

A CONTINUOUS CULTURING DEVICE
AND ITS UNIQUE POSSIBILITIES

A Senior Thesis

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and its Unique Possibilities

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A Continuous Culturing Device and its Unique Possibilities.

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Diatoms (marine algae) are the largest contributor of biological productivity in the Antarctic. As the siliceous diatoms die their tests begin to accumulate on the sea floor, and preserve a record of how environmental conditions change through time. However, studies of diatom tests in seafloor sediments are hindered by a significant problem: the uncertainty about the relationship between diatom growth rates, diatom test characteristics and environmental conditions. A continuously culturing turbidostat was built in order help solve this problem. Continuous culturing provides critical advantages over commonly employed batch culturing. It allows for the diatom growth rate and seawater constituents to be maintained precisely at desired levels, and it yields enough diatom tests for geochemical analysis. Conditions that can be tested include temperature, nutrient levels, isotopic and trace metal levels, light intensity, day/night patterns, or other environmental conditions that occur, or could occur in nature with such factors as ozone depletion or global warming can be explored.

Section 1: Introduction

The waters around Antarctica are among the most biologically productive in the world and one of the two sites where deep ocean waters form; therefore, these waters play critical roles in the global carbon and other nutrient cycles, as well as the global heat budget. To better understand these roles, scientists are trying to reconstruct how environmental conditions in the Antarctic evolved together with the earth's climate during the last 15,000 years (e.g., Froelich et al., 1992; Mortlock, 1991; Shemesh, 1988). Therefore, we need to better understand how past diatom tests were affected by climatic variability. The first step in doing this is to better understand how different environmental conditions affect present-day diatoms.

The Antarctic food web, as well as global food web, begins with the phytoplankton, which use solar energy to undergo photosynthesis, a process by which chlorophyll-containing organisms convert the energy of light into the chemical energy of organic molecules. The oceans produce about half of the world's organic biomass, calculated as about 6×10^{14} kg, so even a slight decrease in primary production would have very serious repercussions (Acevedo et al., 1993).

In the Antarctic Southern Ocean, unique conditions exist that make their waters some of the most productive in the world. Circumpolar winds create ocean currents that move around the Antarctic continent, causing the nutrient rich water from the ocean depths rise to the surface where plants and animals alike can use it (Thurman, 1988).

The reason this cold, and seemingly inhospitable place (at least to humans) has such a high level of productivity is because of the unique nature of the ocean currents found there (e.g., Thurman, 1988). The world's oceans, though it may look like a fairly static environment, are constantly in motion, both horizontally and vertically. A nutrient mass of water called the Atlantic Deep Water is formed in the cold North Atlantic and sinks to the ocean floor and moves southward. Since it has been away from the surface for a long period of time, usually around 300 years; it has a relatively low oxygen content

by Atlantic standards, but high concentrations of nutrients that have built up in the layer over hundreds of years beneath the photosynthetic zone. This deep water then upwells in the Southern Ocean due to two processes. Surface currents driven by the East Wind Drift cause surface flow of water away from the area. Also, the sinking of Antarctic Bottom water near the coast and Antarctic Intermediate water further north. In both instances, this water is replaced at the ocean surface, in part, by nutrient-rich waters deep waters from below. This continual supply of nutrients is one of the major reasons why this region has such high phytoplankton growth (Pickard et.al., 1990). A general map of phytoplankton productivity is shown in Figure 1 (Fryxell, 1991).

For all of these reasons, the study of diatoms in the present and the past are of vital importance to understanding the ecology of the Antarctic, as well as worldwide paleoclimatic conditions. Current methods of studying diatom test characteristics have proven to be problematic in trying to attain geochemical data that simulates the true environmental responses, due to the fact that the diatom cultures are kept in a closed environment. Therefore, a new method must be employed in order to study these relationships further. My goals for this research project were the following: (1) Learn how to culture diatoms using traditional batch culturing methods. (2) Help design and build a modified Patterson-style turbidostat/chemostat, referred to as a continuous culturing device. (3) Design and carry out an experiment using the continuous culturing device to understand how different light levels affect Antarctic diatoms.

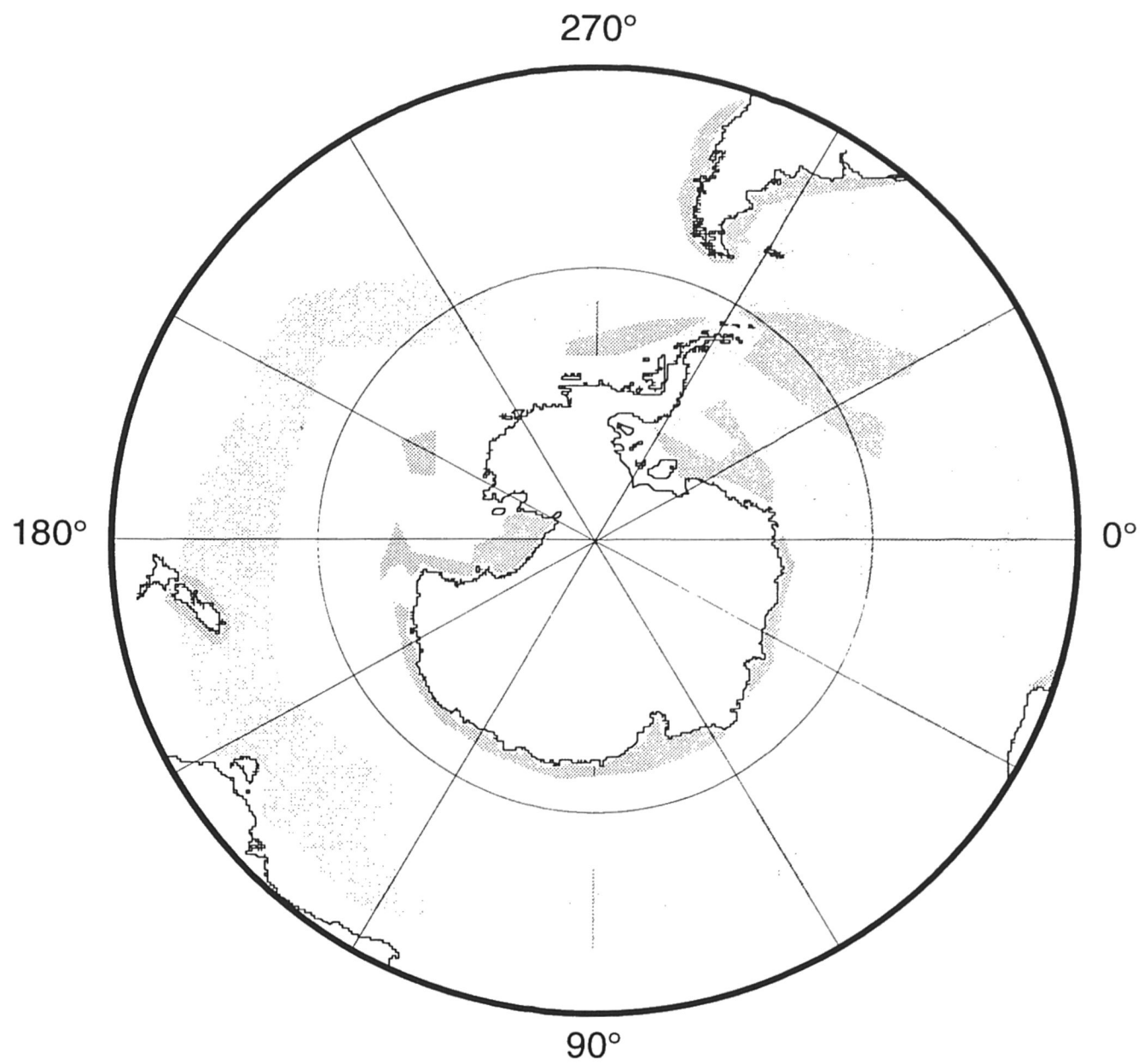


Figure 1. Approximate location of high amounts of phytoplankton production (dark areas) in the Southern Ocean (redrawn from Fryxell, 1991).

Section 2: Biological Materials and Culturing

Diatoms multiply very rapidly compared to other types of phytoplankton, sometimes showing rates of division on the order of one division per day in optimal conditions (Raymont, 1963). This makes them excellent laboratory species to work with.

The Antarctic species *Thalassiosira gravida* will be used for the initial set of experiments for the continuous culturing device (discussed below). This species within genus *Thalassiosira* is rather simple in design. It has disc-like cells with the valves sculptured with hexagonal or pit-like markings and they are covered with numerous fine spines, that are often larger than the cell itself (Newell, 1966). *T. gravida* is a species that tends to do best in the upper two meters of the water column, but it is also found extending down to about 5 meters. This suggests that it has a higher light optimum than other species found in the same location, like *T. nordenskioldii* or *Lauderia* (Raymont, 1963). In a natural phytoplankton community there is usually a marked variation in chlorophyll content, with those found in shallow waters having the highest chlorophyll content, so it is not surprising that *T. gravida* has one of the highest chlorophyll contents of any species of phytoplankton (Raymont, 1963).

This is also important to the seasonal variability and succession in relation to species composition. *T. gravida* tends to succeed *T. nordenskioldii* in the late spring when light levels begin to approach their highest amounts (Raymont, 1963). The yearly transition in light and temperature, which is quite extreme in Antarctic waters, has allowed its species to adapt to a wide range of environmental conditions, which makes their ecology of unique interest.

The sum of the environmental conditions in which diatoms live have profound impacts on their growth, morphology, species composition, etc. (Newell, 1966). For many years scientists have relied on what is known as batch culturing to understand the effects of environmental conditions on diatoms. This method allows you to study diatoms in a controlled environment, as well as propagate them in the lab. The diatoms

are kept in closed test tubes in a refrigeration unit (simulating Antarctic temperatures in our case). The test tubes and flasks are sterilized and initially filled with pure media.

I have learned to batch culture Antarctic diatoms and am now share in the responsibility of maintaining Texas A&M University's stock cultures. I use techniques and procedures taught to me by Marylin Yeager, Texas A&M University Dept. of Oceanography, and developed by Dr. Greta Fryxell.

The media I used to grow the Antarctic diatoms in is a Fritz Stock Nutrient Solution. Fritz Stock Solution is a well-known type of media for diatoms which I made using the following procedure:

1. Filter seawater from large carboys with Gelman Capsule Filter (.2 micron).
2. Determine the salinity of the filtered seawater using a refractometer in order to calculate the amount of double distilled water is needed to dilute the seawater to the desired level of final media salinity(in our case 30%).
3. Autoclave the calculated volume of filtered seawater in a large flask. After it is autoclaved place it in a cool room to allow it to equilibrate for 24 hours. It is now referred to as sterile filtered seawater (SFSW).
4. Measure the determined amount of double distilled water into a small flask. Add the recommended amount of solutions A and B (nutrients) and meta-silicate (also a nutrient). The solution is now referred to as enriched double distilled water (EDDW). Autoclave the EDDW and allow to sit for 24 hours.
5. Mix the SFSW and EDDW and allow to equilibrate for 24-48 hours.

An important part of successful diatom culturing is simply the cleaning of the glassware (test tubes, flasks, pipettes, etc.). It is well known that the sensitive nature of the cultures requires that contamination by outside sources be kept to an absolute minimum. To avoid such contamination I used the following procedure:

1. Clean the used glassware well with a wire brush using normal tap water.

2. Rinse the glassware 3 times with distilled water.
3. Place the glassware in a bin filled with a 10% hydrochloric acid for at least 24 hours.
4. Remove the glassware from the 10% HCl and place it in a bin filled with distilled water (this reacts with the residual HCl) for an 24 hours.
5. Remove the glassware from the bin and rinse well with distilled water.
6. Rinse the glassware with double distilled water.
7. For test tubes, lightly cap them, leaving a small amount of double distilled water in the bottom.
8. For flasks, cover the top with aluminum foil, leaving a small amount of double distilled water in the bottom.
9. Autoclave the glassware and place in a closed cabinet for the next use.

Once the glassware is cleaned and sterilized, and the media is prepared and chilled to the temperature of the current diatom media, the transfer process can begin. All of the transfers must be done in a walk-in refrigeration unit to maintain the required temperature. With a pipette, transfer the diatoms suspended in the solution of the previous test tube to the newly prepared solution in the sterile test tube. This process allows them to grow unimpeded by competition as the nutrients in the previous test tube begin to become limited. For *T. gravida*, and the other cultures currently being kept, the transfers are done by myself every 14 days. At each transfer the diatoms of the previous transfer are studied under a microscope to check their growth rate and to assess their general health. This data is then recorded in order to monitor the history of the cultures. Backup cultures are extremely vital since the samples sometimes show low growth at the end of a transfer cycle and it is necessary to go back to a previous sample that still shows abundant diatom density and transfer from it also.

The Antarctic samples we have are very unique. They represent 3 different species that have been isolated and prepared for batch culturing. They are among only a few such samples being kept alive in the world, much less the United States. They represent a unique opportunity to understand the ecology and environmental responses of Antarctic diatoms.

Though batch culturing is good for maintaining stocks of diatoms and for studying general responses to different conditions, it suffers from a major flaw, it is essentially a closed system. Once the conditions within the test tube are set they do not remain constant, but over time evolve from their initial conditions. Therefore it is difficult to relate the geochemical composition of diatom tests to the chemistry of the solution in which they grew.

An area where the study of diatoms is potentially very useful is in the field of paleoclimatology. For example, the incorporation of germanium (Ge) and silicon (Si) into the siliceous shells of Antarctic diatoms, may provide a quantitative record of nutrient uptake. Diatoms incorporate inorganic germanium from seawater into their siliceous shells as if it were a heavy stable isotope of silicon. The ratio of Ge/Si in their shells is fixed by three factors: the Ge/Si ratio of upwelled seawater, the apparent isotopic fractionation factor (fractionation due to metabolic processes within the diatoms), and the fraction of upwelled silica removed from the solution into the diatoms, referred to as Rayleigh distillation (Mortlock et al., 1991). Knowledge of the ratio at which the Ge and silicon Si are incorporated in diatom tests allows study of their growth rates, whether growing exponentially or in a stationary phase. This ratio is also preserved in the geologic record, so studying the rate of deposition and ratio of Ge/Si in fossil diatoms can help us understand past growth rates and environmental conditions (e.g., Froelich et al., 1992).

Froelich et al. (1992) used batch culture experiments to better understand the factors controlling the Ge/Si ratio of diatoms. The main assumption of their experiments is that in batch cultures, silica incorporated by the diatoms does not dissolve from the

shells of past diatoms. The simplest model to estimate of the Ge/Si ratio during biological uptake is to treat a diatom batch culture as a Rayleigh distillation process. This allows the ratio to be modeled as an isotope ratio. It must be assumed that the silicon is continuously removed from the solution and deposited into a solid (opal) which no longer has any exchange with the solution. If exchange does occur, the effect is that of diluting the solution toward the original Ge/Si ratio. Since there is no way to directly estimate the amount of dissolution of prior diatom generations in a closed batch culture environment, the data is likely not to produce clear and correct results (Froelich et al., 1992). One of the goals of this project was to design a system that better mimics open ocean conditions, while still being able to study diatoms in a controlled lab environment in order to help alleviate this problem.

Section 3: Culturing Device Modifications

Dr. C. O. Patterson and co-workers of Texas A&M's Biology Department have built a computer-controlled turbidostat which is capable on continuously culturing algae under controlled conditions (Hearn, 1994). While very capable, this system does not meet all of the needs of anticipated for experimentation on Antarctic diatom species. To meet these needs Brian Edwards (Biology Department), Dr. Niall Slowey (Oceanography Department) and I modified the Patterson-style turbidostat suitable for continuously culturing Antarctic diatoms (Fig. 2). The modifications have two primary motivations: They improve the ability of the culturing device to maintain stable concentrations of dissolved nutrients and elements in the culture media. Second, the culturing device is now able to maintain the temperature of cultures at very constantly at the very low levels required by Antarctic diatoms.

This section describes some the existing characteristics of the Patterson-style turbidostat (Hearn, 1994) and the modifications made to them. My contribution to these modifications made up a significant portion of my efforts as a University Undergraduate Research Fellow. In the processes I designed and fabricated physical components, electrical hardware and computer software, all done with an understanding of the biological requirements of the diatoms and the experimental needs of paleoclimatic studies.

The Computer Program

A computer program with subprograms that control the functions of stepper motors, thermistor, water jacket, and photodiodes, and was designed and written by myself and can be found in Appendix 1. It was written using Quickbasic. A Keithley Metrabyte DAS-800 Series I/O board installed in personal computer to control the functions of the respective systems. The DAS-800 is a commercially available computer controller board designed to handle analog and digital input and output (I/O) for IBM

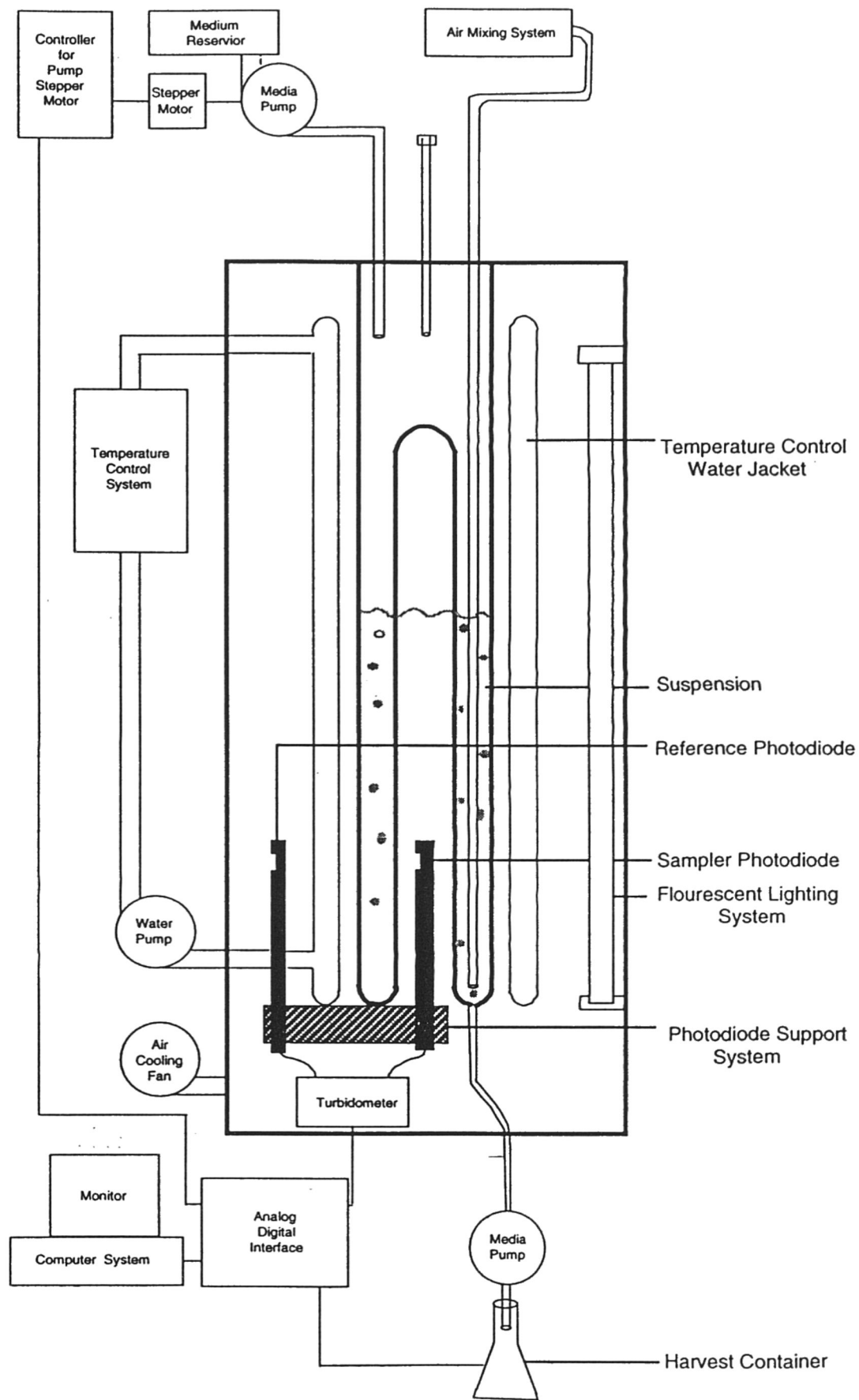


Figure 2. Schematic representation of the original Patterson-style turbidostat prior to modifications made for culturing Antarctic diatoms (from Hearn, 1994).

compatible computers. In essence, the computer program sends signals in the form of calls to the DAS-800 board that are then sent to the respective computer board to do the assigned input task. The output is then returned to the board and recorded using the program..

Flow Through Functions

A turbidostat is by definition a device that is able to maintain solution turbidity, or concentration of a factor, the concentration of algae in the case of the Patterson-style turbidostat. The turbidostat detects changes in turbidity by measuring changes in the transmission of light through the culture media. As the algae multiply in the culture media, the overall solution becomes increasingly turbid and the amount of transmitted light decreases. To maintain a constant turbidity it is necessary to add media to the solution. This is accomplished using a pump head/stepper motor assembly. The actual working of this assembly is shown and discussed in Figure 3. This approach is very useful for maintaining diatom cultures at constant growth rates.

As the culture media remains in the turbidostat it is possible that concentrations of dissolved nutrients or elements might change. To minimize this possibility, important for experiments to better understand the geochemistry of Antarctic diatom tests, it was necessary to modify the the Patterson-style turbidostat to decrease the amount of time any given aliquot of media remains in the culturing device. Basically, the incorporation of a second function, a chemostat, was required. A chemostat is a device that maintains the chemistry of media by flowing it through the culture vessel faster than the organisms being cultured can noticeably alter its chemistry. This essentially maintains the environment that the diatoms are grown in and avoids pitfalls of batch culturing. In order to maintain concentration (turbidostat), and solution conditions (chemostat), a second stepper-pump head assembly had to be used. While incorporating the second stepper-pump head assembly I also redesigned the mounting for both assemblies (Fig. 4). All of

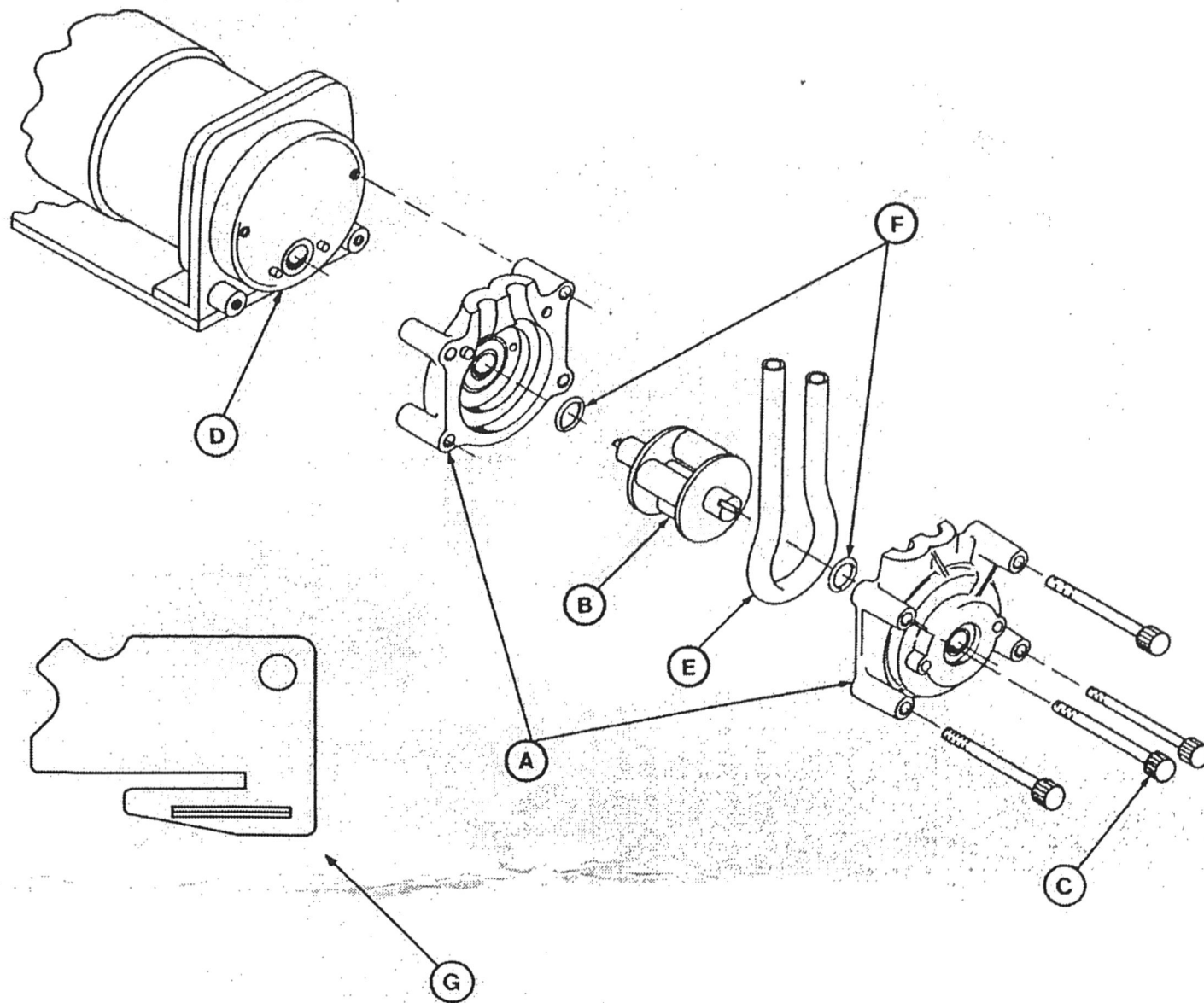


Figure 3. Pump Head Parts Diagram from the Cole-Parmer Masterflex series manual

- A. Pump Head Half with Bearing (End Bell)
- B. Rotor with Rollers and Shaft
- C. Pump Head Mounting Hardware
- D. Drive (Stepper Motor)
- E. Tubing
- F. Thrust Washer
- G. Tubing Key Cat. No. 7013-90

The stepper motor (D) is an electronic device that, when attached to a computer, turns a rod at a specific time and rate. A pump head itself is a plastic piece of machinery (A), that is attached to a stepper motor, and is responsible for transferring a specific amount of liquid to a desired location. The pump head uses a section of plastic tubing (E) attached from a liquid source through the pump head to the desired location. A rotor with 4 rollers (B) is the part that is actually used to deliver the correct amount by “squeezing” the liquid through as each roller moves over the tubing sending the liquid through to the suspension in the turbidostat.

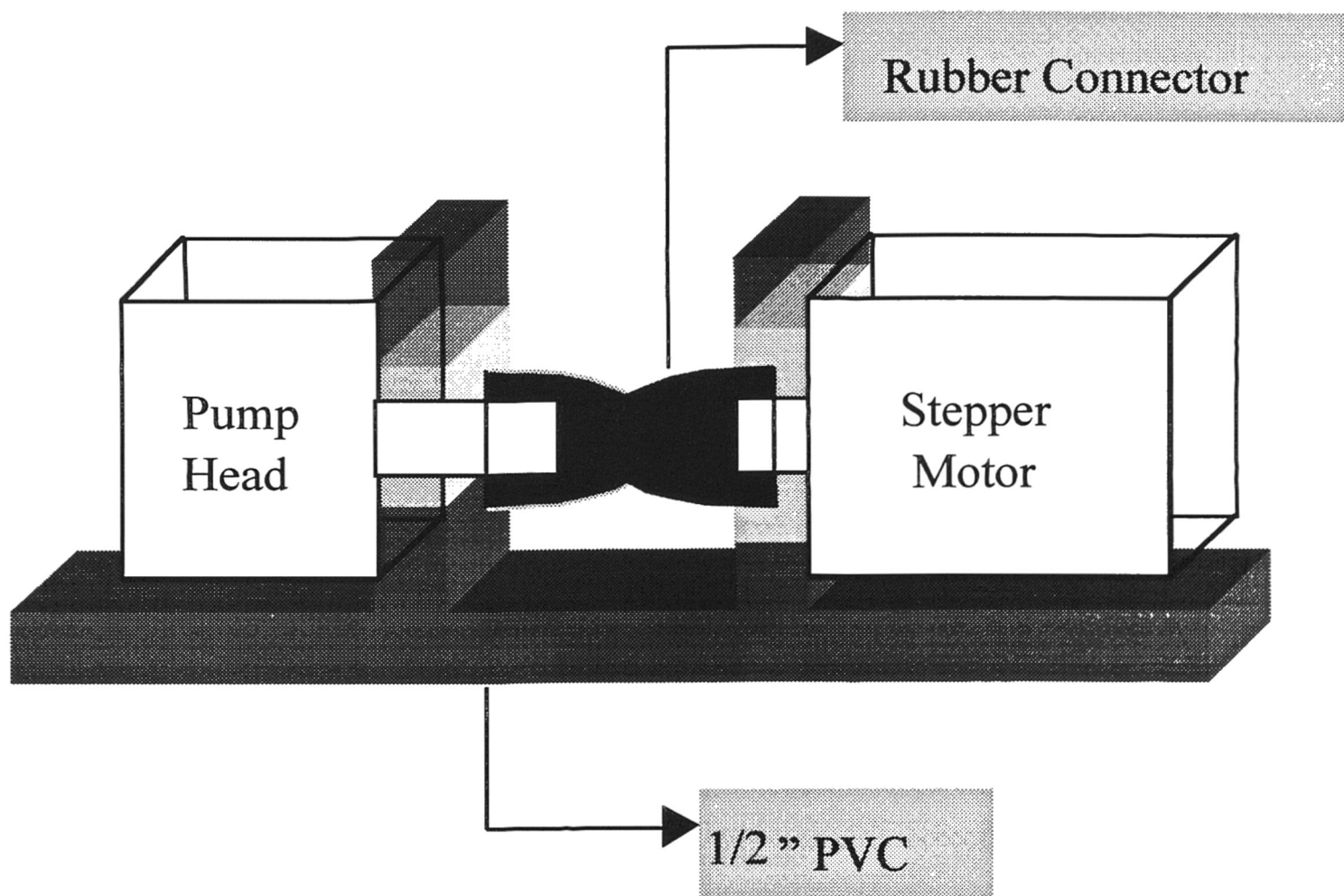


Figure 4. Stepper motor / Pump-head Assembly used for the addition and removal of media from the culturing device.

the stepper motor activity is controlled by a specially designed computer board (Fig. 5) which is controlled by the DAS-800 I/O controller to the computer.

As with the original Patterson-style turbidostat, the first assembly is responsible for adding fresh media in order to maintain the target turbidity as the diatoms grow. As the media is added, the solution level of course rises. In the original Patterson-style turbidostat excess media was removed manually by a lab technician at somewhat regular intervals. Now the second pump head/stepper motor assembly is used to take the turbidostat also act like a chemostat. As the fresh media is added, the second assembly using the same method in reverse, removes the same amount of solution from the turbidostat. The rate of flow of media through the culture can be set to whatever rate required to maintain the chemistry of the media at a constant level. Thus, the problem of solution evolution in batch culturing is overcome.

As with the original Patterson-style turbidostat design, the entire device (Fig. 2) is housed in a wooden box painted white on the inside to help reflect the light throughout the chamber. A single 60 watt fluorescent light attached to the side of the housing. Three photodiodes are used to measure the light level and thus density of the solution, which I will come back to shortly. A photodiode is a small sensor that is able to see light, and to calculate its intensity. They are wired to another specially designed computer board (Fig. 6) that collects the raw data, and transfers it to the computer via a DB-9 connector and DAS-800 controller. The digital values returned by the photodiodes are then modified by the computer program into numerical values that can be recorded. The locations of the three photodiodes (Fig. 2 and Fig. 7) are important because they allow the density of the solution, as well as the solution level to be monitored. The first photodiode, the reference photodiode is placed outside the chamber. It is used to get the initial light level that the other two are compared against. The second, the sample photodiode, is located in the hollow area in the center of the culturing device. Values returned from the sample

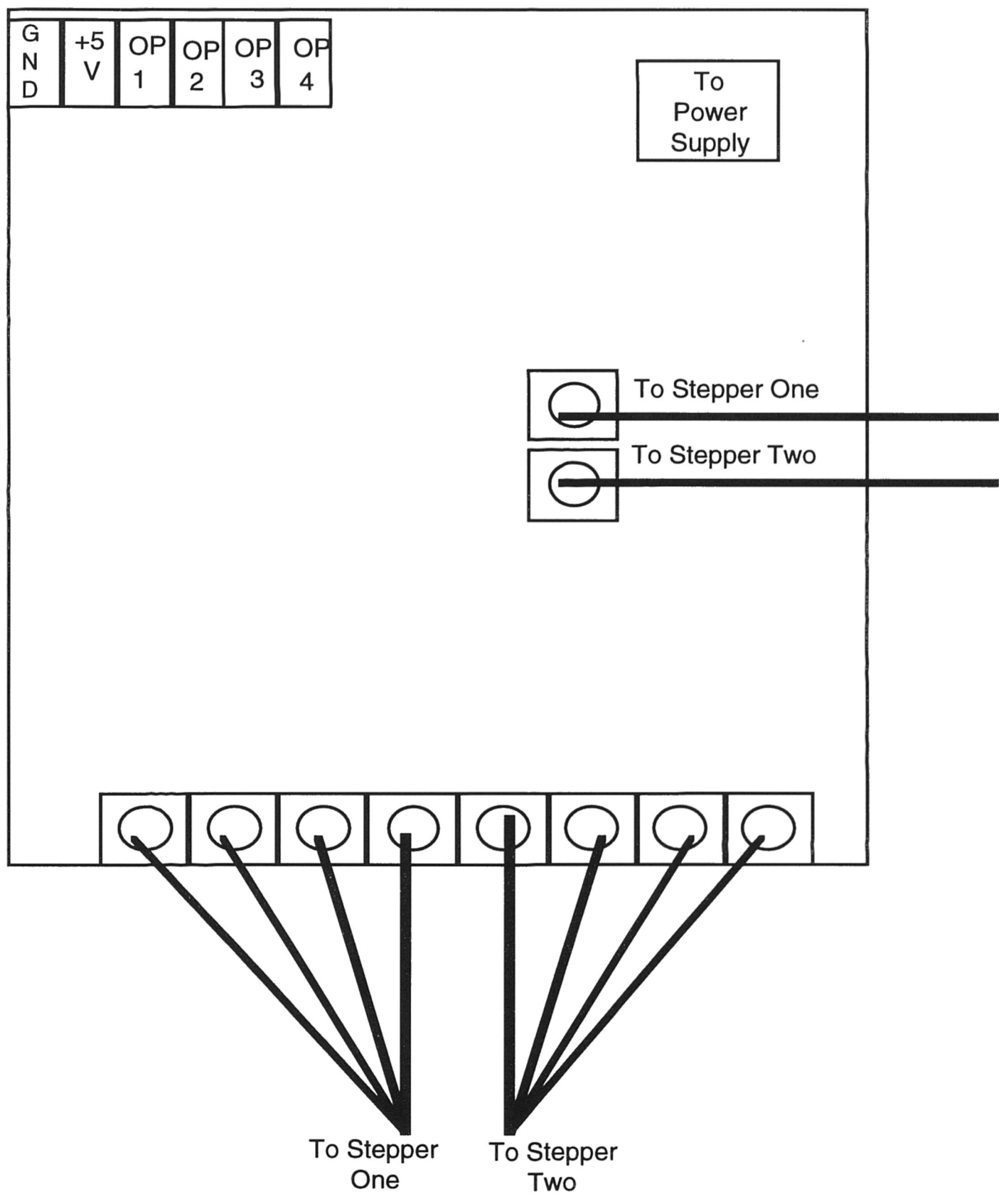


Figure 5. Schematic diagram of the specially designed computer board for controlling the stepper motor activity.

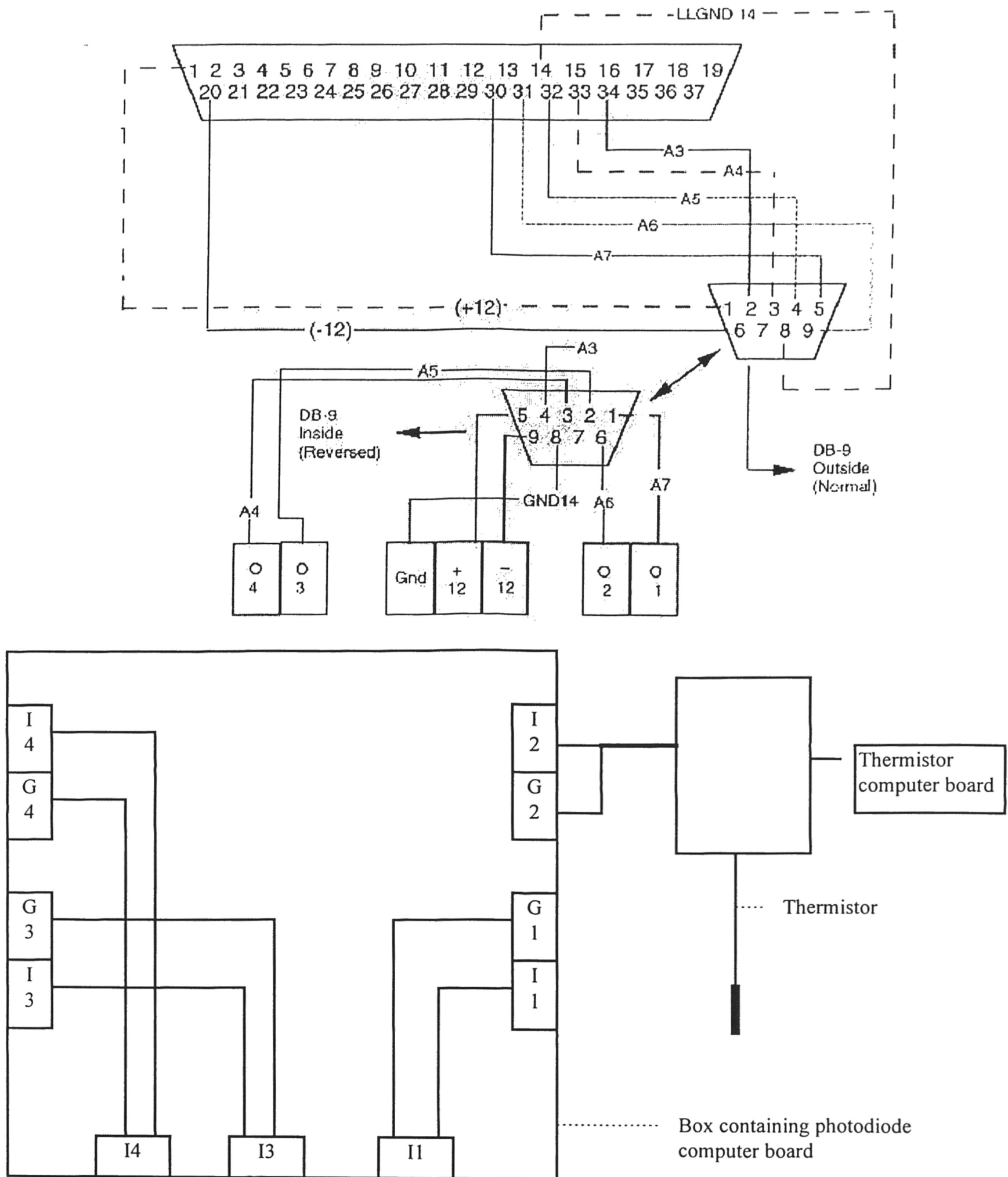


Figure 6. Schematic of the connector for the DAS-800 (top), connections to the inside and outside of the DB-9 connector (middle), and the actual specially designed photodiode and thermistor computer boards.

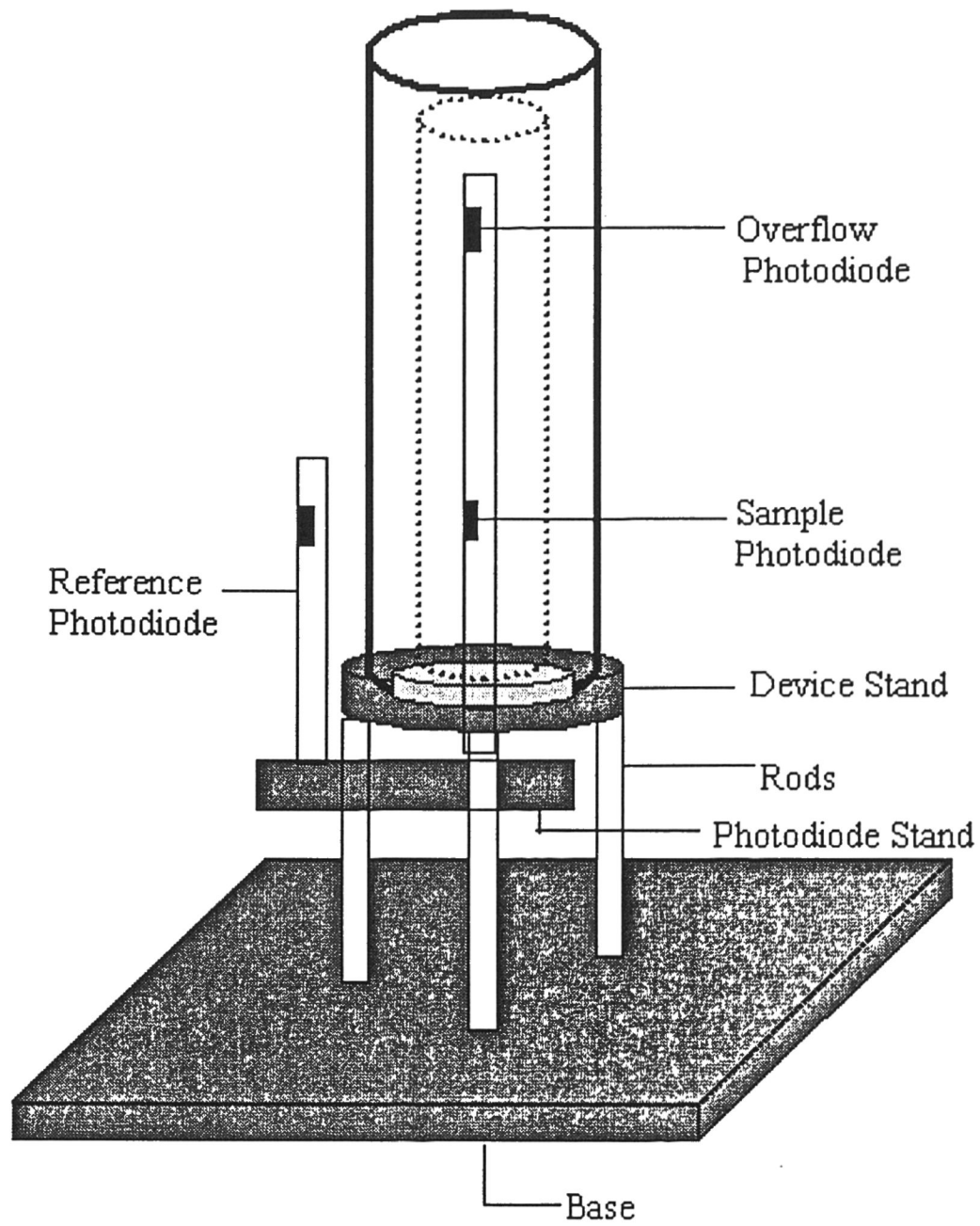


Figure 7. Schematic representation of the stand for the culturing device, along with the locations of the two Patterson-style turbidostat photodiodes and the modified (Overflow) photodiode.

photodiode in comparison to the values returned from the reference photodiode outside the chamber gives you the density *in situ*..

The combination of these two photodiodes is what is used in the Patterson-style turbidostat to maintain turbidity. Use of the third photodiode is a modification to the original design. It is placed at the top inside of the chamber directly above the sample photodiode. This photodiode controls the second stepper-pump head assembly, removing a desired amount of solution to bring the solution level back down within normal operating tolerances. The addition of the third photodiode to the modified design is important because it keeps the solution from overflowing. This is a possibility since there can be variability in the addition and removal from the culturing device. This function adds another layer of control and security to the system when it is being run in chemostat mode.

The addition of the third photodiode required that the associated structural and electronic hardware be redesigned and rebuilt. The computer software which runs the culturing device also had to be written to accommodate the third photodiode and to correct problems with the original software were discovered during this process.

The Culturing Device Stand

In order to keep the culturing device stable and immobile, as well as hold the photodiodes in place, a special stand is required. Another modification made to the original Patterson-style turbidostat was to redesign and build a new stand (Fig. 7). The base and photodiode stand were constructed of 1/2" thick sheet PVC. The rods were made of aluminum and screwed directly into the PVC. The Device Stand is beveled out to accommodate the the culturing device. The light gray inner section on the device stand in Figure 7 represents the inner section where the device actually sits. The dark gray represents the outer lip that holds the device in place. A hole has been made in the center

to allow the rod with the sample and overflow photodiodes to fit through and clamp on to one of the base rods.

Temperature Controls

The Patterson-style turbidostat possesses a Temperature Control Water Jacket jacket to facilitate control of media temperature. However, for their experiments Patterson and co-workers have not needed to maintain the culture temperatures at the very low Antarctic values (-2 to +2°C), nor maintain temperatures precisely as required by our experimental needs (to 0.05°C), nor directly monitor and record culture temperatures. It was therefore necessary to modify the Patterson-style turbidostat so it would have these capabilities.

The first step in this process was to measure the temperature of the media within the turbidostat. A thermistor, a device able to monitor temperature values, was used for this purpose. The actual thermistor we used was an Omega Co. ON-901-44032. This model was selected because it is designed to measure best a temperatures at or just above freezing to within 0.02 degrees Celsius. A thermistor is able to measure temperature by measuring the amount of voltage that can be passed through it, which is dependent on temperature. The voltage from the thermistor is measured and digitized by the same computer board that controls the photodiodes (Fig. 6) and transferred to a personal computer. A DB-9 connector is used in order to be able to attach/remove the box with the computer board from the DAS-800 controller and computer.

Once in the computer, the raw data needs to be processed and recorded. Giving the culturing device this capability proved to be a difficult task since the raw data received was not in the form of temperature values, but in digital voltage values that had to be translated to temperature values and then recorded in a usable form using a computer program. This was done by using a finely calibrated electronic temperature probe and comparing the actual temperature of the probe to the returned digital value from the

thermistor. A plot of these two values was then used to modify the thermistor values using a computer program to temperature values.

Once the data was converted, the measurements of the temperature of the media are passed from the computer to a Haake Co. Flow Through Temperature Control Device, or water bath, which regulates the temperature of the continuous culturing device. Water in the bath is heated or chilled to the desired level and then it is transported to the culturing device via a hose. The water is run through the outer layer of the culturing device, called the Temperature Control Water Jacket, in order to regulate the temperature of the sample in the suspension inside the second layer (Fig. 2).

Section 4: Initial Experimental Design

The first experiment is to investigate the relationship between light level and the growth of *T. gravida* to better understand how it adapts to seasonal changes in the light regime of the Antarctic. This will also begin to provide insight into why different species of diatoms succeed better at different times of the year.

Hypothesis

Since *T. gravida* has such a high chlorophyll content, and is most prevalent in the early-mid spring, it should grow best at high light levels, but have a difficult time adjusting quickly to change in the light environment since it is often succeeded by early summer. It would seem that their high chlorophyll level would allow them to keep their dominance throughout the summer, when light levels are at their maximum. However, other species that have less chlorophyll, have a lower energy requirement for their metabolic processes than *T. gravida* and thus can ultimately out compete *T. gravida*. This also suggest that the initial Photosynthesis-Irradiance curve will be steeper that that of other species, but it will level off at a lower rate than those that succeed it (See Prediction Section below).

Experimental Method

- Grow *T. gravida* in the culturing device at constant light level for 7 days to allow it to adapt to the conditions.
- After this acclimation time, the density of the solution will be used as the baseline for further additions.
- The culturing device will constantly measure the turbidity (diatom cell density) of the solution, and will add Fritz Stock solution (media) as needed to keep the solution at the desired level.
- A known volume of culture solution will be removed and the mass of diatoms that corresponds to the set turbidity level will be determined.

- The frequency at which the turbidostat has to add media depends directly on diatom growth rate. Figure 8a illustrates possible addition schedules for different growth rates.
- The light levels will be increased at regular increments from a baseline value to a maximum value that will depend upon the actual experimental results. This is illustrated in Figure 8b.
- Using a Turner Fluorometer, the amount of chlorophyll per unit solution can be calculated.
- If both the mass of diatoms and the chlorophyll content of the overall solution is known, then the amount of chlorophyll change per diatom over the course of the experiment can be calculated

Prediction

If my hypothesis is correct then the photosynthesis-irradiance curve should have the following characteristics: *T. gravida* will do well in a high light environment (steeper initial curve) but due to its metabolic needs, it will not do as well in the long run as a species with a lower light requirement that is slower to adapt (Fig. 8c).

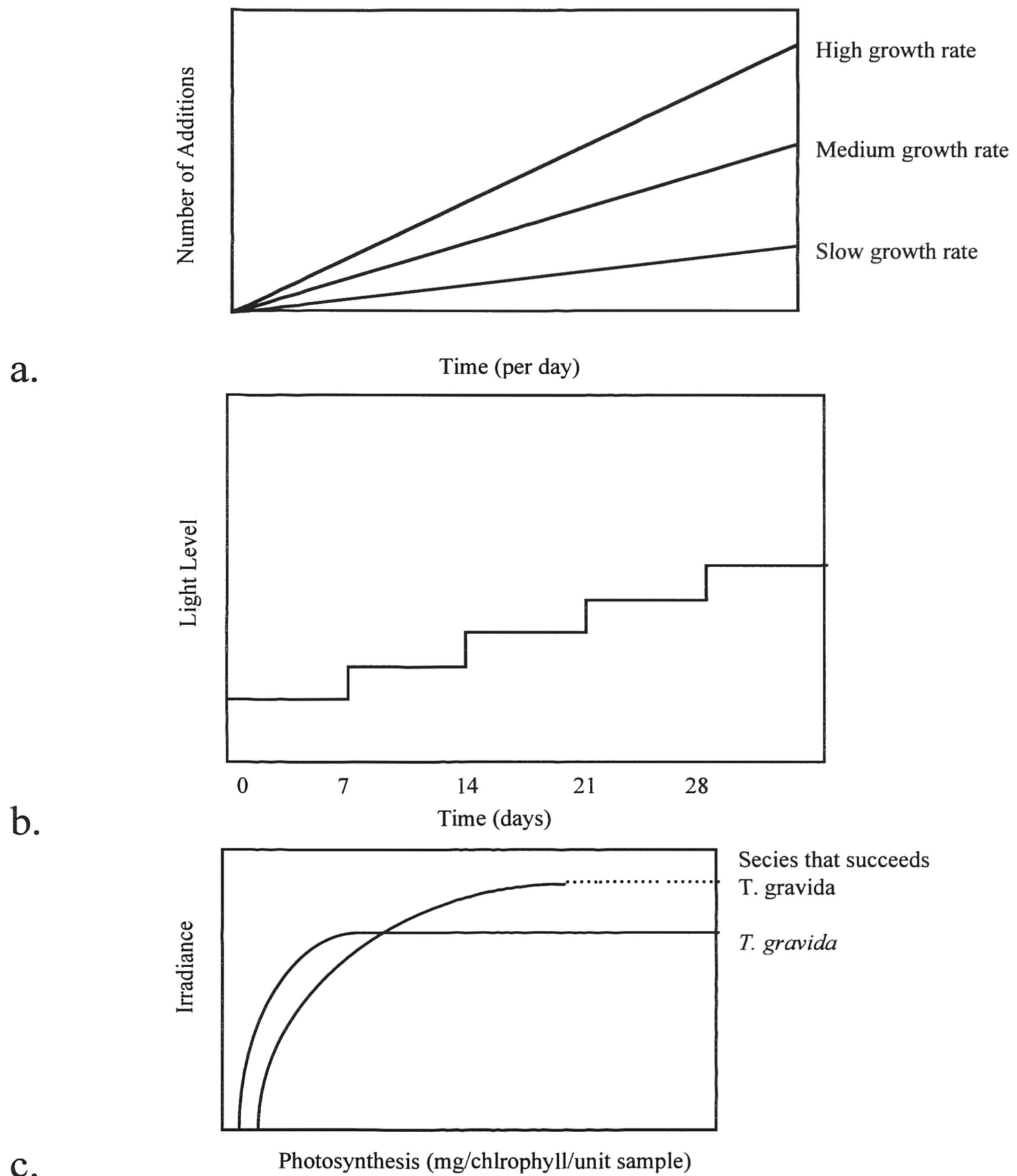


Figure 8. (a) Projected growth curves of *T. gravida* depending on differential growth rates. (b) Projected schedule for change in the light regime of the culturing device during the experiment. (c) Photosynthesis-Irradiance curve showing how *T. gravida* and its successor species' photosynthetic level might change with changes in the irradiance level.

Section 5: Summary of Current Status and Future Work

This project, in my mind at least, was geared in the beginning toward getting scientific results, in the form of carrying out experiments using the culturing device. The focus as the months progressed, however, slowly became that of scientific understanding and invention. The physical and mental preparation and construction associated with modifying the design of the culturing device for Antarctic diatom experiments proved to be a difficult and time consuming task in itself. One of my initial goals was to learn how to grow and maintain the diatom cultures, which I have achieved. The experience I gained doing this allowed me to look at the design of the culturing device with different eyes than I would have otherwise.

The multi-disciplinary nature of a process required modify the Patterson-style turbidostat for Antarctic diatom experiments--it required knowledge from the fields of biology, engineering, computer science, and electronics--though often overwhelming, proved to be very rewarding. There is definitely something to be said for doing a project from the bottom-up. I am happy that I have successfully completed these modifications and have achieved my second goal.

With the design of my initial experiment, using all of my knowledge of batch culturing and the culturing device, I have met part of my final goal, leaving the actual experiment carrying out of the experiment left to do. I would be lying if I said I was not disappointed that I did not accomplish all of my goals in the time I had available, but the knowledge I have gained was well worth the effort. Scientific research in this case couldn't even begin without the scientific invention, of which I played an integral part. I also have more realistic expectations about the rate of progress in a scientific project. It is important to mention that I am not ending this project with the end of my Fellows program. I plan to continue it this summer and next fall. I have put too much work into it not to see it to completion!

Acknowledgements

Without the support of several people, there is no way I could have gotten as far on this project as I have. First, I would like to thank Niall Slowey for all he has done for me. His advice and support was of utmost importance to me. Brian Edwards was responsible for all of the electronics construction for the culturing device, as well as giving me much advice on the programming of the system. His work with Dr. Patterson's turbidostat, and vast knowledge of electrical engineering was invaluable. His effort, though he was not being compensated in any way, was above and beyond the call of duty. Marylin Yeager taught me how to do all of the batch culturing, media preparation, and everything else having to do with maintaining the Antarctic diatom cultures. Her support throughout the year is one of the reasons for my success. Finally, I thank Dr. C. O. Patterson and his students for kindly teaching me about features of their turbidostat's design and showing me the device in operation.

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Appendix 1: Turbidostat Control Routines

```

DIM SHARED densityray%(200)      'stored densitydata
DIM SHARED timeray%(200)        'stored total time values
DIM SHARED timeray2%(200)       'stored clock value
'-----
' Fixed Parameters
'-----
'
Pray$(0, 2) = "C:\BACKUP"        'Backup path
Pray$(0, 3) = "PARAMETE.RS1"    'Backup filename
Pray$(0, 6) = MKI$(60)          'Number of samples
before saving to disk
Pray$(0, 9) = MKS$(60)          'Mu integral time
length (m)
Pray$(0, 10) = MKS$(60)         'Time between
turbidometer samplings (s)
'
'Error messages
'AOK$ = "OFF"                   'Print negative type
errors only

ERRORFLAGSWITCH:                'ON FOR POSITIVE
CONFIRMATION OF CIO8 CALLS

AOK$ = "OFF"                     'OFF FOR NEGATIVE RESULTS
ONLY

'-----
'                                     'Strings for main
MM0$ = "Last sample at"
MM1$ = ", next in"
MM2$ = "seconds."
MM3$ = "F5 to stop."

'-----
'-----
'                                     M A I N   P R O G R A M
'
AA:                               'Initials
' ON TIMER(60) GOSUB RR
'ON ERROR GOTO ER
CLS
CALL Chapter100

```



```

PRINT "got thru 100"
time1 = 0
time2 = 0
md% = 14
op% = 0
flag% = 0
CALL cio8(md%, VARPTR(op%), flag%)
PRINT "this is the first error code ", flag%

aazzdd:
C%(0) = 5
C%(1) = 5
md% = 1
      CALL cio8(md%, VARPTR(C%(0)), flag%)
PRINT "1flag", flag%
PRINT ""
PRINT "Hit return to begin sampling....."
starttime$ = TIME$
startdate$ = DATE$
PRINT "The Start time is "; starttime$;
PRINT " On "; startdate$
TIMER ON
timeron = TIMER
timercheck = TIMER
runtime = timercheck - timeron
PRINT "The program has been running for "; runtime;
PRINT " seconds"
SLEEP 5

C%(2) = 4096
C%(3) = 4096
C%(4) = 4096
dc%(0) = 0
dc%(1) = 0
dc%(2) = 0
dc%(3) = 0

e%(1) = 0
e%(2) = 0
e%(3) = 0
e%(4) = 0
d%(21) = 0

WHILE runtime < 5
d%(18) = 0
m = m + 1

```

```

FOR q = 1 TO 50

d%(0) = 0
d%(1) = 0
d%(2) = 0
d%(3) = 0
d%(4) = 0
d%(5) = 0
d%(6) = 0
d%(7) = 0
d%(8) = 0
d%(9) = 0
d%(10) = 0
d%(11) = 0
d%(12) = 0
d%(13) = 0
d%(14) = 0
d%(15) = 0
d%(16) = 0
d%(17) = 0
d%(19) = 0
avg%(1) = 0
avg%(2) = 0
f%(0) = 0
f%(12) = 0
f%(3) = 0

md% = 1
C%(0) = 0
C%(1) = 0
flag% = 0
CALL cio8(md%, VARPTR(C%(0)), flag%)
md% = 2
C%(0) = 0
CALL cio8(md%, VARPTR(C%(0)), flag%)

md% = 4
flag% = 0

dc%(0) = 0
dc%(1) = 0
dc%(2) = 0
dc%(3) = 0

FOR x = 1 TO 200

```

```

CALL cio8(md%, VARPTR(d%(0)), flag%)
CALL cio8(md%, VARPTR(d%(1)), flag%)
CALL cio8(md%, VARPTR(d%(2)), flag%)
CALL cio8(md%, VARPTR(d%(3)), flag%)
IF x = 40 THEN C%(2) = d%(0) + 2048
IF x = 40 THEN C%(5) = d%(0) + 2048
IF x = 40 THEN cx%(2) = d%(0) + 2048
IF x = 40 THEN cx%(5) = d%(0) + 2048

d%(0) = d%(0) / 10
d%(1) = d%(1) / 10
d%(2) = d%(2) / 10
d%(3) = d%(3) / 10

dc%(0) = d%(0) + dc%(0)
dc%(1) = d%(1) + dc%(1)
dc%(2) = d%(2) + dc%(2)
dc%(3) = d%(3) + dc%(3)
NEXT x
dc%(0) = dc%(0) / 20
d%(0) = dc%(0) + 2048
IF C%(2) > d%(0) THEN C%(2) = d%(0)
IF C%(5) < d%(0) THEN C%(5) = d%(0)

dc%(1) = dc%(1) / 20

d%(1) = dc%(1) + 2048
IF C%(3) > d%(1) THEN C%(3) = d%(1)
IF C%(6) < d%(1) THEN C%(6) = d%(1)
dc%(2) = dc%(2) / 20

d%(2) = dc%(2) + 2048

dc%(3) = dc%(3) / 20

d%(3) = dc%(3) + 2048

md% = 1
C%(0) = 3
C%(1) = 3

flag% = 0
CALL cio8(md%, VARPTR(C%(0)), flag%)
md% = 2
C%(0) = 3
CALL cio8(md%, VARPTR(C%(0)), flag%)

```

```

md% = 4
dc%(0) = 0
dc%(1) = 0
dc%(2) = 0
dc%(3) = 0

FOR x = 1 TO 100

    CALL cio8(md%, VARPTR(d%(8)), flag%)
    CALL cio8(md%, VARPTR(d%(9)), flag%)
    CALL cio8(md%, VARPTR(d%(10)), flag%)
    CALL cio8(md%, VARPTR(d%(11)), flag%)

d%(8) = d%(8) / 10
d%(9) = d%(9) / 10
d%(10) = d%(10) / 10
d%(11) = d%(11) / 10

dc%(0) = d%(8) + dc%(0)
dc%(1) = d%(9) + dc%(1)
dc%(2) = d%(10) + dc%(2)
dc%(3) = d%(11) + dc%(3)
NEXT x

dc%(0) = dc%(0) / 10
d%(8) = dc%(0) + 2048

dc%(1) = dc%(1) / 10
d%(9) = dc%(1) + 2048
dc%(2) = dc%(2) / 10
d%(10) = dc%(2) + 2048
dc%(3) = dc%(3) / 10
d%(11) = dc%(3) + 2048

IF C%(4) > d%(8) THEN C%(4) = d%(8)
IF C%(7) < d%(8) THEN C%(7) = d%(8)

md% = 1
md% = 1
C%(0) = 1
C%(1) = 1
flag% = 0
CALL cio8(md%, VARPTR(C%(0)), flag%)
md% = 2
C%(0) = 1
CALL cio8(md%, VARPTR(C%(0)), flag%)

```

```

md% = 4
  CALL cio8(md%, VARPTR(d%(4)), flag%)
  CALL cio8(md%, VARPTR(d%(5)), flag%)
  CALL cio8(md%, VARPTR(d%(6)), flag%)
  CALL cio8(md%, VARPTR(d%(7)), flag%)
d%(4) = d%(4) + 2048
d%(5) = d%(5) + 2048
d%(6) = d%(6) + 2048
d%(7) = d%(7) + 2048

md% = 1
C%(0) = 4
C%(1) = 4
flag% = 0
CALL cio8(md%, VARPTR(C%(0)), flag%)

md% = 2
C%(0) = 4
CALL cio8(md%, VARPTR(C%(0)), flag%)
md% = 4
  CALL cio8(md%, VARPTR(d%(13)), flag%)
  CALL cio8(md%, VARPTR(d%(14)), flag%)
  CALL cio8(md%, VARPTR(d%(15)), flag%)
  CALL cio8(md%, VARPTR(d%(16)), flag%)
d%(13) = d%(13) + 2048
d%(14) = d%(14) + 2048
d%(15) = d%(15) + 2048
d%(16) = d%(16) + 2048
CLS
'PRINT "avg pd[ 4] { ch 0} ", d%(0), d%(1), d%(2), d%(3)
'PRINT "avg pd[ 5] { ch 1-2} ", d%(8), d%(9), d%(10), d%(11)
'PRINT "avg pd[ 6] { ch 3} ", d%(4), d%(5), d%(6), d%(7)
'PRINT ""
'PRINT "Thermistor", d%(13), d%(14), d%(15), d%(16)

f%(0) = d%(0) + d%(1) + d%(2) + d%(3)
f%(12) = d%(8) + d%(9) + d%(10) + d%(11)
f%(3) = d%(4) + d%(5) + d%(6) + d%(7)

f%(0) = f%(0) / 4
f%(12) = f%(12) / 4
f%(3) = f%(3) / 4

avg%(1) = f%(12) - f%(3)          'Solution Density
avg%(2) = f%(12) - f%(0)        'Overflow Check

```

```

PRINT "Average for Ch 0=", f%(0)
PRINT "Average for Ch 1-2=", f%(12)
PRINT "Average for Ch 3=", f%(3)

PRINT "Solution Density (Uncompensated, Avg. 150 below
actual)", avg%(1)
PRINT "Overflow check", avg%(2)
PRINT ""
PRINT "Current time is "; TIME$
PRINT " On "; DATE$
timercheck = TIMER
runtime = (timercheck - timeron) / 60
PRINT "The program has been running for "; runtime;
PRINT " minutes"

d%(18) = d%(18) + 1
d%(21) = d%(21) + 1
e%(1) = e%(1) + avg%(1)      'Solution Density
e%(2) = e%(2) + avg%(2)      'Overflow Check

d%(19) = 0
d%(20) = 0
PRINT ""
NEXT q 'end testing loop

e%(2) = e%(2) / d%(18)
e%(1) = e%(1) / d%(18)
e%(1) = e%(1) + 150
PRINT "Compensating for light difference"
PRINT "Average Density", e%(1)
PRINT "Average Overflow Check", e%(2)
SLEEP 5
densityray%(m) = e%(1)      'stored densitydata
timeray%(m) = runtime      'stored time values

SLEEP 5
e%(1) = 0
e%(2) = 0
timercheck = TIMER
runtime = (timercheck - timeron) / 60

SLEEP 5
IF e%(2) >= e%(1) THEN
PRINT "The Solution has reached the upper limit"
PRINT "Density is too high, stepping out media"
SLEEP 2

```

```

FOR i = 0 TO 100

  md% = 14
  op% = 1
  PRINT "Step Out first signal "
  flag% = 0
  CALL cio8(md%, VARPTR(op%), flag%)

  md% = 14
  op% = 2
  PRINT "Step Out 12 signal"
  flag% = 0
  CALL cio8(md%, VARPTR(op%), flag%)

  md% = 14
  op% = 3
  PRINT "Step Out zero signal"
  CALL cio8(md%, VARPTR(op%), flag%)

  md% = 14
  op% = 4
  PRINT "Step Out one signal"
  CALL cio8(md%, VARPTR(op%), flag%)

NEXT i
  md% = 14
  op% = 0
  PRINT "stop signal"
  flag% = 0
  CALL cio8(md%, VARPTR(op%), flag%)

END IF

IF e%(1) >= 450 THEN
PRINT "Density is too high, stepping out media"
SLEEP 5

FOR i = 0 TO 100

  md% = 14
  op% = 1
  PRINT "Step Out first signal "
  flag% = 0
  CALL cio8(md%, VARPTR(op%), flag%)

```

```

md% = 14
  op% = 2
  PRINT "Step Out 12 signal"
  flag% = 0
  CALL cio8(md%, VARPTR(op%), flag%)

md% = 14
  op% = 3
  PRINT "Step Out zero signal"
  CALL cio8(md%, VARPTR(op%), flag%)

md% = 14
  op% = 4
  PRINT "Step Out one signal"
  CALL cio8(md%, VARPTR(op%), flag%)

NEXT i

PRINT "Stepping In Fresh Media to Compensate"
SLEEP 5

FOR r = 1 TO 50
FOR q = 1 TO 2

  md% = 14
  op% = 7
  PRINT "Step In 1st"
  flag% = 0
  CALL cio8(md%, VARPTR(op%), flag%)
NEXT q
FOR q = 1 TO 2

  md% = 14
  op% = 8
  PRINT "Step In 2nd"
  flag% = 0
  CALL cio8(md%, VARPTR(op%), flag%)
NEXT q
FOR q = 1 TO 2

  md% = 14
  op% = 9
  PRINT "Step In 3rd"
  flag% = 0
  CALL cio8(md%, VARPTR(op%), flag%)
NEXT q

```



```

FOR q = 1 TO 2

    md% = 14
    op% = 10
    CALL cio8(md%, VARPTR(op%), flag%)
    PRINT "Step In 4th"

NEXT q

    md% = 14
    op% = 0
    PRINT "stop signal"
    flag% = 0
    CALL cio8(md%, VARPTR(op%), flag%)
NEXT r
END IF
WEND
PRINT "Current time is "; TIME$
PRINT " On "; DATE$
timercheck = TIMER
runtime = timercheck - timeron
PRINT "The program has been running for "; runtime;
PRINT " seconds"
timertotal = (timeron - timercheck) / 60
PRINT "The entire series took "; timertotal;
PRINT " minutes"
PRINT "Hit enter to see array"
SLEEP
FOR x = 1 TO 5
PRINT "Density was "; x; "="; densityray%(x);
PRINT " At time "; x; "="; timeray%(x)
OPEN "Density.DAT" FOR OUTPUT AS x           'Open
Density.DAT for writing

WRITE x, densityray%(x)                     'Store A$ and B$ in
second record with WRITE #.
CLOSE x

NEXT x

END

(whoop!)

```