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Abstract approved: Herbert C. Curl, Jr.

A small-scale laboratory representation of the oceanic water column was constructed and operated as a means of investigating the population dynamics of some marine phytoplankton. The apparatus consisted of a Plexiglas® cylinder 150 cm in height and 10 cm in diameter. Physical conditions found in the open ocean were simulated as a function of depth and time. These included: an exponential light gradient, a thermocline, surface turbulence and a source of nutrient input.

Two species of diatom were used in these studies: Thalassiosira nordenskjoldii and Cyclotella nana. In two of the three experiments reported here each diatom was grown in the apparatus separately. In a final experiment both species were grown concomitantly.

It was found that the miniature sea was, for the most part, qualitatively faithful to the real ocean. The population dynamics

showed the same general trends in the model as in the real ocean. T. nordenskjoldii was found to have a positive interaction effect on the relative growth of C. nana.

Simulating Phytoplankton Population Dynamics in a Miniature Sea

by

James Welt Haefner

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Typed by Mary Jo Stratton for James Welt Haefner

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It all started a while back when Dr. Herbert C. Curl casually mentioned a plexiglas cylinder gathering dust in one of the more inaccessible corners of his office. From then on events, more or less, got out of hand and became, euphemistically speaking, a learning experience (i. e., an eye opener) into the never-never land of jury-rigged laboratory research. Dr. Curl's continuing funding, patience and (in a manner of speaking) cosmic sense of humor are necessary and sufficient to account for the completion of this project.

All of my friends had either a hand or a foot in this project at one time or another. Notable among them are Dave Menzies and Harold O'Connors. I am also greatful to John Pequegnat for the use of his phonograph motor, without which surface turbulence would not have been so elegantly simulated. As for the others: May you find peace and prosperity.

This study was kindly supported by FWQA training grant 5T1-WP-111 and NOAA grant number 2-35187. Three cheers for the bureaucracy.

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INTRODUCTION

Due to the excessive intractability of the ocean arising from its size, its surface dynamics, and the distribution of its properties in space and time biological oceanographers are in need of some means by which they may control the critical parameters that govern the behavior of the marine ecosystem. However, as the complexity of the system increases, the necessary number of controlled experiments grows as an increasing power series. As a result, means have been sought that allow the number of required experiments to be reduced. There are two essentially different ways of studying the operation of unknown systems by a reduction in complexity.

By the first means, an oceanographer may impose upon the sea artificial simplifications of his own design (e. g., in situ isolation and control of marine variables). Secondly, an oceanographer may forego a study of naturally occurring systems altogether and impose simplifications in the form and operation of secondarily derived systems designed to represent naturally occurring situations (e. g., "models," utilizing either mathematical equations, physical replicas, or both).

Biological oceanographers have shown an interest in attempts to impose in situ simplifications upon the marine environment. Notable

among these are the so-called "big-bag" experiments. These studies entail the construction of a large, light-weight container that may be deployed at sea and from which samples may be drawn. Historically they have been constructed in two shapes: spherical and cylindrical. Containers of spherical shape have been constructed by Strickland and Terhune (1961), McAllister et al. (1961), and Anita et al. (1963). None of these studies attempted to encompass heterogeneities in the spatial distribution of physical and biological parameters. Those experiments employing cylindrical shapes have been Goldman (1962) and Hirota (1967). These latter studies have attempted to study variables as a function of depth. All studies of this sort have not been very successful due primarily to the complexity of the structural design problems involved (Strickland et al., 1969). In short, in situ simplifications have not been adequate for experimental control.

The failure of this sort of investigation should not, however, deter future work in this area. I believe the problems involved are probably resolvable given sufficient interest and energy extended by those in a position to study these problems in their entirety. These structural design problems fall naturally within the realm of ocean engineers.

In order to acquire sufficient experimental control it became apparent that control over all relevant physical parameters was necessary. Consequently, for this study the second method of

simplification was chosen. This method took the form of the construction and operation of a small-scale laboratory representation of the oceanic water column (as it pertains to phytoplankton population dynamics). Such an idea is not new. Margalef (1963a, b) seems to have been the first to construct a physical replica of the oceanic water column for marine biology studies. In a detailed report, Margalef (1963b) describes the construction, operation and results obtained from a replica similar to the one used in the present experiments. Margalef made use of a Plexiglas® cylinder 2 m in height and 15 cm in diameter. Both thermal and photic structures were introduced to the system as a means of simulating the actual oceanic situation. A non-exponential light gradient was established by placing high intensity lights near the surface and low intensity lights midway down along the column. At no depth in the column was the light intensity zero. A thermocline was induced by the differential heat output of the lights. The thermocline occurred approximately 25 cm from the surface. Water temperatures, as a result, reached 34° C at the surface. Margalef maintained surface turbulence by repeated vertical displacements of a circular net. These displacements occurred just below the surface and were a few centimeters in extent. Sampling was managed by lowering a small instrument package suspended by tubing to the desired depth and extracting water through the tubing at that depth. Some additional, deep turbulence was reported to have been introduced by this technique.

A similar experiment was attempted on a much larger scale by Strickland et al. (1969). Although the size of the tank (10 m deep and 3 m in diameter) would have allowed the study of vertically varying processes, this was not attempted. The increased size of the experiment creates many additional problems such as required manpower, operating cost and installation difficulties. There are obvious advantages, however. The impact of sampling is minimized, as is the affect of the container on the experiment. Also, longer, more complex experiments may be attempted. Nonetheless, under the circumstances of the present study a physical replica was used which was much smaller than that used by Strickland, more nearly the size constructed by Margalef (1963b). To construct such a model it was necessary to both describe which oceanic variables were to be controlled and the manner by which the variables were to be simulated. As a motivation for these descriptions a brief overview of marine phytoplankton ecology is necessary.

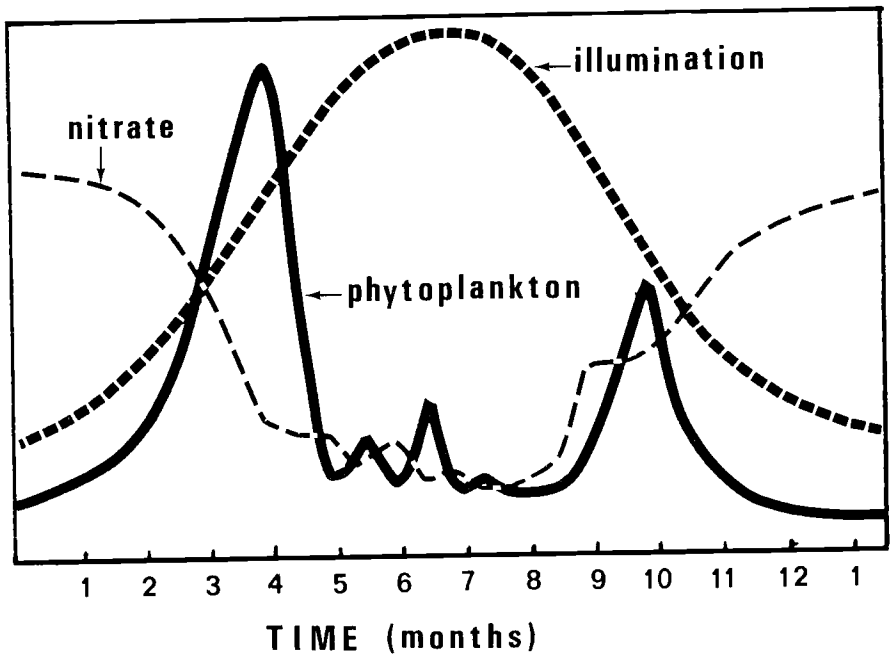
In the pelagic situation, the dynamics of phytoplankton populations are dominated by a remarkably consistent annual cycle. Although the classical description given below is highly idealized the existence of the cycle cannot be doubted (Hardy, 1959; Raymont, 1963; Fogg, 1965; Strickland, 1965).

In early spring, as the intensity of solar radiation incident on the ocean surface increases, the relative stability of the water column

also increases. The increased stability in conjunction with a reduction in the intensity of surface turbulence create the conditions for the spring bloom (Gran and Braarud, 1935). Production then decreases during the summer as the nutrients are depleted in the photic zone. An additional, secondary bloom may occur later in the year as surface turbulence increases in the autumn. This situation is ideally represented in Figure 1 (a). Iverson (1972) has recently reviewed the attempts to quantify the effects of stabilization on phytoplankton growth.

This classical picture, however, obscures changes that occur as a function of space as well as of time. Changes in space can occur both horizontally and vertically. Horizontal structure of phytoplankton populations is generally referred to as "patchiness." Although patches may have subtle ecological significance (Margalef, 1968; Levins, 1968), they are currently considered a statistical problem (e.g., Pielou, 1969). Vertical variation in population dynamics is mechanistically determined. The critical variables in this case are the exponential decrease of light transmittance with increasing depth and the availability of nutrients. Figure 1 (b) shows the response of production as a function of depth and light intensity alone.

These two general relationships, time and vertical space, determine the ingredients that must be included in the laboratory model. These ingredients include: a vertically positioned volume of



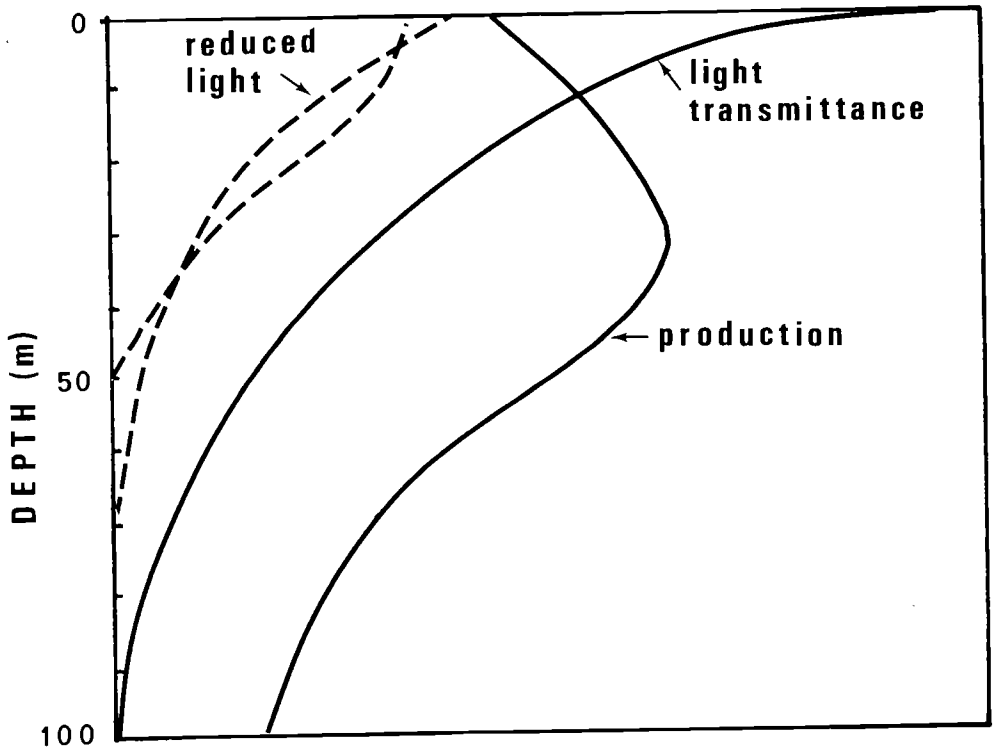


Figure 1. Idealized marine phytoplankton population dynamics.
a) temporal sequence (after Raymont, 1963, p. 194)
b) vertical distribution (after Raymont, 1963, p. 141)

sea water, a means of creating a well-defined thermocline, a means of mixing (both low intensity, daily mixing and occasional, high intensity mixing), an exponential light gradient, and a means by which sea water may be removed or added. In addition we require that all these properties operate simultaneously and independently so that the influence of each may be controlled and studied in the laboratory.

The objectives of the experiments were two-fold. I wished to see how closely the population dynamics of a single species of phytoplankton grown in the miniature sea could mimic the spatio-temporal distributions found in nature and diagrammed in Figure 1. Secondly, I wished to investigate the possibility of studying in the miniature sea the phenomenon of niche differentiation in either space or time when two species of phytoplankton were competing for limited resources.

Because previously reported work is scarce, the experiments reported here must be regarded as feasibility studies. As such the experiments must answer two questions: (a) can the model be made to work (be any criteria), and (b) ought the model be made to work? The questions are not independent. The criteria we choose to describe the success of the model will depend on the rationalizations we invoke in order to justify (to ourselves, if no one else) the effort extended to perform the experiment.

MATERIALS

The scaled-down model was comprised of a complex of sub-systems constructed so as to simulate various interacting physical properties operating in nature. These sub-systems consisted of: (a) the scaled-down column, (b) a thermocline, (c) an exponential light gradient, and (d) surface turbulence.

The container used to model the ocean column was a Plexiglas® cylinder measuring 150 cm in height with an inside diameter of 10 cm. The volume of this cylinder used in the final experiments was approximately 11 liters. Since realistic pressure changes with depth in the ocean were not simulated, the top of the column was open to atmospheric exchange. The bottom of the column was sealed with a threaded cap. Attached to this cap and inserted into the column was a short funnel covered with a fine-mesh screen. This arrangement allowed water to be introduced to the system during operation.

Arrayed along the length of the column, vertically and colinearly, were ten sampling ports. To construct these sampling ports, ten holes were drilled, tapped and each fitted with a 'T'-shaped 1/4 inch Swagelok® fitting (Figure 2). One arm of the 'T' was threaded from the outside through the wall of the column. A thermister was inserted through both arms of the 'T' approximately 5 cm into the interior of the column. The diameter of the thermister was 1/8 inch. This allowed the passage of a water sample from the column, through the

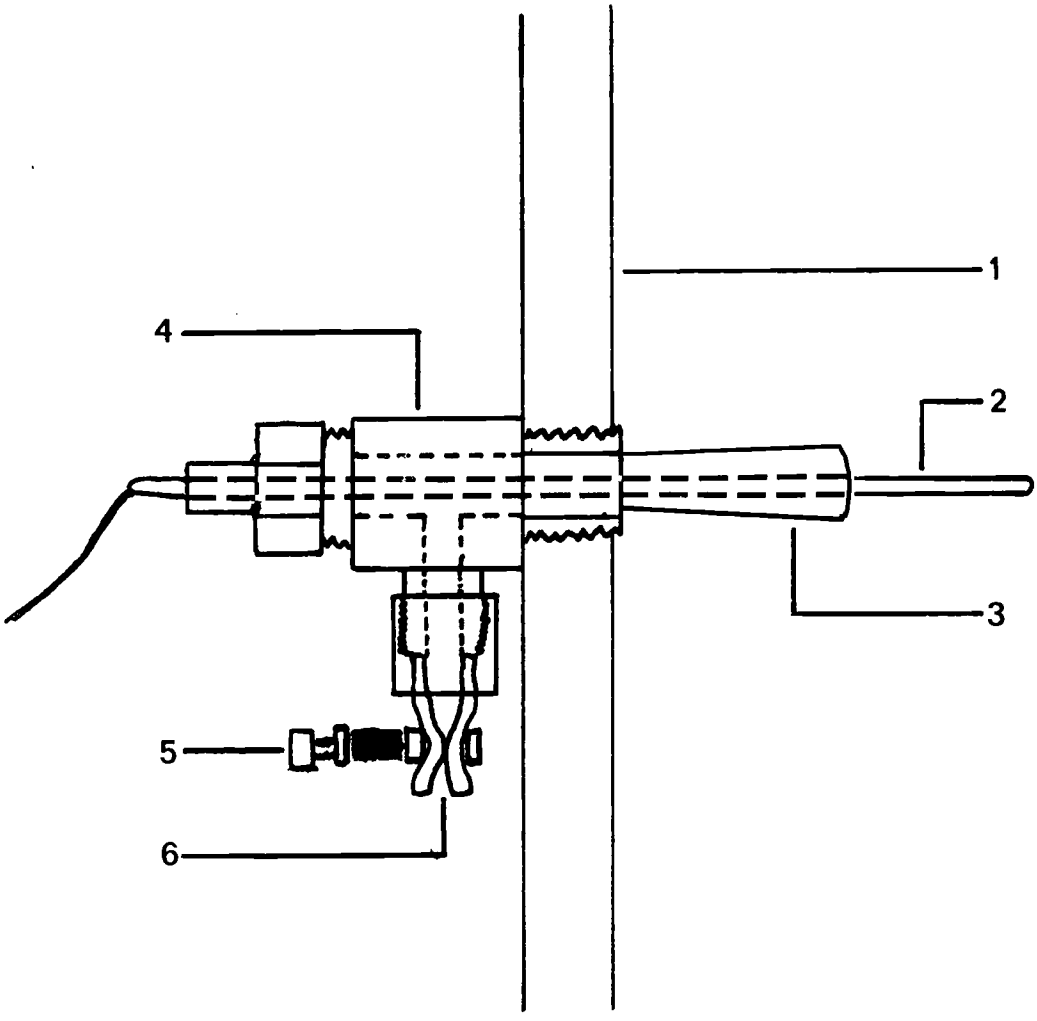


Figure 2. Schematic diagram of a sampling port.
(1) inside wall of plexiglas column
(2) thermistor
(3) sampling extension
(4) Swagelok fitting
(5) Hoffman clamp
(6) sampling exit point

adjacent arm of the 'T' and out the stem of the 'T'. Fitted over the stem was a section of Tygon® tubing closed with an Hoffman pinch clamp. Samples were drawn by opening this clamp. To avoid collecting samples from the immediate vicinity of the column's inside wall, a section of glass tubing was attached to the 'T', extending approximately 3 cm into the interior. The ten sampling ports were located at the following depths (in centimeters) from the surface: 5, 20, 30, 40, 55, 70, 85, 100, 115, and 130.

For the sake of realism it was desired that a definite thermal structure exist so that a relatively large reservoir of cold bottom water would be separated from a warmer, upper reservoir by a sharp thermocline. To simulate such a thermal structure the column was immersed in a metal container measuring 70 cm in height and 30 cm in diameter. The height of the container coincided with the depth of the thermocline and with the level of 1% light transmittance. The container was filled with water and cooled to 7° C by a refrigeration unit. This arrangement created a temperature change of 7-8° C over a vertical distance of 30 cm extending from a depth of 70 cm to a depth of 100 cm below the surface.

The development of an adequate simulation of light transmittance as a function of depth in the ocean proved to be relatively difficult. A light gradient was sought which exponentially attenuated surface intensity to 1% over a distance of 75 cm of sea water. Initially, an

overhead light source was used, attenuation being induced by artificially augmenting the natural absorption of the sea water. Various attenuating substances were employed in this regard, among them India ink and methylene blue. India ink had previously been used for similar purposes in fresh water studies (Harris and Wolfe, 1955). However, it was found that the ionic characteristics of sea water inhibit a fine suspension of the carbon particles. To avoid these problems, methylene blue, a biologic stain that dissolves in sea water, was used. Unfortunately, such quantities of the dye were required for proper attenuation that preliminary culture experiments revealed the dye's detrimental effects on phytoplankton growth.

As a consequence of these difficulties, an overhead light source was rejected in favor of side illumination from two pairs of fluorescent tubes. Each pair of lights consisted of one Sylvania® very high output daylight lamp (F48T12-DVHO) and one General Electric® cool white lamp (F40CW). Each pair was vertically oriented and faced opposite one another on either side of the column. A reflective parabolic surface was inserted behind and along the length of each pair of lights. Unfortunately, the heat output of this lighting arrangement raised the temperature of the water within the column to undesirably high levels. To counter this effect, two infra-red filters were constructed. Each filter was made of two sheets of 1/4 inch plate glass separated by one-inch Plexiglas® spacers and sealed with waterproof cement. The

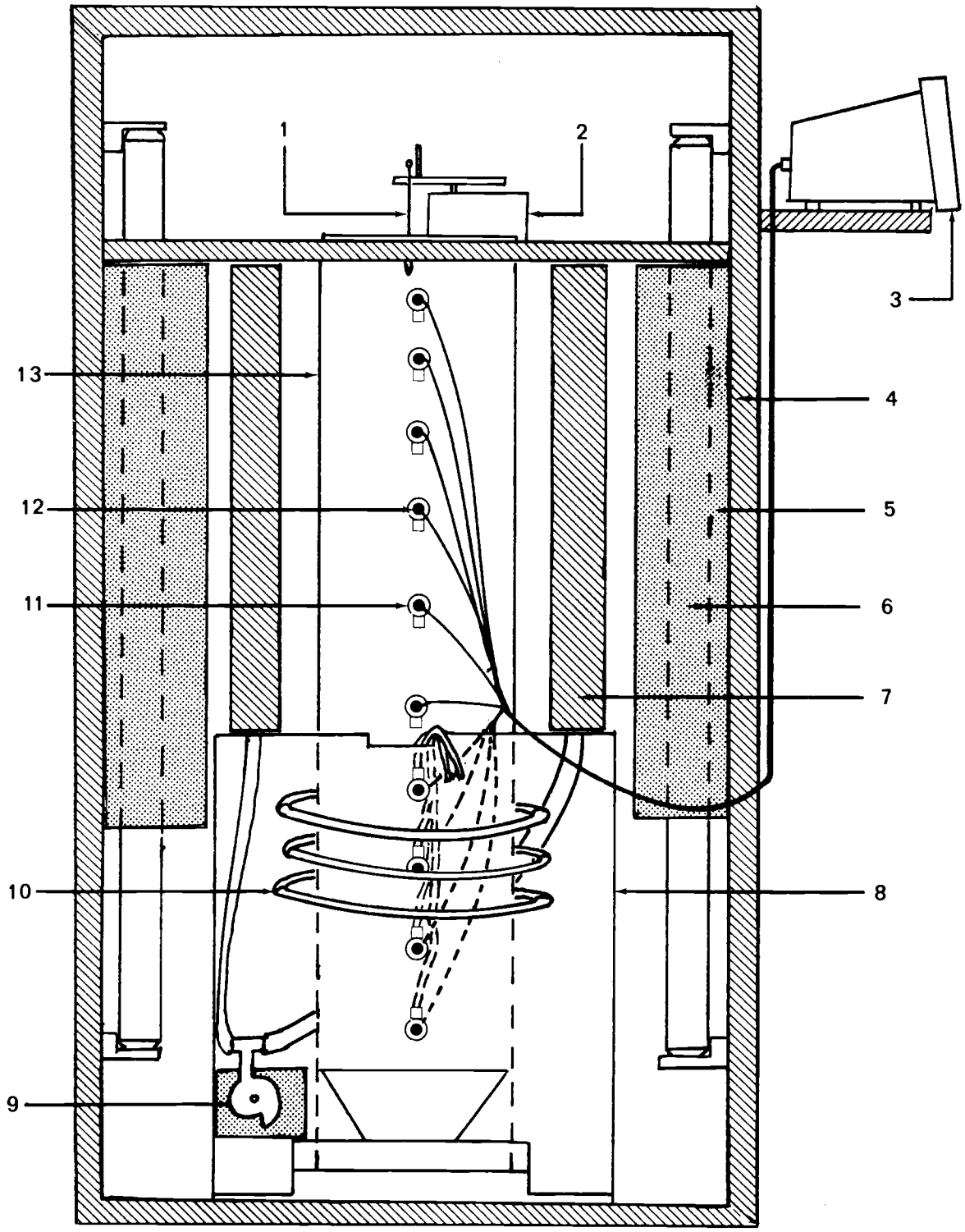
filters were positioned between each pair of lights and the column. Refrigerated water was circulated within each filter by a pump submersed in the metal container. A diagram of the miniature sea is presented in Figure 3.

Horizontal illumination, however, required the construction of a vertically oriented screen whose transmittance was exponential over its length. Initial efforts attempted to achieve exponential decay exterior to the column by placing filters between the lights and the column. These attempts failed for a number of reasons but chiefly due to the fact that the walls of the column behaved as a "light pipe" and counteracted the exterior exponential attenuation. The ultimate solution consisted of applying a layer of black paint to one side of a sheet of clear Mylar® acetate. This sheet of flexible acetate measured 150 cm by 32 cm. Applied to the top 75 cm of the sheet was a gradient of paint that transmitted light exponentially over that distance. The lower 75 cm was given a heavy coat of paint that transmitted no light. The sheet was then rolled into a long cylinder and inserted into the column so that the painted side was contiguous with the inside wall of the column.

Once the gradient had been inserted, but prior to the addition of sea water, the amount of light penetrating the column at depth was measured with the remote sensing ISCO® spectroradiometer. Measurements were made with the IR filters in place by masking one set of

Figure 3. Schematic diagram of the miniature sea.

(1) stirring rod
(2) phonograph motor
(3) temperature readout
(4) lightproof box (curtain not shown)
(5) parabolic reflector
(6) lights
(7) IR filter
(8) water bath
(9) submersible pump
(10) refrigeration coils (compressor not shown)
(11) sampling port
(12) thermister
(13) column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lights and placing the sensor against the inside wall nearest the light source at the desired depth. The results of these measurements are indicated in Figures 4 and 5. Figure 5 shows k to be about 10.0. This can be compared with a value k = 0.2 for an estuary (Iverson, 1972). Integrating the curve for 5 cm from Figure 4 shows that surface illumination was about 0.2 ly/minute. This can be compared with an oceanic average of 1.5 ly/minute (Strickland, 1965).

Wind mixing was simulated in this model using rapid, short, horizontal motions at the surface of the column. A small, stainless steel blade attached to a thin, flexible rod was shaped and fixed to the inside of the box so that only the blade was immersed in the water. On a shelf beside the rod was placed a modified phonograph motor. To the drive wheel of this motor a two-inch post was securely fastened. As the drive wheel was turned by the motor, the post periodically struck and displaced the flexible rod and blade. The motor was of a type allowing variable playing speeds and consequently various intensities of "wind mixing" were possible. The playing speeds were calibrated in the column by visual observations of dye. It was found that various speeds mixed in a convective cell pattern to the following depths: 16 rpm: 8 cm; 33-1/3 rpm: 20 cm; 45 rpm: 30 cm, and 78 rpm: 30 cm. All experiments reported below were conducted with a constant "wind speed" of "45 rpm," that is, mixing to a depth of 30 cm.

Once the component sub-systems had been assembled, they were

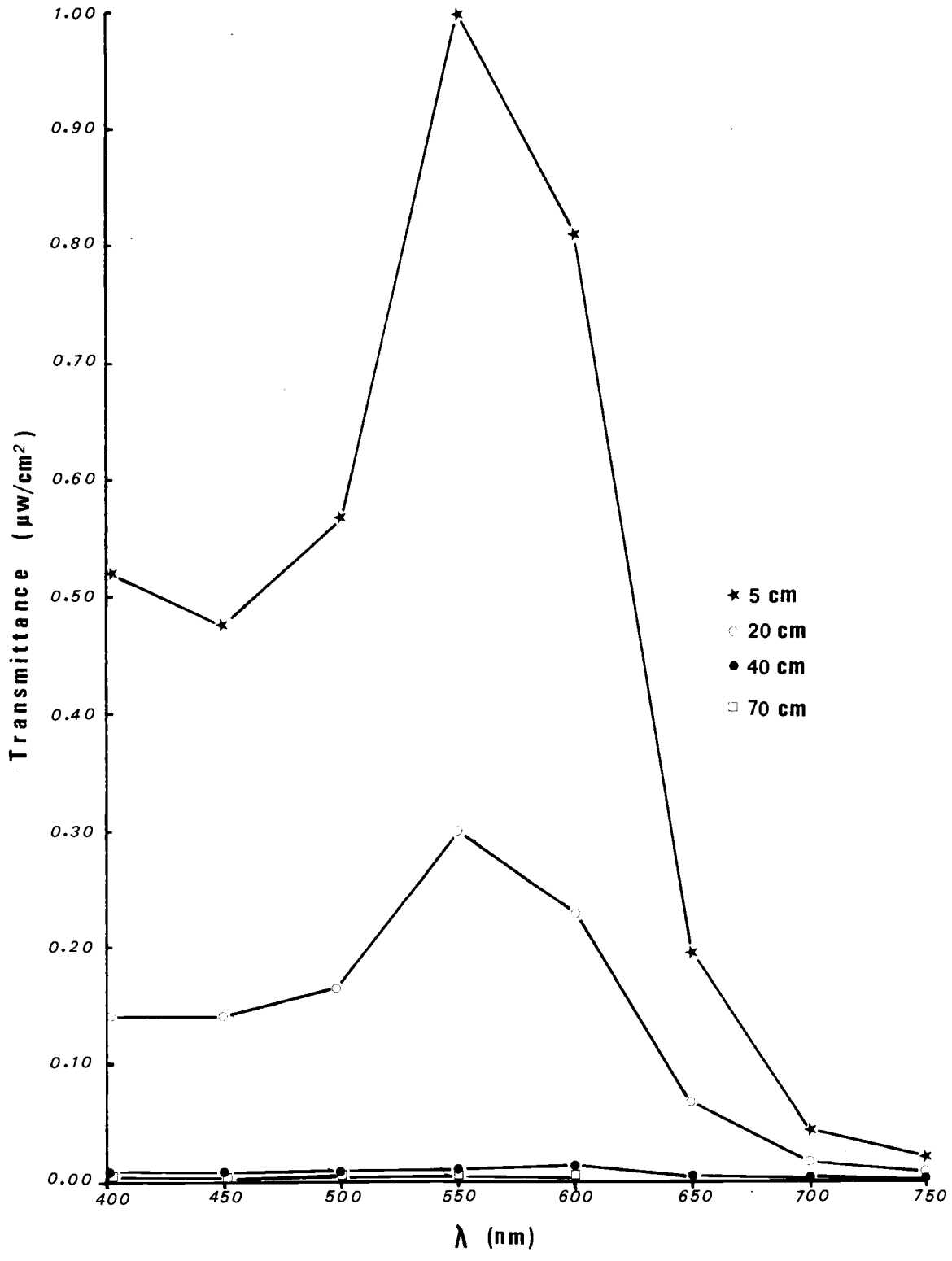

Figure 4. Light transmittance as a function of depth within the minature sea.

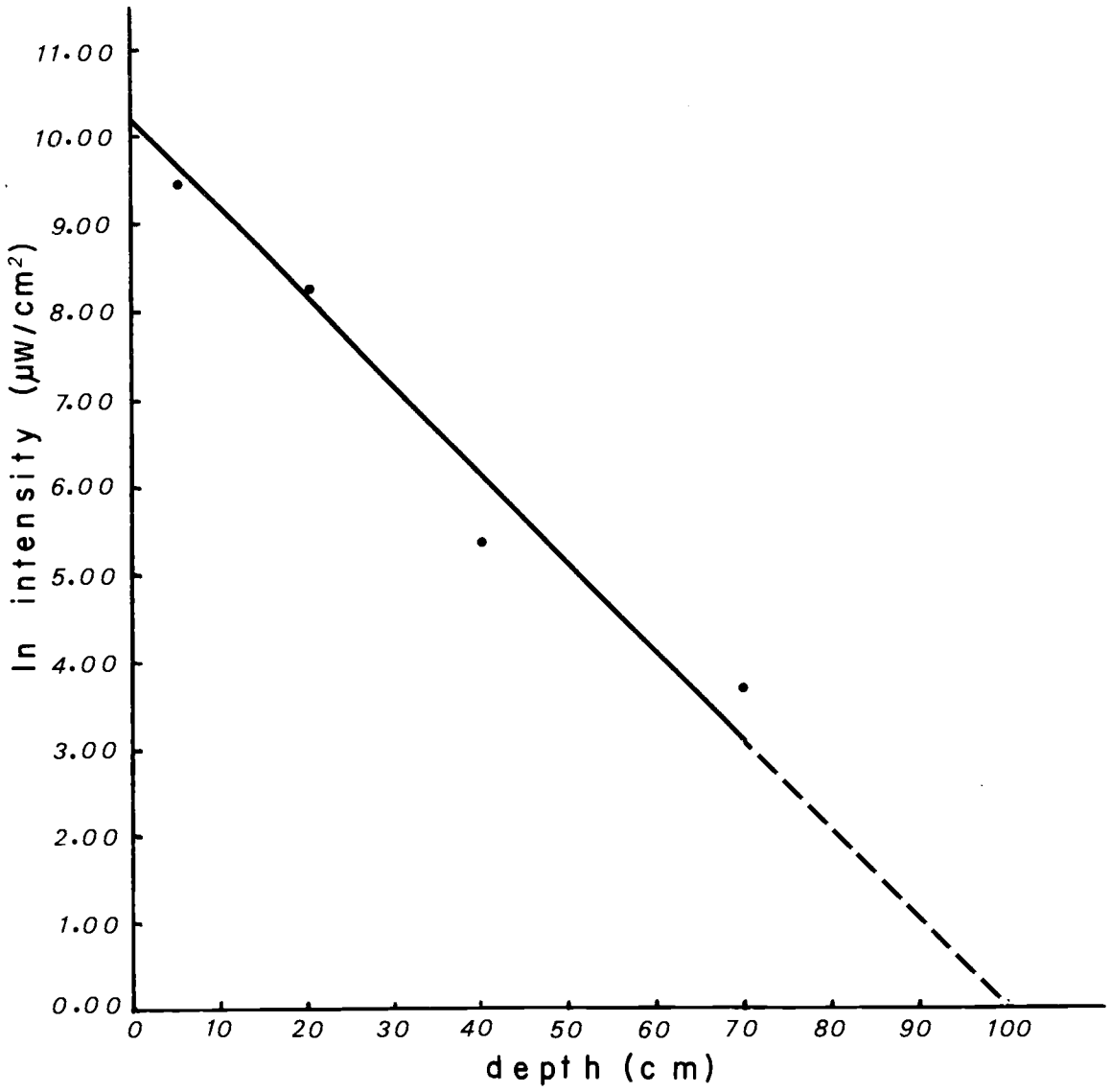

Figure 5. The natural logarithm of the integrated transmittance as a function of depth within the miniature sea.

placed in a large lightproof box. Access to the column was provided by a black felt curtain at the front. The refrigeration employed to cool the lower depths of the column was external to the box. The entire assembly was located in a constant temperature room in order to improve temperature control.

METHODS

After the model had been constructed, experiments were performed by introducing phytoplankton into the model and monitoring their growth dynamics. The monitoring process consisted of daily measurements of temperature, cell numbers and nitrate concentration. Cell numbers were counted on a Coulter Counter®, using a method described by Strickland and Parsons (1968). Nitrate analyses were performed on samples filtered by gravity through glass fiber filters. Nitrate concentrations of the filtered samples were determined using a Technicon Autoanalyzer® by the method of Armstrong given by Hager, Gordon and Park (1968). Two species of diatom were used: Thalassiosira nordenskjoldii and Cyclotella nana. These two organisms were chosen primarily for their ability to remain in suspension in the water column at low nitrate concentrations. Other species, with much higher sinking rates, for example, Ditylum brightwellii, were also investigated but with unsatisfactory results.

Prior to each experiment, the populations of organisms were grown in 12 liter carboys containing filtered, autoclaved sea water. The medium employed in these studies is indicated in Table 1 as concentrations per liter. In these experiments, the basic nitrate concentrations were 50 μM. The cells were grown in constant light during all phases of these experiments in the miniature sea. When the

Table 1. Culture medium.

$NaNO_3$	4.25	mg/l
$NaH_2PO_4 \cdot H_2O$	10.0	mg/l
Fe sequestrene	1.0	mg/l
$Na_2SiO_3 \cdot 9H_2O$	45.0	mg/l
$NaHCO_3$	0.2	g/l
Thiamin HCl	0.2	mg/l
Biotin	1.0	µg/l
B_{12}	1.0	µg/l
$CuSO_4 \cdot 5H_2O$	0.0196	mg/l
$ZnSO_4 \cdot 7H_2O$	0.044	mg/l
$CoCl_2 \cdot 6H_2O$	0.020	mg/l
$MnCl_2 \cdot 4H_2O$	0.360	µg/l
$Na_2MoO_4 \cdot 2H_2O$	0.013	µg/l

number of organisms had reached the desired level for the initiation of the experiment, the nitrate concentration in the carboy was measured and readjusted to 50 µM. No other nutrients were added at this time. In the case of the third experiment, utilizing two species of diatoms, each species was raised separately in 12-liter carboys. After sufficient quantities of each were obtained, the two species were mixed in the ratio of 12 C. nana cells to one T. nordenskjoldii cell. This particular ratio was chosen because it is the reciprocal of the ratio of their respective mean volumes. It was rationalized that equal volumes was a better measure of identical competitive advantage than was, for instance, equal numbers of individuals.

Once the inoculum had been prepared, the column itself was then cleaned as effectively as possible, but not autoclaved. The thermisters and sampling ports were then inserted and the column placed in the lightproof box. The inoculum was siphoned into the column with the aim of producing as little trauma to the cells as possible. All of the various sub-systems were then started; that is, the refrigerator, submersible pump, lights, ventilation fan, and stirring motor were all turned on. Immediately after filling the column, the system was sampled and the samples analyzed in order to precisely determine the initial conditions of the experiment.

The phytoplankton population, allowed to grow and develop, was monitored daily as previously stated. Daily sampling and analysis required approximately 10-15 ml of sea water per station. This amounts to about 200 ml daily and as such represents a significant fraction of the total volume of the model. As a result, this material had to be replaced following sampling. It was replaced in the following way. After the entire sampling procedure had been completed, sufficient quantities of sea water of similar salinity as the original inoculum were added to the column through the bottom. This water was added slowly so as to allow its temperature to come to equilibrium with that of the water within the column. During the early stages of the experiments, this additional water contained 50 μM NO_3. In the later stages, as the nitrate concentration of the deeper depths of the

column decreased, higher concentrations of nitrate were added in this manner. The purpose of this procedure was to maintain the nitrate concentrations of the deeper depths at a constant, higher level than in the shallower depths, as is the case in the open ocean. A review of the relevant data included here indicates that this procedure did not have the desired effects.

In the course of the experiment, the nitrate concentration in the well-lighted, upper depths of the column was reduced to zero. When this occurred, the entire column was artificially mixed until all its measured properties (temperature, cell numbers and nitrate concentration) were homogeneous over the length of the column. This was accomplished by alternatively raising and lowering a heavy, semi-circular plate attached to nylon thread. This procedure was conducted as a means to simulate occasional intensive turbulence which mixes to a depth below the photic zone. In the open ocean this turbulence, in the form of storms, replenishes the depleted supply of available nutrients, thereby providing conditions for an additional, secondary population bloom.

The experiments presented here were terminated once the secondary population bloom had (for a second time) depleted the nitrate concentration in the photic zone to zero. The system was then dismantled, cleansed and prepared for another experiment.

In the following discussion, the three experiments performed

are referred to as I, II, and III. Experiment I was a single species experiment performed with T. nordenskjoldii. It was terminated following the tenth day of operation. Experiment II was also a single species experiment, but utilizing C. nana. It lasted for 14 days. Experiment III used both T. nordenskjoldii and C. nana in the initial ratio of 1:12. This last experiment was terminated after 15 days.

RESULTS

The data are presented in two forms, as tables by experiment and as surfaces. Since the abscissas of the surfaces are space (depth) and time, they do not strictly qualify as "response surfaces" in the sense of Box (1954). Depth and time only implicitly cause a response in the organisms in that the actual causative agents (light, temperature, nutrients, etc.) vary with depth and time. As a result, these surfaces have, primarily, descriptive value and only non-quantitative explanatory value.

A number of factors placed constraints on the extent to which these results have been statistically analyzed. Among these are: (1) the lengthy analyses of the daily samples precluded multiple sampling or analysis, (2) the restrictions on sample size also precluded multiple sampling and analysis, and (3) since a single sample represented a substantial fraction of the total "ocean" (about 2%), repeated sampling without replacement was deemed statistically unjustifiable. As a result, data points represent the result of a single sample, single analysis. Cell counts are excepted; they represent a three count mean of a single sample. However, a non-rigorous approximation of the variability of the sampling plus analyses can be made. Assume the column was homogeneous at the beginning of the experiments and when it was vigorously stirred near the end of the

experiments. Taking the mean over all ten stations in the column at these times allows a 90% confidence interval to be computed and expressed as a percent of the mean (Table 2).

Table 2. A non-rigorous measure of statistical variation.

Analysis	90% confidence interval as % of mean
T. nordenskjoldii (cells/ml)	± 5%
C. nana (cells/ml)	± 2%
nitrate (μM)	± 2%

These computations, although not statistically meaningful, may give an intuitive feeling for the amount of variability involved.

Experiment I

Temperature

The thermocline was established rapidly, requiring only a few hours. The temperature surface (Figure 6, Table 3) reveals that the thermal structure is not a simple step function. Between the surface and the top of the thermocline, at a depth of 70 cm, the temperature decreases slightly. This decrease is approximately 2-3° C. Between

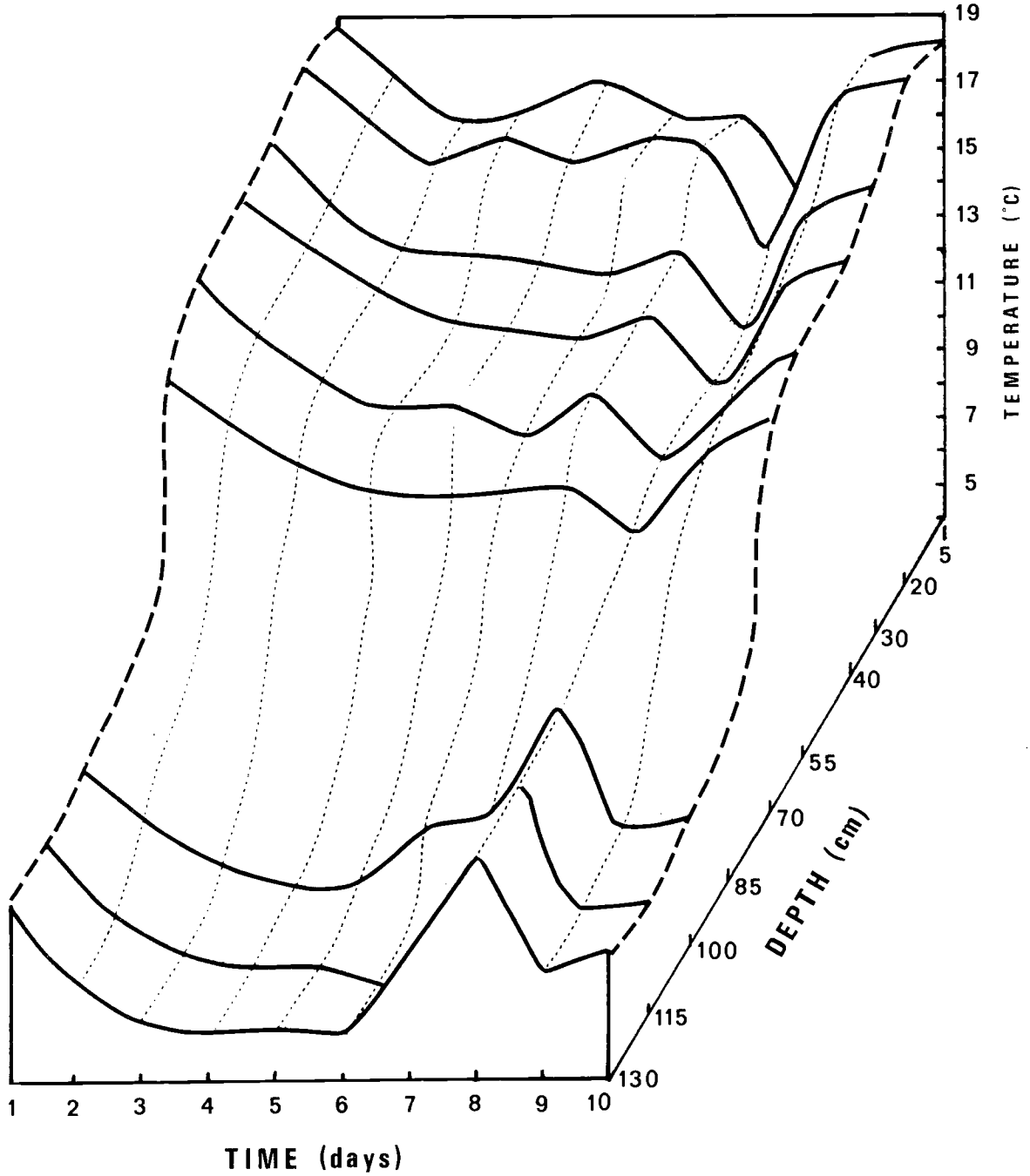

Figure 6. Experiment I: temperature as a function of depth and time.

Table 3. Data for experiment I.

Days	cm 5	20	30	40	55	70	85	100	115	130
1	6026[a]	5685	5600	5892	5679	5228	5616	4358	5273	4301
	48.7[b]	52.1	57.7	41.1	35.6	32.8	30.5	39.1	40.2	41.4
	18.7[c]	18.8	18.7	18.5	18.3	17.3	10.2	9.7	9.3	9.6
2	6881	6734	6842	--	6522	6707	5721	5090	5159	4995
	31.6	29.6	47.0	34.1	36.0	32.9	30.0	38.4	31.6	33.4
	--	--	--	--	--	--	--	--	--	--
3	9480	10568	10256	9662	10280	10149	7719	5946	6065	6818
	21.3	27.8	21.2	27.7	21.7	24.7	27.7	30.5	34.7	27.4
	15.7	15.9	15.5	15.8	15.2	14.5	--	6.7	5.9	6.7
4	20708	--	13700	14520	13870	14760	12215	8126	7922	8034
	13.7	13.1	--	19.1	19.8	18.4	30.0	36.1	35.0	34.4
	16.4	16.8	15.4	14.7	14.3	13.7	--	6.0	5.5	5.8
5	21088	21634	23664	19471	19586	20027	22173	13293	13244	13416
	4.1	9.7	13.4	12.9	13.8	10.9	28.4	33.4	33.2	33.1
	17.2	15.9	15.1	14.7	14.5	--	--	5.9	5.5	5.9
6	17476	17794	15541	20731	20433	20724	21987	17966	17220	19859
	0.0	0.0	3.9	4.5	5.3	2.6	23.6	29.4	29.6	32.3
	15.9	16.6	14.7	14.1	13.3	--	--	5.1	5.0	5.6

(Continued on next page)

Table 3. (Continued)

Days	cm 5	20	30	40	55	70	85	100	115	130
7	19303	18655	16884	22096	22740	24983	26527	28177	23954	26534
	0.0	0.0	0.0	0.0	0.0	0.0	16.4	22.1	22.5	22.9
	16.1	16.5	15.6	15.1	14.7	14.0	--	8.0	7.6	8.1
8	26411	25214	26758	26187	26845	27542	26278	27276	26390	27454
	17.4	17.6	17.5	17.7	17.0	17.0	44.8	16.6	16.8	17.3
	12.7	13.2	12.9	12.8	12.6	12.4	--	11.7	11.0	11.0
9	33219	33814	21249	27094	29285	28354	47856	36781	31381	33530
	0.0	0.0	0.7	0.5	1.7	0.6	6.5	9.3	9.9	9.3
	17.9	18.0	16.7	15.8	14.4	15.0	--	7.7	7.2	7.5
10	24336	25463	24731	29418	32165	34097	45238	35612	35070	37352
	0.0	0.0	0.0	0.0	0.6	0.0	4.4	11.1	11.0	14.0
	18.2	18.2	17.4	16.4	15.9	15.7	--	8.2	7.5	8.2

[a] Thalassiosira nordenskjoldii: cell numbers/ml

[b] Nitrate concentration: μM

[c] Temperature: Centigrade

the third and seventh days the temperature regime was relatively constant, with only mild daily fluctuations. On the eighth day the column was completely mixed, as described earlier. This caused a uniform temperature structure over the column's entire length. By the next day, and for the remainder of the experiment, the thermocline was present.

Nitrate

Difficulties while establishing this first experiment caused the variability of nitrate concentration (Figure 7, note axis rotation) on the first day. By the fourth day an increasing gradient of nitrate concentration was detectable at depths deeper than 75 cm, with a sharp decrease above the thermocline. This basic relationship is stable for several days as the nitrate concentration in the photic zone continued to decline. It will be noted that nitrate concentration also declined in the dark regions. Eppley and Coatsworth (1968) report nitrate uptake in the dark by Ditylum brightwellii. Also, due to the nitrate gradient across the thermocline, a positive diffusion upwards will contribute to decreasing nitrate concentrations below the thermocline.

As previously noted, the column was homogeneously mixed on the eighth day of the experiment. The high nitrate concentration at 70 cm on this day is unexplained. The increase of cell numbers at this depth on the following days suggests that the nitrate concentration

Figure 7. Experiment I: nitrate concentration as a function of depth and time (note axes rotation).

is real. After mixing, nitrate concentration decreases at all depths, being zero in the photic zone after 24 hours. Concentrations in the aphotic zone do not reach zero on the ninth day and increase on the tenth day. This increase was caused by an attempt to maintain high nitrate concentrations in the aphotic zone.

Cell numbers/ml

In general, cell numbers/ml increase at all depths (Figure 8, Table 3). But the rate of increase in the photic zone is greater than in the aphotic zone. By the fifth day a well developed gradient of cells is established in the vicinity of the thermocline. The moderately sharp decline in cell numbers below the thermocline is attributed to a reduction in the growth rate of T. nordenskjoldii at zero light levels. This structure persisted for three days. From the sixth to the seventh days, the top of the gradient shifted from 85 cm to 100 cm. This shift is apparently due to the sinking of the population. An additional, very transient population structure was present in the photic zone on the fourth and fifth days. This was manifested as a region of very fast growth in the upper 30 cm of the column. The nitrate concentration was high at the beginning of this period and zero at its end. This suggests that this transient structure represents a real population increase. Since surface turbulence also extended to 30 cm, cells in the upper 30 cm of the column were all subjected, for

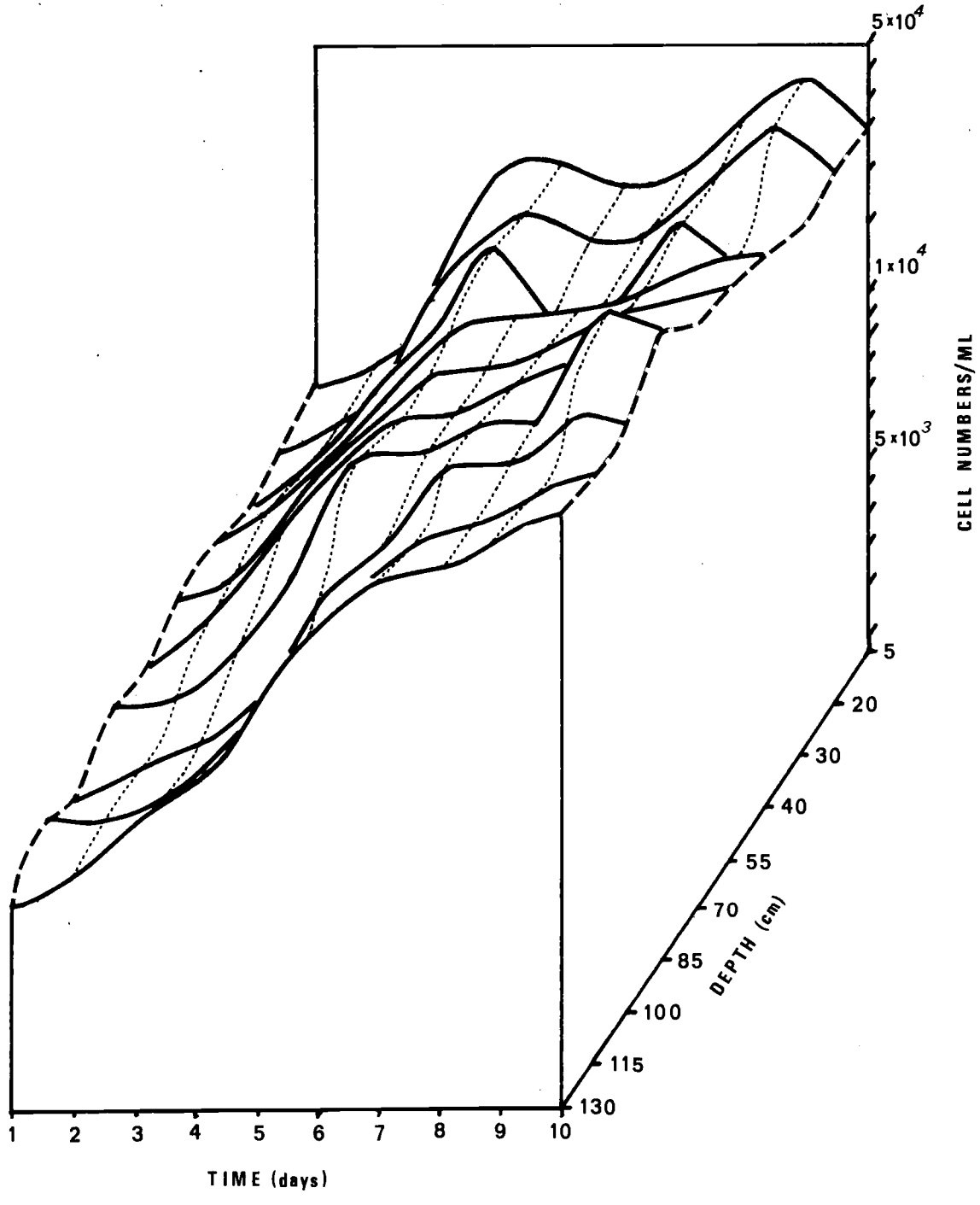

Figure 8. Experiment I: cell numbers/ml as a function of depth and time.

some period of time, to illumination intensities at the surface. Cells below 30 cm were not.

Lastly, the behavior of the population at 85 cm on the ninth and tenth days will be noted. The relatively large increase in cell numbers may be in response to the abnormally high nitrate concentration at that depth resulting from the mixing of the previous day. Decline of this "bloom" on the tenth day is ascribed to sinking.

Experiment II

Temperature

The temperature surface (Figure 9, Table 4) for this experiment is almost identical to the previous experiment. A notable difference, however, exists on the third day of the present experiment. On this day a failure of the refrigeration unit resulted in a uniform temperature structure. The profile returned to its normal stratified state within 24 hours. The subsequent effects of this event on the behavior of the population will be examined below.

Nitrate

The most obvious characteristic of these data (Figure 10, Table 4) is the fact that nitrate concentrations were depleted to zero μM over the entire length of the column. The ability of C. nana to consume nitrate in the dark has been noticed by other investigators (Phil Larsen,

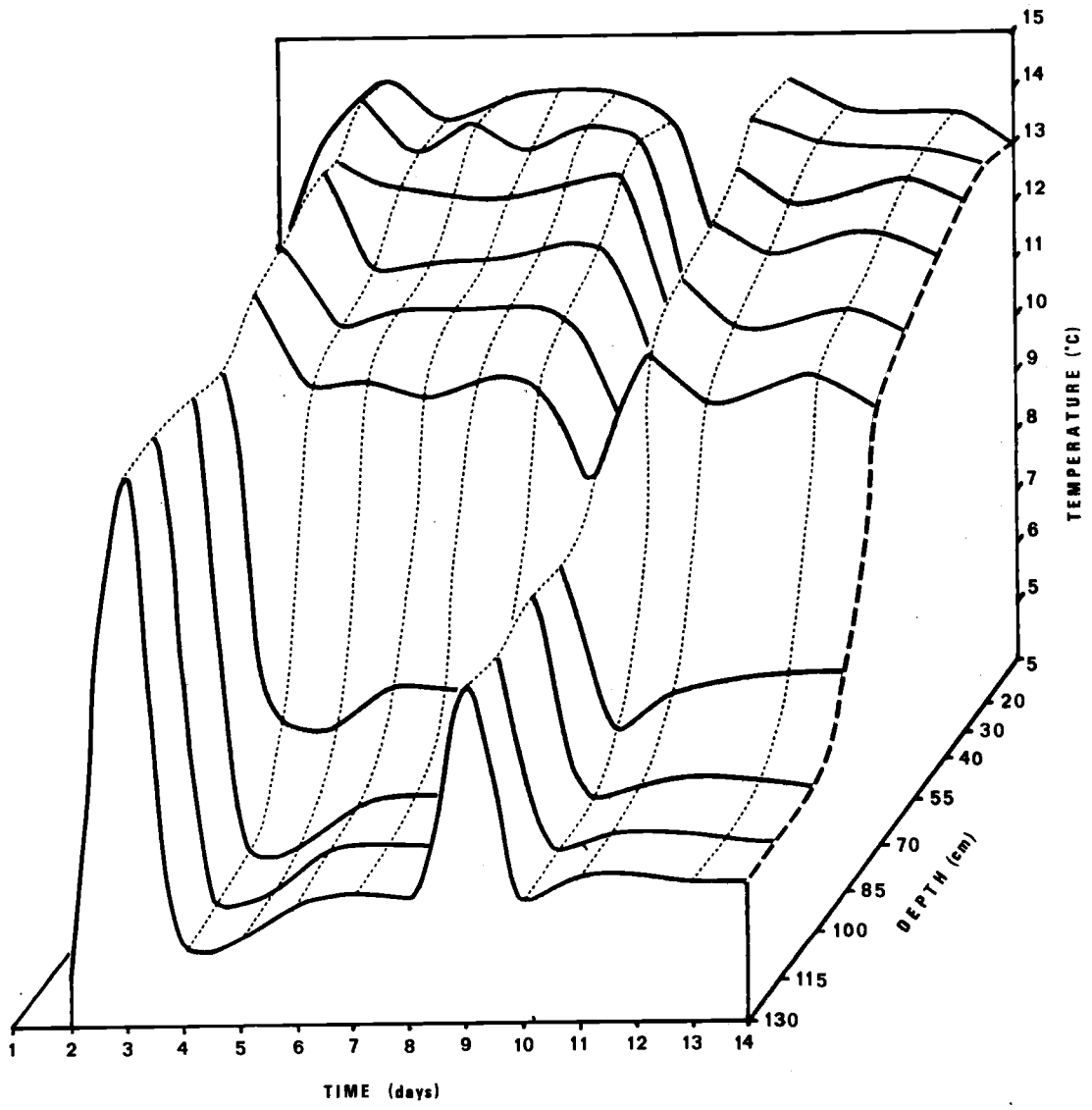

Figure 9. Experiment II: temperature as a function of depth and time.

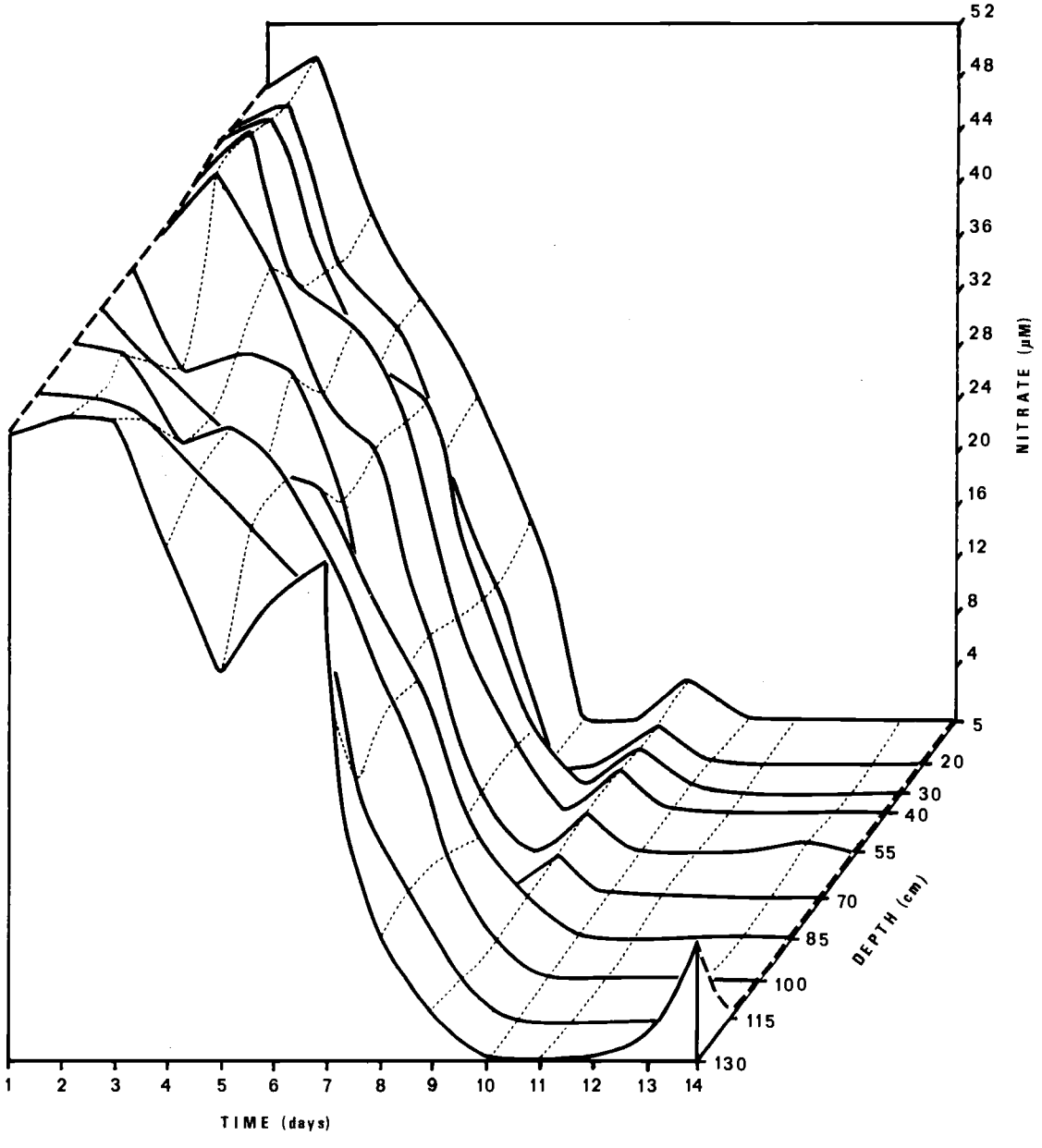

Figure 10. Experiment II: nitrate concentration as a function of depth and time.

Table 4. Data for experiment II

Days	cm 5	20	30	40	55	70	85	100	115	130
1	25380[a]	--	24460	--	24534	--	24966	--	25720	--
	-- [b]	--	48.3	--	46.5	--	--	--	--	--
	-- [c]	--	--	--	--	--	--	--	--	--
2	38126	34974	31040	26794	28337	28646	26566	26486	25994	26086
	49.7	49.2	50.1	51.2	51.2	38.1	43.3	46.6	46.2	48.1
	12.5	13.2	12.2	12.0	11.8	11.4	6.8	4.8	5.2	5.2
3	40760	38600	44406	41714	42154	39274	38646	39020	39660	38246
	37.9	37.1	38.7	38.9	43.9	40.5	39.0	40.2	45.2	48.0
	14.3	14.7	14.1	14.4	13.8	13.8	13.2	13.5	13.7	13.7
4	73366	70600	59954	58574	60206	59074	40646	37566	44226	42934
	31.2	32.9	31.1	36.4	33.8	38.0	34.9	41.4	41.1	36.9
	13.5	13.7	13.6	12.6	12.4	12.1	7.0	5.4	5.4	5.4
5	125534	127966	107866	96014	101460	98200	59700	53894	54894	65260
	23.9	22.8	28.7	29.0	30.8	29.4	34.0	37.5	37.0	29.0
	13.9	14.3	13.4	12.8	12.7	12.3	6.9	5.6	5.6	5.6
6	164354	162186	205734	156386	157426	155274	128906	114486	114234	43020
	15.1	13.2	14.2	14.9	16.7	15.5	25.7	30.0	31.6	34.7
	14.0	13.7	13.4	12.8	12.7	11.9	7.7	6.3	6.4	6.4
7	286520	274692	225708	207012	205554	204546	166440	158180	156500	41080
	0.0	0.0	4.2	5.7	4.6	3.0	19.3	20.7	17.8	37.0
	14.0	14.2	13.6	13.0	12.8	12.3	7.6	6.4	6.4	6.4

(Continued on next page)

Table 4. (Continued)

Days	cm 5	20	30	40	55	70	85	100	115	130
8	367970	354600	360400	327270	321430	358570	271170	256700	253800	235270
	0.0	0.0	0.0	0.0	0.0	0.5	7.6	9.6	9.9	9.5
	13.5	14.5	13.8	13.1	12.6	12.2	7.7	6.4	6.4	6.4
9	338670	332570	321800	323670	330030	331370	334130	333330	336370	337130
	3.3	3.3	3.2	3.3	3.4	3.1	3.2	3.2	3.2	3.3
	10.7	10.7	10.8	10.7	10.4	10.4	9.7	10.0	9.7	9.7
10	393800	401000	412100	402230	400670	412430	369530	370730	371970	374170
	0.6	0.6	0.5	0.8	0.5	0.0	0.0	0.0	0.6	0.5
	14.2	14.2	13.9	13.4	13.2	12.7	6.8	6.3	6.3	6.3
11	431500	442300	411770	434970	427130	448070	398640	387330	394100	369270
	0.6	0.6	0.7	0.5	0.7	0.0	0.5	0.6	0.0	0.6
	13.7	13.8	13.2	12.7	12.3	11.8	7.5	6.6	6.6	6.6
12	--	--	--	--	--	--	--	--	--	--
	--	--	--	--	--	--	--	--	--	--
	--	--	--	--	--	--	--	--	--	--
13	465730	476300	459630	473270	500600	504130	435900	438499	421030	363730
	0.8	0.8	0.7	0.6	1.0	0.5	0.6	0.6	0.6	1.5
	13.0	13.7	13.7	13.1	12.6	12.3	7.8	6.6	6.5	6.5
14	428800	451430	432000	439470	443130	452270	407730	412130	408170	280230
	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	13.0	13.4	13.3	12.8	12.2	11.7	7.8	6.5	6.5	6.5

[a] *Cyclotella nana*: cell numbers/ml [b] Nitrate concentration: μM [c] Temperature: Centigrade

personal communication). At 130 cm, the sharp increase following the fifth day was caused by adding nitrate after sampling. This operation had no affect upon the nitrate concentrations of the immediately shallower depths. The nitrate concentration declined sharply after the third and seventh days. This may be the result of cellular uptake in the dark.

Nitrate concentration was stratified across the thermocline from the fourth to the eighth days, when the column was homogeneously mixed. However, such low levels of nitrate were present at the time of complete mixing that a secondary stratification of nitrate was not established prior to final nitrate depletion. Following sampling on the eleventh day, nitrate was included in the medium added to the column. This resulted in an increase in nitrate at 130 cm on the thirteenth and fourteenth days. A period of three days did not show a subsequent increase at any other depths.

Cell numbers/ml

The surface (Figure 11, Table 4) for these data does not adequately depict actual events during the course of the experiment. One notable feature that does appear, however, is the anomalous behavior at 130 cm. The inverse correlation with nitrate concentration at this depth is obvious. Since the anomalous behavior of nitrate was previously attributed to experimental control, it is tempting to

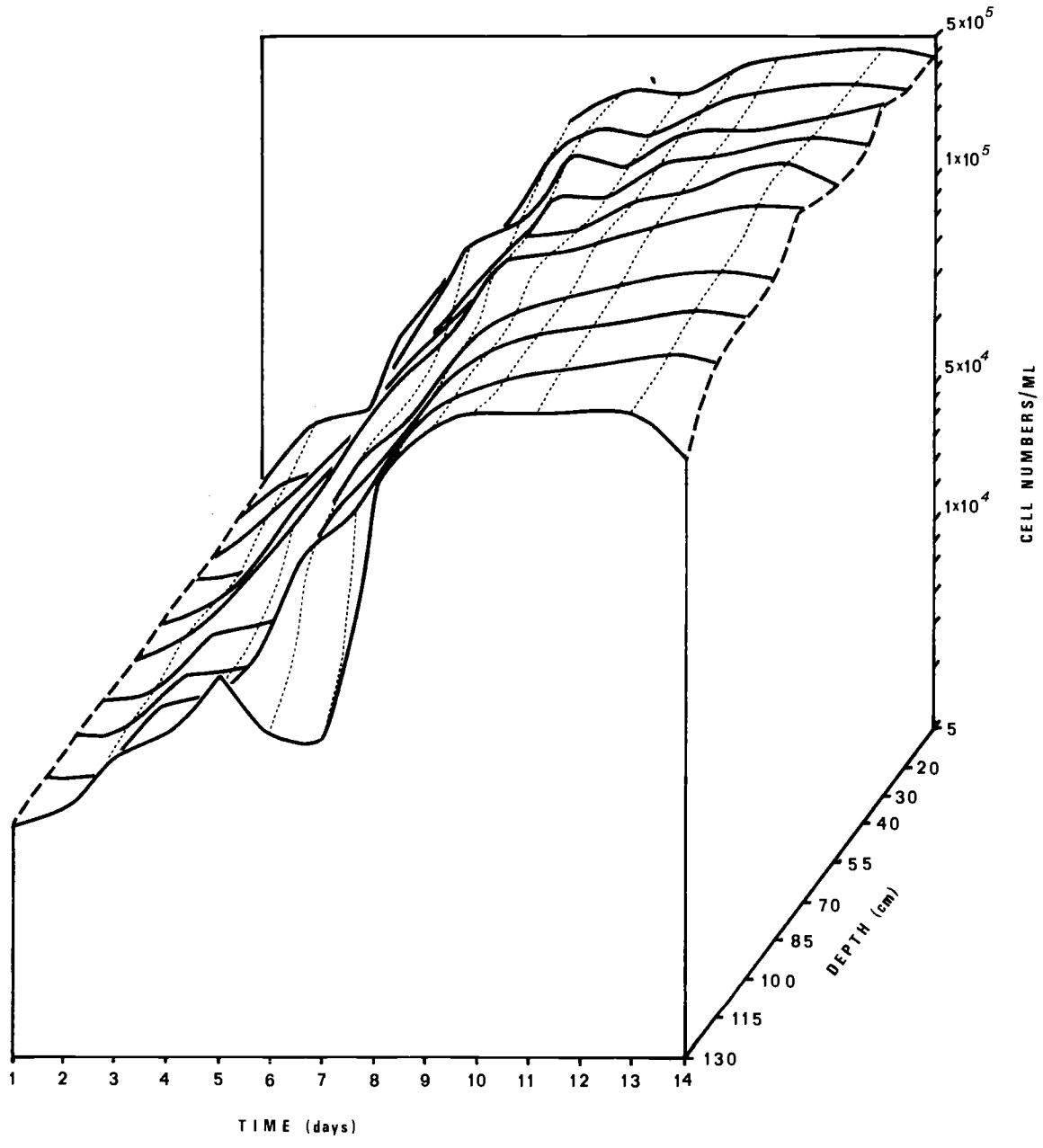

Figure 11. Experiment II: cell numbers/ml as a function of depth and time.

explain cell numbers at this depth in a similar fashion. Two contradictory, mechanistic explanations satisfy the data. The explanations are simply that (a) increased nitrate increased the sinking rate of the cells at 130 cm, and (b) increased nitrate increased the buoyancy of the cells at this depth. The latter mechanism is, of course, of greater selective advantage than the former (Steele and Yentsch, 1960). In the latter case, a population of cells might deplete the surface waters of nitrate, sink, encounter nitrate-enriched water near the thermocline and float to the surface again. Further experimental research is required in order to eliminate one or both of these suggested explanations.

Further examination of the data reveals the effects of the temperature perturbation noted above. As early as the second day, a stratification in the upper depths was detectable. However, on the third day, when the refrigeration system failed, the column was homogeneous with respect to cell numbers. On the fourth day, after normal temperature structure had returned to the column, stratification also returned and remained as a detectable feature until the day of complete mixing. As in the previous experiment, the gradient of this stratification is steepest across the thermocline, from 70-85 cm.

An interesting event occurred as a result of completely mixing the column. The tabular data show that a secondary bloom once again occurred in the column and that a certain stratification of cell numbers

resulted from this bloom. This stratification can be seen as early as the day after complete mixing. However, examination of the nitrate tabular data shows that nitrate concentration was zero at all depths on the tenth day, the day following mixing. Furthermore, since cell numbers increased at 5 cm until the thirteenth day of the experiment, three days following depletion of nitrate at that depth, the existence of an internal pool of nitrate within the organisms (Caperon, 1968) is substantiated.

Experiment III

Temperature

These data (Figure 12, Table 5) are, once again, similar to the preceding temperature surfaces. Two temperature pulses, however, can be seen on the fourth and eighth days. The pulse on the eighth day is attributed to temporary malfunction of the daily surface turbulence system. The pulse on the fourth day has no clear explanation. The importance of these two pulses will be discussed below.

Nitrate

This surface (Figure 13, Table 5) is incomplete in that data from 130 cm are omitted. Following sampling on the fifth day, large quantities of nitrate were added to the column at the bottom. The nitrate behavior of station 9 (115 cm) over time indicates that this

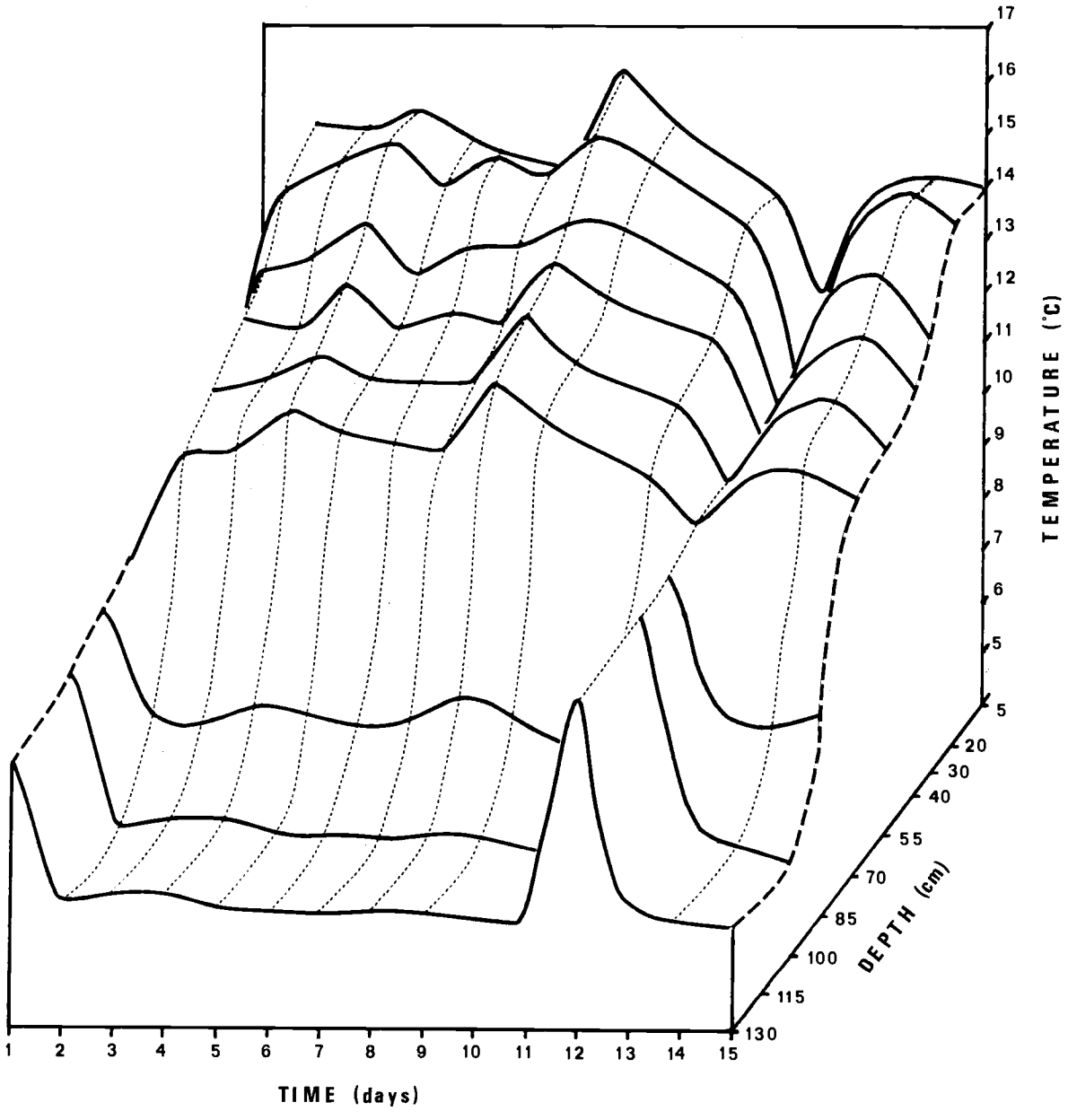

Figure 12. Experiment III: temperature as a function of depth and time.

Table 5. Data for experiment III.

Days	cm 5	20	30	40	55	70	85	100	115	130
1	2060[a]	2032	1996	2108	2010	2016	2026	2010	2120	1942
	24496[b]	27658	27534	27928	27936	27944	27440	28264	28796	28592
	52.7[c]	40.9	--	47.4	44.7	55.2	44.7	46.7	47.0	46.8
	11.1[d]	11.3	11.1	10.8	10.4	10.0	9.8	9.3	--	9.3
2	3768	3336	3108	3042	2892	2976	2466	2430	2580	2418
	60544	62994	49254	40674	40110	42288	36108	30780	30966	35508
	47.0	43.3	44.9	47.0	48.4	43.7	47.9	46.5	43.0	47.4
	15.1	14.6	13.6	13.1	12.5	12.1	7.8	6.4	--	6.5
3	--	3576	3432	--	--	3560	2848	2876	2752	2692
	107436	99384	71952	69756	71736	66660	51300	47448	45408	48096
	38.2	39.7	43.9	43.2	41.9	41.4	48.9	48.0	45.7	47.3
	15.0	14.1	13.8	12.9	12.7	12.1	7.6	6.6	--	6.7
4	4644	4596	3908	4028	4314	4012	3586	3526	3682	3452
	183326	173734	121682	115632	116600	123838	97878	80630	76428	76648
	30.9	31.3	38.0	41.6	39.8	40.4	41.2	45.8	50.0	46.8
	15.4	15.6	14.5	13.8	13.2	12.9	8.0	6.6	--	6.7
5	4688	4664	4722	4660	4478	4678	4452	4604	4372	4396
	242336	238560	215488	201334	203584	196388	181632	160352	157888	135136
	20.0	21.7	23.9	24.7	25.0	28.4	34.4	36.5	38.4	58.9
	14.8	14.7	13.5	12.9	12.7	12.5	7.8	6.3	--	6.4

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Table 5. (Continued)

Days	cm 5	20	30	40	55	70	85	100	115	130
6	6096	5644	5240	5382	5460	5340	5170	5182	5282	4576
	407778	388752	309330	295386	294084	288960	270816	246078	247758	127638
	1.6	2.2	13.3	17.7	17.7	15.2	25.2	--	28.8	275.3
	14.5	15.3	14.1	13.2	12.7	12.3	7.6	6.3	--	6.4
7	7176	6894	6120	6286	6372	6154	5826	5846	5822	4670
	552864	484484	449956	437424	437164	454324	399672	383812	386724	158912
	2.3	0.9	1.1	2.2	1.7	2.3	16.5	18.6	17.9	316.3
	14.3	14.9	14.0	13.0	12.6	12.1	7.7	6.2	--	6.3
8	8418	8582	8036	8412	8348	8472	7686	7542	7702	5224
	593340	595262	583358	608344	588876	590860	560108	525822	532208	163122
	4.5	1.4	3.9	2.5	2.3	1.9	6.8	4.8	10.0	341.4
	16.3	15.7	14.6	14.1	14.0	13.5	8.2	6.3	--	6.4
9	9988	9558	9120	9390	9544	9084	8768	9168	8950	6296
	674322	697476	644742	714000	656778	658512	629850	630258	628218	214302
	0.6	0.0	0.9	0.7	1.3	0.8	0.0	0.0	0.8	332.2
	15.1	15.2	14.4	13.5	13.0	12.7	7.7	6.2	--	6.3
10	--	--	--	--	--	--	--	--	--	--
	--	--	--	--	--	--	--	--	--	--
	--	--	--	--	--	--	--	--	--	--
	--	--	--	--	--	--	--	--	--	--

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Table 5. (Continued)

Days	cm 5	20	30	40	55	70	85	100	115	130
11	10776	10054	9865	10796	11592	10822	11228	11694	10150	8862
	712878	703596	724812	740112	762552	752250	750822	828444	710022	374952
	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	292.6
	13.8	14.1	13.5	12.9	12.4	11.7	7.2	5.8	--	6.1
12	--	--	--	--	--	--	--	--	--	--
	--	--	--	--	--	--	--	--	--	--
	--	--	--	--	--	--	--	--	--	--
	11.1	11.0	10.8	10.9	10.6	10.7	10.5	10.5	--	10.5
13	--	--	--	--	--	--	--	--	--	--
	--	--	--	--	--	--	--	--	--	--
	--	--	--	--	--	--	--	--	--	--
	--	--	--	--	--	--	--	--	--	--
14	17180	17076	17586	17654	18202	27198	17270	18168	17400	17582
	965022	983178	1022652	966042	1023978	1021836	983688	1013472	1020510	1003578
	0.0	0.6	1.4	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	14.1	14.6	13.5	12.8	12.4	11.8	7.6	6.1	--	6.2
15	18080	15184	17400	17778	18236	17260	18066	20184	19182	17880
	971244	1021938	1026630	1062738	1085178	1020204	1011228	1103334	1051110	987768
	0.8	0.6	1.7	0.0	0.7	0.0	0.0	0.0	1.1	2.0
	13.9	14.0	12.3	11.7	11.3	11.2	7.9	5.8	--	6.2

[a] Thalassiosira nordenskjoldii: cell numbers/ml

[b] Cyclotella nana: cell numbers/ml

[c] Nitrate concentration: μM

[d] Temperature: Centigrade

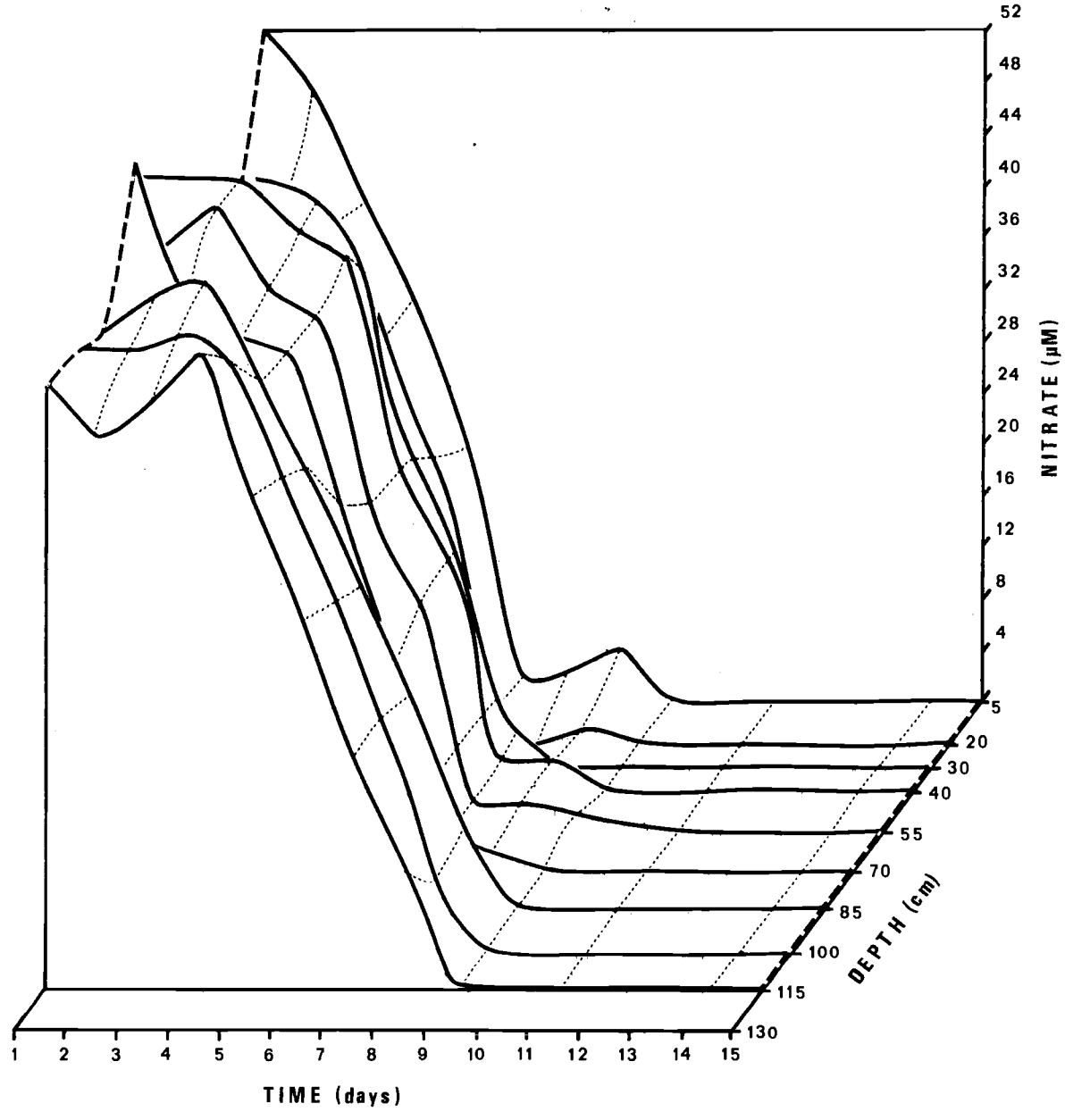

Figure 13. Experiment III: nitrate concentration as a function of depth and time.

method of nitrate addition had no perceivable affect upon the shallower depths of the column. Hence, as a means of maintaining high nitrate concentrations over the entire aphotic zone this methodology was unsuccessful.

Considering the surface as it is plotted, the variability over depth of the initial conditions will be noted. It might be questioned whether these differences between depths actually existed or were attributable to errors in the analysis. The fact that the differences were almost completely eliminated by the second day might lend credence to the latter alternative. However, daily standardization of the nitrate analysis technique was acceptably precise over the entire duration of the experiment, a fact which tends to support the conjecture that the variability was a real phenomenon. If so, this variability is unexplained.

With regard to the remainder of the surface, it is, in general, quite similar to the nitrate data of the second experiment. In particular, nitrate concentration was depleted to zero at all depths by the ninth day (with the exception of 130 cm, which had an external input). Also interesting is the nitrate response on the fourth and eighth days. On the fourth day, at depths 40, 55, and 70 cm, a marked reduction in the depletion rate of nitrate is apparent. To a lesser extent this phenomenon is present at 30 and 100 cm. The increase at 115 cm is so extensive it is suggested that it is not a manifestation of the same

phenomenon. On the eighth day a similar event occurred. On this day, however, the change was much more intense, causing a positive increase in the nitrate present in the water, and was much more extensive, occurring at depths 5, 20, 40, 55, and 70 cm.

There are two explanations of these nitrate concentration increases which are essentially different and rest, respectively, upon two events that preceded or accompanied the nitrate increase. These two events are: (a) the temperature pulse, and (b) the absence of turbulence.

Appealing to small, short-term temperature increases as an explanation for nitrate excretion assumes the validity of several reported facts. Among these are: (1) nitrate reductase requires a co-enzyme $NADH_2$ (Kessler, 1964), (2) an internal pool of nitrate sometimes exists within the cell (Caperon, 1968), and (3) nitrate uptake from the internal pool follows Michaelis-Menton kinetics (Caperon, 1968). Given these facts, an explanation for nitrate excretion might be the following. Suppose nitrate freely diffuses across cell membranes, but once inside forms a weak bond with nitrate reductase in the absence of $NADH_2$. The association with nitrate reductase prevents the nitrate molecule from diffusing to the outside of the cell, thereby creating an internal pool of nitrate. The kinetics of nitrate uptake and assimilation will be dependent on the availability of nitrate reductase and $NADH_2$, respectively. These

conditions should indicate a Michaelis-Menton process, both for uptake and assimilation (where the latter is a function of the internal pool) (Roelofs, 1971). The presence of the cell membrane may, however, mediate the kinetics of uptake. Since a weak bond between nitrate and nitrate reductase is hypothesized, the effect of temperature pulses may be quite dramatic. If such a pulse were sufficient to disrupt this bond, releasing free nitrate into the interior of the cell, then in low nitrate environments a positive gradient toward the exterior of the cell would be established and nitrate would diffuse out.

An alternative explanation based on the absence of turbulence is also constructable. It does not seem likely that the motion itself, as movement through water, will be a factor controlling the physiological state of the cell. However, within the upper 30 cm of the column, turbulence served to prolong the duration a given cell spent in high light conditions. Hence, the absence of turbulence would indirectly, by increasing sinking rates, subject the cells to worsened light conditions, thereby affecting their physiological states. Although it is almost certain that these effects did occur concomitantly with a temperature increase, describing a mechanism whereby the former, independent of temperature, may have occasioned an excretion of nitrate is not trivial. Rather than searching for independent explanations, a more realistic viewpoint would be to suppose that both increased temperatures and worsened light conditions played a role in

causing this nitrate excretion. Obviously, this problem is subject to experimental enquiry; the most important enquiry being directed to the question of the reproducibility of the results.

Cell numbers /ml: Thalassiosira nordenskjoldii

The slightly depressive effect of high nitrate concentrations on the number of cells /ml at 130 cm is apparent on the tabular data (Figure 14, Table 5). The effect seems to be less pronounced than in the case of C. nana, below. The data show only a relatively weak stratification of the population with depth. The photic zone remained essentially homogeneous throughout the experiment. A slight gradient of cell numbers across the thermocline is evident, but the difference between the aphotic and photic zones rarely exceeded 1000 cells /ml. From the seventh to the ninth day, there was a relative increase in the rate of cell number accumulation at almost every depth. This is followed by a decline in the rate of increase between the ninth and eleventh days. This effect correlates well with the failure of the surface stirring system and may perhaps be accounted for by increased sinking rates.

To approximately quantify this effect, consider station 7 at 85 cm. Between the sixth and seventh days, cell numbers /ml increased by about 650 cells /ml. Between the seventh and eighth days, cell numbers increased by about 1650 cells /ml. Granting that station to

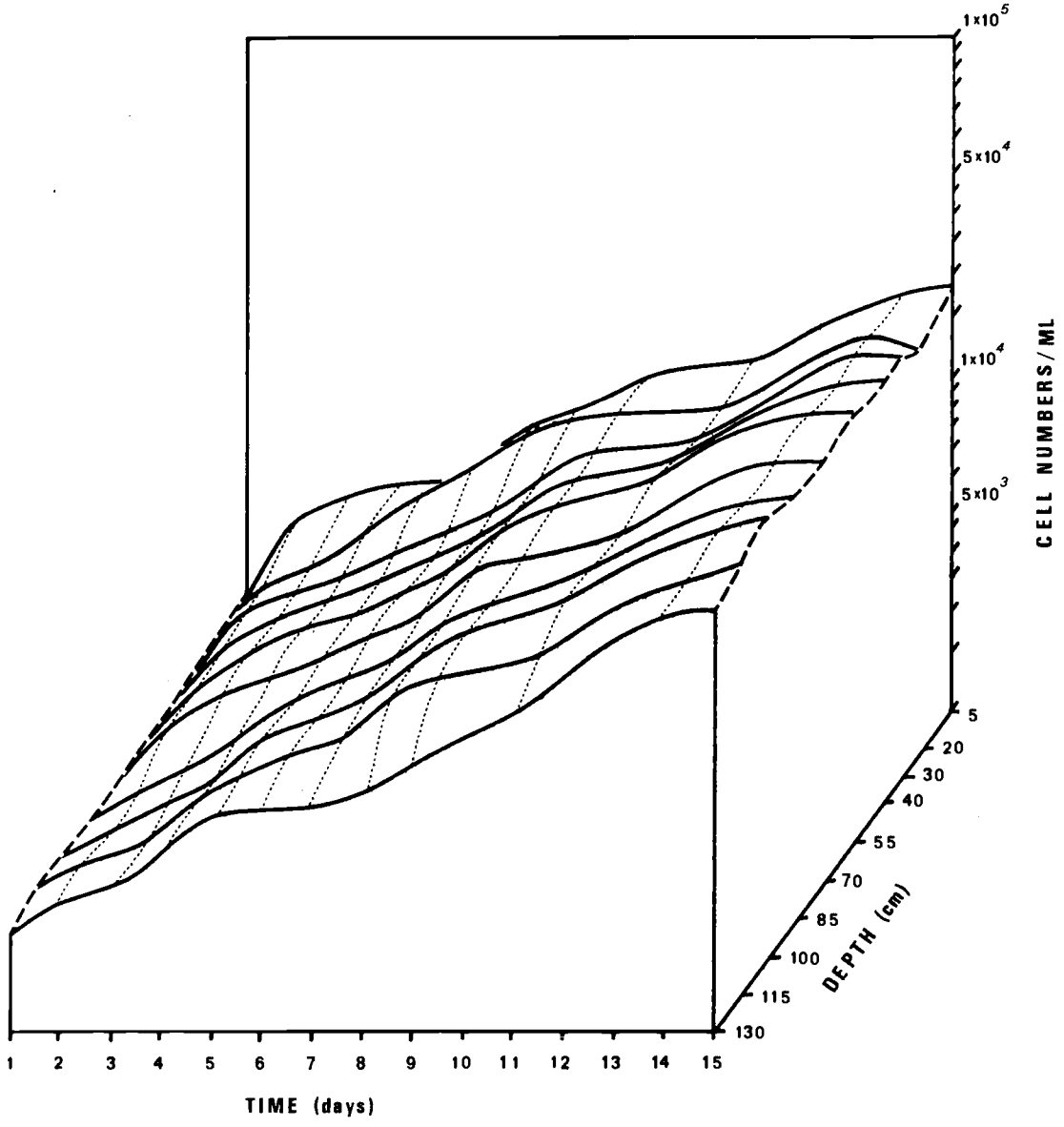

Figure 14. Experiment III: cell numbers/ml: T. nordenskjoldii as a function of depth and time.

station variability is important, it nonetheless appears that failure of the stirrer caused an increase in the rate of increase at 85 cm by about 1000 cells/ml $(days)^{-1}$. By forming the ratio between the before and after increase rates, we may compare the effects with C. nana below. For T. nordenskjoldii, the ratio is 1650/650 = 2.53.

Cell numbers/ml: Cyclotella nana

The surface for these data (Figure 15, Table 5) shows the previously noted effect that high nitrate concentrations depress cell numbers at 130 cm. Nitrate concentration increased at 130 cm following the fifth day. Also, although not well represented on the surface, significant population structure existed over the length of the column until the seventh day. This structure took the expected form of very high cell numbers in the upper 30 cm underlain by moderately high numbers 30-70 cm. Below both these cells and the thermocline, there were populations within the depth interval 70-140 cm which had the fewest cells/ml.

On the eighth day, when the stirring mechanism failed, the deepest stations showed a more rapid rate of accumulation as compared with previous days. As a rough measure of this effect, again consider station 7 at 85 cm. From day 6 to day 7, cell numbers/ml increased by about 129,000 cells/ml. From day 7 to day 8, this increase was about 161,000 cells/ml. So at least for the time periods under

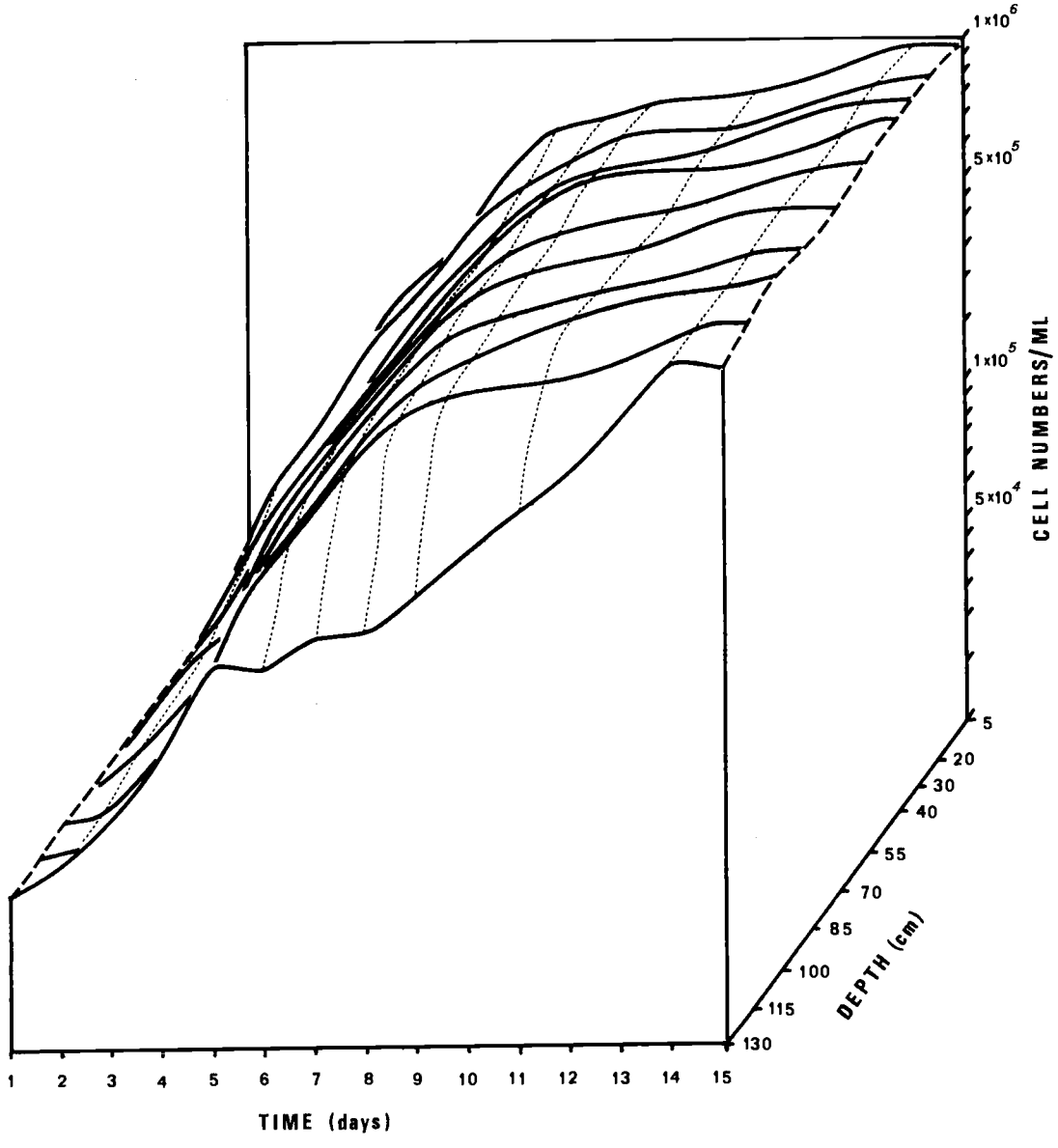

Figure 15. Experiment III: cell numbers/ml: C. nana as a function of depth and time.

consideration the failure of the stirrer apparently increased the rate of increase at 85 cm by 32,000 cells/ml(day)$^{-1}$. Similar calculations could be made at other depths. In order to compare this effect with T. nordenskjoldii, note that 161,000/129,000 = 1.24. Since the same ratio for T. nordenskjoldii was 2.53, it seems that the absence of surface turbulence had a greater affect on T. nordenskjoldii than on C. nana. This conforms with the fact that the latter has a much lower mean sinking rate.

DISCUSSION

The experiments presented here suggest that meaningful comparisons between them can be made. In what follows, comparisons of the nitrate and cell numbers/ml data are made between the experiments. In addition, the results of Margalef (1963b) are compared with those presented here.

Nitrate

The difference between the data surfaces of experiments I and II indicates the fundamental nitrate uptake characteristics of the two species. This difference manifests itself most visibly in the differing abilities of the two species to take up nitrate in low light conditions. In addition, after starting from almost identical initial conditions the nitrate concentration in the photic zone of experiment I was zero after seven days and in experiment II was zero after eight days. As will be indicated below, since C. nana had a much higher rate of increase of cell numbers for the seven day period than did T. nordenskjoldii, the slower rate of nitrate depletion for C. nana may appear anomalous. However, it will be recalled that T. nordenskjoldii has a mean cell volume 12 times that of C. nana. As a consequence of this fact, the T. nordenskjoldii population increased its volume almost four times the volume increase of the C. nana population. It has been suggested

that environmental nitrate depletion is a function of an internal pool, the size of which is presumably related to total cell volume. Increased surface area, of course, accompanies increased volume so it is not surprising that T. nordenskjoldii depleted the environmental nitrate more rapidly.

In experiment III, the nitrate surface resembles quite closely that of experiment II. This would seem to indicate that C. nana in this instance had the greater impact on the environment. It will be noted below that C. nana had a much higher rate of increase of cells / ml in experiment III than in experiment II. Since T. nordenskjoldii did not show a similar proportional improvement from experiment I to experiment III, the cell volume increase for C. nana in experiment III is more nearly equal to the volume increase of T. nordenskjoldii in experiment III.

Cell numbers /ml

Some interesting comparisons can be made if the assumption is made that at station 1 (5 cm) the increase of cell numbers is a result of growth only. That is, there was no sinking from a higher level. This allows the computation and comparison of relative amounts of increase over a given period of time. By calculating the relative increase as N_7/N_0, where N_0 is the cell numbers /ml at the initiation of the experiment and N_7 is the cell numbers /ml at the end of seven days, the three experiments show the following trends (Table 6).

Table 6. Comparison between experiments of relative increase (cell numbers/ml).

Experiment	Organism	Relative increase
I	T. nordenskjoldii	3.3
II	C. nana	11.2
III	T. nordenskjoldii	3.5
III	C. nana	22.5

The remarkable change in the relative increase of C. nana from experiment II to experiment III is suggested to be a positive species interaction. The mechanism for this proposed interaction is not known. Fogg (1965) suggests that glycolic acid has a positive affect upon growth. Further experimental analysis is required in order to determine what mechanism, if any, is operating in this case.

Margalef (1963b)

Comparisons with the paper by Margalef are difficult to make. The spatio-temporal distribution of phytoplankton is strongly dependent upon the distribution of physical parameters. Margalef's distributions were significantly different from those presented here. Notable among these differences are light and temperature. Hence, it will not be surprising if phytoplankton distribution differences exist. In addition, Margalef was interested primarily in species interactions, so none of his experiments are done with only one species of phytoplankton.

One clear result from Margalef's work that is consistent with my data is the problem of sinking rates. Working with much quantitatively smaller inoculations of Skeletonema costatum, Margalef reports that about two weeks after inoculation the population is in decline. He shows that adding nutrients and turbulence allows the population to increase at almost all depths. These results are similar to the ones reported here.

Comparison of Margalef's multi-species results with experiment III reported here shows two different pictures. Margalef indicates a clear example of species succession in one of his experiments. The sequence of succeeding organisms is S. costatum, Nitzschia closterium, Chlorella sp. Although I noted a positive species interaction in experiment III, the results do not show succession. This does not imply that even under experimental control identical to Margalef's we would not see succession. These experiments with the present miniature sea remain to be done.

In summary, then, the results of Margalef (1963b) do not contradict those reported here. Furthermore, although the experiments conducted by Margalef were of greater ambition it is felt that the design and construction of this more recent model more accurately approximates the scope of the present apparatus.

CONCLUSIONS

In the introduction, two basic questions were raised: "Can the model be made to work?" and "Ought the model be made to work?". Before attempting to answer these questions a brief review of what had been done seems advisable.

A scaled-down model of the oceanic water column has been constructed that incorporates some of the physical features of the real ocean. It has been shown that two species of diatom will grow under the conditions present in the model. Moreover, the apparent rates at which the numbers of diatoms increased were not constant over the length of the column nor over the period of the experiment. In particular, the rates of increase were highest in the upper regions of the column and during the early phases of each of the experiments. In addition, the presence of T. nordenskjoldii apparently has a positive affect on the growth characteristics of C. nana, but that C. nana had no affect on T. nordenskjoldii.

Now, in order to answer the first question we must explicate what we mean by 'to work'. That is, we must establish criteria by which we judge success or failure. There are two general categories of relevant criteria: (a) how closely the model mimics the natural system, and (b) the use to which we may put the model (Warren, 1971). When judging the fidelity of the model we may consider generally its

construction and its response. Clearly the construction of this miniature sea does not embody complete fidelity to nature, as the appellation "miniature" implies. In addition to its small size and the resulting abnormally steep light and thermal gradients, there was no day-night cycle. A more serious abstraction from biological reality is the fact that these experiments were at best autecological studies, making no attempt to incorporate either herbivores or carnivores. The final, and perhaps most important, infidelity was one we originally set out to create. This is the fact that the model is an "almost closed" system to material (Warren, 1971). In the ocean there is a continual flux of matter at almost all depths, but this model assiduously avoids this condition, attempting to restrict the influx of material to only one depth and in the most inobtrusive way possible.

On the other hand, the construction is accurate in some respects. The gradients, although steep, are relationally accurate. That is, the light gradient approaches zero somewhere between the surface and the bottom, and the thermocline is stable near the depth of 1% light transmittance. In addition, the nature and extent of daily surface turbulence is approximately correct. It was horizontal in nature and the turbulence so induced extended to a depth which did not erode the thermocline.

Considering the response of the biological variables in the model it was seen that both realistic and unrealistic results were obtained.

In the latter group, the affect of sinking was greatly exaggerated. Estimates of sinking rates in the ocean are many and varied. Riley, Stommel and Bumpus (1949) reviewed values from the literature ranging from 30 m/day to 0.6 m/day. Steele (1956) suggested a more realistic range is from 13 m/day to 1.5 m/day. Two results are clear. First, sinking is almost certainly a function of both species and physiological state. Secondly, no matter what the actual rates may be, even as little sinking as 0.5 m/day will drastically affect the cells in the model ocean. In one day a cell, starting at the surface and sinking 0.5 m/day, will be subjected to an illumination change in excess of 50%. One more day will carry the cell well below the thermocline into a region of zero illumination. In addition to the infidelity of what to the cell appears as accelerated sinking is the problem of maintaining high nitrate concentrations below the thermocline. It was previously noted that adding nitrate to the column at the bottom increased the nitrate concentration at 130 cm but not at any other depths. Eddy diffusivity coefficients are apparently quite low at the bottom of the column.

The phytoplankton response was realistic in some ways. The relative temporal sequence of events duplicated nature in a qualitative sense. That is, the population increased until the nitrate was depleted and then sank. The population response to turbulence was also similar to that which occurs at sea. Daily, moderate stirring kept

some of the cells in suspension near the surface and the occasional, intense stirring created a secondary bloom.

With regard to the second category of applicable criteria, the model's utility, I perceive three possible uses for such a model. Briefly they are: (1) the model allows us to avoid at least some of the problems of the ocean, (2) the model aids us in theory building by suggesting relevant sampling and laboratory experiments, and (3) the model creates data from which we may obtain advanced degrees or publish papers in professional journals. I believe the model presented here meets, to varying degrees, all of these utility criteria. For example, the model eliminates the necessity of coping with the discomfort of the sea's surface dynamics. Also, the spatial localization of the population eliminates the difficulty of returning successively to the location of a specific population. The model also suggests laboratory experiments, e. g., the studies of sinking rates and the uptake of nitrate in the dark. Lastly, the third utility criterion is met trivially, as this thesis reveals.

It seems, then, that the model is only partially faithful to nature, either by accident or design. And that it seems to have some practical uses. However, the final question yet remains: Should we do it?

If the model was perfectly faithful to the real ocean then I think we might be safe in concluding that this type of research ought to be

done. Since the model is obviously not perfectly faithful (but is useful), we are faced, as always, with deciding whether or not we should pursue an approach which requires time and energy and only returns partially correct answers. If the use to which we put the answers is only toward the generation of degrees and papers, then the value of the approach may be rejected out of hand. If, by the first utility criterion, the data are valuable solely because they are easier to obtain than "real" data, then it seems our effort might be more profitably spent developing technology applicable to the ocean itself. For example, we might develop more effective means of following populations in the field. In this regard, the earlier discussion of big-bag experiments is directly relevant and may represent a more valuable technique than laboratory models. The second utility criterion is the most crucial to our question.

If we use the model to formulate testable hypotheses then the ratio between the energy exerted and the number of hypotheses generated must be kept below some critical value for the model to be worthwhile. What sorts of hypotheses could be expect from the present model? What amount of energy would be required to test the hypotheses in the model?

One of the first problems we might wish to investigate would be the measure of the statistical variability of sampling in the model. By the arguments presented earlier the best solution is the construction

of a larger system. This would require a large effort, not only in the initial construction but in daily operation as well. It might even require a team of investigators. In my opinion such an expenditure of energy is not offset by the number of hypotheses that accrue. The statistical problem may, however, be overcome in the present model by multiple sampling at only a few stations every day. This would require far less energy than an enlarged model.

Another problem requiring investigation is the sinking rates of the organisms. A solution to this problem is to empirically measure sinking rates in the laboratory outside the column (perhaps photometrically as in Steele and Yentsch, 1960) for each depth, every day. Essentially this adds another analysis to the daily procedure, but it is necessary in order to calculate growth rates.

There are also a whole host of hypotheses that arise and require repeated experiments. Among these are the effects of variable surface turbulence on population dynamics. Also we are interested in nitrate uptake in the dark by phytoplankton. What is the affect of a day-night cycle on the population dynamics? Further, it is desirable to know the effects of varying the initial conditions of the experiments. Particularly, the effects of different rates of the onset of the thermal structure and the effects of different numbers of organisms are of interest. The prospects of synecological studies also raise interesting hypotheses concerning species succession and trophic relations.

Ultimately, if we rule out the construction of a larger model as inefficient, the problem reduces to some number of repeated experiments for every hypothesis. The number of experiments is dependent on the question asked. Certainly the sinking rate must always be measured. Turbulence studies would require two or three experiments for every mixing intensity and species. Variable initial conditions would require more experiments since interaction effects undoubtedly arise. Synecological studies are the most complex. I suggest these latter types of experiments, with the possible exception of simple multiple phytoplankton species studies, represent investigations beyond the scope of the present apparatus.

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