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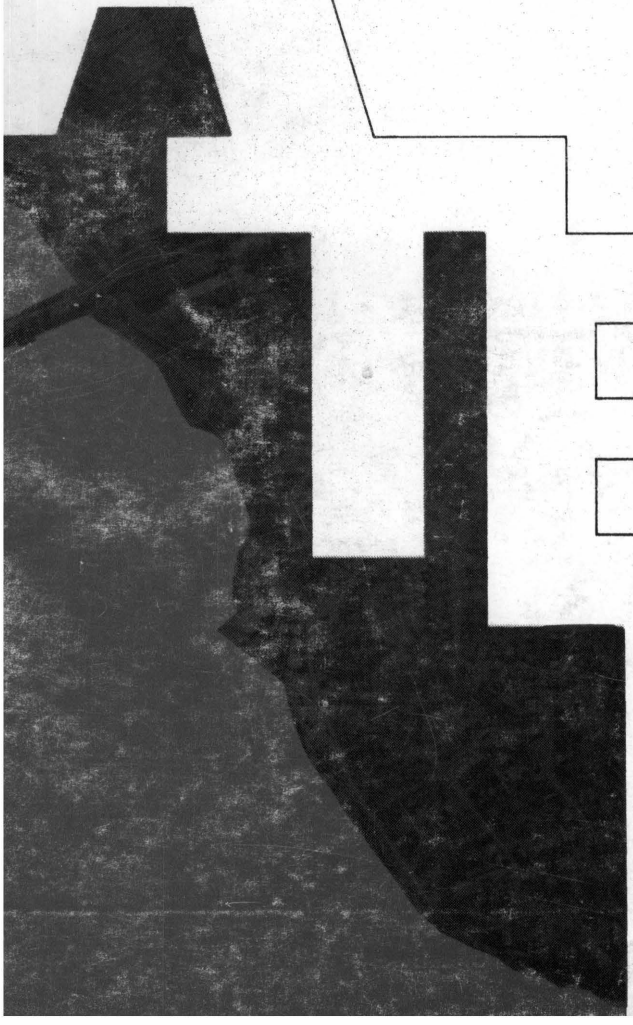

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PREFACE

This research effort was directed toward decreasing the substantial void existing in the area of the effects of heated waste waters on aquatic microorganisms. The National Technical Advisory Committee on Water Quality Criteria to the Water Quality Office of the Environmental Protection Agency has produced recommendations with regard to water temperature for both freshwater organisms and marine and estuarine organisms (fish) but not microorganisms. Protozoans, algae, and fungi fill different but related critical roles in aquatic communities and, thus, it is essential to learn more on the ultimate effects of heated water effluents on the structure and function of microbial communities. Hopefully, the knowledge produced by these investigations can help in improving our understanding and use of the natural environment.

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ABSTRACT

The effects of thermal stress on selected aquatic microorganisms were investigated in these experiments. Numerous categories of temperature shock were studied with emphasis on (1) general simulation of the passage of water through a condensing system of a steam electric power plant to determine the effects of this exposure and (2) the effects of such entrainment upon the microbial system below the discharge point. Three general groups of organisms were examined: protozoans, algae, and chytridiaceous fungi. New equipment developed specifically to aid in these studies (a simple apparatus for delivering heat shock to microorganisms) and a new fluorescent survey technique to characterize stress induced cellular alterations are described in detail.

Key Words: Thermal stress, Thermal pollution, Microorganisms, Fluorescence

INTRODUCTION

The problem of heated discharge waters is becoming more and more critical as the demand for electric power increases. The Water Quality Office of the Environmental Protection Agency considers thermal pollution a major problem and suggests research be carried out under controlled laboratory conditions to determine the effects of temperature increases. In these studies, temperatures were varied under such controlled conditions to show effects ranging from complete lethality to complete survival without change.

Protozoans and diatoms, as groups of microorganisms, assume different but related critical roles in the aquatic community. Diatoms are active at the primary producer level of food webs while protozoans are the primary regulators for controlling bacterial activity. Protozoans also ingest organic pollutants.

The majority of bacteria live on preformed organic matter at an optimum temperature of 22 to 28°C (70 to 82°F) while the parasitic bacteria, including those pathogenic to man, have optimum temperatures near 37°C (98.6°F). The temperature of natural waters is usually below the optimum for pollution-associated bacteria; increasing the temperature in a favorable environment with abundant food could increase the bacteria's reproductive and growth rate. Thus, it is essential to learn how heated water will affect all microbes or their environment.

It is estimated that by 1985 one quarter of all surface water will pass through steam-electric power generator condensers. Clearly, some basic decisions regarding the management of heated discharge waters should be made as soon as possible. Unfortunately, very little information concerning the effects of heated discharge waters is now available. Much of this has been obtained in laboratory situations which may not be easily related to natural conditions, and much of the information available from natural situations is clouded by the presence of other stresses or is concerned with vertebrates, particularly fish, and the higher invertebrates. Very little information seems to be available regarding the effects of heated discharge waters on microbial communities.

The specific aim of this project was to expand the information regarding the effects of heated discharge waters upon communities of protozoans and algae with some emphasis upon aquatic fungal and algal relationships. The study was initially divided into two broad areas: (1) simulation of the passage of water through a condensing system to determine the effect of this exposure upon unicellular organisms and (2) the effect of simulated passage through the condenser upon the microbial system below the discharge point.

PART I
PROTOZOAN STUDIES

PROTOZOAN STUDIES

Among the information gathered concerning the effects of heat on protozoans, very little is applicable to a study of the effects of heated waste water produced by steam electric power stations upon natural protozoan communities. Some protozoologists have confined many of their studies to pure cultures of organisms maintained under controlled laboratory conditions. Another method of determining responses is to observe an entire community as it reacts to environmental changes. Temperature changes in the cooling water of steam electric stations (SES) are unlike any temperature changes previously studied by ecologists. A common approach has been to gradually increase the ambient temperature of cultures of organisms until growth is no longer observed or some other endpoint was reached. Another approach (Cairns, 1969) involved the exposure of protozoan communities associated with a substrate to abrupt high increases in temperature. In these experiments the protozoan communities were exposed *in situ* rather than being pumped through a pipe during the heating process as would occur during actual passage through an SES cooling unit. Clearly, the heating rate and nature and duration of the thermal shock are critical parameters to control and measure in any experiment simulating passage of water through cooling condensers. Because of this, we have attempted to simulate the heating of stream and lake water by a steam electric power station and to monitor the effects of this shock in protozoan community development downstream.

The condenser of a large power station uses cooling water at a very great rate. For example, the condenser of a typical nuclear powered plant of 1700 megawatts capacity is a large chamber 18 m long x 12.2 m high x 6.1 m wide. Pipes inside the chamber carry the cooling water which must condense the turbine exhaust steam back to water. Surface temperature of the interior of these pipes is estimated to be 48.9°C. The cooling water passes through the condenser with a temperature increase of 9 to 10°C or more. Normal flow is 6,380,000 liters per minute. If there is no provision for cooling this water, it is then directly discharged back into a stream where it may be diluted by the cooler stream water to some temperature determined by the ratio of the quantity of effluent flow to the quantity of the stream flow. The warmed

stream then cools until, at some distance downstream, the water temperature may approach the stream's original temperature.

There are several ways in which the heated waste water discharge of an electric power generating station could influence the protozoan communities below the point of discharge:

(1) The heating of water and microorganisms as they pass through the steam condenser, and the physical agitation and abrupt pressure changes.

(2) An increase in the ambient temperature of the stream below the discharge point with consequent changes in water quality characteristics.

(3) Possible reduction in number or a change in kinds of microorganisms invading downstream habitats, which might interfere with the normal succession patterns of the community.

One purpose of this study was to develop an apparatus which permitted continued observation of the responses of microscopic organisms to acute thermal shock. We felt the apparatus should (1) permit continuous observation of the test organisms and (2) be capable of heating the water containing test organisms quickly as well as maintaining nearly constant temperature. Other experiments explored the possible effects of the addition of heated effluents on protozoan community structure below the point of discharge.

MATERIALS AND METHODS

A test chamber was fashioned from two 1/8-in. thick rectangles of Plexiglas* acrylic sheet of approximately the same dimensions as a standard microscope slide. A 3/4-in. diameter hole was drilled through the center of one plate, which was then cemented face to face on top of the other, using 1,2-dichloroethane as a solvent. This created a depression with vertical walls 1/8 in. high and a base formed by part of the top surface of the bottom rectangle. Four

*"Plexiglas" is a trade name of the Rohm and Haas Company.

troughs 1/16 in. deep were cut from the four edges into the well and pointing toward its center (Figure 1). In these troughs were placed the four sets of wires described below.

To heat the water in the cell, a resistance heater was constructed by bending a short length of 0.0035-in. diameter insulated nichrome wire into the shape shown in Figure 1. This was done after first laying out the desired pattern of the heater by imbedding tiny dissecting pins into a block of warm paraffin. The wire was then pulled around the pins and stretched tight to give it the desired shape. It was then carefully removed and placed into the bottom of the well. Three thermocouples were assembled from 0.005-in. diameter insulated constantan and 0.0035 in. insulated nichrome wire with a thermocouple welder.

All four sets of wires, three thermocouples and the heater, were then placed in the troughs and fixed with epoxy cement which was smoothed flush with the top surface of the slide. Care was taken to avoid placing thermocouple wires near the heating wire. Such incorrect placement could induce an additional current in the thermocouple, thereby causing an incorrect temperature reading. A thermocouple switch permitted monitoring any of the three thermocouple junctions in the cell (Figure 1).

The heating wire was connected, at the point where it emerged from the cell, to the five-volt leads of a filament transformer. A Variac* autotransformer was connected to the input of the filament transformer. This combination permitted fine adjustment of heat input. The range of attainable temperatures is dependent on the type of transformers used and varies as the length of the resistance wire. Therefore, the resistance wire length was varied to obtain the desired range of heating characteristics. The heating characteristics of a typical cell at highest heat input are given in Figure 2.

To demonstrate one of the possible applications of this instrument, a description of a typical run describing the techniques used in determining median survival temperatures follows: The micro-

*"Variac" is a trade name of the General Radio Company.

scope objective is racked as far as possible from the stage to provide sufficient work space. With the cell on the stage of the microscope, the culture is introduced into the well until it "bulges" above the surface of the slide. Next, a cover slip is placed over the well so that air bubbles are not trapped and excess fluid is removed with a pipette until the cover slip rests on the surface of the slide. This insures fairly constant volume of test fluid for each test.

All organisms were obtained from the Carolina Biological Supply Company and were acclimated to 25 ± 1°C for at least 72 hours with daily aeration prior to use in an experiment.

No attempts were made to calculate the concentrations of organisms in the cell during actual tests. However, density measurements of the cultures used have been made and are presented here.

Species	Density (cells/ml)
Colpidium colpoda	2,000 - 3,000
Euglena gracilis	10,000 - 12,000
Paramecium multimicronucleatum	30 - 100
Spirostomum ambiguum	30 - 100
Stentor coeruleus	4 - 20
Tetrahymena pyriformis	2,000 - 5,000

While the concentrations of organisms in the test cell were probably within the same orders of magnitude as the above values, the actual concentration of cells in the test chamber probably varied slightly during the repetitions of each test. However, this factor probably did not alter the response of the individual organisms because of the short duration of the test.

To determine acute shock temperature thresholds, the test fluid containing one of the species was heated at the maximum rate until an estimated 50% of the organisms were dead; the temperature at that point was recorded. This was repeated ten times, and the resulting temperatures were averaged and time was determined from Figure 2. For tests at specific temperatures, the fluid in the

cell was heated at the maximum rate until the desired temperature was reached. The temperature was then stabilized and the organisms were observed until an estimated 50% were dead. Time for 50% death was recorded and the average was recorded as median survival time at that temperature.

To simulate the thermal shock of passage through a steam condenser, water containing protozoans was pumped by peristaltic pumps (Masterflex* Tubing Pump) from a reservoir through a 0.6 cm (inside diameter) copper coil† immersed in a 2300-watt hot water bath. Retention time in the coil was about 10 seconds. Immediately after passing through the heater, the water was cooled to its original temperature by passage through a copper tube in a cold water bath. The water was then distributed to three troughs containing the experimental communities as well as to two control communities which were maintained in an identical manner except for the thermal shock. Figure 3 is a schematic of the apparatus.

The reservoir was constructed of 1.9 cm plywood, assembled with wood screws and coated inside and outside with white, epoxy paint. It was 2.4 m long x 0.9 m wide x 1.2 m high. An 8.2 cm x 8.2 cm strut connecting the top centers of the long sides of the tank provided internal support against the pressure exerted by the water and supported the five test troughs. A hinged top provided a relatively air tight container and supported the light sources which consisted of two 40 watt Vita lite+ fluorescent elements and two 100-watt household incandescent light bulbs. The photoperiod was adjusted to coincide with the local periods of light and darkness (approximately 14 hours light, 10 hours darkness).

The bed of the reservoir was composed of rubble, sand, and other particulate material taken from a local stream. The depth was maintained at 60 cm by a gravity drain as water flowed in at a rate of 500 to 2000 ml/min from an activated charcoal dechlorination unit.

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*"Masterflex" is a trade name of the Cole-Parmer Instrument Company.

†The toxicity of copper was not ignored in the design of this experiment, but copper tubing was the only tubing available that was feasible for the project budget. Frequent testing of the water's copper content by atomic absorption spectrophotometry showed no detectable copper in any water during the experiment.

+"Vita lite" is a trade name of the Duro-Test Corporation.

Each of the three experimental and two control communities was contained in a trough constructed of Plexiglas sheet acrylic plastic, similar to those described by Cairns and Yongue (1968) and by Cairns (1969). Each trough was 79 cm long, 5 cm wide, and 7.5 cm high and held water at a depth of 4 cm. Water flowed into one end at a rate of approximately 300 ml/min and was deflected by a baffle to insure mixing.

Two "constant head boxes" were constructed to regulate the flow of water to the experimental and control communities. Each constant head box was a rectangular container of approximately 3 liters capacity. A stand pipe from the bottom of each box, all at the same level (4 cm depth), supplied water to each of the communities. This allowed individual adjustment of flow rate through each trough, without affecting flow rates in other troughs and without requiring a separate metering pump for each community.

The cold water bath consisted of an 8-meter length of 2.6-cm inside diameter plastic pipe containing the copper tube which extended 15 cm from both ends of the pipe. A countercurrent cooling design was used with the water to be cooled in the copper tube flowing in the opposite direction to the cooling water in the plastic pipe. The final temperature of the cooled water and the initial temperature of the cooling water were always nearly identical. Because the water that flowed through the cooler and to the reservoir was from the same tap, the water in the experimental and control communities and in the reservoir were always at nearly identical temperatures.

The sample area was defined as the bottom surface of each trough from the overflow endplate to a line parallel to the endplate and 20 cm upstream from it. This established an identical area of 100 square centimeters for each community. After water began to flow through the troughs, a deposit of minute sand grains and fine particles of detritus settled to the bottom of each sample area. After several days, the bottom and sides of each trough were coated with a moderate growth of diatoms, (predominately *Navicula* sp.) and green algae, (predominately *Scenedesmus* sp.). After this initial growth appeared, sampling was begun, day zero being 14 days after flow had been initiated.

Each trough was sampled once a week during the seven weeks of the experiment. Diversity (number of species) of each community and estimates of population density of six species were recorded. Each community was sampled with a heat sterilized pipette and the same volume of fluid was withdrawn for each sample which was taken so that the entire length and breadth of the sample area was included. The fluid was then mixed in the pipette and four drops were placed on a microscope slide, and the entire area below the cover slip was scanned.

Estimates of the relative density of six species were made in the following manner. If the density of a species was low, i.e., 1 to 50 cells per four drops of fluid, the number of cells in the entire sample was counted. In the case of greater densities, i.e., greater than 50 cells per 4 drops of fluid, the number of cells under a predetermined fraction of the cover slip was counted and then multiplied by the reciprocal of that fraction.

On the occasion of a taxon's first appearance, it was either identified to species or described as accurately as possible using generic names, diagrams, and notes.

Tests of the following qualities of the reservoir water were made at least once weekly: alkalinity (as $CaCO_3$), total hardness, dissolved oxygen concentration, and pH. All tests were made with the equipment and reagents in the Hach model AC-36 WR water testing kit, except for alkalinity for which the brom cresol-methyl red-phenolphthalein titration method was used. The following ranges in water quality were recorded: alkalinity — 35 to 43 ppm; total hardness — 51.3 to 68.4 ppm; pH — 7.5 to 8.1; dissolved oxygen concentration — 9 to 13 ppm.

Both the water in the reservoirs and the influent water from the charcoal filter were tested at least twice weekly for chlorine with a Hellige color comparator test kit. The only detectable chlorine (0.05 ppm) was found in the inflowing water on Day 28. The charcoal filter was immediately changed and no chlorine was then detected in the influent water.

Measurements of the flow rate and water temperature in each trough and water temperature in the reservoir were made at least every other day during the experiment. The differences in

temperatures between the warmest and coolest of the five troughs and the reservoir water never exceeded 1.5°C. No trough was observed to be consistently warmer or cooler than any of the others in the experiment.

Water delivery tubes were regularly cleaned and flow rates were adjusted regularly by either changing the position of the tubes or by using clamps. Flow rates were measured by intercepting the stream of water flowing from the delivery tube with a small container for a period of 10 sec. The water was then transferred to a graduated cylinder and rates of flow in ml/min were calculated. This method proved to be accurate to ± 6 ml/min. Because the flow rates did vary, we decided to calculate the total volume of water which passed through each trough during the course of the experiment. This would show whether or not any trough had consistently received more water containing potential colonizing species than other troughs. The total volume of water passing through each trough was calculated by multiplying the flow rate measured at the end of each time period by the appropriate number of days in the period. The results of these calculations follow:

Trough E1	26,565 liters
Trough C1	26,196 liters
Trough E2	27,911 liters
Trough C2	27,761 liters
Trough E3	27,644 liters

These calculations indicate that each community received a comparable volume of water during the course of the experiment.

A tap was placed in the system between the heater and the cooler, and the temperature increase produced by the heater was calculated by subtracting the reservoir temperature from the temperature of the water removed from this tap. Temperature increases averaged 20.6°C and ranged from 16 to 26°C. This variation was due to changes in the pumping rate of the peristaltic pumps. These pumps required frequent changes of plastic tubing; and as the tubing wore and lost resilience, pumping rate fell. It is unfortunate that this parameter of the experiment could not have been controlled more closely.

Several species were selected for quantitative analysis because they were conspicuous members of the communities and because they were found rather abundantly in all or many of the communities through the greater part of the experiment. Transient habitants of the test troughs and inconspicuous organisms would not be as suitable for quantitative analysis. We do not, however, imply that they are therefore unimportant. It should be noted that the density figures given are relative estimates and are rather subjective. In some cases, the figures given are the products of two estimated values. We feel, however, that these estimates furnish some information about the relative changes in densities among control and experimental communities.

RESULTS

Results of typical experiments utilizing the heat shock apparatus are given in Tables 1, 2, 3, and 4. Ten runs were made for each determination of acute shock temperature experiments.

The apparatus was adequate for producing abrupt heat shocks and continually observing their effects upon microbial species. The apparatus should also be useful for observing more subtle effects. Because of evaporation from the well and the loss of heat to the plastic slide, the voltage required to maintain a constant temperature will change throughout the course of the longer tests thus complicating temperature control.

The extent of microenvironmental temperature gradients within the test chamber was not evaluated because it required more sophisticated equipment than we had available. Other environmental conditions, such as dissolved oxygen concentration and pH, were also undetermined for the same reason. However, it should be noted that most of the reports of environmental conditions tolerated by microbial species contain information about the general environment rather than the microenvironment actually inhabited.

The results of the experiments on the effect of simulated passage of potential colonizing protozoans through the condenser of an electric power generating facility on downstream protozoan community development are summarized in Figures 4 through 10.

The number of species in the two control and three experimental communities throughout the course of the experiment is given in Figure 4. The estimated number of cells (or individuals) of particular species throughout the course of the experiment are shown in Figures 5 through 10.

Figures 5, 6, and 7 show a definite trend, with the control communities usually establishing earlier or to higher densities, or remaining at high densities longer than the experimental communities. In the case of the *Cyclidium* sp. (Fig. 5), the two control curves show greater diversity, earlier establishment, and greater peak population densities than the populations in the experi-

mental troughs. The two control curves for *Aspidisca lynceus* (Fig. 6), also show earlier establishment and greater densities than two of the populations in the experimental troughs which fluctuate between zero and one cell throughout most of the experiment. The curves for *Acanthamoeba* sp. show higher peak densities in the control communities than in the experimental communities (Fig. 7).

The next two figures — Fig. 8, *Vorticella campanula*, and Fig. 9, *Chlamydomyxa montana* (?) — show no pronounced difference between control and experimental populations. Figure 10 — *Pseudodifflugia gracilis* (?) — shows that the experimental populations were larger and appeared earlier than the control populations.

There is no apparent difference in the number of species between experimental and control troughs. However, the experiments were run only once so it is quite possible that a number of replications might reveal subtle differences between experimental and control troughs. In addition, since each recorded species in a sample may be represented by only one or up to thousands of individuals, a detailed quantitative examination might also reveal differences between experimental and control troughs. Unfortunately, the attempts made to gather quantitative information for a few species indicate that adequate quantitative data gathering for all species present would be beyond the scope of this project since we would first have to establish normal variation in quantity of a number of species in a complex community — a difficult task indeed.

It is also possible that there might be substantial qualitative differences between two protozoan communities each with the same or similar numbers of species. However, much more information than is currently available about particular species would be necessary before one could say that one of these communities was superior to, or more "natural" than, the other.

The results of the population density estimates for selected species suggest that some species may have lowered density as a result of simulated passage of upstream water through the condenser of a steam electric power generating plant while in one case the reverse was true. At best these preliminary results indicate that this is an area of complex response patterns which will require intensive investigation before definitive results are available.

PART II
ALGAL STUDIES

ALGAL STUDIES

Research effort relating to the exposure of aquatic microorganisms to artificial temperature increases has been, in certain instances, concerned with the effects on components of the primary producer level of food chains. The critical ecological significance of such autotrophic organisms is well known; diatoms are commonly found as important community components of the periphyton and phytoplankton of aquatic ecosystems.

These experiments involved both autecological and synecological investigations on diatoms subjected to defined abrupt temperature increases under controlled laboratory conditions. The effects of temperature increases on viable diatom populations and communities isolated from other stress factors, e.g., chlorine, heavy metals, turbulence, pressure, etc., commonly encountered at electric power generating facilities were thus ascertained. Experimental design permitted the use of large numbers of physiologically similar cells at various ambient temperatures in two general approaches to assess effects. First, a new technique utilizing fluorescent patterns of diatom cells was developed and initially tested. The procedure involved the establishment of "normal" patterns of autofluorescence of cellular chlorophyll and accessory carotenoids and the inducement of lipid fluorescence with 3,4 Benzpyrene-Caffeine fluorochrome. Deviations from such established "normal" patterns could thus be used to measure major physio-morphological alterations following temperature stress. The technique also provides early detection of sublethal cellular alterations prior to severe disruptions of cellular structure, i.e., as the cell approaches the upper limits of temperature tolerance but prior to total damage encountered beyond such limits. Investigation of the effects of temperature shocks on diatom populations and communities already under multiple stress due to suboptimal culture (nutrient deficiency, aging, density, etc.) was also carried out. Secondly, quantitative estimates of the reproduction of diatom populations subjected to some of the same temperature increases were obtained.

In addition, a biological model was selected for the study of certain effects of defined temperature increases on algal-chytridiaceous fungal relationships under controlled conditions in the laboratory.

MATERIALS AND METHODS

A series of test experiments was conducted prior to the beginning of any studies in order to establish and develop application methodology for the temperature stresses, handling procedures, and microscopic techniques used in the diatom investigations. Autecological investigations of diatoms consisted of a total of 14 additional experiments covering various stress applications to populations with ambient temperatures of 18, 23, and 29°C.

Several different fluorochromes for various cellular components were considered (Neutral Red, Phosphine 3R, 3,4 Benzpyrene-Caffeine, Acriflavine hydrochloride, etc.) with emphasis upon cellular lipids due to their susceptibility to temperature alteration.

Since characterization of lipid in unstained materials is often inconclusive, it was decided to use a lipid fluorochrome. Initial laboratory trials indicated that maximum fluorescence intensity was achieved with 3,4 Benzpyrene-Caffeine (Berg, 1951), a non-differential lipid fluorochrome.

Autecological Studies

All diatom populations were cultured in synthetic water media as described by Cairns (1968). All media were prepared and used within 48 hours. The pH was adjusted to 7.2 with 0.5 N HCl and 0.5 NaOH prior to sterilization in an autoclave. Soil extract used in the media was prepared as described by Barr and Hickman (1967). The same soil batch was used to prepare all extracts for these experiments. All culture flasks used were Pyrex 250 ml Erlenmeyer No. 4980 to minimize differences in glass thickness and subsequently the heat retention of flask walls during periods of thermal shock. All glassware was cleaned by the method of Chu (1943). Test flasks were filled with 50 ml of media (to provide good surface to volume ratio for gas exchange), cotton stoppered, and autoclaved.

Inoculum

Initially the test organisms used were unialgal cultures of *Navicula seminulum* var. *Hustedtii* Patr., a moderately sensitive freshwater

diatom common to many unpolluted streams. All cultured populations, including stocks used for inoculum, were maintained in Shirer Constant Environment Rooms at controlled temperatures with accuracy to ± 1 °C around each ambient temperature. Constant agitation and aeration was provided by Burrel wrist-action shakers and photoperiod of 16 hours light at an intensity of approximately 2690 to 3228 lux at the culture flask surface was provided by two General Electric daylight fluorescent lights. During the remainder of each 24-hour period, there was no measurable illumination. Prior to any use in the preparation of inocula, stock populations were maintained in the laboratory for several months with weekly transfers to assure an active division rate characteristic of the species during periods of rapid growth.

Population cell lines at each of three ambient temperatures, 18, 23, and 29°C, within and spanning the normal optimum growth range of the species were later established. Continuous culture with weekly transfers for a period exceeding five consecutive weeks at each temperature provided actively dividing diatom populations for use as inoculum.

Replicate populations were prepared for various temperature exposures in each experiment by inoculating each flask with 1 ml of diatoms in uniform suspension to insure uniform numbers. A Waring blender equipped with a sterilized mini-container (110 ml) was used to insure uniform suspensions of diatoms at a relatively constant temperature. Since blending diatom suspensions generates considerable heat, temperature increases were controlled during this process by packing the exterior base reservoir of the blender mini-container with crushed ice and blending the diatoms for exactly two minutes. Previous tests using this technique demonstrated temperature control at ambient ± 2°C during blending. This procedure was adhered to throughout all phases of these experiments. Each flask was inoculated with 1 ml of a uniform diatom suspension. At the time of inoculation, a cell count was made using the ASTM technique developed by Patrick (1968) to determine the number of diatoms per microscopic field introduced into each culture. A total of 60 fields was counted and statistically analyzed. Since both the cell age and density affect the number of divisions occurring, all inocula used were composed of cells harvested on the seventh day of growth, adjusted to similar starting densities (mean number of cells per microscopic field).

Cell counts consisting of 100 fields per population were made at the end of each 7-day experiment and statistically analyzed to estimate the amount of cell division which had occurred in all populations.

Autecological investigations involved duplicate experiments with replicate populations at each of three ambient temperatures, 18, 23, and 29°C. Experimental design permitted simultaneous inoculation of all populations used in exposure to Stresses 1, 2, and 3* on Day 3 for use in evaluating immediate and delayed effects. Immediate effects of Stress 1 applied on Day 7 to the 18, 23, and 29°C populations were also measured in separate experiments. Stress 4 was applied in experiments utilizing 29°C populations only.

Temperature Stress

Temperature stress was applied to normal diatom populations in four general categories designed for maximum simplicity and reproducibility. The categories represent certain types of defined abrupt temperature shocks to which aquatic microorganisms might be subjected due to the presence of electric power generating facilities on aquatic ecosystems. The technique used allowed the exposure of intact, actively growing populations at several ambient temperatures within the optimum growth range of the test organism. Temperature stress categories utilized were defined as follows: (1) Stress 1, increases of approximately 10 to 12°C in less than 20 seconds with a return to approximately ambient in less than 60 seconds; (2) Stress 2, increases of approximately 7°C in less than 1 hour with maintenance at this increase for the duration of the growth period, approximately 96 hours; (3) Stress 3, a combination of Stresses 1 and 2; and (4) Stress 4, increases of approximately 4°C in less than 1 hour with maintenance at this increase for the duration of the growth period, approximately 96 hours.

General simulation of certain types of temperature shocks which could possibly result from entrainment through steam condenser cooling lines and overall downstream addition of thermal effluents

*A description of Stresses 1, 2, 3, and 4 follows later.

were thus attained. All experiments were run for 7 days with stress application within this period. The experimental design measured both immediate and delayed effects of stress on replicate populations with stress application on either Day 7 or Day 3, respectively. Since cultured diatom populations may exhibit a lag phase up to 48 hours, stress application between Days 3 and 7 assured exposure of actively growing replicate cell lines in the exponential growth phase. Also, the third to the seventh day is the time when the growth rate can be most accurately correlated with the effects of the test medium, thus assuring maximum numbers of physiologically similar cells. Temperature stress was applied in two basic ways selected for their simplicity and control. The first, Stress 1, involved flasks fitted with sterile thermometer-equipped stoppers for visual temperature monitoring. Stress 1 was effected by immersing the flask containing an intact diatom population into a 100°C water bath for exactly 8 seconds. Removal of the flask allowed a continued temperature rise due to the heat retention of the glass until 9 to 10°C above ambient was attained. The flask was immediately immersed into a cold water bath at 5°C for 20 seconds and removed. The temperature continued to rise 1 to 2°C during this immersion and then leveled off beginning the descent to approximately ambient which occurred in less than 60 seconds. Stress 2 was attained by simply transferring intact diatom populations to a second environmental growth chamber maintained at 7°C above the ambient temperature under test. Such a transfer permitted the temperature of the intact population to reach the 7°C increase in less than 1 hour. Stress 3 involved exposure of populations to a combination of both Stresses 1 and 2, just described above. The last category, Stress 4, was added to provide information relative to the 29°C ambient populations (upper growth range) only. It became evident in the preceding experiments that these populations experienced severe damage following Stress 2. As a result, experiments involving a lower but equally abrupt temperature stress, Stress 4, were carried out. The purpose of these experiments was to measure any sublethal transitional effects occurring prior to severe destruction of 29°C populations following Stress 2. Stress 4 was effected by transferring 29°C populations to another environmental growth chamber maintained at 4°C above ambient.

In addition, experiments were conducted on older, less viable diatom populations under prolonged culture at 20°C in the same

media, i.e., 14 to 30 days. These studies involved two organisms, *Navicula seminulum* var. *Hustedtii* Pat. and *N. pelliculosa* (Breb.) Hilse. Fogg (1959) suggested in discussing laboratory studies of diatoms and other algae that fat accumulated in nitrogen deficient cultures. Prolonged culture in the same media with high density populations was carried out here to produce large numbers of diatoms under multiple stress, including nutrient deficiency, crowding, etc. These diatom populations were investigated as to (1) changes in their "normal" fluorescent patterns due in part to increased fat resulting from such stress, and (2) changes following additional stress from temperature shocks. Temperature stresses in these experiments were administered in controlled temperature water baths as increases above ambient of (1) 60°C for 2 hours, (2) 20°C for 24 hours, and (3) 10°C for 24 hours. The extremely abrupt temperature shock of 60°C for 2 hours was designed to induce extreme cellular alterations as a reference point in appraising lower, more realistic temperature shocks. The effects of the various stress categories were examined relative to immediate and delayed responses of diatom populations. Stress 1 was applied on Days 2 and 7 in different experiments. Stresses 2, 3, and 4 were always applied on Day 3. All population sampling for examination of effects was done on Day 7.

Synecological Studies

Collection and Culture

Synecological studies were conducted to assure positive correlations between cellular fluorescence patterns of laboratory populations and dominant species components of mixed natural field communities. Various different ponds and streams were initially sampled for suitable test material. However, all community collections used in test cultures in the described experiments were collected in the New River in Giles County, Virginia. Since diatoms are usually a dominant group in the fall, spring, and winter, samples were taken from diatometers (Patrick, Hohn, and Wallace, 1954) at appropriate times in the winter and spring months at various existing ambient temperatures. Diatometer slides were placed in the river to permit colonization by existing species for 7 to 21 day periods at the same collection site for use in various experiments. Once colonized, all collections were returned to the laboratory immediately and prepared for

flow and static culture maintenance. Water chemistry consisting of dissolved oxygen, alkalinity (as $CaCO_3$), total hardness, carbon dioxide, pH, and temperature were taken at collection time using a Hach model AC-36-WR water testing kit. While such water chemistry does not provide definitive evidence, it supplies general information about the environmental conditions under which the diatoms were collected.

Continuous flow culture provided a reliable means of in-laboratory maintenance of intact diatom communities on the original field collection substrate. This permitted a study of the normal fluorescent patterns of dominant community components. Such patterns could then be compared to those seen in populations under normal autecological culture. In addition some limited studies of the immediate effects of one type of short-term defined temperature shock could be evaluated. The continuous flow culture system consisted of maintaining colonized slides exposed to a continuous flow of water of the same general physio-chemical characteristics of the collection site. Communities were kept in artificial Plexiglas streams in the laboratory ecosystem described previously in detail in the protozoan studies. The reservoir tank of the continuous flow laboratory ecosystem contained circulating dechlorinated New River water. Additional water chemistry was carried out in the laboratory ecosystem and compared to the field site on each collection day to confirm physio-chemical similarity. Flow rates and temperatures from each culture trough in use were checked daily and recorded.

Static cultures were designed to expose natural diatom communities to conditions generally similar to the prolonged, high density cultures previously described in the autecological studies. The general effects of temperature shocks as an added stress on these communities relative to light and ultraviolet microscopic surveys could, thus, be compared to those seen in autecological studies.

Static cultures were prepared as follows. The contents of diatometer slides were scraped into water in which they were collected, passed quickly through nylon bolting cloth to remove extraneous material, and poured into 16 1/2 x 10 1/8 x 2 1/4-in. enamel pans filled with water from the collection site. Static cultures were stored in Shirer Environmental Chambers to acclimate communities to the laboratory ambient temperature of

20 ± 1°C. A photoperiod of 16 hours light (at an intensity of 2690 lux at the surface of the culture) and 8 hours with no measurable illumination per 24 hours was also maintained. Cultures were maintained for a maximum of 7 days; older cultures were discarded.

Temperature Stress

Numerous fluorescent surveys on dominant community components consisting of normal diatom communities and those from suboptimal cultures were carried out to establish characteristic cellular patterns prior to their use in any experiments. Normal diatom communities examined came from two sources: (1) samples collected from the river site and immediately returned to the laboratory and placed on test and (2) samples maintained in the laboratory under continuous flow culture.

Because of the difficulty of simulating temperature shocks of extended duration under continuous flow conditions, only abrupt, extremely short-term types could be effected. The purpose of these limited studies was to try to detect immediate and short-term delayed effects. Long-term effects were not considered. Here, immediate temperature increases of 16 to 18°C for less than 30 seconds with immediate return to ambient were accomplished as follows. Diatometer slides (16 total) seeded with a 7-day-old community were collected and transferred to the laboratory. In the laboratory, the slide communities were maintained under continuous flow as previously described for an additional 7 days for general acclimation prior to testing. The temperatures at the river collecting site and the laboratory ecosystem were identical, i.e., 22°C. Two troughs served as controls while another two were exposed to increased temperature. Temperature stress was applied by pumping heated water from the artificial ecosystem's head tank into the two experimental area troughs until the temperature stabilized at 18 to 21°C. Four slides from the holding trough at 22 to 23°C were quickly transferred to each of the experimental troughs and left for 25 seconds. The slides were then quickly returned to the control trough and continued in culture at ambient. Four control slides were handled similarly by transfer from troughs to 22 to 23°C to different troughs at the same temperature. Samples consisting of two slides were taken from each trough at 1 and 24 hours following shock and examined for

immediate and delayed effects, respectively. Community components were scraped from slides into beakers of distilled water and wet preparations were made from the beaker contents as needed for microscopic surveys. The surveys consisted of examining 1000 diatoms at random and noting the general cellular patterns of the majority of community components. The remainder of the beaker contents was preserved in 4% formalin for later identification of dominant community components.

Static community cultures were exposed to the same temperature stresses described previously for prolonged culture, high density population studies. Test cultures were prepared by taking 50 ml aliquots of diatom suspensions from stock pan cultures and dispensing them into 250 ml cotton stoppered Erlenmeyer test flasks on the day the experiment commenced. Counts were done in a Sedwick Rafter Chamber to estimate the number of diatoms per milliliter in the inoculum. Replicate communities were run in each treatment group. Experimental communities growing at 20°C were placed in water baths at various elevated temperatures for the indicated time periods. Control communities were also kept in water baths at ambient temperatures during experimental periods. At the conclusion of the temperature stress periods, each culture was sampled and examined. Surveys were carried out in the same fashion described for the continuous flow communities.

Examination Criteria

A total of 36 experiments was conducted to determine the feasibility of utilizing the following criteria in measuring changes in diatoms resulting from exposure to defined abrupt temperature shocks: (1) General surveys carried out under the light microscope to note major changes in total cellular content, pigmentation, and general structure; (2) Autofluorescence surveys to note major physio-morphological alterations in the cell metalloporphyrin content (chlorophyll a + c) as expressed by chloroplast content and ultraviolet fluorescence. Thermal damage to the labile carotenoid accessory pigments, characteristic of the Chrysophyta, would also undoubtedly be manifested through the cell's metalloporphyrin fluorescence pattern; (3) Induced fluorescence surveys on total visible cellular lipid patterns using 3,4 Benzpyrene-Caffiene lipid fluorochrome; and (4) The ability of diatoms to survive and reproduce (autecological studies only).

Fluorochroming and Fluorescent Surveys

All diatom samples were taken from a uniform suspension immediately following mixing in a Waring mini-container blender packed with ice as described previously. Cells were concentrated by centrifugation at approximately 3100 rpm in 15 ml conical centrifuge tubes for 2 minutes. After removal of the supernatant media, cells were fluorochromed by the method of Berg (1951) with 3,4 Benzpyrene complexed to Caffeine in aqueous solution. Cells remained in the fluorochrome for 20 minutes followed by reconcentration by centrifugation after which supernatant fluorochrome was removed. Cells were given three successive rinses by dilution through resuspension in sterile distilled water. After reconcentration by centrifugation, cells were immediately examined to assure maximum fluorescence. Samples were also prepared lacking the fluorochrome to serve as background fluorescence controls. Wet preparations were made on standard 3 x 1 in. microscope slides rinsed in distilled water with 24 x 50 mm cover slips and scanned with a random recording of fluorescence patterns. A total of 1000 cells from an aliquot representing approximately one third of the total culture population or community were examined.

In autecological experiments involving normal *N. seminulum* cells (Experiments 1 through 14), results were recorded as to the specific percent displaying altered fluorescent patterns. In all synecological and suboptimal culture studies, the major fluorescent trends noted in 1000-cell surveys were recorded. Altered cells represented any readily visible deviation from the established normal patterns of autofluorescence and induced fluorescence. These were described accordingly in different shock categories.

Ultraviolet microscopic surveys were conducted on a Leitz Ortholux microscope equipped with an ultraviolet light source from an HBO 200 mercury lamp with BG 38 and UG 1 filters. Additional surveys on chlorophyll autofluorescence were also conducted with BG 38 and BG 12 filters.

Studies of the effects of defined temperature shocks on the algal-chytridiaceous fungal relationship were approached as follows.

Numerous collections from several natural habitats in Montgomery and Giles Counties, Virginia, were conducted in an attempt to locate algae naturally infested with chytridaceous fungi. One such chytrid, *Chytridium hemicysta*, was isolated into unifungal culture for use as a laboratory inoculum source. Unialgal cultures of the Chlorophyta, *Netrium digitus*, were also obtained and subcultured in the laboratory on standard media until an actively growing cell line was preadapted to an ambient temperature of 23°C.

Experiments were conducted by preparing replicate cultures of *Netrium digitus* of fixed volume and known cell density and exposing them to infestation by viable zoospores of *Chytridium hemicysta*. These combined populations of algae and chytrids were then subjected to the standard temperature stress regimen described above in the diatom studies. The algal test inoculum was adjusted so as to provide both live and expired *Netrium* sp. cells on the test commencement day. The purpose of expired cells was to provide suitable host material for infestation by viable zoospores in order to estimate the effects of temperature shocks on the chytrid alone. Live cells of *Netrium* sp. were added to provide an estimate of the effects of the various temperature shocks on the algae alone (i.e., morphology and survival of live cells). Cells were considered live if they appeared visually normal (i.e., intact membrane structures, chloroplast, etc.). All counts were done utilizing a hanging drop depression slide under low power. Temperature stress was applied shortly after the inoculation of all culture populations of algae and chytrids on Day 1. On Day 3 all cultures in each experiment were sampled and counts were made of the number of *Netrium* sp. cells clearly demonstrating infestation by *Chytridium* sp. These were then recorded as a percent of the total cells observed. On Day 5, terminal counts of the live *Netrium* sp. cells were carried out on all culture populations and recorded as the average number of cells per milliliter.

RESULTS

All results of the diatom experiments are based on the application of the previously described examination criteria on autecological and synecological studies. There are several difficulties in identifying and evaluating algal cellular physiological alterations prior

to or after cell expiration utilizing light microscopic surveys. Severe, consistent morphological damage afflicting the majority of species is usually accompanied by cell expiration and offers limited assistance in detecting sublethal cellular abberations. Since only certain species of diatoms exhibit motility, movement as a criterion for cellular damage is generally inadequate. Reproduction, as a manifestation of growth, and primary productivity have been used with some success in partial evaluations of various pollutional stresses.

Light microscopic examinations to provide reference data on control diatoms from regular autecological and continuous flow synecological culture revealed similar general cellular characteristics. Normal cellular content, pigmentation, and intact structure prevailed in the majority of diatoms. Light microscopic appraisals of control suboptimal cultures, i.e., prolonged high density autecological and static synecological cultures, revealed certain general differences. Cellular content and pigmentation were somewhat reduced in many dominant community components of the control static synecological cultures. Autecological cultures, while displaying little visible decrease in total content and pigmentation, did appear more opaque with many cells displaying deformed structures usually manifested by flattened or blunt cell ends.

Attempts were made here to develop and evaluate the use of new criteria, i.e., total cellular fluorescent patterns with emphasis on lipids and metalloporphyrins, as a measurement of lethal and sublethal cellular alterations following stress. Hopefully, the data generated here can provide insight as to major physio-morphological alterations resulting from the exposure of diatoms to abrupt thermal stress.

Initial ultraviolet microscopic surveys on control diatoms established normal cellular fluorescent patterns under various culture conditions for reference purposes. Comparison of control cells from regular autecological and continuous flow synecological culture revealed great similarity. Control diatoms were consistently dominated by a characteristic red autofluorescence of the cell chloroplast. Accompanying this was limited faint blue 3,4 Benzpyrene-Caffeine induced lipid fluroescence. The majority of lipid fluorescence surrounded the chloroplast in close proximity to

cellular poriferal membranes and represented a minute percentage of the total cellular fluorescence. Additional blue to blue-white lipid as a "grainy" scattered pattern within the red chloroplast tended to impart a red to red-pink (blue-white + red) fluorescence to the chloroplast. The apparent lack of visible cell lipid after fluorochroming is in agreement with normal log phase physiology; i.e., cells in the exponential growth phase possess high quantities of protein, chlorophyll, and nucleic acids and low quantities of carbohydrate and fat. In other studies *Navicula pelliculosa*, following photosynthesis for 2 minutes in the presence of C^{14}-labeled bicarbonate, demonstrated less than 20% entering the fat fraction (Fogg, 1966). While control cells cultured at 18, 23, and 29°C were generally similar in gross fluorescent characteristics, 29°C diatoms indicated a possible slight decrease in chloroplast content.

Surveys of control cells from populations under suboptimal culture, i.e., prolonged, high density autecological and static synecological culture, also revealed greatly similar fluorescent patterns when compared. In addition, major deviations from the patterns just described for cells under more optimum culture were clearly evident. Marked increases in visible cellular lipid fluorescence were seen in the vast majority of diatoms observed. One noticeable consistent exception was *Diatoma vulgare* Bory. var. *Breve* Grun which often lacked such visible increase in induced lipid fluorescence. In most other species, however, high concentrations as blue-white spherical deposits on either side of the visible central chloroplast were characteristically present. The increased lipid probably can be attributed to cell degeneration resulting in increased lipid metabolism as described by Fogg (1966).

Autecological Studies

Experiments 1 and 2 (Table V) involved the exposure of replicate diatom populations at an ambient temperature of 18°C to Stresses 1, 2, and 3 on Day 3 of the growth period. Light microscopic surveys demonstrated no consistent differences between any control or experimental populations with reference to cellular content, pigmentation, and general cellular structure. Ultraviolet fluorescence microscopic surveys also failed to demonstrate any consistent differences between control and experimental popu-

lations. The vast majority of cells displayed patterns characteristic of viable log growth phase diatoms. Of the very few altered cells seen in all populations, the majority possessed increased lipid. A great many of these could be the original cells introduced as inoculum at the beginning of the growth period. Reproduction data relative to all replicate populations in Experiments 1 and 2 (Figs. 11 and 12) indicated no consistent decreases resulting from exposure to Stresses 1, 2, and 3. Reproduction was expressed as an estimate of the number of divisions occurring in the 7-day growth period. However, the possibility of some irregular growth stimulation in certain populations following Stress 2 was evident. This is probably best explained by the fact that the populations were moved closer to their optimum growth temperature by the 7°C increase without any noticeable effects of the abruptness of the increase (less than 1 hour). Patrick (1969) has demonstrated similar stimulation with gradual temperature increases on components of glass slide diatom communities in controlled greenhouse studies. Here, increasing temperatures over the naturally occurring temperature resulted in biomass increases until temperatures of 29 to 30°C were attained.

Experiments 3 and 4 (Table VI) were designed to investigate the immediate effects of Stress 1 on Day 7 of the growth period relative to cell physio-morphology. Both light and ultraviolet miscroscope surveys failed to indicate any differences between control and experimental populations following stress.

Populations midway in the growth range at an ambient temperature of 23°C were investigated following Stresses 1, 2, and 3 on Day 3 in Experiments 5 and 6. All populations examined did not contain any consistent differences under all microscopic surveys. Table VII summarizes data from the fluorescent surveys and displays extremely small numbers of cells with physio-morphological alterations. The few cells with deviated fluorescent patterns were like those described for 18°C populations.

Population growth data for 23°C ambient diatoms is summarized in Figures 13 and 14. Here, similar estimates of reproduction expressed as number of divisions can be seen between all populations following all stresses. Experiments 7 and 8 (Table VIII) involved a study of the immediate effects on 23°C populations exposed to Stress 1 on Day 7. No significant

differences in visibly altered diatoms relative to fluorescent surveys were noted.

Experiments 9 and 10 involved studies of diatoms acclimated near the upper threshold of the optimum growth range of the species, i.e., 29°C. Here (Table IX) exposure of populations of *N. seminulum* to Stress 1 produced no significant differences, immediate or delayed, in the total percent of altered cells seen in all microscopic surveys. Only normal numbers of cells with altered fluorescent patterns were noted. Exposure to Stresses 2 and 3, however, produced a marked effect on all diatom populations tested. The vast majority of stressed cells viewed had major cellular alterations. Light microscopic comparisons of experimental and control populations revealed reductions in cellular content and the appearance of internal opaque areas in many experimental cells. The pigmentation of the majority of stressed cells was characterized by a green coloration as opposed to the yellow-brown coloration characteristic of normal diatoms. This striking brown to green color change undoubtedly was the result of temperature induced destruction of the labile carotenoids characteristic of diatoms as a group.

Fluorescent surveys aided in better defining the physio-morphological damage seen under the light microscope. Stressed cells revealed various lipid fluorescent patterns ranging from increases like those seen in the few altered cells of 18 and 23°C populations, to decreases causing cells to be barely visible as faint blue "ghosts." Autofluorescence surveys of chlorophyll in cells following Stresses 2 and 3 also demonstrated marked differences from control diatoms. A total lack of the characteristic red fluorescence was seen, indicating degradation of the cellular chlorophyll content in most of the populations.

Experiments 11 and 12 (Table X) involved exposing 29°C populations to Stress 1 on Day 7 to measure any immediate effects. Light and ultraviolet microscopic surveys failed to detect increased numbers of cellular alterations following the standard criteria.

Growth studies of populations acclimated to an ambient temperature of 29°C are summarized in Figures 15 and 16. Exposure of diatoms to all categories of stress produced negative effects upon

population growth. Stress 1 alone, which had no measurable effect upon the majority of diatoms acclimated at 18 and 23°C, caused a decrease in division in most upper threshold populations as shown in data estimating final population numbers. Here, an extremely abrupt temperature shock of very short duration, i.e., 10 to 12°C increases above ambient in less than 20 seconds with a return to ambient in less than 60 seconds, produced results of possible considerable ecological significance. Temperature shocks of this general type alone and accompanied by other shocks can be anticipated in new high volume cooling systems of power generating facilities. Stresses 2 and 3 also produced population reductions, but of a more severe nature than Stress 1 alone. Stress 2 alone and Stress 3 (Stresses 1 + 2) produced the same general results in decreased divisions. The overall results of upper threshold populations exposed to abrupt temperature stress can be summarized as follows:

(1) A general decrease in the number of divisions occurring in the normal growth period resulting in a lower number of individuals following all stresses and

(2) Severe physio-morphological damage to the diatoms produced during decreased division following Stresses 2 and 3.

The dramatic shift in total cellular fluorescent patterns in 29°C populations following Stresses 2 and 3 prompted further experiments. These were designed to demonstrate the possible existence of a transitional phase of stress induced physio-morphological alterations occurring at a lower abrupt temperature increase. Stress 4 was designed to fill this need and provided a 4°C increase in less than 1 hour.

Experiments 13 and 14 (Table XI) involved exposing 29°C populations to Stress 4 alone on Day 3. Light microscopic surveys indicated no general decreases in total cell content or consistent, easily noted structural changes. However, opaque areas and some reduction in the intensity of pigmentation predominated in many of the cells examined. Interestingly, general total cellular fluorescent patterns of the vast majority of these stressed cells were like those seen and described for diatoms stressed in suboptimal culture prior to any temperature shock. Markedly increased lipid

fluorescence (3,4 Benzpyrene-Caffeine induced) accompanied by decreased visible red chlorophyll autofluorescence was the common pattern.

The results of Experiments 13 and 14 indicate a transitional or intermediate pattern in total cellular fluorescence lying between diatoms growing in log phase at 29°C with no stress and those receiving severe damage following Stress 2 or 3. As noted previously, the vast majority of cells growing at 29°C possess a dominance of red chlorophyll autofluorescence with little, faint blue induced lipid (3,4 Benzpyrene-Caffeine) fluorescence. Following Stress 4 (abrupt 4°C increase), a change in pattern to decreased red chlorophyll autofluorescence and increased blue to blue-white induced lipid fluorescence occurs. A greater total increase, i.e., Stress 2 (7°C increase) or Stress 3 (Stress 1 + Stress 2), results in a general decrease in total cellular fluorescence, both red chlorophyll autofluorescence and blue-white induced lipid fluorescence.

Figures 5 and 6 summarize growth studies of 29°C acclimated populations following Stress 4. Here, a lower number of divisions was noted at the end of the growth period.

The differences of up to approximately one division evident between certain control populations in separate experiments at 18°C (Experiments 1 and 2) and 29°C (Experiments 9, 10, 13, and 14) were found to be normal for the culture system employed. Many factors inherent in the technique may be considered in explaining such differences as the variability in the duration of the lag phase or slightly irregular division due to the close proximity of the ambient temperatures to both extremes of the growth range.

Experiments 15 through 24 were designed to test different temperature shocks upon diatom populations already revealing stress resulting from suboptimal culture. Cells from prolonged high density autecological culture at 20°C bearing increased lipid content were studied for major deviations from normal patterns. Experiments 15 and 16 involved exposures of *N. pelliculosa* to 60°C increases for 2 hours followed by immediate microscopic surveys. Experiments 17 and 18 involved the same treatment with *N. seminulum*. Light microscopic examinations of all populations

revealed severe cellular damage in most cases with structural alterations of cell contents (coagulation) and destruction of normal pigmentation patterns, i.e., the characteristic brown-yellow color of the cells was changed to green. Ultraviolet microscopic examinations indicated that a large part of the internal cellular damage was manifested by a decrease in visible cellular lipid and subsequent induced fluorescence. Varying degrees of decreased red chlorophyll autofluorescence were also seen. Similar results were seen in Experiments 19 through 22 where 20°C prestressed populations of *N. seminulum* (Experiments 19 and 20) and *N. pelliculosa* (Experiments 21 and 22) were exposed to increases of 20°C (to 40°C) for 24 hours. The major visible difference between the two shocks under the ultraviolet microscope was more variable damage in Experiments 19 through 22 than in Experiments 15 through 18.

Generally, a range in degrees of fluorescence from typical control patterns to an almost complete lack of fluorescence was characteristic of cells stressed in Experiments 19 through 22 while more severe, consistent decreased fluorescence was noted in Experiments 15 through 18. Populations exposed to temperature shocks of 10°C above ambient for 24 hours were also observed in Experiments 23 and 24 using populations of *N. seminulum*. No consistent major differences between controls and experimentals were noted in these surveys.

Synecological Studies

Experiments 25 and 26 were carried out on communities collected in the field at 20°C and immediately returned to the laboratory for studies of normal fluorescent patterns and those occurring after temperature stress. Table XII lists the dominant species characterizing their communities. Control cell patterns under the light and ultraviolet microscopes were like those described for the normal diatoms from autecological culture. Here, exposure to increases of 20°C for 24 hours produced overall decreases in total cellular fluorescence also similar to those noted in the autecological studies.

Dominant components of diatom communities in continuous flow culture experiments are listed in Table XIII. In these limited investigations emphasis was directed towards establishing normal

cellular patterns for comparison to laboratory cultured autecological diatoms. Some effort went towards a partial appraisal of one type of temperature stress. Experiments 27 and 28 involved the exposure of communities growing at 22 to 24°C to abrupt temperature elevations of 16 to 18°C for 30 seconds. Dominant species were surveyed immediately after shock for visible effects upon cellular patterns only. No investigations of reproductive or delayed physio-morphological effects manifesting beyond the short 24-hour study period were carried out. General comparisons were made to the effects of Stress 1 on Day 7 as applied to *N. seminulum* in autecological culture at 23°C. Examinations of 1000 cells selected randomly of the various species in these communities like those in the *N. seminulum* studies did not reveal significant differences between control and stressed groups. It should be noted that examinations to characterize the structure of the communities used in these experiments indicated a predominance of *Gomphonema* sp., certain species of which are known to be broadly temperature tolerant (Wallace, 1955; Patrick, 1969).

Experiments 29 through 36 involved evaluating the effects of temperature stresses on static synecological cultures collected during the winter and early spring at various ambient temperatures. Stresses were the same as those previously described for suboptimal autecological studies. As in previous efforts, all experiments were repeated for confirmation at different times with similar communities from the same collection. Dominant components characterizing the community structure of cultures used in Experiments 29 through 34 are listed in Table XIV. Experiments 29 and 30, 31 and 32, and 33 and 34 received increases of 60°C for 2 hours and 20 and 10°C increases for 24 hours, respectively. Immediate microscopic surveys revealed results generally similar to those discussed for the suboptimal autecological studies with the exception of the 10°C increase. The majority of dominant community components receiving 60 and 20°C stresses displayed severe cellular damage at the conclusion of the stress period. A degradation of cellular content and pigmentation resulting in the brown-yellow to green color transformation previously noted along with a general overall decrease of cellular lipid and chlorophyll fluorescence were evident. Communities subjected to the 10°C increase, however, had a general decrease in cellular fluorescence which was not evident in the autecological

studies. This may be due to the fact that the communities were originally collected at an ambient temperature of 9 to 10°C prior to pretest laboratory acclimation at 20°C. While acclimation in the laboratory at a 10°C increase above the natural site ambient could be tolerated by the dominant community components, the additional test increase of 10°C was undoubtedly in excess of their upper threshold. This view is supported by later studies, Experiments 35 and 36, using communities (Table XV) collected at the same site at an ambient temperature of 15 to 16°C and acclimated to 20°C prior to testing. Here, temperature stresses of 10°C for 24 hours produced results similar to those seen in autecological studies, i.e., no consistent significant differences were noted.

The results of defined temperature stress on the algal-chytridiaceous fungal relationship are summarized in Tables XVI through XIX. No significant difference in the ability of *Chytridium hemicysta* to infest expired *Netrium* sp. cells were seen in these data when populations exposed to Stress 1 alone were evaluated. Exposure to Stress 2, alone or in combination with Stress 1, demonstrated decreased infestation of *Netrium* sp. cells by *Chytridium hemicysta* in all observed populations. Live cell counts of *Netrium digitus* at the termination of each experiment provided data with reference to the effect of each stress category on cell condition and survival. Exposure of populations to Stress 1 alone, Stress 2 alone, and Stress 3 produced various degrees of cellular damage and a suppression of division resulting in a general decrease in the mean number of live *Netrium* sp. seen at termination time.

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FIGURES

FIGURE 1

SCHEMATIC OF TEMPERATURE SHOCK TEST CELL APPARATUS.

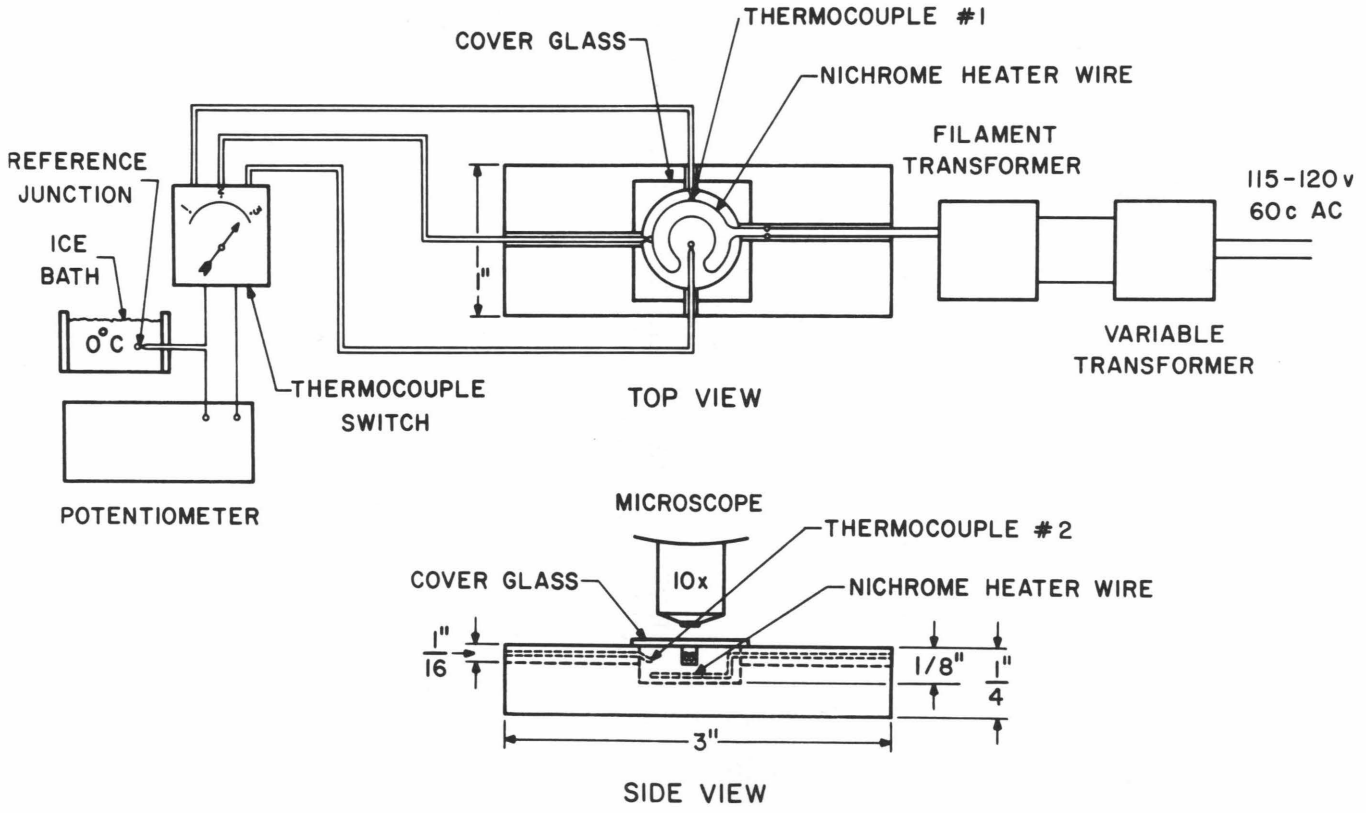

COVER GLASS
THERMOCOUPLE #1
NICHROME HEATER WIRE
FILAMENT TRANSFORMER
115-120v 60c AC
REFERENCE JUNCTION
ICE BATH
0°C
1"
VARIABLE TRANSFORMER
THERMOCOUPLE SWITCH
TOP VIEW
POTENTIOMETER
MICROSCOPE
THERMOCOUPLE #2
COVER GLASS
10x
NICHROME HEATER WIRE
1" 16
1/8"
1" 4
3"
SIDE VIEW

FIGURE 2

HEATING CHARACTERISTICS OF THE CELL AT THE MAXIMUM RATE.

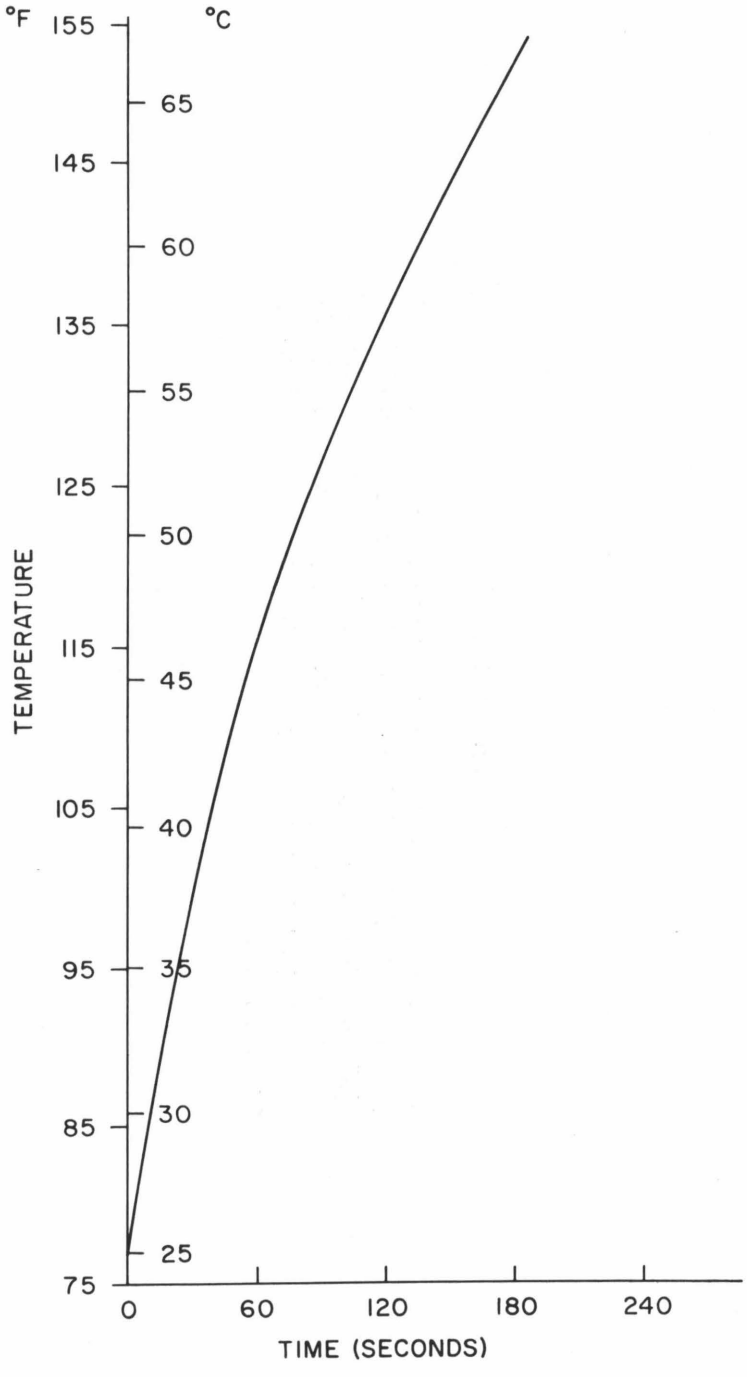

°F
°C
155
145
135
125
115
105
95
85
75
65
60
55
50
45
40
35
30
25
TEMPERATURE
0
60
120
180
240
TIME (SECONDS)

FIGURE 3

SCHEMATIC DIAGRAM OF APPARATUS USED AS LABORATORY ECOSYSTEM, TO SIMULATE PASSAGE OF WATER THROUGH THE CONDENSER OF A STEAM ELECTRIC POWER STATION, AND TO OBSERVE ''DOWNSTREAM'' PROTOZOAN COMMUNITY DEVELOPMENT.

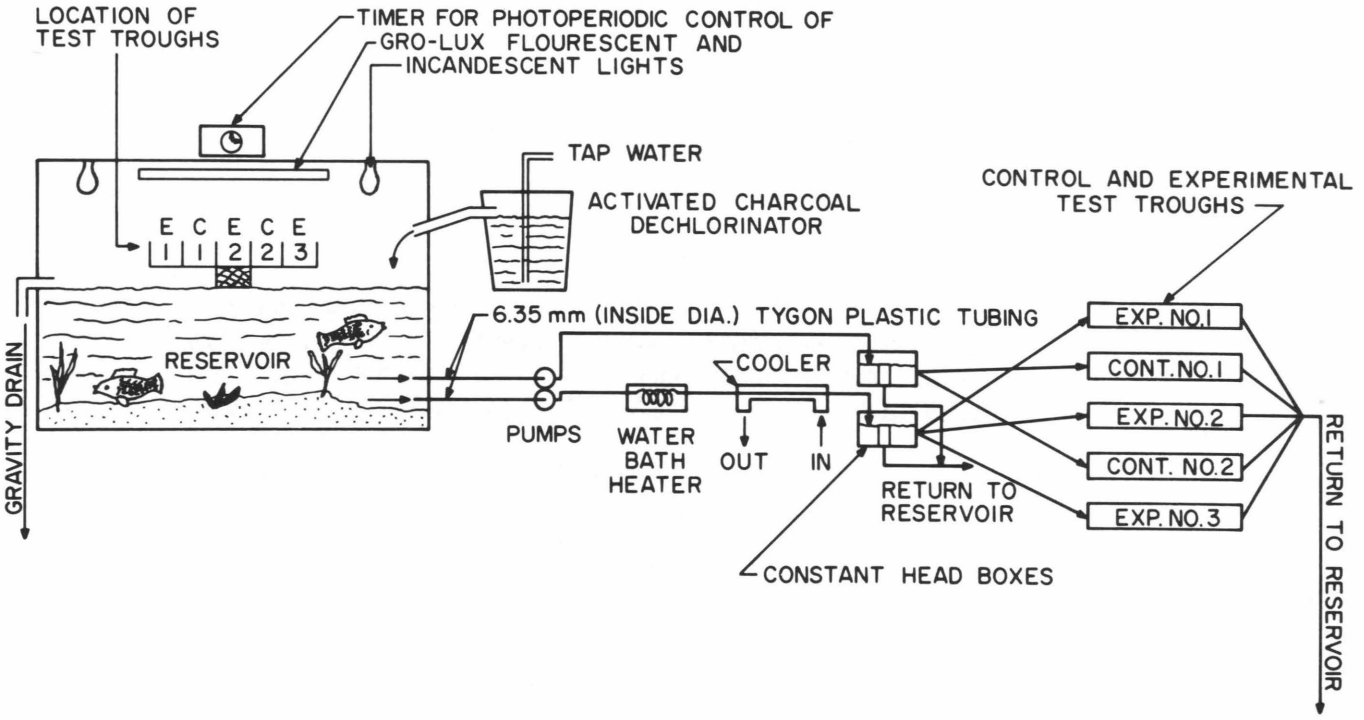

LOCATION OF TEST TROUGHS
TIMER FOR PHOTOPERIODIC CONTROL OF GRO-LUX FLOURESCENT AND INCANDESCENT LIGHTS
TAP WATER
ACTIVATED CHARCOAL DECHLORINATOR
CONTROL AND EXPERIMENTAL TEST TROUGHS
E C E C E
1 1 2 2 3
RESERVOIR
GRAVITY DRAIN
6.35 mm (INSIDE DIA.) TYGON PLASTIC TUBING
COOLER
PUMPS
WATER BATH HEATER
OUT
IN
RETURN TO RESERVOIR
EXP. NO.1
CONT. NO.1
EXP. NO.2
CONT. NO.2
EXP. NO.3
CONSTANT HEAD BOXES
RETURN TO RESERVOIR

FIGURE 4

CHANGES IN DIVERSITY (NUMBER OF TAXA) RELATIVE TO TIME FOR THE TWO CONTROL AND THREE EXPERIMENTAL COMMUNITIES THROUGHOUT THE PROTOZOAN STUDY.

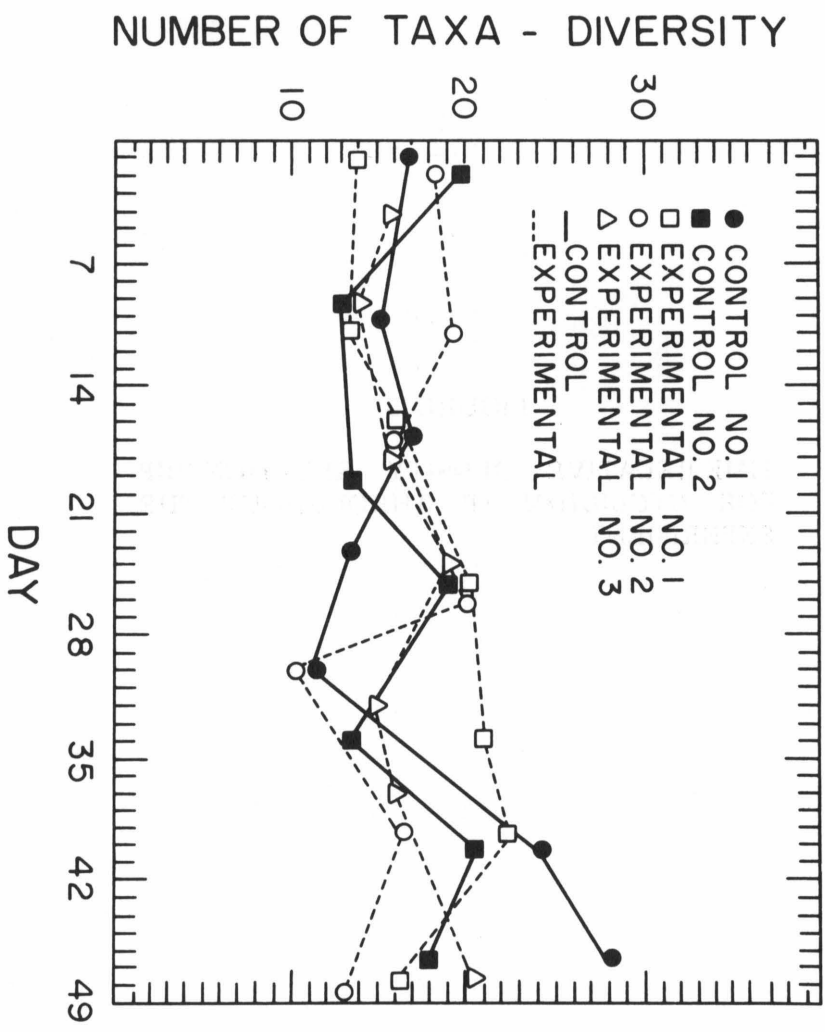

NUMBER OF TAXA - DIVERSITY
10
20
30
7
14
21
28
35
42
49
DAY
● CONTROL NO. 1
■ CONTROL NO. 2
□ EXPERIMENTAL NO. 1
○ EXPERIMENTAL NO. 2
△ EXPERIMENTAL NO. 3
—CONTROL
---EXPERIMENTAL

FIGURE 5

TIME-RELATIVE DENSITY RELATIONSHIP FOR *CYCLIDIUM* SP. THROUGHOUT THE EXPERIMENT.

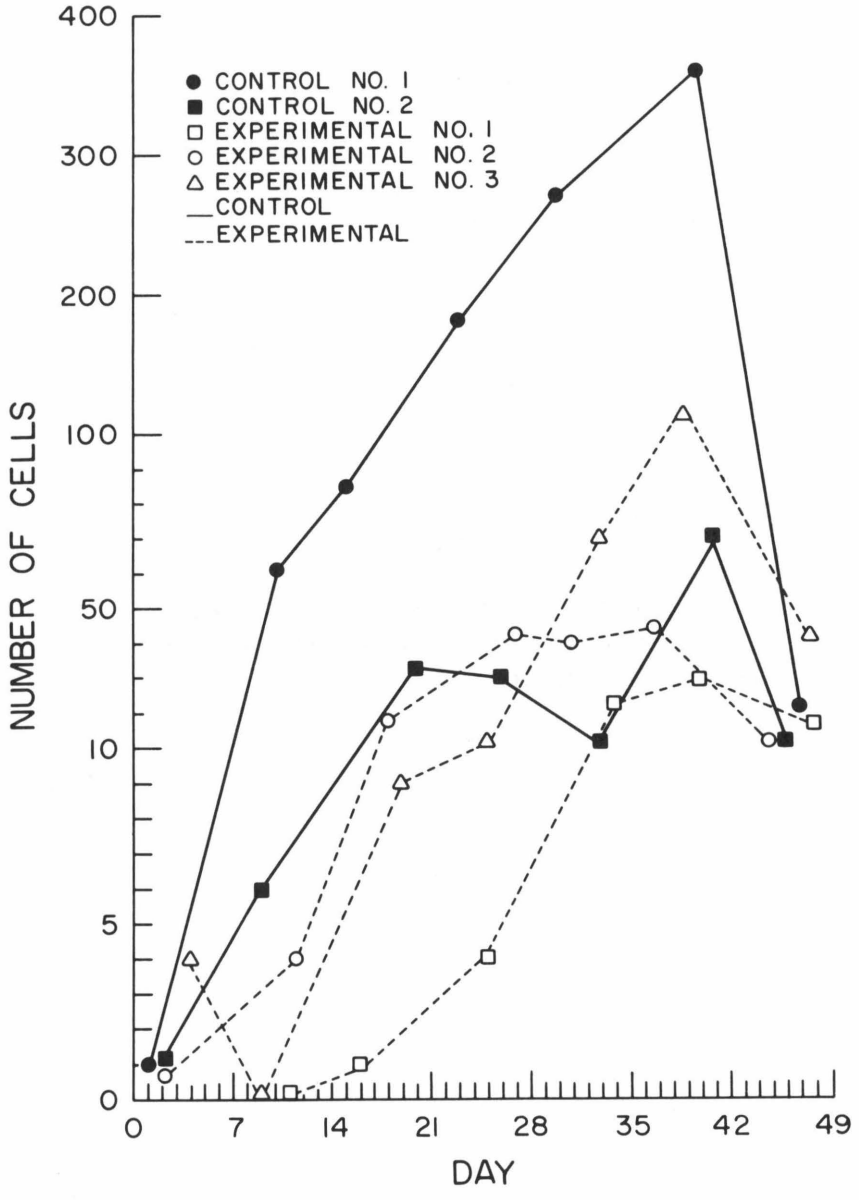

400
300
200
100
50
10
5
0
NUMBER OF CELLS
● CONTROL NO. 1
■ CONTROL NO. 2
□ EXPERIMENTAL NO. 1
○ EXPERIMENTAL NO. 2
△ EXPERIMENTAL NO. 3
__CONTROL
---EXPERIMENTAL
0
7
14
21
28
35
42
49
DAY

FIGURE 6

TIME-RELATIVE DENSITY RELATIONSHIP FOR *ASPIDISCA LYNCEUS* THROUGHOUT THE EXPERIMENT.

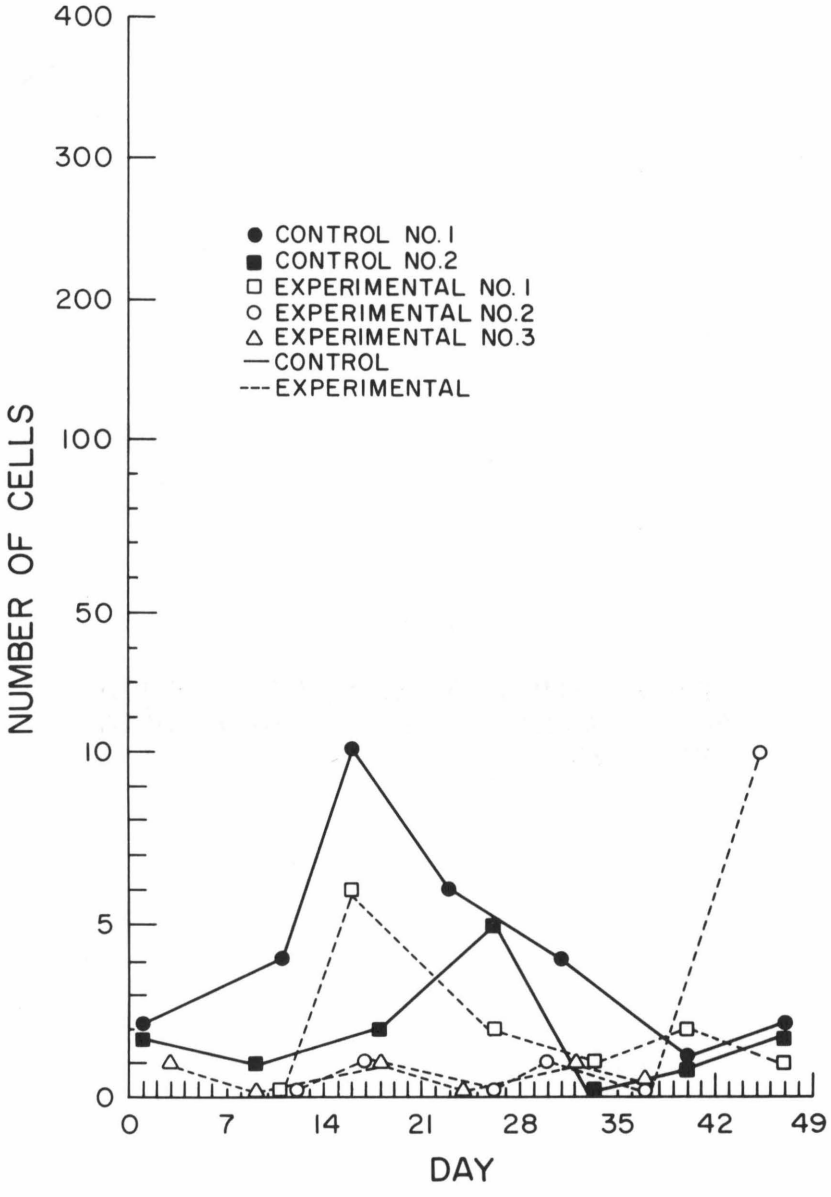

400
300
200
100
50
10
5
0
NUMBER OF CELLS
CONTROL NO. 1
CONTROL NO.2
EXPERIMENTAL NO. 1
EXPERIMENTAL NO.2
EXPERIMENTAL NO.3
CONTROL
EXPERIMENTAL
0
7
14
21
28
35
42
49
DAY

FIGURE 7

TIME-RELATIVE DENSITY RELATIONSHIP FOR *ACANTHAMOEBA* SP. THROUGHOUT THE EXPERIMENT.

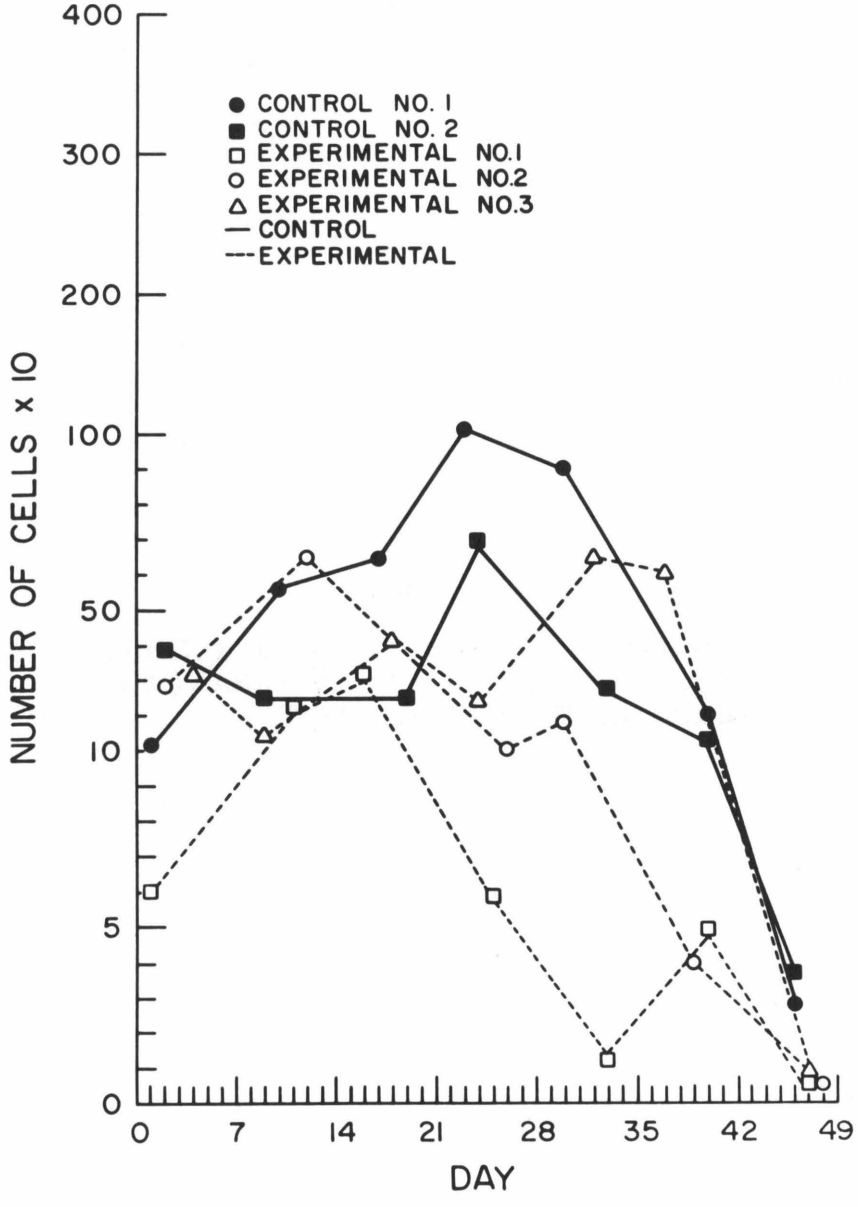

400
300
200
100
50
10
5
0
NUMBER OF CELLS x 10
CONTROL NO. 1
CONTROL NO. 2
EXPERIMENTAL NO.1
EXPERIMENTAL NO.2
EXPERIMENTAL NO.3
CONTROL
EXPERIMENTAL
0
7
14
21
28
35
42
49
DAY

FIGURE 8

TIME-RELATIVE DENSITY RELATIONSHIP FOR *VORTICELLA CAMPANULA* THROUGHOUT THE EXPERIMENT.

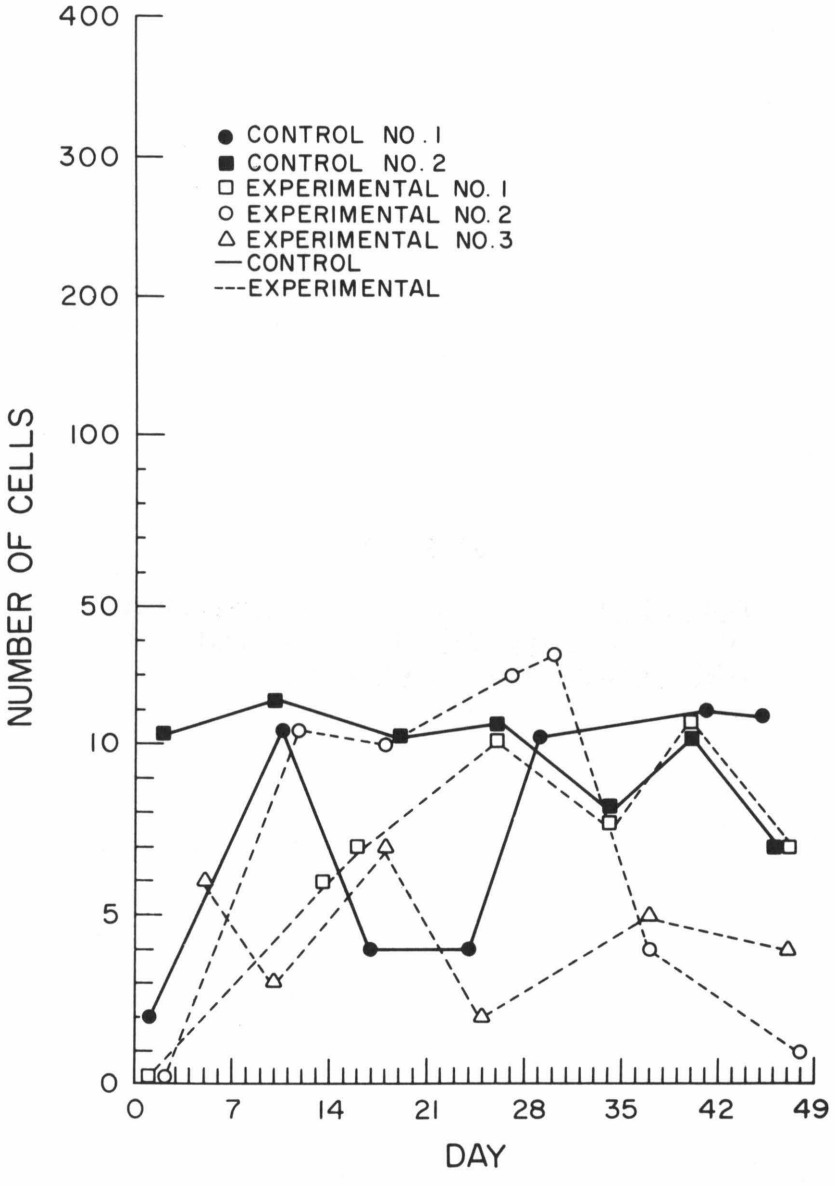

400
300
200
100
50
10
5
0
NUMBER OF CELLS
CONTROL NO. 1
CONTROL NO. 2
EXPERIMENTAL NO. 1
EXPERIMENTAL NO. 2
EXPERIMENTAL NO. 3
CONTROL
EXPERIMENTAL
0
7
14
21
28
35
42
49
DAY

FIGURE 9

TIME-RELATIVE DENSITY RELATIONSHIP FOR *CHLAMYDOMYXA MONTANA* (?) THROUGHOUT THE EXPERIMENT.

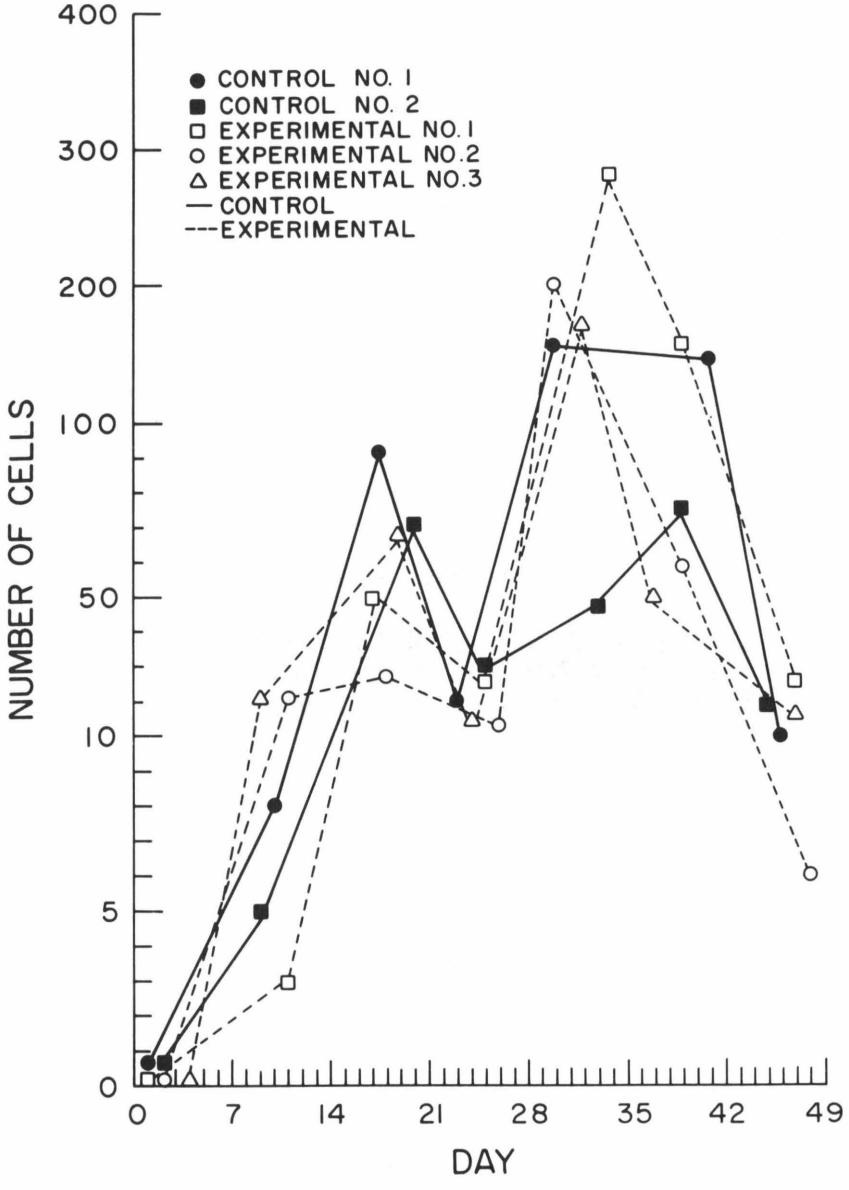

400
300
200
100
50
10
5
0
NUMBER OF CELLS
● CONTROL NO. 1
■ CONTROL NO. 2
□ EXPERIMENTAL NO. 1
○ EXPERIMENTAL NO. 2
△ EXPERIMENTAL NO. 3
— CONTROL
---EXPERIMENTAL
0
7
14
21
28
35
42
49
DAY

FIGURE 10

TIME-RELATIVE DENSITY RELATIONSHIP FOR *PSEUDODIFFLUGIA GRACILIS* (?) THROUGHOUT THE EXPERIMENT.

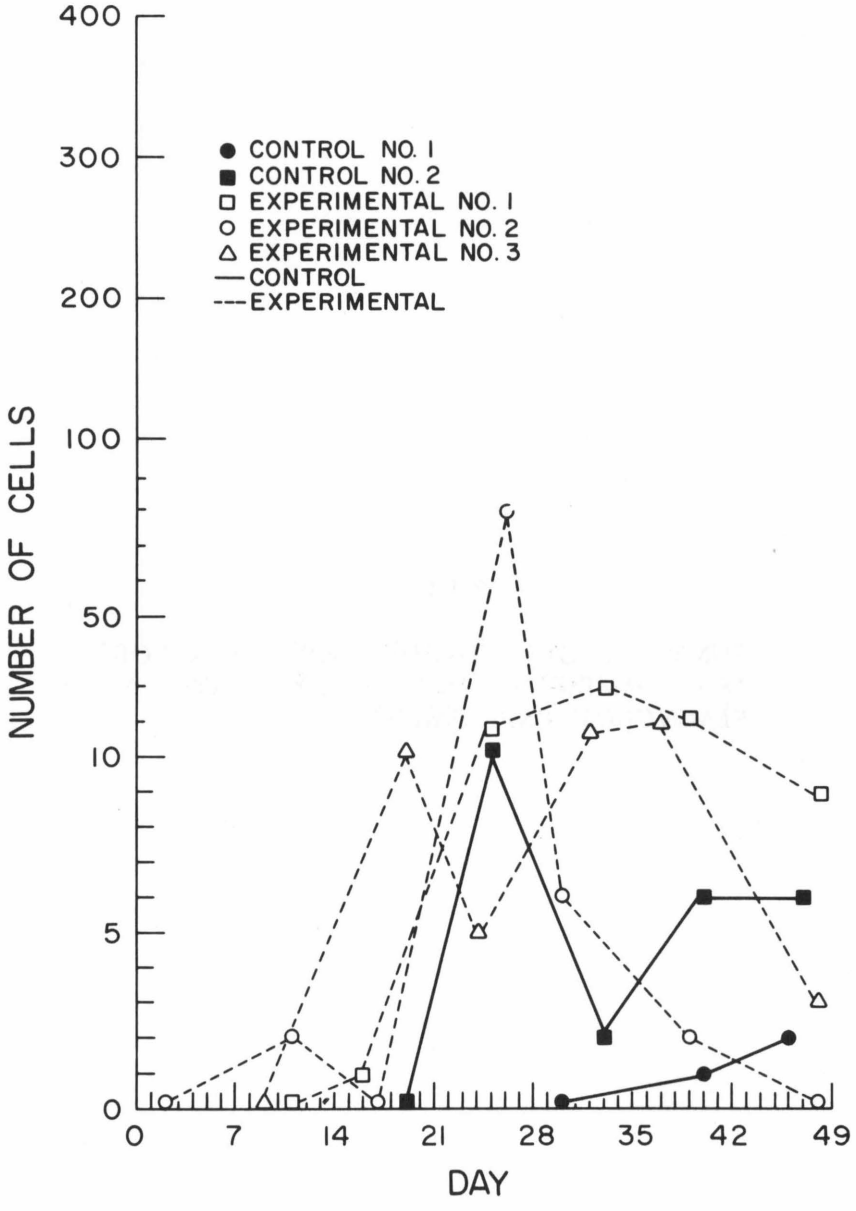

400
300
200
100
50
10
5
0
NUMBER OF CELLS
CONTROL NO. 1
CONTROL NO. 2
EXPERIMENTAL NO. 1
EXPERIMENTAL NO. 2
EXPERIMENTAL NO. 3
CONTROL
EXPERIMENTAL
0
7
14
21
28
35
42
49
DAY

FIGURE 11

SUMMARY OF REPRODUCTION DATA FOR 18°C AMBIENT POPULATIONS OF *N. SEMINULUM*. EXPERIMENT 1.

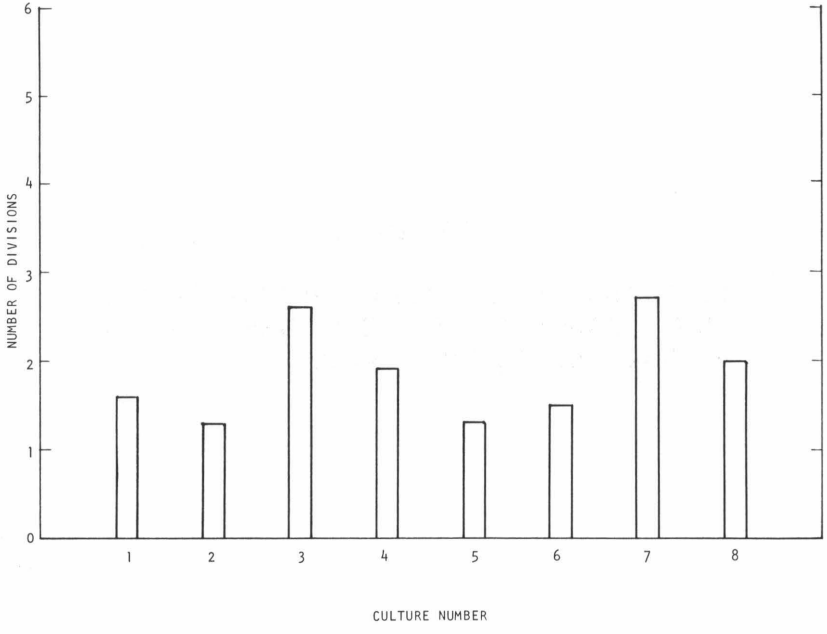

NUMBER OF DIVISIONS
0
1
2
3
4
5
6
1
2
3
4
5
6
7
8
CULTURE NUMBER

FIGURE 12

SUMMARY OF REPRODUCTION DATA FOR 18°C AMBIENT POPULATIONS OF *N. SEMINULUM*. EXPERIMENT 2.

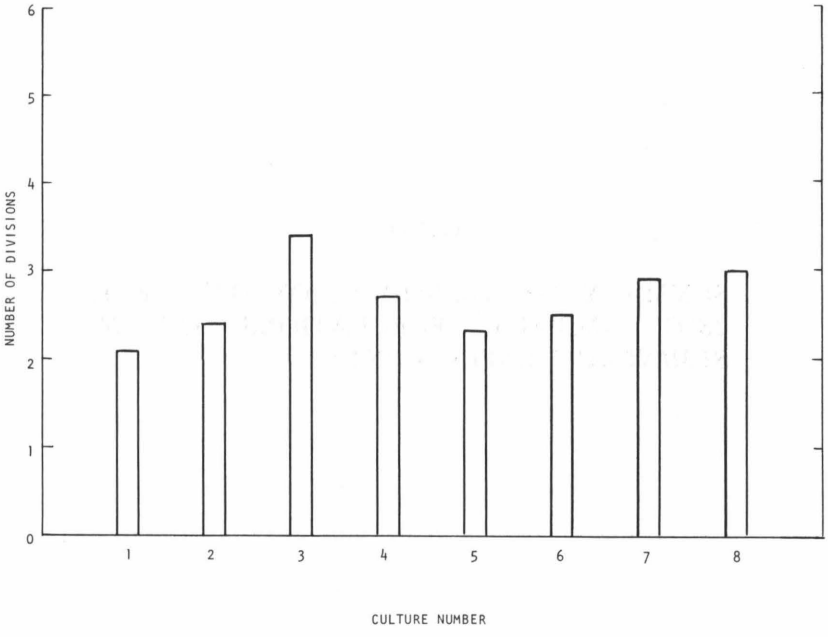

NUMBER OF DIVISIONS
0
1
2
3
4
5
6
1
2
3
4
5
6
7
8
CULTURE NUMBER

FIGURE 13

SUMMARY OF REPRODUCTION DATA FOR 23°C AMBIENT POPULATIONS OF *N. SEMINULUM*. EXPERIMENT 5.

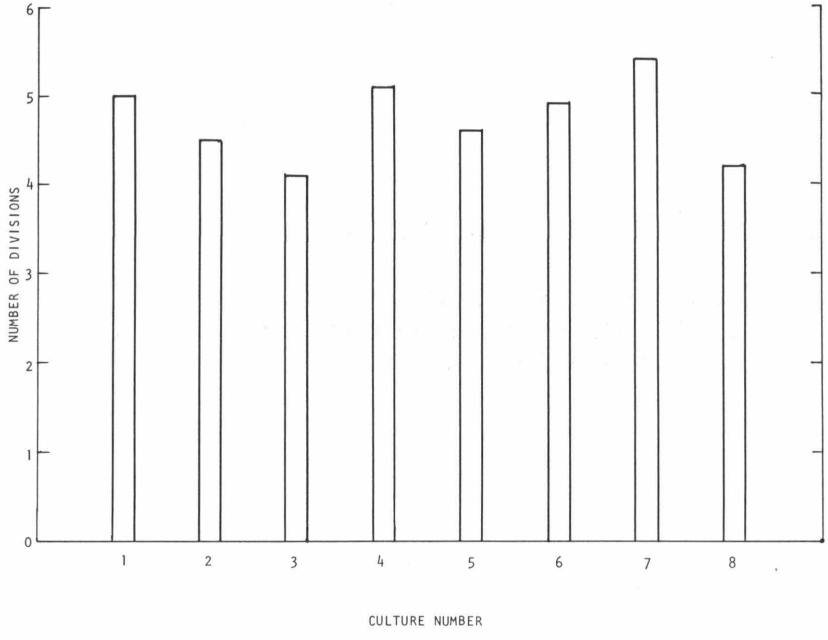

NUMBER OF DIVISIONS
0
1
2
3
4
5
6
1
2
3
4
5
6
7
8
CULTURE NUMBER

FIGURE 14

SUMMARY OF REPRODUCTION DATA FOR 23°C AMBIENT POPULATIONS OF *N. SEMINULUM*. EXPERIMENT 6.

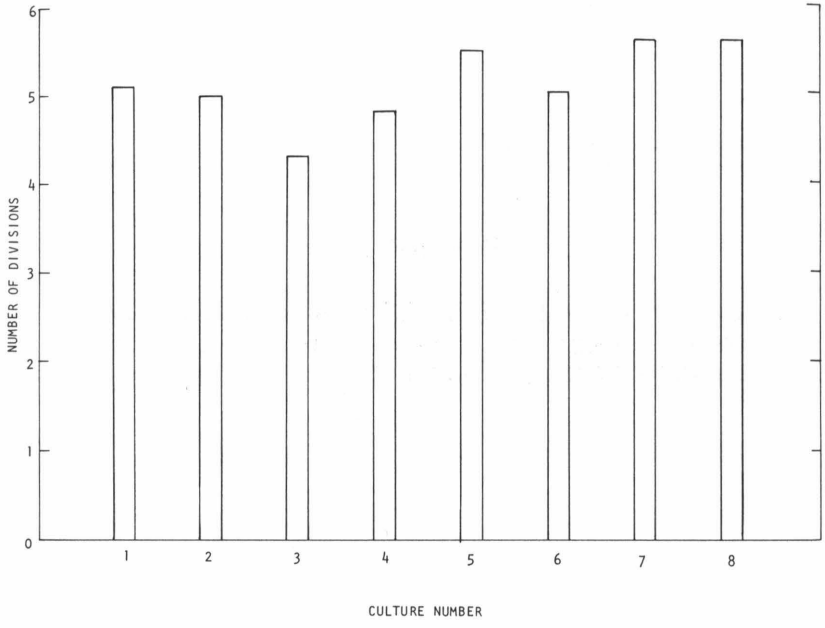

NUMBER OF DIVISIONS
0
1
2
3
4
5
6
1
2
3
4
5
6
7
8
CULTURE NUMBER

FIGURE 15

SUMMARY OF REPRODUCTION DATA FOR 29°C AMBIENT POPULATIONS OF *N. SEMINULUM*. EXPERIMENT 9.

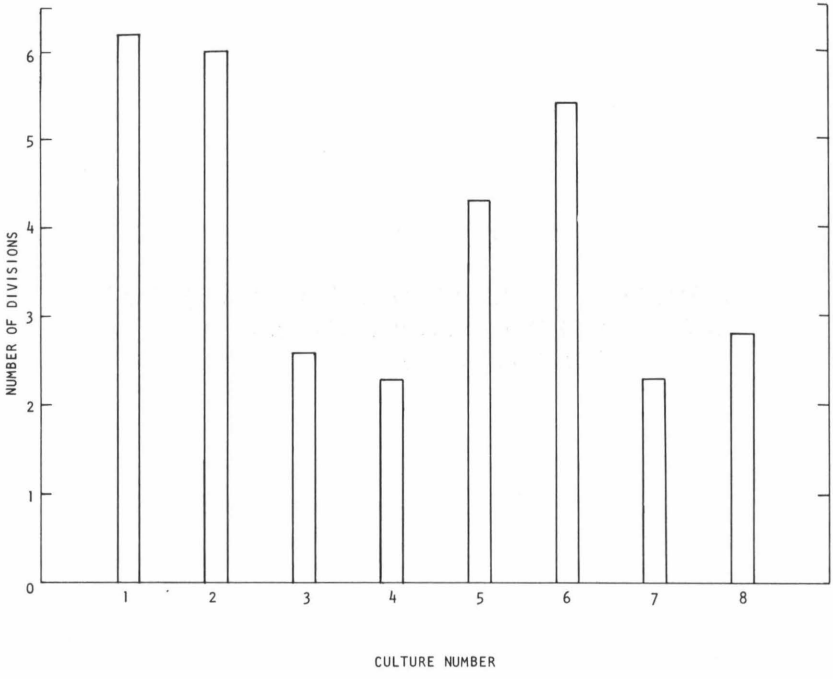

NUMBER OF DIVISIONS
0
1
2
3
4
5
6
1
2
3
4
5
6
7
8
CULTURE NUMBER

FIGURE 16

SUMMARY OF REPRODUCTION DATA FOR 29°C AMBIENT POPULATIONS OF *N. SEMINULUM*. EXPERIMENT 10.

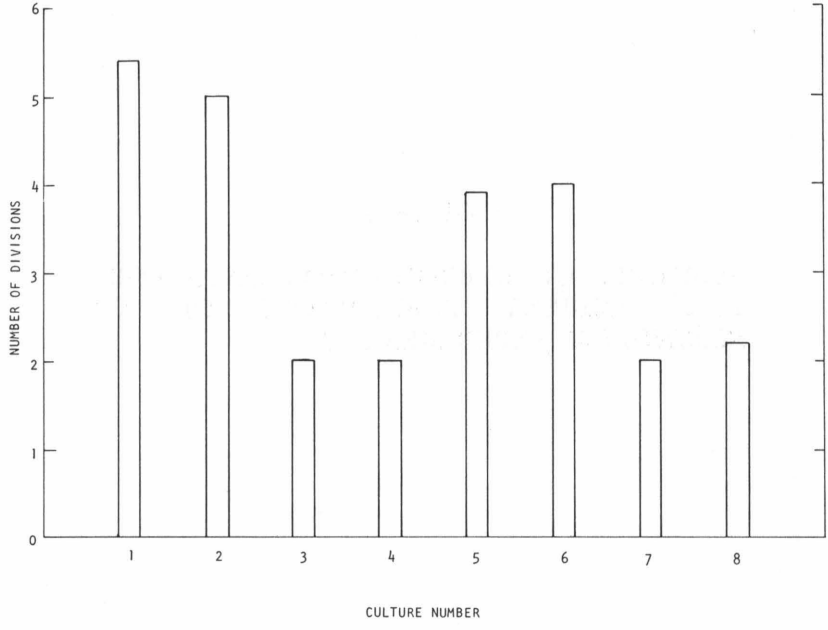

NUMBER OF DIVISIONS
0
1
2
3
4
5
6
1
2
3
4
5
6
7
8
CULTURE NUMBER

FIGURE 17

SUMMARY OF REPRODUCTION DATA FOR 29°C AMBIENT POPULATIONS OF *N. SEMINULUM*. EXPERIMENT 13.

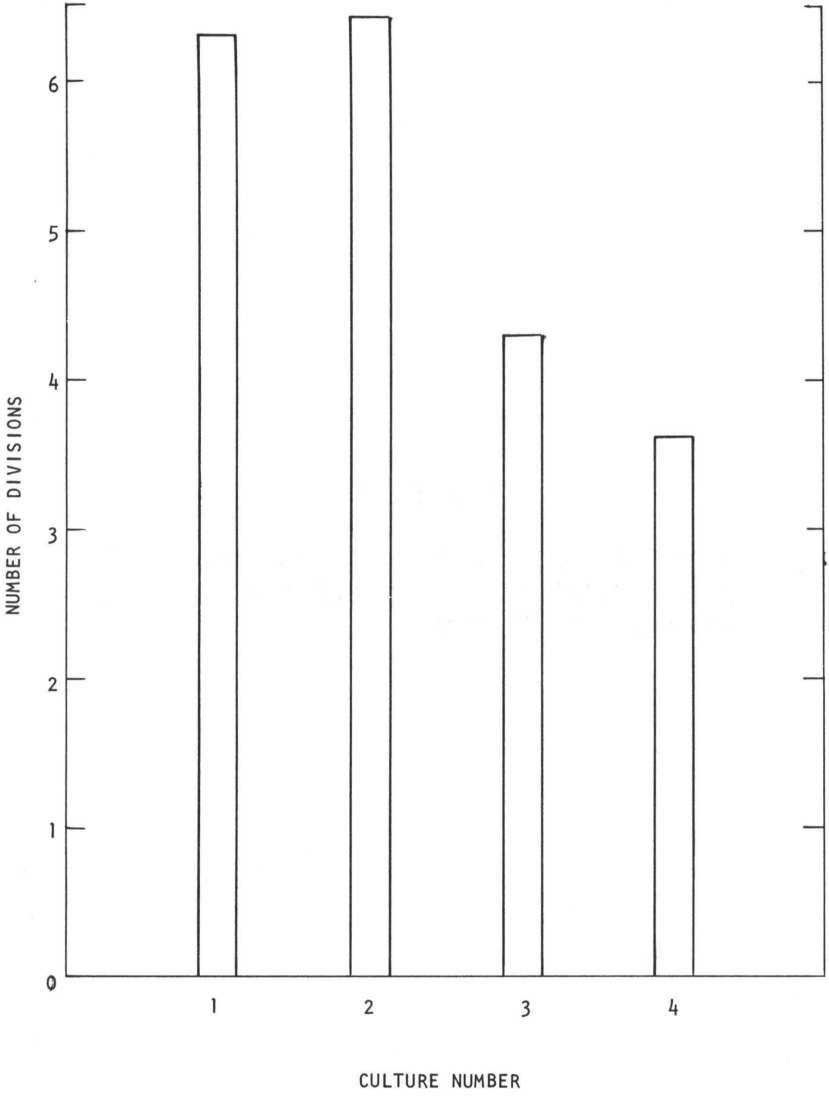
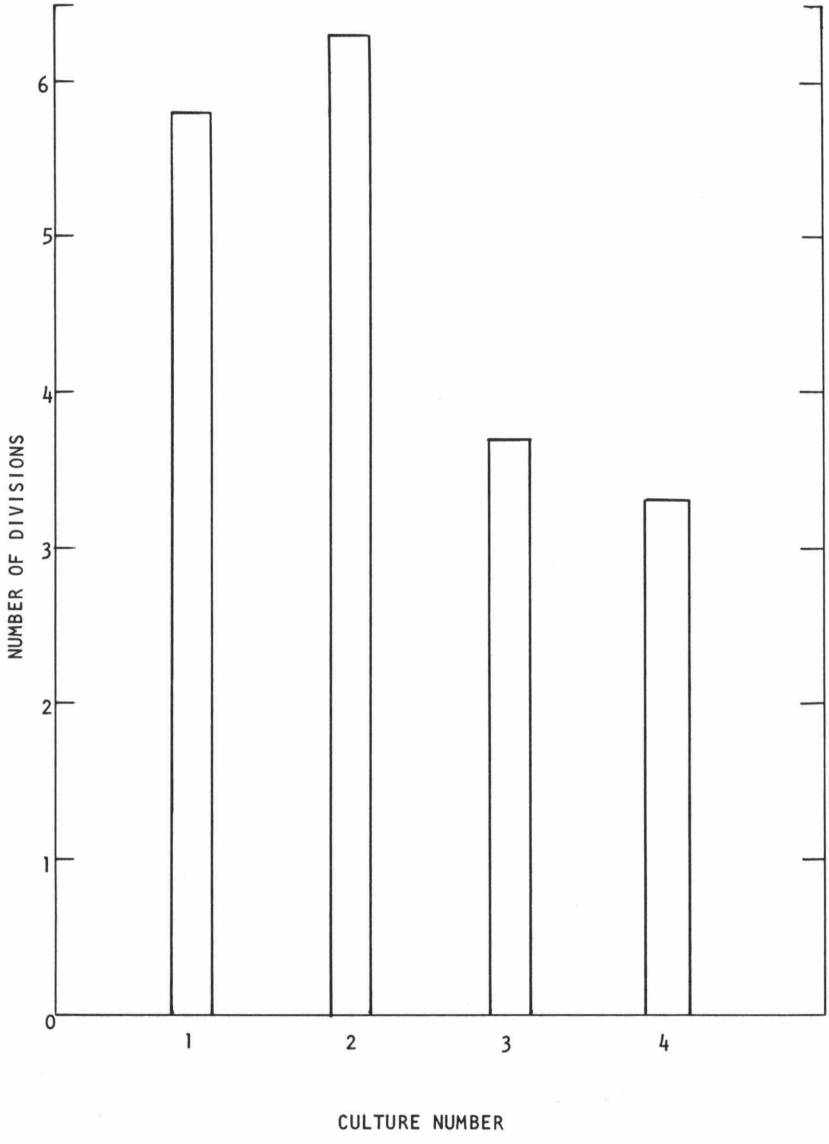

NUMBER OF DIVISIONS
0
1
2
3
4
5
6
1
2
3
4
CULTURE NUMBER

FIGURE 18

SUMMARY OF REPRODUCTION DATA FOR 29°C AMBIENT POPULATIONS OF *N. SEMINULUM*. EXPERIMENT 14.


NUMBER OF DIVISIONS
0
1
2
3
4
5
6
1
2
3
4
CULTURE NUMBER

TABLES

TABLE I

ACUTE SHOCK TEMPERATURE THRESHOLDS AND TIME UNTIL 50% MORTALITY OF *EUGLENA GRACILIS* AT THREE TEMPERATURES.

Acute constant temperature thresholds were determined by heating fluid at the maximum rate from 25°C until an estimated 50% of the organisms were dead. Total exposure time may be determined from figure.

Acute Shock Temperature Thresholds	
Run No.	Temp. (°C)
1	44.0
2	44.0
3	38.8
4	42.3
5	44.5
6	44.0
7	44.0
8	44.0
9	44.0
10	44.0

Test Temperature 37.8°C	
Run No.	Survival Time (min:sec)
1	2:03
2	2:02
3	2:06
4	2:30
5	2:15

Test Temperature 36.6°C	
Run No.	Survival Time (min:sec)
1	6:14
2	7:48
3	6:54
4	14:14
5	11:53

Test Temperature 35°C	
Run No.	Survival Time (min)
1	41
2	42
3	58
4	30
5	29

TABLE II

ACUTE SHOCK TEMPERATURE THRESHOLDS (*) AND TIME UNTIL 50% MORTALITY OF *SPIROSTOMUM AMBIGUUM* AT FOUR TEST TEMPERATURES.

Acute Shock Temperature Thresholds

Run No.	Temp. (°C)
1	41.8†
2	55.0
3	44.5
4	46.1
5	48.3
6	47.8
7	46.1
8	46.1
9	46.1
10	46.7

Test Temperature 42.8°C

Run No.	Survival Time (min:sec)
1	2:27
2	2:13
3	3:33
4	2:05
5	2:08

Test Temperature 38.9°C

Run No.	Survival Time (min:sec)
1	4:26
2	3:06
3	5:43
4	6:20
5	5:23

Test Temperature 35°C

Run No.	Survival Time (min)
1	29
2	25
3	31
4	32
5	26

Test Temperature 34.4°C

Run No.	Survival Time (min)
1	47
2	51
3	60
4	60
5	46

*See text or Table I caption for details.
†Converted from °F — not accurate to first decimal place.

TABLE III

ACUTE SHOCK TEMPERATURE THRESHOLDS (*) AND TIME UNTIL 50% MORTALITY OF *COLPIDIUM COLPODA* AT THREE TEST TEMPERATURES.

Acute Shock Temperature Thresholds	
Run No.	Temp. (°C)
1	45.5†
2	44.5
3	42.3
4	43.4
5	44.0
6	43.4
7	44.0
8	44.5
9	41.1
10	41.6

Test Temperature 37.8°C	
Run No.	Survival Time (min:sec)
1	2:02
2	2:52
3	2:27
4	2:24
5	2:49

Test Temperature 35.0°C	
Run No.	Survival Time (min)
1	19
2	22
3	19
4	16
5	13

Test Temperature 33.4°C	
Run No.	Survival Time (min)
1	35
2	33
3	56
4	54
5	42

*See text or Table I caption for details.
†Converted from °F — not accurate to first decimal place.

TABLE IV

ACUTE SHOCK TEMPERATURE THRESHOLDS (*) FOR *STENTOR COERULEUS*, *TETRAHYMENA PYRIFORMIS* AND *PARAMECIUM MULTIMICRONUCLEATUM*.

Stentor coeruleus		*Tetrahymena pyriformis*	
Run No.	Temp. (°C)	Run No.	Temp. (°C)
1	48.9†	1	50.5
2	46.1	2	45.0
3	44.5	3	47.8
4	42.3	4	48.9
5	50.0	5	48.3
6	52.2	6	46.1
7	48.9	7	43.4
8	48.3	8	42.8
9	52.2	9	42.3
10	48.9	10	44.0

Paramecium multimicronucleatum	
Run No.	Temp. (°C)
1	42.8
2	42.8
3	42.8
4	43.4
5	42.8
6	42.8
7	42.8
8	42.8
9	43.4
10	42.8

*See text or Table I caption for details.
†Converted from °F — not accurate to first decimal place.

TABLE V

DATA SUMMARY OF ULTRAVIOLET MICROSCOPIC SURVEYS OF 18°C AMBIENT POPULATIONS OF *N. SEMINULUM* RECEIVING STRESSES 1 THROUGH 3 ON DAY 3. EXPERIMENTS 1 AND 2.

Population Number	Treatment		Treatment Day	Percent Altered Cells per 1000 Experiment No.	
	Stress 1	Stress 2		1	2
1	--	--	--	3.0	1.2
2	--	--	--	1.0	3.3
3	--	+	3	2.2	2.4
4	--	+	3	1.4	1.6
5	+	--	3	3.2	2.3
6	+	--	3	1.5	1.6
7	+	+	3	2.4	2.8
8	+	+	3	3.6	0.4

TABLE VI

DATA SUMMARY OF ULTRAVIOLET MICROSCOPIC SURVEYS OF 18°C AMBIENT POPULATIONS OF *N. SEMINULUM* RECEIVING STRESS 1 ON DAY 7. EXPERIMENTS 3 AND 4.

Population Number	Treatment Stress 1	Treatment Day	Percent Altered Cells per 1000 Experiment No. 3	4
1	--	--	1.3	3.1
2	--	--	4.2	0.2
3	+	7	0.8	2.3
4	+	7	1.8	1.0

TABLE VII

DATA SUMMARY OF ULTRAVIOLET MICROSCOPIC SURVEYS OF 23°C AMBIENT POPULATIONS OF *N. SEMINULUM* RECEIVING STRESSES 1 THROUGH 3 ON DAY 3. EXPERIMENTS 5 AND 6.

Population Number	Treatment Stress 1	Stress 2	Treatment Day	Percent Altered Cells per 1000 Experiment No. 5	6
1	--	--	--	1.3	2.5
2	--	--	--	0.0	0.5
3	--	+	3	1.5	1.7
4	--	+	3	1.2	0.3
5	+	--	3	1.3	2.0
6	+	--	3	2.2	0.5
7	+	+	3	1.0	1.2
8	+	+	3	3.2	0.3

TABLE VIII

DATA SUMMARY OF ULTRAVIOLET MICROSCOPIC SURVEYS OF 23°C AMBIENT POPULATIONS OF *N. SEMINULUM* RECEIVING STRESS 1 ON DAY 7. EXPERIMENTS 7 AND 8.

Population Number	Treatment Stress 1	Treatment Day	Percent Altered Cells per 1000 Experiment No. 7	8
1	--	--	0.2	0.3
2	--	--	0.1	1.3
3	+	7	0.0	0.4
4	+	7	0.1	0.2

TABLE IX

DATA SUMMARY OF ULTRAVIOLET MICROSCOPIC SURVEYS OF 29°C AMBIENT POPULATIONS OF *N. SEMINULUM* RECEIVING STRESSES 1 THROUGH 3 ON DAY 3. EXPERIMENTS 9 and 10.

Population Number	Treatment Stress 1	Stress 2	Treatment Day	Percent Altered Cells per 1000 Experiment No. 9	10
1	--	--	--	2.9	0.9
2	--	--	--	0.5	0.3
3	--	+	3	99.5	99.5
4	--	+	3	99.8	99.8
5	+	--	3	0.3	0.3
6	+	--	3	0.1	1.0
7	+	+	3	99.7	99.8
8	+	+	3	100.0	99.3

TABLE X

DATA SUMMARY OF ULTRAVIOLET MICROSCOPIC SURVEYS OF 29°C AMBIENT POPULATIONS OF *N. SEMINULUM* RECEIVING STRESS 1 ON DAY 7. EXPERIMENTS 11 and 12.

Population Number	Treatment Stress 1	Treatment Day	Percent Altered Cells per 1000 Experiment No. 11	12
1	--	--	1.5	0.3
2	--	--	2.1	0.8
3	+	7	0.8	0.1
4	+	7	1.2	0.5

TABLE XI

DATA SUMMARY OF ULTRAVIOLET MICROSCOPIC SURVEYS OF 29°C AMBIENT POPULATIONS OF *N. SEMINULUM* RECEIVING STRESS 4 ON DAY 3. EXPERIMENTS 13 and 14.

Population Number	Treatment Stress 4	Treatment Day	Percent Altered Cells per 1000 Experiment No. 13	14
1	--	--	0.8	0.2
2	--	--	0.5	1.1
3	+	3	99.7	99.1
4	+	3	87.2	96.6

TABLE XII

DOMINANT DIATOM COMMUNITY COMPONENTS COLLECTED AT 20°C IN THE NEW RIVER, GILES COUNTY, VIRGINIA, 1970. EXPERIMENTS 25 and 26.

Species

1. *Gomphonema parvulum*
2. *Nitzschia frustulum*
3. *Cocconeis placentula* var. *euglypta*
4. *Fragilaria capucina*
5. *Synedra rumpens*
6. *Navicula salinarum* var. *intermedia*

TABLE XIII

DOMINANT DIATOM COMMUNITY COMPONENTS COLLECTED AT 22°C IN THE NEW RIVER, GILES COUNTY, VIRGINIA, 1970. EXPERIMENTS 27 and 28.

Species

1. *Gomphonema parvulum*
2. *Cocconeis placentula* var. *euglypta*
3. *Nitzschia amphibia*
4. *Gomphonema longiceps* f. *gracilus* Hustedt.
5. *Navicula* sp. 1
6. *Navicula* sp. 2
7. *Fragilaria construens* var. *subsalina* Hustedt.
8. *Gomphonema* sp.
9. *Nitzschia frustulum*

TABLE XIV

DOMINANT DIATOM COMMUNITY COMPONENTS COLLECTED AT 9 TO 10°C IN THE NEW RIVER, GILES COUNTY, VIRGINIA, 1970. EXPERIMENTS 29 THROUGH 34.

Species

1. *Diatoma vulgare* Bory. var. *breve* Grun.
2. *Fragilaria capucina* Desmazieres
3. *Achnanthes lanceolata* (Breb.) Grun.
4. *Nitzschia thermalis* (Kuetzing) Grun.
5. *Navicula cryptocephala* var. veneta (Kuetz.) Rabh.

6. *Cymbella trugida* (Gregory) Cleve.
7. *Gomphonema parvulum* (Kutz.)
8. *Fragilaria brevistriata* Grun. var. *brevistrista*
9. *Fragilaria intermedia* Grunow
10. *Synedra rumpens* var. *familiaris* (Kuetz.) Grun.

11. *Nitzschia amphibia*
12. *Synedra tabulata* Pant.
13. *Nitzschia frustulum*
14. *Achnanthes biasolettiana* (Kuetz.) Grun.
15. *Cocconeis placentula* var. *euglypta*

16. *Achnanthes linearis* f. *curta* H. L. Sm.
17. *Navicula salinarum* var. *intermedia* (Grun.) Cl.
18. *Cocconeis placentula* var. *lineata* (Ehr.) V. H.
19. *Achnanthes lanceolata* var. *rostrata* (Ostrups) Hustedt.
20. *Navicula tripunctata* var. *schizonemoides* (V. H.) Patrick

21. *Gomphonema helveticum* Brun.

TABLE XV

DOMINANT DIATOM COMMUNITY COMPONENTS COLLECTED AT 15 TO 16°C IN THE NEW RIVER, GILES COUNTY, VIRGINIA, 1970. EXPERIMENTS 35 AND 36.

Species

1. *Fragilaria capucina*
2. *Gomphonema parvulum*
3. *Nitzschia frustulum*
4. *Cymbella turgida*
5. *Achnanthes lanceolata*

6. *Fragilaria intermedia*
7. *Gomphonema ventricosum* Gregory
8. *Synedra tabulata* (Ag.) Kuetz.
9. *Cyclotella glomerata* Bachmann
10. *Cocconeis placentula* var. *euglypta*

11. *Synedra hyperborea* var. *rostellata* Grunow and Wien

TABLE XVI

DATA SUMMARY OF CHYTRIDIACEOUS-FUNGAL INFESTATION.

Culture No.	Treatment		Treatment Day	Percent Infestation Expired Cells
	Stress 1	Stress 2		
1	--	--	--	43
2	--	--	--	19
3	--	+	1	3
4	--	+	1	1
5	+	--	1	24
6	+	--	1	16
7	+	+	1	1
8	+	+	1	0

TABLE XVII

DATA SUMMARY OF CHYTRIDIACEOUS-FUNGAL INFESTATION.

Culture No.	Treatment		Treatment Day	Percent Infestation Expired Cells
	Stress 1	Stress 2		
1	--	--	--	10
2	--	--	--	14
3	--	+	1	5
4	--	+	1	2
5	+	--	1	3
6	+	--	1	9
7	+	+	1	1
8	+	+	1	0

TABLE XVIII

DATA SUMMARY OF *NETRIUM* SP. RECEIVING STRESSES 1 THROUGH 3. EXPERIMENT 1.

Culture No.	Treatment		Treatment Day	No. Intact *Netrium* sp. (per ml)
	Stress 1	Stress 2		
1	--	--	--	210
2	--	--	--	210
3	--	+	1	70
4	--	+	1	60
5	+	--	1	100
6	+	--	1	110
7	+	+	1	0
8	+	+	1	30

TABLE XIX

DATA SUMMARY OF *NETRIUM* SP. RECEIVING STRESSES 1 THROUGH 3. EXPERIMENT 2.

Culture No.	Treatment		Treatment Day	No. Intact *Netrium* sp. (per ml)
	Stress 1	Stress 2		
1	--	--	--	240
2	--	--	--	210
3	--	+	1	130
4	--	+	1	180
5	+	--	1	90
6	+	--	1	70
7	+	+	1	60
8	+	+	1	7