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The Impact of Filter Techniques On Chlorophyll Determinations and Size Fractionation

Alison Baker, Karen S. Baker, and Maria Vernet

with investigations regarding:
-Sample Volume
-Filter Rig
-Suction Type
-Biomass Reconstruction

with appendices:
-Phytoplankton Growth, 1996
-Phytoplankton Cellular Changes in Response to Light, 1997

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Alison Baker

University of California, San Diego
Scripps Institution of Oceanography
UC Marine Bio Optics Group
La Jolla, CA 92093-0218

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Introduction

This study investigates the impacts of filter techniques on chlorophyll size fractionation methods used in the determination of phytoplankton biomass. Although the experiments were carried out in a laboratory, they demonstrate the validity of currently used field measurement techniques. Ocean water samples were taken on three separate occasions for the purpose of conducting a set of similar studies on different natural phytoplankton populations.

Chlorophyll measurements in general (Clarke and Leakey, 1996; Weber and El-Sayed, 1987) and size fractionation in particular (Glover et al, 1985; Jacques and Panhouse, 1991; Jochem et al, 1995; Parsons, 1972, Sieburth and Smetacek, 1978; Xiuren et al, 1996) have been discussed in the literature over time. Previous work by the author on phytoplankton growth with additional references is found in the appendices (Baker, 1996; Baker, 1997).

Experiments were designed to test some chlorophyll sampling techniques commonly used in the field. Each of the three studies reported here consists of a set of similar experiments involving filter type, filter size, sample rig fritz type, suction type, size fractionation reconstruction, and filter loading.
The samples for this study were taken at times that can be related to a red tide event.

Methods

Water samples were taken from the ocean at the end of the Scripps Institution of Oceanography pier (latitude 32° 52'North and longitude of 117° 15.4' West). Immediately before filtering the samples, the sample bottles were stirred to assure an even amount of phytoplankton in each sample. Both glass and gel filter towers and fritz stems were used for in order to identify any differences due to equipment. There were three filter setups that produced four data records (Figure 1) so results were coded as 1 through 4 when using the glass fritz and 5 through 8 when using the Gelman fritz. The first filter setup put the sample through a .45u filter using a pump. The second setup put the sample through a 20u filter using a pump as well, and the third setup put the sample first through a 20u filter using gravity and the filtrate then through a .45 u filter under vacuum. A Millipore vacuum/pressure pump was used throughout the experiment. The pump was kept at a low pressure (<10psi) so as not to break cells or pull them through the filter.

Three different volumes of sample (25mL, 50mL, 100mL) were used for these investigations in order to consider the impact of sample size on filter loading.

After the chlorophyll from a sample was concentrated on a filter, the filter was placed into a test tube containing 10 mL of acetone. The tube was placed in a cold, dark environment for 24 hours to ensure complete extraction. Undissolved filters were removed from the solution and disposed. A Turner Designs Field

fluorometer (Model TD10) was then used in discrete mode to determine the chlorophyll amount extracted from the sample. Standard chlorophyll fluorescence procedures were followed (Smith et al, 1981; Strickland and Parsons, 1968).

Materials

Filters are classified as either depth or surface filters. Surface filters are known as screen or membrane filters. These are a continuous mesh with a defined pore size, which limits the largest particle that can pass through. The filters retain little liquid but are known to have low particle load capacity, so they clog with high concentration. For this work, two types of Millipore membrane surface filters were used: a nylon net with a pore size of 20 um and a HA mixed cellulose with pore size .45 mm.

Two types of filter towers and bases were used during this experiment: polysulphone made by Gelman (Pall Gelman Sciences) and borosilicate glass made by Millipore (Millipore Corporation). They are called 'gel' and 'glass' for the purposes of this experiment. The Gelman filter funnels have a capacity of 200mL. The standard glass Millipore funnels have 150 mL capacity, but the 300 mL capacity glass funnels used in these experiments were blown by a glass blower. Both towers have fritz bases with a tapered stem that fits into a standard size stopper. The glass tower rests upon the glass fritz and is held in place by an aluminum clamp. The Gelman tower twist-locks onto its matching fritz. Both filter rigs hold 25 mm diameter filters.

Results

Each one of the three studies was run with a sample collected during different oceanic conditions giving a range of biomass and cell types. The first sample was taken in March of 1997 before a red tide; the next sample was taken in May during the peak of a red tide when large dinoflagellates dominate phytoplankton populations; and the last sample was taken in June after the red tide. A red tide, one of the many natural environment oceanic conditions, is a bloom of a phytoplankton species known as Gonyaulax.

The results of the three studies are summarized in Tables 1 through 3. The columns in the tables represent the following: The sample number, the year, month, and day the sample was taken, the amount of chlorophyll determined to be in the sample, the amount of phaeopigment determined to be in the sample, volume of sample filtered, the volume of acetone used for extraction, the scale and door used on the fluorometer during sample reading, the machine calibration and Tau factors, the fluorescence before and after acid (HCl) was added, the code indicating setup type from Figure 1, and a comment column indicating setup and sample volume. These columns are followed by the average chlorophyll and standard deviation for the three replicates. The eight coded experiments are listed in order giving each of the three sampled volumes (25,50,100mL) for each code type.

In general, each measurement was made with three replicates in order to assure reliable results. Table 4 gives a summary of the three sample results. The replicate averages are shown in the last two columns of Tables 1-3. The March replicates are biased by settling that is know to have occurred with time. This lack of sample mixing resulted in an average standard deviation of 0.49 (range:

1.65-0.07) given an average chlorophyll for the March samples of 2.14. The average chlorophyll for the May sample was 27.88 with an average standard deviation of 1.35 (range: 5.78-0.05). The average chlorophyll for the June sample was 1.28 with an average standard deviation of .09 (range: 0.30-0.02).

The full summary of Tables 1 through 3 is given in Table 5 in order to examine the effects of filter loading, compare the use of glass and Gelman fritz, to consider the use of gravity versus that of a pump, and the reconstruction from filter fractionation. The initial columns of Table 5 are: the date on which the sample was taken, the type or code of the experiment, and then five columns describing the glass experiments, followed by the same information for the Gelman experiments. The five columns are: the chlorophyll of the three different sub sample sizes, followed by the average, and the standard deviation. For each of the three dates, five rows are included representing the results of the four types of experiments run plus a reconstruction as described below.

Because of the incomplete mixing of samples in March, these results are suspect and have been separated in the table as a reminder. The March results are not included in the subsequent analysis.

Sample Volume: Figures 2 show the chlorophyll for the four code types for glass (codes 1-4) and for Gelman (codes 5-8). The two sampling timeframes are represented with results for May glass and Gelman (Figures 2a and 2b, respectively) and June (Figures 2c and 2d. May samples from Table 5, when biomass was high (approximately 30 mg/m3) and cells were large (97%), show that the use of higher volumes of sample (100 ml rather than 25 ml) results in a decrease in chlorophyll determination of approximately 20%. The increased flow through the filters increases the amount of chlorophyll that escapes through the filter. In June, when biomass is lower (approximately 1 mg/m3) and cells are smaller (i.e. 28% large cells), this is not the case.

Filter Rig Type: Figures 3a and 3b show that the use of glass versus Gelman type combination filter tower and fritz does not have a significant impact on experimental results. A linear regression in Figure 4 shows the results are in agreement for both high and low biomass sampling.

Suction Type: Comparing the retrieval of biomass on the 20 micron filter under vacuum of 10 psi (codes 2 and 6) versus gravity (codes 4 and 8) shows that gravity gives higher values ranging from approximately 5% higher values for high biomass samples and 40% more for low biomass sampling. One must consider that a vacuum may pull on cells causing breakage and release of chlorophyll through the filter resulting in a lower biomass estimate. Further, smaller cells may be stacked in samples so that a vacuum dislodges the cells.

Reconstruction: Size fractioned samples are frequently added to give a total biomass estimate. In this work two identical samples were filtered, one directly on a single filter and the other size fractioned onto two filters. That is, one sample was filtered onto a 0.45 micron filter (code 1 for glass and 5 for Gelman) to give total biomass. Another sample was filtered using a 20 micron filter and the filtrate was then filtered through a 0.45 micron filter (code 4 then 5 for glass; code 8 then 7 for Gelman). These two cases are shown in Table 6 by the table lines labeled [0.45u] and the calculated total of [(0.45u - 20u) + 20u]. A comparison for May and June shows good agreement for both high and low biomass with the maximum discrepancy being 5% higher values using the reconstruction method (and gravity feed) in the high biomass case.

Table 6 further characterizes the three separate sampling events by taking the ratio of the biomass found on the 20u filter to that found on the 0.45 filter giving an indication of the percent of large cells present. Note that 61% of the cells were large just prior to the red tide, 97% during the red tide and 28% after the red tide.

Discussion

The sample design permitted a variety of lessons to be learned with respect to chlorophyll filter techniques:

- Samples must be well mixed before a given amount is sub sampled for chlorophyll extraction.
- Caution is necessary with respect to the volume of sample filtered (i.e. 25 ml vs 100 ml) when biomass values are high (>10 mg/m3).
- Glass and Gelman filter rigs give similar results for biomass sampling.
- Attention to maintain low vacuum pressure (<10 psi) is important. With the vacuum pump adjusted to 10 psi, it was shown that particulate chlorophyll is underestimated by 5-20% compared to filtering by gravity.
- Estimation of total biomass is good in the case of reconstruction by size fractions using the two sizes of [>0.45 to 20 microns] and [>20 microns].
- Differences are small in size fractionation results using stacked and nonstacked configurations especially if filter loading is avoided by keeping sample volumes small in high biomass situations.

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Tables

Table 1. Filter Experiments (27Mar97)

SIO pier sample

#	Year	Mo	Day	Chla	Phaeo	VolF	VolA	Scale	Door	Fd	Tau	Rb	Ra	Code	Comments	Avg	Std
1	1997	03	27	4.39	1.38	25	10	1	31.6	0.144	1.929	2.80	1.64	1	.45u glass (25)	4.09	0.26
2	1997	03	27	3.90	1.45	25	10	1	31.6	0.144	1.929	2.55	1.52	1			
3	1997	03	27	3.97	1.05	25	10	1	31.6	0.144	1.929	2.48	1.43	1			
4	1997	03	27	3.53	1.19	50	10	1	10	0.144	1.929	1.44	0.85	1	.45u glass (50)	3.83	0.26
5	1997	03	27	4.01	1.05	50	10	1	10	0.144	1.929	1.58	0.91	1			
6	1997	03	27	3.95	1.28	50	10	1	10	0.144	1.929	1.60	0.94	1			
7	1997	03	27	4.54	0.38	100	10	1	3.16	0.144	1.929	1.04	0.56	1	.45u glass (100)	4.22	0.47
8	1997	03	27	3.68	1.24	100	10	1	10	0.144	1.929	3.00	1.77	1			
9	1997	03	27	4.45	1.35	100	10	1	3.16	0.144	1.929	1.13	0.66	1			
10	1997	03	27	2.65	0.23	25	10	1	31.6	0.144	1.929	1.52	0.82	2	20u glass (25)	2.30	0.34
11	1997	03	27	2.27	0.12	25	10	1	31.6	0.144	1.929	1.28	0.68	2			
12	1997	03	27	1.97	0.07	25	10	1	31.6	0.144	1.929	1.10	0.58	2			
13	1997	03	27	2.29	0.17	50	10	1	31.6	0.144	1.929	2.61	1.40	2	20u glass (50)	2.38	0.21
14	1997	03	27	2.23	0.11	50	10	1	31.6	0.144	1.929	2.51	1.33	2			
15	1997	03	27	2.63	0.24	50	10	1	31.6	0.144	1.929	3.02	1.63	2			
16	1997	03	27	3.59	0.38	100	10	1	10	0.144	1.929	2.63	1.43	2	20u glass (100)	3.13	0.41
17	1997	03	27	2.78	0.36	100	10	1	10	0.144	1.929	2.06	1.13	2			
18	1997	03	27	3.02	0.34	100	10	1	10	0.144	1.929	2.22	1.21	2			
19	1997	03	27	1.36	1.24	25	10	1	31.6	0.144	1.929	1.10	0.74	3	.45u-20u glass (25)	1.20	0.27
20	1997	03	27	0.89	0.91	25	10	100	1	0.144	1.929	2.36	1.62	3			
21	1997	03	27	1.34	1.47	25	10	100	1	0.144	1.929	3.65	2.53	3			
22	1997	03	27	0.91	1.83	50	10	1	31.6	0.144	1.929	2.04	1.56	3	.45u-20u glass (50)	0.87	0.07
23	1997	03	27	0.91	1.27	50	10	1	31.6	0.144	1.929	1.72	1.24	3			
24	1997	03	27	0.79	0.72	50	10	1	31.6	0.144	1.929	1.28	0.86	3			
25	1997	03	27	0.54	0.69	100	10	1	31.6	0.144	1.929	1.97	1.40	3	.45u-20u glass (100	0.48	0.08
26	1997	03	27	0.51	0.75	100	10	1	31.6	0.144	1.929	1.97	1.43	3			
27	1997	03	27	0.40	0.66	100	10	1	31.6	0.144	1.929	1.62	1.20	3			
28	1997	03	27	1.85	0.29	25	10	1	31.6	0.144	1.929	1.10	0.61	4	20u gravity (25)	1.62	0.31
29	1997	03	27	1.74	0.05	25	10	1	31.6	0.144	1.929	0.97	0.51	4			
30	1997	03	27	1.27	0.20	25	10	100	1	0.144	1.929	2.38	1.32	4			
31	1997	03	27	1.42	0.11	50	10	1	31.6	0.144	1.929	1.62	0.87	4	20u gravity (50)	1.73	0.84
32	1997	03	27	2.69	0.32	50	10	1	31.6	0.144	1.929	3.13	1.71	4			
33	1997	03	27	1.10	0.12	50	10	1	31.6	0.144	1.929	1.27	0.69	4			
34	1997	03	27	1.08	0.13	100	10	1	31.6	0.144	1.929	2.51	1.37	4	20u gravity (100)	0.67	0.35
35	1997	03	27	0.47	0.07	100	10	1	31.6	0.144	1.929	1.12	0.62	4			
36	1997	03	27	0.46	0.08	100	10	1	31.6	0.144	1.929	1.11	0.62	4			
37	1997	03	27	3.25	1.25	25	10	1	31.6	0.144	1.929	2.14	1.28	5	.45u gel (25)	2.51	0.92
38	1997	03	27	2.80	1.31	25	10	1	31.6	0.144	1.929	1.91	1.17	5			
39	1997	03	27	1.48	2.00	25	10	1	31.6	0.144	1.929	1.38	0.99	5			
40	1997	03	27	0.44	3.15	50	10	1	31.6	0.144	1.929	2.27	2.04	5	.45u gel (50)	1.51	1.65
41	1997	03	27	0.68	0.67	50	10	1	31.6	0.144	1.929	1.13	0.77	5			
42	1997	03	27	3.41	2.81	50	10	1	10	0.144	1.929	1.69	1.12	5			
43	1997	03	27	3.69	1.67	100	10	1	3.16	0.144	1.929	1.00	0.61	5	.45u gel (100)	2.88	0.83
44	1997	03	27	2.93	0.04	100	10	1	10	0.144	1.929	2.05	1.07	5			
45	1997	03	27	2.03	1.05	100	10	1	10	0.144	1.929	1.79	1.11	5			
46	1997	03	27	2.61	0.45	25	10	1	31.6	0.144	1.929	1.56	0.87	6	20u gel (25)	2.17	0.48
47	1997	03	27	1.67	0.23	25	10	1	31.6	0.144	1.929	0.98	0.54	6			
48	1997	03	27	2.23	0.19	25	10	1	31.6	0.144	1.929	1.28	0.69	6			
49	1997	03	27	2.25	0.24	50	10	1	31.6	0.144	1.929	2.61	1.42	6	20u gel (50)	1.77	0.55
50	1997	03	27	1.87	0.18	50	10	1	31.6	0.144	1.929	2.16	1.17	6			
51	1997	03	27	1.17	0.07	50	10	1	31.6	0.144	1.929	1.33	0.71	6			
52	1997	03	27	1.50	0.20	100	10	1	10	0.144	1.929	1.11	0.61	6	20u gel (100)	1.66	0.20
53	1997	03	27	1.61	0.16	100	10	1	10	0.144	1.929	1.18	0.64	6			
54	1997	03	27	1.88	0.09	100	10	1	10	0.144	1.929	1.34	0.71	6			
55	1997	03	27	1.80	0.91	50	10	1	31.6	0.144	1.929	2.49	1.54	7	.45-20u gel (50)	1.53	0.23
56	1997	03	27	1.42	1.43	50	10	1	31.6	0.144	1.929	2.37	1.62	7			
57	1997	03	27	1.38	1.85	50	10	1	31.6	0.144	1.929	2.57	1.84	7			
58	1997	03	27	1.14	1.81	100	10	1	10	0.144	1.929	1.44	1.06	7	.45-20u gel (100)	1.24	0.09
59	1997	03	27	1.26	1.55	100	10	1	10	0.144	1.929	1.43	1.01	7			
60	1997	03	27	1.32	1.91	100	10	1	10	0.144	1.929	1.60	1.16	7			
61	1997	03	27	0.63	0.06	50	10	100	1	0.144	1.929	2.28	1.23	8	20u gravity (50)	1.36	0.64
62	1997	03	27	1.65	0.43	50	10	1	31.6	0.144	1.929	2.05	1.18	8			
63	1997	03	27	1.80	0.29	50	10	1	31.6	0.144	1.929	2.14	1.19	8			
64	1997	03	27	2.51	0.60	100	10	1	10	0.144	1.929	1.96	1.12	8	20u gravity (100)	3.99	1.31
65	1997	03	27	5.01	0.79	100	10	1	3.16	0.144	1.929	1.19	0.66	8			
66	1997	03	27	4.45	1.27	100	10	1	3.16	0.144	1.929	1.12	0.65	8			
				2.176												2.143	0.4901

Table 2. Filter Experiments (12May97)

SIO pier sample (during red tide)

#	Year	Mo	Day	Chla	Phaeo	VolF	VolA	Scale	Door	Fd	Tau	Rb	Ra	Code	Comments	Avg	Std	
64	1997	05	12	37.98	2.09	25	10	1	10	0.155	1.929	6.3	3.35	Ar	.45u glass (25)	39.96	1.25	
60	1997	05	12	39.78	1.60	25	10	1	10	0.155	1.929	6.55	3.46	A				
48	1997	05	12	39.97	1.05	25	10	1	10	0.155	1.929	6.54	3.43	Ar				
46	1997	05	12	40.94	0.92	25	10	1	10	0.155	1.929	6.68	3.5	A				
10	1997	05	12	41.13	2.04	25	10	1	10	0.155	1.929	6.81	3.61	A				
65	1997	05	12	36.77	1.93	50	10	1	3.16	0.155	1.929	3.85	2.05	A	.45u glass (50)	37.43	2.11	
59	1997	05	12	35.85	3.51	50	10	1	3.16	0.155	1.929	3.84	2.08	Ar				
55	1997	05	12	36.56	3.55	50	10	1	3.16	0.155	1.929	3.92	2.12	A				
1	1997	05	12	40.54	1.47	50	10	1	3.16	0.155	1.929	4.21	2.22	A				
73	1997	05	12	32.85	2.54	100	10	1	3.16	0.155	1.929	6.97	3.74	Ar	.45u glass (100)	33.45	0.96	
69	1997	05	12	33.66	2.25	100	10	1	3.16	0.155	1.929	7.1	3.8	A				
5	1997	05	12	33.10	0.96	100	10	1	3.16	0.155	1.929	6.85	3.6	A				
7	1997	05	12	33.79	1.04	100	10	1	1	0.155	1.929	2.22	1.17	Ar				
42	1997	05	12	31.83	2.23	100	10	1	3.16	0.155	1.929	6.73	3.6	A				
51	1997	05	12	34.22	1.92	100	10	1	3.16	0.155	1.929	7.18	3.82	A				
54B	1997	05	12	34.73	0.75	100	10	1	3.16	0.155	1.929	7.16	3.75	Ar				
11	1997	05	12	33.67	0.24	25	10	1	10	0.155	1.929	5.45	2.84	B	20u glass (25)	35.63	2.78	
61	1997	05	12	37.59	0.80	25	10	1	10	0.155	1.929	6.13	3.21	B				
47	1997	05	12	28.13	7.27	25	10	1	10	0.155	1.929	5.15	2.96	B	**			
56	1997	05	12	38.70	-0.29	50	10	1	3.16	0.155	1.929	3.93	2.03	B	20u glass (50)	37.21	1.53	
39	1997	05	12	35.65	1.82	50	10	1	3.16	0.155	1.929	3.73	1.98	B				
2	1997	05	12	37.28	1.52	50	10	1	3.16	0.155	1.929	3.88	2.05	B				
66	1997	05	12	23.53	0.79	50	10	1	3.16	0.155	1.929	2.44	1.29	B	**			
52	1997	05	12	34.22	1.54	100	10	1	3.16	0.155	1.929	7.14	3.78	B	20u glass (100)	32.68	1.12	
6	1997	05	12	31.83	0.72	100	10	1	3.16	0.155	1.929	6.57	3.44	B				
43	1997	05	12	32.80	0.51	100	10	1	3.16	0.155	1.929	6.74	3.52	B				
70	1997	05	12	31.88	2.09	100	10	1	3.16	0.155	1.929	6.72	3.59	B				
12	1997	05	12	1.66	0.66	25	10	100	1	0.155	1.929	3.23	1.94	C	.45u-20u glass (25)	1.28	0.33	
50	1997	05	12	1.12	0.50	25	10	100	3.16	0.155	1.929	7.01	4.27	C				
62	1997	05	12	1.06	0.49	25	10	100	3.16	0.155	1.929	6.67	4.08	C				
3	1997	05	12	1.45	0.64	50	10	1	31.6	0.155	1.929	1.81	1.1	C	.45u-20u glass (50)	1.10	0.24	
40	1997	05	12	1.01	0.48	50	10	100	1	0.155	1.929	4.07	2.5	C				
57	1997	05	12	1.03	0.57	50	10	100	1	0.155	1.929	4.26	2.67	C				
67	1997	05	12	0.92	0.47	50	10	100	1	0.155	1.929	3.74	2.32	C				
8	1997	05	12	1.31	0.56	100	10	1	31.6	0.155	1.929	3.27	1.98	C	.45u-20u glass (100	1.08	0.16	
44	1997	05	12	0.96	0.52	100	10	1	31.6	0.155	1.929	2.5	1.56	C				
53	1997	05	12	0.97	0.53	100	10	1	31.6	0.155	1.929	2.54	1.59	C				
71	1997	05	12	1.08	0.53	100	10	1	31.6	0.155	1.929	2.77	1.71	C				
13	1997	05	12	45.96	-0.63	25	10	1	10	0.155	1.929	7.36	3.79	D	20u gravity (25)	43.16	3.96	
49	1997	05	12	40.36	0.90	25	10	1	10	0.155	1.929	6.59	3.45	D				
63	1997	05	12	37.66	0.85	25	10	1	10	0.155	1.929	6.15	3.22	D	**			
41	1997	05	12	36.77	0.98	50	10	1	3.16	0.155	1.929	3.8	2	D	20u gravity (50)	36.29	1.10	
58	1997	05	12	37.07	1.91	50	10	1	3.16	0.155	1.929	3.88	2.06	D				
68	1997	05	12	35.04	0.35	50	10	1	3.16	0.155	1.929	3.59	1.87	D				
4	1997	05	12	50.31	1.35	50	10	1	3.16	0.155	1.929	5.2	2.73	D	**			
9	1997	05	12	31.68	1.02	100	10	1	3.16	0.155	1.929	6.57	3.46	D	20u gravity (100)	33.29	1.38	
45	1997	05	12	32.90	0.98	100	10	1	3.16	0.155	1.929	6.81	3.58	D				
54	1997	05	12	33.61	1.02	100	10	1	3.16	0.155	1.929	6.96	3.66	D				
72	1997	05	12	34.99	1.40	100	10	1	3.16	0.155	1.929	7.28	3.85	D				
22	1997	05	12	40.94	3.43	25	10	1	10	0.155	1.929	6.89	3.71	A	.45u gel (25)	40.97	0.05	<
34	1997	05	12	41.00	1.75	25	10	1	10	0.155	1.929	6.76	3.58	A				
14	1997	05	12	39.52	1.55	50	10	1	3.16	0.155	1.929	4.11	2.17	A	.45u gel (50)	38.80	0.64	
26	1997	05	12	38.60	0.85	50	10	1	3.16	0.155	1.929	3.98	2.09	A				
74	1997	05	12	38.30	1.25	50	10	1	3.16	0.155	1.929	3.97	2.09	A				
78	1997	05	12	32.34	2.62	100	10	1	3.16	0.155	1.929	6.87	3.7	A	.45u gel (100)	33.68	1.17	
18	1997	05	12	34.48	1.43	100	10	1	3.16	0.155	1.929	7.18	3.8	A				
30	1997	05	12	34.22	1.02	100	10	1	3.16	0.155	1.929	7.09	3.73	A				
23	1997	05	12	36.43	1.72	25	10	1	10	0.155	1.929	6.02	3.19	B	20u gel (25)	36.82	0.55	
35	1997	05	12	37.21	2.14	25	10	1	10	0.155	1.929	6.18	3.29	B				
75	1997	05	12	36.87	1.36	50	10	1	3.16	0.155	1.929	3.83	2.02	B	20u gel (50)	37.85	2.54	
15	1997	05	12	40.74	-0.43	50	10	1	3.16	0.155	1.929	4.13	2.13	B				
27	1997	05	12	35.95	1.52	50	10	1	3.16	0.155	1.929	3.75	1.98	B				
79	1997	05	12	34.31	1.27	100	10	1	1	0.155	1.929	2.26	1.19	B	20u gel (100)	33.45	0.98	
19	1997	05	12	32.39	0.63	100	10	1	3.16	0.155	1.929	6.67	3.49	B				
31	1997	05	12	33.66	1.54	100	10	1	3.16	0.155	1.929	7.03	3.72	B				
24	1997	05	12	2.16	0.56	25	10	100	1	0.155	1.929	3.95	2.28	C	.45u-20u gel (25)	1.82	0.47	
36	1997	05	12	1.49	0.65	25	10	100	1	0.155	1.929	2.96	1.8	C				
16	1997	05	12	1.60	0.65	50	10	100	1	0.155	1.929	6.26	3.77	C	.45u-20u gel (50)	1.29	0.34	
28	1997	05	12	1.35	0.56	50	10	100	1	0.155	1.929	5.28	3.19	C				
76	1997	05	12	0.93	0.57	50	10	100	1	0.155	1.929	3.96	2.51	C				
20	1997	05	12	1.40	0.50	100	10	1	31.6	0.155	1.929	3.39	2.01	C	.45u-20u gel (100)	1.31	0.12	
32	1997	05	12	1.34	0.58	100	10	1	31.6	0.155	1.929	3.36	2.04	C				
80	1997	05	12	1.18	0.49	100	10	1	31.6	0.155	1.929	2.92	1.77	C				
25	1997	05	12	35.98	0.85	25	10	1	10	0.155	1.929	5.88	3.08	D	20u gravity (25)	40.07	5.78	>
37	1997	05	12	44.16	-0.62	25	10	1	10	0.155	1.929	7.07	3.64	D				
17	1997	05	12	38.09	0.13	50	10	1	3.16	0.155	1.929	3.89	2.02	D	20u gravity (50)	37.55	2.29	
29	1997	05	12	39.52	0.98	50	10	1	3.16	0.155	1.929	4.08	2.14	D				
77	1997	05	12	35.04	1.30	50	10	1	3.16	0.155	1.929	3.64	1.92	D				
21	1997	05	12	33.30	0.95	100	10	1	3.16	0.155	1.929	6.89	3.62	D	20u gravity (100)	33.02	0.64	
33	1997	05	12	32.29	0.17	100	10	1	3.16	0.155	1.929	6.6	3.43	D				
81	1997	05	12	33.46	0.94	100	10	1	3.16	0.155	1.929	6.92	3.64	D				
				28.35												27.88	1.35	

Table 3. Filter Experiments (14Jun97)

SIO pier sample (post red tide)

#	Year	Mo	Day	Chla	Phaeo	VolF	VolA	Scale	Door	Fd	Tau	Rb	Ra	Code	Comments	Avg	Std	
1	1997	6	14	2.53	0.76	25	10	1	31.6	0.151	1.934	1.53	0.89	1	.45 glass (25)	2.49	0.04	
2	1997	6	14	2.45	0.76	25	10	1	31.6	0.151	1.934	1.49	0.87	1				
3	1997	6	14	2.49	0.76	25	10	1	31.6	0.151	1.934	1.51	0.88	1				
7	1997	6	14	2.43	0.73	50	10	1	31.6	0.151	1.934	2.94	1.71	1	.45 glass (50)	2.37	0.06	
8	1997	6	14	2.37	0.62	50	10	1	31.6	0.151	1.934	2.82	1.62	1				
9	1997	6	14	2.32	0.75	50	10	1	31.6	0.151	1.934	2.83	1.66	1				
13	1997	6	14	2.44	0.86	100	10	1	10	0.151	1.934	1.91	1.13	1	.45 glass (100)	2.39	0.09	
14	1997	6	14	2.44	0.95	100	10	1	10	0.151	1.934	1.94	1.16	1				
15	1997	6	14	2.28	0.75	100	10	1	10	0.151	1.934	1.77	1.04	1				0.063
19	1997	6	14	0.03	0.44	25	10	100	3.16	0.151	1.934	1.33	1.26	2	20 glass (25) *	0.42	0.12	
20	1997	6	14	0.42	0.16	25	10	100	3.16	0.151	1.934	2.65	1.58	2				
21	1997	6	14	0.03	0.52	25	10	100	3.16	0.151	1.934	1.56	1.48	2	**			
25	1997	6	14	0.14	0.08	50	10	100	3.16	0.151	1.934	1.93	1.20	2	20 glass (50)	0.23	0.07	
26	1997	6	14	0.28	0.11	50	10	100	3.16	0.151	1.934	3.50	2.09	2				
27	1997	6	14	0.26	0.09	50	10	100	3.16	0.151	1.934	3.17	1.88	2				
31	1997	6	14	0.32	0.10	100	10	100	1	0.151	1.934	2.49	1.46	2	20 glass (100)	0.29	0.11	
32	1997	6	14	0.16	0.07	100	10	100	3.16	0.151	1.934	4.04	2.43	2				
33	1997	6	14	0.38	0.17	100	10	100	1	0.151	1.934	3.09	1.88	2				0.103
40	1997	6	14	1.56	0.55	25	10	100	1	0.151	1.934	3.06	1.81	3	.45-20 glass (25)	1.61	0.06	
41	1997	6	14	1.68	0.50	25	10	100	1	0.151	1.934	3.20	1.86	3				
42	1997	6	14	1.60	0.53	25	10	100	1	0.151	1.934	3.10	1.82	3				
46	1997	6	14	1.78	0.53	50	10	1	31.6	0.151	1.934	2.15	1.25	3	.45-20 glass (50)	1.72	0.07	
47	1997	6	14	1.74	0.53	50	10	1	31.6	0.151	1.934	2.11	1.23	3				
48	1997	6	14	1.64	0.56	50	10	1	31.6	0.151	1.934	2.02	1.19	3				
52	1997	6	14	1.72	0.71	100	10	1	31.6	0.151	1.934	4.37	2.63	3	.45-20 glass (100)	1.69	0.05	
53	1997	6	14	1.72	0.63	100	10	1	31.6	0.151	1.934	4.29	2.55	3				
54	1997	6	14	1.63	0.74	100	10	1	31.6	0.151	1.934	4.22	2.57	3				0.06
37	1997	6	14	0.68	0.12	25	10	100	3.16	0.151	1.934	3.88	2.16	4	20 gravity (25)	0.64	0.08	
38	1997	6	14	0.55	0.10	25	10	100	3.16	0.151	1.934	3.15	1.76	4				
39	1997	6	14	0.70	0.15	25	10	100	3.16	0.151	1.934	4.05	2.29	4				
43	1997	6	14	0.71	0.17	50	10	100	1	0.151	1.934	2.65	1.51	4	20 gravity (50)	0.70	0.04	
44	1997	6	14	0.66	0.18	50	10	100	1	0.151	1.934	2.49	1.44	4				
45	1997	6	14	0.74	0.20	50	10	100	1	0.151	1.934	2.78	1.60	4				
49	1997	6	14	0.59	0.09	100	10	100	1	0.151	1.934	4.22	2.33	4	20 gravity(100)	0.64	0.06	
50	1997	6	14	0.61	0.14	100	10	100	1	0.151	1.934	4.54	2.58	4				
51	1997	6	14	0.71	0.17	100	10	1	31.6	0.151	1.934	1.67	0.95	4				0.062
4	1997	6	14	2.30	0.77	25	10	1	31.6	0.151	1.934	1.41	0.83	5	.45 gel (25)	2.24	0.06	
5	1997	6	14	2.26	0.78	25	10	1	31.6	0.151	1.934	1.39	0.82	5				
6	1997	6	14	2.18	0.67	25	10	1	31.6	0.151	1.934	1.32	0.77	5				
10	1997	6	14	2.35	0.69	50	10	1	31.6	0.151	1.934	2.84	1.65	5	.45 gel (50)	2.43	0.07	
11	1997	6	14	2.49	0.70	50	10	1	31.6	0.151	1.934	2.99	1.73	5				
12	1997	6	14	2.43	0.71	50	10	1	31.6	0.151	1.934	2.93	1.70	5				
16	1997	6	14	2.60	0.35	100	10	1	10	0.151	1.934	1.84	1.01	5	.45 gel (100)	2.44	0.14	
17	1997	6	14	2.35	0.81	100	10	1	10	0.151	1.934	1.83	1.08	5				
18	1997	6	14	2.38	0.72	100	10	1	10	0.151	1.934	1.82	1.06	5				0.089
22	1997	6	14	0.38	0.19	25	10	100	3.16	0.151	1.934	2.51	1.54	6	20 gel (25)	0.32	0.06	
23	1997	6	14	0.27	0.16	25	10	100	3.16	0.151	1.934	1.87	1.18	6				
24	1997	6	14	0.32	0.11	25	10	100	3.16	0.151	1.934	1.96	1.16	6				
28	1997	6	14	0.29	0.11	50	10	100	3.16	0.151	1.934	3.58	2.13	6	20 gel (50)	0.27	0.02	
29	1997	6	14	0.26	0.07	50	10	100	3.16	0.151	1.934	3.07	1.77	6				
30	1997	6	14	0.28	0.11	50	10	100	3.16	0.151	1.934	3.53	2.12	6				
34	1997	6	14	0.25	0.09	100	10	100	1	0.151	1.934	1.97	1.17	6	20 gel (100)	0.27	0.02	
35	1997	6	14	0.27	0.07	100	10	100	1	0.151	1.934	2.01	1.16	6				
36	1997	6	14	0.29	0.08	100	10	100	1	0.151	1.934	2.22	1.28	6				
58	1997	6	14	1.86	0.57	25	10	100	1	0.151	1.934	3.57	2.08	7	.45-20 gel (25)	1.89	0.16	
59	1997	6	14	2.06	0.59	25	10	100	1	0.151	1.934	3.92	2.27	7				
60	1997	6	14	1.75	0.54	25	10	100	1	0.151	1.934	3.36	1.96	7				
64	1997	6	14	1.68	0.57	50	10	1	31.6	0.151	1.934	2.07	1.22	7	.45-20 gel (50)	1.91	0.30	
65	1997	6	14	1.80	0.55	50	10	1	31.6	0.151	1.934	2.18	1.27	7				
66	1997	6	14	2.26	0.65	50	10	1	31.6	0.151	1.934	2.71	1.57	7				
70	1997	6	14	1.82	0.57	100	10	1	31.6	0.151	1.934	4.43	2.59	7	.45-20 gel (100)	1.88	0.05	
71	1997	6	14	1.92	0.53	100	10	1	31.6	0.151	1.934	4.59	2.65	7				
72	1997	6	14	1.89	0.81	100	10	1	31.6	0.151	1.934	4.83	2.92	7				0.171
55	1997	6	14	0.89	0.19	25	10	100	3.16	0.151	1.934	5.15	2.91	8	20 (25)	0.70	0.19	
56	1997	6	14	0.70	0.19	25	10	100	3.16	0.151	1.934	4.19	2.41	8				
57	1997	6	14	0.50	0.12	25	10	100	3.16	0.151	1.934	2.95	1.68	8				
61	1997	6	14	0.72	0.16	50	10	100	1	0.151	1.934	2.66	1.51	8	20 (50)	0.65	0.06	
62	1997	6	14	0.63	0.12	50	10	100	1	0.151	1.934	2.28	1.28	8				
63	1997	6	14	0.60	0.12	50	10	100	1	0.151	1.934	2.20	1.24	8				
67	1997	6	14	0.53	0.09	100	10	100	1	0.151	1.934	3.77	2.09	8	20 (100)	0.60	0.09	
68	1997	6	14	0.59	0.12	100	10	100	1	0.151	1.934	4.33	2.44	8				
69	1997	6	14	0.70	0.15	100	10	100	1	0.151	1.934	5.13	2.90	8				
				1.29													0.114	0.114

Table 4. Chlorophyll Summary

date	chl	stdev	stdev/chl	max std	min std
Mar-97	2.14	0.49	0.228972	1.65	0.07
May-97	27.88	1.35	0.048508	5.78	0.05
Jun-97	1.28	0.09	0.06746	0.3	0.02

Table 5. Filter Experiment Averages

		glass							gel						
date	type	code	25	50	100	avg	std	csv	code	25	50	100	avg	std	csv
27-Mar-97	0.45u	1	4.09	3.83	4.22	4.05	0.20	4.938	5	2.51	1.51	2.88	2.30	0.71	30.9
pre	20u(vacuum)	2	2.30	2.38	3.13	2.60	0.46	17.69	6	2.17	1.77	1.66	1.87	0.27	14.4
redtide	.45u-20u(g)	3	1.20	0.87	0.48	0.85	0.36	42.35	7	-	1.53	1.24	1.39	0.21	15.1
	20u(gravity)	4	1.62	1.73	0.67	1.34	0.58	43.28	8	-	1.36	3.99	2.68	1.86	69.4
	(.45u-20u)+20u(g)	3+4	2.82	2.60	1.15	2.19	0.91	41.55	7+8	-	2.89	5.23	4.06	1.65	40.6
12-May-97	0.45u	1	39.96	37.42	33.45	36.94	3.28	8.879	5	40.97	38.80	33.68	37.82	3.74	9.89
mid	20u(vacuum)	2	35.63	37.21	32.68	35.17	2.30	6.54	6	36.82	37.85	33.45	36.04	2.30	6.38
redtide	.45u-20u(g)	3	1.28	1.10	1.08	1.15	0.11	9.565	7	1.82	1.29	1.31	1.47	0.30	20.4
	20u(gravity)	4	43.16	36.29	33.29	37.58	5.06	13.46	8	40.07	37.55	33.02	36.88	3.57	9.68
	(.45u-20u)+20u(g)	3+4	44.44	37.39	34.37	38.73	5.17	13.35	7+8	41.89	38.84	34.33	38.35	3.80	9.91
14-Jun-97	0.45u	1	2.49	2.37	2.39	2.42	0.06	2.479	5	2.24	2.43	2.44	2.37	0.11	4.64
post	20u(vacuum)	2	0.42	0.23	0.29	0.31	0.10	32.26	6	0.32	0.27	0.27	0.29	0.03	10.3
redtide	.45u-20u(g)	3	1.61	1.72	1.69	1.67	0.06	3.593	7	1.89	1.91	1.88	1.89	0.02	1.06
	20u(gravity)	4	0.64	0.70	0.64	0.66	0.03	4.545	8	0.70	0.65	0.60	0.65	0.05	7.69
	(.45u-20u)+20u(g)	3+4	2.25	2.42	2.33	2.33	0.09	3.863	7+8	2.59	2.56	2.48	2.54	0.06	2.36

Table 6. % Large Cells

date	red tide development	mean chl	% large cells
27-Mar-97	pre	2.18	0.61
12-May-97	mid	28.35	0.97
14-Jun-97	post	1.29	0.28

Figures

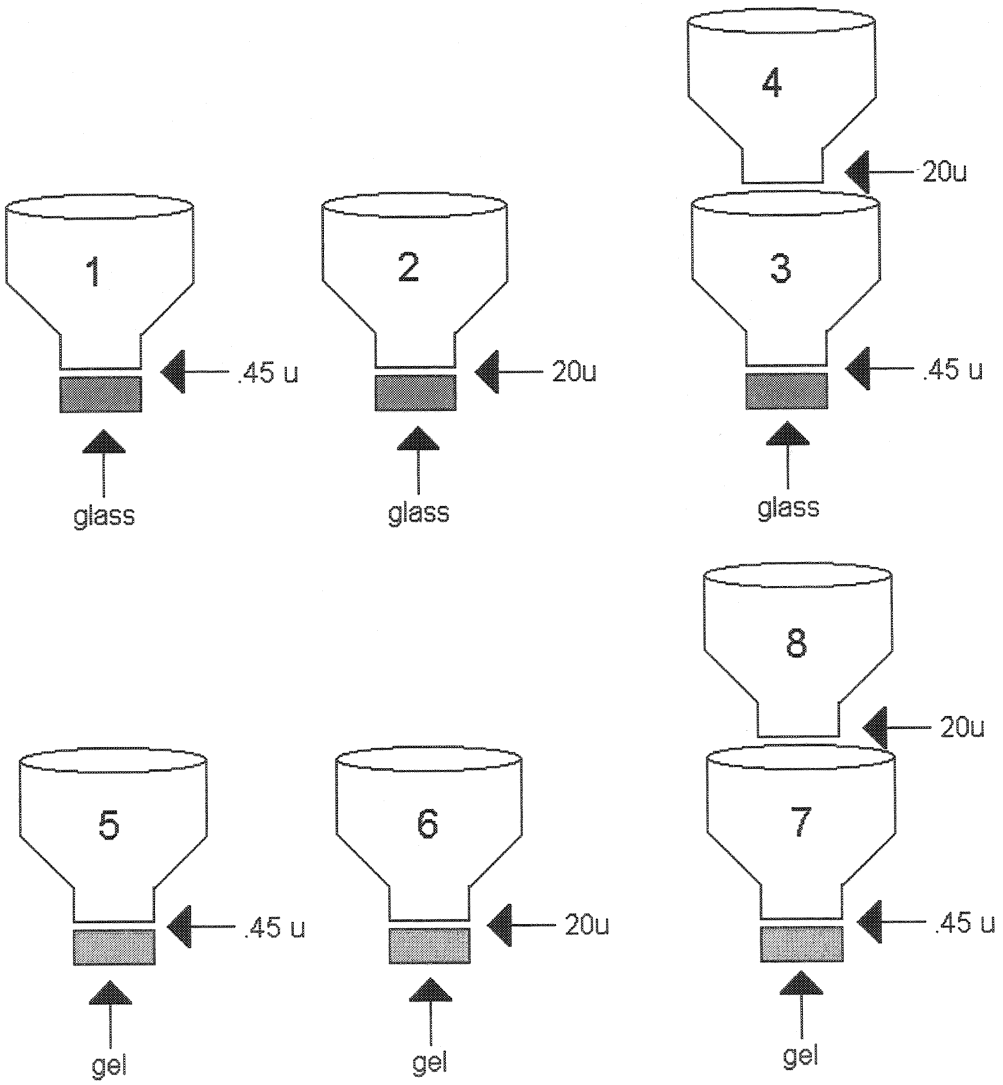
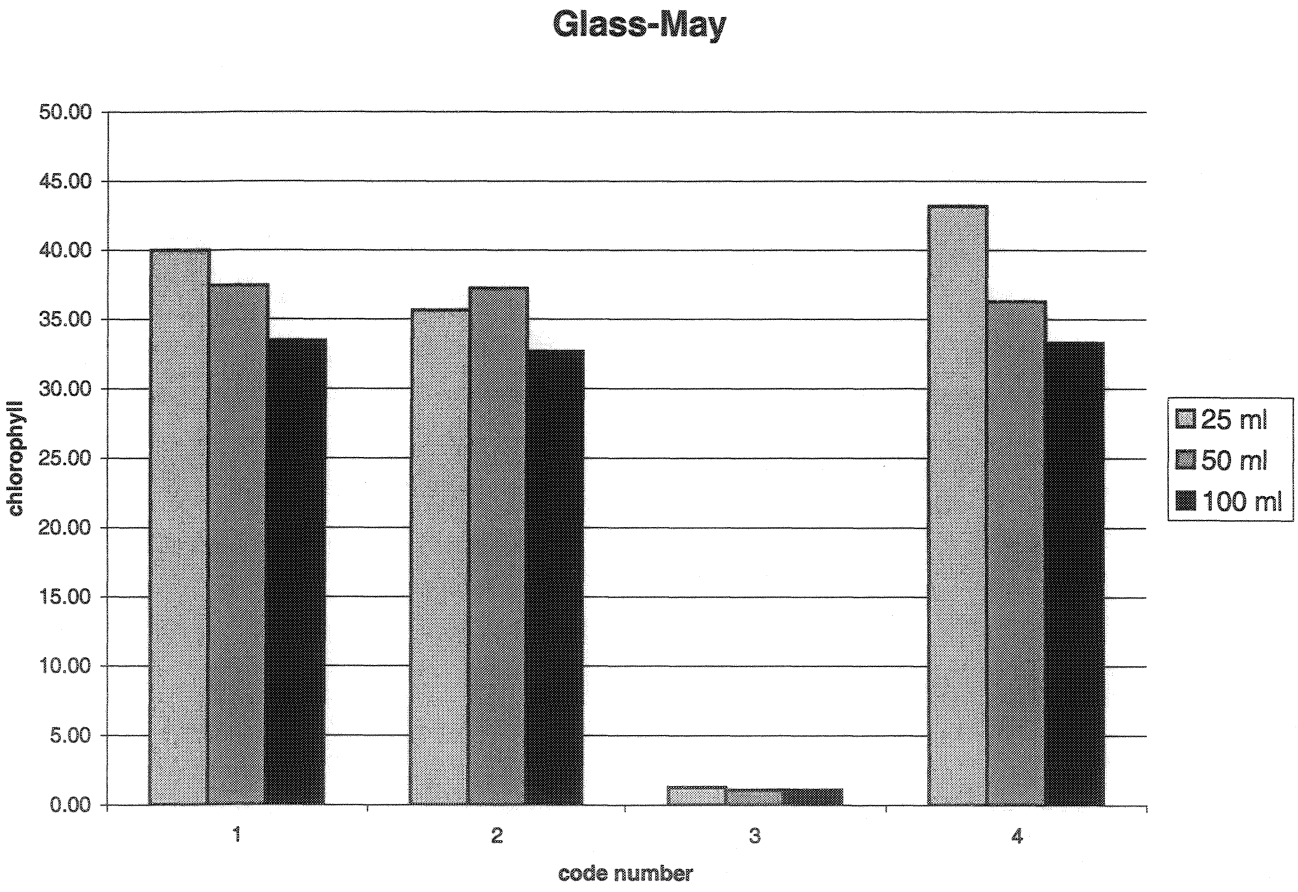

1
.45 u
glass
2
20u
glass
4
20u
3
.45 u
glass
5
.45 u
gel
6
20u
gel
8
20u
7
.45 u
gel

Figure 1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45.00
40.00
35.00
30.00
25.00
20.00
15.00
10.00
5.00
0.00
chlorophyll
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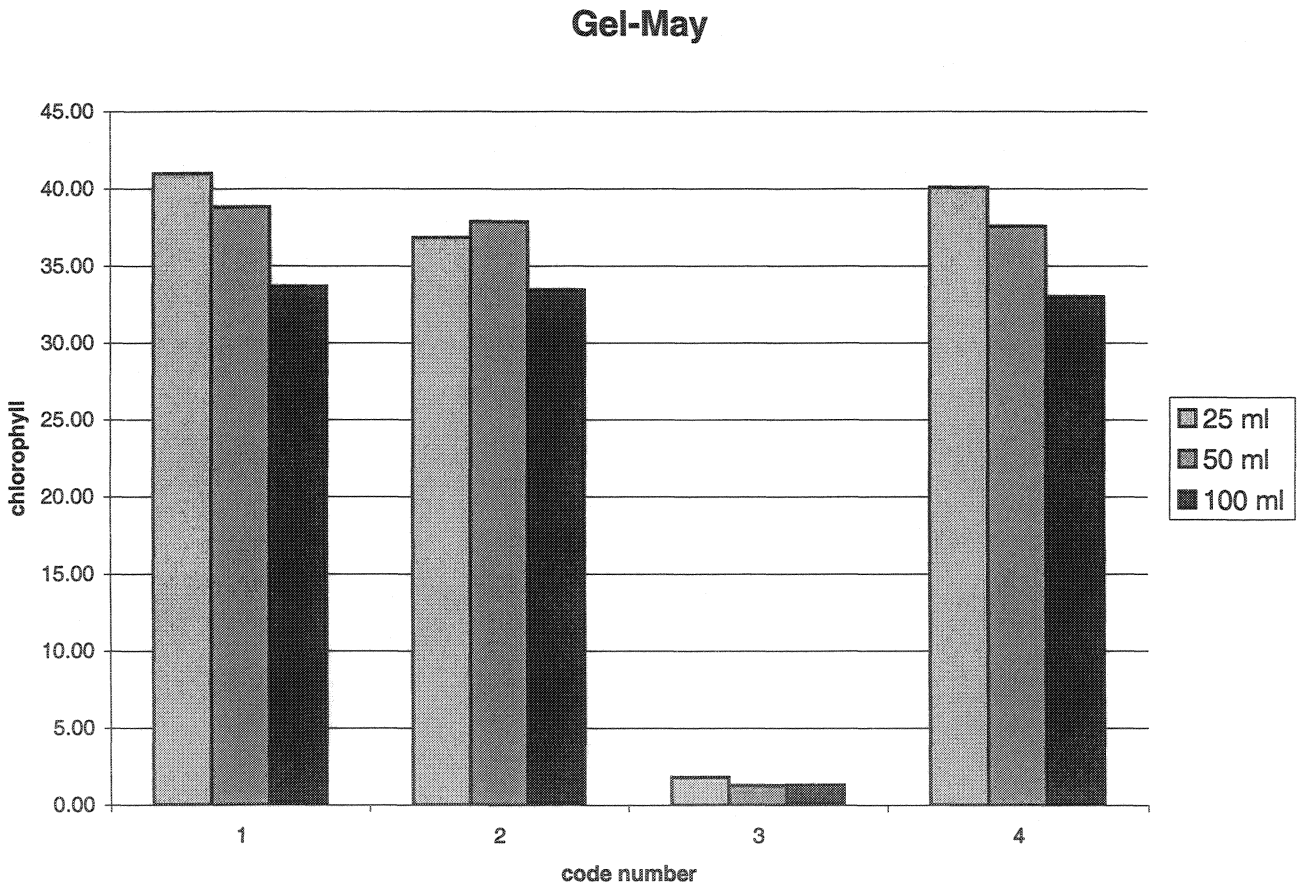
2
3
4
code number
25 ml
50 ml
100 ml

Figure 2a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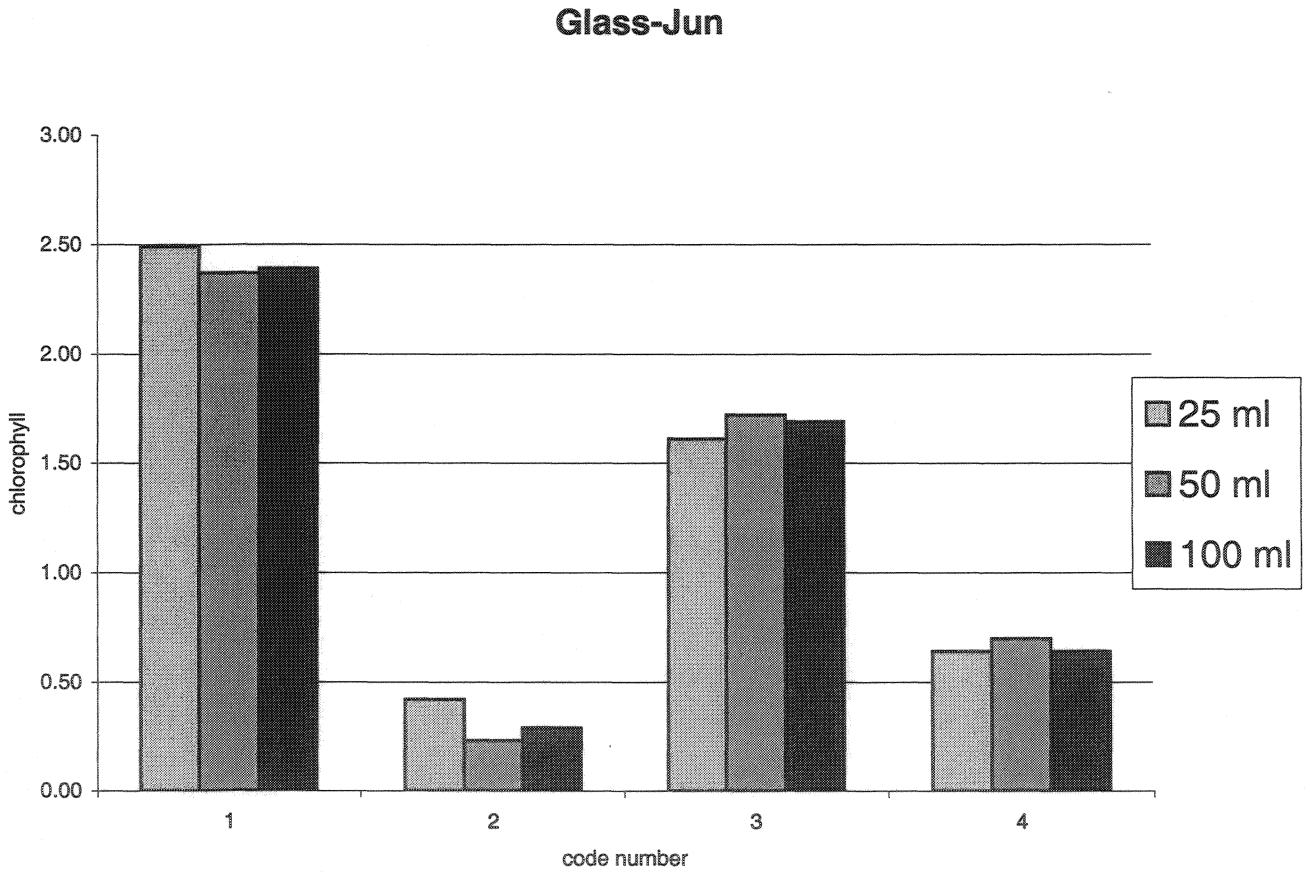
40.00
35.00
30.00
25.00
20.00
15.00
10.00
5.00
0.00
chlorophyll
1
2
3
4
code number
25 ml
50 ml
100 ml

Figure 2b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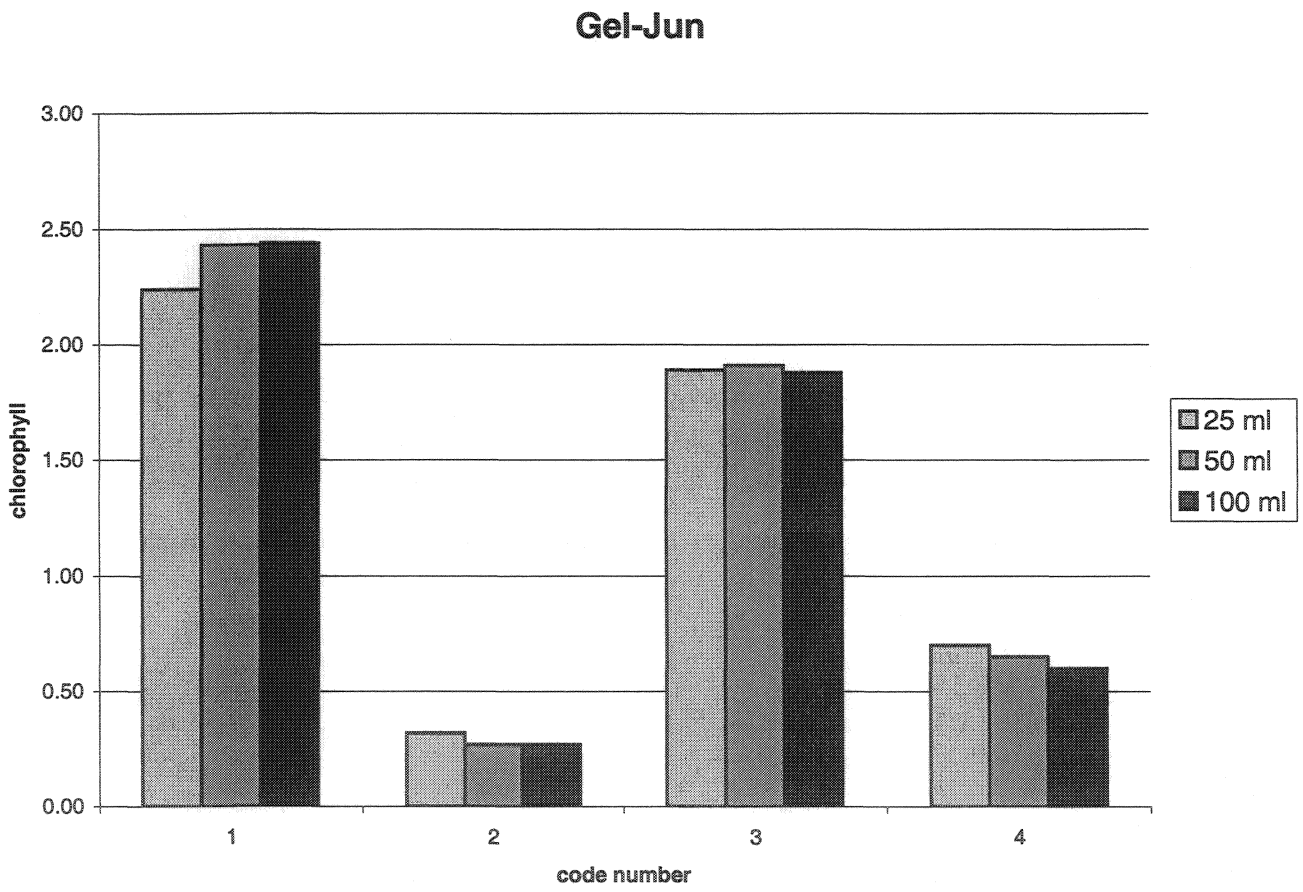
2.50
2.00
1.50
1.00
0.50
0.00
chlorophyll
1
2
3
4
code number
25 ml
50 ml
100 ml

Figure 2c


Gel-Jun
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2.50
2.00
1.50
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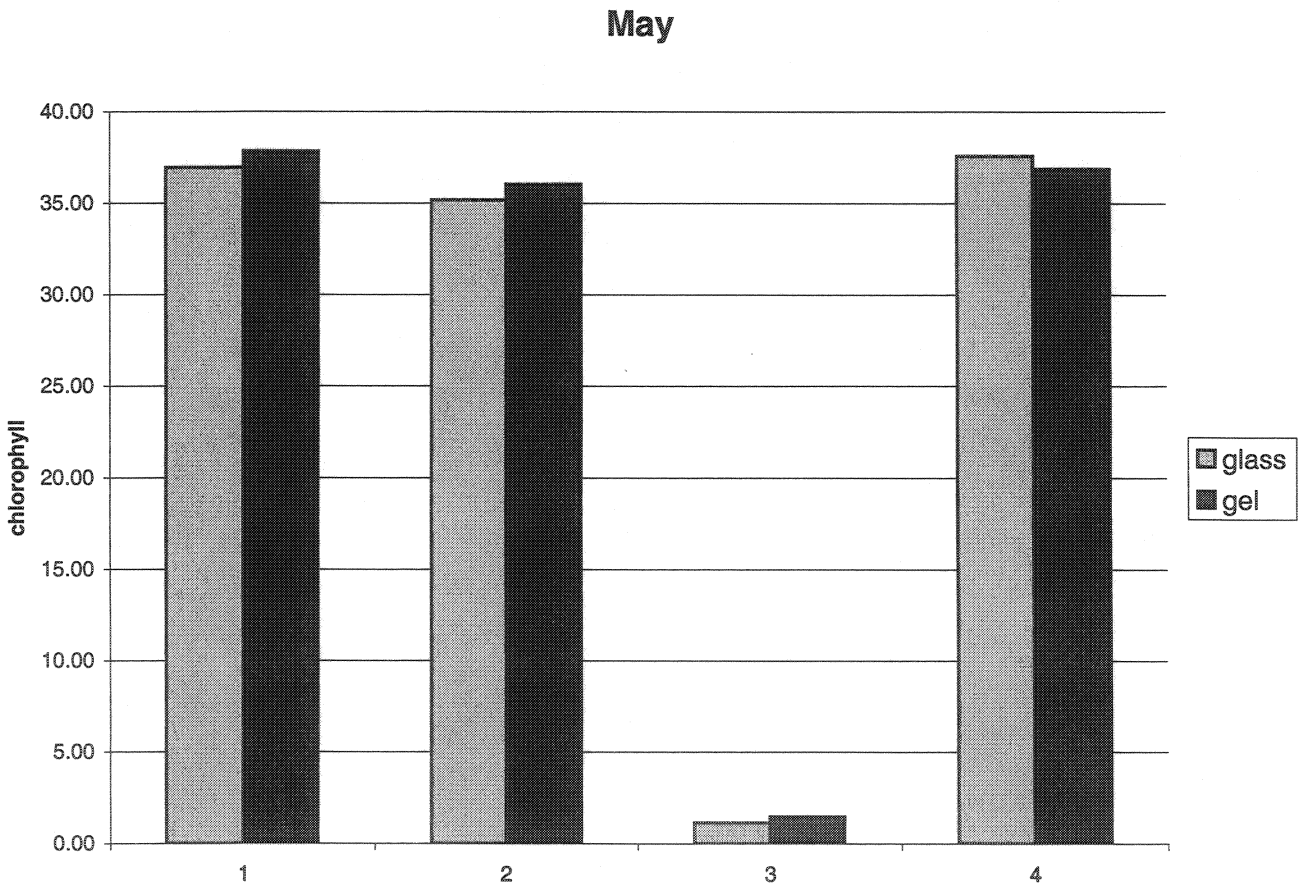
0.50
0.00
chlorophyll
1
2
3
4
code number
25 ml
50 ml
100 ml

Figure 2d


May
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35.00
30.00
25.00
20.00
15.00
10.00
5.00
0.00
chlorophyll
1
2
3
4
glass
gel

Figure 3a

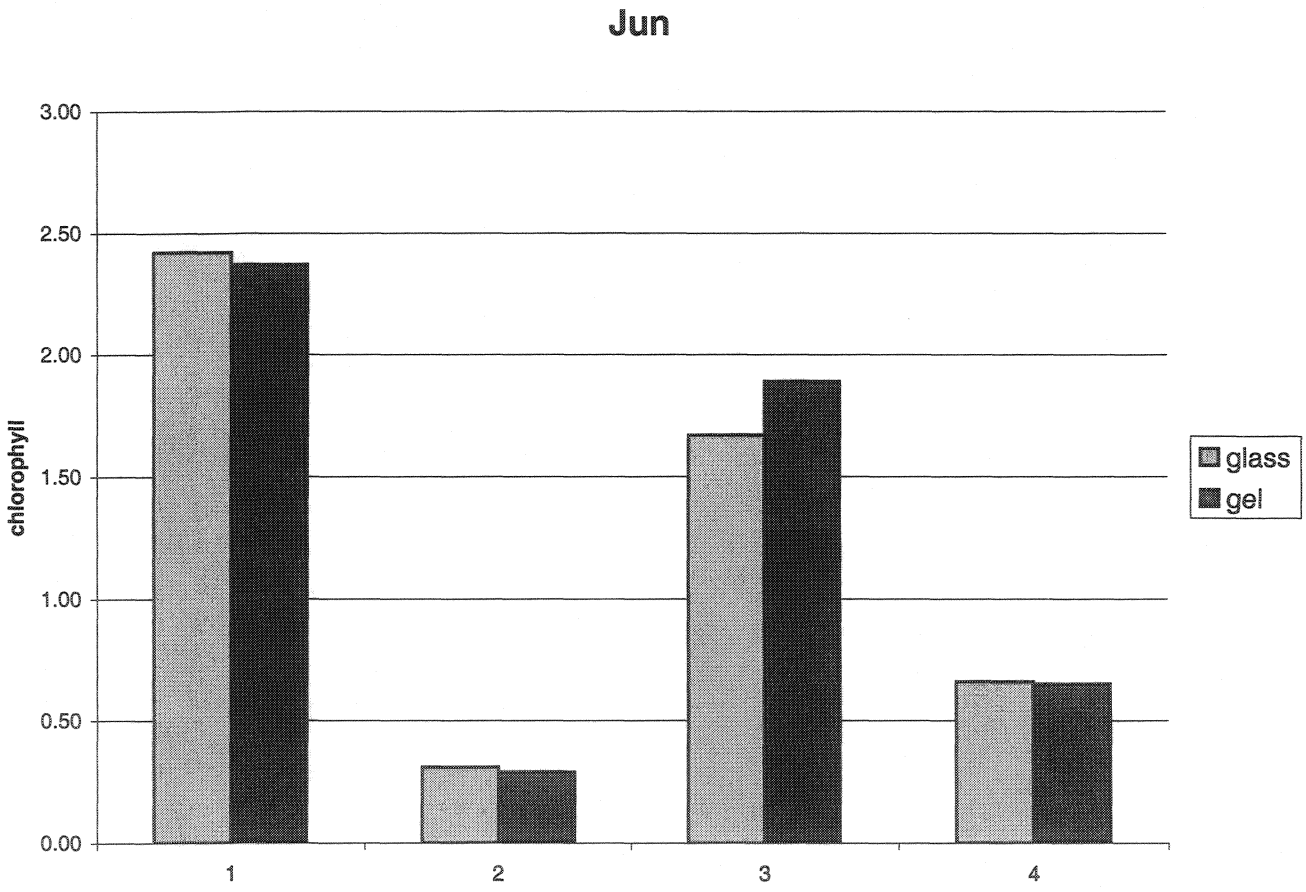
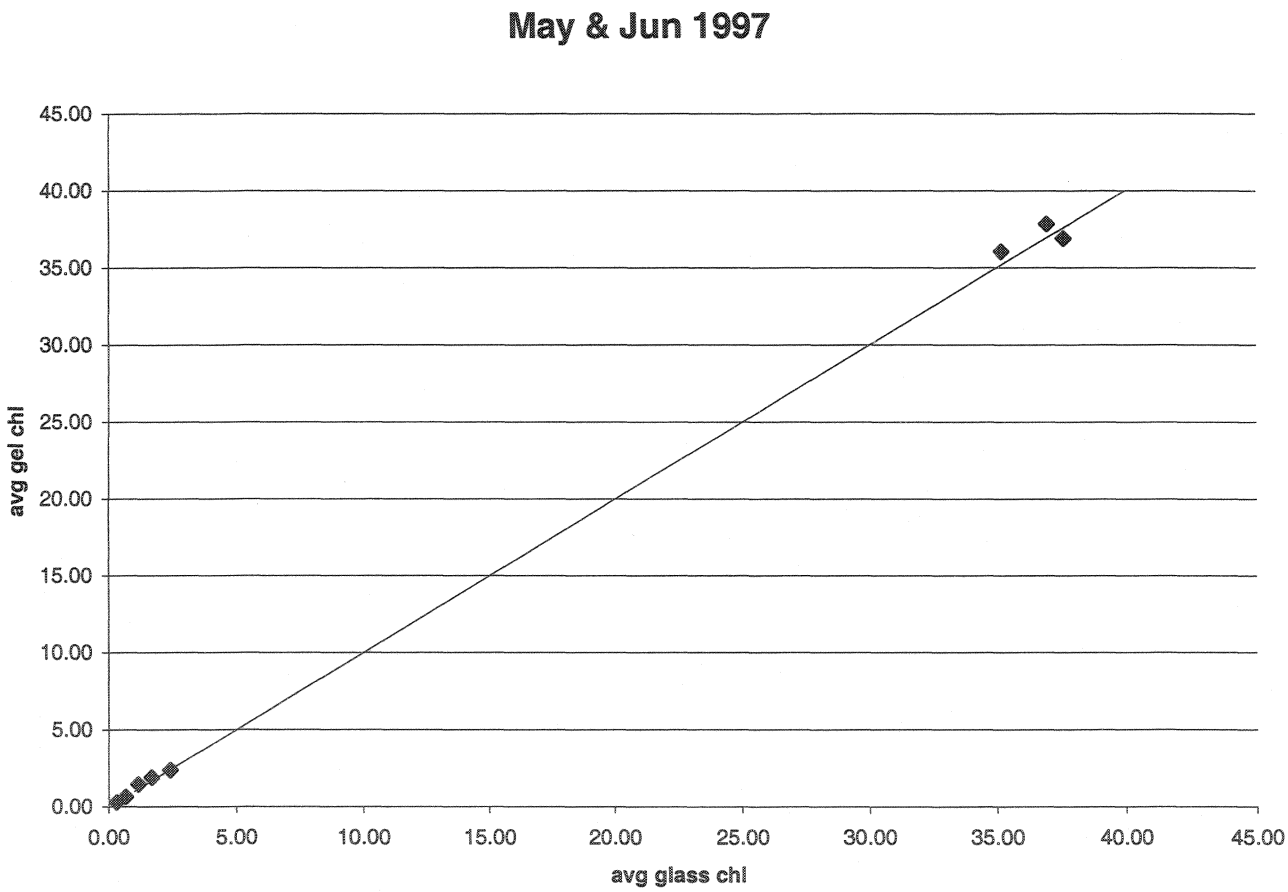


Figure 3b


May & Jun 1997
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40.00
35.00
30.00
25.00
20.00
15.00
10.00
5.00
0.00
avg gel chl
0.00
5.00
10.00
15.00
20.00
25.00
30.00
35.00
40.00
45.00
avg glass chl

Figure 4

Appendix 1

PHYTOPLANKTON GROWTH

Alison Baker
Standley Middle School, 7th grade

Greater San Diego Science and Engineering Fair 1996

Abstract

It has been shown that the size of a kind of phytoplankton affects its growth. In this experiment, a Chlorophycea Dunaliella tertiolecta (size = 10 microns) and a Dinophycea Gonyaulax polyedra (size = 50 microns) were cultured and grown for 3-4 weeks under non limiting conditions. This means that there were no grazers, but there were plenty of nutrients and light. Looking under the microscope showed how the cells changed in time. Using an equation that included only the size of the cell, I calculated that the smaller cells would grow 1.7 times faster than the bigger cells. My experimental finding was that the smaller cells actully grew 5 times faster than the big cells. To explain the result being 3 times bigger than expected, a new calculation was made including the size and the surface area of a cell.

Acknowledgments

I thank Kenia Whitehead who supervised this project. I also thank Maria Vernet for the use of her laboratory at Scripps Institution of Oceanography and Frieda Reid and Tiffany Moisan for their suggestions. I also thank Karen Baker for helping with this project.

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Introduction

All life on land as well as in the sea depends on plants. The floating microscopic plants and animals in the ocean are called plankton. What importance are these tiny creatures to us? They use sunlight to make food. The plants make nearly all of the food in the sea. They make food from carbon dioxide, water, and small amounts of trace metals as well as the energy from the sun. They are also at the bottom of the food web, which means that all of the other animals that live in the ocean rely on them to survive. Think of how many animals wouldn't be able to survive without these creatures. In fact the distribution of fish depends upon the distribution of plankton.

Plankton are divided into two groups: the plants are called phytoplankton; the animals are called zooplankton. There are many different species of phytoplankton that are part of the ocean system. To understand how this system functions and stays balanced, it is important to look more closely at each type of phytoplankton and what makes it grow. In this experiment I will look at two different kinds of phytoplankton and compare their growth rates in a controlled laboratory environment.

The growth rate of a phytoplankton culture can be determined by measuring the amount of phytoplankton present over a period of time. The amount of phytoplankton can be found in several different ways. The two ways that are used in this project are 1) measuring the amount of chlorophyll in a sample and 2) counting the cells in a fixed volume using a microscope.

STATEMENT OF THE PROBLEM: Do different kinds of phytoplankton grow at different rates?

HYPOTHESIS: A Chlorophycea phytoplankton culture of Dunaliella tertiolecta (about 10 micron diameter) will grow 1.7 times faster than a Dinophycea phytoplankton culture of Gonyaulax polyedra (about 50 micron diameter) because of the difference in their size.

Review of the Literature

Phytoplankton are the plants of the sea. They are microscopic plants that are the main diet for many sea animals. Common types of phytoplankton are dinoflagellates and diatoms which grow in the ocean and in cultures too.

Taxonomists organize different species into different kingdoms which are divided into different phylums which are divided into different classes, orders, families and geneses. Scientists sometimes divide kingdoms into the plant and the animal kingdom. Whitker in 1969 suggested that there should be five kingdoms instead of two (figure 1). One of the reasons was that there were so many single cells that didn't just fit in two categories. Phytoplankton are single cells that are in the Protista kingdom in the five kingdom organization.

Dinoflagellates (figure 2) are members of the yellow-green algae class Dinophycea. They got their name from the Greek word "dino" which means whirling. This is how they move. They have two flagella: one trailing and one coming out of the side at a 90 degree angle. The flagella are attached at the girdle. The cell is covered by thecal plates. Dinoflagellates are unicellular, and are about 5-100 micrometers. Since they are a plant, they require light for photosynthesis. They contain the pigments chlorophyll a, chlorophyll c, and carotenoid peredinin. They have a golden pigment, but when they are abundant in one area, they make the water look red. This is called a red tide. A transfer of energy in some dinoflagellates creates bioluminescence. Therefore when there is a red tide, you can see a glow in the water at night. This is why they are part of the phylum Purrophyta meaning flame colored plant. Dinoflagellates are not only found in the ocean, but they are also found in fresh water lakes and ponds.

The class of green algae called Chlorophycea (figure 3), have some of the same characteristics as the dinoflagellates. They have flagella. Also, they require light for photosynthesis, and they react to light. They contain the pigments chlorophyll a, chlorophyll b, and cartenoid lutein. Although most species of phytoplankton like either high or low salinity water, several species in this class are able to live in both low and high salinity water.

Another common type of phytoplankton found in the ocean are the diatoms (figure 4). Diatom is Greek for two cuts meaning two walls or shells which on the diatom are made of silica. They make the diatom practically indestructible. The diatom, unlike the dinoflagellate, has no way to propel itself. It just floats in the water and goes with the current. The diatom is also unicellular, and they are 3-300 micrometers. Diatoms need light to grow for they are also plants. Diatoms have a yellowish brown pigmentation. Scientists have found that the shell of the diatom of frustule creates fossils. Scientists look at the fossils as part of the historical record of the ocean. The diatoms are in a class know as Chryosphycea meaning golden plants in Greek. They are in a subclass known as Bacillariophyceae.

One way people study dinoflagellates, chlorophytes, and diatoms is by growing them in a culture. There are three types of cultures: batch cultures, continuous cultures, and diffusion cultures. In a batch culture the nutrient medium isn't replenished after you begin. It is simple and convenient. The continuous culture is a culture in which nutrients are always flowing in and out so that the growth rate and cell division become nearly constant at times. In a diffusion culture the walls of the container that the culture is in are semi-penetrable. So the cells stay in, but the nutrients can flow through and replace used up nutrients. In all of these cultures the living animals have been left out, so that there isn't anything that can eat the phytoplankton. There are many different kinds of medium that are used for cultures. They have different amounts of salt, water, and nutrients. It is known that nitrogen (N) and phosphorus (P) are needed for growth, and that the DNA is affected if there is not enough.

Some types of phytoplankton multiply by cell division. The way that cell division works is that a cell splits in half. One half will grow another the same size, and the other half will grow a half that is a little bit smaller (figure 5). The most accurate way to find the total population of cells is to count them. Another way to estimate the growth of cells is to determine the amount of chlorophyll present. Chlorophyll is the photosynthetic pigment that is inside each of the phytoplankton cells. It can be extracted from a sample and the amount measured using an instrument called a fluorometer.

If you graph the growth rate, it makes a curve called an exponential growth curve (figure 6). It is called this because the cells double so the numbers go 2,4,8,16,32 instead of 1,2,3,4,5,6,7,8,9 which is called linear

growth. Unlimited growth is impossible because the cells will run out of space or nutrients. After the cells run out of something, the exponential curve flattens out and has an S shape. It is possible to look at what affects the growth rate of phytoplankton. If you change the amount of light that it gets, the kind of light that it gets, or the temperature in the surrounding area, it will change the growth rate. Different phytoplankton species grow at different rates. A lot is still not known about what affects the growth rate of phytoplankton, but scientists are always trying to learn more about phytoplankton and what affects it.

It has been found (figure 7) by Chrisholm in 1992 that there is a relationship between cell growth and cell size where

$$\text{growth} = (0.231)(\text{cell size})^{(-0.32)} \qquad (1)$$

so that smaller cells grow faster than larger cells.

A microscope can be used to count phytoplankton cells. There are different kinds of slides to hold different amounts of the sample depending on how dense the sample is.

size	slide	microscope
1.0-5.0 ml	Utermohl settling chambers	inverted
1.0 ml	Sedgewick Rafter chamber	inverted or compound
0.1 ml	Palmer Malony	compound

There should be a single layer of cells on the slide for observation. Counts can be made of a specific area marked by lines on the slide or a random count of the microscope field can be used.

Procedures

To monitor the growth rate of a kind of phytoplankton, a medium is needed. In these experiments a GPM medium was used which was developed by F. Haxo at Scripps Institution of Oceanography. The medium contains the following water, trace metals, and vitamins.

375 mL filtered sea water (28-32 o/oo salinity)
112.5 mL distilled water
1 mL KNO3
0.1 mL K2HPO4
2.5 mL trace metals
0.5 mL B12
1 mL Thiamin-HCL
1 mL Biotin

The water was put in a ferenbauch flask and the other ingredients were put into a smaller flask. After sitting for 24 hours, they are put into the autoclave (photo 1) to heat to a high temperature in order to kill all living things that might be in the water. After spending 20 minutes in the autoclave, the medium cools.

After cooling for 15 minutes, the phytoplankton from another culture is added to begin the new culture (photo 2). In this case Gonyaulax polyedra and dunaliella were added. A sample of each culture was put into a 20 mL vials and fixed with lugal so the cells could be counted at a later time with a microscope. Lugal is iodine in water. This solution kills, stains and fixes cells. This sampling was repeated at intervals in order to observe the change in the number of cells at different times in the growth.

A sample was taken at the start and every day for several weeks in order to find out the amount of chlorophyll in the cultures. At first 50 mLs were sampled, but that was reduced to 1 mL after a couple weeks. Before the cover to the flask is taken off, the samples are moved to the clean room (photo 3). The neck of the flask is heated with a Bunsen burner (photo 4) so that not as much non sterile air will go in the flask. The sample is poured into a filter tower (photo 5) over a filter which catches the phytoplankton and lets the water through. The water is pulled through the filter by a pump.

Using a pair of tweezers, the filter is removed from filter base and put into a culture tube. Then the culture tube is filled with acetone which extracts the chlorophyll. It is covered with parafilm and placed in the refrigerator so the cold can help the extraction. The tube is removed after 24 hours in a room where the lights have been turned off and the only light on is a yellow light which will not degrade the chlorophyll. The sample is then mechanically stimulated (shaken) (photo 6) in order to break any last cells that might have stayed intact. Then the samples are covered with foil to keep out light, and carried to the fluorometer. The sample (acetone+chlorophyll) is then poured into a culture tube leaving the filter behind. This is put into the fluorometer (photo 7) which measures the fluorescence in volts. Then 10% HCL, an acid, is added to the tube and the fluorescence is again measured. From this information the chlorophyll can be calculated (table 1).

For each measurement, two samples were taken in case one got lost and also to make sure they agreed. When the results agree, it is a check on the measurement.

After the sampling is finished, everything is washed with water and soap (photo 8), then put into the acid bath. The acid bath helps kill germs and bacteria. It also makes sure that acetone doesn't stay in the tube.

A check of the samples on two different days was made with the microscope (photo 9) in order to see by eye what the difference in the culture would be. In my case, I used a sedgewick rafter chamber with an inverted microscope, and counted 14 evenly distributed areas on the slide.

Results

THEORETICAL RESULTS
RELATIONSHIP OF GROWTH TO SIZE

From the previous theory shown in equation 1, the growth rate of both Dunaliella (DUN) and Gonyaulax polyedra (GP) can be calculated.

In the case of DUN with a size = 10 microns

growth(DUN)=0.231(10)^(-0.32)=0.111

In the case of GP with a size = 50 microns

growth(GP)=0.231(50)^(-0.32)=0.066

So that

$$\text{ratio} = \frac{\text{growth(DUN)}}{\text{growth(GP)}} = \frac{.111}{.066} = 1.68 \qquad (2)$$

So the growth of the smaller DUN (.111) is 1.7 times faster than the GP (.066).

EXPERIMENTAL RESULTS

The daily measurements of chlorophyll from the experimental cultures are recorded in a log, and entered into a spreadsheet (table 1).The experiments that were run include:

experiment #1/ culture = GP, avg light
experiment #2A/ culture = GP, low light
experiment #2B/ culture = GP, avg light
experiment #2C/ culture = DUN, low light
experiment #2D/ culture = DUN, avg light

Light has a big effect on phytoplankton growth. I tried some experiements where the amount of light was changed by wrapping the culture with screen mesh. A light measuring instrument was used to find out how much light really got inside the culture vessel. The numbers that I got were very similar, so I concluded that the mesh didn't make a difference because the holes were too big. The results are displayed in figures 8a and 8b where chlorophyll is on the y axis and time is one the x axis. Time is the number of days since the beginning of the experiment. In the Gonyaulax polyedra curve the cells ran out of nutrients after about 20 days so the curve started going down.

The slope of the line in the figure 8, shows how fast the phytoplankton grows since it gives the increase in biomass per time.

$$\text{growth(GP)} \sim \frac{\text{change in y}}{\text{change in x}} \sim \frac{(.7-.25)}{(17-10)} = \frac{.45}{7} = .064$$

$$\text{growth(DUN)} \sim \frac{\text{change in y}}{\text{change in x}} \sim \frac{(2.3-.5)}{(13-7)} = \frac{1.8}{6} = .30$$

$$\text{ratio} = \frac{\text{growth(DUN)}}{\text{growth(GP)}} = \frac{.30}{.064} = 5 \qquad (3)$$

Actually, there were several attempts to grow the cultures: several succeeded and one failed. This is not too unusual. Perhaps the media was contaminated somehow by mistake. The seperate results are shown in figures 8a and 8b and they are combined in 8c.

In order to learn to use a microscope to observe the cells, a few Dunaliella culture samples were prepared for observation. A microscope check on the culture was done on the day that it was started and eight days later. There were approximately 15 cells in each circular viewing area on day one. There were approximately 30 cells in each circular viewing area on day eight.

THEORETICAL RESULTS
RELATIONSHIP OF GROWTH TO SIZE AND SURFACE AREA.

Another theory can take into account both size and surface area
Assuming that

1) cell surface area is related to nutrients
2) cell volume is related to chlorophyll

Then

$$\text{growth} \sim \frac{\text{nutrients}}{\text{chlorophyll}} \sim \frac{\text{surface area}}{\text{volume}} \sim \frac{4*pi*r^2}{4/3*pi*r^3} \sim \frac{3}{r} \quad (4)$$

growth(GP)= 3/25= .12
growth(DUN)= 3/5= .60

$$\frac{\text{growth(DUN)}}{\text{growth(GP)}} = \frac{.60}{.12} = 5 \quad (5)$$

So the new theory shows that Dunaliella grows even faster than was originally shown by the first theory.

Conclusions

Gonyaulax polyedra and Dunaliella tertiolecta are types of phytoplankton of different sizes. Although from different classes, they are both motile with two flagella. Dunaliella is known to grow in a wider variety of situations, but both grow in a lighted culture.

When growth is related only to cell size as in Equation 1, the smaller phytoplankton, Dunaliella, is calculated to grow 1.7 times faster than the bigger phytoplankton, Gonyaulax. The experimental result shown in figure 8 and described in equation 3 shows, however, that the smaller phytoplankton, Dunaliella, grew 5 times faster than the bigger phytoplankton, Gonyaulax. Therefore I made a new theory. An equation to determine the growth rate that incorporates not just the size but also the surface area is shown in equation 4. Calculations with this equation predict that the smaller phytoplankton would grow 5 times faster than the bigger phytoplankton which agrees with my experimental result. For these species this new equation agrees with the experimental result.

The microscope counting of cells showed that after a week the Dunaliella cells had doubled. The distribution of cells on the slide was very uneven, consequently when this method is used several places on the slide should be counted, and the method of making the slide should be considered. Phytoplankton growth can be measured by counting cells but the amount of chlorophyll in each cell can vary greatly thus even with double the cells there can be many more times the amount of chlorophyll.

Recommendations

Before any further conclusions can be made from the microscope work, more samples need to be fixed and counts need to be done. This would be a good study to understand more about the number of cells and the amount of chlorophyll in each cell.

It would be important to check how general equation 4 is. It explains the growth for the two species that I cultured, but it should be checked for other species. It would be very interesting to run this experiment with diatoms because they are different than the species I worked with. They

don't have flagella and they have silica plates. Also, it would be interesting to use a species of phytoplankton from a natural habitat and try to grow them in a culture. This would most likely be a mix of species unless it was possible to sample during a bloom when a single species dominates.

Next year I want to repeat this experiment using different amounts and different kinds of light. I could also change the temperatures in the surrounding area. It would be very interesting to investigate the results with the diatom culture since it does not have flagella like the two cultures I used this year. With so many things that affect phytoplankton, there are still a lot of experiments to do.

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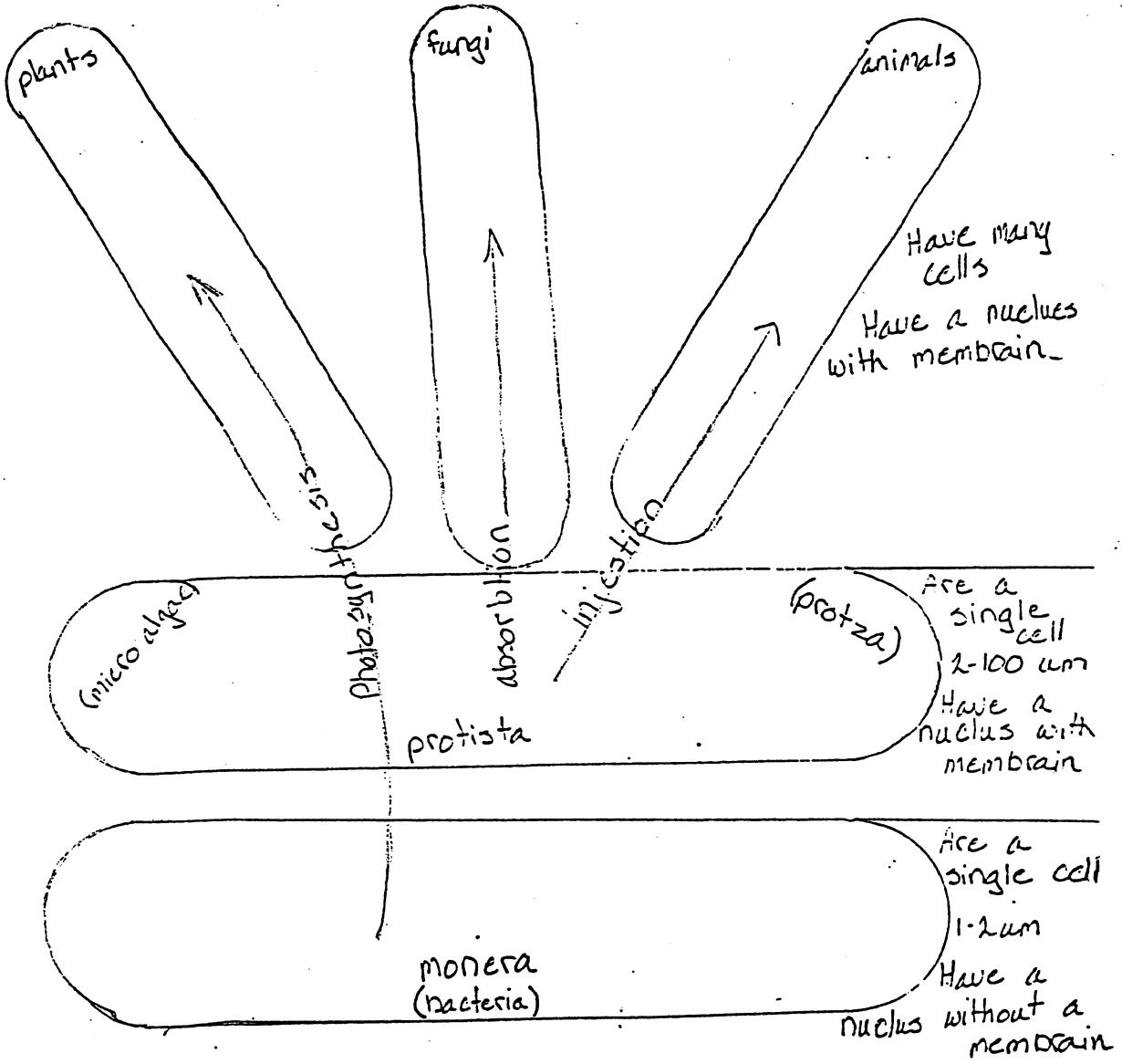


Figure 1: Classification of species into five kingdoms.

Dinophycea
Gonyaulax polyedra

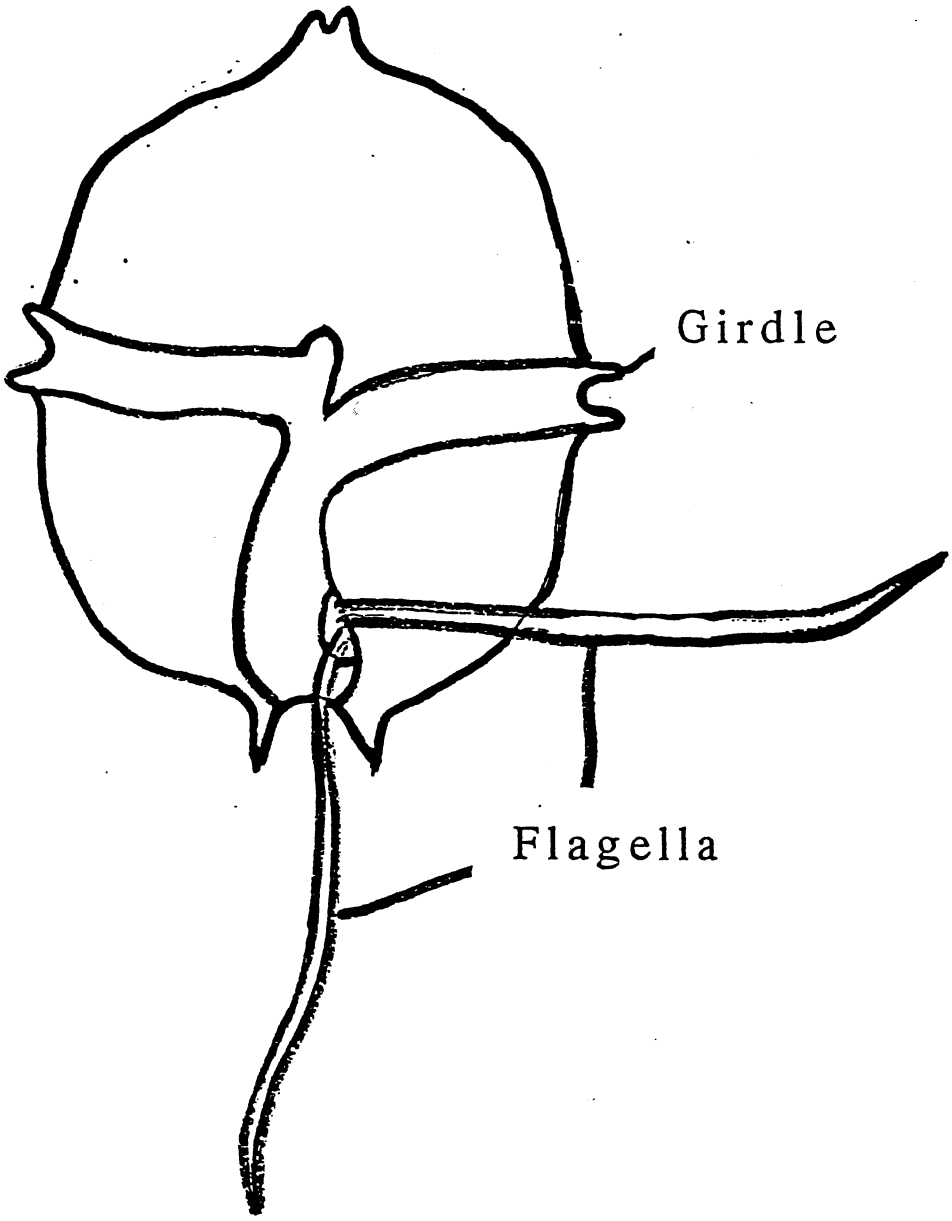


Figure 2: Dinophycea Gonyaulax polyedra.

Chlorophycea
Duneliella tertiolecta

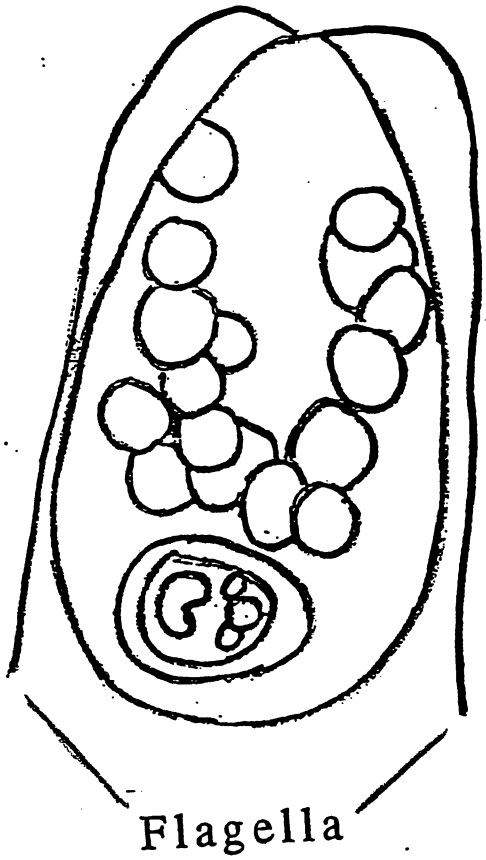
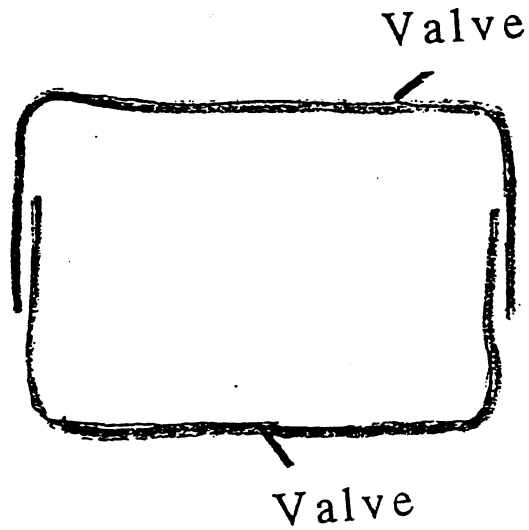
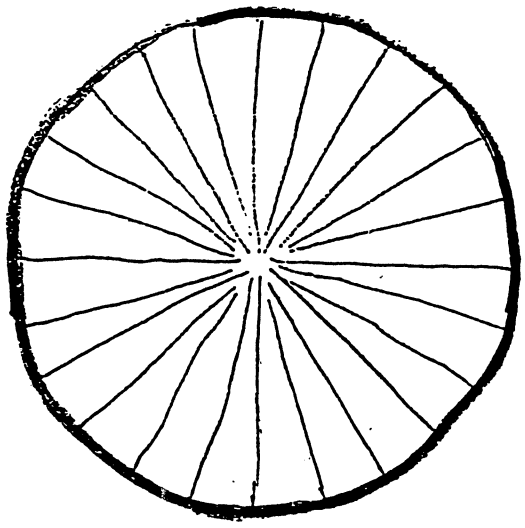


Figure 3: Chlorophycea Duneliella teriolecta.

Bacillariophycea Diatom



Side



Top

Figure 4: Bacillariophycea Diatom.

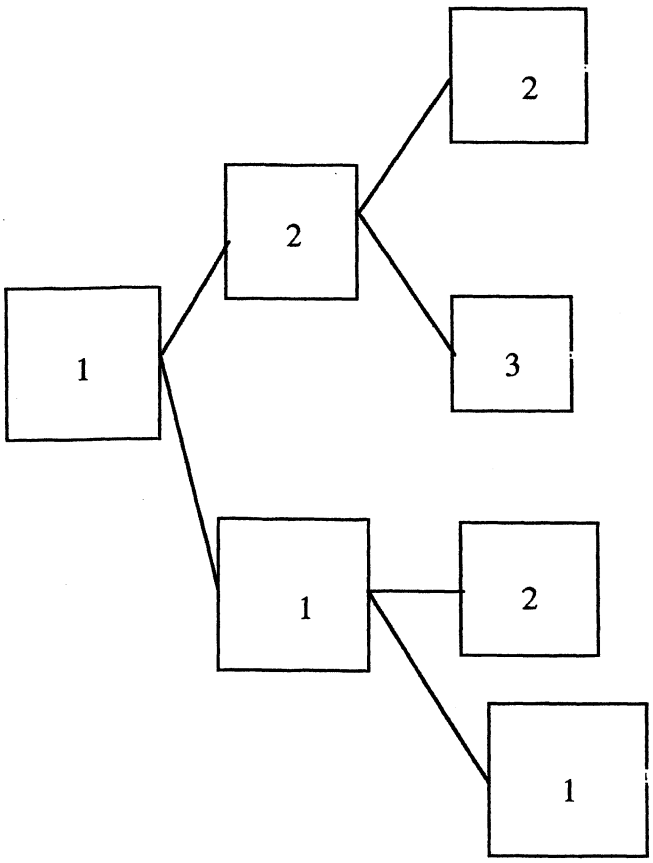


Figure 5. Shows cell growth by division and the change in size of cells where there are three sizes (1, 2, and 3).

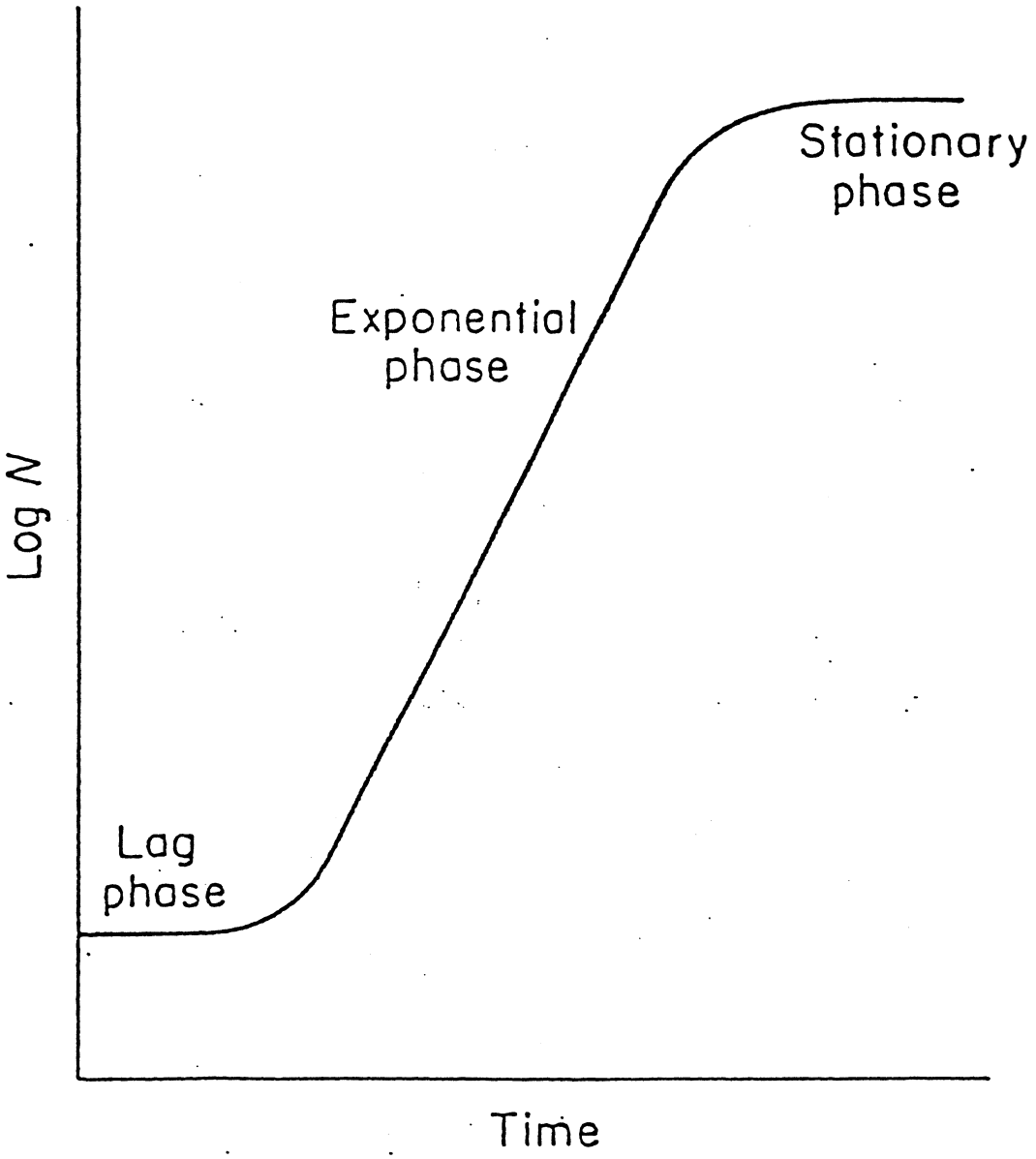


Figure 6: An exponential growth rate curve.

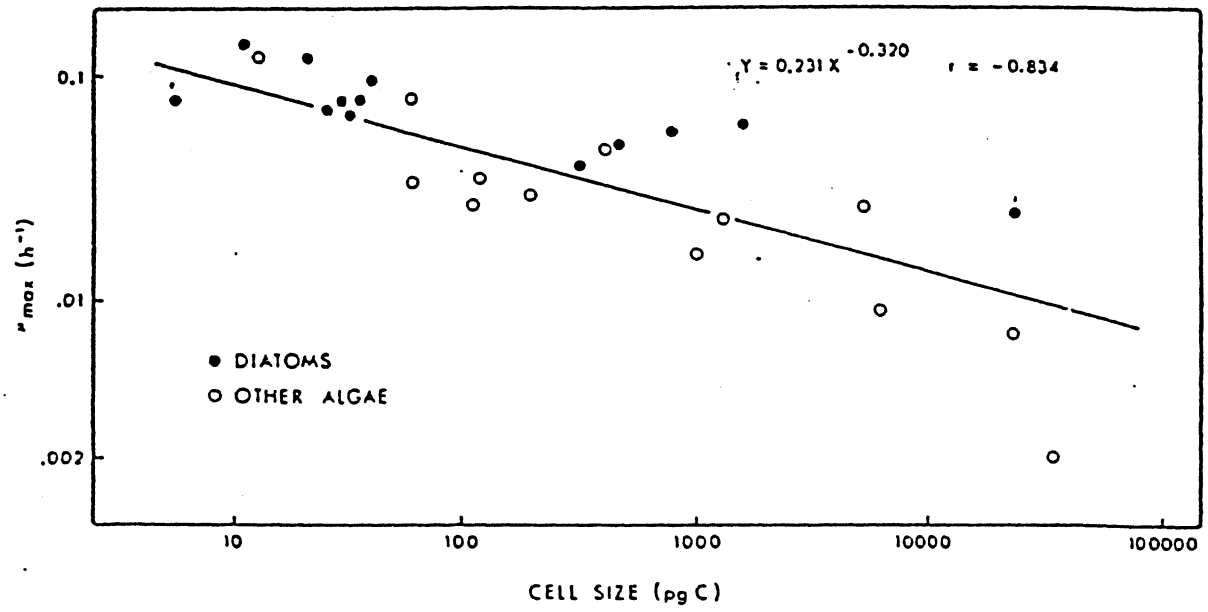


Figure 7: The relationship between cell growth and cell size published by Chriholm in 1992.

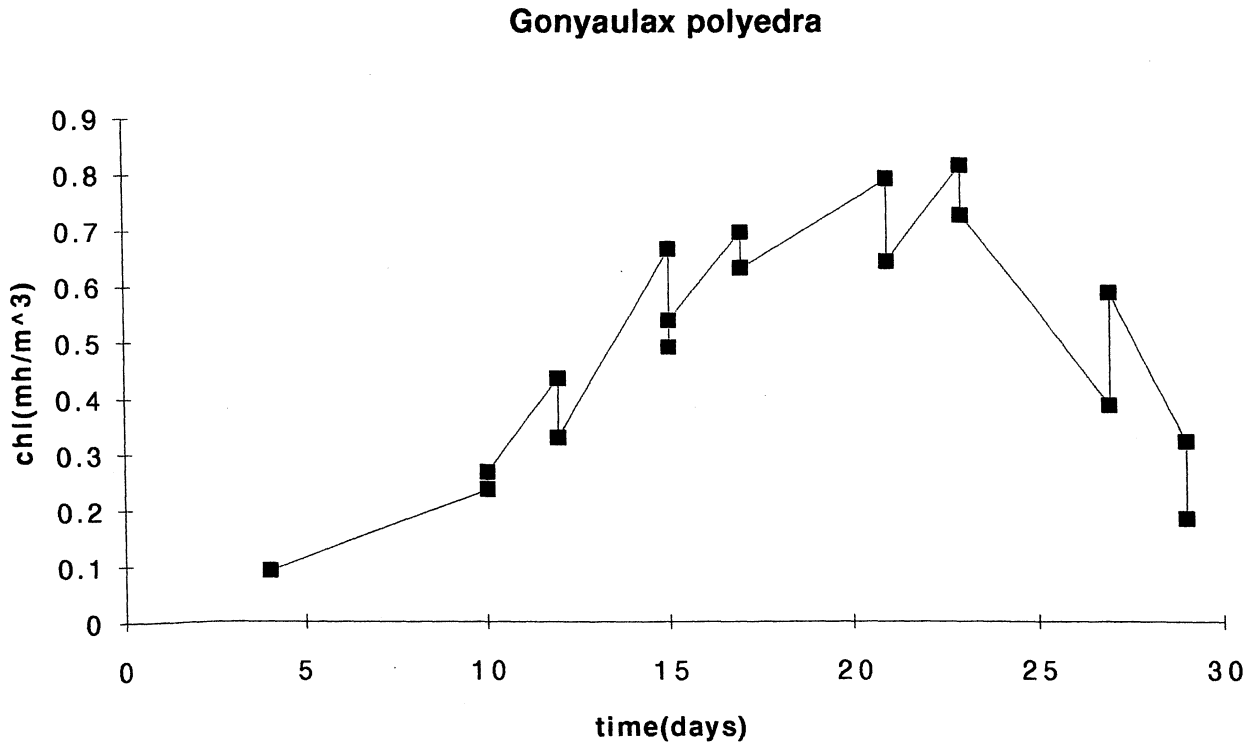


Figure 8a. Gonyaulax polyedra

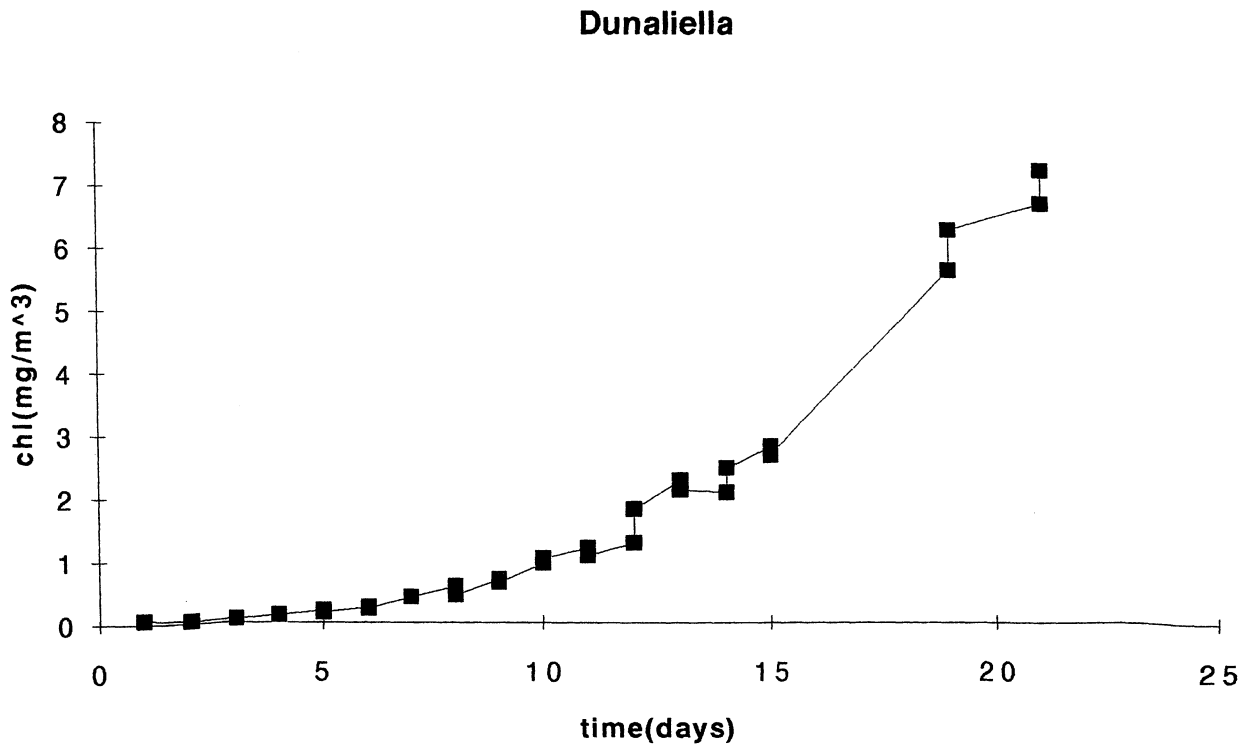


Figure 8b. Dunaliella

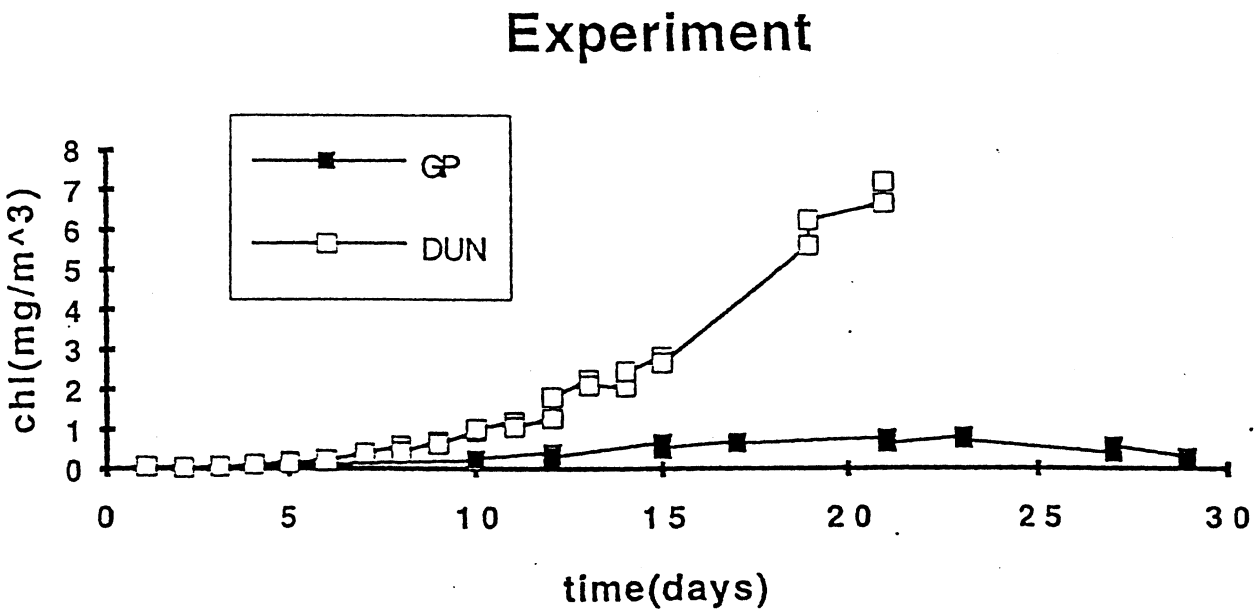


Figure 8: The experimental result showing the change in chlorophyll as a function of time.

Table 1. Experimental Results of Phytoplankton Growth Measured by Chlorophyll

fluorometer info

tau= 1.93

chl = [f* [tau/(tau-1)]*(Rb-Ra)*Volext]/(Vol filt*door*scale)

phae=[f* [tau/(tau-1)]*(tau*Ra-Rb)*Volext]/(Vol filt*door*scale)

EXPERIMENT #1/ CULTURE=GP

	month	day	sample #	vol filte	volext	s1 scal	s2 door	f	Rb	Ra	blanl	Rb-blan	Ra-blan	day	chl	pha	comments
test																	
	9	27	GP1	10	8	1	1	0.143	3.25	1.72	0	3.25	1.72		0.3632	0.02065	
	9	27	GP2	8.9	8	1	1	0.143	2.46	1.29	0	2.46	1.29		0.3121	0.00881	filter on table
culture																	
	9	31	GP1	15	8	1	3.16	0.143	3.77	1.97	0	3.77	1.97	4	0.0902	0.00301	
	9	31	GP2	15	8	1	3.16	0.143	3.9	2.07	0	3.9	2.07	4	0.0917	0.00893	
	10	6	GP1	15	8	1	1	0.143	3.15	1.66	0	3.15	1.66	10	0.2358	0.01597	
	10	6	GP2	15	8	1	1	0.143	3.55	1.87	0	3.55	1.87	10	0.2659	0.01754	
	10	8	GP1	15	16	1	1	0.143	2.77	1.4	0	2.77	1.4	12	0.4337	-0.02018	
	10	8	GP2	11	16	1	1	0.143	1.6	0.84	0	1.6	0.84	12	0.3281	0.00629	
	10	11	GP2	5	8	1	1	0.143	3	1.6	0	3	1.6	15	0.6648	0.02612	
	10	11	GP2	5	8	1	1	0.143	2.29	1.26	0	2.29	1.26	15	0.4891	0.04208	cotten wet pipet
	10	11	GP3	5	8	1	1	0.143	2.47	1.34	0	2.47	1.34	15	0.5365	0.03448	
	10	13	GP1	5	8	1	1	0.143	3.11	1.65	0	3.11	1.65	17	0.6932	0.02211	
	10	13	GP2	4.9	8	1	1	0.143	2.8	1.5	0	2.8	1.5	17	0.6299	0.02819	
	10	17	GP1	4	8	1	1	0.143	2.83	1.5	0	2.83	1.5	21	0.7894	0.01929	
	10	17	GP2	4	8	1	1	0.143	2.33	1.25	0	2.33	1.25	21	0.641	0.02448	
	10	19	GP1	4	8	1	1	0.143	2.95	1.58	0	2.95	1.58	23	0.8131	0.0295	
	10	19	GP2	4	8	1	1	0.143	2.58	1.36	0	2.58	1.36	23	0.7241	0.0133	
	10	23	GP1	4	8	1	1	0.143	1.37	0.72	0	1.37	0.72	27	0.3858	0.00582	
	10	23	GP2	4	8	1	1	0.143	2.39	1.4	0	2.39	1.4	27	0.5876	0.09259	
	10	25	GP1	4	8	1	1	0.143	1.15	0.61	0	1.15	0.61	29	0.3205	0.0081	
	10	25	GP2	4	8	1	1	0.143	0.66	0.35	0	0.66	0.35	29	0.184	0.0046	

EXPERIMENT #2A/CULTURE=GP/low light

	month	day	sample #	vol filte	volext	scal	door	f	Rb	Ra	blanl	Rb-blan	Ra-blan	day	chl	pha	comments
	11	8	GP 1A	50	8	1	3.16	0.143	2.91	1.53	0	2.91	1.53	1	0.0207	0.00403	
	11	10	GP 1A	20	8	1	1	0.143	0.3	0.16	0	0.3	0.16	3	0.0166	0.00261	
	11	10	GP 1B	20	8	1	1	0.143	0.31	0.17	0	0.31	0.17	3	0.0166	0.00537	
	11	11	GP1A	20	8	1	10	0.143	2.95	1.58	0	2.95	1.58	4	0.0163	0.00295	
	11	11	GP1B	20	8	1	10	0.143	2.7	1.44	0	2.7	1.44	4	0.015	0.00235	
	11	12	GP1A	20	8	1	10	0.143	2.86	1.68	0	2.86	1.68	5	0.014	0.01135	
	11	12	GP1B	20	8	1	10	0.143	1.95	1.11	0	1.95	1.11	5	0.01	0.00571	
	11	13	GP1A	20	8	1	10	0.143	2.45	1.36	0	2.45	1.36	6	0.0129	0.00519	
	11	13	GP1B	20	8	1	10	0.143	3.28	1.77	0	3.28	1.77	6	0.0179	0.00404	
	11	14	GP1A	20	8	1	3.16	0.143	1.05	0.57	0	1.05	0.57	7	0.018	0.00471	
	11	14	GP1B	20	8	1	3.16	0.143	1.32	0.7	0	1.32	0.7	7	0.0233	0.00291	
	11	19	GP1A	20	8	1	3.16	0.143	0.69	0.39	0	0.69	0.39	12	0.0113	0.00589	
	11	19	GP1B	20	8	1	10	0.143	1.83	1.01	0	1.83	1.01	12	0.0097	0.00354	

EXPERIMENT #2B/CULTURE=GP/high light

	month	day	sample #	vol filte	volext	scal	door	f	Rb	Ra	blanl	Rb-blan	Ra-blan	day	chl	pha	comments
	11	8	GP 2A	50	8	1	3.16	0.143	4	2.09	0	4	2.09	1	0.0287	0.00316	
	11	10	GP 2A	20	8	1	1	0.143	0.43	0.23	0	0.43	0.23	3	0.0237	0.00413	
	11	10	GP 2B	20	8	1	1	0.143	0.42	0.22	0	0.42	0.22	3	0.0237	0.00137	
	11	11	GP2A	20	8	1	3.16	0.143	1.37	0.74	0	1.37	0.74	4	0.0237	0.00547	
	11	11	GP2B	20	8	1	3.16	0.143	1.05	0.55	0	1.05	0.55	4	0.0188	0.00108	
	11	12	GP2A	20	8	1	3.16	0.143	1.23	0.64	0	1.23	0.64	5	0.0222	0.00049	
	11	12	GP2B	20	8	1	10	0.143	3.73	2.05	0	3.73	2.05	5	0.0199	0.00672	
	11	13	GP2A	20	8	1	3.16	0.143	1.44	0.78	0	1.44	0.78	6	0.0248	0.00614	
	11	13	GP2B	20	8	1	3.16	0.143	1.65	0.92	0	1.65	0.92	6	0.0274	0.0118	
	11	14	GP2A	20	8	1	3.16	0.143	1.7	1.01	0	1.7	1.01	7	0.0259	0.02341	
	11	14	GP2B	20	8	1	10	0.143	1.9	1.06	0	1.9	1.06	7	0.01	0.00433	
	11	19	GP2A	20	8	1	3.16	0.143	1.83	0.96	0	1.83	0.96	12	0.0327	0.00214	
	11	19	GP2B	20	8	1	3.16	0.143	2	1.08	0	2	1.08	12	0.0346	0.00793	

EXPERIMENT #2C/CULTURE=DUN/low light																	
	month	day	sample #	vol filte	volext	s1	s2	f	Rb	Ra	blk	Rb-blk	Ra-blk	day	chl	pha	comments
	11	8	DUN1A	50	8	1	3.16	0.143	5.63	1.53	0	5.63	1.53	1	0.0616	-0.25141	
	11	9	DUN 1A	20	8	1	1	0.143	0.85	0.49	0	0.85	0.49	2	0.0427	0.0284	
	11	9	DUN 1B	20	8	1	1	0.143	0.81	0.47	0	0.81	0.47	2	0.0404	0.02882	
	11	10	DUN 1A	20	8	1	1	0.143	1.29	0.78	0	1.29	0.78	3	0.0605	0.06392	
	11	10	DUN 1B	20	8	1	1	0.143	1.25	0.72	0	1.25	0.72	3	0.0629	0.04143	
	11	11	DUN1A	20	8	1	1	0.143	2.66	1.59	0	2.66	1.59	4	0.127	0.12129	
	11	11	DUN1B	20	8	1	1	0.143	2.6	1.54	0	2.6	1.54	4	0.1258	0.11046	
	11	12	DUN1A	20	28	1	1	0.143	1.21	0.73	0	1.21	0.73	5	0.1994	0.05903	
	11	12	DUN1B	20	8	1	1	0.143	3.56	2.19	0	3.56	2.19	5	0.1626	0.19785	
	11	13	DUN1A	20	8	1	1	0.143	5.18	3.15	0	5.18	3.15	6	0.241	0.26694	
	11	13	DUN1B	20	8	1	1	0.143	5.14	3.21	0	5.14	3.21	6	0.2291	0.31317	
	11	14	DUN1A	10	8	1	1	0.143	4.13	2.42	0	4.13	2.42	7	0.406	0.16043	
	11	14	DUN1B	10	8	1	1	0.143	4.26	2.53	0	4.26	2.53	7	0.4107	0.18485	
	11	15	DUN 1A	10	8	1	1	0.143	5.96	3.5	0	5.96	3.5	8	0.584	0.23593	
	11	15	DUN 1B	10	8	1	1	0.143	4.63	2.8	0	4.63	2.8	8	0.4345	0.22969	
	11	16	DUN 1A	10	8	1	1	0.143	7.08	4.16	0	7.08	4.16	9	0.6932	0.28157	
	11	16	DUN 1B	10	8	1	1	0.143	7.8	4.12	0	6.8	4.12	9	0.6363	0.34175	
	11	17	DUN 1A	5	8	1	1	0.143	4.83	2.85	0	4.83	2.85	10	0.9401	0.19898	
	11	17	DUN 1B	5	8	1	1	0.143	5.19	3.04	0	5.19	3.04	10	1.0209	0.20097	
	11	18	DUN 1A	5	8	1	1	0.143	6.19	3.7	0	6.19	3.7	11	1.1823	0.28222	
	11	18	DUN 1B	5	8	1	1	0.143	5.6	3.36	0	5.6	3.36	11	1.0636	0.26258	
	11	19	DUN 1A	5	8	1	1	0.143	6.85	4.18	0	6.85	4.18	12	1.2678	0.36128	
	11	19	DUN 1B	5	13	1	1	0.143	5.89	3.55	0	5.89	3.55	12	1.8055	0.28534	
	11	20	DUN 1A	2	8	1	1	0.143	4.82	2.92	0	4.82	2.92	13	2.2554	0.24204	
	11	20	DUN 1B	2	8	1	1	0.143	4.42	2.65	0	4.42	2.65	13	2.1011	0.2061	
	11	21	DUN 1A	2	8	1	1	0.143	4.92	3.18	0	4.92	3.18	14	2.0655	0.36128	
	11	21	DUN 1B	2	8	1	1	0.143	5.22	3.16	0	5.22	3.16	14	2.4453	0.2608	
	11	22	DUN 1A	2	8	1	1	0.143	5.9	3.55	0	5.9	3.55	15	2.7896	0.28237	
	11	22	DUN 1B	2	8	1	1	0.143	5.62	3.39	0	5.62	3.39	15	2.6471	0.27382	
	11	26	DUN 1A	1	8	1	1	0.143	5.87	3.52	0	5.87	3.52	19	5.5792	0.27409	
	11	26	DUN 1B	1	8	1	1	0.143	6.77	4.15	0	6.77	4.15	19	6.2202	0.36784	
	11	28	DUN 1A	1	8	1	1	0.143	7	4.2	0	7	4.2	21	6.6475	0.32822	
	11	28	DUN 1B	1	8	1	1	0.143	7.42	4.4	0	7.42	4.4	21	7.1698	0.31813	

EXPERIMENT #2D/CULTURE=DUN/high light

month	day	sample #	vol filte	volext	s1	s2	f	Rb	Ra	blk	Rb-blk	Ra-blk	day	chl	pha	comments
11	8	DUN 2A	50	8	1	3.16	0.143	5.75	2.09	0	5.75	2.09	1	0.055	-0.16118	
11	9	DUN 2A	20	8	1	1	0.143	0.83	0.48	0	0.83	0.48	2	0.0415	0.02861	
11	9	DUN 2B	20	8	1	1	0.143	0.83	0.48	0	0.83	0.48	2	0.0415	0.02861	
11	10	DUN 2A	20	8	1	1	0.143	1.43	0.83	0	1.43	0.83	3	0.0712	0.05101	Fell on the table
11	10	DUN 2B	20	8	1	1	0.143	1.23	0.74	0	1.23	0.74	3	0.0582	0.05882	
11	11	DUN2A	20	8	1	1	0.143	2.33	1.4	0	2.33	1.4	4	0.1104	0.1104	
11	11	DUN2B	20	8	1	1	0.143	2.28	1.33	0	2.28	1.33	4	0.1128	0.08514	
11	12	DUN2A	20	8	1	1	0.143	3.11	2	0	3.11	2	5	0.1318	0.22257	
11	12	DUN2B	20	8	1	1	0.143	3.05	1.93	0	3.05	1.93	5	0.133	0.20029	
11	13	DUN2A	20	8	1	1	0.143	4.32	2.6	0	4.32	2.6	6	0.2042	0.20714	
11	13	DUN2B	20	8	1	1	0.143	4.1	2.49	0	4.1	2.49	6	0.1911	0.20943	
11	14	DUN2A	10	8	1	1	0.143	3.23	1.89	0	3.23	1.89	7	0.3181	0.12396	
11	14	DUN2B	10	8	1	1	0.143	3.09	1.84	0	3.09	1.84	7	0.2968	0.13687	
11	15	DUN 2A	10	8	1	1	0.143	3.96	2.35	0	3.96	2.35	8	0.3822	0.17079	
11	15	DUN 2B	10	8	1	1	0.143	3.7	2.2	0	3.7	2.2	8	0.3561	0.16203	
11	16	DUN 2A	10	8	1	1	0.143	4.84	3	0	4.84	3	9	0.4368	0.28193	

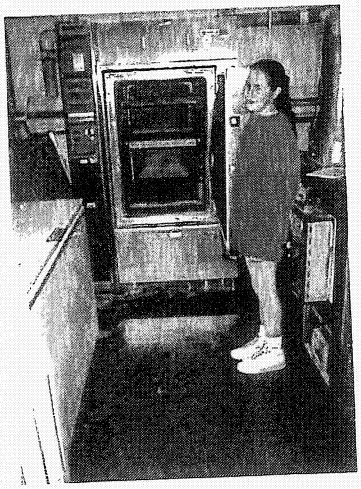


Photo 1: Autoclave for heating the cultures.

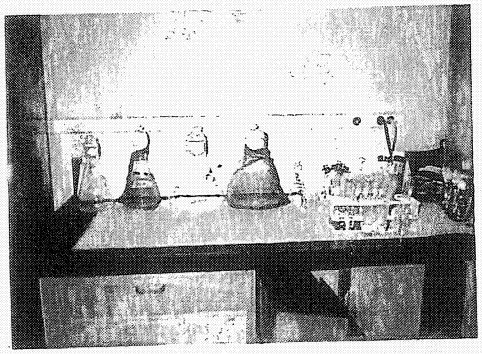


Photo 2: Cultures in front of the light where they grow.

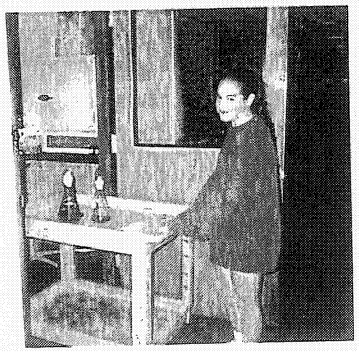


Photo 3: Moving culture flasks to the clean room.

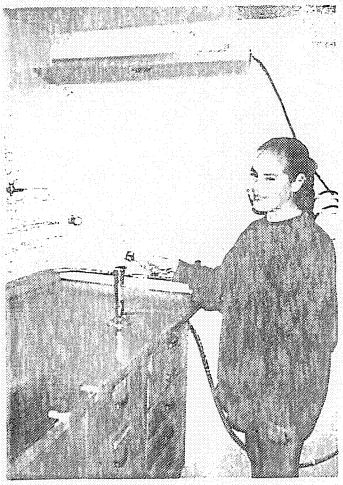


Photo 4: Bunsen Burner used to heat flask.

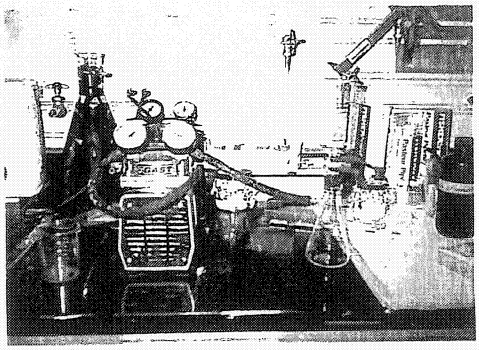


Photo 5: Filter tower.

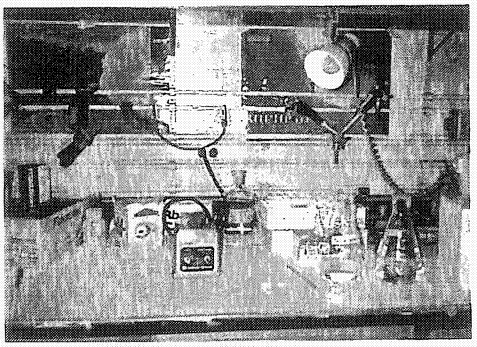


Photo 6: Mechanical stimulator for breaking cells.

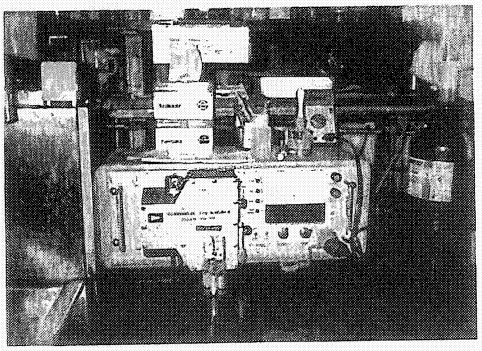


Photo 7: Fluorometer used to measure fluorescence.

Photo 8: Area for washing culture tubes.

Photo 9: Microscope used for viewing cells on slide.

Appendix 2

Phytoplankton Cellular Changes in Response to Light

Alison Baker
Standley Middle School, 8th grade

Greater San Diego Science and Engineering Fair 1997

Table of Contents

Introduction

The floating microscopic plants and animals in the ocean are called plankton. Plankton are divided into two groups: the plants are called phytoplankton; the animals are called zooplankton. Phytoplankton do not have as great a diversity as the zooplankton in the ocean (Sverdrup, 762). What importance are these tiny creatures to us? The plants provide nearly all of the food in the sea. They are at the bottom of the food web, which means that all of the other animals that live in the ocean rely on them as food. Think of how much life wouldn't be able to survive without these plants.

To understand how the ocean functions and stays balanced, it is important to look more closely at each type of phytoplankton and what makes it grow. Past experiments have looked at some species of phytoplankton (see Table 1) and have compared growth rates and absorption rates in controlled laboratory environments (Chan 1, 396). Phytoplankton grows under many sky conditions from sunny (high light) to cloudy (low light) skies. The amount of light affects their growth.

The growth rate of a phytoplankton culture can be determined by measuring the amount of phytoplankton present over a period of time. The amount of phytoplankton can be found in several different ways. The two ways that are often used are 1) measuring the amount of chlorophyll in a sample and 2) counting the cells in a fixed volume using a microscope. Measuring sample absorption tells how much light is absorbed by the phytoplankton and depends on how many cells are in the sample and how much chlorophyll is in each cell. These things are affected by whether it grows under high or low light levels. Absorption is measured using a spectrophotometer.

Phytoplankton growth can be controlled by factors such as light and nutrients. Phytoplankton aren't usually found competing for nutrients because physiological differences allow them to occupy different niches (Chan 2, 428). Most experiments use continuous light for energy.

Scientists have not found out everything about all the kinds of phytoplankton and their different light requirements for photosynthesis.

Background

All the passively floating plants of the ocean are phytoplankton. Phytoplankton are very important to us even though they are microscopic plants. Phytoplankton are single cells that are in the Protista kingdom in the five kingdom organization (Sieberth, Fig. 1). A sample of sea water contains many different types of phytoplankton (Fig. 2). Certain types of scientists spend time sorting through these microscopic creatures and counting them.

The most common types of phytoplankton are dinoflagellates and diatoms which grow in the ocean and in laboratory cultures too. Different types of phytoplankton have many different characteristics. The diatoms in general usually bloom in the spring, and are the chief component of phytoplankton then. The dinoflagellates in general grow best in the summer, and are the second main component of phytoplankton (Chan 2, 430). Phytoplankton are also often distinguished by their size (see Tables 1&3).

Dinoflagellates are members of the yellow-green algae class Dinophycea (Dodge, 1). They have two flagella: one trailing and one coming out of the side at a 90 degree angle. The flagella are attached at the girdle. Dinoflagellates are unicellular and are less than 5-100 micrometers. When they are abundant in one area, they make the water look red. This is called a red tide. Gonyaulax Polyedra (GP), micro plankton in size, is a type of dinoflagellate that creates the red tide (Fig. 2).

The diatom, unlike the dinoflagellate, has no way to propel itself. It just floats in the water and goes with the current. The diatom is also unicellular, and they are 3-300 micrometers. The diatom Thalassiosira Weissflogii (TW) is a centric diatom. It is also a micro plankton of 10 microns in size (Fig. 2). This diatom is very ecologically important, and is prevalent in the Gulf Stream and in Antarctica (Fryxell, 41).

The class of green algae called Chlorophycea, have some of the same characteristics as the dinoflagellates (Butcher, 65). They also have flagella,

The equation that is used to find the growth rate of phytoplankton is:

$$Nt=No*e(\mu*t) \qquad (1)$$

μ=growth rate which is proportional to rate of cell division and N=number of cells (Banse, 1886). The growth rate measurement is something that is done in the laboratory, but it applies to growth in the field as well (see Table 4). The growth is effected by changes in nutrients, temperature, and light. The growth of a cell is dependent on many things including the amount of nutrients in the media, and the surface area of the cell. It is possible to calculate the number of cells at a later time with equation 1 if the initial number of cells is known and the growth rate (Fig.4c). Also, if the end number of cells is known, it is possible to calculate the number of cells needed to start in order to get the desired concentration after t days (Fig. 4d).

Methods

In order to investigate phytoplankton growth, the following methods are used:

1. Media preparation to grow cultures
2. Light regime definition
3. Culture growth measured
4. Fluorescence measurements to determine chlorophyll biomass
5. Microscope cell counts on fixed cultures
6. Absorption measurements to measure absorption by pigments

1. Media (f/2 & GPM)

There are many different kinds of medium that are used for cultures. They have different amounts of salt, water, and nutrients. There are three kinds of media used at Scripps Institute of Oceanography: IMR, GPM, and F/2. The IMR (Institute of Marine Resources) is a medium good for dinoflagellates in general. However, a media specifically made for Gonyaulax Polyedra is

GPM (Gonyaulax Polyedra Media) (Baker, 5). It is made by adding the following to 700 mL of GFF SW (filtered sea water of 28-32 o/oo salinity):

225 mL distilled water
2 mL KNO_3
.2 mL K_2HPO_4
5 mL trace metals
1 mL B_{12}
1 mL Thiamin-HCL
1 mL Biotin

The F/2 media is used for diatoms which need silicon and is made by adding the following to 1 liter of GFF SW:

1 mL NaH_2PO_4*H2O
1 mL $NaNO_3$
1 mL $NaSiO_3$
1 mL vitamins
100 uL trace metals

Dinoflagellates, diatoms and chlorophytes can be studied by growing them in a culture. There are three types of cultures: batch cultures, continuous cultures, and diffusion cultures (Sieburth, 128). In a batch culture the nutrient medium isn't replenished after beginning. It is a simple and convenient culture to use. In all of these cultures the living animals have been left out so that there isn't anything that can eat the phytoplankton.

The autoclave (Fig. 5) is a large oven that sterilizes the chemicals, water, or containers using steam and water to kill all bacteria that might affect phytoplankton growth in cultures (Fig. 5). A dial selects whether you are heating dry flasks containers or heating containers with liquids in them. The temperature will go from 54°C to 123°C, and the pressure goes from 0 to 20 PSU. The autoclave will take from 40 minutes to 100 minutes to cool down

2. Light Regimes

Screen is used to wrap different flasks so that experiments can be done using different amounts of light. A spectrophotometer is used to measure the amount of light that can go through the screen. It isn't known for all phytoplankton if they have different minimums of amount of light required for photosynthesis. Most of the tests on phytoplankton have been done with maximum light, although many scientists are interested in tests done with different amounts of light.

Lights used in experiments are chosen to be as much like sunlight as possible (Fig. 4). The amount of light for visible wavelengths (350-750nm) for sunlight and the amount of light for the lamps used in this work is shown in Fig 4a and 4b.

3. Culture Growth

A beginning culture is grown up at 100% light and then an estimated growth rate (see Table 4) was used in the growth rate equation to calculate what should be the beginning amount of inoculation culture to add to the three flasks with media. Two flasks are wrapped in screens to give 60% and 30% light. The growth should continue for several generations and end with a culture density that is optimum for the absorption curve analysis. This takes approximately one month.

4. Fluorescence Measurements

A fluorometer (Fig. 6) measures the amount of chlorophyll in a sample. The chlorophyll molecule is fluorescent which means it absorbs light at one wavelength and emits light at a new, longer wavelength (Fig. 6). In a fluorometer, there is an exciting light (white daylight) from a lamp that goes through a colored filter and transmits light of chosen wavelength (436nm). The light then passes through the sample and the sample emits light at 676 nm. This light is proportional to the fluorescent material or the chlorophyll present (Turner, A2-1).

5. Microscope Cell Counts

Fixation is when a chemical, usually Lugol, is dropped into a container of phytoplankton, to fix or preserve the cells at their present state. This is convenient for transportation. Lugal is iodine dissolved in a potassium iodine solution. It is often used in its acid version for marine phytoplankton. This version was first proposed for freshwater studies. The preserved sample remains good to view under a microscope for up to six months. There are a variety of slide types used to count cells depending on their density and size (see Table 5, Fig. 7).

I used a standard microscope for all the counts. The Palmer-Malony slide was used for GP cells; the Haemacytomer slide was used for the denser DUN cell counts (Fig. 8). A defined grid like the one in Fig 8 was used to count cells. To get a statistical sampling, five different grid blocks were counted (Fig. 7). The results were averaged and the average number of cells per volume can be calculated.

6. Absorption Measurements

There are many kinds of spectrophotometers including a Perkin-Elmer (Perkin Elmer, 1) and a Cary (Cary, 1). All spectrophotometers (Fig. 9) have a monochrometer that splits light into single wavelengths, and a dual beam where one beam goes through sample and one through the reference simultaneously (Fig. 9). The spectrophotometer sends the information to a computer, that uses software collects data so the spectral absorption curve can be calculated. Chlorophyll molecules absorb some wavelengths of light more than others for use in photosynthesis (Fig. 10 & 11). The absorption spectrum shows how much of each wavelength is absorbed the most (violet 436nm and red 676nm) and the least (green 550nm).

The spectral data recorded is the optical density for each sample.
The following equation has been developed to convert from the optical density to the absorption (Mitchell, 138-139):

$$A_p(wl) = 2.3 * OD_f(wl)/(lg\ B) = 2.3\ OD_S(wl)/lg \qquad (2)$$

wl = wavelength
A_p = Particulate Absorption
OD_f = measured optical density from a filter
OD_S = measured optical density from a suspension
lg = V_f/A_c
V_f = Volume filtered
r = filter radius
A_c = Circular filter area = $Pi*r^2$ = 3.46 cm^2

The optical density for a suspension (OD_S) is determined from the optical density of a filter using the following equation:

$$OD_S\,(wl) = a*OD_f\,(wl) + b(OD_f(wl))^2 \qquad (3)$$

wl = wavelength
OD_f = measured optical density from a filter
OD_S = measured optical density from a suspension

Where for filters of the type GFF in general from table 1

$$a = .392 \qquad b = .655 \qquad (4)$$

and for filters of the type GFF and for Dun, TW, and GP in particular from table 2

$$a = .415 \qquad b = .690 \text{ (used)} \qquad (5)$$

Combining equations (2), (3), and (5) gives an equation from which the absorption can be calculated if the optical density is known.

$$Ap=2.2(3.46/V_f)(.415\ OD_f + .69OD_f^2) \qquad (6)$$

or

$$Ap=1/Vf(3.3OD_f + 5.5\ OD_f^2) \quad (7)$$

This equation was used and the result normalized by both chlorophyll and by number of cells to give the absorption per biomass and per cell.

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Problem

Does the amount of light affect the amount of absorption of different types of phytoplankton?

The growth of the phytoplankton will be monitored over time by determining the biomass, the number of cells and the absorption rate of the different phytoplankton. The growth is monitored by measuring the fluorescence of cultures by taking a daily sample for about one month. The number of cells in each culture will be determined by counting under a microscope at three stages of growth. The absorption is also determined at three stages of growth by putting a sample in a spectrophotometer and calculating the spectral absorption.

Hypothesis

I think that a phytoplankton culture's growth as well as absorption will be affected by the amount of light present and the cells will adapt to the light. The smaller phytoplankton (Dun) and the growth rate of the smaller phytoplankton will be more sensitive to changes in the amount of light than the larger phytoplankton (GP). The importance of size was shown in my experiments last year on phytoplankton growth, which demonstrated that smaller cells grow faster than larger cells. Since cells adapt to light, I think that the faster growing phytoplankton (Dun) will be more affected by the change in the amount of light because they multiply faster, therefore there will be more generations that have been grown up at the different light levels.

Procedures

The project timeline in given in Table 2. Media is prepared, autoclaved, and cultures are prepared as described below. The lights were also prepared. The first set of experiments were growth rates fro each phytoplankton species where the chlorophyll was measured. After this an experiment at three light levels was done, and the chlorophyll, cell counts, and absorption measurements were made as described below.

Media preparation:

1) For each 1L batch of media a 2L flask and a 125 mL flask were used.
2) The filtered sea water and distilled water were measured out into the 2L flask.
3) The GPM medium for making 500 mL's uses the following ingredients measured into a 125mL flask:

375 mL filter sea water (28-32 o/oo salinity)
112.5 mL distilled water
1 mL KNO3
0.1 mL K2HpO4
2.5 mL trace metals
0.5 mL B12
1 mL Thiamin-HCL
1 mL Biotin

The GPM sea water is put in one flask and the nutrients in another smaller flask so that they can be heated separately before they are mixed. Otherwise, it has been found that the nutrients precipitate out.

4) The F/2 medium for making 1L contains the following ingredients:

1 mL $NaH_2PO_4*H_2O$
1mL $NaNO_3$
1mL $NaSiO_3$
1mL vitamins
100uL trace metals

5) Cotton stoppers were placed on all flasks so they were ready to be autoclaved. (See steps on autoclave use)

6) When the flasks were taken out of the autoclave they were taken into the clean room along with a syringe and a filter

Autoclave use:
Heating is done in an autoclave which reaches a temperature of 105 deg Celsius for 20 minutes (GPM) or 15 minutes (F/2). Cooling then takes about an hour.

Putting things in the autoclave:
1) Put the flasks on a tray so that if they overflow the tray will catch all of the water, also it makes them easier to take out.
2) Put the tray in the Autoclave.
3) Shut the door until the Door Open light turns off (For this light to be on, the Power switch and the Control switch must be turned on).
4) Lift up the top metal covering for the water and steam knobs. Turn these two knobs as much as you can.
5) Set the timer for as long as you would like to heat the flasks (I heat 20 minutes for cultures, and 15 minutes for dry flasks).
6) Wait until the jacket pressure is at 20, then close the metal covering.
7) If you are heating cultures or flasks that have liquids in them then press the liquid button, if you are heating empty flasks then press the gravity button.
   Temp goes from 54degC to 123degC.
   Pressure goes from 0 to 20 PSU.
   Lights go from condition to sterilize.

Taking things out of the Autoclave:
1) Immediately after the set time lift up the metal covering and turn off the water and steam supply.
2) Wait until the temperature goes down to 40-60 degrees, this will take about 40 min-1hour and 15 min.
3) Then crack the door. There will be a big puff of steam, wait until it is all out before completely opening the door.
4) Use the orange lab gloves to remove your culture from the Autoclave(they will still be quite hot).

Light regimes:
1) Several types of screens were purchased to use as light filters on the culture flasks.

2) The light transmittance of the screens was measured by putting the screens in a spectrophotometer.
3) The screens that allowed 60% and 30% light were selected.
4) Three 1,000 mL flasks were autoclaved to kill any living cells that might hurt the phytoplankton.
5) Two of the three culture flasks were wrapped with screens to give three different light regimes: 100%, 60% and 30%.
6) The lamps in the cold room growth area were placed on the side so that the light would filter through the screens and not be obstructed by the cotton tops.

Culture Preparation:
1) A beginning culture was grown up at 100% light and then an estimated growth rate was used in the growth rate equation to calculate what should be the beginning amount of inoculation culture to add to the three flasks with media. The growth should continue for several generations and end with a culture density that is optimum for the absorption curve analysis.
2) The already started culture of phytoplankton, the three flasks that had been autoclaved (culture flasks), and the flask containing 1,500 mL's of the autoclaved media were taken into the clean room.
3) The neck of the flask that contains media, and the neck of one of the culture flasks were heated using a Bunsen burner. The cotton top was then removed from the neck of the flask. This prevents unsterial air from entering the flask during the transfer of liquids.
4) 500 mL's was then poured from the flask containing the media into the already opened culture flask.
5) The necks of both bottles were reheated, and the cotton stoppers were replaced in the neck of the flask.
12) Repeat steps 9-11 for the remaining two culture flasks.
13) The neck of the flask that contained the already growing culture, and the neck of one of the culture flasks was heated using a Bunsen burner.
6) The decided amount of inoculum was measured from the growing culture, and was placed into the 500 mL's of media in the already opened culture flask.
7) The necks of both bottles were reheated, and the cotton stoppers were replaced in the neck of the flask.

8) Steps 13-15 were repeated for all of the three culture flasks.
9) The three culture flasks were then removed from the clean room, and placed in the cold (18 deg C) room in front of a light to begin growing.

Chlorophyll sampling:
1) After each culture (500 mL volume) was begun by putting the inoculation in the media, two culture sub-samples were taken every day for several weeks in order to find out the amount of chlorophyll in the cultures and to determine how fast these cultures were growing.
2) Before the cover to the flask was taken off to sample, the samples are moved to the clean room.
3) The neck of the flask was heated with a Bunsen burner so that not as much non sterile air would go in the flask.
4) The amount of culture that was to be filtered was poured into a plastic container.
5) Each sample is poured into a filter tower over a 0.45 micron filter which catches most of the phytoplankton but lets the water through.
6) The water was pulled through the filter by a pump.
7) A pair of tweezers was used so that oil from my hand wouldn't contaminate the sample, the filter is removed from the filter base and put into a culture tube.
8) Then the culture tube is filled with 10 mL of 90% acetone which extracts the chlorophyll from the phytoplankton cells.
9) This culture tube is covered with parafilm and placed in the refrigerator because the cold can help the extraction.
10) The tube is removed after 24 hours in a room where the normal lights have been turned off and the only light on is a yellow light which will not degrade the chlorophyll.
11) The sample was then mechanically stimulated (shaken) in order to break any last cells that might have stayed intact.
12) Then the samples were covered with foil to keep out light, and carried to the fluorometer.
13) The sample (acetone+chlorophyll) was poured into a fluorometer glass culture tube leaving the filter behind.
14) This was put into the fluorometer which measures the fluorescence in volts.

15) Then 10% HCL, an acid, was added to the tube and the fluorescence was again measured.
16) From this information the chlorophyll was calculated because the fluorescence of a sample is proportional to the chlorophyll present.
17) All of the used culture tubes were taken back to the lab, they were washed with water and soap, and then they were put into the acid bath. The acid bath helped to kill germs and bacteria.

Cell Counts:
1) 5 mLs of the culture were taken and put into a screw top vial every time that an AP was taken.
2) 1 mL of Lugols solution is added to the sample of the culture. Lugol is iodine in water. This solution kills, stains and fixes the phytoplankton cells so they can be viewed under a microscope at a later time.
3) When the cells were to be counted they were taken to the microscope room and put into one of two types of slides. For the dense cells, a hemocytometer slide holding .01 mL can be used with a standard upright microscope. Five representative sections are counted and averaged to give the final number of cells. For the less dense culture samples, a .1 mL sample on a Sedgewick Rafter is used with an either a standard or inverted microscope.
4) When all of the samples had been counted, the slides were emptied and cleaned.

Absorption sampling:
To find the absorption rate and the number of cells, I took further sub-samples of each culture at three different times: at the beginning of the experiment, at the end of it's exponential growth, and when the culture has become nutrient limited.
1) Repeat the steps 1-6 for taking a normal sample of the culture.
2) Tweezers were used to place the filter on a 2.5 cm grated plastic container.
3) Steps 1 and 2 were repeated for all of the samples.
4) Each of these are labeled and dropped into a container of liquid nitrogen. The purpose of the liquid nitrogen is to preserve the samples until they are run on the spectrophotometer.

5) When the samples were ready to be run on the spectrophotometer, the filters were place on a filter holder, and into the spectrophotometer which shines light through the sample and compares the transmitted light with light that goes through an empty filter holder. The difference between these two spectral curves gives the amount of light absorbed by the cells.

Variables and Controls

1) Variables to be Changed:

The variables that I have in my project are the two types of phytoplankton and three different amounts of light.

2) Variables to be Measured:

The chlorophyll, absorption, and the number of the cells will be measured through out the experiments. This information will tell how different types of phytoplankton are affected by light.

3) Controls:

- The temperature
- The nutrients in each media
- The flasks
- The set up

4) Control Group:

Before each light experiment there was an experiment done at 100% light. This culture was used as a control group. Also during each experiment there was a 100% light done again for each group.

Materials

Media:

•6 ferenbauch flasks (Fisher)
•6 cotton stoppers (Custom Made)
• GPM ingredients:
750 mL of filtered sea water, 225 mL distilled water, 2 mL KNO_3, .2 mL K_2HPO_4, 5 mL trace metals, 1 mL B_{12}, 1 mL Thiamin-HCL, 1 mL Biotin
•F/2 ingredients:
1 L of GFF SW, 1 mL NaH_2PO_4*H2O, 1mL $NaNO_3$, 1mL $NaSiO_3$, 1mL vitamins, 100uL trace metals
•Autoclave
•Bunsen burner

Chlorophyll Measurements:

•fluorometer (Turner Designs)
•fluorometer culture tubes (Fisher)
•GF/F filters 25mm (Fisher)
•tweezers
•air pump
•culture tubes (Fisher)
•90% acetone
•Parafilm (Fisher)
•Colored labeling tape (Fisher)
•10% HCL
•mechanical stimulator

Absorption Measurements:

•Perkin-Elmer Spectrophotometer
•quartz sample cuvette (Fisher)
•PC computer w/ spec software

•liquid Nitrogen
•cool white fluorescent lights
•screens (for 30%, and 60% lights)

Cell Counts:

•Microscope

•Microscope slides
•Lugol solution
•screw top vials
•disposible glass pipettes (Fisher)

Theory

There is a relationship between cell growth and cell size (Chrisholm, 214) where

$$\text{growth} = (0.231)(\text{cell size})^{(-0.32)} \qquad (8)$$

so that smaller cells grow faster than larger cells. Another theory takes into account both size and surface area (Baker, 9) where

1) cell surface area is related to nutrients
2) cell volume is related to chlorophyll.

So

$$\text{growth} \sim \frac{\text{nutrients}}{\text{chlorophyll}} \sim \frac{\text{surface area}}{\text{volume}} \sim \frac{4*pi*r^2}{4/3*pi*r^3} \sim \frac{3}{r} \qquad (9)$$

would mean for the cultures grown in the study:

growth(GP)= 3/25= .12
growth(DUN)= 3/5= .60
growth(TW)= 3/10=.30

So it is expected that DUN (10 microns in diameter) will grow faster than GP(50 microns in diameter) when cultures are grown with constant temperature and light. DUN have more surface area for their volume so they can absorb more nutrients (Baker, 10). TW grows more rapidly than GP (Chan 1, 398).

Table 4 shows the results of using equation 1 to calculate the culture density to begin with at the start of each experiment in order to obtain an end culture value of 150 ug/l while in the exponential phase of the growth. This end value was chosen to be not too dense for making the final absorption measurements and cell counts. Figure 12 plots the beginning cell values versus the time it takes to reach the 150 ug/l point. The results depend on the specific growth rate for each species so a range of growth rates was

chosen to show the range of beginning values needed. The range chosen was based on the values shown in Table 1 that were obtained last year for the growth rate of GP and Dun.

The growth rate of GP, Dun and TW as estimated from the size as given in equation 8 is listed in Table 1 on the "growth calc by size" line which ranges from 0.31 to 0.51. Equation 9 developed last year was also used and the results are listed in Table 1 on the line labeled "growth calc by size/nut". The values range from 0.16 to 0.75. The growth rate curves and the cell counts from this year allow actual growth rates to be measured and these can be compared with the theoretical values.

Experimental Results

In order to calculate growth rates, the chlorophyll was measured daily over several weeks in 100% light for three species of phytoplankton Gonyaulax polyedra (GP) , Thalassiosira Weisflogii (TW) , and Duneliella Tertiolecta (Dun) cultures. The growth is shown in Figures 13a, 13b and 13c. The data is shown in Tables 6a, 6b, and 6c. The results from last year are labeled '95 in the legends.

In order to look at the effects of light once the growth curves under 100% light were done, batch cultures of phytoplankton were grown again but under three different light levels. The beginning chlorophyll of the cultures was measured again every 1-3 days (Tables 6a, 6b, and 6c). The absorption measurements and the cell counts were taken three times during each experiment which lasted up to a month for each (Figs 13a,13b and 13c) in order to have growth past the exponential phase into the stationary phase. The growth from the different light regimes is labeled 100%, 60% and 30% in Figures 13. In these figures arrows mark the points where sub samples for cell counts and absorption measurements were taken.

The first light experiment was done with Gonyaulax polyedra. The culture in the 30% light did not grow. It is thought that the 30% light was too low a light level for the amount of innoculum (starting density of cells) that was added to the new media. The exponential growth phases ended on about the 20th sample day. The culture under 60% light grew less and reached a lower stationary phase value (or carrying capacity) than the culture under 100% light. That is, the chlorophyll in the 100% culture was higher at all times, but the 60% culture peaked at the same time as the culture under 100% light. The culture was sub sampled for cell counts and for absorption measurements on days 1, 18 and 25. The cell counts for GP showed that the 60% light also had less cells than the 100%, a difference which increased with time through the experiment (Fig. 14a).

Thalassiosira Weisflogii was grown under the same light conditions as the Gonyaulax polyedra. The Thalassiosira cultures did not grow during the

different light experiments. Therefore only the growth rate results for TW are included. A different medium had to be prepared for growing the TW cultures. Since it was suspected that the nutrients in the medium were not the correct proportions, a new medium was prepared. The cultures also did not grow this time. It is likely that the initial innoculum was not high enough.

After the Thalassiosira Weisflogii, Duneliella Tertiolecta was grown under 100%, 60% and 30% light. The experiment with the Duneliella worked very well. The results were very different from the results of the Gonyaulax experiment. Figure 13b shows that the different amounts of light didn't make as a large a difference in chlorophyll values during the exponential growth phase which ended at about day 17. The culture in 30% light grew less rapidly but continued to grow to a higher stationary growth level (Fig. 13b). The cultures under 60% light and 100% light grew about the same amount. The cell counts led to the same conclusion as the Gonyaulax, that the higher the light, the more cells there are (Fig. 14b). The difference in cell numbers between the 60% and 30% cultures was not as big as the difference between the 60% and the 100% light which has the most cells.

Since both chlorophyll and cells were measured, it is possible to look at how much chlorophyll is in each cell. Table 7 shows the chloropyll and the cell counts for each culture, for each light regime, and for each of the three sample days counted. Fig. 15 shows that after two weeks when both cultures are growing well that the less the amount of light, the more chlorophyll per cell. The chlorophyll per cell in the Gonyaulax changes more than it does in the Duneliella. Another way to see this same information but which emphasizes that the number of cells present in the DUN cultures is more than 100 times as many as the cells in the GP cultures is shown in Fig. 16 where the chlorophyll is plotted versus the amount of light.

In addition to measuring chlorophyll and cell numbers, the spectrophotometer measurements done three times during each experiment give an indication of how light was absorbed by each species in the visible region every one nanometer. The visible region is wavelengths 350-750 nm

Figure 17 shows a comparison of the absorption in the visible region by GP and DUN.

Normalizing the absorption by the number of cells present (Fig. 18a,18b) shows the same thing as the normal absorption curves, except that the y-axis is scaled a lot lower. These curves show how much each individual cell absorbs. Earlier it was shown that the lower light level cultures contain more chlorophyll per cell. This affects the absorptions, where the more chlorophyll per cell, the more absorption per cell. Therefore the more light, the less absorption per cell.

Chlorophyll absorbs the most in the red (676nm), so the absorption at this wavelength was divided by the number of cells to show (Fig. 19) the absorption per cell of GP and Dun at different light levels. It is clear that the absorption per cell is higher for less light in both species. Normalizing the absorption by the amount of chlorophyll present is shown in figures 20 and 21. In Figure 21 the absorption at 676 normalized to chlorophyll shows that the lower the light level, the more absorption at wavelength 676nm.

Conclusion

In conclusion light does affect the growth rate, cell number, and absorption of all species of phytoplankton grown during these experiments. Although all of the cultures were affected, they were affected in different ways.

These growth curves show a shape similar to the exponential growth curve in Fig. 4. All cultures grew cells faster in the higher light environment but the different species grew at different rates. Duneliella was the species that grew the fastest, and Gonyaulax was the slowest as is summarized in Table 1. This is due to the difference in their size as shown by the calculations from equations 1 and 2. The calculated growth rates based on just size ranged from 0.31 to 0.51 and the calculations based on size and nutrients ranged from 0.16 to 0.75 for GP, Dun and TW. The growth rates based on the cell numbers counted ranged from 0.38 to 0.47. The different amounts of light affected the GP growth rate much more than it affected the others.

When the amount of light was decreased for a culture, the growth was limited so the culture did not reach as high of a stationary state. The higher the light level, the more the chlorophyll in the culture. Also, the number of cells increased with time more for the higher light levels. But in both cultures, Gonyaulax and Duneliella, the chlorophyll per cell was greater with less light. So although the number of cells is less in a culture grown at lower light, the cells adapt to lower light by containing more chlorophyll in each cell as is shown in Figure 22.

The absorption per cell at 676nm also showed there was more cellular absorption with less light. This is expected because the chlorophyll per cell was higher and the more chlorophyll per cell, the more absorption per cell. At the 60% light the chlorophyll per cell in GP is about 200 times the chlorophyll per cell in Dun. The cultures adjusted to the lower light by making more cells and more chlorophyll in each cell. Although the hypothesis was that the cells that grow fastest would change the most, Figure 22 shows that the cells that are the biggest changed the most. So the

absorption per chlorophyll by these two different species relates to the size of the cell.

The absorption curves for the GP and Dun are very different and show how other pigments in the cells are also absorbing light. These pigments are also being affected by the change in light in addition to the chlorophyll. Since absorption was measured at many different wavelengths, figure 23 shows the absorption at 436nm where chlorophyll and other pigments absorb light and compares with the absorption of just chlorophyll at 676nm. At 436nm there is more absorption than at 676nm. In the GP culture some pigments besides chlorophyll are doing a lot of the absorbing while in the Dun culture the chlorophyll does most of the absorbing. Also, in Dun the absorption curves show that are some pigments absorbing strongly in the UV part of the spectrum (350nm)

The growth rates showed that the smaller cells of Dun grew faster than the larger cells of GP. The results also showed that the chlorophyll per cell and the absorption per cell are less for the smaller sized Dun than they are for GP. However the absorption per chlorophyll for Dun is greater than the absorption per cell for GP. There was a difference in how light affected both species, but the affect of less light for both species was to increase the chlorophyll in each cell.

There are large changes that happen at the cell level in response to light. Scientists know a lot about phytoplankton, but there is still a lot that is not known about them including a complete understanding of the response to light. It was hard working with light and cultures, in the end there were more variables than I originally expected. I would like to continue this project next year and look more at the other wavelengths that might give more information about changes in the cells. The TW was inoculated at a higher start level last month, and is growing so can be added to this experiment. It is very different from the GP and Dun. Since it is a diatom so perhaps its adaptation to changes in light will be quite different. Phytoplankton are very important because they are at the base of the food chain. Although phytoplankton are small, they are interesting and they have an important role in the oceans.

Acknowledgments

I thank Kenia Whitehead for working with me last summer at Scripps Institution of Oceanography to begin this project and Tiffany Moisan who continued to supervise experiments during the school year. I also thank Maria Vernet for the use of her laboratory and Frieda Reid for the use of her microscopes and references. I also thank Karen Baker for helping. All of these people contributed their suggestions and have improved the project greatly.

Table 1. Phytoplankton Characteristics

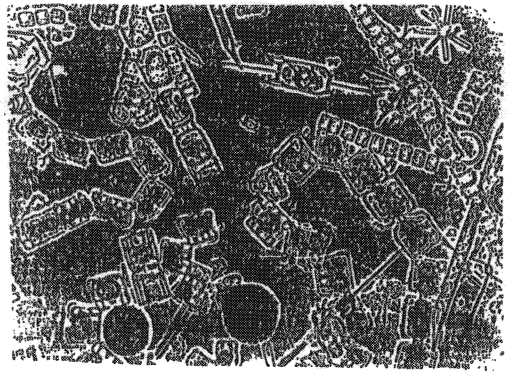
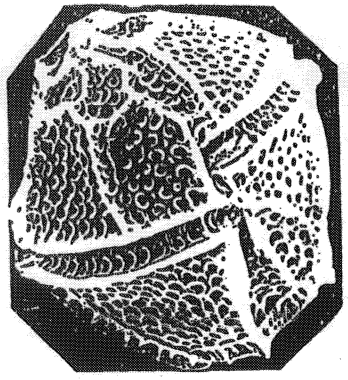
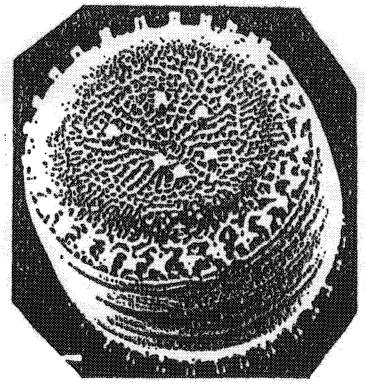
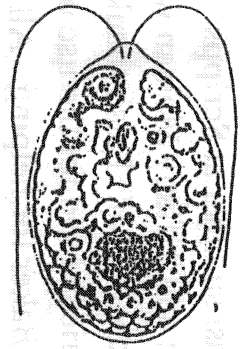
name	Gonyaulax Polyedra	Thalassiosira Weisflogii	Duneliella Tertiolecta
				
abbreviation	GP	TW	Dun
type	dinoflagelate	diatom	green algae
	diophycea	bacillariophycea	Chlorophycea
	pynophyta		
color	yellow-green	yellow-green	green
size category	micro	micro	nano
size from literature	5u-100u	2u-300u	5u-10u
size measured	38u	25u	8u
growth calc by size	0.31	0.36	0.51
growth calc by size/nut	0.16	0.23	0.75
growth calculated by slope	0.064/.11	-	0.7
specific growth by cell#	0.38	-	0.47
culture media	GPM	F/2	GPM
characteristics	summer bloom	spring bloom	culture weed
	flagella	silica plates	
	redtide		

Table 2. PROJECT TIMELINE

DATE	TYPE	TYPE	LENGTH	PAGES	COMMENTS
1995	PREP	media			make GPM media for cultures
	TOOL	chl			measure chl
	SAMPLE	GP-growth		25.38	GP growth rate
	SAMPLE	DUN-growth		38	DUN growth rate
	TOOL	cell			cell counts
	THEORY	growth			growth =F(size.nutrients)
Jun-96	PREP			44	discuss/design new experiment
	PREP				gather materials & tools
	PREP	TW.GP.DUN			choose species
Jul-96	TOOL	lights			set up lights
	PREP	media			make F/2 media for diatoms
	SAMPLE	time series	2 days		take ocean samples SD to Catalina
Aug-96	PREP			61	water budget design
	CALC	growth		64	growth calculations
	SAMPLE	TW-growth	25days	57	sample TW-good growth
Sep-96	TECH	abs		50	learn absorption spectrophotometer
	TECH	screens		52	measure screen transmittance
	SAMPLE	TW-light	30 days	57	sample for light 100% 60%: Fail
	SAMPLE	GP-light	30 days	77	sample GP light100%.60%.30%-> chl.abs.cell
Oct-96	SAMPLE	TW-fail	18 days	81	sample light TW-> growth fail
	PREP	media			new TW culture stock
	SAMPLE	TW-fail	9 days		sample TW->growth fail
	SAMPLE	TW-fail	5 days		sample TW->growth fail
Nov-96	TECH	cells			learn cell count techniques
	ANALYSIS	GP-abs		89	run GP abs and cell counts
	PREP	media			new TW F/2 media
	ANALYSIS	GP		103	summary
	SAMPLE	TW-fail	15 days		sample TW light 100%.60%.30%
Dec-96	SAMPLE	DUN-growth	6 days		sample for growth curve DUN
	SAMPLE	DUN-light	22 days	106	sample DUN light100%.60%.30%->chl.abs.cell
	SAMPLE	TW-growth	16 days	93	growth TW -> growth OK
Jan-97	ANALYSIS	DUN		112	run DUN abs and cell counts
	PREP	TW			transfer TW stock (next year!)
	TECH	light		114	measure light
	ANALYSIS	DUN		115	summary

Table 3. Phytoplankton Size

name	size
Pento	.02-0.2u
Pico	0.20-2.0u
Ultra	<2.0u
Nanno	2.0-20u
Net	10u
Micro	20-200u
Meso	0.2-20mm (200-20,000u)
Macro	20-200mm

where
1mm=1000u

Table 4. Growth Rate Calculations

Nt=end number of cells(chl concentration)
No=starting number of cells
u=growth rate=doublings per day
u(diatom TW) ~1
u(dino GP)~ 0.2
t=time

Given starting No, calculate Nt

Nt=No*exp(u*t)

		u=.166	u=.20	u=.325	u=.70	u=1.0
No	t	Nt	Nt	Nt	Nt	Nt
10	0.5	10.865418	11.0517092	11.76448318	14.19067549	16.4872127
10	1	11.805731	12.2140276	13.84030646	20.13752707	27.1828183
10	2	13.937528	14.918247	19.15540829	40.55199967	73.890561
10	3	16.454271	18.221188	26.51167211	81.66169913	200.855369
10	4	19.42547	22.2554093	36.69296668	164.4464677	545.9815

Given Nt, calculate starting No

No=Nt/exp(u*t)

		u=.166 (GP)	u=.20	u=.325 (TW)	u=.7	u=1
Nt	t	No	No	No	No	No
150	0	150	150	150	150	150
150	0.5	138.0527	135.7256	127.5024	105.7032	90.9796
150	1	127.0569	122.8096	108.3791	74.4878	55.1819
150	2	107.6231	100.5480	78.3069	36.9895	20.3003
150	3	91.1617	82.3217	56.5789	18.3685	7.4681
150	4	77.2182	67.3993	40.8798	9.1215	2.7473
150	5	65.4074	55.1819	29.5368	4.5296	1.0107
150	6	55.4031	45.1791	21.3411	2.2493	0.3718
150	7	46.9290	36.9895	15.4195	1.1170	0.1368
150	8	39.7510	30.2845	11.1410	0.5547	0.0503
150	9	33.6709	24.7948	8.0497	0.2754	0.0185
150	10	28.5208	20.3003	5.8161	0.1368	0.0068
150	11	24.1585	16.6205	4.2023	0.0679	0.0025
150	12	20.4633	13.6077	3.0363	0.0337	0.0009
150	13	17.3334	11.1410	2.1938	.016751	.000341
150	14	14.6822	9.1215	1.5851	.008321	.000121
150	15	12.4365	7.4681	1.1453	.004131	.000051
150	16	10.5343	6.1143	0.8275	.002051	.000021
150	17	8.9230	5.0060	0.5979	.001021	.000011
150	18	7.5582	4.0986	0.4320	.000511	.000001
150	19	6.4022	3.3556	0.3121	.000251	.000001
150	20	5.4229	2.7473	0.2255	.000121	.000001

Table 5. Microscope Slide Types

name	vol(ml)	culture	microscope
Petroff-Hausser	.002	bacteria	
Haemacytometer	.01	dense, DUN	standard
Palmer-Maloney	0.1	dense, GP	standard
Sedgewick Rafter	1.0	dilute	standard&inverted
Utermohl Settling Chamber	1-5	dilute	inverted

Table 6a. GP Growth Experiments

chla= (Fd *(tau/(tau-1))*(vol. acetone (ml))*(Rb-Ra)) / (vol. filtered (ml))
phae= (Fd *(tau/(tau-1))*(vol. acetone (ml))*(tau*Ra-Rb)) / (vol. filtered (ml))

						mg/m3	mg/m3					ug/ul				
#	Code	Year	Month	Day	Plotday	chla	phaeo	VolFil	VolA	Scale	door	Fd	Tau	Rb	Ra	Comments
1	GP1	1996	07	17	01	27.416	0.533	39	10	3.16	1	0.144	1.929	2.37	1.24	
2	GP1	1996	08	07	22	463.65	-6.55	2	10	3.16	1	0.144	1.929	2.02	1.04	
3	GP1	1996	08	10	25	709.66	28.73	1	10	3.16	1	0.144	1.929	1.59	0.84	
4	GP1	1996	08	14	29	986.72	-14.5	2	10	1	1	0.144	1.929	1.36	0.7	
5	GP1	1996	08	19	31	496.77	-39.7	2	10	3.16	1	0.144	1.929	2.09	1.04	
6	GP1	1996	08	25	37	662.35	5.715	2	10	3.16	1	0.144	1.929	2.92	1.52	
1	GP2	1996	08	25	01	189.24	18.21	5	10	3.16	1	0.144	1.929	2.18	1.18	
2	GP2	1996	08	28	04	326.45	7.589	4	10	3.16	1	0.144	1.929	2.9	1.52	
3	GP2	1996	09	02	09	529.88	54.68	2	10	3.16	1	0.144	1.929	2.45	1.33	
1	GP3	1996	09	02	09			2	10			0.144	1.929			
1	GP3	1996	09	09	16	259.26	9.721	5	10	3.16	1	0.144	1.929	2.9	1.53	
GP																
1	100A	1996	09	11	01	2.9475	0.068	20	7	31.6	1	0.144	1.929	1.87	0.98	
1	100B	1996	09	11	01	1.4439	0.094	50	7	31.6	1	0.144	1.929	2.34	1.25	
2	100A	1996	09	15	05	37.849	0.389	20	10	3.16	1	0.144	1.929	1.67	0.87	
2	100B	1996	09	15	05	14.567	0.818	19.5	10	10	1	0.144	1.929	2.03	1.08	
3	100A	1996	09	18	08	62.45	-2.68	10	10	3.16	1	0.144	1.929	1.34	0.68	
3	100B	1996	09	18	08	65.289	0.639	10	10	3.16	1	0.144	1.929	1.44	0.75	
4	100A	1996	09	22	12	281.07	18.93	10	10	1	1	0.144	1.929	2.02	1.08	
4	100B	1996	09	22	12	202.49	4.962	5	10	3.16	1	0.144	1.929	2.25	1.18	
5	100A	1996	09	25	15	157.7	108.9	3	10	3.16	1	0.144	1.929	1.41	0.91	
5	100B	1996	09	25	15	321.71	50.41	3	10	3.16	1	0.144	1.929	2.29	1.27	
6	100A	1996	09	27	17	170.32	11.35	3	10	3.16	1	0.144	1.929	1.16	0.62	
6	100B	1996	09	27	17	473.11	10.36	3	10	3.16	1	0.144	1.929	3.15	1.65	
7	100A	1996	09	28	18	416.34	53.95	2	10	3.16	1	0.144	1.929	1.95	1.07	
7	100B	1996	09	28	18	458.92	15.76	2	10	3.16	1	0.144	1.929	2.05	1.08	
8	100A	1996	09	30	20	1001.7	12.21	2	10	1	1	0.144	1.929	1.4	0.73	
8	100B	1996	09	30	20	539.35	14.45	2	10	3.16	1	0.144	1.929	2.4	1.26	
9	100A	1996	10	2	23	435.26	52.61	2	10	3.16	1	0.144	1.929	2.03	1.11	
9	100B	1996	10	2	23	908.37	-20.5	1	10	3.16	1	0.144	1.929	1.97	1.01	
10	100A	1996	10	4	25	652.89	-55.1	1	10	3.16	1	0.144	1.929	1.37	0.68	
10	100B	1996	10	4	25	539.35	-11.9	1	10	3.16	1	0.144	1.929	1.17	0.6	
11	100A	1996	10	8	29	1021.9	-24.2	2	10	3.16	1	0.144	1.929	4.43	2.27	
11	100B	1996	10	8	29	1807.3	12.33	1	10	3.16	1	0.144	1.929	3.98	2.07	
1	60A	1996	09	11	1	6.8652	0.057	50	7	10	1	0.144	1.929	3.42	1.78	
1	60B	1996	09	11	1	7.7861	0.186	50	7	10	1	0.144	1.929	3.91	2.05	
2	60A	1996	09	15	5	27.44	1.128	20	10	3.16	1	0.144	1.929	1.23	0.65	
2	60B	1996	09	15	5	30.752	0.014	20	10	3.16	1	0.144	1.929	1.35	0.7	
3	60A	1996	09	18	8	54.881	1.378	10	10	3.16	1	0.144	1.929	1.22	0.64	
3	60B	1996	09	18	8	70.966	-0.64	10	10	3.16	1	0.144	1.929	1.55	0.8	
4	60A	1996	09	20	10	141.93	11.9	10	10	3.16	1	0.144	1.929	3.25	1.75	
4	60B	1996	09	20	10	97.461	5.387	10	10	3.16	1	0.144	1.929	2.2	1.17	
5	60A	1996	09	22	12	227.09	10.25	5	10	3.16	1	0.144	1.929	2.55	1.35	
5	60B	1996	09	22	12	287.65	14.74	5	10	3.16	1	0.144	1.929	3.24	1.72	
6	60A	1996	09	25	15	145.52	62.82	3	10	10	1	0.144	1.929	3.71	2.25	
6	60B	1996	09	25	15	233.4	3.939	3	10	3.16	1	0.144	1.929	1.55	0.81	
7	60A	1996	09	27	17	362.72	-5.24	3	10	3.16	1	0.144	1.929	2.37	1.22	
7	60B	1996	09	27	17	438.42	4.034	3	10	3.16	1	0.144	1.929	2.9	1.51	
8	60A	1996	09	28	18	516.28	-75.5	3	7	1	1	0.144	1.929	1.42	0.68	
8	60B	1996	09	28	18	432.56	1.695	3	7	1	1	0.144	1.929	1.29	0.67	
9	60A	1996	09	30	20	1225.9	-3.71	2	10	1	1	0.144	1.929	1.7	0.88	
9	60B	1996	09	30	20	719.13	19.27	1	10	3.16	1	0.144	1.929	1.6	0.84	
10	60A	1996	10	2	23	1016.6	-30.5	2	10	1	1	0.144	1.929	1.39	0.71	
10	60B	1996	10	2	23	889.45	-19.2	1	10	3.16	1	0.144	1.929	1.93	0.99	
11	60A	1996	10	4	25	842.14	-7.05	1	10	3.16	1	0.144	1.929	1.84	0.95	
11	60B	1996	10	4	25	898.91	-55	1	10	3.16	1	0.144	1.929	1.91	0.96	
11	60A	1996	10	8	29	2469.6	105.9	1	10	3.16	1	0.144	1.929	5.54	2.93	
11	60B	1996	10	8	29	624.5	43.56	1	10	3.16	1	0.144	1.929	1.42	0.76	
2																
1	30A	1996	09	11	1	1.126	0.043	50	7	31.6	1	0.144	1.929	1.8	0.95	

1	30B	1996	09	11	1	1.8016	-0.05	50	7	31.6	1	0.144	1.929	2.78	1.42	
2	30A	1996	09	15	5	5.1569	0.249	20	10	31.6	1	0.144	1.929	2.32	1.23	
2	30B	1996	09	15	5	3.3118	0.292	20	10	31.6	1	0.144	1.929	1.52	0.82	
3	30A	1996	09	18	8	5.2988	0.239	10	10	31.6	1	0.144	1.929	1.19	0.63	
3	30B	1996	09	18	8	3.4984	0.196	10	10	1	100	0.144	1.929	2.5	1.33	
4	30A	1996	09	20	10	1.9435	0.279	10	10	1	100	0.144	1.929	1.45	0.8	
4	30B	1996	09	20	10	1.8538	0.257	10	10	1	100	0.144	1.929	1.38	0.76	
5	30A	1996	09	22	12	5.2042	0.158	10	10	31.6	1	0.144	1.929	1.16	0.61	
5	30B	1996	09	22	12	2.3023	0.031	10	10	1	100	0.144	1.929	1.61	0.84	
6	30A	1996	09	25	15	4.0687	1.381	10	10	31.6	1	0.144	1.929	1.05	0.62	
6	30B	1996	09	25	15	1.1355	0.183	10	10	3.16	100	0.144	1.929	2.7	1.5	
7	30A	1996	09	27	17	4.814	0.936	10	10	1	100	0.144	1.929	3.68	2.07	
7	30B	1996	09	27	17	6.3987	-2.87	10	10	1	100	0.144	1.929	3.41	1.27	
7	30A	1996	09	28	18	4.5283	0.306	7	5	31.6	1	0.144	1.929	1.44	0.77	
7	30B	1996	09	28	18	2.9303	0.736	7	7	1	100	0.144	1.929	2.3	1.32	
8	30A	1996	09	30	20	3.02	0.147	10	10	1	100	0.144	1.929	2.15	1.14	
8	30B	1996	09	30	20	1.0692	0.17	10	10	3.16	100	0.144	1.929	2.54	1.41	
9	30A	1996	10	2	23	5.8666	0.111	10	10	31.6	1	0.144	1.929	1.3	0.68	
9	30B	1996	10	2	23	2.3023	0.142	10	10	1	100	0.144	1.929	1.65	0.88	
10	30A	1996	10	4	25	8.6106	-0.08	10	10	31.6	1	0.144	1.929	1.88	0.97	
10	30B	1996	10	4	25	2.1528	0.153	10	10	1	100	0.144	1.929	1.55	0.83	

Table 6b. Dun Growth Experiments

chl= (Fd *(tau/(tau-1))*(vol. acetone (ml))*(Rb-Ra)) / (vol. filtered (ml))

pha= (Fd *(tau/(tau-1))*(vol. acetone (ml))*(tau*Ra-Rb)) / (vol. filtered (ml))

						mg/m3	mg/m3					ug/ul					
#	Code	Year	Month	Day	Plotda	chla	phaeo	VolFilt	VolAce	Scale	door	Fd	Tau	Rb	Ra	Plotd:	Comments
1	DUN	1996	12	15	1	47.424	159.71	10	10	3.16	1	0.155	1.946	2.64	2.17	1	
2	DUN	1996	12	17	3	207.86	80.41	5	10	3.16	1	0.155	1.946	2.54	1.51	3	
3	DUN	1996	12	17	3	204.06	88.519	2	10	10	1	0.155	1.946	3.22	1.94	3	
4	DUN	1996	12	18	4	1004.4	443.45	2	10	1	1	0.155	1.946	1.59	0.96	4	
5	DUN	1996	12	18	4	876.83	224.12	2	10	1	1	0.155	1.946	1.28	0.73	4	
6	DUN	1996	12	20	6	2646.4	822.31	1	10	1	1	0.155	1.946	1.98	1.15	6	
7	DUN	1996	12	20	6	2263.8	933.46	1	10	1	1	0.155	1.946	1.77	1.06	6	
100A	DUN	1996	12	20	1	2646.4	822.31	1	10	1	1	0.155	1.946	1.98	1.15	1	
100B	DUN	1996	12	20	1	2263.8	933.46	1	10	1	1	0.155	1.946	1.77	1.06	1	
					1	121.4										1	
					1	103.84										1	
100A	DUN	1996	12	23	4	616.44	258.29	3	10	1	1	0.155	1.946	1.45	0.87	4	
100B	DUN	1996	12	23	4	680.21	214.63	3	10	1	1	0.155	1.946	1.53	0.89	4	
100A	DUN	1996	12	24	5	908.72	343.05	2	10	1	1	0.155	1.946	1.4	0.83	5	
100B	DUN	1996	12	24	5	908.72	433.54	2	10	1	1	0.155	1.946	1.46	0.89	5	
100A	DUN	1996	12	25	6	1009.7	448.19	3	10	1	1	0.155	1.946	2.4	1.45	6	
100B	DUN	1996	12	25	6	1286	-19.17	3	10	1	1	0.155	1.946	2.47	1.26	6	
100A	DUN	1996	12	31	12	1881.2	893.79	1	10	1	1	0.155	1.946	1.51	0.92	12	
100B	DUN	1996	12	31	12	2104.4	700.76	1	10	1	1	0.155	1.946	1.59	0.93	12	
100A	DUN	1996	01	1	13	2136.3	397.41	1	10	1	1	0.155	1.946	1.51	0.84	13	
100B	DUN	1996	01	1	13	1976.9	767.98	1	10	1	1	0.155	1.946	1.53	0.91	13	
100A	DUN	1996	01	2	14	2774	1117.1	1	10	1	1	0.155	1.946	2.16	1.29	14	
100B	DUN	1996	01	2	14	2678.3	1061.9	1	10	1	1	0.155	1.946	2.08	1.24	14	
100A	DUN	1996	01	3	15	2933.4	1198.9	1	10	1	1	0.155	1.946	2.29	1.37	15	
100B	DUN	1996	01	3	15	2997.2	954.18	1	10	1	1	0.155	1.946	2.25	1.31	15	
100A	DUN	1996	01	5	17	3571.1	1647.1	1	10	1	1	0.155	1.946	2.85	1.73	17	
100B	DUN	1996	01	5	17	3571.1	1797.9	1	10	1	1	0.155	1.946	2.9	1.78	17	
100A	DUN	1996	01	7	19	3921.8	1205.9	1	10	1	1	0.155	1.946	2.93	1.7	19	
100B	DUN	1996	01	7	19	3730.5	1427.4	1	10	1	1	0.155	1.946	2.88	1.71	19	
100A	DUN	1996	01	9	21	4400.1	2296.1	1	10	1	1	0.155	1.946	3.6	2.22	21	
100B	DUN	1996	01	9	21	4368.2	2177.2	1	10	1	1	0.155	1.946	3.54	2.17	21	
100A	DUN	1996	01	12	22	4081.3	1951.3	1	10	1	1	0.155	1.946	3.28	2	22	
100B	DUN	1996	01	12	22	3826.2	1965.1	1	10	1	1	0.155	1.946	3.12	1.92	22	
					1	187.21										1	
					1	175.51										1	
60A	DUN	1996	12	23	4	459.14	192.38	5	10	1	1	0.155	1.946	1.8	1.08	4	
60B	DUN	1996	12	23	4	484.65	166.87	5	10	1	1	0.155	1.946	1.84	1.08	4	
60A	DUN	1996	12	24	5	871.52	395.33	3	10	1	1	0.155	1.946	2.08	1.26	5	
60B	DUN	1996	12	24	5	775.86	480.93	3	10	1	1	0.155	1.946	1.98	1.25	5	
60A	DUN	1996	12	25	6	1062.8	415.16	3	10	1	1	0.155	1.946	2.47	1.47	6	
60B	DUN	1996	12	25	6	945.92	501.91	3	10	1	1	0.155	1.946	2.33	1.44	6	
60A	DUN	1996	12	31	12	2040.6	704.21	2	10	1	1	0.155	1.946	3.1	1.82	12	
60B	DUN	1996	12	31	12	2327.6	930.02	1	10	1	1	0.155	1.946	1.81	1.08	12	
60A	DUN	1996	01	1	13	2168.2	697.32	1	10	1	1	0.155	1.946	1.63	0.95	13	
60B	DUN	1996	01	1	13	2008.7	1037.7	1	10	1	1	0.155	1.946	1.64	1.01	13	
60A	DUN	1996	01	2	14	2933.4	1198.9	1	10	1	1	0.155	1.946	2.29	1.37	14	
60B	DUN	1996	01	2	14	3603	1494.6	1	10	1	1	0.155	1.946	2.82	1.69	14	
60A	DUN	1996	01	3	15	2997.2	1527.3	1	10	1	1	0.155	1.946	2.44	1.5	15	
60B	DUN	1996	01	3	15	2869.6	1383.4	1	10	1	1	0.155	1.946	2.31	1.41	15	
60A	DUN	1996	01	5	17	3794.3	1695.4	1	10	1	1	0.155	1.946	3.01	1.82	17	
60B	DUN	1996	01	5	17	3634.9	1915.1	1	10	1	1	0.155	1.946	2.98	1.84	17	
60A	DUN	1996	01	7	19	3634.9	2096.1	0.5	10	1	1	0.155	1.946	1.52	0.95	19	
60B	DUN	1996	01	7	19	3188.5	1901.5	0.8	10	1	1	0.155	1.946	2.15	1.35	19	
60A	DUN	1996	01	9	21	4910.3	2328.9	0.5	10	1	1	0.155	1.946	1.97	1.2	21	
60B	DUN	1996	01	9	21	4742.9	2307.7	0.8	10	1	1	0.155	1.946	3.06	1.87	21	
60A	DUN	1996	01	12	22	3603	1705.7	1	10	1	1	0.155	1.946	2.89	1.76	22	
60B	DUN	1996	01	12	22	3626.9	1538.5	0.8	10	1	1	0.155	1.946	2.28	1.37	22	

					1	165.27										1	
					1	166.37										1	
30A	DUN	1996	12	23	4	286.96	129.29	5	10	1	1	0.155	1.946	1.14	0.69	4	
30B	DUN	1996	12	23	4	299.72	110.5	5	10	1	1	0.155	1.946	1.15	0.68	4	
30A	DUN	1996	12	24	5	552.67	251.68	3	10	1	1	0.155	1.946	1.32	0.8	5	
30B	DUN	1996	12	24	5	584.55	249.96	3	10	1	1	0.155	1.946	1.38	0.83	5	
30A	DUN	1996	12	25	6	573.93	270.64	3	10	1	1	0.155	1.946	1.38	0.84	6	
30B	DUN	1996	12	25	6	563.3	220.94	3	10	1	1	0.155	1.946	1.31	0.78	6	
30A	DUN	1996	12	31	12	1934.3	669.73	3	10	1	1	0.155	1.946	4.41	2.59	12	
30B	DUN	1996	12	31	12	2072.5	536.59	2	10	1	1	0.155	1.946	3.03	1.73	12	
30A	DUN	1996	01	1	13	2279.8	766.7	2	10	1	1	0.155	1.946	3.45	2.02	13	
30B	DUN	1996	01	1	13	2263.8	903.3	2	10	1	1	0.155	1.946	3.52	2.1	13	
30A	DUN	1996	01	2	14	2805.9	1371.7	2	10	1	1	0.155	1.946	4.53	2.77	14	
30B	DUN	1996	01	2	14	2439.2	1406.6	2	10	1	1	0.155	1.946	4.08	2.55	14	
30A	DUN	1996	01	3	15	4049.4	1138.7	1	10	1	1	0.155	1.946	2.99	1.72	15	
30B	DUN	1996	01	3	15	2901.5	1562.6	1	10	1	1	0.155	1.946	2.39	1.48	15	
30A	DUN	1996	01	5	17	4559.5	2106.5	1	10	1	1	0.155	1.946	3.64	2.21	17	
30B	DUN	1996	01	5	17	4623.3	2374.5	1	10	1	1	0.155	1.946	3.77	2.32	17	
30A	DUN	1996	01	7	19	6950.9	2821.9	0.5	10	1	1	0.155	1.946	2.71	1.62	19	
30B	DUN	1996	01	7	19	5101.6	2499.5	0.5	10	1	1	0.155	1.946	2.06	1.26	19	
30A	DUN	1996	01	9	21	7333.5	3344.2	0.5	10	1	1	0.155	1.946	2.92	1.77	21	
30B	DUN	1996	01	9	21	6568.3	3385.5	0.5	10	1	1	0.155	1.946	2.68	1.65	21	
30A	DUN	1996	01	12	22	5133.4	2497.8	1	10	1	1	0.155	1.946	4.14	2.53	22	
30B	DUN	1996	01	12	22	5500.1	2832.4	0.8	10	1	1	0.155	1.946	3.59	2.21	22	

Table 6c. TW Growth Experiments

chl= (Fd *(tau/(tau-1))*(vol. acetone (ml))*(Rb-Ra)) / (vol. filtered (ml))

pha= (Fd *(tau/(tau-1))*(vol. acetone (ml))*(tau*Ra-Rb)) / (vol. filtered (ml))

#	Code	Year	Month	Day	Plotday	mg/m3 chla	mg/m3 phaeo	VolFilt	VolAce	Scale	door	ug/ul Fd	Tau	Rb	Ra	Comments
1	T1	1996	08	08	01	50.831	-1.526	8	10	10	1	0.144	1.929	2.78	1.42	
2	T1	1996	08	10	03	88.705	2.4982	6	10	10	1	0.144	1.929	3.75	1.97	
3	T1	1996	08	11	04	142.03	11.444	4	10	10	1	0.144	1.929	4.11	2.21	
4	T1	1996	08	12	05	194.35	-4.077	2	10	10	1	0.144	1.929	2.67	1.37	
5	T1	1996	08	13	06	236.21	-0.105	2	10	10	1	0.144	1.929	3.28	1.7	
6	T1	1996	08	14	07	242.19	7.804	2	10	10	1	0.144	1.929	3.42	1.8	
7	T1	1996	08	15	08	251.16	-31.72	2	10	10	1	0.144	1.929	3.26	1.58	
8	T1	1996	08	16	09	264.62	-9.066	2	10	10	1	0.144	1.929	3.61	1.84	
9	T1	1996	08	18	11	269.1	3.1156	2	10	10	1	0.144	1.929	3.76	1.96	
10	T1	1996	08	19	12	288.54	-17.71	2	10	10	1	0.144	1.929	3.88	1.95	
11	T1	1996	08	25	18	230.23	12.82	2	10	10	1	0.144	1.929	3.29	1.75	Transfered
12	T1	1996	09	02	25	224.25	0.7445	2	10	10	1	0.144	1.929	3.12	1.62	Transfered
1	T2	1996	08	25	01	21.827	-0.439	10	10	10	1	0.144	1.929	1.5	0.77	Transfered
2	T2	1996	08	28	04	18.688	0.4093	8	10	10	1	0.144	1.929	1.05	0.55	
3	T2	1996	09	02	11	12.869	2.0751	10	10	31.6	1	0.144	1.929	3.06	1.7	Transfered
1	T3a	1996	09	02	01	#DIV/0!	#DIV/0!	2	10			0.144	1.929			from T1
1	T3b	1996	09	02	01	#DIV/0!	#DIV/0!	10	10			0.144	1.929			from T2
1	T3b	1996	09	09	08	89.702	4.7422	5	10	10	1	0.144	1.929	3.2	1.7	from T2
1	T5	1996	09	29	01	180	-4.03	2	7	10	1	0.144	1.929	3.53	1.81	
1	T5	1996	09	29	01	171.63	3.3698	3	7	10	1	0.144	1.929	5.16	2.7	
2	T5	1996	10	06	09	18.239	20.927	10	10	10	1	0.144	1.929	2.02	1.41	
2	T5	1996	10	06	09	9.2692	39.619	10	10	10	1	0.144	1.929	2.07	1.76	
1	T6	1996	10	07	01	1345.5	-67.75	2	10	1	1	0.144	1.929	1.82	0.92	
1	T6	1996	10	07	01	1554.8	0.7176	1	10	1	1	0.144	1.929	1.08	0.56	
2	T6	1996	10	08	02	362.72	-2.312	3	10	3.16	1	0.144	1.929	2.38	1.23	
2	T6	1996	10	08	02	350.1	-4.346	3	10	3.16	1	0.144	1.929	2.29	1.18	
3	T6	1996	10	11	05	487.3	4.9582	2	10	3.16	1	0.144	1.929	2.15	1.12	
1	T-100	1996	10	12	02	548.81	-12.59	2	10	3.16	1	0.144	1.929	2.38	1.22	first day in
1	T-100	1996	10	12	02	288.6	-11.7	2	10	3.16	1	0.144	1.929	1.24	0.63	
1	T-100	1996	10	12	01	5	0					0.144	1.929			
2	T-100	1996	10	13	02	8.6106	0.2677	10	10	1	31.6	0.144	1.929	1.92	1.01	
2	T-100	1996	10	13	02	8.3267	0.3757	10	10	1	31.6	0.144	1.929	1.87	0.99	
3	T-100	1996	10	14	03	12.395	0.5264	10	10	1	31.6	0.144	1.929	2.78	1.47	
3	T-100	1996	10	14	03	13.531	-0.785	10	10	1	31.6	0.144	1.929	2.88	1.45	
4	T-100	1996	10	15	04	16.937	-0.851	10	10	1	31.6	0.144	1.929	3.62	1.83	
4	T-100	1996	10	15	04	14.856	-0.527	10	10	1	31.6	0.144	1.929	3.2	1.63	
5	T-100	1996	10	16	05	22.425	-0.759	10	10	1	10	0.144	1.929	1.53	0.78	
5	T-100	1996	10	16	05	19.734	1.3766	10	10	1	10	0.144	1.929	1.42	0.76	
6	T-100	1996	10	17	06	25.116	-0.117	10	10	1	10	0.144	1.929	1.74	0.9	
6	T-100	1996	10	17	06	23.322	1.6774	10	10	1	10	0.144	1.929	1.68	0.9	
7	T-100	1996	10	18	07	24.696	-0.435	10	10	1	31.6	0.144	1.929	5.37	2.76	
7	T-100	1996	10	18	07	30.09	-0.378	10	10	1	31.6	0.144	1.929	6.56	3.38	
8	T-100	1996	10	19	07	31.695	0.2497	10	10	1	10	0.144	1.929	2.21	1.15	
8	T-100	1996	10	19	07	31.695	-0.306	10	10	1	10	0.144	1.929	2.19	1.13	
9	T-100	1996	10	20	07	37.675	0.3806	10	10	1	10	0.144	1.929	2.63	1.37	
9	T-100	1996	10	20	07	35.881	0.508	10	10	1	10	0.144	1.929	2.51	1.31	
10	T-100	1996	10	21	07	36.479	1.5767	10	10	1	10	0.144	1.929	2.59	1.37	
10	T-100	1996	10	21	07	40.366	-0.366	10	10	1	10	0.144	1.929	2.79	1.44	
11	T-100	1996	10	22	07	39.17	3.33	10	10	1	10	0.144	1.929	2.84	1.53	
11	T-100	1996	10	22	07	37.974	0.3594	10	10	1	10	0.144	1.929	2.65	1.38	
12	T-100	1996	10	23	07	42.16	1.1733	10	10	1	10	0.144	1.929	2.97	1.56	
12	T-100	1996	10	23	07	48.439	-6.217	10	10	1	10	0.144	1.929	3.14	1.52	
13	T-100	1996	10	24	07	41.861	3.3771	7	10	1	10	0.144	1.929	2.12	1.14	
13	T-100	1996	10	24	07	44.851	1.9743	7	10	1	10	0.144	1.929	2.23	1.18	
14	T-100	1996	10	25	07	49.549	-6.693	7	10	1	10	0.144	1.929	2.24	1.08	
14	T-100	1996	10	25	07	41.861	-2.178	7	10	1	10	0.144	1.929	1.98	1	

1	T-100	1996	10	12	01	5.5	0					0.144	1.929			
2	T-60	1996	10	13	02	10.692	0.1199	10	10	1	31.6	0.144	1.929	2.36	1.23	
2	T-60	1996	10	13	02	9.3676	0.0381	10	10	1	31.6	0.144	1.929	2.06	1.07	
3	T-60	1996	10	14	03	9.6514	-0.246	10	10	1	31.6	0.144	1.929	2.09	1.07	
3	T-60	1996	10	14	03	9.273	0.1328	10	10	1	31.6	0.144	1.929	2.05	1.07	
4	T-60	1996	10	15	04	10.692	-0.759	10	10	1	31.6	0.144	1.929	2.26	1.13	
4	T-60	1996	10	15	04	11.922	-2.517	10	10	1	31.6	0.144	1.929	2.33	1.07	
5	T-60	1996	10	16	05	11.449	0.4178	10	10	1	31.6	0.144	1.929	2.56	1.35	
5	T-60	1996	10	16	05	10.503	0.8366	10	10	1	31.6	0.144	1.929	2.4	1.29	
6	T-60	1996	10	17	06	11.071	0.1809	10	10	1	31.6	0.144	1.929	2.45	1.28	
6	T-60	1996	10	17	06	11.228	-0.094	15	10	1	31.6	0.144	1.929	3.68	1.9	
7	T-60	1996	10	18	07	10.787	-0.502	10	10	1	31.6	0.144	1.929	2.31	1.17	
7	T-60	1996	10	18	07	10.503	-0.746	10	10	1	31.6	0.144	1.929	2.22	1.11	
8	T-60	1996	10	19	07	10.787	-0.063	10	10	1	31.6	0.144	1.929	2.36	1.22	
8	T-60	1996	10	19	07	10.408	0.4038	10	10	1	31.6	0.144	1.929	2.33	1.23	
9	T-60	1996	10	20	07	9.273	0.5723	10	10	1	31.6	0.144	1.929	2.1	1.12	
9	T-60	1996	10	20	07	9.8407	0.0924	10	10	1	31.6	0.144	1.929	2.17	1.13	
10	T-60	1996	10	21	07	9.6514	0.2817	10	10	1	31.6	0.144	1.929	2.15	1.13	
10	T-60	1996	10	21	07	8.2321	2.0526	10	10	1	31.6	0.144	1.929	2.04	1.17	
11	T-60	1996	10	22	07	8.0429	-0.483	10	10	1	31.6	0.144	1.929	1.71	0.86	
11	T-60	1996	10	22	07	7.8536	-0.03	10	10	1	31.6	0.144	1.929	1.72	0.89	
12	T-60	1996	10	23	07	7.8063	0.3248	8	10	1	31.6	0.144	1.929	1.4	0.74	
12	T-60	1996	10	23	07	8.0429	-0.241	8	10	1	31.6	0.144	1.929	1.39	0.71	
13	T-60	1996	10	24	07	7.2994	0.1096	7	10	1	31.6	0.144	1.929	1.13	0.59	
13	T-60	1996	10	24	07	7.0291	0.2544	7	10	1	31.6	0.144	1.929	1.1	0.58	
14	T-60	1996	10	25	07	17.94	-0.083	7	10	1	10	0.144	1.929	0.87	0.45	
14	T-60	1996	10	25	07	7.0053	-0.537	7	10	100	1	0.144	1.929	3.27	1.63	
1	T-100	1996	10	12	01	6						0.144	1.929			
2	T-30	1996	10	13	02	11.355	0.3366	10	10	1	31.6	0.144	1.929	2.53	1.33	
2	T-30	1996	10	13	02	9.9353	0.1736	10	10	1	31.6	0.144	1.929	2.2	1.15	
3	T-30	1996	10	14	03	9.9353	-0.266	10	10	1	31.6	0.144	1.929	2.15	1.1	
3	T-30	1996	10	14	03	9.5568	0.3763	10	10	1	31.6	0.144	1.929	2.14	1.13	
4	T-30	1996	10	15	04	13.152	-1.637	10	10	1	31.6	0.144	1.929	2.7	1.31	
4	T-30	1996	10	15	04	10.503	-0.394	10	10	1	31.6	0.144	1.929	2.26	1.15	
5	T-30	1996	10	16	05	13.152	0.2089	10	10	1	31.6	0.144	1.929	2.91	1.52	
5	T-30	1996	10	16	05	12.585	0.1613	10	10	1	31.6	0.144	1.929	2.78	1.45	
6	T-30	1996	10	17	06	10.882	-0.069	10	10	1	31.6	0.144	1.929	2.38	1.23	
6	T-30	1996	10	17	06	12.869	0.0533	10	10	1	31.6	0.144	1.929	2.83	1.47	
7	T-30	1996	10	18	07	14.477	-1.292	10	10	1	31.6	0.144	1.929	3.03	1.5	
7	T-30	1996	10	18	07	13.152	-0.582	10	10	1	31.6	0.144	1.929	2.82	1.43	
8	T-30	1996	10	19	08	14.193	0.3987	10	10	1	31.6	0.144	1.929	3.16	1.66	
8	T-30	1996	10	19	08	13.436	-0.602	10	10	1	31.6	0.144	1.929	2.88	1.46	
9	T-30	1996	10	20	09	14.004	0.2364	10	10	1	31.6	0.144	1.929	3.1	1.62	
9	T-30	1996	10	20	09	10.976	-0.164	10	10	1	31.6	0.144	1.929	2.39	1.23	
10	T-30	1996	10	21	10	13.436	0.6283	10	10	1	31.6	0.144	1.929	3.02	1.6	
10	T-30	1996	10	21	10	12.49	-0.096	10	10	1	31.6	0.144	1.929	2.73	1.41	
11	T-30	1996	10	22	11	13.342	-0.068	10	10	1	31.6	0.144	1.929	2.92	1.51	
11	T-30	1996	10	22	11	10.503	-0.482	10	10	1	31.6	0.144	1.929	2.25	1.14	
12	T-30	1996	10	23	12	13.909	0.2431	10	10	1	31.6	0.144	1.929	3.08	1.61	
12	T-30	1996	10	23	12	12.774	-1.259	10	10	1	31.6	0.144	1.929	2.66	1.31	
13	T-30	1996	10	24	13	10.787	0.9043	10	10	1	31.6	0.144	1.929	2.47	1.33	
13	T-30	1996	10	24	13	10.408	0.4038	10	10	1	31.6	0.144	1.929	2.33	1.23	
14	T-30	1996	10	25	14	12.679	-1.428	10	10	1	31.6	0.144	1.929	2.62	1.28	
14	T-30	1996	10	25	14	10.125	-2.741	10	10	1	31.6	0.144	1.929	1.91	0.84	
1	tw	1996	11	18	01	88.945	4.2334	10	10	1	3.16	0.144	1.929	2	1.06	
1	tw	1996	11	18	01	81.375	3.8918	10	10	1	3.16	0.144	1.929	1.83	0.97	
2	tw	1996	11	21	04	73.805	-1.724	5	10	1	3.16	0.144	1.929	0.8	0.41	
2	tw	1996	11	21	04	87.998	2.5425	10	10	1	3.16	0.144	1.929	1.96	1.03	
3	tw	1996	11	22	05	128.69	1.4118	5	10	1	3.16	0.144	1.929	1.42	0.74	
3	tw	1996	11	22	05	119.22	5.5997	5	10	1	3.16	0.144	1.929	1.34	0.71	
4	tw	1996	11	23	06	130.58	4.7935	5	10	1	3.16	0.144	1.929	1.46	0.77	
4	tw	1996	11	23	06	123.01	3.5729	5	10	1	3.16	0.144	1.929	1.37	0.72	
4	tw	1996	11	24	07	122.59	0.5552	3	10	1	10	0.144	1.929	2.56	1.33	
4	tw	1996	11	24	07	125.58	3.1206	3	10	1	10	0.144	1.929	2.65	1.39	
1	twold	1996	12	1	01	89.891	-1.108	10	10	1	3.16	0.144	1.929	1.96	1.01	
1	twold	1996	12	1	1	89.891	1.5291	10	10	1	3.16	0.144	1.929	1.99	1.04	
2	twold	1996	12	3	3	88.506	1.4938	5	10	1	10	0.144	1.929	3.1	1.62	

2	twold	1996	12	3	3	89.702	0.8534	5	10	1	10	0.144	1.929	3.13	1.63	
1	twnew	1996	12	1	01	54.718	0.0039	10	10	1	10	0.144	1.929	3.8	1.97	
1	twnew	1996	12	1	01	48.439	1.0053	10	10	1	10	0.144	1.929	3.4	1.78	
2	twnew	1996	12	3	03	70.565	-0.01	5	10	1	10	0.144	1.929	2.45	1.27	
2	twnew	1996	12	3	03	58.605	0.839	5	10	1	10	0.144	1.929	2.05	1.07	
3	twnew	1996	12	8	8	92.094	1.2391	5	10	1	10	0.144	1.929	3.22	1.68	
3	twnew	1996	12	8	8	81.927	0.2942	5	10	1	10	0.144	1.929	2.85	1.48	
4	twnew	1996	12	10	10	94.486	-1.153	5	10	1	10	0.144	1.929	3.26	1.68	
4	twnew	1996	12	10	10	86.213	-1.492	6	10	1	10	0.144	1.929	3.56	1.83	
TWA	fr. tiff	1996	12	23	1	430.57	-2.793	5	10	1	1	0.144	1.929	1.49	0.77	
TWB	fr. tiff	1996	12	23	1	441.57	6.7402	3	10	1	3.16	0.144	1.929	2.93	1.53	
TWA	fr. tiff	1996	12	25	3	422.64	16.874	3	10	1	3.16	0.144	1.929	2.84	1.5	
TWB	fr. tiff	1996	12	25	3	422.64	16.874	3	10	1	3.16	0.144	1.929	2.84	1.5	
TWA	fr. tiff	1996	12	31	9	637.88	-82.33	3	10	1	1	0.144	1.929	1.24	0.6	
TWB	fr. tiff	1996	12	31	9	662.35	1.32	2	10	1	3.16	0.144	1.929	2.91	1.51	
TWA	fr. tiff	1996	1	1	10	837.22	-17.78	2	10	1	1	0.144	1.929	1.15	0.59	
TWB	fr. tiff	1996	1	1	10	837.22	-101.1	2	10	1	1	0.144	1.929	1.09	0.53	
TWA	fr. tiff	1996	1	7	16	1121.3	59.278	2	10	1	1	0.144	1.929	1.6	0.85	
TWB	fr. tiff	1996	1	7	16	1001.7	67.77	2	10	1	1	0.144	1.929	1.44	0.77	

Table 7. Summary of Results

Gonyaulax polyedra

GP=35u		orig	dilution	dilution
	day		34.5	29.4
			100%	60%
chl	1	259	7.51	8.81
mg/m3=ug/l	18		438	474
	28		1414	1547
#cells	1	0.037	0.0011	0.0013
10^4cells/ml	18		0.5132	0.2218
10^7cell/l	28		1.9480	0.5548
chl/cell	1	7000	7000.00	7000.00
10^-7ug/cell	18		853.47	2137.06
	28		725.87	2788.39
A676	1		0.38	0.38
	18		3.24	4.83
	28		8.2	10.65
A676/chl	1		0.0015	0.0015
	18		0.0074	0.01018
	28		0.0058	0.00689
A676/cell	1		354.32	301.95
	18		6.31	21.76
	28		4.21	19.2
A436	1		1.85	1.85
	18		5.67	7.6
	28		15.65	20.24

irradiance	Eo	2300
uE/m2sec	E	
	100lamp	90
	60lamp	70
	30lamp	30

Duneliella teriolecta

DUN=8u			dilution	dilution	
	day		22.7	22.7	
			100%	60%	30
chl	1	2455	108.1	108	108
mg/m3=ug/l	18		1148	1005	569
	28		3954	3615	5377
#cells	1	74	3.26	3.4	3.4
10^4 cells/ml	18			86	15
10^7cell/l	28		576	214	191
chl/cell	1	33.2	33.18	31.76	31.76
10^-7ug/cell	18			11.69	37.93
	28		06.86	16.89	28.15
A676	1		2.25	2.25	2.25
	18		22.35	25.8	13.58
	28		106.4	94.18	124.25
A676/chl	1		0.021	0.0208	0.0208
	18		0.02	0.0257	0.0239
	28		0.027	0.0261	0.0231
A676/cell	1		0.662	0.662	0.662
	18			0.727	0.887
	28		0.185	0.439	0.65
A436	1		3.53	3.53	3.53
	18		34.86	38.79	20.2
	28		192	154.4	179.98

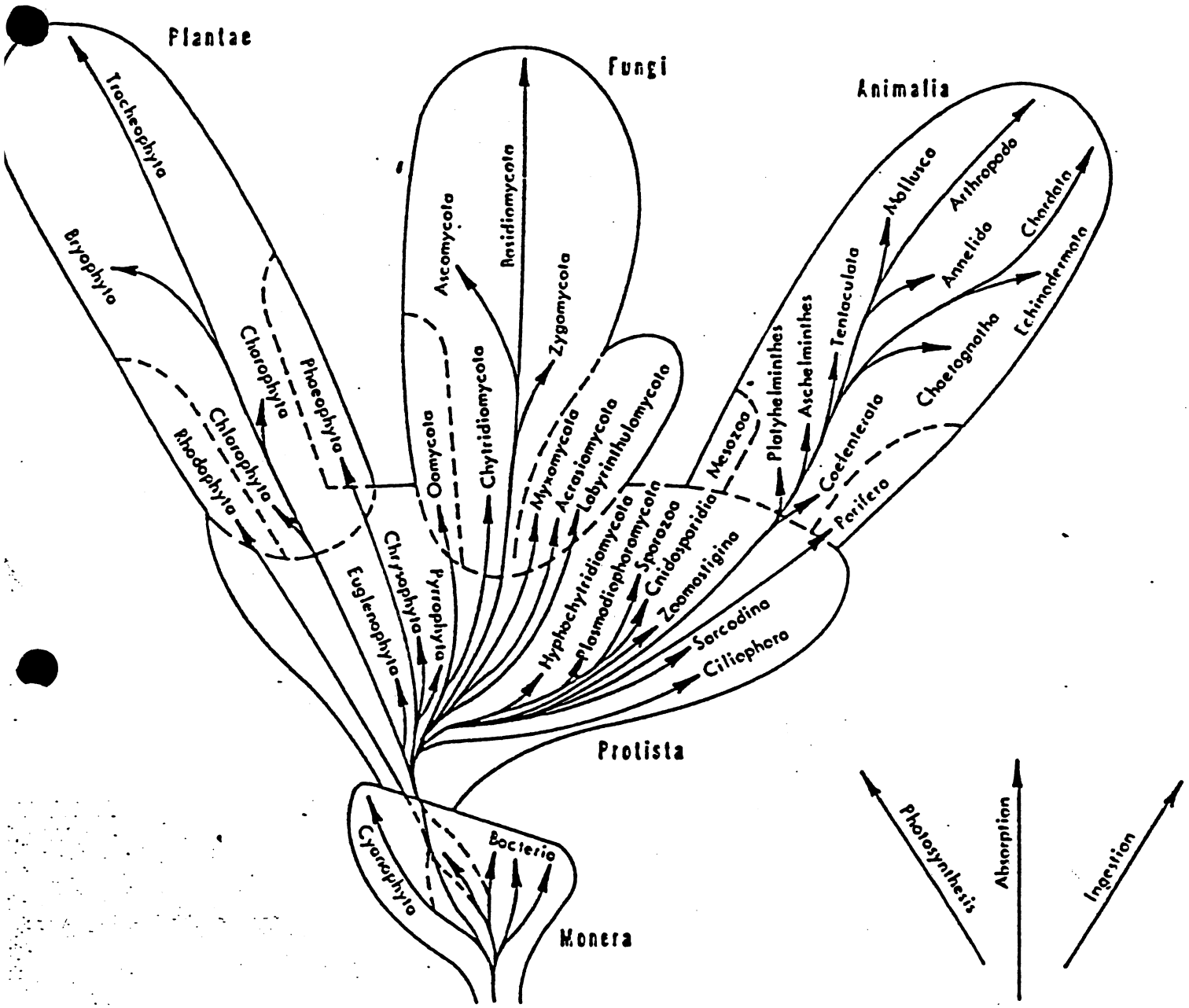
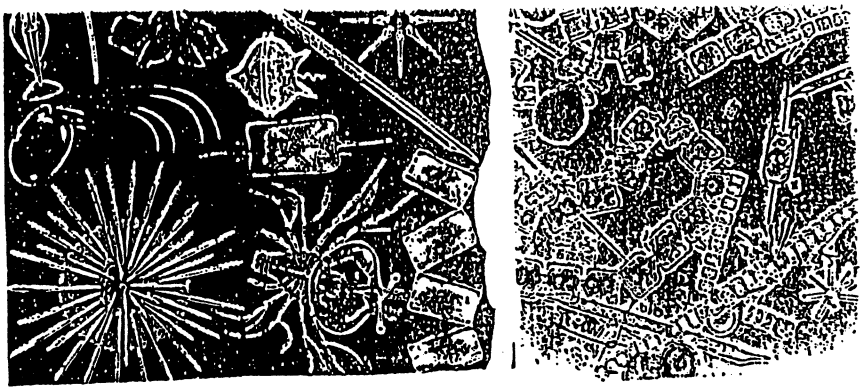
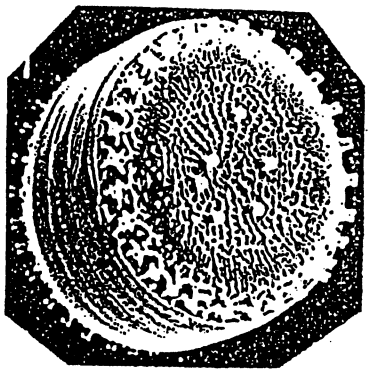


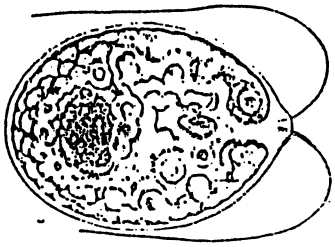
Figure 1. Animal and Plant Classifications



Dinoflagelate Gonyaulax polyedra

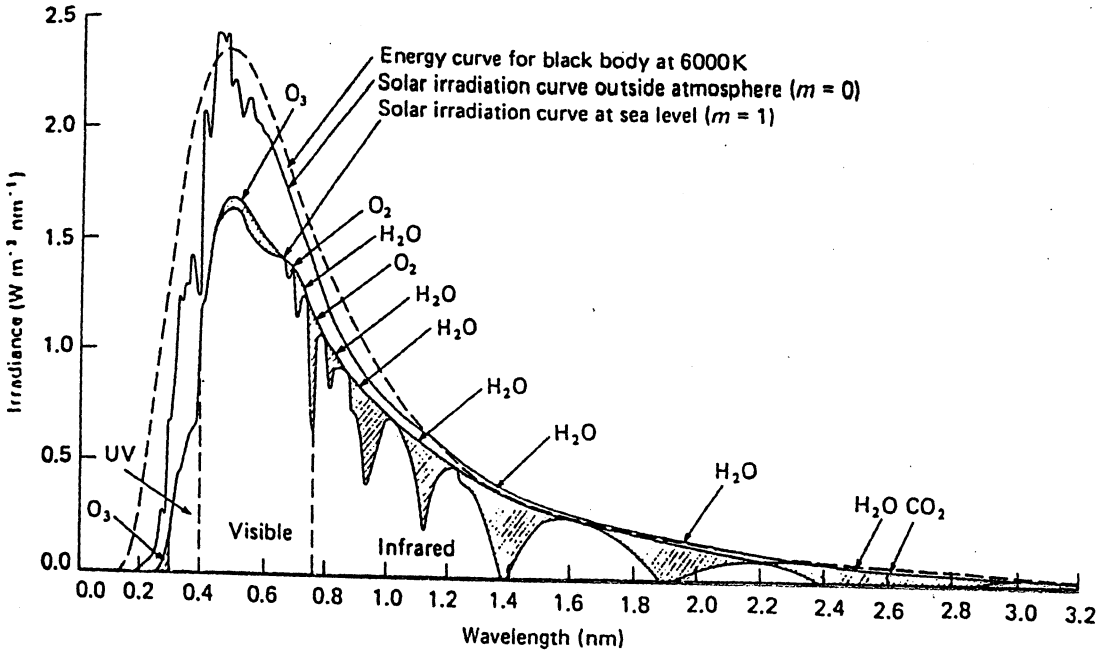


Diatom Thalassiosira Weisflogii



Green Algae Dunelliella Tertiolecta

Figure 2. Mixed and individual cultures of phytoplankton.



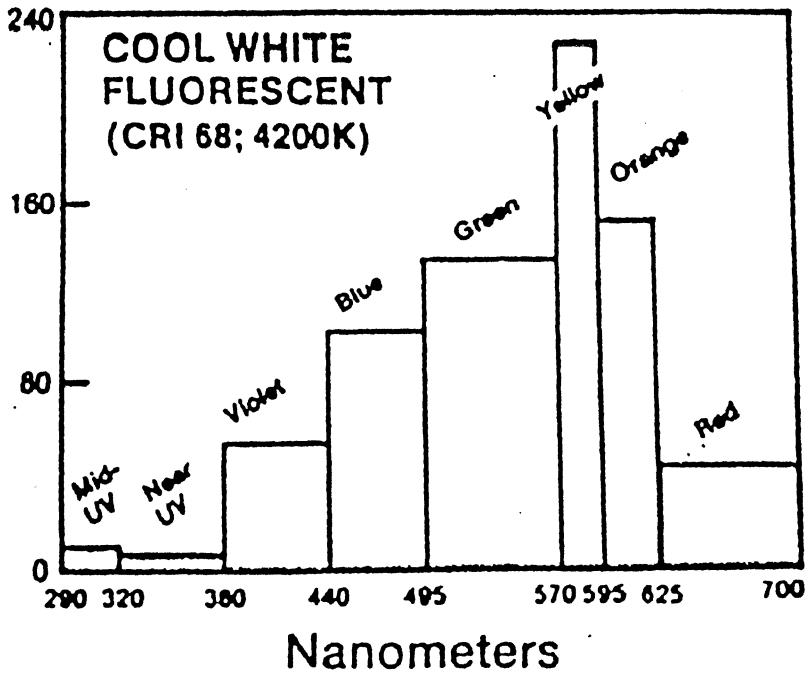
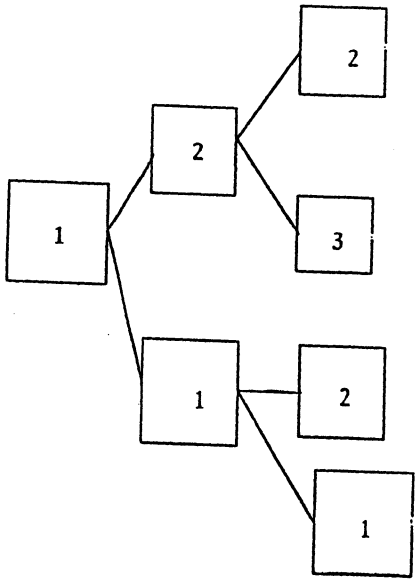


Figure 3a. Sunlight wavelength spectrum
Figure 3b. Light wavelength spectrum.....



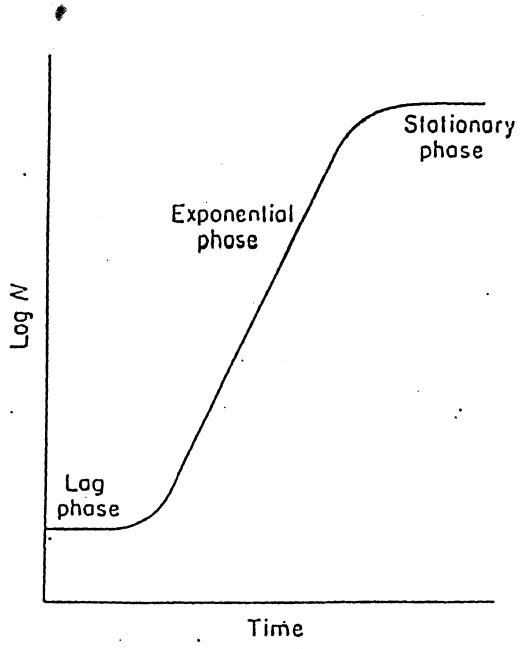


Figure 4a. Cell division

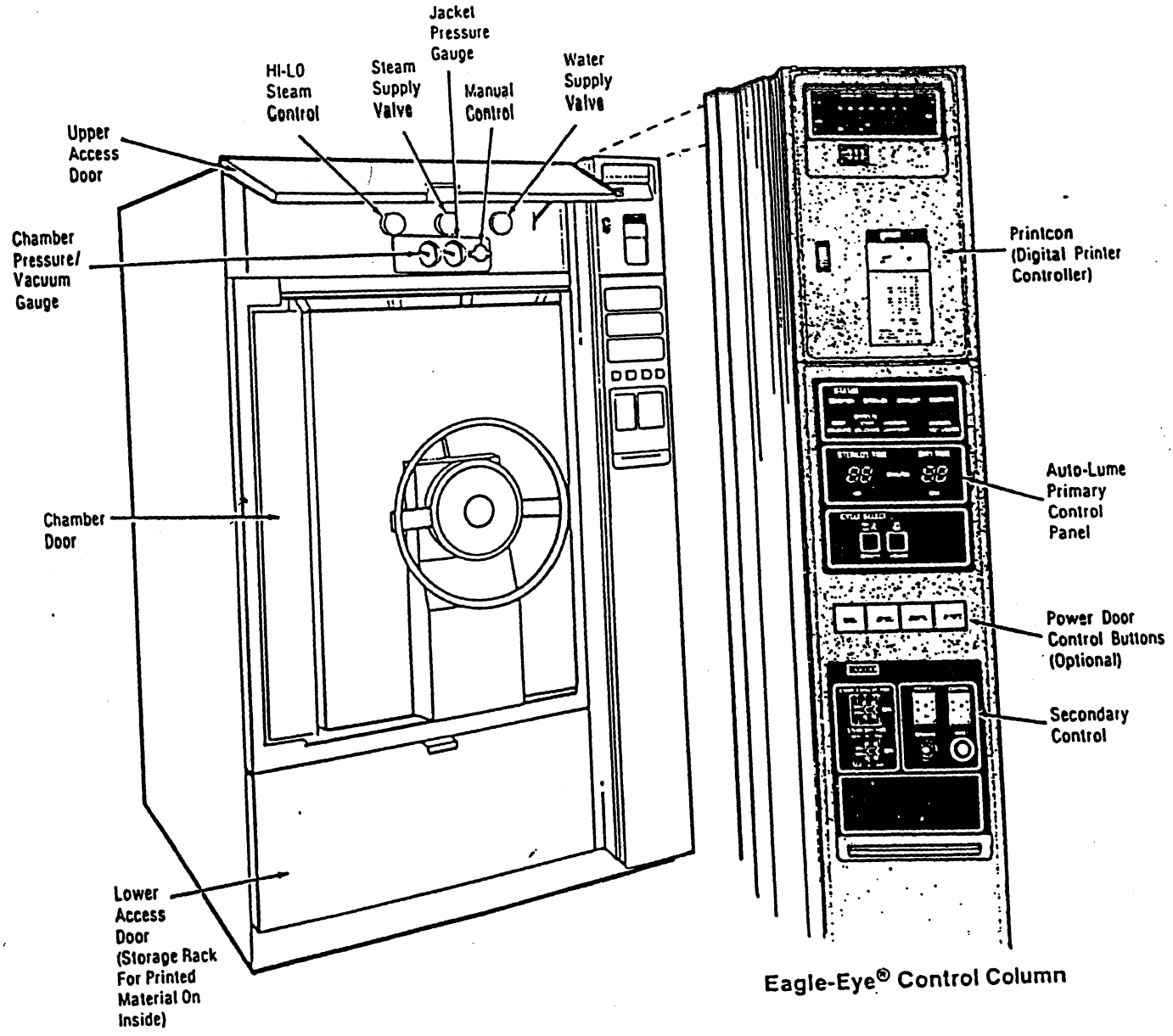
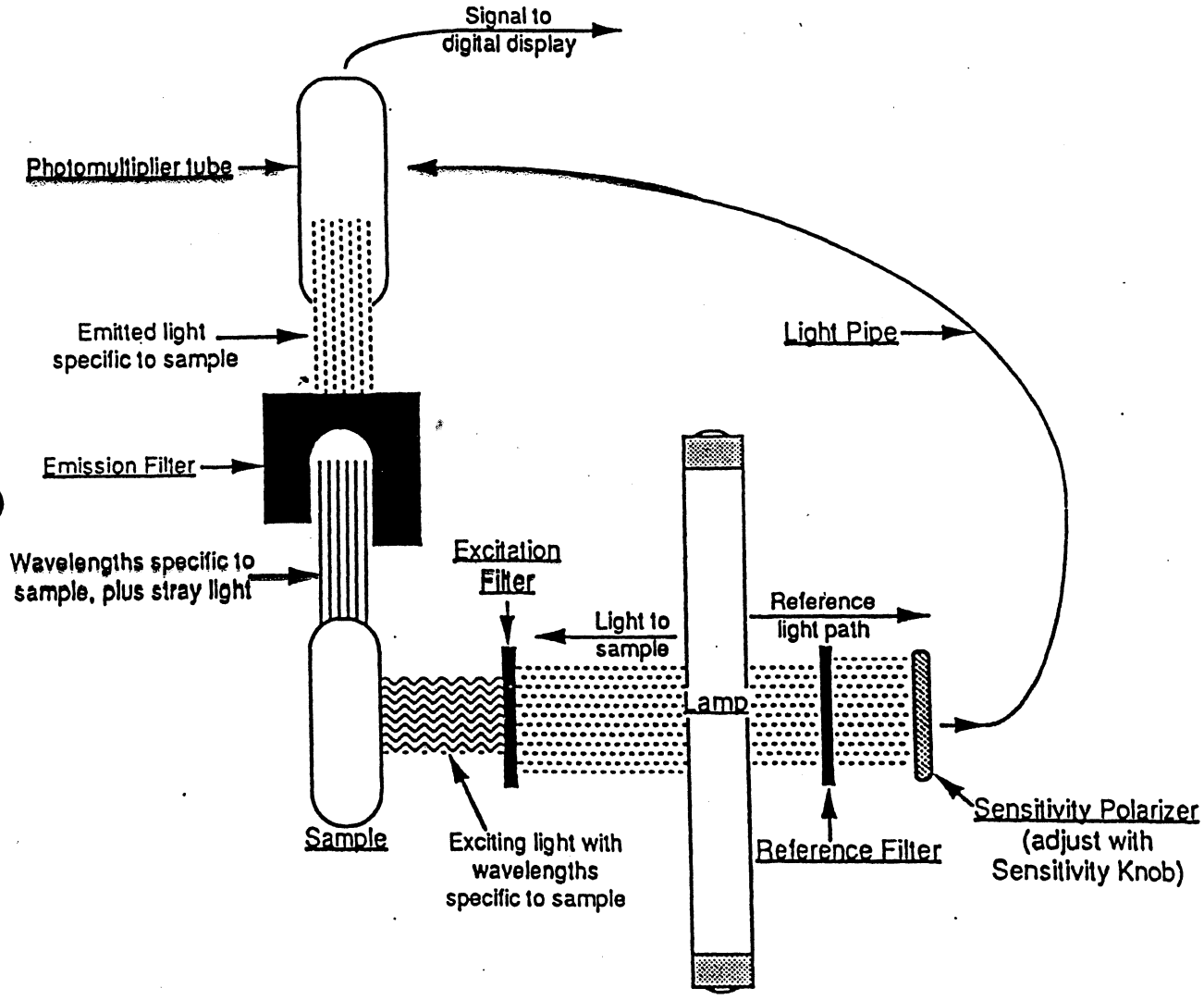


Figure 5. Autoclave for sterilizing


Signal to
digital display
Photomultiplier tube
Emitted light
specific to sample
Light Pipe
Emission Filter
Wavelengths specific to
sample, plus stray light
Excitation
Filter
Light to
sample
Reference
light path
Lamp
Sample
Exciting light with
wavelengths
specific to sample
Reference Filter
Sensitivity Polarizer
(adjust with
Sensitivity Knob)

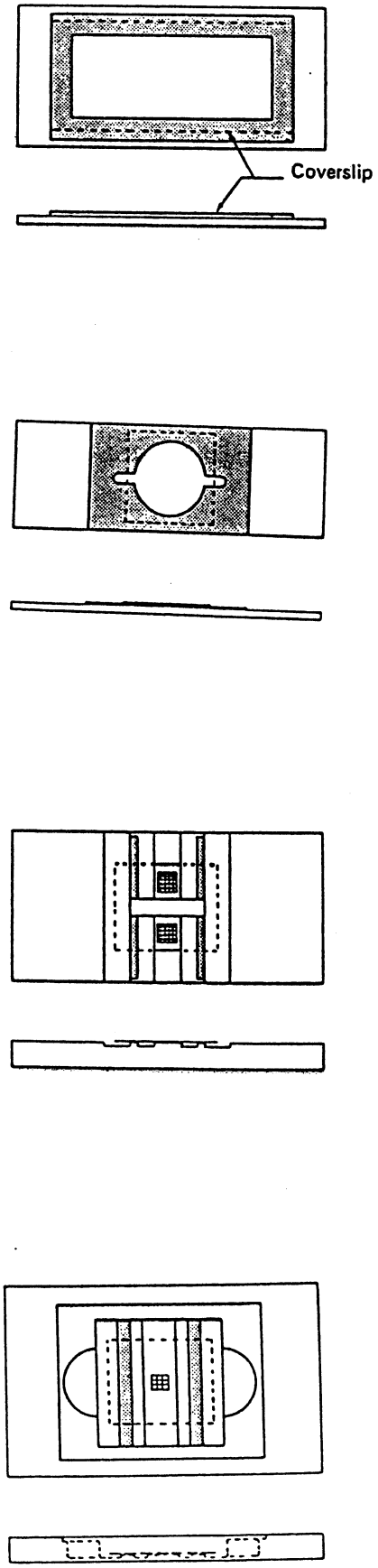
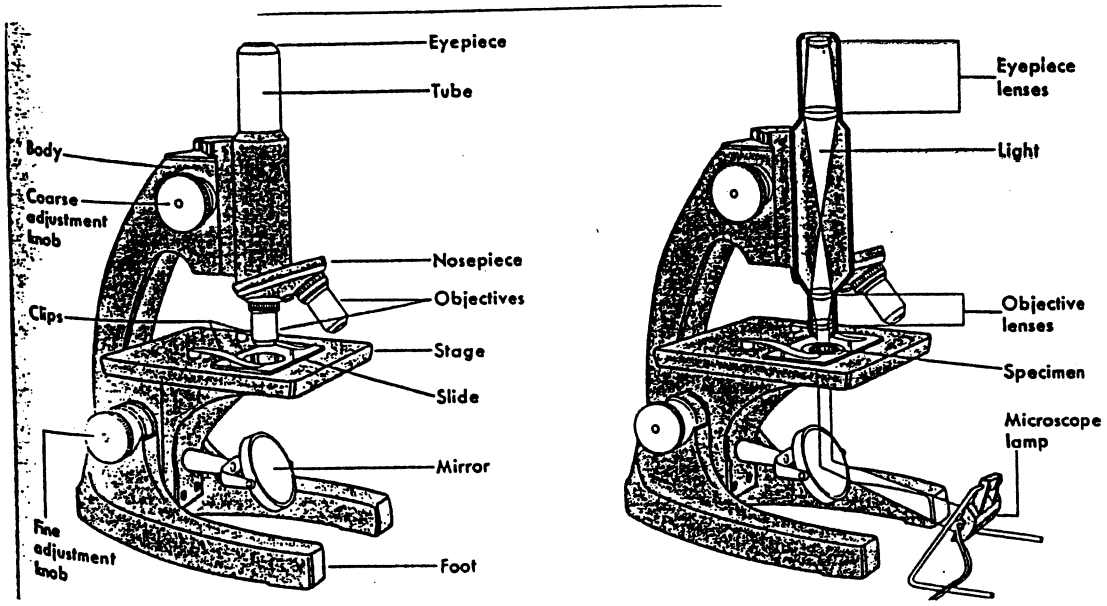


Figure 7- Counting Chambers: Sedgwick-Rafter chamber; Palmer-Maloney chamber; one type of haemacytometer (blood counting



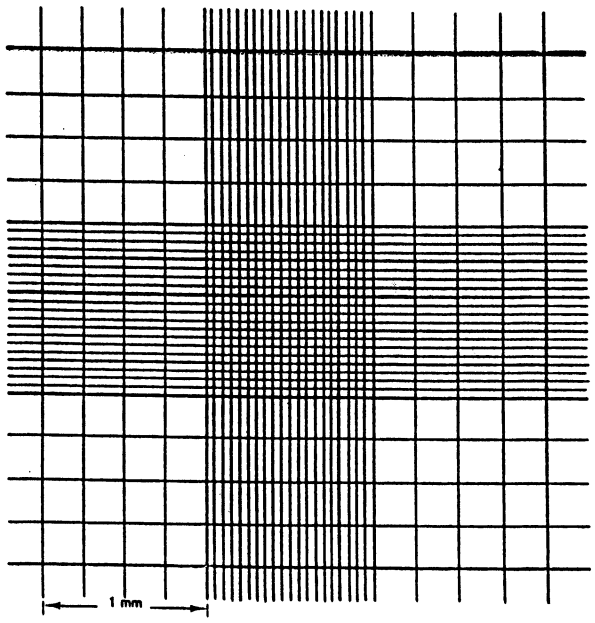


Figure 8a. Standard upright microscope
Figure 8b. Standard counting grid

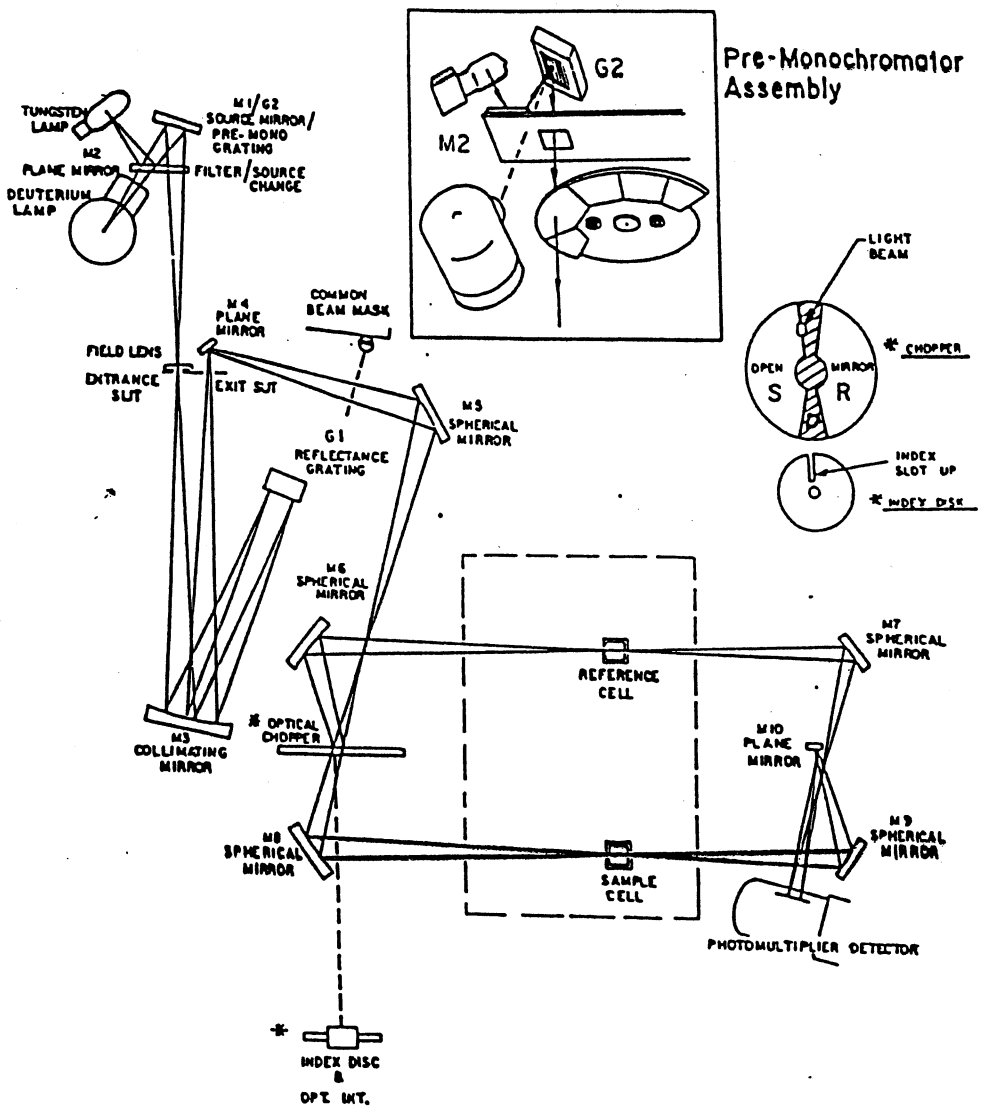


Figure 9. Schematic of Spectrophotometer

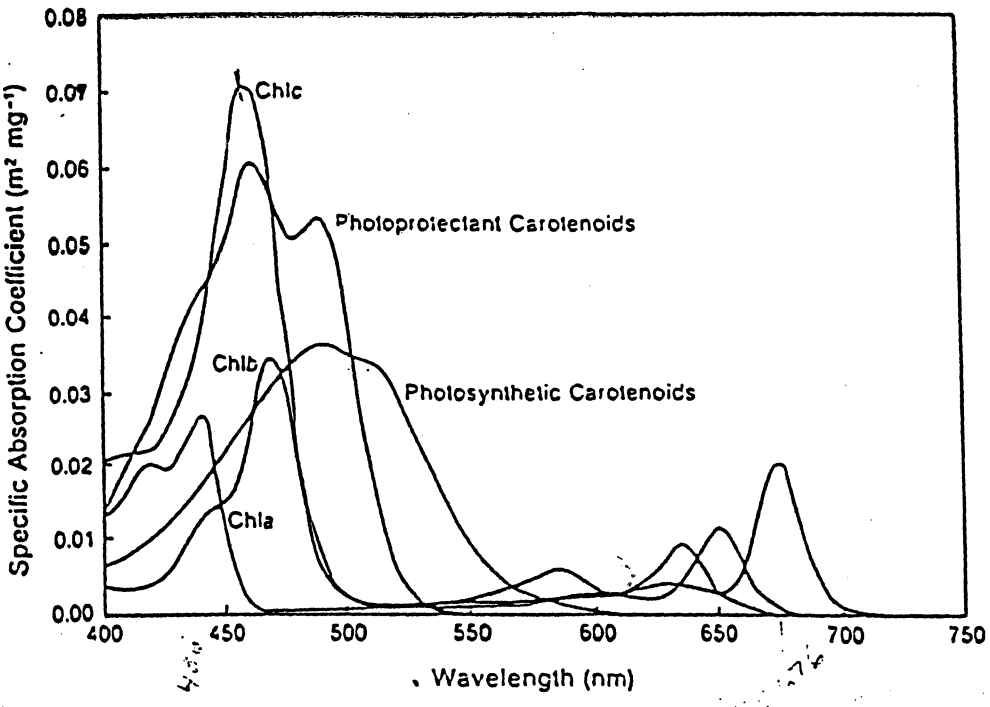


Figure 10 Absorption spectrum of chlorophyll pigments

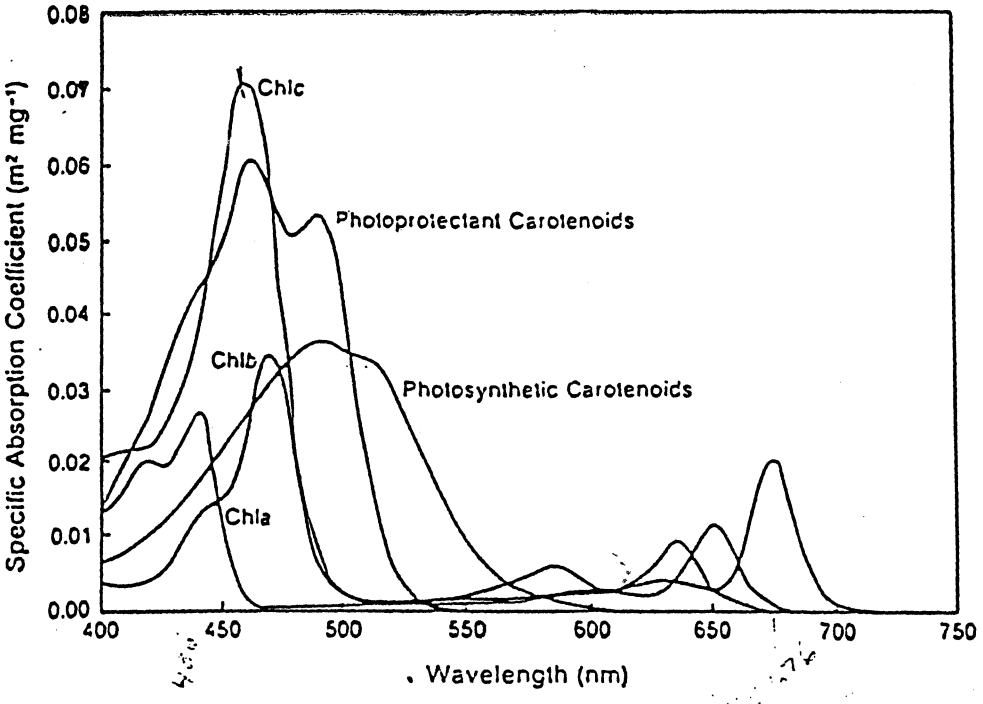


Figure 10. Absorption spectrum of chlorophyll pigments

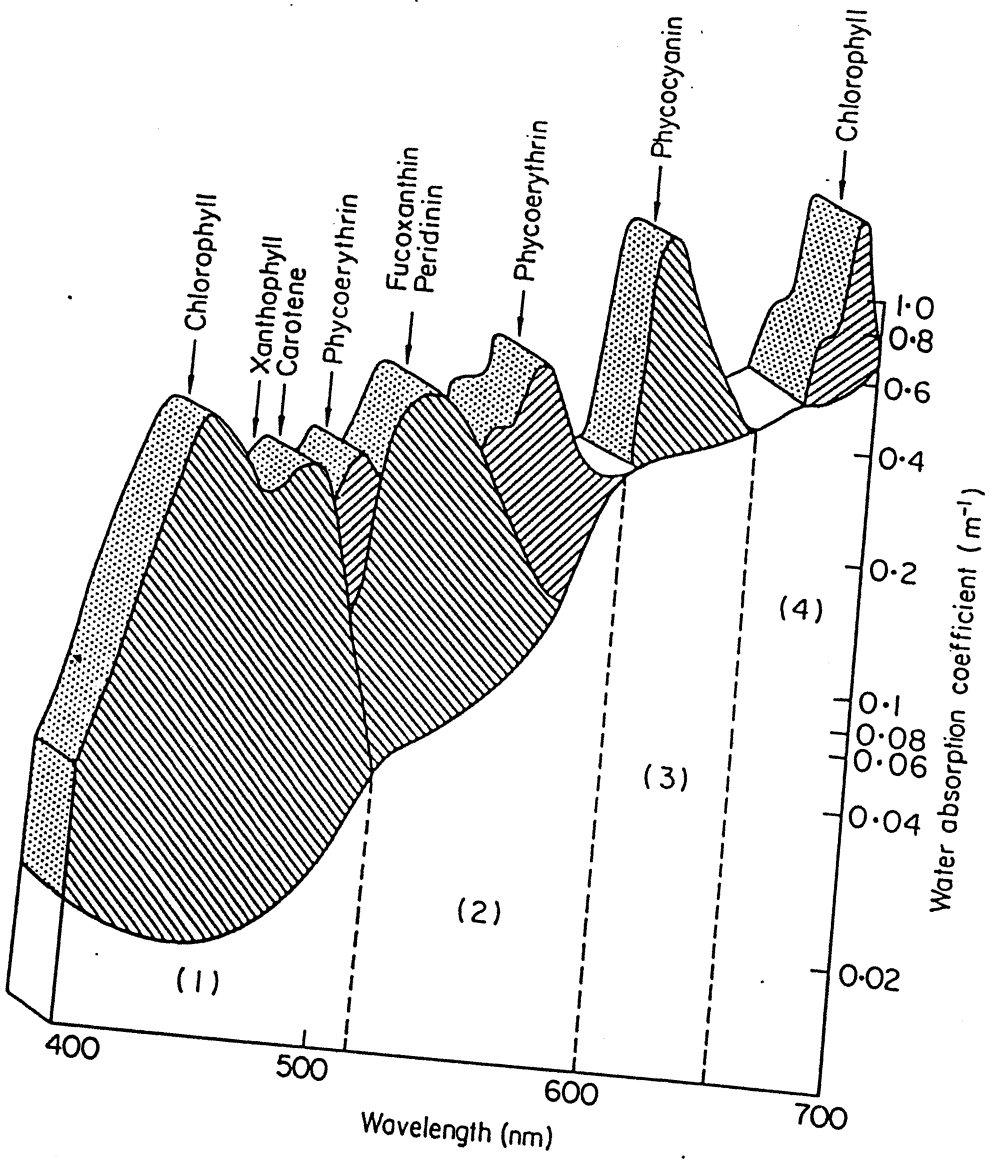
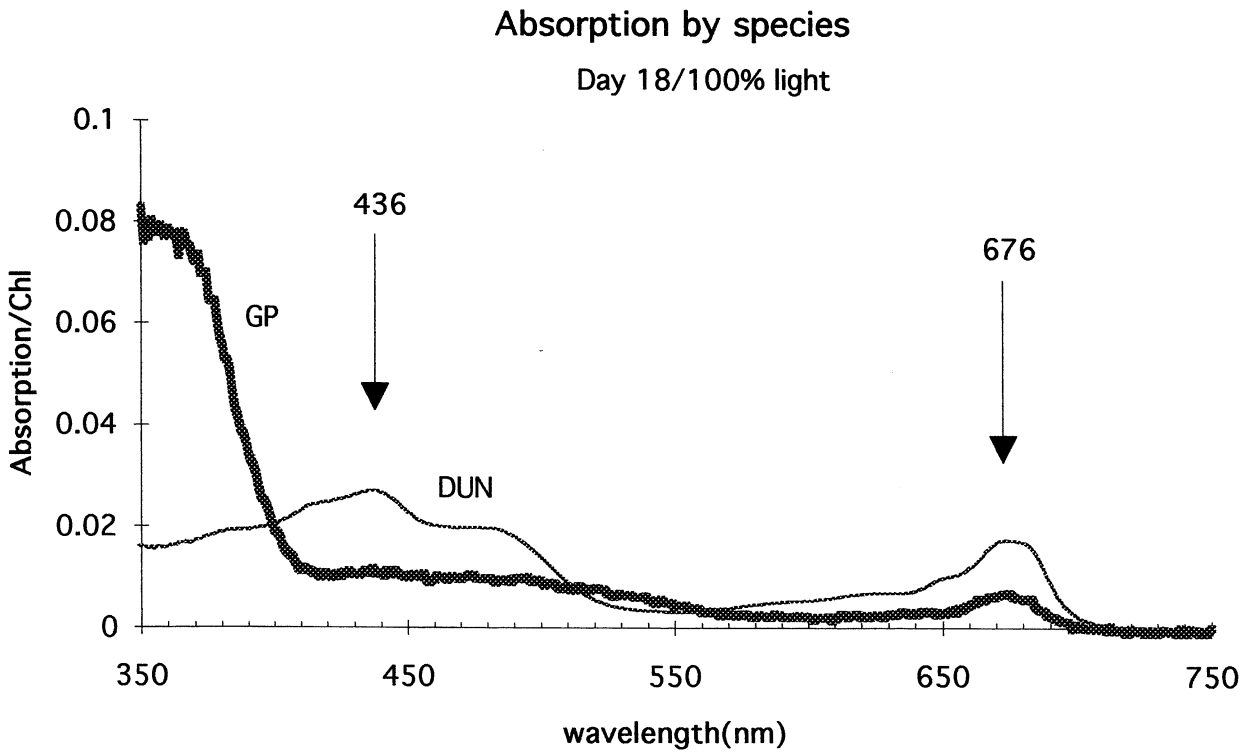

Chlorophyll
Xanthophyll
Carotene
Phycoerythrin
Fucoxanthin
Peridinin
Phycoerythrin
Phycocyanin
Chlorophyll
(1)
(2)
(3)
(4)
400
500
600
700
Wavelength (nm)
1·0
0·8
0·6
0·4
0·2
0·1
0·08
0·06
0·04
0·02
Water absorption coefficient (m⁻¹)

Figure 11 Ab


Absorption by species
Day 18/100% light
0.1
0.08
0.06
0.04
0.02
0
Absorption/Chl
436
676
GP
DUN
350
450
550
650
750
wavelength(nm)

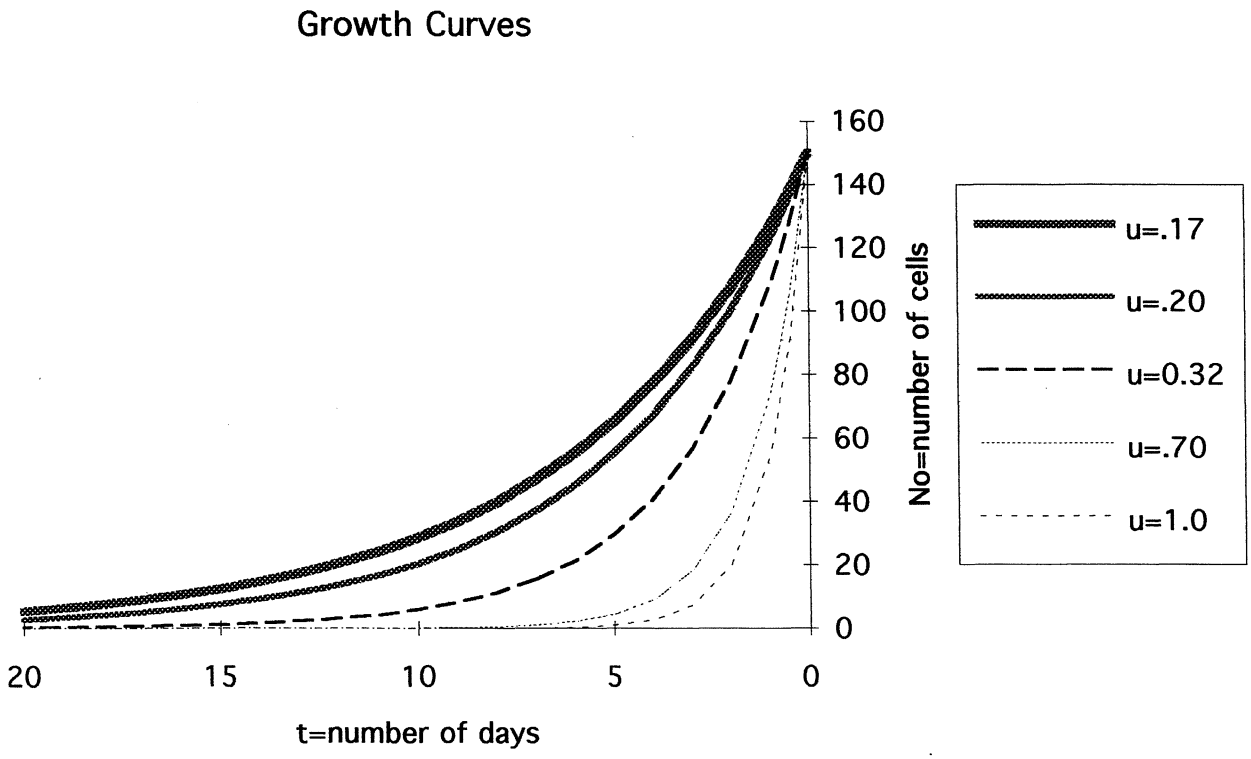

Growth Curves
160
140
120
100
80
60
40
20
0
No=number of cells
u=.17
u=.20
u=0.32
u=.70
u=1.0
20
15
10
5
0
t=number of days

Figure 4c

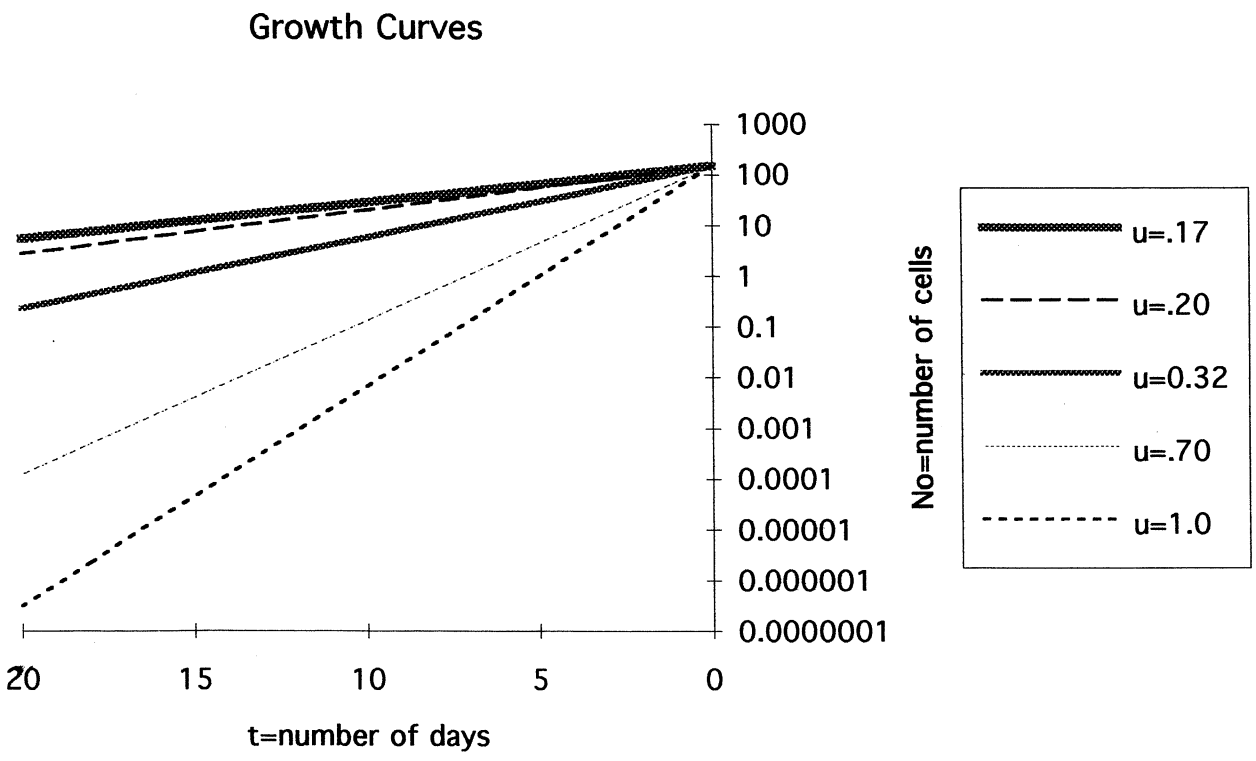

Growth Curves
1000
100
10
1
0.1
0.01
0.001
0.0001
0.00001
0.000001
0.0000001
20
15
10
5
0
t=number of days
No=number of cells
u=.17
u=.20
u=0.32
u=.70
u=1.0

Figure 4d

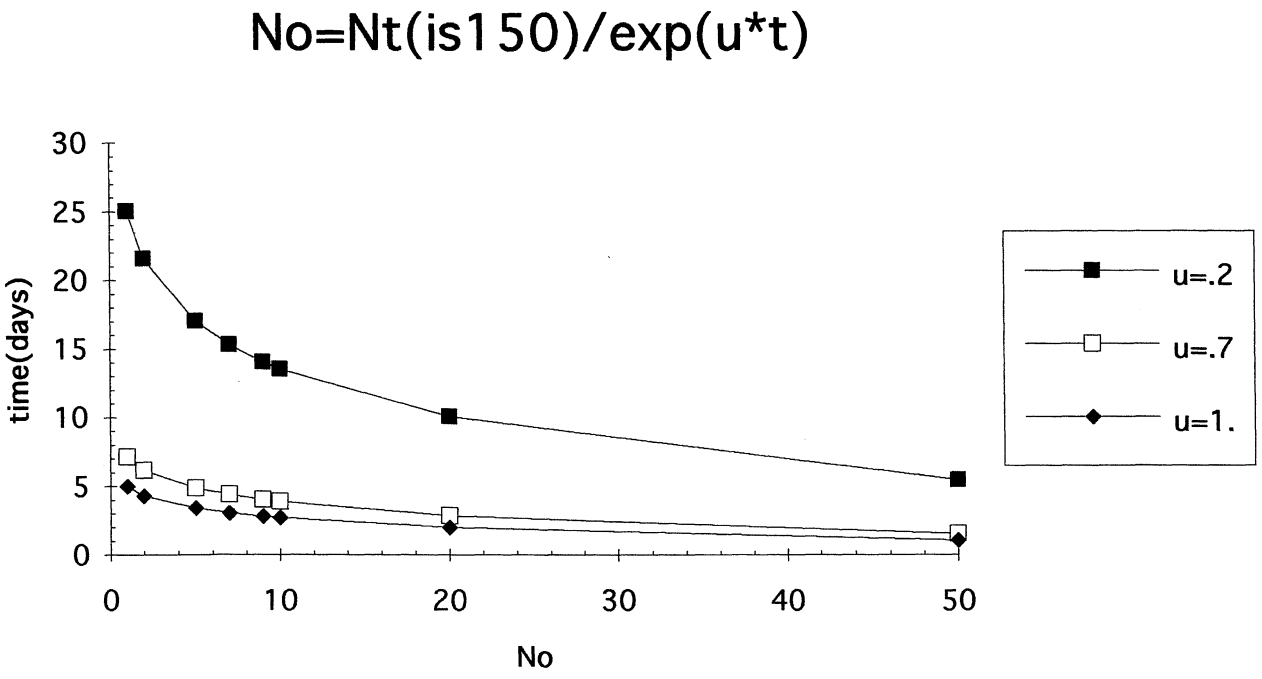

No=Nt(is150)/exp(u*t)
time(days)
30
25
20
15
10
5
0
0
10
20
30
40
50
No
u=.2
u=.7
u=1.

Figure 12

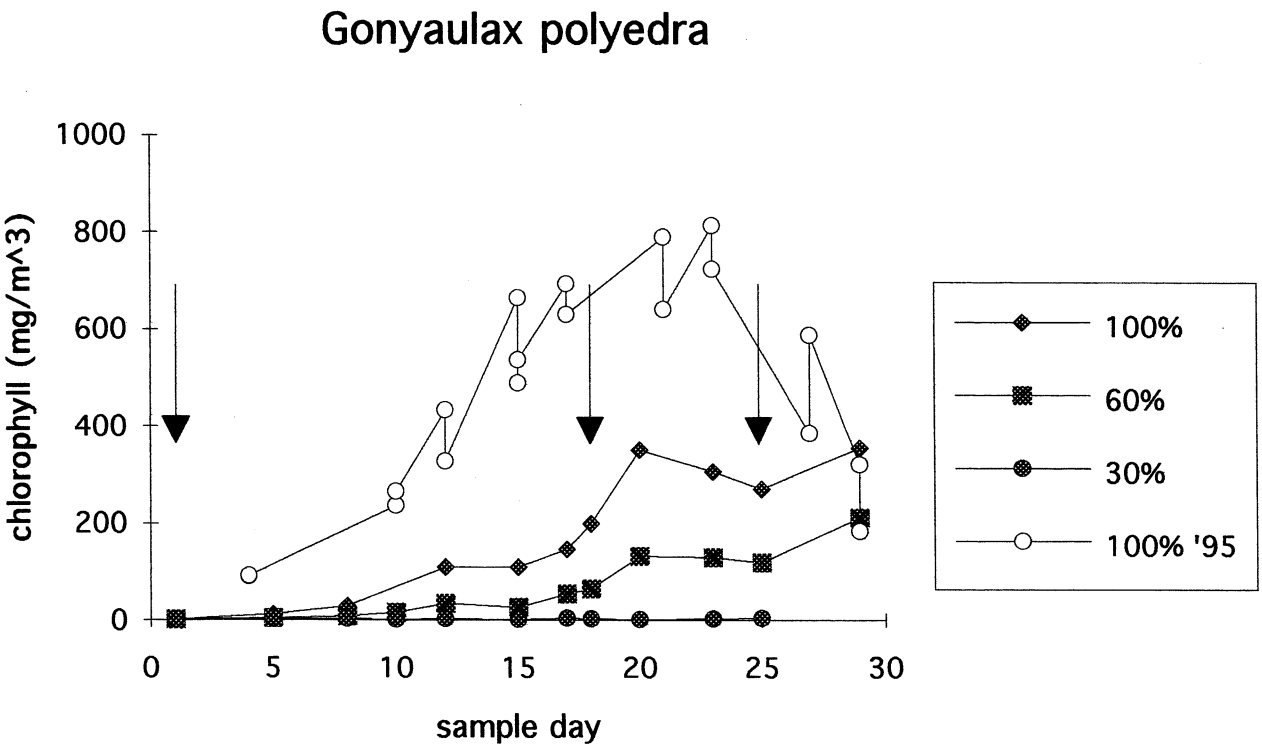

Gonyaulax polyedra
chlorophyll (mg/m^3)
1000
800
600
400
200
0
0
5
10
15
20
25
30
sample day
100%
60%
30%
100% '95

Figure 13a

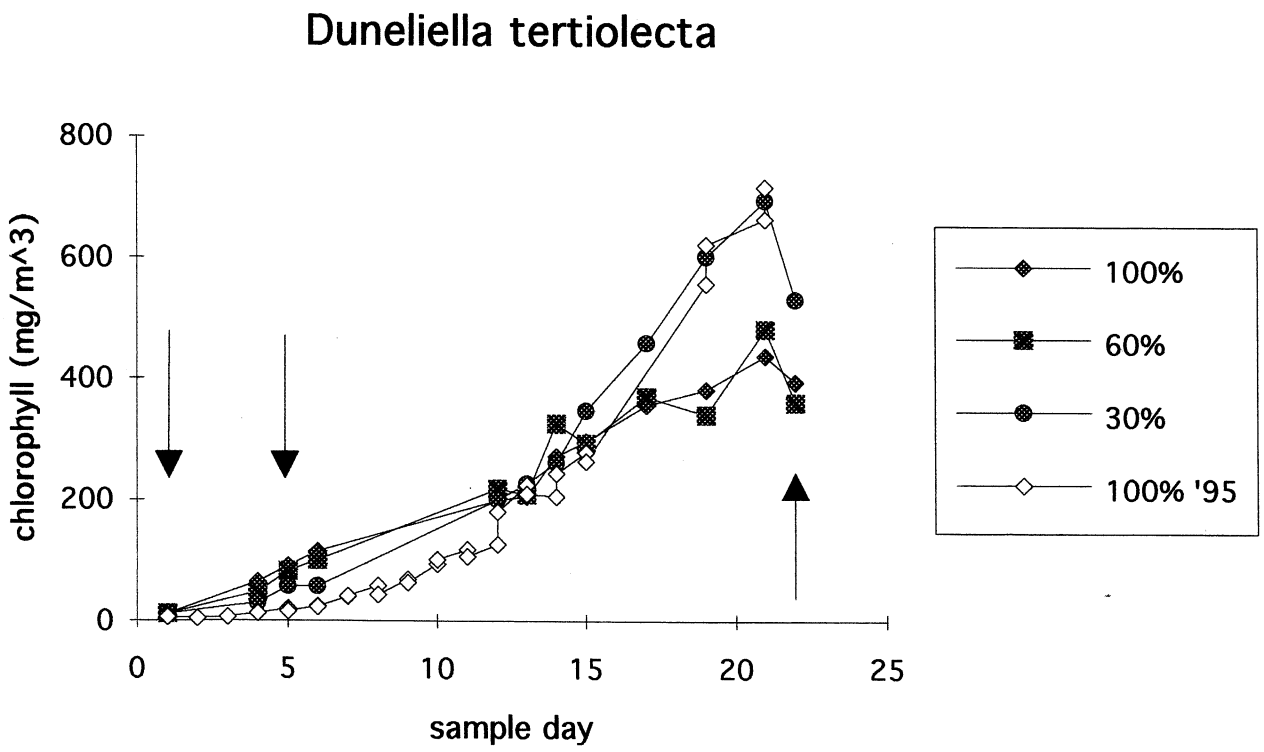

Duneliella tertiolecta
chlorophyll (mg/m^3)
800
600
400
200
0
0
5
10
15
20
25
sample day
100%
60%
30%
100% '95

Figure 13b

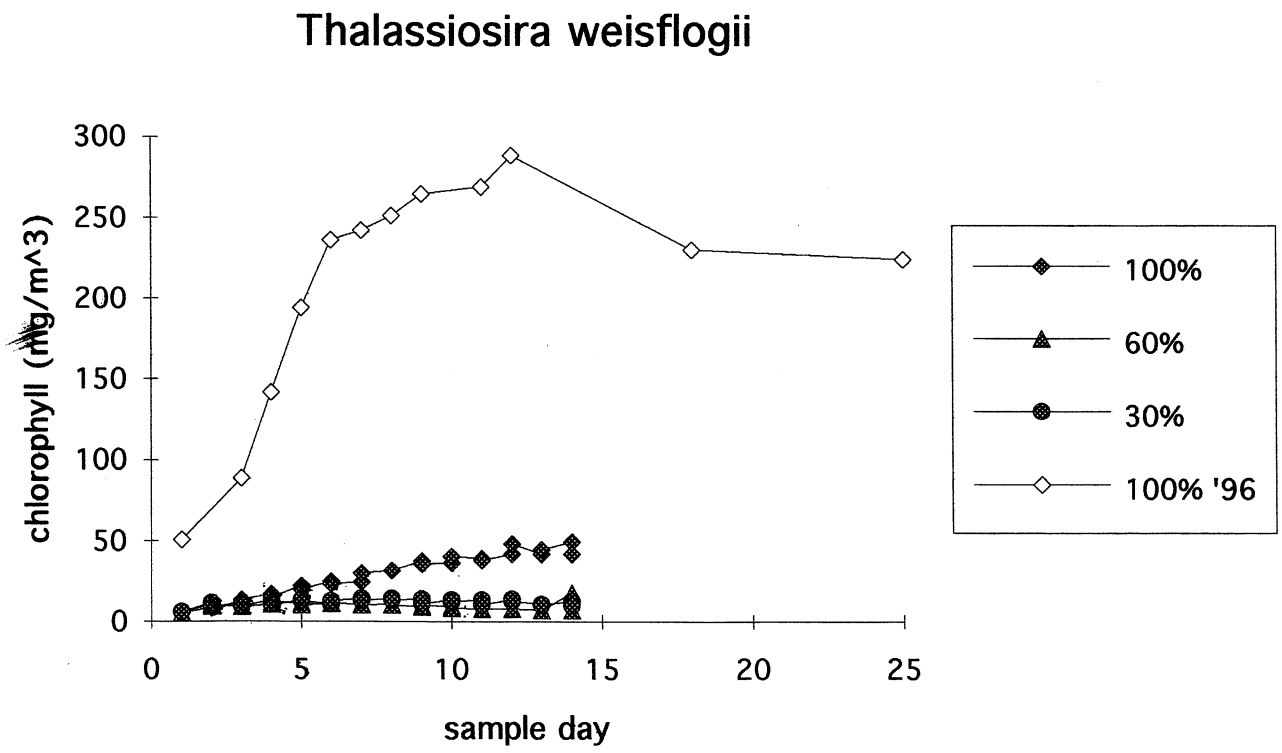

Thalassiosira weisflogii
chlorophyll (mg/m^3)
300
250
200
150
100
50
0
0
5
10
15
20
25
sample day
100%
60%
30%
100% '96

Figure 13c

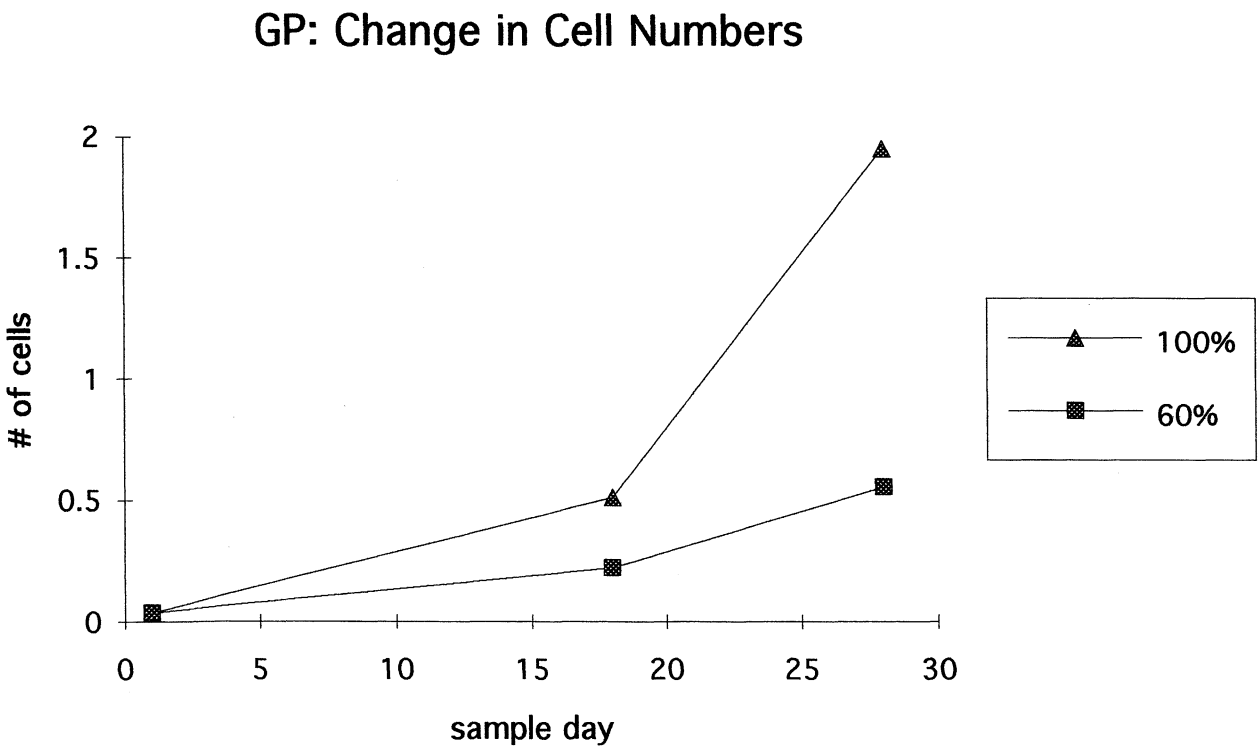

GP: Change in Cell Numbers
# of cells
2
1.5
1
0.5
0
0
5
10
15
20
25
30
sample day
100%
60%

Figure 14a

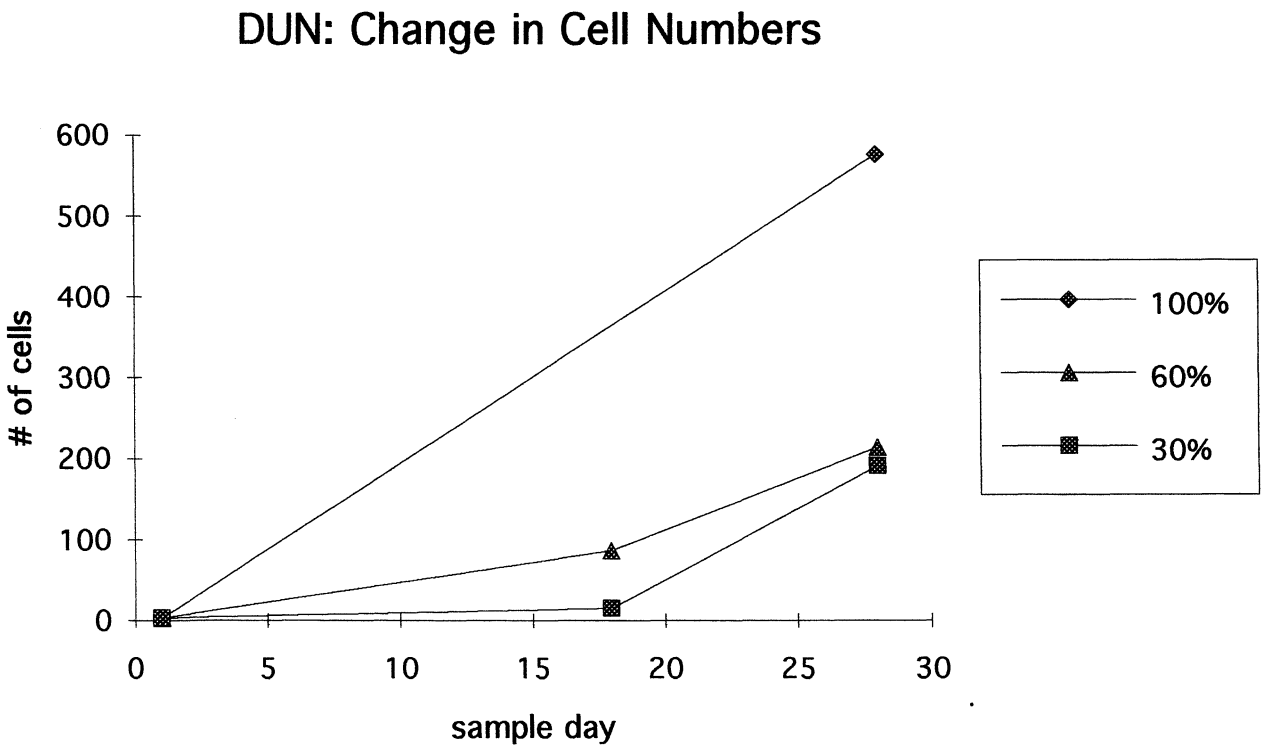

DUN: Change in Cell Numbers
600
500
400
300
200
100
0
# of cells
0
5
10
15
20
25
30
sample day
100%
60%
30%

Figure 14b

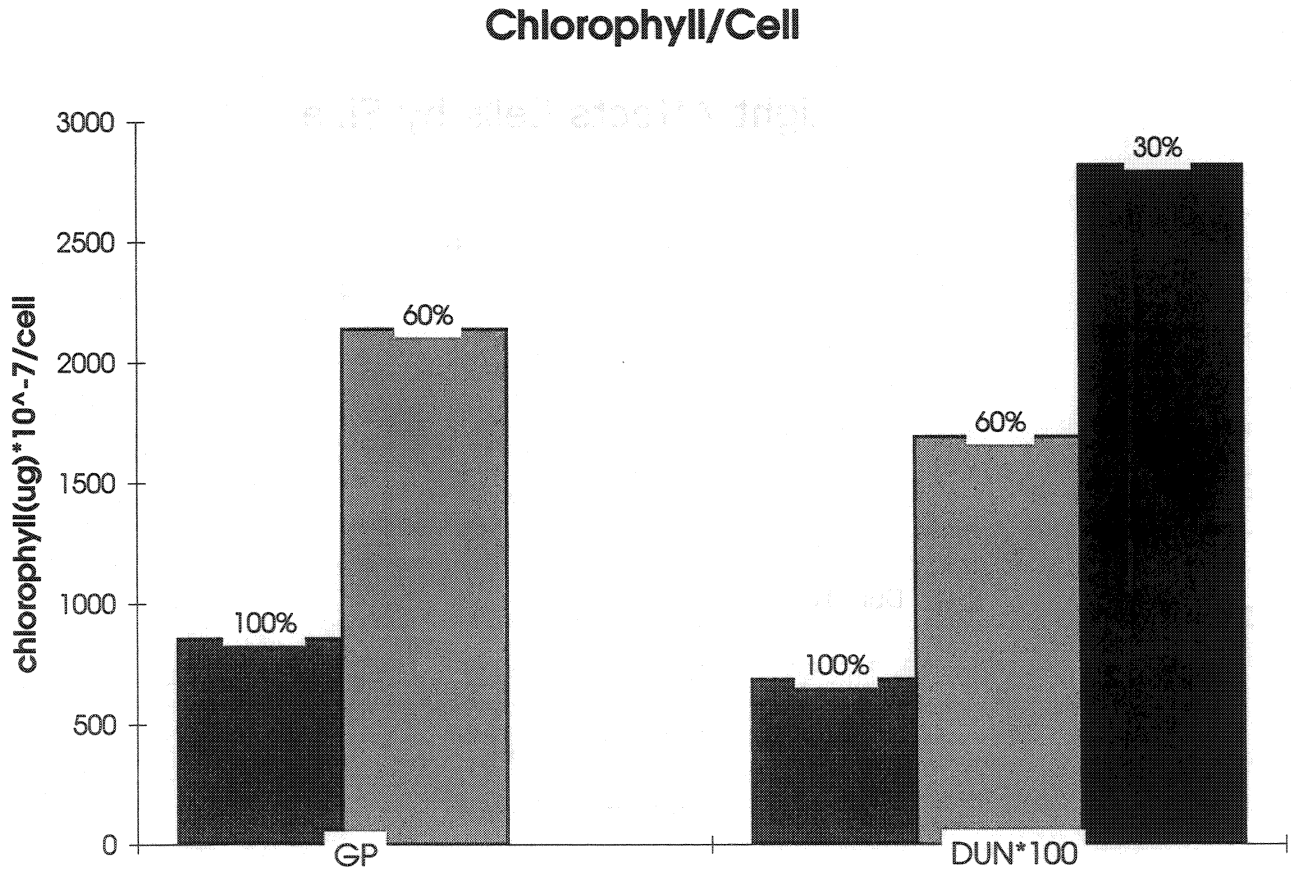

Chlorophyll/Cell
chlorophyll(ug)*10^-7/cell
3000
2500
2000
1500
1000
500
0
100%
60%
100%
60%
30%
GP
DUN*100

Figure 15

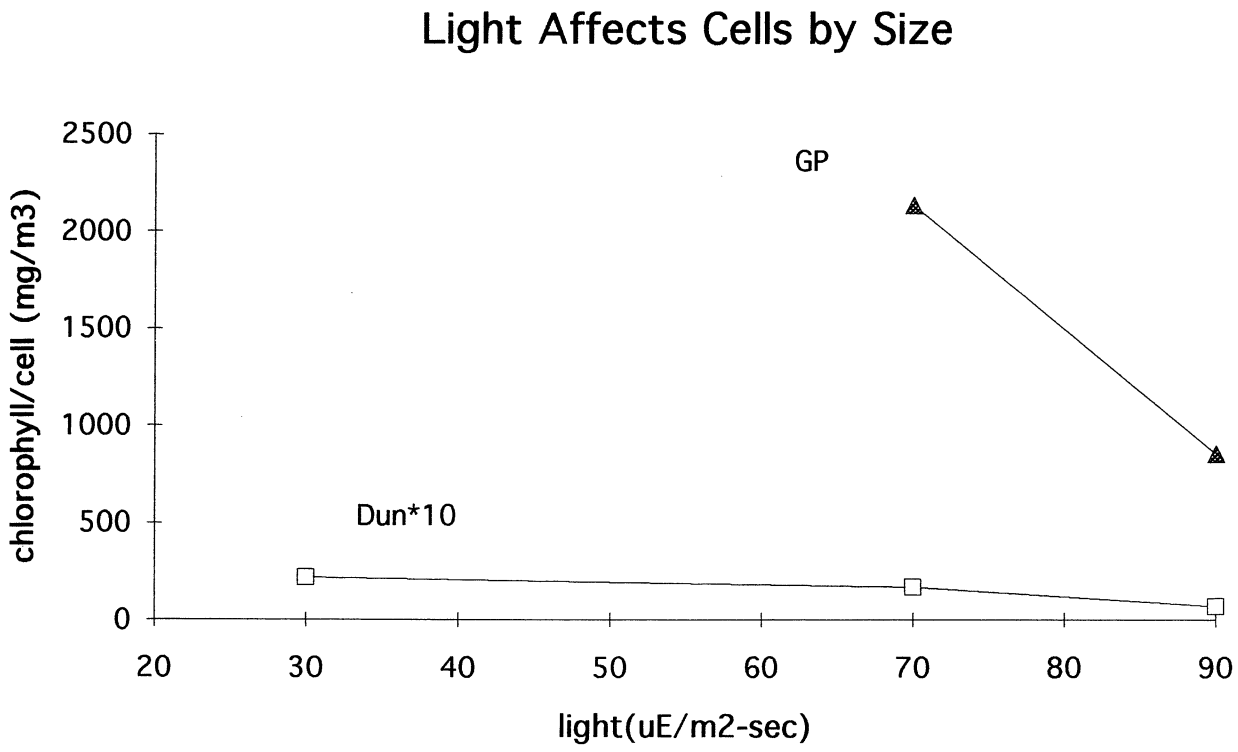

Light Affects Cells by Size
chlorophyll/cell (mg/m3)
2500
2000
1500
1000
500
0
GP
Dun*10
20
30
40
50
60
70
80
90
light(uE/m2-sec)

Figure 16

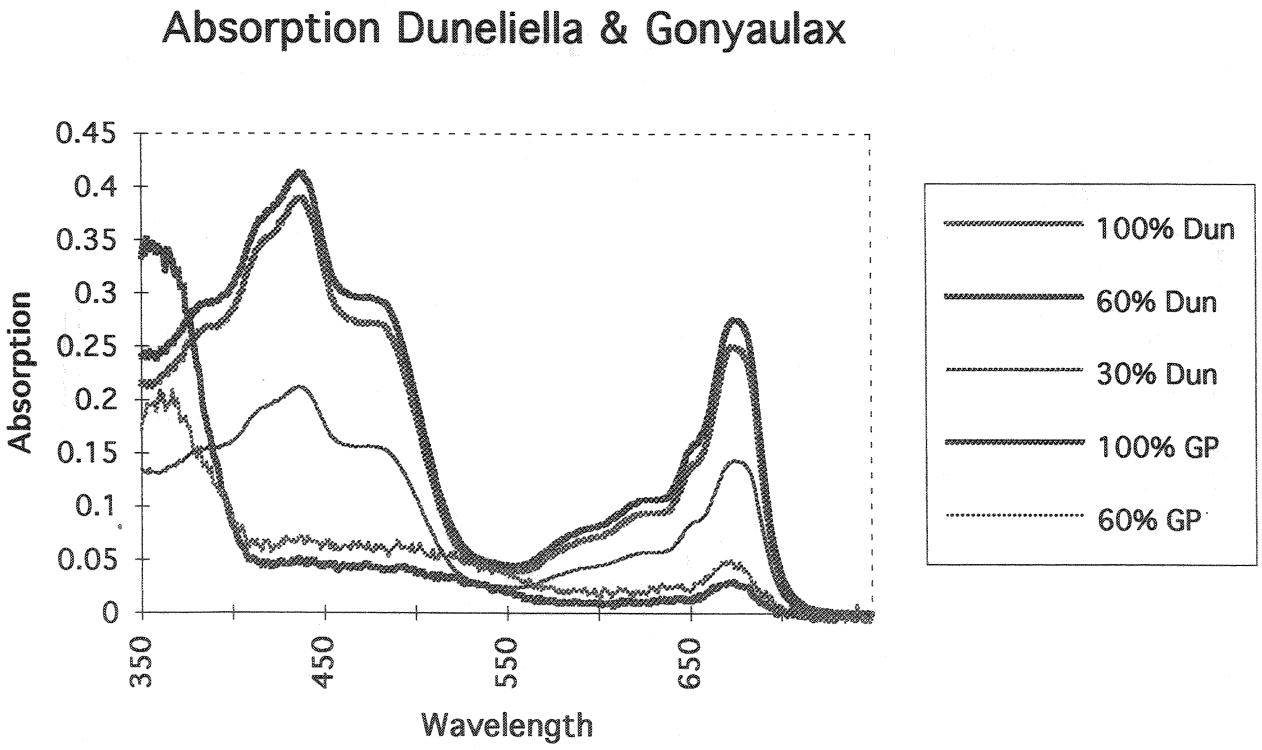

Absorption Duneliella & Gonyaulax
0.45
0.4
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
Absorption
350
450
550
650
Wavelength
100% Dun
60% Dun
30% Dun
100% GP
60% GP

Figure 17

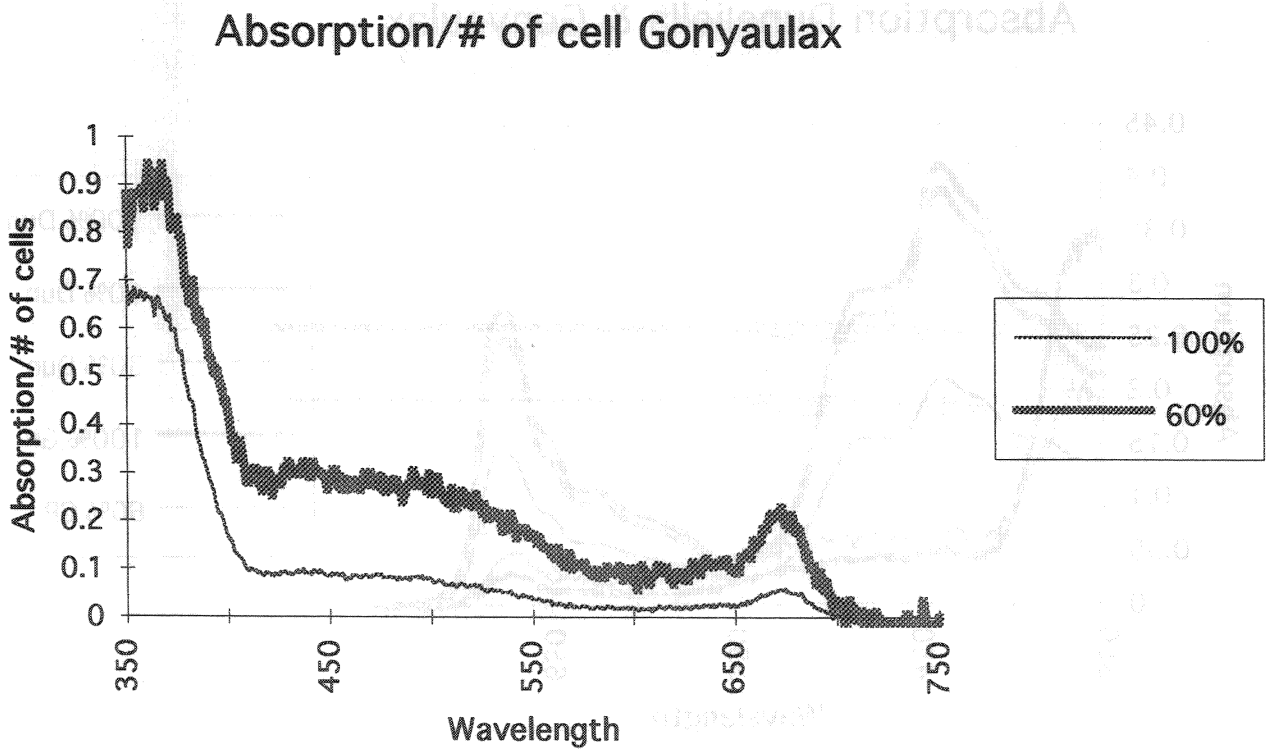

Absorption/# of cell Gonyaulax
Absorption/# of cells
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
350
450
550
650
750
Wavelength
100%
60%

Figure 18a

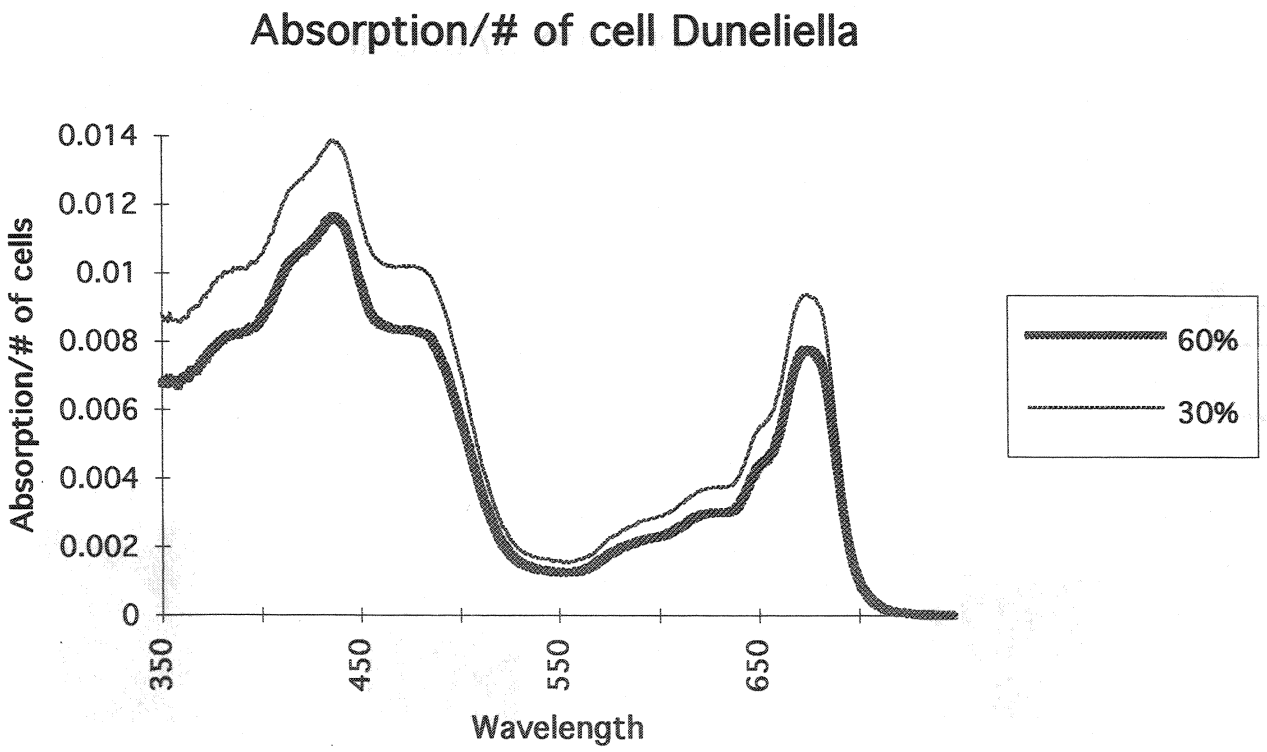

Absorption/# of cell Duneliella
Absorption/# of cells
0.014
0.012
0.01
0.008
0.006
0.004
0.002
0
350
450
550
650
Wavelength
60%
30%

Figure 18b

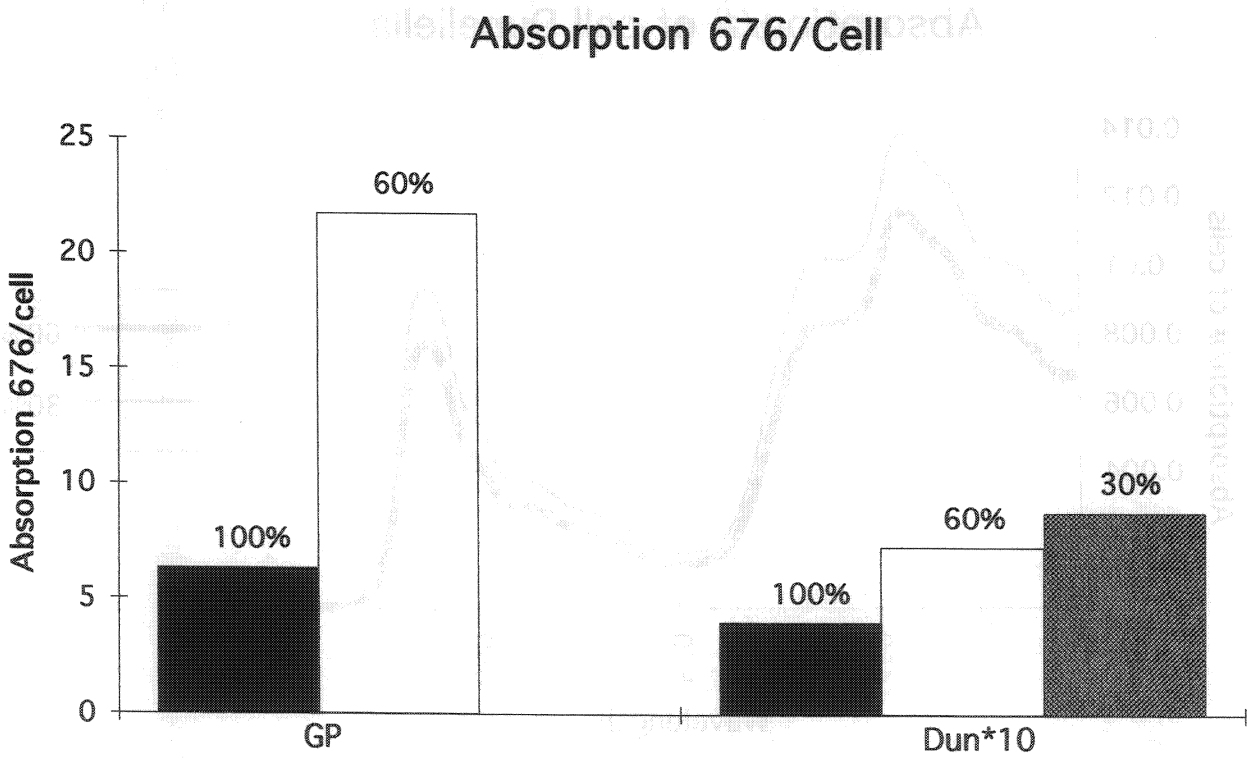

Absorption 676/Cell
Absorption 676/cell
25
20
15
10
5
0
60%
100%
100%
60%
30%
GP
Dun*10

Figure 19

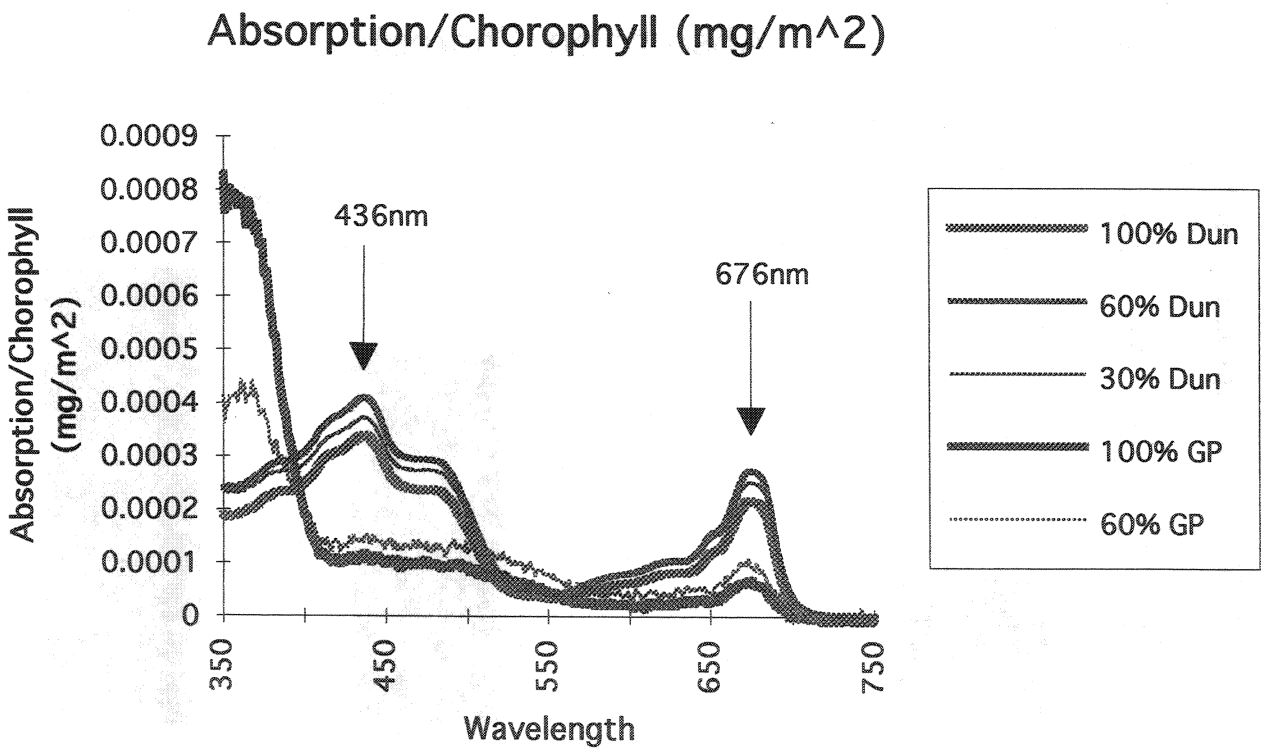

Absorption/Chorophyll (mg/m^2)
Absorption/Chorophyll (mg/m^2)
0.0009
0.0008
0.0007
0.0006
0.0005
0.0004
0.0003
0.0002
0.0001
0
436nm
676nm
350
450
550
650
750
Wavelength
100% Dun
60% Dun
30% Dun
100% GP
60% GP

Figure 20

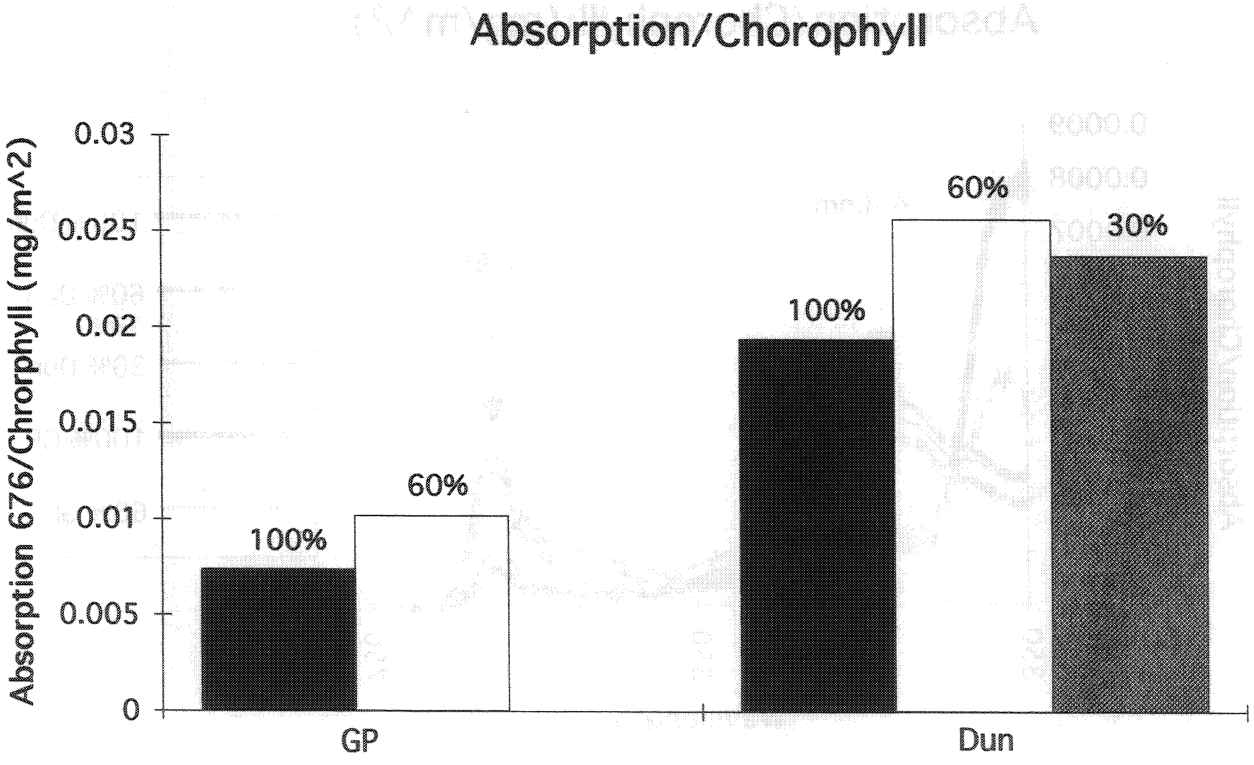

Absorption/Chorophyll
Absorption 676/Chrorphyll (mg/m^2)
0.03
0.025
0.02
0.015
0.01
0.005
0
100%
60%
100%
60%
30%
GP
Dun

Figure 21

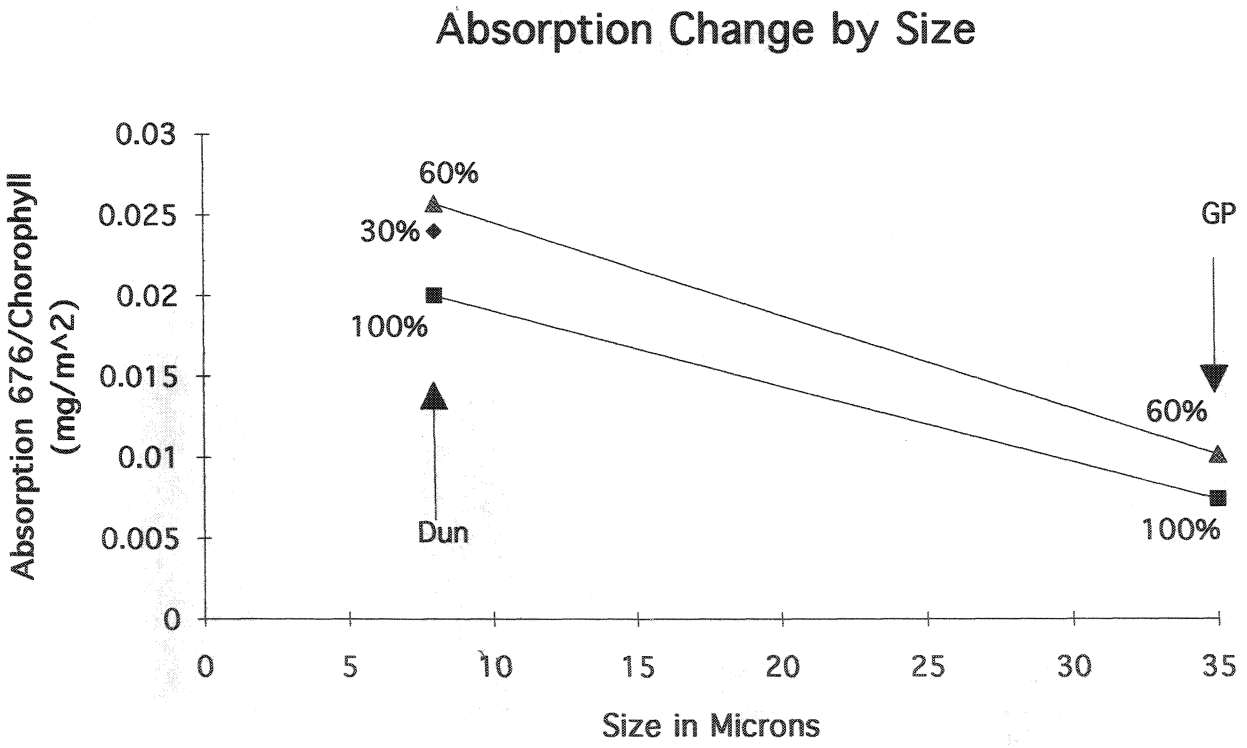

Absorption Change by Size
Absorption 676/Chorophyll (mg/m^2)
0.03
0.025
0.02
0.015
0.01
0.005
0
60%
30%
100%
Dun
GP
60%
100%
0
5
10
15
20
25
30
35
Size in Microns

Figure 22

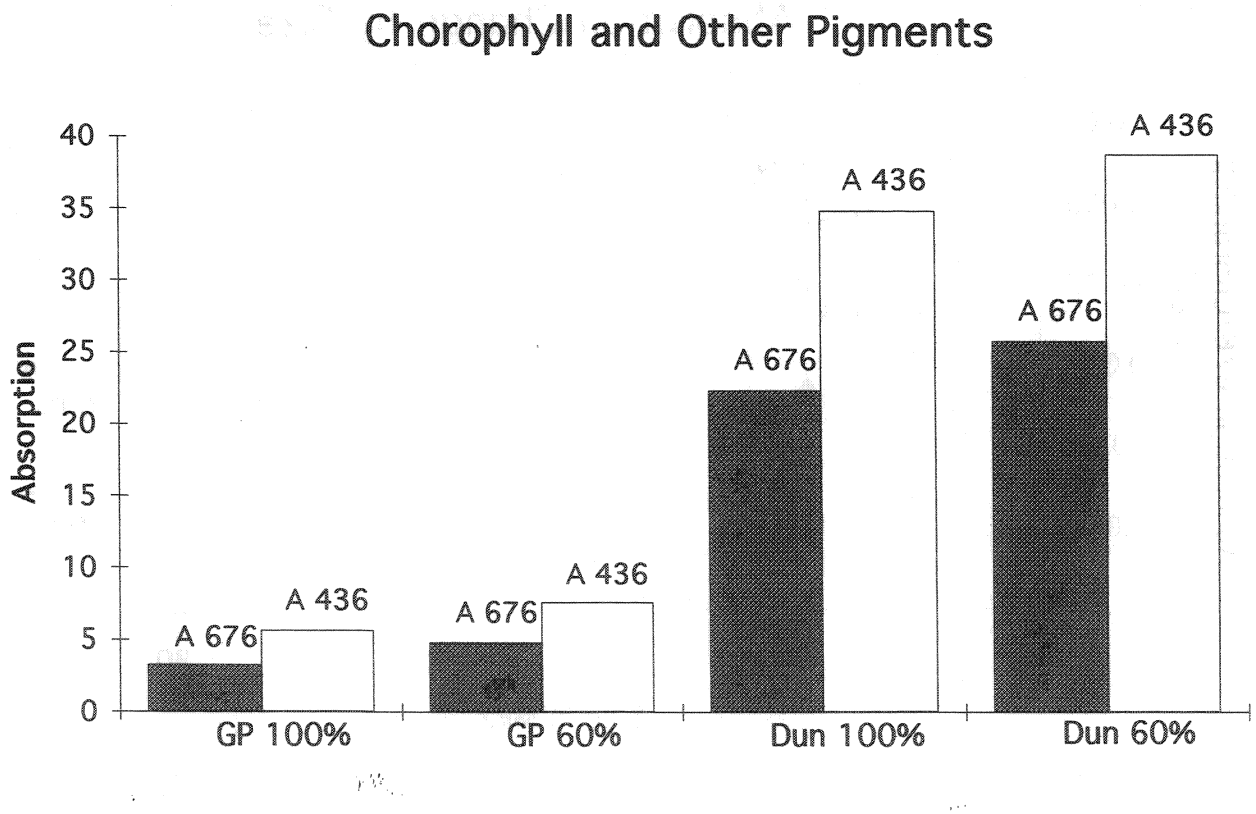

Chorophyll and Other Pigments
Absorption
40
35
30
25
20
15
10
5
0
A 676
A 436
A 676
A 436
A 676
A 436
A 676
A 436
GP 100%
GP 60%
Dun 100%
Dun 60%

Figure 23

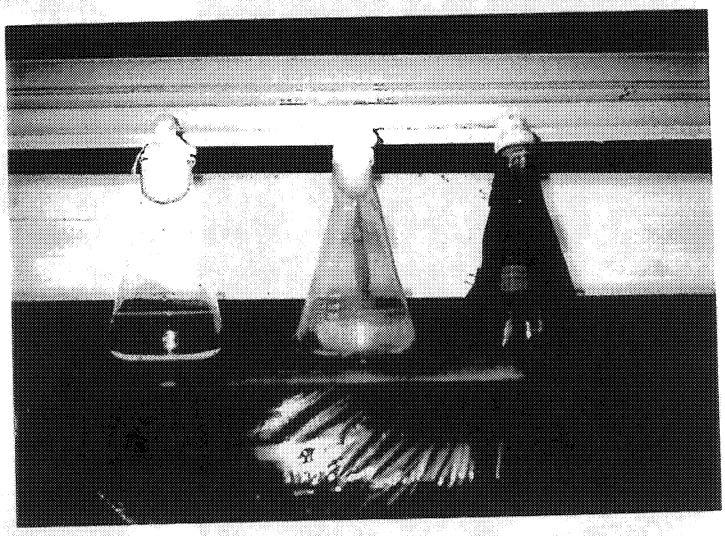
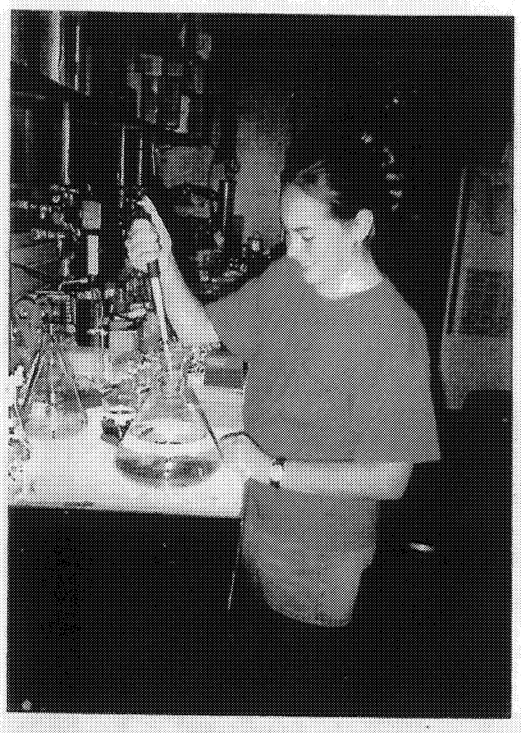


Photo 1a. Light set up for culture growth.
Photo 1b. Flasks with screens, which create the three different light regimes.



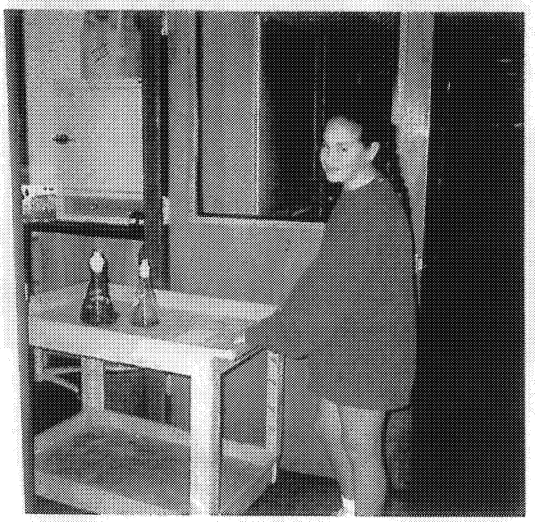
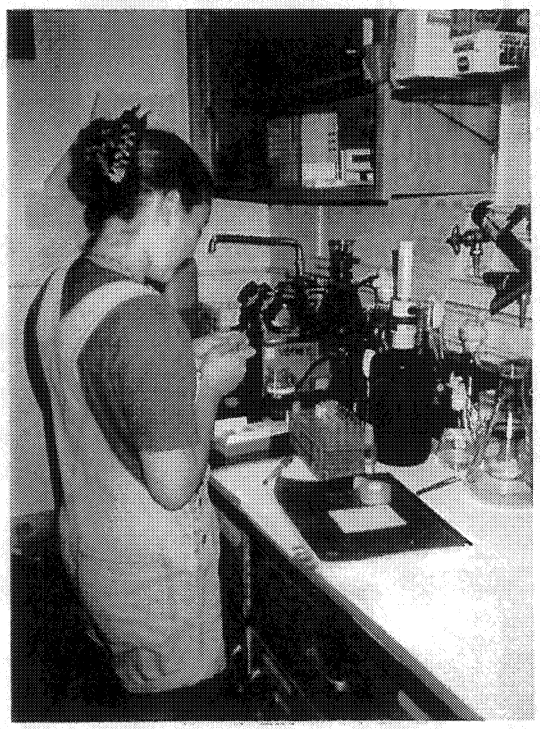
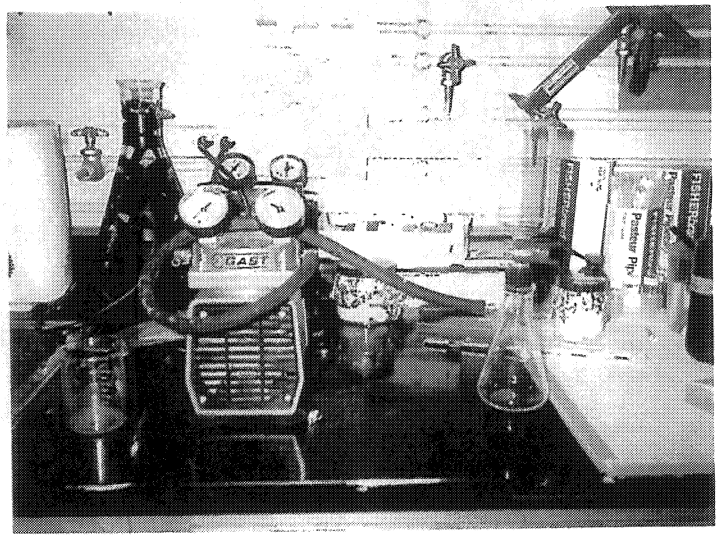
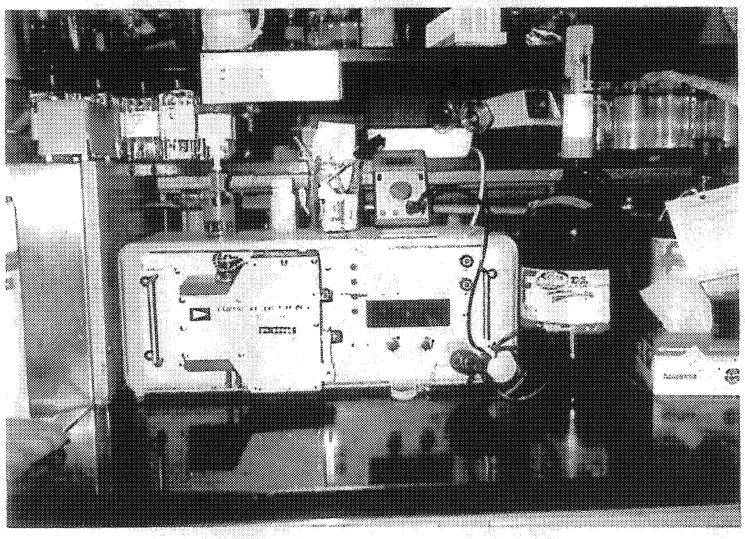


Photo 2a. Measuring the nutrients into the media.
Photo 2b. Autoclave with media flasks to be sterilized.
Photo 2c. Moving the culture flasks into the clean room.
Photo 2d. Lighting the bunson buner to heat the necks of the flasks to avoid contamination when subsampling culture.







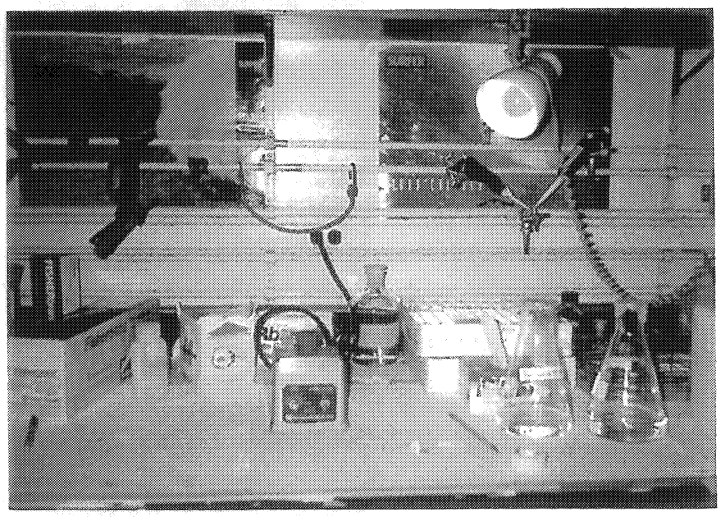
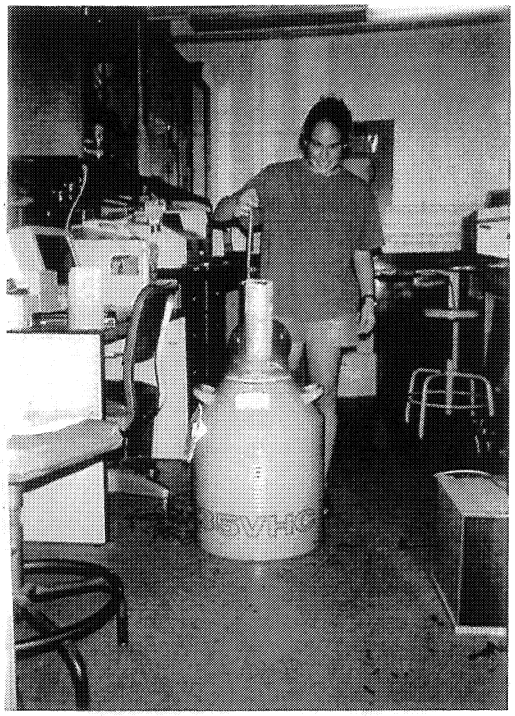


Photo 3a. Labeling each culture tube for subsamples.
Photo 3b. Filter tower attached to a pump.
Photo 3c. Flourometer for measuring chlorophyll fluorescence.
Photo 3d. Mechanical stimulator to break open cells for chlorophyll extraction.



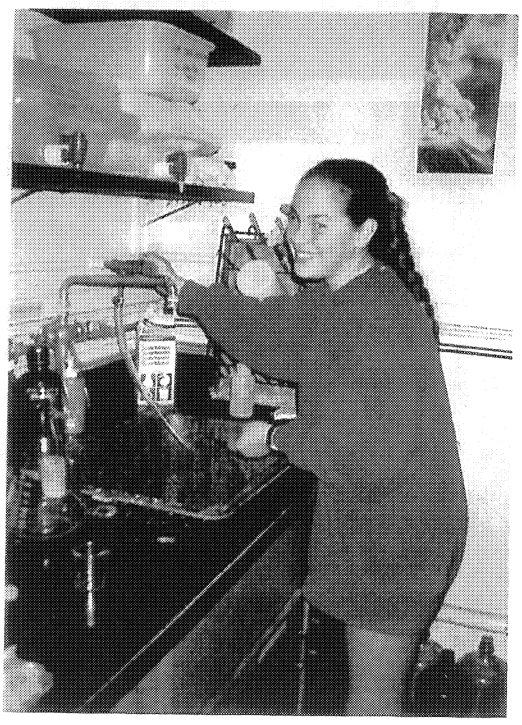


Photo 4a. Spectroflourometer for measuring spectral absorption.
Photo 4b. Removing filters from the liquid nitrogen.
Photo 4c. Microscope for cell counts.
Photo 4d. Cleaning up all of the culture glassware