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ESTUARINE ECOSYSTEMS AND HIGH TEMPERATURES

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ABSTRACT

A study was designed to investigate the responses of estuarine community structure, respiration and production to added heat, sewage and their combination. The study was conducted in plastic pools containing transplanted ecosystems from South Creek Estuary, N. C. Temperature replication was achieved. Temperature in the heated pools was regulated at 5 C (9°F) above that of the ambient pools; but due to local and short-term weather variations, the actual differences were 2-5 C, 3-5 C and 0-12 C during spring, summer and winter, respectively.

Thermal treatment increased the nutrient regeneration rates, yielding slightly higher algal biomass; although, seasonal differences were more significant. Gross community productivity was regulated by ammonia, light, and temperature levels and total respiration was regulated by temperature and primary productivity. Sewage addition substantially increased the ammonia levels, particularly during the winter. Community metabolism responded positively to thermal treatment, but not to sewage treatment. Thermal treatment and the combination of sewage and thermal treatments increased the photosynthesis/respiration ratios (P/R) during spring and summer, but decreased the P/R when temperature was limiting during winter.

Temperature had very little effect on phytoplankton composition during the spring. Blue-green algae and coccoid green algae dominated in the heated and sewage-treated pools during summer.

Nekton and benthic (blue crabs, grass shrimp, widgeon grass, bay clams, and fish) increased to higher biomass in the heated pools during spring and achieved a lower biomass in the heated pools during summer than in the ambient pools. No significant differences were observed during winter among heated and ambient pools. Sewage addition did not substantially alter the patterns between heated and ambient systems. Oysters, bay clams and widgeon grass reached higher biomass in the heated pools during winter than in the ambient pools.

A flow-through experiment was conducted during the 1971 summer to test more realistic estuarine conditions. The results of this experiment were substantially the same as obtained the previous summer under quiescent conditions.

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I. SUMMARY AND RECOMMENDATIONS

I. A. Summary and Conclusions

A study was designed to investigate the response of estuarine community structure, respiration and production to added heat, sewage and their combination. This study was conducted at the Pamlico Marine Laboratory, N. C. State University, Aurora, N. C. during 1970-71 in plastic pools containing water and biota from the South Creek Estuary.

The study had the following objectives:

a) to establish the feasibility of studying whole estuarine ecosystems under controlled conditions with thermal loading;

b) to measure the ecosystem response to a 5 C phytoplankton, zooplankton, nekton and benthos;

c) to measure the ecosystem response to a combination of added heat and sewage; and

d) to establish data for setting criteria to assess the effects of thermal loading on estuarine ecosystems.

Temperature replication was achieved among replicate pools in all experiments. Diurnal temperature changes were 3-4 C, but annual temperature variation was about 26 C in the ambient pools and about 22 C in the heated pools. Temperature in the heated pools was regulated at 5 C above that of the ambient pools, but due to local and short-term weather variations the actual differences were 2-5 C, 3-5 C and 0-12 C during the spring, summer and winter, respectively.

The addition of sewage during the winter increased the nutrient concentrations in those pools. No differences were observed in the nutrient concentrations because of added heat. Thermal treatment did increase the nutrient regeneration rates, yielding slightly higher algal biomass; although, seasonal differences were more significant. Thus, heat treatment did not substantially change nutrient conditions in these experimental systems.

The following conclusions were determined.

1. Gross community productivity appeared to be regulated by ammonia, light, and temperature levels and rates of total respiration appeared to be regulated by the temperature and primary production. The level of ammonia appeared to be regulated by the heterotrophic activity and the addition of primary sewage. During the spring experiment, respiration and ammonia levels increased with temperature and spring blooms followed. During the summer experiment, the ammonia levels increased and respiration and photosynthetic pulses followed. During the winter experiment, ammonia was available in the sewage-treated pools, but production or respiration did not increase. Also, temperature was high in the heat+sewage-treated pools, but production or respiration did not increase. Therefore, it is considered that a combination of light, temperature, and ammonia levels and possibly the nitrate levels limited the winter ecosystems; a combination of temperature and ammonia and possibly nitrate levels limited the spring ecosystems; and, ammonia and possibly nitrate levels limited the summer ecosystems.

2. Community metabolism responded significantly to thermal treatment, but not to sewage treatment. A 2-5 C temperature elevation significantly increased the amount of respiration and primary production during the spring and summer experiments, but a 0-12 C temperature elevation significantly inhibited primary production by preventing succession to a more productive phytoplankton community during the winter experiment. On the other hand, weekly additions of ammonia-poor sewage did not affect respiration or primary production.

3. Thermal treatment and the combination of sewage and thermal treatments increased the P/R ratios when temperature was not limiting, (spring and summer) but decreased the P/R ratios when temperature was limiting (winter). Yet, sewage treatment alone did not affect the P/R ratios. Thus, increased temperatures caused the systems

to be more autotrophic during the spring and summer (exceeding present temperature standards of 32 C) and more heterotrophic during winter. This would create a tendency to more algal blooms during spring and summer in heated estuaries.

4. Chrysophytes dominated the phytoplankton in both heated and ambient pools during the spring experiment. The percent diatom composition of the phytoplankton was larger in the heated pools than in the ambient pools. Thus, temperature had very little effect on phytoplankton composition during the spring, except for the tendency of diatom blooms in the heated pools.

5. Concentration of phytoplankton was less during the summer experiment than during the spring. Chrysophytes dominated in the heated pools during August, but were succeeded by dinoflagellates and green algae during September. In the heated+sewage-treated pools a diverse community of blue-greens and small greens and diatoms existed during the summer experiment. Blue-green algae also dominated in the sewage-treated pools. Thus, both the addition of sewage and/or heat increased the possibility for dominance by blue-green algae or coccoid green algae.

6. Larger amounts of algae accumulated on the bottoms of pools receiving sewage than in those without sewage during the summer, reflecting the increased net photosynthesis. Heat also caused larger accumulations than were found in the ambient pools. During the winter, the largest algal accumulations occurred in the heated pools, regardless of sewage treatment. Thus, heat and sewage additions resulted in larger accumulation of algae on pool bottoms, which is in agreement with the pattern of net photosynthesis.

7. During the spring experiment, nekton (blue crabs, grass shrimp and fish) increased to higher biomass in the heated pools than in the ambient pools.

Temperature during this period was 18 to 30 C in the heated pools and 13 to 25 C in the ambient pools. At these intermediate temperatures, it is clear that the additional 5 C of water temperature significantly increased nektonic biomass in the heated pools.

8. During the summer experiment, nekton (blue crabs, grass shrimp and fish) achieved a lower biomass in the heated pools than in the ambient pools. Temperature was 25 to 33 C in the heated pools and 20 to 28 C in the ambient pools. These warmer temperatures were detrimental to the production of nekton biomass when the water was already near maximum temperature ordinarily attained in North Carolina estuaries. The addition of sewage during the summer experiment did not significantly alter the nekton growth in ambient or heated pools.

9. During the winter experiment, blue crabs exhibited no significant growth and fish decreased in biomass in heated and ambient pools. Mean water temperature was 2 to 10 C in the ambient pools and 9 to 15 C in the heated pools. The addition of sewage did not significantly alter the growth or survival patterns during the winter. Apparently, at these cooler temperatures the addition of only 5 C was not enough to cause increased growth.

10. *Ruppia maritima* increased in biomass in the heated pools during the spring experiment (18 to 30 C), but decreased in biomass in the ambient pools (13 - 25 C). The increased temperature apparently caused the *Ruppia* to initiate spring growth earlier in the season (March) than is normally expected (April). *Ruppia* biomass decreased to almost zero in all pools during the summer and winter, regardless of heated and/or sewage treatments. There was significantly more *Ruppia* in the ambient pools at the end of the summer experiment and in the heated pools at the end of the winter experiment. Thus, the higher temperatures were detrimental to *Ruppia* growth during summer and beneficial during winter and spring.

11. There were significant decreases in the biomass of bay clams (*Rangia cuneata*) and oysters (*Crassostrea virginica*) in the heated and heat+sewage-treated pools during the winter experiment. No other significant differences were observed.

12. Several species of benthic macrofauna became established in the experimental pools during the course of experiments. Except for two freshwater species (*Hydaticus* sp. and *Trichocorixa* sp.) there were significantly more biomass in the heated pools than in the ambient pools during spring and winter. In all cases there was a larger biomass in ambient pools than in heated pools during summer. The addition of sewage eliminated some species, but did not significantly alter the biomass of those surviving. Thus, the water temperatures in the heated pools (33 C) during summer was detrimental to benthic macrofauna.

A flow-through experiment was conducted during the 1971 summer. The same temperature conditions were maintained as in the quiescent pools, but water flowed through the pools at a rate that exchanged the pools volumes about every 6-10 days. Temperatures in the ambient pools were 23 to 28 C and 29 to 33 C in the heated pools. The following conclusions were noted.

1. Phytoplankton increased in the heated pools, was highly variable, and was dominated by blue-green algae. The more diverse phytoplankton population in the ambient pools remained at about the same concentration through the experiment. Zooplankton was dominated by the copepod, *Acartia tonsa*, with smaller populations in the heated pools than in the ambient pools.

2. Due to the large variability between replicates (an inherent problem in dynamic situations), statistical significance could not be obtained for the nekton and benthos outputs. However, the general trend was toward lower biomass in the heated pools than in the ambient pools for blue crabs, grass shrimp, fish and *Rangia*

clams. No differences were observed between experiments for oysters and widgeon grass and they steadily decreased in biomass during the experiment. Benthic macrofauna reached lower biomass/pool in the heated pools than in the ambient pools, except for the highly-adaptable warm, Glycera sp., which had a higher biomass in the heated pools. Thus, it is apparent that the heated pool temperatures were detrimental under flow-through conditions as well as quiescent conditions.

I. B. Recommendations

In view of the findings in this project, the following recommendations seem appropriate.

1. Any studies of the effects of heated effluents in natural waters should be from the system point of view. The many subtle interactions and competition among organisms in nature are too complex to approach temperature effects on an individual organism basis.
2. More detailed work needs to be done with community metabolism, because it is an integrative measurement of ecosystem response to stresses. More detailed studies should be conducted with nutrient cycling and availability; perhaps with radioisotopes.
3. Replicability in flow-through experiments was difficult. Thus, in future studies of this type more replicates need to be used so that normal statistical analyses can be performed with the data.
4. The state water quality standards for temperature are as follows:

   > "The water temperature shall not be increased above natural water temperature by more than 1.5°F. during the months of June, July, and August, nor more than 4.0°F. during other months and in no case to exceed 90°F. . . ."

   The 1.5°F. limit does not appear to be restrictive during June to August, but the natural water temperature oftens exceeds 90°F. during this time. The

4.0°F. limit does appear to be restrictive during other times of the year (*i.e.*, findings of this study indicate that +9.0°F. is not harmful to estuarine ecosystems). Thus, it is recommended that criteria for establishing water quality standards for heat should be reviewed in relation to these study findings.

5. Experiments should be conducted, using variable temperature regulation, to study response to increases less than 5 C (experimental conditions).

II. INTRODUCTION

II. A. Introductory Statement

Thermal loading is rapidly growing since increasing numbers of power plants and factories are discharging heated water into estuaries and waterways. Furthermore, Gaucher (1) predicts that the number of nuclear power plants will increase exponentially until the year 2200. Because the effects of thermal changes in the aquatic environment are not well understood, proper laws controlling thermal loading cannot be developed. In the past ten years, there have been many studies on the metabolic influence of heated water on fish and moderate number of studies on other organisms (for literature reviews see 2, 3, 4). Despite this attention, there are only a few studies on the effects of heated water on whole communities.

The decision was made to investigate the effects of increased temperatures on community structure, production and respiration in estuarine systems in North Carolina, since the Cape Fear and Neuse River Estuaries are potential sites for future nuclear power facilities and the thermal effects on these systems are not understood. This study characterizes community studies in 4 to 8 pools that were treated with heat, sewage, and a combination of heat plus sewage during the spring, late summer, and winter.

Before 1963, there were a number of studies on the effects of heated water on aquatic invertebrates and plants. One of the first thermal studies was conducted by Dallinger (5) on the response of protozoans to gradual temperature increases. Since Dallinger's study, there have been many facets of thermal research. Of these, six are directly related to our study of community metabolism. Firstly, the effect of temperature on the toxicity of poisons was studied by Carpenter (6). A second phase of thermal research was initiated by Sayle (7) on the respiratory responses and acclimatization periods of aquatic organisms in heated waters. A third phase began with Walshe's work (8) on succession in thermally stressed communities. A fourth phase concerns the photosynthetic responses to increased temperatures and the dependency on nutrient levels (9). A fifth phase of thermal research deals with field studies on the effects of thermal effluents on the aquatic environment. Van Vliet (10) was one of the first to conduct a field study. Finally, a sixth phase was initiated by Beyers (11) on community metabolism in heated systems.

The toxicity of poisons and diseases in fish is at least doubled by a 10 C temperature elevation, but other organisms occupying lower trophic levels such as invertebrates are even more sensitive. The survival times of poisoned fishes (12, 13) decreased 50 percent for a 10 C temperature elevation. In addition, Hynes (14) contends that fish are less affected by poisons than invertebrates. However, only a few studies have been conducted on the sensitivity of invertebrates to poisons at high temperatures since it is difficult to keep these organisms alive under optimal laboratory conditions. De Sylva (15) pointed out that diseases in fish and some invertebrates are common at high temperatures since high temperatures generally favor the pathogen but not the host. For example, Stewart, Cormick, and Zwicker (16) showed that survival periods of diseased American lobsters are reduced ten-fold for a 10 C increase.

Community structures are changed by unnatural temperature increases, but not always destructively. For example, Mann (17) found that thermal loading increased the abundance of a tropical tubificid oligochaete (Branchiura sowerbyi) in a temperate system. Also, Naylor (18) found that the abundance of a semi-tropical copepod (Acartia tonsa) increased in a temperate system heated by thermal effluents. However, Heinle (19) concluded that two copepods, (A. tonsa and Eurytemora affinis) may be eliminated from temperate systems during sustained periods of temperatures above 30 C. Furthermore, Trembley (20) found that blue-green algae increased in areas affected by a thermal effluent during the summer. In addition, under thermal loadings, insects, worms, and crustaceans declined during the summer, but increased during the winter. Anderson (21) found that the pond weed (Potamogeton) replaces a heat sensitive pond weed (Ruppia) in thermally stressed weed beds in the Patuxent River. Similarly, the density and number of plant species declined in the heated discharge at Morro Bay, California (22). Despite these trends towards a reduction in the diversity, Poltoracka (23) stated that phytoplankton diversity increased 50 percent in a Polish lake heated 4 C above ambient.

Maximum growth rates of invertebrates occur in heated waters at temperatures near 30-32 C, but decline at higher temperatures. For example, Cory and Nauman (24) found that barnacles, hydroids, tunicates, and tube amphipods grow three times faster than normal in effluent waters that are 6 C above ambient temperatures in the Patuxent River. Cook and Murphy (25) stated that the growth rate of brown shrimp larvae (Penaeus aztecus) increased with temperatures up to approximately 32 C. Furthermore, growth rates of the copepod (Acartia tonsa) increased with temperature to 30 C, but decreased at higher temperatures (19).

Respiration rates of individual plants and invertebrates increased with temperature, but respiration decreased as temperatures rose above the thermal maxima. For instance, Siefken and Armitage (26), as cited by Heinle (19), showed that the respiration rates of five populations of freshwater copepods increased with temperature to 22-26 C, but the respiration rates of three populations declined above about 24 C. Respiration rates of the pond weed (Potamogeton perfoliatus) increased with temperature to 40 C, but decreased at 45 C (21). Furthermore, Vernberg and Vernberg (27) showed that the respiration rates in a marine tremetode (Saccacoelium beauforti from mullet) increased with temperature to 34 C and decreased at higher temperatures.

Primary productivity is increased by short periods of heating at winter temperatures when nutrients are available, but is inhibited at summer temperatures when nutrients are limiting. For instance, Hutner *et al.* (9), found that photosynthesis in phytoplankton cultures decreased at high temperatures and at low nitrogen and phosphorus levels. However, by increasing the nitrogen and phosphorus levels the cultures could be heated to higher temperatures without inhibiting photosynthesis. In field studies, Warinner and Brehmer (28) and Morgan and Stross (29) concluded that raising the temperature 3-8 C at high temperatures inhibits photosynthesis. However, at low temperatures they found that a 3-8 C rise stimulates photosynthesis.

Beyers (11) showed that heat affects the community metabolism in experimental ecosystems. He contends that the magnitude of metabolic responses to a 7 C temperature rise decreases as the diversity increases. This could be caused by the buffering effect that energy pathways have on metabolic responses to heat. Furthermore, it is likely that the number of energy pathways increases with diversity, as the metabolic responses by single organisms are much greater than the metabolic responses of communities.

II. B. Objectives

The study had the following objectives:

1) To establish the feasibility of studying whole estuarine ecosystems under controlled conditions with thermal loading;
2) To measure the ecosystem response to a 5 C temperature increase, including community metabolism, phytoplankton, zooplankton, nekton and benthos;
3) To measure the ecosystem response to a combination of added heat and sewage; and
4) To establish data for setting criteria to assess the effects of thermal loading on estuarine ecosystems.

II. C. Scope

Since there has been little study of the response of whole estuarine ecosystems to elevated temperatures, we attempted to measure ecosystem structure, productivity and respiration in heated systems under controlled conditions. The philosophy was to obtain data on whole ecosystems so that realistic water quality criteria may be better set. Thermal loading was regulated at 5 C above ambient because this is a realistic temperature increase in estuarine ecosystems receiving power generation station effluents. Finally, water from the Pamlico River Estuary with its attendent microorganisms and larger organisms from a nearby area were used as experimental ecosystems because of the need for thermal response studies in North Carolina waters.

Although the findings of this study will be directly applicable to North Carolina estuaries it is hoped that the findings will be useful for other estuaries in the temperate zone. Certainly, the need for meaningful criteria for setting temperature standards is apparent, especially in view of the proliferation of power plant construction and the use of many millions of gallons of water each day for cooling purposes.

III. EXPERIMENTAL DESIGN

III. A. Artificial Systems

Heat induced changes were followed in estuarine communities contained in 4000-gallon, plastic pools, 4.5 m (15 ft) in diameter and 1.2 m (4 ft) deep. Eight pools (Fig. 1) were set up on a bulldozed area covered with sand near the Pamlico Marine Laboratory (Aurora, North Carolina).

The pools were all filled at the same time with estuary water from South Creek Estuary (a tributary of the Pamlico River Estuary) and its attendent phytoplankton and zooplankton. Small plastic basins were filled with sediment plus oysters, clams and aquatic plants (*Ruppia* and *Potomogeton*). The number and volume of all the organisms were recorded before they were randomly placed in the basin and the basins randomly placed in the pools. In addition to these animals and plants, several blue crabs (*Callinectes* sp.), a large number of grass shrimp (*Palaemonetes pugio*) and some small fish (flounder, killifish, pinfish and spot) were placed in the pools. All these plants and animals are found in the Pamlico River Estuary.

To study the effects of thermal loading on these artificial estuarine ecosystems, half of the pools were heated 5 C above ambient temperature. The remaining pools were used for control (*i.e.*, they were allowed to remain at ambient temperature). Domestic sewage was added to the heated ponds and to ambient ponds to study the interaction of sewage with heat.

III. B. Heated Water Circulating System

Hot water, circulated from a 1200-gallon per hour industrial water heater, provided the heating for thermal loading (Fig. 2). Heat exchangers were made

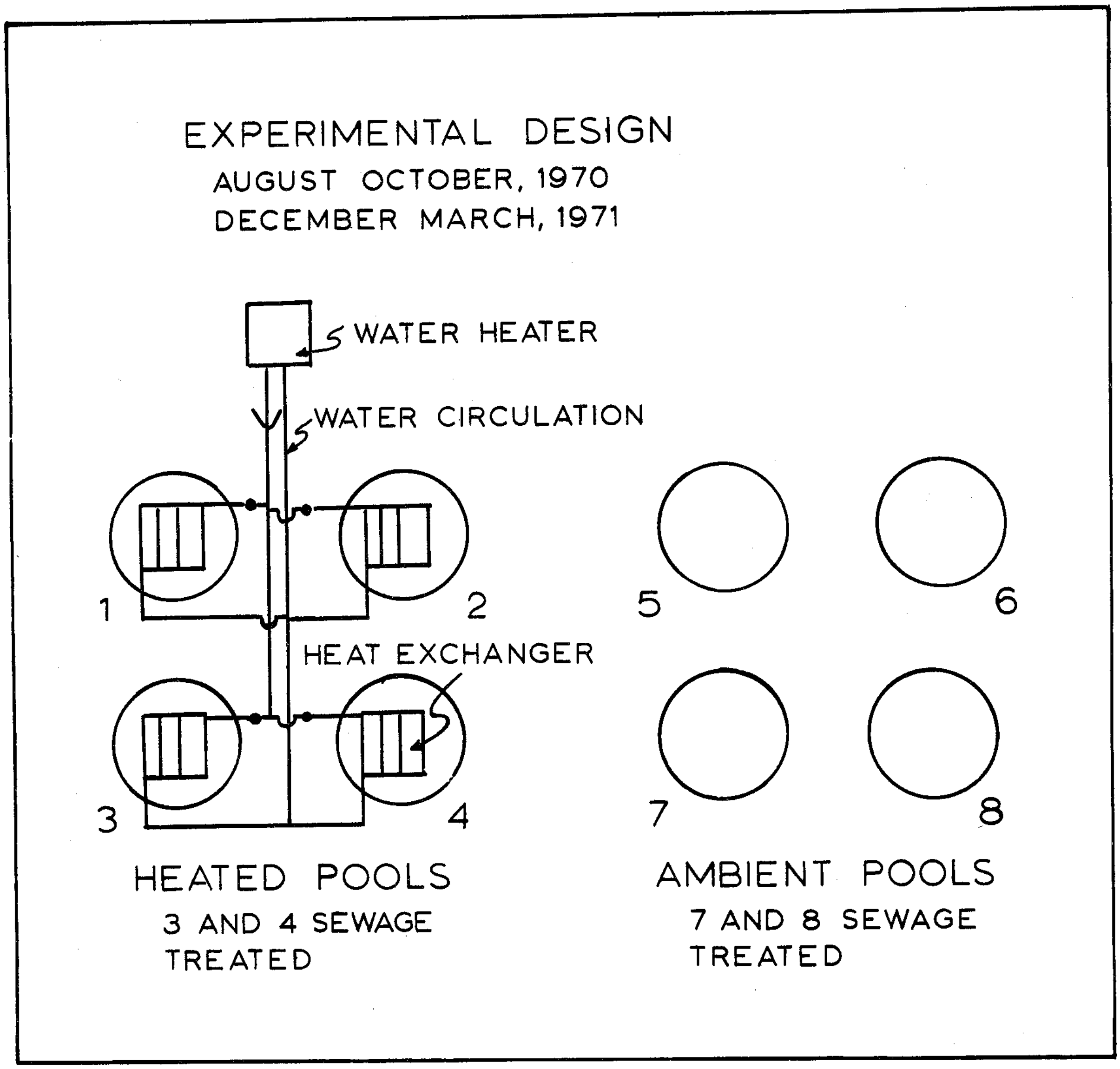

Figure 1. Diagram of experimental estuarine ecosystems for heated water experiments.

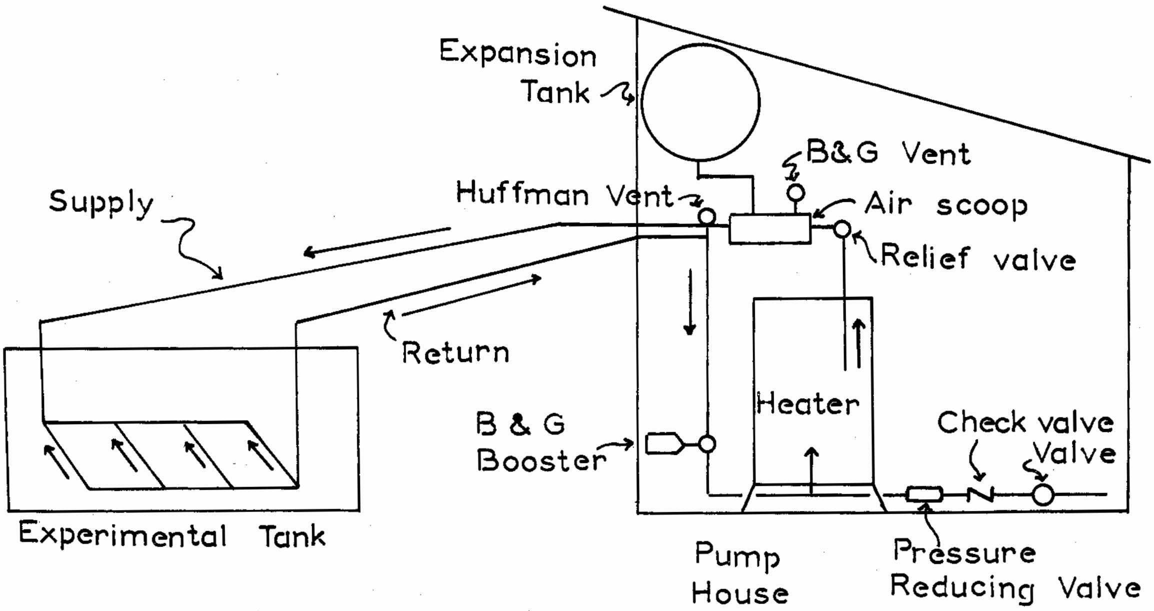

Figure 2. Design of heated water circulating system.

of galvanized pipe (scraped and cleaned) coated with several layers of epoxy. Chemical analyses for zinc in the water verified that the epoxy effectively sealed the pipes. Circulation of hot water between the water heater and heat exchangers in the experimental pools was faciliated by the installation of an air scoop equipped with a Huffman vent and B & G vent. A mercury thermostat control placed in the experimental pools was connected to a circulating pump (B & G Booster in Fig. 2) to enable to ± 0.5 C temperature control in the heated pools. Temperature measurements in all pools verified that temperature in the heated pools could be maintained at 5 C above ambient, even under fluctuating ambient conditions.

III. C. Experiments

In order to detect seasonal differences and acclimation of organisms in different species (as well as to temperature levels), experiments were conducted over an annual cycle; _i.e._, February 1970-August 1971. A brief description of each experiment follows.

1. <u>Spring conditions</u> -- This experiment was conducted during February-May 1970 and covers the late winter-early spring time span and serves as a measurement of heat effects during times of intermediate temperatures. Only four pools were used in this experiment--two of them heated and two remaining at ambient.
2. <u>Summer conditions</u> -- This experiment was conducted to determine the effects of thermal loading during the time that estuarine waters are normally relatively warm. The experiment was initiated on 1 August 1971 and carried through the middle of October 1971. Eight pools were filled with water and estuarine systems established; four receiving heat and four remaining at ambient. In addition to the heat stress, sewage from the settling pond at the Aurora, N. C. Sewage Plant was added at the rate of 10 gallons per pool per week in two of

the heated pools and two of the ambient pools. This was done to test the interacting effects of both temperature and sewage on estuarine systems.

3. Winter conditions -- In order to test effects of normally low temperatures, an experiment was conducted during the winter of 1970-71 (1 December 1970 to the middle of March 1971). Eight pools were used in the experiment, four heated and four ambient. The untreated sewage from the Aurora Sewage Plant was added at the rate of 10 gallons per pool per week to two of the heated pools and two of the ambient pools.

4. Flow-through experiments -- In the upper reaches of estuaries where the dominent biological community is made up of attached and benthic organisms unable to avoid changes in the water above them, any heated water effluent would tend to flow through the system. Thus, we needed to be able to assess the ecosystem response to heated water passing over these organisms. During the summer of 1970 (June-August), four of the pools were modified to enable flow-through conditions (*i.e.*, a siphon drain and a continuous input from South Creek Estuary). Two of the flow-through systems were heated 5 C above ambient and two remained at ambient conditions. Thus, we were able to test the effects of heat in an estuarine system with continuously moving water.

III. D. Techniques

III. D. 1. Temperature and Salinity

Temperature was monitored in each pool with a thermister probe. Temperature was measured at 8:00 AM each day the experiments were in progress. On special occasions (such as the measurement of cambium metabolism) temperature was monitored on hourly basis for one day at a time. Several measurements were taken at the surface and near the bottom to verify the absence thermocline.

Salinity was measured with a Beckman RS5-3 Induction Salinometer at weekly intervals in each pool. Occasional sample were taken from the surface and near the bottom to establish possible differences with depth.

III. D. 2. Nutrient Chemistry

Water samples for phosphorus analysis were taken at two week intervals from each pool. Analysis methods followed in general those proposed by Strickland and Parsons (30). Part of the sample was filtered through Gelman Type A glass fiber filters prior to analysis and part of the unfiltered and part of the filtered sample was digested by potassium sulfate oxidation method of Menzel and Corwin (31), using an autoclave. All three samples were then analyzed by the mixed reagent method of adding molybdic acid, ascorbic acid and trivalent antimony. The three types of phosphorus were as follows:

1. not filtered-digested-analyzed=total unfiltered P;
2. filtered-digested-analyzed=total filtered P; and
3. not filtered-not digested-analyzed=reactive P.

The color development was read on a Beckman DU II Spectrophotometer and calibrated against standards. The phosphorus concentrations were corrected for differences in salinity.

Water samples for nitrogen analysis were taken every two weeks from each pool and analysed in the laboratory following the procedures of Strickland and Parsons (30). Nitrate, nitrite and ammonia (+ amino acids) nitrogen were analyzed. Color development was read on a Beckman DU II Spectrophotometer and compared to standards.

III. D. 3. Community Metabolism

Community metabolism was measured by following diel changes in the partial pressure of carbon dioxide in the pool water. Carbon dioxide partial pressure (pCO_2) was measured to an accuracy of $\pm$ 1.5 ppm by volume in an equilibrated air phase after vigorous mixing with a water sample (see 32 for detailed discussion of methods). An equilibrator mixed the air phase with the water sample recirculating air through the water sample as illustrated in Figure 3). The water sample was continuously pumped through the equilibrator and discarded through an overflow. The equilibrated air was next recirculated through a magnesium perchlorate drying chamber and an infra-red CO_2 gas analyzer, Beckman IR 215 with 13.5 inch sample cells, at a flow rate near 100 ml/min. Water was pumped through the quilibrator at a flow rate of 0.8-1.0 liter/min. In addition, water and air flow were metered and adjusted when necessary. Also, a recorder monitored the pCO_2 that plateaued as the air phase reached equilibrium with the water phase. Generally, the required time for equilibrium was five minutes, but it depended on the pCO_2 difference between the sample and the previous sample. Since there was instrument drift, the gas analyser was standardized at 15 min. intervals with 1,000, 500, and 0 ppm by volume CO_2 standard gases.

Diel changes in total CO_2 were calculated from observed temperature, pCO_2, and diel pH curves. The diel pCO_2 curves were used to calculate diel total CO_2 durves for pCO_2 levels above 10 ppm (32) and they were of acceptable precision and accuracy for the pCO_2 range of 10-50 ppm. However, diel pH curves were used for pCO_2 levels below 10 ppm since pH changes per unit metabolism are large and pCO_2 changes are negligible per unit metabolism (32).

Community metabolism (photosynthetic productivity and community respiration) was estimated by calculating rates of total CO_2 change over a 24-hour cycle.

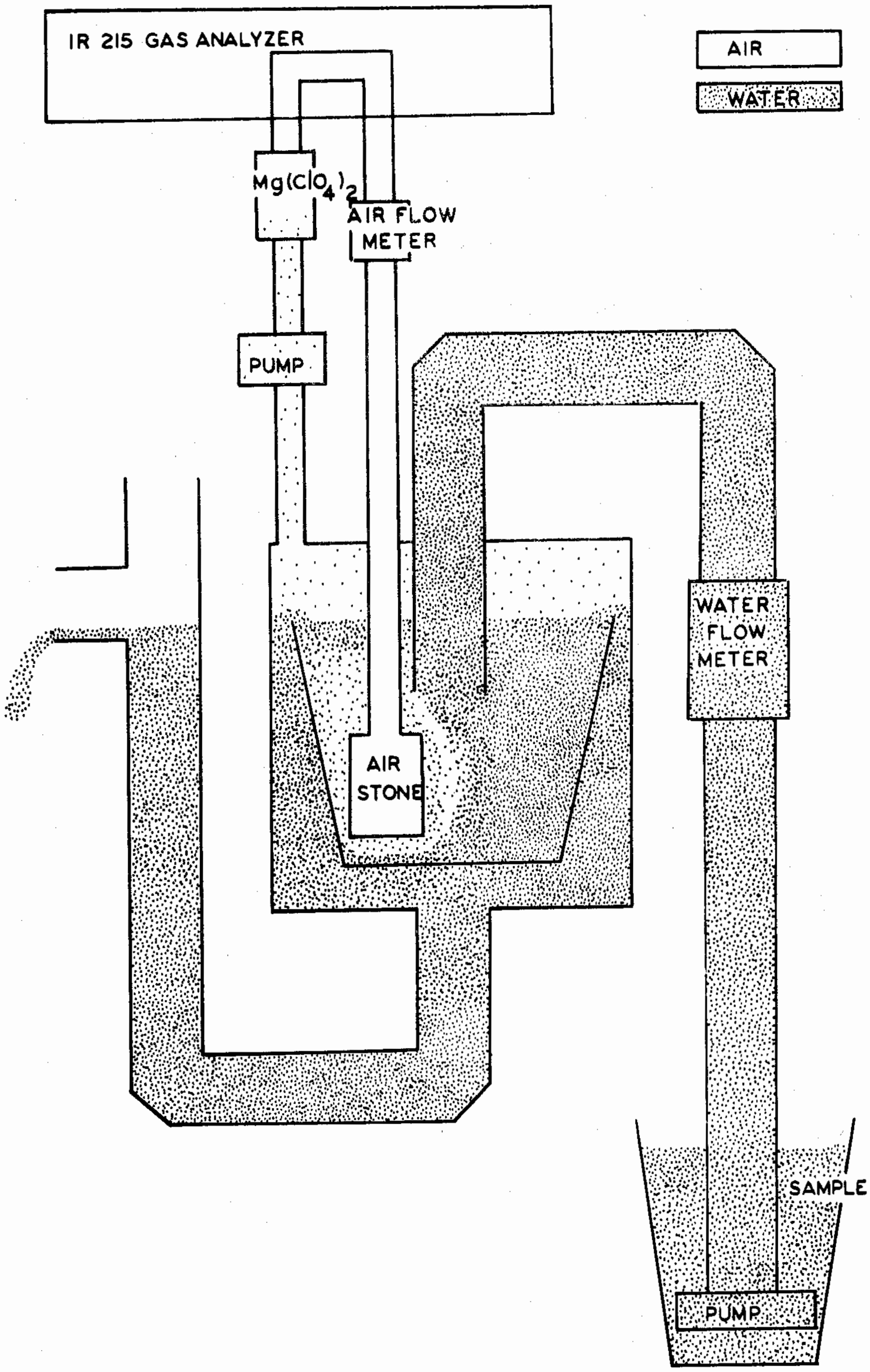

Figure 3. Schematic diagram of the equilibrator used in the determination of carbon dioxide partial pressure (pCO_2) in estuarine water.

In this study, the definition for the rate of community metabolism is categorized into gross photosynthetic productivity, total community respiration, and net community metabolism. Gross photosynthetic productivity is sometimes referred to as just productivity in this report and is defined as the rate of photosynthetic and chemosynthetic assimilation of carbon dioxide and/or bicarbonate by the community within a pool. This includes losses of assimilated carbon dioxide and bicarbonate by respiration, death, grazing, or predation. The rate of total community respiration is sometimes referred to as just total respiration in this report and is defined as the rate of respiration of the aquatic plants, animals, and bacteria within a pool. The rate of net community metabolism is defined as the difference between gross photosynthetic productivity and the rate of total community respiration. Hence, net metabolism may be net productivity in which there is a gain in the amount of organic matter within a pool or it may be net respiration in which there is a loss in the amount of organic matter in a pool. The photosynthetic productivity/community respiration (P/R) ratio is defined as the ratio of gross photosynthetic productivity to the rate of total respiration.

III. D. 4. Organism Response

Phytoplankton were collected by collecting 100 ml of water and adding 2 ml of Lugol's solution as a preservative. Phytoplankton were identified and enumerated under an inverted microscope according to the techniques outlined by Hobbie (33). Dr. Edward Carpenter, Woods Hole Oceanographic Institute, Woods Hole, Massachusetts, a former graduate student at the Pamlico Marine Lab, assisted in the analysis of phytoplankton. Chlorophyll *a* was measured according to the procedure outlined by Lorenzen (34).

Zooplankton were sampled by pouring 10 1 of water through a number 20 plankton net and saving the filtered organisms. Zooplankton were identified and enumerated under a binocular dissecting microscope.

Various animals and plants were inserted into the pools at random. The volume of the organisms going into each station in each pool was determined in graduate cylinders prior to placing the organisms in the pools. Five clusters of oysters, 10 individuals in each cluster, were placed around the perimeter of each pool. Five groups of *Rangia* clams were placed in mud contained in five plastic containers, consisting of 15 clams per container. Several complete plants (blades and rhizomes) of *Ruppia* grass were placed in sediment within plastic containers and inserted at five places around the perimeter of each pool. Several individuals of blue crabs (*Callinectes sapidus*), grass shrimp (*Palaemonetes pugio*), and fish were measured and placed at random in each pool. The species of fish differed from season to season: *i.e.*, flounder (*Paralichthyes* sp.) for the spring experiment; pinfish (*Lagodon rhomboides*), silversides (*Menidia* sp.), spot (*Leiostomus xanthurus*), croaker (*Micropogon undulatus*) and gobie (*Gobiosoma* sp.) were used in the summer experiment; pinfish (*Lagodon rhomboides*) were used in the winter experiment; and spot (*Leiostomus xanthurus*) and gobie (*Gobiosoma* sp.) were used in the flow-through experiment (summer 1971).

At the end of each experiment the organisms were removed and the volume of each group obtained. The organisms were dried in a drying oven at 65 C and dry weight determined with a Mettler top-loading balance. The pools were then drained and all small organisms filtered from the water. In addition to the grass shrimp and fish, these included polychaete worms, midge larvae and several other types of organisms, apparently resulting from eggs and larvae in the water at the beginning of the experiment. Changes in volume were regarded as the criteria for growth and changes in numbers were used as criteria for survival.

A conversion factor was obtained to relate changes in volume to changes in dry weight for all organisms (Table 1). The standard deviation was relatively small for each conversion factor although the conversion factors did change seasonally. The volume to weight conversion factors will enable conversion of all growth reported in the following pages to changes in biomass for energy calculations.

IV. TEMPERATURE AND NUTRIENTS

IV. A. Temperature

The temperature in the pools showed small diurnal changes (Fig. 4), but larger annual changes (Fig. 5). Diurnal temperature changes were 3-4 C, but annual temperature changes were approximately 26 C in the ambient pools and approximately 22 C in the heated pools.

Generally there were only small temperature differences within treatments (heated or ambient pools), but larger differences between treatments. The temperatures in one of the sewage treated pools were as much as 6 C less than the other ambient pools since the black liner increased the rates of back radiation. Otherwise temperature differences between heated pools and between ambient pools were less than 1 C. In contrast, temperatures in the heated pools were 2-5 C, 3-5 C, and 0-12 C higher than the temperatures in the ambient pools during the spring, summer and winter experiments, respectively.

The thermal treatment was slightly irregular since the thermostat control needed frequent adjustment.

IV. B. Nutrients

Comparison of phosphorus and ammonia levels in the sewage pools with the non-sewage pools suggests that nutrient-poor sewage was used during the summer

Table 1: Conversion factors for volume to gms dry weight for each organismal group and each experiment.

Organism	Mean gm/ml	Standard Deviation	Range	Sample size
Experiment 1 - February-June, 1970				
Blue Crab	0.3106	0.0780	0.2247 to 0.3770	3
Flounder	0.8543	0.5704	0.2398 to 1.3667	3
Rangia	1.3521	0.0696	1.22 to 1.469	12
Oyster	1.0427	0.2038	0.6913 to 1.2667	11
Ruppia	0.0888	0.0657	0.010 to 0.250	15
Palaemonetes	0.0917	0.0256	0.0632 to 0.1193	4
Experiment 2 - August-October 1970				
Blue Crab	0.2051	0.0825	0.1122 to 0.3164	8
Fish				
Pinfish	0.2735	0.0322	0.2367 to 0.320	8
Silversides	0.2395	0.0242	0.1983 to 0.2645	8
Spot, Croaker	0.2144	0.0608	0.0865 to 0.2968	10
Gobie	0.1941	0.0739	0.1312 to 0.3110	5
Rangia	1.3656	0.0411	1.2514 to 1.4568	40
Oyster	1.2670	0.1136	1.0265 to 1.4320	40
Ruppia	0.1891	0.0703	0.0820 to 0.3520	22
Palaemonetes	0.1596	0.0289	0.1176 to 0.1985	7
Experiment 3 - December 1970-March 1971				
Blue Crab	0.2659	0.0374	0.2194 to 0.3293	8
All Fish	0.2647	0.0618	0.1829 to 0.3470	6
Rangia	1.3192	0.0423	1.1895 to 1.3971	35
Oyster	1.2652	0.1342	0.9977 to 1.4773	35
Ruppia	0.1061	0.0318	0.0540 to 0.2011	34
Experiment 4 - June-August 1971				
Blue Crab	0.0145	0.0053	0.0085 to 0.0184	3
Fish				
Spot	0.2152	0.0213	0.1840 to 0.2312	4
Gobie	0.1283	0.0335	0.090 to 0.1520	3
Rangia	1.3354	0.1091	1.2534 to 1.7535	20
Oyster	1.2817	0.1235	0.8859 to 1.4606	21
Ruppia	0.0869	0.0596	0.0049 to 0.2520	16
Palaemonetes	0.1373	0.0322	0.0946 to 0.1667	4

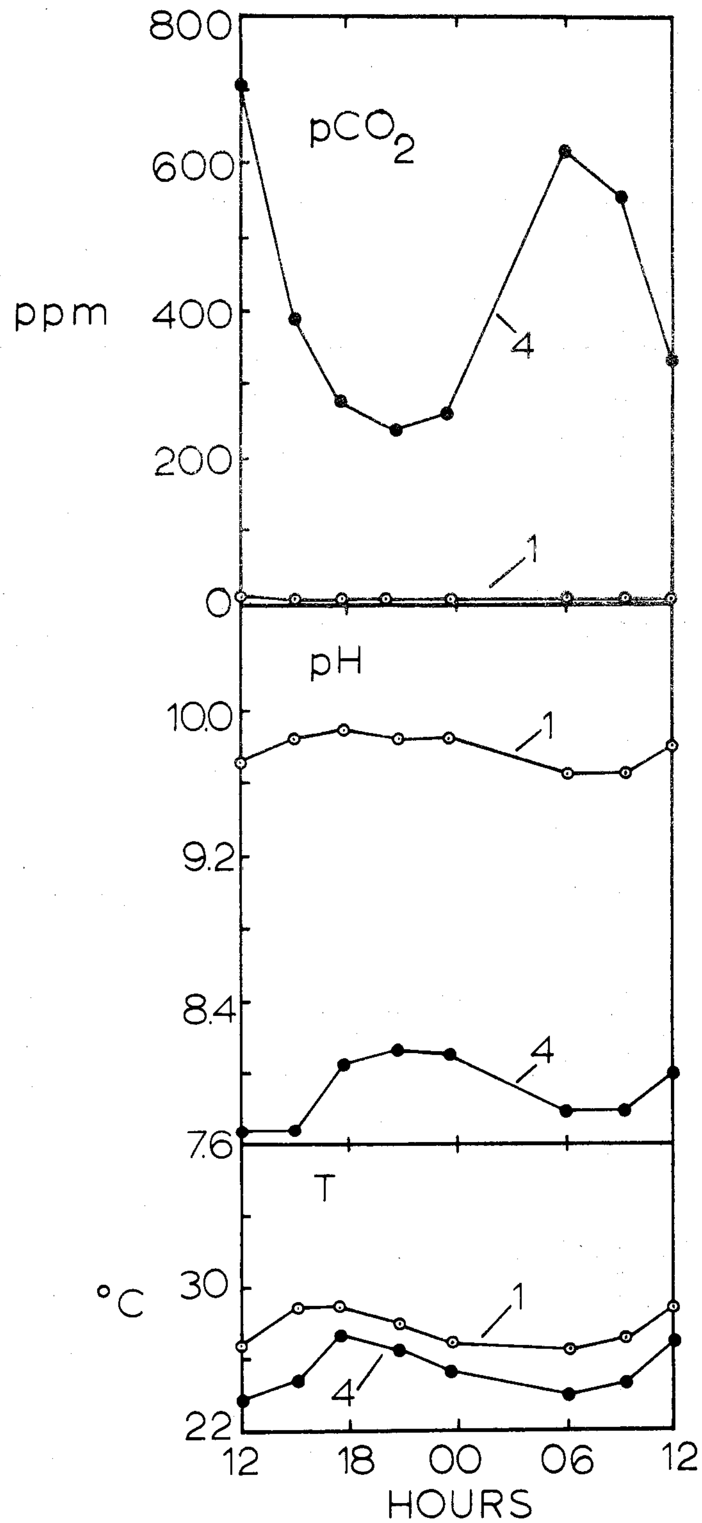

Figure 4. Comparison of diel pCO_2, pH, and temperature in heated (pool 1) and ambient (pool 4) pools on 20 May 1970.

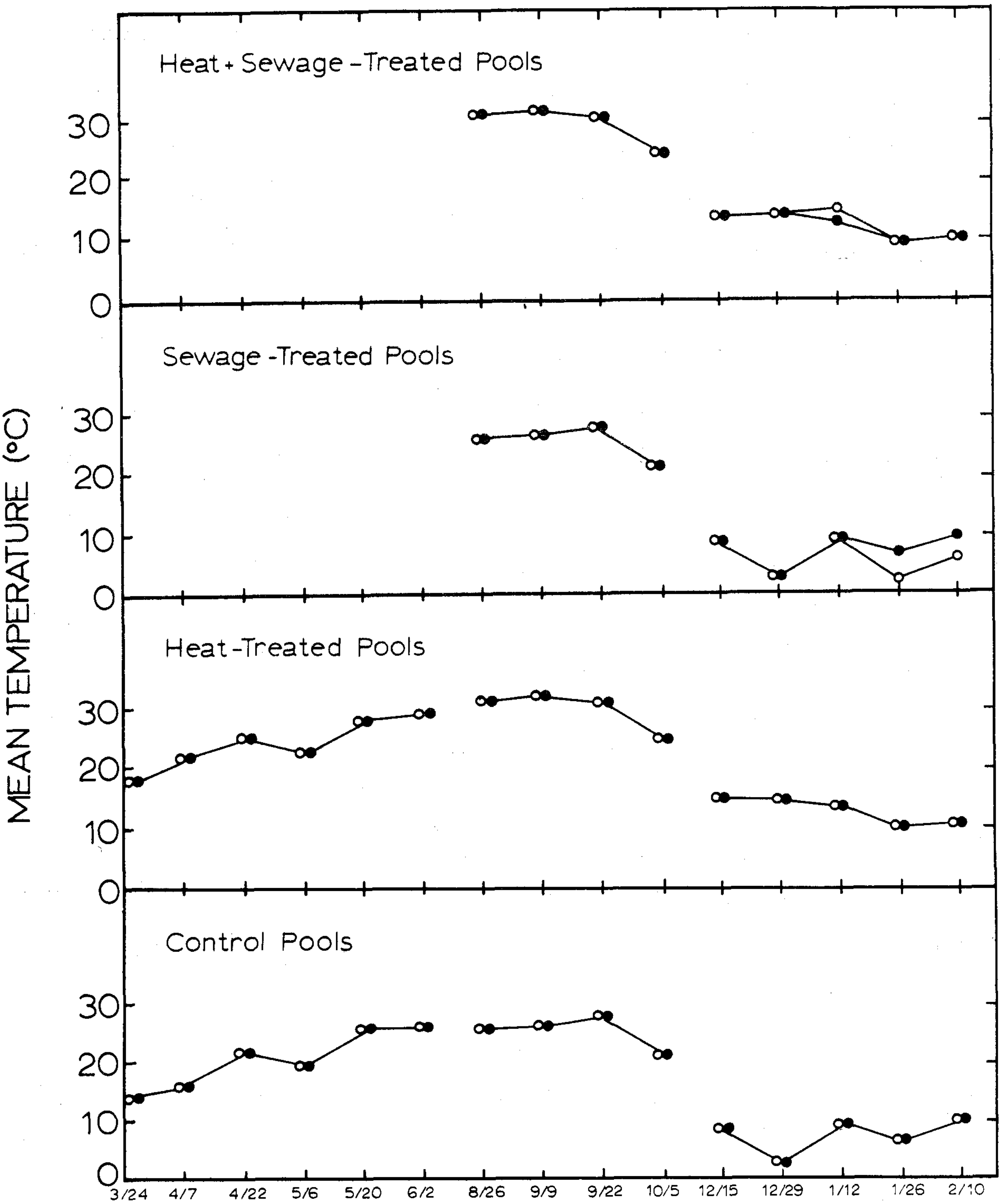

Figure 5. Mean daily temperatures at two-week intervals in the ambient (control), heated, sewage-treated, and heated+sewage-treated pools during the spring, summer and winter experiments. Points indicate temperature for each replicate.

experiment, but nutrient-rich sewage was used during the winter experiment (Figs. 6, 7, 8 and Table 2). Water from a secondary settling pond was used during the summer experiment, but sewage from a primary settling pond was used during the winter experiment. In addition, there were no differences between reactive phosphates and ammonia levels in the sewage and non-sewage pools during the summer experiment (Table 2). Also, there were no significant differences between total phosphorus levels in non-sewage and sewage pools during the summer experiment, but there were significant differences between total phosphorus levels in the sewage and non-sewage pools during the winter experiment (Table 2) according to Tukey's w test (35). Furthermore, the reactive phosphate levels in the sewage pools were 1-4 mg-at P/m^3 higher than the phosphate levels in the non-sewage pools and ammonia levels in the "sewage treated" pools were 1-10 mg-at N/m^3 higher than the other pools during the winter experiment.

IV. C. Discussion

Thermal treatment probably increased the nutrient regeneration rates during the spring experiment and possibly during the winter experiment, but not during the summer experiment (Figs. 6 and 7). During the winter experiment, the reactive phosphate levels in the heated pools were similar to the controls, but they were higher than the controls during the summer and spring experiments. In addition, the ammonia levels in the heated pools were similar to the controls during the summer and winter experiments, but they were higher than the controls during the spring experiments.

The nutrient regeneration rates and cycles were dependent upon the temperature and light levels. For example, during the spring experiment temperature and respiration rates gradually increased (Section V), and, in turn, the decomposition

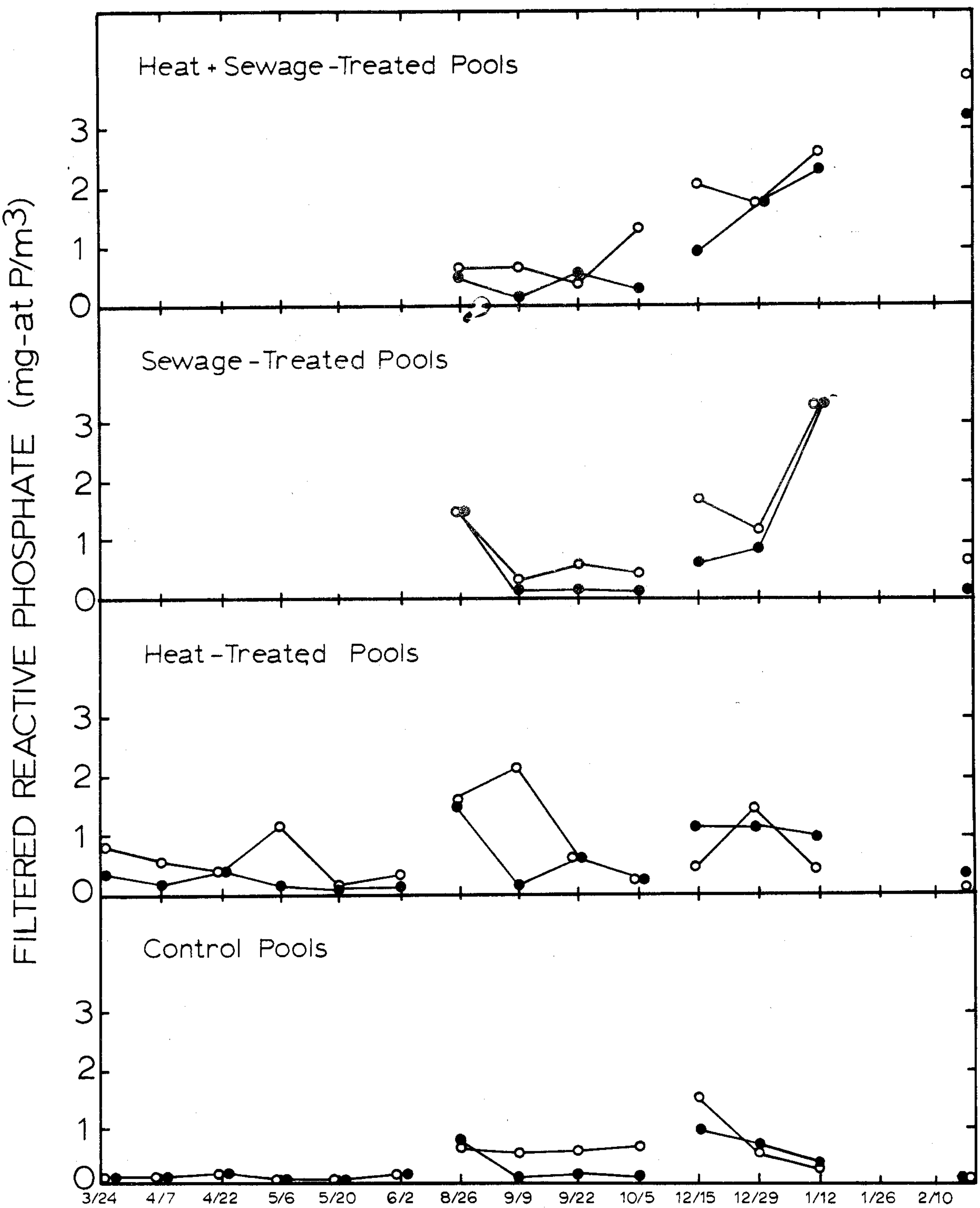

Figure 6. Filtered reactive phosphorus at two-week intervals in the ambient (control), heated, sewage-treated, and heated+sewage treated pools.

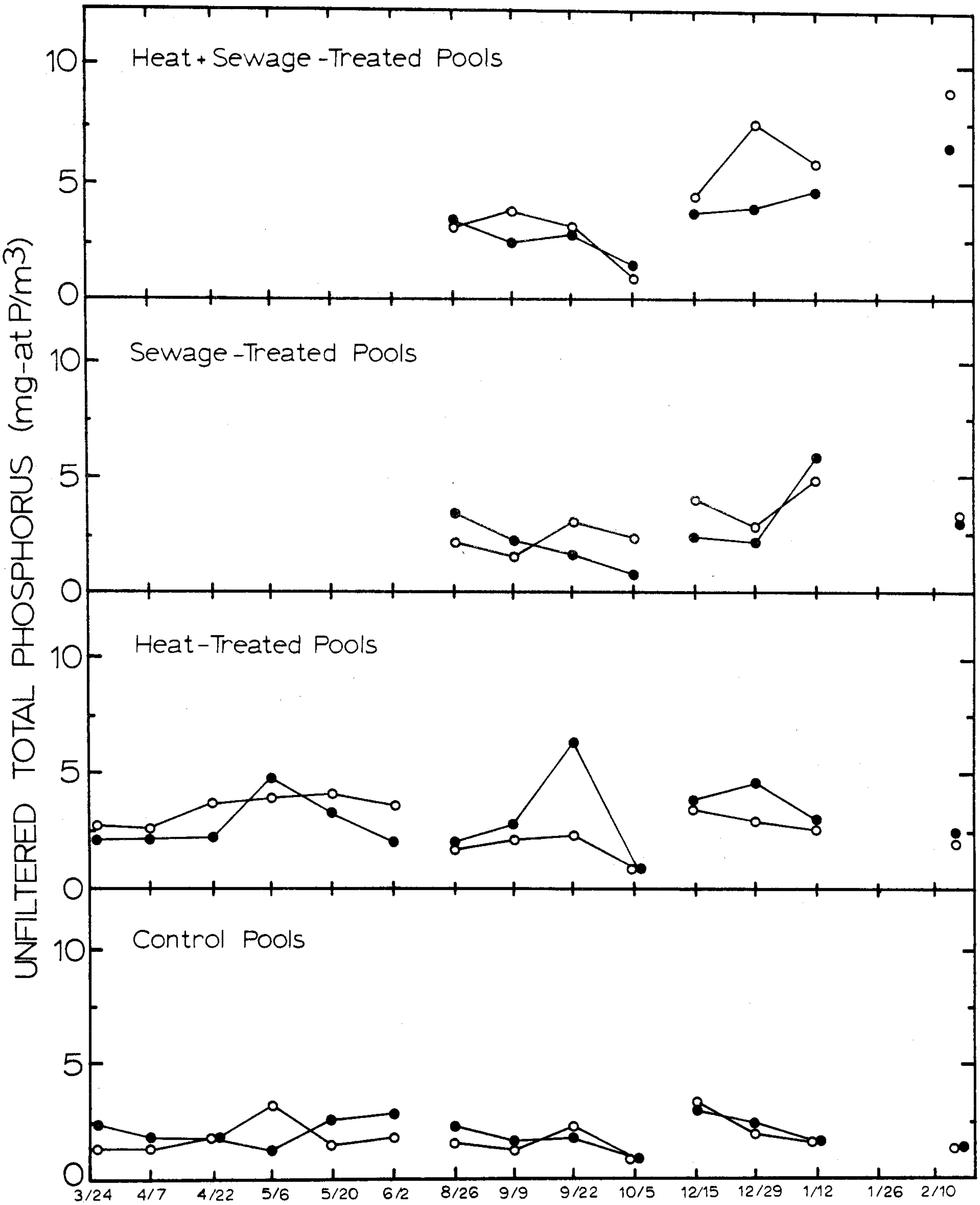

Figure 7. Unfiltered total phosphorus at two-week intervals in the ambient (control), heated, sewage-treated, and heated+sewage-treated pools.

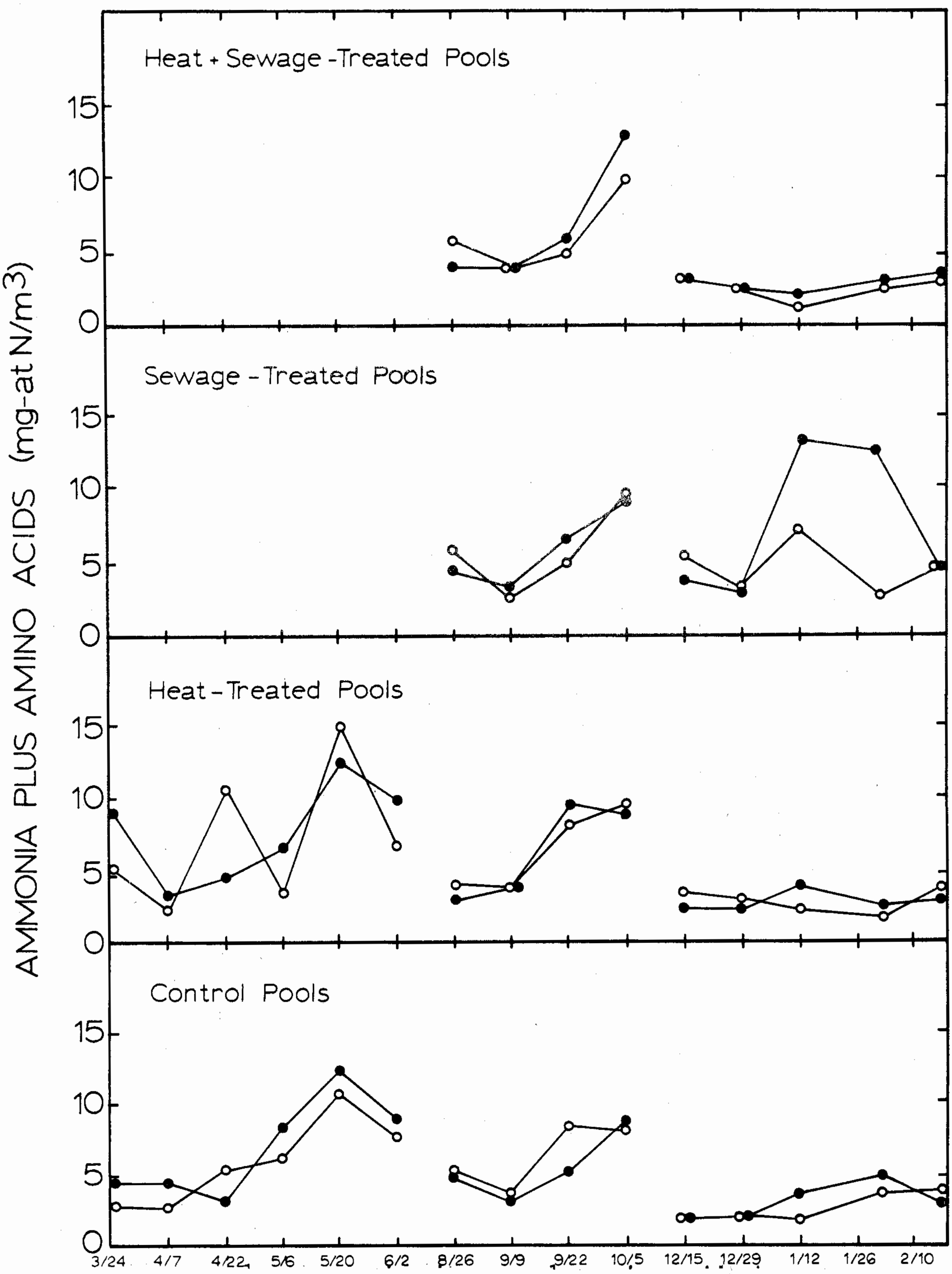

Figure 8. Ammonia (+ amino acids) nitrogen at two-week intervals in the ambient (control), heated, sewage-treated, and heated + sewage-treated pools.

Table 2. Tukey's w values calculated for total unfiltered phosphorous levels and hypothesis accepted using the 95 percent confidence interval.

Null hypothesis	w	Significance level
Pooled sewage systems (S) = Pooled non-sewage systems (S)	0.193	0.05, N. S.
Pooled sewage systems (W) = Pooled non-sewage systems (W)	4.415	0.05, sewage (W) > non-sewage (W)
Pooled sewage systems (W) = Pooled sewage systems (S)	4.999	0.05, sewage (W) > sewage (S)
Pooled sewage systems (W) = Pooled non-sewage systems (S)	5.192	0.05, sewage (W) > non-sewage (S)
Pooled sewage systems (S) = Pooled non-sewage systems (W)	-0.584	0.05, N. S.
Pooled non-sewage systems (S) = Pooled non-sewage systems (W)	-0.777	0.05, N. S.

Note: The abbreviation (S) refers to summer systems and (W) refers to winter systems of organic matter increased the pCO_2 and ammonia levels. After high ammonia levels accumulated, primary production increased rapidly and reduced the ammonia levels. Simultaneously, feeding rates of heterotrophs probably multiplied as the supply of organic carbon rose during the bloom. Consequently, there was a rapid increase in respiration followed by rises in pCO_2 and ammonia levels as heterotrophs oxidized the organic matter produced during the bloom. As temperatures and light increased to maximal levels, a second cycle occurred during the spring experiment, and then two cycles were observed during the summer experiment. Yet, only one cycle occurred during the winter experiment as temperatures declined to minimal levels. In conclusion, the nutrient regeneration cycles followed sinusoidal patterns that had frequencies of six weeks when temperatures were above 20 C, but the frequencies were eight weeks for lower temperatures.

The added heat affected nutrient regeneration rates during the early part of the spring experiment and during the winter experiment, but it did not affect the nutrient regeneration rates during the latter part of the spring experiment or during the summer experiment. Thermal treatment probably increased the decomposition rates of organic matter to ammonia, phosphate, and carbon dioxide, since both concentrations of these compounds and respiratory rates increased during the spring experiment. As a result, accumulated nutrients triggered an early spring bloom. On the other hand, thermal treatment apparently did not affect the nutrient regeneration rates during the late part of the spring experiment or the summer experiment since metabolism was not affected by the thermal treatment. At that time, metabolism may have been independent of small temperature changes since the upper tolerance temperature had probably been approached and the peak of the metabolic-temperature had probably been approached and the peak of the metabolic-temperature response curve had been reached. However, it is likely that the nutrient regeneration rates will be increased by thermal treatment during the late fall as normal temperatures decline to limiting levels. Yet, thermal treatment (0-12 C) stabilized the nutrient regeneration rates during the winter experiment since the higher temperatures were more constant.

The nutrient regeneration rates were possibly reduced by the sewage treatment during the summer experiment, but not during the winter experiment. The sewage treatment caused blue-green algae to replace the natural phytoplankton (Section VI); consequently, the heterotrophs were forced to utilize the blue-green algae as a source of organic carbon during the summer-fall experiment. This source of carbon may have been undesirable for the heterotrophs since the summer ecosystem was programmed to utilize organic carbon fixed by a diverse community of diatoms, greens, blue-greens, and Chrysophytes. Ryther (36) suggested that duck farm manure leaching into Great South Bay and Moriches Bay fertilized and changed the N/P ratio; and therefore, caused the natural phytoplankton community to be almost replaced by new species of algae. Since the oysters were unable to digest these new species, they starved. Therefore, a decrease in usable organic matter in the sewage treated pools could have reduced the oxidation rates of organic matter and availability of nutrients. As a result, the productivities declined during the second half of the summer experiment. To the contrary, sewage treatment probably did not affect the nutrient regeneration rates during the winter experiment since the metabolic rates in the two sewage treated pools were similar to the controls.

Thermal treatment may have increased the nutrient regeneration rates in the heat+sewage-treated pools during the summer experiment, but stabilized the nutrient regeneration rates during the winter experiment. An increase in the community metabolism associated with the treatments suggested that the nutrient regeneration rates increased during the summer experiment. In contrast, the community metabolism during the winter experiment remained constant possibly because the nutrient regeneration rates were stabilized by the relatively constant temperature. These effects may be explained partly by the slight temperature increase that stimulated metabolism during the summer-fall experiment and the constant temperature that probably stabilized the phytoplankton community during the winter experiment. In conclusion, sewage treatment probably had little affect on the nutrient regeneration rates during the summer experiment since secondary sewage was used; yet, sewage treatment may have interacted with heat treatment during the winter experiment when primary sewage was used.

V. COMMUNITY METABOLISM

Community metabolism, consisting of photosynthetic productivity and respiration, is a useful means of evaluating ecosystem response (37). Photosynthetic productivity is a measure of the ecosystem's rate of fixing solar energy into chemical energy to support food chains. If this parameter is affected, then the basic function within an ecosystem is jeopardized. Respiration is a measure of the ecosystem's comsumption rate, which is the rate at which work is performed. If this parameter is affected, then the structure of the ecosystem is in jeopardy. Ratio and/or magnitudes of the two are basic means of evaluating changes in function and structure of ecosystems in relation to stress.

V. A. Gross Photosynthetic Productivity

During the spring experiment, photosynthetic rates were probably limited by the combination of low ammonia levels and low temperatures, but probably not by the reactive phosphate levels (Fig. 9). Reactive phosphate levels were not

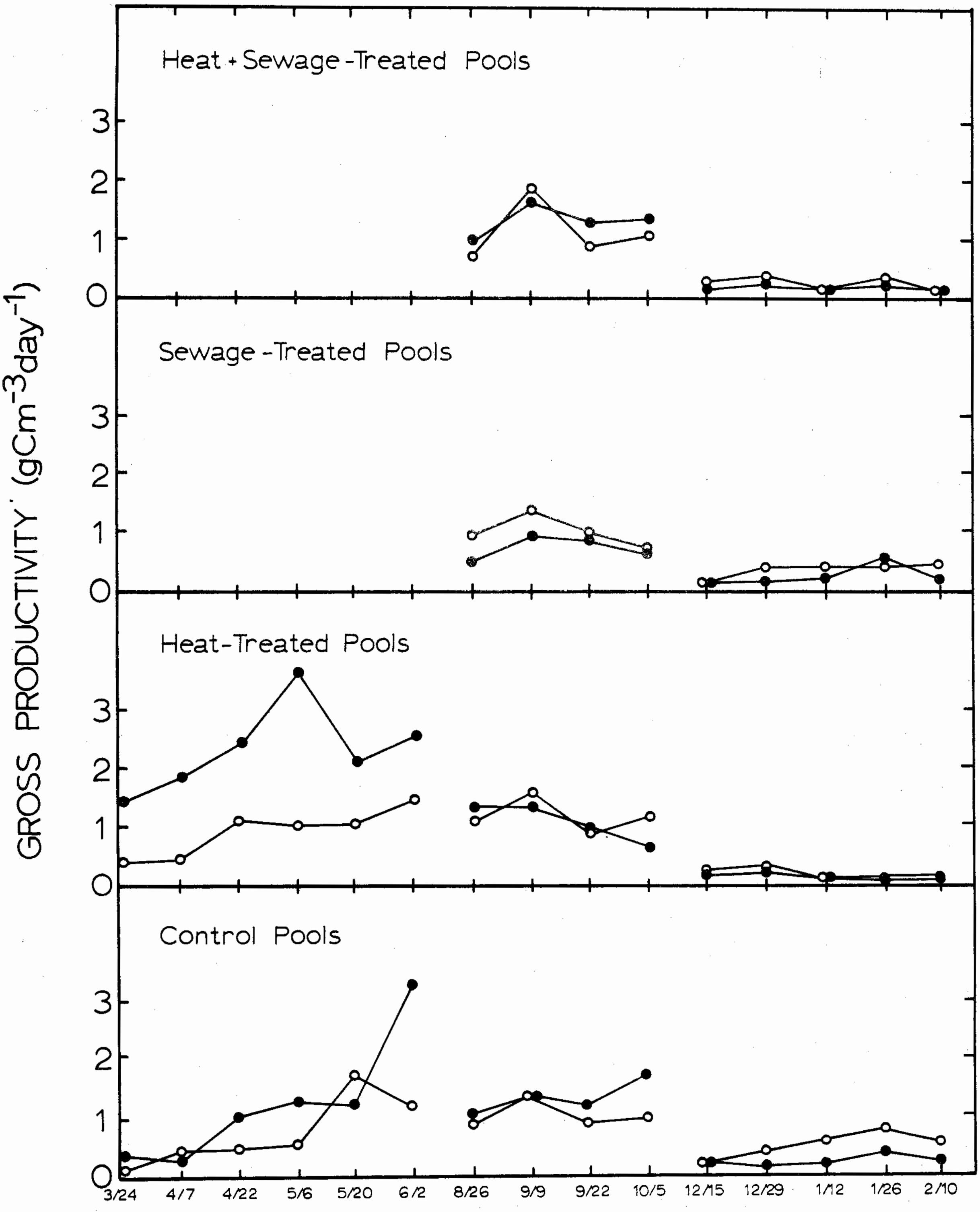

Figure 9. Gross photosynthetic productivity at two-week intervals in the ambient (control), heated, sewage-treated, and heated+sewage-treated pools.

correlated with productivity in the controls. In addition, reactive phosphate levels in the heated pools were correlated with the productivity only to the extent that they were lower in the more active pool. On the other hand, there were many correlations between the productivity and the ammonia levels and temperatures in the heated and ambient pools. Relatively high productivities, temperatures, and ammonia levels occurred in the heated pools from late March to May. In contrast, relatively low productivities, temperatures, and ammonia levels occurred in the ambient pools from late March to May. The productivities in the ambient pools increased as ammonia peaks were followed by photosynthetic pulses.

During the summer experiment, productivities may have been limited by low ammonia and reactive phosphate levels, but probably not by high temperatures (Fig. 9). Temperatures were not correlated with productivities during August and September and possible trends during October were obscured by the variability in the productivities within and between treatments. Furthermore, the thermal addition was only associated with a faint increase in photosynthetic productivity in the two heat+sewage-treated pools during early September. In contrast, many correlations existed between ammonia, reactive phosphate, and productivity. For example, ammonia declined to minimal levels as productivities peaked during early September and then increased productivities decreased during late September. This was followed by an increase in photosynthetic productivity during October. In addition, reactive phosphate decreased to minimal levels as productivities peaked in one ambient pool, one heated pool, one sewage-treated pool, and one heat+sewage-treated pool during early September.

During the winter experiment, productivities may have been limited by the combination of low ammonia levels and low temperatures, but probably not by the reactive phosphate levels (Fig. 9). The reactive phosphate levels were relatively high during the winter experiment, but the productivities and temperatures were the lowest of anytime during the year. Also, the two sewage-treated pools that had high ammonia levels showed only faint increases in production. Similarly, production in the controls slightly increased as temperatures and ammonia levels increased during January. On the other hand, production in the four heated pools was less than in the ambient pools.

Heat treatment significantly increased the response of production to temperature (regression coefficient of the productivity-temperature curve) in the artificial ecosystems during the three experiments (Table 3, Fig. 10). Heat treatment increased the productivities during the spring and summer experiments and caused them to remain relatively low and constant during the winter experiment.

Sewage treatment did not significantly change the response of production to temperature in the artificial ecosystems during the summer and winter experiments (Table 3, Fig. 11).

V. B. Community Respiration

The previous statement that heat treatment probably increased the nutrient regeneration rates is supported by correlations of increased respiration with increased temperatures, reactive phosphate and ammonia levels during the spring experiment, but not during the summer or winter experiments (Fig. 12). For example, temperatures, reactive phosphate and ammonia levels as well as respiration were simultaneously higher in the heated pools than in the ambient pools during late March, April and early May. However, respiration, reactive phosphate and ammonia levels in the heated pools were not simultaneously higher than in the ambient pools during the summer and winter experiments.

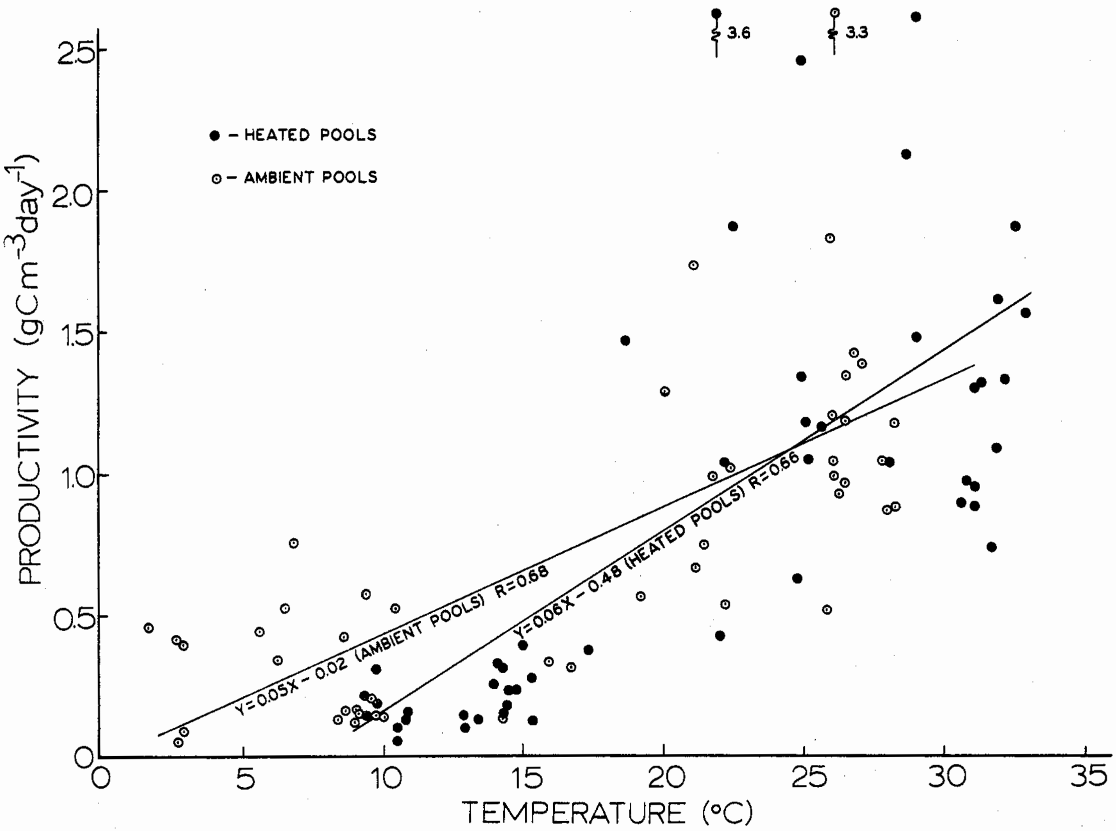

Figure 10. Gross photosynthetic productivity at different temperatures in ambient and heated pools during the spring, summer and winter experiments.

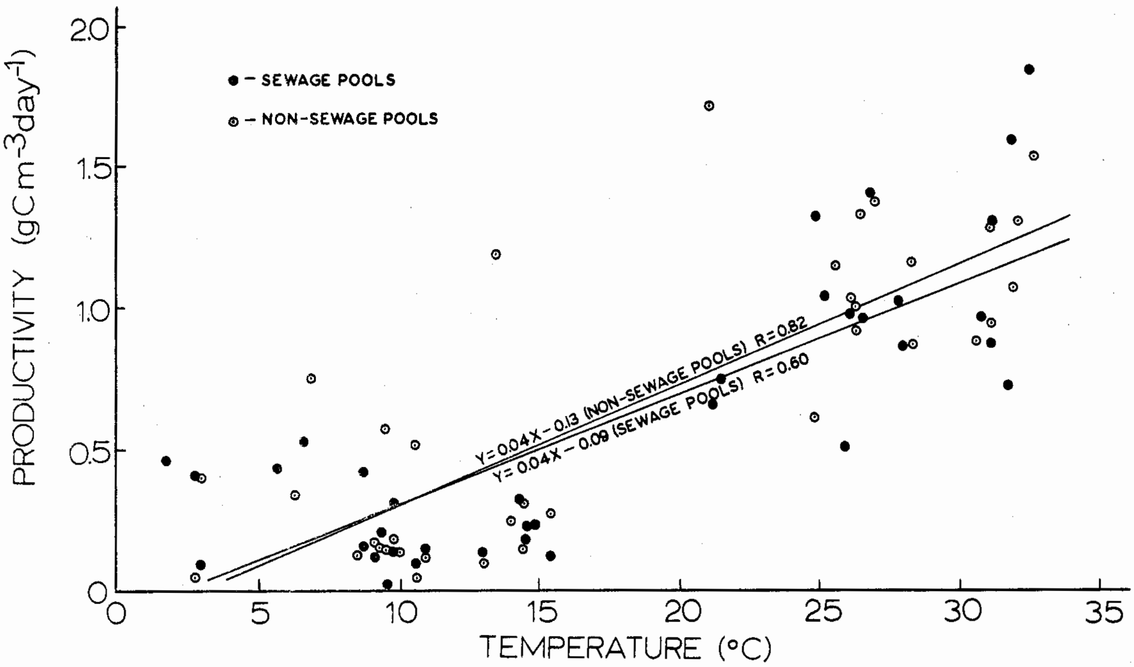

Figure 11. Gross photosynthetic productivity at different temperatures in the non-sewage and sewage-treated pools during summer and winter experiments.

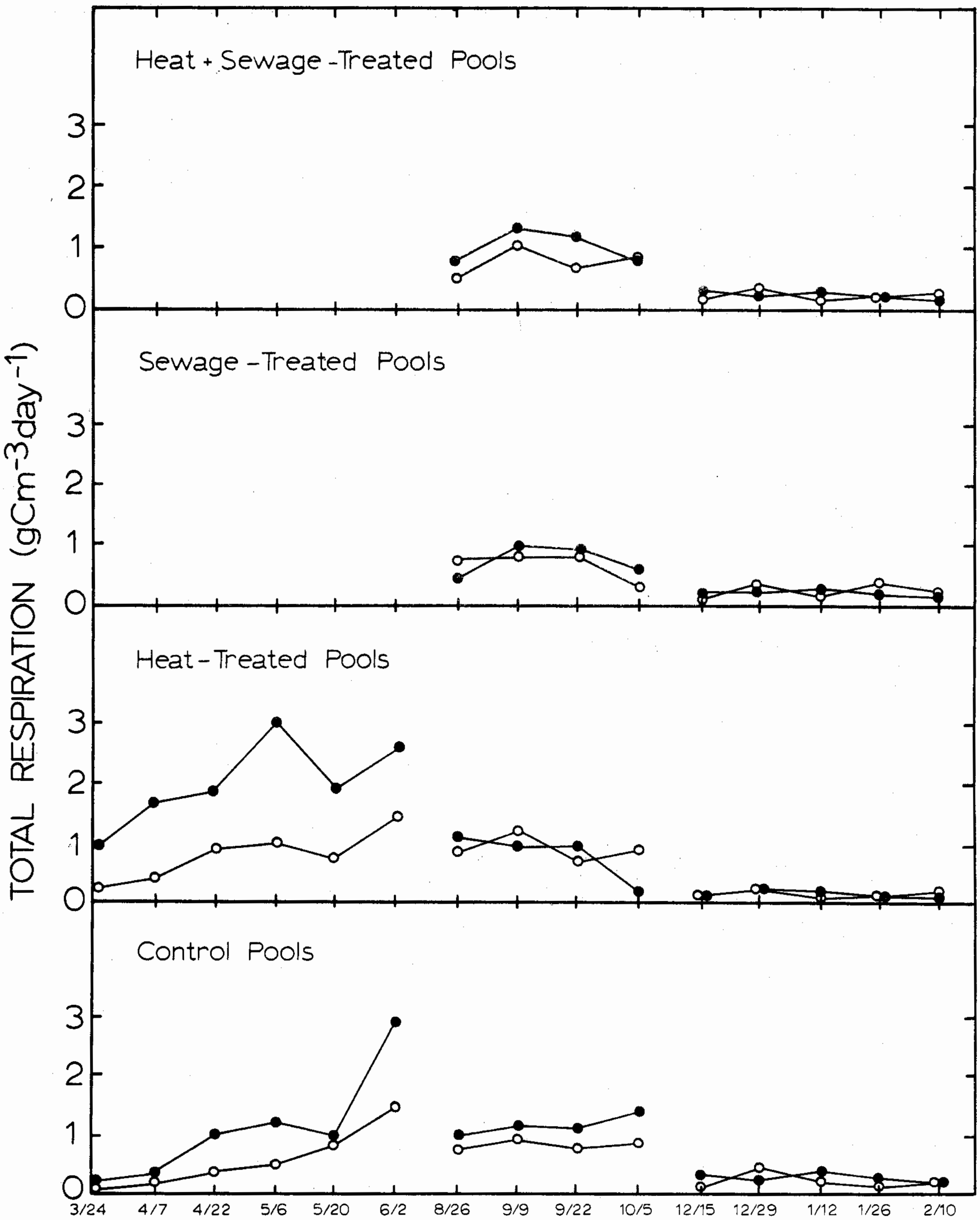

Figure 12. Community respiration at two-week intervals in the ambient (control), heated, sewage-treated, and heated+sewage-treated pools.

Table 3. Student's t values calculated for metabolism regression coefficients and hypothesis accepted using the 95 percent confidence interval.

Null hypothesis	t	Significance
Temperature vs Respiration		
Pooled ambient systems = Pooled heated systems	2.281	0.05, heated > ambient
Pooled non-sewage systems = Pooled sewage systems	1.993	0.05, N. S.
Temperature vs Gross Photosynthesis		
Pooled ambient systems = Pooled heated systems	3.491	0.05, heat > ambient
Pooled non-sewage systems = Pooled sewage systems	1.096	0.05, N. S.
Respiration vs Gross Photosynthesis		
Heated system = Heat + sewage system	1.763	0.05, N. S.
Heated system = Ambient system	1.341	0.05, N. S.
Heated system = Sewage system	1.787	0.05, N. S.
Heat + sewage system = Ambient system	2.898	0.05, heat + sewage> control
Heat + sewage system = Sewage system	2.900	0.05, heat + sewage > sewage
Ambient system = Sewage system	0.038	0.05, N. S.

Statistically greater responses of respiration to temperature were observed in the pools receiving heat treatment (Table 3, Fig. 13). Heat treatment increased the respiration rates during the spring and summer experiments, but did not change the respiration rates during the winter experiments.

Sewage treatment did not significantly affect the response of respiration to temperature in the artificial ecosystems during the summer and winter experiments (Table 3, Fig. 14).

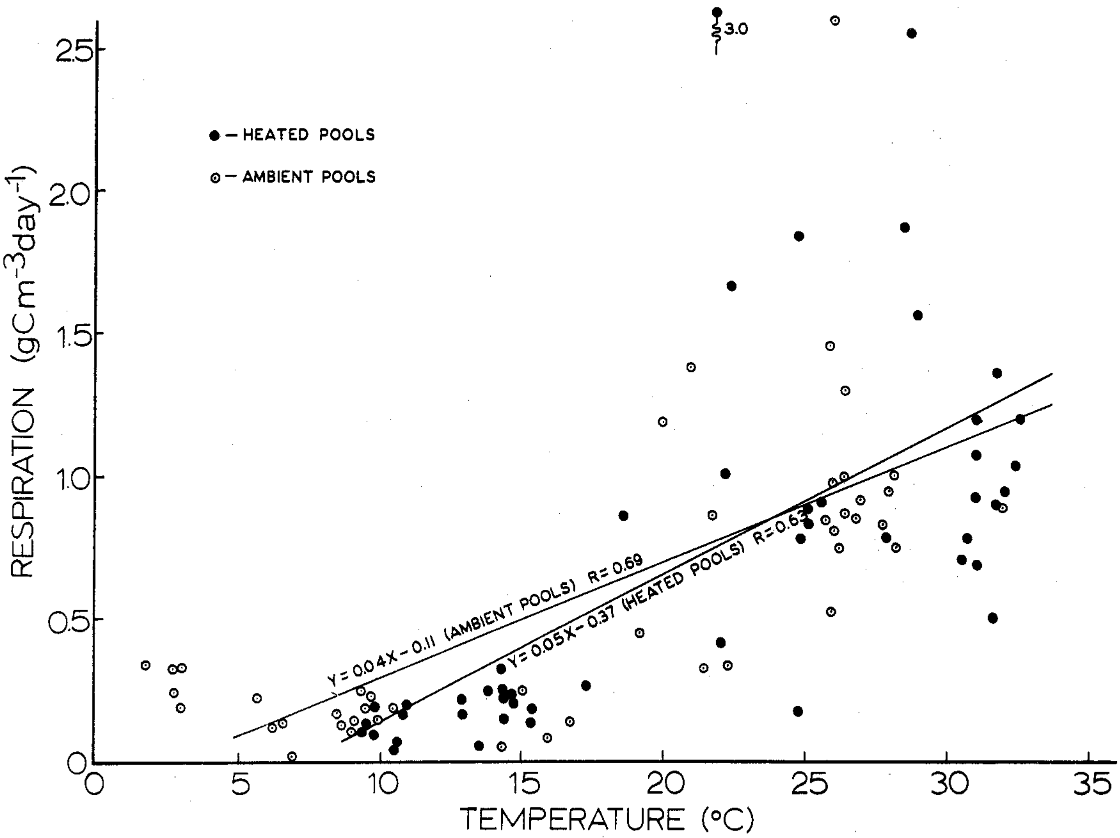

Figure 13. Community respiration at different temperatures in the ambient and heated pools during the spring, summer and winter experiments.

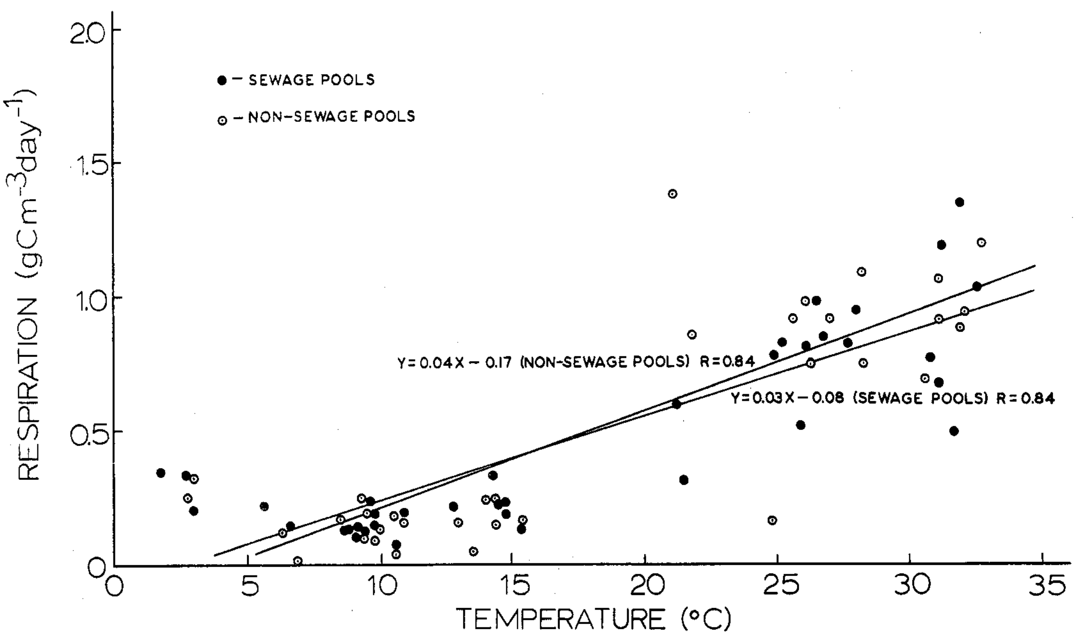

Figure 14. Community respiration at different temperatures in the non-sewage and sewage-treated pools during summer and winter experiments.

V. C. P/R Ratio and Net Metabolism Parameters

During the last half of the spring experiment and the first half of the summer experiment, the heated pools were more autotrophic than the ambient pools; i.e., the P/R ratios were greater than unity and higher in the heated pools than in the ambient pools (Fig. 15) . However, the ambient pools were more autotrophic than the heated pools during the first half of the spring experiment, but both were similarly autotrophic during the last half of the summer experiment. In addition, the heated pools were heterotrophic (P/R ratios less than unity), and the ambient pools were autotrophic during the winter experiment.

The pCO_2 and chlorophyll a levels are influenced by the net community metabolism (compare Figs. 16 and 17). Negative net community metabolism as respiration results in an increase in the pCO_2 level, but a decrease in the chlorophyll a level. For example, the chlorophyll a level decreased and the pCO_2 level increased in one of the ambient pools as the rate of respiration exceeded the productivity from late May to early June. In contrast, net community metabolism as production results in a decrease in the pCO_2 level, but an increase in the chlorophyll a level. For example, the chlorophyll a level increased, but the pCO_2 level decreased as the productivity exceeded the rate of respiration in the other ambient pool during the same period.

Comparisons of pCO_2 levels between treatments suggests that heat treatment, but not sewage treatment, influenced net metabolism (Fig. 16). The pCO_2 levels in the heated pools were higher than in the ambient pools during the winter experiment, and the first half of the spring experiment, but they were lower than in the ambient pools during the first half of the summer experiment. In addition, the pCO_2 levels peaked in one of the heated pools and both ambient pools during the last half of the spring experiment. Still, the pCO_2 levels in the sewage-treated pools were similar to those in the ambient pools and the heat+sewage-

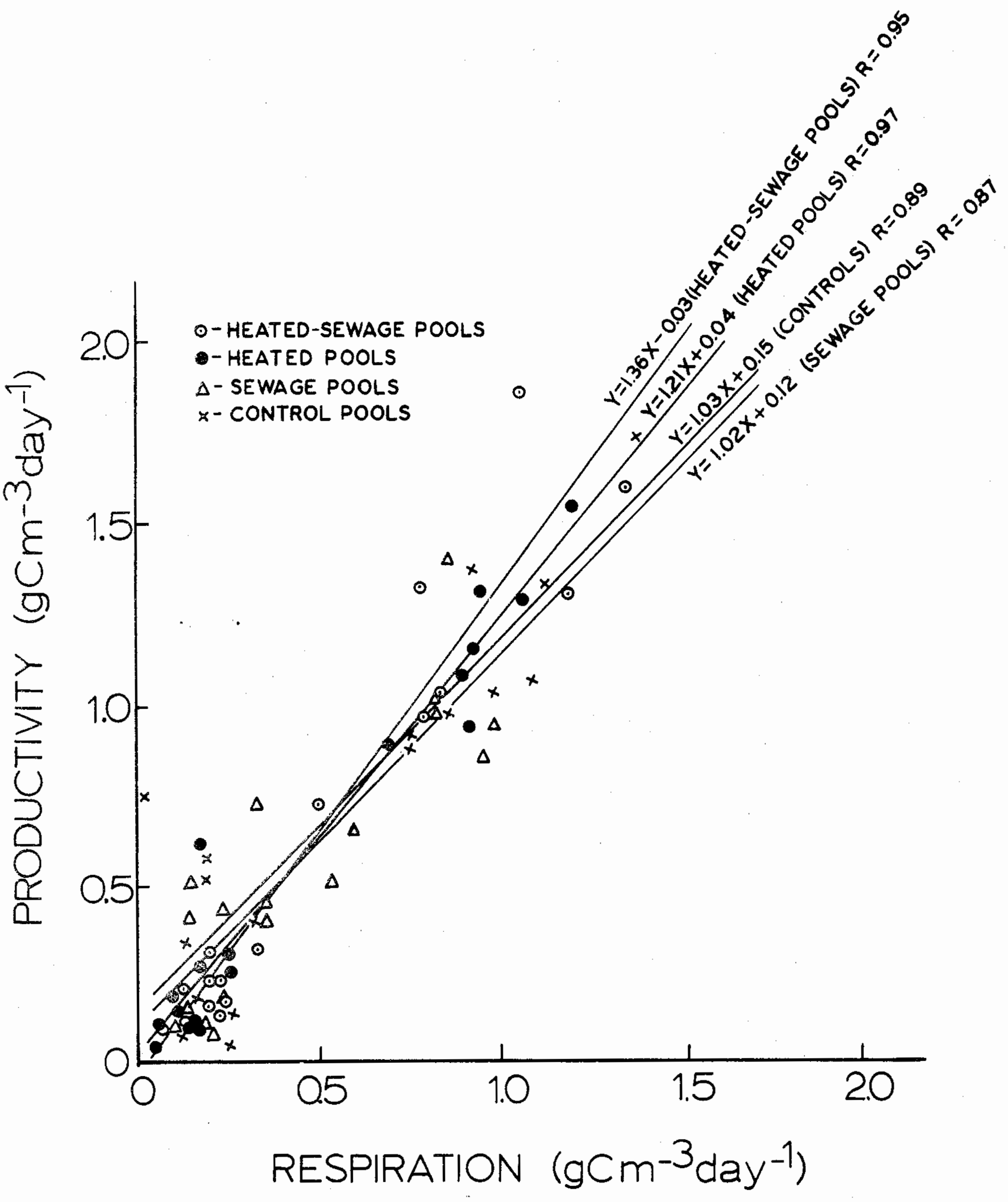

Figure 15. Gross photosynthetic productivity versus community respiration in the ambient (control), heated, sewage-treated, and heated + sewage-treated pools.

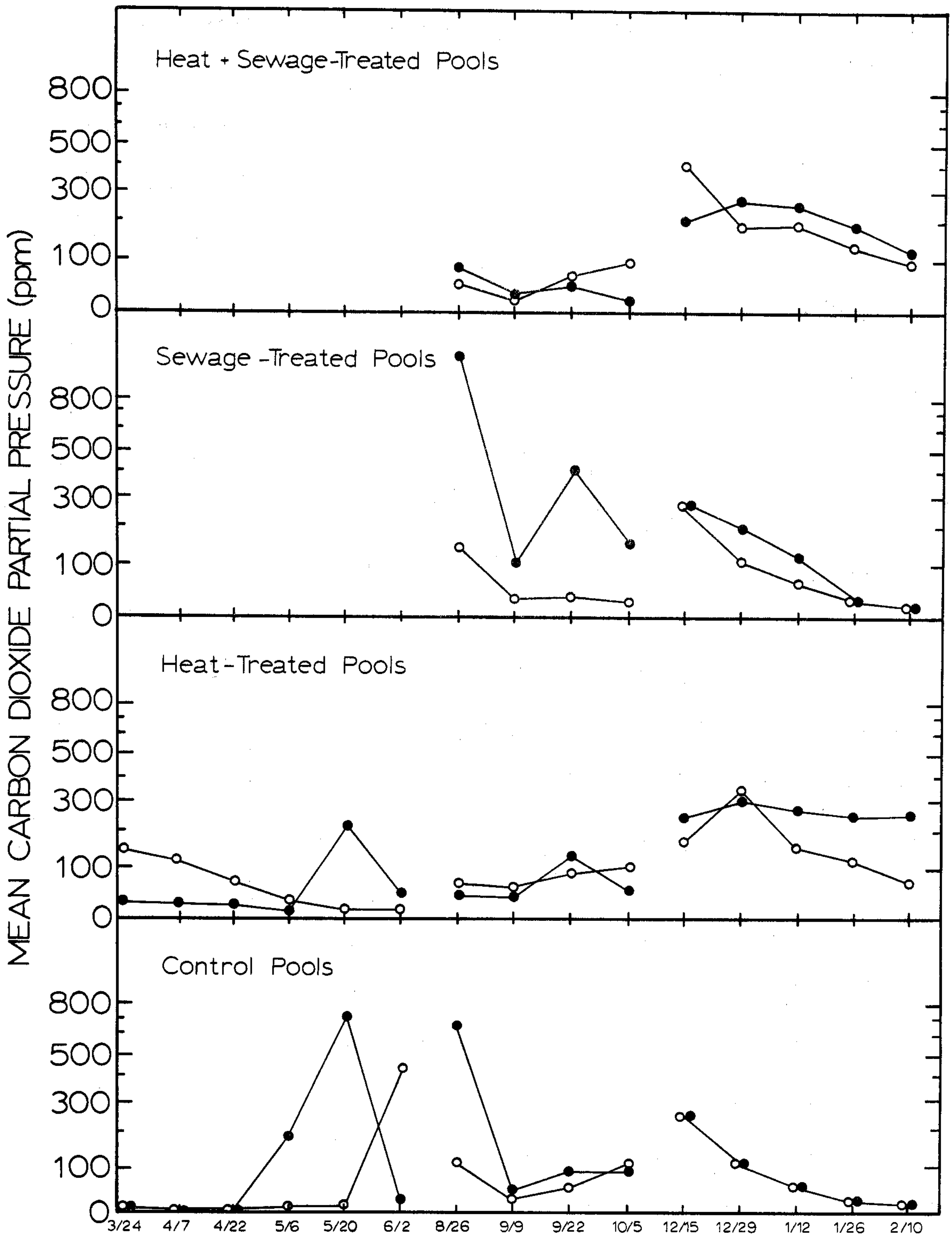

Figure 16. Mean partial pressure of carbon dioxide (ppm) at two-week intervals in the ambient (control), heated, sewage-treated, and heat+sewage-treated pools.

treated pools were similar to the heated pools during the summer and winter experiments.

Comparisons of chlorophyll a levels between treatments suggests that heat treatment influenced the standing crop (Fig. 17). First, the chlorophyll a levels in the heated pools were lower than in the ambient pools during the first half of the spring experiment and higher than in the ambient pools during the first half of the summer experiment and during the entire winter experiment. During the last half of the spring experiment, the chlorophyll a levels in one of the heated pools and both ambient pools peaked, however no two on the same date. Secondly, the chlorophyll a levels in the heat+sewage-treated pools were higher than in the ambient pools during the summer and winter experiments.

Other comparisons of chlorophyll a levels between treatments suggests that sewage treatment [possibly] influenced the standing crop during the winter experiment but not during the summer experiment (Fig. 17). Chlorophyll a levels in the sewage-treated pools were higher than in the ambient pools during the winter experiment but during the summer experiment, the chlorophyll a levels in replicate pools reacted to sewage treatment in opposite directions.

In light of these results, statistical tests showed that a combination of heat and sewage treatments and possibly heat treatment affected the P/R ratios, but sewage treatment alone had no significant affect (Table 3, Fig. 15). A combination of heat+sewage-treatments significantly increased the P/R ratio during the summer experiment as metabolism was greater than 0.5 $gCm^{-3}day^{-1}$. In contrast, a combination of heat+sewage treatment significantly decreased the P/R ratio during the winter experiment as metabolism was less than 0.5 $gCm^{-3}day^{-1}$. Yet, no significant differences in the P/R ratios occurred between the ambient and sewage-treated pools or heated pools. In the same way, no significant differences in P/R ratios

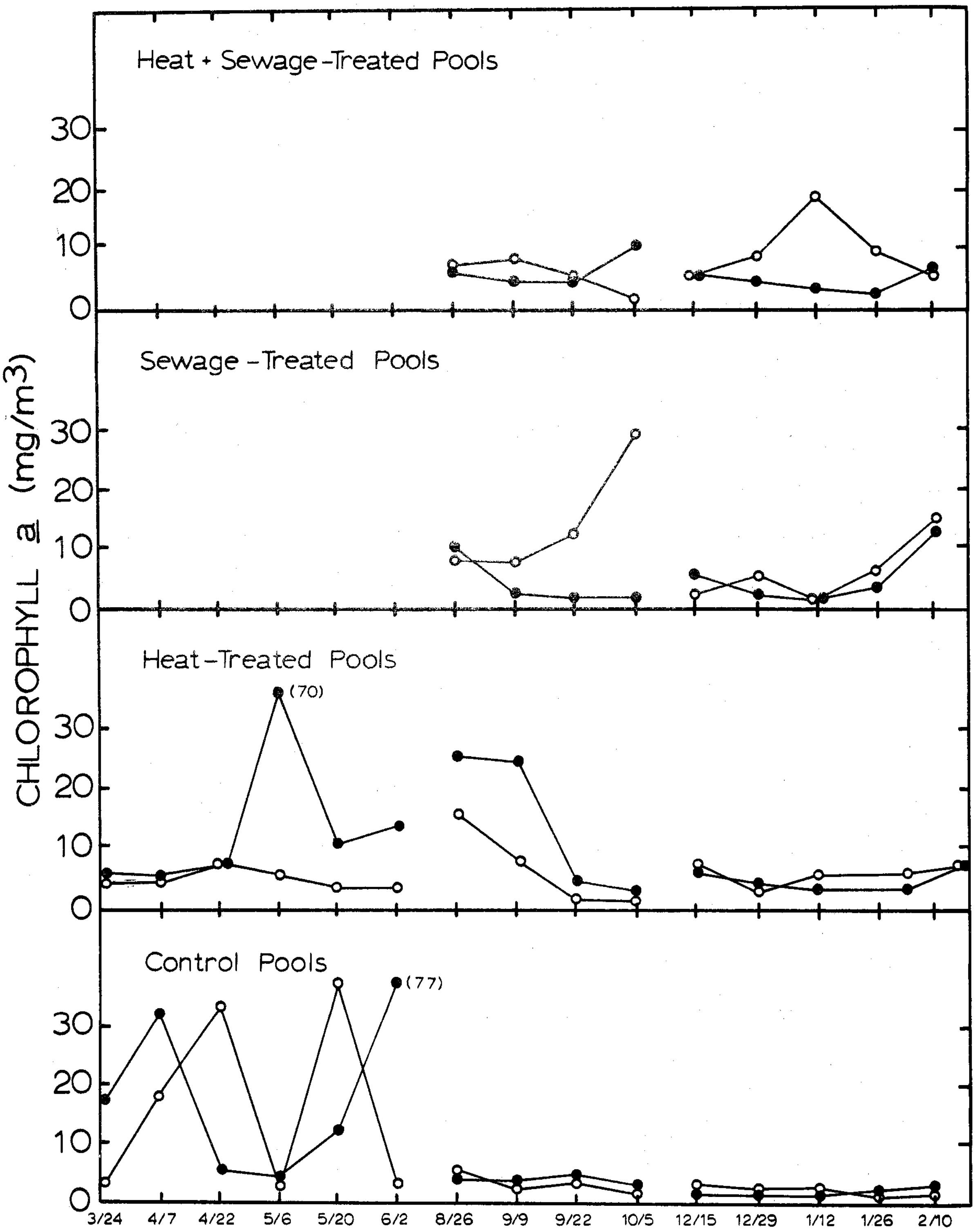

Figure 17. Chlorophyll a at two-week intervals in the ambient (control), heated, sewage-treated, and heated+sewage-treated pools.

occurred between the heat+sewage-treated pools and heated pools, but there were significant differences between the heat+sewage-treated pools and sewage-treated pools.

V. D. Discussion

V. D. 1. Community Metabolism

Respiration and production were increased by the thermal treatment during the spring and summer experiments, but they were stabilized by the thermal treatment during the winter experiment. One explanation for the metabolic increases during the spring and summer experiments may be that metabolic rates are increased to maximal levels by increases in temperature to the upper tolerance limit and further increases in temperature inhibit (38). Similarly, Vernberg and Vernberg (27) concluded that heat tolerant and cold tolerant invertebrates have parabolic shaped respiratory-temperature response curves; however, the curves are shifted to higher temperatures for heat-tolerant species. This observation may also apply to the phytoplankton and cold-blooded vertebrates as well as the invertebrates. During the winter experiments, the thermal treatment stabilized the temperature and probably prevented succession to a more productive phytoplankton community.

The metabolic-temperature responses in the heated ecosystems were significantly higher than those in the ambient ecosystems since the diversities may have been lower in the heated pools. We have shown (Section VI) that the phytoplankton communities in the heated pools were composed of fewer taxa than in the controls. Also, Beyers (11) found that the metabolic-temperature responses increased as the diversity decreased, since the lower diversity ecosystems have fewer energy pathways to absorb thermal stresses.

The metabolism was less sensitive to heat during the winter experiment than during the spring and summer experiments since the systems may have been limited by the low light level during the winter. First, metabolic rates in the heated pools were higher than those in the ambient pools during spring and summer experiments, but they were slightly lower during the winter experiment. Secondly, it is assumed that tht light level was much lower during the winter experiment. Vollenweider (39) as cited by Strickland (40) found that photosynthesis is dependent on temperature at high light intensities, but independent of temperature at low light intensities. Findenegg (41) contends that a combination of light and temperature governs the structure of the phytoplankton community.

The added heat may have increased productivities during the summer and spring experiments since the levels of ammonia-nitrogen and reactive phosphate were higher in the heated pools than the controls. Moreover, it has been shown (9) that temperature increases may cause a reduction in productivities when ammonia and orthophosphate levels are low, but a rise in temperature may cause an increase in production when ammonia and orthophosphate are not limiting.

Metabolic-temperature responses were not significantly affected by the sewage treatment; however, the diversity was affected. For example, an increase in blue-green algae occurred (Section VI), but diatoms decreased in the sewage pools. Similarly, Carpenter (42) and Sherk (43) concluded that nitrogen-poor sewage did not increase metabolic rates, but increased the abundance of blue-green algae in experimental estuaries on the Pamlico River. While primary sewage increased the ammonia-nitrogen levels in the two sewage-treated pools during the winter experiment, metabolism was not affected since a combination of temperature and light were probably limiting. Also metabolism was not affected by sewage treatment in the two heat+sewage-treated pools since a combination of ammonia and light were probably limiting.

Pathogenic bacteria, fungi, viruses, and other parasites associated with the sewage treatment may have interacted with the thermal treatment and reduced the nekton diversity during the winter experiment, but not during the summer experiment. Many dead fish and crabs were observed in the heat+sewage-treated pools during the winter experiment when primary sewage was added to the pools and the temperatures were as much as 12 C above ambient temperatures. Yet, very few dead fish and crabs were observed in the heat+sewage-treated pools during the summer experiment. Furthermore, diseases are possibly more lethal above optimal temperatures according to Stewart et al. (16).

V. D. 2. Net Community Metabolism

High organic levels increased net community respiration while high ammonia levels increased net community production in the experimental pools. Net community production occurred at relatively high rates as photosynthetic pulses and chlorophyll a levels increased, but the pCO_2 and ammonia levels declined. On the other hand, net community respiration occurred at relatively high rates as the photosynthetic pulses and the chlorophyll a levels declined, but the pCO_2 and the ammonia levels increased. As a result, the supply of organic matter increased with net community production, but it was consumed as net community respiration occurred during the decline of the pulse.

In the ambient pools, high temperatures decreased net community production while low temperatures increased net community production. Net community productivity was relatively high during the winter and the early part of the spring experiments as pCO_2 levels were minimal, but the P/R ratios were maximal. Therefore, the low temperatures encountered during these cold months probably inhibit respiration more than primary production and, thereby, increased net community production. On the other hand, net community productivity was relatively low during the summer and the latter part of the spring experiments as pCO_2 levels were maximal, but the P/R ratios were minimal. Consequently, the high temperatures encountered during these warm months stimulated respiration more than photosynthesis and, therefore, decreased net community production.

Thermal treatment may have increased net community production during the summer and the latter half of the spring experiments and stabilized net production at a relatively low rate during the winter and the first half of the spring experiments. In the heat-treated pools, the P/R ratios were higher, but the pCO_2 levels were lower than in the ambient pools since thermal treatment stimulated photosynthesis more than respiration during the summer and the latter half of the spring experiments. In contrast, the P/R ratios were lower, but the pCO_2 levels were higher than in the ambient pools since thermal treatment probably had a stabilizing effect on the structure of the phytoplankton community and the productivity during the winter and the first half of the spring experiments. Consequently, the growth rates of zooplankton and grass shrimp (Section VI) were increased by thermal treatment since ambient temperatures were limiting during the first half of the spring experiment.

Similarly, the combination of sewage and thermal treatments increased net community production during the summer experiment and stabilized net community production at a relatively low rate during the winter experiment. In the heat+sewage-treated pools during the summer experiment, the P/R ratios were significantly higher, but the pCO_2 levels were lower than in the ambient pools since the thermal treatment stimulated photosynthesis more than respiration. On the other hand, the P/R ratios in the heat+sewage-treated pools were significantly lower, but the pCO_2 levels were higher than in the ambient pools since thermal treatment probably had a stabilizing effect on the structure of the phytoplankton community and the productivity during the winter experiment.

Sewage treatment did not affect net community metabolism during the summer or winter experiments since the P/R ratios and the pCO_2 levels were the same in the sewage-treated pools as in the ambient pools. Furthermore, the addition of sewage did not increase the ammonia levels or stimulate gross production during the summer experiment. Temperature and light were limiting when sewage treatment increased the ammonia levels during the winter experiment.

VI. ORGANISMAL RESPONSE

Among the many ways to examine ecosystem response to high temperatures are the measurements of more subtle aspects, such as community metabolism or physiological response of community organisms, and less subtle aspects such as survival and growth of populations of organisms. In Section V we have examined the subtle response of community metabolism in the pools subjected to high temperatures. While the subtle aspects of the ecosystem are important, it is just as important to examine the overall ecosystem response in terms of survival and growth of organisms.

In contrast to the usual methodology of examining individual organisms in rather artificial situations, we have attempted to evaluate organismal response to high temperatures in terms of the whole ecosystem approach. We have transplanted estuarine ecosystems to plastic pools and controlled the inputs of ecosystem components and temperature. We view this approach as a more realistic means of examining the effects of high temperatures with respect to the interacting system of biotic and abiotic materials as they would exist in nature. Perhaps, with this approach we will be more able to realistically establish water quality criteria as it applies to the effects of high temperatures on estuarine ecosystems.

VI. A. Plankton

VI. A. 1. Phytoplankton

Phytoplankton samples were taken from each pool ecosystem during the spring and summer experiments. The samples were taken about every two weeks. Dr. Edward J. Carpenter, Woods Hole Oceanographic Institution, Woods Hole, Massachusetts, examined the phytoplankton and provided the data reported in subsequent pages.

Considerable variation among phytoplankton populations in replicate pools was observed (Fig. 18). However, in terms of numbers and biomass, the differences between replicate pools was less than the difference between treatments by the end of the experiment (Fig. 18). There were larger populations of phytoplankton in the ambient pools than in the heat treated pools during the spring experiment.

Chrysophytes dominated the phytoplankton community in both heated and ambient pools during the spring experiment (Fig. 19). One notable characteristic of the heated pools during the spring experiment was the large variability in types of phytoplankton and numbers of phytoplankton between replicate pools. As noted in Figure 19 (for sample 6), at the end of the experiment the ambient pools (pools 3 and 4) were quite similar in species and in numbers. The heated pools, on the other hand, were different in that the Chrysophytes (small diatoms) dominated the phytoplankton community in pool 2. There was a noticeable lack of large diatoms in the pools during the spring experiment (Fig. 19), but this is in agreement with the findings of Hobbie (33) in his study of the phytoplankton communities of the Pamlico River Estuary. It should be noted, however, that the percent diatom composition of the phytoplankton community was larger in the heated pools than in the ambient pools.

The concentration of the phytoplankton was less during the summer experiment than during the spring (compare Figs. 18 and 20). This is in agreement with most

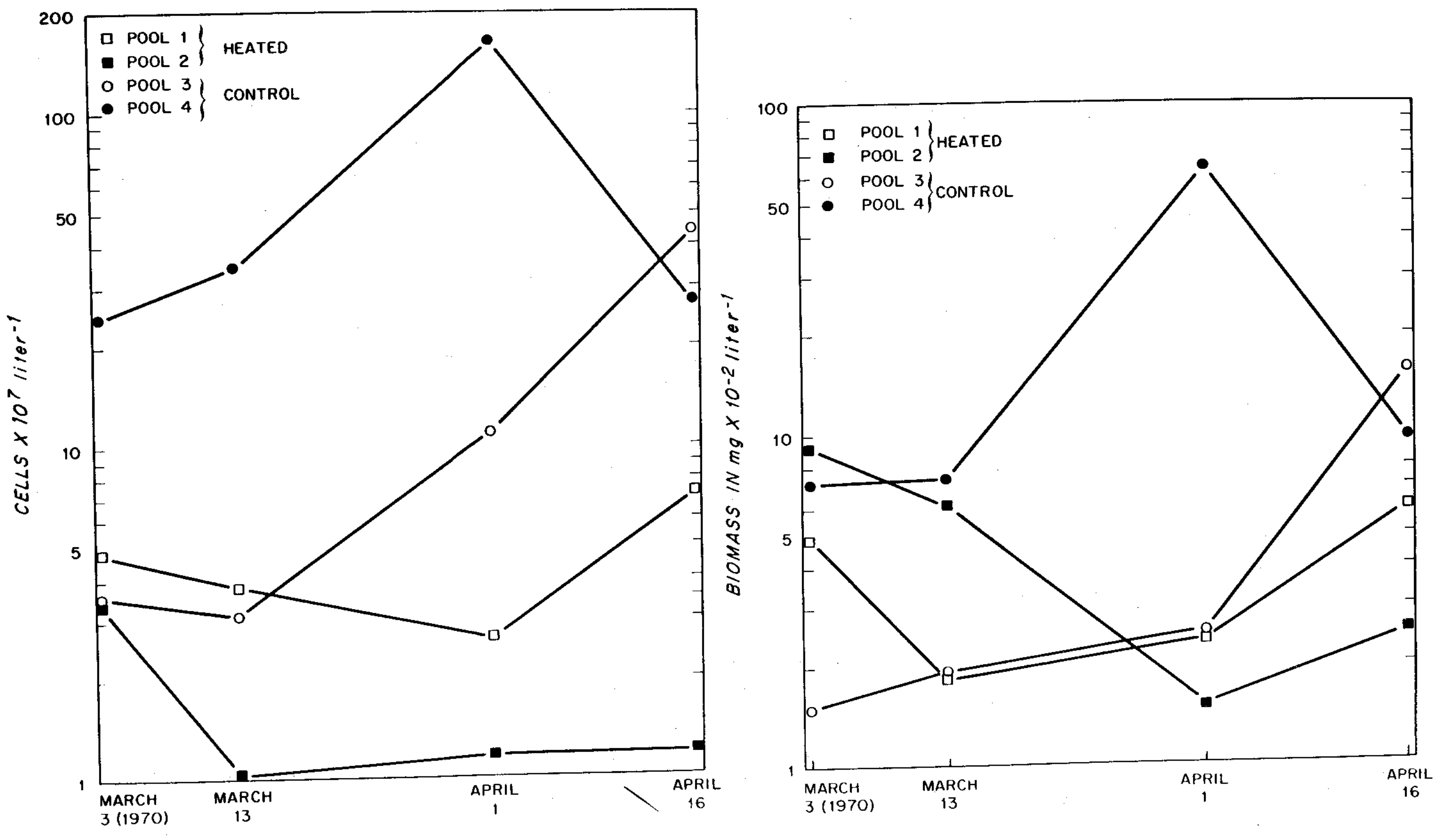

Figure 18. Numbers of phytoplankton cells and biomass per liter in each of the four pools during the spring experiment. The points correspond to samples 2, 3, 4 and 5, respectively, shown in Figure 19.

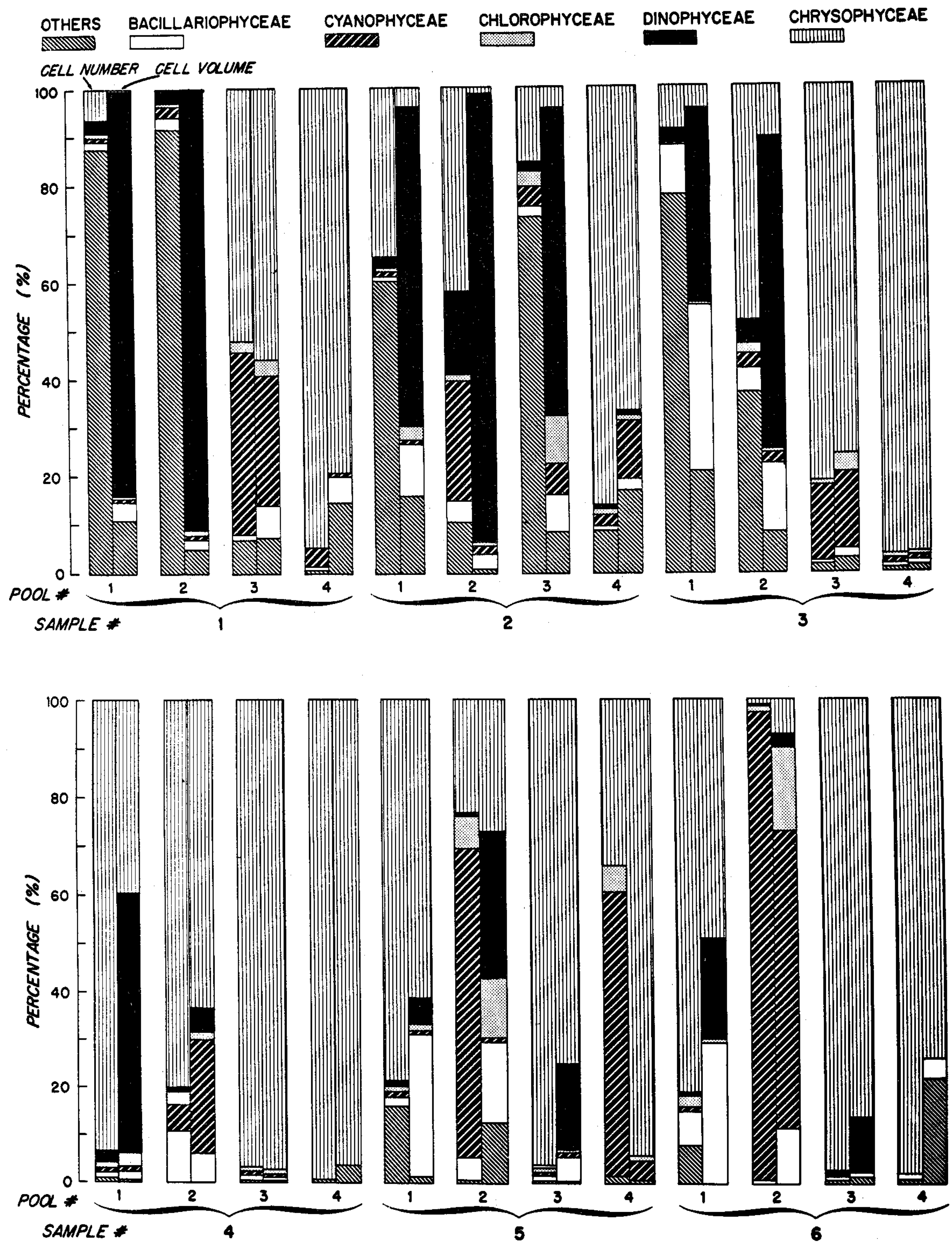

Figure 19. Percent composition, by major class, of phytoplankton samples during the spring experiment. Sample 1 was taken at the beginning of the experiment (1 Feb 1970) and sample 6 was taken at the end of the experiment (10 June 1970).

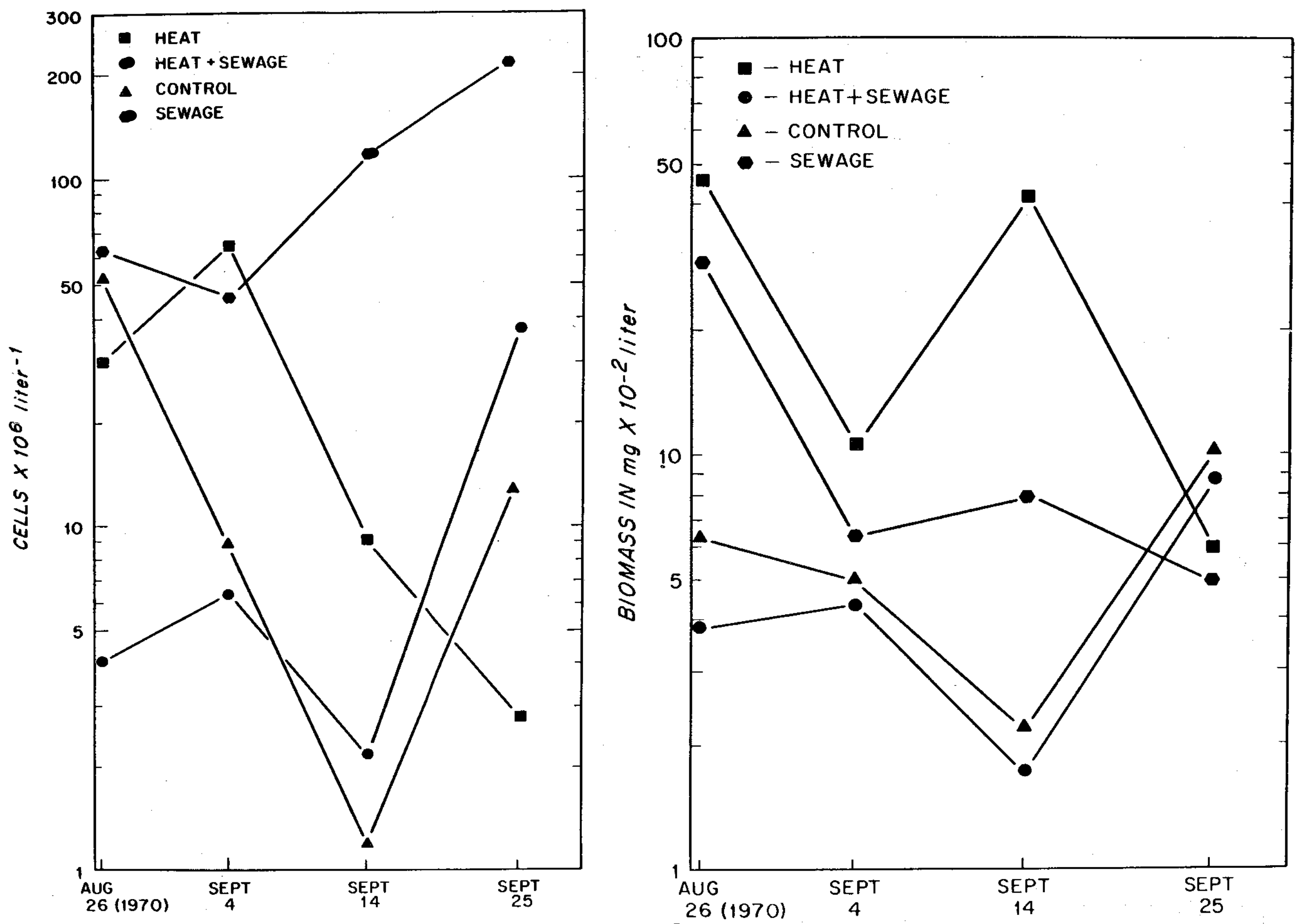

Figure 20. Numbers of phytoplankton cells and biomass per liter in each of the eight pools during the summer experiment. The points are averages of the two replicates and correspond to samples 1, 2, 3 and 4, respectively, shown in Figure 21.

phytoplankton studies of east coast estuaries, wherein the summer population is always less than the spring-bloom populations (33).

The concentration of phytoplankton in terms of cells per liter was greatest in the pools receiving sewage and the least in the pools receiving heat alone. (Fig. 20). It seems that the addition of sewage during the summer experiments resulted in an increase in population numbers. However, the cells in the sewage treated pools were apparently very small and the biomass calculations (Fig. 20) indicated much lower concentrations than in the ambient pools.

In the heated pools, Chrysophytes dominated during August but were succeeded by dinoflagellates and green algae during September (Fig. 21). In the heat+sewage-treated pools a diverse community of blue-greens, greens and diatoms existed during the summer experiment. Green algae dominated the phytoplankton community from August to mid-September, but blue-greens dominated from mid-September to October. In comparison, diatoms were less abundant but blue-greens were more abundant than in the controls. It should also be noted that a large portion of the phytoplankton community in the heated+sewage-treated ponds were coccoid blue-greens and greens. In the pools that received sewage, but not heat, blue-green algae were the dominant group throughout the summer experiment (pools 7 and 8 in Fig. 21).

In summary, the Chrysophytes dominated phytoplankton in the ambient pools during the spring, but a more diverse community of diatoms, greens, blue-greens and Chrysophytes existed during the summer experiments. In the heated pools, Chrysophytes dominated the first spring bloom, but blue-greens dominated in the second spring bloom. During the summer experiment Chrysophytes dominated during August and were succeeded by dinoflagellates and green algae during September. Blue-green algae were the predominant group of phytoplankton in the sewage-treated

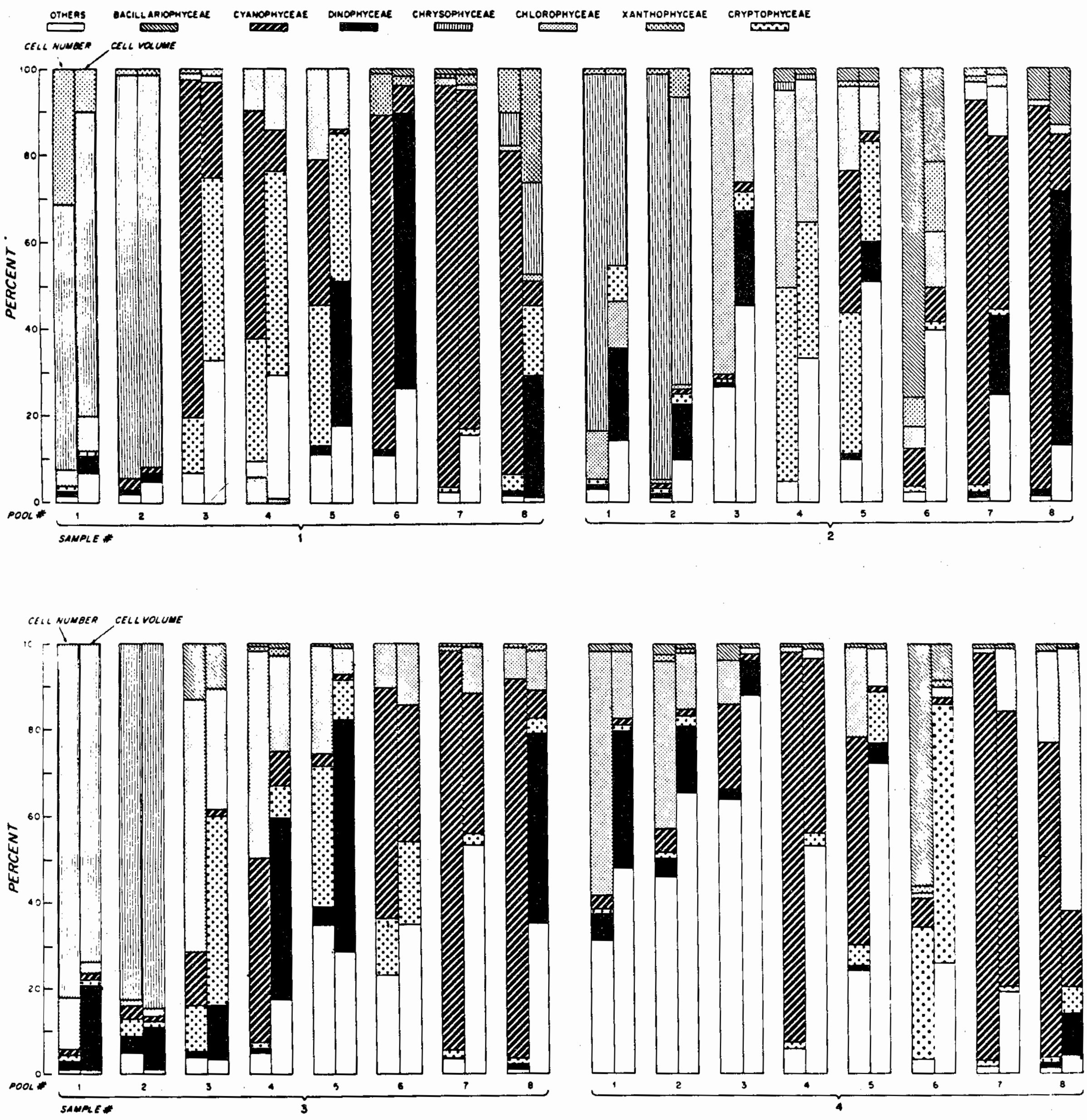

Figure 21. Percent composition, by major class, of phytoplankton samples during the summer experiment. Sample 1 was taken at the beginning of the experiment (1 August 1970).

pools throughout the summer experiment. A diverse community of blue-greens, greens and diatoms existed in the heated+sewage-treated pools during the summer experiment. Green algae dominated the phytoplankton community from August to mid-September, but blue-greens dominated from mid-September to October. It appears that the sewage addition resulted in dominance by small-celled blue-green algae and green algae; whereas, larger blue-green algae and green algae dominated in the heat+sewage treatment. These results are in agreement with those found by other investigators (2, 36, 42).

VI. A. 2. Bottom Algae

Algae settling to the bottom of the pools during the experiments were collected by a siphon, which sampled a known area of the bottom. Each pool was clean at the beginning of each experiment, but algae had accumulated on the bottom by the end of the experiment. Dry weights of the algae samples were determined (Table 4).

The amount of algae that accumulated on the bottom of the plastic pools was about four times as great during the summer than during the winter experiments (Table 4). This is in agreement with the amount of net productivity that is reported in Section V; i.e., the net productivity during the winter was considerably lower than during the spring or summer.

During the summer experiment the accumulation of algae on the bottom of the experimental pools was significantly greater in those receiving sewage than in those that did not (Table 4). The accumulation of algae was significantly greater in the heated pools than in the ambient pools, but the addition of heat did not result in a significant difference in the sewage-treated pools. Heating during the winter experiments resulted in a larger accumulation of algae on [illegible]

Table 4. Dry weight of algae on the bottoms of pools during the summer and winter experiments.

Treatment	Mean gm/sample	Range gm/sample	Mean gm/m^2
Summer			
Ambient	3.90	3.60-4.20	390.2
Heated	4.41	4.12-4.70	440.8
Sewage	5.77	2.78-8.75*	576.5
Heat+Sewage	5.65	4.52-6.77*	564.8
Winter			
Ambient	1.01	1.00-1.01	100.9
Heated	1.73	1.54-1.91	172.8
Sewage	1.42	1.35-1.49	141.7
Heat+Sewage	1.65	1.46-1.84	165.1

*Note: These samples contained one or more gm of sand.

the bottom of the plastic pools than in the unheated ones. Algal accumulation was greater in the sewage-treated pools than in the ambient pools during the winter experiment.

VI. A. 3. Zooplankton

Zooplankton samples were taken from all of the plastic pools during the experiments, but analysis was very difficult due to the small numbers of zooplankton that were present. Acartia tonsa was the only species of zooplankton encountered during the summer and winter experiments.

VI. B. Nekton

VI. B. 1. Blue Crabs

Blue crabs (Callinectes sapidus) are common components of the estuarine ecosystems of coastal North Carolina. They are present all year in the estuarine environment and have very wide temperature tolerances (44).

In the heated versus ambient pool experiments the survival of blue crabs was not significantly different among the experimental conditions. There was a large increase in the volume of blue crabs per pool during the spring experiment particularly in the heated pool (Fig. 22). The amount of volume increase of blue crabs in the heated pools was more than twice the volume increase in the ambient pools during the spring experiment.

In the summer and winter experiments the change in volume of blue crabs was less than during the spring experiment. The blue crab biomass in the ambient pools was greater than that in the heated pools during the summer experiment, and the difference was statistically significant. There was no significant change in the volume per pool of blue crabs in either heated or ambient pools during the winter experiment.

When sewage was added to the experimental conditions (Fig. 23) no significant differences from ambient could be determined for blue crab growth. The survival of blue crabs in the pools that received sewage were the same as survival in pools that did not receive sewage. The final volume of blue crabs in the pools receiving sewage treatment, both heated and unheated, were significantly larger than the final volume in the pools receiving heat alone during the summer experiment. This may be related to the additional food available to the blue crabs in the pools receiving sewage.

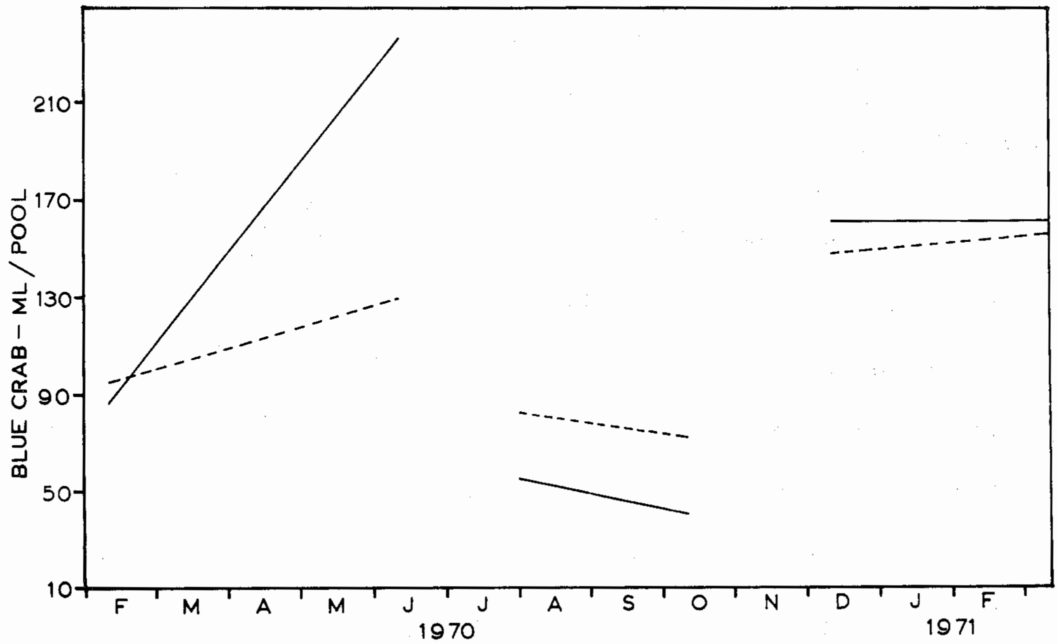

Figure 22. Mean volume (ml/pool) of blue crabs (C. sapidus) in the heated vs. ambient experiments. Solid line is for heated pools and dashed line is for ambient pools.

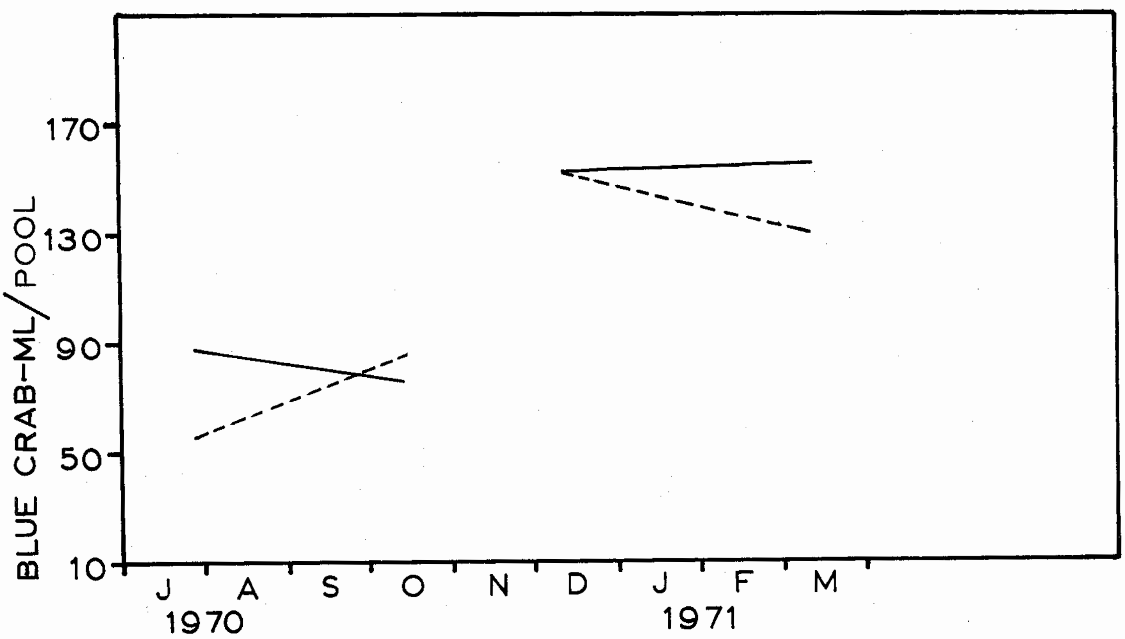

Figure 23. Mean volume (ml/pools) of blue crabs (C. sapidus) in the sewage-treated pools. Solid line is for heated+sewage-treated pools and dashed line is for ambient+sewage-treated pools.

VI. B. 2. Grass Shrimp

The grass shrimp (*Palaemonetes pugio*) are very important fish-food organisms in the estuaries of the east and gulf coasts. These organisms are very common among the underwater grass growing in the oligohaline sections of estuaries. This species is usually most abundant during the spring and summer in North Carolina estuaries.

During the spring experiment grass shrimp in the heated pools increased their volume per pool at a much faster rate than occurred in the ambient pools (Fig. 24). The final grass shrimp volume in the heated pools at the end of the spring experiment was more than twice as much as the final volume in the ambient pools. This large difference can be attributed to the fact that the grass shrimp spawn when the water temperature reaches about 20 C (45). The water temperature in the heated pools reached 20 C about one month earlier than in the ambient pools during the spring experiment. Thus, the reproductive capacity of the grass shrimp yielded a much larger volume in the heated pools than in the ambient ones.

There was a net decrease in volume of grass shrimp in all pools during the summer experiment (Figs. 24 and 25). The final volumes of grass shrimp in the ambient pools, heated pools, and sewage-treated pools was significantly larger (95%) than the volume in the heated pools. The decrease in volume in the sewage-treated pools at ambient temperature was less than in the pools not receiving sewage, but not statistically so.

No grass shrimp were available for the experiment during the winter.

VI. B. 3. Fish

Fish that were common to the immediate estuarine area during each season were used in the pool experiments. Obviously, fish are more prominant members of

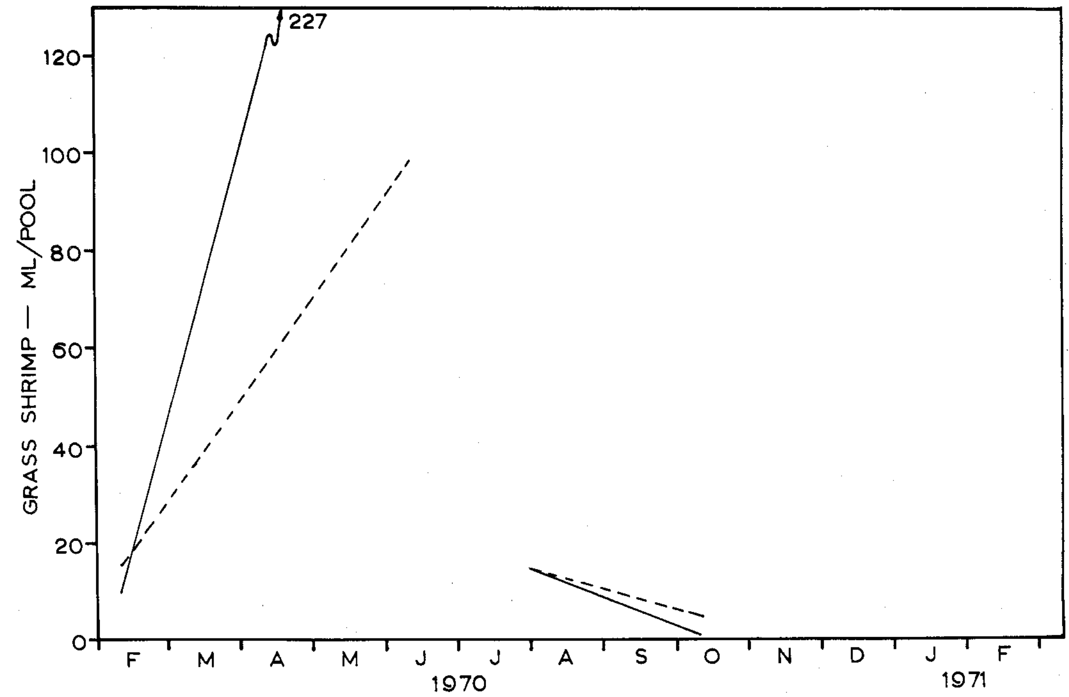

Figure 24. Mean volume (ml/pool) of grass shrimp (P. pugio) in the heated vs. ambient experiments. Solid line is for heated, pools and dashed line is for ambient pools.

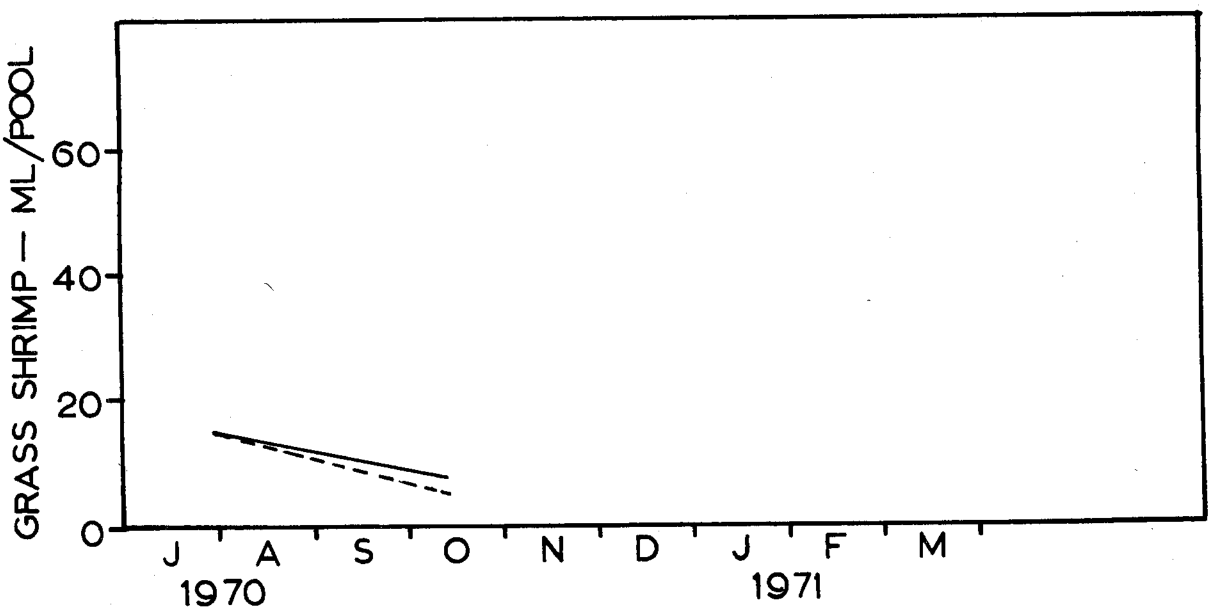

Figure 25. Mean volume (ml/pools) of grass shrimp (P. pugio) in the sewage-treated pools. Solid line is for heated+sewage-treated pools and dashed line is for ambient+sewage-treated pools.

estuarine ecosystems and receive considerable attention in most investigations of heat stresses. Experiments using individuals of various species of fish in relation to heat have been summarized by Raney and Menzel (46). In our experiments flounder were used in the spring, pinfish, spot and killifish during the summer, and pinfish during the winter experiments.

The flounder volume increased in the heated pools during the spring experiment, whereas in the ambient pools the increase was only very slight (Fig. 26). Although the total volume of flounder at the end of the spring experiment was greater in the heated pools than in the ambient pools, the different was not statistically significant.

The total volume of fish in the experimental pools during the summer increased under all experimental conditions (Figs. 26 and 27). The increase in volume was significantly larger in the ambient pools than in the heated pools; regardless of whether sewage was added or not. Comparing the final volumes in the heated pools, the fish volumes in pools that received sewage were significantly higher than volumes in those pools that did not, but the difference was not statistically significant. All species of fish involved in the summer experiment increased in volume.

There were significant decreases in total volumes of fish during the winter experiment in all experimental pools (Figs. 26 and 27), with those at ambient decreasing at a significantly greater rate. There were no statistical differences, regardless of treatment, between the outputs of any of the pools. The decrease in total volume can be attributed to the mortality of some of the fish.

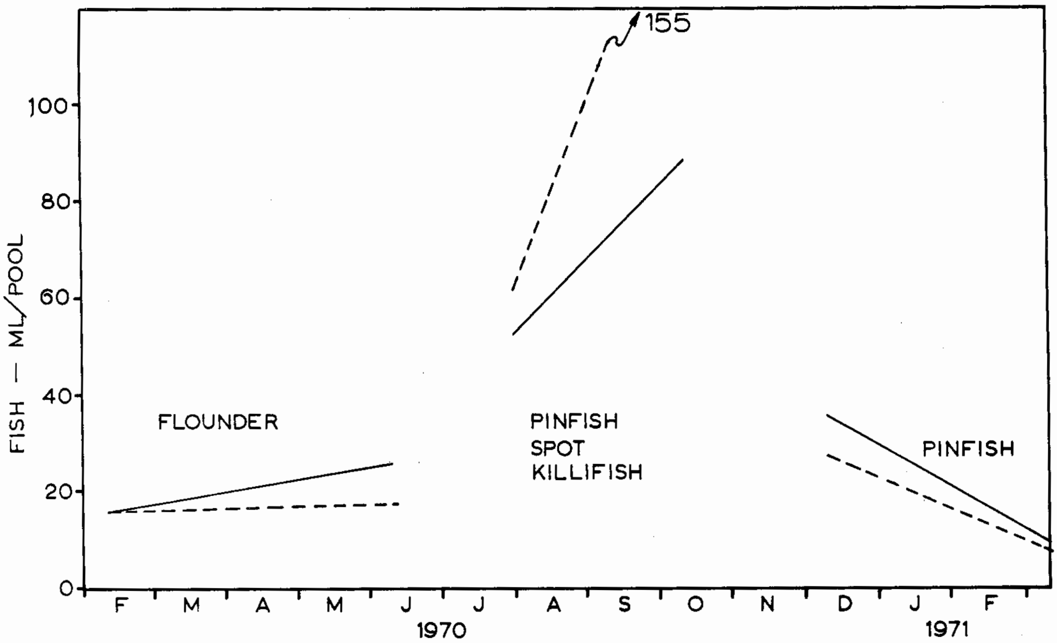

Figure 26. Mean volume (ml/pool) of fish in the heated vs ambient experiments. Solid line is for heated pools and dashed line is for ambient pools. Species are indicated for each experiment.

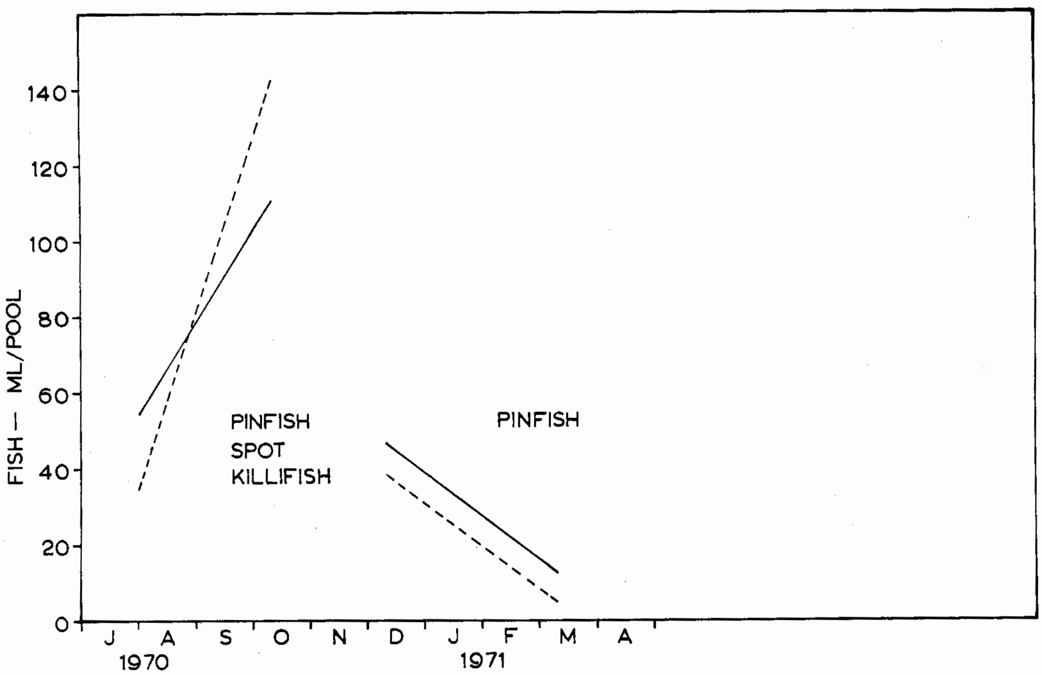

Figure 27. Mean volume (ml/pool) of fish in the sewage-treated pools. Solid line is for heated+sewage-treated pools and dashed line is for ambient+sewage-treated pools. Species are indicated for each experiment.

VI. C. Benthos

VI. C. 1. Widgeon Grass

Widgeon grass (*Ruppia maritima*) is an important underwater grass that grows typically in oligohaline estuarine systems along the east and gulf coasts of the United States. This grass contributes a considerable amount of organic detritus to estuarine ecosystems through its growth during the spring and decay in the fall. Widgeon grass is very difficult to transplant in artificial situations such as the experimental pools. We were, however, successful in transplanting the grass to these pools although considerable loss of grass occurred just from the transplanting.

During the spring experiments *Ruppia* increased in volume in the heated pools and decreased in volume in the ambient pools (Fig. 28). Statistically, the *Ruppia* volume in the heated pools was significantly larger than in the ambient pools. Apparently, the application of heat to the pools enabled the *Ruppia* to grow; whereas, in the ambient pools the net volume of *Ruppia* decreased.

In the summer and winter experiments the total volume of *Ruppia* at each pool station decreased from the beginning to the end of the experiments (Figs. 28 and 29). This may be a reflection of the added turbidity in the pools due to phytoplankton growth during the summer experiments and low solar inputs during winter. There was significantly more *Ruppia* in the ambient pools than in the heated pools during the summer experiment. There were no significant difference in the volume of *Ruppia* at the end of the summer experiments in the sewage-treated pools, regardless of heat application.

Although *Ruppia* decreased in volume throughout the winter experiment, it decreased less in the heated pools than in either the ambient or sewage-treated pools (Figs. 28 and 29). There was significantly greater *Ruppia* volume in the heated pools than in the other three experimental conditions. When sewage was

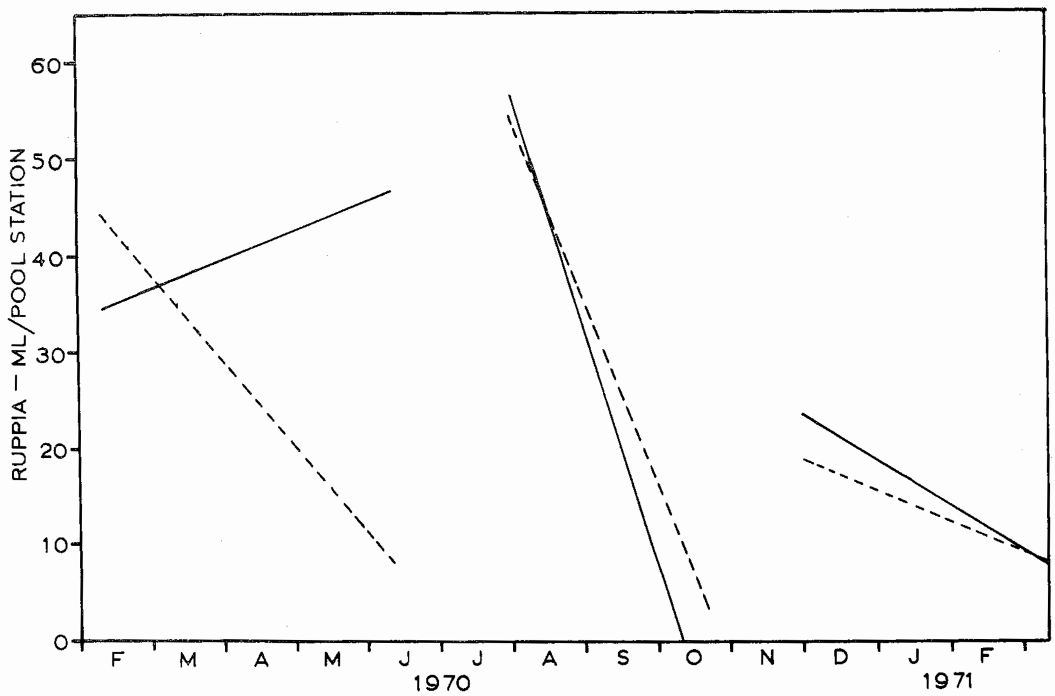

Figure 28. Total volume (ml/pool station) of widgeon grass (Ruppia maritima) in heated vs. ambient experimental pools. Solid lines indicate heated and dashed lines indicate ambient conditions.

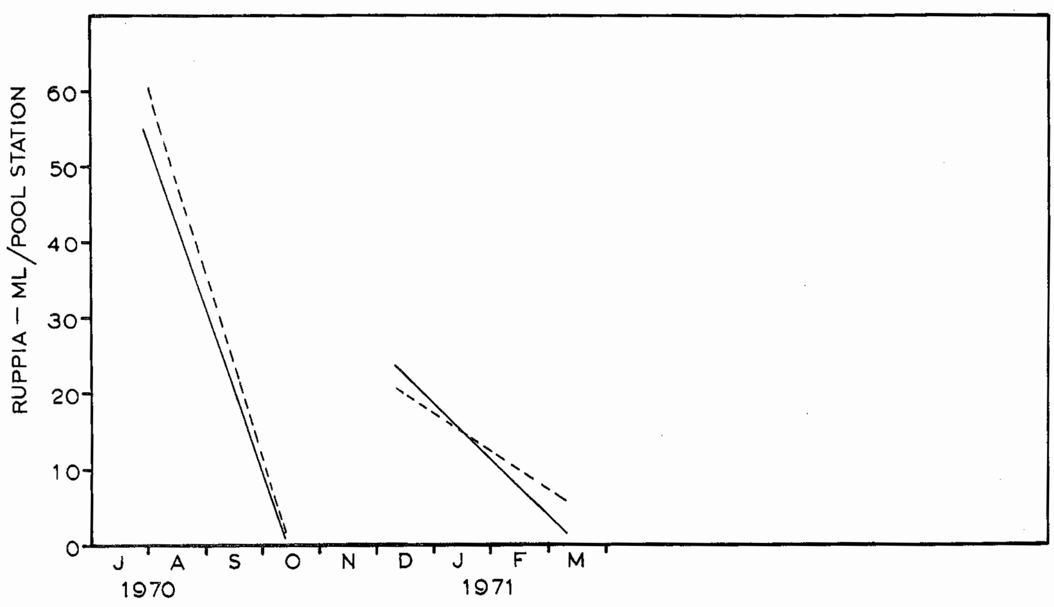

Figure 29. Total volume (ml/pool station) of widgeon grass (Ruppia maritima) in the sewage-treated pools. Solid lines indicate heated and dashed lines indicate ambient conditions.

added to pools, those at ambient temperature had less decrease in volume of Ruppia than those with heat.

VI. C. 2. Bay Clams

Bay clams (Rangia cuneata) are the dominant benthic macroorganisms in North Carolina estuaries (47). They sometimes reach large concentrations in the shallow mud bottom area.

There was a significant decrease in the total volume of Rangia clams in the experimental pools during the spring experiments (Fig. 30), with the volume in the ambient pools larger than in the heated pools. The survival was almost 100% in all pools throughout the experimental period.

During the summer experiment there was a significant decrease in the total volume of Rangia clams from the beginning to the end of the experiment (Figs. 30 and 31). A greater decrease in volume was observed in the heated pools than in the ambient pools, but the difference was not statistically significant except between heated vs. ambient (less total volume in the heated pools). Survival was statistically the same and was greater than 90% in all pools regardless of sewage or heat treatments.

During the winter experiment there was a significant decrease in the volume of Rangia clams in the heated pools and the heated+sewage-treated pools (Figs. 30 and 31). There was no significant decrease in volume of Rangia clams during the winter experiment in the ambient pools, regardless of sewage treatment (Figs. 30 and 31). The final total volume of Rangia clams in the heated+sewage-treated pools was significantly less than in any other experimental combination.

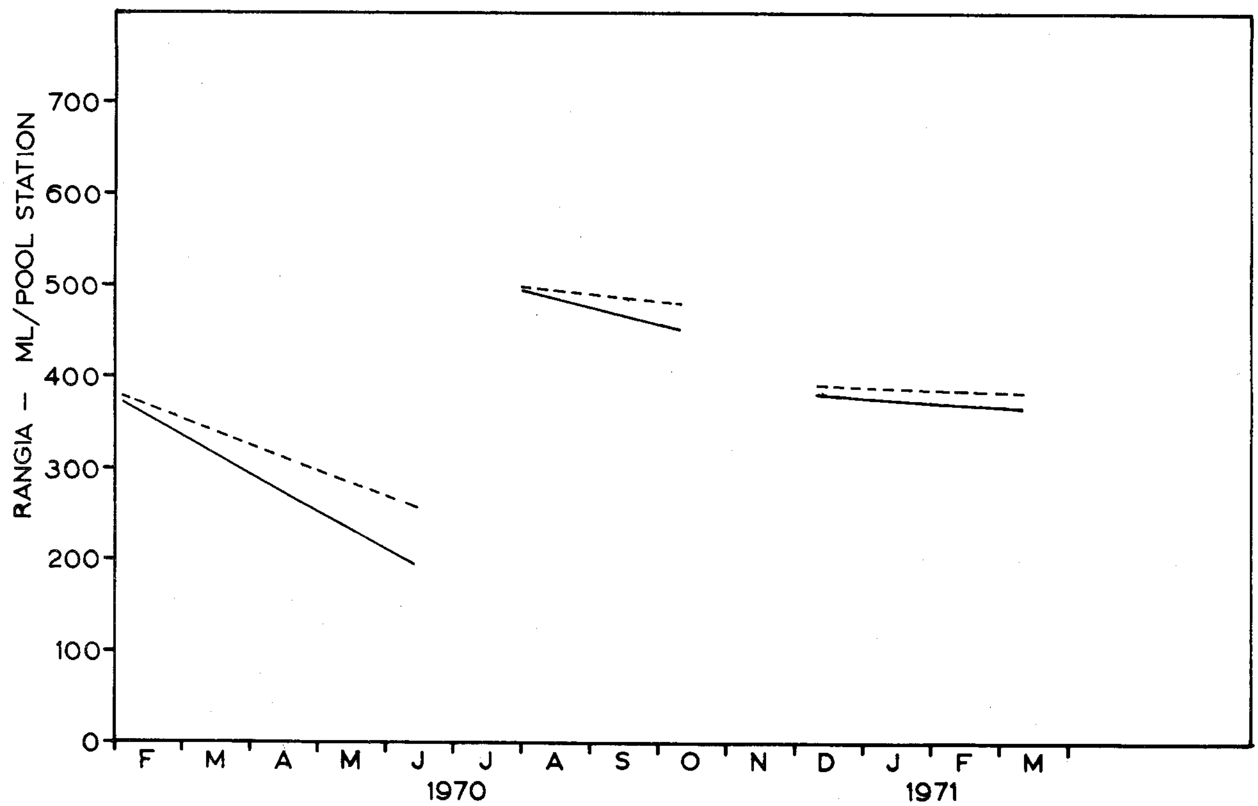

Figure 30. Total volume (ml/pool station) of bay clams (Rangia cuneata) in heated vs. ambient experimental pools. Solid lines indicate heated and dashed lines indicate ambient conditions.

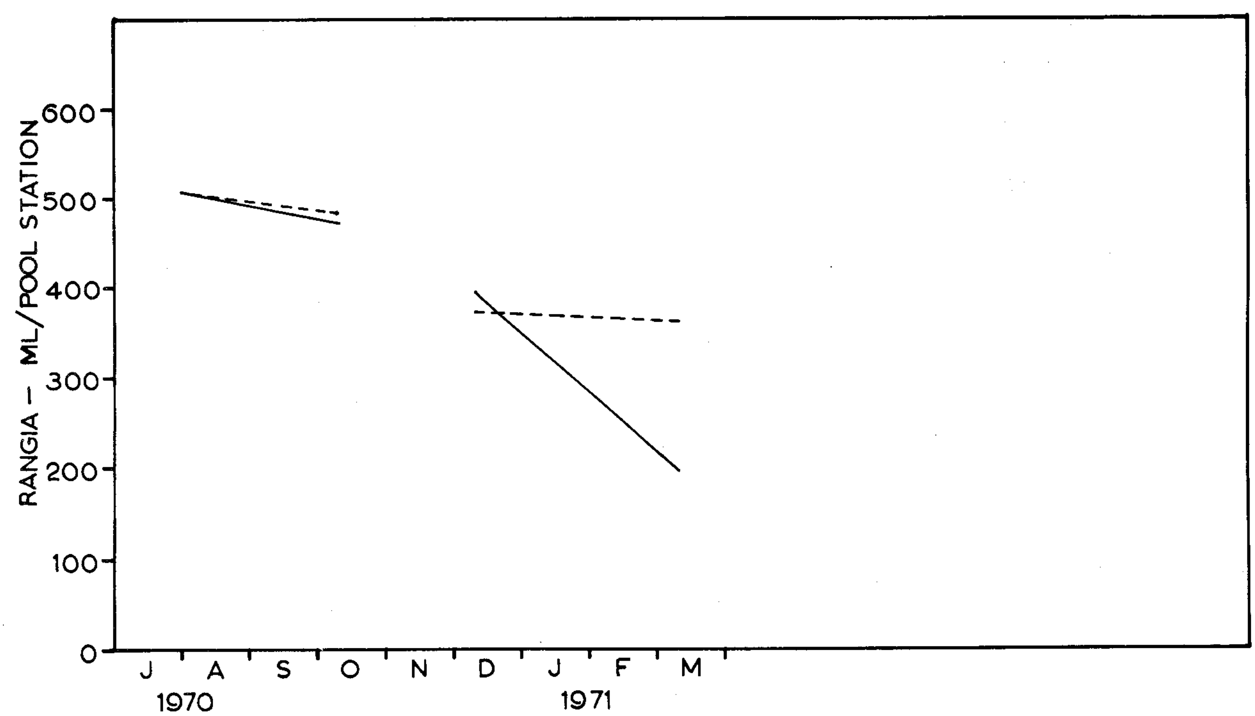

Figure 31. Total volume (ml/pool station) of bay clams (Rangia cuneata) in the sewage-treated pools. Solid lines indicate heated and dashed lines indicate ambient conditions.

VI. C. 3. Oysters

Oyster reefs, composed of the American oyster (*Crassostrea virginica*), are wide-spread throughout the estuaries of North Carolina. Because of their commercial importance they have received considerable attention. We included the oysters in our heat experiments.

No significant differences could be determined for changes in the volume of oysters in the heated pool experiments under every experimental condition during the spring and summer. Survival was almost 100% in all pools during both spring and summer.

During the winter experiments there was a significant difference in the volume of oysters at the end of the experiment, with oyster volume in the heated pools significantly larger than the oyster volume in the pools receiving the heat+sewage-treatment. The oyster volume in the ambient pools were significantly larger than in the pools receiving heat+sewage, and those receiving heat alone also had a larger volume than the pools receiving heat+sewage-treatment. There was no significant difference between oyster volumes in the ambient pools, regardless of sewage treatment.

VI. C. 4. Other Benthos

Several species of benthic organisms were found in the experimental pools at the end of each experiment. None of these organisms were placed in the pools at the beginning of the experiment, but apparently arose from entrapped larvae and eggs in the original water. These benthic organisms make up a significant portion of fish-food organisms in natural estuarine systems (47).

The mean dry weights in grams per pool for benthic macrofauna are given in Table 5. Theoretically, at the beginning of each experiment the potential for the development of benthic macrofauna was the same since water was randomly placed in all pools from the same source (i.e., South Creek Estuary).

Chironomous larvae were present in some pools during all experiments (Table 5). During the spring the mean dry weight of midge fly larvae was almost twice as much in the heated pools than in the ambient pools. During the summer the ambient pools produced a significant amount of Chironomous larvae while those receiving heat and/or sewage treatments produced considerably less. During the winter experiment the concentrations of Chironomous larvae were not statistically different between experiments, although those pools receiving sewage produced slightly more.

Polychaete worms (Nereis sp.) were present in all pools during all experiments (Table 5). Large concentrations of these worms were present in the heated pools during spring, ambient pools during winter, and sewage-treated pools during winter. During the spring experiment Nereis sp. was almost one order of magnitude larger in the heated pools than in the ambient pools, indicating the beneficial effect of heat. During the summer the concentration of polychaete worms was larger in the ambient pools than in the heated or sewage-treated pools. The relatively high concentration in the heated+sewage-treated pools during the summer experiment is inexplicable. The mean dry weight of polychaete worms during the winter experiment was considerably larger in the pools not receiving heat than in those receiving heat, regardless of the sewage treatment.

Five other species of benthic macro-fauna were present on a seasonal basis (Table 5). The amphipod, Gammarus sp., was present during spring in the heated pools and during winter in ambient pools. Hydaticus sp. (a freshwater beetle larva) was present during the spring experiment, with significantly larger concentrations

Table 5: Mean dry weight (gm/pool) of benthic organisms at the end of each experiment.

Species	Ambient	Heated	Sewage-Treated	Heat + Sewage-Treatec
<u>Chironomous</u> larvae				
Spring	0.97	1.61		
Summer	0.24	0.03	0.002	0.02
Winter	0.01	0	0.03	0.001
<u>Nereis</u> sp.				
Spring	0.61	5.60		
Summer	0.43	0.12	0.07	0.43
Winter	2.52	0.12	2.38	0.05
<u>Gammarus</u> sp.				
Spring	0	0.01		
Winter	0.001	0	0	0
<u>Hydaticus</u> sp.				
Spring	504.9	22.3		
<u>Glycera</u> sp.				
Spring	0.17	0.87		
Summer	0.35	0.13	1.01	0.91
<u>Trichocorixa</u> sp.				
Spring	1.74	0.96		
<u>Rangia</u> <u>cuneata</u> (immature)				
Summer	0.35	0	1.01	4.36

in the ambient pools than in the heated pools. <u>Glycera</u> sp. were present during the spring and summer experiments. Significantly larger concentrations were found in response to the added heat during the spring experiment and in response to sewage treatment during the summer experiment. <u>Trichocorixa</u> sp. was present during the spring experiment, with the larger concentration found in the ambient pools. Immature specimens of the bay clam, <u>Rangia</u> <u>cuneata</u>, were collected during the summer experiment. The largest concentrations occurred in the sewage-treated pools, probably in response to the additional food materials.

VII. FLOW-THROUGH EXPERIMENT

A flow-through experiment was conducted during the summer of 1971 to test the response to increased temperatures under more realistic estuarine conditions. Water was allowed to flow directly from South Creek Estuary through all experimental pools with a pump-siphon arrangement. Other than the flow-through mechanism, the experiments were conducted in the same manner as those discussed in previous sections.

Rate of flow was adjusted so that the volume of each pool was replaced every six-ten days. The flow-rate was measured by determining the amount of discharge from each pool through the siphon drain. The measured flow-rates are given in Table 6 and indicate the variability with time and the necessity for constant adjustment. Although the rate of flow through the pools was erratic, it is not unlike that of natural situations.

VII. A. Temperature, Salinity and Nutrients

The temperature of surface and bottom of the experimental pools were similar throughout the flow-through experiment (Figs. 32 and 33). The maximum temperature during the flow-through experiments was 33.5 C in the heated pools. The heated pools were maintained at 5 C warmer than the ambient pools, except during cooler weather (*e.g.*, July 7, July 13, July 22) when the temperature in the ambient pools would decrease 3 to 5 C and the difference between heated and ambient would be about 8 to 9 C.

Salinity increased throughout the experiment beginning at 5.5 ppt and getting up to about 7.3 ppt at the end of the flow-through experiment (Fig. 34). This salinity increase is a reflection of evaporation during the warm summer months.

Table 6: Rate of Water input into the pools during the flow-through experiments.

Date	Pool*	Gal/min(approx)	ml/min(2 liter graduated cylinder)
23 June 1971	1	0.40	1588
	2	0.40	1440
	3	0.36	1376
	4	0.37	1390
25 June 1971-(Manual Change)			
	1	0.50	1930
	2	0.49	1880
	3	0.50	1940
	4	0.51	2020
28 June 1971-(Cleaned and reset)			
	1	0.49	1826
	2	0.50	1943
	3	0.50	1905
	4	0.50	1895
30 June 1971			
	1	0.50	1915
	2	0.51	2020
	3	0.50	1935
	4	0.51	1995
2 July 1971-(Manual Change)			
	1	0.49	1850
	2	0.51	2020
	3	0.50	1910
	4	0.50	1950
7 July 1971			
	1	0.49	1840
	2	0.51	1985
	3	0.50	1900
	4	0.50	1935
28 July 1971			
	1	0.50	1915
	2	0.51	2020
	3	0.49	1870
	4	0.49	1835

* Pools 1 and 2 were heated and pools 3 and 4 were at ambient temperatures.

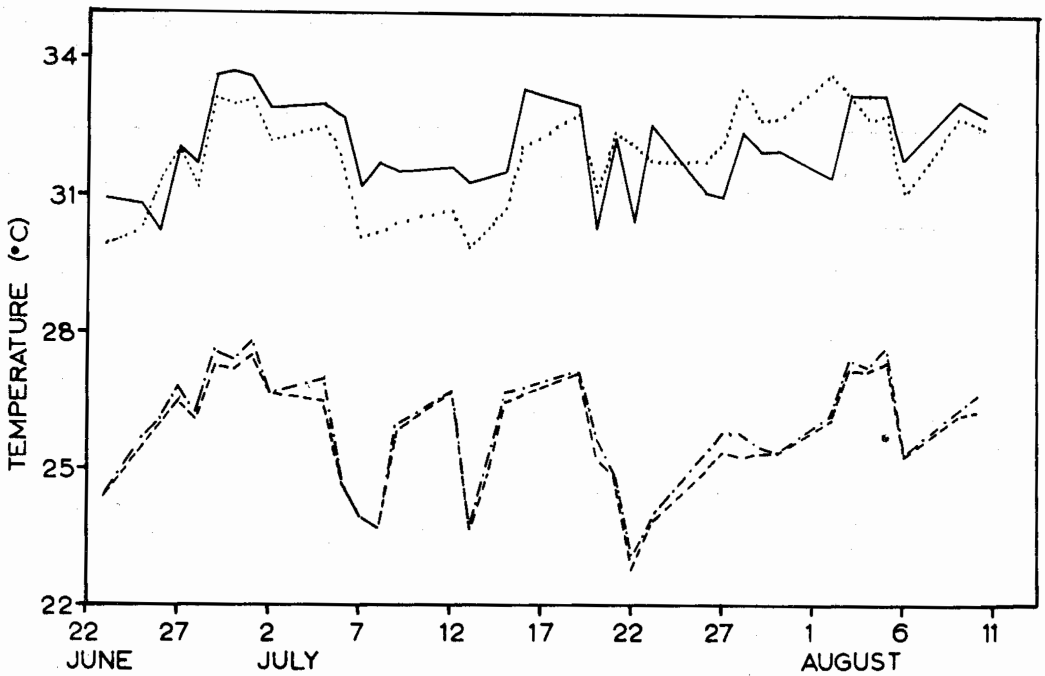

Figure 32. Surface temperatures (C) for all pools for the flow-through experiment. The solid and dotted lines are for replicate heated pools and the dashed and dash-dot lines are for replicate ambient pools.

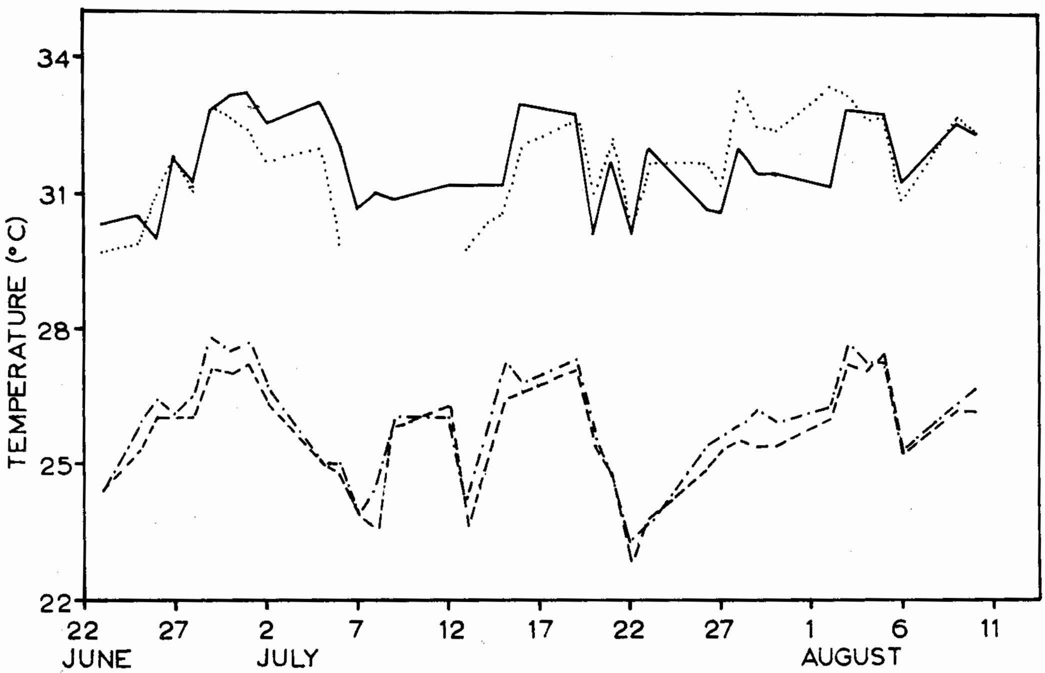

Figure 33. Bottom temperatures (C) for all pools in the flow-through experiment. The solid and dotted lines are for replicate heated pools and the dashed and dash-dot lines are for replicate ambient pools.

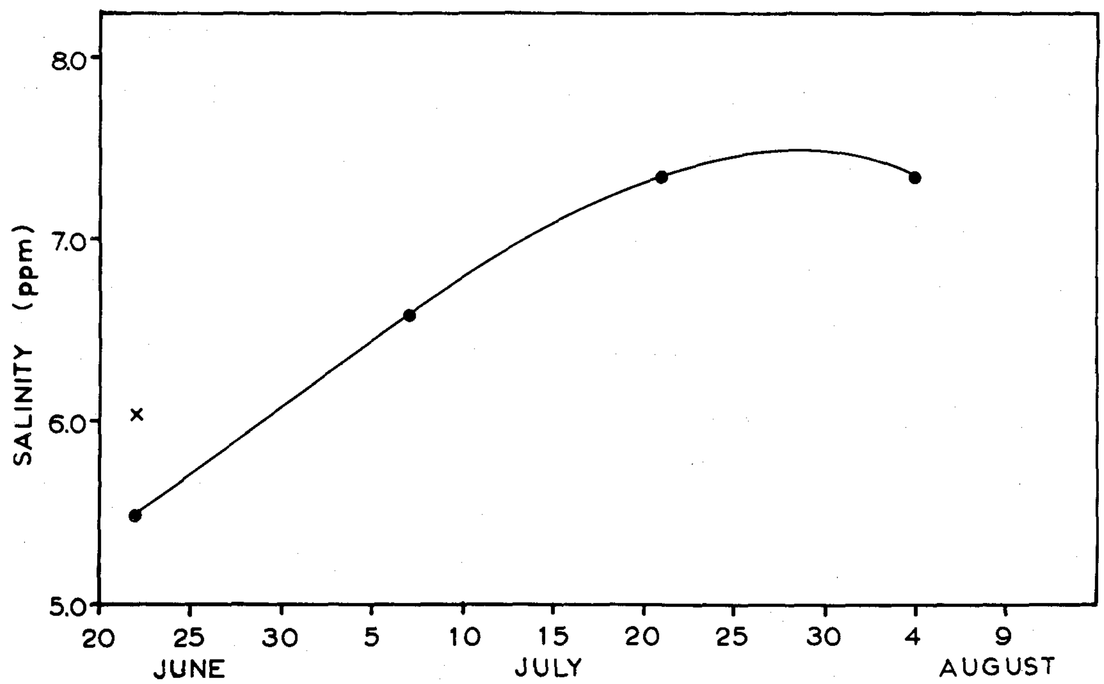

Figure 34. Salinity (ppt) in the flow-through experiment. The x represents bottom salinity in one ambient pool. All other values were the same for each sampling data in all pools.

The salinity in the pools, however, was very similar to that of South Creek Estuary. Salinity was almost the same in all four experimental pools on each sampling date, except on 22 June salinity at the bottom of one of the ambient pools was about 0.5 ppt higher than in the other three pools.

Reactive phosphorus concentrations (Fig. 35) increased in three of the pools throughout the flow-through experiment, ranging in concentrations from 0.1 ug-at P/1 on 26 June to 2.6 ug-at P/1 on 5 August. In one of the heated pools the reactive phosphorus concentration began to decrease after 20 July and continued to decrease throughout the tenure of the experiment. This significant decrease in reactive phosphorus concentration in that one heated pool is undoubtedly a reflection of the phytoplankton activity in that pool (i.e., chlorophyll concentration increased significantly during the same time period). The concentration of total phosphorus in the pools remained very similar throughout the experiment, with the value ranging from about 3 ug-at P/1 to 6 ug-at P/1.

Nitrate-nitrogen concentrations (Fig. 36) were relatively low throughout the flow-through experiment, ranging in values from about 0.25 ug-at N/1 up to about 0.7 ug-at N/1. With the exception of one sampling date (7 July), the concentrations of nitrate-nitrogen were similar in all four pools. Because the ammonia-nitrogen concentrations were relatively large (i.e., between 5 and 10 ug-at N/1), there was no drastic decrease in the nitrate-nitrogen concentration in the heated pool at the end of the experiment as there was with phosphorus concentration. The phytoplankton in the pools were undoubtedly using ammonia-nitrogen as their main nitrogen source rather than nitrate-nitrogen.

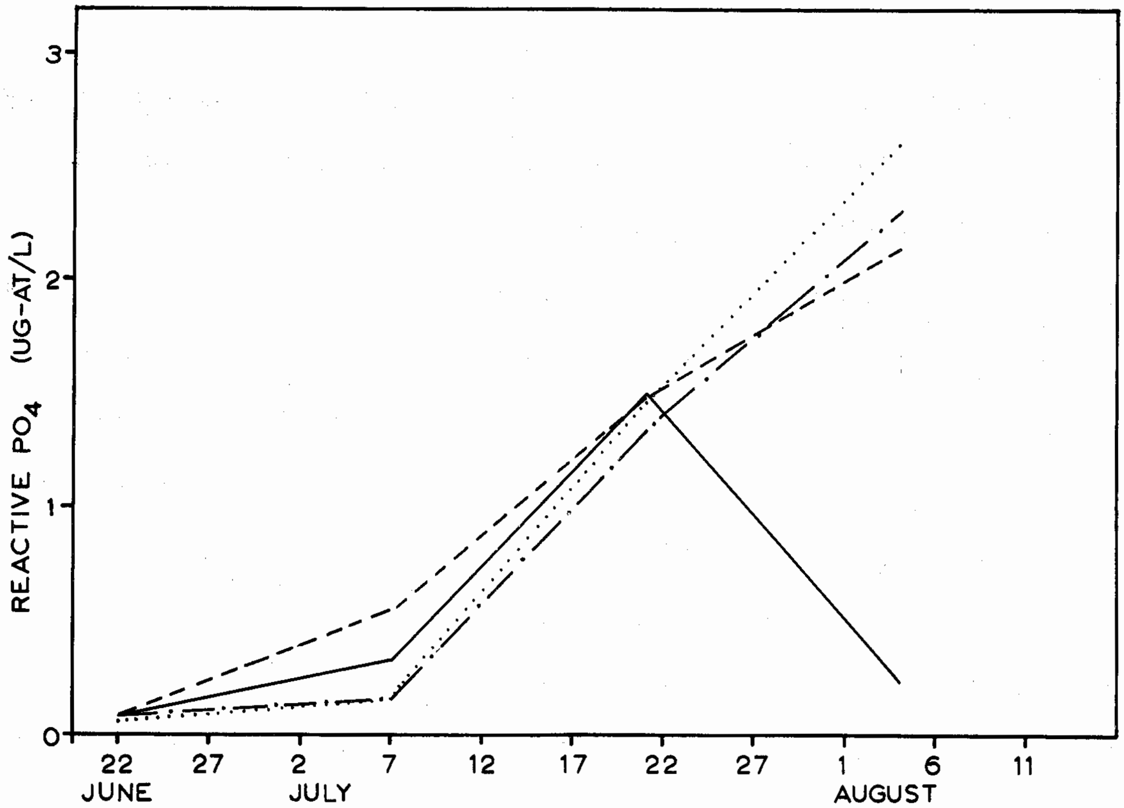

Figure 35. Reactive phosphorus (ug-at P/l) in the flow-through experiment. The solid and dotted lines are for replicate heated pools and the dashed and dash-dot lines are for replicate ambient pools.

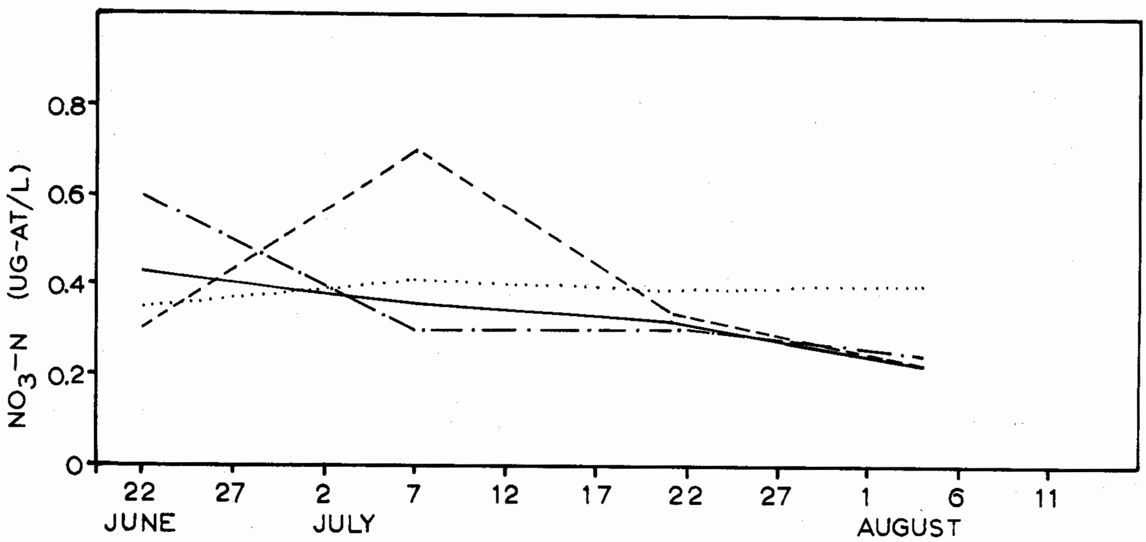

Figure 36. Nitrate (ug-at N/l) in the flow-through experiment. The solid and dotted lines are for replicate heated pools and the dashed and dash-dot lines are for replicate ambient pools.

VII. B. Plankton

Phytoplankton samples were not quantified during the flow-through experiment, but blue-green algae dominated in the heated pools and diatoms dominated in the ambient pools. The dominance of blue-greens in the heated pools was expected in view of the utilization of nutrients and the additional heat (See Section VI).

Chlorophyll concentrations were monitored during the flow-through experiment to provide information concerning concentrations of phytoplankton in the pools (Fig. 37). Chlorophyll concentrations were higher in the heated pools than in the ambient pools, with one of the heated pools achieving very high concentrations (*i.e.*, 50 mg/m^3) at the end of the experiment. This was the same pool in which the reactive phosphorus concentration decreased drastically during the last two weeks of experimental conditions. Another characteristic of the heated pool was the large phytoplankton population variability in the plankton concentrations as was discussed in Section VI for previous experiments.

Algae that had settled to the bottom of pools ranged from a mean of 324 gm/m^2 in the heated pools to 446 gm/m^2 in the ambient pools (Table 7). The amount of algae on the bottom was greater in the ambient than in the heated pools, which is opposite of the effect observed the previous summer without flow-through capabilities (Table 4). Perhaps, this is due to the fact that most of the algae produced in the heated pools during the flow-through experiment were still suspended in the water at the time the pools were drained.

Zooplankton were sampled at the beginning and the end of the flow-through experiment, and the samples were quantified (Table 8). The zooplankton was composed entirely of the copepod, *Acartia tonsa*, both adults and young. The total number of zooplankton per 10-liter sample in the heated pools decreased with time between 22 June and 10 August 1971; whereas, in the ambient pools the number of adults increased. The number of eggs in the zooplankton samples on 22 June 1971

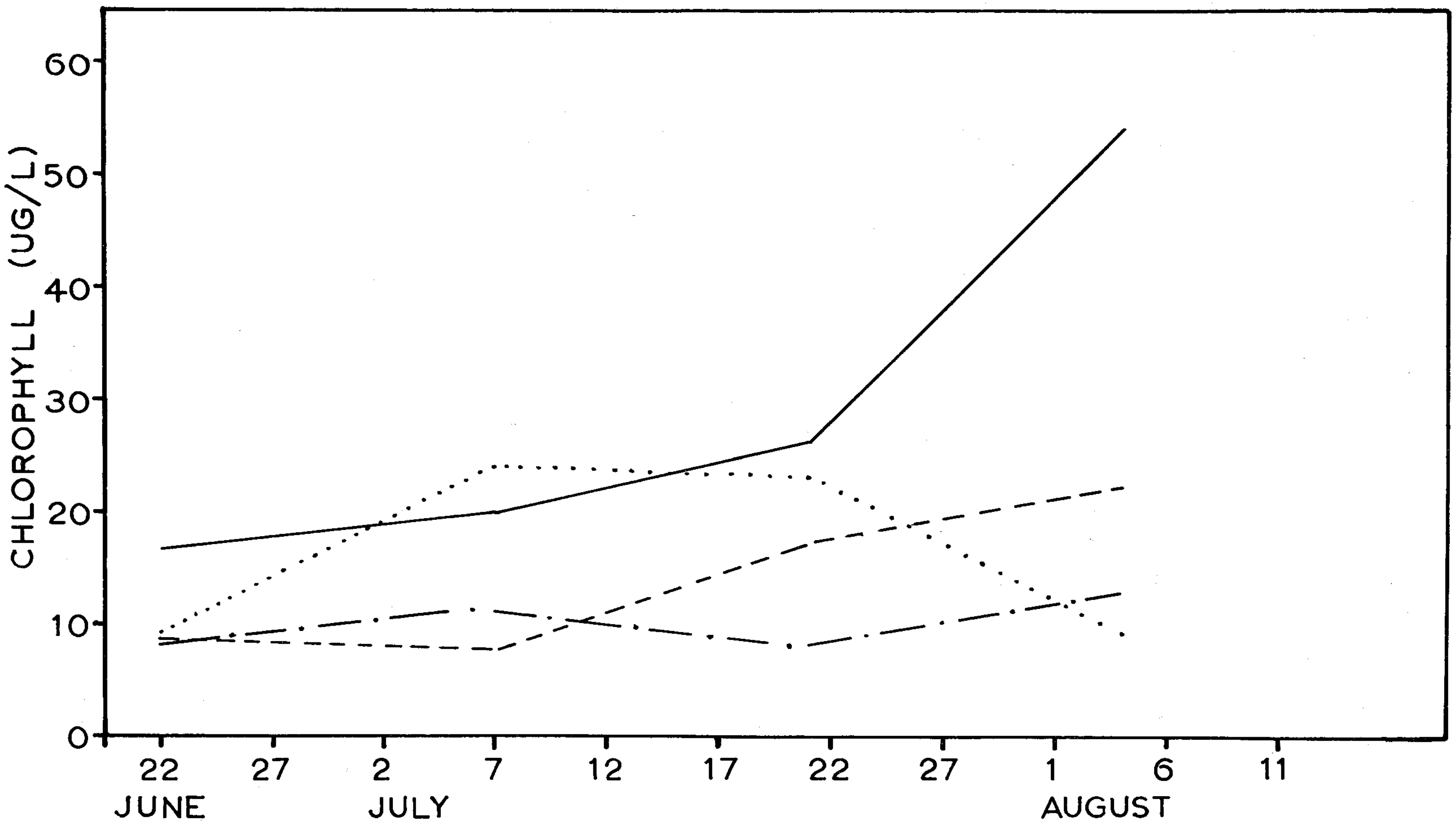

Figure 37. Chlorophyll a (ug/l) in the flow-through experiment. The solid and dotted lines are for replicate heated pools and the dashed and dash-dot lines are for replicate ambient pools.

Table 7: Mean dry weight of algae on the bottom at the end of the flow-through experiment.

Pools	Mean gm/sample	Range	Mean gm/m^2
Heated	3.24	3.11 - 3.37	324.0
Ambient	4.47	4.08 - 4.85	446.5

Table 8: Zooplankton in the flow-through experiment.

Pool	Organism	Number/10 liter sample	
		Input 22 June 71	Output 10 August 71
Heated			
	Acartia tonsa	21,290.5 (20,982-21,599)	5,352 (1,141-9,563)
	Nauplii	595 (577-613)	0
	Eggs	0	1,169.5 (0-2,339)
Ambient			
	Acartia tonsa	16,234 (13,767-18,701)	28,680 (4,742-52,618)
	Nauplii	2,054 (0-4,108)	0
	Eggs	0	7,668.5 (347-14,990)

was zero, but a substantial number (particularly in the ambient pools) had developed by the end of the experiment. All the nauplii that were present at the beginning of the experiment had disappeared by the end of the experiment.

VII. C. Nekton

Large variability existed between replicates in the flow-through experiments. This variability was the greatest in ambient pools for the nekton, although the phytoplankton was highly variable in both heated and ambient pools. Because of this large variability, the usual statistical analysis could not be used and, thus, the following discussion of results does not have statistical significance. Even though statistical significance did not exist for these experiments some trends are apparent.

The volume of blue crabs (C. sapidus) per pool (Fig. 38) decreased throughout the experiment. These decreases were influenced by the escapement of blue crabs during the course of the experiment (i.e., the flow-through conditions maintained water levels to the top of the plastic pools and allowed the swimming crabs to escape). The decrease in the volume of blue crabs was less in the ambient pools than in the heated pools, although the difference was not significant.

Grass shrimp (P. pugio) volume increased in the ambient pools during the flow-through experiment (Fig. 38). This large increase was in contrast to the grass shrimp data from the previous summer for quiescent conditions. The difference can possibly be attributed to the incoming food under flow-through conditions. There was a slight decrease in grass shrimp volume in the heated pools. The grass shrimp continued to spawn in the ambient pools, but discontinued spawning activity in the heated pools. Apparently, the 33-34 C temperatures in the heated pools discouraged further spawning activity.

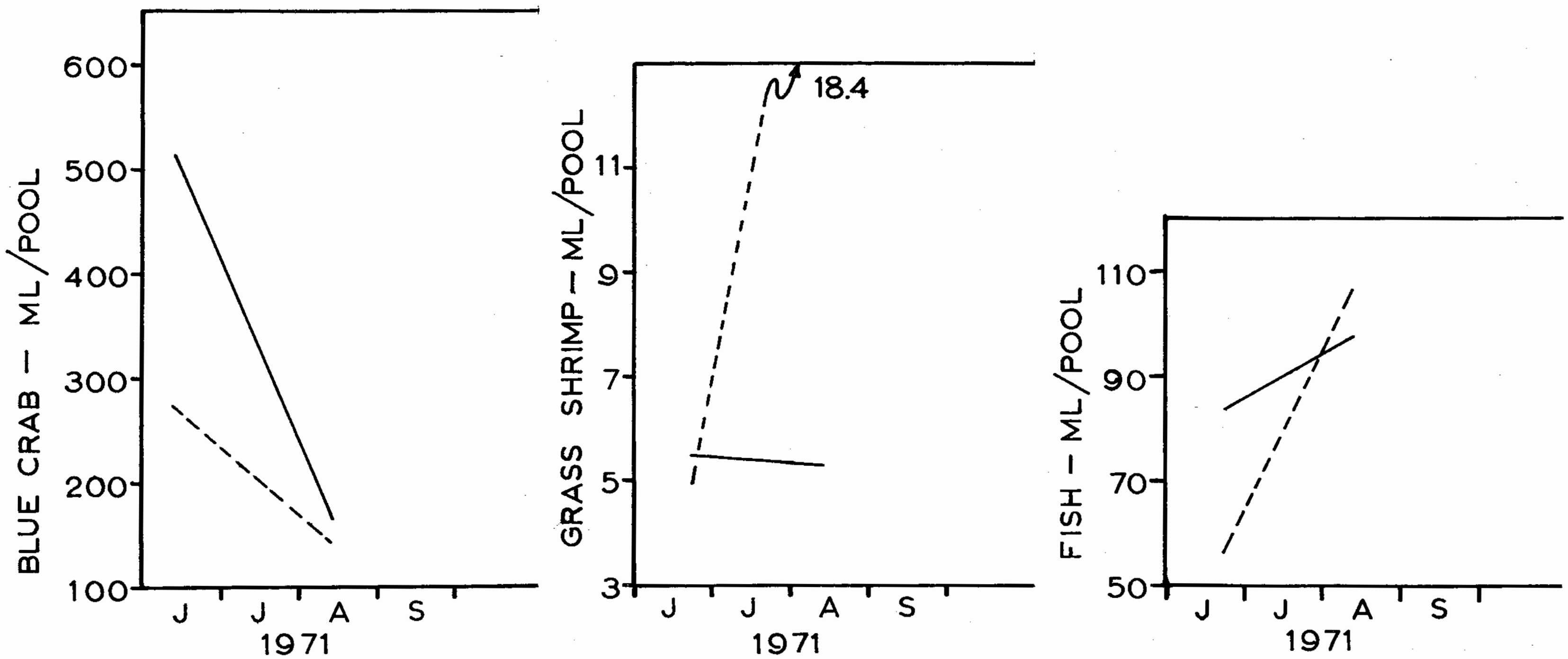

Figure 38. Mean volume (ml/pool) of blue crabs, grass shrimp and fish in the flow-through experiment. Solid lines are for heated pools and the dashed lines are for ambient pools.

The fish volume per pool increased in both the heated and ambient pools throughout the flow-through experiment (Fig. 38). The fish involved in this experiment consisted of croakers (Micropogon undulatus) and killifish (Fundulus grandis). Percent survival was the same in all pools, regardless of heat treatments. The increase in volume of fish in the ambient pools was considerably larger than the increase in volume in the heated pools, but because of the large variability in the ambient pools the differences were not statistically significant. The changes in volume of fish in the flow-through experiment were similar to the changes in fish volume the previous summer under quiescent conditions (see Fig. 26).

VII. D. Benthos

The volume of oysters per pool station decreased in both the ambient and heated pools during the flow-through experiment (Fig. 39). This decrease was due to the mortality of several oysters in all pools. The percent survival was the same under both heated and ambient conditions.

The volume of Rangia clams in the heated pools decreased; whereas, the volume in the ambient pools increased (Fig. 39). This is similar to the response noted for the pools during the previous summer under quiescent conditions. Apparently, the temperature in the heated pools exceeded the ability to survive for some of Rangia in all of the pools. The differences in Rangia volumes in this experiment were statistically significant.

Widgeon grass (R. maritima) volume decreased in all pools during the flow-through experiment (Fig. 39). This is a normal behavior of the grass and is similar to the results obtained during the previous summer under quiescent conditions. Apparently, temperatures under both ambient and heated conditions are too high for growth of Ruppia, which is similar to observations in natural estuaries. There was no statistical difference between Ruppia in the heated pools and in the ambient pools.

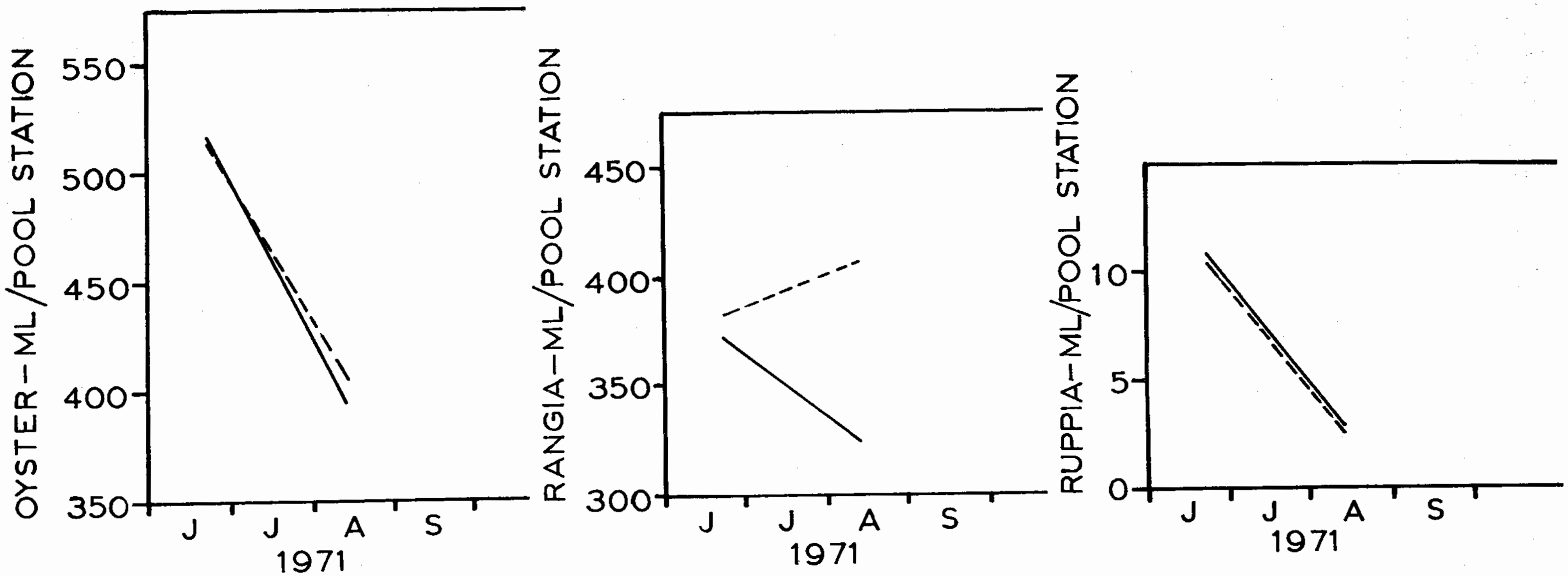

Figure 39. Mean volume (ml/pool station) of oysters, bay clams and widgeon grass in the flow-through experiment. Solid lines are for heated pools and the dashed lines are for ambient pools.

Table 9: Mean volumes (ml/pool) and dry weights (gm/pool) of benthic macrofauna at the end of the flow-through experiment.

Organisms	Pool	Organism/pool(range)	ml/pool(range)	gm/pool(range)
Nereis sp.	heated	80 (75-85)	8.5 (8.3-8.7)	.6495(.557-.742)
	ambient	99 (70-128)	12.95 (6.1-19.8)	1.2745(.405-2.144)
Glycera sp.	heated	83 (25-141)	9.75(4.2-15.3)	2.6505(.987-4.314)
	ambient	54 (11-97)	6.85(1.1-12.6)	1.4325(.375-2.490)
Chironomous sp.	heated	307 (205-409)	1.95(1.9-2.0)	.1005(.1-.101)
	ambient	301 (150-452)	1.7 (1.0-2.4)	.089 (.032-.146)
Gobiosoma sp.	heated	2 (1-3)	.65(1.0-0.3)	.085 (.027-.143)
	ambient	8.5 (0-17)	2.75(0-5.5)	.418 (0-.836)
Rangia cuneata (immatures)	heated	0	0	0
	ambient	145 (0-290)	3.2 (0-6.4)	2.5125(0-5.025)

Benthic macrofauna, which appeared on the bottoms of the pools at the end of the flow-through experiment, are summarized in Table 9. None of these organisms were specifically placed in the pools at the beginning of the experiment, although they were allowed to enter as eggs and larvae during the course of the experiment. Larger concentrations of *Nereis* sp. and *Gobiosoma* sp. (Gobies) were observed in the ambient pools than in the heated pools. *Glycera* sp. had a greater concentration in the heated pools than in the ambient pools. These worms are found in the Pamlico River Estuary during the summer and apparently survive high temperatures. *Rangia cuneata* immatures were found in the ambient pools in large concentrations, but not in the heated pools. These clams are known to spawn in the estuary during early summer (47) and undoubtedly came into the pools as eggs in the water, and survived in the ambient pools only. No difference could be detected in the concentrations of *Chironomous* sp. between the ambient and heated pools.

VIII. LITERATURE CITED

1. Gaucher, L. P., "Energy in Perspective," Chem. Tech., March (1971).

2. Coutant, C. C., "Thermal Pollution - Biological Effects, A Review of the Literature of 1969," Rpt. Battelle Northwest, Richland, Wash., BNWL-SA 3255, 1-90 (1970).

3. Sinha, E. S., "Coastal/Estuarine Pollution," Ocean Engineering Information Series, 3, 87 pp (1970).

4. Wurtz, C. B., and Renn, C. E., "Cooling Water Studies for Edison Electric Institute," Research project of Sanitary Engineering and Water Resources of the Johns Hopkins University, RP-49, 99 pp (1965).

5. Dallinger, W. H., "The President's Address," J. Roy. Micro. Soc., 2, 185-199 (1887).

6. Carpenter, K. E., "The Lethal Action of Soluble Metallic Salts on Fishes," Brit. J. Exp. Biol., 4, 378-390 (1927).

7. Sayle, M. H., "Factors Influencing the Rate of Metabolism of Aeschna umbrosa Nymphs," Biol. Bull., 54, 212-225 (1928).

8. Walshe, B. M., "Oxygen Requirements and Thermal Resistance of Chironomid Larvae from Flowing and Still Waters," J. Exp. Biol., 25, 35-44 (1948).

9. Hutner, S. H., Baker, H., Aaronson, S., Nathan, N. A., Rodriquez, E., Lockwood, S., Saunders, M., and Petersen, R. A., "Growing Ochromonas malhamensis above 35° C," J. Protozool., 4, 259-269 (1957).

10. Van Vliet, V., "Effect of Heated Condenser Discharge Water upon Aquatic Life," Am. Soc. Mech. Angnr., 57, 1-10 (1957).

11. Beyers, B. J., "Relationship Between Temperature and the Metabolism of Experimental Ecosystems," Science, 136, 980-982 (1962).

12. Douboroff, P., and Katz, M., "Critical Review of the Literature on the Toxicity of Industrial Wastes and Their Components to Fish, II, The Metals and Salts," Sewage Industr. Wastes, 25, 802-839 (1953).

13. Herbert, D. W. M., Downing, K. M., and Merkens, J. C., "Studies on the Survival of Fish in Poisonous Solutions," Verh. int. Ver. Limnol., 12, 789-794 (1955).

14. Hynes, H. B. N., "Biological Effects of Poisons, p. 70-85. In Hynes, H. B. N., (ed.) The Biology of Polluted Waters," Liverpool University Press, Liverpool (1966).

15. De Sylva, D. P., "The Unseen Problems of Thermal Pollution," Oceans, 1, 37-41 (1969).

16. Stewart, J. E., Cormick, J. W., and Zwicker, B. M., "Influence of Temperature on Gaffkemia, a Bacterial Disease of the Lobster Homarus americanus," Jour. Fish. Res. Bd. Can., 26, 2503-2510 (1969).

17. Mann, K. H., "Occurrence of an Exotic Oligochaete Branchiura sowerbyi Beddard, 1892, in the River Thames," Nature Lond., 182, 732 (1958).

18. Naylor, E., "Effects of Heated Effluents upon Marine and Estuarine Organisms, p. 63-103. In Russell, F. S., (ed.) Advances in Marine Biology," Academic Press, London, Vol. 3 (1965).

19. Heinle, D. R., "Temperature and Zooplankton," Chesapeake Sci., 10, 186-209 (1969).

20. Trembley, F. J., "Research Project on Effects of Condenser Discharge Water on Aquatic Life," Progress Rpt. of the Institute of Research, Lehigh University, 141 pp (1960).

21. Anderson, R. R., "Temperature and Rooted Aquatic Plants, "Chesapeake Sci., 10, 157-164 (1969).

22. North, W. J., "Biological Effects of a Heated Water Discharge at Morro Bay, California," Sixth International Seaweed Symposium, Madrid (1969).

23. Poltoracka, J., "Specific Composition of Phytoplankton in a Lake Warmed by Wastewater from a Thermoelectric Plant and Lakes with Normal Temperature," Biol. Abs., 50, 23148 (1969).

24. Cory, R. L., and Nauman, J. W., "Epifaunal and Thermal Addition in the Upper Patuxent River Estuary," Chesapeake Sci., 10, 210-217 (1969).

25. Cook, H. L., and Murphy, M. A., "The Culture of Larvae Penaeid Shrimp," Trans. Amer. Fish. Soc., 98, 751-754 (1969).

26. Siefken, M., and Armitage, K. B., "Seasonal Variation of Metabolism and Organic Nutrients in Three Diaptomus (Crustacea: Copepoda), "Comp. Biochem. Physiol., 24, 591-609 (1968).

27. Vernberg, F. J., and Vernberg, W. B., "Thermal Influence on Invertebrate Respiration," Chesapeake Sci., 10, 234-240 (1969).

28. Warinner, J. E., and Brehmer, M. L., "The Effects of Thermal Effluents on Marine Organisms," Intern. J. Air Water Poll., 10, 277-289 (1966).

29. Morgan, R. P., and Stross, R. G., "Destruction of Phytoplankton in the Cooling Water Supply of a Steam Electric Station," Chesapeake Sci., 10, 165-171 (1969).

30. Strickland, J. D. H., and Parsons, T. R., "A Practical Handbook of Seawater Analysis," Fish. Res. Bd., Canada, 167, 1-311 (1968).

31. Menzel, D. W., and Corwin, N., "The Measurement of Total Phosphorus in Sea Water Based on the Liberation of Organically Bound Fractions by Persulfate Oxidation," Limnol. Oceanogr., 10, 280-282 (1965).

32. Davis, H. L., "Evaluation and Use of the Partial Pressure of Carbon Dioxide in Studying Community Metabolism in Heated Experimental Ecosystems," Unpubl. Ph.D. Thesis, Dept. of Zoology, N. C. State Univ., Raleigh, 90 pp (1971).

33. Hobbie. J. E., "Phytoplankton Species and Populations in the Pamlico River Estuary of North Carolina," Rpt. of Water Resources Research Institute, Univ. of N. C., 56, 1-147 (1971).

34. Lorenzen, C. J., "Determination of Chlorophyll and Pheo-pigments: Spectrophotometric Equations," Limnol. Oceanogr., 12, 343-346 (1967).

35. Steele, R. G. D., and Torrie, J. H., Principles and Procedures of Statistics McGraw-Hill Book Co., New York (1960).

36. Ryther, J. H., "The Ecology of Phytoplankton Blooms in Moriches Bay and Great South Bay, Long Island, New York," Biol. Bull., 106, 198-209 (1954).

37. Cooper. D. C., and Copeland, B. J., "Responses of Continuous-Series Estuarine Microecosystems to Point-Source Input Variations," Ecol. Monogr., 42, In Press (1972).

38. Odum, E. P., "Principles Pertaining to Limiting Factors, p. 88-144. In Odum, E. P., (ed.) Fundamentals of Ecology," W. B. Saunders Co., Philadelphia (1959).

39. Vollenweider, R. A., A Manual on Methods for Measuring Primary Productivity in Aquatic Environments, Blackwell, Oxford (1969).

40. Strickland, J. D. H., "Production of Organic Matter in the Primary Stages of the Marine Food Chain, p. 477-610. In Riley, J. P., and Skirrow, G., (eds.) Chemical Oceanography," Academic Press, New York, Vol. 1 (1965).

41. Findenegg, I., "Untersuchungen uber die Ökologia und die Produktionsverhältniss des Planktons in Karntver Seengebiete," Int. Rev. Hydrobiol., 43, 368-429 (1943).

42. Carpenter, E. J., "Effects of Phosphorus Mining Wastes on the Growth of Phytoplankton in the Pamlico River Estuary," Chesapeake Sci., 12, 85-94 (1971).

43. Sherk, J. A., Effects of Low Levels of Phosphate Mining Effluent on Periphyton in Controlled Artificial Estuaries," Unpubl. Ph. D. Thesis, Dept. of Zoology, N. C. State Univ., Raleigh (1969).

44. Copeland, B. J., and Bechtel, T. J.," Some Environmental Limits of Six Important Galveston Bay Species," Rpt. Galveston Bay Study Program, Texas Water Quality Board, Austin (1970).

45. Wood, C. E., "Physioecology of the Grass Shrimp, Palaemonetes pugio, in the Galveston Bay Estuarine System," Contri. Mar. Sci., 12, 54-79 (1967).

46. Raney, E. C., and Menzel, B. W., "Heated Effluents and Effects on Aquatic Life with Emphasis on Fishes, A Bibliography," Bull., Cornell Univ. Water Resources and Marine Sciences Center, Philadelphia Electric Company, and Ichthyological Associates, 2, 1-470 (1969).

47. Tenore, K. R., "The macrobenthos of the Pamlico River Estuary, North Carolina," Rpt. of Water Resources Research Institute, Univ. of N. C., 40, 1-113 (1970).