERVISION
INDIVIDUAL NEIGHT (G)
TUTAL PROD. CUSTS
DAYS TO HARVEST
ANNUAL CAP. CUST (DUN)
UUTPUT (KG/YEAR)
TUTAL CAPITAL (000)
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DEEP-SEA WATER

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^ $40313=274
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# DESCRIPTION OF THE VARIABLES USED IN THE PROGRAM

NOTE: ASSUMPTIONS ARE IDENTIFIED BY (\*)

```
346.
A903(1) SURVIVAL AT END OF DAY (I)
         = EXP(I*ALOG(VAR(899))/180.) [I=1,900]
          SURVIVAL, CURRECTED FOR INIT. SURVIVAL
         = A903(I) *VAK(902)
                                       [I=1,IMAX]
VAR( 2)* SET TO 1. TO DELETE DS" COSTS
VAR( 3)* TIME LIMIT UN GRONTH
                                                              DAY
         = 900.
      5)* INLET NH3 CONCENTRATION
VAR (
                                                         UG=N/L
         و ژبا
VAR ( 14)* CUST OF DEEP SEA WATER
                                                             5/43
         S 12 .
VAR ( 15)* TUTAL WET/WET MEAT WEIGHT
         = 2.65
VAR( 16)* DRY MEAT/MEAT PROTEIN
         = 2.5
VAR( 17)* RESERVE PHYTO POUL CAPACITY
         = 0,
VAR( 10)* NEI/ORY MEAT NEIGHT RATIO
         = 5.
VAK( 19) PUUL DEPTH
                                                              CM
         = VAK( 70)
VAR( 21) * NUTRIENT CONCENT., DEEP SEA WATER G-N/CM3
         = 4.48E-37
VAR( 22) ENFICHMENT
         = (VAR(21)+VAR(645))/VAR(21)
VAR( 23)* CUMPENSATION DEPTH, DEEP SEA WATER
                                                              CM
        = 400.
         CUMP. DEPTH, CURRECTED FOR ENRICHMENT
                                                              CM
         = VAR(23)/VAR(22)
                                                    CM3/SEC/CM2
         DSW FLOW PER UNIT POUL AREA
        = VAR( 72) * VAR( 19) / 86400.
VAR( 27)* PHYTO CONVERSION EFFICIENCY
         = ,9
VAR( 30) * FEEDING RATE/BASELINE
        = 3.
VAR( 32)* BASELINE FEEDING CRITERION
                                                G-PROT/SEC/1G-AN
        = 1.15E-48
VAR( 35)* SHELLFISH CONVERSION EFFICIENCY
        = .158
                                                           (STX)
        = .242
                                                        (EXTRAP)
VAK ( 30)*
VAR( 48)* CUST OF SPAT
                                                          $/HEAD
        = . 1111
VAR( 52) MINIMUM OF ACTUAL OR COMP. DEPTH
                                                              CM
        = AMIN1 (VAR( 23), VAR( 19))
VAR( 54) PHYTO CONVERSION EFFICIENCY
        = VAR( 27) * VAR( 19) / VAR( 52)
         PROTEIN EQUIV. OF NUTRIENT CONCENTRATION G-PROT/CM3
VAR( 55)
        = 6.25 * (VAR(21) + VAR(645))
VAR ( 50)
        PRUTEIN PRUDUCTION RATE
                                                G-PRUT/CM2/SEC
        = VAR(55) * VAR(54) * VAR(25)
                                                     G-PROT/SEC
VAH ( 57) SPECIFIC FEEDING RATE
        = VAR( 30) * VAR( 32)
VAR ( 58) SHELLFISH CONVERSION EFFICIENCY
        = VAR( 35)
        TUTAL WET/PROTEIN RATIO
VAK ( 59)
        = VAR( 15) * VAR( 10) * VAR( 18)
                                                     G-PRUT/SEC
VAR ( DO) FEEDING RATE/ANIMAL-IN
```

DAY

VAR( 01) \* INTERVAL BETWEEN TRANSFERS

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347.
VAK ( 62) x
VAR( 63)*
VAR( 04)*
VAR ( 65) *
VAK( 65)*
VAK( 67)*
VAK ( 68)*
V4K( 69)*
VAR( /0) * POUL DEPTH
                                                                   CM
         = 100.
                                                                (STX)
         = 300.
                                                             (EXTRAP)
VAR( /1)*
VAM ( 72)* TURNUVER RATE
                                                                1/DAY
         = 1.15
                                                                (STX)
         = ,75
                                                             (EXTRAP)
VAR ( 73)*
VAR ( 74)* TRAY AREA/FLOW
                                                         CM2/CM3/SEC
         = 74.
           TRAY AREA/FLOW, CORRECTED FOR ENRICHMENT CM2/CM3/SEC
         = VAR(74)/VAR(22)
VAR( 75)* FLUM REGULATION MEAD MULTIPLIER
         = 2.
VAR ( 76)* CHANNEL FLOW VELUCITY
                                                                MISEC
         = 1.
VAR( 77)* RUUGHNESS FACTUR
         = .N2
VAK( 70)*
VAR ( 74)*
VAR( 80) *
VAR(102)* CUST OF LAND
                                                                 S/HA
         = 10000.
VAR(103)* AREA USABLE BY BLDG. OR STRUCTURE
                                                               M2/HA
         = BNNA.
VAR(104)* CAPITAL INVESTED, PHYTO POOL LINER
                                                                 $/M2
         = 10.
VAR(165)* LIFE OF POOLS
                                                                 YEAR
         = 5.
VAR (106) * COST OF COVER
                                                                 5/M2
         = U.
VAR(107)* LIFE OF COVER
                                                                 YEAR
VAR(108)* OFFICE/STURAGE AREA REG. PER POOL AREA
         = .41063
VAR(109)* CAPITAL INVESTED, ANIMAL TRAYS
                                                                5/M2
         = 20.
VAR(110)* LIFE OF TRAYS
                                                                YEAR
         = 5.
VAR(111)* SHELLFISH STACKING FACTOR
         = 1.
VAR(112)
           COST OF USABLE LAND
                                                                5/M2
         = VAR(102)/VAR(103)
           RETURN ON CAPITAL INV. IN LAND
VAK(113)
                                                                SM/8
        = VAR(112) * VAR(929)
VAR (114)
           AMORTIZATION OF POOL LINER
                                                           S/MZ/YEAR
         = VAR(104)*(VAR(928)/((VAR(928)+1.)**VAR(105)=1.)+VAR(929)
vAH(110)
           AMORTIZATION OF COVER
         = VAR(106)*(VAR(926)/((VAR(928)+1.)**VAR(107)=1.)+VAR(929)
           CAPITAL COST OF OFFICE/STORAGE SPACE/M2-POOL
VAH (117)
         = VAR(642)*VAR(188)
           AMORTIZATION OF OFFICE/STORAGE SPACE
VAR(118)
                                                           5/M2/YEAR
         = VAR(117)/(VAR(920)/((VAR(928)%1.)**VAR(643)=1.)+VAR(929)
```

```
VAR(119)
           AMURIZATIUN UF TRAYS
                                                              S/M2/YEAR
          = VAR(109)*(VAR(920)/((VAR(928)+1.)**VAR(110)=1.)+VAR(929)
 VAK (120)
            TUTAL POUL CAPITAL INVESTMENT
                                                                   3/42
          = VAR(112) + VAR(104) + VAR(117) + VAR(127)
            AMBRITIZATION OF PUOLS
 VAR (121)
                                                              S/MZ/YEAR
          = VAR(113)+VAR(114)+VAR(116)+VAR(118)+VAR(128)
           TOTAL TRAY CAPITAL INVESTMENT
 VAK (122)
                                                                   $/M2
          = VAR(112)+VAR(100)+VAR(117)+VAR(109)
            AMORTIZATION OF TRAYS
 VAR (123)
                                                            $/MZ/YEAR
          = VAR(113)+VAR(110)+VAR(118)+VAR(119)
            LENGTH OF PUUL WALLS
 VAR (125)
                                                                      M
          = (3.*VAR(804)+1.)*SQRT(VAR(663))/100.
 VAR(120)
           CHUSS-SECTION OF POUL MALLS
                                                                     M2
          = 4.*vAR(19)/100.+2.*(VAR(19)/100.)**2
VAK(127)
            EXCAVATION CUSTS OF MALLS/POOL AREA
                                                                   5/M2
          = VAR(805) * VAR(125) * VAR(126) * 10000./VAR(663)
            AMURTIZATION OF PUOL WALLS
VAR (128)
                                                             S/M2/YEAR
          = VAR(127) * VAR(929)
VAR (150)
            INTAKE CHANNEL LENGTH
                                                                      M
          = SURT (VAR (002))/200.
VAK (151)
           CHANNEL CROSS-SECTION
                                                                     MS
          = VAR(664)/VAR( 76)
VAR(152)
            HYDRAULIC RADIUS
                                                                     M
          = SWRT(2.*VAR(151)/3.)/2.
VAK (153)
           CHANNEL SLUPE
          = (VAR( 76)*VAR( 77)°)**2./VAR(152)**(4./3.)
            EXCAVATION CUSTS OF CHANNELS
VAR (155)
                                                                      5
          = VAR(150) * VAR(151) * VAR(805)
            AMURIIZATION OF CHANNEL EXCAV.
VAR (150)
                                                                S/YEAR
         = VAR(155) * VAR(929)
VAR (163)
            TUTAL CHANNEL CAPITAL INVEST.
                                                                      $
            VAR(154)*(VAR(112)+VAR(164)+VAR(117))+VAR(155)
VAR (161)
            CHANNEL AMURTIZATION
                                                                S/YEAR
         = VAR(154)*(VAR(113)+VAR(114)+VAR(118))+VAR(156)
VAR(202)* MEAN AMBIENT SEAWATER TEMPERATURE
                                                                     C
         = 200
VAR(203)* OPERATING TEMPERATURE OF POOLS
                                                                     C
         = 211.
VAR(212)* MAINTENANCE ENGINEER WAGE
                                                                  $/HR
         = 6.
(PSS) MAY
VAR (240) * NUMBER OF PIPELINES
         = 1.
VAR(241)* PIPELINE LENGTH
         = 1000.
VAR(242)* LIFE OF PIPELINE
                                                                  YEAR
         = 20%
VAR(243)* PIPELINE DUTY CYCLE
         = 1.
VAR(244)* PIPELINE MAINTENANCE FACTOR
                                                              P/YEAR
         = . 13
VAR(245)* FRICTION HEAD AT 143/SEC IN 14 DIAM. PIPE
                                                                     r
         = .0001
VAR (240) * FLU. EXPONENT
                                                                     P
         = 1.5
VAR(247) * F CULFFICIENT, PIPELINE COST
                                                                     $
         = 1400mt.
VAR(248)* G COEFFICIENT, PIPELINE COST
                                                                     r)
         = 37JAJA.
VAR(249) * K CUEFFICIENT, PIPELINE COST
                                                                     钟
         = 740.
VAR(250) PIPELINE DIAMETER
```

M

```
ANNUALIZATION FACTOR (INTAKE)
      VAR (251)
                                                                              349.
               = vAR(928)/((VAR(928)+1.)**VAR(242)=1.) +
                                                                     M/YELR
                 VAR(929) + VAR(244)
                YEARLY OPERATING COST FOR UNIT FLOW/DIAM
      VAR (252)
               = VAR(243) *8760. *VAR(414) *9.807*VAR(245) /VAR(382)
                                                                      SIYEAR
                 CAPITAL INVESTMENT (INTAKE)
      VAK (255)
               = VAR(247) + VAR(248) * VAR(256) +
                 VAR(246) * VAR(241) * VAR(249) * VAR(250)**1.8
                 YEARLY CAPITAL AND OPERATING COSTS (INTAKE)
      VAK (259)
               = VAR(251) * VAR(256) + VAR(240) * VAR(241) * VAR(252) *
                                                                     1/YEAR
                 (VAR(004) / VAR(240))**(VAR(246) + 1.) /
                 (VAR(250) ** (VAR(246) + 3.))
      VAR(302) * EFFICIENCY OF PUMPS
               = .05
      VAR (303)
                 DISTRIBUTION PUMP LIFT
               = VAR(15u) * VAR(153) * VAR(-75)
      VAR (364) * STANDBY REGULKED
               = 100.
      VAN (305) * LIFE UF PUMPS
                                                                       YEAR
               = 10.
      VAR (SUO) * PUMP MAINTENANCE
                                                                       +CAP
               = 3.
     VAR (300)
                 CAPITAL INVEST. EACH PUMPING STAGE
                                                                           3
               = (560.*VAR(1)**£.6)*(1.+VAR(304)/160.)*VAR(402)/2006.
                     [1=327,330]
     VAR (309)
                 AMURTIZATION, EACH PUMPING STAGE
                                                                     S/YEAR
               = VAR(308)*(VAR(928)/((VAR(928)+1.)**VAR(305)-1.)+VAR(929))
     VAR (31a)
                MAINTENANCE AND POWER, EACH PUMP. STAGE
                                                                     $/YEAR
               = VAR(308) * VAR(306) / 100 . + VAR(327) * 8760 . * VAR(414)
     VAR (311)
                 SHELLFISH TRAY RECIRCULATION COSTS
                                                                     $/YEAR
     VAR (312)
     VAR(315)* SHELLFISH TRAY RECIRCULATION POWER
                                                                     KW/42
              ≕ v¹ .
     VAR (314)
     VAR (321)
                PUUL PUMPING COSTS
                                                                     $/YEAR
              = VAR(309)+VAR(310)
                                        [I=327]
                DIRECT USA TO SHELLFISH PUMP. COSTS
     VAR (322)
                                                                     S/YEAR
              = VAR(309)+VAR(310)
                                     [1=328]
     VAK (323)
                SHELLFISH THAY RECIRCULATION COSTS
                                                                     S/YEAR
= VAR (369) + VAR (310)
                                       [1=329]
     VAn (324)
               PUOL RECIRCULATION COSTS
                                                                    $/YEAR
              = VAR (308)+VAR (318)
                                       [1=330]
     VAR (327)
                PUMPING CAPACITY, INTAKE TO POOLS, NET
                                                                         KN
              = 9.607*VAR(664)*VAR(303)
                PUMPING CAPACITY, INTAKE TO POOLS, GROSS
                                                                         KW
              = VAR(327)/VAR(342)
     VAR (328)
                PUMPING CAPACITY, DS# TO SHESH, NET
                                                                         KW
                PUMPING CAPACITY, DSW TO SHESH, GROSS
                                                                         KW
              = VAR(326)/VAR(362)
     VAR (329)
                PUAPING CAPACITY, SFSH RECIRCULATION, NET
                                                                         KVi
              = VAR(313) * VAR(949)
                PUMPING CAPACITY, SFSH RECIRCULATION, GROSS
                                                                        KW
              = VAR(329)/VAR(302)
     VAR (330)
               PUMPING CAPACITY, POOL RECIRCULATION, NET
                                                                         K'N
              = VAR(002) xVAR(005)
                PUMPING CAPACITY, POOL RECIRCULATION, GROSS
                                                                        KIN
              = VAR(338)/VAR(382)
    VAn (331)
                PUMPS CAPITAL INVEST. (SUMMATION)
                                                                          3
              = VAR (351) + VAR (368)
```

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TO TO MINORITATION TOUR PLACES
                                                                 water is some process
                                                                        350.
         = VA4(332)+VAX(309)
VAR (402) * ENG. WENS RECORD COST INDEX
         = 2400.
VAR (414) * ELECTRICAL POWER COSTS
                                                                  SIKNH
         = . . . .
VAR (451)
          MASTE TREATMENT
VAR (444) *
VAK (440) *
VAR (506)* ELECTRIC MOTUR EFFICIENCY
                                                                      r)
         = 1.
VAR (501)*
VAR (500) *
VAR (510)*
                                                                S/YEAR
VAK(515) AERATION CUSTS
                                                                  KW/M3
VAR(602)* PHYTU POOL RECIRCULATION POWER
VAR(643)*
VAR (OUL) *
VAR (beb)
VAR(611)* CUST OF ADDED NUTRIENTS
                                                                 5/KG=N
         ≅ V'•
                                                                   S/HR
           "AGE RATE
VAR(612)*
         = 4,
                                                        GDAY-PROT/SEC
           SUMMATION OF DAILY FOUD FLOWRATES
VAR(013)
         = VAR(013)+VAR( 64) xC962(1)
           ADDITIONAL FOUD TO SHELLFISH
                                                               $/KG-OUT
VAR (017)
           TUTAL PLANT FUOD FLOW/ANIMAL-IN G-PROT/SEC/ANIMAL-IN
VAK (022)
         = VAR(013)/VAR(867)
                                                            G-PROT/SEC
          TUTAL PLANT FUUD FLOW
VAK (041)
         = VAR(927) * VAR(622)
                                                                   5/M2
VAR(642)* COST OF UFFICE/STORAGE SPACE
         = 500.
VAR (643) * LIFE OF OFFICE/STORAGE SPACE
                                                                   YEAR
         = 15.
                                                               G-N/CM3
VAR(645)* NUTRIENTS ADDED TO DEEP SEA WATER
         2 V) .
                                                                    CM2
           PHYTOPLANKTON PUUL AREA
(200) HAV
         = VAR(041)/VAR( 50)
VAR(663) PHYTO. POOL AREA, INCL. RESERVE
                                                                    CWS
         = VAR(002)*(1.+VAR(17))
                                                                M3/SEC
          TOTAL FLOW INTO PUOLS
VAR (004)
         = VAR(602) * VAR( 25)/10000000.
                                                                     M3
           TUTAL POOL VOLUME, INCL. RESERVE
VAR (605)
         = VAH(663) * VAR( 19)/1800000.
           MALL TO CELL RATIO
VAK (670)
         = WL/CEL
                                                              S/KG-OUT
VAR(702)* COST OF LABOR, SUPERVISION
         = .1
                                                                 M2/HR
VAR (704) * RATE OF TRANSFER AND MARVEST
         = 20.
VAR(7.5)* PHYTO POOL CYCLE DURATION
                                                                   DAY
         = 15.
                                                                 M2/HR
VAR (7.10) * HATE UF POUL CLEANING
         = 5.0.
                                                               M2/YEAR
VAR(737)
          SHELLFISH AREA TO BE CLEANED
         = VAR(949) * 365. / VAR( 61)
                                                                S/YEAR
           SHELLFISH AREA CLEANING COSTS
         = (VAR(747) * VAR(612))/VAR(704)
                                                                5/YEAR
          PHITO. POUL CLEANING COSTS
VAK (7/9)
         = VAR(602) * VAR(012) * 365./(VAR(705) * VAR(706) * 10000.)
```

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VAK(Ous) * RETABOLIC CHEFFICIENT
                                                                       351.
         = 2./5.
VAR(004)* NUMBER OF PHYTU. PUBLS
         = 4.
                                                                (STX)
                                                             (EXTRAP)
VAR(005)* EXCAVATING COSTS
                                                                 5/43
         = 1.
VAR(000) * INTITAL METGTH/ANIMAL
                                                                    G
         = .001
VAR(bu7)* BATCH INTERVAL
                                                                  DAY
         = 5.
          DAILY GRUNTH INCREMENT/ANIMAL-IN
v Ar (5:15)
                                                                G/DAY
         = VAR( 59) * VAR( 58) * VAR( 60) * 86400.
VAR(864)* FRACTION SURVIVING AFTER 188 DAYS
         = .8
VAR(902)* STARIING PUPULATION SURVIVAL
         = 1.
VAR (916)
           INDIVIDUAL MEIGTH, AT HARVEST
                                                                    G
         NUMBER OF ANIMALS/ANIMAL-IN AT HARVEST
VAR (917)
         = C942(1) = [1=IMAX]
          CUMUL. WEIGTH, CURRECT. FOR SURVIVAL
VAR (918)
                                                                GDAY
         = VAR(918) + wT(I+1) *C902(I) [I=1,1MAX]
           CUMUL. MEIGTH, SIMULTAN. IN PLANT
                                                                    G
         = VAR(918)/VAR(807)
           CUMUL. NUMBER, CORRECT. FOR SURVIVAL
VAH (919)
                                                                PDAY
         = VAR(919) + C982(1) [I=1, IMAX]
           CUMUL. NUMBER, SIMULTAN. IN PLANT
         = VAR(919)/VAR(807)
VAR(921)* TARGET INDIVID. WEIGTH AT HARVEST
                                                                    G
         = 10.
VAR(922)* TARGET OUTPUT/BATCH
                                                                   ΚG
         = 507%.
VAR (423)
          NUMBER OF DAYS TO HARVEST (+.1)
                                                                  DAY
         = l+.1  [I=I^{MAX}]
VAR (927)
          NUMBER OF ANIMALS INTRODUCED/BATCH
         = 1000.*VAR(922)/(VAR(916)*VAR(917))
VAR(926)* INTEREST RATE UN SINKING FUND
                                                              ⇒/YEAR
         = 1
VAR(929)*
          RETURN ON CAPITAL
                                                              #/YEAR
         = 1
          DEEP SEA WATER COSTS
VAR (932)
                                                             S/KG-UUT
         = (VAR(259)+VAR(14)*VAR(664)*VAR(243)*31536000.)/VAL
VAR (933)
          DEEP SEA WATER FLOW
                                                              M3/SEC
         = VAR (664)
VAK (934)
           DEEP SEA MATER DISTRIBUTION COSTS
                                                            $/KG-OUT
         = VAR(321)/VAL
VAR (935)
          NUMBER OF INTAKE PIPELINES
         = VAR (240)
VAK (936)
          PHYTU SPACE COSTS
                                                            $/KG-OUT
         = VAR(121) * VAR(663)/(10000, *VAL)
VAR (937)
           PIPELINE DIAMETER
         = VAR (250)
VAR (938)
           PHYTU LABOR CUSTS
                                                            S/KG-OUT
         = VAR(739)/VAL
VAR (939)
           PIPELINE LENGIH
         = VAR(241)
VAR (940)
           PHYIO TOTAL CUSTS
                                                            3/KG-UUT
         = VAR(932) + VAR(934) + VAR(936) + VAR(938)
VAR (941)
          NUMBER OF PUOLS
         = VAR (BU4)
VAR (942)
           SHELLFISH SPACE CUSTS
                                                            3/KG-DUT
```

		TO STAND AND AND AND AND AND AND AND AND AND		
VAR (945)		PHYTO POOL VOLUME	м3	352.
		VAR (665)		
		SHELLFISH LADUR COSTS	S/kG=OUT	
-		VAK(7h7)/VAL		
VAR (945)		PHYTU POUL AREA	S M	
	<b>89</b>	VAR (603)/10000.		
VAK (446)		SHELLFISH LARVAE COSTS	D/KKG-OUT	
	60r 600	VAR( 48) * 1000./(VAR(916) * VAF(917))		
VAR (947)		PHYTU PUOL DEPTH	M	
		VAR( 19)/100.		
VAK (446)		SHELLFISH SPECIFIC TOTAL COSTS	S/KG-OUT	
		VAR (942) + VAR (944) + VAR (946)		
VAK (949)		SHELLFISH TRAY AREA	M2	
		VAR(062) * VAR( 25) * VAK( 74)/10000.		
VAR (950)		SUPERVISION LABOR	\$/KG-OUT	
		VAR (702)		
vak (951)		INDIVIDUAL WEIGTH AT HARVEST	G	
		VAK (916)		
VAR (952)		TOTAL UNIT COST	\$/KG-OUT	
		VAR (940) + VAR (948) + VAR (950)		
VAR (953)		DAYS TO HARVEST	DAY	
		AINI(VAR(923))		
VAR (954)		ANNUAL CAPITAL AND OPERATING COSTS	5/YEAR	
	em)	(VAR(934) *VAL+VAR(123) *VAR(949)		
		+VAR(161)+VAR(332)+VAR(259))/1000.	VC 445 45	
VAK (955)			KG/YEAR	
		VAL	д.	
VAR (956)		CAPITAL INVESTED	\$	
		(VAR(12b) *VAR(6b3)/10b00,+V, H(122) *VAR(949)		
	-	+VAR(15%)+VAR(331)+VAR(258)1/1000.	V6 145 15	
VAL		OUTPUT	KG/YEAR	
		VAR(922)*365./VAR(807)	,~	
1:T(I+1)		INUIVIDUAL MEIGHT, START DAY (I+1)	G	
	40	$w(I) + VAR(868) \qquad [I=1,I \cap FX]$		

## APPENDIX C

A FACTORIAL NUTRIENT ENRICHMENT STUDY

OF <u>CHAETOCEROS CURVISETUS</u>, STX-167<sup>-1</sup>

bу

S. M. Anderson and O. A. Roels

A FACTORIAL NUTRIENT ENRICHMENT STUDY OF CHAETOCEROS CURVISETUS, STX-1671

by

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## ABSTRACT

Nutrient-limitation of batch cultures of the diatom

Chaetoceros curvisetus, STX-167, grown in Antarctic Intermediate seawater, was investigated. A factorial nutrient
enrichment experiment indicated simultaneous nutrient-limitation by phosphate and nitrate with synergistic involvement
of various vitamins and trace metals. It is suggested that
the factorial design nutrient enrichment experiment may be
an excellent tool for investigating occurrences of multiple
nutrient-limitation.

## INTRODUCTION:

Any essential phytoplankton nutrient will stop growth when its concentration declines below levels that can be utilized by the organism, sometimes approaching the lower limits of detection by normal chemical analysis. The limiting role of nitrate, phosphate, and silicate in primary production has been studied extensively (Caperon and Meyer, 1972; Eppley and Renger, 1974; Chu, 1946; Kilham et al., 1977; Kuenzler and Ketchum, 1962; Paasche, 1973(a), 1973(b)). Trace metals and vitamins can also limit phytoplankton growth. Menzel and Ryther (1961) observed increased primary production when iron was added to Sargasso Sea water. Molybdenum, mangamese, copper, and zinc have been found to be essential to algal growth (O'Kelly, 1974). Vitamins B12, thiamine, and biotin appear to be the main vitamins required by certain algae and are potentially capable of limiting production (Carlucci and Bowes, 1970).

Most studies have not examined the problem of multiple nutrient limitation or the synergistic and antagonistic effects of nutrient enrichment. The factorial experiment addresses these complex problems of nutrient limitation. Each macronutrient or micronutrient examined is considered a factor that can affect phytoplankton growth. The effect of two or more concentrations of each nutrient, singly and in all of the various combinations, to determine which nutrient or combination of nutrients will give enhanced growth, can be studied with this method.

The technique is also well suited to determining the optimal enrichment of culture media in mass culture of phytoplankton. The factorial design was used in this study to determine nutrients limiting primary production in the St. Croix Artificial Upwelling Mariculture system in the U. S. Virgin Islands. This mariculture operation utilizes Antarctic Intermediate water pumped from a depth of 870 meters as a culture medium to grow the diatom, Chaetoceros curvisetus, clone STX-167, isolated by Dr. Kenneth Haines. This alga is utilized by filter-feeders in a controlled food chain.

## METHODS:

Factorial Experimental Cultures: Factorial design experiments are classified by the number of factors (nutrients) and their concentrations. The classification of this factorial experiment was a 2<sup>n</sup> design (two concentrations of n nutrients). The factors considered in this study were nitrate, phosphate, silicate, iron, trace metals, vitamins, and a chelator, EDTA. The vitamins, biotin, thiamine, and B<sub>12</sub>, were combined and the trace metals, copper, zinc, cobalt, manganese, and molybdenum, were also combined. Each of these nutrients, when examined, was used in two different concentrations. The lower concentrations were those naturally occurring in the Antarctic Intermediate water. The enriched concentrations were approximately 50% above the Antarctic Intermediate seawater levels or, in

the case where these were not known, as with the vitamins and trace metals, Guillard and Ryther's (1962) F/4 levels were used (Table #1). The F/4 medium has been shown previously to be an excellent medium for algal growth. The nutrient factors were considered in two separate experiments. In Experiment #1, phosphate, silicate, iron, EDTA, vitamins, and trace metal enrichments were considered (Table #1). Experiment #2 examined the effects of phosphate, vitamins and trace metals again, along with nitrate enrichment. All enrichments were done in duplicate.

The culture methods were those of Picard (1976). The nutrients under study were added in all possible enrichment combinations to 93 ml aliquots of glass-fiber filtered Antarctic Intermediate water in individual 125 ml Erlenmeyer flasks. The volume was brought to 99 mls in each flask with Antarctic Intermediate water and the cultures were autoclaved. Chaetoceros curvisetus, STX-167, was grown axenically in F/4 medium until cell densities were high enough for inoculation (circa 5 x 10<sup>5</sup> cells/ml). The cultures were then centrifuged and the pellet washed twice with 10 ml portions of sterile Antarctic Intermediate water. One ml aliquots of the washed cell suspension were then added to each of the experimental flasks. The cultures were then incubated at 23°C with a 12L:12D light cycle. Cool-white fluorescent bulbs provided a light intensity of 0.03 ly/min as measured with a Lambda

light meter. The culture flasks were shaken twice a day to keep the algae from settling out of suspension. At approximately 1000 hours of each day, the cultures were sampled aseptically, the samples were preserved with Basic Lugol's preservative, and cell counts were done with a Speirs-Levy hemacytometer until stationary growth phase was reached. Stationary growth was considered to have been reached when cell counts were the same for at least two successive days. The experiment was terminated when a drop in cell numbers occurred.

Data Analysis: The final cell yield was defined as the average cell density when stationary growth was reached, or as the highest cell density attained when stationary growth did not occur and the cell number declined within one day after reaching its peak.

Changes in cell yield due to culture enrichment with a single nutrient were considered primary effects, while enrichment of two nutrients involved first-order interactions. The higher order interactions were enrichments of three or more nutrients. For cultures enriched with phosphate, nitrate, and silicate, the interactions compared the effect of phosphate on the nitrate-silicate interaction, the effect of nitrate on the phosphate-silicate interaction, and the effect of silicate on the phosphate-nitrate interaction. The major objective of these experiments was to compare two treatments.

The yield resulting from enrichment was compared to the yield of all other treatments. For example, when phosphate enrichment was considered, all phosphate enriched cultures were compared to all treatments without phosphate enrichment. It is assumed, therefore, that the effect of phosphate on cell yield will be neither hidden nor enhanced by the other enrichments, and all of the phosphate enrichments were considered replicates of each other. For the t-test to compare all the enrichment combinations, the equation of Freund (1970) was used:

$$t = \frac{\overline{x}_{1} - \overline{x}_{2}}{\sqrt{\frac{(n_{1}-1)s_{1}^{2} + (n_{2}-1)s_{2}^{2}}{n_{1} + n_{2} - 2}}} \times \sqrt{\frac{1}{n_{1} + 1/n_{2}}}$$

 $n_1$  = number of yields of the enrichment treatment of interest

n<sub>2</sub> = remaining number of yields (not including treatment of interest)

 $\overline{X}_1$  = mean yield of the treatment of interest

 $\overline{X}_{2}$  = mean yield of the remaining treatments

 $s_1^2 = \text{variance of } \overline{X}_1$ 

 $S_2^2 = \text{variance of } \overline{X}_2$ 

 $n_1 + n_2 - 2 = degrees of freedom for t-test$ 

A computer program generated a t-value for each nutrient combination compared to the remaining treatments.

The t-test values were then used to determine which treatments produced significant results at the 95-99% confidence levels. A positive t-value indicated enhanced growth, while a negative t-value indicated reduced growth. This type of data analysis cannot give the rank importance of the various nutrients if more than single nutrient limitation is occurring. It will, however, indicate the presence of multiple nutrient limitation.

#### RESULTS:

In the first experimental run where phosphate, silicate, iron, EDTA, vitamins, and trace metals were considered, phosphate enrichment produced significantly higher yields than any other enrichment at the 99% confidence level (Table #2). The vitamins and trace metals, when added singly to the phosphate enrichment, appeared to enhance the effect of phosphate as is noted by the depression of the phosphate t-test value when vitamins or trace metals are removed from the comparisons. The vitamins and trace metals in combination, however, seemed to depress the effects of the phosphate enrichment. The toxic effect of silicate enrichment was amplified by the addition of vitamins to the medium. Final cell yields in experiment #1 ranged from 2.0-4.2 x 10<sup>5</sup> cells/ml (Table #3).

The second experimental run examined the effect of enrichment with nitrate, phosphate, vitamins and trace metals.

Phosphate enrichment again produced significantly higher cell yields at the 99% confidence level (Table #4). In addition, nitrate also produced significantly increased yields. Phosphate and nitrate, when added in combination, produced the most significant results. Phosphate enrichment appears to enhance the effect of nitrate enrichment, while mitrate enrichment similarily enhances the effect of phosphate enrichment. The vitamins and trace metals again are enhancing the effect of nitrate and phosphate enrichment though not significantly affecting the final cell yield by themselves. In both experiments, unenriched Antarctic Intermediate water did not have significantly lower cell yields. A maximum cell yield of 4.4 x 10<sup>5</sup> cells/ml was obtained with the phosphate-nitrate-vitamin-trace metal enrichment combination (Table #5).

#### DISCUSSION:

In Antarctic Intermediate seawater, under the experimental conditions used, Chaetoceros curvisetus, STX-167, appears to be experiencing dual-nutrient limitation from phosphate and nitrate, indicating that adequate amounts of silicate, vitamins, and trace metals are present in the Antarctic Intermediate water to ensure maximum utilization of existing nitrate and phosphate supplies. Enrichment with phosphate causes nitrate limitation, while nitrate enrichment induces

phosphate limitation. The vitamins and trace metals, though not limiting in themselves, are involved synergistically with this dual limitation. Because nitrate and phosphate appear to be limiting to growth simultaneously, the N:P ratio in the Antarctic Intermediate water (approximately 15:1) may well be identical to the intracellular N:P ratio of Chaetoceros curvisetus. Further investigations will verify this hypothesis.

Multiple-nutrient limitation has been discarded as a possibility by some investigators (Droop, 1974; Rhee, 1973). The culture methods and means of analysis used, however, were different from those presented here and may lack the sensitivity to perceive the subtle effects of certain types of nutrient limitation.

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Expt. 2 Enrichment Concentration	50.0 JM	3.0 JM	22.5 JIM	ŧ	į		0.25 µg/1	$0.25  \mu g/1$	0.25 µg/1		$7.86 \times 10^{-4}  \mu M$	$1.53 \times 10^{-3}  \mu M$	$8.50 \times 10^{-4}$ JM	$1.83 \times 10^{-2}  \mu M$	$5.20 \times 10^{-4}  \mu M$
Expt. 1 Enrichment Concentration	31.8 µM	3.0 pM	33.5 pM	1.0 JM	1.0 JM		0.25 µg/1	0.25 µg/1	0.25 µg/1		$7.86 \times 10^{-4}  \text{µM}$	1.53 x 10 <sup>-3</sup> µM	8.50 × 10 <sup>-4</sup> µM	$1.83 \times 10^{-2}  \mu M$	$5.20 \times 10^{-4}  \mu M$
Antarctic Intermediate Seawater Concentration	31.8 µM	1.9 MM	22.5 µM	i	f		1	ı	ł		į	·	i	1	ı
Nutrient	T CON	PO_13	Sio <sub>3</sub> -2	70	EDIA	Vitamins	Biotin	B	Thiamine	Trace Metals	cu <sup>+2</sup>	Zn+2	co+2	Mn+2	MO+6
Expt. 2 Code	Z	C.	dia	duate	eg	<b>&gt;</b>									
Expt. 1 Exp Code CC	a a a a a a a a a a a a a a a a a a a	C4	Ø	ļo;	U	>				Sound ton					

Table #2

	t	t	t
Enrichment Treatment	(all treatments)	(no V)	(no T)
_		Control of the Contro	
P	2.783	1.664	1.723
S	-1.543	1.151	-1.484
F	-1.236	-2.393	-0.076
C	0.137	-0.210	1.108
Λ	0.104	****	0.979
T	0.974	2.335	****
PS	-0.656	0.629	-0.632
PF	0.545	-0.372	1.248
PC	1.406	0.773	1.965
PV	2.215	****	2.373
PT	2.166	2.964	****
SF	-0.850	-0.230	-0.681
SC	0.041	1.435	-0.044
SV	-2.489	***	-2.007
ST	0.118	1.434	****
FC	-1.439	-2.318	0.102
FV	0.080	****	0.795
FT	-0.811	-0.951	***
CV	351	***	1.449
CI	-0.307	0.414	****
VI	-0.230	****	****
PSF	-0.404	0.131	0.099
PSC	0.407	1.364	0.421
PSV	-1.273	***	-1.001
PST	0.153	0.883	***
PFC	-0.150	-0.431	0.742
PFV	1.024	****	1.914
PFT	-0.201	0.130	****
PCV	1.333	****	1.903
PCI	0.305	0.506	****
PVI	0.865	****	****
SFC	0.002	-0.527	0.420

# (continued)

Enrichment Treatment	t (all treatments)	(no V)	(no T)
SFV	-0.913	****	-0.738
SFT	-0.049	0.244	オヤマナカ
SCV	-0.914	***	-0.739
SCT	0.457	1.175	<b>有实现实</b>
SVI	-0.810	****	<b>マカマカガ</b>
FCV	-0.252	****	7.938
FCT	-1.635	-1.488	****
FVI	-0.353	****	2444
CVI	-0.658	****	マヤマヤヤ
PSFC	0.625	0.473	- 9 - 9 - 9
PSFV	-0.624	****	-0.064
PSFT	-0.414	0.089	大学专宾宫
PSCV	-0.416	****	-0.330
PSCT	0.347	0.730	***
PSVI	-0.414	****	****
PFCV	0.140	****	0.818
PFCT	-0.76l	-0.679	****
PFVI	-0.345	****	****
PCVI	0.070	***	****
SFCV	0.417	****	0.730
SFCI	-0.206	-0.296	***
SFVI	-0.206	****	****
SCVT	-0.206	****	****
FCVI	-1.112	****	****
PSFCV	0.532	****	1.070
PSFCT	-0.240	-0.027	****
PSFVT	-0.625	****	***
PSCVT	-0.047	****	****
PFCVT	-0.529	****	****
SFCVT	-0.047	****	****
PSFCVT	<del>-</del> 0.303	****	****
CONTROL	-0.819	-1.072	-0.589

Yield	2.2	2.7	2.0	3.0	2.6	2.6	2.4	2.9	2.9	2.6	2.6	2.8	2.6	2.0	3.0	2.6	2.4	2.8	2.5	2.8	2.6	2.5
Enrichment Treatment	PSFV	PSFT	PSCV	PSCI	PSVI	pfcv	PFCF	PFVI	PCVI	SFCV	SFCT	SFVI	SCVI	FCVF	PSFCV	PSFCT	PSFVF	PSCVI	PFCVF	SFCVI	PSFCVI	CONTROL
Yield	2.5	2.9	2.5	3.0	2.7	2.6	3.0	4.2	2.9	3.5	2,3	2.1	2.8	2.2	3.7	2.2	2.9	2.2	3.0	2.7	2.8	
Enrichment Treatment	PSF	PSC	PSV	PST	PFC	PFV	PFT	PCV	PCI	PVT	SFC	SFV	SFT	SCV	SCI	SVI	FCV	FCI	FVI	CVF	PSFC	
Yield	2.5	2.7	2.6	2.4	2.6	2.8	2.4	2.5	2.6	2.7	3.9	2.7	3.0	2.3	2.7	2.2	2,3	2,3	2.8	2.8	2.2	
Enrichment Treatment	Q.	S	Ē.	O.	Λ	E	PS	PF	PC	PV	Z	Ş	SC	SV	ST	E	F	20 P.	Ç	む	\rangle I	

Table #4

	<b>=</b>		t	t	t
Enrichment Treatment	(all treatments)	(no P)	(no N)	(no V)	(no T)
Audio dibiliti di amini di antico di mandio della solo di ancio di ancio di antico di mandio antico di ant			Microsoppine 200 Miles de considere de l'assessimente en la consideration de l'acceptance de l		
P	5.117	****	3.796	4.051	4.284
N	3.184	2.540	***	1.909	1.973
7	0.937	1.324	0.443	****	1.476
ry's	0.439	0.522	-C.110	1.304	****
PN	7.797	***	****	4.561	5.469
PV	3.209	***	1.720	****	2.713
PT	2.804	中有女女女	1.595	2.281	****
NA	2.726	2.303	****	***	2.029
NI	2.353	2.061	****	1.671	***
VI	0.312	-0.211	-0.699	***	****
PNV	5.024	****	****	****	2.634
PNI	4.389	女女女女女	****	2.058	****
	2.091	****	0.041	****	****
PVI	2.003	0.413	****	****	****
NVI		マッツエン	****	****	***
PNVI	4.005				
CONTROL	-1.751	-1.687	-1.725	-1.748	-2.001

# Table #5

Enrichment Treatment	Yield
P	2.5
N	2.0
V	2.3
Ţ	2.1
PN	3.6
PV	3.0
PT	2.9
NV	2.8
MI	2.8
VI	2.2
PNV	3.7
PNI	3.5
PVI	2.4
PNVI	4.4
CONTINCT.	2.0

Table #1 Factorial Nutrient Enrichment Concentrations

Table #2 Factorial Enrichment t-test Values - Experiment
#1. P = phosphate S = silicate F = iron t = 2.3 (99%
confidence level; 120 degrees freedom) C = EDTA V = vitamins
T = trace metals

Table #3 Final Cell Yields (cells/ml x  $10^5$ ) - Experiment #1. P = phosphate S = silicate F = iron C = EDTA V = vitamins T = trace metals

Table #4 Factorial Enrichment t-test Values - Experiment #2. P = phosphate N = nitrate t = 2.75 (99% confidence level; all compared) t = 2.04 (95% confidence level; all compared) t = 2.98 (99% confidence level; one treatment removed) t = 2.14 (95% confidence level; one treatment removed)

Table #5 Final Cell Yields (cells/ml x  $10^5$ ) - Experiment #2. P = phosphate N = nitrate V = vitamins T = trace metals

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# APPENDIX D

THE CULTURE OF TAPES JAPONICA (DESHAYES)
(BIVALVIA: VENERIDAE) IN A TROPICAL HATCHERY

bу

J. B. Sunderlin, S. Laurence, O. A. Francis, and O. A. Roels

THE CULTURE OF TAPES JAPONICA (DESHAYES)

(BIVALVIA: VENERIDAE) IN A TROPICAL HATCHERY

by

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## ABSTRACT:

An experimental shellfish hatchery was established in April 1974 at the "Artificial Upwelling" mariculture system on St. Croix to test the feasibility of operating a hatchery in the tropics for rearing temperate species and to augment the supply of commercial shellfish seed which is not normally available on a year round basis. From August 1976 through October 1978, the hatchery reared <a href="Tapes japonica">Tapes japonica</a> (DESHAYES), on a regular basis, to supply pilot plant Models I and II. During the 4 1/2 years that the hatchery was operated, 50 batches of <a href="Tapes">Tapes</a> were spawned and 45 of these were reared in the system. Out of these 45 batches, there are data from 40 batches for percent survival to metamorphosis; the average for all these populations is 40%. The range of survival was 0.4% to 90%. For the 18 batches introduced into the Pilot Plants, the percent survival averaged 51%.

After rearing 45 batches of <u>Tapes</u>, including five generations, in the hatchery system, it is apparent that the feasibility of a hatchery in the tropics has been demonstrated. Refinements and improvements in the system are substantiated by the increased survival in the last 18 batches of Tapes reared.

#### INTRODUCTION:

A hatchery facility was developed in April 1974 at the St. Croix "Artificial Upwelling" mariculture system to (1) test the feasibility of operating a hatchery in the tropics using predominantly temperate shellfish species (2) insure a supply of hatchery seed when needed instead of having to rely on a supply of commercial hatchery seed that is not constant and which is usually geared seasonally to the temperate climate and (3) eliminate acclimation procedures to the tropical environment that are essential with purchased commercial seed.

In addition to five generations of <u>Tapes japonica</u>, <u>Crassostrea</u> gigas (Thumberg) and <u>Ostrea edulis</u> (Linne) have also been successfully spawned and reared through metamorphosis to adults in St. Croix. The kumamoto variety of <u>C. gigas</u> has been successfully spawned 10 times but the species never metamorphosed in the St. Croix hatchery.

A critical factor to consider when establishing a pelecypod shellfish hatchery is water quality. Most hatcheries must filter or centrifuge their available water to remove undesirable organisms and even then water quality varies seasonally (Bardach et al., 1972, Chanley, 1975). In the "Artificial Upwelling" mariculture system in St. Croix, U.S. Virgin Islands, water of excellent quality and constancy is available since Antarctic Intermediate water is pumped from a depth of 870 m to shore. Nutrient concentrations are stable and do not vary seasonally (Roels et al., 1975) and deep water is free of man made pollutants, diseases and predators harmful to shellfish.

Larval feeding studies conducted in St. Croix on Tapes

japonica larvae indicated the best diet to be a mixture of three

algal species (Thalassiosira pseudonana, Bellerochea polymorpha

and S-1, an undetermined cryptophyte flagellate. All three of

these algal species can be grown in large scale culture in

St. Croix. When these algal species are fed singly or in

combinations of two but not as a mixture of three, the larval

growth rate is reduced (Sunderlin et al., 1976).

Survival for 40 populations of <u>Tapes</u> will be presented.

However more detailed analysis was carried out on data generated from the 18 batches of <u>Tapes</u> used for the pilot plant Models I and II from August 1976 through October 1978.

This paper reports the hatchery procedures, hatchery growth data, and some pilot plant data for 5 generations of <a href="Tapes">Tapes</a> japonica.

## MATERIALS AND METHODS:

Brood stock used to produce the <u>Tapes japonica</u> were from two sources: clams from the "Artificial Upwelling" mariculture station which were originally purchased as 3mm juveniles from Pacific Mariculture, Inc., Pescadero, California; and adult clams obtained from the State of Washington (Ellison Oyster Co., Olympia, Washington, 98502). The same procedures were used for the spawning and rearing of the <u>Tapes</u> larvae for all 45 populations. The hatchery techniques, essentially the Milford method (Loosanoff and Davis, 1963), employed were the following:

Shellfish brood stock were subjected to thermal stimulation to induce spawning. Deep water was circulated through a glass-lined, gas-fired water heater to raise the temperature in the spawning dishes from 22 to 32°C within 10 minutes. Stripped gonad solution (sperm or eggs) was added to the dishes to stimulate spawning. When brood stock were not stimulated to spawn on a specified date, in order to keep the pilot plant time schedule (spawning every 28 days), eggs and sperm from spontaneous spawnings in our pilot plant were collected.

Larval cultures at an initial concentration of 10 larvae/ml were reared in 379 l conical fiberglass tanks with moderate aeration. Prior to Spring 1976, 15, 30 or 50 l polyethylene containers were used for larval rearing. The algal culture was removed three times/week by filtering the larvae tanks. The larval cultures were filtered through a graduated series of three 25.4-cm diameter Nitex sieves (nylon-monofilament bolting cloth; Tobler, Ernst and Traber, Inc., Elmsford, N.Y.). Clumped food and debris were

trapped on the top sieve and discarded, while larvae were collected on the bottom two sieves. Larvae were rinsed from sieves and combined in a 10-12 liter concentrate. Sieve sizes were increased on the next filtration day if a large percentage (>90%) of larvae accumulated on the middle sieve.

A 15 ml sample was taken from the 10-12 l concentrate of larvae after it was thoroughly mixed. Data on larval growth and survival were obtained from this 15 ml sample. On each filtration day, at least 10 larvae were measured (length and width in microns (Loosanoff et al., 1966) using an occular micrometer. On day 2, day 10 and day 14, a Sedgwick-Rafter cell was used to determine the number of larvae/ml; duplicate or triplicate 1 ml aliquots of the sample were counted.

After filtering, the 379 liter larval containers were refilled with deep water and 0.2 ml of "Vetstrep" (Streptomycin sulfate, Merck and Co., Rahway, N.J.) per liter of larval culture was added (equivalent to 50 mg/liter). A mixed diet of two diatoms (Thalassiosira pseudonana and Bellerochea polymorpha) and one unknown Cryptophyte flagellate (S-1) was fed to the larvae most of the time, but on occasion only two of these species were available. The initial food concentration in the larval cultures ranged from 8 x 10<sup>4</sup> to 2 x 10<sup>5</sup> cells/ml.

The yearly temperature range in the larval cultures varied between 23-28°C and the salinity was 34.75 to 34.95 ppt.

On day 14, when metamorphosis began the larvae were transferred into flumes measuring 10'  $\times$  1'  $\times$  5 1/2".

Until day 21, the larvae/juveniles were filtered three times per week and batch fed. One million to 1.5 x 10<sup>6</sup> larvae were placed in each setting flume and again "Vetstrep" and food were added at proportional concentrations. All food for the hatchery until day 21 was cultured in an air conditioned laboratory in 15 liter carboys. On day 21, when the majority of the Tapes population had metamorphosed, the flume was placed on continuous flow. The juveniles then received a mixed algal diet of 2 or more species from outdoor 2,000 liter reactor cultures. Densities in reactor cultures range from 1 x 104 to  $1 \times 10^6$  cells/ml depending on the age and species in culture. Prior to February 18, 1978, flow to the flumes was 1.38 1/min from day 21-35 and 2.76-4.14 1/min from day 35-56. Thereafter Day 21-35 flow was 2.76 1/min and Day 35-56 flow was 5.52 1/min. Three air lines were placed in each flume beginning on Day 14 to aid in mixing.

On Day 56, the hatchery population was introduced into the pilot plant facility. Two hundred gm of clams were placed in a tank. However, if the population weighed >200 gm, sieving was done to select 200 gm of the larger and faster growers for the pilot plant. If less than 200 gm were available, the entire hatchery population was placed in the pilot plant without sieving. The clams were fed STX-167 (Chaetoceros curvisetus) exclusively after introduction into the pilot level facility.

#### RESULTS AND DISCUSSION:

# Operation of the Hatchery

Forty-five populations of <u>Tapes japonica</u> were reared in the hatchery over a 4 1/2 year period and data for 40 of these populations are given in Table 1. The average % survival to metamorphosis for all 40 batches is 40%. The range of survival was 0.4% to 90%. The 18 batches of <u>Tapes</u> introduced into pilot plant Model I and Model II are marked with an \* in Table 1 and more detailed growth data are given for these populations in Table 2.

Eighteen populations of <u>Tapes japonica</u> were spawned in the St. Croix hatchery and subsequently introduced into the pilot plant. The larval period for this species averaged 14 days and percent survival to metamorphosis averaged 51%. Consistent growth results were obtained from Day 0-Day 14 for all 18 populations (Table 2); standardized techniques and a steady food supply account for this similarity in growth.

When the length-width relations of 18 populations of <u>Tapes</u>

japonica, from Day 2 through metamorphosis, are graphed, a

similar curve to that described by Loosanoff et al., (1966) results.

Figure 1 depicts this length-width relationship.

For populations of <u>Tapes</u> #34 through <u>Tapes</u> #43, the weights produced on Day 35 and Day 56 were erratic. The disappointing weights for populations #42 and #43 were specifically believed to be related to food (availability of poor quality reactor cultures consisting primarily of S-l during their hatchery rearing). Lower temperatures in winter (23°C vs. 25-27°C in summer) also may have contributed to slower growth.

On February 18, 1978, starting with <u>Tapes</u> #44, several changes were made in the hatchery procedures:

- a) at least  $6-8\times10^6$  fertilized eggs were obtained on spawning days.
- b) On Day 14, populations were culled to  $2-3\times10^6$  larvae and  $1-1.5\times10^6$  larvae were placed in each of two setting flumes.
- c) On Day 21, the two flumes were combined and placed on continuous flow and the flow to the flume was increased by a factor of 2.
  - d) On Day 35, populations were culled to 25 gm.
- e) On Day 56, populations were culled by sieving and 200 gm of the largest clams were introduced into the pilot plant.

Since the aim of the hatchery is to produce juvenile <u>Tapes</u> for introduction into the pilot plant at 28-day intervals, it is expected that on Day 56 the hatchery will provide a homogenously distributed population of 20,000-25,000 animals, each 4.3 mm in shell length and 0.01 gm in individual whole wet weight. Ten populations of individuals weighing 0.01 gm or more were introduced into the pilot plant. The last four populations <u>Tapes</u> #47 through <u>Tapes</u> #50 exceeded this weight by a factor of 2 to 3 and averaged over 7 mm in length (Table 2).

One disappointment was the number of spontaneous spawnings that had to be used to supplement induced spawnings (7 out of 18) to provide the 18 populations destined for the pilot plant. Brood stock were not always ripe, primarily due to poor holding tank facilities and lack of constant food supply. We did learn over the last two years that <u>Tapes</u> grown in the pilot plant will undergo gametogenesis and can be easily spawned by manipulating

temperature, flow rate or a variety of stress inducing variables. Clams can be kept in a ripe condition at all times if sufficient care and feeding is maintained. No artificial lowering of temperature below the  $21^{\circ}-23^{\circ}$ C range supplied by the deep water is necessary. Regular cleaning of the brood stock facilities is very important.

Generally, data collected indicates that a separate brood facility is not required and enough animals of a preselected size, condition and genetic history can be removed from the pilot plant and induced to spawn. In the present pilot plant, such a procedure has provided spat more reliably than has the use of a separate brood stock operation.

# Use of Antibiotics in Hatchery Operations

The role of antibiotics in the hatchery has been reviewed and several studies that we conducted are mentioned at this time. The first batch of <u>Tapes</u>, destined for the pilot plant, that was grown entirely without antibiotics was population #36. Prior to being placed into the setting flume, this batch grew well and had good survival (76%) to metamorphosis. However, several days after transfer to the setting flumes, very high mortality (>90%) occurred. This was tentatively traced to bacterial slime on the bottom and sides of the flume probably due to the lack of "Vetstrep" in the flume. Hence, it was decided to add "Vetstrep" to the larval cultures through Day 21, at which time, the flume is placed on continuous flow.

Tapes population #39 was divided into two groups; one group with "Vetstrep" at 50 mg/l and one group without antibiotics.

On May 14 the "Vetstrep" group larvae were larger, but survival

was higher in the no antibiotic group. Again, during metamorphosis, the no antibiotic group experienced a mortality of over 90% and the "Vetstrep" group's survival was close to 40%.

Tapes population #40 was divided into two groups: the "Vetstrep" group and a no antibiotic group, and up until Day 13 when the larvae were transferred to flumes, the results for survival and growth were similar to <u>Tapes</u> population #39 and other batches grown in this manner. On Day 13 both batches were treated similarly but separately ("Vetstrep" was added to each flume at 50 mg/l). On Day 20 there was very little difference between the groups and nearly 300,000 clams had set in both groups. Based on the results obtained in the <u>Tapes</u> #40 study, it appears possible that antibiotics can be eliminated through Day 13 and that adequate survival can be maintained during Days 14-21 with the addition of "Vetstrep" (50 mg/l) to the flumes.

It may be possible to use considerably less than 50 mg/l of "Vetstrep" as indicated by an experiment run on <u>Tapes</u> population #41. One group from this population had 50 mg/l "Vetstrep" added and the other group, 25 mg/l. Survival to metamorphosis for both groups was excellent and there was virtually no difference in the size of the larvae at the time of setting. A good percentage of clams for both groups metamorphosed.

# The Influence of Number of Generations in the System and Culling Procedures on Survival and Growth in the System

Despite the fact that no definite conclusions can be drawn at this time as to whether our fifth generation clams exhibited enhanced growth and survival when compared to preceeding generations, there is sufficient preliminary data available to warrant a discussion of the potential influence of long term selection on growth rates.

The present study employed animals from five (5) generations of <u>Tapes</u> and two mixtures (two each combined generations 1 and 3 and 1 and 4). In addition to the mixtures there were four (4) 1st generation populations, three (3) 2nd generation populations, three (3) 3rd generation populations, two (2) 4th generation populations, and two (2) 5th generation populations (18 populations total). Although there was no systematic attempt to control the influence of genotype and culling in the pilot plant, animals selected for brood stock for the production of each successive generation were selected from groups of individuals judged as the largest (generally, by shell length, but also by weight). Assuming some significant degree of heritability of growth rate for the species and a significant correlation between adult and larval growth rate, an increase in mean larval size with each successive generation would be expected.

Table 3 illustrates the relationship of 1st, 2nd, 3rd, 4th, 5th and mixed populations of <u>Tapes</u> grown in the hatchery.

There is a trend for increased survival from 3rd to the 5th generation, and the 5th generation attained the highest average weight of a population on both Day 35 and Day 56.

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Table 4 illustrates the mean s.d. of the product of larval length and width on day 14 for the 5 generations and two mixtures. A general positive trend with generation exists with the exception of the 3rd. There is good evidence to indicate the 3rd generation population should be excluded from analysis, as they were spawned in August 1976 within three weeks of one another, and were the only groups fed non-sterile algal culture prior to day 14. Further, only two instead of the usual three algal species were fed to them consistently (Sunderlin, 1978). The mean, s.d., survival for this generation on day 14 was 19  $\pm$  12.3%, compared with 57.1  $\pm$  14.2% for the remaining 15 populations.

There is strong evidence to indicate that selective culling of individuals from any of the populations is of greater importance than is the relatively weak trend with generation. Table 4 presents a comparison of observed length and width measurements for the 5 generations with a simulation of what these values would be if all but the largest 50% and 10% of the population were culled. Note that if the top 10% of the seed from the 3rd generation are selected, the mean length is  $250.06\mu$ compared with 225.5µ for all of the 5th generation with no culling. Table 5 presents a similar calculation for each Tapes population. These values may be compared with the actual length and width measurements on day 14 presented in Table 2. Over the long term, selective breeding will probably be more advantageous and lead to significant improvement in the seed. For the short term, a combination of high production and rigorous selection will provide sufficient numbers of fast growing seed. The growth rate of the final animals selected as well as their numbers will involve a trade off between production cost and growth rate.

#### ALLOMETRICS:

Allometric data from pilot plant Model II best illustrate some aspects of the continued growth of <a href="Tapes">Tapes</a> populations after leaving the hatchery. Despite the fact that all individual populations exhibit varying growth rates and wet weights relative to their chronological ages, various allometric parameters show very constant relationships. Figure 2 demonstrates the consistent relationship of length, width and depth which exists through market size for the <a href="Tapes">Tapes</a> populations grown in pilot plant Model II. The linear relationships illustrated in Figure 2 also serve to demonstrate that our measurement techniques for the clams were good. Similarly, Figure 3 demonstrates the constancy of wet weight protein content of pilot plant <a href="Tapes">Tapes</a> populations. Percent protein content remained at a level of 3 to 4% of whole wet weight through an animal weight of 5.7 gms.

Figure 4 displays the relationship between shell length or width and shell depth of <u>Tapes</u> populations grown in pilot plant Model II. While the relationship between shell width and length remains linear throughout <u>Tapes</u> life span (Figure 1), when shell width reaches 10 mm, and shell length reaches 13-14 mm, depth of shell begins to increase relative to both these dimensions, indicating that after this point in time, an increasing proportion of weight gain is due to increase in shell depth.

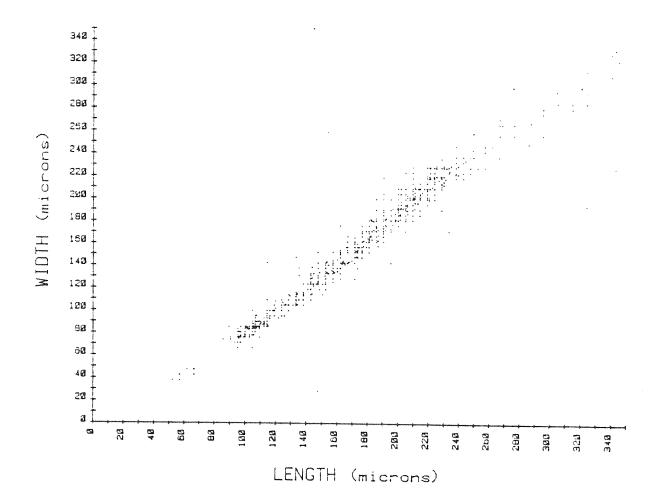
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Figure 1. Length-Width Relationship of <a href="Tapes">Tapes</a> japonica.



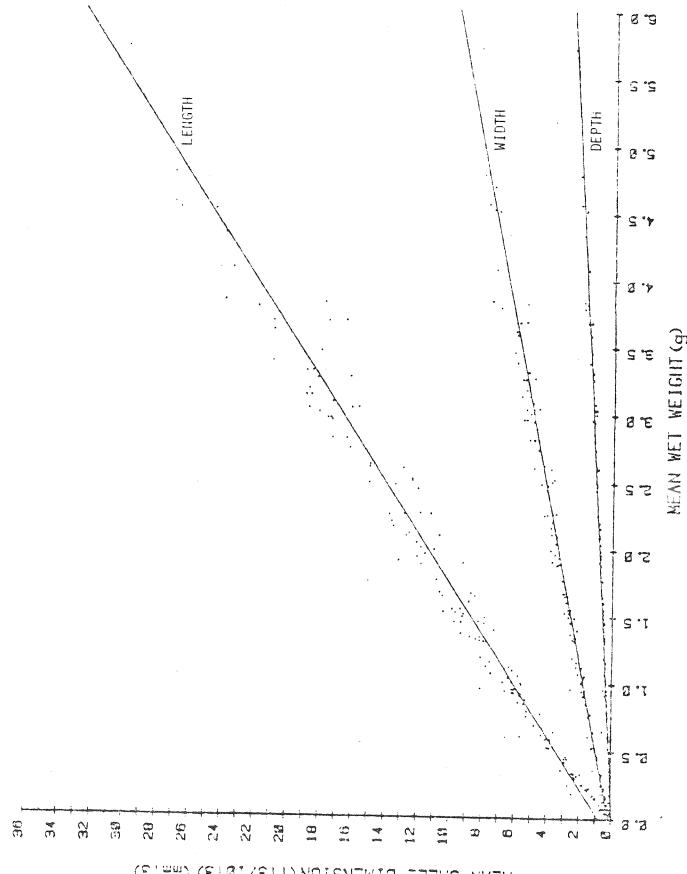
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FIGURE 2. The relationship between length, width and depth of  $\underline{\text{Tapes}}$  japonica grown to market size in the Model II pilot plant.

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MEAN SHELL DIMENSION (Y13/1813) (mm.13)

Figure 3. The relationship Between <a href="Tapes">Tapes</a> japonica live wet weight, and protein content.

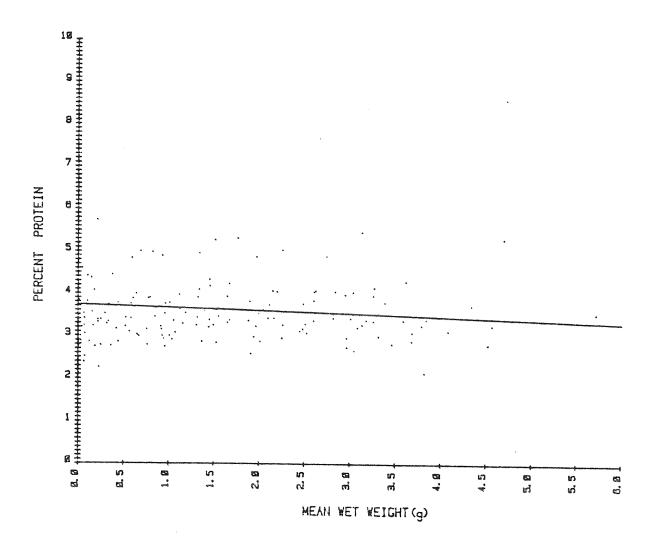
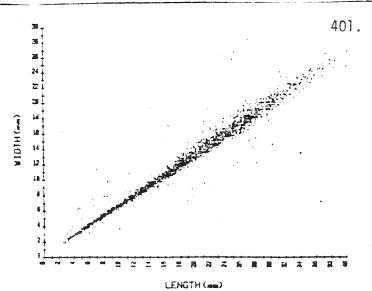
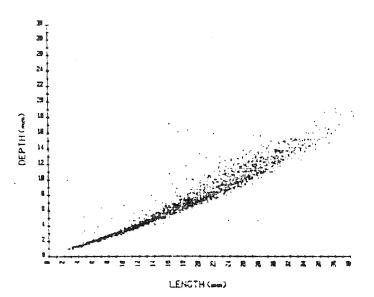


Figure 4. The Relationship of Shell Depth fo Shell Length and Width of <a href="Tapes japonica">Tapes japonica</a> Cultured in the Model II Pilot Plant.





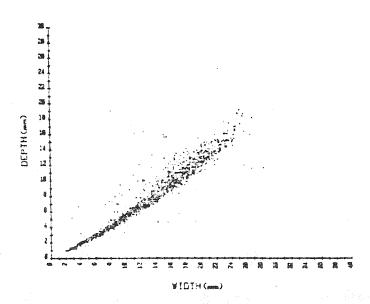


Table 1. Percent survival to metamosphosis for 40 <u>Tapes</u>
japonica populations.

Tapes		Spawning	
population number	date spawned	induced or spontaneous	% survival to metamorphosis
5	2/04/75	spontaneous	41.
6	3/05/75	induced	44.
7	4/05/75	spontaneous	63.
9	7/16/75	induced	34.
10	10/16/75	induced	36.
11	12/30/75	induced	4.
12	1/02/76	induced	51.
13	1/27/76	induced	46.
16	5/12/76	induced	60.
17	6/02/76	spontaneous	30.
18	6/18/76	spontaneous	63.
20*	8/03/76	induced	33.
21*	8/16/76	induced	14.
22*	8/24/76	induced	10.
23	8/31/76	induced	11.
24	1/03/77	spontaneous	6.
25	1/24/77	spontaneous	4.5
26	1/31/77	spontaneous	0.4
27	2/08/77	spontaneous	15.
28	2/28/77	induced	2.
30	3/16/77	spontaneous	40.

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Table 1 (cont.)

Tapes			
population	date spawned	induced or spontaneous	% survival to metamorphosis
31	3/30/77	spontaneous	6.
32	4/01/77	spontaneous	13.
33	4/25/77	induced	68.
34*	5/23/77	induced	63.
35*	6/17/77	induced	57.
36	7/18/77	induced	76.
37*	8/15/77	induced	71.
39*	9/14/77	induced	59.
40*	10/11/77	spontaneous	56.
41*	11/07/77	induced	90.
42*	12/6&7/77	spontaneous	58.
43*	1/02/78	induced	38.
44*	1/31/78	spontaneous	46.
45*	2/27/78	spontaneous	43.
46*	3/27/78	spontaneous	71.
47*	4/25/78	spontaneous	66.
48*	5/23/78	spontaneous	40.
49*	6/19/78	induced	57.
50*	7/17/78	induced	41.

<sup>\*</sup>populations introduced into Pilot Plants Model I and Model II.

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SUMMARY DATA OF TAPES POPULATIONS PRODUCED IN THE HATCHERY TABLE 6.2

			* CHONTAR			der Charles (Bricks, parent Laboratoria	DAY 14	14			DAY 35	DAY 56	INTRODUK	INTRODUCED TO PILOT PLAN	OT PLANT
SHOW		eng diam	TO MFIA-		LENGIH (micron	microns)			WIDIII (microns	crons.)	13	2		MINOED	AVERAGE
POPULATION	GENERATION	(105)	MORPHOSIS	ı×	SD	RANGE	ايبا	1×	SD	RANGE	(July)	(dll)	(dm)	CLAMS	M. IGH PER CLAM
000	Š	A RO	(**	207 &	17 62	171 A	226 6	100	זע ענ		6		000	221	2010
3 6	; ;		) ·	200	30.18	20 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1	2.03	200	2		5		07/	00.7	0070.0
2	3rd	2,52		212.5	28.27	154.2 +	257.0	201.6	24.09		5.0.	! !	215	9,000	0.0238
22	320	13.84		194.	33.42	152.3 +	266.6	178.5	30.08		6.	! !	200	00000	0.0222
34	C C C C C C C C C C C C C C C C C C C	29.84	63	213.3	10.66	190.4 +	228.5	206.7			12.7	142.4	1 1	{	1 1
S.		16.40	57	206.4	8.09	190.4 +	219.0	107.1	7.52		2,0	166.4		1	1 1
37	ر الاسم الاسم	12.78		210.6	6.56	199.9 +	220.9	202.7	8.94		20.0	642.3**	202	12,000	0.0118
39	mix (183)	10.56	59	195.2	8.03	180.9 +	209.4	184.7	2.7	161.8 - 199.9	<20.0	142.0***	142	142,000	0.00.0
40	70	7.62	26	215.8	7.23	204.7 +	228.5	214.5	13.23		67.59*	1434.79	250	25,000	0.0100
4	154	30.96	96	206.4	8.75	+ \$.061	219.0	200.9	9.04		, e	327.5	175	47,500	0.0036
42	45	4.32	58	224.2	17.42	199.9 +	247.5	217.2	14.56		22.67	86.48	86	13,500	0.0063
43	Znd	96.9	38	221.7	13.99	195.2 →	247.5	211.3	15.89		7.44	27.0	27	5,312	0.0050
	2nd	13.76	46	219.0	18.84	180.9	238.0	206.5	18.65		12.38	162.33	162	29,167	0.0055
, 45	mix (164)	25.40	43	208.8	6.60	196.4 →	220.3	198.1	7.47		14.88	128.16	128	19,375	0.0066
3	215	27.40	~	215.6	9.77	199.3	237.6	207.7	10.34		61.96	752.25	246.6	21,493	0.0114
	2	31.90	99	232.0	10.34	210.7 +	249.1	222.9	16.11		128.10	1325.4	363	9,722	0.0373
800 800	mix (163)	19.20	40	206.1	15.99	176.1 +	223.7	187.1	16.94		48.49	2722.43	200	(a)	0.0244
49	2	15.80	2	239.0	36.66	180.9	247.5	211.7	15.99		132.53	2994.45	400	9,886	0.0404
20	mtx (183)	27.40		206.1	17.80	161.8	219.0	196.3	17.99	152.3 + 209.4	93.04	5026.48	!	1	1 1
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Tapes 20, 21 and 22 were spawned on 8/3/76, 8/10/76 and 8/24/76 respectively and were reared in the 1976-77 (Model I) pilot plant and transferred to the Model II configuration 10/04/77.

Japes 34 and Japes 35 were spawned on 5/23/77 and 6/17/77 and introduced into the Model I plant and transferred to Model II on 10/04/77. Japes 36 was a poor population and was not introduced to the pilot plant. Japes 37 was the first population which spent days 56 ff entirely in the Model II pilot plant. Excess animals from Japes 37 were used to occupy the tray positions intended for Japes 36. MOTE:

Populations 20, 21 and 22 were not introduced on day 56 into pilot plant Model I, whereas populations 37-50 were introduced on day 56 into pilot plant Model II.

Table 3. Comparison of the 5 generations of <a href="Tapes japonica">Tapes japonica</a> reared in the St. Croix hatchery.

generation	average % survival to metamorphosis	average weight on D35 of population (gm)	average weight on D56 of population (gm)	average weiç per clam on D56 (gm)
lst	70	15.53	319.40	0.0077
2nd	52	27.26	313.86	0.0073
3rd	19	N/A	N/A	0.0222
4th	57	45.13	760.64	0.0081
5th	62	109.54	2159.93	0.0388
mixed	46	44.30	2004.76	0.0106

Comparison of Observed Average Length and Width of Tapes Generations on Day 14 with Predicted Values had all but the Largest 50% and 10% of the Animals been Culled. Table 4.

	Observed	l Averages	Observed Averages for Generations	tions	Averages for Generations if all but the top 50%	Generations the top 50%	Averages for Generatic if all but the top 10	Generation 10
Generation	Length µ	່ວໍ	Width µ	ი	are culled Length µ	on day 14 Width µ	are culled on day Length μ Width	on day 1' Width µ
a T	209.2	8.52	201.8	9.28	215.75	208.68	221.82	216.58
2nd	218.8	14.20	208.5	14.96	229.25	220.06	241.03	228.96
, 3rd	204.7	. 26.40	192.8	23.61	221.82	208.55	250.06	227.85
4	220.0	12.33	215.9	13.90	229.62	226.39	238.00	234.67
N T	225.5	13.50	217.3	13.98	232.27	227.02	248.30	241.15
m;X	204.1	12.17	191.5	13.03	212.39	199.77	218.12	203.81
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Average length and width  $(\mu)$  for each Tapes population stocked in the hatchery if the smallest 50% or 90% are culled on Day 14. Table 5.

	and a control of the	50% C1	Culled	908 CI	Culled
Population	Generation	Length µ	Width µ	Length μ	Width µ
20	3rd	220.10	211.53	226.58	217.06
21	3rd	232,29	216.10	257.04	228.48
22	3rd	213.06	198.02	266.56	238.00
34	lst	221.44	216.10	228.48	218.96
35	lst	212,30	202.78	218.96	209.44
37	lst	215.53	208.30	220.86	223.72
39	mix	200.87	190.40	209,44	199.92
40	4th	221.24	223.34	228.48	236.09
41	lst	213.72	207.54	218.96	214.20
42	4th	238.00	229.43	247.52	233.24
43	2nd	232.29	224.20	247.52	233.24
44	2nd	232,05	220.15	238.00	223.72
45	mix	213.63	203.10	220.34	201.18
46	2nd	223.41	215,84	237.58	229.92
47	5th	236.05	231.84	249.08	244.29
48	mix	217.06	198.02	223.72	204.68
49	5th	228.48	222.20	247.52	238.00
50	mix	218.01	207.54	218.96	209.44
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## APPENDIX E

#### CULTURE COLLAPSE EXPERIMENTATION

To date, our research in the causes of Chaetoceros curvisetus (STX-167) pool culture collapse has been directed toward the following general areas:

- 1) The possible influences of bacterial and/or viral contamination on culture collapse.
- 2) The possible influences of autotrophic competition, or zooplankton predation on culture collapse.
  - 3) The influence of turnover rate on culture longevity.
- 4) The influence of nutrient enrichment at 2x and 4x levels on culture longevity.
- 5) Testing of other clones (notably STX-200) of <u>Chaetoceros curvisetus</u> for improved culture longevity in the system.

A study of viruses and/or bacteria as causative agents in culture collapse indicated that although both groups of organisms could be isolated from our 50,000 litre culture pools no consistent relationship between their occurrence and the onset of collapse could be identified. Similarly, a 173 day study on the influence of autrophic and heterotrophic contaminants in deep sea water and phytoplankton pools demonstrated no consistent relationship between the occurrence of contaminants and collapse of the STX-167 population. On the contrary, collapse of the STX-167 population was generally accompanied by a reduction in the numbers of all contaminant

species present in the pool.

Studies on the influence of turnover rate on culture longevity, use of <u>Chaetoceros curvisetus</u> clone STX-200 in pools, nutrient enrichment of deep water and an analysis of routine pool culture parameters for the period January 1979 - November 1979 all point to the probablility that pool culture collapse is a light/temperature effect. Comparison of reactor cultures operated at turnover rates of 0.75, 1.15, and 1.30 day<sup>-1</sup> with pool cultures operated at a turnover rate of 1.15 day<sup>-1</sup> for the same period indicated that culture temperature (a function of turnover rate in 2,000 liter reactors) rather than the turnover rate per se had most effect on culture longevity; the coolest cultures lasting for the greatest length of time. These results are amplified by a seasonal analysis of pool cultures which demonstrated a strong correlation (0.64, 0.59 for pools 1 and 2 respectively) between depressed culture temperatures and increased culture longevity and average STX-167 cell density.

Light effects may be equally as important as temperature in determing STX-167 culture longevity. When clone STX-200, which had demonstrated excellent tolerance to high-light conditions in laboratory experiments, was introduced into routine culture in Pool 2, average pool longevity, and average cell density increased significantly in comparison to the performance of STX-167 in Pool 1. Similarly, complete nutrient enrichment to 2x and 4x levels in reactor cultures caused a nearly 100% increase in

culture longevity as compared to unenriched DSW reactor cultures, possibly due to the effects of increased self shading.

While we have yet to identify the definitive causes of culture collapse, we are now in a position to partially mitigate their effects, either through the exclusive use of Clone STX-200, and/or through the introduction of a nutrient enrichment scheme in our 50,000 liter pool cultures. This latter approach might have the additional ancillary benefits of providing an adequate test of the concept of operating deep sea water phytoplankton pools in a light limited mode to optimize areal production while at the same time providing additional production capabilities within the framework of the existing facility in St. Croix.

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