Shallow Algal Mass Culture Systems for the Production of Oils

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SUMMARY

Objective

The objective of this project was to improve the technology of outdoor mass culture of microalgae for oil production by investigation of species/strains, optimization of culture conditions, and development of strategies that increase efficiency and improve yield. Specific objectives of the subcontract were to determine if selected marine strains of microalgae could grow in water types found in the southwestern U.S., to evaluate and optimize yields of desert oil producing strains in outdoor culture, and to evaluate management strategies that will improve the efficiency or increase yields in outdoor flume culture systems.

Discussion

A shallow flume system for the mass culture of microalgae has been developed and constructed by the University of Hawaii. Briefly, the system concept includes shallow channels circulated at a high flow rate containing very dense algal cultures. Airlift pumps, restricted within 15 cm PVC pipes, are used for recirculation and gas exchange. A major contribution of the Hawaii project has been the development of a system that introduces ordered vertical mixing into the culture as a byproduct of stream flow. Small foils, similar in design to airplane wings, are suspended in the flowstream at a relatively high angle of attack. Vortices are generated at the tips of each of these foils and are propagated downstream. Arrays of foils are positioned every 1.2m along the culture flume. Observations of the vortex rotation rates have indicated that cells are exposed to a light-dark cycle time of 1 to 2 seconds in this mixing regime. This light-dark cycle avoids prolonged exposure of the cells to bright light and associated low photosynthetic efficiencies. A major accomplishment of the past ten months' work was the identification of a microalgal species which can be grown successfully in the system on a 12-month basis without temperature control, and which produces large quantities of lipids when silicate stressed. This species is a diatom of the genus Cyclotella which was isolated from an estuary in the southeastern U.S. The species grows well in both seawater and water types characteristic of saline ground-waters found in the southwestern U.S. at salinities from 7 ^{o}/oo to 50 ^{o}/oo, and is not adversely affected by the warm temperatures (30 - 34°C) encountered in a shallow culture system during the summer months. Yields of about 34 g AFDW/m^2/d were obtained with this organism during ten-day factorial growth experiments. The composition of Cyclotella was found to change from about 25% lipid carbon to over 50% lipid carbon over a time period of one day when the silicate concentration of the growth medium dropped to 0.5 - 1.0 uM. This increase in cellular lipid content occurred at cell densities typical of operating conditions in the outdoor flumes, and is associated with a large increase in the rate of lipid synthesis rather than merely a conversion of other cellular constituents to lipids. The mechanism responsible for this rapid increase in lipid synthesis is apparently the blockage of cell division

due to the lack of silicate required for frustule formation. During the period of silicate stress the diatom frustules appear to become fragile and subject to significant damage caused by the buffeting in the airlift-driven flume system. As a result it appears that the "flattening up" process will have to be performed in a less turbulent system.

An analysis of the efficiency of the mass culture system revealed that operation of the airlift at full power during the evening hours was almost certainly unnecessary, and that a reduction in the supply of air to the airlifts by as much as 90% at night had no significant effect on production. Reducing the input of CO_2 to the system by allowing the pH to rise to 8.5 rather than 7.5 resulted in no significant change in photosynthetic efficiency, but eliminating CO_2 additions altogether reduced photosynthetic efficiency by more than a factor of two in a culture of T. suecica. The cost effectiveness of CO_2 input reduction will depend on the cost of CO_2 versus the value of the product algae.

Conclusions

The project is making good progress toward identifying a suitable microalgal species and associated culture system which can be used for the mass culture of oil-producing microalgae. Silicate starvation appears to be an effective way to trigger lipid synthesis in diatoms, as evidenced by the need to add CO_2 to a dense culture in order to bring about lipid synthesis during silicate starvation. This approach appears to have the potential for nearly doubling the rate of lipid synthesis over that attainable in a silicate-replete medium. A strain of Cyclotella isolated from an estuary in the southeastern U.S. increases its lipid content to over 50% on a carbon basis during only one day of silicate starvation, produces biomass yields (34 g AFDW/m^2/d) comparable to the high yields previously achieved with Tetraselmis suecica, and is virtually unaffected by predator or competitor problems in outdoor mass culture. This species appears to be a very strong candidate for use as an oil-producing microalgal strain.

SECTION 1.0

INTRODUCTION

1.1 HISTORICAL BACKGROUND

Microalgae are attractive as biomass producers because they generally exhibit higher yields and photosynthetic efficiencies than terrestrial plants. In addition, many species of microalgae produce high concentrations of intracellular oils as energy storage compounds. Most pilot-scale mass culture systems, operated at depths of greater than 20 cm, have shown peak average yields of around 15 dry ash free grams $m^{-2}d^{-1}$. This experimental facility was designed on the premise that operation at less than 15 cm depth will result in significantly higher productivities.

Research on this problem began at the University of Hawaii under SERI subcontract on February 15, 1980 under the title "Research, Development, and Demonstration of Algal Production Raceway (APR) Systems for the Production of Hydrocarbon Resources." Work during the first two and one-half years resulted in the collection of a great deal of laboratory data on the performance of Phaeodactylum tricornutum; the design, construction, and operation of one 48m² continuous flume; the development of predator control techniques and baseline performance characteristics for the flume; and the construction of four 9.2m² experimental flumes (33).

A major contribution of the project during these early years was the development of a system that introduced ordered vertical mixing into the culture as a by-product of stream flow. Small foils, similar in design to airplane wings, were suspended in the flowstream at a relatively high angle of attack. Vortices were generated at the tips of each of these foils and were propagated downstream. Arrays of foils were positioned every 1.2 m along the culture flume. Observations of the vortex rotation rates indicated that cells were exposed to a light-dark cycle time of 1 to 2 seconds in this mixing regime. Productivity in the system employing the foils was increased by a factor of 2.2 over the productivity in the same system without foils (33).

During the 1982-83 funding period the four 9.2m² experimental flumes were operated in a factorial design experiment to determine the maximum sustainable biomass yields achievable with P. tricornutum cultured in shallow flume systems. Dilution rate and CO_2 supply were found to be the most important system parameters (34). Maximum production was found to be about 25 g ash-free dry wt $m^{-2}d^{-1}$. This production corresponded to a photosynthetic efficiency of 5.6% (34). These figures were 50-100% better than the production rates achieved in earlier P. tricornutum cultures using conventional culture techniques. The results were found to be consistent with a theoretical model of the impact of the flashing light effect on algal mass culture production (34). This model predicated that at the typical irradiances in Hawaii, full utilization of the flashing light effect should enhance production by 70% to over 200%. It was concluded that the use of foil arrays in the experimental flume created systematic vertical mixing on a time scale suitable for utilizing the flashing light effect. It was found that harvesting P. tricornutum cells could be accomplished straightforwardly by adding approximately 0.5 mM NaOH to the culture. With this addition the cells rapidly flocculated and settled out with almost 100% efficiency (34).

Although high yields were achieved with P. tricornutum during this period of study, a serious problem with the use of P. tricornutum in outdoor algal cultures became apparent. P. tricornutum grows poorly if at all when temperatures exceed 25°C. In parts of the U.S. best suited for algal mass cultural growth, water temperatures during the summer months, the prime production season, will routinely exceed 25°C. If the water were cooled in order to permit growth of P. tricornutum, the energy inputs to the system would exceed the energy produced. It therefore became obvious that strains of microalgae capable of growing in the temperature range 25-35° would have to be identified, and their production capability in shallow outdoor mass culture systems determined. The primary goal of the next phase of the research was therefore to determine maximum sustainable biomass yields achievable with

thermophilic species of marine microalgae cultured in outdoor shallow flume systems. Secondary goals were to gain an understanding of the mechanisms and conditions which lead to high productivity, photosynthetic efficiency, and lipid yield, and to analyze the effect on production of growing thermophilic strains during warm weather and P. tricornutum during cooler weather as a means of minimizing biomass production costs.

A major accomplishment of this phase of the research was the identification of a microalgal species which could be grown in the system on a 12-month basis without temperature control. The most promising species identified was Tetraselmis suecica. This species grew rapidly at temperatures from 20 to 34°C, and at salinities from 15 to 35°/oo. A factorial experiment designed to determine optimum growth conditions indicated that the optimum culture depth was 10 - 12 cm, the optimum pH about 7.5, and the optimum flow rate abut 30 cm/sec. A major discovery was that diluting the culture every third day greatly enhanced production (35). In this dilution mode daily yields averaged over 40 g/m² ash-free dry weight (AFDW) over a one-month period, and photosynthetic efficiencies averaged 8 - 11% (based on visible light energy). The former figure is over twice the best long-term yields achieved in microalgal mass culture systems grown exclusively on inorganic nutrients. An analysis of variance on the experimental results showed photosynthetic efficiencies for the three-day dilution cycle to be significantly different from those for the two and four-day dilution cycles at $p = 0.05$ (36).

During the present funding period three general problem areas were addressed. First, there was a desire to know whether the species being studied were suitable for growth in water types representative of the southwestern U.S. Second, there was a desire to reduce production costs both in terms of energy and dollars. Finally, there was a desire to find a species which would produce large quantities of lipids under appropriate growth conditions. The first problem area was addressed by growing both marine species and algae isolated from the southwestern U.S. in seawater and characteristic southwestern U.S. water types to determine the optimum water type for each species. The second problem was addressed by various manipulations with the experimental flumes during production runs with T. suecica. The third problem was addressed by studying the effects of silicate starvation on lipid production in marine and brackish water diatoms. Lack of silicate prevents cell division in diatoms, and under appropriate conditions silicate-starved diatoms manufacture and store large quantities of lipids. The question to be answered was whether this phenomenon could be utilized conveniently in a mass culture system. It was expected that these problems would be addressed through work on the following four tasks.

I. Evaluating the growth response of a least three thermophilic marine microalgae in water types representative of saline waters found in the southwestern United States.

II. Optimization of the yields of three species in outdoor flumes, at least two of the species to be selected from microalgae collected from the southwestern region of the U.S., and the third species to be a marine thermophilic strain that had been shown to grow in southwestern U.S. water types.

III. Evaluation of the management strategies that might improve the culture efficiency (lower costs) or increase yields in the outdoor flume systems.

IV. Optimization of the lipid yield from one strain of oil producing microalgae in the outdoor flume (optional).

1.2 THEORETICAL BACKGROUND

It has been known for a number of years that the efficiency of light utilization by phytoplankton can be markedly increased by exposing the cells to alternating periods of light and dark (1-7). More recently, a number of investigators have explored this phenomenon both experimentally and theoretically (8-15). Although early work (2-4) indicated that flash periods as short as 10 us were needed to take maximum advantage of the flashing light effect, it is now clear that much longer flash periods may still result in significant enhancements of photosynthetic efficiencies (14-16). Marra (13) for example has reported increases up to 87% in photosynthesis by simply modulating the irradiance on algal cells on a time scale ranging from minutes to hours. The most directly relevant and recent work has been that of Terry and Hock (17), who showed experimentally that significant enhancements of photosynthetic efficiency could be achieved with modulation periods on the order of seconds. It seems reasonable to postulate that the physiological mechanisms responsible for these observed photosynthetic enhancements differ substantially over the vast range of time scales involved. However, regardless of the physiological mechanisms involved, it is clear that photosynthetic efficiencies may be enhanced by a factor of two or more by modulating or flashing the incident light, it is apparent that the maximum duration of the light pulse which produces the highest photosynthetic efficiency will be negatively correlated with light intensity at light intensities greater than or equal to I_K, the light intensity above which photosynthetic efficiency begins to decrease under constant illumination (18), and that the duration of the dark period must lie somewhere in the approximate range 0.1-10s (19).

A critical question in algal mass culture work is whether a physical mechanism exists for taking advantage of the flashing light effect in a practical way. As noted by Phillips and Myers (14), "Any attempt to grow algae in sunlight will experience some gain by turbulence. The feasibility of increasing the turbulence will depend upon the extent of the gain in growth as compared to the increased power requirements of stirring or pumping the suspension." Unfortunately, it does not appear that merely producing random turbulence is sufficient to take advantage of the flashing light effect to any significant degree in a mass culture system. Powell, Chaddock, and Dixon (15) reached this conclusion based on theoretical calculations, and Miller et al. (20) commented that, "Utilization of the flashing light effect for improvement of photosynthetic efficiency of dense algal cultures requires a nonrandom mixing pattern - one in which cells are exposed to regular sequences of light and darkness . . ."

In the past, two types of experimental systems have been studied as possible means of utilizing the flashing light effect. A so-called Couette device was used by Miller et al. (20), Davis et al. (21), and Howell, Fredrickson, and Tsuchiya (22). The Couette device consists of two concentric, circular cylinders, the inner of which can be rotated at a selected speed. The system is illuminated either from the outside or inside, and the culture is grown in the gap between the cylinders. The flow pattern in the culture medium is complex, but sufficiently nonrandom that a systematic flashing light effect is achieved. Howell, Fredrickson, and Tsuchiya (22) estimated the period of the light-dark cycles in their system to be about 40 msec. More recently, Oswald et al. (23) and Shelef, Sabanas, and Oswald (24) have described a chemostat-type system called an algatron, which consists of a drum that is rotated about its vertical axis. The algal culture is contained within the drum as a film a few centimeters thick on the inside wall of the drum, and is retained against the wall by centrifugal force. A light source is mounted inside the drum, and a row of steel strips extending to within 0.5-1.0 mm of the inside wall produces highly turbulent wakes, which extend in spirals around the culture. The photosynthetic efficiencies which can be achieved with the Couette device and algatron are on the order of 10% (18).

It seems unlikely that either the Couette device or the algatron will prove to be a practical means of utilizing the flashing light effect. Both devices are mechanically complex, and the power requirements for operating them may well offset any increase in production, both from a financial and an energetic standpoint. As noted by Fredrickson and Tsuchiya (18), "Clearly some device that is mechanically more simple than the foregoing devices is required." The mechanism we have devised to produce systematic vertical mixing in our flume is illustrated in Figures 1-1 and 1-2. The device consists of a series of foils similar in design to airplane wings placed across the flume. As water flows over and under these foils, a pressure difference is created as illustrated in Figure 1-1. At the tips of the foil, the flow of water from the high pressure region below the foil to the low pressure region above the foil creates a vortex off each tip of the foil. If the foils are properly spaced along a suitable supporting structure (Figure 1-2), the vortices on adjacent foils rotate in opposite directions and thus reinforce each other. The width of each foil and the gap between foils are equal to the depth of the culture, so that circular vortices created by the foils effectively mix the culture from top to bottom (Figure 1-2). The system of vortices with rotational axes parallel to the direction of flow produces the sort of systematic mixing necessary to produce the flashing light effect (33).

Recent mass culture results obtained by Laws et al. (25) with T. suecica indicated that photosynthetic efficiencies (based on visible light) as high as 7% could be achieved in an outdoor shallow flume system, and that the efficiencies could be pushed into the 8-11% range if the cells were grown on a three-day dilution cycle. The physiological mechanism responsible for this dilution interval effect is a matter of speculation, and will be a subject of investigation in future research. Relevant to the research results reported here is the report of Enright (26) concerning the effect of silicate starvation on lipid accumulation in diatoms. She reported a 232% higher lipid content in silicate depleted cultures of the diatom Chaetoceros gracilis versus controls, but gave no indication of the increase (if any) in actual

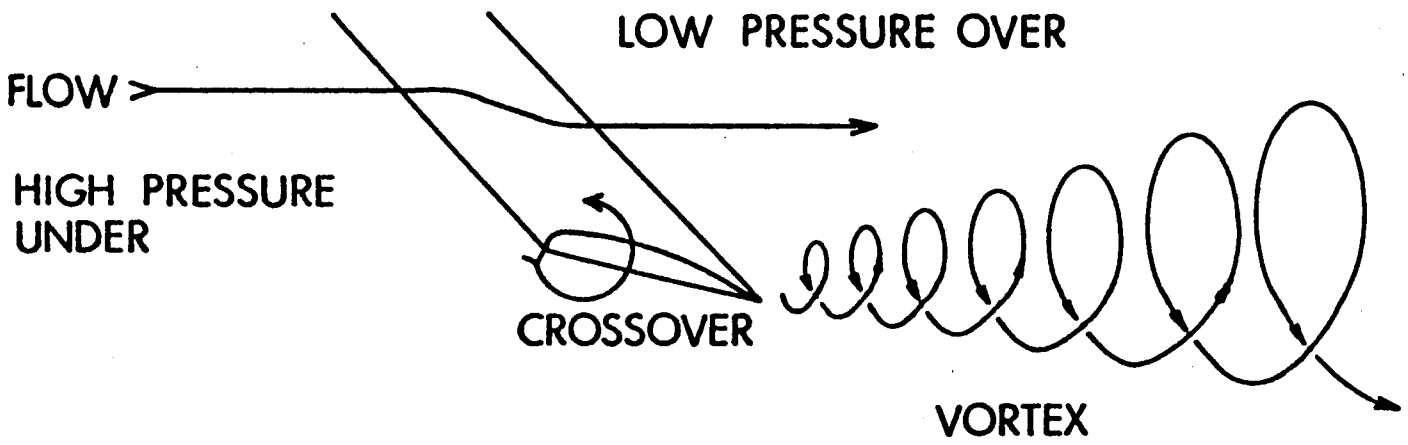


Figure 1-1. Design of a single foil indicating mechanism of vortex production.

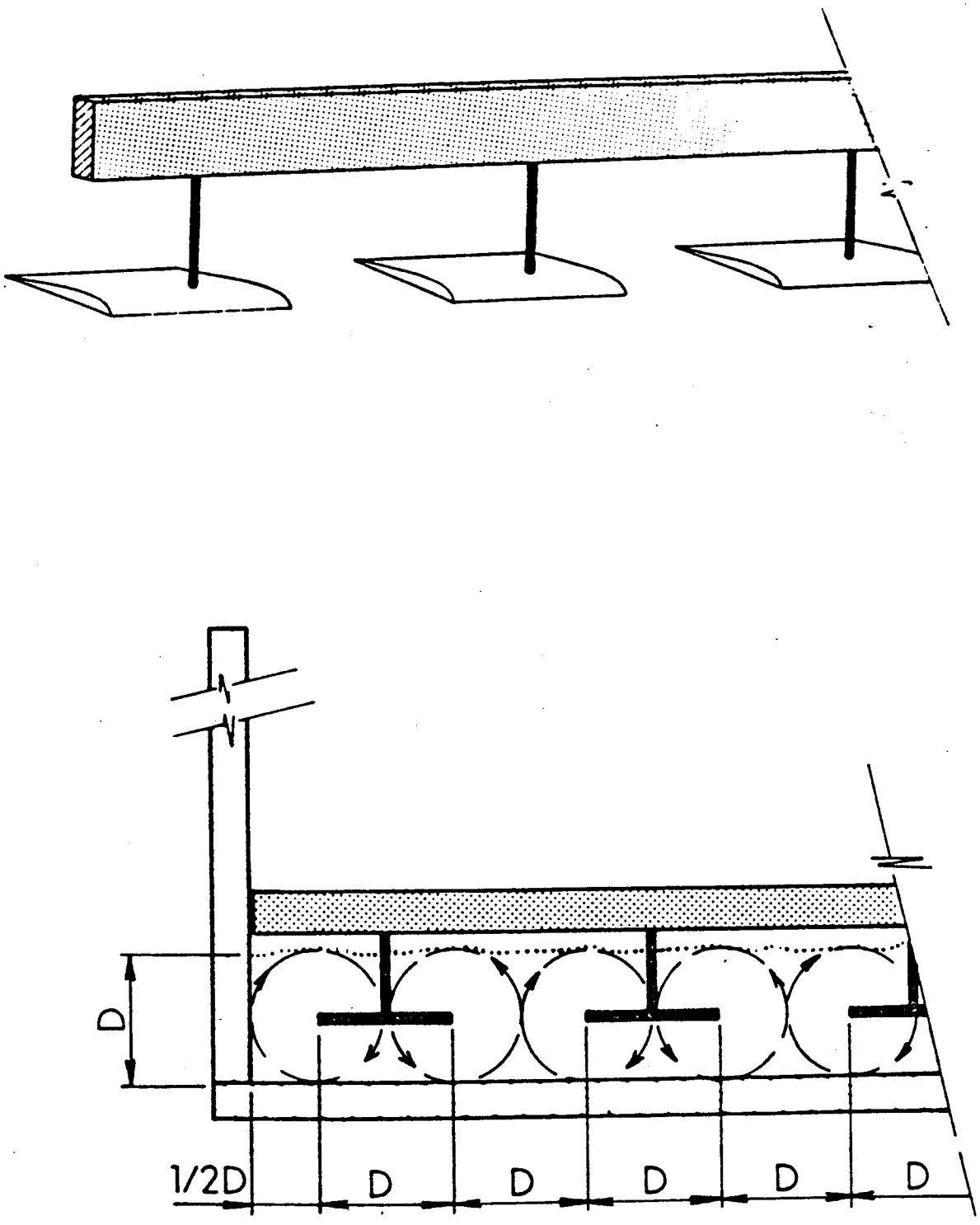


Figure 1-2. Positioning of individual foils in foil array. Lower figure indicates positioning of array in the flume. D is the depth of the water. Arrows indicate rotational direction of vortices.

lipid production. The physiological explanation for this effect is presumably related to a blockage of the cell division process. A fundamental question addressed in this year's research was whether a diatom could be found which would give high biomass yields in an outdoor culture system and at the same time produce a high percentage of lipids as the medium became silicate depleted.

2.1 THE CULTURE SYSTEM

The flumes utilized in our studies consisted of one 48m² continuous flume and four 9.2m² continuous flumes. The 48m² flume is illustrated in Figure 2-1, and specifications for the 48m² flume appear in Table 2-1. The flume is of a modular construction. The base channel module is a 1.2 x 2.4m molded fiberglass trough with 10 cm sides. The sides are raised an additional 23 cm with a plywood extension coated with acrylic resin (Figure 2-2). All seams are covered with fiberglass cloth and resin. The channel modules are reinforced with split 7.5 cm diameter polyvinyl chloride (PVC) pipe, and are mounted on 5 x 10 cm wooden rails supported by concrete blocks.

In addition to the channel modules, there are two end-box modules and a turning box. One end box serves as a drain box, the other as a lift box, and they are connected by three recirculation pipes with airlifts. The drain box (Figure 2-2) includes a small 15-cm deep sump to provide for even flow. The airlifts discharge directly onto the level surface of the flume in the lift box (Figure 2-2). The recirculation pipes (Figure 2-3) are 15 cm outside diameter PVC pipe turned in a U-shaped using 45° PVC elbows to reduce flow resistance over that which would be introduced by 90° elbows. Each of the two inner recirculation pipes has a small drain port at the bottom of the U; there is a 15 cm valve and drain line located in the outermost recirculation pipe which allows the culture to be transfered to the culture storage tank (Figure 2-4). The airlifts are fed by 2.4 cm PVC pipes which discharge 1.4 m below the water surface. Oil-free air is suppled by a Fuller Co. 7.5 horsepower model 4 MV-F air blower. CO_2 gas additions are made utilizing an aquarium airstone at a depth of 1 m in the downward-flowing portion of the recirculation pipes.

A seawater system (Figure 2-4) provides capabilities for adding seawater to the flume, obtaining seawater for laboratory use, partially or completely draining the flume, and storing the culture or seawater. Seawater is drawn from a 45-m-deep well. The seawater is filtered through coral rubble, and is therefore very low in particulate organic matter. Levels of major inorganic nutrients are typically 50 uM ammonium and 0.8 uM phosphate. The inlet to the seawater pump is from the saltwater well or from the storage tanks. The outlet is to the flume, to the utility outlet, or to waste. The flume can be drained into the culture storage tank through the 15-cm PVC pipe using a manual value, and pumped from that tank to any of the outlet ports, or discarded by gravity through the waste port. Wastewater is discharged into an underground sump excavated in the coral rubble.

The flume incorporates a computer-based system that monitors and controls the physical and chemical environment of the culture. The system controller is a

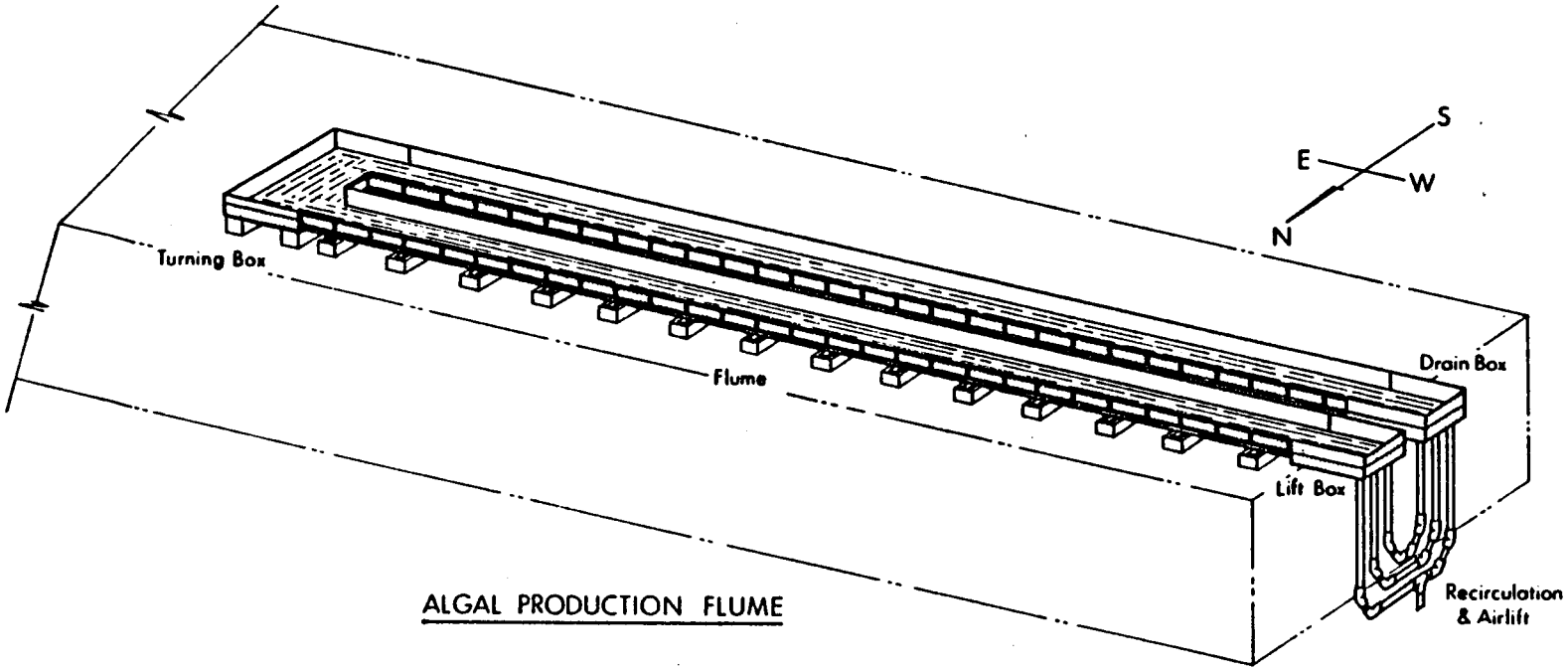


Figure 2-1. Perspective of algal production flume showing location of turning box, lift box, drain box, and airlift system.

Table 2-1.

Flume Specifications	
Construction	Modular: 1 liftbox module 1 turning box/heat exchanger 1 drainbox 14 channel modules 3 recirculation pipes/airlifts
Centerline channel length	37.2 m
Area	48.39 m^2
Depth range	5-28 cm
Operating depth	7.75 cm (standard deviation 0.74 cm, 91 measurements), with upper level electrode set at 7.6 cm.
Volume at operating depth	4150 L
Flow rate	30 cm/s (circuit time 120 s)
Slope	0.16 cm/m
Module Specifications	
Liftbox	
Construction	2-cm plywood painted with fiberglas resin
Area	1.49 m^2
Volume at operating depth	115 L
Turning box	
Construction	2-cm plywood painted with fiberglas resin
Area	3.73 m^2
Volume at operating depth	290 L
Drainbox	
Construction	2-cm plywood painted with epoxy resin
Area	1.49 m^2
Volume at operating depth	220 L
Channel modules	
Construction	Spray-molded fiberglas PVC reinforcements 2-cm plywood painted with fiberglas resin
Area	2.98 m^2 each module, 41.67 m^2 total
Volume at operating depth	230 L each module, 3225 L total
Recirculation	
Construction	15 cm i.d. PVC pipe PVC fittings epoxy resin silicone glue (three separate pipes, nested, with separate airlifts)
Volume	300 L
Airlift pumping rate	690 liter min^{-1} each lift, 2070 liter min^{-1} total
Airlift life height	163 cm (air outlet to water surface at operating depth)
Airlift air consumption rate	550 liters min^{-1}

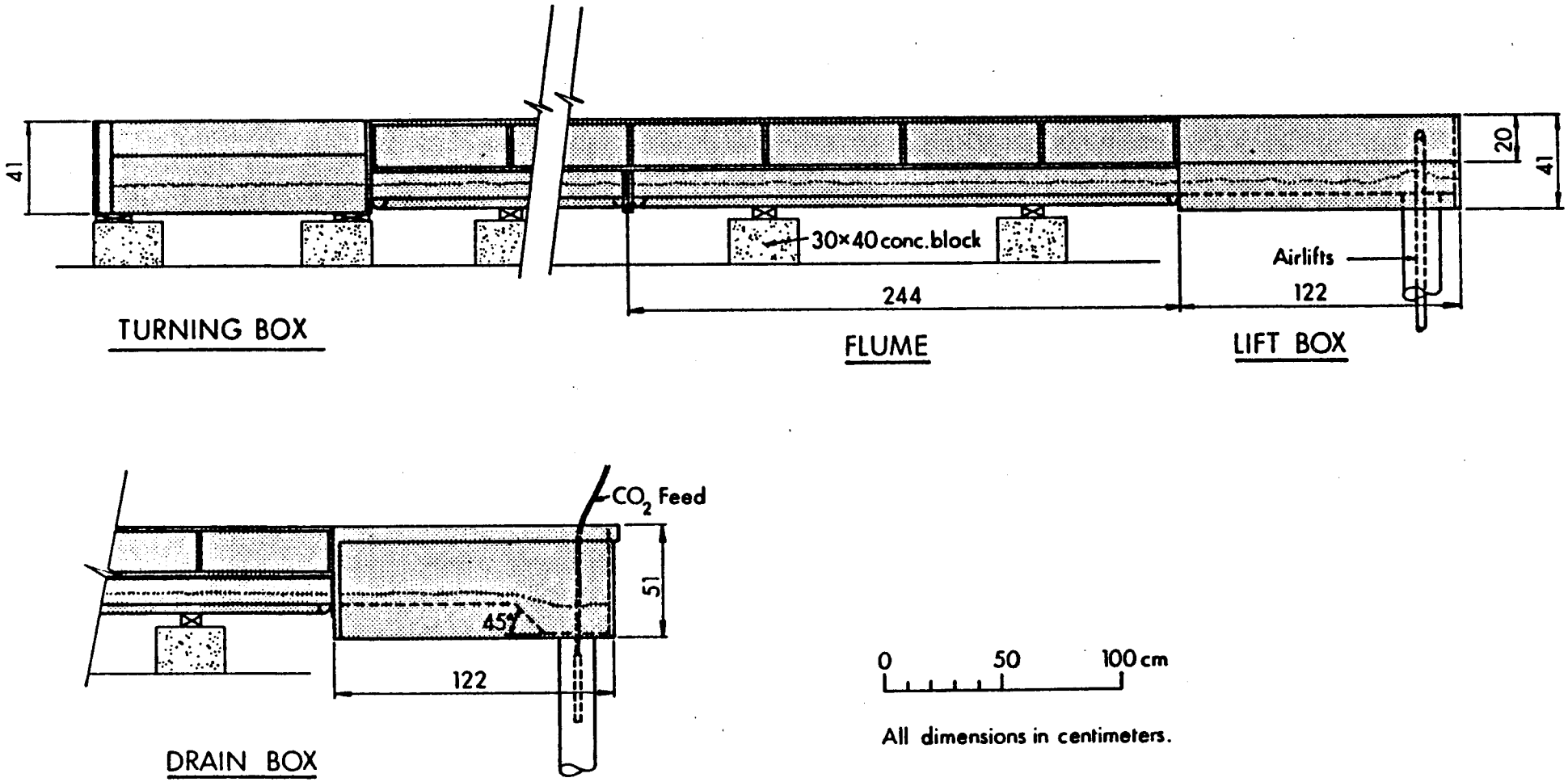
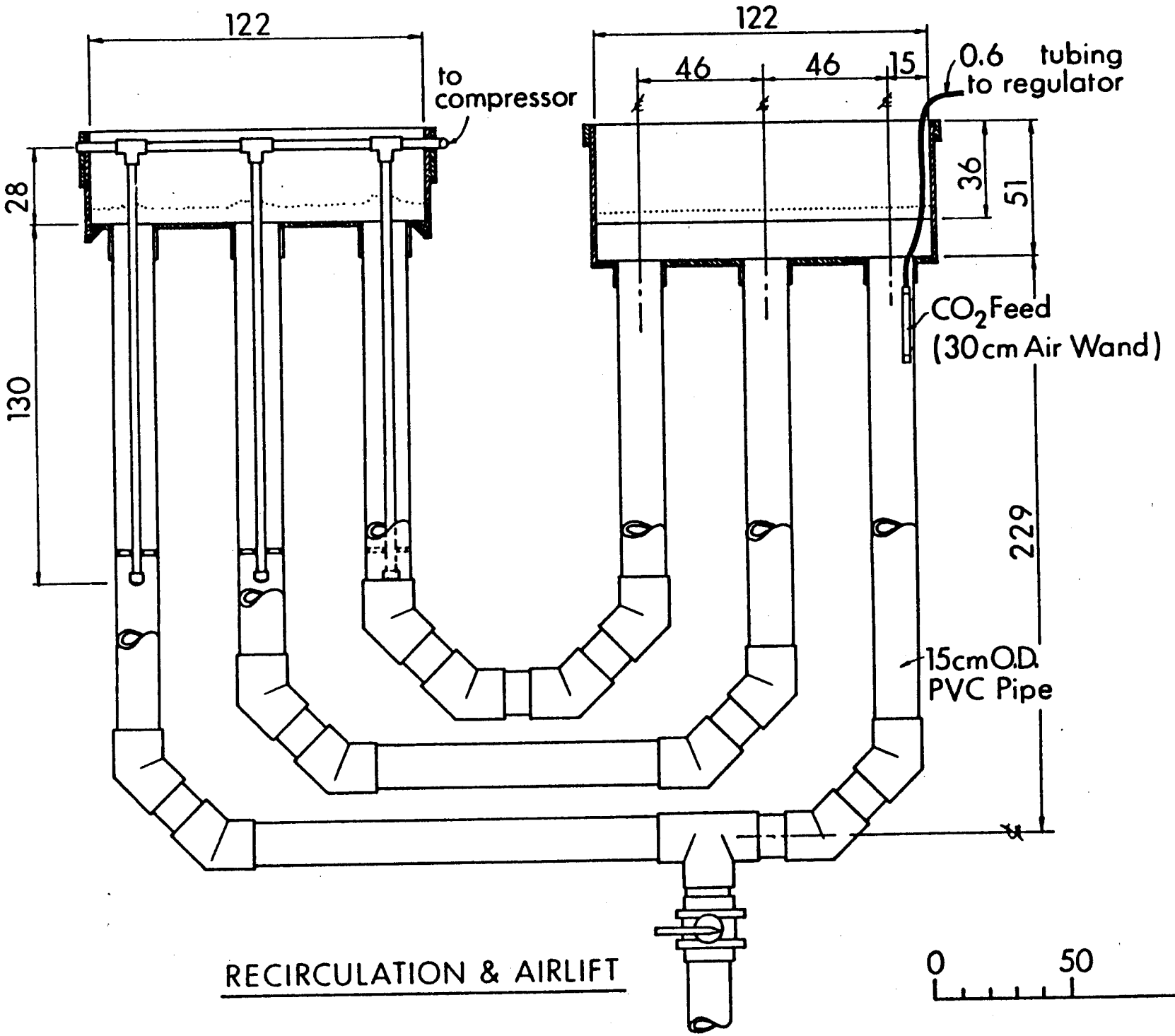


Figure 2-2. Cross section of flume modules indicating positioning of airlift and CO_2 feed.


122
122
to
compressor
46
46
15
0.6 tubing
to regulator
28
130
36
51
CO_2 Feed
(30 cm Air Wand)
229
15cm O.D.
PVC Pipe
RECIRCULATION & AIRLIFT
0
50
100 cm
All dimensions in centimeters.

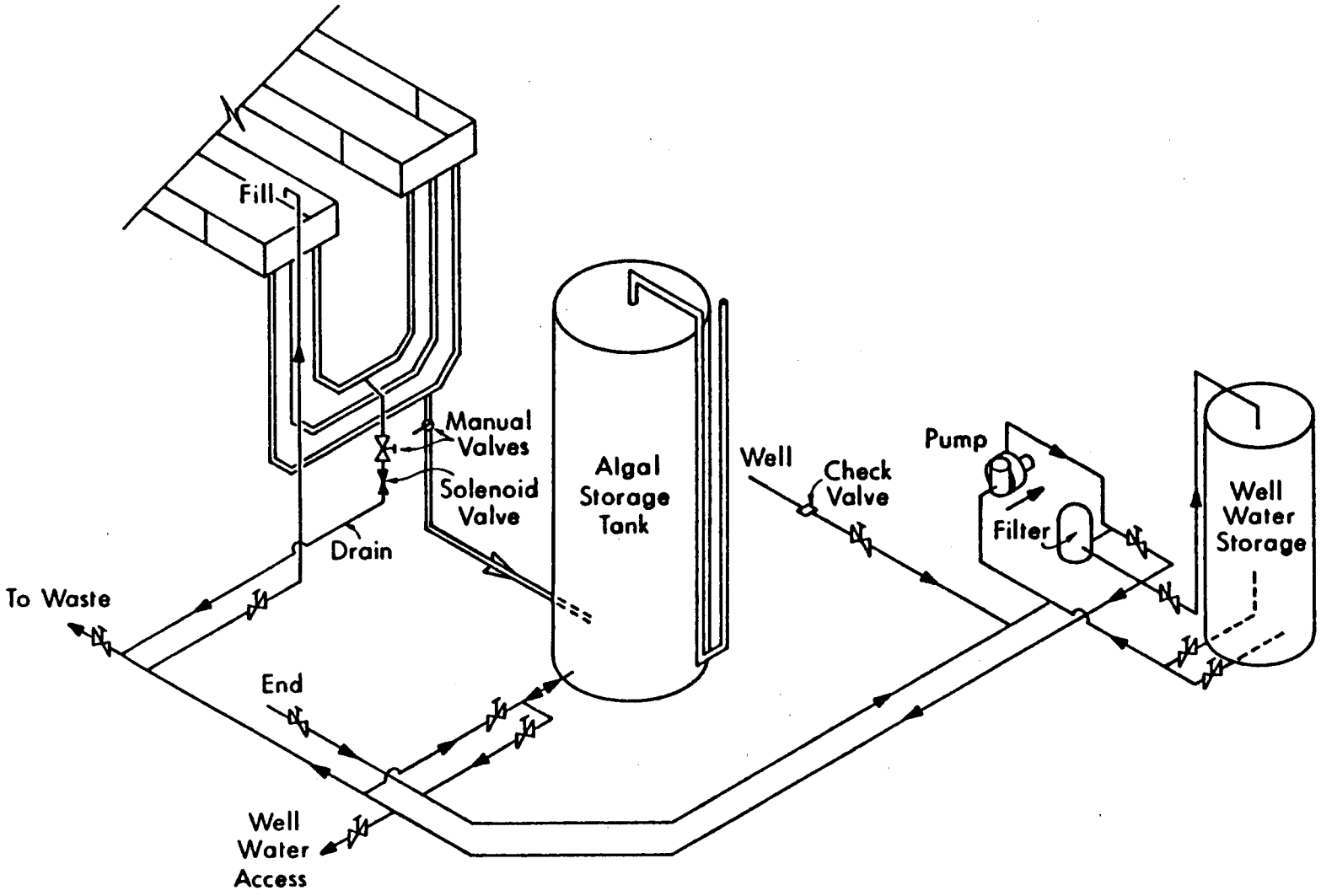


Figure 2-4. Schematic of seawater supply system and algal storage tank.

Hewlett-Packard model 9845B computer, which acquires data from the sensors and manipulates the control devices based on the information received. The seawater pump is controlled on the basis of the operator input and the output of the level detector. Carbon dioxide gas (100% pure) addition is controlled on the basis of pH. Draining is controlled on a time-of-day basis.

Laboratory culture experiments were performed using IMR medium (27) sterile-filtered through 0.22 um Millipore membrane filters. Borosilicate glass culture tubes (25mm x 150mm) were placed in a temperature controlled (± 0.1°C) water bath in front of a bank of daylight fluorescent bulbs which supplied light at an irradiance of 450 uEinst $m^{-2}s^{-1}$. Light was provided on a 12:12 L:D illumination cycle. The biomass of the cultures in each tube was monitored through measurements of in vivo fluorescence on a Turner model 10 fluorometer. Fluorescence measurements were made at the same time each day to avoid artifacts associated with light:dark periodicity. All experiments were run in duplicate.

The four 9.2m^2 flumes are similar in design to the 48m^2 flume, but consist of two channels 7.6m long by 0.6m wide by 0.15 deep. The channels are connected by 20 cm recirculation pipes; one of the two recirculation pipes of each culture contains an airlift pump which drives culture recirculation. Each 9.2m^2 flume is equipped with a CO_2 supply line. pH sensors in the cultures supply data to the Hewlett-Packard 9845B computer, which in turn controls the CO_2 supply solenoids.

2.2 ANALYTICAL DATA

Production in the flume was calculated from daily changes in particulate carbon (PC) concentrations corrected for dilution due to drainage and introduction of fresh medium. Changes in carbon concentration were converted to areal carbon production rates by multiplying the change in carbon concentration by the average depth of the culture system. The average depth of the culture system is somewhat greater than the depth of water in the two arms of the flume due to the presence of the deep U-tubes at one or both ends of the arms (Figure 2-1). Mathematically the average depth of the culture system is equal to the volume of water in the system divided by the surface area. In the small flumes the average depth of the water is 11.7 cm when the depth of water in the two arms is 10.0 cm, and in the large flume the average depth is 10.8 cm when the depth of water in the arms is 10.0 cm. Samples for PC analyses were collected on glass-fiber (GF/C) filters and analyzed on a Hewlett-Packard model 185B CHN analyzer following procedures recommended by Sharp (28). These carbon production numbers were converted to ash-free dry weight production by multiplying by the experimentally determined ratio of ash-free dry weight:PC. The proximate composition of the algae was obtained by partitioning the ash-free dry weight (AFDW) between lipid, protein and carbohydrate. The lipid content was estimated using a modified Bligh-Dyer extraction. The protein content was estimated to be 6.25 times the nitrogen content (29). Carbohydrate content was estimated by difference, i.e., carbohydrate = AFDW - lipid - protein.

Solar irradiance was measured with a Lambda model LI-190B quantum sensor, which has a nearly flat response to light in the wave-length range 400-700 nm (i.e., photosynthetically active radiation or PAR). Visible quantum fluxes were converted to energy fluxes using the spectral distribution of sunlight (30). The conversion ratio was 21.4 uEinst per calorie. The caloric content of the microalgae was calculated from the proximate analysis and energy contents of 9.3 kcal g^{-1} for lipid, 4.2 kcal g^{-1} for carbohydrate, and 5.7 kcal g^{-1} for protein (31). Solar energy conversion efficiencies were calculated based on this caloric content and the incident energy flux of visible light.

SECTION 3.0

RESULTS

3.1 TASK I: Evaluate the growth response of at least three thermophilic marine microalgae in water types representative of saline waters found in the southwestern United States.

Purpose o To determine if sites in the southwest can be used to culture marine species of microalgae.

The chemical compositions of water types representative of southwestern U.S. water were provided by SERI. Two basic water types, identified as Type I and Type II water, were defined. The chemical composition of these water types as indicated in the subcontract are listed in Table 3-1. Type I water is relatively high in Ca^{2+} and SO_4^{2-} and relatively low in Na^+, Cl^-, and HCO_3^- compared to type II water. Seawater, which has a very high concentration of NaCl, is considered a limiting case of type II water.

Unfortunately Type I low ionic strength water as defined in Table 3-1 proved impossible to prepare, because a precipitate always developed when the chemical reagents were added. It was suspected that natural waters with a composition similar to Type I low ionic strength water may have been supersaturated with CO_2 due to the breakdown of naturally occurring organics. In any case the directions for preparing Type I and Type II water were eventually revised, and the revised composition information is listed in Table 3-2.

A total of seven species, listed in Table 3-3, were studied to determine their growth potential in southwestern U.S. water. Of the seven species, four were marine species (C. gracilis, Cyclotella sp., Hantzchia sp., and T. suecica) and three were collected from saline waters in the southwestern U.S. (Amphora sp., Boekelovia sp., and Chlorella sp.). Growth rates were estimated from log-linear plots of fluorescence versus time in duplicate culture tubes. In most cases at least 5-6 data points were included from the linear portion of these plots. Correlation coefficients were in all cases significant at $P = 0.05$. The coefficient of variation of the duplicate growth rate estimates averaged 7.3 ± 5.4%.

Table 3-1. Ionic composition of the two major classes of saline groundwater identified by factor analysis of saline groundwater data from areas of New Mexico suitable for algal biomass production systems.

Ion Species	Type I Low	Type I Moderate	Type I High	Type II Low	Type II Moderate	(Seawater)
	IONIC COMPOSITION (ppm)					
Na	709	3552	7692	1929	4646	10762
K	46	278	405	205	194	399
Ca	1034	678	633	256	89	411
Mg	438	303	250	171	392	1293
HCO_3	499	867	1605	1622	2100	142
SO_4	4284	2973	4735	2025	805	2709
Cl	990	6349	9680	1782	6775	19353
TDS	8000	15000	25000	8000	15000	35069

Table 3-2. Revised definitions of southwestern U.S. water types. All concentrations are mg/l.

Type I

Salt	Conductivity (umho) 10,000	25,000	40,000	55,000	70,000
$CaCl_2$	------	3,932	5,618	7,610	8,430
$MgCl_2 \cdot 6H_2O$	4,114	11,844	22,789	35,305	42,230
Na_2SO_4	------	2,925	3,310	3,705	3,620
KCL	194	407	662	960	1,186
$NaHCO_3$	184	168	168	168	168
NaCl	2,118	3,845	14,132	13,023	16,039
$CaSO_4$	1,686	------	------	------	------

Type II

Salt	Conductivity (umho) 10,000	25,000	40,000	55,000	70,000
$CaCl_2$	28	28	28	28	28
$MgCl_2 \cdot 6H_2O$	1,953	3,026	3,920	4,362	4,230
Na_2SO_4	2,671	5,870	15,720	23,305	28,360
KCL	466	965	2,028	3,044	3,673
$NaHCO_3$	1,208	2,315	2,855	3,234	3,245
Na_2CO_3	231	876	1,234	1,492	1,527
NaCl	1,511	8,078	12,963	20,588	26,075

Table 3-3. Species studied on laboratory scale for growth potential in southwestern U.S. water.

Species	Source:	Comments
Amphora sp.	W. Barclay, SERI	Hot springs diatom from Utah/Colorado
Boekelovia sp.	W. Barclay, SERI	
Chaetaceros gracilis	R. York, Hawaii	
Chlorella sp.	W. Thomas, Scripps	
Cyclotella sp.	M. Tadros, Alabama A&M	Isolated from estuary
Hantzschia sp.	M. Tadros, Alabama A&M	Isolated from estuary
Tetraselmis suecica	Hawaii	Contaminant in *P. tricornutum* culture

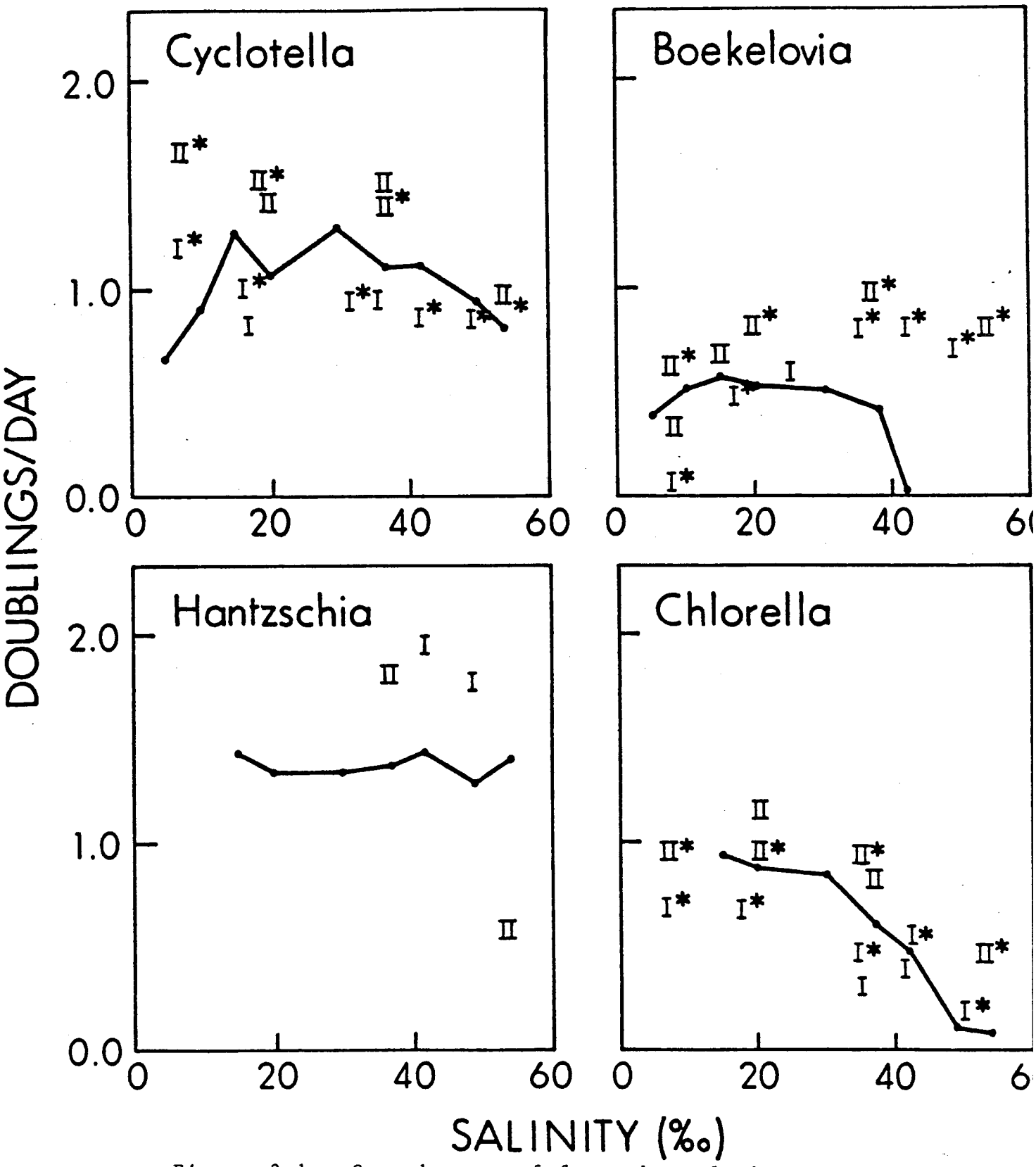


Figure 3-1. Growth rate of four phytoplankton species as a function of salinity at 28°C. Solid lines connect data collected using seawater medium. Roman numerals indicate data collected in type I and type II water as defined in

A major concern which motivated the work in both Tasks I and II was the fact that the mass culture facility in Hawaii has in the past routinely used a seawater base medium for growing the microalgae, and that the species studied have often been of marine origin. SERI has considered that the southwestern U.S. is a likely site for the mass culture of microalgae for energy production. If mass culture facilities were established in the southwest, the water types would be saline groundwater from that area. A question then naturally arises as to whether marine microalgae can grow as well in southwestern U.S. water types as in seawater, and/or whether microalgae isolated from the southwest grow as well in seawater as in typical southwestern U.S. water types. Tasks I and II were aimed in part at answering these questions.

Task I was envisioned as a crude screening procedure to avoid wasting time in Task II. Obviously there was no point in trying to grow a particular species in a certain water type outdoors if the species would not grow or would grow very poorly in that water type. Therefore Task I was designed to screen species for their ability to grow in various water types. Neither the number of personnel nor the facilities available for Task I permitted attempting to stimulate conditions in the outdoor flumes (e.g., high cell concentration, pH control, natural sunlight) in the laboratory. In large part because of this fact, areal production, which is the criterion for comparing results outdoors, was not used in evaluating the indoor culture results. Instead, the performance of the cultures was based on the growth rate of the cells during log-phase growth in batch culture. Because the cell concentrations were low (10^3 - 10^4 cells/ml), the photosynthetic activities of the cells had no significant effect on the chemical composition of the medium. A species was considered to grow satisfactorily in a particular medium in the laboratory if the log phase growth rate was at least one to two doublings per day. If such growth rates could be achieved, the species was judged suitable for further testing in the outdoor flumes. The purpose of Task I was not to determine the temperature, water type, and salinity at which growth rate was a maximum, because maximum areal production is not achieved at maximum growth rate (37).

The most intensive studies were performed at 28°C, a temperature typical of operating conditions in our flumes during the summer months. The growth rate results, expressed as doublings per day during log phase growth, are shown in Figures 3-1 and 3-2. Cyclotella, Chlorella, and Bockelovia all seemed to prefer Type II water to Type I water, while C. gracilis, T. suecica, Amphora, and Hantzschia seemed to prefer Type I to Type II. Except in the case of Amphora, each species grew at least as well in either Type I or Type II water as in seawater. There was a rather well defined negative correlation between salinity and growth rate for Chlorella, and both Cyclotella and Boekelovia exhibited fairly well defined salinity optima regions.

Additional growth rate studies were carried out at two or three additional temperatures with T. suecica, Amphora, C. gracilis, and Boekelovia in Type I and Type II water, and the results are shown in Figures 3-3 to 3-6. A general trend observed in these studies was that the species tended to grow most rapidly in low salinity Type I water at lower temperatures and most rapidly at higher salinity or in Type II water at the higher temperatures. In the case of T. suecica for example (Figure 3-3), there is an obvious positive correlation between growth rate and temperature for Type II water with a salinity

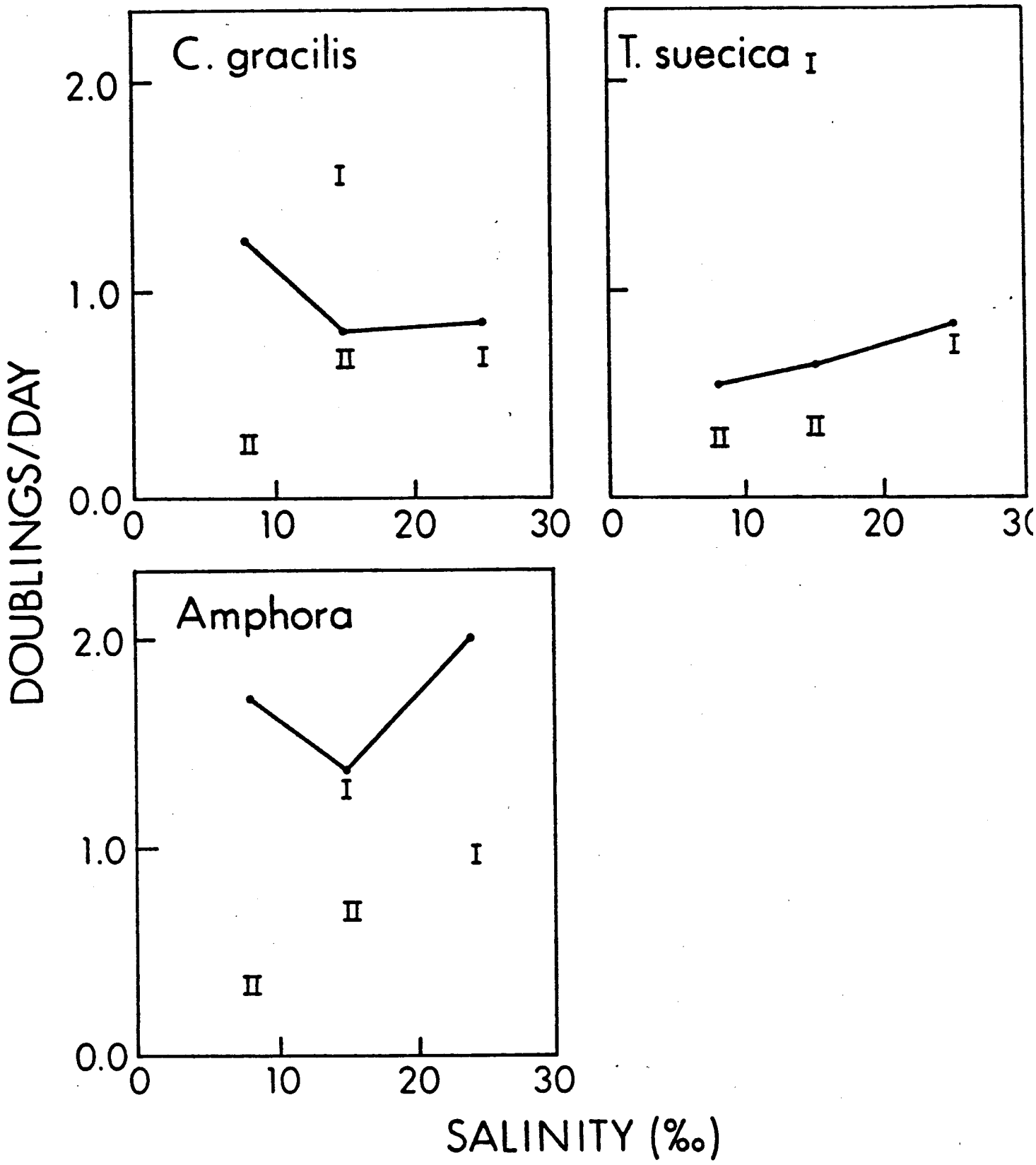


Figure 3-2. Growth rates as in Fig. 3-1.

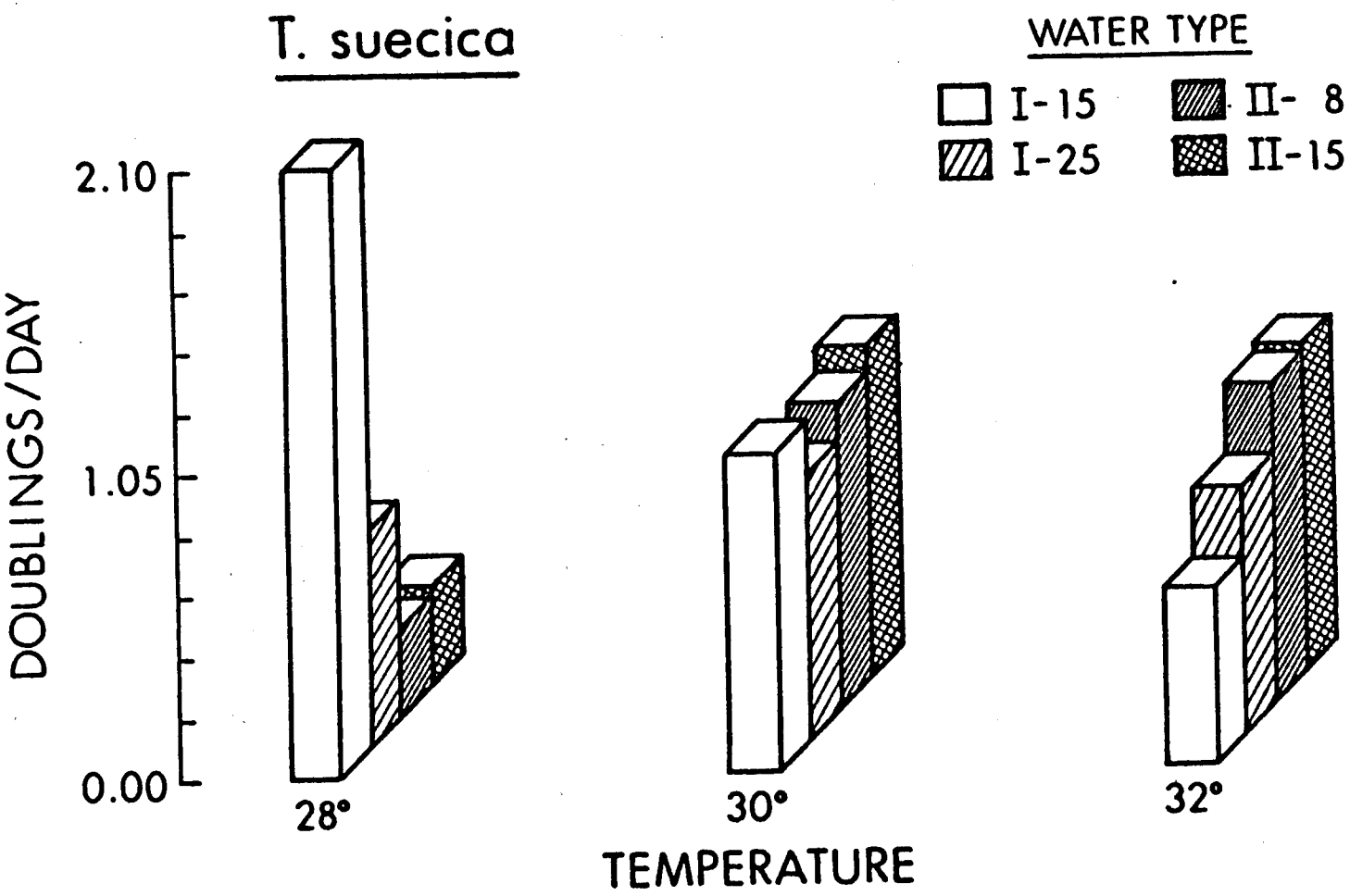


Figure 3-3. Growth rate of T. suecica as a function of temperature and water type in laboratory-scale experiments.

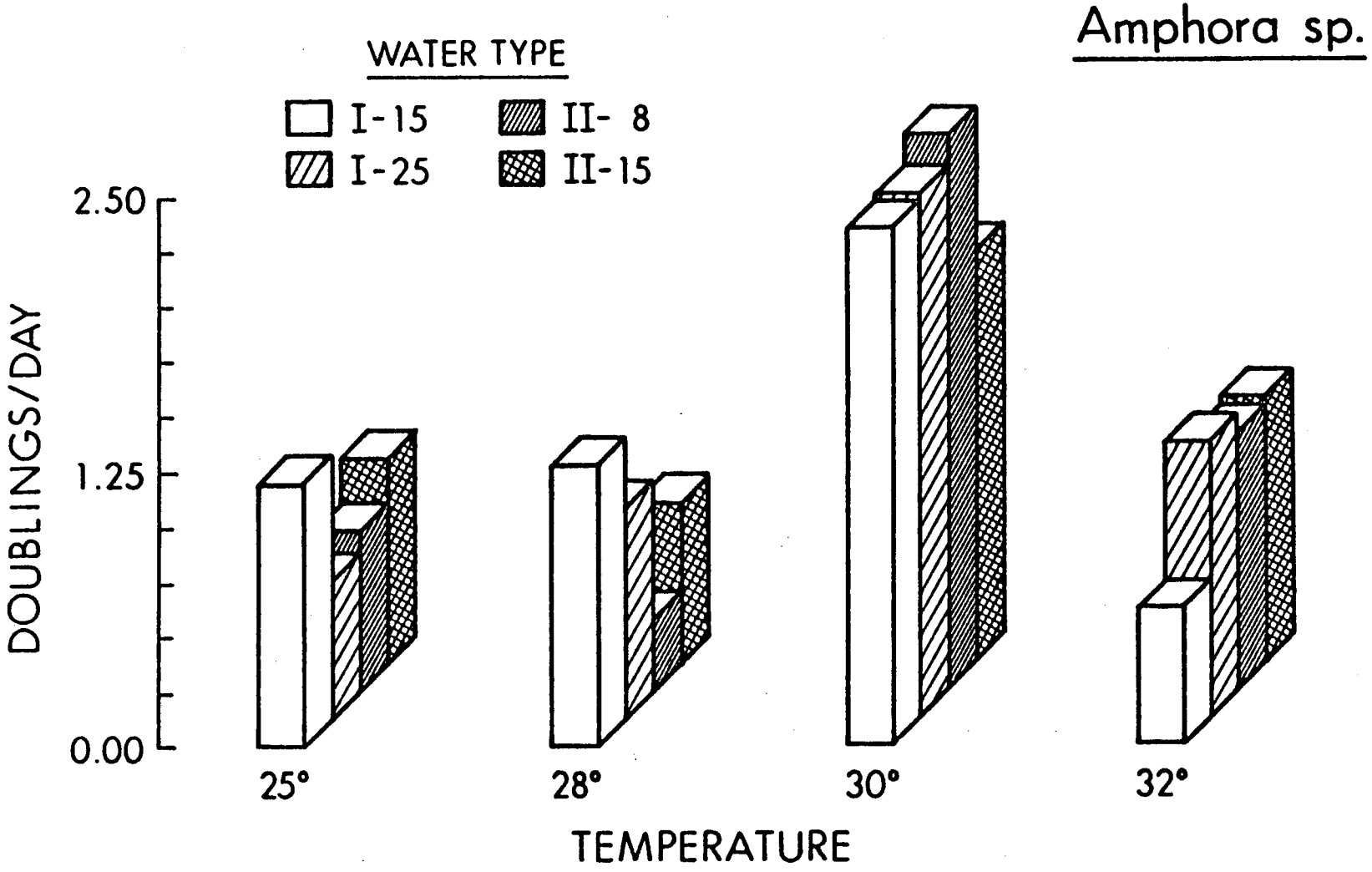


Figure 3-4. Growth rate of Amphora sp. as a function of temperature and water type in laboratory-scale experiments.

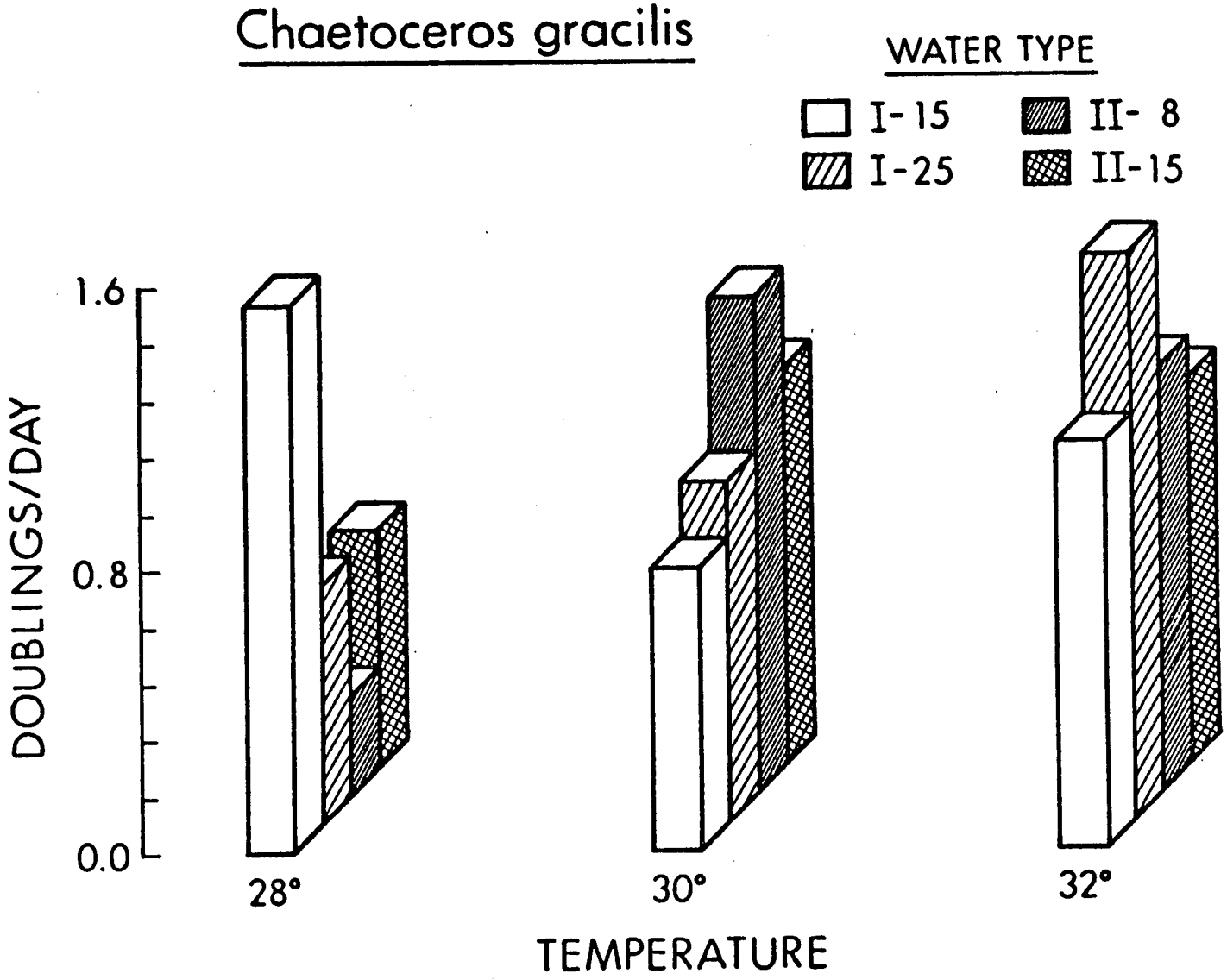


Figure 3-5. Growth rate of C. gracilis as a function of temperature and water type in laboratory-scale experiments.

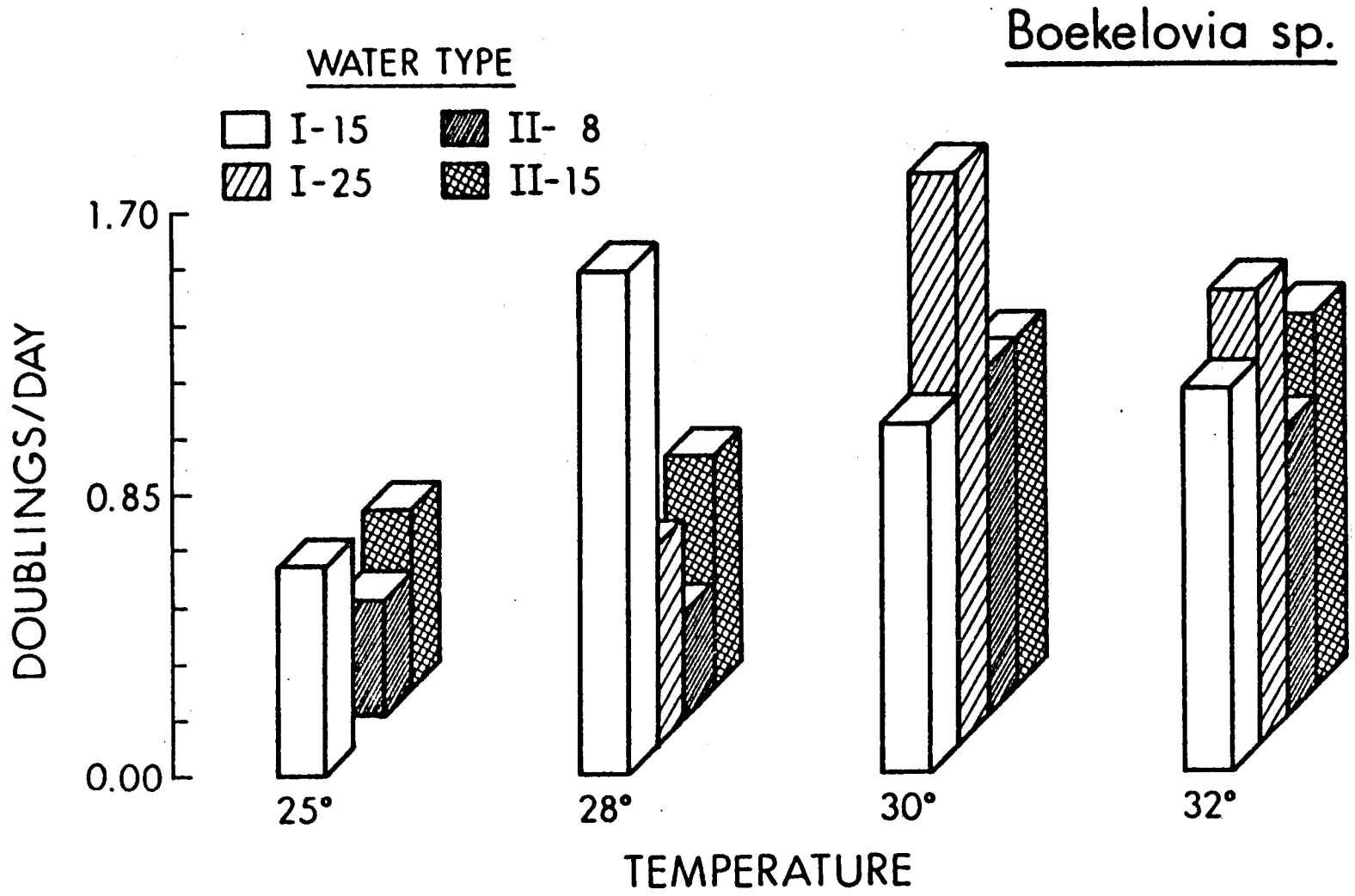


Figure 3-6. Growth rate of Boekelovia sp. as a function of temperature and water type in laboratory-scale experiments.

of 15 °/oo and a negative correlation for Type I water at 15 °/oo. Similarly, Amphora, C. gracilis, and Boekelovia grew most rapidly in Type I, 15°/oo water at the one or two lowest temperatures studied, but most rapidly in Type II water or Type I 25 °/oo water at the two highest temperatures studied. The physiological cause of this trend is unclear.

The general conclusion that can be drawn from this study is that both the marine and brackish water species studied were all capable of growing in Type I water, Type II water, and seawater. The preferred water type and salinity varied between species and as a function of temperature. With the exception of Chlorella, all the species showed a capability of consistently growing at over one doubling per day in one or more southwestern U.S. water types.

3.2 TASK II. Optimize yields of three species in the outdoor flumes, at least two of the species selected from microalgae that have been collected from the southwestern region of the U.S. The third species shall be a marine thermophilic strain that has been shown to grow in southwestern water types.

Purpose o To define optional culture conditions of oil yielding microalgae species that would be suitable for culture in the southwest and determine potential outdoor yields of these species.

A total of four species were studied in the outdoor flumes. The species intended for study included T. suecica, Cyclotella, C. gracilis, and Boekelovia. T. suecica was chosen because of the excellent production it had given in our previous outdoor flume work (25). Cyclotella and C. gracilis were chosen because our laboratory studies showed that these species would store large quantities of lipids if silicate starved (see Task IV). Boekelovia was chosen because work at SERI had shown that this species would store large amounts of lipids if nitrogen stressed (32). The results of these studies more-or-less ran the gamet of what can happen when one tries to mass culture a particular organism in an outdoor system. Both T. suecica and Cyclotella produced dense monocultures and impressive yields under favorable management strategy. C. gracilis grew well over short periods, but predation by a protozoan made maintenance of the culture over long periods of time difficult, and to a certain extent it was difficult to isolate treatment effects from predation effects. The Boekelovia culture was immediately taken over by an unidentified green flagellate. Laboratory work and correspondence with SERI revealed that the flagellate contaminant was present in the original culture from SERI. The laboratory studies revealed that when Boekelovia and the flagellate are present in the same culture, the winner in a competition experiment depends very much on the growth conditions (e.g., bath versus continuous culture). In the outdoor flumes the flagellate always overwhelmed Boekelovia. We elected to go ahead and study production by the flagellate in the flumes, since the organism clearly came from the same southwestern U.S. water sample as did Boekelovia, and was obviously a good competitor in an outdoor mass culture.

3.3 MASS CULTURE RESULTS WITH T. SUECICA

Our previously reported extensive studies with T. suecica (25) had clearly defined the optimum growth conditions for this species in our outdoor flumes, and the purpose of our studies under Task II was to compare production results in seawater, Type I and Type II water. These studies were performed at a time when in effect only two salinities of Type I and Type II water were defined (Table 3-1), and the results are summarized in Figure 3-7 and Table 3-4. The data were analyzed using an analysis of variance (ANOVA) to determine whether there were any significant differences between treatments. The results showed a significant effect of salinity ($p = 0.002$), with results at $25^{0}/oo$ significantly different then results at $15^{0}/oo$ or $8^{0}/oo$. Results at $8^{0}/oo$ and $15^{0}/oo$ were not significantly different from each other, based on a Duncan's Multiple Range test (p greater than 0.05). The ANOVA revealed no significant treatment effect from water type ($p = 0.11$), although Duncan's Multiple Range test showed type I water to be significantly different (better) than seawater or type II water ($p = 0.05$). The best production was achieved in Type I water at a salinity of $25\ ^{0}/oo$. It is clear from an examination of Figure 3-7 that the limited salinity range of Type I and Type II water was unfortunate, because there is clearly a positive correlation between photosynthetic efficiency and salinity for each water type and for all water types taken together over the range of salinities studied. Therefore there is reason to suspect that even higher photosynthetic efficiencies (PE's) might have been achieved at higher salinities.

3.4 MASS CULTURE RESULTS WITH THE FLAGELLATE

Although we were initially optimistic about obtaining high production rates with the flagellate because of its ability to outcompete Boekelovia, production results were disappointing, and it did not seem worthwhile to embark on an extensive factorial study. The work performed was similar to that reported for T. suecica in that a two way factorial study was used to examine the effects of salinity and water type on photosynthetic efficiency. The study was restricted due to the fact that no formula for type II water at $25\ ^{0}/oo$ was provided by SERI, and because at the time of the study, SERI had provided no satisfactory formula for type I water at $8\ ^{0}/oo$. The results of the study are shown in Figure 3-8 and Table 3-5. The results were analyzed using an ANOVA exactly as in the case of the T. suecica data. The ANOVA revealed no significant difference between any of the treatments. The range of PE's over all treatments was 4.6 - 5.8%. The best PE of 5.8% achieved with the flagellate (Figure 3-8) is far below the PE of 10.8% achieved with T. suecica (Figure 3-7), and the difference is significant at $p = 0.05$ by a standard t-test. Given these results, we felt it was not worthwhile to perform additional studies with the flagellate, but instead elected to pursue studies with other species which we hoped would achieve photosynthetic efficiencies comparable to those achieved with T. suecica and at the same time have the capacity to store large quantities of lipids.

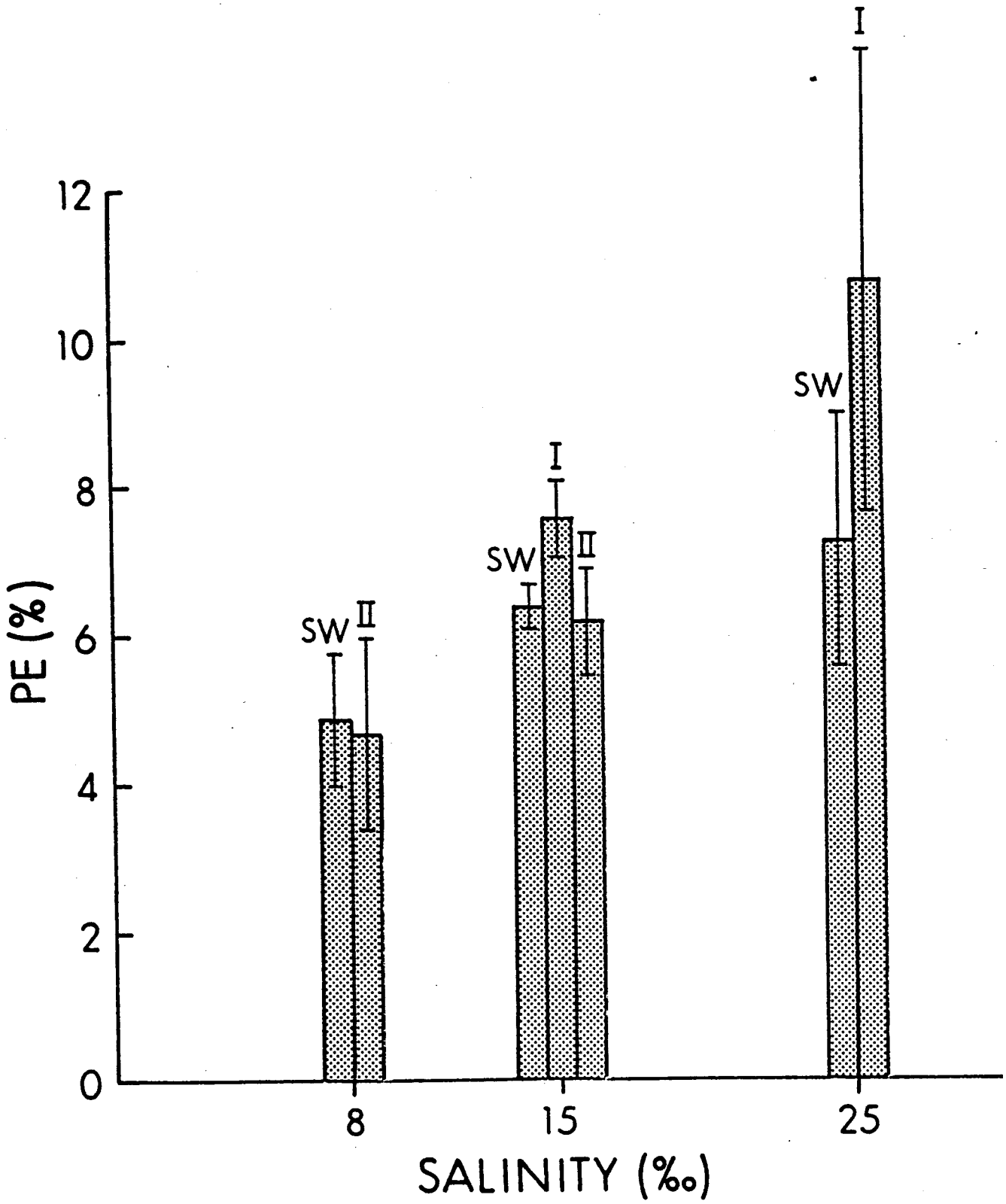


Figure 3-7. Photosynthetic efficiency of T. suecica culture grown in outdoor flumes as a function of salinity and water type. SW = seawater. Error bars are standard

Table 3-4. Best production results achieved with T. suecica in the outdoor flume systems and culture characteristics. Data collected from 11/3/84 to 11/16/84. Error bars are 95% CI.

average culture depth	10.7 cm
pH	7.4-7.6
dilution	culture diluted every 3 days to $2x10^6$ cells per ml.
cell concentration range	$2x10^6$ - $15x10^6$ per ml.
O_2 range	8-12 ppm
mixing speed	0.3 m/s
salinity	$25^o/oo$
water type	I
temperature range	22.9 - 31.1°C
biomass concentration	0.17 - 1.05 g AFDW/1
productivity	31.5 ± 19 g AFDW/m²/d
PE	10.8 ± 6.7
C/N of cells	5.79
AFDW/C	1.9
lipid/AFDW	0.175
protein/AFDW	0.560
carbohydrate/AFDW	0.265
lipid productivity	5.5 g/m²/d
irradiance	34.7 ± 4.3 E/m²/d

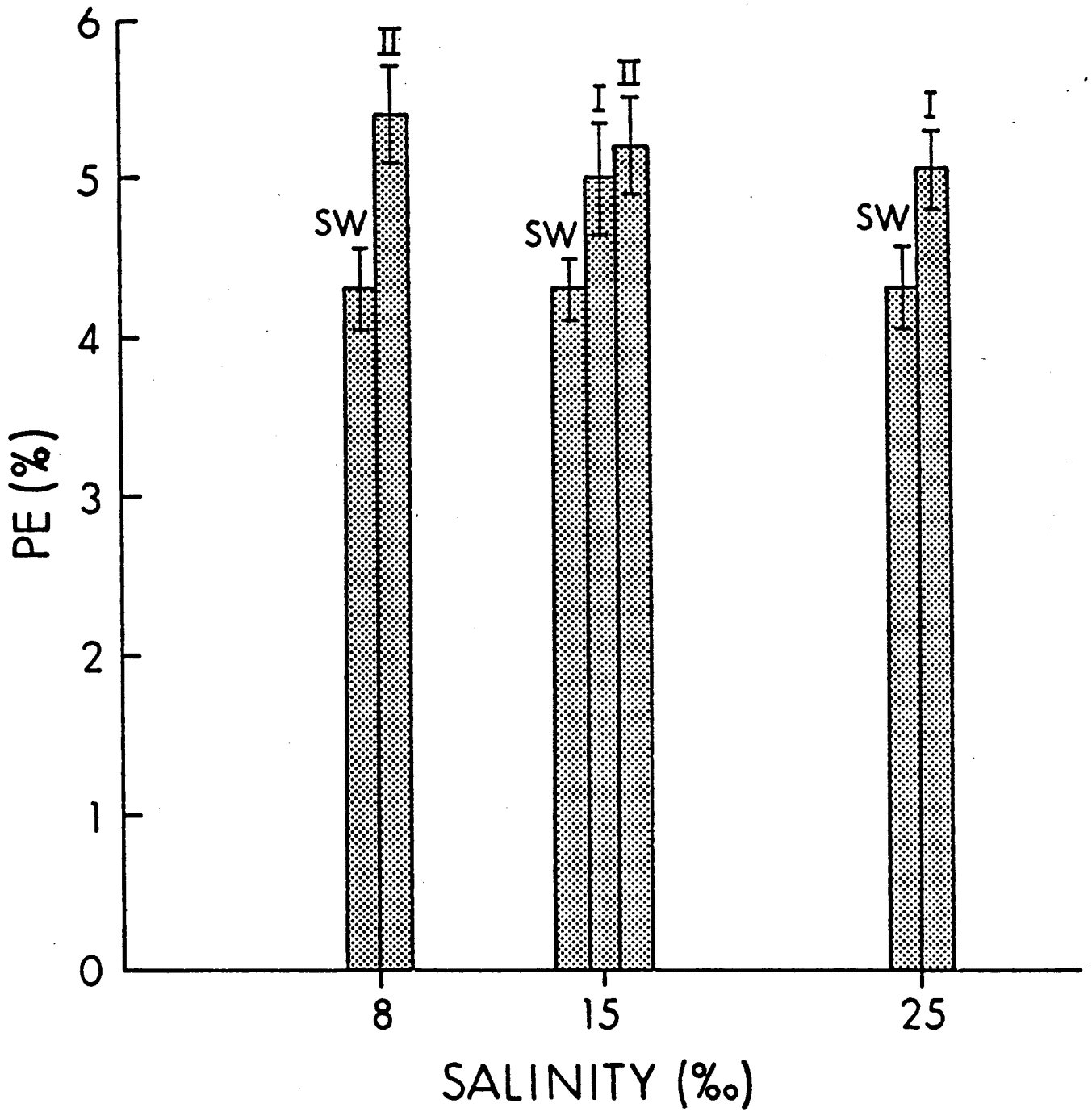


Figure 3-8. Photosynthetic efficiency of the flagellate grown in outdoor flumes as a function of salinity and

Table 3-5. Best production results achieved with the flagellate in the outdoor flume systems and culture characteristics during 3/12/85 - 3/23/85. Error bars are 95% CI.

average culture depth	12.9 cm
pH	7.4-7.6
dilution	culture diluted by 75% every 3 days
cell concentration range	3.2×10^6 - 12.6×10^6 per ml
O_2 range	8-11 ppm
mixing speed	0.3 m/s
salinity	$8^o/oo$
water type	II
temperature range	19.5 - 29.6°C
biomass concentration range	0.17 - 0.58 g AFDW/I
productivity	17.8 ± 2.0 g AFDW/m^2/d
PE	5.8 ± 1.4%
C/N of cells	4.97
AFDW/C	1.8
lipid/AFDW	0.250
protein/AFDW	0.699
carbohydrate/AFDW	0.051
lipid productivity	4.5 g/m^2/dm
irradiance	45.4 ± 5.1 E/m^2/d

3.5 MASS CULTURE RESULTS WITH C. GRACILIS

Two experiments at a dilution interval of four days and two at a dilution interval of one day were run and are indicated by square data points in Figure 3-9. An ANOVA on these four data points revealed treatment effects significant at $p = 0.0009$, and it therefore seemed worthwhile to proceed with the factorial. A first order least square fit to the four data points produced an equation which predicted lines of constant PE as indicated by the solid lines in Figure 3-9. The line of steepest ascent is perpendicular to these solid lines, and is indicated by the dashed line in Figure 3-9. Two lines parallel to the line of steepest ascent, indicated by the dash-dot lines were also studied. Since it was impractical to run dilution cycles for a non-integral number of days, experimental runs actually followed horizontal lines, but were chosen so as to be close to the line of steepest ascent and the two dash-dot lines and within the region of interest. A second-order least squares fit to all the experimental data points yielded a predicted maximum PE of 10.2% at a dilution interval of 2.4 days and a percent dilution of 50%. Since as indicated a non-integral value for the dilution interval was impractical, separate calculations were made for integral values of the dilution interval. The best predicted PE at an integral dilution interval was 10.1% at a percent dilution of 51% and dilution interval of two days. Note that these predicted PE's are actually slightly less than the best observed PE of 10.5% at a dilution interval of two days and percent dilution of 72% (Table 3-6).

Given these results, we ran an additional experiment with seawater, type I and type II water at a salinity of $15^{0}/oo$, dilution interval of two days, and dilution percentage of 72% to compare the three water types. The experiments were run over a period of 10 days in January, 1985, with the following results:

Water Type	PE (± 95% Confidence)
Seawater	7.2 ± 1.0
Type I	6.8 ± 1.8
Type II	8.3 ± 3.4

An ANOVA showed no significant difference between these results. The lower PE's achieved during this last experiment may be due to the fact that temperatures were suboptimal; the original factorial (Figure 3-9) was run in July and August.

Outdoor culture studies on C. gracilis were terminated at this time because of protozoan predation problems. The most effective method for dealing with this problem was dilution of the culture to about 10^{5} cells/ml. At this cell concentration the density of C. gracilis may have been too low to permit effective grazing by the protozoa. In any case a considerable amount of time was lost due to predation, and a number of experiments had to be prematurely terminated. It is clear from our results that impressive PE's can be achieved with this species over time periods of 10-14 days. If a satisfactory means of predator control could be found, this species appears to have great potential as a mass culture organism. However, because of the predator problem, we

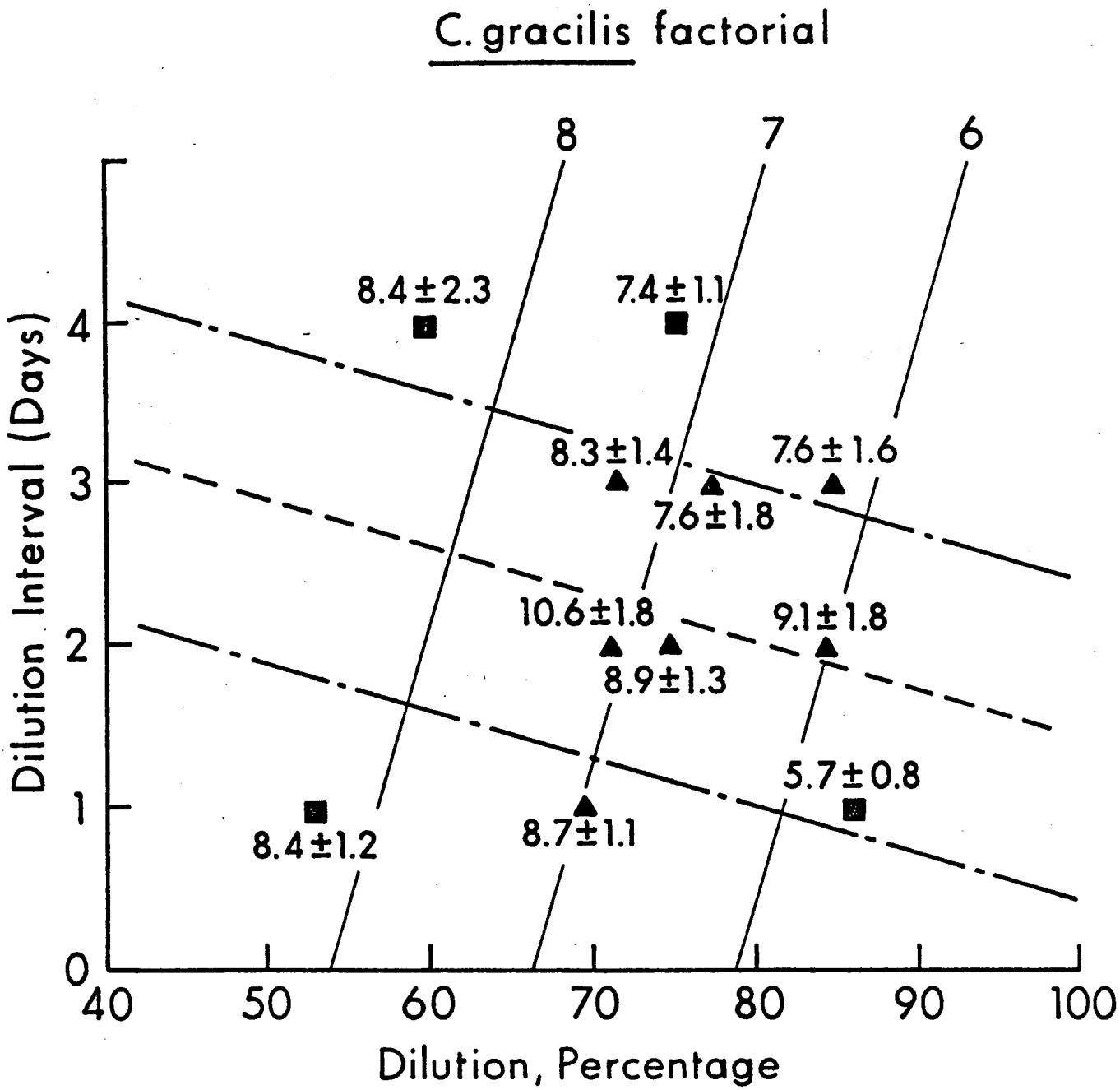


Figure 3-9. Photosynthetic efficiencies obtained in C. gracilis factorial study as a function of dilution percentage and interval between dilutions. Errors bars are 95% confidence intervals.

Table 3-6. Best production results achieved with *C. gracilis* in the outdoor flume systems and culture characteristics. Data collected from 7/29/84 to 8/9/84.

cultural depth	11.8 cm
pH	7.4-7.6
dilution	culture diluted by 75% every two days
cell concentration range	4.7×10^6 - 17.1×10^6 per ml
O_2	8 - 12 ppm
mixing speed	0.3 m/s
salinity	$15^o/oo$
water type	seawater
temperature range	20.6 - 28.8°C
biomass concentration range	0.28 - 1.02 g AFDW/1
productivity	43.1 ± 8.0 g AFDW/m^2/d
PE	10.6 ± 1.7%
C/N of cells	5.13
AFDW/C	1.9
lipid/AFDW	0.245
protein/AFDW	0.641
carbohydrate/AFDW	0.114
lipid productivity	10.6 g/m^2/d
irradiance	51.5 ± 3.5 E/m^2/d

elected to terminate experiments with C. gracilis in January, 1985, and move on to work with Cyclotella.

3.6 MASS CULTURE RESULTS WITH CYCLOTELLA

An attempt was made to carry out a thorough factorial optimization study with Cyclotella, but because the experiments did not get underway until early April, it was impossible to complete the optimization study before the termination of the contract on June 15. Table 3.7 indicates the parameters which we elected to vary and the experimental values assigned to those parameters during the analyses. A complete first-order factorial involving all of these variables would have required $(2)^4(3)^2 = 144$ separate experiments. Our earlier experience with T. suecica however had clearly indicated that a 10 cm depth and pH of 7.5 were superior to a 5 cm depth and pH of 8.5. We therefore set up two small two-way factorials to examine the effects of pH and depth variation on photosynthetic efficiency for Cyclotella. The cells were grown in the $9.2m^2$ flumes in a seawater medium at a pH of 7 $^{o}/oo$ and dilution interval of two days. Tables 3.8 and 3.9 summarize the experimental results. A depth of 10 cm was preferable to a depth of 5 cm at $p = 0.0001$, and a pH of 7.5 was preferable to a pH of 8.5 at $p = 0.0001$. There was no significant effect of dilution percentage on photosynthetic efficiency. Given these statistically highly significant results, we did not consider it worthwhile to include a depth of 5 cm or pH of 8.5 in the experimental design for the rest of the study. The depth and pH were therefore fixed at 10 cm and 7.5, respectively.

With depth and pH thus fixed, a complete first-order factorial on the remaining parameters in Table 3.7 would have required a total of $(2)^2(3)^2 = 36$ experiments. The various combinations of dilution percentage, interval between dilutions, salinity, and water type are indicated in Table 3.10. It was impossible to complete this factorial within the period of the contract, and in order to avoid as much as possible block interactions, the sequence of experimental runs was chosen using a random number generator. Results from the nine experiments which were completed are indicated in Table 3.10. These data were collected over a time period from 4/2/85 to 5/28/85. During the last month of the contract, our efforts were concentrated on inducing lipid storage through silicate starvation of the same species.

Since we were unable to complete even a first-order factorial with Cyclotella, an analysis similar to that employed with C. gracilis was impossible. However, the PE results were subjected to an ANOVA, and the results are indicated in Table 3.11. While treatment effects were highly significant ($p = 0.019$), no one or two treatments could be singled out as being clearly superior to all others. However, in looking over the Duncan's Multiple Range analysis, we noted that the three lowest PE's, which were significantly lower than the two highest PE's, all three involved a dilution interval of three days. In fact the six highest PE's were all achieved on a two-day dilution interval cycle. Thus for this species at least a two-day dilution interval seems superior to a three-day dilution interval. Salinity did not seem to be critical; the highest PE was achieved at $7^{o}/oo$ and the second highest PE at $35^{o}/oo$. Similarly, there appeared to be no convincing reason to prefer seawater over

Table 3-7. Parameter and assigned values during optimization studies with Cyclotella.

Parameter	Values
culture depth	5 cm, 10 cm
pH	7.5, 8.5
dilution percentage	75%, 87%
interval between dilution	2 days, 3 days
salinity	7^{o}/oo, 17^{o}/oo, 35^{o}/oo
water type	seawater, type I, type II

Table 3-8. Two-way analysis of variance on experiments to determine effect of culture depth and dilution percentage on Cyclotella photosynthetic efficiency. All experiments run in seawater medium, s = 7°/oo, pH = 7.5, at a dilution interval of two days. Results are reported as photosynthetic efficiency and AFDW production. Error bars are 95% CI.

		Depth	
		5 cm	10 cm
Dilution Percentage	75%	10.3 ± 3.9 g/m²/d 2.7 ± 0.9%	29.5 ± 5.1 g/m²/d 7.3 ± 1.2%
	87%	8.0 ± 3.3 g/m²/d 2.3 ± 0.8%	24.9 ± 4.3 g/m²/d 6.6 ± 1.2%
ANOVA results:	depth - p = 0.0001 dilution % - p = 0.28		

Table 3-9. Two-way analysis of variance on experiments to determine effect of culture pH and dilution percentage on Cyclotella photosynthetic efficiency. All experiments run in seawater medium, s = 7°/oo, at a dilution interval of two days and depth of 10 cm. Results are reported as photosynthetic efficiency and AFDW production. Error bars are 95% CI.

		pH	
		7.5	8.5
Dilution Percentage	75%	29.5 ± 5.2 g/m²/d 7.3 ± 1.2%	16.3 ± 4.3 g/m²/d 4.3 ± 1.2%
	85%	24.9 ± 4.3 g/m²/d 6.6 ± 1.2%	12.6 ± 5.2 g/m²/d 3.6 ± 2.3%
ANOVA results:	ph - p = 0.0001 dilution % - p = 0.31		

Table 3-10. Possible combinations of salinity, time interval between dilutions (Δt), water type, and % dilution in a complete first-order factorial of these parameters. Experimental results indicated where experiments were run. Error bars are 95% CI.

S (°/oo)			Δt Days		Water Type			% Dilution		g AFDW/m²/d	PE
7	17	35	2	3	sw	I	II	75	87		
+			+		+			+		29.5 ± 5.2	7.3 ± 1.2
+			+		+				+	24.9 ± 4.3	6.6 ± 1.2
+			+			+		+			
+			+			+			+		
+			+				+	+		25.8 ± 9.3	6.5 ± 2.2
+			+				+		+		
+				+	+			+			
+				+	+				+		
+				+		+		+			
+				+		+			+		
+				+			+	+			
+				+			+		+		
	+		+		+			+		19.0 ± 5.6	5.3 ± 1.6
	+		+		+				+		
	+		+			+		+		17.1 ± 4.3	5.0 ± 0.9
	+		+			+			+		
	+		+				+	+			
	+		+				+		+		
	+			+	+			+		15.5 ± 3.3	4.6 ± 0.9
	+			+	+				+		
	+			+		+		+			
	+			+		+			+		
	+			+			+	+		17.1 ± 6.6	4.7 ± 1.7
	+			+			+		+		
		+	+		+			+		23.3 ± 4.7	5.8 ± 0.9
		+	+		+				+		
		+	+			+		+			
		+	+			+			+		
		+	+				+	+		27.4 ± 5.6	6.9 ± 1.4
		+	+				+		+		
		+		+	+			+			
		+		+	+				+		
		+		+		+		+			
		+		+		+			+		
		+		+			+	+			
		+		+			+		+		

Table 3-11. ANOVA results from analysis of PE data in Table 3-10. Treatment effects were significant at p = 0.019. PE's with the same letter of the alphabet were not significantly different from each other based on Duncan's Multiple Range Test.

Photosynthetic Efficiency			
7.3 ± 1.2	A		
6.9 ± 1.4	A	B	
6.6 ± 1.2	A	B	C
6.5 ± 2.2	A	B	C
5.8 ± 0.9	A	B	C
5.3 ± 1.6	A	B	C
5.0 ± 0.9		B	C
4.7 ± 1.7			C
4.6 ± 0.9			C

type II water. Of the four highest PE's, two were achieved in seawater and two in type I water. There were simply too few data to judge the relative merits of diluting by $75^{o}/oo$ versus $87^{o}/oo$. Although the data in Tables 3.8 and 3.9 consistently show higher PE's at $75^{o}/oo$ dilution, the differences were not statistically significant.

Although we were unable to complete the optimization study with Cyclotella, a final run was made in one of the flumes following completion of the silicate starvation work. Although the duration of the run (6/12/85 - 6/22/85) extended by one week into the next contract period, we report the results here for the sake of completeness. The culture conditions were identical to those which gave the best PE in Table 3.11. The estimated dilution percentage, based on changes in particulate carbon, was 79± 5%. The results are summarized in Table 3.12. The PE of 8.6% was not significantly different (ANOVA $p = 0.05$) from the PE of 7.3% obtained under identical management strategy in late April (Table 3.10).

3.7 TASK III: Evaluate management strategies that will improve the efficiency of microalgae culture in the flume system.

Purpose o To examine and research areas that will improve the efficiency of microalgae culture in the flume system.

Research under this task centered on three possible mechanisms for improving on the efficiency of culture operations. These mechanisms included (1) reduction in the supply of air to the air lift system at night, (2) increasing the distance between foil arrays in the flume, and (3) cutting back on the supply of CO_2 to the culture. All the experiments were performed with T. suecica in the $48m^2$ flume, and the results are summarized in Tables 3-13 to 3-15. Based on an ANOVA, reducing the aeration rate at night (Table 3-13) had no significant effect on production results. While this result may seem surprising, there is certainly a possible logical explanation. Since photosynthesis occurs only during the photoperiod, there is no need from the standpoint of light utilization to run the airlift system at night. The major concern would be a reduction of O_2 due to the respiratory activities of the culture, but significant O_2 problems did not develop even when aeration was cut back by 90%. The potential advantage of reducing aeration at night is the reduction in physical agitation to the cells. Although T. suecica is obviously capable of withstanding this buffeting to a degree sufficient to permit impressive production, other species cannot. For example, Dunaliella will literally not grow in an airlift driven mass culture system (A. Ben-Amotz, pers. comm.). It is reasonable to assume that the physical buffeting caused by the airlift is a source of stress to T. suecica, and at night this stress may well outweigh any advantages associated with running the airlift at full power. Therefore reducing the aeration rate at night appears to be a real source of increased efficiency to the culture operation.

An ANOVA revealed no significant effect on PE's due to changing the distance between foil arrays. The results are summarized in Table 3-14. The trend in the data do however indicate a negative correlation between PE and the distance between foil arrays. The lack of statistical significance in the

Table 3-12. Best production results achieved with Cyclotella in the outdoor flume systems and culture characteristics during 6/12/85 - 6/22/85. Error bars are 95% CI.

culture depth	10 cm
pH	7.4 - 7.6
dilution	culture diluted by 79% every two days
cell concentration range	1.9×10^6 - 9.8×10^6 per ml
O_2 range	9 - 13 ppm
mixing speed	0.3 m/s
salinity	$7^o/oo$
water type	seawater
temperature range	22.6 - 33.1°C
biomass concentration range	0.16 - 0.76 g AFDW/l
productivity	35.4 ± 4.7 g AFDW/m²/d
PE	8.6 ± 1.1
C/N of cells	4.88
AFDW/C	2.06
lipid/AFDW	0.241
protein/AFDW	0.622
carbohydrate/AFDW	0.137
lipid productivity	8.5 g/m²/d
irradiance	56.0 ± 3.0 E/m²/d

TABLE 3-13. Effect of reducing aeration rate at night on production of *T. suecica* in the 48 m^2 flume. At full power the aeration rate is 27.5 cfm. Error bars are 95% CI.

Culture Condition	g AFDW/m^2/d	E(E/m^2/d)	PE
full air (3/26/85-4/10/85)	20.5 ± 3.6	41.2 ± 5.8	6.3 ± 0.6%
75% reduction at night (4/11/85-4/19/85)	26.4 ± 10.8	51.4 ± 6.2	6.2 ± 2.3%
90% reduction at night (4/25/85-5/14/85)	23.8 ± 4.4	53.9 ± 3.5	5.6 ± 1.0%
full air (5/15/85-5/25/85)	17.3 ± 4.2	54.8 ± 8.0	4.7 ± 1.3%

Table 3-14. Effect of varying the distance between foil arrays on production of T. suecica in 48m² flume. Production results are averages over periods of at least two weeks. Error bars are 95% CI.

Distance Between Foil Arrays	g AFDW/m²/d	E(E/m²/d)	PE
4 ft (12/9/84-12/24/84)	19.4 ± 4.6	32.6 ± 3.1	8.1 ± 2.5%
8 ft (12/25/84-1/13/85)	16.7 ± 3.5	29.2 ± 4.3	7.7 ± 1.6%
12 ft (2/14/85-2/25/85)	21.9 ± 4.1	45.3 ± 4.5	6.7 ± 2.0%
(11/16/84-12/8/85)	17.5 ± 3.6	31.1 ± 3.7	6.8 ± 2.2%

results in Table 3-14 may therefore simply reflect a signal-to-noise problem. More extensive data are being collected during the present contract to explore the foil effect further.

Changing the rate of CO_2 addition to the culture had a dramatic effect on production (Table 3-15).[2] An ANOVA indicated treatment effects to be significant at $p = 0.006$. Production was over two times higher in the system when the pH was held at 7.5 versus no CO_2 addition. However, most of this increased production was achieved by holding the pH at only 8.5. Controlling the pH at 8.5 required turning on the CO_2 for only about one hour during midday hours. As was the case with aeration, the cost effectiveness of allowing the pH to rise above 7.5 would depend on the cost of CO_2 versus the value of the product sacrificed by allowing the pH to increase above 7.5. Furthermore, the results obtained here with T. suecica are not necessarily applicable to other species of microalgae, because phytoplankton differ greatly in their ability to utilize CO_2 and bicarbonate in photosynthesis.

3.8. TASK IV. Optimize the lipid yield from one strain of oil producing microalgae in the outdoor flume.

Lipid accumulation studies were completed with three diatoms, C. gracilis, Hantzschia, and Cyclotella. Most of this work was conducted in the laboratory with cultures uniformly labeled with C-14. However, several experiments were conducted with Cyclotella in the outdoor flumes. In all cases, the mechanism used to induce lipid accumulation was silicate starvation.

Table 3-16 summarizes the results of a laboratory scale silicate starvation experiment with C. gracilis. The culture was grown in IMR medium (27) with five times the normal nitrogen and phosphorus concentrations. As can be seen from Table 3-16, the percentage of lipid carbon in the cells increased from about 14% during log-phase growth to over 50% during silicate starvation as the silicate concentration dropped from over 7.0 uM to less than 1.0 uM. The actual rate of lipid production increased dramatically during this time period. Therefore the increase in the percentage of lipid carbon did not merely represent a conversion of other cellular components into lipids as the cells became silicate starved. In a similar experiment with Hantzschia, the percentage of lipid carbon increased from 20-25% (four replicate cultures) to over 50% within one day as the silicate concentration dropped to about 0.7 uM, and to 65-70% within three days. In Cyclotella the log phase lipid carbon percentage of 22 ± 5% increased to about 50% in 1-2 days when the silicate concentration dropped to about 0.7 uM, and to 60-65% after 4-5 days. In both the Hantzschia and Cyclotella experiments the percentage lipid carbon dropped back to 25-30% within about two days when silicate was reintroduced into the medium.

Attempts to produce similar results in the outdoor flumes with Cyclotella were initially unsuccessful. When the culture was initially diluted with fresh medium containing no added silicate, the cells reduced the silicate concentration from about 500 uM to less than 5 uM in one day, but at that point the silicate concentration began to increase rather than to further decrease, and there was no evidence of an increase in the percentage of cellular lipids.

Table 3-15. Effect of reducing CO_2 supply to T. suecica culture in 48 m² flume. Error bars are 95% CI.

Culture Condition	g AFDW/m²/d	E(E/m²/d)	PE
no CO_2 addition (1/26/85-2/6/85)	6.8 ± 4.2	28.0 ± 6.8	3.4 ± 2.0%
hold pH at 8.5 (1/13/85-1/23/85)	14.6 ± 3.0	30.8 ± 4.3	6.1 ± 1.5%
hold pH at 7.5 (12/9/84-12/23/84)	19.4 ± 4.6	32.6 ± 3.1	8.1 ± 2.5%

Table 3-16. Lipid carbon production during late log phase and early stationary phase in a C. gracilis culture in which silicate limited biomass production.

Day	um Si	% Lipid	Lipid C ($ug\ l^{-1}$)	Average Lipid C Production Rate Since Day 1 $ug\ C \times l^{-1} \times d^{-1}$
1	7.6	14.4	1.63	---
2	5.5	13.8	3.49	1.86
3	5.0	13.0	9.63	4.00
4	5.0	24.6	33.8	10.70
5	3.8	28.0	96	23.6
6	0.9	42.3	261	51.9
7	1.2	52.9	455	75.6
8	1.3	54.9	546	77.8
9	1.4	54.7	582	72.5
10	0.5	54.3	614	68.2
11	1.0	57.1	665	66.3

One possible explanation for this observation is that the diatom frustules become fragile as the cells become silicate depleted, and the frustules begin to break apart and subsequently dissolve due to the buffeting caused by the airlift.

In order to check this hypothesis, we harvested log phase Cyclotella cells from the flume by centrifugation when the cell concentration had reached 6×10^6 per ml. The cells were resuspended in 7 °/oo offshore seawater diluted with distilled water and enriched with all IMR nutrients except silicate. The cells were incubated in duplicate polycarbonate flasks at concentrations of either 2.25×10^6/ml or 4.5×10^6/ml. One flask of each pair was bubbled with CO_2; the other received no CO_2 additions. Monitoring of the culture over the subsequent three-day period produced the results in Table 3-17. Neither cell counts nor carbon concentration increased significantly in the flasks which were not bubbled with CO_2. In the flasks bubbled with CO_2, cell numbers remained essentially unchanged, but particulate carbon, C/N ratios, and the percentage of lipids increased dramatically.

It is apparent from this study that inducing lipid accumulation at these high cell densities will require CO_2 additions. Depriving the cells of silicate effectively blocks cell division. The dramatic increase in the C/N ratio of both flasks bubbled with CO_2 is reflected in the increased lipid content of the treatment 1b flask, where lipid increased to 48% in two days as a percent of cell carbon (38% of cell AFDW). The lipid analyses on the third day were unfortunately lost in the treatment 1a experiment, but the similarity in the C/N ratio changes in treatments 1a and 1b strongly suggests that the lipid content of the treatment 1a cells increased in a manner similar to that observed in the treatment 1b cells. In treatment 1b the lipid concentration increased from (2)(123)(0.2515) = 61.9 mg/l to (2)(248)(0.3795) = 188.2 mg/l in one day, an increase of 188.2 - 61.9 = 126.3 mg/l. It seems likely that with additional experimentation this lipid production can be further enhanced. However, it seems evident from the work completed to date that this "fattening up" process will have to occur in a system separate from the flumes so as to avoid damage to the cells from the buffeting in the airlift system.

Table 3-17. Results of S starvation experiment with Cyclotella culture taken from the outdoor flume.

Date	Treatment	Cell No.	mg C/l	C/N	% lipid AFDW	% lipid Total C	M Si
6-20	1a*	4.49×10^6	164	5.54	22.49	25.23	3.1
6-21		4.54×10^6	229	6.45	22.27	20.96	---
6-22		4.93×10^6	390	11.45	-----	-----	0.4
6-20	1b*	2.26×10^6	83	5.54	24.49	25.23	3.1
6-21		2.28×10^6	123	6.35	25.15	29.27	---
6-22		2.36×10^6	248	12.88	37.95	48.39	0.5
6-20	2a*	4.49×10^6	164	5.54	24.49	25.23	3.1
6-21		4.39×10^6	177	5.86	27.02	31.64	---
6-22		4.69×10^6	183	5.75	27.71	27.32	7.8
6-20	2b*	2.26×10^6	83	5.54	24.49	25.23	3.1
6-21		2.20×10^6	97	5.95	28.64	37.02	---
6-22		2.49×10^6	112	6.06	29.70	24.82	1.9

* 1a, 1b with CO_2

* 2a, 2b without CO_2

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