

THE INTERACTIVE EFFECTS OF
TEMPERATURE AND NUTRIENTS
ON PERIPHYTON ASSEMBLAGES FROM A
LOW-PH SOUTH CAROLINA
STREAM

MASTER

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The interactive effects of temperature and nutrients on periphyton assemblages from a low pH South Carolina stream.

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Periphyton that accumulated on glass slides in a flow-through, artificial stream system at the Thermal Effects Laboratory, Savannah River Laboratory, were studied for their response to continuously elevated temperatures and to nutrient additions. Although the stream water used in these experiments was low in nutrients, addition of nutrients rarely increased periphyton biomass or carbon-uptake rates. The most consistent stimulation occurred in July at 12°C above ambient temperature. Large day-to-day variations in chlorophyll a and rate of carbon-uptake of the assemblages were observed at ambient temperatures and at elevated temperatures. Temperature and available carbon varied on approximately the same time scale as the algal parameters. It is concluded that nutrient limitation, if it exists in this system, is masked by the effects of temperature and, in summer, possibly by limitation of carbon to the periphyton.

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INTRODUCTION

Algae are a major group of organisms found in every habitat on earth. They form the bases of aquatic and marine food webs. The maintenance and productivity of algal populations are as important to man as the food he harvests and the recreation he derives from aquatic areas. Ecosystems should be studied to understand man's impact on them.

Pollution of waterways is an ever-increasing problem; industries, cities, and power plants dump their wastes into rivers, lakes, and coastal areas (Krenkel and Parker, 1969; Reish et al., 1978). Effects of toxic compounds, heavy metals, sewage and waste heat on aquatic communities are sometimes difficult to detect (Buck and Rankin, 1972; Burton et al., 1976), sometimes impossible to overlook (Thorhaug, 1974; Rice and Ferguson, 1975).

Many single-variable experiments have been carried out with single species or defined communities to determine the response of algae to light, temperature, and nutrients. These studies provide valuable information on the reactions of these species and communities to changes in the variable considered. But since a change in one variable can alter a community's response to a second variable (e.g. Goldman, 1979), more studies are now needed to look at effects of the interactions of variables on communities, incorporating as much as possible fluctuations similar to those found naturally in the environment.

The purpose of this dissertation is to explore the effects of consistent temperature elevation and nutrient additions on algal communities. I hypothesize that enrichment of an oligotrophic system will in general result in higher biomass and carbon-uptake rates than normal, and that enrichment will decrease inhibition of algal growth or metabolism caused by thermal stress.

LITERATURE REVIEW

Temperature

The use of rivers and estuarine waters for cooling purposes increases daily - the volume used approaches 1/5 the total run-off of the US (Krenkel and Parker, 1969). The effects of temperature on algal species and communities have been studied in many systems. In general, as summarized by Patrick (1971, 1974), in temperate aquatic communities a moderate increase in temperature towards the optimum of most of the species tends to increase growth rate, biomass, and diversity statistics. As the upper limit to growth is approached, temperature increases alter community structure from an assemblage dominated by diatoms (20°C) to one dominated by green algae (30°C), to one dominated by blue-green algae (35°C and above).

Although the argument continues whether ecosystems with many or few species are more stable (e.g. Pomeroy, 1975; McNaughton, 1977), communities do differ in their response to temperature fluctuations. Some systems showed little change when the temperature was raised or lowered a few degrees from ambient (Beyers, 1962; Cairns et al., 1970; Rankin et al., 1974). Other communities exhibited a drastic shift in structure

(Carpenter, 1973) or biomass (Thorhaug, 1974) when the temperature was raised a few degrees. Still other systems responded to temperature manipulation only at certain seasons (Flemer and Sherk, 1977) or at certain ambient temperatures (Fox and Moyer, 1973).

Variation among communities in response to temperature manipulation can be due to any of several reasons. Systems that are highly integrated have alternate routes of energy flow, and their functional response to a change in temperature is small compared to that of a less complex system (Beyers, 1962; Cooke, 1971). Some communities may be adapted to moderate temperature fluctuations which they normally encounter and thus may appear impervious to experimental temperature fluctuations. Communities adapted to stenothermal conditions, conversely, may exhibit noticeable changes in structure or function with a moderate temperature change (Naylor, 1965). Still another consideration is how close to the upper or lower lethal limit the temperature fluctuation takes the community. If a community is living close to its upper temperature limit, a small increase in temperature will decrease biomass or productivity (Thorhaug, 1974; Naylor, 1965). Changing proximity to the upper temperature limit could well account for seasonal responses to temperature change; inhibition of algal communities by elevated temperatures is greatest when the ambient temperature is closest to the upper limit (Flemer and Sherk, 1977).

Elevated temperature is not the only thermal alteration which can affect communities. Deep release dams may eliminate much of the temperature fluctuation below them, resulting in cool summers and warm winters. The thermal stimulæ for physiological processes may be removed, thus delaying or reducing feeding or reproduction (Ward, 1976).

Since reproduction of many species occurs in only a narrow temperature range, with diurnal fluctuations contributing to the cues, any alteration of the normal temperature cycle may affect the survival of populations (Naylor, 1965; Sweeney and Schnack, 1977). Elimination of a population from one segment of a river may reduce migration and interfere with community dynamics in other areas of the river (Gore, 1980).

Regular short-term temperature fluctuations may have little effect on algal communities. The noon peak temperature may be tolerated by organisms only the one or two hours it normally lasts - it could be lethal if imposed constantly (Soeder and Stengel, 1974; Lanza and Cairns, 1972).

The manner in which temperature affects the algal cell is the key to the response of populations and communities to temperature change. Eppley (1972) suggested that temperature dictates the maximum possible growth rate for algae, but that its effect is often masked by nutrient or light limitation. If nutrients or light are at suboptimal or high, inhibitory levels, the algal populations cannot acquire materials or energy for division fast enough to exhibit their maximum possible growth rate.

Several studies have shown that algal cells contained more nitrogen and carbon while growing at a temperature other than optimal (Ahlgren, 1978; Goldman, 1977b). At optimum temperature, cell division and nutrient uptake may be uncoupled (DeManche et al., 1979), or growth may be so fast that nutrients do not accumulate before cell division occurs. Algal cells have been found to be smaller when growing at optimum temperature, larger at suboptimal temperature (Margalef, 1954; Goldman, 1977a), possibly also related to the rate of division.

While Margalef (1954) found dry weight/cell to be unaffected by temperature, Morris and Glover (1974) found less dry weight/cell at elevated temperatures, but more chlorophyll a. Protein synthesis by algae was conserved at nonoptimal temperatures (Morris et al., 1974); at optimum temperatures one would expect to find more carbohydrate and lipid production in relation to protein synthesis than at nonoptimal temperatures.

As the temperature rises, reactions in the cell take place more quickly (Marré, 1962) and the structure and/or activity of membranes may change (Soeder and Stengel, 1974). The liquid structure of water may also change (Drost-Hansen, 1969). An algal cell must therefore be able to continue functioning as reactions take place faster, its cellular structure changes, and the shape of its environment (the water) changes. The temperature limits shown by most algae are no doubt an indication of the failure of the cells to accommodate so many changes. The rates of successive reactions respond differently to temperature change, and syntheses may be slow or incomplete (Marré, 1962). The activity of the membranes is altered and uptake of nutrients can change, also affecting syntheses (Soeder and Stengel, 1974). At very high temperatures, respiration may outstrip photosynthesis and the cell starve to death, or enzymes and cell walls may be altered beyond the ability to function.

Some algae, notably the procaryotic blue-greens, can live at very high temperatures. Schizothrix calcicola and Phormidium sp. were found at 37°C (Stockner, 1968), Calothrix up to 50°C (Brock, 1967a), and Synechococcus from 50°C to 60°C (Brock, 1967a; Brock and Brock, 1969 a and b). The optimum temperatures for these algae appear to be at those elevated temperatures; carbon fixation studies showed high rates of

photosynthesis at high temperatures (Brock, 1967b; Brock and Brock 1966 and 1969b). Mastigocladus laminosus has been found in thermal streams from 35-60°C, with occasional surges up to 73°C which it appeared to survive (Castenholz, 1969a). Several reviews of high temperature environments (Brock, 1970; Castenholz, 1969b) discuss the occurrence of various taxonomic groups in these areas.

The physiological and biochemical basis for life at these high temperatures, or even at a wide range of lower temperatures, has been studied. Somero (1978) reviewed work done on the temperature adaptation of enzymes. Although he dealt with animal and higher plant enzymes, the compromises for existence at high temperatures that he listed (conservation of apparent K_m values, temperature-dependent enzyme concentrations, heat stability differences among enzyme homologues, modification of structural stability, multiple isozymes, and eurythermality) probably account for much of the success of algal survival over a range of temperatures. Membrane lipids and fatty acids are also often cited as important to thermotolerance; thermophilic organisms tended to have fatty acids with longer, straighter, high melting point chains (McElhaney, 1976; Welker, 1976), and the proportion of saturated to unsaturated fatty acids decreased as the temperature rose (Holton et al., 1964).

The term thermal stress is used here as the existence of temperature conditions far enough away from the optimal range, the range of maximum photosynthesis, growth, and reproduction of an organism, to reduce the organism's growth and/or photosynthesis rate. It is obviously different for different species and communities. Thermal spring algae would experience thermal stress at temperatures below 50-60°C and

above 70-75°C; Antarctic algae would experience thermal stress above 3-5°C. Temperate zone algae, depending on the range of temperatures they tolerate (Patrick, 1971), would be expected to experience thermal stress below 5-10°C and above 25-30°C.

Light

Light intensity and periodicity is another important factor in autotrophic algal growth. Light provides the energy source for photosynthesis and energy-requiring processes. Chlorophylls and accessory pigments absorb energy between 400 and 700 nm wavelength and transfer it to reaction centers (Govindjee and Braun, 1974). The pigmentation of an algal population - the amount of chlorophyll a present and the ratio of accessory pigments to chlorophyll a - is dependent on its light history (Carreto and Catoggio, 1976). Laboratory cultures and natural populations grown at light intensities approaching full sunlight had less chlorophyll a/cell than did cultures grown at lower light intensities (Brown and Richardson, 1968; Steeman-Nielsen and Jorgensen, 1968a and b; Sheridan, 1976; Beardall and Morris, 1976). Macroalgae in deep water contained more accessory pigments/chlorophyll a than did algae growing in shallow water (Ramus et al., 1976). Since chlorophyll a is more stable than other pigments at high light intensity, algae growing in full sunlight had more chlorophyll a/accessory pigments (Brown and Richardson, 1968).

Many workers have reported diel variations in algal cell activity and contents. Chlorophyll content (Yentsch and Ryther, 1957) and activity (Sournia, 1973) rose and fell throughout the day as well as dry weight/cell, cell size, division rate (Gons and Mur, 1979), and

photosynthetic rate (Harris and Piccinin, 1977). Fluorescence/ chlorophyll a was highest at night, lowest at noon (Kiefer, 1973a and b). Daylength (photoperiod) has often been directly correlated with algal productivity (Jewson, 1976; Castenholz, 1964; Stepanek, 1965), although some algae grew faster at suboptimal temperatures with a short, rather than long, day (Admiraal, 1977a).

Photosynthesis has been shown to vary with varying light intensity. Talling (1957) first introduced the terms I_k and P_{max} : the lowest saturating light intensity and the maximum rate of photosynthesis, respectively. Photosynthesis at low light is directly correlated with light intensity, indicating a photochemical rate limiting step. Above I_k , light saturated photosynthesis is enzyme regulated and dependent on temperature (Jorgensen and Steeman-Nielsen, 1965; Steeman-Nielsen and Jorgensen, 1968a), although there is some temperature effect on the photochemical reactions through the enzymes involved (Yentsch, 1974). P_{max} therefore changes with changing temperature (Talling, 1957), and I_k changes with it by extension or truncation of the linear part of the curve (Gargas et al., 1979; Beardall and Morris, 1976; Phinney and McIntire, 1965; Steeman-Nielsen and Hansen, 1961).

Some discussion has centered on whether α , the slope of the initial part of the P vs I curve, can change. Platt and Jassby (1976) hypothesized that the slope should remain constant since it was photochemically defined; light capture by the cells regulates the photochemical reactions, however, and is affected by pigmentation, history of the cells (Bannister, 1974; Platt and Jassby, 1976), and nutrient limitation (Strickland, 1958). Photosynthesis is often light-saturated at much

higher light intensities than is growth (McAllister et al., 1964; Prézelin and Sweeney, 1978).

Nutrients

The study of the nutrient requirements of algae began with Miquel's (1890-1893, cited by Provasoli et al., 1957) attempts to culture diatoms. Chu (1942, 1943) and Provasoli (1958; Provasoli et al., 1957) increased knowledge in this area with their work on media for the growth of algae. Droop (1957, 1958, 1961) and Carlucci and Silbernagel (1966a and b, 1967, 1969) contributed to an understanding of the needs of some algae for vitamins. Although today the macronutrients, and the micronutrients within analytical measurement, needed by most algae for growth and reproduction, and their use in the cell, are known (see O'Kelley, 1974); the amounts needed by different taxonomic groups are still in doubt. The requirements of individual species for one or more nutrients (Ahlgren, 1977; Eyster, 1978; Wolfe, 1954; Rhee, 1973 and 1974; Myklestad, 1977) is an area of continuing study.

Nutrient deficiency or limitation is usually regarded as the reduction of full potential photosynthesis and/or growth rate by the low level of one or more nutrients in the environment. It can be detected by increased carbon-uptake or growth rates of natural populations in enriched conditions (Gerhart and Likens, 1975) or by the rate of carbon-uptake of a bioassay alga (Gerhold and Otto, 1976). Communities in lakes (Schindler et al., 1971, 1973, and 1978; Lean, 1973a and b; Goldman, 1960a and b, 1964, 1965, 1972; Schelske et al., 1972, 1974, and 1978) and marine waters (Admiraal, 1977b, Ignatiades, 1977; McCarthy et al., 1977; Ryther and Dunstan, 1971; Thomas, 1969, 1970a and b) have

been studied for nitrogen, phosphorus, silicate, or trace element deficiency by nutrient addition, bioassay, or nutrient uptake studies.

Other workers have looked at nutrient uptake rates of individual species (Conway et al., 1976; Davies, 1970; Davis et al., 1978; Eppley et al., 1969; Goldman and Peavey, 1979; Guillard et al., 1973; Harrison et al., 1976) and/or intact communities (Caperon and Meyer, 1972; Eppley et al., 1969). Nutrient kinetic studies have begun to explain the distribution of some species and the interactions of species in some ecosystems (D'Elia et al., 1979; Titman, 1977; Tilman, 1978). Since rapid uptake of nutrients may precede increased growth rates, consideration of both external and internal nutrient concentrations is important to an understanding of nutrient effects (DeManche et al., 1979; Goldman, 1979).

Phosphorus has often been found to limit freshwater communities (Dillon and Rigler, 1974; Kerekes, 1974, 1975; Sakamoto, 1971; Schindler et al., 1973; Welch et al., 1978), while nitrogen is frequently cited as the limiting nutrient in coastal marine waters (Harrison and Davis, 1977; Nasev et al., 1977; Ryther and Dunstan, 1971; Thomas, 1969, 1970a and b; Yentsch et al., 1977), although nitrogen has been found to be limiting in some freshwaters (Lannergren, 1978) and phosphorus deficiency has been found in some marine areas (Thayer, 1974). Micronutrients stimulated productivity of some algal communities (Goldman, 1964, 1965, 1972; Ryther and Guillard, 1959), as did silicon (Ryther and Guillard, 1959). Carbon has been thought to be a limiting nutrient in some systems (Adams et al., 1978; Church et al., 1978; King, 1970; Kuentzel, 1969; Dickman, 1973), although rapid atmospheric CO₂ invasion of aquatic systems and low K_s values for inorganic carbon

uptake are arguments used against that hypothesis (Goldman et al., 1974; Loftus et al., 1979).

In some cases several nutrients at once - nitrogen + phosphorus + trace elements - are necessary to increase algal biomass or productivity (Maslin and Boles, 1978; Menzel et al., 1963; Polisini et al., 1970; Schelske et al., 1972, 1974, 1978; Brockway et al., 1970; Stoermer et al., 1978, Viner, 1977). Sometimes no nutrient tested has been found to increase the productivity of an oligotrophic system (Ignatiades, 1977; Larson, 1972; Patrick, 1966). These results may depend on what nutrients were tested on the communities and in what combinations. If only nitrogen or phosphorus deficiency is suspected and tested, a trace metal deficiency can go undetected.

Synergisms

The interaction of environmental factors make the study of single variables difficult, especially under natural conditions. Laboratory studies must consider what constant conditions to use, and results may be influenced by the constants chosen. The uptake of a nutrient is dependent on the concentrations of other nutrients (Blankenship and Wilbur, 1975; Mohleji and Verhoff, 1980), on the temperature (Goldman, 1979), and on light (Nelson and Conway, 1979; Harvey and Caperon, 1976; Eppley et al., 1971). Nonsimultaneous uptake of nutrients, such as carbon and nitrogen, may result in preferential uptake of one at first, so that apparently no carbon is being fixed while N_2 is fixed or NO_3 is taken up (Falkowski and Stone, 1975; Paerl, 1979). The amount of a nutrient required is affected by temperature (Jacobs and Lind, 1977). At supraoptimal temperatures, growth is often inversely proportional to

light intensity (Gross and Jahn, 1962), with light stress occurring at intensities which are saturating at optimal temperature (Durbin, 1974). Since the effects of the interaction of environmental factors vary among species, different combinations of temperature, light, and nutrient levels can obviously affect species composition (Patrick et al., 1969).

Although a change in temperature theoretically affects only the solution of gases in natural waters (Hawkes, 1969), this change can affect the action of toxicants on algal species and communities. As temperature approaches the upper or lower limit of growth of a species or system, toxicants such as chlorine (Flemer and Sherk, 1977) and PCBs (Fisher and Wurster, 1973) inhibit growth and productivity at low concentrations. Since nutrient-assimilation rates can be lowered by toxicants (Harrison et al., 1977), increasing amounts of nutrients are needed to maintain normal rates of cell growth and production in polluted waters.

Eppley (1972) suggested that temperature determines the maximum growth rate possible by algae, and that light and nutrient limitations (or toxicity effects) are responsible for growth rates less than maximum. A few studies on interactions of variables have begun to explore this hypothesis. Maddux and Jones (1964) considered all three major factors - light, temperature, and nutrients - and found that saturating light intensity was higher with higher nitrogen and phosphorus levels (I_k increased as P_{max} increased), and that the optimum temperature was higher under enriched conditions for Nitzschia closterium and Tetraselmis sp. McCombie (1960) found that, when nutrients were doubled, the temperature range for growth narrowed and the optimum temperature fell from 28°C to 18°C for Chlamydomonas reinhardtii. The doubling

of all nutrients (Chu #10 medium) in this case may have introduced some inhibitory effects.

The theory of one limiting factor is rarely accepted. Algal communities adapt to the conditions in their environment. Unless one or more nutrients are extremely low, algae are adapted to slow growth at low light and low temperatures in the winter (Wallen 1977). P_{max} and I_k increase with increasing temperature; coincidentally, temperature and light intensity increase through the spring. Summer populations are at saturating levels for growth at high light intensities and tolerate warm temperatures (Moore, 1977a). Although one nutrient at a time may be at a limiting level, a change in temperature may still stimulate the carbon-uptake rate of a community under that limitation (Stanley and Daley, 1976). The length of time one nutrient is at a limiting level for a community can vary from several days to several months (Harris, 1980).

Community Effects

Temperature rise or nutrient addition can affect a community in three ways. The normal species composition may stay the same, with only biomass and productivity changing; the species composition may change, with no noticeable change in biomass and productivity; or species composition, productivity, and biomass may all change.

Many studies have been restricted to either functional aspects of a community, such as carbon fixation rates or biomass measurements (Beyers, 1962; Flemer and Sherk, 1977), or structural aspects, by taxonomic methods and/or diversity calculations (Patrick, 1971, 1974; Saks et al., 1974; Carpenter, 1973). This segregation of techniques presents

a problem in interpretation of results. While Beyers (1962) indicated no change in respiratory rate with a 7°C increase in temperature due to alternate pathways for energy flow, this change in temperature could well have resulted in a change in species composition if continued long enough. Likewise, the change in community structure of phytoplankton with a 5.5°C increase in temperature (Carpenter, 1973) might well have been accompanied by no change in rate of carbon fixation. Although such partial results have been treated here as indicating effect or no effect on the system, future studies should attempt to consider both aspects of community response (Stoermer et al., 1978).

Upper Three Runs offers an interesting site for a study of the interaction of temperature and nutrients on algal communities. It is an oligotrophic, low pH stream in the National Environmental Research Park of the Savannah River Plant, Aiken, South Carolina. The Thermal Effects Laboratory is a system of six artificial streams in a greenhouse (Harvey, 1973) fed by Upper Three Runs (U3R), with the capacity to elevate the temperature of those streams. Past projects on the effects of elevated temperatures on stream communities offer some background on the physical, chemical, and biological history of Upper Three Runs.

Smaller streams have little in the way of true phytoplankton. Algae suspended in the current are believed to originate from periphytic populations, or "aufwuchs" (Hynes, 1970; Roeder, 1977). The major energy input into such systems is leaf detritus in autumn (Cummins, 1974; Fisher and Likens, 1972). Processing of detritus by the stream community has been studied in many systems (Anderson and Sedell, 1979; Cummins et al., 1972, 1973; Kaushik and Hynes, 1968; Lush and Hynes, 1978). Study of the regulation of nutrient concentrations by

angiosperms and periphyton has also recently begun (Mickle and Wetzel, 1978a and b; Stockner and Shortreed, 1978).

Periphyton communities have for years been a useful research tool in the assessment of water quality. Patrick et al. (1954) introduced a floating slide holder (diatometer), which helped sample periphyton on glass slides; analysis of the structure of the periphyton communities yielded information on water quality (Patrick, 1963, 1973; Collins and Weber, 1978). PH (Patrick et al., 1968), temperature (Patrick et al., 1969; Patrick, 1971), light (Patrick, 1971), toxicants (Gerhart et al., 1977), and nutrient changes (Patrick, 1966) all caused changes in the periphyton communities.

Most studies of periphyton communities have used glass slides as artificial substrates (Patrick, 1973). Patrick (1967) showed that the species pool, invasion rate, and area of the artificial substrate can affect the community which develops on the slides. Other workers have found that weakly attaching algae commonly found on macrophytes are rare on glass slides (Brown, 1976; Cattaneo and Kalff, 1978; Tippett, 1970). Despite the differences in algal communities on natural and artificial substrates, the use of glass slides to collect periphyton enables a quantitative sample (in terms of area) to be taken (Kevern et al., 1966; Crossman and Cairns, 1974), and provides material for experimentation.

Temperature effects on periphyton communities had been studied for several years at the Thermal Effects Laboratory. Large variances among replicates had contributed to finding no effect of elevated temperatures up to 12°C above ambient (Tilly, pers. comm.). A 1-6°C ΔT from a power plant in New Jersey, however, had resulted in fewer species and lower diversity statistics in heated effluent relative to ambient temperature

communities (Hein and Koppen, 1979); the frequency of temperatures over 29°C was believed to cause the change in community structure. Tuchman and Blinn (1979) found different algal dominants on slides and on natural substrates when the temperature was above 26°C, as opposed to when it was below 26°C.

The effects of added nutrients on periphyton communities has been studied in a few cases. Stockner and Shortreed (1978) found phosphorus, or nitrogen plus phosphorus, additions to a Canadian stream increased periphyton biomass May through October. Dickman (1973) showed that a 2.5X increase in bicarbonate concentration in a similar stream (pH 6.8-7.0) also increased biomass in the summer.

Water flow over periphyton communities is important to their metabolism. Uptake of nutrients and respiration rate are higher for attached algae in a current rather than in static medium (Schumacher and Whitford, 1965; Whitford and Schumacher, 1961, 1964). Nutrients are more available to algae in a current because the diffusion gradient around the cell is kept steep by water movement (Whitford and Schumacher, 1964). Low population levels or productivity rates in slow flow conditions (McIntire, 1966, 1968) may be a result of diminished nutrient availability (Douglas, 1958). At higher nutrient concentrations, stimulation of nutrient uptake by current is less (Lock and John, 1979).

The current which enhances algal growth by its effect on nutrient availability also regulates standing crop. Storm surges scour substrates and flush nutrients, algae, and detritus out of the stream (Cummins, 1974; Long and Cooke, 1978; Wetzel et al., 1977; Moore, 1977b). Streams with fast flow-through are variable environments.

Light and temperature fluctuate on diel and seasonal cycles, nutrients and current on an irregular, storm related cycle. The rapid change of conditions makes determination of factors correlated with algal parameters difficult (Moore, 1977b).

Periphyton communities established on glass slides in heated and unheated, enriched and unenriched, streams of the Thermal Effects Laboratory (SRL) were used to study the interaction of temperature and nutrients on algal communities. Preset temperature elevations of +5°C and +12°C were used. Enriched conditions were a 2-5X increase over Upper Three Runs ambient nutrient levels for N, P, Ca, Mg, Mo, Co, K, S. N and P were added before the other nutrients to see if a macro-nutrient limitation existed. Algal biomass (as shown by chlorophyll a), total biomass (as shown by ash-free dry weight and protein), net $^{14}\text{CO}_2$ uptake rate, and community composition were sampled in all treatments. As stated before, change in species composition, biomass, metabolic rate, or any combination of the three may occur. The type of effect on the periphytic assemblages of the experimental conditions may indicate effects on other trophic levels.

METHODS AND MATERIALS

Flowing Streams Laboratory

Research was conducted at the Flowing Streams Laboratory (Thermal Effects Laboratory), a Department of Energy facility at the Savannah River Laboratory, Aiken, South Carolina. This facility has been described by Harvey (1973). The Flowing Streams Lab is a system of six "artificial streams" inside a greenhouse (Figures 1 and 2). Water from Upper Three Runs is pumped into holding and heater tanks. Consistently elevated temperatures are achieved by mixing heated and unheated water. At the time of this study, ambient temperature water flowed through two of the artificial streams; heated water flowed through the other four. Temperature elevations of 2.5, 5.0, 7.5, and 12.5°C were in use at the time.

Experimental System

The algal communities used in this study were the assemblages which became established on regular microscope slides (2.5 x 7.5 cm). These slides were held in the water current by slide holders (Figure 3) consisting of two grooved Plexiglas™ plates (9.5 x 4.0 cm) and four screws (6.5 cm). The six grooves evenly spaced across the plates were two-thirds filled with Silastic sealant (Dow Corning 723 RTV) to ensure a firm but cushioned seating for the slides. The Plexiglas plates were pressed against the middle of the two long edges of the six slides; nuts on the screws held the Plexiglas plates firmly against the slides. The holders therefore held the slides in the current with the short axis of the slide vertical and the long axis parallel to the stream flow. This orientation exposed the slide to direct current, gave

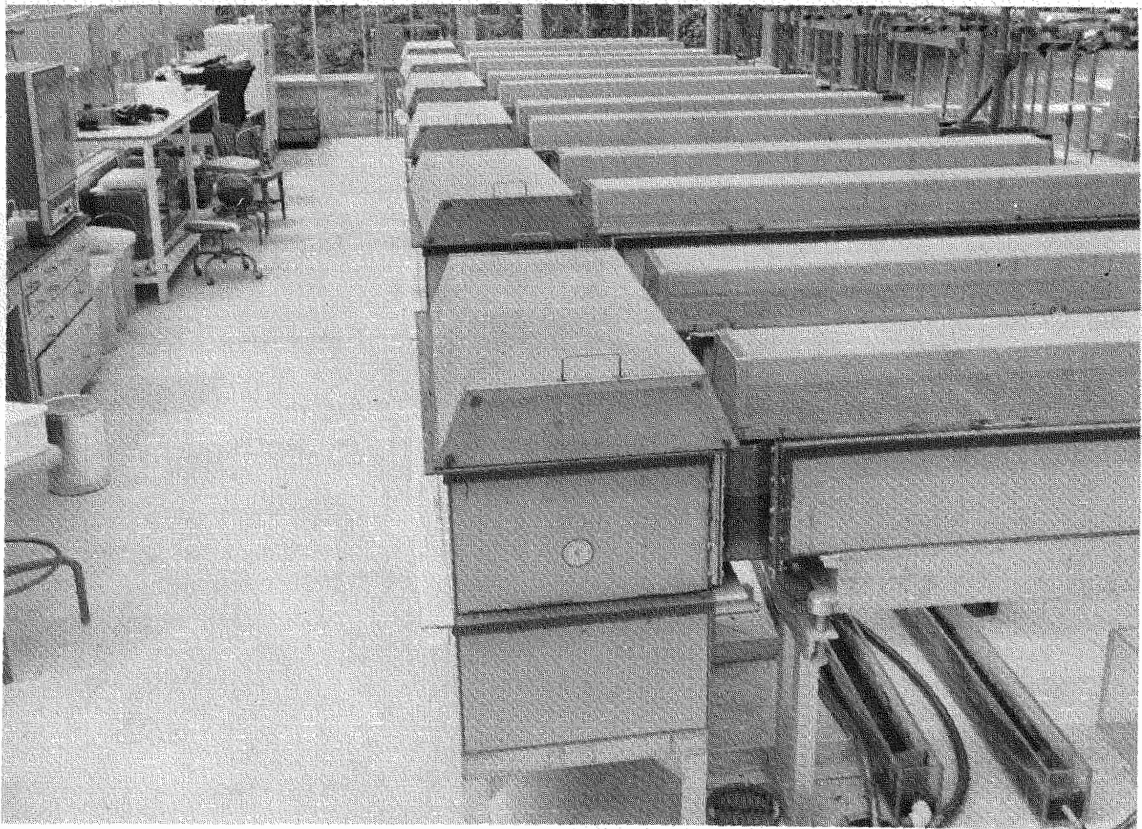


FIGURE 1. Flowing Streams Laboratory

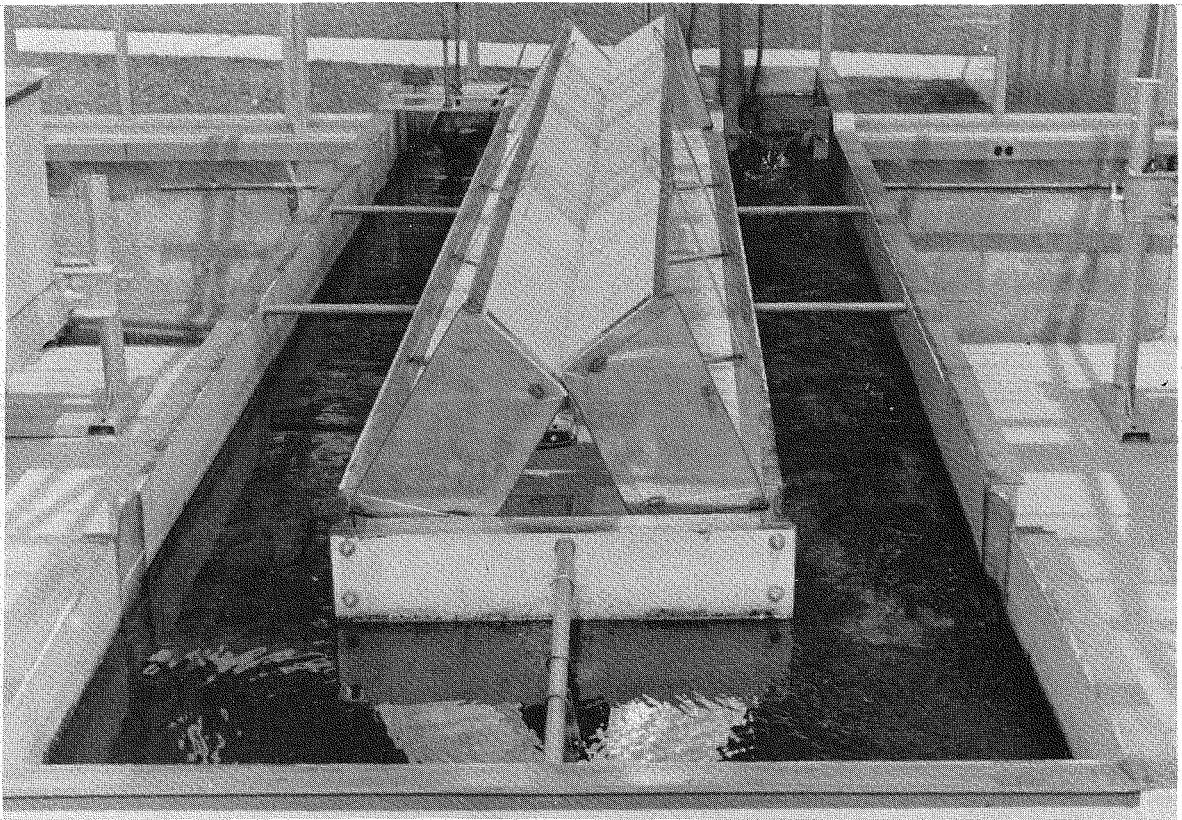


FIGURE 2. Artificial stream in FSL.

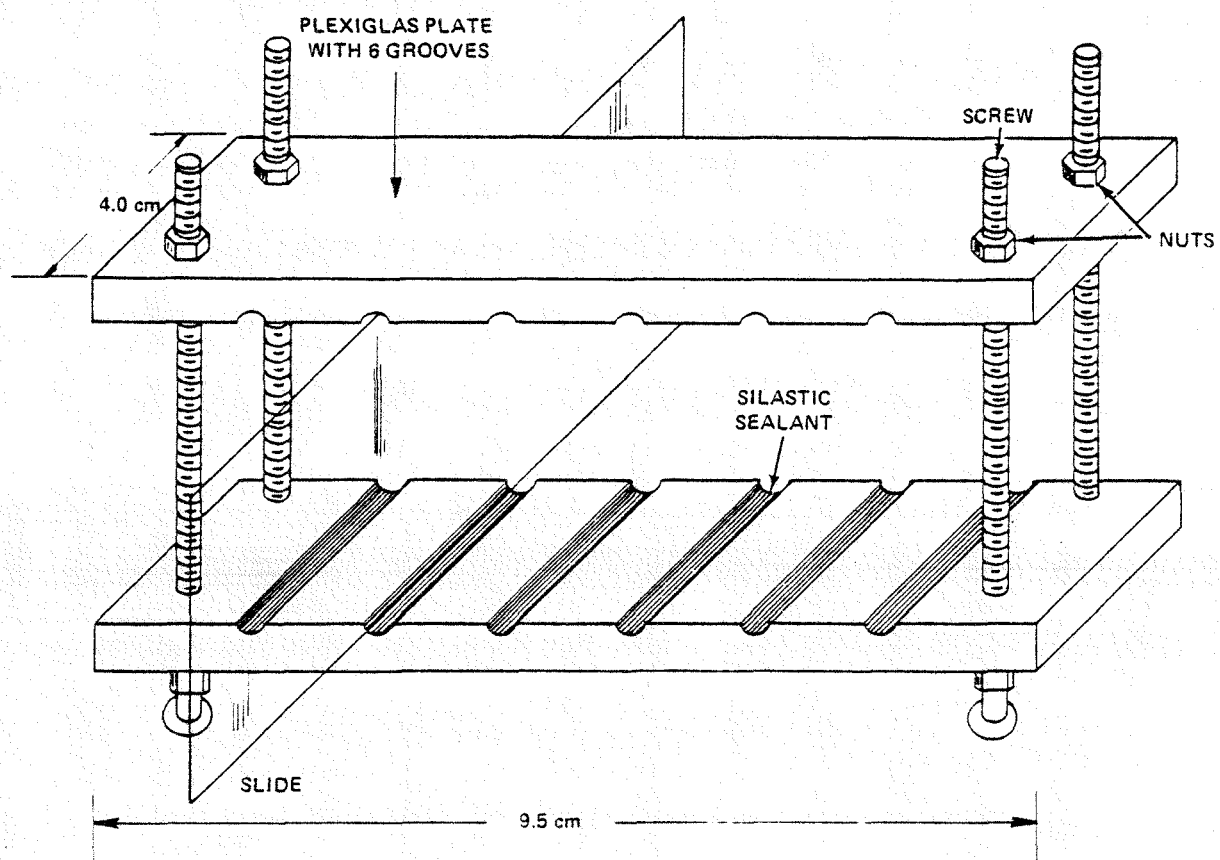


FIGURE 3. Plexiglas slide holder (grooves are approximately 2/3 full of Silastic sealant).

similar flow along both sides of the slide, and prevented silt buildup directly on the slide. Silt that accumulated on the upper surfaces of the Plexiglas plates was removed every two or three days by tilting the holder in the current and letting the silt fall out. The upper surface of the top plate was cleared more often with a small brush to prevent shading of the algal assemblages.

Clear plastic troughs 92.2 cm long, 9.8 cm wide, and 13.0 cm deep were constructed from .64 and .32 cm thick Plexiglas for use as enrichment streams (Figure 4). A rectangular weir 6.5 cm high separated the 81.5 cm long upstream end from the 7.5 cm long outflow end in each trough. Water was gravity fed through 2.5 cm (inner diameter) tubing and connectors. Water flow was directed through the slides in their slide holder, and the amount of water necessary for a water velocity of .25 cm/min was minimized to keep total amounts of nutrients added low. Average flow through the troughs was 15 L/min. Flow through the artificial streams was 140 L/min., at an average velocity of 1 cm/min.

A downstream portion of the artificial streams was deepened in January 1978, in an attempt to slow water velocity to match the highest velocity possible in the troughs. The water in the artificial streams could diverge around the slide holders, however, while in the troughs the holders were as wide as the troughs, so current was directed through the slide array. Early experiments showed that chlorophyll a/slide and carbon-uptake/slide/h differed greatly between artificial stream assemblages and trough assemblages. Reference (unenriched) as well as enriched treatments were therefore sampled from the troughs.

Early experiments also showed that there was sometimes an effect of position in the slide holder on chlorophyll a/slide. To prevent

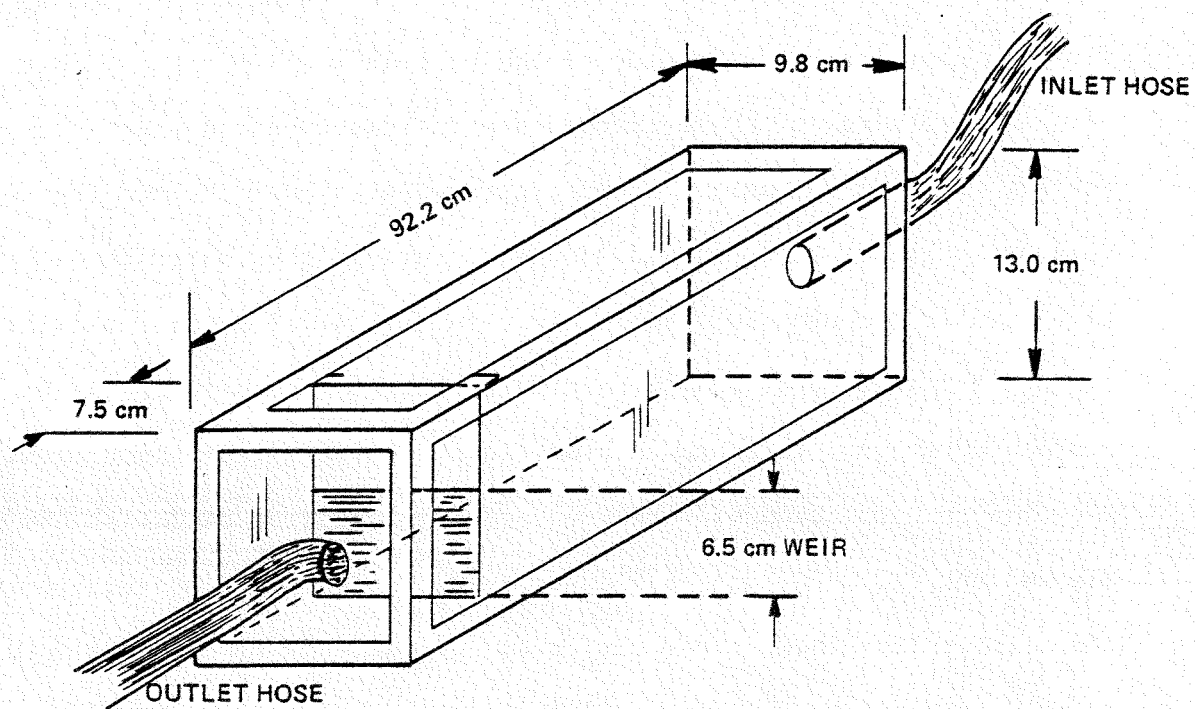


FIGURE 4. Plexiglas trough.

this effect from influencing comparisons among treatments, one set of random samples was decided upon (using a random numbers table) for each sampling and used in each trough sampled. If chlorophyll samples were taken from the three leftmost positions in the slide holder at ambient temperature, the three leftmost slides in the +5°C and +12°C treatments were also used for chlorophyll analysis. Other parameters were sampled from the same positions in each trough in this way.

A 3 x 3 array of these troughs was the experimental area (Figure 5). Gravity-fed ambient temperature water flowed through the left sequence of three troughs, +12.5°C water through the center three, and +5.0°C water through the right three troughs. A 7.5 cm drop between the three upstream troughs and three middle troughs, and between the three middle troughs and three downstream troughs, maintained a head on water flowthrough. The troughs were numbered from upstream to downstream for the ambient, +12.5°C, and +5.0°C sequence (Table 1). The periphytic communities from these three temperature treatments will be referred to as +0°C (ambient), +5°C, and +12°C assemblages.

In this study, a sample refers to the periphytic accumulation from an individual slide. A sampling is the collecting and processing of one set of samples (usually triplicates for each parameter). This was a one day event for much of the background data, but a two day event for the enrichment experiments and a few of the background experiments. In the samplings lasting two days, chlorophyll, taxonomy, and carbon-uptake samples were taken one day, dry weight and protein the next. An experiment is one to three samplings within eight days, or an accumulation experiment.

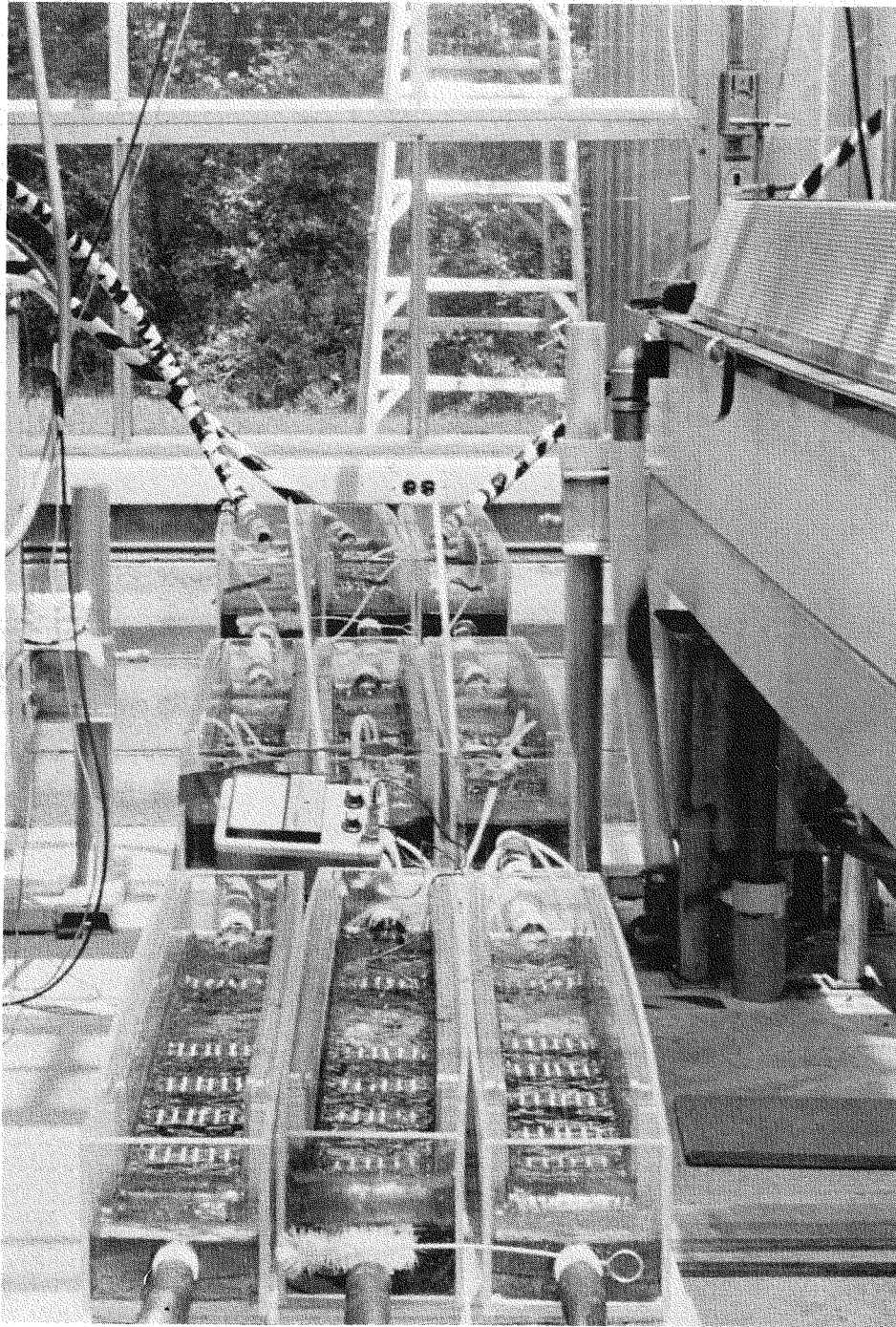


FIGURE 5. Trough array.

TABLE 1

Trough numbers, temperature and nutrient treatments

<u>Nutrients Added</u>	<u>Ambient</u>	<u>+12.5°C</u>	<u>+5.0°C</u>	
None	T1	T4	T7	Upstream Set
N+P (or TE in Feb. and April)	T2	T5	T8	Midstream Set
TE (or N+P) + S	T3	T6	T9	Downstream Set

Outline of Experiments

Background experiments were run from January 1978 through March 1979. Each quarter upstream-downstream effect (difference among troughs) was tested at each temperature. The effect of incubation time, light intensity, and accumulation time was also tested at different seasons. In the among-troughs experiments, triplicate samples (duplicates in January 1978) for chlorophyll a, ash-free dry weight, and carbon-uptake rate were taken from each trough. For carbon-uptake rate vs light intensity and vs incubation time, one set of triplicate samples for ash-free dry weight and chlorophyll a was taken at each temperature while enough samples for triplicate carbon-uptake rate at varying light intensities or for varying times were taken. For the light intensity experiments, the carbon-uptake incubation bottles were wrapped in varying thicknesses of cheesecloth to reduce light intensity. Full, 1/2, 1/4, and 1/8 noon sunlight levels were used. Samples were taken from, and incubated at, +0°C, +5°C, and +12°C. For the incubation time experiments, the incubation time of each set of replicates was nested around noon. The 1.0 h incubation time set was incubated from 11:30 a.m. to 12:30 p.m.; the 6.0 h set was incubated from 9:00 a.m. to 3:00 p.m. In the accumulation experiments, triplicate samples for each parameter were taken at 4 to 7 day intervals for up to 42 days.

Three short-term nutrient experiments (STNE) were run, although these were not considered as informative as long-term experiments. For one STNE, slides from unenriched treatments were placed in beakers with enriched stream water for 24 h before carbon-uptake rates were measured. The enriched waters were kept stirred by magnetic mixers for the pre-incubation period, and slides were kept at the temperature from

which they were sampled. For the two other STNE, the accumulation experiments were timed to overlap the beginning of nutrient additions for the quarterly enrichment experiments. Around day 32 of the colonization period, half the remaining slides were left in unenriched troughs (T1, T4, T7); the other half were put in the "complete" enrichment troughs (T3, T6, T9) at each temperature. On day 35 or 42, the last accumulation sampling showed normal 35 (or 42) day development and also that as a result of 3 days of nutrient enrichment.

Enrichment experiments were done in February, April, July, October 1978 and January 1979. Each experiment consisted of three samplings in an 8 day period. For these experiments, nutrient solutions were pumped into the middle and downstream troughs at each temperature using a 10-channel Masterflex pump (Cole Parmer #7568). Table 2 shows the compounds and amounts used. These nutrients were added in three solutions - a nitrate plus phosphate solution (N + P), a trace element solution (TE), and a sulfate solution (S). The upstream trough at each temperature was left as a reference (unenriched) treatment. One solution (TE in February and April, N + P the next three experiments) was added to the outflows of the upstream troughs. The remaining two solutions were added to the outflow of the middle troughs. This resulted in a partial enrichment (middle troughs) and a "complete" enrichment (CE; downstream troughs) at each temperature (see Table 1).

Enrichment experiments were performed quarterly, and timed to coincide with maximum and minimum water temperatures (July and January respectively), and with most fluctuating water temperatures (April and October). All slides were in the assigned nutrient addition treatment (none, N + P, CE) for the 14 days of accumulation.

TABLE 2

Nutrients added in enrichment experiments. Fe added only in Feb. 1978. Concentrations in parentheses used in Feb. and April; other concentrations used in last three enrichment experiments.

<u>Solution</u>	<u>Nutrient</u>	<u>Added as</u>	<u>Enrichment Concentration</u>	
			<u>mg/L</u>	<u>μM</u>
N+P	NO ₃ -N	NaNO ₃	0.75 (1.33)	35.0 (71.4)
	Ortho-PO ₄	K ₂ HPO ₄	0.075 (0.35)	2.0 (10.0)
	K	K ₂ HPO ₄	0.156 (0.78)	4.0 (20.0)
TE	Ca	CaCl ₂ ·2H ₂ O	3.3 (1.4)	7.5 (3.2)
	Mn	MnCl ₂ ·4H ₂ O	0.1	1.82
	Co	CoCl ₂ ·6H ₂ O	2.5 x 10 ⁻³	.042
	Cl	(above 3)	0.332	9.362
	Mo	Na ₂ MoO ₄ ·2H ₂ O	2.5 x 10 ⁻³	.026
	Fe	FeCl ₂ ·6H ₂ O	0.5	9.0
S	SO ₄ -S	MgSO ₄ ·7H ₂ O	0.96	30.0
	Mg	MgSO ₄ ·7H ₂ O	0.73	30.0

Laboratory Analyses

When a sample was taken, the slide was gently removed from the holder and shaken twice in the stream water, to remove loose cells and silt. The periphyton was scraped off with a clean glass slide; no other tool did as thorough a job of clearing the slides, and few broken cells resulted.

Samples for dry weight/slide (DW) and ash-free dry weight/slide (AFDW) were taken in triplicate between 7 and 9 a.m. for each sampling. The periphytic accumulation was scraped from a slide, as soon as it was removed from the holder, into an 8 ml crucible, which had been pre-ashed and weighed to .01 mg. Deionized water (from a Milli-R040 plus Milli-Q, 4 housing deionizer) was used to rinse loosened material into the crucible. Samples were dried 8-24 h at 65°C, then removed to a dessicator for at least one hour. After weighing for DW, the samples were ashed at 500°C for 3 h. After cooling, the samples were replaced in the dessicator for at least one hour. A final weighing for ash-weight was made. AFDW was found by subtracting ash-weight from dry weight. Fraction organic material was found by dividing AFDW by DW.

Samples for chlorophyll a/slide (CHLA) were taken between 8 and 10 a.m. for each sampling. Each slide was removed from the holder and its periphyton scraped into a 50 ml glass beaker. 90% acetone (buffered with MgCO_3) was used to wash loosened material into the beaker. The acetone/periphyton slurry was transferred to a 10 mL graduated screwcap centrifuge tube and the volume made up to 10 mL with more 90% acetone. Samples were placed in a foil-shaded test tube rack as soon as possible. After all samples were taken, the contents of each centrifuge tube were sonicated 30 sec (Lab-Line Ultratip Labsonic System (#9100) to disrupt

membranes. The rack of tubes was kept refrigerated 1-6 h. As soon as possible, samples were centrifuged and the acetone-chlorophyll extracts read using a spectrophotometer (Beckman Model 25) at 750, 665, 645, and 630 nm. After acidification of the sample with 5% HCl, absorbance at 750 and 655 nm was reread. Strickland and Parsons (1972) trichromatic equations were used to calculate chlorophyll a to .01 $\mu\text{g}/\text{sample}$.

Samples for protein/slide (PROT) analysis were taken between 10 and 12 a.m. for each sampling. The scraping procedure for chlorophylls was followed, using deionized water instead of acetone. The 10 mL samples in centrifuge tubes were held in a foil-covered test tube rack until all had been taken, and then were centrifuged. A modified Lowry's (Lowry et al., 1951) protein analysis was used as follows: 6 mL of supernatant was removed, leaving 4 mL supernatant plus cell pellet in the centrifuge tube. 1.0 mL of 2.5 N NaOH was added to each tube. Samples were then boiled 30 min to solubilize the proteins. After cooling, 1.0 mL of the protein solution was removed to a clean centrifuge tube and the volume made up to 5.0 mL with deionized water. 1.0 mL of mixed reagent (100 mL 2% Na_2CO_3 in .1 N NaOH + 2.0 mL 2% $\text{NaKC}_4\text{H}_4\text{O}_6 \cdot 4\text{H}_2\text{O}$ + 2.0 mL 1% $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$) was added to each sample and the mixture vortexed immediately. After 15 min, 0.5 mL of 1 N Folin phenol reagent (Fisher Folin Ciocalteu SO-P-24) was added to each sample and the mixture stirred immediately. After 30 min, optical density of the samples was read at 550 nm in a spectrophotometer. Bovine Serum albumin (Difco Bacto Bovine Albumin 5%) was used as a standard. The BSA was diluted to cover the range of proteins expected in the samples and a standard line calculated from the OD readings of four levels of BSA. Protein/slide was

calculated to .001 mg by comparison of spectrophotometric readings of samples to the standard line.

Samples for rate of carbon-uptake/slide (C-UP) were incubated between 11:15 a.m and 12:45 p.m. (EST) for each experiment. Each slide was put into a wide-mouth bottle containing a 2.5 cm magnetic stirring bar and 100 mL incubation water. Incubation water was at the temperature and nutrient addition level from which the slide was sampled. Approximately 0.05 $\mu\text{Ci NaH}^{14}\text{CO}_3$ (New England Nuclear, 10 $\mu\text{Ci}/100 \mu\text{gm}$) was added to each incubation bottle. The same amount of ^{14}C solution (50 μL) was immediately added to a vial of scintillation fluid. The incubation bottles were then filled with the proper incubation water (final volume 130 mL) and stoppered. Incubation time was 1.5 h. Samples were incubated in full sunlight in water baths at their respective temperatures, and stirred by magnetic mixers. Stirring of carbon-uptake incubation samples of periphyton from streams has been shown to result in higher and more consistent carbon-uptake rates than nonstirred samples (Rodgers and Harvey, 1976).

Twelve places for bottles in the water baths above the stirrers were available at each of the three temperature treatments. C-UP incubations were run only between 11 a.m. and 1 p.m. to avoid shaded conditions which occurred earlier and later in the day. Incubation was ended by placing all bottles in a light-tight box insulated with styrofoam. Comparison of replicates filtered immediately and those held in this box 3 h showed little change in retained ^{14}C . The incubation water and periphyton from the slide in each bottle was filtered through a 0.45 μm Millepore (HAWP) filter at 1 atm. A glass slide was used to scrape as described before. Deionized water (pH 4) was used to rinse out

incubation bottle and to wash loosened periphyton from the slide. The low pH of the rinse water ensured that no inorganic ^{14}C was left on the filter. Three hours were necessary to process all 36 carbon-uptake samples. Each sample on a filter was wrapped in aluminum foil and placed in a dessicator. Between one day and one week later, samples were oxidized in a Packard Tri-Carb oxidizer (B306), which automatically transfers the combustion gases into absorbant and scintillation fluid in vials. Scintillation samples were counted in a Beckman LS-250 liquid scintillation counter with teletype. Sample channels ratios were employed to check for chemical quench; none was ever observed.

Carbon-uptake rate was calculated according to Strickland and Parsons (1972) to 0.01 μg carbon/slide/h. Alkalinity was determined by low level alkalinity titration (A.P.H.A., 1976). Available carbon was found by multiplying alkalinity by a conversion factor determined by pH and temperature (Saunders et al., 1962). Although at low alkalinity humic acids are reported to cause an overestimate of alkalinity from 7% to 25% (Wilson, 1979), the method used here of calculating available carbon agreed well with samples measured on a carbon analyzer (Tilly, pers. comm.; Loftus et al., 1979).

Samples for organism counts and identification were removed from the slide holders and put immediately into individual, 60 mL jars of 2.5% glutaraldehyde (Electron Microscopy Sciences Biological grade). These samples were refrigerated until processed. The periphyton on each slide was scraped back into its sample jar. Scraped slides were scanned under 16X power on a Zeiss inverted microscope (Invertoscope D) with fluorescent lighting to estimate the number of cells left on the slide; less than 1% failed to be removed. A 5.0 mL aliquot of the

periphyton suspension from the sample jar was pipetted into a 10 mL settling chamber. Deionized water was added to fill the chamber. Samples were allowed to settle 14 to 48 h. The number of filaments in each sample was estimated from a scan at 16X, using normal lighting. The number of organisms in each sample was estimated from at least 400 organisms counted in each sample (Lund et al., 1958; Hobro and Willen, 1977); normal and fluorescent lighting were used at 63X, oil immersion (Brock, 1978). Numbers of organisms per slide = number counted x 60 mL total sample volume/5 mL aliquot settled x 4.9876 mm² settling surface/area counted mm². Diatoms were identified to species using Hyrex mounts. Filaments were identified to genus, and coccoids were separated into greens and blue-greens. Green and blue-green algae could be distinguished by the wavelength of maximum excitation under fluorescence (Caldwell, 1977). Few broken cells were observed during these counts, indicating that relatively few cells were ruptured during the scraping process.

Chemical analyses were done on water samples twice during each enrichment experiment, from all three enrichment types (unenriched, N + P, and CE). Water samples were run through 0.45 µm Millepore filters which had been soaked 24 h in deionized water. These samples were refrigerated immediately (Florence, 1977). Water samples for metals were acidified with nitric acid (Batley and Gardner, 1977). All water analyses were made by Envirochem, Aiken, South Carolina.

Precision of slide scraping was checked by the scans on the scraped taxonomy slides; consistently less than 1% of the number of cells was left on the slides. Chlorophyll a samples reread up to 24 h later differed by less than 10%. Carbon-uptake samples read several times on

the scintillation counter differed by less than 5%. Alkalinity titrations were made at least twice for each different water sample, to ensure a correct measurement. Lab error was much lower than slide-to-slide variability.

Parametric statistics were applied to the data, where applicable. Means (\bar{x}), variances (s^2), and standard deviations (s) were calculated for each set of replicates within a treatment. Normality of data was assumed. Where homogeneity of variances was accepted as tested by the F_{\max} test or Bartlett's test (Sokal and Rohlf, 1969), one- and two-way analyses of variance (1 x and 2 x ANOVA) tests were used to find if differences existed among treatment means. A multiple range test (SNK; Zar, 1974) was used to determine which means differed. Significance level of (α) = .05 was always used. Often an ANOVA would indicate a difference among means which the SNK could not detect. This is a function of the large variances in relation to the small number of replicates.

As noted above, number of replicates could not be expanded, so more than one sampling per experiment was planned. Since large day-to-day variation appeared, a nonparametric ranking test (Friedman's; Sokal and Rohlf, 1969) was used to compare results among samplings within an experiment. This test is useful when the ranks of treatment parameters are similar among samplings (if the +12°C AFDW is always greater than the +0°C AFDW, for example).

Since seasonal effects were observed background and enrichment experiments will be presented first from each season. Overall effects will be summarized in the Discussion.

RESULTS

Background Experiments

Among Troughs

Homogeneity among all 9 troughs was tested at the beginning and end of experimentaion. All troughs were at ambient temperature; accumulation period was 14 days; and two or three replicates for AFDW, CHLA, and C-UP were taken. The data from the first experiment, Jan. 8/9 (Table 3), show that, although differences did exist among troughs, the difference was primarily an upstream-downstream effect. The mean squares for the upstream-downstream difference is twice the mean squares for the side-to-side difference for CHLA, and C-UP differed solely upstream-downstream.

On the basis of this experiment, experiments to check upstream-downstream effect were scheduled to precede each enrichment experiment at all three temperatures. The small or nonexistent side-to-side mean squares indicated that the three series of troughs were suitable for use as the temperature treatments. The enrichment experiments were designed to consist of three separate samplings to verify a pattern of response from the treatments.

The March 4 trough homogeneity experiment confirmed the results of the Jan. 8/9 experiment. Difference among troughs in AFDW was upstream-downstream, and in CHLA the upstream-downstream mean squares is three times the side-to-side mean squares. Note that the upstream-downstream effect was caused by low values in the upstream troughs, as opposed to high values in the first trough-homogeneity experiment. This reversal of the Jan. 8/9 results indicated that a seasonal effect might appear in the quarterly among-troughs experiments.

TABLE 3

Trough homogeneity experiments; ambient temperature, 14-day colonization period. Troughs ranked from highest to lowest; underscore denotes equality.

January 8 - 9, 1978

	<u>n</u>	<u>$\bar{x} \pm 1 \text{ s.d.}$</u>								<u>Range</u>		
AFDW (mg/slide)	2	1.12 \pm 0.276								0.66	-	2.14
ORG(AFDW/DW)	2	0.4980 \pm 0.0928								0.4115	-	0.6110
CHLA ($\mu\text{g/slide}$)	3	5.34 \pm 1.462								2.10	-	12.51
		<u>T1</u>	<u>T4</u>	<u>T2</u>	<u>T7</u>	<u>T3</u>	<u>T6</u>	<u>T8</u>	<u>T5</u>	<u>T9</u>		
C-UP($\mu\text{g/slide/h}$)	2	24.48, 11.61 \pm 2.665								5.58	-	27.76
		<u>T1</u>	<u>T4</u>	<u>T7</u>	<u>T6</u>	<u>T8</u>	<u>T9</u>	<u>T3</u>	<u>T5</u>	<u>T2</u>		
C-UP/CHLA	9	2.41 \pm 0.559								1.61	-	3.19
<u>March 4, 1979</u>												
AFDW(mg/slide)	3	1.73 \pm 0.230								1.09	-	2.41
		<u>T3</u>	<u>T6</u>	<u>T5</u>	<u>T9</u>	<u>T8</u>	<u>T2</u>	<u>T4</u>	<u>T1</u>	<u>T7</u>		
ORG(AFDW/DW)	3	0.3234 \pm 0.03702								0.2902	-	0.3562
CHLA($\mu\text{g/slide}$)	3	9.22, 6.94, 4.58 \pm 0.780								3.93	-	10.45
		<u>T8</u>	<u>T6</u>	<u>T3</u>	<u>T5</u>	<u>T9</u>	<u>T7</u>	<u>T2</u>	<u>T4</u>	<u>T1</u>		
C-UP($\mu\text{g/slide/h}$)	3	10.43 \pm 4.144								4.63	-	23.96
C-UP/CHLA	9	1.61 \pm 0.4012								1.32	-	2.09

Table 4 shows the results of the among-troughs experiments. On Jan. 24/26, T7 was low in CHLA once and in C-UP twice relative to T8 and T9 (+5°C). On March 27/30, the downstream troughs had high values in some cases: CHLA for the +5°C series twice, organic for all the series once, AFDW for the +5°C series once, C-UP for both the +5°C and +12°C series once. On June 20, the upstream trough in the +5°C series had high values for organic and CHLA; the midstream trough at +0°C was low in organic. On Oct. 4, the upstream trough at +0°C was high in CHLA and C-UP. On Jan. 4, the downstream trough at +5°C was low in AFDW, while the upstream trough at +0°C was low in CHLA.

The +5°C series appeared most susceptible to differences among troughs. The high values for CHLA and C-UP in upstream troughs were probably due to filaments hanging up on the first sets of slides. Although all parameters at any season did not show differences among troughs for any temperature series, some effect of differences among troughs will be considered in interpreting the results of the enrichment experiments, especially for organic in July for the +0°C and +12°C series, for CHLA in April for the +5°C series and in Oct. for the +0°C series, and for C-UP in Feb. for the +5°C series.

C-UP vs Light Intensity

The adaptation of the periphyton assemblages to seasonal light levels was tested March 22, May 24, Aug. 30, and Nov. 14. Results of these experiments are shown in Table 5. Only when light intensity was below 100 $\mu\text{Einstein}/\text{m}^2/\text{sec}$, as in November, was C-UP significantly reduced by low light levels. The Nov. 14 experiment was the only one done under cloudy light conditions (i.e., inconstant light intensity).

TABLE 4

Among-troughs experiments. T1, T2, T3 = +0°C series; T7, T8, T9 = +5°C series; T4, T5, T6 = +12°C. Mean (1 s.d.) for temperature treatments given, n = 3, except where noted. Where significant differences exist, outlying trough and mean given.

	+0°C	+5°C	+12°C
<u>January 24</u>			
AFDW mg/slide	0.97 (0.152)	1.47 (0.156)	3.29 (0.307)
ORGANIC	0.6211 (0.082)	0.4702 (0.082)	0.3537 (0.082)
CHLA µg/slide	4.14 (0.524)	3.58 (T7) 6.52 (0.836)	9.57 (2.950)
C-UP µg/slide/h	10.62 (2.28)	5.46 (T7) 14.00 (2.28)	30.70 (T4) 16.51 (2.28)
C-UP/CHLA	2.86 (0.172)	2.17 (0.269)	2.47 (1.022)
<u>January 26</u>			
AFDW mg/slide	1.08 (0.151)	1.59 (0.235)	3.53 (0.538)
ORGANIC	0.3654 (0.028)	0.3245 (0.028)	0.2996 (0.028)
CHLA µg/slide	5.06 (0.605)	6.44 (1.008)	10.39 (2.496)
C-UP µg/slide/h (n = 2)	15.20 (2.403)	9.18 (T7) 19.51 (2.731)	33.72 (12.234)
C-UP/CHLA	3.26 (0.692)	2.54 (0.670)	3.28 (0.254)

TABLE 4 (Continued)

	+0°C	+5°C	+12°C
<u>March 27</u>			
AFDW mg/slide	5.37 (1.473)	5.63 (T7) 8.65 (T8) (1.064) 11.11 (T9)	4.88 (2.248)
ORGANIC	0.3358 (T1) 0.3940 (.0279)	0.3938 (.0279)	0.3995 (0.0279)
CHLA µg/slide	15.11 (1.473)	15.92 (4.290)	9.35 (5.513)
C-UP µg/slide/h	50.05 (11.098)	58.42 (10.390)	29.00 (6.058)
C-UP/CHLA	3.09 (0.101)	4.03 (1.320)	3.49 (1.183)
<u>March 30</u>			
AFDW mg/slide	8.25 (1.494)	13.54 (3.430)	5.52 (1.127)
ORGANIC	0.4172 (0.0246)	0.4037 (T4) 0.4725 (0.0246)	0.4226 (0.0246) 0.4682 (T8)
CHLA µg/slide	11.99 (7.402)	16.24 (5.103)	10.44 (5.724)
C-UP µg/slide/h	53.56 (12.416)	50.72 (T7) 72.93 (5.326)	30.32 (T4) 57.73 (7.853)
C-UP/CHLA	4.67 (1.712)	4.48 (1.626)	4.70 (0.509)

TABLE 4 (Continued)

	+0°C	+5°C	+12°C
<u>June 20</u>			
AFDW mg/slide	4.51 (1.011)	3.32 (1.011)	4.45 (1.011)
ORGANIC	0.4632 (0.0244) 0.3772 (T2)	0.4274 (T7) 0.3697 (0.0244)	0.4266 (0.0244)
CHLA µg/slide	45.50 (12.40)	80.94 (T7) 29.32 (12.40)	46.53 (12.40)
C-UP µg/slide/h	87.01 (19.339)	83.09 (19.339)	84.50 (19.339)
C-UP/CHLA	1.80 (0.227)	1.98 (0.536)	2.97 (0.073)
<u>October 4</u>			
AFDW mg/slide	3.60 (0.671)	4.21 (1.365)	3.42 (1.456)
ORGANIC	0.5038 (0.0378)	0.4485 (0.0378)	0.4495 (0.0378)
CHLA µg/slide	47.38 (T1) 17.99 (7.460)	32.98 (17.015)	14.94 (4.091)
C-UP µg/slide/h	26.43 (T1) 11.86 (3.217)	26.10 (6.739)	23.06 (3.676)
C-UP/CHLA	0.62 (0.095)	0.85 (0.309)	1.52 (0.156)
<u>January 4</u>			
AFDW mg/slide	0.96 (0.339)	2.62 (0.468) 1.34 (T9)	2.38 (0.588)
ORGANIC	0.4245 (.0490)	0.4879 (0.0498)	0.4314 (0.0490)
CHLA µg/slide	3.04 (T1) 7.96 (1.448)	16.81 (3.566)	42.28 (13.686)
C-UP µg/slide/h	4.30 (3.106)	19.45 (3.996)	45.03 (25.714)
C-UP/CHLA	0.75 (0.357)	1.16 (0.108)	1.02 (0.244)

A continuous record of light intensity in Nov. showed the range 120-300 $\mu\text{Ein}/\text{m}^2/\text{sec}$, with an average light intensity of 180 $\mu\text{Ein}/\text{m}^2/\text{sec}$. All carbon-uptake experiments were carried out at light intensities greater than 100 $\mu\text{Ein}/\text{m}^2/\text{sec}$. All rates are therefore assumed to be light-saturated and are not normalized to light intensity.

C-UP vs Incubation Time

The incubation time of a carbon-uptake experiment must be long enough for sufficient ^{14}C to be incorporated into the cells for reliable counting but not so long that inorganic carbon becomes limiting in the incubation vessels or that much organic carbon is released from the cells. The effect of length of incubation time on C-UP was tested twice, Dec. 6 and June 7. In the Dec. 6 experiment, an incubation time of 1-3 h appeared optimal (Table 6). In June, the rate of carbon-uptake was the same for 1-3 h incubations. An incubation time of 1.5 h was used in all other experiments to avoid potential problems of longer incubation times.

Accumulation experiments

The progress of accumulation of material on the glass slides, a function of colonization, sedimentation, and growth, and the photosynthetic activity of the algal accumulations were measured quarterly. The Feb. accumulation experiment (1-17 Feb.) results show the state of 14 day-old assemblages, in preparation for the first enrichment experiment. AFDW at all three temperatures accumulated very slowly for 8 days, then increased rapidly in the two high temperature treatments for the next 8 days (Figure 6). The periphyton at all three temperatures appeared to be in a phase of rapid accumulation at day 14, with distinct

TABLE 5

C-UP vs. light intensity. Carbon uptake ($\mu\text{g}/\text{slide}/\text{h}$), $\bar{x} \pm 1 \text{ s.d.}$, $n = 3$. Grand mean given where no difference exists ($\alpha = .05$). Vertical lines denote equality.

Date	Light ($\mu\text{Ein}/\text{m}^2/\text{sec}$)	+0°C	+5°C	+12°C
3/22	820-102	25.32 \pm 5.208	31.09 \pm 5.208	23.84 \pm 5.208
5/24	200-25	44.28 \pm 11.581	59.55 \pm 11.581	52.31 \pm 11.581
8/30	660-82	97.70 \pm 21.907	125.47 \pm 21.907	49.54 \pm 21.907
11/14	180	26.68	37.64	14.68 \pm 5.414
	(120-300)			
	45	16.70 \pm 5.414	21.44 \pm 5.414	
	22	9.39	26.16	

TABLE 6

C-UP vs. incubation time. Carbon uptake ($\mu\text{g}/\text{slide}/\text{h}$). $\bar{x} \pm 1 \text{ s.d.}$; $n = 2$ in December, $n = 3$ in June. Vertical lines denote equality.

December 6, 1977

<u>Incubation Time (h)</u>	<u>+0°C</u>
.5	6.26 \pm 9.618
1.0	61.58
1.5	
2.0	
2.5	30.94
3.0	
4.0	20.81
5.0	
6.0	

June 7, 1978

<u>Incubation Time (h)</u>	<u>+0°C</u>	<u>+5°C</u>	<u>+12°C</u>
1.0	76.52 \pm 26.172	60.11 \pm 26.172	34.77 \pm 26.172
2.0			
3.0			

differences among temperatures by day 16. CHLA (Figure 7) also lagged for 4-8 days, then increased rapidly at all three temperature treatments. By day 14, +0°C and +5°C assemblages had ceased CHLA accumulation while that of the +12°C assemblages was still increasing. By day 16, differences among the temperature treatments were again distinct (+12°C >+5°C >+0°C).

The pattern of C-UP (Figure 8) resembled that of CHLA: there was an 8 day lag, followed by increasing C-UP in the two higher temperature treatments for the next 8 days, and differences existed among treatments by day 16. C-UP on the +0°C slides was low at all four samplings. By day 14, the +0°C assemblages were in plateau phase, while the +5°C and +12°C assemblages were still increasing in C-UP. The assimilation numbers (ASSIMNO) (Figure 9) over the 16-day period in Feb. rose rapidly and by day 8 were close to maximum for this experiment (1.0-1.5).

In the June accumulation experiment (8 June-13 July), the pattern of AFDW accumulation was similar for all three temperature treatments through day 8 (Figure 10) - a jump in AFDW between days 4 and 8. The +12°C assemblages oscillated around 4 mg AFDW after day 8, while the +5°C assemblages stabilized around 7 mg/slide and the +0°C assemblages increased in AFDW throughout the accumulation period to 8 mg/slide. From day 20 to day 35, the +12°C assemblages had significantly less AFDW than did the other two temperature treatments.

CHLA (Figure 11) of the +0°C assemblages rose to 100 µg/slide by day 28; the +5°C and +12°C assemblages had maximum CHLA around day 16, and leveled off at 25 µg/slide days 20-35. CHLA was the same for all three temperature treatments through day 16 (40-50 µg/slide). Past day

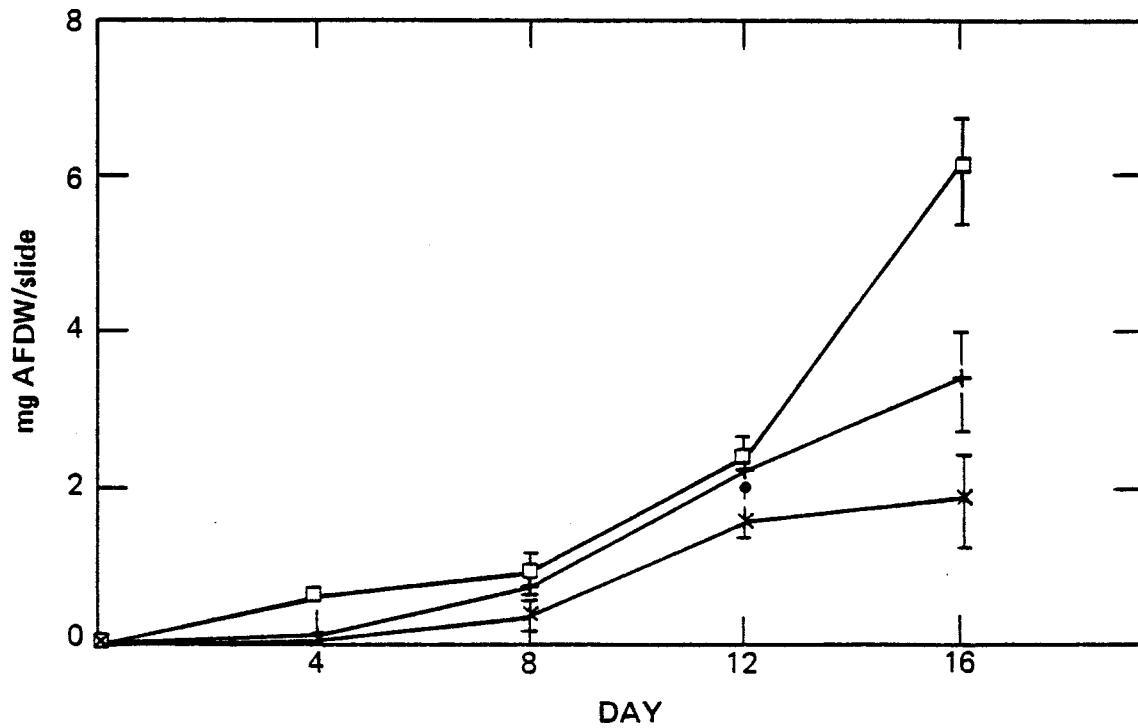


FIGURE 6. AFDW accumulation, February 1978. Ambient temperature (X), +5°C (+), +12°C (□). Means of three replicates shown (where no difference among treatments exists, a common mean is shown by a dot), ± 1 s.d..

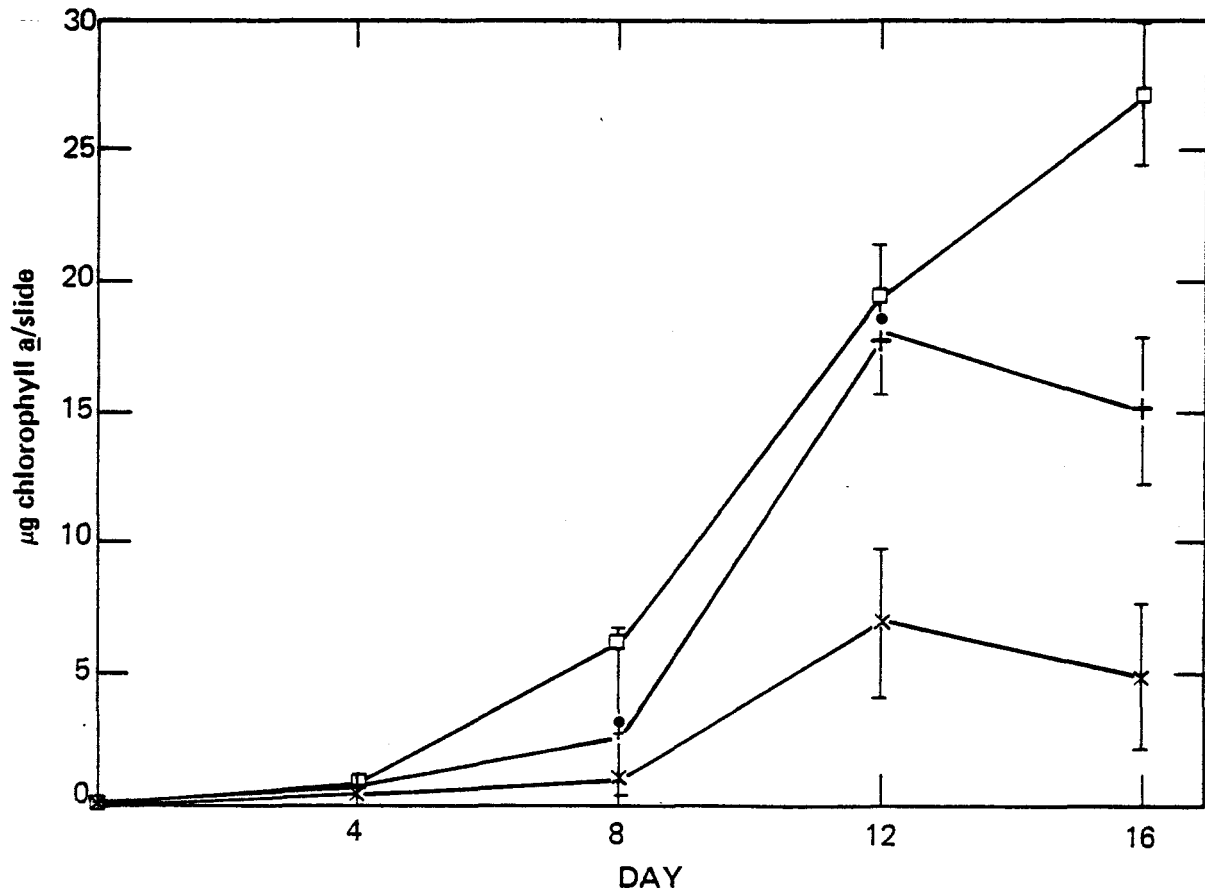


FIGURE 7. CHLA accumulation, February 1978. Explanation as in FIG. 6. +0°C (X), +5°C (+), +12°C (□).

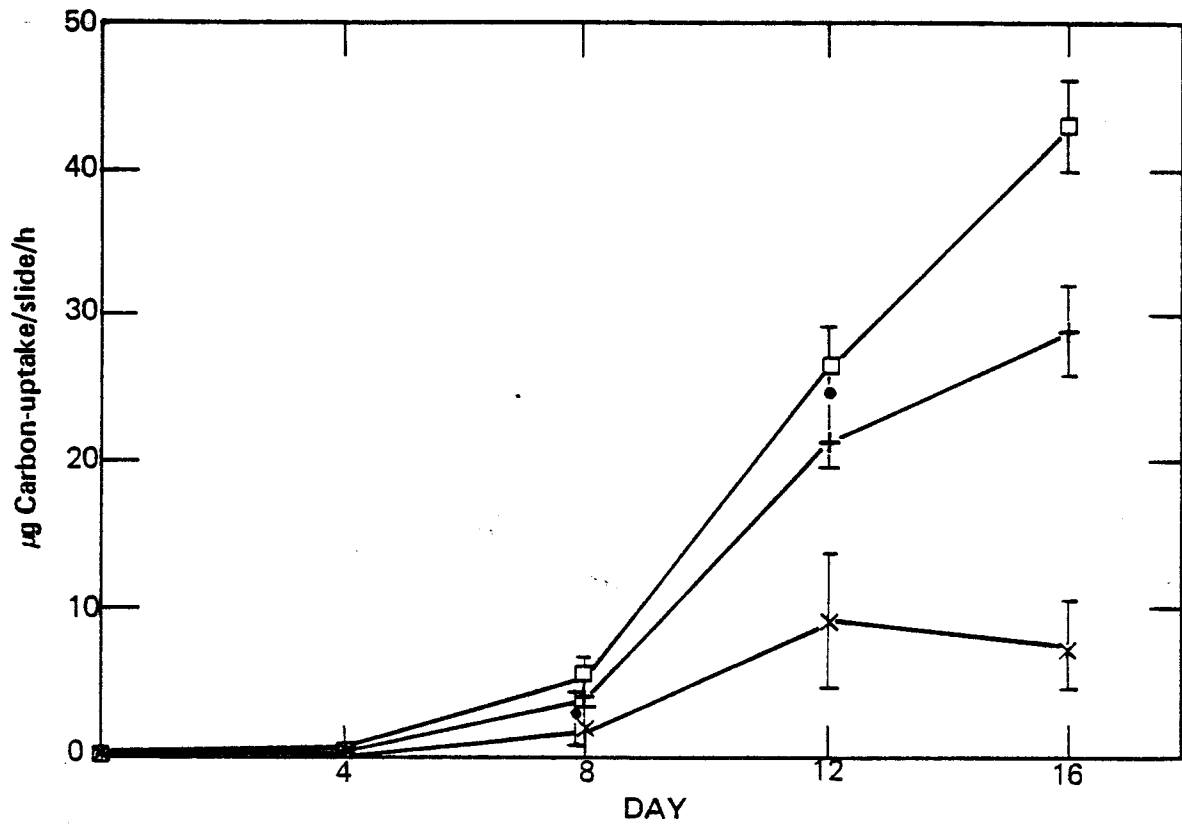


FIGURE 8. C-UP vs time of accumulation, February 1978. Explanation as in FIG. 6. +0°C (X), +5°C (+), +12°C (□).

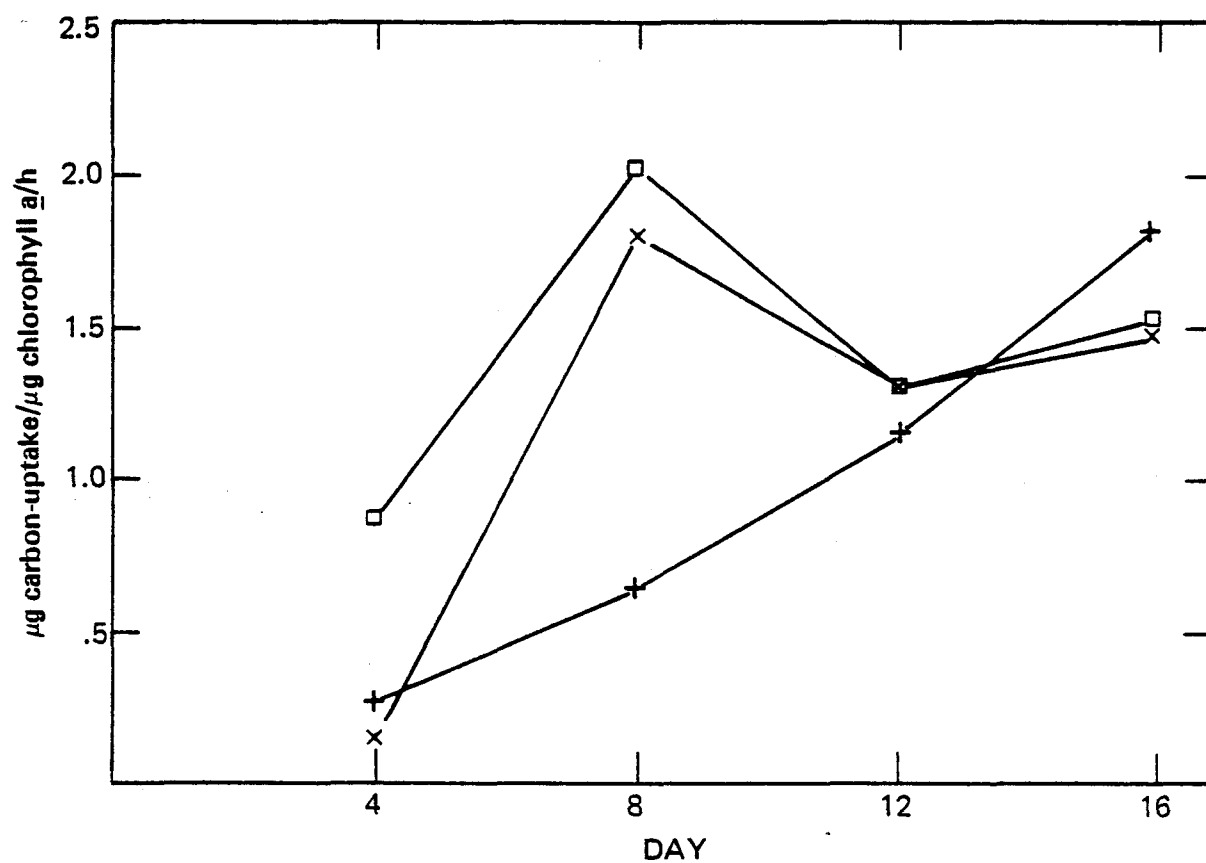


FIGURE 9. ASSIMNO vs time of accumulation, February 1978. $+0^\circ\text{C}$ (X), $+5^\circ\text{C}$ (+), $+12^\circ\text{C}$ (\square). Points are average C-UP values divided by average CHLA values for the three replicates at each time and temperature.

16, the +0°C assemblages had 1.5 to 3 times the CHLA as did the other two assemblages.

C-UP was similar for all three temperature treatments in June (Figure 12). All three assemblages had a maximum C-UP of 150 µg/slide/h on day 8. This rate then dropped to 50-75 µg/slide/h days 12-28. The ambient temperature assemblages were highest in CHLA and C-UP past day 8, although not significantly so.

The ASSIMNO (Figure 13) of these assemblages were greatest on day 4 (+12°C) or day 8, then were between 1 and 5 for the rest of the accumulation period. Note that past day 8, the ASSIMNO of the three assemblages were ranged as follows: +12°C >+5°C >+0°C. Although the +12°C assemblages had less CHLA and C-UP than did the other two temperature assemblages in the later days of the accumulation experiment, the ASSIMNO were always higher for the highest temperature assemblages.

Day 14 sampling in the summer enrichment experiment should occur when AFDW and CHLA are close to maximum for the two higher temperature treatments, but still increasing at ambient temperature. C-UP and ASSIMNO should be in plateau phase for all three temperature assemblages.

During the Sept. accumulation experiment (6 Sept. - 11 Oct.), AFDW was the same for the three treatments, except on day 20 when +12°C assemblages differed from the +5°C assemblages. No AFDW samples were taken on day 35. AFDW accumulated on the slides at a rate of approximately .25 mg/slide/day (Figure 14).

CHLA (Figure 15) of the +0°C and +5°C assemblages increased during the first 12 days, then was fairly constant through day 28. An approximately two-fold increase occurred by day 35. The +12°C assemblages

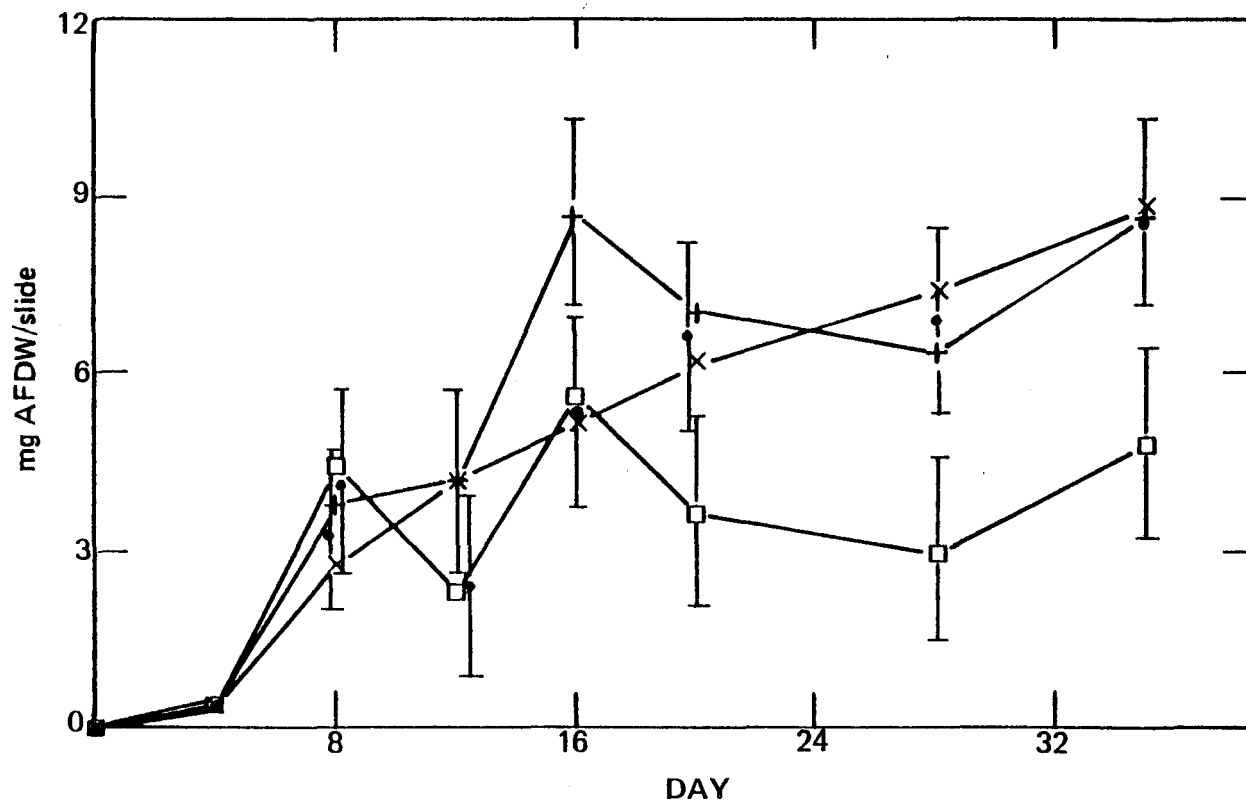


FIGURE 10. AFDW accumulation, June 1978. +0°C (X), +5°C (+), +12°C (□). Means of three replicates shown, ± 1 s.d.. Common means shown by a dot.

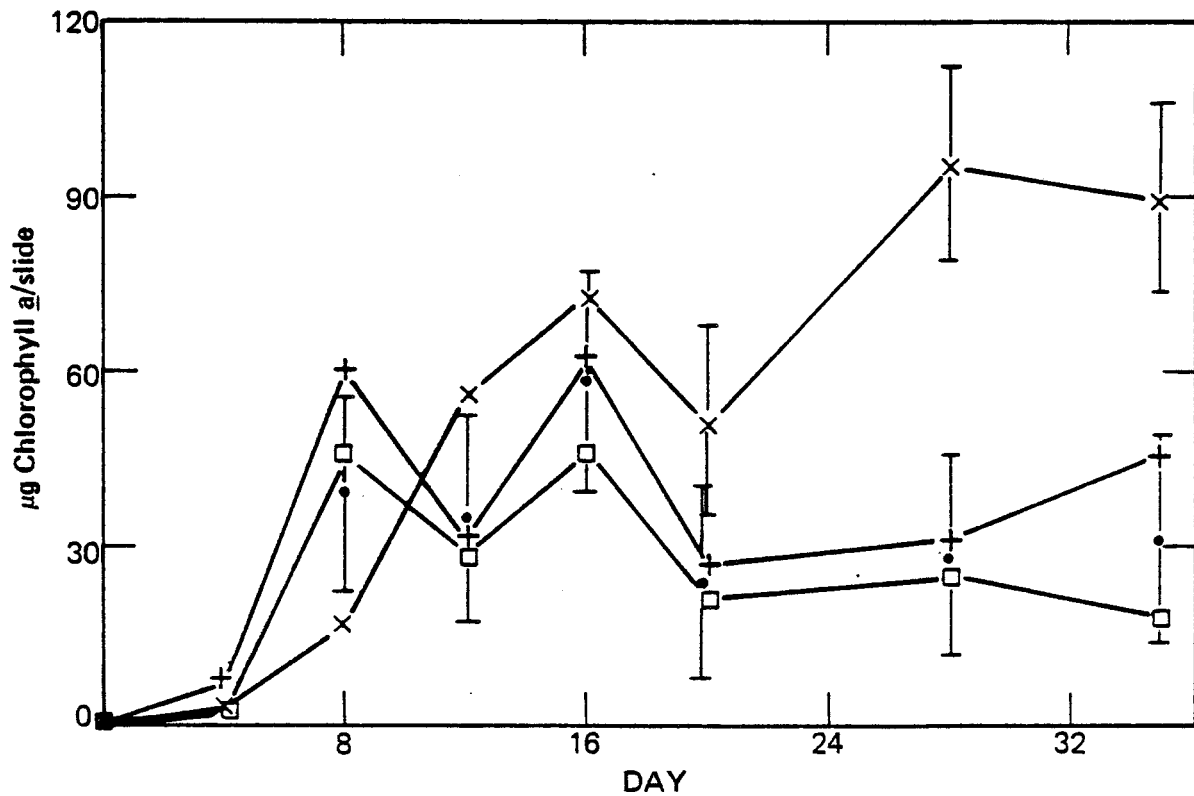


FIGURE 11. CHLA accumulation, June 1978. Explanation as in FIG. 10. +0°C (X), +5°C (+), +12°C (□).

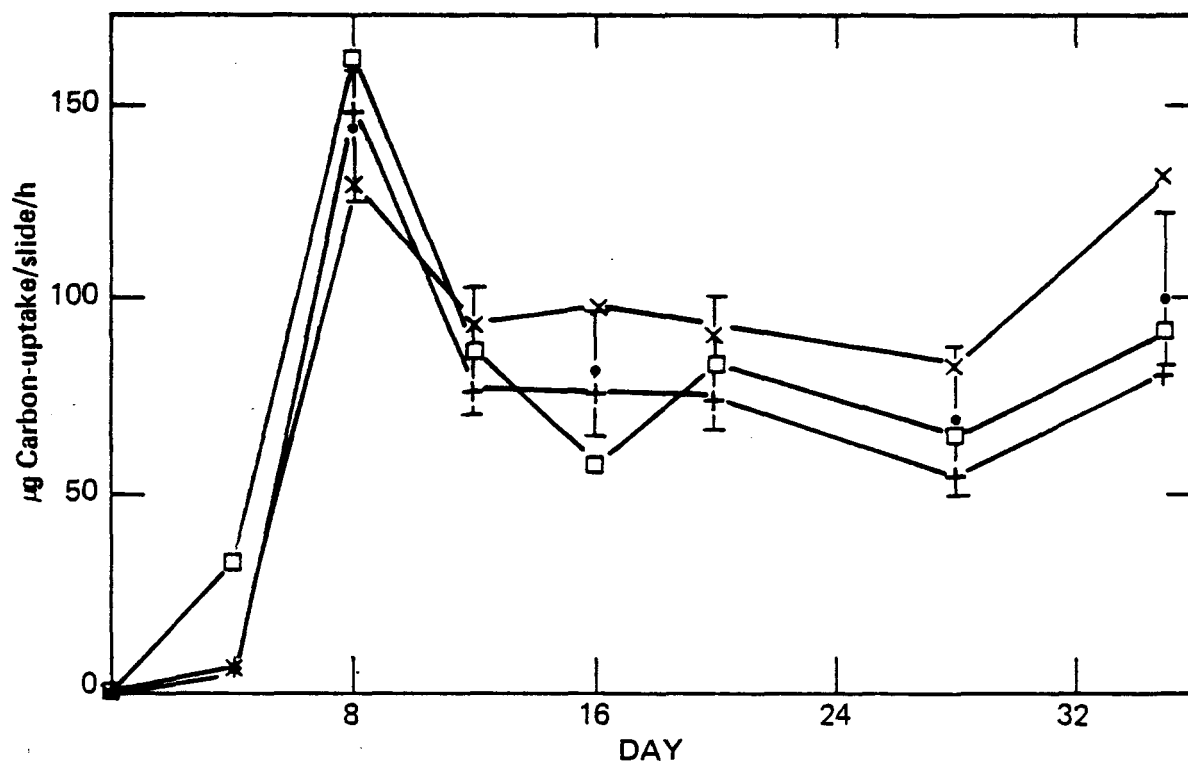


FIGURE 12. C-UP vs day of accumulation, June 1978. Explanation as in FIG. 10. +0°C (X), +5°C (+), +12°C (□).

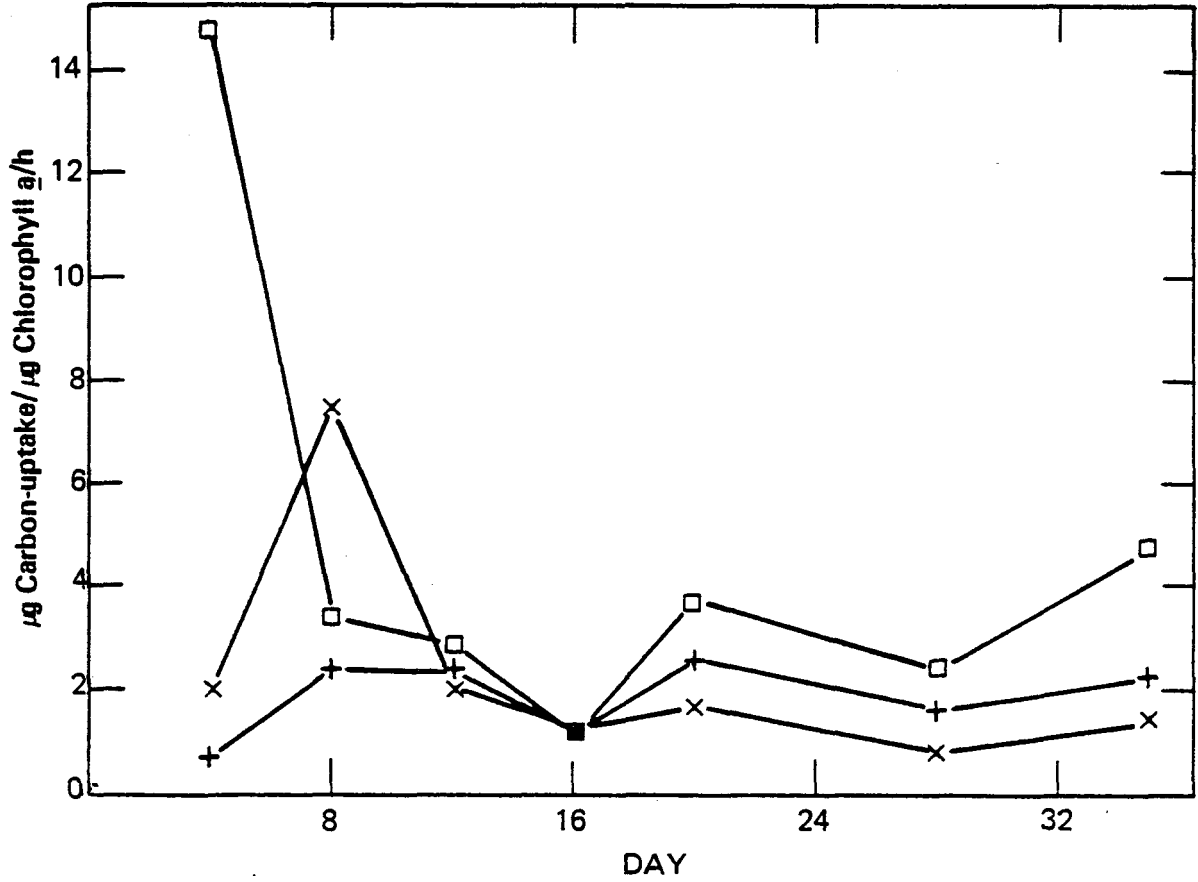


FIGURE 13. ASSIMNO vs time of accumulation, June 1978. $+0^\circ\text{C}$ (X), $+5^\circ\text{C}$ (+), $+12^\circ\text{C}$ (\square). Average C-UP/average CHLA for each time and temperature.

were low in CHLA (10 mg/slide) through day 28; CHLA for this highest temperature treatment also increased greatly on day 35 (35 mg/slide).

C-UP was low for all three temperature treatments in Sept. (Figure 16). The +0°C assemblages reached a maximum C-UP of 22 μg C/slide/h on day 16, with an average of 16 μg C/slide/h days 20-35. The +5°C assemblages had a similar average maximum value between days 4 and 16, but by day 35 their C-UP had increased to 35 μg C/slide/h, just as their CHLA had increased. The C-UP of the 12°C assemblages fluctuated between 5 and 22 μg C/slide/h until day 28, then rose to 27 μg C/slide/h on day 35. There was little difference among the three temperature assemblages during most of the accumulation period. Any difference, such as on day 20 (+12°C >+0°C >+5°C), was transitory.

ASSIMNO (Figure 17) were highest on day 4 for the +0°C and +5°C assemblages, on days 8 and 16 for the +12°C assemblages. Values were lower (maximum ~2) than in June, and dropped to .5 to 1.0 from day 20 to 35.

Day 14 sampling in the fall enrichment experiment should occur when AFDW is increasing steadily in all the treatments, CHLA and C-UP are in plateau phase, and ASSIMNO are still relatively high.

AFDW in the Dec. accumulation experiment (1 Dec. 1978 - 11 Jan. 1979) rose throughout the 42-day period for the three temperature treatments (Figure 18). The +12°C assemblages had significantly more AFDW than did the other two treatments from day 8 through day 20. The +0°C and +5°C assemblages were similar in AFDW throughout the period except days 35 and 42.

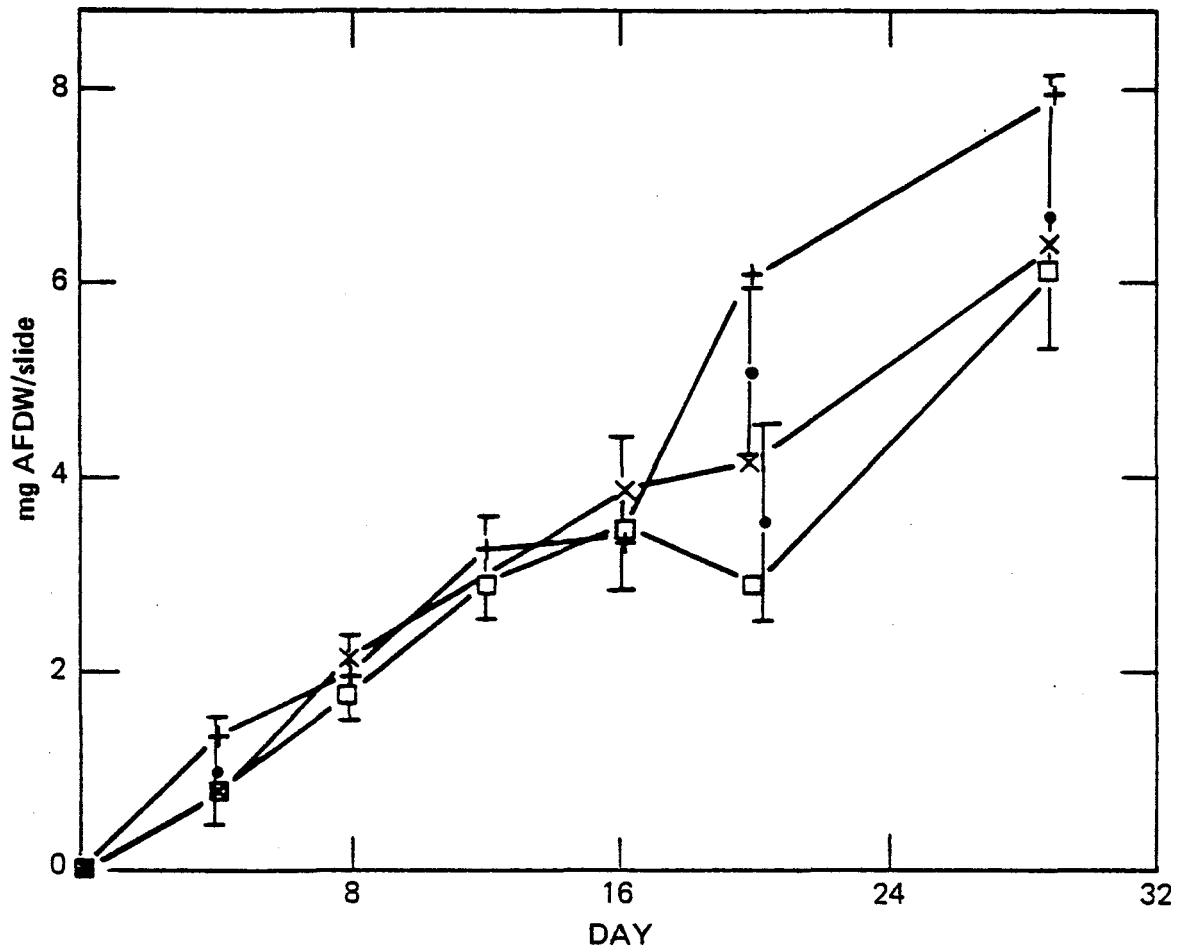


FIGURE 14. AFDW accumulation, September 1978. +0°C (X), +5°C (+), +12°C (□). Means of three replicates shown, ± 1 s.d.. Common means shown by dot.

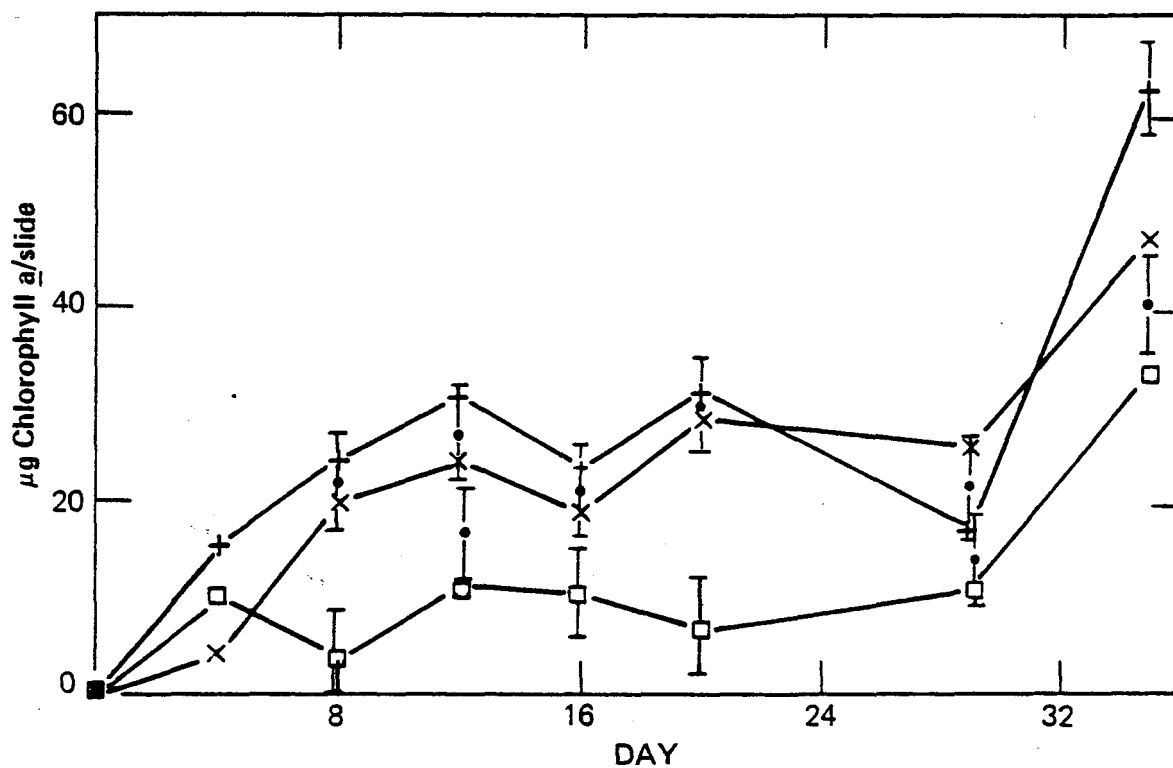


FIGURE 15. CHLA accumulation, September 1978. Explanation as in FIG. 14. +0°C (X), +5°C (+), +12°C (□).

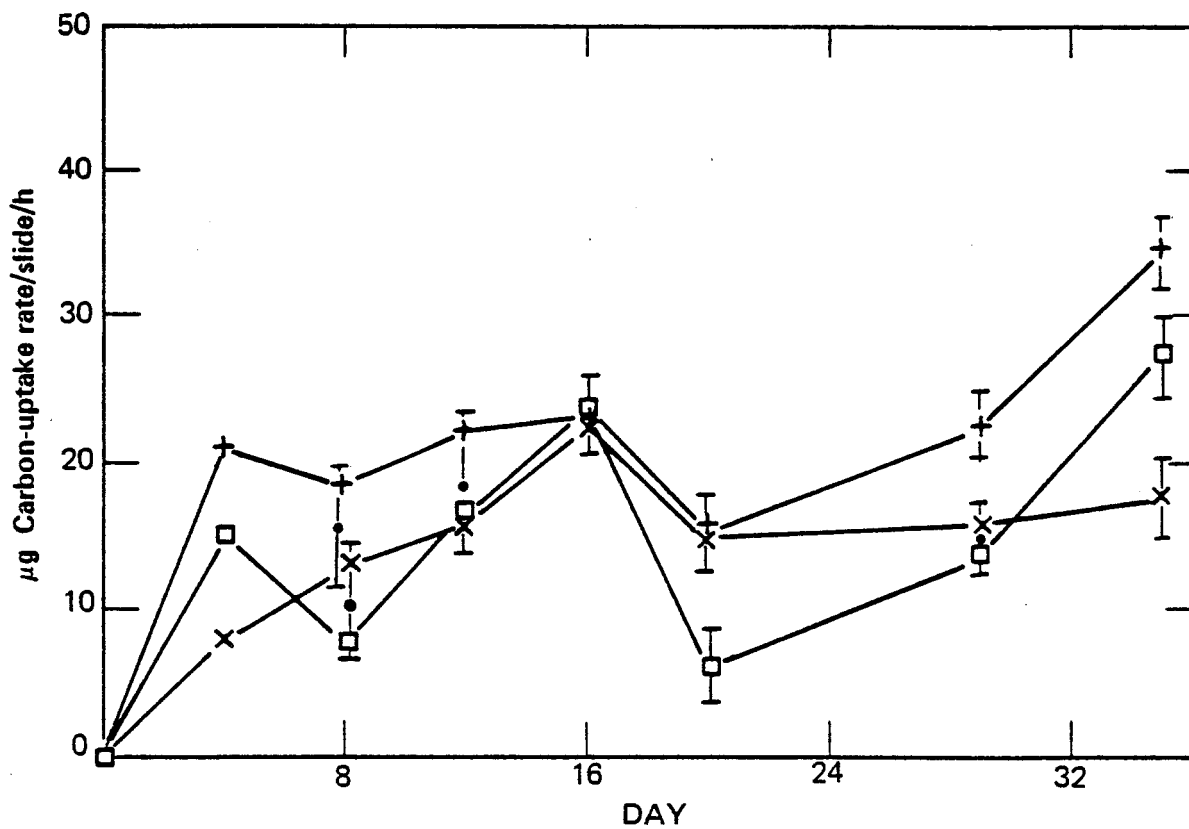


FIGURE 16. C-UP vs time of accumulation, September 1978. Explanation as in FIG. 14. +0°C (X), +5°C (+), +12°C (□).

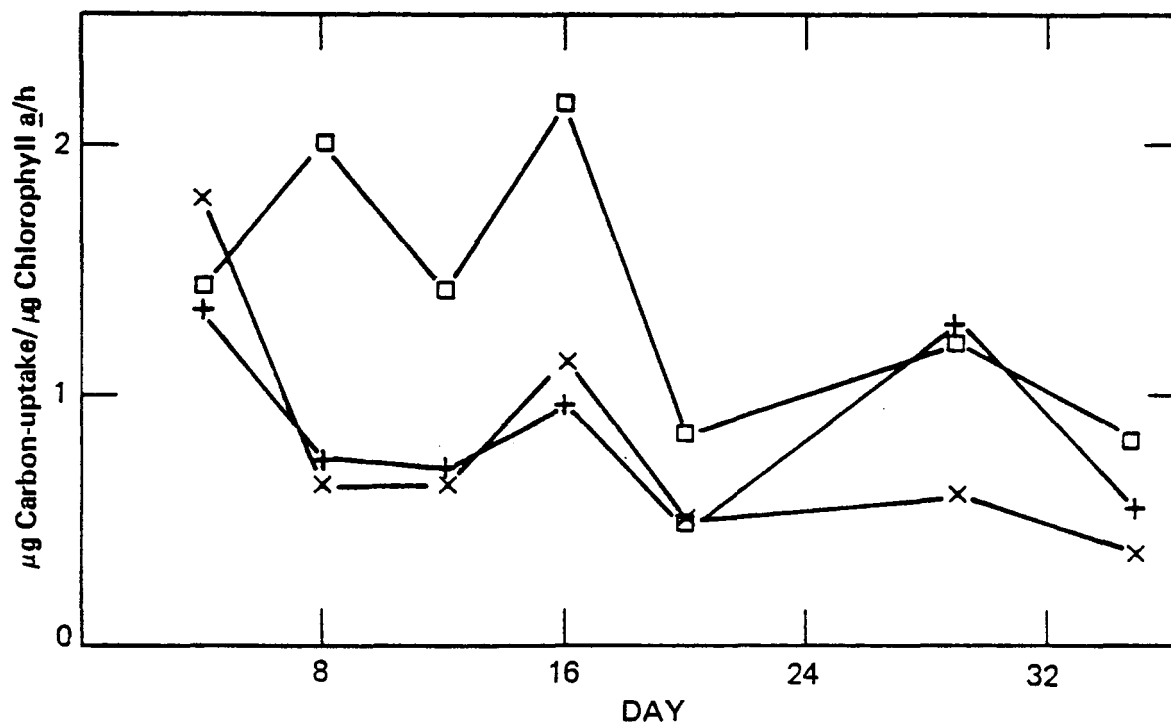


FIGURE 17. ASSIMNO vs time of accumulation, September 1978. +0°C (X), +5°C (+), +12°C (□). Average C-UP/average CHLA for each time and temperature.

CHLA (Figure 19) followed a pattern similar to that of AFDW: a steady rise for 42 days. The +12°C assemblages were only distinct from the other two assemblages on days 16 and 20.

As material accumulated on these slides in Dec., C-UP rose to a maximum on day 16 for +5°C and +12°C assemblages, on day 20 for +0°C assemblages (Figure 20). C-UP then fell for 10-15 days, and showed a second peak on day 42 for all three temperature treatments. ASSIMNO (Figure 21) were high (2-5) from day 8 to day 20, then fell to around 1 for all three assemblages.

Day 14 sampling in the winter enrichment experiments should occur while AFDW and CHLA are still accumulating on the slides. C-UP would be near maximum for all three assemblages, as would ASSIMNO.

The proportion of organic material (AFDW/DW; ORGANIC) showed the same accumulation pattern in all seasons. ORGANIC was high on days 4 and 8, then leveled off for the rest of the accumulation periods. In Feb., there was much variation in ORGANIC on day 4, but no difference among treatments days 8 to 16. ORGANIC was 35-40% in this experiment (Figure 22). In June (Figure 23), the +12°C assemblages were 40-42% organic between days 12 and 35; the +5°C assemblages were also 40% organic, but this value increased slightly toward day 35. The +0°C assemblages varied between 40% and 60% organic from day 8 to day 35. In Sept. (Figure 24), ORGANIC dropped from 47% to 40% for all three treatments. In Jan. (Figure 25), the +12°C assemblages were 40-42% organic from day 12 to day 42. The +0°C and +5°C assemblages were significantly higher in ORGANIC, dropping from 55% to 40% from day 8 to day 32.

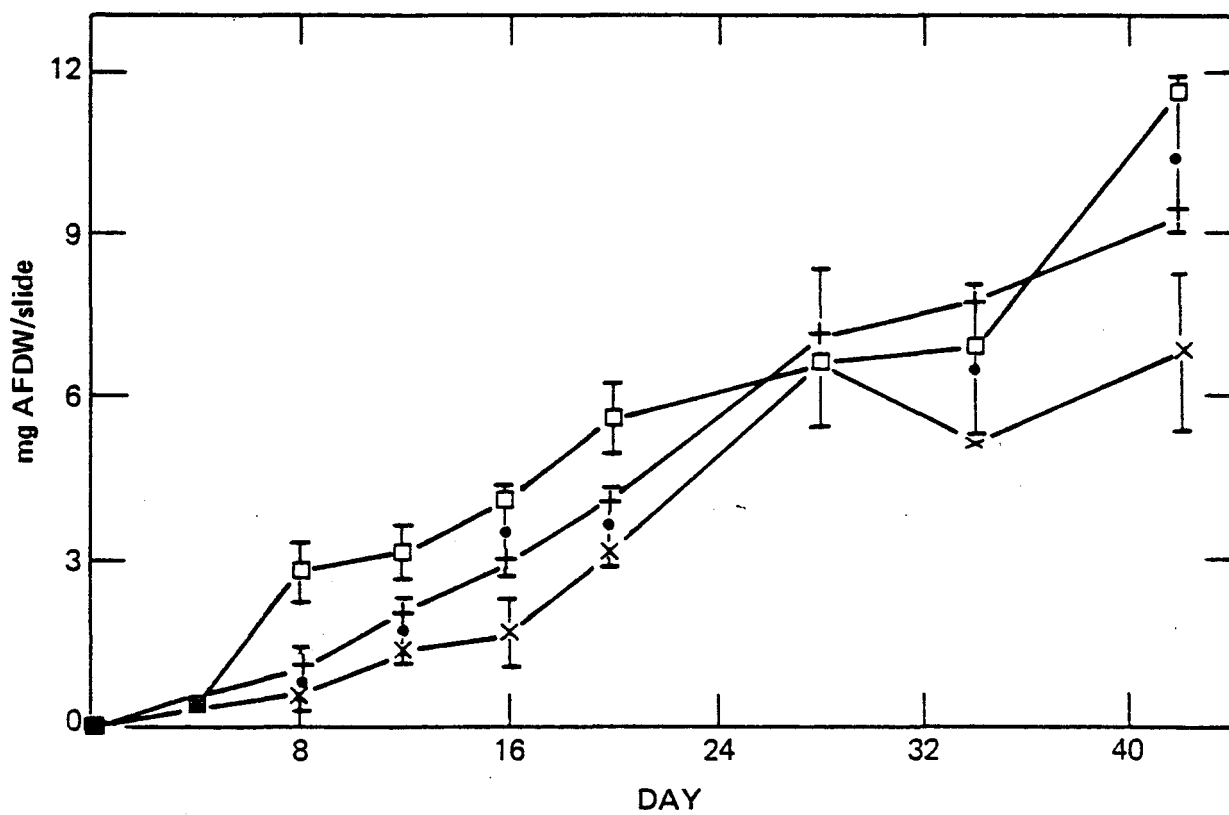


FIGURE 18. AFDW accumulation, December 1978. +0°C (X), +5°C (+), +12°C (□). Means of three replicates shown, ± 1 s.d.. Common mean shown by dot.

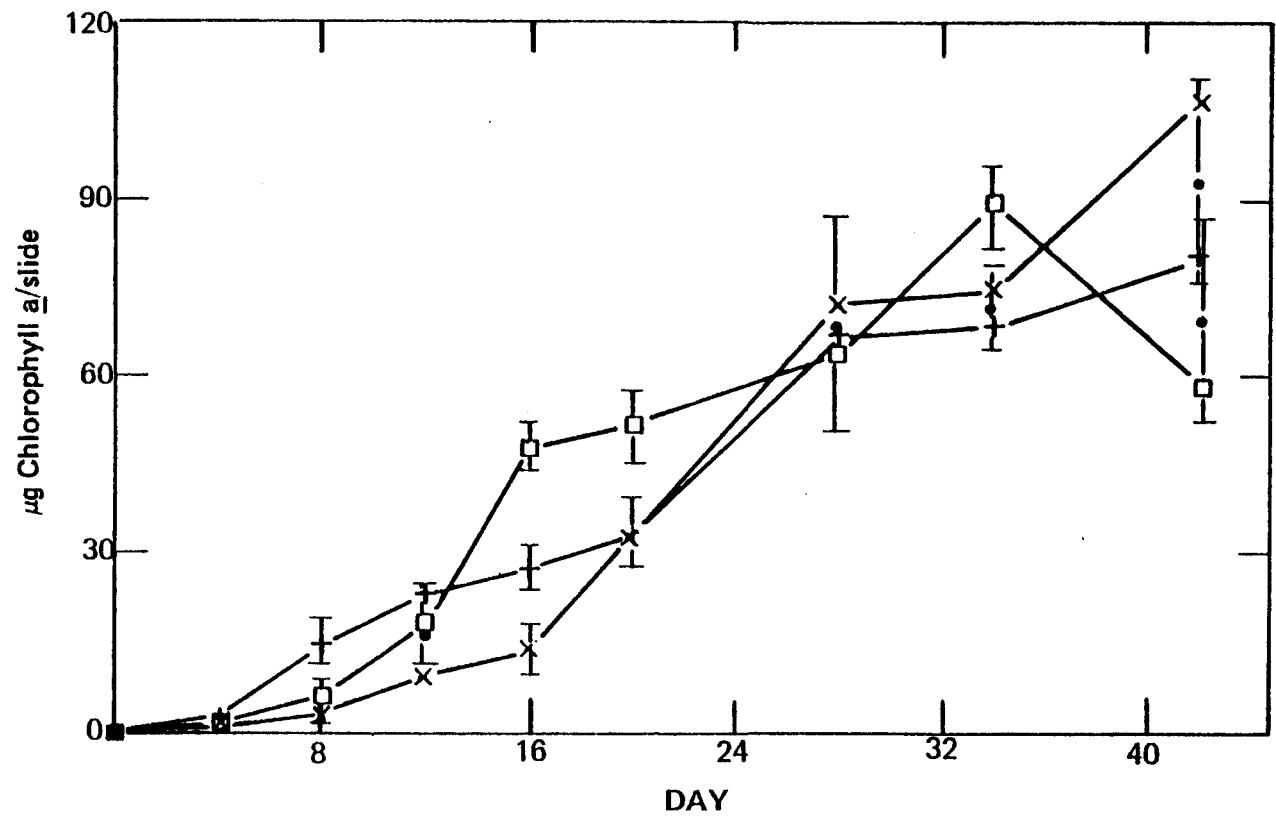


FIGURE 19. CHLA accumulation, December 1978. Explanations as in FIG. 18. +0°C (X), +5°C (+), +12°C (□).

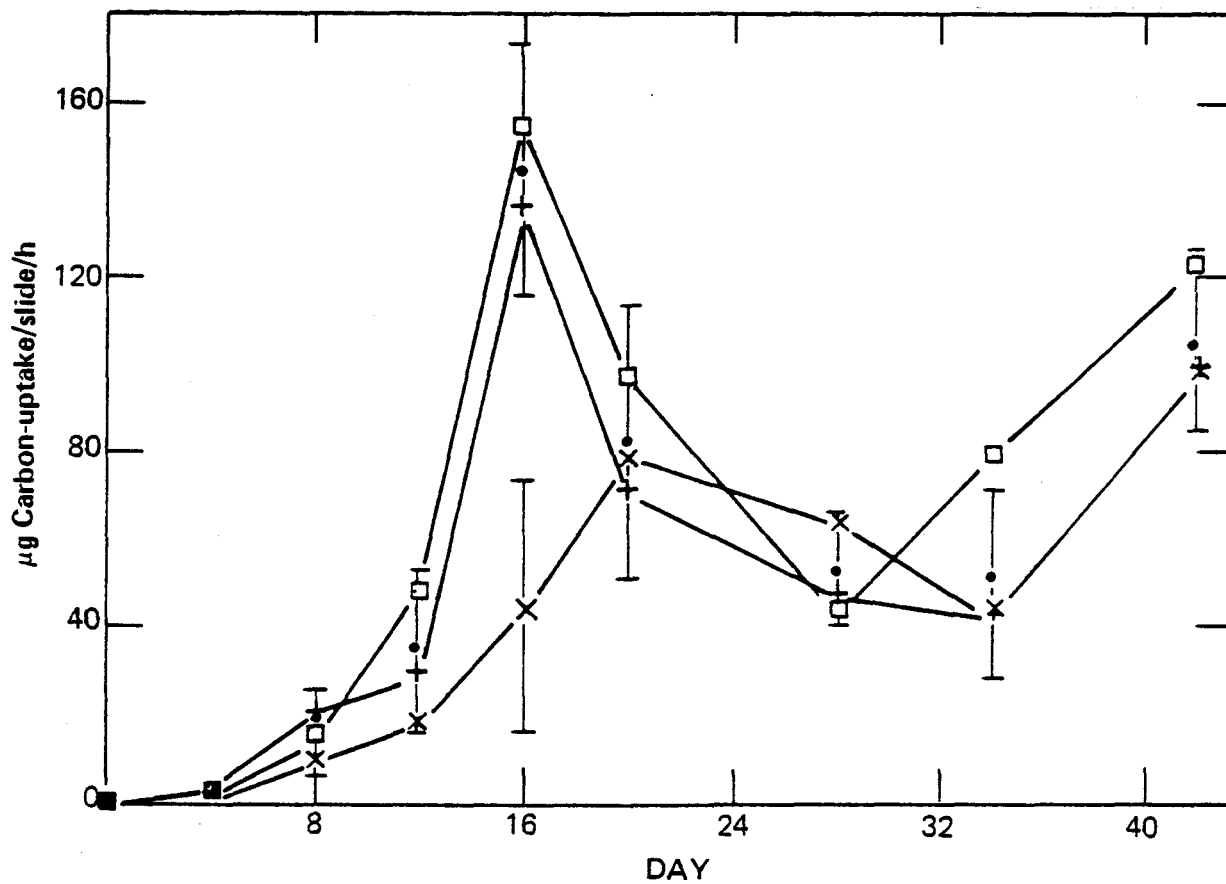


FIGURE 20. C-UP vs time of accumulation, December 1978. Explanation as in FIG. 18. +0°C (X), +5°C (+), +12°C (□).

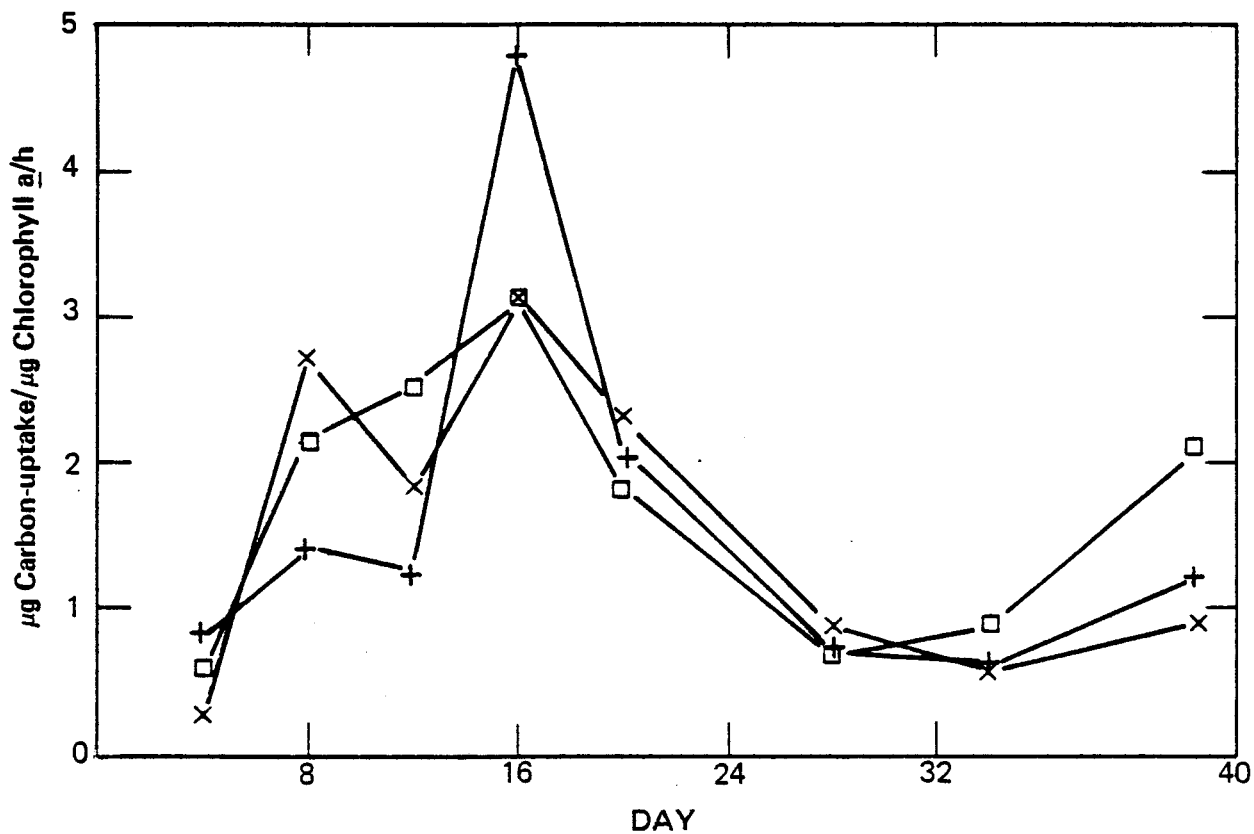


FIGURE 21. ASSIMNO vs time of accumulation, December 1978. $+0^\circ\text{C}$ (X), $+5^\circ\text{C}$ (+), $+12^\circ\text{C}$ (\square). Average C-UP/average CHLA for each time and treatment.

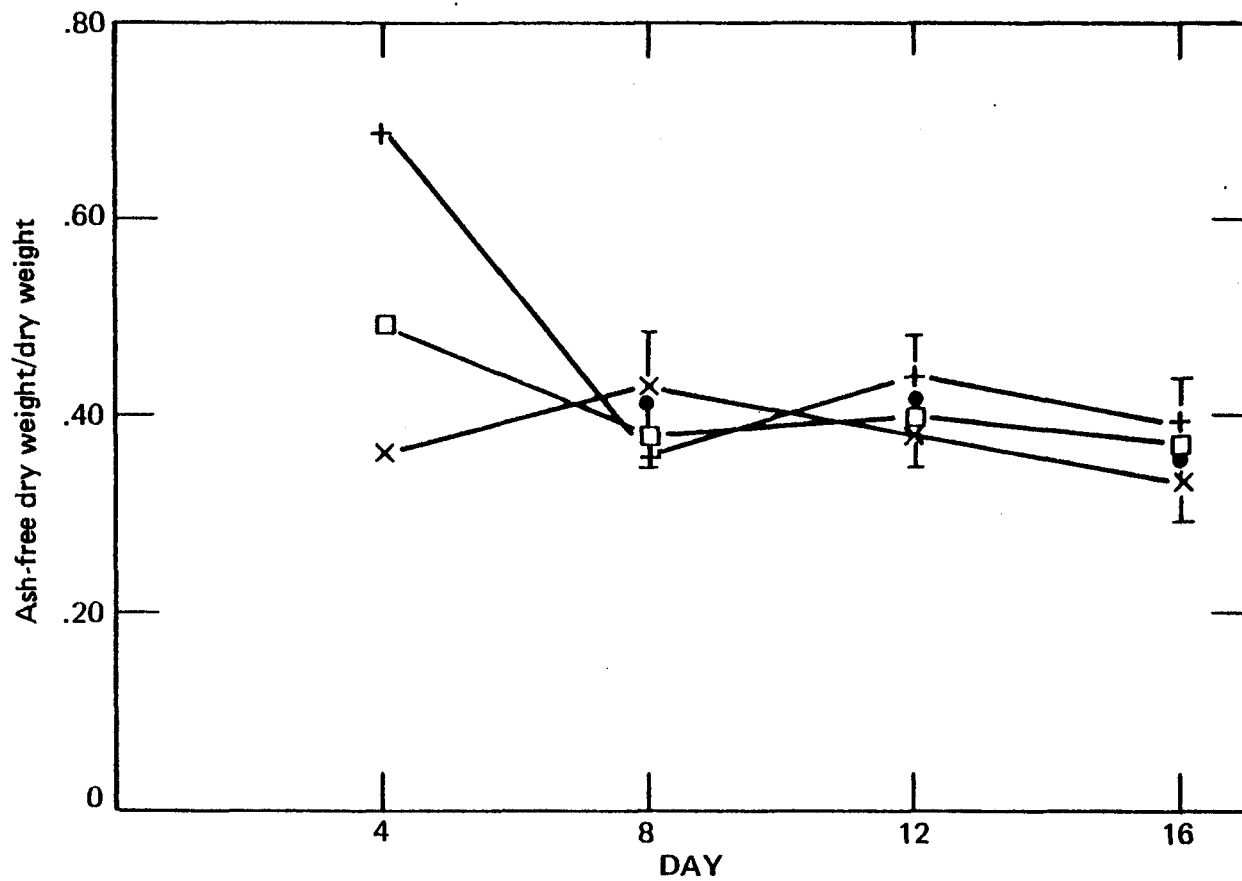


FIGURE 22. ORGANIC accumulation, February 1978. +0°C (X), +5°C (+), +12°C (□). Means of three replicates shown, ± 1 s.d.. Common mean indicated by a dot. (No error bars are put on day 4 because of large variation early in accumulation period.)

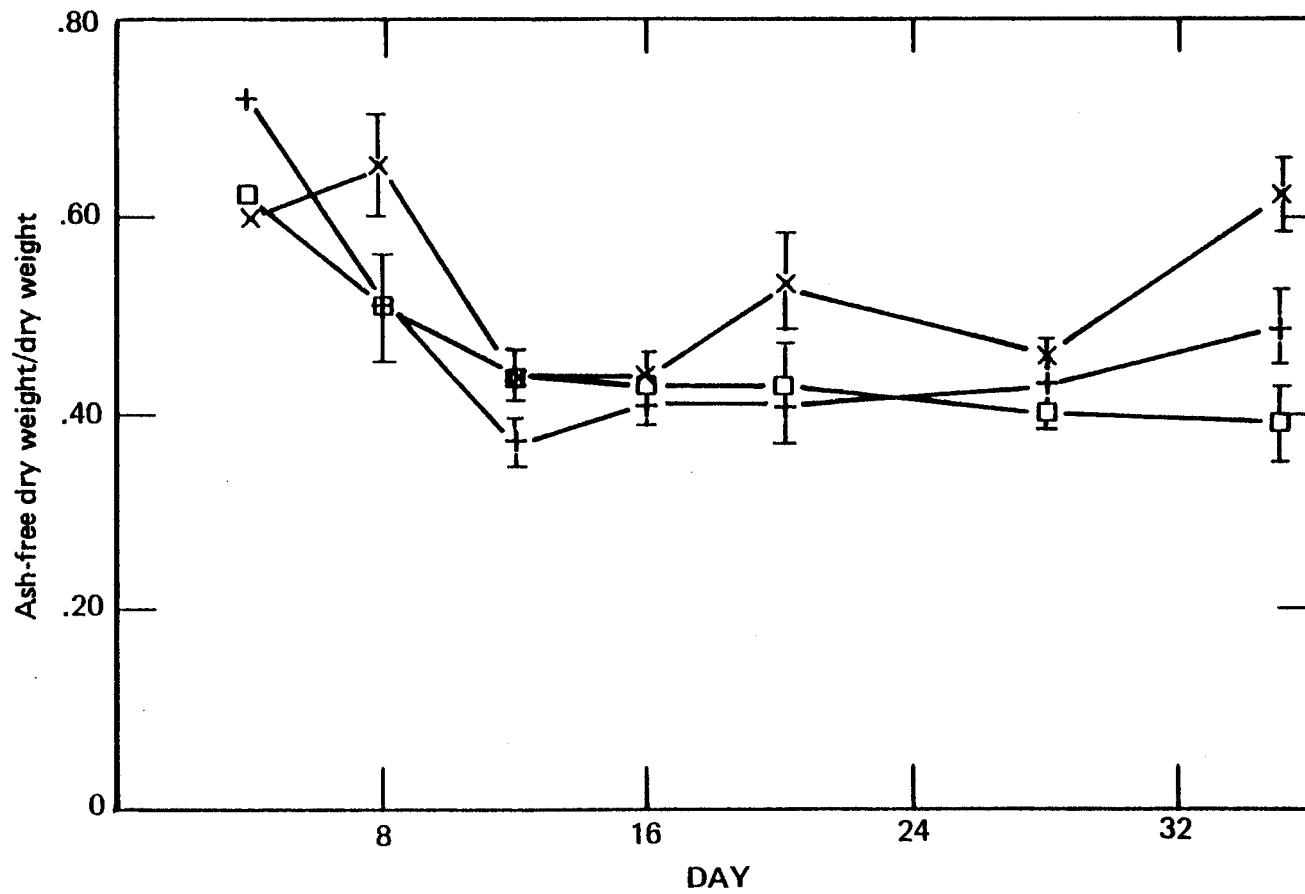


FIGURE 23. ORGANIC accumulation, June 1978. Explanation as in FIG. 22.
 +0°C (X), +5°C (+), +12°C (□).

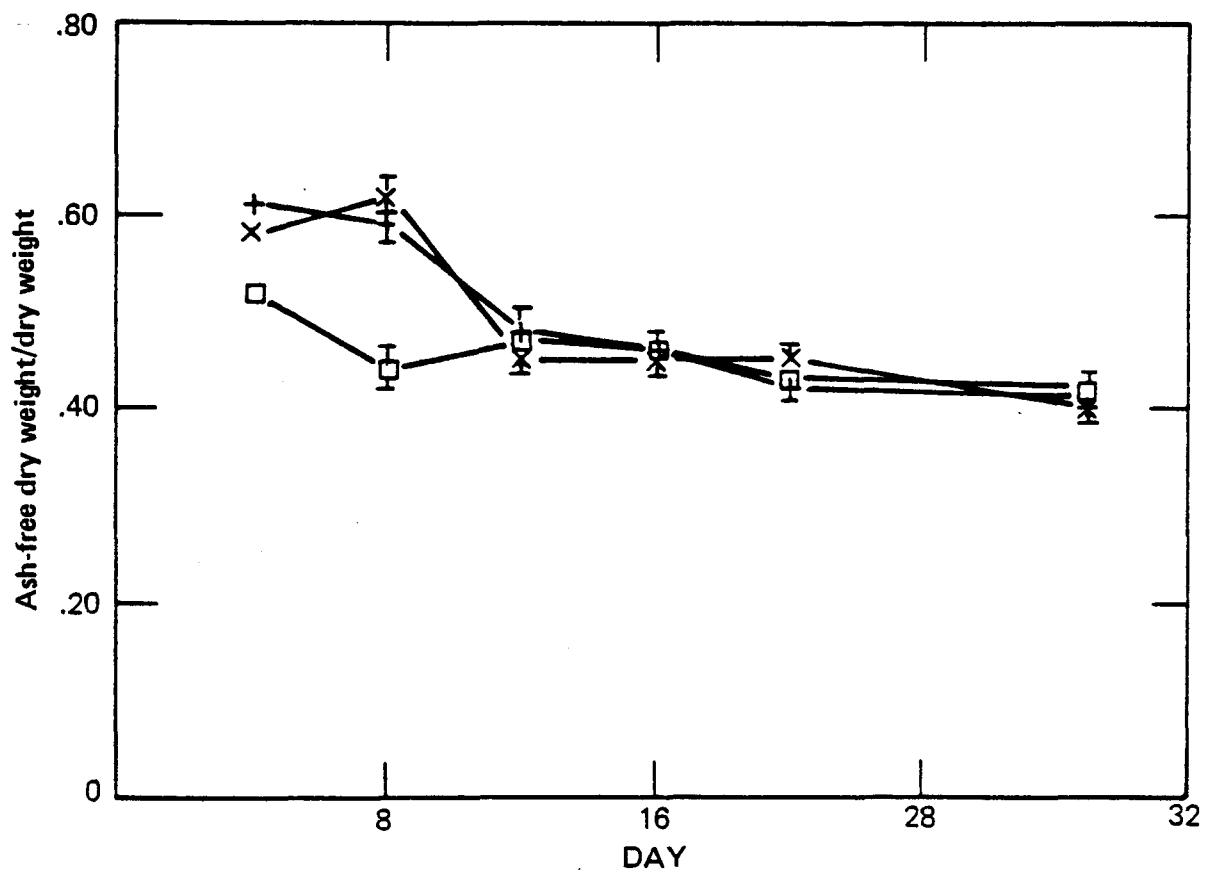


FIGURE 24. ORGANIC accumulation, September 1978. Explanation as in FIG. 22. +0°C (X), +5°C (+), +12°C (□).

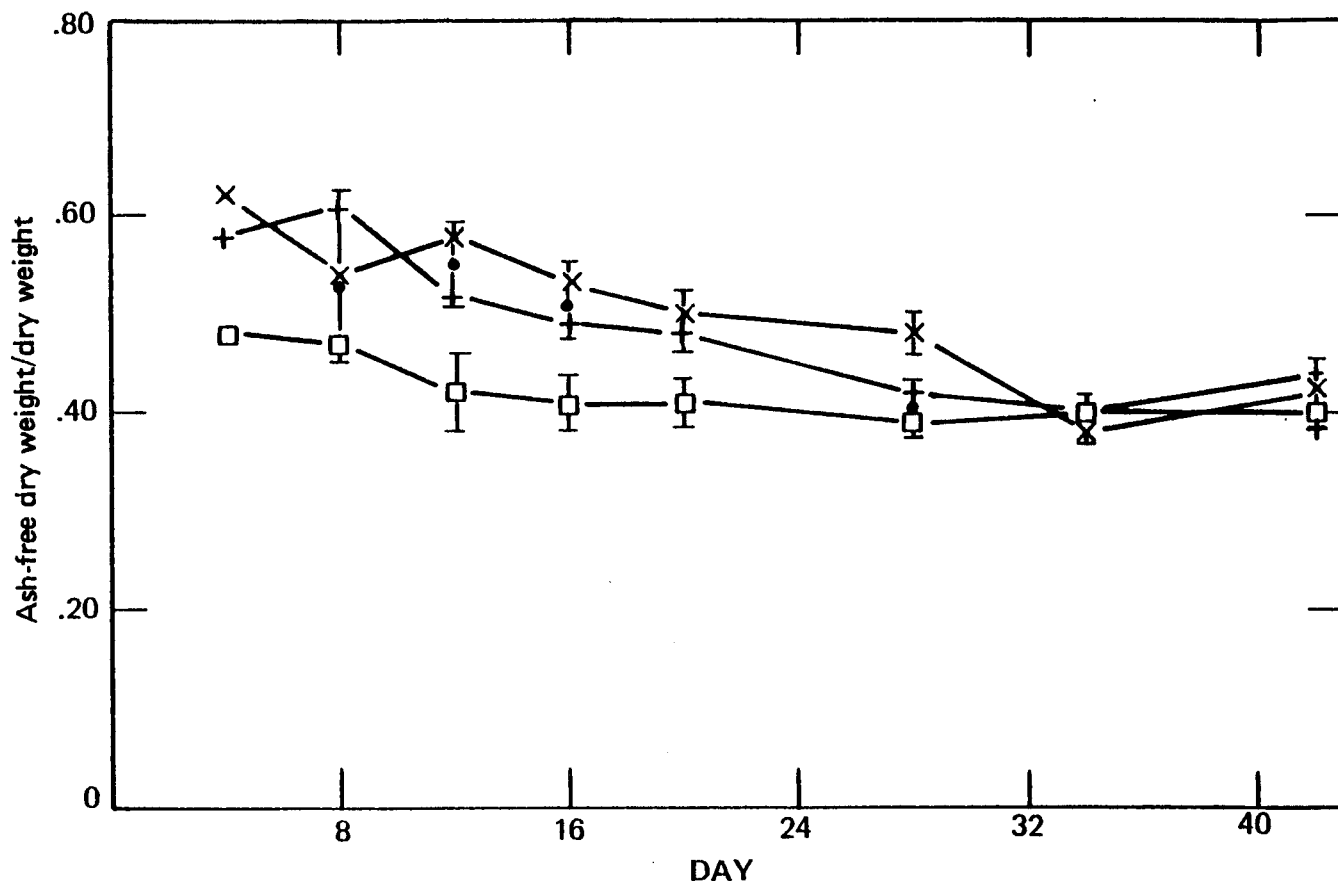


FIGURE 25. ORGANIC accumulation, December 1978. Explanation as in FIG. 22. +0°C (X), +5°C (+), +12°C (□).

Day 14 sampling for enrichment experiments was chosen not for its historical aspects but because a 2-week accumulation period allowed close to maximum levels of material to grow on the slides but avoided a long stationary phase. Carbon-uptake rates of algae were better correlated with change in particulate carbon for communities which were still growing rapidly (Peterson, 1978), perhaps because extracellular release of photosynthate was lower in "younger" populations (Berman and Holm-Hansen, 1974).

Seasonal Summary

The water temperature of Upper Three Runs followed a sinusoidal path through the year (Figure 26). Highest temperatures (20-25°C ambient) occurred in July and August, when there was also the least fluctuation in water temperature (Table 7) but a month after longest photoperiods and highest light intensities. Lowest temperatures occurred in January and February (5-10°C ambient), again a month after the solstice. From September to May, there was considerable fluctuation in water temperature - a 5 to 10°C change in 2-3 days was recorded several times. The pH of the stream responded to rainfall (Figure 27), presumably due to input of humic acid-laden water from swamps along the creek.

The levels of some nutrients varied over the year (Figure 28). Inorganic phosphorus ranged from 5 µg/L in winter to 15 µg/L in summer. Inorganic nitrogen varied less. Variation in chloride, manganese, and calcium appears to be due to high levels in one season's sampling and not to cyclic effects. Rainfall affected abundance of nutrients as well as pH level. In July, the two samplings for water chemistry were taken 1 week apart. The first sampling occurred the day after a heavy

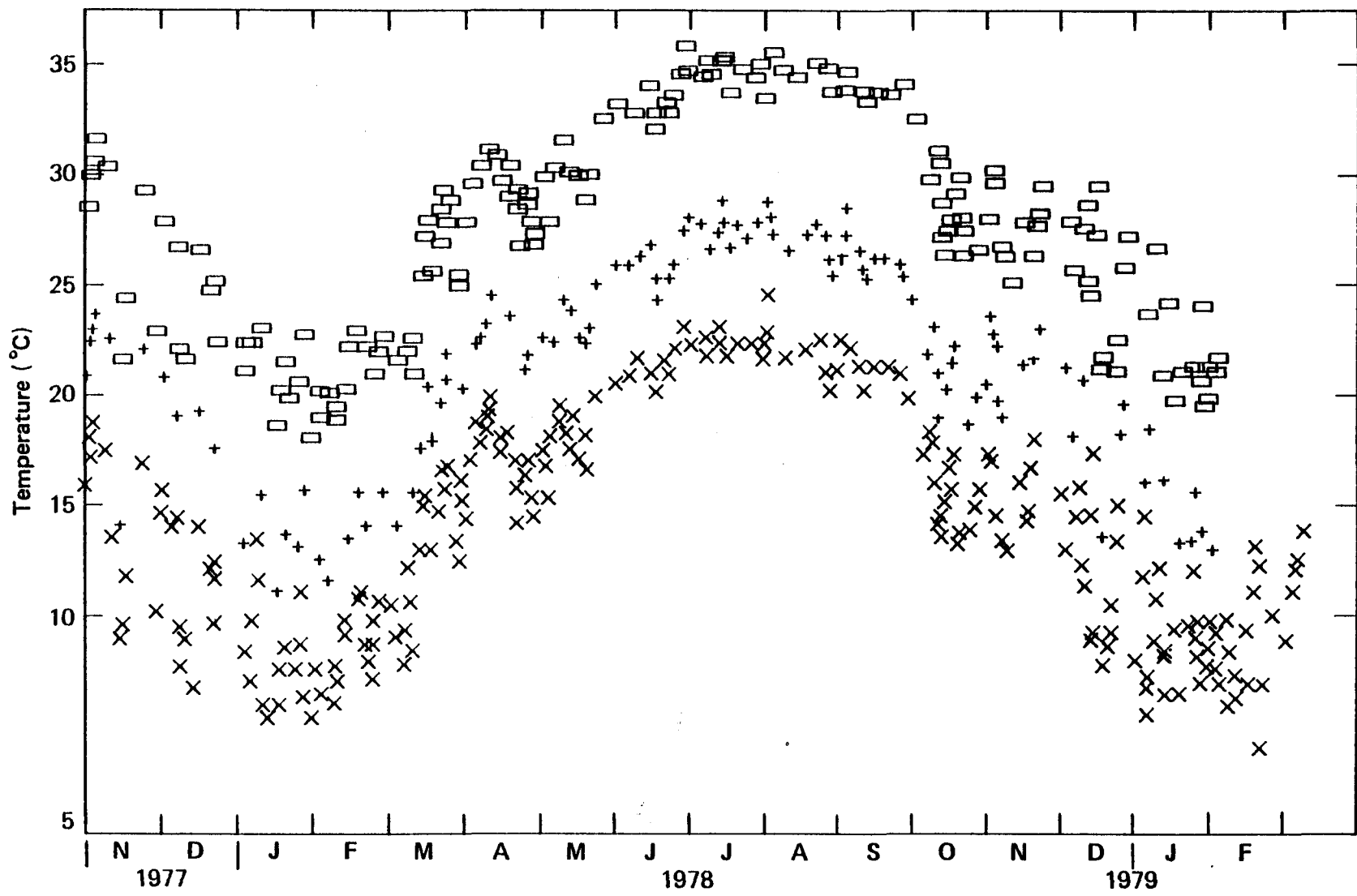


FIGURE 26. Daily temperature data over experimentation period.
 +0°C (X), +5°C (+), +12°C (□).

TABLE 7

Seasonal summary of temperature and pH data. See text for explanation of seasons.

		<u>+0</u>	<u>°C +5</u>	<u>+12</u>	<u>pH</u>
WINTER I	n=	65	62	63	63
	x=	9.27	14.07	21.48	5.72
	s=	2.6891	2.6710	2.6745	0.2987
	s ² =	7.2313	7.1341	7.1528	0.0893
SPRING I	n=	56	56	56	36
	x=	16.68	21.22	28.87	5.78
	s=	1.8330	2.0723	1.6501	0.3694
	s ² =	3.3598	4.2943	2.7227	0.1364
SUMMER	n=	84	83	82	74
	x=	21.87	26.76	34.13	5.71
	s=	0.8744	1.0143	0.8725	0.5434
	s ² =	0.7647	1.0288	0.7613	0.2953
FALL	n=	48	48	48	8
	x=	15.73	20.66	28.02	6.20
	s=	1.6844	1.6747	1.5385	0.6392
	s ² =	2.8372	2.8046	2.3670	0.4086
WINTER II	n=	61	40	40	17
	x=	9.59	13.88	21.72	5.89
	s=	2.1810	2.1986	2.2103	0.4723
	s ² =	4.7567	4.8338	4.8854	0.2231
SPRING II	n=	4			1
	x=	13.44			5.70
	s=	1.1150			
	s ² =	1.2433			

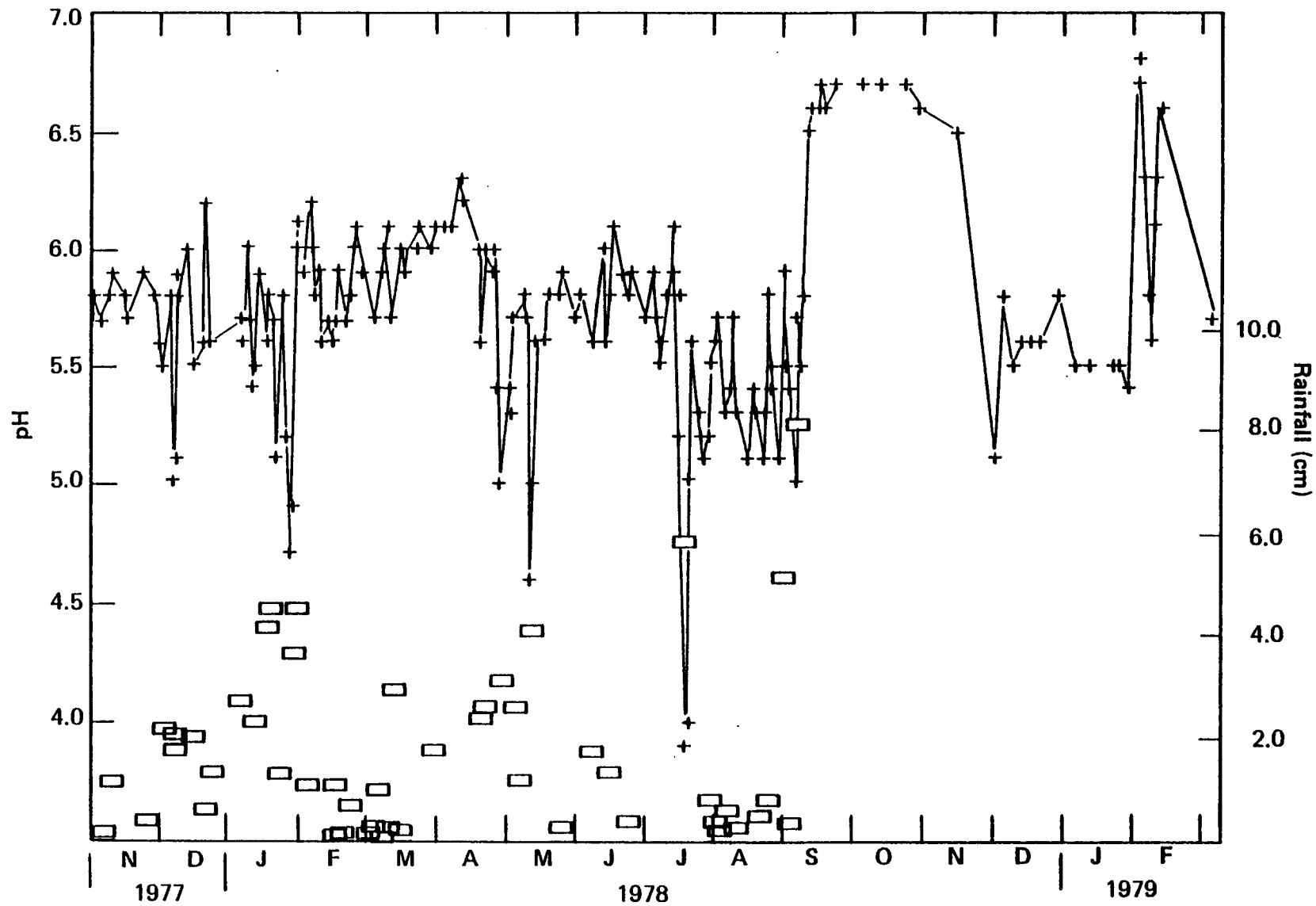


FIGURE 27. Daily pH and rainfall data over experimentation period. Rainfall (□), pH (+).

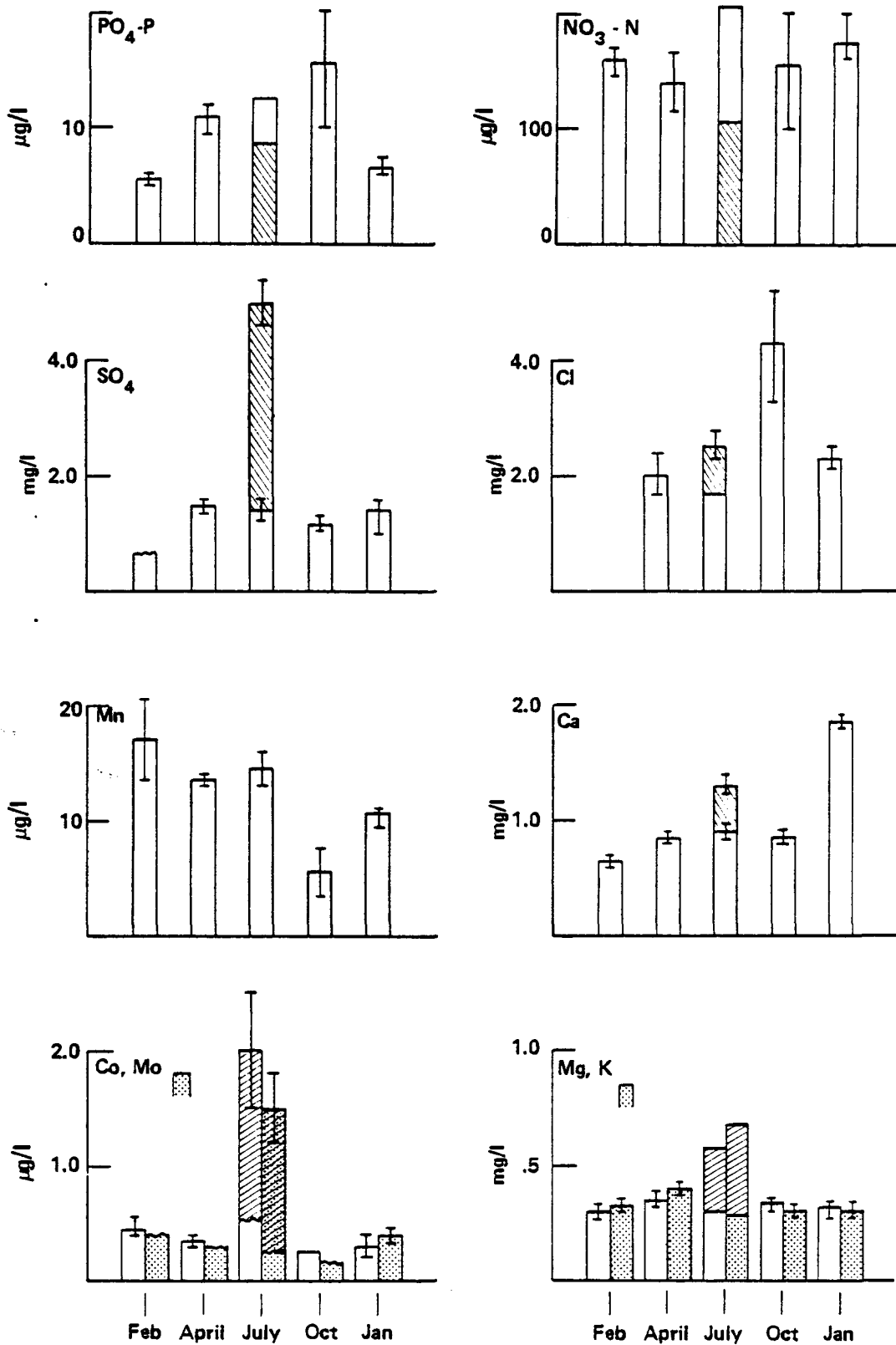


FIGURE 28. Seasonal variation in water chemistry of Upper Three Runs. Height of bar is mean of 2-6 replicates, \pm s.d.. In July, hatched vs open bar shows storm-influenced difference in values sampled one week apart (hatched = before storm).

rainfall, when water level was 1 m above normal. The second sample was taken at normal flow conditions. The hatched bars in Figure 28 show the effect of this storm surge. Nitrogen and phosphorus dropped 25-50%; and sulfate, chloride, calcium, cobalt, molybdenum, magnesium, and potassium increased 50-400%. The increased run-off and swamp input, perhaps combined with lower pH levels, altered the usual balance of nutrients in Upper Three Runs.

There was sometimes large variation from day-to-day in AFDW, CHLA and C-UP, as shown in the two-sampling among-troughs experiments. To look for an annual trend in the data, the experiments were grouped into seasons, which were defined as follows: Winter I, 14/11/77 to 10/3/78 - from the time the water temperature first dropped below 10°C (during the day) in the fall until it rose above 10°C for the last time in the spring; Spring I, 11/3/78 to 19/5/78 - from the time the water temperature rose above 10°C for the last time at the end of the winter until it rose above 20°C for the first time; Summer, 22/5/78 to 26/9/78 - from the time the water temperature first rose above 20°C at the end of the spring until it dropped below 20°C in the fall; Fall, 3/10/78 to 10/12/78 - from the time the water temperature fell below 20°C at the end of the summer until it first began dipping below 10°C in the winter; Winter II, 11/12/78 to 28/2/79, and Spring II, 1/3/79 to 4/3/79 - same definitions as Winter I and Spring I, respectively.

The seasonally grouped data are shown in Figures 29-34. The vertical lines in these figures, representing the ranges of means for the three temperature assemblages in each season, show the large within season variation found in periphyton assemblages in Upper Three Runs. Despite the large ranges, there is a decipherable seasonal and treatment

effect on the parameters measured, both through comparison of the means of experiments bounded by ± 1 standard error and by Friedman's ranking test applied to each season (Table 9). The experiments included are day 14, unenriched treatments from the entire data set (See Enrichment Section for the data taken from that set).

AFDW (Figure 29) during both winters was ranked $+12^{\circ}\text{C} > +5^{\circ}\text{C} > +0^{\circ}\text{C}$. Spring I AFDW was higher than that for winter for all temperature treatments, and although the mean of the ambient temperature assemblages' AFDW was lower in spring than that of the $+5^{\circ}\text{C}$ and $+12^{\circ}\text{C}$ assemblages (4.85 vs 7.35 mg AFDW/slide), the ranking test does not detect a consistent ordering of the temperature treatments. In summer, AFDW means were ranked $+0^{\circ}\text{C} > +5^{\circ}\text{C} > +12^{\circ}\text{C}$, while Friedman's test finds $+12^{\circ}\text{C}$ AFDW to be consistently lower than that of $+0^{\circ}\text{C}$ and $+5^{\circ}\text{C}$. In the fall, the $+5^{\circ}\text{C}$ assemblages were higher in AFDW than the $+0^{\circ}\text{C}$ and $+12^{\circ}\text{C}$ assemblages.

ORGANIC (Figure 30) did not vary as much among treatments as did AFDW. This is evident from the closeness of most of the seasonal means. There was no distinguishable difference in ranks in Winter I and Summer. In Spring I, the rankings of ORGANIC were $+12^{\circ}\text{C} > +5^{\circ}\text{C} > +0^{\circ}\text{C}$; in Fall, $+12^{\circ}\text{C}$ was lower in ORGANIC than the other two treatments. ORGANIC ranking sometimes differed from that of AFDW, as in Winter I and Fall, indicating that total dry weight was high in relation to AFDW in some treatments ($+12^{\circ}\text{C}$).

Protein, which was measured only during the last three enrichment experiments, is shown in Figure 31. The rankings were the same as for AFDW, with $+0^{\circ}\text{C}$ assemblages being the highest in PROT of the three temperature treatments in summer, the lowest in fall and winter.

CHLA (Figure 32) and C-UP (Figure 33) followed the same rankings through the seasons as AFDW. The $+12^{\circ}\text{C} > +5^{\circ}\text{C} > +0^{\circ}\text{C}$ pattern of winter was reversed in summer, with the $+5^{\circ}\text{C}$ assemblages having greatest levels in fall. For $+0^{\circ}\text{C}$ assemblages, AFDW, CHLA and C-UP were greatest in summer and lowest in winter. For the $+5^{\circ}\text{C}$ assemblages, AFDW was highest in spring and lowest in winter, while CHLA was highest in fall and lowest in winter. C-UP for the $+5^{\circ}\text{C}$ treatment followed the same pattern as the $+0^{\circ}\text{C}$ treatment - high in summer and low in winter. The $+12^{\circ}\text{C}$ assemblages had more AFDW in spring than in the other seasons, with no well-defined minimum. Average CHLA was fairly constant over the year at the highest temperature treatment, and along with C-UP showed a slight minimum in the fall.

The means and ranges of ASSIMNO are shown in Figure 34. There was little difference among temperature treatments except in summer when $+12^{\circ}\text{C}$ assemblages had consistently higher ASSIMNO than did $+0^{\circ}\text{C}$ and $+5^{\circ}\text{C}$ assemblages. All three temperature treatments had minimum assimilation numbers in the fall.

Enrichment Experiments

The results of the Feb. enrichment experiment show little besides temperature effect on the periphyton assemblages (Tables 9-13). ORGANIC was the same for all treatments (nutrient and temperature) at each sampling. Occasionally one of the enriched treatments at $+12^{\circ}\text{C}$ was lower in AFDW or C-UP than the reference treatment. Only once, in the third sampling, did the trace element enriched assemblages have significantly more CHLA than the unenriched assemblages at $+12^{\circ}\text{C}$.

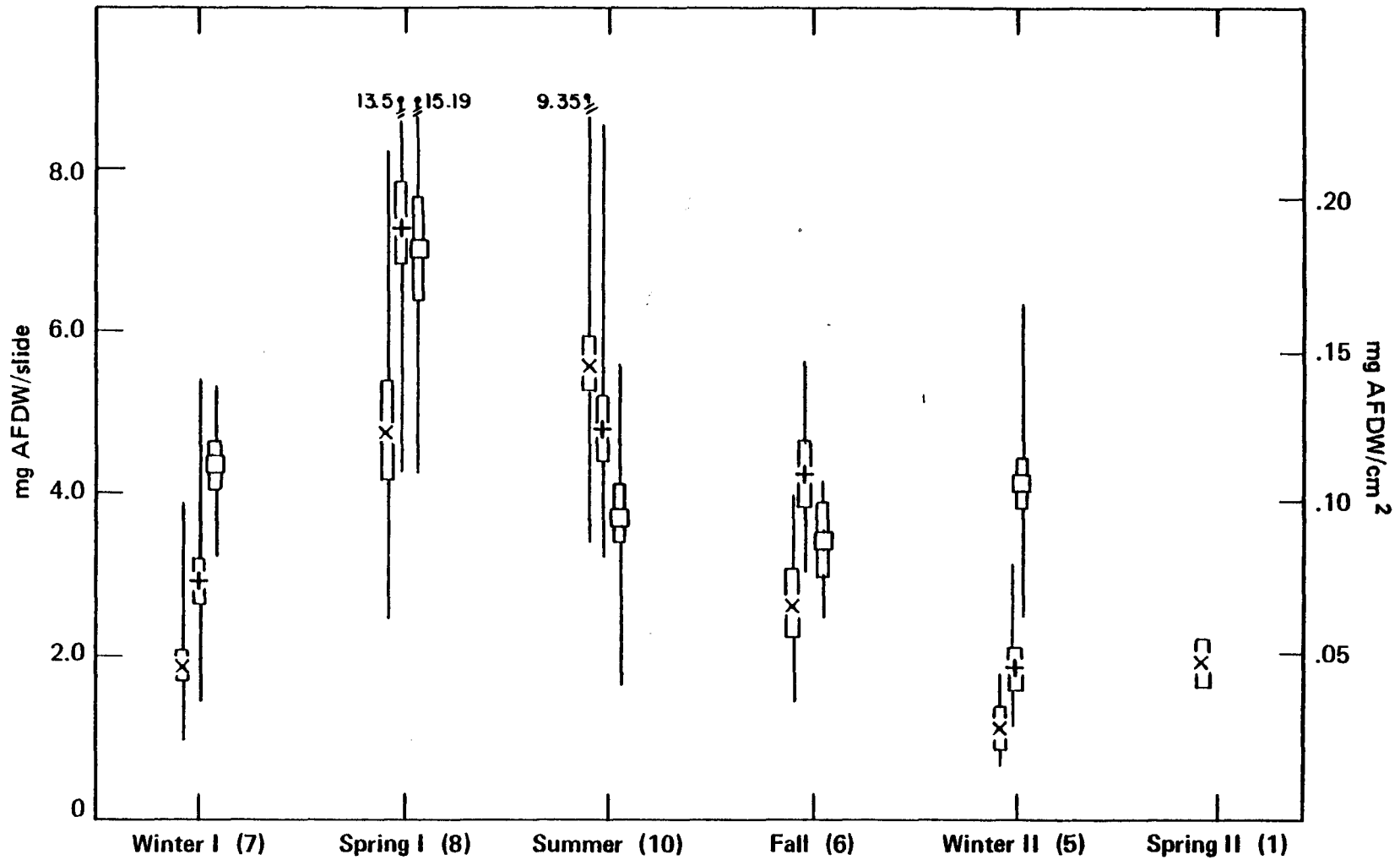


FIGURE 29. Seasonal summary of AFDW. +0°C (X), +5°C (+), +12°C (□); means of experiment means, grouped by season (see text for explanation of seasons). Rectangle is ± 1 standard error; vertical line is range of means. Temperature treatments only. Number of experiments in parentheses after season.

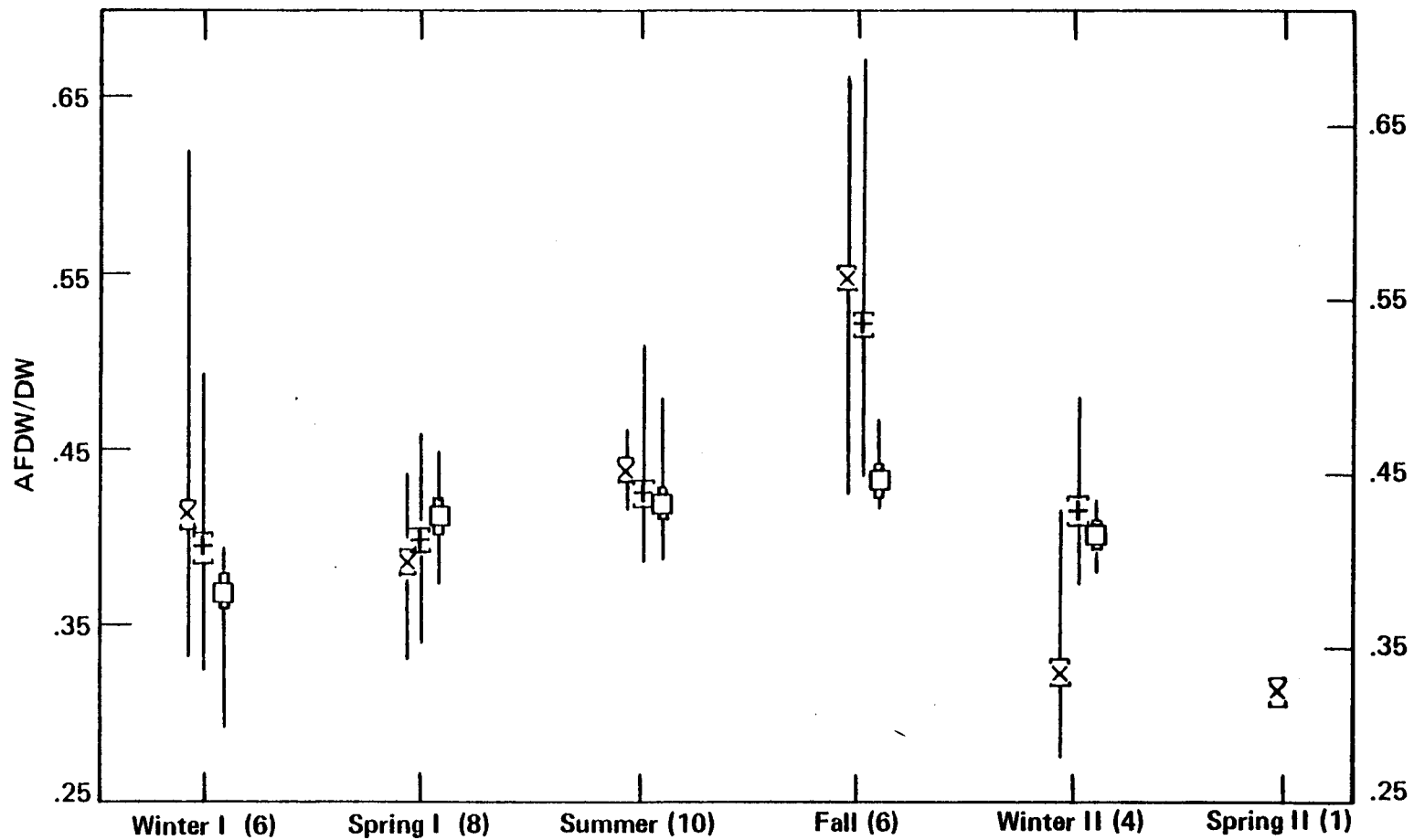


FIGURE 30. Seasonal summary of ORGANIC . Explanation as in FIG. 29.
 +0°C (X), +5°C (+), +12°C (□).

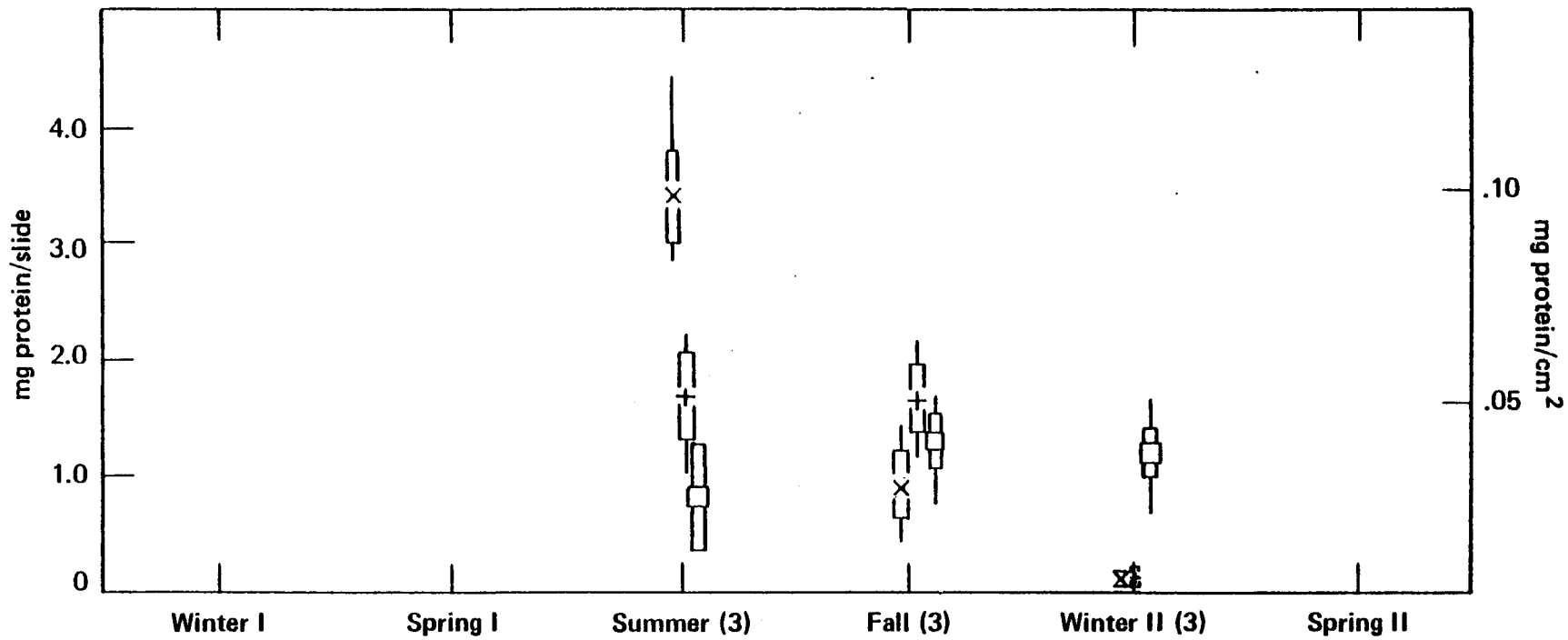


FIGURE 31. Seasonal summary of PROT. Explanation as in FIG. 29. +0°C (X), +5°C (+), +12°C (□).

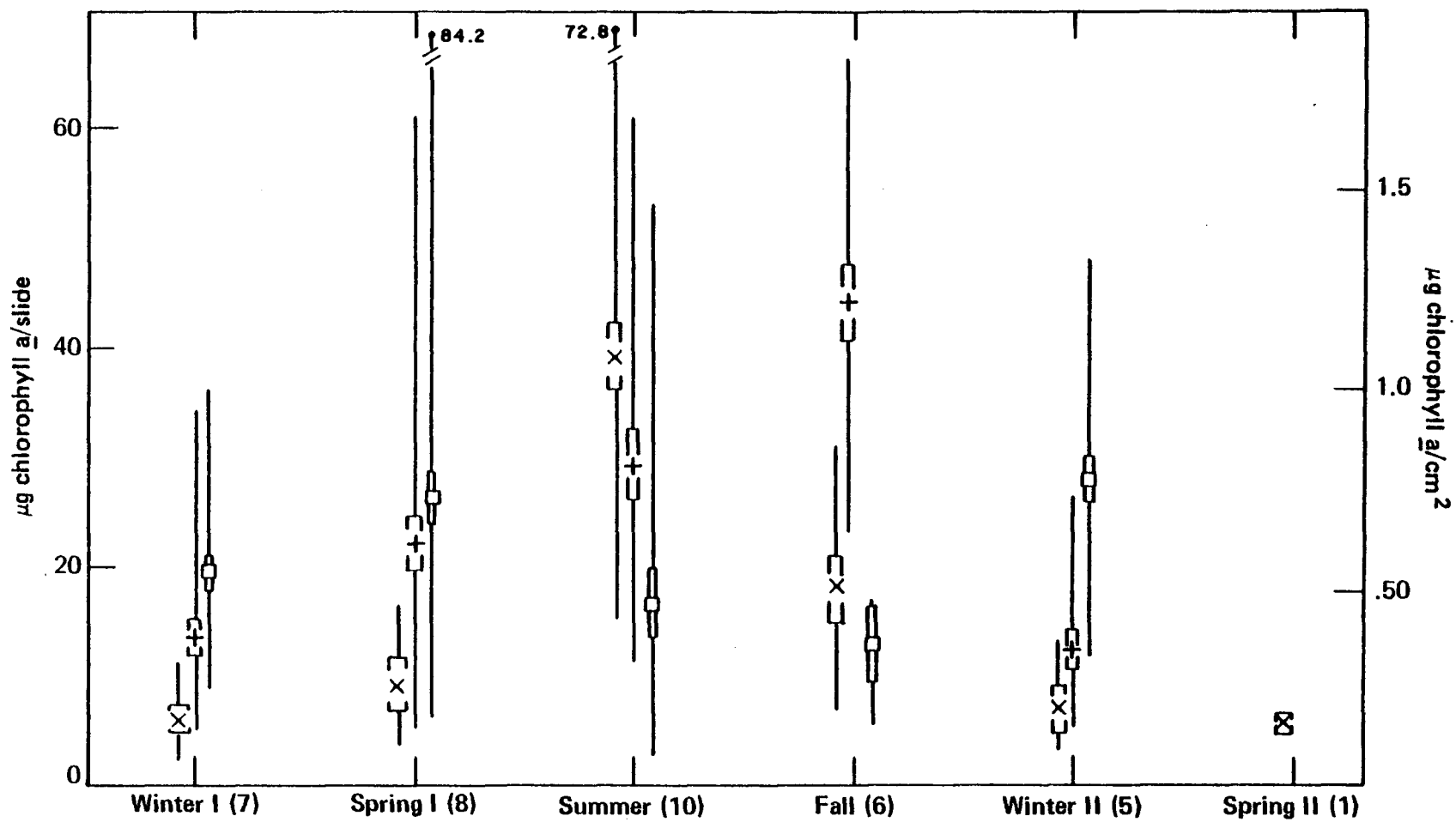


FIGURE 32. Seasonal summary of CHLA. Explanation as in FIG. 29. +0°C (X), +5°C (+), +12°C (□).

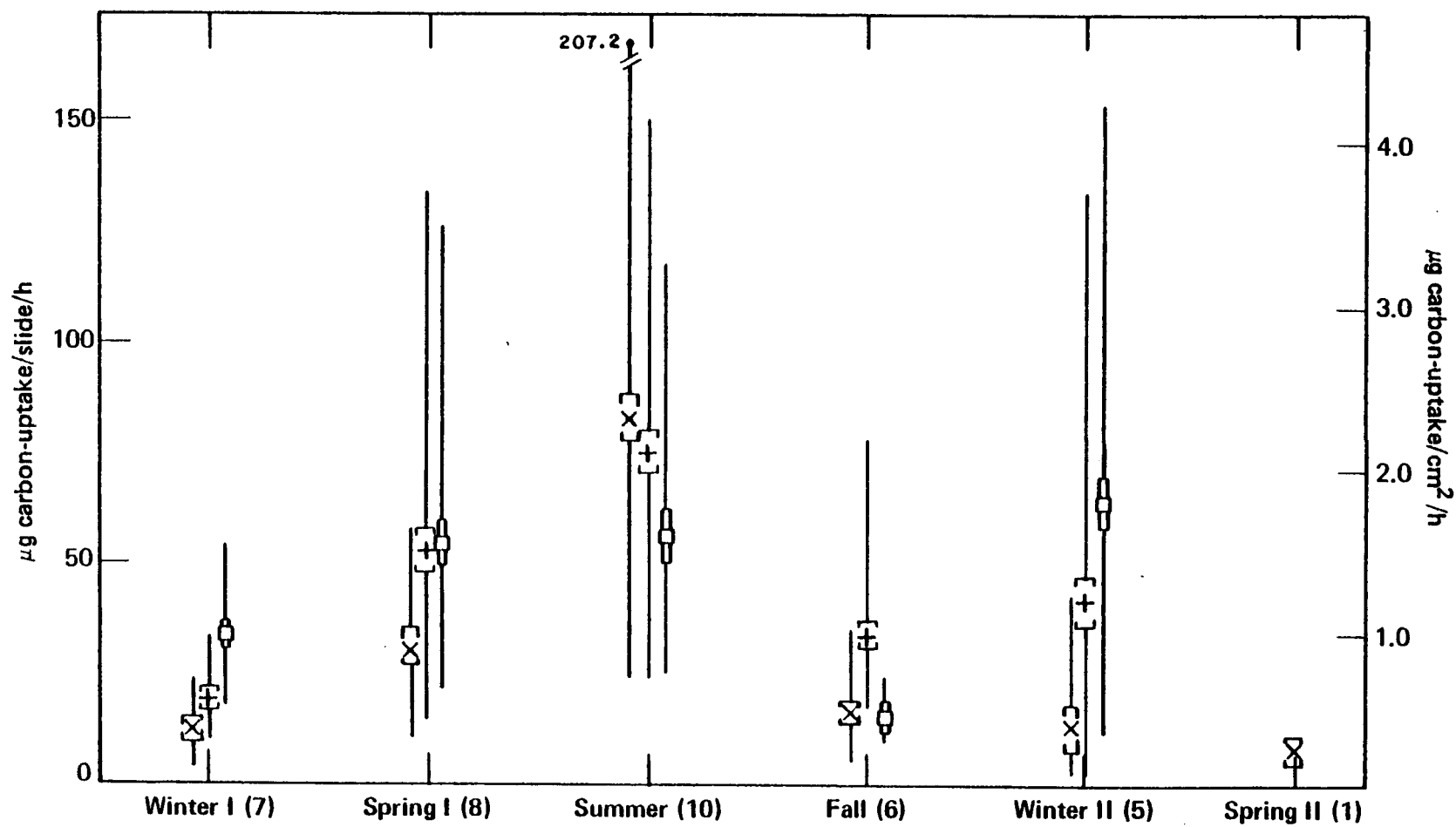


FIGURE 33. Seasonal summary of C-UP. Explanation as in FIG. 29. +0°C (X), +5°C (+), +12°C (□).

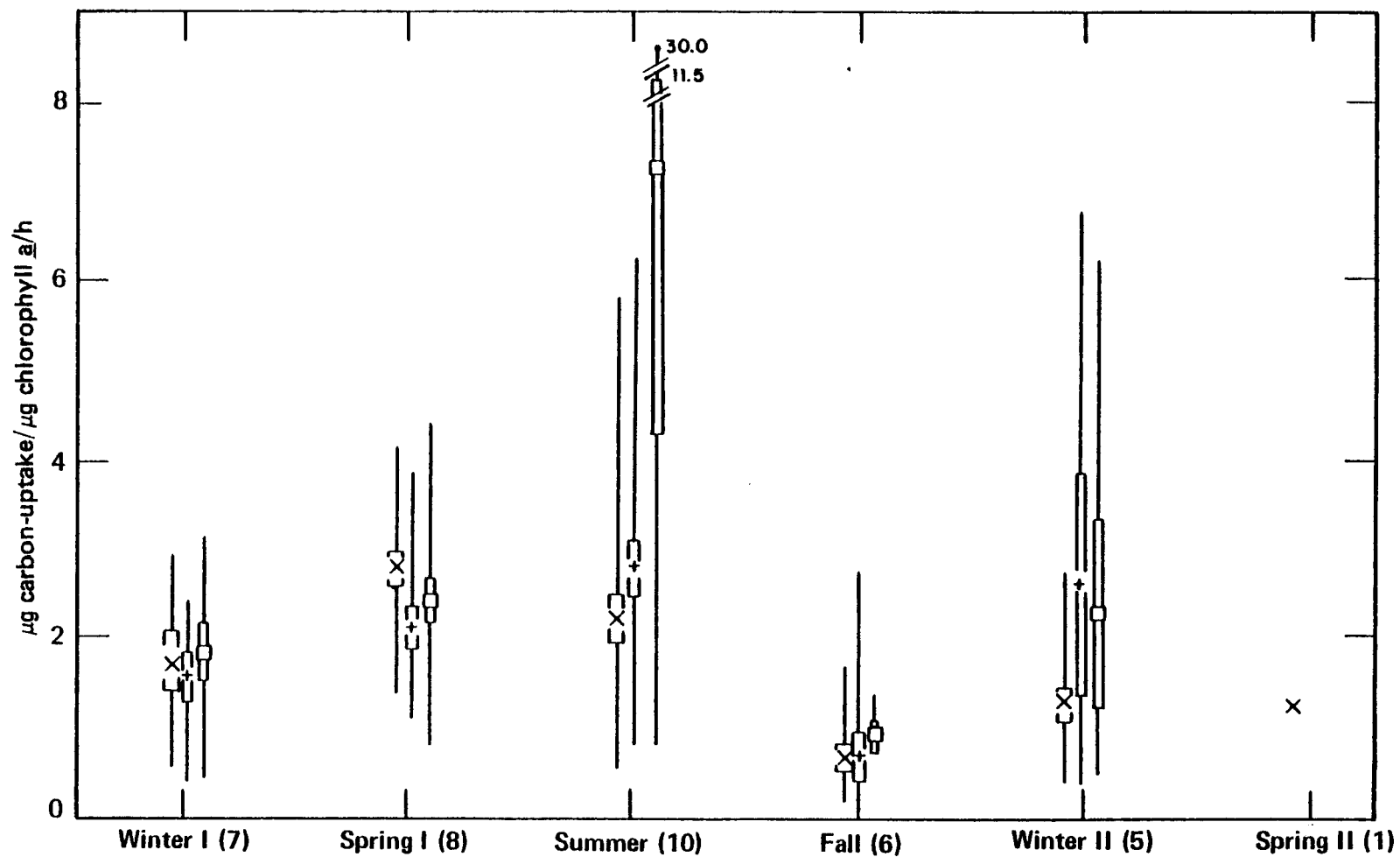


FIGURE 34. Seasonal summary of ASSIMNO. +0°C (X), +5°C (+), +12°C (□), means of experiment assimilation numbers. Rectangle is ± 1 standard deviation; vertical line is range of experimental ASSIMNO. Temperature treatments only.

TABLE 8

Friedman's Chi Square statistic, with rankings of treatments and probability levels, for seasonal values. Three treatments for all tests; b = number of blocks. P = probability range for significant ranking. Overscore denotes equality.

		AFDW	ORG	PROT	CHLA	C-UP	ASSIMNO
WINTER I		<u>+12>+5>+0</u>	<u>+0=+5>+12</u>		<u>+12>+5>+0</u>	<u>+12>+5>+0</u>	<u>+0=+5>+12</u>
b=7	X ² =	12.29	2.00		14.00	14.00	3.43
	P=	.005-.001	.95-.90		.001	.001	.25-.10
SPRING I		<u>+5>+12>+0</u>	<u>+12>+5>+0</u>		<u>+5>+12>+0</u>	<u>+5>+12>+0</u>	<u>+0>+12>+5</u>
b=8	X ² =	3.00	7.75		3.25	3.25	1.75
	P=	.25-.10	.025-.01		.25-.10	.25-.10	.50-.25
SUMMER		<u>+0>+5>+12</u>	<u>+0>+5=+12</u>	+0>+5>+12	<u>+0>+5>+12</u>	<u>+0>+5>+12</u>	<u>+12>+5>+0</u>
b=10	X ² =	8.60	.60		7.13	2.60	9.80
	P=	.025-.01	.50-.25		.05-.025	.50-.025	.01-.005
FALL		<u>+5>+12>+0</u>	<u>+0>+5>+12</u>	+5>+12>+0	<u>+5>+12>+0</u>	<u>+5>+0>+12</u>	<u>+12>0>+5</u>
b=6	X ² =	9.33	7.00		9.33	9.00	4.33
	P=	.01-.005	.01-.005		.01-.005	.025-.01	.25-.10
WINTER II		<u>+12>+5>+0</u>	<u>+5>+12>+0</u>	+12>+5=+0	<u>+12>+5>+0</u>	<u>+12>+5>+0</u>	<u>+12=+5>+0</u>
b=5	X ² =	10.00	9.00		8.40	10.00	1.20
	P=	.005-.001	.05-.01		.005-.001	.005-.001	.50-.25

Figure 35 summarizes a comparison of the two enriched treatments against the unenriched treatment, for the three temperature treatments, in AFDW, CHLA, C-UP, and ASSIMNO. Most enriched treatments were 20-60% below the reference values, although the differences are not significant at $\alpha = .05$ (ANOVA). The few positive differences occurred most often in the third sampling, when the ambient temperature had risen from 7.2°C to 10.7°C (+5°C = 15.4°C; +12°C = 22.6°C). Friedman's test on the ranks of means for all three samplings (AFDW, CHLA, and C-UP) within each temperature treatment revealed a significant ordering only for +12°C: reference > TE > CE.

The taxonomic composition of the Feb. assemblages also reflected primarily a temperature effect (Figure 36 (after Lamacraft, 1979); Table 14). The +0°C assemblages were 95% green coccoids. The +5°C assemblages consisted of fewer green coccoids, more Eunotia pectinalis and Gomphonema parvulum. The +12°C assemblages were 1/3 E. pectinalis, with substantial proportions of G. parvulum and Navicula cryptocephala. Friedman's ranking test on total cells/slide and on diatoms/slide shows a significant enrichment effect only for the diatoms of the +0°C assemblages. The trace element treatment was lower in diatoms/slide than the other two ambient temperature treatments - from three-fourths to one-half the number on the reference slides. Although the +0°C assemblages were lowest in AFDW and CHLA in Feb., they had consistently more (two to three times) total cells/slide than the other temperature treatments. The +12°C assemblages had more diatoms/slide than the ambient temperature treatment; the +5°C assemblages had as many or more diatoms/slide as the +0°C assemblages.

TABLE 9

AFDW data, FEBRUARY enrichment experiment. Means (\bar{X}) of individual troughs for each sampling: first sampling on first line, second sampling on second line, third sampling on third line for each temperature and nutrient treatment. Pooled \bar{X} (where indicated by 1X ANOVA) given within temperature treatment for each sampling. Below pooled \bar{X} , temperature treatments ranked from largest to smallest, left to right. Underscore denotes equality. Square root of 2X ANOVA error mean square ($\sqrt{\text{EMS}}$) given for each sampling. (N=nitrogen; P=phosphorus; TE=trace element).

	mg AFDW/slide		
	<u>+0°C</u>	<u>+5°C</u>	<u>+12°C</u>
REFERENCE	1.99	2.51	3.88
	2.36	2.71	3.77
	3.37	3.22	6.43
+TE	1.79	2.74	3.74
	2.48	2.95	3.78
	3.49	4.08	5.81
CE	1.97	2.13	2.74
	1.72	2.24	2.23
	2.74	3.90	3.81
Pooled \bar{X}	1.92	2.46	3.46
		<u>+12</u> <u>+5</u> <u>+0</u>	
	2.19	2.64	3.77 (T4,5) 2.23 (T6)
		<u>T4,5</u> <u>+5</u> <u>T6</u> <u>+0</u>	
	3.20	3.73	5.35
		<u>+12</u> <u>+5</u> <u>+0</u>	
$\sqrt{\text{EMS}}$		0.6879	
		0.6496	
		0.9678	

TABLE 10

Proportion organic, FEBRUARY enrichment experiment.
Explanation as in Table 9.

	AFDW/DW		
	<u>+0°C</u>	<u>+5°C</u>	<u>+12°C</u>
REFERENCE	0.3962	0.3510	0.4232
	0.3623	0.3806	0.3918
	0.3979	0.3531	0.3728
+TE	0.4155	0.4153	0.3838
	0.3767	0.3950	0.3974
	0.4055	0.4151	0.3736
CE	0.3988	0.3939	0.3760
	0.4019	0.3938	0.3622
	0.3601	0.4443	0.3736
Pooled \bar{X}		0.3949	
		0.3846	
		0.3884	
$\sqrt{\text{EMS}}$		0.050825	
		0.016370	
		0.041892	

TABLE 11

Chlorophyll a data, FEBRUARY enrichment experiment.
Explanation as in Table 9.

	ug Chlorophyll <u>a</u> /slide			
	<u>+0°C</u>	<u>+5°C</u>	<u>+12°C</u>	
REFERENCE	4.50	9.87	18.30	
	6.97	11.15	21.30	
	16.33	18.83	18.62	
+TE	3.85	7.56	12.10	
	7.05	9.63	14.00	
	10.08	14.52	44.47	
CE	6.46	8.20	15.18	
	9.16	11.53	15.77	
	9.45	14.03	29.56	
Pooled \bar{X}	4.94	8.54	15.20	
		<u>+12</u> <u>+5</u> <u>+0</u>		
	7.73	10.77	17.03	
		<u>+12</u> <u>+5</u> <u>+0</u>		
	11.95	15.79	44.47 (T5) 24.09 (T4,6)	
		<u>T5</u> <u>T4,6</u> <u>+5</u> <u>+0</u>		
$\sqrt{\text{EMS}}$		3.4193		
		4.2453		
		6.9552		

TABLE 12

Carbon-uptake rate data, FEBRUARY enrichment experiment.
Explanation as in Table 9.

	$\mu\text{g Carbon-uptake/slide/h}$			
	<u>+0°C</u>	<u>+5°C</u>	<u>+12°C</u>	
REFERENCE	11.94	20.17	27.41	
	11.17	17.91	38.16	
	28.76	38.79	56.82	
+TE	8.43	11.39	18.13	
	7.77	12.21	37.54	
	25.55	30.36	51.64	
CE	6.17	13.53	11.84	
	7.97	18.01	14.79	
	17.93	31.95	26.15	
Pooled \bar{X}	8.46	15.03		
		<u>T4</u> <u>T5</u> <u>+5</u> <u>T6</u> <u>+0</u>		
	8.97	16.05	37.85 (T4,5)	14.79 (T6)
		<u>T4,5</u> <u>+5</u> <u>T6</u> <u>+0</u>		
	24.08	33.70	54.23 (T4,5)	26.15 (T6)
		<u>T4,5</u> <u>+5</u> <u>T6</u> <u>+0</u>		
$\sqrt{\text{EMS}}$		2.9451		
		3.2384		
		6.0325		

TABLE 13

Assimilation numbers, FEBRUARY enrichment experiment. For each sampling and each temperature x nutrient treatment, ASSIMNO is average C-UP/average CHLA. Results of Friedman's nonparametric analysis over ASSIMNO within temperature treatments given below data. Other explanation as in Table 9.

	C-UP/CHLA		
	<u>+0°C</u>	<u>+5°C</u>	<u>+12°C</u>
REFERENCE	2.56	1.98	1.45
	1.49	1.55	1.71
	1.67	2.00	2.94
+TE	2.12	1.46	1.44
	1.03	1.23	2.54
	2.44	1.99	1.17
CE	0.92	1.61	0.76
	0.82	1.52	0.90
	1.84	2.19	0.86
X ²	2.00	4.67	4.67
Probability	0.50	.20	0.20

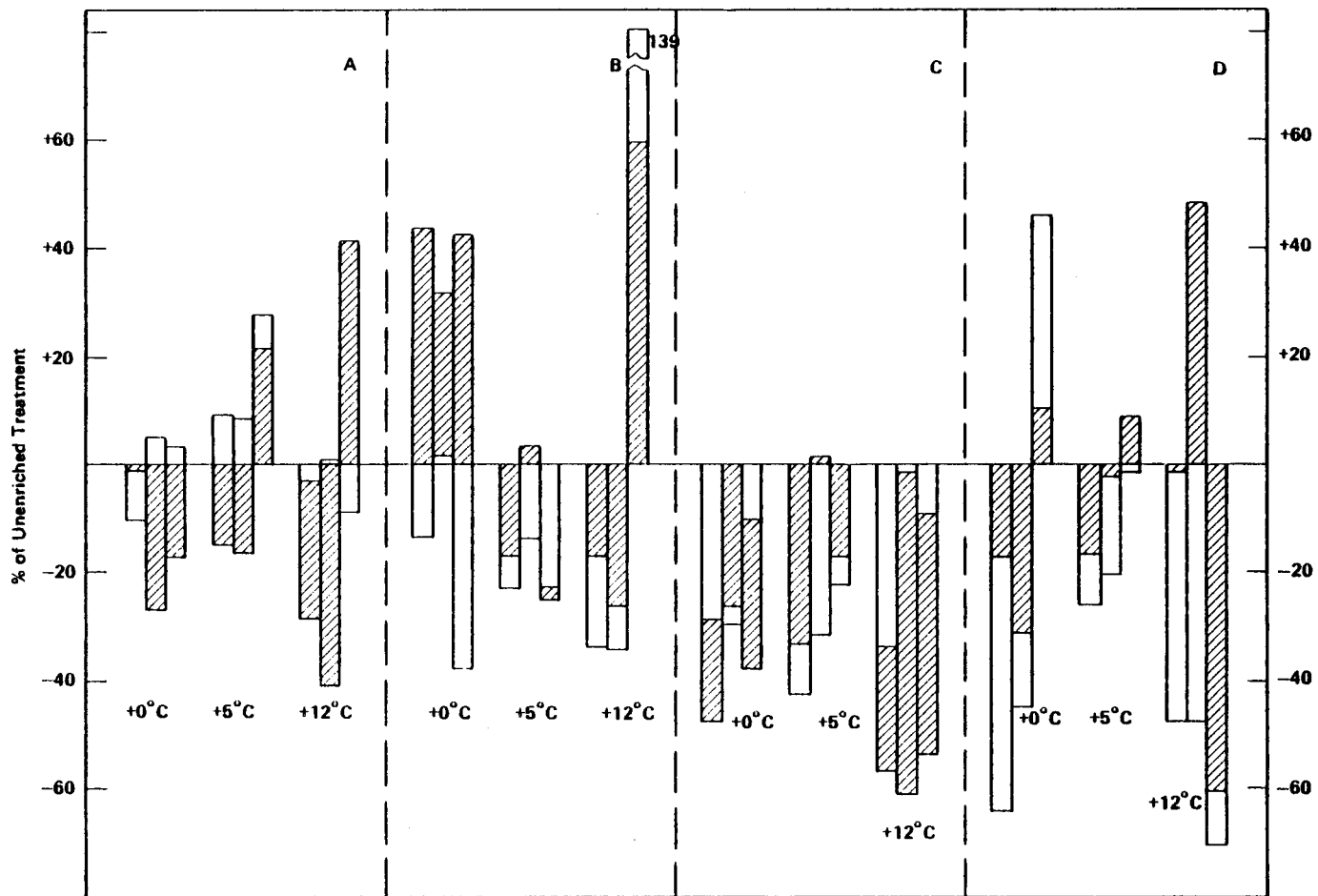


FIGURE 35. February enrichment experiment, treatments normalized to reference values for partial (open part of bar) and complete enrichment (hatched part of bar). Where both are negative or positive, end of each (open or hatched) bar is actual percent. Three bars at each temperature are 3 samplings per experiment, in order from left to right. A. AFDW B. CHLA C. C-UP D. ASSIMNO

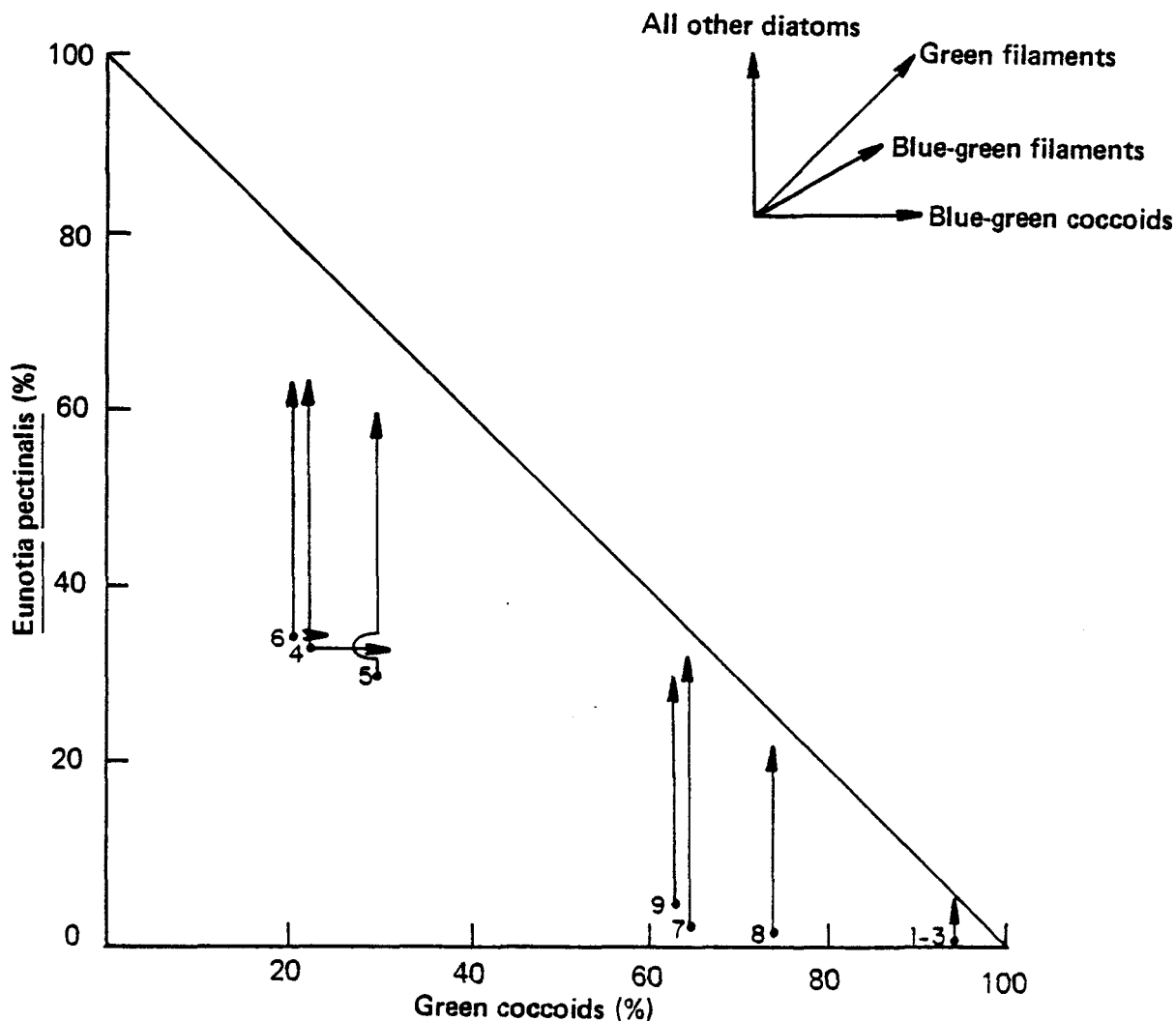


FIGURE 36. Taxonomic composition, February enrichment experiment. Treatments (identified by trough number-see TABLE 1) plotted by percent green coccoids (x-axis) and percent *Eunotia pectinalis* (y-axis). Arrows (on same scale as axes) indicate percent of other groups of algae; north, all other diatoms; east, blue-green coccoids; northeast, green filaments; east-northeast, blue-green filaments. (After Lamcraft, 1979)

TABLE 14

FEBRUARY enrichment experiment taxonomic data. Percent of type of algae present on treatment slides. Results of first sampling given on first line (for each trough and alga) or before slash; results of second sampling given on second line or after slash. Numbers are average % of three replicates; p = present in at least two of three replicates. Percent given (instead of p) when type is more than 5% of total in at least one sample.

	+0°C			+5°C			+12°C		
	T1	T2	T3	T7	T8	T9	T4	T5	T6
<u>Eunotia</u>	p/-	p/p	p/p	p	p	p	37.75	32.11	34.07
<u>pectinalis</u>				4.35	3.83	7.57	28.07	28.80	35.62
<u>Gomphonema</u>	4.56	p/p	p	8.39	4.37	4.78	18.92	6.90	6.60
<u>parvulum</u>	2.82		4.89	25.43	16.97	26.96	10.43	21.94	11.54
<u>Navicula</u>									
<u>capitata</u>		-/p				-/p	p/p	p/-	p/p
<u>N. cryptocephala</u>	p/-	-/p	p/-	p/p	p	p/p	8.08	8.29	8.23
					4.68		6.09	5.71	7.16
<u>Nitzschia</u> spp	p/p	p/p	p/p	p/p	p	p	6.02	6.43	6.91
					3.97	3.69	6.43	p	10.11
Other diatoms*	1	1		1,2,4	1,2,4	3,5	1,3,5	1,2,4,5	1,2,5
Total diatoms	7.92	2.78	3.70	19.29	11.27	14.73	73.21	55.01	58.18
	4.50	5.77	6.95	41.06	31.77	45.73	53.93	64.48	66.60
<u>Spirogyra</u> spp		p/-	p/-	p/p	p/p	p/-	p/p	p/p	-/p
<u>Stigeoclonium</u> spp	p/p	p/p	p/p	p/p	p/p	p/p	p/p	p/p	p/p
<u>Mougeotia</u> spp		p/-		p/p	p/p	p/-	p/p		p/p
<u>Schizothrix</u> sp	p/p	p/p	p/p	p/p	p/p	p/p	p/p	p/p	6.53/10.44
<u>Anabaena</u> spp	p/p	-/p		p/p	p/p	p/p	p/p	p/p	p/p
Blue-green coccoids		-/p	p/p	4.95	p/p	p/p	3.50	p/p	8.14
				p			15.06		p
Green coccoids	91.89	96.81	95.33	74.49	85.82	79.51	19.51	35.02	24.73
	93.85	93.16	92.40	55.59	63.82	48.97	26.14	26.68	18.13
Total cells	x = 3.854	3.877	4.6069	1.8156	1.5317	1.5325	1.5839	1.0432	1.4532
per	s = 1.0936	1.0685	2.1821	0.0719	0.1676	0.1750	0.2745	0.0995	0.2861
slide	x = 6.999	5.2063	7.1686	2.8581	2.5582	2.5715	2.4057	2.5135	1.9328
x10 ⁶	s = 1.6066	0.9216	1.1618	0.2049	0.3068	0.6824	1.0302	0.5253	0.5017

* Other diatoms: 1-Navicula aikenensis; 2-Achnanthes minutissima; 3-Tabellaria spp;
4-Frustulia spp; 5-Cymbella minuta.

In the April enrichment experiment, there was no apparent effect of added nutrients and little effect of temperature on AFDW (Table 15). Only in the third sampling did the +12°C assemblages have more (twice the) AFDW than the other two temperature treatments. Also in the third sampling, the enriched +0°C treatments had more AFDW than did the unenriched treatment, but the SNK failed to distinguish among them. The +0°C and +5°C enriched treatments had more ORGANIC than did their unenriched treatments, indicating less dry weight for the same amount of AFDW (Table 16). CHLA and C-UP showed temperature effect on the second and third samplings (+12°C >+5°C >+0°C; Tables 17 and 18). The complete enrichment treatment at +5°C was higher in CHLA and C-UP than the other two nutrient treatments in the first sampling, but this is probably upstream-downstream effect. The complete enrichment treatment at +0°C was higher in CHLA and C-UP than the other two treatments in the third sampling (not always distinguished by the SNK, due to large variances). ASSIMNO varied randomly with treatment (Table 19.)

A comparison of enriched treatments to unenriched (Figure 37) shows a definite effect of sampling sequence on the +0°C assemblages' response to nutrients. Ambient temperature during this experiment dipped from 18°C to 14.4°C, then rose to 17.2°C over the 8 days. The +5°C and +12°C assemblages also showed some change in their response to nutrient enrichment through the three samplings, although it was not a consistent increase as with the +0°C assemblages. The only significant ranking occurred for CHLA and C-UP, where the partial enrichment treatment was consistently lower than the other two nutrient treatments.

Taxonomic composition of the April slides (Figure 38; Table 20) showed more nutrient than temperature effect. All the unenriched

TABLE 15

AFDW Data, APRIL enrichment experiment. Means (\bar{X}) of individual troughs for each sampling: first sampling on first line, second sampling on second line, third sampling on third line for each temperature and nutrient treatment. Pooled \bar{X} (where indicated by 1X ANOVA) given within temperature treatment for each sampling. Below pooled \bar{X} , temperature treatments ranked from largest to smallest, left to right. Underscore denotes equality. Square root of 2X ANOVA error mean square ($\sqrt{\text{EMS}}$) given for each sampling. (N=nitrogen; P=phosphorus; TE=trace elements)

	mg AFDW/slide				
	<u>+0°C</u>	<u>+5°C</u>	<u>+12°C</u>		
REFERENCE	5.13	5.58	4.10		
	5.50	8.14	5.64		
	1.12	3.72	8.56		
+TE	2.99	4.41	5.17		
	6.61	7.96	7.52		
	2.18	5.16	12.49		
CE	3.89	4.17	3.70		
	5.38	6.68	7.31		
	5.83	6.03	13.03		
Pooled \bar{X}	4.35	4.35	4.35		
	6.75	6.75	6.75		
		4.97	11.36		
				<u>+12</u>	<u>T3</u>
				<u>+5</u>	<u>T2</u>
				<u>T1</u>	<u>T1</u>
$\sqrt{\text{EMS}}$.9720			
		1.5990			
		2.1972			

TABLE 16

Organic proportion, APRIL enrichment experiment. Explanation as in Table 15.

	AFDW/DW		
	<u>+0°C</u>	<u>+5°C</u>	<u>+12°C</u>
REFERENCE	0.3958 0.3952 0.3414	0.3740 0.3937 0.3314	0.4026 0.4443 0.3745
+TE	0.4311 0.4196 0.3124	0.4307 0.4413 0.3501	0.4036 0.4232 0.3372
CE	0.4478 0.4716 0.3518	0.4466 0.4500 0.3392	0.4001 0.4360 0.3786
Pooled \bar{X}	0.4394 (T2,3),	0.3958 0.4387 (T8,9)	0.3740 0.4021
	<u>T2,3</u>	<u>T8,9</u>	<u>+12</u> <u>T1</u> <u>T7</u>
	0.4456 (T8,9)	0.3937	0.4345
	<u>T3</u>	<u>T8,9</u>	<u>+12</u> <u>T2</u> <u>T1</u> <u>T7</u>
	0.3501,	0.3353 (T7,9)	0.3765 (T4,6) 0.3372
	<u>T4,6</u>	<u>T3</u> <u>T8</u>	<u>T1</u> <u>T5</u> <u>T7,9</u> <u>T2</u>
$\sqrt{\text{EMS}}$		0.018806 0.018962 0.022689	

TABLE 17

Chlorophyll a, APRIL enrichment experiment. Explanation as in Table 15.

	$\mu\text{g Chlorophyll } \underline{a}/\text{slide}$			
	<u>+0°C</u>		<u>+5°C</u>	<u>+12°C</u>
REFERENCE	7.92		6.14	10.08
	10.20		13.84	24.28
	4.00		12.59	41.25
+TE	7.43		6.09	5.21
	9.86		12.04	9.65
	5.20		12.77	30.01
CE	6.92		11.06	6.82
	9.68		12.34	16.46
	16.26		12.56	46.74
Pooled \bar{X}	7.42	11.06 (T9)	6.11 (T7,8)	7.37
		<u>T9</u>	<u>+0</u> <u>+12</u> <u>T7,8</u>	
	9.91		12.74	16.80
		<u>+12</u> <u>+5</u> <u>+0</u>		
	16.26 (T3)	4.60 (T1,2)	12.64	39.33
		<u>+12</u> <u>T3</u> <u>+5</u> <u>T1,2</u>		
$\sqrt{\text{EMS}}$			1.5874	
			4.3938	
			7.0384	

TABLE 18

Carbon-uptake rate, APRIL enrichment experiment. Explanation as in Table 15.

	$\mu\text{g Carbon-uptake/slide/h}$			
	<u>+0°C</u>		<u>+5°C</u>	<u>+12°C</u>
REFERENCE	27.46		15.75	25.40
	18.52		21.28	49.11
	9.84		27.65	70.06
+TE	11.84		15.05	20.02
	18.11		24.84	31.60
	13.98		39.80	68.00
CE	19.29		29.16	17.14
	18.29		23.81	37.75
	27.27		25.66	61.79
Pooled \bar{X}	19.53	15.40 (T7,8)	29.16 (T9)	20.85
		<u>T9</u>	<u>+12</u>	<u>+0</u>
			<u>T7,8</u>	
	18.31		23.31	39.49
		<u>+12</u>	<u>+5</u>	<u>+0</u>
	11.91 (T1,2)	27.27 (T3)		66.62
		39.80 (T8)	26.66 (T7,9)	
		<u>+12</u>	<u>T8</u>	<u>T3</u>
			<u>T7,9</u>	<u>T1,2</u>
$\sqrt{\text{EMS}}$			4.6302	
			6.3578	
			8.2350	

TABLE 19

Assimilation numbers, APRIL enrichment experiment. For each sampling and each temperature X nutrient treatment, ASSIMNO is average C-UP/average CHLA. Results of Friedman's nonparametric analysis over ASSIMNO within temperature treatments given below data. Other explanation as in Table 15.

	C-UP/CHLA		
	<u>+0°C</u>	<u>+5°C</u>	<u>+12°C</u>
REFERENCE	3.36 1.76 2.40	2.50 1.49 2.14	2.44 1.96 1.64
+TE	1.52 1.77 2.61	2.40 1.99 3.02	3.74 3.14 2.18
CE	2.69 1.82 1.62	2.56 1.86 1.98	2.44 2.21 1.27
Chi Square	0	.67	4.50
Probability	>.50	>.50	.50-.20

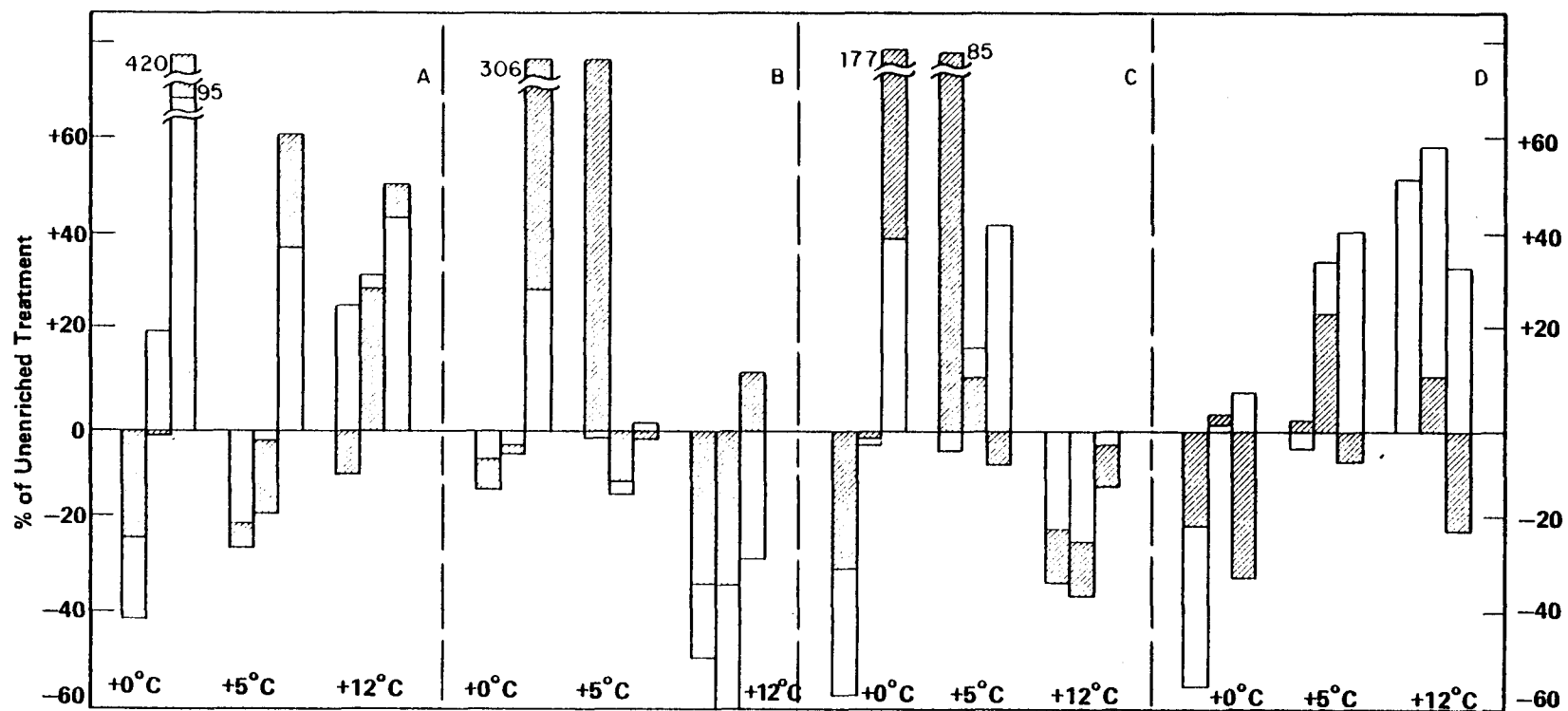


FIGURE 37. April enrichment experiments, treatments normalized to reference samples. Explanation as in FIG. 35. A. AFDW B. CHLA C. C-UP D. ASSIMNO

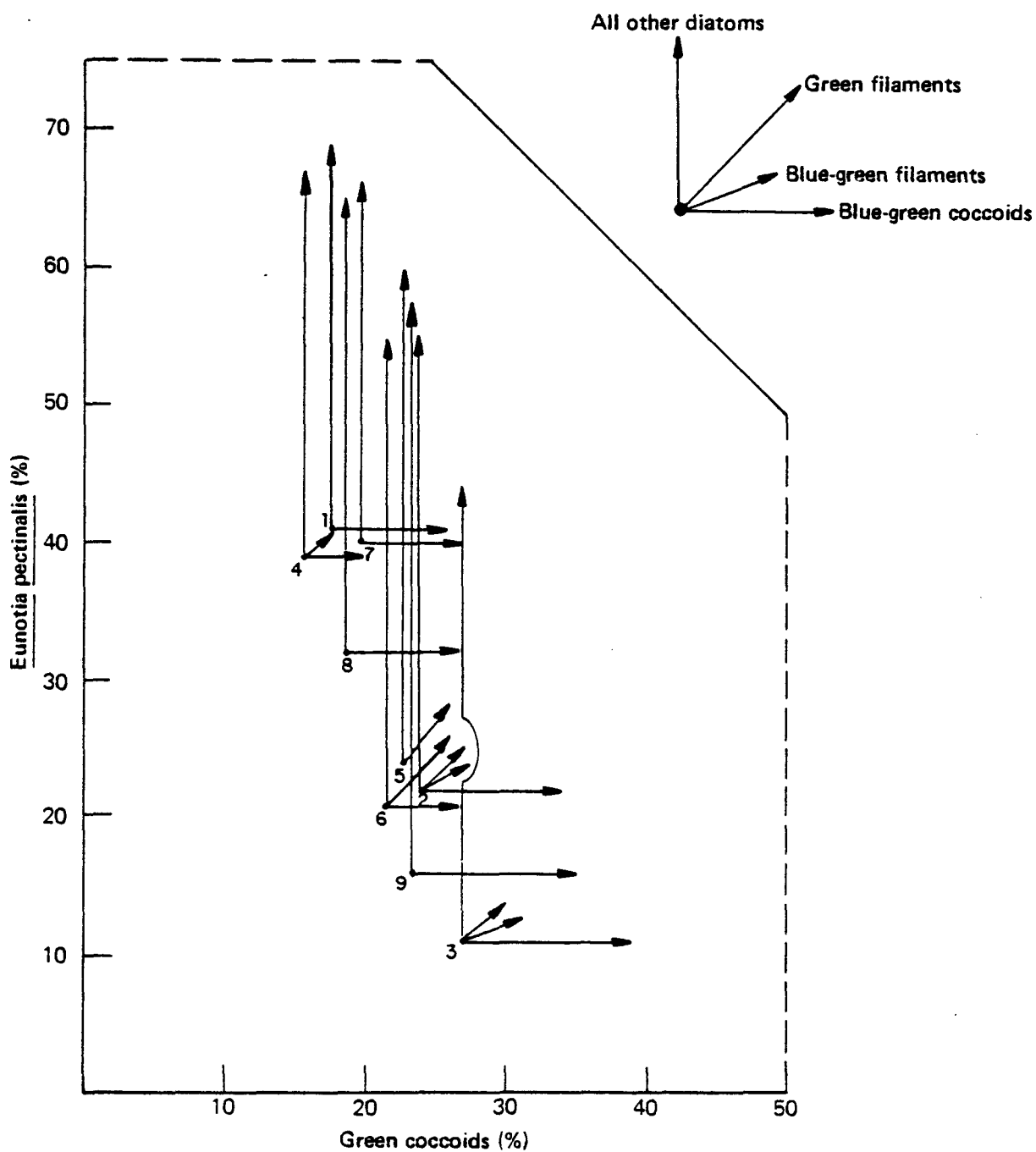


FIGURE 38. Taxonomic composition, April enrichment experiment. Explanation as in FIG. 36. (Triangle truncated to show detail.)

TABLE 20

APRIL enrichment experiment taxonomic data. Percent of type of algae present on treatment slides. Results of first sampling on first line (for each trough and algae) or before slash; results of second sampling on second line or after slash. Numbers are average % of three replicates; p = present in at least two of three replicates. Percent given (instead of p) when type is more than 5% of total in at least one replicate.

	+0°C			+5°C			+12°C		
	T1	T2	T3	T7	T8	T9	T4	T5	T6
<u>Eunotia</u>	38.52	13.33	10.21	21.96	19.20	14.78	43.34	18.98	22.97
<u>pectinalis</u>	44.94	32.25	13.73	60.47	46.53	17.75	35.01	29.69	21.17
<u>Gomphonema</u>	p	p	p/p	p/p	p/p	p/p	p/p	p/p	p
<u>parvulum</u>	14.52	3.83							3.33
<u>Navicula</u>	p/-	p/p	2.39	3.85	3.26	4.27	7.19	13.55	9.17
<u>capitata</u>			p	p	5.55	5.01	15.61	16.82	16.47
<u>N. cryptocephala</u>	9.63	9.25	9.22	10.88	13.34	11.69	5.48	5.74	5.79
	5.00	11.57	16.29	p	6.64	9.11	p	5.67	p
<u>Nitzschia</u> spp	7.36	9.76	9.19	11.32	14.31	19.76	8.14	6.90	7.89
	4.08	7.32	9.50	p	4.35	7.26	4.72	4.89	p
Other diatoms *	1-5	1-5	1-5	1-5	1-5	1-5	1-5	1-5	1-5
Total diatoms	64.39	46.44	46.28	57.53	58.19	61.15	69.68	51.11	53.34
	71.91	66.66	53.20	75.57	71.38	50.35	64.86	66.06	58.19
<u>Spirogyra</u> spp	p/p	p/p	p/p	p/p	p/p	p/p	p/p	p/p	p/p
<u>Stigeoclonium</u> spp	p/p	3.53	3.44	p/p	p/p	p/p	3.33	8.57	10.06
		p	p				p	4.50	5.58
<u>Mougeotia</u> spp			-/p				-/p	-/p	-/p
<u>Schizothrix</u> spp	p/p	4.51/p	4.85/p	p/p	p/p	p/p	p/p	p/p	p/p
<u>Anabaena</u> spp	p/p	p/p	p/p	p/p	p/p	p/p	p/p	p/p	p/p
Blue-green	9.62	12.09	15.05	9.52	9.49	10.93	p	p	4.13
coccolids	6.31	7.17	9.02	6.92	7.65	13.28	5.34	p	5.98
Green coccolids	19.16	29.40	26.32	24.09	24.56	19.36	17.31	31.49	25.85
	17.96	19.10	28.20	15.62	14.51	26.88	16.84	17.10	20.37
Total cells	x = 0.3428	0.3000	0.3177	0.4477	0.3410	0.4788	0.5908	0.3756	0.7105
per	s = 0.0128	0.1125	0.0248	0.3510	0.0551	0.0358	0.0810	0.1195	0.02181
slide	x = 0.5317	0.3565	0.4401	0.7730	0.6343	0.5442	0.8836	0.6681	1.1198
x10 ⁶	s = 0.1066	0.0612	0.0591	0.0517	0.1305	0.0508	0.1307	0.1091	0.1242

* Other diatoms are: 1-Navicula aikenensis; 2-Achnanthes minutissima; 3-Tabellaria spp; 4-Frustulia spp; 5-Cymbella minuta.

treatments were similar with ~20% green coccoids, 40% E. pectinalis, and 5-10% each N. cryptocephala and Nitzschia spp. The two enriched treatments at +0°C had slightly higher percentages of green and blue-green coccoids, lower percentages of E. pectinalis, than did the unenriched treatment. The +5°C and +12°C enriched treatments decreased in proportion of E. pectinalis relative to their reference treatments.

Total cells/slide were consistently increased by "complete" enrichment in the +12°C treatment. Total cells/slide decreased with enrichment at ambient temperature, although not significantly so. There were more total cells/slide in the +12°C treatment than in the other temperature treatments. The +5°C assemblages had as many or more cells/slide as the +0°C assemblages. Diatoms/slide were unaffected by enrichment; there were more at the higher temperature treatments than at ambient temperature.

In July, both temperature and nutrient effects were observed. The temperature treatments in all parameters were ranked as follows: +0°C > +5°C > +12°C (Tables 21-25). One or both enriched treatments at +12°C were significantly higher than the unenriched treatment in AFDW and ORGANIC (once), PROT and C-UP (twice), and CHLA (three times). At +5°C, the N + P treatment was lower than the other two enrichment treatments in AFDW and ORGANIC once and in C-UP twice; the high values for the unenriched treatment could be upstream-downstream effect. As the summary comparison of enriched to unenriched treatments (Figure 39) and Friedman's test show, the +12°C treatments were more often stimulated by nutrients, and to a greater extent, than were the other two temperature treatments ($X^2_T = 9.5$, prob. = .01-.005).

TABLE 21

AFDW data, JULY enrichment experiment. Means (\bar{X}) of individual troughs for each sampling: first sampling on first line, second sampling on second line, third sampling on third line for each temperature and nutrient treatment. Pooled \bar{X} (where indicated by 1X ANOVA) given within temperature treatment for each sampling. Below pooled \bar{X} , temperature treatments ranked from largest to smallest, left to right. Underscore denotes equality. Square root of 2X ANOVA error mean square ($\sqrt{\text{EMS}}$) given for each sampling. (N=nitrogen; P=phosphorus; TE=trace elements)

	mg AFDW/slide			
	<u>+0°C</u>	<u>+5°C</u>	<u>+12°C</u>	
REFERENCE	6.69	3.09	1.53	
	6.05	3.93	2.33	
	5.90	5.10	3.04	
+N+P	7.38	3.41	1.85	
	5.84	3.38	2.50	
	5.66	3.84	3.23	
CE	5.66	3.30	2.52	
	6.55	3.64	2.76	
	6.33	6.77	3.97	
Pooled \bar{X}	6.58	3.26	2.52 (T6)	1.68 (T4,5)
		<u>+0</u>	<u>+5</u>	<u>T6</u> <u>T4,5</u>
	6.14		3.65	2.53
		<u>+0</u>	<u>+5</u>	<u>+12</u>
	5.96	6.77 (T9)	4.47 (T7,8)	3.41
		<u>T9</u>	<u>+0</u>	<u>T7,8</u> <u>+12</u>
$\sqrt{\text{EMS}}$			0.8433	
			0.9748	
			0.6709	

TABLE 22

Organic proportion, JULY enrichment experiment. Explanation as in Table 21.

	AFDW/DW		
	<u>+0°C</u>	<u>+5°C</u>	<u>+12°C</u>
REFERENCE	0.4061 0.4255 0.4517	0.5119 0.4281 0.4640	0.4545 0.3756 0.3904
N+P	0.4377 0.4082 0.4288	0.4378 0.4168 0.4216	0.4710 0.3912 0.4674
CE	0.4076 0.3979 0.4314	0.4266 0.4002 0.4624	0.4573 0.4020 0.4753
Pooled X	0.4170	0.5119, 0.4322 (T8,9) <u>T7 +12 T8,9 +0</u>	0.4609
	0.4105	0.4150 <u>+5 +0 +12</u>	0.3896
	0.4517, 0.4301 (T2,3)	0.4714 (T5,6)	0.3904
		0.4632 (T7,9)	0.4216
	<u>T5,6 T7,9 T1</u>	<u>T2,3 T8</u>	<u>T4</u>
$\sqrt{\text{EMS}}$		0.034992 0.020307 0.016053	

TABLE 23

Protein data, JULY enrichment experiment. Explanation as in Table 21.

	mg protein/slide		
	<u>+0°C</u>	<u>+5°C</u>	<u>+12°C</u>
REFERENCE	4.65	1.58	0.95
	2.47	1.09	0.89
	3.06	1.85	0.90
N+P	4.39	1.92	0.93
	2.49	0.90	0.76
	2.57	1.88	0.94
CE	4.37	2.21	1.29
	3.77	1.12	0.68
	3.02	2.75	1.87
Pooled \bar{X}	4.47	1.90	1.29 (T6) 0.94 (T4,5)
		<u>+0</u> <u>+5</u> <u>T6</u> <u>T4,5</u>	
	2.91	1.04	0.72
		<u>+0</u> <u>+5</u> <u>+12</u>	
	2.88	2.16	1.87 (T6) 0.92 (T4,5)
		<u>+0</u> <u>+5</u> <u>T6</u> <u>T4,5</u>	
$\sqrt{\text{EMS}}$		0.6813	
		0.8006	
		0.5751	

TABLE 24.

Chlorophyll a, JULY enrichment experiment. Explanation as in Table 21.

	$\mu\text{Chlorophyll } \underline{a}/\text{slide}$				
	<u>+0°C</u>	<u>+5°C</u>	<u>+12°C</u>		
REFERENCE	52.57	26.00	5.79		
	21.63	13.88	7.12		
	49.71	25.13	5.95		
+N+P	44.34	15.58	7.46		
	29.24	10.13	14.55		
	52.44	15.97	12.10		
CE	54.65	28.11	10.39		
	25.23	12.67	22.56		
	57.06	26.31	21.22		
Pooled \bar{X}	50.52	23.23	6.62(T4,5)	10.39 (T6)	
		<u>+0</u>	<u>+5</u>	<u>T6</u>	<u>T4,5</u>
	25.37		12.22	7.12(T4)	18.56(T5,6)
		<u>+0</u>	<u>T5,6</u>	<u>+5</u>	<u>T4</u>
	53.06		22.47	21.22(T6)	9.02(T4,5)
		<u>+0</u>	<u>+5</u>	<u>T6</u>	<u>T4,5</u>
$\sqrt{\text{EMS}}$			9.8333		
			5.1745		
			5.4172		

TABLE 25

Carbon-uptake rate data, JULY enrichment experiment. Explanation as in Table 21.

	$\mu\text{g Carbon-uptake/slide/h}$						
	<u>+0°C</u>	<u>+5°C</u>	<u>+12°C</u>				
REFERENCE	207.18	150.95	112.40				
	34.15	34.61	26.98				
	45.92	42.76	20.11				
+N+P	98.13	79.57	71.54				
	38.77	27.00	39.57				
	34.92	28.63	27.21				
CE	112.02	94.68	65.16				
	37.53	28.26	42.48				
	33.65	42.22	39.08				
Pooled \bar{x}	207.18 (T1)	105.07 (T2,3)	112.40 (T4)	68.35 (T5,6)			
		150.95 (T7)	87.13 (T8,9)				
	<u>T1</u>	<u>T7</u>	<u>T4</u>	<u>T2,3</u>	<u>T8,9</u>	<u>T5,6</u>	
	36.82		29.96		41.03 (T5,6)	26.98 (T4)	
			<u>T5,6</u>	<u>+0</u>	<u>+5</u>	<u>T4</u>	
	38.16				39.08(T6)	23.66(T4,5)	
			28.63(T8)		42.48(T7,9)		
			<u>T7,9</u>	<u>T6</u>	<u>+0</u>	<u>T8</u>	<u>T4,5</u>
$\sqrt{\text{EMS}}$			17.3365				
			6.6777				
			5.9598				

TABLE 26

Assimilation numbers, JULY enrichment experiment. For each sampling and each temperature X nutrient treatment, ASSIMNO is average C-UP/average CHLA. Results of Friedman's nonparametric analysis over ASSIMNO within temperature treatments given below data. Other explanation as in Table 21.

	C-UP/CHLA		
	<u>+0°C</u>	<u>+5°C</u>	<u>+12°C</u>
REFERENCE	3.94	5.81	19.42
	1.58	2.49	3.79
	0.92	1.70	3.38
N+P	2.21	5.11	9.59
	1.32	2.67	2.72
	0.66	1.79	2.25
CE	2.05	3.37	6.27
	1.49	2.23	1.88
	0.59	1.60	1.84
χ^2	4.67	4.67	6.00
Probability	0.20	0.20	0.05

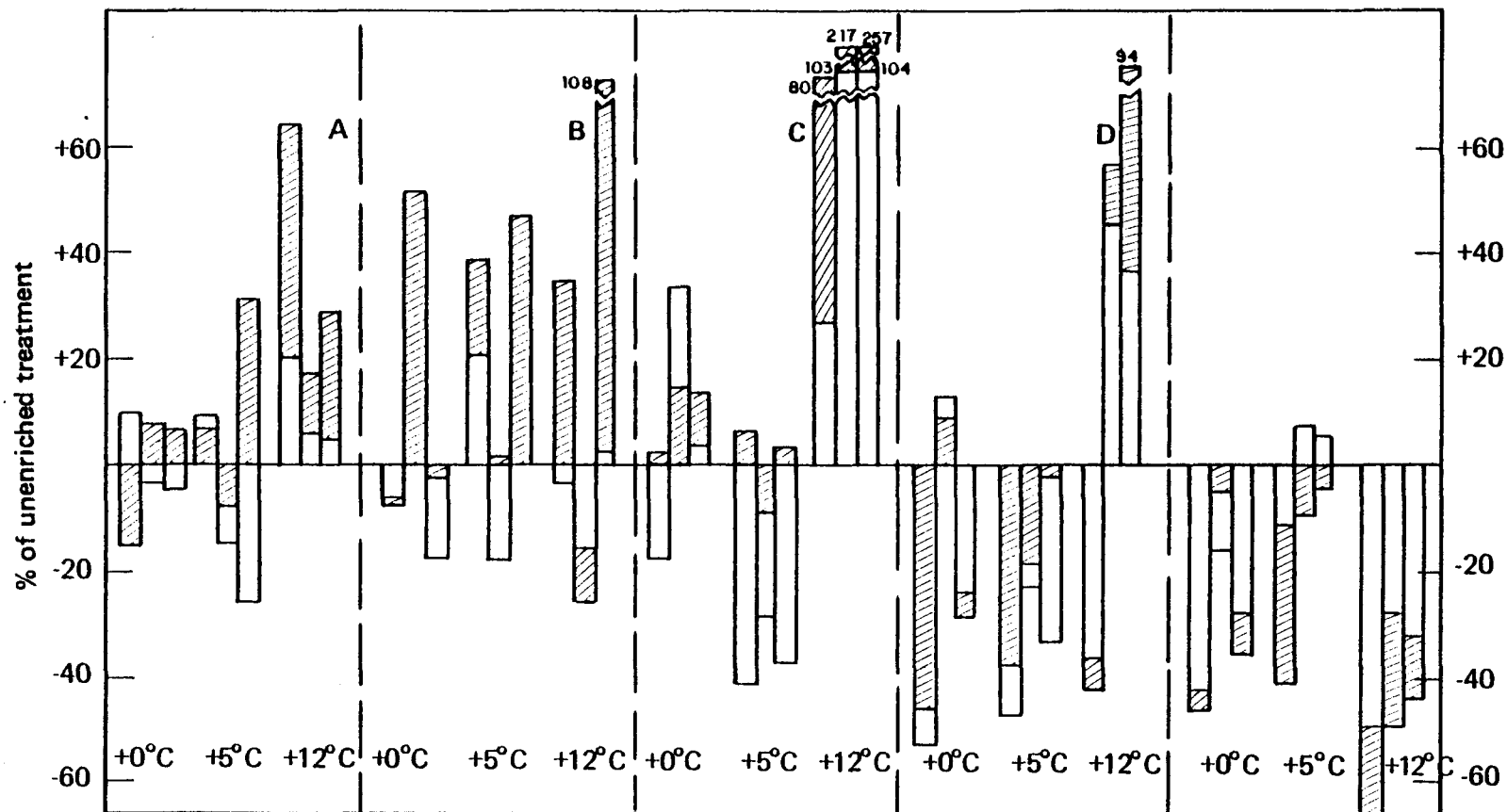


FIGURE 39. July enrichment experiment, treatments normalized to reference samples. Explanation as in FIG. 35. A. AFDW B. PROT
 C. CHLA D. C-UP E. ASSIMNO

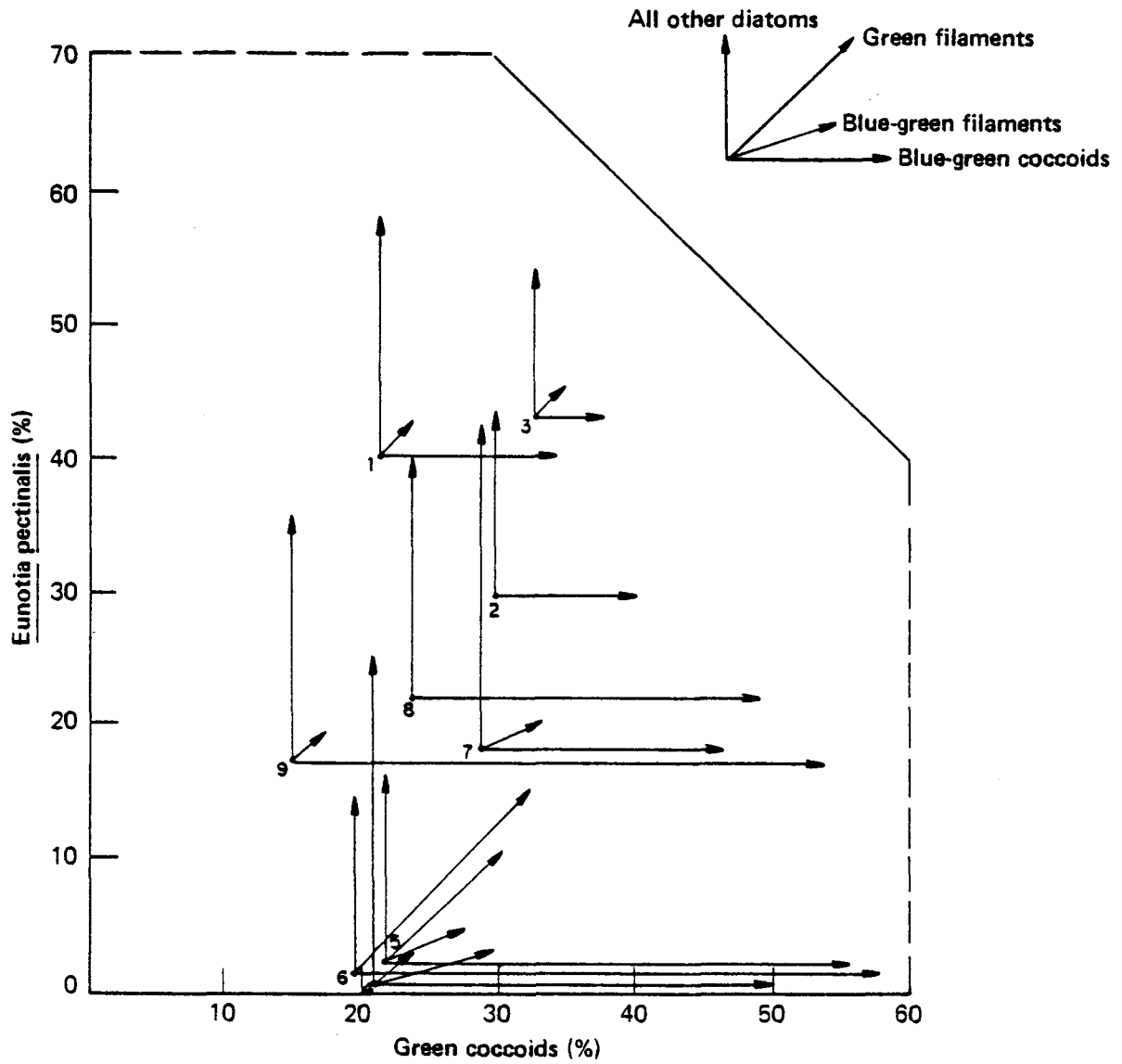


FIGURE 40. Taxonomic composition, July enrichment experiment. Explanation as in FIG. 36. (Triangle truncated to show detail.)

TABLE 27

JULY enrichment experiment taxonomic data. Percent of type of algae present on treatment slides. Results of first sampling on first line (for each trough and algae) or before slash; results of second sampling on second line or after slash. Numbers are average % of three replicates; p = present in at least two of the three replicates. Percent given (instead of p) when type is more than 5% of total in at least one replicate.

	+0°C			+5°C			+12°C		
	T1	T2	T3	T7	T8	T9	T4	T5	T6
<u>Eunotia</u>	36.86	24.24	30.71	14.33	17.70	15.52	p	p	p
<u>pectinalis</u>	43.82	35.37	55.23	22.56	27.40	19.35	p	p	p
<u>Gomphonema</u>	p/11.28	p/p	4.27/5.63	p/4.17	p/p	p/p			p/-
<u>parvulum</u>									
<u>Navicula</u>	p/p	p/p	p/p	12.07	6.06	7.59	p/p	p/-	p/p
<u>capitata</u>				5.72	6.20	7.65			
<u>N. cryptocephala</u>	2.82/p	p/p	p/p	4.27/p	p/p	p/p	p/p	p/p	
<u>Nitzschia</u> spp	6.72/p	p/p	p/p	p/p	p/p	p/p	12.52/p	p/p	p/-
<u>Achnanthes</u>	p/p	p/p	p/p	p/p	3.97	5.32	21.28	16.73	15.08
<u>minutissima</u>					3.12	p	10.96	8.80	4.40
Other diatoms*	1,3,4,5	1,4,5	1,4	1,4,5	1,4,5	1,4,5	4		
Total diatoms	54.18	37.53	42.50	41.34	37.81	35.30	39.67	21.24	20.79
	63.50	46.49	66.12	44.21	42.66	36.73	14.78	10.56	5.58
<u>Spirogyra</u> spp	p/p	p/p	p/p	p/p	p/p	p/p	p/-		p/p
<u>Stigeoclonium</u> spp	7.24	p	4.02	p	p	2.61	8.01	13.49	19.23
	p	p	p	p	p	3.83	p	10.83	19.20
<u>Mougeotia</u> spp	p/	p/p	p/p			p/p	p/-	p/-	
<u>Schizothrix</u> spp	p/p	p/p	p/p	p/p	p/p	p/p	7.65/p	5.15/3.64	p/p
<u>Anabaena</u> spp	p/p	p/p	p/p	p/4.81	p/p	p/p	p/14.13	p/4.50	p/p
Blue-green	13.02	12.84	7.32	26.24	32.98	45.32	20.47	24.17	26.22
coccolids	12.70	8.49	p	8.43	18.62	33.56	37.41	46.20	51.19
Green coccolids	22.91	41.33	43.00	23.00	19.71	10.58	17.24	24.06	22.36
	19.93	21.20	24.14	37.12	29.92	20.84	24.60	19.43	18.46
Total cells x =	1.1434	1.5301	2.4544	0.4194	0.4200	0.9029	0.6838	0.936	1.9309
per s =	0.2960	0.2924	0.6123	0.0174	0.1015	0.0165	0.0819	0.1695	0.4146
slide x =	1.0224	1.2022	1.2309	0.5411	0.8050	1.2068	1.2211	2.0321	2.9467
x10 ⁶ s =	0.3453	0.2610	0.1285	0.0841	0.0556	0.3988	0.1916	0.2330	0.4536

* Other diatoms: 1-Navicula aikenensis; 2-Achnanthes minutissima; 3-Tabellaria spp; 4-Frustulia spp; 5-Cymbella minuta.

The taxonomic composition of the July slides (Figure 40; Table 27) showed temperature and nutrient effects. At +0°C, the assemblages consisted of ~20% green coccoids, 40% E. pectinalis, and 12% bluegreen coccoids. Navicula capitata appeared in significant proportions (5-12%) at +5°C, and a small Achnanthes (minutissima) appeared above 5% in some samples. At +12°C, E. pectinalis was less than 5% of all samples and green coccoids were ~20%. A. minutissima was most prevalent at +12°C, in unenriched treatments. Blue-green coccoids and Stigeoclonium spp. increased with enrichment (28% to 39%, and 8% to 19%, respectively). Total cells/slide increased with enrichment at +5°C and +12°C. Diatoms/slide were increased by enrichment only at +5°C. The +0°C temperature treatments had the most cells and diatoms/slide, +5°C the fewest.

In the Oct. enrichment experiment, the overall temperature effect was +5°C > +12°C > +0°C (Tables 28-33). For AFDW and PROT, the "complete" enrichment at +12°C was higher than the other two enrichment treatments. The enriched treatments at +0°C and +5°C had low AFDW values, relative to their unenriched treatments, in the third sampling; this could be due to upstream-downstream effect. CHLA showed several differences: the total enrichment treatments at +12°C and +5°C were lower than the other nutrient treatments twice, and both enriched treatments at +0°C were lower than the unenriched treatment. ORGANIC was higher in the +0°C and +5°C unenriched treatments than in the enriched treatments twice. C-UP showed only temperature effect, except in the second sampling when the unenriched treatment at +5°C was higher than the enriched treatments.

The summary of enrichment effects (Figure 41) and Friedman's test show most of the enrichment effect to be negative. The consistency of

TABLE 28

AFDW data, OCTOBER enrichment experiment. Means (\bar{X}) of individual troughs for each sampling; first sampling on first line, second sampling on second line, third sampling on third line for each temperature and nutrient treatment. Pooled \bar{X} (where indicated by 1X ANOVA) given within temperature treatments for each sampling. Below pooled \bar{X} , temperature treatments ranked from largest to smallest, left to right. Underscore denotes equality. Square root of 2X ANOVA error mean square ($\sqrt{\text{EMS}}$) given for each sampling. (N=nitrogen; P=phosphorus; TE=trace element)

	mg AFDW/slide		
	<u>+0°C</u>	<u>+5°C</u>	<u>+12°C</u>
REFERENCE	1.47	3.06	2.24
	1.76	3.85	3.88
	2.69	4.56	3.87
+N+P	1.33	2.70	2.69
	1.53	2.82	1.95
	1.43	3.03	3.31
CE	1.44	3.27	3.80
	1.65	5.20	4.96
	1.89	5.02	5.27
Pooled \bar{X}	1.41	3.01	2.46(T4,5) 3.80(T6)
		<u>T6</u> <u>+5</u> <u>T4,5</u> <u>+0</u>	
	1.65	3.96	3.60
		<u>+5</u> <u>+12</u> <u>+0</u>	
	2.69(T1)	1.66 (T2,3)	3.03(T8) 4.79(T7,9) 4.15
		<u>T7,9</u> <u>+12</u> <u>T8</u> <u>T1</u> <u>T2,3</u>	
$\sqrt{\text{EMS}}$		0.3846	
		1.2179	
		1.0977	

TABLE 29

Organic proportion, OCTOBER enrichment experiment. Explanation as in Table 28.

	AFDW/DW						
	<u>+0°C</u>		<u>+5°C</u>		<u>+12°C</u>		
REFERENCE	0.5502		0.5162		0.4464		
	0.4341		0.5578		0.4262		
	0.5388		0.5136		0.4206		
N+P	0.4740		0.4742		0.4532		
	0.4692		0.4711		0.4357		
	0.3754		0.4848		0.4172		
CE	0.4883		0.4296		0.4242		
	0.4362		0.4317		0.4742		
	0.4131		0.4279		0.4008		
Pooled \bar{X}	0.5502, 0.4811 (T2,3)				0.4413		
		<u>T1</u>	<u>T7</u>	<u>T2,3</u>	<u>T8</u>	<u>+12</u>	<u>T9</u>
		0.4596		0.4596		0.4596	
	0.5388, 0.3942 (T2,3)					0.4129	
				0.4993 (T7,8)	0.4279		
		<u>T1</u>	<u>T7,8</u>	<u>T9</u>	<u>+12</u>	<u>T2,3</u>	
$\sqrt{\text{EMS}}$				0.023988			
				0.055027			
				0.037098			

TABLE 30

Protein data, OCTOBER enrichment experiment. Explanation as in Table 28.

	mg protein/slide		
	<u>+0°C</u>	<u>+5°C</u>	<u>+12°C</u>
REFERENCE	0.68	0.04	0.79
	0.84	2.47	1.57
	2.01	2.01	1.98
+N+P	0.50	0.86	1.19
	0.69	1.50	1.50
	1.04	2.17	1.75
CE	0.70	1.45	1.68
	0.98	1.52	1.89
	1.18	1.98	1.29
Pooled \bar{X}	0.62	1.12	
		<u>T6</u> <u>T5</u> <u>+5</u> <u>T4</u> <u>+0</u>	
	0.83	1.83	1.65
		<u>+5</u> <u>+12</u> <u>+0</u>	
	1.41	2.05	1.67
		<u>+5</u> <u>+12</u> <u>+0</u>	
$\sqrt{\text{EMS}}$		0.2173	
		0.3724	
		0.4968	

TABLE 31

Chlorophyll a data, OCTOBER enrichment experiment. Explanation as in TABLE 28.

	<u>µg Chlorophyll a/slide</u>					
	<u>+0°C</u>		<u>+5°C</u>		<u>+12°C</u>	
REFERENCE	12.55		38.96		13.64	
	10.51		54.42		12.36	
	15.82		52.20		5.29	
+N+P	9.14		41.75		14.11	
	7.12		23.42		10.96	
	7.04		17.61		9.65	
CE	11.56		16.99		5.64	
	6.25		10.42		5.75	
	5.74		13.26		5.13	
Pooled X	11.50	16.99 (T9)	40.35	5.64 (T6)	13.87	
		<u>T7,8</u>	<u>T9</u>	<u>T4,5</u>	<u>+0</u>	<u>T6</u>
	7.96				5.75 (T6)	11.67
		<u>T7</u>	<u>T8</u>	<u>T4,5</u>	<u>T9</u>	<u>+0</u>
	15.83 (T1)	6.39	52.20 (T7)	15.44	6.69	
			<u>T7</u>	<u>T1</u>	<u>T8,9</u>	<u>+12</u>
						<u>T2,3</u>
$\sqrt{\text{EMS}}$			5.6993			
			3.2823			
			5.4883			

TABLE 32

Carbon-uptake data, OCTOBER enrichment experiment. Explanation as in TABLE 28.

	$\mu\text{g Carbon-uptake/slide/h}$		
	<u>+0°C</u>	<u>+5°C</u>	<u>+12°C</u>
REFERENCE	11.60	18.58	13.52
	4.54	23.83	13.24
	10.87	23.87	5.62
+N+P	6.00	19.45	14.75
	4.52	10.78	14.54
	4.78	15.70	6.99
CE	6.98	18.03	8.97
	6.70	15.53	9.06
	4.08	11.81	4.33
Pooled \bar{X}	8.19	18.69	12.41
		<u>+5</u> <u>+12</u> <u>+0</u>	
	5.25	23.83 (T7) 13.16	12.28
		<u>T7</u> <u>T8,9</u> <u>+12</u> <u>+0</u>	
	6.58	17.12	5.65
		<u>+5</u> <u>+0</u> <u>+12</u>	
$\sqrt{\text{EMS}}$		2.7621	
		3.7042	
		5.3958	

TABLE 33

Assimilation numbers, OCTOBER enrichment experiment. For each sampling and each temperature X nutrient treatment, ASSIMNO is average C-UP/average CHLA. Results of Friedman's nonparametric analysis over ASSIMNO within temperature treatments given below data. Other explanation as in Table 28.

	C-UP/CHLA		
	<u>+0°C</u>	<u>+5°C</u>	<u>+12°C</u>
REFERENCE	0.79	0.46	0.91
	0.40	0.42	1.00
	0.64	0.43	0.90
+N+P	0.61	0.44	0.96
	0.59	0.44	1.23
	0.61	0.83	0.65
CE	0.56	0.99	1.38
	0.98	1.38	1.43
	0.61	0.82	0.71
χ^2	0.5	2.67	2.00
Probability	>.50	>.50	>.50

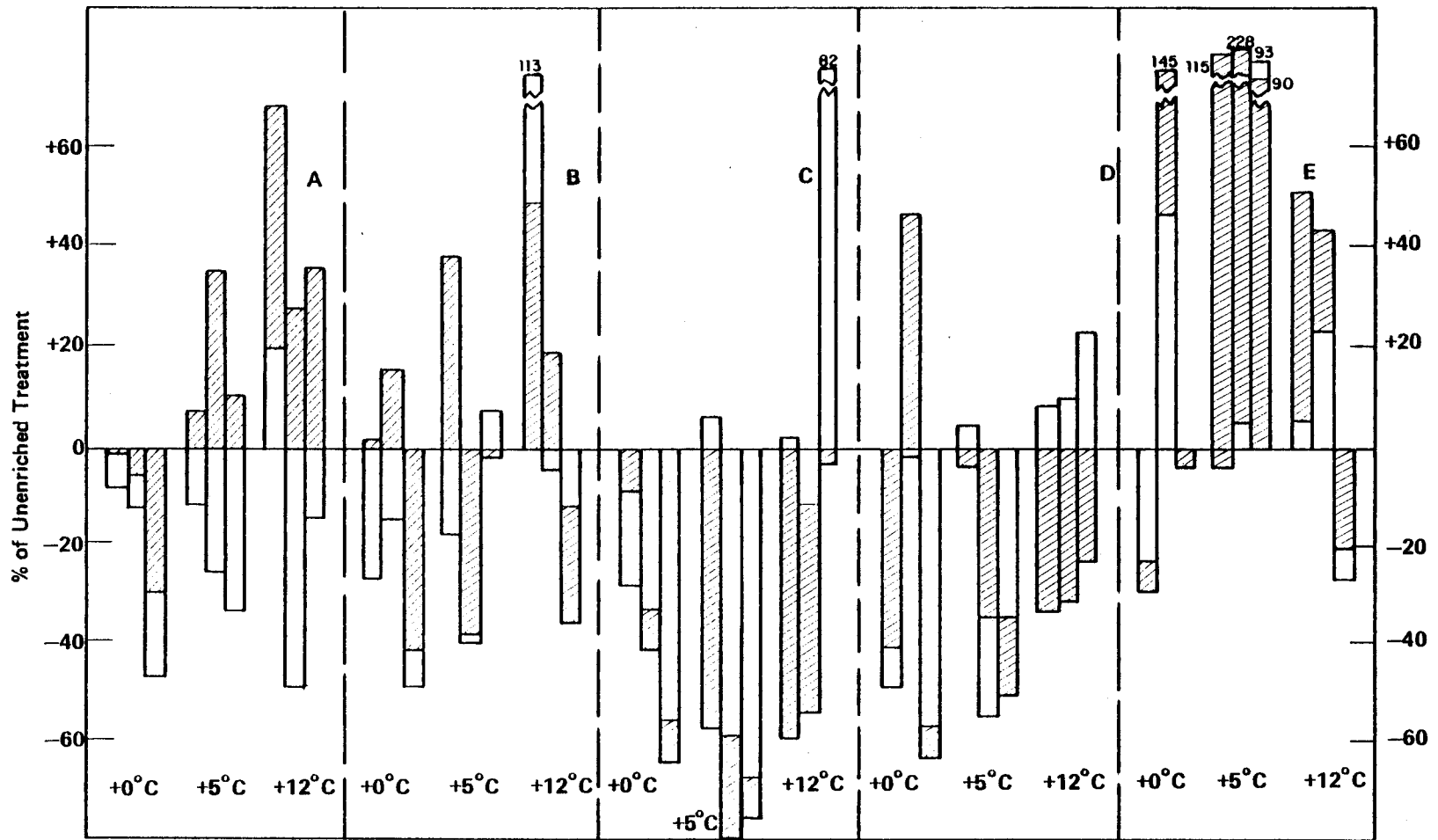


FIGURE 41. October enrichment experiment, treatments normalized to reference samples. Explanation as in FIG. 35. A. AFDW B. PROT C. CHLA D. C-UP E. ASSIMNO

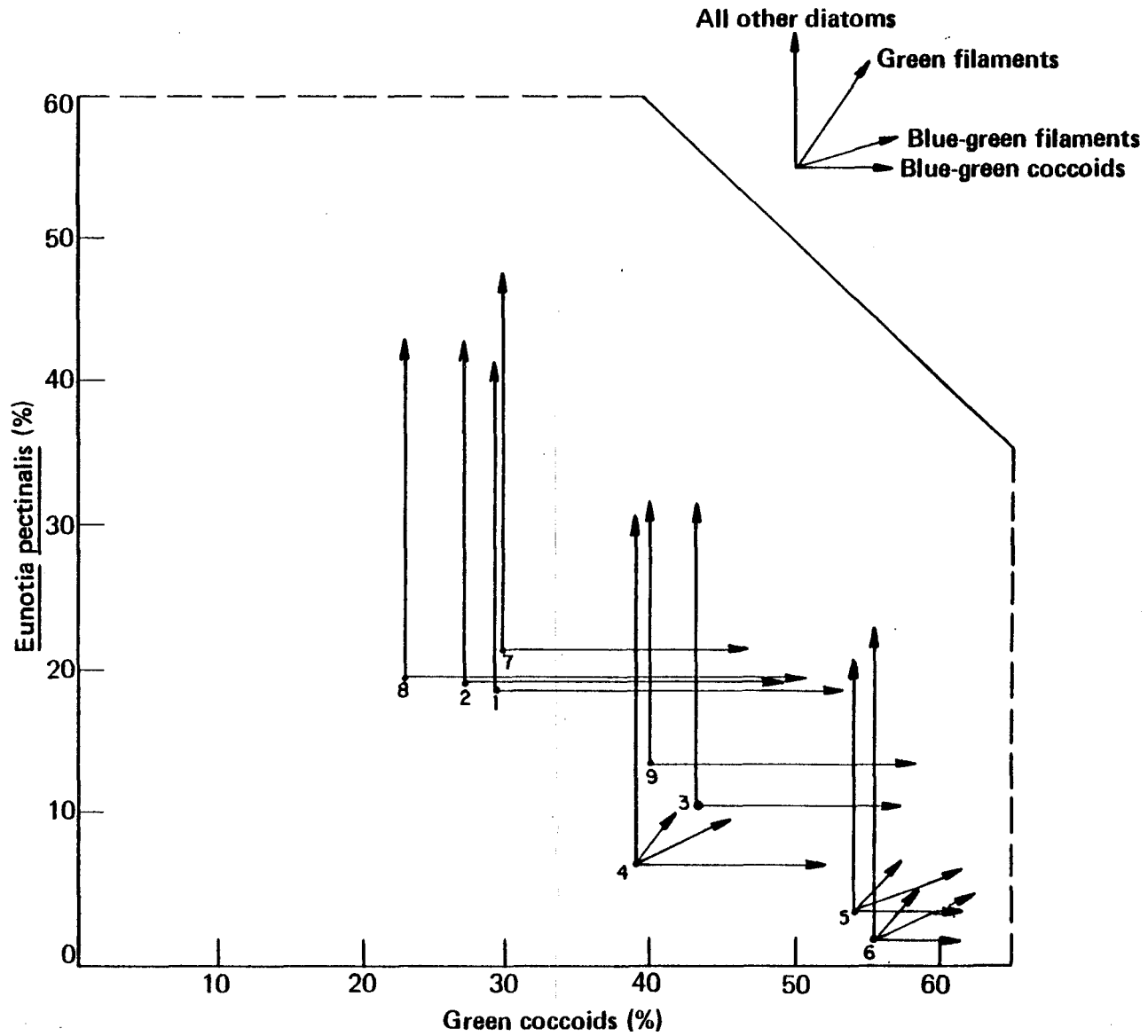


FIGURE 42. Taxonomic composition, October enrichment experiment. Explanation as in FIG. 36. (Triangle truncated to show detail.)

TABLE 34

OCTOBER enrichment experiment taxonomic data. Percent of type of algae present on treatment slides. Results of first sampling on first line (for each trough and algae) or before slash; results of second sampling on second line or after slash. Numbers are average % of three replicates; p = present in at least two of the three replicates. Percent given (instead of p) when type is more than 5% of total in at least one replicate.

	+0°C			+5°C			+12°C		
	T1	T2	T3	T7	T8	T9	T4	T5	T6
<u>Eunocia</u>	21.46	24.94	12.15	23.39	22.54	16.32	6.74	4.37	p
<u>pectinalis</u>	16.54	14.12	10.64	22.87	17.36	11.44	7.83	3.60	p
<u>Gomphonema</u>	12.83	12.21	12.97	6.64	4.08	p	3.46	p	p
<u>parvulum</u>	5.84	8.69	5.59	4.45	p	p	p	p	p
<u>Navicula</u>	6.61	5.30	4.21	13.33	11.11	5.44	9.71	5.65	5.23
<u>capitata</u>	7.43	5.51	3.39	9.69	11.32	8.52	6.76	4.56	4.33
<u>N. cryptocephala</u>	p	p	p	p	5.97	p	6.32	8.78	9.06
	p	p	p	p	p	p	p	3.68	4.28
<u>Nitzschia</u> spp	p/p	p/p	p/p	p/p	p/p	p/p	4.82/p	4.92/p	5.90/p
Total diatoms	47.39	48.55	38.77	52.83	50.53	33.73	32.83	26.13	25.63
	36.18	37.51	26.13	41.89	35.36	29.29	26.87	15.68	17.79
<u>Spirogyra</u> spp	p/p	p/p	p/p	p/p	p/p	p/p	p/p	p/p	p/p
<u>Stigeoclonium</u> spp	p	p	p	p	p	p	5.41	5.53	5.39
	p	p	p	p	p	p	4.36	5.26	5.39
<u>Mougeotia</u> spp			/p	p/p	p/p	p/p	p/-	p/p	p/p
<u>Schizothrix</u> spp	p	p	p	p	p	p	6.44	8.48	6.42
	p	p	p	p	p	p	8.91	8.73	10.08
<u>Anabaena</u> spp		-/p	-/p	p/-	p/-	p/p	p/-	p/p	p/p
Blue-green coccolids	14.55	23.89	9.37	10.51	22.42	19.73	6.58	6.77	7.63
	33.11	22.00	19.00	23.36	31.52	18.42	21.04	8.46	5.68
Green coccolids	33.93	22.86	45.44	32.41	20.93	39.46	45.87	48.29	52.72
	24.93	32.13	41.52	26.69	25.37	41.57	33.63	59.07	57.08
Total cells	x = 0.4197	0.3978	0.6349	1.2453	0.8219	0.6301	0.7460	0.6980	0.6038
per	s = 0.1756	0.1394	0.2127	0.5444	0.1576	0.1708	0.1031	0.2356	0.0417
slide	x = 0.3733	0.4105	0.3289	0.6106	0.4570	0.4560	0.2764	0.5679	0.3026
x10 ⁶	s = 0.0623	0.0771	0.0226	0.0718	0.0158	0.0928	0.0382	0.0772	0.0095

the negative effect suggests an "inhibitory" action by nutrients, rather than upstream-downstream effect, which was not prevalent in the Oct. among-troughs experiment.

The taxonomic composition (Figure 42; Table 34) showed some change with nutrients and with temperature. At ambient temperature, the unenriched and N + P treatments were similar with ~25% green coccoids, 18% E. pectinalis, 20% blue-green coccoids, and 5-10% G. parvulum and N. capitata. With "complete" enrichment, green coccoids rose to 40% E. pectinalis dropped to 10%. The three enrichment treatments at +5°C followed the pattern of the ambient temperature enrichment treatments. The unenriched +12°C assemblages were 40% green coccoids and 7% E. pectinalis. With enrichment, E. pectinalis was present in smaller proportions. The +5°C assemblages had the greatest number of total cells and diatoms/slide more frequently than the other two temperature treatments. With enrichment, however, the +5°C assemblages decreased in diatoms/slide, resulting in no significant ranking of total cells or diatoms/slide at "complete" enrichment for the three temperature treatments.

In the Jan. enrichment experiment, the temperature treatments were ranked +12°C >+5°C >+0°C in biomass and C-UP as in the Feb. enrichment experiment (Tables 35-40). AFDW and PROT were lower in the enriched treatments at +12°C than in the unenriched treatment. CHLA was higher in the enriched treatments, at +0°C and +5°C twice and at +12°C once, than in the unenriched treatments. C-UP was higher in the enriched treatments at +0°C and +12°C once, relative to the unenriched treatments.

The summary of enrichment effect (Figure 43) shows more negative effects in AFDW and PROT, more positive effects in CHLA and C-UP. Friedman's test agrees, with enriched +0°C and +12°C assemblages having consistently higher values of CHLA and C-UP than unenriched treatments, and with AFDW and PROT showing the opposite trend.

The taxonomic composition (Figure 44; Table 41), while not as distinct in temperature treatments as the Feb. results, is still in the same pattern. The ambient temperature treatment was 95% green coccoids; the +5°C assemblages had greater percentages of E. pectinalis, lower percentages of green coccoids; and the +12°C assemblages had the greatest proportion of E. pectinalis (20%) and the smallest proportion of green coccoids of the three temperature treatments. The +0°C and +5°C treatments showed increased number of total cells and diatoms/slide with enrichment (ranking test). The +0°C assemblages again had the most cells/slide of the three temperature treatments, regardless of enrichment, but diatoms/slide were not ranked significantly.

Short-Term Enrichments

Three short-term enrichment experiments (STNE) were performed in conjunction with accumulation experiments; a fourth STNE was done alone, using C-UP as the measured parameter. For the description of the set-up of these experiments, see Methods section.

Large variances associated with the older assemblages in the accumulation experiments made a definite nutrient effect hard to decipher. In general, in July (Table 42) the +12°C assemblages increased in AFDW, CHLA and C-UP with enrichment. The other two temperature treatments changed little with enrichment; there was some drop in C-UP with

TABLE 35

AFDW data, JANUARY enrichment experiment. Means (\bar{X}) of individual troughs for each sampling: first sampling on first line, second sampling on second line, third sampling on third line for each temperature and nutrient treatment. Pooled \bar{X} (where indicated by 1X ANOVA) given within temperature treatments for each sampling. Below pooled \bar{X} , temperature treatments ranked from largest to smallest, left to right. Underscore denotes equality. Square root of 2X ANOVA error mean square ($\sqrt{\text{EMS}}$) given for each sampling. (N=nitrogen; P=phosphorus; TE=trace element)

	mg AFDW/slide		
	<u>+0°C</u>	<u>+5°C</u>	<u>+12°C</u>
REFERENCE	0.59	0.91	3.43
	1.19	1.07	6.24
	1.15	1.22	4.45
+N+P	0.49	1.00	2.13
	0.86	0.93	3.15
	0.94	1.91	4.69
CE	0.50	1.02	1.79
	0.98	1.20	2.73
	1.05	1.34	3.41
Pooled \bar{X}	0.53	0.98	3.43 (T4) 1.96
		<u>T4</u> <u>T5,6</u> <u>+5</u> <u>+0</u>	
	1.01	1.07	6.25 (T4) 2.94
		<u>T4</u> <u>T5,6</u> <u>+5</u> <u>+0</u>	
	1.04	1.45	4.18
		<u>+12</u> <u>+5</u> <u>+0</u>	
$\sqrt{\text{EMS}}$		0.3262	
		0.6876	
		0.5647	

TABLE 36

Organic proportion, JANUARY enrichment experiment. Explanation as in Table 35.

	AFDW/DW		
	<u>+0°C</u>	<u>+5°C</u>	<u>+12°C</u>
REFERENCE	0.2827	0.3884	0.4002
	0.3318	0.4516	0.3887
	0.2620	0.4121	0.3643
+N+P	0.3196	0.3822	0.3860
	0.3480	0.4274	0.4227
	0.2948	0.4277	0.3894
CE	0.3377	0.3603	0.4070
	0.3417	0.3895	0.4292
	0.2958	0.3949	0.3919
Pooled \bar{X}	0.3133	0.3770	0.3977
		<u>+12</u> <u>+5</u> <u>+0</u>	
	0.3405	0.4228	0.4136
		<u>+5</u> <u>+12</u> <u>+0</u>	
	0.2842	0.4116	0.3819
		<u>+5</u> <u>+12</u> <u>+0</u>	
$\sqrt{\text{EMS}}$		0.042116	
		0.049850	
		0.050733	

TABLE 37

Protein data, JANUARY enrichment experiment. Explanation as in Table 35.

	mg protein/slide			
	<u>+0°C</u>	<u>+5°C</u>	<u>+12°C</u>	
REFERENCE	0.062	0.147	0.908	
	0.086	0.108	1.213	
	0.214	0.331	1.807	
+N+P	0.025	0.089	0.568	
	0.146	0.186	0.908	
	0.292	0.263	1.335	
CE	0.072	0.180	0.391	
	0.205	0.146	0.791	
	0.159	0.253	1.125	
Pooled \bar{X}	0.048	0.137	0.908 (T4)	0.480
		<u>T4</u> <u>T5,6</u>	<u>+5</u>	<u>+0</u>
	0.146	0.146	1.213 (T4)	0.850
		<u>T4</u> <u>T5,6</u>	<u>+5</u>	<u>+0</u>
	0.222	0.283	1.810 (T4)	1.230
		<u>T4</u> <u>T5,6</u>	<u>+5</u>	<u>+0</u>
$\sqrt{\text{EMS}}$		0.0672		
		0.0942		
		0.1599		

TABLE 38

Chlorophyll a data, JANUARY enrichment experiment. Explanation as in Table 35.

	ug Chlorophyll <u>a</u> /slide				
	<u>+0°C</u>	<u>+5°C</u>	<u>+12°C</u>		
REFERENCE	5.44	7.13	20.19		
	3.83	4.07	15.57		
	7.14	6.22	12.28		
+N+P	6.12	7.43	13.62		
	6.62	6.35	15.42		
	10.15	6.19	19.52		
CE	6.75	14.53	24.88		
	7.63	7.73	18.17		
	9.43	8.59	25.34		
Pooled \bar{X}	6.08	14.53(T9)	7.28(T7,8)	13.62(T5)	22.54
		<u>T4,6</u>	<u>T9</u> <u>T5</u>	<u>T7,8</u>	<u>+0</u>
	3.83(T1)	7.13(T2,3)	6.05		16.38
			<u>+12</u>	<u>T2,3</u>	<u>+5</u> <u>T1</u>
	7.14(T1)	9.79 (T2,3)		12.28(T4)	22.43(T5,6)
			8.59(T9)	6.21(T7,8)	
			<u>T5,6</u>	<u>T4</u> <u>T2,3</u>	<u>T9</u> <u>T1</u> <u>T7,8</u>
$\sqrt{\text{EMS}}$			2.6588		
			1.7665		
			2.1092		

TABLE 39

Carbon-uptake data, JANUARY enrichment experiment. Explanation as in Table 35.

	$\mu\text{g Carbon-uptake/slide/h}$				
	<u>+0°C</u>	<u>+5°C</u>	<u>+12°C</u>		
REFERENCE	5.86	8.76	27.76		
	2.68	2.71	14.53		
	5.56	44.87	80.73		
+N+P	8.50	4.24	22.70		
	4.04	4.55	11.50		
	10.91	23.86	115.46		
CE	7.16	11.02	30.36		
	9.24	3.98	12.91		
	16.04	40.96	125.46		
Pooled \bar{x}	7.17	8.00	26.94		
		<u>+12</u> <u>+5</u> <u>+0</u>			
	3.36(T1,2)	9.24(T3)	3.74		12.78
		<u>+12</u> <u>T3</u> <u>+5</u> <u>T1,2</u>			
	10.84	23.86(T8)	42.92(T7,9)	80.73(T4)	120.46(T5,6)
		<u>T5,6</u> <u>T4</u> <u>T7,9</u> <u>T8</u> <u>+0</u>			
$\sqrt{\text{EMS}}$		7.0249			
		3.8093			
		9.0756			

TABLE 40

Assimilation numbers, JANUARY enrichment experiment. For each sampling and each temperature X nutrient treatment, ASSIMNO is average C-UP/average CHLA. Results of Friedman's nonparametric analysis over ASSIMNO within temperature treatments given below data. Other explanation as in Table 35.

	C-UP/CHLA		
	<u>+0°C</u>	<u>+5°C</u>	<u>+12°C</u>
REFERENCE	1.08 0.70 0.78	1.23 0.67 7.21	1.38 0.93 6.57
+N+P	1.39 0.61 1.08	0.57 0.72 3.85	1.67 0.74 5.92
CE	1.06 1.21 1.70	0.76 0.51 4.77	1.22 0.71 4.95
χ^2	0.67	2.00	4.67
Probability	>.50	>.50	.20

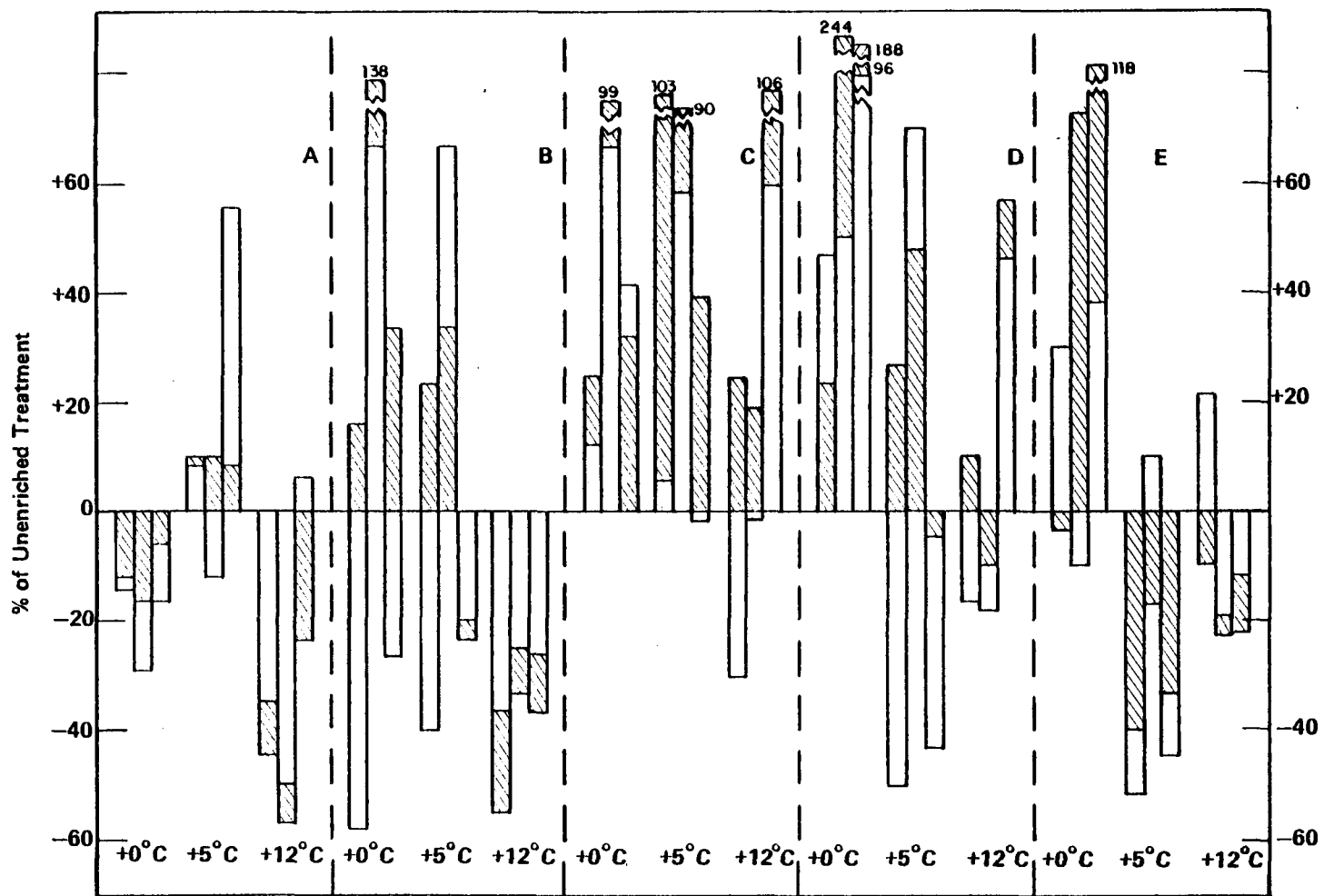


FIGURE 43. January enrichment experiment, treatments normalized to reference samples. Explanation as in FIG. 34. A. AFDW B. PROT C. CHLA D. C-UP E. ASSIMNO

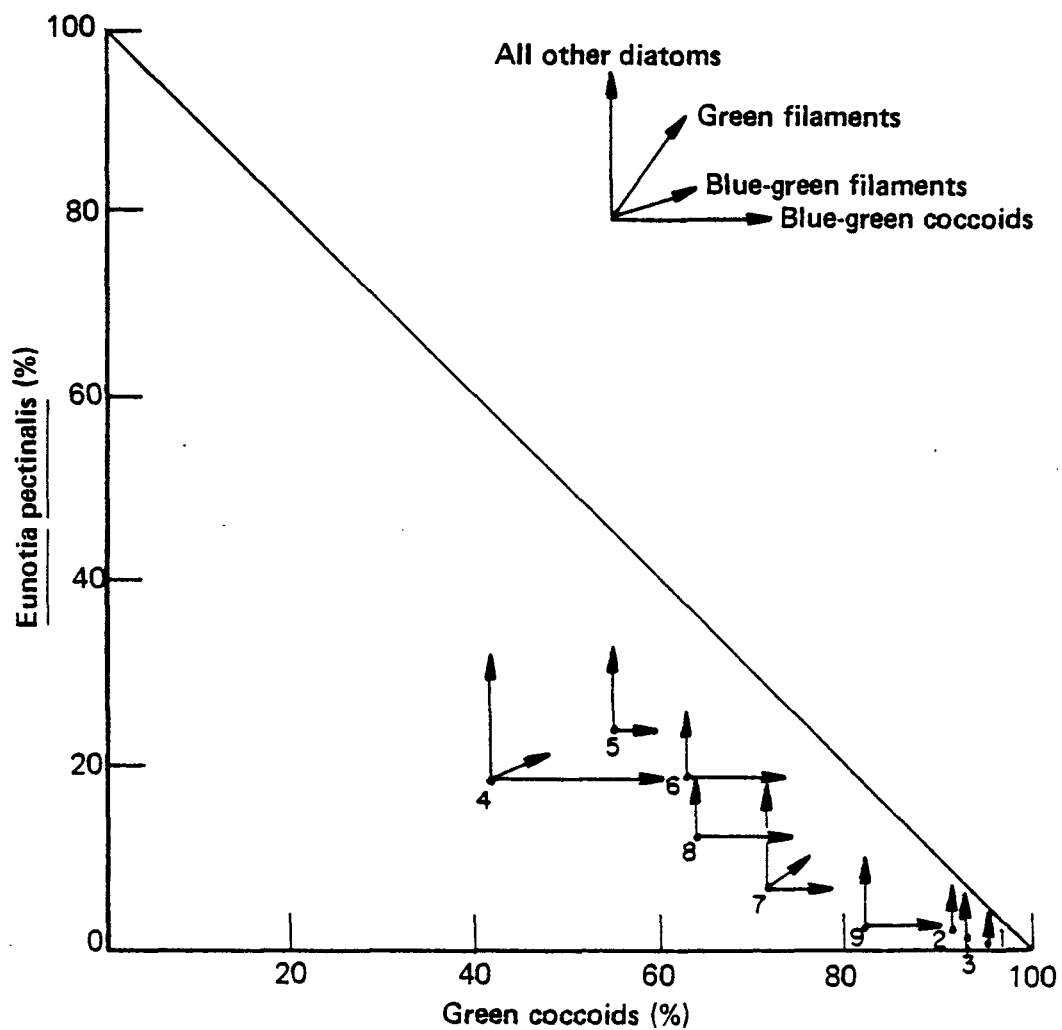


FIGURE 44. Taxonomic composition, January enrichment experiment. Explanation as in FIG. 36.

TABLE 41

JANUARY enrichment experiment taxonomic data. Percent of type of algae present on treatment slides. Results of first sampling on first line (for each trough and algae) or before slash; results of second sampling on second line or after slash. Numbers are average % of three replicates; p = present in at least two of the three replicates. Percent given (instead of p) when type is more than 5% of at least one replicate.

	+0°C			+5°C			+12°C			
	T1	T2	T3	T7	T8	T9	T4	T5	T6	
<u>Eunotia</u>	p	5.98	p	5.25	16.74	6.32	23.47	34.33	24.82	
<u>pectinalis</u>	p	p	4.08	9.41	8.38	p	15.42	13.77	14.43	
<u>Gomphonema</u>	p	p	p	4.17	2.85	p	p	p	p	
<u>parvulum</u>	p	p	p	8.59	4.59	4.51	3.08	p	p	
<u>Navicula</u>	-	-	-	p/p	p/p	-/p	p/p	p/p	p/p	
<u>capitata</u>										
<u>N. cryptocephala</u>	-	p/p	-/p	p/p	p/p	p/p	p/4.61	p/4.48	p/3.74	
Total diatoms	2.12 3.05	8.60 4.67	5.95 6.06	12.17 20.93	22.69 14.95	11.10 9.58	34.76 28.61	42.03 25.36	28.56 24.50	
<u>Spirogyra</u> spp	-	-	-	p/p	p/p	p/p	p/p	p/p	p/p	
<u>Stigeoclonium</u> spp	p/p	p/p	p/p	p/4.09	p/p	p/p	-	-	-	
<u>Mougeotia</u> spp	-	-	-/p	-	-	-	-	-	-	
<u>Schizothrix</u> spp	p/p	p/p	p/-	p/p	p/-	p/p	p/7.18	p/p	p/p	
<u>Anabaena</u> spp							p/-	p/p	p/-	
Blue-green coccoids	- -	p -	p p	5.35 7.54	8.34 13.53	5.99 2.98	13.89 23.88	p 8.03	p 22.96	
Green coccoids	96.63 96.45	90.11 94.31	92.50 93.55	80.64 63.53	66.08 62.53	81.32 84.45	49.28 36.56	48.32 62.11	66.77 59.07	
Total cells	x =	3.6645	2.5834	5.1372	0.9796	1.0744	1.8780	0.7019	0.5834	1.0753
per	s =	0.4511	1.0507	2.3258	0.1978	0.8053	0.7158	0.2590	0.1910	0.2333
slide	x =	3.1923	4.9599	7.5647	0.4543	0.9877	2.6746	0.3184	0.6486	0.6631
x10 ⁶	s =	1.1246	0.8632	2.1259	0.0157	0.5721	1.1366	0.1444	0.1922	0.2460

enrichment for both. In October, AFDW samples were not collected because an insufficient number of slides remained. CHLA decreased slightly with enrichment while C-UP increased significantly for the +0°C and the +12°C assemblages. There was therefore a doubling of assimilation numbers with enrichment. In January, enrichment resulted in slight increases in AFDW, CHLA, and C-UP for the +0°C and +5°C assemblages.

The fourth STNE experiment consisted of a 24-h preincubation with nutrients with 14 day-old assemblages before C-UP were measured. CHLA and AFDW were sampled before the preincubation. Trace element levels were the same as for the long-term enrichment experiments. Nitrogen was added at .5, 1.0, and 2.0 ppm ($\text{NO}_3\text{-N}$), phosphorus at .05, .10, and .20 ppm ($\text{PO}_4\text{-P}$). The four treatments were as follows: no nutrients added (-); .05P + .5N + TE ("1.5"); .10P + 1.0N + TE ("1.0"); and .20P + 2.0N + TE ("2.0"). The 1.0 treatment was approximately the same as the complete enrichment for the long-term experiments. The 1.0 treatments yielded the greatest rate of C-UP (Table 42) for the +0°C and +12°C assemblages, but only the 1.0 treatment at +0°C is distinguishable from the rest.

TABLE 42

Short-term nutrient enrichment experiments. - = reference treatment, + = enriched treatment. Trough numbers included for use in SNK. n=3, except where noted. $\sqrt{\text{EMS}}$ for each group of data in parentheses below parameter name.

	+0°C		+5°C				+12°C	
	T2,-	T3,+	T7,-	T9,+	T4,-	T6,+		
	13 JULY (t=35)							
AFDW mg/slide (1.5568)	8.81	8.85	8.23	8.65	4.85	7.32		
		<u>T3</u>	<u>T1</u>	<u>T9</u>	<u>T7</u>	<u>T6</u>	<u>T4</u>	
ORG AFDW/DW (0.03766)	<u>0.6218</u>	<u>0.5282</u>	<u>0.4885</u>	<u>0.4526</u>	<u>0.3899</u>	<u>0.4018</u>		
CHLA µg/slide (18.3596)	<u>89.83</u>	<u>106.86</u>	<u>36.89</u>	<u>36.37</u>	<u>18.17</u>	<u>46.48</u>		
C-UP µg/slide/h (17.6803)	<u>131.80</u>	<u>96.16</u>	<u>88.51</u>	<u>80.74</u>	<u>91.53</u>	<u>111.01</u>		
ASSIMNO C-UP/CHLA	1.43	.88	2.30	2.13	4.79	2.28		
	11 OCTOBER (t=35)							
CHLA µg/slide (6.0268)	46.78	32.78	62.14	39.16	32.87	25.94		
		<u>T7</u>	<u>T1</u>	<u>T9</u>	<u>T4</u>	<u>T3</u>	<u>T6</u>	
C-UP µg/slide/h (2.8298)	17.76	23.80	34.59	38.59	27.44	40.86		
		<u>T6</u>	<u>T9</u>	<u>T7</u>	<u>T4</u>	<u>T3</u>	<u>T1</u>	
ASSIMNO C-UP/CHLA	0.37	0.72	0.54	0.97	0.82	1.54		
	11 JANUARY (t=42)							
AFDW mg/slide (1.2899)	<u>6.84</u>	<u>7.56</u>	<u>9.32</u>	<u>11.23</u>	<u>11.66</u>	<u>10.70</u>		
ORG AFDW/DW (0.02915)	<u>.4222</u>	<u>.4059</u>	<u>.4359</u>	<u>.4265</u>	<u>.4048</u>	<u>.4002</u>		

TABLE 42. (cont)

CHLA	107.26	136.22	80.38	97.18	59.14	54.12					
μg/slide			T3	T1	T9	T7 T4 T6					
(18.5467)											
C-UP	98.04	107.91	97.28	120.70	122.31	106.52					
μg/slide/h											
(29.8486)											
ASSIMNO	0.91	0.79	1.21	1.24	2.07	1.97					
C-UP/CHLA											
2 SEPTEMBER (t=15)											
C-UP	-	116.18		87.16		117.14					
μg/slide/h	.5	108.25		59.38		103.71					
(579.6239)	1.0	191.91*		83.30		137.36(?)					
	2.0	49.18		49.14		62.34					
ASSIMNO	-	4.54		5.79		24.97					
C-UP/CHLA	.5	4.23		3.94		22.11					
	1.0	7.49		5.53		29.28					
	2.0	1.92		3.26		13.29					
+0	+12	+12	+0	+0	+12	+5	+5	+12	+5	+0	+5
1.0	1.0	-	-	.5	.5	-	1.0	2.0	.5	2.0	2.0

DISCUSSION

The periphyton of the system studied did not respond to added nutrients with increased biomass and C-UP. The first part of the hypothesis is rejected at $\alpha = .05$. The second part of the hypothesis - apparent alleviation of thermal stress by the addition of nutrients - is rejected for all seasons except summer, also at $\alpha = .05$. In summer, the +12°C assemblages with added nutrients attained biomass and C-UP levels almost as high as those of the ambient temperature assemblages; the +12°C assemblages without added nutrients had biomass and C-UP levels much lower than those of the ambient temperature assemblages.

Temperature

The effect of constantly elevated temperatures on periphyton in this system was more pronounced in the winter and summer than in the spring and fall. In the winter, +5°C and +12°C temperature elevations resulted in more rapid accumulation of biomass; higher maxima of AFDW, CHLA and C-UP; and more diatoms per slide than were found at ambient temperatures. In the summer, the same temperature elevations resulted in lower maxima of the same three parameters and fewer diatoms per slide relative to ambient temperatures.

In winter, +12°C conditions (17 to 22°C) supported the most diatoms and the highest C-UP. In the spring and fall, +5°C conditions (19 to 23°C) supported the largest and fastest growing assemblages. Ambient temperature, (20 to 25°C) in summer supported the most cells per slide and highest C-UP. From these results, temperature appears to be an overall limiting factor to periphyton in Upper Three Runs from fall through spring.

Most studies of the effect of temperature on communities have been short-term, such as on the entrainment of phytoplankton in cooling water through power plants. These studies often agree with the results here, i.e., that stimulation by elevated temperatures (increased biomass and productivity) occurs primarily when ambient temperatures are low, and that inhibitory effects occur when ambient temperatures are above 25°C (see Introduction).

Studies of periphyton in thermal plumes or gradients (i.e., constant temperature elevations) have reported similarly increased accumulation rates of biomass or increased species number at elevated temperatures compared to ambient temperature in winter (Patrick, 1971, Tuchman and Blinn, 1979) and detrimental effects of elevated temperatures in summer (Hein and Koppen, 1979). Tilly (1974), studying Par Pond reservoir near Upper Three Runs, found CHLA and AFDW to be higher at an elevated temperature station than at an ambient temperature station only in the winter.

Since natural sunlight was used in all experiments, the effects of photoperiod and light intensity are difficult to separate from those of temperature. Highest light levels and longest days occurred when temperature was approaching the summer maximum. The saturating light level of 100 $\mu\text{Ein}/\text{m}^2/\text{sec}$ agreed with other studies on light adaptation (Harris and Piccinin, 1977).

Seasonal Summary

The assemblages of periphyton were strongly influenced by the seasonal changes in light and temperature. Those at ambient temperature

had maximal biomass, both algal (CHLA) and total (AFDW), in summer, minimal in winter, similar to other periphyton communities (Lowe and Gale, 1980; Kaufman, 1980). Spring (as defined earlier) did not produce a rapid increase in biomass or maximum C-UP as others have found (Cummins, 1974; Moore, 1977a). Mean summer CHLA and C-UP were much greater than those of all other seasons. AFDW biomass, however, did show a spring burst that was close to the mean summer level.

This concentration of productivity and chlorophyll a in summer, with little spring increase, may be due to low ambient temperatures in other seasons. The +5°C assemblages showed a more gradual increase in CHLA and AFDW from winter through spring to summer. The effects of grazers, which could account for low spring periphyton biomass at ambient temperature, could not be quantified since few grazers were seen in the taxonomy samples. The assimilation numbers of periphyton assemblages at ambient temperatures were a little higher in spring than in winter or summer. This suggests that the sparse spring algal assemblages (as shown by CHLA) were photosynthesizing rapidly, but the standing crop was kept low by wash-off, by grazing, or both.

The process of accumulation of periphyton on a bare substrate is analogous to the colonization of new islands; it is the continuous arrival of new species and "extinction" (wash-off, removal by grazers or death) of previous colonizers (Simberloff and Wilson, 1970; Bazzaz, 1979). For benthic organisms, the scarcest resource is usually space (Cairns, 1956). A cell that can attach to a substrate in a current - glass slide or sand grain - to take advantage of a continuous supply of nutrients would have a better chance for growth and reproduction in a low nutrient environment than a floating cell. A shift in environmental

conditions (temperature or pH, for example) away from the cell's optimal range might affect its activity, growth, or even its ability to remain attached to the substrate.

The progress of accumulation of total and algal biomass on the slides also varied with season. The accumulation rate in September was generally slower than that during June or December, and lowest maxima were reached in fall. Most rapid colonization in spring and summer has often been reported (Kaufman, 1980; Lowe and Gale, 1980), as has the increasing variation of replicates with increasing accumulation period (Tilley and Haushild, 1975) as was found here. In June and December, although C-UP peaked at day 8 or 16 and then dropped, CHLA and AFDW biomass continued to rise, as others have observed in other systems (Jones, 1978).

The accumulation process found in any season in this study may not necessarily be representative of that season in general. The increases in CHLA and C-UP at the end of the accumulation periods in September and December indicate that conditions may change and cause a "bloom" on slides that had otherwise apparently reached stationary biomass levels.

Although the limited taxonomic data does not show gradual changes because of infrequent samplings, spring may have been a period of increasing proportion of diatoms, decreasing blue-greens and greens. Fall may have been a period of diatom die-off or wash-off, with green coccoids approaching their winter maximum. The disappearance of diatoms in the fall, perhaps triggered by the shortening days, may account for the low biomass and C-UP by day 28, and the low accumulation rate seen in ambient temperature assemblages in September.

Nutrients

The levels of nitrogen and phosphorus used in the enrichment experiments (0.75 and 0.075 mg/L, respectively), although originally patterned after Savannah River water, are similar to those in many published studies of nutrients in fresh waters. Goldman's (1978) use of 10-25 mg/L $\text{NO}_3\text{-N}$ appears to be the maximum used in temperate systems. Most workers have used 0.1-1.0 mg/L $\text{NO}_3\text{-N}$, 0.01-0.1 mg/L $\text{PO}_4\text{-P}$ (Larsson, 1972; Maslin and Boles, 1978). An N/P ratio of 17, as used here, is between the ratios other workers have found to be balancing between nitrogen and phosphorus deficiency, 12 (Chiaudani and Vighi, 1976; Mykkestad, 1977), and 30 (Rhee, 1978; Stockner and Shortreed, 1978).

Micronutrient concentrations were 10 to 50 times less than some other studies (Goldman, 1965; Polisini et al., 1970). Since trace elements can reach toxic concentrations at low levels of addition in some systems (Goldman, 1965), and since an individual study of each trace element was impractical, it was decided to use low levels in the enrichment experiments.

An increase in C-UP and biomass with enrichment occurred most consistently in July in the +12°C temperature treatments. At other seasons, one or two parameters showed stimulation or inhibition from added nutrients in one or two of the samplings; these inconsistent effects were probably due to the unanticipated close correlation of periphyton biomass and C-UP with temperature and available carbon (see below).

The increase in diatoms per slide and C-UP with enrichment at +12°C in summer tends to support the hypothesis that nutrients can "alleviate" temperature stress. Further studies are needed to determine if the

algae of Upper Three Runs at 35-37°C have a higher nutrient requirement or if they have reduced uptake ability.

Several other studies have shown changes in stimulation of algal communities by nutrients with season. Where nitrogen and phosphorus have been tested separately as well as together, nitrogen addition stimulated CHLA biomass in fall and winter, phosphorus in spring and summer (White and Payne, 1977; Gerhold and Otto, 1976).

The change in nutrient limitation with season (at least for the +12°C assemblages) could be a reflection of the variation in amounts of nutrients available with season. In Upper Three Runs, phosphate showed the most seasonal variation of the nutrients measured. Change in nutrient limitation could also be indicative of the requirement of winter communities for different amounts or ratios of N:P as compared to summer populations. Finally, algal species present year-round could require different amounts of nutrients in different seasons. There was a noticeable shift in algae present through the year in all three temperature treatments.

Assimilation numbers have sometimes been used as indicators of nutrient deficiency. As assimilation number of less than 1 indicated extreme deficiency, while 2.5 to 5.0 indicated moderate deficiency (Thomas and Dodson, 1972). Assimilation numbers in these experiments were 1 to 2 in winter, rose to 2 to 3 in spring, and for the +12°C assemblages were between 4 and 11 in summer. Fall assimilation numbers were less than 1, but rose somewhat in the second winter. In the enrichment experiments, assimilation numbers generally fell with enrichment, a function of slight increases in CHLA combined with slight decreases in C-UP. C-UP per unit CHLA appeared here to rise and fall

seasonally. If this ratio is truly indicative of nutrient deficiency, then the conditions in Upper Three Runs in winter led to assemblages that were nutrient deficient, while summer assemblages were not limited by nutrients. Other workers have, however, found assimilation numbers to correspond more to temperature conditions than to nutrient levels (Schelske et al., 1974).

Two nutrients omitted from the enrichments were silicate and inorganic carbon. Silicate is believed to limit diatom growth below 100 g/L (Wallen and Tuppling, 1977; Schelske and Stoermer, 1971). The level of silicate in Upper Three Runs, 2.5-5.3 mg/L, should not have limited diatom growth.

Possible limitation of C-UP by low levels of inorganic carbon has been studied several times. Schindler (1971) showed that addition of inorganic carbon to a whole lake did not increase productivity, but another study in the Experimental Lakes Area showed that carbon could become limiting in incubation bottles (Sakamoto, 1971). Although bicarbonate additions to some systems or cultures did increase C-UP (Loftus et al., 1979; Dickman, 1973), the carbonate equilibrium shift of bicarbonate to carbon dioxide takes place more quickly than algae can use up CO₂. Therefore an open system should not be carbon limited (Goldman et al., 1974; Lehman, 1978).

The strong correlation of C-UP with available carbon in summer (see next section) does suggest that inorganic carbon levels may sometimes affect algal photosynthesis rates. The effect of bicarbonate additions to the periphyton of Upper Three Runs will be reported elsewhere (Brown and Zingmark, MS in prep.). Briefly, no stimulation from added carbon on the three temperature treatments was observed.

Carbon limitation in incubation bottles appears likely in low alkalinity waters. The short ^{14}C incubation time minimized the possibility of that occurrence here. Only in rare situations in summer, if low available carbon levels were to coincide with high rates of C-UP, would carbon become limiting in the incubations. The correlation of available carbon and C-UP could be an indirect indication of the effect of pH on taxonomic composition, and not a true indication of carbon limitation.

Synergisms

The large day-to-day variations in algal biomass and C-UP indicated that some combination of variables also changes on a similar time scale to affect the periphyton. As others have shown, algae can respond rapidly to changes in light intensity (Gallegos et al., 1977) and inorganic carbon levels (Schindler and Fee, 1973). Many workers have observed maximum photosynthetic rates in the morning, followed by an afternoon slump (Lorenzen, 1963; Doty and Oguri, 1957; Tilzer and Horne, 1979). Rates of nutrient-uptake may likewise be inconstant over a day (Lorenzen, 1963; MacIsaac, 1978). These variations may be caused by variations in rates of uptake of different species (Stross and Pemrick, 1974; Paerl and MacKenzie, 1977), which in turn are responding to changes in light and nutrient levels. The state of activity of individual algal cells may change from hour to hour, as well as with season, leading to changes in the activity of the community (Soeder, 1965). The scale of environmental variation, which in turn cues variation in algal communities, must be known to adequately test limiting factors (Harris, 1980). If limitations occur only at a certain point of the cycle of

environmental variation, a study which does not consider this variation might miss the limitation.

Upper Three Runs is shallow and is warmed by solar radiation and cooled by air temperature rapidly. Temperature, pH, and available carbon were the most variable environmental factors measured. Multiple regression analyses of physical and chemical variables on CHLA and C-UP showed that variation of temperature, available carbon, and sometimes photoperiod accounted for 30-80% of the variation in these parameters, depending on the season.

Temperature and available carbon are weakly correlated with C-UP and CHLA of the two elevated temperature assemblages in spring, no doubt because these elevated temperatures would be close to optimum temperature for the periphyton. Available carbon is strongly correlated with summer C-UP: 50% of the variation of C-UP of the +0°C assemblages and 75-80% of the variation in C-UP of the elevated temperature assemblages is accounted for by variation in available carbon. CHLA and C-UP of ambient temperature assemblages are also correlated with the temperature at the time of sampling for summer data. Variations in available carbon are correlated with data from the +12°C assemblages: 80% of the variation of the fall set, 20% of the variation of the winter set. Fall data from the +0°C and +5°C assemblages are correlated with photoperiod (70-80%), while the winter data are correlated (33%) with average temperature over the accumulation period.

Although this is a condensed review of the correlations, and while the applicability of linear regression models to some sets of biological data has been questioned (Green, 1974; Green and Vascotto, 1978; Levins, 1979), the large proportion of the seasonal variation in CHLA and C-UP

which can be attributed to temperature and available carbon suggests that these variables strongly affect periphyton communities in Upper Three Runs (Brown, MS in prep.).

Water chemistry, although only measured twice quarterly, is a variable which was found to fluctuate on a longer time-scale than temperature and available carbon. Because both water samples in all seasons except summer were similar, nutrient levels probably do not fluctuate much from day-to-day and would therefore account for little of the day-to-day variation in biomass and C-UP. Heavy rainfall, however, did repeatedly lower the pH of the creek for 2-3 days, and, as the July water samples indicated, altered the nutrient concentrations. A similar rainfall event that occurred before the July enrichment experiment depressed the pH, raised available carbon levels, and probably was responsible for C-UP measurements that were four to five times above normal.

Community Composition

Although this study was primarily concerned with the function of the intact assemblages, a knowledge of the composition of the assemblages was also sought. A shift in species or type of algae may be correlated with changes in biomass or C-UP among the temperature or nutrient treatments.

The importance of an alga cannot always be determined from its size; some algae have much less biovolume than their apparent volume (Sicko-Goad et al., 1977). Studies on the activity of different algal species in communities have shown that smaller cells may fix carbon more rapidly (per cell carbon) than larger cells (Faust and Correll, 1977;

Maguire and Neill, 1971; Watt, 1971). Diatoms have been found to be major producers in some systems (Thomas et al., 1978). The green coccoids in winter, and perhaps the filamentous algae throughout the year, may not be as active as the diatoms. Some of the apparently inhibitory effects of nutrient addition may actually be the effect of competition for space - coccoids may grow over the slide and prevent settling of diatoms, thus keeping CHLA levels and C-UP low.

The assemblages on glass slides in these experiments were dominated by green coccoid cells in the winter, and by diatoms and blue-green algae in the summer and spring. The green coccoids which may have been zoo- spores of some of the green filamentous algae (Stigeoclonium, Spirogyra, Mougeotia), boosted the number of cells per slide to 4-7 million at ambient temperature in February and January. The proportion of green cells was lower at other seasons and at elevated temperatures in winter. The presence of these cells in large numbers when CHLA and C-UP were low suggests that they are indeed reproductive or dormant cells with minimal CHLA and low C-UP.

Eunotia pectinalis, the most common diatom found on the slides, was rare at ambient temperature in January and February. E. pectinalis rose to 40% of total cells in April and July, and fell back to 20% in October. Other workers have reported E. pectinalis to be common in summer and fall (Hargraves and Wood, 1967). The +12°C temperature elevation enabled E. pectinalis to constitute 20-30% of the assemblages in the winter. In spring, the elevated temperatures affected the proportions of E. pectinalis little. In summer and fall, however, few E. pectinalis were found at +12°C; +0°C and +5°C assemblages had similar proportions of this diatom.

Nutrient additions decreased the proportions of *E. pectinalis* in spring and fall at all three temperature treatments; there was little effect at other seasons. Although Tippett (1970) reported that *E. pectinalis* did not grow as well on slides as on natural substrates, Schumacher and Whitford (1965) found it to grow better in high flow conditions, similar to flow rates in this study.

Gomphonema parvulum was 5-15% of the ambient temperature assemblages the whole year. In winter, elevated temperatures resulted in 10-25% *G. parvulum*; at other seasons elevated temperatures had little effect. *G. parvulum*, although common on sand grains in Upper Three Runs (Ed Wilde, pers. comm.), was persistent but not common on the experimental slides. This diatom is more common in acidic water (Patrick et al., 1968) and was reportedly stimulated in a eutrophic creek by an addition of nitrate + ammonium + phosphate + glucose (Patrick, 1966). No stimulation by nutrients was seen here. In batch culture its optimum temperature was reported to be 22°C, and it tolerated up to 34°C (Wallace, 1955). The only discernible temperature preference seen here was for temperatures above 10°C.

The other diatoms which were present 5% or more at one time or another were *Navicula cryptocephala*, *N. capitata*, *Nitzschia* spp., and *Achnanthes minutissima*. Various species of *Navicula* and *Nitzschia* have been found on natural and artificial substrates in flowing water (Hein and Koppen, 1979; Lowe and Gale, 1980). On artificial substrates and in culture they are more common at warmer temperatures, especially under enriched conditions (Lowe and Gale, 1980; Hargraves and Wood, 1967; Patrick, 1966; Maddux and Jones, 1964; Wallace, 1955). These observations agree with the effects of temperature seen in this study.

Navicula and Nitzschia species were most often found on slides in the summer at ambient temperature, and in cooler months at elevated temperatures. A. minutissima appeared in appreciable numbers only at +12°C in summer - at a temperature of ~35°C; nutrient additions seemed to adversely affect its abundance.

Blue-green coccoids were also warm-water constituents of the assemblages, but in summer and fall the +12°C treatments had lower proportions of blue-greens than the other two temperature treatments. The only possible stimulation by nutrients was at +5°C in July.

Although filamentous algae were surveyed and counted under low magnification as well as under high magnification and fluorescence microscopy, the numbers are too variable to draw many conclusions as to the effects of temperature and nutrients. Stigeoclonium spp. appeared to prefer warmer temperatures, being in greatest proportions at +12°C in July and October.

Although blue-green algae are often associated with eutrophic conditions (Shapiro, 1973), there was no detectable stimulation by nutrients of blue-green algae. The usual decrease in proportions of some blue-greens occurred with nutrient addition, as was found by Wall and Briand (1980). Some filamentous blue-greens have been observed to have optimal light levels below 100 $\mu\text{Ein}/\text{m}^2/\text{sec}$ or above 800 $\mu\text{Ein}/\text{m}^2/\text{sec}$ (Ahlgren, 1977; Senft, 1978). Light conditions in the experimental troughs may have affected their growth more than temperature or nutrients.

Conclusion

Periphyton in Upper Three Runs are not nutrient limited. Day-to-day fluctuations in temperature and pH correlate closely with variations

in periphyton biomass and C-UP. Only in summer do added nutrients appear to alleviate temperature stress by increasing biomass and productivity of +12°C assemblages (37°C).

Although many workers assume that nutrient additions (especially phosphorus) to oligotrophic systems will result in increased algal biomass and productivity, this generalization cannot be extended to all freshwater systems. Nutrient addition studies on a pond with water chemistry similar to that of Upper Three Runs (Polisini et al., 1970) showed results opposite to those found here. The present study indicates that 1) not all types of oligotrophic freshwater systems are stimulated by nutrient additions and 2) not all freshwater systems within a type (Southern coastal plain) respond to nutrient additions in the same way.

Limnologists have concentrated their attention on lakes and ponds; these systems are larger and more heavily used by the public than are flowing systems. The processes of streams and rivers are important, however, both to the basic science of ecology and in relation to their impact on ponds and lakes. Upper Three Runs may represent a "typhoid Mary" type of stream - a carrier that does not show symptoms of the problem. If we assume that other compartments of this system are as oblivious to nutrient additions as the periphyton, cultural eutrophication could have little effect on Upper Three Runs but could greatly affect downstream pool areas. Although the algal assemblages in this stream offer an interesting study of the importance of environmental fluctuation to algal biomass and productivity, the lack of nutrient limitation cannot be interpreted to mean that nutrient levels are of no

importance to this system or to the systems influenced by Upper Three
Runs.

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