1992


Kelly Ann Rusch
Louisiana State University and Agricultural & Mechanical College

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Demonstration of a control strategy for sustained algal growth at a full-scale level under computer-automated, continuous culture conditions

Rusch, Kelly Ann, Ph.D.
The Louisiana State University and Agricultural and Mechanical Col., 1992

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DEMONSTRATION OF A CONTROL STRATEGY FOR SUSTAINED ALGAL GROWTH AT A FULL-SCALE LEVEL UNDER COMPUTER AUTOMATED, CONTINUOUS CULTURE CONDITIONS

A Dissertation

Submitted to the Graduate Faculty of the
Louisiana State University and
Agricultural and Mechanical College
in partial fulfillment of the
requirements for the degree of
Doctor of Philosophy

in

The Department of Civil Engineering

by
Kelly Ann Rusch
B.S., University of Wisconsin-LaCrosse, 1986
M.S. in Civil Engineering, Louisiana State University, 1989
December 1992
ACKNOWLEDGEMENTS

This research was supported by the Louisiana Sea Grant College Program, an element of the National Sea Grant College Program, under the auspices of the National Oceanic and Atmospheric Administration, U.S. Department of Commerce. Special thanks is extended to Mr. Ronald Becker, Associate Director of the Louisiana Sea Grant College Program, who has continually supported this research effort throughout the past two and one-half years. The author would also like to thank Dr. James R. Nelson of the Skidaway Institute of Oceanography, Savannah, Georgia, for supplying the stock algal cultures used in this research. Technical site testing was performed in a greenhouse bivalve hatchery under the direction of Dr. Peter B. Heffernan of the University of Georgia, Marine Extension Service, Savannah, Georgia. Special thanks goes to David Head for providing the technical support of the Georgia facility. Additional thanks goes to Mr. Yan Huang for analytical assistance and Mr. John Nelson for general laboratory support.

I would like to sincerely thank Dr. Ron Malone for his assistance, guidance, encouragement and support as my major professor, as well as being a very close friend. The numerous accomplishments I have achieved over the past six years reflect positively on our working relationship. Without the structure of the Civil Engineering Aquatic System Laboratory (CEASL) research group, I do not feel that I would have been as prepared to enter the "real" world as I am. In other words Ron, thanks; you have been a great inspiration for me to pursue an academic career. I would also like to thank my other committee members, Drs. Dean Adrian, Dipak Roy, Chuck Wilson and Russ Chapman.
I would like to thank all of my close friends and old college roommates whom encouraged and supported me during my tenure as a graduate student at Louisiana State University: Mary Hoeltke, Cary Leider, Kristi Gunderson, Cathy Carroll, Bobbie Jones and Lorie McGee. I would also like to acknowledge the members of the CEASL research group for their technical and editorial support over my last years here as a Ph.D. student: Dr. Shulin Chen, Douglas Drennan, Lihua Wang, Pamela Rupert, Dave Coffin, Chandra Theegala, Babu Chitta and Craig Dalferes.

I hope the next student who occupies Desk No. 32 enjoys the same working environment I was lucky enough to be part of for six years. Thanks CEASL. However, I am glad I finally made it through.
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ABSTRACT

A computerized algal turbidostat was designed and constructed for use in aquaculture facilities requiring a continuous feed source. Continuous cultures, maintained in the exponential growth phase by the continual introduction of fresh media, exhibit a steadystate nature easily adaptable to computer automation and optimization processes. These characteristics, in contrast to batch culture methods, result in both reduced land and labor requirements; an important consideration for commercial applications wherein algal production occupies as much as 40 percent of a facility in both space and cost. The turbidostat system consisted of two 0.6 m³ growth chambers supported by a central monitoring block and pressurized dosing apparatus, and operated from a single air/vacuum piston pump. System processes were executed by a computer control system interfaced to input and output devices through an analog to digital converter. The software program, "Supervisor", written in Turbo Pascal 4.0, provided: 1) system control and monitoring, 2) high rate data collection and storage and 3) reduced operational costs through the displacement of manual labor. The core control and monitoring algorithm contained a central supervisor that mitigated the temporal demands of simultaneous processes. System operation depended on the interaction between the photocell and computer to maintain high standing crop concentrations and appropriate harvest rates leading to high production levels. Investigations were undertaken to: 1) demonstrate the capabilities of the control system in maintaining growth conditions conducive to high production levels and 2) collect baseline optimization data for Chaetoceros muelleri (Chaet 10) under varying temperature and lighting conditions. No significant
difference (P<0.05) was detected between computer estimated standing crop concentrations and analytically measured total suspended solids (TSS). This result indicated the reliability of the computer/photocell combination. An average production level of 221 g/m³/day-dry wt was obtained for Chaetoceros muelleri (Chaet 10) under continuous lighting from 250W metal halide lamps and at a temperature of 30°C, which resulted in a 400 - 500% increase in production levels over traditional outdoor pond cultures. The turbidostat system has been implemented and is undergoing "semi-commercial" evaluation within a greenhouse bivalve hatchery located on Skidaway Island, Savannah, Georgia; an affiliation of the University of Georgia, Marine Extension Service.
CHAPTER I
INTRODUCTION

The United States' consumption of fisheries products stood at 15.5 pounds per capita in 1990, and is forecasted to rise to 20 pounds per capita by the year 2000 (NCAE, 1990). Unfortunately, natural fisheries supplies, which are estimated to be at their maximum sustainable yield, will not be able to meet this demand. Overfishing, increasing pollution legal restrictions, and in some areas (Louisiana), loss of suitable wetland habitats through erosion and saltwater intrusion will further restrict natural harvests.

This growing demand from within the United States has placed pressure on the domestic aquaculture industry to make great strides towards meeting consumer needs. Even though the American aquaculture industry has grown 15 percent annually since 1980, and has provided about 12 percent of the consumed fish and shellfish in 1990, the U.S. still remains a weak competitor in the world market place, importing over 40 percent of all fisheries products (NCAE, 1990). This corresponded to a trade deficit of $3.2 billion in 1989, surpassed only by petroleum imports (NCAE, 1990).

While the U.S. continues to move forward in its efforts to establish a sound, economic aquaculture industry, the nation remains overshadowed by the tremendous growth of low-technology, extensive systems in a number of the poorly developed countries. This is attributed to the more favorable climates, abundant water resources, low land prices, loose environmental regulations and, most importantly, inexpensive labor. Therefore, the establishment of a strong domestic
industry within the international market requires an intelligently planned strategy in many segments of aquaculture to decrease the cost/benefit ratio through insightful and prudent technological advances encompassing: 1) intensive rearing systems, 2) cost-effective, high production techniques and 3) computer automation to optimize the level of system control.

While research interests have been directed towards the development of optimal closed recirculating system designs for the intensive rearing of aquatic animals, little has been done to change the status quo of the state-of-the-art technology for algal production or for the implementation of basic computer control and monitoring systems. For facilities producing algae as a feed source (largely, shellfish and shrimp operations), the major issues remain to be: 1) the economics associated with the large-scale production of algae and 2) the dependability and consistency of large culture volumes. Shellfish operations are especially targeted because algae are required through all life stages. Additionally, most facilities rearing crustaceans or finfish utilize either brine shrimp (Artemia sp.) and/or rotifers (Brachionus sp.) as part of their dietary plan. The nutritional quality of these feeder organisms is enhanced by rearing them on cultured algae. The bottom line is that algae, which are the base of the food chain, dictates the carrying capacity of many facilities; and, the securement of this base will provide a sound foundation for broad aquacultural development.

The state-of-the-art technology for algal production throughout the world relies almost exclusively on manually operated batch cultures and induced algal blooms. Batch culture technologies for algal production, although well established, contain a
multitude of inherent limitations, most of them directly linked to the nature of the culture itself (Table 1). First, low production levels must be offset by large culture volumes, which, in turn, require a vast amount of space. This is a significant problem in areas with high land prices. Second, the manual labor required to maintain a batch system is extremely high and increases with every additional culture tank put into operation. Finally, extensive space requirements limit most batch cultures to outdoor conditions, eliminating the ability to control environmental parameters and contamination from predators and competing algal species; the primary cause of all batch culture collapses.

Table 1. Advantages and limitations of batch culture technologies for algal production in aquaculture facilities.

<table>
<thead>
<tr>
<th>Advantage</th>
<th>Disadvantage</th>
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<tr>
<td>Compatible with training of aquaculturists</td>
<td>High capital cost</td>
</tr>
<tr>
<td>&quot;Reliable&quot; production</td>
<td>Labor intensive</td>
</tr>
<tr>
<td></td>
<td>Land intensive</td>
</tr>
<tr>
<td></td>
<td>Low productivity per unit volume</td>
</tr>
<tr>
<td></td>
<td>Poor environmental control</td>
</tr>
<tr>
<td></td>
<td>Intermittent production/supply</td>
</tr>
<tr>
<td></td>
<td>Varying nutritional quality</td>
</tr>
<tr>
<td></td>
<td>Subject to frequent contamination</td>
</tr>
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<td></td>
<td>Very low potential for optimization</td>
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With low labor and standard of living costs, many of the Southeast Asian and South American countries execute batch algal production at substantially lower costs than are feasible in the United States. It has been estimated that as much as 20 - 40 percent of an American shellfish hatchery is devoted to algae production (Taub, 1975). DePauw (1981) estimated the cost for indoor production of monospecific cultures to be $160 - 200/kg-dry weight of unharvested algae. Due to the high cost of indoor cultures, facilities requiring large quantities of algae are forced to rely on natural phytoplankton blooms that cost $4 - $20/kg-dry weight of unharvested algae (DePauw et al., 1983). However, labor still constitutes 50 - 85 percent of this total cost (DePauw et al., 1983). While the use of natural blooms may reduce production costs, they are unreliable and often do not produce the quantity or quality of algae required. Additionally, the utilization of natural phytoplankton is restricted to temperate climates, which limits a commercial operation to the warmer periods of the year (Persoone and Claus, 1980). With high land and labor costs in the United States, reliance on large-scale algal production by batch culture technology will seriously impede the future development of certain species in aquaculture.

Although all countries face the problem of dependable and consistent algae production, less developed countries are able to cost-effectively maintain a series of back-up cultures. This facilitates increased carrying capacities and, subsequently, realization of higher profits. "For reasons of high labor costs, algal culture systems should be automated as much as possible" (DePauw and Persoone, 1988); particularly in countries with higher scales of economy.
Some of the economic burden constraining the expansion of American aquaculture facilities may be mitigated by the implementation of computer automated, continuous algal production systems (turbidostats). Such systems offer a number of advantages vis-a-vis batch cultures (Table 2). Continuous cultures, with a continual input of media and outflow of algal cells and expended media, are maintained in the exponential growth phase (high specific growth rates).

Table 2. Advantages and limitations of computer-automated, continuous culture technologies for algal production in aquaculture facilities.

<table>
<thead>
<tr>
<th>Advantage</th>
<th>Disadvantage</th>
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<tr>
<td>High productivity per unit volume</td>
<td>Technology unfamiliar to aquaculturists</td>
</tr>
<tr>
<td>&quot;Reliable&quot; production</td>
<td>High capital cost</td>
</tr>
<tr>
<td>Precise environmental control</td>
<td></td>
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<tr>
<td>Reduced labor requirements</td>
<td></td>
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<tr>
<td>Reduced land requirements</td>
<td></td>
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<tr>
<td>Constant nutritional quality</td>
<td></td>
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<tr>
<td>Continuous production/supply</td>
<td></td>
</tr>
<tr>
<td>High potential for optimization</td>
<td></td>
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<tr>
<td>Easily computer automated</td>
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Subsequently, the turnover time (the period between the initial start-up and the point at which the chambers are completely harvested and disinfected) of the cultures is dramatically extended. Continuous cultures can be maintained for months at high production levels as long as all parameters influencing growth are provided
in excess. In contrast, the life of a batch culture is typically less than two weeks from start-up to harvest. High specific growth rates result in rapidly dividing cells, allowing biomass concentrations within continuous cultures to reach levels not readily sustainable by batch methods. This combination of high specific growth rates, high biomass concentrations (not necessarily maximum levels) and continuous production results in high production levels per unit volume, directly reducing space and labor requirements. Computer automation further alleviates the tremendous labor requirements and provides a level of control and monitoring not obtainable by manual methods.

**Dissertation Objectives and Approach**

This dissertation consists of three manuscripts, each focusing on a specific part of a two-year research project directed towards developing and optimizing production-scale algal turbidostats for feed production within aquacultural facilities. Although the complete optimization of an algal production system must address both production levels and nutritional quality, this research focused on demonstrating the computer's capability to maintain high production levels and collect baseline operational data for temperature and lighting conditions. Total algal lipid and protein percentages were estimated during each investigation to detect trend differences under varying environmental conditions. The main focus centered on the initial optimization of production levels within the system. This research covered three areas: 1) development of computer algorithms for the control and monitoring of the system, 2) development and construction of the production-scale turbidostat and 3) the integration and evaluation of the computer automated algal
turbidostat. The first investigation dealt with the development of a software package that: 1) provided for the complete control and monitoring of the turbidostat, 2) created a programming environment, easily modified to fit the needs of individual experiments and 3) facilitated the collection and storage of all monitored data. The Turbo Pascal 4.0 program, "Supervisor", contained three core elements: 1) a "stack" or chronologically ordered array of commands and associated execution times, 2) a "stack sorter" that positions new operations within the "stack" and 3) a "supervisor" that continuously polled the execution time of the top command and the internal clock. The software had complete control of all processes and monitoring requirements of the turbidostat. Information transmitted to the computer from monitoring devices was used to make procedural decisions concerning temperature, harvest rate and pH. Chapter II, "A Micro-Computer Control and Monitoring Algorithm with Application to Aquaculture Systems - Algal Turbidostat", presents an in-depth discussion of the programming approach and support hardware necessary to execute the control and monitoring algorithm. Included are results from initial production studies showing the reliability of the software to estimate standing crop concentrations within the culture chambers and to maintain the environmental conditions within the realms of initial boundary conditions. This manuscript has been submitted to The Progressive Fish-Culturist.

The second portion of this research involved the development of a production-scale algal turbidostat. Based on the results of investigations with bench-scale turbidostats, a production-scale system was designed to incorporate overhead illumination, a single air/vacuum pump for bulk solution movement and centralized
monitoring and dosing systems to reduce component redundancy. Chapter III, "The Development of a Computerized Turbidostat for the Continuous Production of Algae", discusses the rationale for the design of the algal turbidostat, presents a description of the system and summarizes initial optimization studies under varying temperature and lighting conditions. This chapter has been submitted to the *Journal of Applied Aquaculture*.

Finally, Chapter IV, "Baseline Optimization of *Chaetoceros muelleri* (Chaet 10) Within a Computerized Turbidostat", presents the results of six initial studies and a one month baseline optimization study using the optimal operational parameters selected from the first six studies. The effects of temperature and lighting conditions were investigated to establish a baseline range from which to perform an in-depth computerized search for optimal operating conditions. Based on these results, a one month optimization study was performed to determine the production capabilities and stability of the system under specified operating conditions. Along with this, a computer model was developed to project production levels over extended time periods. This manuscript has been submitted to the *Journal of Aquaculture*. 
CHAPTER II
A MICRO-COMPUTER CONTROL AND MONITORING SYSTEM WITH APPLICATION TO AQUACULTURE SYSTEMS: ALGAL TURBIDOSTATS

INTRODUCTION

Aquaculture facilities relying on live microalgae as a feed source have yet to establish a production methodology that balances adequate control over algal quantity and quality with production costs. Outdoor methods, namely induced blooms and cultivation ponds, provide an economic method for obtaining large quantities of algae, but without the benefit of quality/quantity control (Persoone and Claus, 1980; Ukeles, 1980; Claus, 1981; DePauw, 1981; Riva and Lelong, 1981; DePauw et al., 1983). Indoor technologies, mostly batch cultures, facilitate more than adequate control over culture quality. However, the operating cost is often times an order of magnitude greater than outdoor cultures (DePauw, 1981). In recent years, small-scale automated systems for the continuous culture of algae have been reported in the literature (Sorgeloos et al., 1976; James et al., 1987) As complexity and demands increase with system expansion, automated systems based on timers may not accommodate the level of control required. Development of cost-effective, high production, indoor algal technologies for large-scale aquaculture applications may require the utilization of computer based control and monitoring systems to reduce labor requirements and accommodate all routine processes. Scientifically based management of turbidostats will require precise control of a variety of parameters affecting temperature, lighting conditions, harvest
rates, production levels and pH. Additionally, inexpensive monitoring data will be required to provide a database for decision making processes.

The move from an industry based largely on extensive (batch) culture to one utilizing computerized, intensive (continuous) systems has been inhibited by the slow development of aquaculturally oriented software. Yet, both the electronics and programming required for implementing a basic control and monitoring program are basic compared to the state-of-the-art in parallel industries including wastewater treatment and chemical operations (Briggs, 1990; Berg, 1991; Hughson, 1991; Stover and Campana, 1991). One of the key features of a control system is flexibility. Continuous culture systems are computerized to: 1) execute routine operational processes, 2) optimize production levels, 3) monitor and control operational conditions and 4) control cost of operations. Although interest is often focused on a specific area, the complex interactions that occur during the production of microalgae normally dictate management of all these objective areas simultaneously. Therefore, control systems must be carefully designed and loosely configured to permit the use of data from a variety of inputs and to provide control through a series of devices.

This paper presents a programming approach that has been developed and implemented with success in the authors' laboratories for the complete control and baseline optimization of a 1.2 m³ production-scale algal turbidostat. The ease of programming and low cost of support hardware has enhanced the testing and documentation of various algal turbidostat prototypes. The programming approach
presented within provides research and small commercial adventures, with customized needs, a long term cost-effective alternative to the many "CANNED" programs available. Although sophisticated in nature, "CANNED" programs are often inflexible and limited in their operational capabilities, making the introduction of new processes rather difficult. "CANNED" programs are probably more applicable to large, established commercial facilities where relatively few changes are required. Although the presented programming approach could serve a wide range of aquaculture applications, this paper focuses only on the algal turbidostat. Experimental results from the initial testing of the turbidostat system are also presented, including a cost estimate of the control system relative to overall system cost.

Background

The inherent characteristics of batch cultures, including the dynamic growth patterns and nutrient exhaustion with time, result in low production levels per unit volume or area. Compensation for low production levels results in a system that is both space and labor intensive, in as much as 20-40% of an American hatchery may be devoted to the production of algae (Taub, 1975). Unfortunately, the availability of a continuous and reliable feed source combined with the operational costs associated with manual batch culture systems dictates that the carrying capacity of hatcheries will be dependent on microalgal production. Continuous cultures theoretically afford a ten to twentyfold increase in production per unit volume over batch cultures (Herbert et al., 1956), thereby, realizing a reduction in
operating costs through culture volume minimization. The integration of continuous cultures with computer control systems reduces manual supervision requirements through increased monitoring and control support.

The production-scale turbidostat system developed within the Civil Engineering Aquatic Systems Laboratory (CEASL) at Louisiana State University consists of ten basic modules, interrelated to one another through the computer control system and operated using a 0.37 kW piston air/vacuum pump rated for continuous duty (Figure 1 and Table 3). Algae are cultured in two 0.6 m³ enclosed fiberglass chambers housed inside an artificially illuminated, temperature control room. All additions and withdrawals occur through outlets in the acrylic top, eliminating the need for outlets in the chambers themselves, thereby minimizing dead areas. Irrespective of chamber size or number, the entire turbidostat system can be serviced by a single centrally located pressurized dosing apparatus and one centralized monitoring block to mitigate redundancy and reduce system complexity. Through a series of electronic solenoid and check valves, media and disinfectant are pneumatically moved from the dosing apparatus to the growth chambers, eliminating mechanical devices that may be prone to failure with extended use.

During each harvest cycle, algae are removed from the chambers via vacuum and routed through the monitoring block where culture pH, temperature, salinity and turbidity measurements are automatically taken and recorded. The photocell, inexpensively constructed from a 5.1 cm diameter acrylic tube, a fluorescent light and a photovoltaic cell, detects the light transmitted by the algal culture, and sends
Figure 1. The algal turbidostat system consists of two 0.6 m³ growth chambers supported by a central monitoring unit and pressurized dosing apparatus. The system is controlled and monitored by the software program, “Supervisor” (not shown here).
Table 3. Components of the computer automated algal turbidostat and their function.

<table>
<thead>
<tr>
<th>Component</th>
<th>Specifications</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>Culture Chambers (2)</td>
<td>0.6 m³ Fiberglass Chambers; Acrylic Covers; Overhead Illumination</td>
<td>- Provides an enclosed environment for the algal culture</td>
</tr>
<tr>
<td>Lighting Block</td>
<td>250 W Metal Halide</td>
<td>- Provides overhead illumination to culture</td>
</tr>
<tr>
<td>Air/Vacuum Block</td>
<td>1/2 HP, Piston</td>
<td>- Pressurizes entire system</td>
</tr>
<tr>
<td></td>
<td>Vac: 1.93 CFM @ 10&quot; Hg</td>
<td>- Provides aeration to the cultures</td>
</tr>
<tr>
<td></td>
<td>Air: 4.05 CFM @ 0 PSI</td>
<td>- Moves solutions from dosing apparatus to chambers</td>
</tr>
<tr>
<td></td>
<td></td>
<td>- Moves algal culture from chambers to monitoring block and harvesting port</td>
</tr>
<tr>
<td></td>
<td></td>
<td>- Reinoculates disinfected chamber with algae from another culture</td>
</tr>
<tr>
<td>CO₂ Block</td>
<td></td>
<td>- Intermittently injects additional CO₂ into the airline</td>
</tr>
<tr>
<td>Pressurized Dosing Apparatus</td>
<td>Each Dosing Chamber=2.5L; Pneumatically Operated</td>
<td>- Maintains proper level of macro nutrients, trace elements, and vitamins in the culture</td>
</tr>
<tr>
<td></td>
<td></td>
<td>- Doses chambers with a saturated sodium hypochlorite solution during cleaning periods</td>
</tr>
<tr>
<td>Monitoring Block</td>
<td>pH, Conductivity, Temperature, Photocell-Turbidity</td>
<td>- Checks algal culture pH, temperature, conductivity and density during the harvest cycle</td>
</tr>
</tbody>
</table>
Table 3 (Cont'd). Components of the computer automated algal turbidostat and their function.

<table>
<thead>
<tr>
<th>Component</th>
<th>Specifications</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>Harvest Block</td>
<td>0.15 m³ Fiberglass Chamber</td>
<td>- Collects and distributes algae from the chambers at specified times</td>
</tr>
<tr>
<td>Computer Control Block</td>
<td>See Table 1</td>
<td>- Monitors and controls the activities of the turbidostat</td>
</tr>
<tr>
<td>Control Devices</td>
<td>Solenoid Valves</td>
<td>- Activate in response to processes occurring within the system</td>
</tr>
<tr>
<td></td>
<td>Actuated Ball Valves</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Air Conditioner</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Heater</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Photocell Light</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Chamber Lights</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Air/Vacuum Pump</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Level Detectors</td>
<td></td>
</tr>
<tr>
<td></td>
<td>UV Light</td>
<td></td>
</tr>
<tr>
<td>Saltwater Treatment</td>
<td>0.76 m³ Reservoir</td>
<td>- Concentrates algal suspension into paste</td>
</tr>
<tr>
<td></td>
<td>200 GPH Cont. Centrifuge</td>
<td>- Recirculates saltwater through filter and UV for reuse within</td>
</tr>
<tr>
<td></td>
<td>Upflow Sand Filter</td>
<td>culture chambers</td>
</tr>
<tr>
<td></td>
<td>UV Light</td>
<td></td>
</tr>
</tbody>
</table>
this information to the computer where standing crop concentrations are calculated using a linear regression equation correlating the millivolt output of the photovoltaic cell with analytically measured total suspended solids (TSS). After culture conditions are recorded, the algae is collected in the harvesting chamber and distributed to the saltwater conservation system consisting of a continuous flow centrifuge, a 0.76 m³ saltwater reservoir, an upflow sand filter and a UV light. The algal paste is collected and stored in the refrigerator for use as needed. The saltwater circulates through the upflow sand filter to trap any excess algae and the UV light to disinfect the saltwater prior to reuse in the culture chambers. The saltwater reservoir has a turnover rate of 5% per day to mitigate the build-up of metabolic by-products. Being inland, the turbidostat system utilizes artificial seawater (Instant Ocean¹), resulting in salt costs greater than $450 per week. The use of the saltwater conservation system reduced this cost to approximately $70 per week.

SOFTWARE DEVELOPMENT

Of the few automated continuous culture systems reported in the literature, the majority employ a series of timers that activate solenoid valves and pumps to effectuate processes such as CO₂ injection and media addition (Sorgeloos et al., 1976; James et al., 1987). In particular, most turbidostat systems utilize electromechanical systems that detect the electrical imbalance between a sensor and reference voltages to activate relays controlling output devices (Munson, 1970).

¹The use of this product does not represent an endorsement.
Although well established and reliable, this technology requires manual adjustments in response to changing environmental conditions. Subsequently, the development of a micro-computer control and monitoring system, to further advance algal production technologies, must: 1) address and circumvent temporal conflicts of simultaneous processes, 2) provide for information feedback between the software and sensors and probes, with automatic adjustments in routine processes, 3) reduce long term operational costs over present methods through the minimization of manual labor and 4) provide for the collection and storage of information obtained from the system.

Temporal requirements for the normal operation of the 1.2 m³ turbidostat system are not particularly demanding; not many operations require timing resolutions less than one second. Thus, control programs based upon simple serial execution of operations are intuitively apparent (Figure 2). Programs written in a serial format execute commands one at a time; the succeeding command not performed until the one preceding it has been completed. Therefore, these programs do not facilitate the overlapping temporal demands of several simultaneously occurring operations. For instance, timing demands for harvest operations conflict with the simultaneous requirements for culture condition monitoring within the turbidostat system. This inherent structural weakness in serially based programs becomes increasingly difficult to deal with as the complexity of the operations increases. The timing conflicts that appear can be rectified by an ever increasingly sophisticated series of checks; but, the end result is a lengthy program that is rigid in its structure and prone to error upon modification.
Figure 2. Control and monitoring algorithms employing serial execution of operations lead to temporal conflicts.

Computer is inactivated until this loop is completed. No new commands or processes can be executed, causing time delays of nearly simultaneous operations.
Simultaneous or nearly simultaneous operations not executable by serial algorithms may be addressed by a software program containing a central supervisor that manages the potentially conflicting demands of a multitude of operations (Figure 3). The control and monitoring algorithm developed by the CEASL group contains three generic core elements used to sort and execute all processes required for the daily operation of the turbidostat (Figure 4). Central to the three "Supervisor" core elements is a chronologically ordered array of commands and execution times, the "stack". The "stack" consists of a record of elements containing both a real and an integer component. The real element holds the decimal equivalent of the initial execution time in days (calculated from an arbitrary datum) associated with the integer command number. The "stack" is loaded by the "stack sorter", which employs a variation of a bubble sort routine (Miller, 1981; Zaks, 1986) to position new commands chronologically in the "stack", add delayed commands and reload the just executed command for the next cycle. The "supervisor" procedure continuously polls the execution time of the top command against the current time, again expressed as a decimal equivalent of the days since an arbitrary datum. Real times for both the current and command execution times are converted to month, days, hour, minutes and seconds prior to being displayed on the computer screen for operator reading ease.

The control system is actually driven by the operations themselves since the "supervisor" procedure can only execute commands loaded to the "stack". Each command is configured to: 1) execute instantaneous operations, 2) load delayed commands to the "stack" and/or 3) reload itself for the next cycle. Thus, the
Figure 3. Control and monitoring algorithms employing a centralized chronological array of operations mitigates temporal conflicts. Illustrated is the flow diagram of "Supervisor" for the control of the algal turbidostat.
Procedure Start_Supervisor;
Var ih:integer;
BEGIN
  command[1]:= 'Room Environ Check';
  command[2]:= 'Light Output Check'
  command[3]:= 'Harvest 1';
  command[4]:= 'Harvest 2';
  command[5]:= 'Monitoring Unit On';
  command[6]:= 'Up Harv Detect On';
  command[7]:= 'Cham Level Detect On';
  command[8]:= 'End Nutrient Addition';
  command[9]:= 'CO2 Addition On';
  command[10]:= 'CO2 Addition Off';
  command[11]:= 'Culture Lights On';
  command[12]:= 'Culture Lights Off';

Procedure Stack_Sort(tte:real;tta:integer);
Var ir,ie:integer; pass_it:dgt;
BEGIN
  pass_it.time:=tte; pass_it.action:=tta;
  if (pass_it.time<stack[1].time) then ir:=1
  else BEGIN
    ir:=0; Repeat ir:=ir+1; Until (pass_it.time<=stack[ir].time) or (ir=69);
  END;
  for ie:=70 downto ir+1 do stack[ie]:=stack[ie-1];
  stack[ir]:=pass_it;
  if ir=69 then BEGIN
    clrscr; gotoxy(1,21);
    write('ERROR DETECTED IN COMMAND STACK, END OF FILE REACHED');
  END;
END; {of procedure stack_sort}

Procedure Kill_Top;
Var op:integer;
BEGIN
  for op:=1 to 69 do stack[op]:=stack[op+1]; stack[70]:=space;
END; {of procedure kill_top}

Procedure Command_Stack;
Var jk:integer;
BEGIN
  i:=0; gotoxy(1,2); writefLast Command', last_command, ' at ');
  convert_time(last_time);
  gotoxy(1,5); Repeat i:=i+1; convert_time(stack[i].time);
  writeln(' ',command[stack[i].action]);
  Until (stack[i+1].action=0) or (i=10);
  for jk:=i+1 to 10 do writeln(' ');
END; {of command_stack}

Function Stack_Match:boolean;
BEGIN
  if (stack[1].time<=dtime) then stack_match:=true
  else stack_match:=false;
END; {of function stack_match}

Figure 4. The "Supervisor" control and monitoring algorithm consists of three core elements: 1) "stack", 2) "stack sorter" and 3) "supervisor". The integration of these three components creates a flexible programming environment.
"supervisor's" control is relinquished only momentarily as a command is executed. The timing resolution of the "supervisor" is limited to a few hundredths of a second by the analog/digital conversion process (10-20 readings per second) required for control or monitoring of external probes and sensor devices. Therefore, commands are executed at almost precisely the same time everyday. Furthermore, future commands (i.e., execution of a disinfection cycle four months from the present) can be loaded by the user at the initiation of the program. Temporal conflicts, for example between harvesting and culture condition monitoring operations, are avoided by splitting the harvest process into a command ('harvest on') and a delayed command ('harvest off'), with the latter being loaded by the former. Thus, in the case of simultaneous requests, the execution error is limited to the execution time of one command, and not the entire operational process. Serially written algorithms lack the capability to split commands. For instance, the initiation of the harvest cycle would incapacitate the computer from executing any other command until the harvest cycle had been completed. Consequently, simultaneous requests such as culture monitoring during the harvest cycle cannot be met, resulting in a cumulatively increasing execution error with every operation. This cumulative error inhibits the execution of routine processes at a precise time every day.

Operations are related to each other only through the "stack" or through checks of system conditions and programming flags. This creates an extremely friendly programming environment, facilitating the addition of new operational algorithms without increasing the complexity of the program. The core of the program is
generic, permitting the application of this control strategy to a variety of projects (Malone et al., 1986; Manthe et al., 1988; Robin, 1992; Rondelle, 1992; Chen et al., in press; Chen et al., in press;). In fact, this algorithm readily accommodates the control of several physical systems simultaneously.

The core control and monitoring elements have been integrated into the menu-driven, user friendly Turbo Pascal 4.0 (Borland International, 1987) software program, "Supervisor", providing an interactive environment for the operator. The computer program, in addition to providing control and monitoring services, allows for the collection and storage of incoming data and furnishes warning messages pertaining to ensuing system anomalies. At the initiation of a production run, the operator is prompted to input boundary conditions affecting culture pH, salinity, both room and culture temperature, lighting conditions and CO₂ injection frequency. At any time during a run, the operator may adjust any of these parameters without interrupting the normal execution of the program. Harvest rate frequency is automatically set at one harvest per hour (0.32 liters per min) per chamber and remains a variable, adjustable by the software. Additionally, the computer uses feedback information from the various probes and sensors to make stepwise adjustments in room temperature and CO₂ injection frequency to maintain culture temperature and pH within the initial boundary conditions. All information received by the computer is temporarily stored in an array, averaged and dumped to a diskette for permanent storage.
HARDWARE

The software program, "Supervisor", is executed by a computer control system consisting of a micro-computer, an interface device, input devices and output devices (Figure 5 and Table 4). The integration of these physical components with the carefully designed software control strategy produces a management tool that: 1) reduces long term operational costs through manual labor reduction, 2) facilitates high frequency data collection of system conditions and 3) provides a level of system management not easily achievable under manual operation.

The control point for all system components is a Zenith Z-184 Supersport laptop micro-computer (Zenith Data Systems, Inc.), containing an 80C88 CMOS 16-bit processor and 640K RAM. Unless detailed, on-site statistical analyses and graphical data representation are desired, more expensive and sophisticated computers add unnecessary capital costs to system construction. The Z-184 micro-computer is fully capable of executing the control program in addition to collecting and storing monitoring data obtained from system probes and sensors. Irrespective of the type, computers only understand digital information.

Therefore, central to the control system is the analog/digital (A/D) converter, ADC-1-B+12 (Remote Measurement Systems, Inc.), facilitating communication between the computer and the input and output devices through the computer's serial port (RS-232C). Data being transmitted and received by the A/D converter is accomplished using binary code (1 = on, 0 = off), 8 bits per byte. This serial interface connection requires no parity, allowing the full use of 8 bits for data.
Figure 5. The control system consists of a computer interfaced to input and output devices through an analog/digital converter. This schematic exemplifies the system controlling the algal turbidostat system.
Table 4. Major hardware elements required for a basic micro-computer control and monitoring system.

<table>
<thead>
<tr>
<th>Component</th>
<th>Cost</th>
<th>Manufacturer</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>Computer</td>
<td>$900-$1900</td>
<td>Variable</td>
<td>- Provides logical control of all system components</td>
</tr>
<tr>
<td>Analog/Digital Converter</td>
<td></td>
<td>Remote Measurement Systems, Inc.</td>
<td>- Provides primary interface between the computer and input/output devices</td>
</tr>
<tr>
<td>ADC-1-B+12</td>
<td>$613</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Input Devices</td>
<td>See Table 2</td>
<td>Item Dependent</td>
<td>- Probes/sensors providing monitoring data</td>
</tr>
<tr>
<td>Signal Modifier</td>
<td>Input Device</td>
<td>See Table 2 for example</td>
<td>- Electronic components frequently required to assure sensor signal compatibility with analog/digital converter</td>
</tr>
<tr>
<td></td>
<td>Specific</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Output Devices</td>
<td>System Specific</td>
<td>Item Dependent</td>
<td>- Pumps, heaters, valves, and other units which are physically activated by the system</td>
</tr>
<tr>
<td>Multiport Controller 524</td>
<td>$299</td>
<td>Remote Measurement Systems, Inc.</td>
<td>- Permits up to four peripheral devices to be interfaced to the computer’s RS-232 port</td>
</tr>
<tr>
<td>Transformers (optional)</td>
<td>$10 - $40 (each)</td>
<td>Local Electronics Store</td>
<td>- Facilitates voltage decreases from 110 volts to 24 volts</td>
</tr>
<tr>
<td>Solid State Relays 110 volts, 10 amps</td>
<td>$10/each</td>
<td>Local Electronics Store</td>
<td>- Switching devices that use low voltage output from the A/D converter to provide 'on'/'off' control of output devices</td>
</tr>
</tbody>
</table>
Therefore, at an operating baud rate of 9600, the unit is capable of converting 960 characters per second. The initialization of the RS-232C port is accomplished through the software. The ADC-1-B+12 provides for the conversion of analog signals (electronic pulses of constant voltage) produced by input devices to digital (binary) code understood by the computer. Conversely, the analog/digital converter also functions as a control device, facilitating the conversion of digital signals from the computer into electronic pulses that implement control actions. Control of output devices is often accomplished by an intermediate electronic relay that uses low voltage output signals from the converter to execute the desired operation.

The ADC-1-B+12 contains 16 analog input channels, 4 digital input channels and 12 TTL (transistor transistor logic) controlled output channels. The unit consumes only 20 mA at +5VDC. The converter is an integrating slope A/D, using an Intersil 7109 analog/digital conversion chip. For precise control and monitoring of the turbidostat system, the unit is operated at a processing speed of 20 Hz and a resolution of 12 bit, plus sign bit. At this low speed conversion rate, the converter provides for automatic channel zeroing after each sampling, thereby minimizing noise levels and increasing data transfer reliability. This processing speed enables an analog channel sampling frequency of 10-20 readings per second, allowing nearly simultaneous execution of system commands. Due to the 12 bit conversion resolution, information transmitted to the computer following the conversion process is contained in two 8 bit bytes; a high and low byte. Bits 4-7 of the high byte contain conversion information including the status of the conversion process, while bits 0-3 and 0-7 of the low byte contain the actual desired data. Before the
response signal can be transmitted from the ADC-1-B+12 to the computer, bits 3-7 of the high byte must be masked, and the remaining bits combined (Remote Measurement Systems, Inc.).

The fully differential analog channels receive signals from precise input devices (photovoltaic cells, temperature, pH, conductivity, etc.), calculate the voltage difference between the positive and negative posts and transmit this difference to the 12-bit A/D converter. The analog channels have an input voltage range of ±0.4095 V, with a resolution of 100 μV. Most sensors and probes work within the ±0.4095 V range; however, for those probes with higher voltage requirements, voltage dividers, made from two resistors, may be connected to the input channels in question or signal conditioning components may be installed between the sensor and the ADC-1-B+12 to modify the electronic signal produced by the input device to fall within the range of the interface unit. If the majority of the probes have outputs outside the standard voltage range, an internal circuitry modification may be made to increase the voltage level to ± 4.0 V, with a resolution of 1.0 mV. Many of the newer probes and sensors produce voltage outputs tremendously below the ±0.4095 V. In this case, an amplifier can be installed that may increase the output voltage by as much as 50 times.

Table 5 presents a list of the analog input devices utilized for monitoring both room and culture conditions for the turbidostat system. Often, the demise of many algal production systems stems from poor management decisions due to the lack of a well planned monitoring program. Within the turbidostat system, the lack of maintaining the algal culture in the exponential growth phase through harvest rate
Table 5. Common input devices used for monitoring within a micro-computer automated aquaculture system.

<table>
<thead>
<tr>
<th>Device</th>
<th>Cost</th>
<th>Manufacturer</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td></td>
<td>Cole Parmer</td>
<td>maintenance of proper level for animal growth</td>
</tr>
<tr>
<td>Transmitter</td>
<td>$225</td>
<td></td>
<td>maintenance of optimal level for nitrification</td>
</tr>
<tr>
<td>Probe</td>
<td>$98</td>
<td></td>
<td>maintenance of optimal level for algal growth</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>verification of buffer and CO₂ operations</td>
</tr>
<tr>
<td>Conductivity Controller</td>
<td>$249</td>
<td>Cole Parmer</td>
<td>maintenance of salinity control</td>
</tr>
<tr>
<td>Probe</td>
<td>$100</td>
<td></td>
<td>verification of buffer and nutrient additions</td>
</tr>
<tr>
<td>Photovoltaic Cell</td>
<td>$11</td>
<td>Remote Measurement Systems, Inc.</td>
<td>measurement of algal cell density</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>monitoring of long-term source light degradation</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>detection of 'on'/'off' status of lights within a system</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>activate supplemental artificial lighting on cloudy days for systems with natural lighting</td>
</tr>
<tr>
<td>Level Detector Stainless Steel Rods</td>
<td>&lt;$1 ea.</td>
<td>Local Welding Supply</td>
<td>verification of draining/filling operations</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>detection of floods/spills</td>
</tr>
<tr>
<td>Temperature Transducer</td>
<td>$8/ea.</td>
<td>Remote Measurement Systems, Inc.</td>
<td>monitor room and system temperature</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>activate system heaters or chillers</td>
</tr>
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</table>
manipulation undoubtedly leads to culture collapse. Although manually this is a tedious and time-consuming task, implementation of the control and monitoring algorithm allows for the continuous alteration of the harvest rate and subsequent maintenance of steadystate production levels quickly and easily. It is imperative, therefore, that each culture chamber within a facility be closely monitored to detect signs of oncoming failure. In a facility with multiple chambers, the need for redundant probes can be eliminated by using a central monitoring block that draws from each tank. This arrangement not only reduces the number of required probes and sensors, but also decreases the amount of maintenance necessary to keep the probes in working order. Intermittently recirculating a mild chlorox solution through the monitoring chamber has been found to extend the longevity and accuracy of the probes.

While the analog input channels receive information that is converted and transmitted to the computer for decision making processes, the controlled outputs activate external 'on'/off devices either in response to incoming information of as part of a predetermined routine command contained within the "stack". The controlled outputs are latched TTL drivers with a maximum current of 200 mA at 5VDC; the current being supplied by the ADC-1-B+12's internal power supply. Output devices (air/vacuum pump, lights, level detectors, air conditioner, etc.) are actuated by an intermediate switch, a 10 Amp solid state electronic relay. The positive lead of the relay is connected to one of the 12 controlled outputs, while all negative leads are attached to the ADC-1-B+12's ground (GND) terminal. Based on response signals from the computer, the ADC-1-B+12 supplies +5VDC and
approximately a 5mA current from its internal power supply to activate a specific output, closing the normally open circuit and turning 'on' the external device. Similarly, an opposite signal re-opens the circuit, turning off the external device. Determining which controlled outputs are to be activated is accomplished by summing the bit values for all set bits (0-5) and adding the resultant to a base value (Remote Measurement Systems, Inc):

\[
\]

\[
\]

where,

\[ cs = \text{a 12 element array holding the set value for each controlled output} \]

\[ ctoutput = \text{contains the byte value used to activate the controlled outputs} \]

Each bit has a numeric value associated with the binary system, with bit 0 having a numeric value of 1 when set. Output channels 1-6 are differentiated from 7-12 by a base value determined by the set condition of bits 6 and 7. Channels 1-6 have a base value of 64 (6 set, 7 not set), while channels 7 through 12 have a value of 192 (both bits set).

The number of external output devices utilized by the turbidostat system requires the employment of two ADC-1-B+12 units for extended controlled output capabilities. A multiport controller (Bay Technical Associates, Inc., Bay St. Louis, MS) an interface device between the computer and the ADC-1-B+12 units, allows four peripheral units to gain access to the computer's RS-232C serial port. Communication between the computer and a particular ADC-1-B+12 unit is
performed automatically through the software. With this type of supporting technology, multiple systems within an aquaculture facility can be simultaneously controlled by one central computer unit.

APPLICATION OF THE CONTROL AND MONITORING SYSTEM

The software program, "Supervisor" has been integrated with the algal turbidostat, lending extensive control support and providing data collection services not easily achievable under manually operational conditions. The success of system operation stems from the computer's ability to not only execute commands already contained within the "stack", but also to use the information transmitted by the input devices to maintain certain parameters within initial boundary conditions and to make adjustments to other parameters influencing algal growth. Central to the operation of the turbidostat is the maintenance of a dilution rate that results in sustained high production levels. Therefore, harvest frequency, which determines the dilution rate, remains a variable, controllable and adjustable by the software in response to changing conditions within the culture chambers. Decisions concerning harvest frequency modifications is based on feedback information received from the photocell contained within the monitoring block. The harvest frequency is set at one harvest per chamber per hour at the start of a production run. Decisions concerning dilution rate adjustments are made over three consecutive harvest cycles. Standing crop estimates are stored in a temporary array and compared after the third harvest cycle of the decision period is completed. If standing crop concentrations estimated during the third harvest period are the same or greater than those obtained for the first harvest period, the frequency is increased stepwise, resulting in increased
production levels. If however, the reverse situation occurs, the computer reduces the dilution rate in an effort to re-establish stable standing crop concentrations.

The reliability of the interaction between the control system and the monitoring devices (photocell) is exhibited in Figures 6 and 7. The regression between millivolt output from the photocell and analytically measured TSS resulted in an $R^2 = 0.87$ (Figure 6), with no noticeable drift over the ten month study. Weekly calibration checks of the photovoltaic cell did not detect any significant ($P<0.05$) drift in output. A further demonstration of the reliable interaction between the control system and monitoring devices (photocell) is exhibited in Figure 7. *Chaetoceros muelleri* (Chaet 10) was cultured in the turbidostat under continuous lighting from 250W metal halide lamps and subjected to a temperature, salinity and pH of 30±1°C, 35±1 ppt and 7.8±0.2, respectively. During the production run, the computer monitored standing crop concentrations and modified the harvest rate accordingly based on the criteria previously established. The computer estimated standing crop concentrations did not significantly differ from those obtained by analytical TSS measurements (APHA, 1989). This is of importance to commercial facility operators who generally rely on either visual observations or manual counting methods for culture density estimates. Visual observations are quick, but do not give a quantitative measurement of the culture; and in contrast, manual estimates are precise but tedious and time consuming. Harvest rates were maintained between 0.39 - 0.86 days$^{-1}$ and 0.54 - 0.86 days$^{-1}$ for chambers one and two, respectively.
Figure 6. Millivolt signals transmitted from the photovoltaic cell were regressed against analytically measured algal standing crop.

Figure 7. Estimated and analytically measured standing crop concentrations and dilution rates for *Chaetoceros muelleri* (Chaet 10) under continuous lighting at 30°C.
Culture pH and temperature (Figure 8) were maintained within the desired boundaries through automatic adjustments to the CO$_2$ injection frequency and room temperature (Figure 8). Additionally, both chambers exhibited very similar temperature and pH patterns, lending evidence to the computer's ability to maintain the same culture conditions within a series of growth chambers. The observed variations for both pH and temperature may be attributed to the discontinuous monitoring method employed with this system. Culture conditions were measured only during harvesting events; subsequently, software adjustments of these parameters were limited to that same time period. The lower the harvest frequency,
the more pronounced the effects. These variations could be reduced by implementing a more frequent monitoring schedule, particularly for stabilizing pH within the cultures.

The turbidostat system, under complete computer control, is capable of daily production levels of $1 \times 10^{12}$ cells/day of *Chaetoceros muelleri* (Chaet 10) compared to $7.6 \times 10^{10}$ cells/day obtained by a batch culture of similar volume (David Head, personal communication). Increased production levels per unit volume or area facilitates the use of smaller culture volumes, resulting in lower labor requirements for operation and maintenance. The integration of the turbidostat with the versatile software algorithm creates a passive system, reducing manual labor requirements even further, and allowing management efforts to be redirected.

**ISSUES**

The control and monitoring algorithm was developed as a generic software tool to aid in the advancement aquaculture (both research and industry), and has proven to be a reliable and stable management device for the turbidostat during the last four and one-half years. The main advantage of "Supervisor" is the integration of three core elements that results in a flexible programming environment adaptable to a broad spectrum of applications. The independent relation between the various operations creates a programming structure that is easily modified to fit the customized needs of any individual system without altering the core program. "Supervisor" facilitates the type of integrated system designs that can aid in reducing algal production costs within nurseries and grow-out facilities. While the control and monitoring algorithm described in this paper is a powerful control and
management tool, operators must understand the needs of their systems. This tool is not meant to completely replace manual supervision, but to increase operational efficiency and reduce costs through complimentary control and monitoring methods. "Supervisor" is most suited for research activities and customized smaller facilities where frequent programming adjustments are desired. The authors do agree that, at this time, larger commercial facilities that do not require frequent program modifications may find "CANNED" programs to be more cost-effective.

Although "Supervisor" allows programming freedom, it is limited by its one-dimensional, temporal structure. Program execution is based on chronological or prioritized operations, resulting in slight time delays (less than one second) for operations with identical initial execution times. For the majority of applications, this is unnoticeable by the operator. The algorithm does not have the capability to distinguish between the importance of simultaneous operations. Consequently, for these types of situations, execution of one of the operations may not necessarily take place at its exact designated execution time. This situation could be mitigated by the development of a two-dimensional algorithm that would not only sort by chronological time but also by the relative importance of the operation, assuring exact execution.

The overall reliability of this technology depends to a large extent on 1) the stability of the supporting control system and 2) the accuracy and precision of the data obtained from the monitoring instruments. In addressing the first issue, the operator must be concerned with the conditions under which both the control components and the input and output devices are exposed; especially in saltwater
situations where the probability of an electronic failure is dramatically increased due to salt spray. Corrosion of the control system is easily mitigated by maintaining the components in a separate, air-conditioned room. Secondly, data reliability can be assured by 1) developing a weekly calibration routine for all probes and sensors, 2) incorporating a chlorox loop in the monitoring block to mitigate biofouling of the probes and 3) incorporating insightful verification loops and data collection processes.

The cost of implementing this basic control and monitoring system depends on the selection of the supporting technology. Both Tables 4 and 5 include cost estimates for components commonly used in aquaculture systems, while Table 6 provides a capital cost breakdown of the algal turbidostat, showing the relative cost of the control components to the overall system. The core control system for the algal turbidostat, excluding monitoring probes and sensors, can be implemented for less than $3,300 or 20% of the total system set-up cost. As the economy of scale increases, this component of total system cost decreases. Additionally, this 20% may be reclaimed in the long run by reduced manual labor requirements. For instance, the computerized turbidostat requires approximately one-half hour per day of manual supervision. By comparison, a batch culture system of similar size under maximum operation requires 35 hours per week of manual labor (Dave Head, Personal Communication, 1992), an order of magnitude greater than warranted by the turbidostat system. Set-up costs may be reduced by: 1) the use of a centralized monitoring unit and 2) careful selection of measurement devices. Device costs can be lowered by the prudent selection of reliable, yet cost-effective
Table 6. Capital cost estimate for the computer automated algal turbidostat.

<table>
<thead>
<tr>
<th>Component</th>
<th>Cost</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Control System</strong></td>
<td></td>
</tr>
<tr>
<td>Computer</td>
<td>$1,400.00</td>
</tr>
<tr>
<td>ADC-1-B+12 (2)</td>
<td>$1,200.00</td>
</tr>
<tr>
<td>Multiport Controller 524</td>
<td>$299.00</td>
</tr>
<tr>
<td>Relay Boxes (2)</td>
<td>$300.00</td>
</tr>
<tr>
<td>Transformers (7-optimal)</td>
<td>$70.00</td>
</tr>
<tr>
<td><strong>Input Devices</strong></td>
<td>$3,269.00</td>
</tr>
<tr>
<td>pH Transmitter/Probe</td>
<td>$390.00</td>
</tr>
<tr>
<td>Conductivity Transmitter/Probe</td>
<td>$390.00</td>
</tr>
<tr>
<td>Temperature Sensor (3)</td>
<td>$24.00</td>
</tr>
<tr>
<td>Solar Cells (6)</td>
<td>$66.00</td>
</tr>
<tr>
<td>Level Detectors (4)</td>
<td>$&lt;5.00</td>
</tr>
<tr>
<td><strong>Output Devices</strong></td>
<td>$875.00</td>
</tr>
<tr>
<td>Solenoid Valves (5)</td>
<td>$650.00</td>
</tr>
<tr>
<td>(2)</td>
<td>$60.00</td>
</tr>
<tr>
<td>Actuated Ball Valves (4)</td>
<td>$600.00</td>
</tr>
<tr>
<td>Metal Halide Lamps (2)</td>
<td>$340.00</td>
</tr>
<tr>
<td>Air/Vacuum Pump</td>
<td>$320.00</td>
</tr>
<tr>
<td>Air Conditioner</td>
<td>$400.00</td>
</tr>
<tr>
<td>Heater</td>
<td>$30.00</td>
</tr>
<tr>
<td>UV Lights (2)</td>
<td>$240.00</td>
</tr>
<tr>
<td>Pumps</td>
<td>$200.00</td>
</tr>
<tr>
<td>Centrifuge</td>
<td>$7,500.00</td>
</tr>
<tr>
<td><strong>Miscellaneous Components</strong></td>
<td>$10,340.00</td>
</tr>
<tr>
<td>Culture Chamber (2)</td>
<td>$800.00</td>
</tr>
<tr>
<td>Harvest Chamber</td>
<td>$100.00</td>
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<tr>
<td>Activated Carbon Column</td>
<td>$100.00</td>
</tr>
<tr>
<td>Exhaust Fan</td>
<td>$75.00</td>
</tr>
<tr>
<td>Gauges (Vacuum, Pressure) (3)</td>
<td>$65.00</td>
</tr>
<tr>
<td>Pressure Reducer/Relief Valve</td>
<td>$50.00</td>
</tr>
<tr>
<td>Check Valves (6)</td>
<td>$50.00</td>
</tr>
<tr>
<td>PVC Pipe, Acrylic, Fittings</td>
<td>$500.00</td>
</tr>
<tr>
<td>Electronic Supplies</td>
<td>$100.00</td>
</tr>
<tr>
<td></td>
<td>$1,800.00</td>
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<tr>
<td></td>
<td>======</td>
</tr>
<tr>
<td></td>
<td>$16,324.00</td>
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</tbody>
</table>
probes and sensors. For example, the determination of harvest rate depends on information transmitted by an $11.00 photovoltaic cell. As a comparison, the analytical determination of algal biomass costs approximately $1.30 per sample if run within the authors' analytical laboratory. Consequently, continual monitoring of one growth chamber over a single 24 hour period (1 sample per hour) would cost $31.00, the price of three solar cells.

With the aquaculture industry moving in the direction of intensive culture systems, management practices will become crucial. The control and monitoring approach presented in this paper has proved reliable for complete control of the turbidostat, with a substantial reduction in labor requirements. Regardless of the degree of support given by the control system, the end result is a reduction in operational costs over manually operated systems.
CHAPTER III

DEVELOPMENT OF A COMPUTERIZED TURBIDOSTAT FOR THE CONTINUOUS PRODUCTION OF MICROALGAE

INTRODUCTION

Although the aquaculture industry is experiencing a movement from extensive to intensive culture methods in an attempt to address both consumer demands and the present status of the natural fisheries, the replacement of live microalgae with inert foods as a feed source remains unproven. Therefore, the reliance on live microalgae for rearing commercially important species including molluscs, crustaceans and finfish remains an important aquaculture component. Live microalgal production continues to be the economic bottleneck in many operations. This paper presents a description of a computerized production-scale algal turbidostat developed as an alternative to present culture methods and summarizes production capabilities for Chaetoceros muelleri (Chaet 10) cultured under varying temperature and light conditions.

Background

Microalgae are the base of the food chain for several aquacultural species, providing both a direct (molluscs and larval and juvenile crustaceans and finfish) and indirect (rotifers, brine shrimp and copepods) source of nutrition. The aquaculture industry, in particular molluscan operations, has found it difficult to economically culture the large quantities of algae required for nurseries and grow-out facilities without sacrificing nutritional quality and control. The reliance on natural phytoplankton and induced blooms, while cost-effective (U.S. $4 - $23/kg-dry wt, unharvested), suffers several drawbacks including lack of species control,
contamination, predation and poor control over quantity and quality (Persoone and Claus, 1980; Ukeles, 1980; Claus, 1981; DePauw, 1981; Riva and Lelong, 1981; DePauw et al., 1983). The movement to outdoor cultivation ponds mitigates some of these inherent drawbacks. Recently, Walsh et al., (1987) developed a continuous outdoor cultivation pond, resulting in yields of 8 - 10 g/m$^2$/day-dry wt for May through September and 3 g/m$^2$/day-dry wt for November through December. These lower levels of production result in area requirements typically greater than 100 m$^2$ to produce the quantity of algae required by most commercial facilities (Claus, 1981; Witt et al., 1981; DePauw et al., 1983; Walsh et al., 1987; Boussiba et al., 1988). Although the outdoor pond does often suffer from short lived cultures (batch), competition from undesired species and other organisms and poor environmental control, it is to date, the most reliable technology for large-scale algal production.

The inefficiencies of outdoor production methods have been addressed, to a certain extent, by the use of indoor, monospecific batch cultures, however, at higher production costs. Walne (1974) and DePauw (1981) have estimated production costs at U.S. $120 - $200/kg-dry wt (unharvested), limiting this technology to rather low demand applications, such as hatcheries. The use of natural phytoplankton populations, induced blooms and outdoor cultivation ponds for large-scale applications and indoor, monospecific batch cultures for lower quantity demands are well established methods for algal production, but result in characteristically low production levels per unit area or volume. Continuous cultures are more suited for the controlled production of algae because: 1) cell composition remains
homogeneous, 2) the culture remains in the exponential stage, increasing production runs from a few weeks as observed with batch cultures to months and 3) the steadystate nature of the culture lends itself to automation.

Continuous cultures are divided into two types based on the mode of operation: chemostats and turbidostats. However, the theoretical considerations governing the two are the same (Monod, 1942; Herbert et al., 1956; Herbert, 1958; Munson, 1970; Tempest, 1970; Goldman, 1977). Within chemostat cultures, the dilution rate is set by the operator and maintained constant, allowing steadystate conditions to be reached. Hence, the specific growth rate is theoretically equivalent to the dilution rate. In this mode of operation, biomass production is dependent on the set dilution rate. Turbidostat cultures, on the other hand, maintain a constant biomass population within the growth chamber by using a photocell to trigger a harvest response. Under these conditions, growth rates change in response to the harvest events. At some point, usually high dilution rates, steadystate conditions are reached and the specific growth rate will again be equivalent to the dilution rate. Therefore, biomass populations are the independent variable under turbidostat condition; and this factor may provide an advantage over chemostats in aquaculture applications where production levels are one of the main issues.

Although the principles of continuous cultures are well established, the integration of this technology with computer automation and its subsequent dissemination within the aquaculture industry is slow. Recent literature on computer automated continuous cultures is scarce. However, the few documented studies have shown tremendous production increases over traditional batch cultures (Hill et
al., 1985; James et al., 1988; Wangersky et al., 1989). James, et al. (1988), for example, reported average production levels of 108 g/m²/day-dry wt for *Chlorella MFD-1*, a tenfold increase over the production levels obtained by Walsh et al. (1987) for outdoor continuous ponds.

**METHODS AND MATERIALS**

*System Design for Turbidostat*

Development of the turbidostat involved a three-stage design rationale: 1) modularization, 2) contamination minimization and 3) self-cleaning capabilities; all aimed at the reduction of manual labor and optimization of production capabilities and reliability. Modularization mitigates system complexity by facilitating quick and easy expansion and replacement or modification of system components.

Contamination prevention was addressed by four basic control strategies:

1) maintenance of positive air pressure at all points within the system to minimize the entrance of airborne bacteria, zooplankton or other undesirable algal species, 2) prefiltration of the incoming air, 3) maintenance of an air pocket between the culture and any lighted surfaces, reducing the potential for attached growth that could effect light penetration into the culture and 4) utilization of the capabilities of the computer to maintain optimal growth conditions of the cultured algal species within the system at all times, minimizing competition by contaminants, if present.

The production-scale turbidostat contains ten interdependent modules (Figure 9), utilizing a centralized dosing apparatus and monitoring block to minimize redundancy of components. Wherever appropriate, low-cost, yet reliable, components were utilized, reducing overall system cost. The system contains two
Figure 9. The computerized algal turbidostat contains ten interdependent components including a centralized monitoring unit and pressurized dosing apparatus to reduce component redundancy. All system processes are executed by a computer control system not shown here.
fiberglass growth chambers containing 48° conical bottoms to prevent the settlement of algae. Each chamber is 1.22 m (4.00') in diameter and 0.99 m (3.24') deep with an approximate volume of 0.6 m³ (159 gallons). The inside surface is coated with white gelcoat to provide maximum reflectance of light back into the culture and creating a smooth surface, thereby, reducing the probability of attached growth. The chambers are covered with 0.64 cm (0.25") thick clear acrylic, facilitating overhead illumination and preventing the entrance of airborne contaminants. The overhead illumination, combined with the air pocket, provides a buffer zone between the acrylic cover and algal culture, reducing the chance for attached growth on lighted surfaces. A simple airlift system provides the culture with aeration, suspension and mixing. The airlift, constructed from 5.08 cm (2.00") diameter PVC pipe, creates a circular flow pattern, aiding in the reduction of settling and continuously moving individual algal cells to the lighted surface, maximizing photosynthetic potential. Any algae settling out of suspension slide down the sloped sides, collect at the bottom of the cone and are picked up by the airlift and reintroduced into the culture. Excess air is vented out of the chambers through a 1.9 cm (0.75") diameter filtered outlet. The introduction of air and solutions and withdrawal of algae is accomplished using a common line passing through the acrylic cover and connecting into the bottom of the airlift.

The entire system operates from a single 0.37 kW (0.5 HP) piston air/vacuum pump (Thomas, Model No. 1007CK72) rated for continuous duty and a series of electronic solenoid and actuated ball valves, reducing the number of pumps required for solution movement and facilitating the use of a common
manifold line for liquid and air flows. Following filtration through a 0.45 μm prefilter and a 0.2 μm capsule filter, air is delivered to each airlift at a rate of 0.05 m³/min (1.75 SCFM) at less than 0.14 kg/cm² (2 psi), creating a circular flow pattern. Carbon dioxide is intermittently injected into the air manifold at a rate of 3 - 5% of the airflow. The frequency of addition is determined by the computer and is dependent on the culture pH. In addition to aeration, the pressure side of the pump facilitates bulk solution movement into the culture chambers, while the vacuum effects movement of algae from the chambers at an approximate rate of 0.01 m³/min (0.35 SCFM) at 381mm of Hg (15” of Hg).

All solutions required to maintain proper nutritional conditions are introduced into the chambers via a central pressurized dosing apparatus (Figure 10). Each dosing unit is constructed from 10.20 cm (4.00") diameter SCH 40 PVC and contains 2.50 liters of concentrated media. The dosing apparatus operates on basic air displacement principles, eliminating the need for the expensive metering pumps commonly used. Media from stock solution tanks gravity feeds into the dosing units through 1.27 cm (0.50" o.d.) diameter tygon tubing, maintaining the dosing units at full volume capacity at all times. The final volume contained within the dosing unit is 2.50 liters plus that volume contained in the tubing to the level of the stock solution tanks. The amount of each dose is set by a 0.64 cm (0.25" o.d.) diameter adjustable acrylic tube. Each 0.64 cm (0.25") adjustment up or down corresponds to a dosing volume of approximately 50 ml. Air from the primary air manifold is diverted to the secondary air manifold by activating a 0.48 cm (0.19") normally
Figure 10. Fresh media and disinfectant are added to the culture chambers by a centralized dosing apparatus consisting of a series of dosing units operated pneumatically.
closed electronic solenoid valve. Air forced into the dosing units causes the solution to be displaced into the solution manifold which feeds into the main manifold. Once the solution level within the dosing unit falls below the bottom of the acrylic adjustment tube, solution addition stops. Based on the dosage volume, this process takes anywhere from 5 - 15 seconds. During the dosing event, solution from the stock solution tank is prevented from entering the dosing unit by a check valve forced closed by the air pressure. Once the dosing cycle is completed, the units refill and are ready for the next dosing event. The accuracy of the dose is limited mainly by the volume of solution contained within the air inlet and solution outlet tubes. As the dosing units fill, the level rises within the unit and the tubes until an equilibrium is reached with the level within the stock solution tanks, resulting in less than a 5 ml discrepancy between the assumed and actual dosage volumes. Unless the utmost accuracy is required for experimental studies, the use of this type of dosing apparatus has proven to be a reliable and cost-effective means for media addition, with material costs less than $8/unit.

The 0.15 m³ fiberglass harvest chamber serves as the collection and distribution point for algae withdrawn from the culture chambers. The exact configuration of this component is site specific, depending on the end-use of the algae. During harvesting, a vacuum is created within the harvest chamber by closing the 1.90 cm (0.75") normally open electronic solenoid valve located on the vacuum side of the pump and the 1.90 cm (0.75") actuated ball valve on the main manifold. Following deactivation of the vacuum, the check valve on the outlet of the chamber opens, and the algae is pumped outside the room to a 1.12 kW (1.50 HP) continuous flow
centrifuge (A.M.L. Industries, Inc., Model No. B30S) that functions both to concentrate the algae into a paste and to reclaim the saltwater for reuse within the culture system. The two chamber culture system utilizes approximately 710 pounds of salt per week (full strength artificial saltwater, Instant Ocean\(^1\)) at $0.70 per pound; this is by far the highest operating cost. To circumvent part of this expense, a recirculating saltwater conservation system was installed in-line following the centrifuge. The saltwater treatment systems consists of a 0.78 m\(^3\) (200 gallon) reservoir from which the saltwater is recirculated through an upflow sand filter (0.84-1.68 mm sand) to trap excess algae and an UV unit for disinfection prior to re-entering the reservoir. Approximately 5% of the saltwater within the reservoir is replaced daily to mitigate the build-up of metabolites and unused trace elements and vitamins. This process reduces the required salt to about 50 pounds per week, a substantial savings of operating expenses. The use of the centrifuge also enables the algae to be concentrated into a paste that is refrigerated and used as needed.

Conditions within each chamber are monitored using a central monitoring unit containing a temperature sensor, pH probe, conductivity probe and photocell for turbidity measurements. Algae harvested from the chambers are routed through the monitoring unit prior to reaching the harvest chamber. Temperature readings are accomplished using an $8$ solid state temperature transducer (Remote Measurement Systems, Inc., Model No. Ad590JH). pH and conductivity measurements were taken using standard in-line probes. Standing crop estimations

\(^1\)The use of this product does not represent an endorsement.
were made using a photocell that measured the light transmitted by the culture. The photocell was inexpensively constructed from 5.10 cm (2.00" i.d.) diameter clear acrylic tube, an $11 photovoltaic cell (Remote Measurement Systems, Inc., Model No. PVC) and a fluorescent light source (Figure 11). The photocell was calibrated by measuring millivolt output at various analytically determined algal concentrations. A standard curve was calculated and incorporated into the software. During system operation, millivolt (mv) readings corresponding to the amount of light transmitted through the culture are sent to the computer and converted to standing crop concentrations (TSS) by:

\[
\text{Standing Crop (g/m}^3) = 541.1735 - \frac{6.1551848}{\text{mv}} \quad R^2=0.87
\]

providing an inexpensive, but reliable method for estimating culture densities within commercial facilities. The error associated with this photocell can be attributed to the scattered light reaching the photocell. This was minimized as much as possible by focusing the transmitted light on the photovoltaic cell.

Daily processes are executed using a micro-computer based control and monitoring system (Rusch and Malone, 1989; Rusch and Malone, 1990), providing an alternative to control methods described in the literature. Most systems employ photoelectric/electromechanical devices (i.e., the cadmium sulfide photo-conductive cell) that increase in resistance with increasing turbidity until surpassing a high set point which triggers a relay controlling either a solenoid valve or pump for fresh media input (Munson, 1970; Sorgeloos et al., 1976; Skipnes et al., 1980; Laing and Jones, 1983). Although relatively reliable, once the set points are set, manual adjustments are required to change these trigger points in response to changing
Decision making processes concerning harvest rate adjustments are dependent on feedback information received from the photocell, measuring light transmitted from the culture.
external conditions. The micro-computer based system (Figure 12) provides for
information feedback, allowing the computer to make adjustments through the
software in response to changing conditions within the culture, thereby,
circumventing manual manipulation. For instance, the harvest rate is dependent
upon the standing crop concentration within the growth chambers and remains a
random variable controlled by the software. The micro-computer assimilates
information transmitted from the probes and sensors contained within the monitoring
block, adjusting the harvest rate accordingly. Maintaining standing crop
concentrations and appropriate harvest rates helps sustain high production levels.

The turbidostat system is controlled using a Zenith Z-184 Supersport laptop
micro-computer interfaced to input and output devices through an analog to digital
converter (Remote Measurement Systems, Inc., ADC-1-B+12). Two A/D converters,
each containing 16 analog input channels four digital input channels and 12
controlled output channels are used to accommodate increased controlled output
capacity. The units are connected to the RS-232C port of the computer via a
multiport controller (Baytech, Model No. 524) that functions as a switching device,
allowing both ADC units access to the computer. Electronic pulses of constant
voltage are transmitted from the probes and sensors to the analog input channels
which convert these signals to binary code understood by the computer. Incoming
information from the monitoring block and room sensors is used by the software to
effectuate procedural decisions and to activate output devices through the controlled
output channels.
Figure 12. Control and monitoring of the turbidostat is accomplished by a computer-based system interfaced to input and output devices through an analog to digital converter.
The control and monitoring algorithms are executed using the software program, "Supervisor", written in Turbo Pascal 4.0 (Borland, 1987). The program contains three core elements: 1) the "stack", 2) the "stack sorter" and 3) the "supervisor". The integration of these components creates a flexible programming environment that mitigates temporal conflicts from simultaneous or nearly simultaneous procedures. The "stack" contains the list of commands and their associated execution times. The commands are loaded into the "stack" by the "stack sorter", a variation of a bubble sort routine (Miller, 1981; Zaks, 1986). The "stack sorter" also loads delayed commands and reloads just executed commands for the next execution cycle. The "supervisor" watches the time associated with the top command and the internal clock times and executes when the two are equal. The structure of this software program allows procedures and functions to be added and removed without effecting other procedures or the core elements.

System Operation

In principle, turbidostat systems operate by removing a portion of the culture when cell densities (standing crop) reach a predetermined point, and diluting until a low set point concentration is obtained (Munson, 1970; Laing and Jones, 1983; Rhee, 1989). The system described in this paper operates under slightly different criteria, allowing the computer to determine the high or maximum standing crop concentration within the growth chambers. At the start of a production run, the user is prompted to input values for several parameters including desired room temperature range, culture pH range, culture light duration, CO₂ addition frequency
and culture temperature. Once the system is under operation, the room temperature range and CO₂ remain variable, changing in response to culture temperature and pH.

The initial harvest rate is set at 0.32 liters of culture suspension/min/chamber (one harvest per hour). During the harvest cycle, the culture passes through the monitoring block where temperature, pH, conductivity and turbidity readings are taken. Ten measurements per parameter are taken at five second intervals and stored in a record of arrays. Following each harvest episode, the arrays are averaged, and the average values are used by the computer for decision making processes concerning harvest rate, CO₂ addition frequency and room temperature modification. After each harvest event, the data are dumped to a diskette.

Decisions concerning the harvest rate are made over three consecutive harvest cycles. If standing crop displays a stable or increasing trend, the harvest frequency is incrementally increased for the next harvest cycle. Increased harvest frequencies equates to increased production levels. As long as standing crop concentrations continue to increase, harvest frequency increases until an upper limit is reached; this upper boundary determined by the physical limitation of the system. Each chamber can be harvested every 20 minutes, resulting in an upper dilution rate of 2.56 days⁻¹. If, on the other hand, the standing crop displays a decreasing trend, the harvest frequency is incrementally decreased until X becomes stable again.

During normal operation, maintenance of culture volumes and determination of harvested volumes is accomplished using inexpensive level detectors constructed from two 2 mm (0.50") diameter stainless steel welding rods. The positive post of
the level detector was intermittently powered by the +5V controlled output on the ADC-1-B+12 unit. The conductivity of the water was sufficient to close the circuit when both posts are in submerged. Corrosion problems were mitigated by limiting output activation to one-second, with monitoring every five seconds during the harvest cycle and culture refill procedure.

Algal Cultures

Stock subcultures of *Chaetoceros muelleri* (Chaet 10) were obtained from our research collaborator located on Skidaway Island, Savannah, Georgia. Chaet 10 (division Chrysophyta, family Chaetoceraceae) has a reported apical length of 4 - 12 μm, setae length of 5 - 22 μm and temperature tolerance of 13 - 35°C (S.E.R.I., 1986). A two-stage batch culture process using 150 ml and 2 liter Erlenmeyer flasks was employed to provide the inocula for the 0.6 m³ (159 gallons) growth chambers. All glassware and water were disinfected with 20 ppm chlorine prior to use. Inoculum cultures were started by transferring 2 ml of the stock cultures into 150 ml of artificial saltwater (Instant Ocean) containing F/2 silicate, nitrate, phosphate, trace metals and vitamins (Guillard, 1974). Inoculum cultures were maintained on a shaker table at approximately 25°C, 35 ppt salinity and 4.26 W/m² using four 40W cool white fluorescent bulbs placed horizontally above the cultures. Each growth chamber was inoculated with 10 liters of stock culture.

Preliminary Production Studies Using the Turbidostat

The turbidostat was operated under varying temperature and lighting regimes to collect baseline data demonstrating the capabilities of the computer in maintaining standing crop, production and harvest levels for Chaet 10. Studies were performed
under four sets of conditions: 1) 28°C, 40W cool white fluorescent lights, continuous illumination, 2) 30°C, 250W metal halide lamps, continuous illumination, 3) 30°C, 250W metal halide lamps, 20:4 (L:D) and 4) 35°C, 250W metal halide lamps, continuous illumination. Surface illumination averaged 9.26 W/m² and 89.86 W/m² for the cool white fluorescent and metal halide lamps, respectively. Both lighting sources have a color spectrum beneficial for photosynthesis, with emissions in the photosynthetically active radiation (PAR) range (400 - 700 nm). The metal halide lamps have a more broad base spectrum, while the cool white fluorescent tend to be richer in the red range (630 - 700 nm). The actual quality of the light within this range was not investigated for either light source. The two 0.6 m³ culture chambers were subjected to the same environmental conditions and thus considered as replicates for each experiment. The 250W metal halide lamps are contained within High Intensity Discharge (HID) light fixtures (Lumark, Model No. MHSS-SA23-M-250MT). During Experiment 3, harvesting was inactivated during the dark period. For each study, pH was maintained between 7.6 - 8.0 by the computer through adjustments in the CO₂ injection frequency. Salinity was maintained at 35 ppt and was monitored with every harvest. Salinity adjustments were made in the 0.76 m³ recirculating reservoir, manually. After each harvest event nutrients were added at three times the F/2 level to prevent nutrient limitations. At the start of each study, values for room temperature, culture temperature, pH, light duration and CO₂ addition frequency were entered into the computer when prompted and used by the software to maintain the desired conditions within the temperature control room. The computer collected data
included both room temperature and light conditions, light intensity output from the 250W metal halide lamps and photocell source light, culture temperature, pH, salinity, standing crop concentration and harvest rate. Production levels were calculated with respect to both volume (\( P_v = XD \), where \( P_v = g/m^3/day-dry wt \); \( X = \) standing crop, g/m\(^3\)-dry wt; \( D = \) dilution rate, days\(^{-1}\)) and area (\( P_a = P_v/h \), where \( h = \) height of culture chamber, m). Areal production levels can be used to estimate space savings compared to other culture methods.

Culture samples were collected during the harvest cycle from a sampling port located between the harvest chamber and the centrifuge (at approximately 1430), two to three times a week for analytical determination of total suspended (TSS). The TSS data were used as a check against the estimation reliability of the regression equation used by the computer to convert turbidity readings (millivolts) to standing crop concentrations (g/m\(^3\)-dry wt). The collected samples were filtered through GF/C filters and dried at 105°C determine TSS (APHA, 1989). Intermittent samples were also collected from the centrifuge to: 1) calculate a regression equation between biomass wet weight and dry weight and 2) analyze for total lipid and protein analyses. Samples for total lipids and proteins were dried and frozen until analyzed. Lipid samples were analyzed in duplicate according to procedure AOAC 27.006 (AOAC, 1984), using a 2:1 chloroform:methanol solvent mixture.

RESULTS

Each experiment was performed over a 21-day period using *Chaetoceros muelleri* (Chaet 10), during which time standing crop estimates and harvest rates were collected by the computer. Production levels and harvested biomass were
Figure 13. Computer estimated (SC-Est) and analytically measured standing crop (SC-Anal), production and harvest levels for Chaet 10 at 28°C, under continuous lighting from six 40W CW fluorescent bulbs per chamber. (a) chamber 1 and (b) chamber 2.
Figure 14. Computer estimated (SC-Est) and analytically measured standing crop (SC-Anal), production and harvest levels for Chaet 10 at 30°C, under continuous lighting from one 250W metal halide lamp per chamber. (a) chamber 1 and (b) chamber 2.
Figure 15. Computer estimated (SC-Est) and analytically measured standing crop (SC-Anal), production and harvest levels for Chaet 10 at 30°C, under intermittent (20:4, L:D) from one 250W metal halide lamp per chamber. (a) chamber 1 and (b) chamber 2.
Figure 16. Computer estimated (SC-Est) and analytically measured standing crop (SC-Anal), production and harvest levels for Chael 10 at 35°C, under continuous lighting from one 250W metal halide lamp per chamber. (a) chamber 1 and (b) chamber 2.
then calculated based on daily harvest rates. Trends for these two parameters mimicked each other, differing only in magnitude. Figures 13 through 16 illustrate standing crop, production and harvested biomass trends over the specified experimental periods, and Table 7 presents summarized data for each study. The daily data plotted in Figures 13 through 16 was averaged over a six hour time period, resulting in four points per day. No significant difference (P<0.05) was observed between computer estimated standing crop concentrations and analytically measured values, except for chamber 2 (Experiment 3) which exhibited no difference at (P<0.01). However, on the average, the computer based standing crop estimates were 8.8% lower than those measured analytically. Steady state standing crop conditions, once obtained, were maintained throughout the course of Experiments 1 through 3. Cultures grown at 35°C (Experiment 4), however, showed signs of clumping after three weeks; and by the fourth week, standing crop concentrations decreased substantially.

Cultures grown under continuous lighting using 40W cool white fluorescent bulbs (average surface illumination of 9.26 W/m² per bulb) exhibited the lowest average standing crop concentrations (63 ± 15.9 g/m³-dry wt and 75 ± 17.5 g/m³-dry wt for chamber 1 and 2, respectively). Cultures grown at 30°C, under both continuous and intermittent (20:4, L:D) lighting from 250W metal halide lamps (average surface illumination of 89.86 W/m²) exhibited the highest average standing crop concentrations (Table 7), almost three times higher than obtained for cultures grown under fluorescent lighting. Hence, it appears that light intensity is indeed the limiting factor within the turbidostat. Due to the nature of the studies, it would be
Table 7. Production summary of *Chaetoceros muelleri* (Chaet 10) cultured under four different environmental regimes within the turbidostat (mean±STD, (range)). Production, standing crops, harvest, lipids and proteins are reported with respect to dry weights. (#1: 28°C, 40W CWF, continuous; #2: 30°C, 250W metal halide, continuous; #3: 30°C, 250W metal halide, 20:4 (L:D); #4: 35°C, 250W metal halide, continuous).

<table>
<thead>
<tr>
<th>Study</th>
<th>Harvest Rate (1/day)</th>
<th>Production (g/m³/day)</th>
<th>Production (g/m²/day)</th>
<th>Standing Crop (g/m³)</th>
<th>Harvest (g/day)</th>
<th>Lipids (%)</th>
<th>Protein (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>#1</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cham 1</td>
<td>0.86</td>
<td>54.2±13.7</td>
<td>50.9±12.9</td>
<td>63.3±15.9</td>
<td>24.7±6.2</td>
<td>6.7±0.9</td>
<td>27.4±3.1</td>
</tr>
<tr>
<td>Cham 2</td>
<td>0.86</td>
<td>64.7±15.0</td>
<td>60.8±14.0</td>
<td>75.5±17.5</td>
<td>29.5±6.9</td>
<td>9.3</td>
<td>29.6±5.8</td>
</tr>
<tr>
<td><strong>#2</strong></td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cham 1</td>
<td>0.62±0.19</td>
<td>121.7±46.5</td>
<td>114.4±43.8</td>
<td>199.9±47.5</td>
<td>42.3±25.6</td>
<td>9.3</td>
<td>29.6±5.8</td>
</tr>
<tr>
<td>Cham 2</td>
<td>0.65±0.14</td>
<td>124.2±33.5</td>
<td>116.7±31.5</td>
<td>192.2±39.1</td>
<td>44.2±19.0</td>
<td>10.5±1.8</td>
<td>22.2±5.4</td>
</tr>
<tr>
<td><strong>#3</strong></td>
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<tr>
<td>Cham 1</td>
<td>0.47±0.09</td>
<td>93.2±20.6</td>
<td>87.6±19.4</td>
<td>203.2±37.6</td>
<td>23.4±8.8</td>
<td>10.5±1.8</td>
<td>22.2±5.4</td>
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<td>Cham 2</td>
<td>0.45±0.08</td>
<td>88.1±20.6</td>
<td>83.8±19.3</td>
<td>195.1±34.8</td>
<td>21.8±8.6</td>
<td>10.4±1.0</td>
<td>18.5±5.2</td>
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<tr>
<td><strong>#4</strong></td>
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<td></td>
<td></td>
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</tr>
<tr>
<td>Cham 1</td>
<td>0.74±0.23</td>
<td>123.6±50.0</td>
<td>116.2±47.0</td>
<td>176.3±21.9</td>
<td>49.8±37.6</td>
<td>11.7±0.2</td>
<td>30.0±4.5</td>
</tr>
<tr>
<td>Cham 2</td>
<td>0.71±0.19</td>
<td>125.1±39.5</td>
<td>117.6±37.9</td>
<td>184.3±15.9</td>
<td>47.7±28.6</td>
<td>8.9±0.2</td>
<td>26.1±5.6</td>
</tr>
</tbody>
</table>
presumptuous to make definite conclusions related to the quality of light emitted from the two illumination sources. No significant difference (P<0.05) was observed between Experiments 2 and 3; however, standing crop concentrations obtained under intermittent lighting were artificially increased during the dark period in which the harvest cycle was bypassed. Elimination of these concentrations resulted in average concentrations that were significantly lower (P< 0.05) than those obtained under continuous lighting.

Production levels for Chaetoceros muelleri (Chaet 10) were determined using the estimated standing crop values and harvest rates controlled and adjusted by the computer (Table 7). Average volumetric production levels ranged from 54 g/m³/day-dry wt to 125 g/m³/day-dry wt for Experiments 1 and 4, respectively. This corresponds to over a 200% increase between fluorescent vis-a-vis metal halide lighting. Although, there was no significant difference (P<0.05) in estimated production levels at 30°C (123 g/m³/day-dry wt, average for both chambers) and 35°C (124 g/m³/day-dry wt, average for both chambers). Although cultures grown under intermittent lighting at 30°C exhibited the highest average standing crop concentrations, average production levels were only 74% of the levels obtained under continuous lighting from the metal halide lamps. This result, attributed to the residual growth taking place during the early stages of the dark period combined with the lower average harvest rate, provided a greater retention time, thereby allowing the standing crop concentration to increase. Conversely, the lower harvest
rate also reduced the production capabilities of the system. For all experiments, production levels fell within 95% confidence intervals about the means, indicating the scatter about the means can be attributed to random error.

The harvested biomass was a function of standing crop levels and harvest rate. Average harvested values of 27 g/day and 23 g/day were obtained for Experiments 1 and 3, respectively. On the other hand, Experiments 2 and 4 resulted in 43 g/day and 49 g/day, respectively; significantly (P<0.05) different from Experiments 1 and 3. The low harvested biomass observed for Experiment 1 was attributed to the lower standing crop concentrations in comparison with the other experiments, while low harvest rates influenced the decreased harvested biomass during Experiment 3. To provide a quick and easy method for estimating actual harvested biomass, the wet weight of the algae collected from the centrifuge was regressed against analytically measured TSS; the regression curve is illustrated in Figure 17. Dry biomass constituted 22%, on the average, of the wet algae collected from the centrifuge.

Total lipids and proteins were monitored to determine trend differences under the four experimental conditions (Table 7). Total lipids were statistically the same (P<0.05) for Experiments 2 through 4, differing only from Experiment 1. Total proteins, on the other hand, showed no significant difference between Experiments 1, 2, and 4, but did differ from Experiment 3 (P<0.05).
DISCUSSION

The operation of the turbidostat for sustaining production levels not obtainable by most methods currently utilized is influenced by the physical configuration of the apparatus in conjunction with a monitoring system capable of making procedural decisions based on feedback information. The critical element providing this linkage was the photocell. As indicated by the results, the photocell provided reliable and relatively accurate estimations of actual standing crop concentration within the growth chambers. The 8.8% observed average deviation of the computer estimates from analytical measurements can be attributed to: 1) inherent variation or drift in

Figure 17. Algae collected from the centrifuge (gm-wet wt) was dried and regressed against analytically measured TSS (gm-dry wt).

\[ TSS = 0.226254(WET), \quad R^2 = 0.9290. \]
the photovoltaic cell, 2) analytical error during TSS measurements, 3) variation in
the algae itself and perhaps 4) the measurement of transmitted versus scattered
light from the culture. The latter two explanations probably constitute the majority of
the observed variations. Algae are a dynamic, living microorganism, constantly
changing throughout its life cycle. These changes, mainly cell size and shape, do
not have a direct, linear impact on the relationship between total suspended solids
and turbidity. Consequently, deviations between the computer estimated and
analytically measured values cannot be predicted for this source of variation.
During the measurement of transmitted light, a certain amount of forward scattered
light will also be detected by the photovoltaic cell, causing deviations from the norm.
Although it is impossible to completely eliminate this forward scattered light, the
error can be minimized by focusing the light beam on the photovoltaic cell at a point
sufficiently remote from the culture (Munson, 1970). The accuracy and precision
might also be improved by incorporating a reference cell to detect drifts in intensity
output from the fluorescent light, thereby, providing a means for automatic
adjustment of the estimates. Keeping in mind the practical applications of this
system, a conservative deviation from actual standing crop concentrations is fully
acceptable considering many facilities use visual techniques or time consuming cell
counts for density estimates. Only under closely controlled experimental regimes
might the sensitivity of this photocell be inadequate.

The integration of the photocell with the software to maintain steadystate
conditions for standing crop and production through the manipulation of harvest rate
was readily apparent (Table 7). While all four experiments were initiated at a
harvest rate of 0.86 days\(^{-1}\), only Experiment 1 remained at that level. Experiments 2 through 4 ended with average harvest rates of 0.64, 0.46 and 0.73 days\(^{-1}\), respectively. Maximum harvest rates of 1.14 days\(^{-1}\) were obtained in some cases, however, they did not result in sustainable standing crop or production levels. By maintaining harvest rate as a variable, the computer had the freedom to make adjustments in accordance with changing conditions within the culture, a characteristic not feasible with systems controlled by simple electromechanical mechanisms where harvest rate manipulations require manual adjustments.

The monitoring system described in this paper allowed the unbiased control of the four experimental studies. Procedural decisions were based on the same set of criteria for each study. The results show that standing crop concentrations and production levels significantly increased (P<0.05) within the culture chambers when fluorescent lighting was replaced by metal halide lamps. Although both light sources produced relatively the same output per unit watt (79 lumens/watt for the fluorescent and 82 lumens/watt for the metal halide), the overall intensity of the metal halide lamps was ten times that of the fluorescent bulbs, resulting in a larger photic zone within the culture chambers.

Although it appeared that cultures grown at 35°C, under continuous metal halide lighting result in highest productions levels, no significant difference (P<0.05) was observed between 35°C and 30°C. Additionally, the culture grown at 35°C was not sustainable at this temperature, with cell clumping occurring by week 3. Subsequently, 30°C was determined as the best temperature for Chaet 10 within the conditional bounds of the study. This is in contrast to Nelson et al. (in press)
who reported excellent growth for Chaet 10 at temperatures greater than 30°C. However, their results were reported for batch cultures, with no reported timeframe. The excellent growth *Chaetoceros muelleri* (Chaet 10) observed at 30°C facilitates application of this strain in facilities where high temperatures prevail during the majority of the growing season.

Production levels obtained at 30°C and under continuous lighting from the metal halide lamps is substantially higher than observed for traditional culture methods currently utilized in the aquaculture industry. The theoretical production potential for outdoor ponds has been approximated at 30 - 50 g/m²/day-dry wt under light limiting conditions (Goldman, 1980). Actual literature reported values for outdoor pond cultures range from 8 - 60 g/m²/day-dry wt for various algal species, depending on the season and mode of operation (Ryther et al., 1972, 1975; Mann and Ryther, 1977; Walsh et al., 1987). More importantly, the area required to obtain these production levels ranged from 20m² to greater than 100m² (DePauw et al., 1983; Walsh et al., 1987). Our study resulted in sustained mean production levels of 115 g/m²/day-dry wt, with cultures occupying only 1.2 m² of horizontal space. Subsequently, the turbidostat system described in this paper has the potential to reduce the cost associated with the procurement of land for constructing an algal production facility.

Indoor, continuous culture methods reported in the literature have made considerable increases in production levels over the outdoor ponds, but still remain relatively lower than the production levels observed within our system. For instance, Trotta (1981) reported yields of 20 - 30 g/day-wet wt for *Tetraselmis* using
50 liter polyethylene bags operated as a turbidostat, and Palmer et al., (1975) reported yields of 5 g/day dry wt for *Monochrysis* obtained from a 40-liter chemostat. Using the regression relationship illustrated in Figure 9, our system was capable of yielding an average harvested biomass of 200 g/day-wet wt per growth chamber or 43 g/day-dry wt. More recently, James et al., (1988) reported average production levels of 93.8 ± 9.2 g/m²/day-dry wt for *Chlorella MFD-1* under chemostat conditions, 18.4% lower than obtained during our study.

The observed total lipid levels fell within the range (10 - 20%) reported by Goldman (1980) for cultures grown under the presence of excess nutrient and limiting light conditions. Increased lipid content, while desirable for many feeding situations, is incompatible with high production levels. Therefore, within turbidostat cultures, some production must be sacrificed to obtain higher lipid levels. Percent protein corresponded to the range obtained by Taub (1980). The lack of variation expected under different growth conditions may be explained by the light limitation and self-shading which tends to occur in larger volume cultures (Taub, 1980).

Overall, it is concluded that the computerized turbidostat provided an environment conducive to high production levels for microalgae. The system was influenced by light, limiting sustained production levels to an average of 123 g/m³/day-dry wt. Although it has been demonstrated that increasing the intensity of the source light increased production levels, the effect of light quality could not be determined from this study. Stable production was assured by maintaining harvest
rate as a variable, adjustable by the computer based on information transmitted by the photocell. The system provides a practical and cost-effective alternative to present indoor culture methods utilized in aquaculture.
CHAPTER IV

BASELINE OPTIMIZATION OF *CHAETOCEROS MUELLERI* (CHAET 10) WITHIN A COMPUTERIZED TURBIDOSTAT

INTRODUCTION

The consistent and reliable production of microalgae remains an economic variable within the aquaculture industry. Facility operators are concerned with the maintenance of high production levels and consistent nutritional quality characteristics. Traditional culture methods, namely induced blooms and outdoor ponds, provide a cost-effective and well established means for obtaining large quantities of algae. However, production and quality inconsistencies have yet to be fully addressed. Indoor batch cultures facilitate improved control over environmental conditions; unfortunately, production costs limit this culture method to applications of relatively low demand, such as hatcheries. The development of indoor continuous culture methods at a positive cost:benefit ratio would provide facility operators with sustained high production levels and consistent nutritional quality. Production costs may be reduced by optimizing the environmental conditions influencing algal growth and through the use of computer automation to alleviate the majority of the manual labor.

This paper presents: 1) the results of investigations performed using *Chaetoceros muelleri* (Chaet 10) cultured in a computerized turbidostat to establish initial temperature and lighting parameter values resulting in high production levels, 2) a discussion of a one month baseline optimization study performed on Chaet 10
at 30°, under continuous lighting from 250W metal halide lamps and 3) a computer
simulation model used to predict production levels within the computerized
turbidostat system over extended time periods.

Background

More than forty genera of microalgae are currently cultivated as aquaculture
feed, with the largest percentage being the diatom species (Class
Bacillariophyceae) (DePauw and Persoone, 1988). The marine diatom Chaetoceros
t spp. is an important natural food for the zoeal larvae of penaeids, bivalve mollusc
larvae and postlarvae, freshwater prawn larvae and Artemia (Hirata et al., 1972;
Hirata et al., 1975; Wilson, 1978; Simon, 1978; Jones et al., 1979; Webb and Chu,
1981; Yamasaki et al., 1981; Kanazawa et al., 1982; Rodhouse et al., 1983;
DePauw and Persoone, 1988). Barclay et al., (1987) reported that Chaetoceros
muelleri (Chaet 6 and Chaet 14) appeared to be one of the best new strains for
potential aquaculture applications due to its increased tolerance to a combination of
increased light, temperature and salinity. More recently, Nelson et al., (in press)
documented good growth of Chaetoceros muelleri (Chaet 10) at temperatures
between 30 - 34.5°C, with excellent growth at 34.5°C. These results establish this
species as an excellent candidate for culturing under tropical conditions. However,
these results were reported for batch cultures; therefore, the effects of high
temperatures under sustained continuous cultures must still be ascertained.

For outdoor cultures, the use of algal species less sensitive to environmental
conditions is an important consideration for increasing production throughout the
year. Maybe more importantly, as research focuses on reducing the production
costs of indoor cultures, algal strains amenable to satisfactorily growth under high temperatures and lower light conditions could help reduce operating costs through reduced energy inputs. Although using microalgal species that are more adaptable to a wider range of environmental conditions may indeed enhance production capabilities of existing culture systems, optimizing culture techniques with inherent advantages may substantially augment production capabilities.

Continuous cultures provide a controlled environment, facilitating the characterization of production trends under varying conditions. The theoretical considerations for continuous cultures were originally established for the cultivation of bacteria (Monod, 1942; Golle, 1953; Powell, 1958; Ricica, 1958; Herbert et al., 1956; Herbert, 1958; Munson, 1970; Tempest, 1970), and later applied to algal production systems investigating uptake kinetics under substrate limiting conditions (Eppley and Thomas, 1969; Eppley et al., 1969; Caperon and Meyer, 1972; Nelson et al., 1976; Davis et al., 1978). The change in biomass (dX) over an infinitely small time interval (dt) can be expressed as (assuming the decay rate k_d is very small and can be neglected):

\[
\frac{dX}{dt} = X\left\{\mu_m\frac{S}{K_s+S} - D\right\}
\]

where,

- \(X\) = algal biomass (g)
- \(S\) = concentration of limiting nutrient (g/m^3)
- \(K_s\) = half-saturation constant (g/m^3)
- \(\mu_m\) = maximum value of \(\mu\) where \(S\) is no longer limiting (days\(^{-1}\))
- \(D\) = dilution rate (days\(^{-1}\))
For practical aquaculture applications where production is of prime concern, essential nutrients (mainly $\text{NO}_3^{-1}$, $\text{PO}_4^{-3}$ and silicate) are usually supplied in excess. Subsequently, the rate of change in biomass will no longer follow saturation kinetics, but instead, should be linearly proportional to the biomass concentration, assuming no other limiting of inhibitory factors are present:

$$\frac{dX}{dt} = X(\mu - D) \quad (5)$$

Based on principle, the turbidostat minimizes standing crop variations by changing the dilution or harvest rate in response to turbidity values transmitted by a photocell. Specific growth rates, over the long run, reach steadystate levels which approximate the dilution rate. However, during the interim, the culture goes through transient phases where instantaneous specific growth rates can be determined by:

$$\mu = D + \frac{(\ln dX)/dt}{D} = D + \frac{\ln(X_2/X_1)}{(t_2-t_1)} \quad (6)$$

The steadystate conditions achieved within continuous cultures facilitates optimization processes difficult to implement with batch cultures.

**METHODS AND MATERIALS**

*Experimental Apparatus*

Experiments were performed in a computerized turbidostat system consisting of two 0.6 m$^3$ fiberglass growth chambers (depth = 1.0 meters) contained within a temperature control room (Figure 18) and supported by a computer control and monitoring system consisting of a Zenith Z-184 laptop micro-computer, and analog to digital converter and input and output devices (Figure 19). The entire system was monitored and controlled by the software program, "Supervisor" (Rusch and
Figure 18. The computerized turbidostat system consisted of two 0.6 m³ culture chambers supported by a centralized monitoring block and pressurized dosing apparatus and a saltwater conservation system for saltwater reuse. The turbidostat was integrated with a computer control and monitoring system (not shown in the schematic).
Figure 19. Control and monitoring of the turbidostat system was accomplished using a computer based system interfaced for input and output devices through an analog to digital converter.
Malone, 1989; Rusch and Malone, 1990), written in Turbo Pascal 4.0 (Borland, 1987). The chambers contained 48° conical bottoms to prevent settling, and were coated with white gelcoat to maximize the reflectance of light back into the cultures. The culture chambers were supported by a central dosing apparatus and monitoring block, a harvesting chamber, a CO₂ injection system and an air/vacuum pump for operation. Each chamber was covered with 0.64 cm acrylic sheets, facilitating overhead illumination and the placement of outlets for solution and air addition, culture removal and excess air ventilation. Aeration was provided through a 5.1 cm diameter airlift system within each chamber. Contamination was minimized by four mechanisms: 1) sealed covers eliminating the entrance of airborne contaminants, 2) maintenance of positive air pressure within the chambers, 3) filtration of incoming air through a 0.4 μm prefilter followed by a 0.2 μm capsule filter and 4) utilization of the software to maintain optimal culture conditions for Chaet 10, minimizing competition from any contaminants.

Fresh media and disinfectant were added to the chambers through a central pressurized dosing apparatus, activated by opening a normally closed solenoid valve. Based on basic air displacement principles, solutions were moved from each dosing unit into the main manifold and then the designated culture chamber. Following the addition of media, the dosing apparatus automatically refilled from stock solution tanks, resetting them for the next harvest cycle. Intermittent CO₂ injection from a pressurized cylinder was used to maintain culture pH between 7.6 and 8.0 for all experiments. The frequency of injection was determined by the pH measured during each harvest cycle and adjusted by the computer. Culture
volumes were maintained by level detectors constructed from inexpensive 0.2 mm stainless steel welding rods. The positive post of the detector was intermittently energized by the +5V controlled output located on the analog to digital convertor. The conductivity of the water closed the circuit when both posts were submerged, sending an increased millivolt signal to through the second post to the analog channel and deactivating the saltwater valve.

The turbidostat was supported by a saltwater conservation system consisting of a 0.76 m³ reservoir, a continuous operating centrifuge, a upflow sand filter and a UV light. Algal culture from the turbidostat chambers was concentrated into a paste and refrigerated. The saltwater was recirculated through the upflow sand filter and UV light for treatment prior to reuse within the culture chambers, substantially reducing artificial salt requirements.

All routine processes were monitored and executed by the computer control system. The harvest rate was set at once per hour per chamber with the initiation of each production run; however, this parameter remained a random variable, adjustable by the software in response to changing culture conditions. During each harvest cycle, culture temperature, pH, salinity and turbidity measurements were transmitted to the computer, stored and used to make decisions about ensuing procedures.

Baseline Optimization Studies

Stock cultures of (Chaet 10) were obtained from Dr. James Nelson, a research collaborator located on Skidaway Island, Savannah, Georgia. A two-stage batch culture process using 500 ml and 2 liter Erlenmeyer flasks was employed to
provide the inoculum for the turbidostat system. All glassware and water were disinfectected with 20 ppm chlorine and rinsed with sodium thiosulfate prior to use. Inoculum cultures were maintained in F/2 medium (Guillard, 1974) on an Orbital shaker table (Lab Line Instruments, Inc., Model No. 3590). Temperature and continuous lighting conditions were held at 25±1°C and 4.26 W/m², respectively. Salinity was maintained at 35 ppt through the use of artificial sea salts (Instant Ocean¹).

Experiments were performed to: 1) determine initial production optimization values for temperature and light conditions, 2) characterize growth responses under varying temperature conditions ranging from 26 - 33°C, 3) perform a baseline optimization study on Chaetoceros muelleri (Chaet 10) under temperature and lighting conditions obtained from the first objective, 4) compare dilution rates controlled by the computer with instantaneous growth rates calculated from computer estimated biomass concentrations at steadystate conditions and 5) collect data to calibrate and verify the production simulation model.

Four experiments were performed on Chaet 10 to establish temperature and lighting parameter values as the starting basis for further optimization studies: 1) 28°C, continuous lighting from six 40W fluorescent bulbs per chamber, 2) 30°C, continuous lighting from 250W metal halide lamps, 3) 30°C, intermittent (20:4, L:D) lighting from 250W metal halide lamps and 4) 35°C, continuous lighting from 250W metal halide lamps. The temperature range was selected based on the results

¹The use of this product does not represent an endorsement.
obtained by Nelson et al. (in press) for the culture of Chaet 10 under batch conditions. Following this set of experiments, the data was compared to determine which conditions resulted in the best overall production levels. These conditions were then used to perform a one month optimization study during which time the computer used feedback information transmitted from the photocell to make incremental increases or decreases in the harvest rate. The primary objective of this study was to achieve the highest sustainable production levels for the given environmental conditions. The optimization study also served as a demonstration of the computer's capabilities in maintaining stable conditions within both growth chambers. Two experiments were performed to characterize the growth response of Chaet 10 under varying temperatures as might be experienced under commercial conditions where temperature is not controlled. Both experiments were performed at temperatures ranging from 25 - 33°C, with temperature increasing over the study period.

All experiments were carried out at a pH of 7.6 - 8.0 and a salinity of 35±1 ppt using artificial sea salts. Major nutrients (NO$_3$ $^{-1}$, PO$_4$ $^{-3}$ and silicate) were added at three times the F/2 medium concentration, while all other nutrients were added at twice the F/2 level to circumvent nutrient limitation. Standing crop, pH, salinity, temperature and dilution rate data was collected and stored by the computer. Dilution rate was maintained as a random variable, adjustable by the computer based on the standing crop concentration estimates obtained during each harvest event. Instantaneous specific growth rates were calculated using Equation (3) and
the computer estimated standing crop concentrations. Volumetric production levels \( P_v \) \( \text{g/m}^3/\text{day-dry wt} \) were calculated from the standing crop estimates and dilution rates.

Gross lipid and protein composition was determined during each experiment to detect trend differences with varying temperature and light conditions. Chambers one and two were considered replicates; therefore, combined samples were intermittently collected from the centrifuge, dried at 105°C and stored in the freezer until analyzed. Total lipids were analyzed using the soxhlet extraction method described by AOAC 27.006 (AOAC, 1984). A 2:1 chloroform:methanol solvent was used to extract the lipid material from the algal cells. Total protein samples were sent outside of the laboratory for analysis.

**Computer Model**

A computer model was developed as a tool to predict production levels within the turbidostat system over extended time periods, thereby, allowing facility operators to determine culture volumes based on the daily requirements of the target species. Because practical applications of continuous culture systems are concerned with obtaining high production levels, all controllable parameters that may limit algal growth are addressed to whatever extent possible, with light intensity most likely the remaining limiting factor for indoor cultures. The model presented in this paper was, therefore, developed using the first order relationship described in Equation (5). Using the Fourth Order Runge Kutta numerical solution technique, standing crop concentrations were estimated by a two step process:

\[
X := X + \Delta t \left( \frac{1}{6} \left( K_1 + 2 \cdot K_2 + 2 \cdot K_3 + K_4 \right) \right)
\]  

(7)
\[ X := X - HF \cdot X \cdot CF \]

where,

\[ X = \text{standing crop (g/m}^3\text{-dry wt)} \]
\[ \Delta t = \text{time step (days)} \]
\[ K_1, K_2, K_3, K_4 = \text{slopes} = \mu X \]
\[ HF = \text{harvested fraction = dilution rate (days}^{-1}) \]
\[ CF = \text{calibration factor associated with harvest volume} \]

For every time step, a standing crop concentration estimate is calculated, from which daily production levels and harvested biomass determinations are made. Due to the discontinuous nature of the turbidostat, the dilution effects on standing crop levels is exhibited only during the harvest cycle (Equation 8). During a harvest event, standing crop concentrations are calculated both at the time of harvest (Equation 7) and an instantaneous second (\( \Delta t/10 \)) after the harvest (Equation 8). The cumulative harvested biomass is calculated for each harvest by:

\[ HB := HB + HF \cdot X \cdot V \cdot CF \]

where,

\[ HB = \text{cumulative harvested biomass (g-dry wt)} \]
\[ V = \text{culture volume (m}^3\text{)} \]

The model was calibrated using eight data sets from Experiments One through Four and verified from two data sets collected from Experiment Seven. During the calibration process, CF was manipulated to adjust for discrepancies in the actual harvested volume. Using Experiments One through Four, CF was set at 1.07, corresponding to an actual harvest volume seven percent larger than the
assumed volume. Following both the calibration and verification procedures, the model was used to simulate production levels at three harvest rate/specific rate levels.

RESULTS

The results of Experiments One through Four indicated that average production levels were greatest for *Chaetoceros muelleri* (Chaet 10) cultured under continuous lighting from 250W metal halide lamps at 30°C. Cultures grown under 40W cool white fluorescent bulbs exhibited significantly (P<0.05) lower levels (Figure 20). Though Figure 20 does not illustrate any significant difference between cultures grown at 30°C and 35°C, under continuous lighting from metal halide lamps, the latter cultures started clumping by week three, with a substantial decline in standing crop concentrations thereafter.

Chaet 10 grown under continuous lighting from 250W metal halide lamps at temperatures varying from 27°C to 33°C exhibited an increasing trend, though not significant, with increasing temperature (Figure 21). Additionally, there was very little variance in production levels obtained from both chambers, supporting the hypothesis that chambers one and two were replicates and also indicating the reliability of the computer. In a similar experiment, cultures grown at temperatures ranging from 26°C to 31°C, under the same lighting conditions, showed more variance in estimated production levels between the two chambers (Figure 22). Although production levels remained relatively constant, on the average, chamber one exhibited a slight decrease in production beginning at 28.6°C, while chamber two showed a slight increase. Chamber one did, however, exhibit another increase
Figure 20. Comparison of Chaet 10 production levels for Experiments One through Four. (a) chamber one and (b) chamber two.
Figure 21. *Chaetoceros muelleri* (Chaet 10) cultured at temperatures varying between 27°C to 33°C exhibited an increasing trend in production level with increasing temperature.

Figure 22. *Chaetoceros muelleri* (Chaet 10) cultured under temperatures varying between 26°C to 31°C showed no substantial increase in production levels with increasing temperature.
at 30.4°C, as did chamber two. This is in contrast to Experiment Five where an increasing trend was observed for both chambers (Figure 21). In comparison, the temperature increase for Experiment Six occurred over seven days, while the time period was almost doubled for the increase seen in Experiment Five allowing more response time for the algae.

With estimated production levels the highest at 30°C, under 250W metal halide continuous lighting and no significant difference indicated between temperatures ranging from 26° to 32°C, a one month optimization study was performed on *Chaetoceros muelleri* (Chaet 10) at 30°C, under continuous 250W metal halide lighting to optimize production levels. Due to a drastic temperature increase in the laboratory in which the temperature control room was located, the air conditioning unit within the control room was not able to maintain culture temperatures at 30°C; consequently, the optimization study was terminated on Day 24 after which culture temperatures exceeded, 37°C causing a near collapse. Table 8 presents a statistical summary for Experiment Seven, while computer estimated and analytically measured standing crop concentrations, volumetric production levels and harvested biomass levels are illustrated in Figure 23. Estimated and analytically measured standing crop concentrations averaged 143.1 and 144.4 g/m³-dry wt and 139.9 and 140.1 g/m³-dry wt for chambers one and two, respectively, indicating the reliability of the photocell and regression equation for transmitting and converting millivolt signals to biomass. Although standing crop concentrations obtained during Experiment Seven were, on the average, 55 g/m³-dry wt lower than
Table 8. Results of the optimization study for *Chaetoceros muelleri* (Chaet 10) grown under continuous 250W metal halide lighting at 30°C. All biomass values are dry weight (mean±STD, (range)).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Chamber One</th>
<th>Chamber Two</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standing Crop (g/m³)</td>
<td>143.1±27.2 (94-209)</td>
<td>139.9±26.8 (90-237)</td>
</tr>
<tr>
<td>Volumetric Production (g/m³/day)</td>
<td>224.6±62.9 (93-383)</td>
<td>217.2±61.9 (94-350)</td>
</tr>
<tr>
<td>Areal Production (g/m²/day)</td>
<td>211.2±59.2 (88-360)</td>
<td>204.2±58.2 (88-329)</td>
</tr>
<tr>
<td>Harvested Biomass (g/day)</td>
<td>205.7±108.6 (43-451)</td>
<td>198.9±108.2 (44-399)</td>
</tr>
<tr>
<td>Dilution Rate (1/day)</td>
<td>1.60±0.54 (0.86-2.36)</td>
<td>1.59±0.56 (0.86-2.39)</td>
</tr>
<tr>
<td>Specific Growth Rate (1/day)</td>
<td>1.61±0.88 (-1.31-3.65)</td>
<td>1.63±0.85 (-0.87-3.46)</td>
</tr>
<tr>
<td>Temperature (°C)</td>
<td>30.8±1.1 (28.4-33.4)</td>
<td>30.9±1.1 (28.4-33.8)</td>
</tr>
<tr>
<td>Light Intensity (W/m²)</td>
<td>94.9±9.9 (68.5-108.9)</td>
<td>84.9±3.0 (75.9-100.1)</td>
</tr>
<tr>
<td>pH</td>
<td>7.75±0.14 (7.40-8.08)</td>
<td>7.75±0.15 (7.41-8.04)</td>
</tr>
</tbody>
</table>
Figure 23. Computer estimated (SC-Est) and analytically measured standing crop concentration (SC-Anal), production levels and harvested biomass obtained during the optimization of Chaet 10. (a) chamber one and (b) chamber two.
observed under the same conditions during the preliminary investigations (Experiment Two), the average dilution rate almost tripled between Experiment Two and Seven, resulting in a significantly higher production level. By the end of the study, production levels for chamber one and two had reached average steadystate values of 225 g/m³/day-dry wt and 217 g/m³/day-dry wt, respectively, almost double the levels obtained during Experiment Two.

The high production levels obtained during Experiment Seven, were in large part, influenced by the maintenance of high specific growth rates (Figure 24). For continuous cultures operated under steadystate conditions, the dilution rate must equal the specific growth rate. Although the turbidostat was not operated under fully continuous conditions, both chambers averaged 45 harvests per day, resulting in average dilution rates of 1.60 and 1.59 (days⁻¹) for chambers one and two, respectively. In comparison, instantaneous specific growth rates, calculated from Equation (6), averaged 1.61 and 1.63 (days⁻¹) for chambers one and two, respectively, indicating steadystate conditions. On only three occasions did the estimated specific growth rate fall below zero for each chamber, indicating a net negative growth during that period. This average specific growth rate resulted in a culture doubling of 2.34 days⁻¹, with a maximum doubling of 5.27 days⁻¹.

High dilution rates also resulted in high daily harvested biomass levels, the bottom line for commercial facility operators. Harvested biomass values averaged 206 and 199 g/day for chambers one and two, respectively. This represents a fourfold increase over the preliminary study performed under the same conditions. Additionally, although all harvested biomass values remained below volumetric
Figure 24. Computer controlled harvest rates and calculated specific growth rates for Chaet 10 averaged: (a) chamber one, 1.60 and 1.61 days\(^{-1}\) and (b) chamber two, 1.59 and 1.63 days\(^{-1}\).
Production levels during Experiment Two, Experiment Seven exhibited harvested biomass values greater than production levels for harvest rates equal to or greater than 57/day.

In addition to controlling and monitoring biomass concentrations, the computer also controlled culture temperature and pH and monitored lighting conditions (Figure 25). The pH for both chambers was maintained between 7.6 and 8.0, with the exception of a few occasions when the empty CO₂ cylinder was not immediately replaced. The pH variations were attributed to the intermittent sampling and adjustment process used with the turbidostat. Temperature was also maintained at 30±1°C, except during the latter part of the study when a air conditioner problem occurred in the main lab, leading to failure of the conditioner unit in the temperature control room. Figure 25 also illustrates that both temperature and pH remained relatively the same within both chambers, again establishing the reliability of the computer control system. Light intensity averaged 95 W/m² and 85W/m² for chambers one and two, respectively. The observed difference has been attributed to a slight variation in output intensity between the two lamps and may explain the slightly lower production values obtained for chamber two.

Although production levels, specific growth rates and harvested biomass significantly increased for Experiment Seven compared to Experiments One through Four, total proteins showed a decreasing trend, while total lipids remained relatively unchanged, as expected (Figure 26).

The computer model was calibrated using the production data obtained from Experiments One through Four, resulting in eight separate calibration data sets.
Figure 25. For all experiments, temperature and pH were actively monitored and controlled by the control system, while light intensity output was

Figure 26. Gross lipid and protein percentages determined for Experiments One through Seven did not statistically differ from one experiment to another. Bars without lipid percentages represent missing data.
Figure 27 illustrates the calibration curves for production levels determined during Experiment Two (chamber two) and Experiment Three (chamber two), while Figure 28 depicts the verified model using data from Experiment Seven. As shown in Figure 28, the model tended to underestimate production levels during the earlier part of the study when the harvest rate was quickly changing. Towards the end of the study, the model tended to overestimate production levels. Three simulations, representing the lowest, average and highest harvest rates observed during Experiment Seven, were executed, with the results illustrated in Figure 29. All three simulations assumed a starting standing crop concentration of 50 g/m³-dry wt, with a maximum level of 200 g/m³-dry wt. Steadystate production levels were estimated at 169.2 g/m³/day-dry wt, 319.4 g/m³/day-dry wt and 476.9 g/m³/day-dry wt for harvest rates of 0.86 days⁻¹, 1.60 days⁻¹ and 2.37 days⁻¹, respectively. The time required for steadystate levels to be reached ranged from 17 days for a harvest rate of 0.86 days⁻¹ to 1.75 days at a harvest level of 2.37 days⁻¹.

DISCUSSION

The series of experiments performed and discussed in this paper represents the first level optimization of the computer automated algal turbidostat for sustained high production levels. The investigations actually served two functions: 1) refinement of the physical system along and the controlling software and 2) determination of baseline optimal temperature and lighting conditions for *Chaetoceros muelleri* (Chaet 10).

The data obtained from Experiments One through Four indicated that Chaet 10 cultured under continuous lighting from 250W metal halide lamps at 30°C
Figure 27. The computer simulation model was calibrated using production level data obtained from Experiments One through Four. (a) Experiment Two, chamber one and (b) Experiment Three, chamber two.
Figure 28. Data from Experiment Seven was used to verify the computer simulation model. (a) chamber one and (b) chamber two.
resulted in the highest sustained production levels, averaging 122 g/m$^3$/day-dry wt and 124 g/m$^3$/day-dry wt for chambers one and two, respectively. Cultures grown at 35°C, although resulting in similar production levels, started to clump after three weeks, indicating the adverse effects of prolonged exposure to high temperatures. These results were in contrast to Nelson et al., (in press) who reported excellent growth of Chaet 10 at 34.5°C under batch conditions.

*Chaetoceros muelleri* (Chaet 10) cultures grown under the conditions of Experiments One through Four resulted in production levels, on the average, substantially higher than obtained for outdoor cultures. In particular, Barclay et al., (1987) achieved average production levels of 35 g/m$^2$/day-dry wt for Chaet 6 and
Chaet 10, only 70% of the lowest areal production levels obtained within this study (59 g/m²/day-dry wt under 40W cool white fluorescent lighting) and 30% of the highest observed average production levels (116 g/m²/day-dry wt under 250W metal halide lamps).

The results of Experiments One through Four, combined with those obtained for Experiments Five and Six under varying temperatures, suggested the use of the 250W metal halide lamps operated continuously at 30°C for the optimization study. The data also suggested that the algorithm controlling harvest rate adjustments could be further refined to achieve possibly even higher production levels. This was proven to be true by the results of Experiment Seven, performed under the same conditions as Experiment One. Although average estimated standing crop concentrations decreased by 36% during Experiment Seven, volumetric production levels increased by 81% over that observed during Experiment One, indicating the appropriateness of the software algorithm as a tool for production optimization within the turbidostat system.

The average volumetric (221 g/m³/day-dry wt) and areal (208 g/m²/day-dry wt) production levels obtained for Chaet 10 during the optimization study substantially surpassed production levels recorded in literature from both batch and continuous culture methods. The closest production results were reported by James et al., (1988), who obtained average levels of 117 g/m³/day-dry wt and 94 g/m²/day-dry wt for Chlorella grown within an automated chemostat under optimal pH conditions. Traditional outdoor ponds, under solar radiation, have a reported theoretical maximum production level of 30-50 g/m²/day-dry wt (Goldman, 1980).
Most literature reported values show a typical long term average of 10 - 20 g/m²/day-dry wt (Ryther et al., 1972, 1975; Mann and Ryther, 1977), almost two orders of magnitude lower than obtained within this study.

The high production levels observed for Experiment Seven can be attributed to the maintenance of a relatively high standing crop concentration combined with high dilution rates. The average specific growth rate resulted in 2.34 doublings/day, with maximum values of 5.27 and 4.99 doublings/day for chambers one and two, respectively. Although the average doublings/day were slightly lower than the values obtained by Barclay et al., (1987) for Chaet 6 (3.4 doublings/day) and Chaet 14 (4.0 doublings/day), the maximum values fell within the range of highest growth rates (4.5 - 5.0 doublings/day) reported for marine algal species (Thomas, 1966). The results reported here also compare well with Wangersky et al., (1989) who obtained maximum growth rates of 3.0 doublings/day for Chaetoceros gracilis grown within an 0.2 m³ automated turbidostat system. The average dilution rate did not significantly differ (P<0.05) from the average estimated specific growth rates for either chamber, indicating that, on the average, steadystate conditions were obtained over the 24 day study period. Additionally, chambers one and two exhibited almost the same average dilution rates and specific growth rates, supporting the hypothesis that the two chambers could be treated as replicates and indicating the ability of the computer to maintain the same conditions within the two culture.

Both high production levels and specific growth rates resulted in an average daily harvested biomass of 202 g-dry wt, dramatically higher than most values
reported in literature. Under indoor, continuous culture conditions, Laing and Jones (1983) obtained 20 g/day-wet wt of *Tetraselmis*, and Palmer et al., (1975) reported levels of 5 g/day-dry wt for *Monochrysis* within a 40 liter chemostat. This large difference between reported values and those obtained here may be attributed to maintaining the harvest rate as a variable, and allowing the computer to make adjustments in response to changing culture conditions.

The production levels obtained during the optimization study exceeded literature values substantially. The simulation model predicted that the turbidostat was capable of sustaining levels of 319 g/m³/day-dry wt for the average harvest rate observed during the study. This is approximately 98 g/m³/day-dry wt greater than the actual average production level obtained during the study. If only the latter, steadystate stages of growth are considered, the average observed production level for the turbidostat would be 284 g/m³/day-dry wt, only 35 g/m³/day-dry wt less than predicted. Although there is room for further optimization of this system, attention must be paid to the fact that the calculated doublings/day (specific growth rate/ln 2) corresponding to the predicted production level is near the maximum reported value; and, sustaining these high harvest rates over long time periods may result in culture washout.

The ability of the computer to maintain the two culture chambers as replicates was exhibited not only by the dilution rate and specific growth rate, but also by the maintenance of the temperature and pH values over the 24 day experimental period. Although the average pH was maintained at 7.75, the chambers did exhibit a greater variation than observed for temperature. Carbon
dioxide is continuously removed from the culture media and converted to biomass, making pH control more difficult than temperature. This variation may be reduced by increasing the monitoring frequency. Although Chaetoceros muelleri (Chaet 10) responded very well to continuous lighting from 250W metal halide lamps, it was not clearly demonstrated that continuous lighting was, indeed, superior to intermittent lighting. The average production levels obtained during under intermittent light were 25% lower than observed under continuous lighting; however, the inactivation of the harvest cycle during the dark period decreased the harvest rates, which resulted in suppressed production levels.

The advantages of continuous over intermittent lighting are not clearly defined in the literature. General findings suggest that continuous lighting results in faster growth (Castenholz, 1964; Durbin, 1974; Holt and Smayda, 1974; Admiraal, 1977). However, in some cases long exposures to continuous lighting has been reported to cause "photoinhibition" in which there is an apparent decrease in the net photosynthetic rate once the saturation point has been reached (Steeman-Nielson, 1952b, 1962; Vollenweider and Nauwerck 1961; Findenegg 1965; Goldsworthy, 1970). Due to the depth of the culture and the use of artificial lamps, the authors are doubtful that "photoinhibition" influenced the growth of Chaet 10 during any of the experiments.

Although varying temperature and lighting conditions resulted in significantly different standing crop, production and harvest levels, the gross lipid and protein profiles obtained from each study showed little variance, with the exception of Experiment Seven where both total lipids and proteins exhibited decreases. On the
average, gross lipid levels fell within the range reported in literature for continuous cultures. According to Goldman (1980), lipid content will average 10 - 20% of the dry weight of an algal cell under conditions where the only limiting factor is light. More importantly, peak production levels for a turbidostat are obtained by maintaining the algae in the exponential growth phase; although, increased lipid content is possible only under stressful conditions such as nitrogen limitations. Therefore, if high production levels is the major consideration, lipid content will remain low. Production levels must be compromised to increase lipid levels.

Protein levels, which averaged slightly below typically reported levels under excess nutrients, showed more variation than the lipid content. The trends, however, for Experiments One through Six followed typical patterns exhibiting lower protein content with increased retention time (Taub, 1980). The lower observed protein levels for Experiments Seven may be attributed to the increased dilution rate, which resulted in an overall lower substrate concentration.

In summary, the reduction of algal production costs is a main concern within facilities utilizing algae as a live feed source. Therefore, optimizing the parameters influencing high production levels appears to be the most logical answer to reducing production cost. By increasing daily production levels, overall volume and space requirements will be reduced. The baseline optimization of Chaet 10 within the computerized turbidostat did result in production levels significantly higher than seen in literature. The simulation model provides a useful tool for predicting not only
production levels over time, but also for determining the lag time or the period required to reach steady state. Thus, this simulation model allows facility operators to estimate volumetric culture requirements.
CHAPTER V

CONCLUSIONS

The production-scale, computer automated algal turbidostat was proven to be a reliable and effective alternative to the production of algae for aquaculture applications. Initial studies showed that production levels for Chaetoceros muelleri (Chaet 10) were greatest at 30°C and under continuous lighting from 250W metal halide lamps, averaging 208 g/m²/day-dry wt or 400 - 500% greater than observed for typical outdoor pond cultures. Production levels obtained under 40W cool white fluorescent bulbs only averaged 56 g/m²/day-dry wt, indicating that light was indeed the limiting growth factor. An extended baseline optimization study performed at 30°C, under continuous lighting from 250W metal halide lamps resulted in average production levels of 221 g/m³/day-dry wt, 89% higher than the most recently reported literature values under continuous culture conditions. The high sustained production levels was explained by the computer's ability to maintain relatively high standing crop concentrations in combination with high harvest rates, controlled by the software in response to information transmitted by the photocell. Additionally, labor requirements were reduced to approximately four hours per week compared to 35 hours necessary for maintenance of a batch culture system of similar volume under full operation (David Head, personal communication, 1992).

This research underscores the tremendous impact this type of system could have on the aquaculture industry. For industrial implementation, future research should focus on: 1) complete optimization of the system for sustained high production levels, 2) manipulation of the system to obtain varying nutritional quality.
of the algae as specified by individual facilities and 3) technology transfer strategies
to introduce the control and monitoring algorithm to operators within the industry.

**Industrial Impact**

Dissemination of this technology has been occurring since November, 1990 through a collaborative effort with the Shellfish Research Laboratory located on Skidaway Island, Savannah, Georgia; an affiliation of the University of Georgia, Marine Extension Service. The research staff at the facility have installed a system similar to the one located in the LSU laboratory, with two additional chambers. The system is currently undergoing evaluation testing under greenhouse conditions.

The use of the algal turbidostat within the aquaculture industry is a specific application of the technology. The turbidostat system is much more generic and can be applied to the production of algae for pharmaceuticals, retail sales through aquarium stores, food additives, natural dyes and pigments and educational purposes.
REFERENCES CITED


APPENDIX A

SOFTWARE DOCUMENTATION

This appendix provides a listing of the Turbo Pascal program, "Supervisor" described in-depth in Chapter II (Control and Monitoring Algorithm) and referred to in Chapter III (Development of Computerized Turbidostat) and Chapter IV (Production Evaluation). Appendix A-1 presents the listing of the software, "Supervisor", while Appendix A-2 presents supporting documentation corresponding to the ADC units and various parameters utilized in the software.
CONTROL AND MONITORING PROGRAM, "SUPERVISOR"

The control and monitoring program contains three core elements that manage and execute system processes: (1) "stack", (2) "stack sorter" and (3) "supervisor". The program listing also contains all procedures and functions necessary to initiate and communicate with the analog to digital converter and to carry out all control and monitoring processes.
Program SUPERVISOR;  {turbidostat prototype number three}

(This program is a modified version of CHEMOSTAT written by Kelly A. Rusch for prototype number two. The program controls all processes of the algal turbidostat and collects and stores data in a data file.)

uses async,turbo3,crt,dos;

TYPE
  onoff = (on,off);
  burp = string[5];
  status_type = array[1..12] of integer;
  Key = string[80];
  lines = string[70];

  dgt = record  {record of the supervisor procedure}
    time:real;
    action:integer;
  end;

  info = record  {record of analog data}
    name:string[40];
    points:array[1..10] of real;
    stats:array[1..2] of real;
  end;

  conditions = record  {record of initial conditions}
    ADC_set:string[70];
    light_out:array[1..5] of real;
    room_temp:array[1..2] of real;
    params:array[1..9] of real;
  end;

CONST
  PN = $3F8;  {data port address}
  SP = $3FD;  {status port address}
  max_records = 30;  {maximum number of records allowed in data set}
  analog1_names:array[1..16] of string[40] = (   'Room Temperature 1 (deg. C)
    'Room Temperature 2 (deg. C)
    'Monitoring Block - Temperature (deg. C)
    'Monitoring Block - Light Intensity (Mv)
    'Monitoring Block - Intensity Output Check (Lux)
    'Room Intensity 1 (Lux)
    'Room Intensity 2 (Lux)
    'Room Intensity 3 (Lux)
    'Room Intensity 4 (Lux)
    'Room Intensity 5 (Lux)
    'Room Intensity 6 (Lux)
    'Room Intensity 7 (Lux)
    'Room Intensity 8 (Lux)
    'Room Intensity 9 (Lux)
    'Room Intensity 10 (Lux)
    'Room Intensity 11 (Lux)
    'Room Intensity 12 (Lux)
    'Room Intensity 13 (Lux)
    'Room Intensity 14 (Lux)
    'Room Intensity 15 (Lux)
    'Room Intensity 16 (Lux)
  );
'Room Intensity 2 (Lux)
'Chamber 1 Intensity Output Check (Lux)
'Chamber 2 Intensity Output Check (Lux)
'Level Detector 1 (mv)
'Level Detector 2 (mv)
'Upper Harvest Level Detector (mv)
'Monitoring Block - Salinity (ppt)
'Lower Harvest Level Detector (mv)

analog2names:array[1..16] of string[40] = (
  'Monitoring Block - Salinity (ppt) ',
  'Lower Harvest Level Detector (mv) ',
  '); analog2names:array[1..16] of string[40] = (  
  'Monitoring Block - Salinity (ppt) ',
  'Lower Harvest Level Detector (mv) ',
  ');  

menu_names:array[1..12] of string[40] = (  
  'Air Conditioner/heater ',
  'Water Addition ',
  'Harvest Cycle ',
  'Disinfection Cycle ',
  'CO2 ',
  'Room Light Control ',
  'Room Environment Check ',
  'Data File ',
  'Change Operational Parameters ',
  'Check Light Intensity Output ',
  'Flush Manifold ',
  'Valve Diagnostics '),

ctout1_names:array[1..12] of string[40] = (  
  'Level Detectors ',
  'Saltwater close ',
  'Heater ',
  'Saltwater open ',
  ')';
}
'UV Light',
'Solenoid 10',
'Solenoid 3-Disinf',
'Solenoid 4-Nutr',
'Solenoid 6-open-c2',
'Chamber Lights',
'CO2 Solenoid',
'Harvest Pump');
ctout2_names:array[1..12] of string[40] = ('Air Conditioner',
'Solenoid 5-close-m',
'Solenoid 8-main',
'Solenoid 2-water',
'Solenoid 6-close-c2',
'Solenoid 7-close-c1',
'Solenoid 9-A/V pump',
'Photocell Light',
'Solenoid 1-Air',
'Solenoid 5-open-m',
'A/V Pump',
'Solenoid 7-open-c1');
lite:array[1..5] of string[20] = ('Room Intensity 2 (Lux)',
'Room Intensity 2 (Lux)',
'Photocell Output (Lux)',
'Chamber 1 (Lux)',
'Chamber 2 (Lux)');
parameters:array[1..9] of string[40] = ('Low temp',
'High temp',
'Low pH',
'High pH',
'Lights on',
'Lights off',
'CO2 on delay',
'CO2 off delay',
'Cult Temp');
checks:array[1..10] of real = (0.00092592593,0.00005787073,
0.00416666667,0.01388888889,0.00416666667,0.00312500000,
0.00138888889,0.00012731481,0.00013888889,0.00011574074);
one_second = 0.00001157407; {time delay for one second}
two_seconds = 0.00002314815; {two second time delay}
five_seconds = 0.00005787037; {time delay for five seconds}
six_seconds = 0.00006944444; {time to open and close chamber valves}
nine_seconds = 0.00010416667; {nine second time delay}
ten_seconds = 0.00011574074; {time delay for ten seconds}
twelve_seconds = 0.000138888889; {twelve second time delay}
fourteen_seconds = 0.00016203704; {fourteen second time delay}
fifteen_seconds = 0.00017361111; {fifteen second time delay}
nineteen_seconds = 0.00021990741; {nineteen second time delay}
twenty_seconds = 0.00023148148; {twenty second time delay}
twenty_five_seconds = 0.00028935185; {twenty-five second time delay}
thirty_seconds = 0.0003472222; {thirty second time delay}
forty_seconds = 0.00046296296; {forty second time delay}
forty_five_seconds = 0.0005208333; {forty-five second delay}
forty_six_seconds = 0.00053240741; {forty-six second delay}
fifty_one_seconds = 0.00059027778; {fifty-one second delay}
fifty_six_seconds = 0.00064814815; {fifty-six second delay}
one_minute = 0.0006944444; {one minute delay}
seventy_seconds = 0.00081018519; {seventy second delay}
ninety_seconds = 0.00104166667; {ninety second delay}
two_minutes = 0.0013888899; {two minute time delay}
three_minutes = 0.00208333333; {three minute time delay}
five_minutes = 0.0034722222; {five minute time delay}
six_minutes = 0.00416666667; {six minute time delay}
ten_minutes = 0.0069444444; {ten minute time delay}
fifteen_minutes = 0.01041666667; {fifteen minute delay}
twenty_minutes = 0.01388888889; {twenty minute time delay}
thirty_minutes = 0.02083333333; {thirty minute time delay}
one_hour = 0.0416666667; {one hour delay}
one_hour_forty_minutes = 0.06944444444; {one hour and forty minute delay}
four_hours = 0.16666666667; {four hour time delay}
eight_hours = 0.33333333333; {eight hour time delay}
eight_hour_ten = 0.34027777778; {eight hour and ten minute time delay}
one_day = 1.00000000000; {one day time delay}
one_day_ten = 1.00694444444; {one day and ten minute time delay}

cond_slope = 0.91890032751; {slope for conductivity/salinity equation}
cond_int = -1.69206624611; {intercept for conductivity/salinity equation}
pH_slope = 0.96875; {slope for pH equation}
pH_int = 0.21875; {intercept for pH equation}

mv1_slope = 5.44439686118; {slope for room intensity 1}
mv1_int = -26.0701258297; {intercept for room intensity 1}
mv2_slope = 4.00462107542; {slope for room intensity 2}
mv2_int = -34.1105979232; {intercept for room intensity 2}
smv1_slope = 183.95606; {slope for chamber 1 lights}
smv1_int = -9161.6025; {intercept for chamber 1 lights}
smv2_slope = 172.55911; {slope for chamber 2 lights}
smv2_int = -4853.2826; {intercept for chamber 2 lights}

pmv_slope = 33.0609116092; {slope for photocell output check}

mmv_slope = 0.001155118612; {slope for photocell intensity}
mmv_int = 105.393209386; {intercept for photocell intensity}
bio_slope = 6.1551848;  \{slope for biomass conversion\}
bio_int = 541.1735;  \{intercept for biomass conversion\}

VAR
flag:array[1..25] of boolean;
command:array[1..70] of string[50];
flow:array[1..10] of real;
biomass1:array[1..3] of real;
biomass2:array[1..3] of real;
sgr1:array[1..2] of real;
sgr2:array[1..2] of real;
last_command:string[30];
dummy,nexttime:string[18];
stack:array[1..70] of dgt;
space,dum:dgt;
data1,dummy1:array[1..max_records] of info;
init:conditions;
lux,mv,ph,temperat,cond,biomass:real;
status1,status2:status_type;
masterfile:text;
dfile:string[20];
dumm:string[5];
choice:char;
year,month,day,dayofweek,hour,minute,second,sec100:word;
count,mu,bio_time,,c,x,a,t,b,udetector,first_harvest,second_harvest:real;
cotime,etime,etime,entry_time,ldetector,cdetector,dead_time,last_time:real;
mv2,d,xtime,chktime,flow_time,trash,check_time,uhcheck_time,uhcheck_time:real;
num1,num2,rep3,rep1,water,rep2,ans,change,clean,divide,hold,cha,ij,z:integer;
dump1,dump2,dump3,dump4,b1,b2,dump5,dump6,dump7,dump8,sta:integer;
temp1,temp2,temp3,data,cumharv1,cumharv2,harvest_day:integer;
dif,sgr,timer,last_time1,last_time2,harvest1,harvest2:real;

{*********************************************************************}

Procedure Rs_Init;
{this procedure initializes the RS-232 port; the bit field layout is as follows
bits 7-5; Speed
 000 = 110
 001 = 150
 010 = 300
 011 = 600
 100 = 1200
 101 = 2400
 110 = 4800
 111 = 9600

bits 3-4: Parity
  00 = None
  01 = Odd
  10 = None
  11 = Even

bit 2: Stop bits
  0 = 1
  1 = 2

bits 0-1: Word length (bits)
  00 = 5
  01 = 6
  10 = 7
  11 = 8

Bit Position  7  6  5  4  3  2  1  0
Bit Value     1  1  1  0  0  0  1  1
Integer Value 128 64 32 16  8  4  2  1

var
  regs: Registers;
begin
  with regs do
  begin
    ax := 128 + 64 + 32 + 2 + 1;
    {Initialize 9600 baud, no parity, 1 stop bit, 8 data bits}
    dx := 0; {com1}
    intr($14, regs);
  end;
end;

FUNCTION Listen:Integer;
{This function is meant to be used after a command has been sent to the
  ADC-1 unit. The unit's response is returned}
Var i, RcvrState:Integer;
Begin
  Repeat RcvrState := Port[SP]; {Read RR0 of Port #6}
  Until ((RcvrState and 1) = 1); {Check bit #0 of RR0 for high}
  Listen := Port[PN];
End;
Function ReadChannel(CN:Integer):real;
{This function reads the indicated input channel and returns an integer value
  corresponding to mV*2 across the selected channel.
The function LISTEN must precede this procedure!}
Var trash, Digin, LowZot, HighZot, MaskedHZ :integer;
  zot:real;
Begin
  trash:=port[pn]; {clear trash}
  CN:=CN-1; {Set to 0-15 range}
  Port[PN]:=CN; {Select input Channel}
  Digin:=Listen; {Read digital inputs}
  Repeat
    Port[PN]:=129+32; {Request A/D high byte/status}
    HighZot:=Listen; {Get high byte from port #4}
    Until (HighZot AND 128)=0 ; {Check status for A/D done}
    Port[PN]:=129+16; {Request A/D low byte}
    LowZot:=Listen; {Get low byte}
    MaskedHZ:=(HighZot and 15); {Mask off 4 high order bits}
    Zot:=(LowZot+256*MaskedHZ); {Combine all 12 bits}
    If (HighZot and 16)=0 then Zot:=-Zot; {Check for negative: Bit#5}
    Zot:=Zot/divide; {Readings are converted to}
    ReadChannel:=Zot; {millivolts}
End;

Procedure Cursor(Action:OnOff);
{This procedure turns the cursor off and back on again. The following TYPE
declaration must be made in the calling program:
  TYPE
      OnOff = (on,off);
  and the procedure is called thusly: Cursor(on); (turns on the cursor)
      Cursor(off); (turns off the cursor)
Whenever a Cursor(off) is called, a Cursor(on) must be called prior to
  the termination of the program or the cursor will not appear
{created 8/16/87 by MPT}
var
  Regs : Registers;
  Value : Integer;
begin
  Intr($11,regs);
  if lo(regs.ax) and $30 = $30 then
    value := $0C0D {monochrome}
  else

value := $0607; {color}
regs.ax:=0100;
case Action of
  off : Regs.cx := $2607;
on  : Regs.cx := value;
end;
Intr($10,Regs);
end;
{****************************************************************************************************}

Function EXIST(IOP:KEY):BOOLEAN;
{this function tests for the existence of the file named IOP and returns a boolean "true" if the file exists.}

The main program calling the function must include the type statement: TYPE

  KEY=STRING[80];


var
  fil:file;
begin
  assign(fil,iop);
  {$I-}
  reset(fil);
  {$I+}
  exist:=(ioresult=0);
end; {of function EXIST}
{****************************************************************************************************}

Function Read_real(x,y:integer):real;
{This function assumes the input of a real number and full line control }
Var
  trash:string[20]; code:integer; a:real;
BEGIN
  repeat
    gotoxy(x,y); cleol;
    readln(trash); val(trash,a,code);
    if not (code=0) then write(#07) else read_real:=a;
  until code=0;
END; {of function read-real}
Function Read_Integer(x,y:integer):integer;
{This function assumes an input of an integer and full line control.}
Var
    trash:string[20]; code,a:integer;
BEGIN
    repeat
        gotoxy(x,y); clreol;
        readln(trash); val(trash,a,code);
        if not (code = 0) then write (#07) else read_integer:=a;
    until code = 0;
END; {of function read_integer}

Procedure Setup;
var l:integer;
BEGIN
    gotoxy(50,1); clreol; write('begin setup');
    t:=0; b:=0; x:=0; time:=0; etime:=0; stime:=0; entry_time:=0; dead_time:=0; d:=0;
    last_time:=0; chktime:=0; first_harvest:=0; trash:=0; detector:=0; count:=0; rep3:=0;
    water:=0; c:=0; a:=0; ccheck_time:=0; uhcheck_time:=0; divide:=0; clean:=0; j:=0;
    hold:=0; cham:=0; j:=0; z:=0; ihcheck_time:=0; second_harvest:=0; rep1:=0; rep2:=0;
    xtime:=0; flow_time:=0; ans:=0; lux:=0; temperat:=0; biomass:=0; pH:=0; cond:=0;
    sta:=0; udetector:=0; cdetector:=0; num1:=0; num2:=0; dump1:=0; dump2:=0; dump3:=0;
    dump4:=0; data:=0; dump5:=0; dump6:=0; dump7:=0; dump8:=0; temp1:=0; temp2:=0;
    temp3:=0; b1:=0; b2:=0; umahv1:=0; umahv2:=0; harvest1:=0.013888889;
    last_time1:=0.0; last_time2:=0.0; harvest2:=0.013888889; dif:=0.0; sgr:=0.0;
    timer:=0.0;
    dfile:='  ';
    for l:=1 to 2 do
        BEGIN
            sgr1[l]:=0.0;
            sgr2[l]:=0.0;
        END;
    for l:=1 to 10 do
        flow[l]:=0.0;
    for l:=1 to 25 do
        flag[l]:=false;
    flag[10]:=true;
    for l:=1 to 3 do
        BEGIN
            biomass1[l]:=0.0;
            biomass2[l]:=0.0;
        END;
with data1[max_records] do
BEGIN
  for l:=1 to 10 do points[i]:=0;
  for l:=1 to 2 do
    stats[i]:=0;
END;
data1[1].name:='room temp 1 (deg. C)';
data1[2].name:='room temp 2 (deg. C)';
data1[3].name:='room intensity 2 (Lux)';
data1[4].name:='room intensity 1 (Lux)';
data1[5].name:='MB-light output (Lux)';
data1[6].name:='chamber 1 light (Lux)';
data1[7].name:='chamber 2 light (Lux)';
data1[8].name:='Chamber 1 temp (deg. C)';
data1[9].name:='Chamber 1 density (mv)';
data1[10].name:='Chamber 1 pH';
data1[11].name:='Chamber 1 salinity (ppt)';
data1[12].name:='Chamber 1 density (g/m3)';
data1[13].name:='Chamber 2 temp (deg. C)';
data1[14].name:='Chamber 2 density (mv)';
data1[15].name:='Chamber 2 pH';
data1[16].name:='Chamber 2 salinity (ppt)';
data1[17].name:='Chamber 2 density (g/m3)';
with init do
  BEGIN
    ADC_set:=' ';  
    for l:=1 to 5 do light_out[i]:=0;
    for l:=1 to 2 do room_temp[i]:=0;  
    for l:=1 to 9 do params[i]:=0;
    END;
gotoxy(1,21);writeln('1: +/- 400 mv');
gotoxy(1,22);writeln(' 2: +/- 1.023 volts');
gotoxy(1,23);writeln(' 3: +/- 2.047 volts');
gotoxy(1,24); writeln(' 4: +/- 4.095 volts');
gotoxy(17,20); clreol;
write('For what voltage range is the ADC-1 board set? ');  
nans:=read_integer(64,20); 
case ans of
1:BEGIN 
  divide:=10; 
  init.adc_set:='ADC-1 voltage range = +/- 400 mv';
  END;
2:BEGIN 
  divide:=4; 
  init.adc_set:='ADC-1 voltage range = +/- 1.023 volts';
  END;
3:BEGIN
    divide:=2;
    init.adc_set:='ADC-1 voltage range = +/- 2.047 volts';
END;
4:BEGIN
    divide:=1;
    init.adc_set:='ADC-1 voltage range = +/- 4.095 volts';
END;
END; {case of}
gotoxy(17,20); clreol;
gotoxy(50,1); clreol; write('end setup');
END; {of procedure setup}

{******************************************************************************}

Procedure Genscreen;
{This procedure generates the screen when the computer is initially booted.}
Var
    i:integer;
BEGIN
    clrscr;
gotoxy(37,1); write('ACTIVITY ==>');
gotoxy(50,1); clreol; write('begin genscreen ');
gotoxy(1,1); write('CURRENT TIME: ');
gotoxy(36,3); write('TOGGLE DEVICE STATUS');
gotoxy(36,4); write('-------------------------------');
for i:=1 to 12 do
    BEGIN
        gotoxy(36,4+i);
        if (i = 6) then
            write(' ',menu_names[i])
        else
            write(chr(65+i-1):4,' ',menu_names[i]);
        gotoxy(76,4+i);
        write('OFF');
    END;
gotoxy(36,17); clreol;
write(' S SUPERVISOR InActive');
gotoxy(36,18); clreol;
write('Press [ESC] to leave program');
gotoxy(1,20);
write('UPDATE/MESSAGES:');
gotoxy(50,1); clreol; write('end genscreen');
END; {of Procedure genscreen}
**Procedure Display_Time:**

(This procedure calls the library procedure to display the time)

```
BEGIN
  Getdate(year,month,day,dayofweek);
  Gettime(hour,minute,second,sec100);
  gotoxy(15,1);
  write(month:2,'/',day:2,'/',year:2, ' ',hour:2,':',minute:2,':',second:2);
END; {end of display_time}
```

**Function Dtime:real:**

(This function calculates the real time in fractions of day (based on seconds))

```
Var
  dyear,dmonth :real;
  month_days:array[1..12] of integer;
BEGIN
  month_days[1]:=0;
  month_days[2]:=31;
  month_days[3]:=59;
  month_days[4]:=90;
  month_days[5]:=120;
  month_days[6]:=151;
  month_days[7]:=181;
  month_days[8]:=212;
  month_days[9]:=243;
  month_days[10]:=273;
  month_days[11]:=304;
  month_days[12]:=334;
  Display_Time;
  dmonth:=month_days[trunc(month)];
  Dtime:=dmonth-day+((hour*3600.0)+(minute*60.0)+second)/(86400.0);
END; {of function Dtime}
```
Procedure Convert_Time(t:real);
{This procedure converts a real time back to month, day, hour,... }
Var
  mt,dy,temp,hr,mn,sec:integer;
  hrs,mns,secs,ttemp,rtemp:real;
BEGIN
  temp:=trunc(t);
  if temp >= 334 then BEGIN mt:=12; dy:=temp-334; END
  else if temp > 304 then BEGIN mt:=11; dy:=temp-304; END
  else if temp > 273 then BEGIN mt:=10; dy:=temp-273; END
  else if temp > 243 then BEGIN mt:=9; dy:=temp-243; END
  else if temp > 212 then BEGIN mt:=8; dy:=temp-212; END
  else if temp > 181 then BEGIN mt:=7; dy:=temp-181; END
  else if temp > 151 then BEGIN mt:=6; dy:=temp-151; END
  else if temp > 120 then BEGIN mt:=5; dy:=temp-120; END
  else if temp > 90 then BEGIN mt:=4; dy:=temp-90; END
  else if temp > 59 then BEGIN mt:=3; dy:=temp-59; END
  else if temp > 31 then BEGIN mt:=2; dy:=temp-31; END
  else BEGIN mt:=1; dy:=temp; END;
  ttemp:=(t-temp);
  hrs:=ttemp*24; hr:=trunc(hrs);
  mns:=(hrs-hr)*60.0; mn:=trunc(mns);
  secs:=(mns-mn)*60.0; sec:=trunc(secs);
  writeln(mt:',',dy:',',hr:',',mn:',',sec); END;
{end of procedure convert_time}

Procedure Clear_Update;
Var
  i:integer;
BEGIN
  gotoxy(50,1); clreol; write('begin clear_update');
  for i:=1 to 4 do
    BEGIN
      gotoxy(1,20+i);
      clreol;
      END;
  gotoxy(50,1); clreol; write('end clear_update');
END;
Procedure Comments(num:integer);
Const
  cmts:array[1..12] of string[60] = (
    {1} 'Light output intensity below 85%','
    {2} 'Check level detector--not working',
    {3} 'Having problems writing to disk',
    {4} 'Data stored in dummy file',
    {5} 'Data will be dumped to disk at a later time',
    {6} 'Put disk in drive B',
    {7} 'Can"t print dummy file',
    {8} 'Attempting disk write operations....',
    {9} 'Saving data to disk',
    {10} 'Data in dummy file lost',
    {11} 'Problem opening file',
    {12} 'Can"t close file, will try later');
BEGIN  
clear_update;
write(\07);
gotoxy(1,21); write(cmts[num]);
END; {of procedure comments}

Procedure Time_date;
BEGIN
  {+$-}
  if not (ioresult = 0) then
    BEGIN
      comments(3);
    END
  else if (flag[8]) then
    writeln(masterfile,month:2,'/',day:2,'/',year:2,' ',hour:2,':',
      minute:2,':',second:2,'."
  else if (not flag[8]) then
    writeln(month:2,'/',day:2,'/',year:2,' ',hour:2,':',minute:2,':',
      second:2)
  {+$+}
END; {of procedure time_date}
Procedure ADC_1_1;
BEGIN
    async_init;
    if not async_open(1,9600,'N',8,1) then
        BEGIN
            gotoxy(1,24); clrnl;
            writeln('** ERROR: ASYNC_OPEN FAILED');
            halt
        END;
        async_send('#20);
        delay(1000);
        async_send('1');
        delay(1000);
        async_send('#20);
        delay(500);
        async_send('1');
        delay(500);
    END; {of procedure adc_1_1}

Procedure ADC_1_2;
BEGIN
    async_init;
    if not async_open(1,9600,'N',8,1) then
        BEGIN
            gotoxy(1,24); clrnl;
            writeln('** ERROR: ASYNC_OPEN FAILED');
            halt
        END;
        async_send('#20);
        delay(1000);
        async_send('2');
        delay(1000);
        async_send('#20);
        delay(500);
        async_send('2');
        delay(500);
    END; {of procedure adc_1_2}
Procedure ctoutput_signal(cs:status_type);
Var
coutput:integer;
BEGIN
  port[pn]:=coutput;
  trash:=listen;
  port[pn]:=coutput;
  trash:=listen;
END; {of procedure ctoutput_signal}

Procedure All_Off(var status:status_type);
Var
  x:integer;
BEGIN
  gotoxy(50,1); clrdoc; write('begin all off procedure');
  for x:=1 to 12 do
    status[x]:=0;
  coutput_signal(status);
  gotoxy(50,1); clrdoc; write('end all off procedure');
END; {of procedure all_off}

Procedure ctoutput_action(y:integer; var status:status_type);
Var
  yy:integer;
BEGIN
  if (status[y] = 0) then status[y]:=1 else status[y]:=0;
  coutput_signal(status);
END; {of procedure ctoutput_action}
Procedure all_cout_off;
BEGIN
  adc_1_1;
  rs_init;
  all_off(status1);
  adc_1_2;
  rs_init;
  all_off(status2);
  adc_1_1;
  rs_init;
  ctoutput_action(10,status1);
  adc_1_2;
  rs_init;
  ctoutput_action(11,status2);
  flag[10]:=true;
  adc_1_2;
  rs_init;
  ctoutput_action(1,status2);
  for i:=5 to 16 do
    BEGIN
      gotoxy(76,i);
      clreol;
      write('OFF');
    END;
END; {of procedure all cout off}

Function Temperature(j:integer):real;
{This function monitors the Jth analog input channel and returns
a temperature value. A 1000 ohm resistor is used across the analog channels.}
Var
  adjust:array[1..3] of real;
BEGIN
  gotoxy(50,1); clreol; write('begin reading temperature');
  adjust[1]:=0.00; {correction factor for room probe #1}
  adjust[2]:=1.50; {correction factor for room probe #2}
  adjust[3]:=1.50; {correction factor for monitoring probe}
  Temperature:=readchannel(j)-273+adjust[j];
  gotoxy(50,1); clreol; write('end temperature reading');
END; {of function temperature}
Function Intensity(j:integer):real;
{This function monitors the Jth analog input channel and returns a light
intensity reading in mv. A 4700 ohm resistor is used across the analog
channels.}
BEGIN
  gotoxy(50,1); clreol; write('begin reading intensity');
  intensity:=readchannel(j);
  gotoxy(50,1); clreol; write('end intensity reading');
END; {of function intensity}

Procedure data_file;
BEGIN
repeat
  clear_update;
  if (flag[4] = false) then
    BEGIN
      gotoxy(1,21); write('No data files are currently open');
    END
  else if (flag[4] = true) then
    BEGIN
      gotoxy(1,21); writeln(dfile, ' is currently open.');
      gotoxy(1,22); writeln('Please close this file before opening another.');
    END;
  gotoxy(1,23); write('1: Create datafile ');
  gotoxy(1,24); write('2: Close datafile ');
  gotoxy(30,23); write('3: Quit ');
  gotoxy(17,20); clreol;
  write('Which option would you like to choose ? ');
  repeat
    ans:=read_integer(57,20);
  until (ans = 1) or (ans = 2) or (ans = 3);
  case ans of
    1:BEGIN
      {$I-}
      flag[4]:=true;
      repeat
        data:=data+1;
        str(data,dumm);
        dfile:='b:dat'+dumm+'.dat';
      until not EXIST(dfile);
      assign(masterfile,dfile);
      rewrite(masterfile);
if (not ioresult = 0) then comments(11);
gotoxy(17,20); clreol; write('datafile = ',dfile);
{$!+$
END;
2:BEGIN
{$!-$
    flag[4]:=false;
close(masterfile);
if (not ioresult = 0) then comments(12)
else
    data:=data+1;
dfile:='none open';
gotoxy(17,20); clreol; write('datafile = ',dfile);
{$!-$
END;
3:BEGIN
    clear_update;
END;
END; {of case 0f}
until (ans = 3);
clear_update;
gotoxy(17,20); clreol; write('datafile = ',dfile);
END;

{**************************************************}

Procedure Air_Conditioner;
{This procedure turns the air conditioner on or off depending on the room
and culture temperatures.}
BEGIN
    gotoxy(50,1); clreol;
    if (status2[1] = 0) then
    BEGIN
        write('begin air conditioner');
gotoxy(76,5); clreol; write(' ON');
    END
else
    BEGIN
write('end air conditioner');
gotoxy(76,5); clreol; write(' OFF');
END;
adc_1_2;
rs_init;
coutput_action(1,status2);
END; {of procedure air_conditioner}
Procedure Heater;
{This procedure turns the heater on or off depending on the room and
culture temperatures.}
BEGIN
  gotoxy(50,1); clrloc;
  if (status1[3] = 0) then
  BEGIN
    write('begin heater');
    gotoxy(76,5); clrloc; write(' ON');
    END
  else
  BEGIN
    write('end heater');
    gotoxy(76,5); clrloc; write('OFF');
    END;
  adc_1_1;
  rs_init;
  ctoutput_action(3,status1);
END; {of procedure heater}

Procedure Statist(num1,num2,num3:integer);
Var
  x,i:integer;
BEGIN
  if (num3 > 0) then
  BEGIN
    dt:=dtime;
    for i:= num1 to num2 do
    BEGIN
      for x:=1 to num3 do
        data1[i].stats[1]:=data1[i].stats[1]+data1[i].points[x];
        data1[i].stats[1]:=data1[i].stats[1]/num3;
    END;
  END;
END;
Procedure Messages;
{This procedure asks the operator to choose either chamber 1 or 2.}
BEGIN
  gotoxy(50,1); clreol; write('begin messages');
  clear_update;
  repeat
    gotoxy(1,24); clreol;
    write('Which chamber would you like to select => 1 or 2: ');
    cham:=read_integer(51,24);
  Until (cham = 1) or (cham = 2);
  flag[8]:=false;
  gotoxy(1,24); clreol;
  if (flag[19]) or (flag[20]) then
    BEGIN
      case ans of
        1: write('Chamber ',cham,' harvested at ');
        2: write('Chamber ',cham,' refilled at ');
        3: write('Chamber ',cham,' had additional brine added at ');
      END; {of case of}
      time_date
    END
  else if (flag[2]) or (flag[3]) then
    BEGIN
      case ans of
        1: write('Chamber ',cham,' completely harvested at ');
        2: write('Chamber ',cham,' filled with disinfectant at ');
        3: write('The disinfectant was vacuumed from chamber ',cham,' at ');
        4: write('Chamber ',cham,' was rinsed at ');
        5: write('The equilibrium cycle was activated at ');
        6: write('Both chambers were refilled, starting at ');
      END; {of case of}
      time_date;
    END;
  END;
  gotoxy(50,1); clreol; write('end messages');
END; {of procedure messages'};

{*******************************************************************************}

Procedure Temperature_Update(num1,num2:integer);
{This procedure reads the analog input channels and updates the room
  conditions on the screen.}
Var
  i,x:integer;
  mv:real;
BEGIN
    gotoxy(76,11); clrcl; write(' ON');
    gotoxy(50,1); clrcl; write('begin room_environment_update');
    rep1:=rep1+1;
    adc_1_1;
    rs_init;
    x:=num1;
    data1[x].points[rep1]:=temperature(1);x:=x+1;
    data1[x].points[rep1]:=temperature(2);
    if (flag[11]) then
        BEGIN
            i:=0;
            with init do
                BEGIN
                    i:=i+1;
                    room_temp[i]:=data1[x].points[rep1];
                END;
        END;
    if (data1[1].points[rep1]<init.params[1]) and
        (data1[2].points[rep1]<init.params[1]) and (status1[3]=0) then heater;
    if (data1[1].points[rep1]<init.params[1]) and
        (data1[2].points[rep1]<init.params[1]) and (status2[1]=1) then air_conditioner;
    if (data1[1].points[rep1]>init.params[2]) and
        (data1[2].points[rep1]>init.params[2]) and (status1[3]=1) then heater;
    if (data1[1].points[rep1]>init.params[2]) and
        (data1[2].points[rep1]>init.params[2]) and (status2[1]=0) then air_conditioner;
    clear_update;
    i:=0;
    for x:=num1 to num2 do
        BEGIN
            i:=i+1;
            if (i <=4) then
                gotoxy(1,20+i)
            else
                gotoxy(45,15+i);
            write(data1[x].name,'  = ',data1[x].points[rep1]:6:2);
        END;
    if (rep1 >= 10) then
BEGIN
  sta:=1;
  if (flag[5]) then
    BEGIN
      dum pl :=1;
    END
  else
    rep1:=0;;
  END;
  gotoxy(76,11); clreol;
  write('OFF');
  gotoxy(50,1); clreol; write('end temperature_update');
END; {of procedure temperature_update}

BEGIN
  Procedure Light_Output_Check(num1,num2:integer);
  {This procedure checks the light intensity of the culture and photocell lights and gives a message if the intensity has dropped below 85 percent of the initial intensity.}
  Var
    i,x:integer;
  BEGIN
    gotoxy(50,1); clreol; write('begin light_output_check');
    if (flag[18]) and (not flag[9]) then
      BEGIN
        adc_1_2;
        rsinit;
        ctoutput_action(8,status2);
      END;
    adc_1_1;
    rsinit;
    rep2:=rep2+1;
    x:=num1;
    mv:=intensity(7);
    data1[x].points[rep2]:=mv2_slope*mv+mv2_int;x:=x+1;
    mv:=intensity(6);
    data1[x].points[rep2]:=mv1_slope*mv+mv1_int;x:=x+1;
    mv:=intensity(5);
    data1[x].points[rep2]:=pmv_slope*mv+pmv_int;x:=x+1;
    mv:=intensity(8);
    data1[x].points[rep2]:=smv1_slope*mv+smv1_int;x:=x+1;
    mv:=intensity(9);
    data1[x].points[rep2]:=smv2_slope*mv+smv2_int;
    clear_update;
  END;

Procedure Light_Output_Check(num1,num2:integer);
{This procedure checks the light intensity of the culture and photocell lights and gives a message if the intensity has dropped below 85 percent of the initial intensity.}
i:=0;
for x:=num1 to num2 do
BEGIN
  i:=i+1;
  if (i <= 4) then
    gotoxy(1,20+i)
  else
    gotoxy(40,16+i);
  write(data1[x].name,' = data1[x].points[rep2]:6:2);
END;
if (flag[11]) then
BEGIN
  i:=0;
  with init do
  BEGIN
    for x:=num1 to num2 do
    BEGIN
      i:=i+1;
      light_out[i]:=data1[x].points[rep2];
    END;
  END;
END;
if (rep2 >= 10) then
BEGIN
  sta:=2;
  if (flag[5]) then
    BEGIN
      dump5:=1;
    END
  else
    rep2:=0;
END;
gotoxy(76,14); clreol;
write('OFF');
if (flag[18]) and (not flag[9]) then
  BEGIN
    adc_1_2;
    rs_init;
    cloutput_action(8,status2);
  END;
  flag[18]:=false;
gotoxy(50,1); clreol; write('end light_output_check');
END; {of procedure light_output_check}

{**********************************************************************}

Procedure CO2_Addition;
BEGIN
  if (status1[11] = 0) then
    BEGIN
      gotoxy(50,1); clreol; write('begin CO2 addition');
      gotoxy(76,9); clreol; write('ON');
    END
  else
    BEGIN
      gotoxy(50,1); clreol; write('end CO2 addition');
      gotoxy(76,9); clreol; write('OFF');
    END;
  adc_1_1;
  rs_init;
  cloutput_action(11,status1);
END; {of procedure CO2 addition}

{**********************************************************************}

Procedure Init_Cond;
BEGIN
  gotoxy(50,1); clreol; write('begin initial conditions');
  flag[11]:=true;
  clear_update;
  temperature_update(1,2);
  delay(5000);

  flag[18]:=true;
  light_output_check(3,7);
  delay(5000);
  flag[11]:=false;
  gotoxy(50,1); clreol; write('end initial conditions');
END; {of init cond}
Procedure Op_Params;
Var
i: integer;
BEGIN
  gotoxy(50,1); clrln; write('begin operational parameters');
  clear_update;
  with init do BEGIN
    gotoxy(1,21);
    write('1: ',parameters[1],', ',params[1]:4:1);
    gotoxy(1,22);
    write('2: ',parameters[2],', ',params[2]:4:1);
    gotoxy(1,23);
    write('3: ',parameters[3],', ',params[3]:4:1);
    gotoxy(1,24);
    write('4: ',parameters[4],', ',params[4]:4:1);
    gotoxy(22,21);
    write('5: ',parameters[5],', ',params[5]:10:8);
    gotoxy(22,22);
    write('6: ',parameters[6],', ',params[6]:10:8);
    gotoxy(22,23);
    write('7: ',parameters[7],', ',params[7]:10:8);
    gotoxy(22,24);
    write('8: ',parameters[8],', ',params[8]:10:8);
    gotoxy(52,21);
    write('9: ',parameters[9],', ',params[9]:4:1);
    gotoxy(52,22);
    write('10:QUIT');
  END;
  Repeat
    with init do BEGIN
      gotoxy(17,20); clrln;
      write('Press the desired toggle number or "10" to quit');
      change:=read_integer(65,20);
      case change of
      1: BEGIN
        gotoxy(17,20); clrln;
        write('Input new low temp');
        params[1]:=read_real(36,20);
        gotoxy(14,21); write('');
        gotoxy(14,21);
        write(params[1]:4:1);
        END;
2:BEGIN
  gotoxy(17,20); clrsl;
  write('Input new high temp');
  params[2]:=read_real(37,20);
  gotoxy(14,22); write('
');
  gotoxy(14,22);
  write(params[2]:4:1);
END;
3:BEGIN
  gotoxy(17,20); clrsl;
  write('Input new low pH');
  params[3]:=read_real(34,20);
  gotoxy(14,23); writeln('
');
  gotoxy(14,23);
  write(params[3]:4:1);
END;
4:BEGIN
  gotoxy(17,20); clrsl;
  write('Input new high pH');
  params[4]:=read_real(35,20);
  gotoxy(14,24); write('
');
  gotoxy(14,24);
  write(params[4]:4:1);
END;
5:BEGIN
  gotoxy(17,20); clrsl;
  write('Input new lights on time');
  params[5]:=read_real(42,20);
  gotoxy(38,21); write('
');
  gotoxy(38,21);
  write(params[5]:10:8);
END;
6:BEGIN
  gotoxy(17,20); clrsl;
  write('Input new lights off time');
  params[6]:=read_real(43,20);
  gotoxy(38,22); write('
');
  gotoxy(38,22);
  write(params[6]:10:8);
END;
7:BEGIN
  gotoxy(17,20); clrsl;
  write('Input new CO2 on delay');
  params[7]:=read_real(40,20);
  gotoxy(38,23); write('
');
gotoxy(38,23); write(params[7]:10:8); END;
8:BEGIN
  gotoxy(17,20); clreol; write('Input new CO2 off delay '); params[8]:=read_real(41,20);
  gotoxy(38,24); write('  
  
  );
  gotoxy(38,24);
  write(params[8]:10:8);
END;
9:BEGIN
  gotoxy(17,20); clreol; write('Input new culture temperature '); params[9]:=read_real(47,20);
  gotoxy(65,21); write('  
  
  ');
  gotoxy(65,21);
  write(params[9]:4:1);
END;
clear_update;
END; {of case}
END; {of procedure op_params}

{*******************************************************}

Procedure Valve_Diag;
BEGIN
  clear_update;
gotoxy(50,1); clreol; write('begin valve diagnostics');
gotoxy(76,13); clreol; write('  ON');
gotoxy(1,21);
write('1: ',ctout1_names[4]);
gotoxy(1,22);
write('2: ',ctout1_names[7]);
gotoxy(1,23);
write('3: ',ctout1_names[8]);
gotoxy(1,24);
write('4: ',ctout1_names[9]);
gotoxy(20,21);
write('5: ctout1_names[11]);
gotoxy(20,22);
write('6: ctout2_names[2]);
gotoxy(20,23);
write('7: ctout2_names[3]);
gotoxy(20,24);
write('8: ctout2_names[4]);
gotoxy(40,21);
write('9: ctout2_names[5]);
gotoxy(39,22);
write('10: ctout2_names[6]);
gotoxy(39,23);
write('11: ctout2_names[7]);
gotoxy(39,24);
write('12: ctout2_names[9]);
gotoxy(60,21);
write('13: ctout2_names[10]);
gotoxy(60,22);
write('14: ctout2_names[11]);
gotoxy(60,23);
write('15: ctout2_names[12]);
gotoxy(60,24);
write('16: QUIT');
repeat
  gotoxy(17,20); clreol;
  write('Press the desired toggle number or "9" to quit');
  change:=read_integer(64,20);
  case change of
    1,2,3,4,5:BEGIN
      adc_1_1;
      rs_init;
    END;
    1,2,3,5:BEGIN
      ctoutput_action(change,status1);
      gotoxy(17,20); clreol;
      write('Press any key to deactivate controlled output');
      repeat until keypressed;
      if keypressed then
        ctoutput_action(change,status1);
    END;
    4:BEGIN
      ctoutput_action(change,status1);
      delay(5000);
      ctoutput_action(change,status1);
    END;
END;
END;

6,7,8,9,10,11,12,13,14,15:BEGIN
  adc_1_2;
  rs_init;
  case change of
    7,8,11,12,14:BEGIN
      ctoutput_action(change,status2);
      gotoxy(17,20);clreol;
      write('Press any key to deactivate controlled output');
      repeat until keypressed;
      if keypressed then
        ctoutput_action(change,status2);
    END;
    6,9,10,13,15:BEGIN
      ctoutput_action(change,status2);
      delay(5000);
      ctoutput_action(change,status2);
    END;
  END;
END;

END;

16:clear_update
END; {of case of
until (change = 16);
gotoxy(17,20);clreol;write('datafile = ',dfile);
gotoxy(76,16);clreol;
write('OFF');
gotoxy(50,1);clreol; write('end valve diagnostics');
END;

{*******************************************************************************
{*******************************************************************************

Procedure Dump_Data_Disk(num1,num2,num3:integer);
Var
  i,x:integer;
BEGIN
  {$I-}
  if (not ioresult=0) then

    BEGIN
      i:=num1;
      comments(3);comments(4);comments(5);
      while (i >= num1) and (i <=num2) do
BEGIN
  for x:=1 to num3 do
    BEGIN
      dummy1[i].points[x]:=data1[i].points[x];
      data1[i].points[x]:=0;
    END;
    dummy1[i].stats[1]:=data1[i].stats[1];
    data1[i].stats[1]:=0;
    i:=i+1;
  END; {of while}
  flag[16]:=true;
END

else
  BEGIN
    gotoxy(50,1); clreol; write('begin data dump');
    i:=num1;
    flag[16]:=false;
    flag[8]:=true;
    writeln(masterfile,' ':20,'******************************');
    write(masterfile,' ':10,'Data dumped at: ');
    time_date;
    while (i >= num1) and (i <= num2) do
      BEGIN
        write(masterfile,' ':10,datal[i].name,' : ');
        for x:=1 to num3 do
          BEGIN
            if (x = num3) then
              BEGIN
                writeln(masterfile,datal[i].points[x]:10:2,' : MEAN = ';
                data1[i].stats[1]:7:2);
                datal[i].points[x]:=0;
                data1[i].stats[1]:=0;
              END
            else
              BEGIN
                write(masterfile,datal[i].points[x]:10:2);
                datal[i].points[x]:=0;
              END;
            END;
          i:=i+1;
        END;
    gotoxy(50,1); clreol; write('end data dump');
  END;
{$!+}
END; {of procedure data dump}
Procedure Dummy_data_dump_disk(num1,num2,num3:integer);
Var
  i,x:integer;
BEGIN
  if not ioresult=0 then
    BEGIN
      comments(6);
      comments(7);
    END
  else
    BEGIN
      gotoxy(50,1); clrcli; write('begin dummy data dump');
      i:=num1;
      flag[8]:=true;
      writeln(masterfile,' ':20,'***************');
      write(masterfile,' ':10,'Data dumped at: ');
      time_date;
      while (i >= num1) and (i <= num2) do
        BEGIN
          write(masterfile,' ':10,data1[i].name,': ');
          for x:=1 to num3 do
            BEGIN
              if (x = num3) then
                BEGIN
                  writeln(masterfile,dummy1[i].points[x]:10:2,' : MEAN = ',
                          dummy1[i].stats[1]:7:2);
                  dummy1[i].points[x]:=0;
                  dummy1[i].stats[1]:=0;
                END
              else
                BEGIN
                  write(masterfile,dummy1[i].points[x]:10:2);
                  dummy1[i].points[x]:=0;
                END;
            END;
          i:=i+1;
        END;
      flag[16]:=false;
    gotoxy(50,1); clrcli; write('end data dump');
    END;
{$+}$
END; {of procedure data dump}
Procedure Dump_init_data;
Var
i,x:integer;
BEGIN
flag[8]:=true;
BEGIN
if not ioresult=0 then
BEGIN
  comments(3);comments(7);
  flag[15]:=true;
END
else if (not flag[14]) then
BEGIN
  flag[15]:=false;
  writeln(masterfile,'  ': io, '*******************************************');
  writeln(masterfile,'  ': io, 'Data file = ', dfile);
  write(masterfile,'  ': io, 'Initial conditions: ');
  time_date;
  writeln(masterfile);
  with init do
  BEGIN
    writeln(masterfile,'  ': io, 'ADC_set');
    for i:=1 to 5 do
      writeln(masterfile,'  ': io, 'lite[i]', ': ', light_out[i]:7:2);
    for i:=1 to 2 do
      writeln(masterfile,'  ': io, 'Room temperature ', i , ' (deg. C) : ',
                  room_temp[i]:7:2);
    for i:=1 to 9 do
      writeln(masterfile,'  ': io, 'parameters[i]', ': ', params[i]:15:10);
  END;
END
else if (flag[14]) then
BEGIN
  flag[15]:=false;
  writeln(masterfile,'  ': io, '*******************************************');
  writeln(masterfile,'  ': io, 'The operational parameters were changed at: ');
  time_date;
  writeln(masterfile);
  with init do
  BEGIN
    for i:=1 to 9 do
      writeln(masterfile,'  ': io, 'parameters[i]', ': ', params[i]:7:2);
  END;
  flag[14]:=false;
Procedure dump_harvest(f,g:integer;h:real);
BEGIN
  if not ioresult=0 then
    BEGIN
      comments(6);
      comments(7);
    END
  else
    BEGIN
      gotoxy(50,1); clreol; write('begin harvest dump');
      flag[8]:=true;
      writeln(masterfile,' ':20,'');
      time_date;
      writeln(masterfile,' ':10,'Data dumped at: ');
      writeln(masterfile,' ':10,'Chamber ',f,' had a harvest of ',g,
        ' gallons for previous day');
      writeln(masterfile,' ':10,'Chamber ',f,' had a specific growth rate of ',h,
        ' (1/day) for the previous day');
      END;
    gotoxy(50,1); clreol; write('end harvest dump');
END; {of procedure harvest_day}
{The functions and procedures located between the double lines load and shuffle the stack and watch the internal clock and the time associated with the top command.}

Function Stack_Match:boolean;
BEGIN
  if (stack[1].time<=dtime) then
    stack_match:=true
  else
    stack_match:=false;
END; {of function stack_match}
Procedure Stack_Sort(tte:real;tta:integer);
Var
ir,ie:integer;
pas_it:dgt;
BEGIN
  gotoxy(50,1); clreol; write('begin stack_sort');
  pas_it.time:=tte;
  pas_it.action:=tta;
  if (pas_it.time<stack[1].time) then
    ir:=1
  else
    BEGIN
      ir:=0;
      Repeat
        ir:=ir+1;
        Until (pas_it.time<=stack[ir].time) or (ir=69);
      END;
    for ie:=70 downto ir+1 do stack[ie]:=stack[ie-1];
    stack[ir]:=pas_it;
    if ir=69 then
     BEGIN
        clrscr;
        gotoxy(1,21);
        write('ERROR DETECTED IN COMMAND STACK, END OF FILE REACHED');
      END;
  gotoxy(50,1); clreol; write('end stack_sort');
END; {of procedure stack_sort}

Procedure Kill_Top;
{This procedure removes the top command from the stack and moves the
remaining commands up one position.}
Var
  op:integer;
BEGIN
  gotoxy(50,1); clreol; write('begin kill_top');
  for op:=1 to 69 do
    stack[op]:=stack[op+1];
  stack[70]:=space;
  gotoxy(50,1); clreol; write('end kill_top');
END; {of procedure kill_top}
Procedure Command_stack;
Var
  jk:integer;
BEGIN
  gotoxy(50,1); clrcl; write('begin command_stack');
  i:=0;
  gotoxy(1,2);
  write('Last Command:',last_command,' at '); convert_time(last_time);
  gotoxy(1,5);
  Repeat
    i:=i+1;
    convert_time(stack[i].time);
    writelnf(command[stack[i].action]);
  Until (stack[i+1].action=0) or (i=10);
  for jk:=i+1 to 10 do
    writeln('');
  gotoxy(50,1); clrcl; write('end command_stack');
END; {of command_stack}
gotoxy(17,20);clreol;
gotoxy(20,20);write('Chamber ',cham);
gotoxy(1,21);write('Culture temperature = ',temperat:6:2,' (deg. C)');
gotoxy(1,22);write('Culture intensity = ',lux:6:2,' (Lux)');
gotoxy(1,23);write('Culture biomass = ',biomass:6:2,' (g/m3)');
gotoxy(45,21);write('Culture pH = ',pH:6:2);
gotoxy(45,22);write('Culture salinity = ',cond:6:2,' (ppt)');
if (not flag[9]) or (rep3>=10) then
  BEGIN
    gotoxy(17,20);clreol;
    sta:=3;
    if (flag[5]) then
      BEGIN
        if (cham = 1) then dump3:=1
        else if (cham = 2) then dump7:=1;
        stack_sort(dtime+five_seconds,45);
      END
    else
      BEGIN
        adc_1_2;
        rs_init;
        ctoutput_action(8,status2);
        rep3:=0;;
      END;
  END
else if (rep3 < 10) and (flag[9]) then
  BEGIN
    if (flag[5]) then
      stack_sort(dtime+0.00000289352,5);
  END;
end;

gotoxy(50,1); clreol; write('end culture conditions');
END;

{*******************************************************************************}

Function Flow_calc(a,b:integer):real;
{This function calculates the time required for (1) the harvest chamber to
empty and fill and (2) the chamber to empty and fill.}
BEGIN
  gotoxy(50,1); clreol; write('begin flow time');
  flow_calc:=(flow[a] - flow[b])*86400;
END; {of function time_flow}
Procedure Open_Chamber_Valve;
BEGIN
if (flag[12]) then
  BEGIN
    case cham of
      1: BEGIN
        adc_1_2;
        rs_init;
        ctoutput_action(12,status2);
        END;
      2: BEGIN
        adc_1_1;
        rs_init;
        ctoutput_action(9,status1);
        END;
    END; {of case of}
    adc_1_2;
    rs_init;
    ctoutput_action(10,status2);
    flag[12]:=false;
  END
else
  BEGIN
    case cham of
      1: BEGIN
        adc_1_1;
        rs_init;
        ctoutput_action(9,status1);
        END;
      2: BEGIN
        adc_1_2;
        rs_init;
        ctoutput_action(12,status2);
        END;
    END; {of case of}
  END;
END; {of procedure open chamber valve}

{******************************************************************************************}

Procedure Close_Chamber_Valve;
BEGIN
  adc_1_2;
  rs_init;
if (flag[12]) then
BEGIN
   case cham of
      1:BEGIN
         ctoutput_action(6,status2);
         END;
      2:BEGIN
         ctoutput_action(5,status2);
         END;
   END; {of case of}
   adc_1_2;
   rs_init;
   ctoutput_action(2,status2);
END
else
BEGIN
   case cham of
      1:BEGIN
         ctoutput_action(5,status2);
         END;
      2:BEGIN
         ctoutput_action(6,status2);
         END;
   END; {of case of}
   END;
END; {of procedure close chamber valves}

{*******************************************************************************}

Procedure Open_Both_Chamber_Valves;
BEGIN
   adc_1_1;
   rs_init;
   ctoutput_action(9,status1);
   adc_1_2;
   rs_init;
   ctoutput_action(12,status2);
END; {of procedure open both chamber valves}

{*******************************************************************************}

Procedure Close_Both_Chamber_valves;
BEGIN
   adc_1_2;
   rs_init;
   ctoutput_action(5,status2);
Procedure Open_Main_Valve;
BEGIN
  adc_1_2;
  rs_init;
  ctoutput_action(10,status2);
END; {of procedure open main valve}

Procedure Close_Main_Valve;
BEGIN
  adc_1_2;
  rs_init;
  ctoutput_action(2,status2);
END; {of procedure close main valve}

Procedure Harvest_On;
BEGIN
  gotoxy(76,7); clreol; write(' ON');
  adc_1_2;
  rs_init;
  flow[3]:=dttime;
  gotoxy(50,1); clreol;
  write('begin harvest of chamber ',cham);
  uhcheck_time:=dttime+checks[3];
  ctoutput_action(3,status2);
  ctoutput_action(7,status2);
END; {of procedure harvest on}

Procedure Harvest_Off;
BEGIN
  flag[9]:=false;
  if (flag[5]) then
    BEGIN
      if (flag[2]) or (flag[3]) then
        BEGIN
          stack_sort(dttime,33);
        END;
stack_sort(dtime+checks[2],33);
stack_sort(dtime+0.00006365741,21);
END;
if (flag[19]) or (flag[20]) then
BEGIN
    stack_sort(dtime,43);
    stack_sort(dtime+checks[2],43);
    stack_sort(dtime+ten_seconds,23);
END;
END;
adc_1_2;
rsjnit;
ctoutput_action(3,status2);
ctoutput_action(7,status2);
gotoxy(76,7);crlol; write('OFF');
gotoxy(50,1);crlol;
    write('end harvest of chamber ',cham);
END; {of procedure harvest}

{*******************************************************************************}

Procedure Refill_Chambers;
{This procedure refills the culture chambers with nutrients, brine, 
and tap water following the routine harvest cycle.}
BEGIN
    gotoxy(50,1);crlol;
    write('begin chamber ',cham,' refill');
    gotoxy(76,7);crlol; write(' ON');
    ccheck_time:=dtime+checks[5];
    flow[4]:=dtime;
    adc_1_2;
    rsjinit;
    if (status2[9] = 0) then
        ctoutput_action(9,status2);
    adc_1_1;
    rsjinit;
    ctoutput_action(8,status1);
    ctoutput_action(5,status1);
    flag[1]:=false;
    if (flag[5]) then
        BEGIN
            stack_sort(dtime+fifteen_seconds,8);
        END
else
    BEGIN
        delay(20000);
adc_1_1;
rs_init;
ctoutput_action(8,status1);
ctoutput_action(4,status1);
delay(5000);
ctoutput_action(4,status1);
END;
END; {of procedure refill_chambers}

 Procedure End_Refill_Chambers;
{This procedure deactivates the filling procedure once the level detectors signal that the chamber is full.}
BEGIN
gotoxy(50,1);clreol;write('begin end_refill_chambers');
gotoxy(76,7);clreol;write('OFF');
if (not flag[5]) then
BEGIN
  adc_1_1;
  rs_init;
  ctoutput_action(2,status1);
delay(5000);
  ctoutput_action(2,status1);
END;
flow[5]:=dtime;
adc_1_1;
rs_init;
ctoutput_action(5,status1);
flow_time:=flow_calc(5,4);
gotoxy(1,24); clreol; write('Chamber refilled in ',
  flow_time:6:2,' seconds');
if (flag[19]) or (flag[20]) then
BEGIN
  flag[19]:=false;
  flag[20]:=false;
END;
gotoxy(50,1);clreol;write('end end_refill_chambers');
End; {of procedure end_refill_chambers}

 Procedure Disinfectant_discharge;
{This procedure discharges the disinfectant solution from the chamber.}
BEGIN
gotoxy(50,1); clreol; write('begin disinfect discharge');
gotoxy(76,8); clreol; write(' ON');
uhcheck_time:=dtime+checks[3];
adc_1_2;
rs_init;
ctoutput_action(3,status2);
ctoutput_action(7,status2);
if (flag[5]) then
  BEGIN
    adc_1_1;
    rs_init;
    ctoutput_action(1,status1);
    stack_sort(dtime+ten_seconds,6);
  END;
END; {of procedure disinfectant_discharge}

{*******************************************************************************}

Procedure Chamber_Level_Detector;
{This procedure checks the liquid level in the chambers during the refill cycle.}
Var
  i:integer;
BEGIN
  gotoxy(50,1); clreol; write('begin chamber level check');
  adc_1_1;
  rs_init;
  cdetector:=readchannel(hold);
  clear_update;
  gotoxy(1,21);
  write('Chamber ',cham,' level detector = ',cdetector:6:1,' mv');
  if (cdetector>200) or (dtime>ccheck_time) then
    BEGIN
      if (dtime > ccheck_time) then
        BEGIN
          gotoxy(1,21); clreol;
          writeln(#07,'Check the level detector output for chamber ',cham);
        END;
      if (flag[5]) then
        BEGIN
          if (flag[19]) or (flag[20]) then
            BEGIN
              stack_sort(dtime,46);
              stack_sort(dtime+five_seconds,46);
            END;
          stack_sort(dtime,28);
          stack_sort(dtime+five_seconds,28);
        END;
    END;
END;
if (status2[4] = 1) then stack_sort(dtime,27);
    stack_sort(dtime+one_second,49);
    stack_sort(dtime+six_seconds,52);
END;
adc_1_1;
rs_init;
if (status1[1] = 1) then
coutput_action(1,status1);
if (flag[2]) or (flag[3]) and (flag[5]) then
BEGIN
    clean:=clean+1;
    if (clean = 1) then
BEGIN
    stack_sort(dtime+0.01383101852,29);
    stack_sort(dtime+twenty_minutes,29);
    stack_sort(dtime+twenty_minutes,18);
END
else if (clean > 1) and (clean < 5) then
BEGIN
    stack_sort(dtime+0.00341435185,29);
    stack_sort(dtime+five_minutes,29);
    stack_sort(dtime+five_minutes,18);
    END;
if (flag[7]) then
BEGIN
    flag[7]:=false;
    count:=17;
    case cham of
      1:flag[2]:=false;
      2:flag[3]:=false;
    END; {of case of}
END;
END;
END
else if (cdetector <= 200) then
BEGIN
    adc_1_1;
    rs_init;
    if (status1[1] = 1) then
coutput_action(1,status1);
    if (flag[5]) then
    stack_sort(dtime+checks[2],7);
    END;
gotoxy(50,1);clreol;write('end chamber level check');
END; {of procedure chamber_level_detector_check}
{**********************************************************}

Procedure Disinfect_Chambers;
{This procedure fills the culture chamber with disinfectant and tap water
 following the complete harvest of the chamber.}
Var 
  i:integer;
BEGIN
  gotoxy(50,1); clrsl;
  write('begin chamber ',cham,' disinfection');
  gotoxy(76,8); clrsl; write('ON');
  ccheck_tine:=dtime+checks[4];
  adc_1_2;
  rs_init;
  ctoutput_action(9,status2);
  adc_1_1;
  rs_init;
  ctoutput_action(7,status1);
  ctoutput_action(5,status1);
  flag[1]:=false;
  if (flag[5]) then
    BEGIN
      stack_sort(dtime+ten_minutes,7);
      stack_sort(dtime+checks[1],8);
      stack_sort(dtime+checks[1],27);
    END
  else
    BEGIN
      delay(60000);
      adc_1_1;
      rs_init;
      ctoutput_action(7,status1);
      adc_1_2;
      rs_init;
      ctoutput_action(9,status2);
      ctoutput_action(4,status2);
      flag[1]:=true;
    END;
END; {of procedure disinfect_chambers}
Procedure Rinse_Chambers;
{This procedure fills the chamber with tap water following the disinfection cycle.}
Var
  i: integer;
BEGIN
  gotoxy(50,1); clreol; write('begin chamber rinse');
  gotoxy(76,8); clreol; write(' ON');
  adc_1_2;
  rs_init;
  ctoutput_action(4,status2);
  ccheck_time:=dtime+checks[4];
  if (flag[5]) then
      stack_sort(dtime+ten_minutes,7)
END; {of procedure rinse_chamber}

Procedure Chamber_Equilibrium;
{This procedure starts the siphon between the two chambers.}
BEGIN
  ctoutput_action(3,status2);
  ctoutput_action(7,status2);
  if (flag[5]) then
      BEGIN
        stack_sort(dtime+nine_seconds,28);
        stack_sort(dtime+fourteen_seconds,28);
        stack_sort(dtime+fourteen_seconds,24);
      END;
  END;

Procedure Siphon_Chambers;
{This procedure siphons algae from one chamber to the other.}
BEGIN
  gotoxy(50,1); clreol; write('begin start siphon');
  adc_1_2;
  rs_init;
  ctoutput_action(11,status2);
  ctoutput_action(3,status2);
  ctoutput_action(7,status2);
  if (flag[5]) then
      BEGIN
etime:=dtime+twenty_minutes;
stack_sort(etime,20);
END;
END; {of procedure start siphon}

**********

Procedure End_Chamber_Equilibrium;
{This procedure terminates the equilibrium procedure between chambers.}
BEGIN
flag[12]:=false;
adc_1_2;
rs_init;
gotoxy(50,1); clreol; write('end of chamber equilibrium');
case of
1:BEGIN
  stack_sort(dtime,29);
  stack_sort(dtime+checks[2],29);
  stack_sort(dtime+0.00006365741,19);
  END;
2:BEGIN
  stack_sort(dtime+0.02077546296,29);
  stack_sort(dtime+thirty_minutes,29);
  stack_sort(dtime+thirty_minutes,25);
  END;
END; {of case of}
ctoutput_action(11,status2);
END; {of procedure end chamber equilibrium}

**********

Procedure Equilibrium_Refill;
{This procedure refills both chambers after the equilibrium procedure.}
BEGIN
  count:=0;
  flag[7]:=true;
  adc_1_2;
  rs_init;
  ccheck_time:=dtime+checks[6];
  adc_1_1;
  rs_init;
  ctoutput_action(5,status1);
  if (flag[5]) then
    BEGIN
      stack_sort(dtime+five_seconds,26);
      stack_sort(dtime+0.01250000000,26);
    END;
else if (not flag[5]) then
BEGIN
repeat
  water:=0;
repeat
    adc_1_1;
    rs_init;
    ctoutput_action(4,status1);
    ctoutput_action(8,status1);
    adc_1_2;
    rs_init;
    ctoutput_action(9,status2);
    delay(60000);
    count:=count+0.5;
until (count >= 7);
  adc_1_2;
  rs_init;
  ctoutput_action(4,status2);
for i:=1 to 10 do
  delay(30000);
  ctoutput_action(4,status2);
  water:=water+1;
until (water = 2);
END;
gotoxy(50,1); clreol; write('end chamber refill');
END; {of procedure equilibrium refill}

{---------------------------------------------------------------}

Procedure Water_Flush_Manifold;
{This procedure rinses the main manifold with water.}
BEGIN
  gotoxy(50,1); clreol; write('begin flush manifold');
  gotoxy(76,15); clreol; write(' ON');
  flag[13]:=true;
  adc_1_2;
  rs_init;
  ctoutput_action(3,status2);
  ctoutput_action(4,status2);
  flag[1]=false;
stack_sort(dtime+twenty_seconds,22);
END; {of procedure flush manifold}
Procedure End_Water_Flush_Manifold;
{This procedure deactivates the flushing procedure.}
BEGIN
  gotoxy(50,1); clreol; write('begin end flush manifold');
  adc_1_2;
  rs_init;
  ctoutput_action(3,status2);
  ctoutput_action(4,status2);
  ctoutput_action(9,status2);
  flag[1]:=true;
  if (flag[5]) then
    BEGIN
      stack_sort(dtime,47);
    END;
  gotoxy(76,15); clreol; write('OFF');
END; {of procedure end flush manifold

Procedure Air_Flush_Manifold;
{This procedure rinses the main manifold with air.}
BEGIN
  gotoxy(50,1); clreol; write('begin flush manifold');
  gotoxy(76,15); clreol; write('ON');
  flag[13]:=true;
  adc_1_2;
  rs_init;
  ctoutput_action(3,status2);
  stack_sort(dtime+five_seconds,50);
END; {of procedure air flush manifold

Procedure End_Air_Flush_Manifold;
{This procedure deactivates the flushing procedure.}
BEGIN
  gotoxy(50,1); clreol; write('begin end flush manifold');
  adc_1_2;
  rs_init;
  ctoutput_action(3,status2);
  adc_1_1;
  rs_init;
  ctoutput_action(6,status1);
  if (status1[12] = 0) then
ctoutput_action(12,status1);
lhcheck_time:=dtime+checks[6];
if (flag[5]) then
BEGIN
  stack_sort(dtime,32);
  stack_sort(dtime+checks[2],32);
  stack_sort(dtime+five_seconds,16);
END;
gotoxy(76,15); clreol; write('OFF');
END; {of procedure end flush manifold}

***********************

Procedure Lower_Harvest_Level_Detector;
{This procedure checks the lower level in the harvest chamber during
harvesting.}
BEGIN
  clear_update;
gotoxy(50,1); clreol; write('begin low harv level check');
adc_1_1;
rs_init;
ldetector:=readchannel(15);
clear_update;
gotoxy(1,21);
write('The lower harvest level detector = ',ldetector:6:1,' mv');
if (ldetector<200) or (dtime>lhcheck_time) then
BEGIN
  if (dtime > lhcheck_time) then
  BEGIN
    gotoxy(1,21); clreol;
    writeln(#07,'Check the output on the lower harvest level detector');
  END;
ad_1_1;
rs_init;
if (status1[12] = 1) then
coutput_action(12,status1);
if (status1[1] = 1) then
coutput_action(1,status1);
if (flag[13]) or (flag[21]) then
BEGIN
  adc_1_1;
  rs_init;
coutput_action(6,status1);
  flag[13]:=false;
  flag[21]:=false;
END;

flow[2]:=dt ime;
if (flag[19]) or (flag[20]) then
BEGIN
  flow_time:=flow_calc(2,1);
gotoxy(1,24); clreol;
  writeln('The harvest chamber emptied in ',flow_time:6:2,' seconds');
END;
if (flag[2]) or (flag[3]) and (flag[5]) then
BEGIN
 adc_1_1;
  rs_init;
  if (clean > 0) then
    coutput_action(6,status1);
  stack_sort(dtime+0.0000578704,31);
  stack_sort(dtime,28);
  stack_sort(dtime+checks[2],28);
  flow_time:=flow_calc(2,3);
  gotoxy(1,24); clreol;
  writeln('Chamber ',cham,' was completely emptied in ',
          (flow_time/60):6:2,' minutes');
  if (clean = 0) then
    BEGIN
      stack_sort(dtime,29);
      stack_sort(dtime+checks[2],29);
      stack_sort(dtime+0.00006365741,34);
    END
else if (clean > 0) and (clean < 4) then
BEGIN
  stack_sort(dtime,29);
  stack_sort(dtime+checks[2],29);
  stack_sort(dtime+0.00006365741,35);
END
else if (clean >= 4) then
BEGIN
  flag[12]:=true;
  stack_sort(dtime,29);
  stack_sort(dtime+checks[2],29);
  stack_sort(dtime+0.00006365741,15);
END;
END;
END;
else if (Idetector >= 200) then
BEGIN
  adc_1_1;
  rs_init;
  if (status1[1] = 1) then
ctoutput_action(1, status1);
if (flag[5]) then
  stack_sort(dtime + checks[2], 16);
END;
gotoxy(50,1); clr; write('end low harv level check');
END; {of procedure lower harvest level detector}

****************************

Procedure Upper_Harvest_Level_Detector;
{This procedure checks the upper level in the harvest chamber during harvesting.}
BEGIN
  clear_update;
gotoxy(50,1); clr; write('begin up harv level check');
adc_1_1;
rs_init;
udetector:=readchannel(12);
clear_update;
gotoxy(1,21);
writeln('The upper level harvest detector = ',udetector:6:1,' mv');
if (udetector>90) or (dtime>uhcheck_time) then
  BEGIN
    if (dtime>uhcheck_time) then
      BEGIN
        gotoxy(1,21); clr;
        writeln('#07','Check the output on the upper harvest level detector');
      END;
    if (flag[19]) or (flag[20]) then
      BEGIN
        flag[9]:=false;
        stack_sort(dtime, 31);
        stack_sort(dtime + five_seconds, 48);
        flow[1]:=dtime;
        flow_time:=flow_calc(1,3);
        gotoxy(1,24); clr;
        write('Chamber ',cham,' took ',
             'flow_time:6:2,' seconds to harvest');
      END
  ELSE IF (flag[2]) or (flag[3]) AND (flag[5]) then
    BEGIN
      adc_1_1;
      rs_init;
      IF (clean > 0) then
        ctoutput_action(6, status1);
    END;
  adc_1_1;
rs_init;
if (status1[12] = 0) then
  ctoutput_action(12,status1);
lncheck_time:=dtime+checks[6];
if (flag[5]) then
  Begin
    if (status1[1] = 1) then
      ctoutput_action(1,status1);
      stack_sort(dtime+checks[2],16);
  End;
END
else if (udetector <= 90) then
  BEGIN
    adc_1_1;
    rs_init;
    if (status1[1] = 1) then
      ctoutput_action(1,status1);
      if (flag[5]) then
        stack_sort(dtime+checks[2],16);
  END;
gotoxy(50,1);clreol;write('end up harv level check');
END; {of procedure upper harvest_level_detector}

{******************************************************************************}

Procedure Bye;
BEGIN
  gotoxy(50,1); clreol; write('begin bye');
  all_cout_off;
  genscreen;
  gotoxy(36,17);
  write(' S Supervisor INACTIVE ');
  gotoxy(36,18); clreol; write('Press [ESC] to leave program');
  gotoxy(17,20); clreol; write('datafile = ',dfile);
  flag[5]: =false;
  gotoxy(50,1); clreol; write('end bye');
END; {of bye}

{******************************************************************************}

Procedure Start_Supervisor;
Var
  ih:integer;
BEGIN
  gotoxy(50,1); clreol; write('begin start_supervisor ');
command[1] := 'Room Environ Check';
command[2] := 'Light Output Check';
command[3] := 'Harvest 1';
command[5] := 'Monitoring Unit On';
command[7] := 'Cham Level Detect On';
command[8] := 'End Nutrient Addition';
command[9] := 'CO2 Addition On';
command[10] := 'CO2 Addition Off';
command[12] := 'Culture Lights Off';
command[13] := 'Disinfect 1';
command[14] := 'Disinfect 2';
command[16] := 'Low Harv Detect On';
command[17] := 'Dump Data to Disk';
command[18] := 'Disinfect Discharge';
command[19] := 'Equilibrium Refill 1';
command[20] := 'End Chamber Equil';
command[21] := 'Water Flush Manifold';
command[22] := 'End Water Flush Man.';
command[23] := 'Refill Chambers';
command[24] := 'Siphon Chambers';
command[25] := 'Equilibrium Refill 2';
command[26] := 'Equil Dosing App';
command[27] := 'Tap Water';
command[28] := 'Open Cham Valve';
command[29] := 'Close Cham Valve';
command[31] := 'Harvest Off';
command[32] := 'Open Both Cham Valvs';
command[33] := 'Close Both Cham Valvs';
command[34] := 'Disinfect Chambers';
command[35] := 'Rinse Chambers';
command[36] := 'Dump Init Cond-Disk';
command[37] := 'Dump Dummy Data Disk';
command[38] := 'Disinfect Manifold';
command[39] := 'Brine Solution Addn';
command[40] := 'Lights on/off check';
command[41] := 'Disinfectant Addition';
command[42] := 'Saltwater Open';
command[43] := 'Open Main Valve';
command[44] := 'Close Main Valve';
command[45] := 'Statistics';
command[46] := 'Saltwater Close';
command[47]:='Air Flush Manifold '; command[48]:='Photocell Light '; command[49]:='End Refill Chambers '; command[50]:='End Air Flush Man. '; command[51]:='Dump Harvest Rate/Day'; command[52]:='Check Harvest Chamber';
gotoxy(1,3); write('');
gotoxy(1,3); writeln('SUPERVISOR COMMAND SEQUENCE ');
gotoxy(1,4); write(' ');
gotoxy(1,4); writeln('--------------------------------------------------');

space.time:=1e37;
space.action:=0;
for ih:=1 to 70 do
  stack[ih]:=space;
dead_time:=trunc(dtime);
etime:=dtime+one_minute;
stime:=dtime+ten_seconds;
cime:=dtime+ninety_seconds;
stack_sort(stime,1); {room environment check}
stack_sort(stime+one_second,2); {light output check}
stack_sort(time,9); {turn on CO2}
stack_sort(dead_time+first_harvest,3); {harvest chamber #1}
stack_sort(dead_time+second_harvest,4); {harvest chamber #2}
stack_sort(dead_time+30.375000000,13); {disinfect chamber #1 at 9:00 a.m.}
stack_sort(dead_time+init.params[6],12);
stack_sort(dead_time+37.375000000,14); {disinfect chamber #2 at 9:00 a.m.}
stack_sort(etime,36); {dump init data}
gotoxy(50,1); clreol; write('end start_supervisor');
END; {of procedure start_supervisor}

******************************************************************************

Procedure Stack_Supervisor;
{This procedure implements the commands of the supervisor stack.}
BEGIN
  all_cout_off;
  flag[1]:=true;
  flag[10]:=true;
gotoxy(50,1); clreol; write('begin Stack_Supervisor');
clear_update;
gotoxy(1,22);
writeln('Time must be entered in fractions of days');
gotoxy(1,23);
write('Input the hour of the first harvest for chamber 1');
first_harvest:=read_real(51,23);
gotoxy(1,24);
write('Input the hour of the first harvest for chamber 2');
second_harvest:=read_real(51,24);
gotoxy(36,18); clreol; write('Press [ESC] to deactivate SUPERVISOR');
start_supervisor;
Repeat {start of [ESC] key loop release check}
  Repeat
    a:=dtime;
    if stack_match then begin
      last_command:=command[stack[1].action];
      last_time:=stack[1].time;
      case stack[1].action of
        1:BEGIN
          stack_sort(dtime+fifteen_minutes,1);
          temperature_update(1,2);
          if (rep1 >=10) then stack_sort(dtime+five_seconds,45);
          END;
        2:BEGIN
          stack_sort(dtime+fifteen_minutes,2);
          flag[18]:=true;
          light_output_check(3,7);
          if (rep2 >=10) then stack_sort(dtime+five_seconds,45);
          END;
        3:BEGIN
          if (not flag[10]) then
            BEGIN
              stime:=dtime+ten_minutes;
              stack_sort(stime,3);
            END
          else if (flag[2]) or (flag[3]) then
            BEGIN
              etime:=dtime+eight_hours;
              stack_sort(etime,3);
            END
          else if (flag[20]) then
            BEGIN
              etime:=dtime+five_minutes;
              stack_sort(etime,3);
            END
          else if (flag[21]) then
            BEGIN
              etime:=dtime+two_minutes;
              stack_sort(etime,3);
            END
          else if (not flag[2]) or (not flag[3]) and (flag[10]) then
            BEGIN
              cham:=1;
timer:=dtime;
if (((timer - trunc(timer)) > (last_time1 - trunc(last_time1))) then
  BEGIN
    cumharv1:=cumharv1+5;
    last_time1:=timer;
  END
else
  BEGIN
    harvest_day:=cumharv1;
    sgr:=harvest_day/140.0;
    sgr1[2]:=sgr1[1];
    sgr1[1]:=sgr;
    dif:=sgr1[2] - sgr1[1];
    if (dif >= 0.0) and (dif > 0.1) or (sgr < 0.8) then
      BEGIN
        harvest1:=harvest1-two_minutes;
        if (harvest1 < 0.013888889) then harvest1:=0.013888889;
      END;
  END;
stack_sort(dtime+one_second,51);
cumharv1:=0;
cumharv1:=cumharv1+5;
last_time1:=timer;
END;
flag[19]:=true;
flag[9]:=true;
flag[18]:=false;
flag[8]:=true;
stime:=dtime+harvest1;
stack_sort(dtime+one_second,48);
stack_sort(dtime+five_seconds,29);
stack_sort(dtime+six_seconds,44);
stack_sort(dtime+checks[10],29);
stack_sort(dtime+checks[8],44);
hold:=10;
stack_sort(dtime+fifteen_seconds,6);
stack_sort(dtime+checks[9],30);
stack_sort(dtime+thirty_seconds,5);
stack_sort(stime,3);
END;
END;
4:BEGIN
  if (not flag[10]) then
    BEGIN
      stime:=dtime+ten_minutes;
      stack_sort(stime,4);
    END
else if (flag[2]) or (flag[3]) then
  BEGIN
    etime:=dtime+eight_hours;
    stack_sort(etime,4);
  END
else if (flag[19]) then
  BEGIN
    etime:=dtime+ten_minutes;
    stack_sort(etime,4);
  END
else if (flag[21]) then
  BEGIN
    etime:=dtime+two_minutes;
    stack_sort(etime,4);
  END
else if (not flag[2]) or (not flag[3]) and (flag[10]) then
  BEGIN
    cham:=2;
    timer:=dtime;
    if (timer - trunc(timer) > (last_time2 - trunc(last_time2))) then
      BEGIN
        cumharv2:=cumharv2+5;
        last_time2:=timer;
      END
    else
      BEGIN
        harvest_day:=cumharv2;
        sgr:=harvest_day/140.0;
        sgr2[2]:=sgr2[1];
        sgr2[1]:=sgr;
        dif:=sgr2[2] - sgr2[1];
        if (dif >= 0.0) and (dif > 0.1) or (sgr < 0.8) then
          BEGIN
            harvest2:=harvest2-two_minutes;
            if (harvest2 < 0.013888889) then harvest2:=0.013888889;
          END;
        stack_sort(dtime+one_second,51);
        cumharv2:=0;
        cumharv2:=cumharv1+5;
        last_time2:=timer;
      END;
    flag[20]:=true;
    flag[9]:=true;
    flag[18]:=false;
  stime:=dtime+harvest2;
    flag[8]:=true;
stack_sort(dtime+one_second,48);
stack_sort(dtime+five_seconds,29);
stack_sort(dtime+six_seconds,44);
stack_sort(dtime+checks[10],29);
stack_sort(dtime+checks[8],44);
hold:=11;
stack_sort(dtime+fifteen_seconds,6);
stack_sort(dtime+checks[9],30);
stack_sort(dtime+thirty_seconds,5);
stack_sort(stime,4);
END;
END;
5:BEGIN
  if (cham = 1) then culture_conditions(8,9,10,11,12)
  else culture_conditions(13,14,15,16,17);
END;
6:BEGIN
  adc_1_1;
  rs_init;
  if (status1[1] = 0) then
    ctoutput_action(1,status1);
    upper_harvest_level_detector;
END;
7:BEGIN
  adc_1_1;
  rs_init;
  if (status1[1] = 0) then
    ctoutput_action(1,status1);
    chamber_level_detector;
END;
8:BEGIN
  gotoxy(50,1);clreol;write('begin deact dosing apparatus');
  if (flag[19]) or (flag[20]) then
    BEGIN
      adc_1_1;
      rs_init;
      ctoutput_action(8,status1);
    END
  else if (flag[2]) or (flag[3]) or (flag[13]) then
    BEGIN
      adc_1_1;
      rs_init;
      ctoutput_action(7,status1);
    END
  else if (flag[7]) and (flag[2]) or (flag[3]) then
    BEGIN
adc_1_1;
rs_init;
ctoutput_action(8,status1);
stack_sort(dtime+one_minute,26);
END;
adc_1_2;
rs_init;
if (status2[9] = 1) then
coutput_action(9,status2);
flag[1]:=true;
adc_1_1;
rs_init;
if (status1[1] = 0) then
coutput_action(1,status1);
cdetector:=readchannel(hold);
coutput_action(1,status1);
if (cdetector <= 200) then
BEGIN
  stack_sort(dtime,7);
  stack_sort(dtime+one_second,42);
  stack_sort(dtime+six_seconds,42);
END
else
BEGIN
gotoxy(76,7);clreol;write('OFF');
if (flag[19]) or (flag[20]) then
  BEGIN
    flag[19]:=false;
    flag[20]:=false;
  END;
  stack_sort(dtime,28);
  stack_sort(dtime+five_seconds,28);
  stack_sort(dtime+six_seconds,52);
END;
gotoxy(50,1);clreol;write('end deact dosing app');
END;
9:BEGIN
  CO2_addition;
  ctime:=dtime+init.params[7];
  stack_sort(ctime,10);
END;
10:BEGIN
  CO2_addition;
  ctime:=dtime+init.params[8];
  stack_sort(ctime,9);
END;
11:BEGIN
    if (status1[10] = 0) then
        BEGIN
            adc_1_1;
            rs_init;
            coutput_action(10,status1);
        END;
        flag[10]:=true;
    if (init.params[5] > 1.0) then
        etime:=trunc(dtime)+init.params[6]
    else
        etime:=trunc(dtime)+1.0+init.params[6];
    stack_sort(etime,12);
END;
12:BEGIN
    if (init.params[6] <> 0) then
        BEGIN
            if (status1[10] = 1) then
                BEGIN
                    adc_1_1;
                    rs_init;
                    coutput_action(10,status1);
                END;
            flag[10]:=false;
            etime:=trunc(dtime)+init.params[5];
            stack_sort(etime,11);
        END;
    END;
13:BEGIN
    if (flag[19]) or (flag[20]) then
        BEGIN
            stime:=dtime+five_minutes;
            stack_sort(stime,13);
        END
    else if (not flag[19]) or (flag[20]) then
        BEGIN
            dead_time:=trunc(dtime);
            flag[2]:=true;
            flag[15]:=true;
            cham:=1;
            hold:=10;
            stack_sort(dtime,29);
            stack_sort(dtime+checks[2],29);
            clean:=0;
            stack_sort(dtime+fifteen_seconds,6);
stack_sort(dtime+0.00006365741,30);
stack_sort(dtime+thirty_seconds,5);
stack_sort(dead_time+30.37500000000,13);
END;
END;
14:BEGIN
if (flag[19]) or (flag[20]) then
BEGIN
stime:=dtime+five_minutes;
stack_sort(stime,14);
END
else if (not flag[19]) or (flag[20]) then
BEGIN
dead_time:=trunc(dtime);
flag[3]:=true;
flag[15]:=true;
cham:=2;
hold:=11;
stack_sort(dtime,29);
stack_sort(dtime+checks[2],29);
clean:=0;
stack_sort(dtime+fifteen_seconds,6);
stack_sort(dtime+0.00006365741,30);
stack_sort(dtime+thirty_seconds,5);
stack_sort(dead_time+30.37500000000,14);
END;
END;
15:BEGIN
chamber_equilibrium;
END;
16:BEGIN
adc_1_1;
rs_init;
if (statusl [1] = 0) then
coutput_action(1,status1);
lower_harvest_level_detector;
END;
17:BEGIN
if (dump1 = 1) then
BEGIN
Dump_data_disk(1,2,temp1);
dump1:=0;
if (flag[16]) then dump2:=1;
END;
if (dump3 = 1) then
BEGIN
    Dump_data_disk(8,12,temp3);
    dump3:=0;
    if (flag[16]) then dump4:=1;
END;
if (dump5 = 1) then
BEGIN
    Dump_data_disk(3,7,temp2);
    dump5:=0;
    if (flag[16]) then dump6:=1;
END;
if (dump7 = 1) then
BEGIN
    Dump_data_disk(13,17,temp3);
    dump7:=0;
    if (flag[16]) then dump8:=1;
END;
if (flag[16]) then
    stack_sort(dtime+five_minutes,37);
END;
18:BEGIN
    disinfectant_discharge;
END;
19:BEGIN
    equilibrium_refill;
END;
20:BEGIN
    end_chamber_equilibrium;
END;
21:BEGIN
    water_flush_manifold;
END;
22:BEGIN
    end_water_flush_manifold;
END;
23:BEGIN
    refill_chambers;
END;
24:BEGIN
    siphon_chambers;
END;
25:BEGIN
    if (flag[2]) then
        stack_sort(dtime+one_minute,25)
    else
equilibrium_refill;
END;
26:BEGIN
  if (count <= 7) then
    BEGIN
      adc_1_1;
      rs_init;
      ctoutput_action(8,status1);
      ctoutput_action(4,status1);
      adc_1_2;
      rs_init;
      ctoutput_action(9,status2);
      stack_sort(dtime+one_minute,8);
      count:=count+1;
    END;
  END;
27:BEGIN
  adc_1_2;
  rs_init;
  ctoutput_action(4,status2);
END;
28:BEGIN
  Open_Chamber_Valve;
END;
29:BEGIN
  Close_Chamber_Valve;
END;
30:BEGIN
  Harvest_on;
END;
31:BEGIN
  Harvest_off;
END;
32:BEGIN
  Open_both_chamber_valves;
END;
33:BEGIN
  Close_both_chamber_valves;
END;
34:BEGIN
  Disinfect_chambers;
END;
35:BEGIN
  Rinse_chambers;
END;
36:BEGIN
    Dump_init_data;
    if (flag[15]) then
        stack_sort(dtime+two_minutes,36);
END;
37:BEGIN
    if (dump2 = 1) then
        BEGIN
            Dummy_data_dump_disk(1,2,temp1);
            if (flag[16]) then dump2:=1
            else dump2:=0;
        END;
    if (dump4 = 1) then
        BEGIN
            Dummy_data_dump_disk(8,12,temp3);
            if (flag[16]) then dump4:=1
            else dump4:=0;
        END;
    if (dump6 = 1) then
        BEGIN
            Dummy_data_dump_disk(3,7,temp2);
            if (flag[16]) then dump6:=1
            else dump6:=0;
        END;
    if (dump8 = 1) then
        BEGIN
            Dummy_data_dump_disk(13,17,temp3);
            if (flag[16]) then dump8:=1
            else dump6:=0;
        END;
    if (flag[16]) then
        stack_sort(dtime+two_minutes,37);
END;
38:BEGIN
    gotoxy(50,1); clreol; write('begin disinfectant manifold');
    flag[17]:=true;
    stack_sort(dtime,33);
    stack_sort(dtime+five_seconds,33);
    stack_sort(dtime+five_seconds,41);
    stack_sort(dtime+fifteen_seconds,41);
    stack_sort(dtime+fifteen_seconds,21);
END;
39:BEGIN
    adc_1_1;
    rs_init;
    ctoutput_action(4,status1);
40:BEGIN
END;
41:BEGIN
        gotoxy(50,1); clreol; write('begin disinfectant addition');
        adc_1_2;
        rs_init;
        ctoutput_action(9,status2);
        adc_1_1;
        rs_init;
        ctoutput_action(7,status1);
END;
42:BEGIN
        adc_1_1;
        rs_init;
        ctoutput_action(4,status1);
END;
43:BEGIN
    open_main_valve;
END;
44:BEGIN
    close_main_valve;
END;
45:BEGIN
    case sta of
        1:BEGIN
            statist(1,2,rep1);
            temp1:=rep1;
            stack_sort(dtime+one_second,17);
            rep1:=0;
        END;
        2:BEGIN
            statist(3,7,rep2);
            temp2:=rep2;
            stack_sort(dtime+one_second,17);
            rep2:=0;
        END;
        3:BEGIN
            if (cham = 1) then
                BEGIN
                    statist(8,12,rep3);
                    b1:=b1+1;
                    biomass1[b1]:=data1[12].stats[1];
                    if (data1[10].stats[1]<init.params[3]) then
                        init.params[8]:=init.params[8]+two_minutes;
                    if (data1[10].stats[1]>init.params[4]) then
                        
```
if (data1[8].stats[1] < init.params[9]) then
  BEGIN
    init.params[1] := init.params[1] + 0.5;
  END;
if (data1[8].stats[1] > init.params[9]) then
  BEGIN
    init.params[1] := init.params[1] - 0.5;
  END;
if (b1 = 3) then
  BEGIN
    b1 := 1;
    if (biomass1[3] > biomass1[1] - (0.1 * biomass1[1])) and
        (biomass1[3] < biomass1[1] + (0.1 * biomass1[1])) then
      BEGIN
        harvest1 := harvest1 - 0.00034722222;
        if (harvest1 <= 0.0138888889) then
          harvest1 := 0.0138888889;
        biomass1[1] := biomass1[3];
        biomass1[2] := 0;
        biomass1[3] := 0;
      END
    else if (biomass1[3] < biomass1[1] - (0.1 * biomass1[1])) then
      BEGIN
        harvest1 := harvest1 + three_minutes;
        if (harvest1 >= 0.0444444444) then
          harvest1 := 0.0444444444;
        biomass1[1] := biomass1[3];
        biomass1[2] := 0.0;
        biomass1[3] := 0.0;
      END
    else if (biomass1[3] > biomass1[1] + (0.1 * biomass1[1])) then
      BEGIN
        harvest1 := harvest1 - five_minutes;
        if (harvest1 <= 0.013888888889) then
          harvest1 := 0.013888888889;
        biomass1[1] := biomass1[3];
        biomass1[2] := 0.0;
        biomass1[3] := 0.0;
      END;
  END;
temp3 := rep3;
stack_sort(dtime + one_second, 17);
rep3 := 0;
else
BEGIN
    statist(13,17,rep3);
    b2:=b2+1;
    biomass2[b2]:=data[17].stats[1];
    temp3:=rep3;
    if (data[15].stats[1]<init.params[3]) then
        init.params[8]:=init.params[8]+two_minutes;
    if (data[15].stats[1]>init.params[4]) then
        init.params[7]:=init.params[7]+0.00005787037;
    if (data[13].stats[1]<init.params[9]) then
        BEGIN
            init.params[1]:=init.params[1]+0.5;
            init.params[2]:=init.params[2]+0.5;
        END;
    if (data[13].stats[1]>init.params[9]) then
        BEGIN
            init.params[1]:=init.params[1]-0.5;
            init.params[2]:=init.params[2]-0.5;
        END;
    if (b2 = 3) then
    BEGIN
        b2:=1;
        if (biomass3 > biomass1 - (0.1*biomass1)) and
            (biomass3 < biomass1 + (0.1*biomass1)) then
            BEGIN
                harvest:=harvest-0.00034722222;
                if (harvest <= 0.0138888889) then
                    harvest:=0.0138888889;
                    biomass1:=biomass3;
                    biomass2:=0;
                    biomass3:=0;
            END
        else if (biomass3 < biomass1 - (0.1*biomass1)) then
            BEGIN
                harvest:=harvest+three_minutes;
                if (harvest > 0.044444444444) then
                    harvest:=0.044444444444;
                    biomass1:=biomass3;
                    biomass2:=0;
                    biomass3:=0;
            END
        else if (biomass3 > biomass1 + (0.1*biomass1)) then
            BEGIN
                harvest:=harvest-five_minutes;
            END
        END;
    END;
END
if (harvest2 <= 0.013888888889) then
    harvest2:=0.013888888889;
    biomass2[1]:=biomass2[3];
    biomass2[2]:=0.0;
    biomass2[3]:=0.0;
END;
END;
stack_sort(dtime+one_second, 17);
rep3:=0;
END;
END;
end; {of case of}
END;

46:BEGIN
    adc_1_1;
    rs_init;
    ctoutput_action(2,status1);
END;

47:BEGIN
    air_flush_manifold;
END;

48:BEGIN
    adc_1_2;
    rs_init;
    ctoutput_action(8,status2);
END;

49:BEGIN
    end_refill_chambers;
END;

50:BEGIN
    end_airFlush_manifold;
END;

51:BEGIN
    dump_harvest(cham,harvest_day,sgr);
END;

52:BEGIN
    adc_1_1;
    rs_init;
    if (status1[12] = 1) or (flag[19]) or (flag[20]) then
        stack_sort(dtime+one_minute,52)
    else
        BEGIN
            flag[21]:=true;
            stack_sort(dtime,33);
            stack_sort(dtime+five_seconds,33);
            stack_sort(dtime+six_seconds,21);
rsjn it;
if (status2[9] = 0) then
coutput_action(9,status2);
END;
END;
END; {of case}
kil_top;
command_stack;
END; {of if stack_match}
Until keypressed;
if keypressed then
read(kbd,choice);
case choice of
'G','g':BEGIN
gotoxy(76,11); clreol;
write(' ON');
temperature_update(1,2);
END;
'H','h':BEGIN
data_file;
END;
'I','i':BEGIN
gotoxy(76,13); clreol;
write(' ON');
op_params;
END;
'J','j':BEGIN
gotoxy(76,14); clreol;
write(' ON');
flag[18]:=true;
light_output_check(3,7);
END;
END; {of case of}
Until (choice=#27);
bye;
flag[1]:=true;
gotoxy(50,1); clreol; write('end stack_supervisor');
END; {of procedure stack supervisor}

BEGIN {main program}
clrscr;
rs_init;
cursor(off);
genscreen;
all_cout_off;
clear_update;
setup;
data_file;
t:=dtime;
c:=dtime;
l:=dtime;
d:=dtime;
op_params;
init_cond;
flag[1]:=true;
Repeat
  Repeat
    b:=dtime;
  Until keypressed or ((t<=b) or (l<=b) or (c<=b) or (d<=b));
if keypressed then
  read(kbd,choice)
else choice:='e';
case choice of
#27:flag[6]:=true;
'A','a':BEGIN {air conditioner/heater}
clear_update;
gotoxy(1,21); write('1:Air Conditioner');
gotoxy(1,22); write('2:Heater');
repeat
  gotoxy(17,20); clreol;
  write('Which option would you like to choose ? ');
  ans:=read_integer(57,20);
until (ans = 1) or (ans = 2);
gotoxy(17,20); clreol; write('datafile = '+dfile);
clear_update;
case ans of
  1:BEGIN {air conditioner}
    air_conditioner;
  END;
  2:BEGIN
    heater;
  END;
END;
END;
'B','b':BEGIN {water addition}
clear_update;
gotoxy(76,6); clreol; write(' ON');
gotoxy(1,21); write('Tap Water');
gotoxy(1,22); write('Saltwater');
repeat
  gotoxy(17,20); clreol;
write('Which option would you like to choose?');
ans:=read_integer(57,20);
until (ans = 1) or (ans = 2);
gotoxy(17,20); clr; write('datafile = ',dfile);
clear_update;
messages;
close_chamber_valve;
delay(5000);
close_chamber_valve;
case ans of
1:BEGIN
    adc_1_2;
    rs_init;
    ctoutput_action(4,status2);
    repeat
        gotoxy(1,21); clr;
        write('Press any key to end water addition');
        until keypressed;
        ctoutput_action(4,status2);
    END;
2:BEGIN
    adc_1_1;
    rs_init;
    ctoutput_action(4,status1);
    repeat
        gotoxy(1,21); clr;
        write('Press any key to end saltwater addition');
        until keypressed;
        ctoutput_action(4,status1);
    END;
END;
open_chamber_valve;
delay(5000);
open_chamber_valve;
gotoxy(76,6); clr; write('OFF');
clear_update;
END;
'C','c':BEGIN {harvest cycle}
    flag[19]:=true;
    flag[20]:=true;
    clear_update;
    gotoxy(1,21); write('1: Harvest Chambers');
    gotoxy(1,22); write('2: Refill Chambers');
    repeat
        gotoxy(17,20); clr;
        write('Which option would you like to choose?');

ans:=read_integer(57,20);
until (ans = 1) or (ans = 2);
gotoxy(17,20); clr; write('datafile = ',dfile);
clear_update;
case ans of
1:BEGIN  {harvest algae}
gotoxy(76,7); clr; write(' ON');
if (status2[8] = 0) then
  BEGIN
    adc_1_2;
    rs_init;
    coutput_action(8,status2);
  END;
  messages;
close_chamber_valve;
close_main_valve;
delay(5000);
close_chamber_valve;
close_main_valve;
harvest_on;
repeat
  adc_1_1;
  rs_init;
  coutput_action(1,status1);
repeat
  gotoxy(1,21); writeln('Press [ESC] to end harvest ');
  adc_1_1;
  rs_init;
  mv:=intensity(4);
  mv2:=intensity(12);
  gotoxy(1,21); clr; write(mv:7:2);
  gotoxy(1,22);clr; write(mv2:7:2);
  delay(1000);
until keypressed;
  coutput_action(1,status1);
if keypressed then read (kbd,choice);
until (choice = #27);
adc_1_2;
rs_init;
  coutput_action(8,status2);
clear_update;
harvest_off;
open_chamber_valve;
open_main_valve;
delay(5000);
open_chamber_valve;
open_main_valve;
gotoxy(76,7); clreol; write('OFF');
flag[19]:=false;
flag[20]:=false;
adc_1_1;
rs_init;
ctoutput_action(12,status1);
ltcheck_time:=dtime+checks[6];
repeat
  adc_1_1;
  rs_init;
  ctoutput_action(1, status1);
  lower_harvest_level_detector;
until (ldetector < 160) or (dtime > ltcheck_time) or
  (keypressed);
if keypressed then
  BEGIN
    adc_1_1;
    rs_init;
    ctoutput_action(12, status1);
    if (status1[1] = 1) then
      ctoutput_action(1, status1);
  END;
END;
2:BEGIN {refill chambers}
gotoxy(76,7); clreol; write('ON');
messages;
close_chamber_valve;
delay(5000);
close_chamber_valve;
refill_chambers;
clear_update;
repeat
  gotoxy(1,21); writeln('Press any key to end chamber refill');
until keypress;
clear_update;
if (status2[9] = 1) then
  BEGIN
    adc_1_2;
    rs_init;
    ctoutput_action(9, status2);
  END;
if (status1[8] = 1) then
  BEGIN
    adc_1_1;
    rs_init;
ctoutput_action(8, status1);
END;
end_refill_chambers;
open_chamber_valve;
delay(5000);
open_chamber_valve;
gotoxy(76,7); clrdef; write('OFF');
flag[19]:=false;
flag[20]:=false;
END;
END; {of case of}
END;
'D','d':BEGIN {disinfection cycle}
clear_update;
gotoxy(1,21); write('1: Harvest Algae');
gotoxy(1,22); write('2: Disinfect Chambers');
gotoxy(1,23); write('3: Discharge Disinfectant');
gotoxy(30,21); write('4: Rinse Chambers');
gotoxy(30,22); write('5: Chamber Equilibrium');
gotoxy(30,23); write('6: Equilibrium Refill');
gotoxy(17,20); clrdef;
write('Which option would you like to choose?');
repeat
ans:=read_integer(57,20);
until (ans > 0) and (ans < 7);
case ans of
1:BEGIN {harvest algae}
messages;
end_refill_chambers;
open_chamber_valve;
delay(5000);
open_chamber_valve;
set_chamber_valve;
close_main_valve;
x:=dtime+one_minute;
uhcheck_time:=dtime+checks[3];
clear_update;
repeat
repeat
adc_1_1;
rs_init;
ctoutput_action(1, status2);
upper_harvest_level_detector;
gotoxy(1,21); writeln('Press [ESC] to end disinfection/harvest ');
until (keypressed) or (udetector > 60) or
(dtime > uhcheck_time);
if keypressed then read (kbd,choice);
until (choice = #27) or (udetector > 60) or (dtime > uhcheck_time);
if (choice = #27) then
BEGIN
  adc_1_1;
  rs_init;
  ctoutput_action(12,status1);
  repeat
    adc_1_1;
    rs_init;
    ctoutput_action(1,status1);
    lower_harvest_level_detector;
    gotoxy(1,21); writeln('Press any key to end process ');
  until (ldetector < 60) or (keypressed);
  if keypressed then
    BEGIN
      harvest_off;
      open_chamber_valve;
      open_main_valve;
      delay(5000);
      open_chamber_valve;
      open_main_valve;
      adc_1_1;
      rs_init;
      status1[1]:=1;
      ctoutput_action(1,status1);
      ctoutput_action(12,status1);
    END;
  END;
END;

case cham of
  1:flag[2]:=false;
  2:flag[3]:=false;
END; {of case of}
gotoxy(76,8); cleol; write('OFF');
flag[15]:=false;
END;

2:BEGIN {disinfect chambers}
  messages;
  case cham of
    1:BEGIN
      flag[2]:=true;
      hold:=10;

END;
2:BEGIN
   flag[3]:=true;
   hold:=11;
END;
END; {of case of}
close_chamber_valve;
delay(5000);
close_chamber_valve;
ccheck_time:=dtime+checks[4];
disinfect_chambers;
clear_update;
repeat
   adc_1_1;
   rs_init;
   ctoutput_action(1,status1);
   chamber_level_detector;
gotoxy(1,21); writeln('Press any key to end disinfectant fill ');
until (cdetector > 60) or (keypressed) or (dtime > ccheck_time);
if keypressed then
   BEGIN
      if (status2[9] = 0) then
         BEGIN
            adc_1_2;
            rs_init;
            ctoutput_action(9,status2);
         END;
      if (status1[7] = 1) then
         BEGIN
            adc_1_1;
            rs_init;
            ctoutput_action(7,status1);
         END;
      adc_1_2;
      rs_init;
      ctoutput_action(4,status2);
      adc_1_1;
      rs_init;
      ctoutput_action(5,status1);
      open_chamber_valve;
      delay(5000);
      open_chamber_valve;
   END;
   case ch of
      1:BEGIN
         flag[2]:=false;
2:BEGIN
    flag[3]:=false;
    END;
END; {of case of}
clear_update;
gotoxy(76,8); clrrel; write('OFF');
END;
3:BEGIN {Discharge disinfectant}
    flag[15]:=true;
    messages;
case cham of
    1:flag[2]:=true;
    2:flag[3]:=true;
    END; {of case of}
close_chamber_valve;
delay(5000);
close_chamber_valve;
disinfectant_discharge;
clear_update;
uhcheck_time:=dtime+checks[3];
repeat
    adc_1_1;
    rs_init;
    coutput_action(1,status1);
    upper_harvest_level_detector;
    gotoxy(1,21); writeln('Press any key to end discharge ');
until (udetector > 60) or (keypressed) or
    (dtime > uhcheck_time);
clear_update;
if keypressed then
    BEGIN
        adc_1_1;
        rs_init;
        coutput_action(6,status1);
        coutput_action(12,status1);
        repeat
            adc_1_1;
            rs_init;
            coutput_action(1,status1);
            lower_harvest_level_detector;
            gotoxy(1,21);
            writeln('Press any key to discontinue ');
        until (cdetector < 60) or (keypressed);
        if keypressed then
            BEGIN
                adc_1_1;
                rs_init;
                coutput_action(6,status1);
                coutput_action(12,status1);
                repeat
                    adc_1_1;
                    rs_init;
                    coutput_action(1,status1);
                    lower_harvest_level_detector;
                    gotoxy(1,21);
                    writeln('Press any key to discontinue ');
                until (cdetector < 60) or (keypressed);
adc_1_2;
rs_init;
ctoutput_action(3,status2);
ctoutput_action(7,status2);
open_chamber_valve;
delay(5000);
open_chamber_valve;
case cham of
1:BEGIN
  flag[2]:=false;
  END;
2:BEGIN
  flag[3]:=false;
  END;
END; {of case of}
END;
END;
clear_update;
gotoxy(76,8); clreol; write('OFF');
flag[15]:=false;
END;
4:BEGIN {Rinse chambers}
  messages;
  adc_1_2;
  rs_init;
  close_chamber_valve;
  delay(5000);
  close_chamber_valve;
case cham of
1:BEGIN
  flag[2]:=true;
  hold:=10;
  END;
2:BEGIN
  flag[3]:=true;
  hold:=11;
  END;
END; {of case of}
ccheck_time:=dtime+checks[4];
rinse_chambers;
clear_update;
repeat
  adc_1_1;
  rs_init;
  ctoutput_action(1,status1);
  chamber_level_detector;
gotoxy(1,21); writeln('Press any key to end rinse cycle');
until (cdetector > 60) or (keypressed) or (dtime > ccheck_time);
if keypressed then
  BEGIN
    adc_1_2;
    rs_init;
    ctoutput_action(4,status2);
    open_chamber_valve;
    delay(5000);
    open_chamber_valve;
  END;
  case cham of
    1:BEGIN
      flag[2]:=false;
      END;
    2:BEGIN
      flag[3]:=false;
      END;
  END; {of case of}
clear_update;
go toxy(76,8); clreol; write('OFF');
END;
5:BEGIN  {Chamber equilibrium}
  messages;
  case cham of
    1:flag[2]:=true;
    2:flag[3]:=true;
  END; {of case of}
  flag[12]:=true;
  close_chamber_valve;
  delay(5000);
  close_chamber_valve;
  chamber_equilibrium;
  delay(15000);
  open_chamber_valve;
  delay(5000);
  open_chamber_valve;
  siphon_chambers;
  clear_update;
go toxy(1,21);
  write('Press any key to discontinue equilibrium');
  repeat until keypressed;
  flag[12]:=false;
  end_chamber_equilibrium;
  case cham of
1: flag[2]:=false;
2: flag[3]:=false;
END; {of case of}
clear_update;
gotoxy(76,8); clrpl; write('OFF');
END;
6: BEGIN {Equilibrium refill}
messages;
case cham of
1: BEGIN
  flag[2]:=true;
  hold:=10;
  END;
2: BEGIN
  flag[3]:=true;
  hold:=11;
  END;
END; {of case of}
close_chamber_valve;
delay(5000);
close_chamber_valve;
equilibrium_refill;
clear_update;
repeat
  adc_1_1;
  rs_init;
  ctoutput_action(1,status1);
  chamber_level_detector;
gotoxy(1,21);
  writeln('Press any key to end chamber refill ');
until (cdetector > 60) or (keypressed);
if keypressed then
BEGIN
  adc_1_2;
  rs_init;
  ctoutput_action(4,status2);
  open_chamber_valve;
delay(5000);
  open_chamber_valve;
END;
case cham of
1: BEGIN
  flag[2]:=false;
  END;
2: BEGIN
  flag[3]:=false;
clear_update;

'E';'e':BEGIN {CO2/lights/room conditions}
if (t<=b) then
BEGIN
if (status1[11] = 1) then
BEGIN
  t:=dtime+init.params[8];
  CO2_addition;
  gotoxy(1,4);write('  ');
  gotoxy(1,4);
  convert_time(t);
  write('  CO2 ON');
END
else
BEGIN
  t:=dtime+init.params[7];
  CO2_addition;
  gotoxy(1,4);write('  ');
  gotoxy(1,4);
  convert_time(t);
  write('  CO2 OFF');
END;
END;
if (l<=b) then
BEGIN
if (init.params[5] = 0) and (init.params[6] = 0) then
  flag[10]:=true
else
BEGIN
  if (status1[10] = 1) and (dtime>= b) then
  BEGIN
    adc_1_1;
    rs_init;
    ctoutput_action(10,status1);
    flag[10]:=false;
    l:=dtime+init.params[5];
    gotoxy(1,5);write('  ');
    gotoxy(1,5);
    convert_time(b);
    write('  LIGHTS ON');
  END
else if (status1[10] = 0) and (dtime>=b) then
    BEGIN
        adc_1_1;
        rs_init;
        ctoutput_action(10,status1);
        flag[10]:=true;
        l:=dtime+init.params[6];
        gotoxy(1,5);write(' ');
        gotoxy(1,5);
        convert_time(b);
        write(' LIGHTS OFF');
        END;
    END;
    END;

If (c<=b) then
    BEGIN
        temperature_update(1,2);
        c:=dtime+fifteen_minutes;
        gotoxy(1,6); write(' ');
        gotoxy(1,6);
        convert_time(c);
        write(' TEMPERATURE_UPDATE');
        END;
    END;

If (d<=b) then
    BEGIN
        flag[18]:=true;
        light_output_check(3,7);
        d:=dtime+fifteen_minutes;
        gotoxy(1,7); write(' ');
        gotoxy(1,7);
        convert_time(d);
        write(' LIGHT_OUTPUT_CHECK');
        END;
    END;

'G','g':BEGIN
    temperature_update(1,2);
    END;

'H','h':BEGIN
    data_file;
    END;

'I','i':BEGIN
    flag[14]:=true;
    op_params;
    END;

'J','j':BEGIN
    flag[18]:=true;
    END;
Light_Output_Check(3,7);
END;

'K','k':BEGIN {system flush}
clear_update;
gotoxy(1,21); write('1: Water Flush');
gotoxy(1,22); write('2: Disinfectant Flush');
repeat
  gotoxy(17,20); clrnl;
  write('Which option would you like to choose ? ');
  ans:=read_integer(57,20);
until (ans = 1) or (ans = 2);
gotoxy(17,20); clrnl; write('datafile = ',dfile);
clear_update;
case ans of
1:BEGIN {water flush}
  adc_1_2;
  rs_init;
  close_both_chamber_valves;
  delay(5000);
  close_both_chamber_valves;
  water_flush_manifold;
  clear_update;
  repeat
    gotoxy(1,21);
    writeln('Press any key to end manifold flush '); 
  until keypressed;
  if keypressed then
    BEGIN
      end_water_flush_manifold;
      open_both_chamber_valves;
      delay(5000);
      open_both_chamber_valves;
    END;
  END;

END;

2:BEGIN
  adc_1_2;
  rs_init;
  close_both_chamber_valves;
  delay(5000);
  close_both_chamber_valves;
  ctoutput_action(9,status2);
  adc_1_1;
  rs_init;
  ctoutput_action(7,status1);
  delay(10000);
  water_flush_manifold;
adc_1_2;
rs_init;
ctoutput_action(9,status2);
adc_1_1;
rs_init;
ctoutput_action(7,status1);
clear_update;
repeat
gotoxy(1,21);
writeln('Press any key to end manifold flush ');
until keypressed;
if keypressed then
BEGIN
end_water_flush_manifold;
open_both_chamber_valves;
delay(5000);
open_both_chamber_valves;
END;
END; {of case of}
repeat
adc_1_1;
rs_init;
ctoutput_action(1,status1);
lower_harvest_level_detector;
until (Idetector <60) or (dtime > Ihcheck_time) or
(keypressed);
if keypressed then
BEGIN
adc_1_1;
rs_init;
ctoutput_action(12,status1);
ctoutput_action(6,status1);
if (status1[1] = 1) then
cctoutput_action(1,status1);
END;
END;
'L',l':BEGIN
valve_diag;
END;
'S',s':BEGIN
flag[5]:=true;
rep1:=0;rep2:=0;rep3:=0;
gotoxy(36,17); clrhol;
write(' S Supervisor ACTIVE');
clear_update;
gotoxy(1,21); write('You must have an open a data file when ');
goxy(1,22); write('in the supervisor mode!!!');
data_file;
stack_supervisor;
END;
END; {case of}
Until (flag[6]);
data_file;
all_cout_off;
clrscr;
cursor(on);
END. {main program}
APPENDIX A-2

SUPPORTING DOCUMENTATION

Table 1 lists connections to the ADC-1 units and provides the resistors used with each probe and sensor. Tables 2 and 3 provide the connections the the controlled outputs for ADC-1-1 and ADC-1-2, respectively. Table 4 presents a list of flags used within the program.
Table 1. Probe and sensor connections to both ADC-1-1 and ADC-1-2, with corresponding resistor information.

### ADC_1_1

<table>
<thead>
<tr>
<th>Channel</th>
<th>Resistor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Room Temperature 1</td>
<td>1000 Ω</td>
</tr>
<tr>
<td>Room Temperature 2</td>
<td>1000 Ω</td>
</tr>
<tr>
<td>MB - Temperature</td>
<td>1000 Ω</td>
</tr>
<tr>
<td>MB - Light Intensity</td>
<td>4700 Ω</td>
</tr>
<tr>
<td>MB - Light Output</td>
<td>4700 Ω</td>
</tr>
<tr>
<td>Room Light Intensity 1</td>
<td>4700 Ω</td>
</tr>
<tr>
<td>Room Light Intensity 2</td>
<td>4700 Ω</td>
</tr>
<tr>
<td>Intensity Output Check - Chamber 1</td>
<td>4700 Ω</td>
</tr>
<tr>
<td>Intensity Output Check - Chamber 2</td>
<td>4700 Ω</td>
</tr>
<tr>
<td>Level Detector - Chamber 1</td>
<td>470 Ω</td>
</tr>
<tr>
<td>Level Detector - Chamber 2</td>
<td>470 Ω</td>
</tr>
<tr>
<td>Upper Harvest Level Detector</td>
<td>470 Ω</td>
</tr>
<tr>
<td>MB - Conductivity</td>
<td>2200 Ω</td>
</tr>
<tr>
<td>Lower Harvest Level Detector</td>
<td>470 Ω</td>
</tr>
</tbody>
</table>

### ADC_1_2

<table>
<thead>
<tr>
<th>Channel</th>
<th>Resistor</th>
</tr>
</thead>
<tbody>
<tr>
<td>MB - pH</td>
<td>-----</td>
</tr>
</tbody>
</table>
Table 2. Controlled output connections for ADC-1-1.

<table>
<thead>
<tr>
<th>Controlled Output</th>
<th>Relay Number</th>
<th>Item</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>------</td>
<td>Level Detectors</td>
</tr>
<tr>
<td>2</td>
<td>Box 2 - 2</td>
<td>Close Saltwater</td>
</tr>
<tr>
<td>3</td>
<td>2</td>
<td>Heater</td>
</tr>
<tr>
<td>4</td>
<td>4</td>
<td>Open Saltwater</td>
</tr>
<tr>
<td>5</td>
<td>8</td>
<td>UV Light</td>
</tr>
<tr>
<td>6</td>
<td>Box 2 - 4</td>
<td>Solenoids 10</td>
</tr>
<tr>
<td>7</td>
<td>7</td>
<td>Disinf. Soln.</td>
</tr>
<tr>
<td>8</td>
<td>6</td>
<td>Nutrients</td>
</tr>
<tr>
<td>9</td>
<td>5</td>
<td>Open Chamber 2</td>
</tr>
<tr>
<td>10</td>
<td>Box 2 - 6</td>
<td>Chamber Lights</td>
</tr>
<tr>
<td>11</td>
<td>3</td>
<td>CO₂</td>
</tr>
<tr>
<td>12</td>
<td>1</td>
<td>Harvest Pump</td>
</tr>
</tbody>
</table>
Table 3. Controlled output connections for ADC-1-2.

<table>
<thead>
<tr>
<th>Controlled Output</th>
<th>Relay Number</th>
<th>Item</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4</td>
<td>Air Conditioner</td>
</tr>
<tr>
<td>2</td>
<td>8</td>
<td>Close Main Manifold</td>
</tr>
<tr>
<td>3</td>
<td>9</td>
<td>Main Manifold NC</td>
</tr>
<tr>
<td>4</td>
<td>5</td>
<td>Tap Water</td>
</tr>
<tr>
<td>5</td>
<td>1</td>
<td>Close Chamber 2</td>
</tr>
<tr>
<td>6</td>
<td>11</td>
<td>Close Chamber 1</td>
</tr>
<tr>
<td>7</td>
<td>2</td>
<td>A/V Solenoid</td>
</tr>
<tr>
<td>8</td>
<td>6</td>
<td>Photocell Light</td>
</tr>
<tr>
<td>9</td>
<td>7</td>
<td>Air</td>
</tr>
<tr>
<td>10</td>
<td>10</td>
<td>Open Main Manifold</td>
</tr>
<tr>
<td>11</td>
<td>3</td>
<td>A/V Pump</td>
</tr>
<tr>
<td>12</td>
<td>12</td>
<td>Open Chamber 1</td>
</tr>
</tbody>
</table>
Table 4. Conditional flags used in the software program, "Supervisor".

<table>
<thead>
<tr>
<th>Flag Number</th>
<th>Flag Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Air</td>
</tr>
<tr>
<td>2</td>
<td>Disinfect Chamber 1</td>
</tr>
<tr>
<td>3</td>
<td>Disinfect Chamber 2</td>
</tr>
<tr>
<td>4</td>
<td>Supervisor</td>
</tr>
<tr>
<td>5</td>
<td>Escape or Quit</td>
</tr>
<tr>
<td>6</td>
<td>Equilibrium Refill</td>
</tr>
<tr>
<td>7</td>
<td>Write to Textfile for Date/Time</td>
</tr>
<tr>
<td>8</td>
<td>Culture Conditions</td>
</tr>
<tr>
<td>9</td>
<td>Lights</td>
</tr>
<tr>
<td>10</td>
<td>Initial Light Output Conditions</td>
</tr>
<tr>
<td>11</td>
<td>Chamber Equilibrium</td>
</tr>
<tr>
<td>12</td>
<td>Flush Manifold</td>
</tr>
<tr>
<td>13</td>
<td>Operational Parameter Update</td>
</tr>
<tr>
<td>14</td>
<td>Initial Dump</td>
</tr>
<tr>
<td>15</td>
<td>Dummy Dump</td>
</tr>
<tr>
<td>16</td>
<td>Disinfect Manifold</td>
</tr>
<tr>
<td>17</td>
<td>Light Output</td>
</tr>
<tr>
<td>18</td>
<td>Harvest Chamber 1</td>
</tr>
<tr>
<td>19</td>
<td>Harvest Chamber 2</td>
</tr>
<tr>
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APPENDIX B

PRODUCTION DATA FOR CHAETOCEROS MUELLERI (CHAET 10)

This appendix contains a listing of the production data collected for Experiments One through Seven. This data is used in Chapters II, III and IV. The values presented have been averaged over six hour time periods, resulting in four data points per day. Light, temperature, pH, C-Bio and dilution were collected by the computer during each harvest, while harvest, VolProd, AerProd, No. Harv/day, SGR were calculated using the computer collected data. Blank cells represent missing data, with the exception of lipids and protein. Separate data sets are presented for each chamber within each experiment.

Definition of Terms

1) C-Bio: computer estimated standing crop concentration (g/m³)
2) A-Bio: analytically measured TSS, representing standing crop (g/m³)
3) Dilution: dilution rate = (number of harvest/day)(5 gallons/harvest)/140 gallons = (days⁻¹)
4) VolProd: volumetric production level = (C-Bio)(Dilution) = (g/m³/day)
5) AerProd: aerial production level = (VolProd)(0.94 m) = (g/m²/day)
6) SGR: specific growth rate = Dilution+ln(C-Bioₓ/C-Bioₓ₋₁)/0.25 = (days⁻¹)
7) Temperature: (°C)
8) Light: lumens/m²
9) Lipids (total) = percent of total biomass
10) Protein (total) = percent of total biomass
Experiment One was performed at 28°C under continuous lighting from six 40W cool white fluorescent bulbs (for each chamber). pH was maintained between 7.6 and 8.0, while salinity was held at 35±1 ppt. The experiment was performed during the period extending from January 25, 1992 to February 5, 1992.
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| Average | 63.0   | 6.2   | 24.7   | 54.2   | 50.9   | 0.86  | 24   | 0.81  | 27.8   | 7.79   | 5836   | 6.7  | 27.4 |
| STD   | 15.9   | 6.2   | 13.7   | 12.9   | 0      | 0     | 0    | 1.20  | 0.5    | 0.11   | 179    | 3.1  |
| Min   | 41.19  | 89.3  | 16.1   | 35.4   | 33.3   | 0.86  | 24   | -1.32 | 26.7   | 7.54   | 5482   | 6.7  | 24.9 |
| Max   | 122.8  | 94.3  | 48.1   | 105.6  | 99.3   | 0.86  | 24   | 3.67  | 29.5   | 8.00   | 6270   | 6.7  | 39.8 |</p>
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APPENDIX B-2

EXPERIMENT TWO

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| Count   | 57    | 5    | 57     | 57     | 57     | 57    | 57   | 57    | 56     | 52    | 39  | 1    |
| Average | 192.2 | 218.4| 44.2   | 124.2  | 116.7  | 0.65  | 18   | 0.69  | 30.1   | 7.87  | 9.3 | 17.7 |
| STD     | 39.1  | 27.5 | 19.0   | 33.5   | 31.3   | 0.14  | 4    | 0.66  | 0.6    | 9.3   | 14.5 |
| Min     | 104.7 | 184.7| 26.8   | 90.0   | 84.6   | 0.54  | 14   | -0.69 | 28.3   | 6.45  | 9.3 | 0.0  |
| Max     | 294.5 | 256.7| 110.1  | 241.6  | 227.1  | 0.66  | 24   | 3.09  | 31.3   | 9.66  | 9.3 | 31.1 |
APPENDIX B-3

EXPERIMENT THREE

Experiment three was performed at 30°C under intermittent (20:4, L:D) lighting from a 250W metal halide lamp (for each chamber). pH was maintained between 7.6 and 8.0, while salinity was held at 35±1 ppt. The experiment was performed during the time period extending from March 6, 1992 through March 27, 1992.
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| Count  | 87 | 4       | 87     | 87     | 89     | 89    | 86   | 85    | 82     | 3     | 4    |
| Average | 203.2 | 273.1  | 23.4   | 93.2   | 87.6   | 0.47  | 13   | 0.48  | 29.8   | 7.97  | 10.5 | 22.2 |
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Count   | 87      | 4      | 87     | 87     | 87       | 87    | 86   | 84    | 87      | 3      | 4    |
Average | 195.1   | 277.2  | 21.8   | 86.1   | 82.8     | 0.45  | 13   | 0.47  | 30.6    | 7.94   | 10.5 | 22.2 |
STD     | 34.8    | 72.4   | 8.6    | 20.6   | 19.3     | 0.08  | 2    | 0.64  | 0.9     | 0.37   | 1.8  | 5.4  |
Min     | 125.7   | 187.7  | 9.6    | 47.8   | 44.9     | 0.36  | 10   | -1.65 | 28.2    | 7.53   | 8.4  | 15.1 |
APPENDIX B-4

EXPERIMENT FOUR

Experiment Four was performed at 35°C under continuous lighting from a 250W metal halide lamp (for each chamber). pH was maintained between 7.6 and 8.0, while salinity was held at 35±1 ppt. The experiment was performed during the time period extending from April 10, 1992 through April 22, 1992.
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| Average | 184.3 | 46.5 | 123.9 | 116.5 | 0.70 | 20 | 0.54 | 35.1 | 7.73 | 12.2 | 30.0 |
| STD | 15.9 | 117.9 | 37.7 | 35.4 | 0.17 | 5 | 0.41 | 1.0 | 0.11 | 0.2 | 4.5 |
| Min | 153.3 | 162.0 | 26.4 | 87.4 | 62.2 | 0.57 | 16 | -0.30 | 31.4 | -0.71 | 12.0 | 25.4 |
| Max | 228.4 | 227.0 | 104.3 | 196.9 | 185.1 | 1.00 | 28 | 1.36 | 37.9 | 8.17 | 12.4 | 35.6 |
APPENDIX B-5

EXPERIMENT FIVE

Experiment Five was performed under continuous lighting from a 250W metal halide lamp (for each chamber) and at temperatures ranging from 27 - 32°C. pH was maintained between 7.6 and 8.0, while salinity was held at 35±1 ppt. The experiment was performed during the time period ranging from May 5, 1992 through May 16, 1992.
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| Count|        | 42     | 2      | 42      | 42      | 42      | 44     | 44   | 44     | 42.0   | 42.0   | 3    | 3    |
| Average|       | 157.8  | 99.0   | 89.9    | 84.5    | 0.57    | 16     | 0.63 | 30.3   | 7.71   | 8.8    | 8.8  | 8.8  |
| STD  |       | 17.6   | 29.3   | 3.0     | 10.1    | 9.5     | 0      | 0    | 0.24   | 1.0    | 0.08   | 0.7  | 0.7  |
| Min  |       | 125.1  | 168.3  | 21.5    | 71.3    | 67.0    | 0.57   | 16   | 0.09   | 28.4   | 7.50   | 8.30 | 8.3  |
| Max  |       | 193.7  | 209.7  | 33.3    | 110.4   | 103.8   | 0.57   | 15   | 1.17   | 32.4   | 7.84   | 9.6  | 9.6  |
APPENDIX B-6

EXPERIMENT SIX

Experiment Six was performed under continuous lighting from a 250W metal halide lamp (for each chamber) and at temperatures ranging from 26 - 31°C. pH was maintained between 7.6 and 8.0, while salinity was held at 35±1 ppt. The experiment was performed during the time period extending from June 5, 1992 to June 11, 1992.
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APPENDIX B-7

EXPERIMENT SEVEN

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| Count | 82 | 82 | 82 | 82 | 88 | 88 | 88 | 82 | 82 | 92 | 9 | 9 |
| Ave | 139.9 | 140.1 | 189.8 | 217.2 | 204.2 | 1.59 | 45 | 1.63 | 30.9 | 7.75 | 57724 | 7.7 | 14.2 |
| STD | 26.8 | 24.2 | 108.0 | 61.9 | 58.2 | 0.56 | 16 | 0.89 | 1.1 | 0.15 | 2057 | 2.8 | 4.9 |
| Min | 90.1 | 107.7 | 43.6 | 93.6 | 88.0 | 0.86 | 24 | -0.87 | 28.4 | 7.41 | 51621 | 4.9 | 7.2 |
| Max | 236.8 | 183.7 | 359.1 | 350.1 | 329.1 | 2.39 | 67 | 3.46 | 33.8 | 8.04 | 68097 | 13.1 | 22.5 |
APPENDIX C

TOTAL LIPID AND PROTEIN DATA
(Experiments One - Seven)

This appendix presents a list of total lipid and protein data for Experiments One through Seven and discussed in Chapters III and IV. Values for both parameters are expressed as percent of dry biomass (TSS).
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APPENDIX D

REGRESSION ANALYSIS: WET VERSUS DRY BIOMASS

Appendix D presents the wet weights (grams) of the algal paste collected from the centrifuge. The paste was dried, and the dry biomass values were regressed against the wet values. Dry biomass averaged 22 percent of the wet weight. The regression is discussed in Chapter III and used to estimated daily average harvested biomass.
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**Regression Output:**

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- Std Err of Y Est: 3.343067
- R Squared: 0.928993
- No. of Observations: 34
- Degrees of Freedom: 33

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APPENDIX E

REGRESSION ANALYSIS: MILLIVOLTS VERSUS TSS

Appendix E presents the raw millivolt data, obtained from the photovoltaic cell of the photocell and regressed against analytically measured TSS. The regression equation was used by the software to estimate standing crop concentrations within the growth chambers. A discussion of this relationship is presented in Chapters II, III and IV.
DATA FOR TURBIDITY REGRESSION EQUATION
Photovoltaic (mv) versus TSS measurements

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Regression Output:
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- Std Err of Y Est: 23.871
- R Squared: 0.865058
- No. of Observations: 21
- Degrees of Freedom: 19
- X Coefficient(s): -4.35806
- X Coefficient(s): -4.81424
- Std Err of Coef.: 0.436216
FIRST ORDER COMPUTER MODEL TO PREDICT ALGAL PRODUCTION WITHIN THE TURBIDOSTAT SYSTEM

Presented in this appendix is the program listing for the computer model developed to predict production levels within the turbidostat system over extended time periods. This model employs first order kinetics and the Fourth Order Runge Kutta numerical solution technique to estimate standing crop concentrations over time. The model was calibrated using data obtained from Experiments One through Four and verified against data from Experiment Seven. The model is discussed in Chapter IV, along with the presentation of calibration curves for chamber two (Experiments One through Four) and verification curves from Experiment Seven. The remaining calibration curves are included in this appendix.
Program Algae_Production;

(This program, written by Kelly A. Rusch, estimates daily algal production within the computer automated turbidostat.)

uses turbo3, crt, dos;

Type
key = string[80];

Const
V = 0.530; {m3, chamber volume}

Var
ans:char;
X:real; {g/m3, standing crop concentration}
Xo:real; {g/m3, initial standing crop concentration}
t:real; {days, time}
c:real;
Quit_Run:boolean;
NP,PI:real; {days, next_print, print_interval}
NH,Hl:real; {days, next_harvest, harvest_interval}
HB:real; {g, harvested biomass}
HF:real; {harvest_fraction}
Xave:real; {g/m3, average daily standing crop}
Have:real; {g/day, average harvested biomass}
Pave:real; {g/m3/day, average daily production}
DP:real; {g/m3/day, daily production}
DH:real; {g/day, daily harvest}
Delta_t:real; {days, time step}
K1,K2,K3,K4:real; {slopes for 4th order Runge Kutta}
X1,X2,X3:real; {intermediate biomass concentrations for 4th order Runge Kutta}
ttemp,cptemp,chtemp,cxtemp:real;
indata,outdata:text;
ifile,ofile:string[20];
RL:real; {days, run_length of simulation period}
ET:real; {days, end_time}
SGR:real; {1/day, specific growth rate}

Procedure Comments(num:integer);
CONST
   cmts:array[1..4] of string[60] = (
      (1) 'Having problems opening file',
BEGIN
clrscr;
write(#07);
gotoxy(20,10);
write(cmts[num]);
End; {of procedure comments}

Procedure Hold;
Var
  f:char;
BEGIN
  clrscr;
gotoxy(20,10);
  write('Press any key to continue');
  repeat until keypressed;
  read(kbd,f);
END;

Procedure Out_Data_file;
BEGIN
  repeat
    clrscr;
gotoxy(20,8);
    writeln('Input the desired data file to write to '); 
    gotoxy(20,9);
    write('(less than 8 characters, no suffix) '); 
    readln(ofile);
    {$!-}
ofile:='a:'+ofile+'.dat';
until not EXIST(ofile);
assign(outdata.ofile);
rewrite(outdata);
if (not ioresult = 0) then
BEGIN
  comments(1);
  comments(2);
  END;
{$!+}
END;

Procedure In_Data_File;
BEGIN
clrscr;

gotoxy(20,8);
writeln('Input the data file to read from ');
gotoxy(20,9);
write('(less than 8 characters, no suffix) ');
readln(ifile);
ifile:=a.'+ifile+'.fdt';
{$!-}
assign(indata,ifile);
reset(indata);
{$!+}
if (not ioresult = 0) then
BEGIN
  comments(4);
  hold;
  exit;
END;
End; {procedure data file}

Procedure Initial_Information;
BEGIN
  clrscr;
  gotoxy(20,8);
  write('Input data for initial conditions. Please wait.......
';
  delay(3000);
  clrscr;
  gotoxy(1,5);
  write('Condition 1: Fraction of chamber harvested per harvest episode = ');
  readln(HF);
  gotoxy(1,6);
  write('Condition 2: Run length (days) = ');
  readln(RL);
  gotoxy(1,7);
  write('Condition 3: Time step (days) = ');
  readln(Delta_t);
  gotoxy(1,8);
  write('Condition 4: Print Interval (days) = ');
  readln(PI);
  gotoxy(1,9);
  write('Condition 5: Initial Standing Crop Concentration (g/m3) = ');
  readln(Xo);
  NP:=round(PI*100)/100;
  X:=Xo;
END; {of procedure initial information}

Procedure Initialize_Variables;
BEGIN
HB:=0;
DP:=0;
DH:=0;
NP:=0;
NH:=0;
c:=0.25;
X:=0;
Xo:=0;
PI:=0;
HI:=0;
t:=0;
ET:=0;
SGR:=0;
K1:=0;K2:=0;K3:=0;K4:=0;
X1:=0;X2:=0;X3:=0;
ttemp:=0;cxtemp:=0;chtemp:=0;cptemp:=0;
Have:=0;Pave:=0;Xave:=0;
Quit_Run:=false;
END; {of procedure initialize variables}

Procedure Get_Data;
BEGIN
  if EOF(indata) then Quit_Run:=true else
    BEGIN
      readln(indata,SGR,HI);
      HI:=1/Hi;
    END;
END;

Procedure Headings;
BEGIN
  writeln(outdata,' ':5,'Fourth order Runge Kutta model to predict production ');
  writeln(outdata,' ':5,'levels in the algal turbidostat. ');
  writeln(outdata);
  writeln(outdata);
  writeln(outdata,' ':5,'lnput Dta' );
  writeln(outdata,' ':5,'----------');
  writeln(outdata,' ':5,'Harvest Fraction = HF:6:4);
  writeln(outdata,' ':5,'Run length = RL:5:1,'  (days)' );
  writeln(outdata,' ':5,'Time Step = Delta_t:4:2,'  (days)' );
  writeln(outdata,' ':5,'Print Interval = PI:4:2,'  (days)' );
  writeln(outdata,' ':5,'Initial Standing Crop = Xo:5:1,'  (g/m3)' );
  writeln(outdata); writeln(outdata); writeln(outdata);
  writeln(outdata,' ':5,'Day ','HI ','  SGR '.' Standing Crop '.'Production ',' Harvest ','Cum. Harvest');
  writeln(outdata,' ':5,'(Day) ','(1/Day) ','(g/m3) ','(g/m3/d) ','(g/Day) ','(g) ');}
writeln(outdata);
BEGIN;  {of procedure headings}

Procedure Specific_Growth_Rate_Check;
BEGIN
  if (t >= c) then
    BEGIN
      c:=c+0.25;
      get_data;
    END;
END;

Function SC(a,b:real):real;
BEGIN
  SC:=a*b;
END;  {of function SC}

Procedure Standing_crop;  {4th order Runge Kutta}
BEGIN
  K1:=SC(SGR,X);
  X1:=X+K1*delta_t/2;
  K2:=SC(SGR,X1);
  X2:=X1+K2*delta_t/2;
  K3:=SC(SGR,X2);
  X3:=X2+K3*delta_t;
  K4:=SC(SGR,X3);
  X:=X+delta_t/6*(K1+2*K2+2*K3+K4);
  cxtemp:=cxtemp+X;
  DP:=X*HF*(1/HI);
  cptemp:=cptemp+DP;
  DH:=DP*(1/HI)*0.018925;
  chtemp:=chtemp+DH;
  Xave:=xave+X;
  Pave:=Pave+DP;
  Have:=Have+DH;
  if X < 0 then
    BEGIN
      comments(3);
      hold;
    END;
  if X > 300 then X:=300;
END;  {of procedure standing crop}

Procedure Print_Check;
BEGIN
  if (t+delta_t>= NP) then
BEGIN
NP:=round((t+PI)*100)/100;
Xave:=Xave/(PI/delta_t);
Pave:=Pave/(PI/delta_t);
Have:=have/(PI/delta_t);
writeln(outdata,' :5,t:6:3,HI:8:4,SGR:8:2,Xave:10:1,Pave:15:1,Have:15:1);
Xave:=0;
Pave:=0;
Have:=0;
END;
{of procedure print check}

Procedure Harvest_Check;
BEGIN
if (t >= NH) and (t < RL) then
BEGIN
writeln(outdata,' :5,t:6:3,HI:8:4,SGR:8:2,x:10:1,DP:15:1,DH:15:1);
NH:= round((t+HI)*100)/100;
HB:=HB+HF*X*V*1.07;
X:=X-1.07*HF*X;
ttemp:=t+delta_t/10;
DP:=X*HF*(1/HI);
DH:=DP*(1/HI)*0.018925;
writeln(outdata,' :5,ttemp:6:3,HI:8:4,SGR:8:2,x:10:1,DP:15:1,DH:15:1,HB:10:1);
END;
{of procedure harvest check}

BEGIN
clrscr;
Repeat
Initialize_Variables;
Initial_Information;
Out_Data_File;
In_data_file;
Headings;
crscr;
Get_Data;
NH:=HI;
Repeat
t:=t+delta_t;
Specific_Growth_Rate_Check;
Standing_Crop;
Print_Check;
Harvest_Check;
Until (t=RL) or (Quit_run) or (keypressed);
close(indata);
HB := HB + X * V;
X := 0;
DP := 0;
DH := 0;
SGR := 0;
Ht := 0;
ET := t + delta_t;
writeln(outdata, ' ':5, ET:6:3, Ht:8:4, SGR:8:2, X:10:1, DP:15:1, DH:15:1, HB:10:1);
Xave := cxtemp / (t / delta_t);
Have := chtemp / (t / delta_t);
Pave := cptemp / (t / delta_t);
writeln(outdata); writeln(outdata);
writeln(outdata, ' ':5, 'The average daily standing crop = ', Xave:7:1);
writeln(outdata, ' ':5, 'The average harvested mass per day = ', Have:7:1);
writeln(outdata, ' ':5, 'The average daily production = ', Pave:7:1);
close(outdata);
clrscr;
gotoxy(20, 8);
write('Do you wish to continue (Y/N) ? ');
readln(ans);
until UpCase(ans) = 'N';
END.
VITA

Kelly Ann Rusch was born September 21, 1964 in Plymouth, Wisconsin. She graduated with honors from Plymouth Comprehensive High School in 1982. After graduation, she attended the University of Wisconsin-LaCrosse and graduated magna cum laude with a B.S. in Biology and Chemistry. In January, 1987, Kelly entered the Master’s program in Civil Engineering (emphasis in Environmental Engineering) at Louisiana State University. Upon completion of this degree in December, 1989, she continued to pursue her Ph.D. in Civil Engineering at Louisiana State University. In December, 1992, Kelly received her Ph.D. and is presently an Assistant Research Professor in the Department of Civil Engineering at Louisiana State University.
DOCTORAL EXAMINATION AND DISSERTATION REPORT

Candidate:       Kelly Ann Rusch
Major Field:     Civil Engineering

Title of Dissertation: Demonstration of a Control Strategy for Sustained Algal Growth at a Full-Scale Level Under Computer Automated, Continuous Culture Conditions

Approved:

[Signatures]

Major Professor and Chairman
Dean of the Graduate School

EXAMINING COMMITTEE:
[Signatures]

Date of Examination: August 31, 1992