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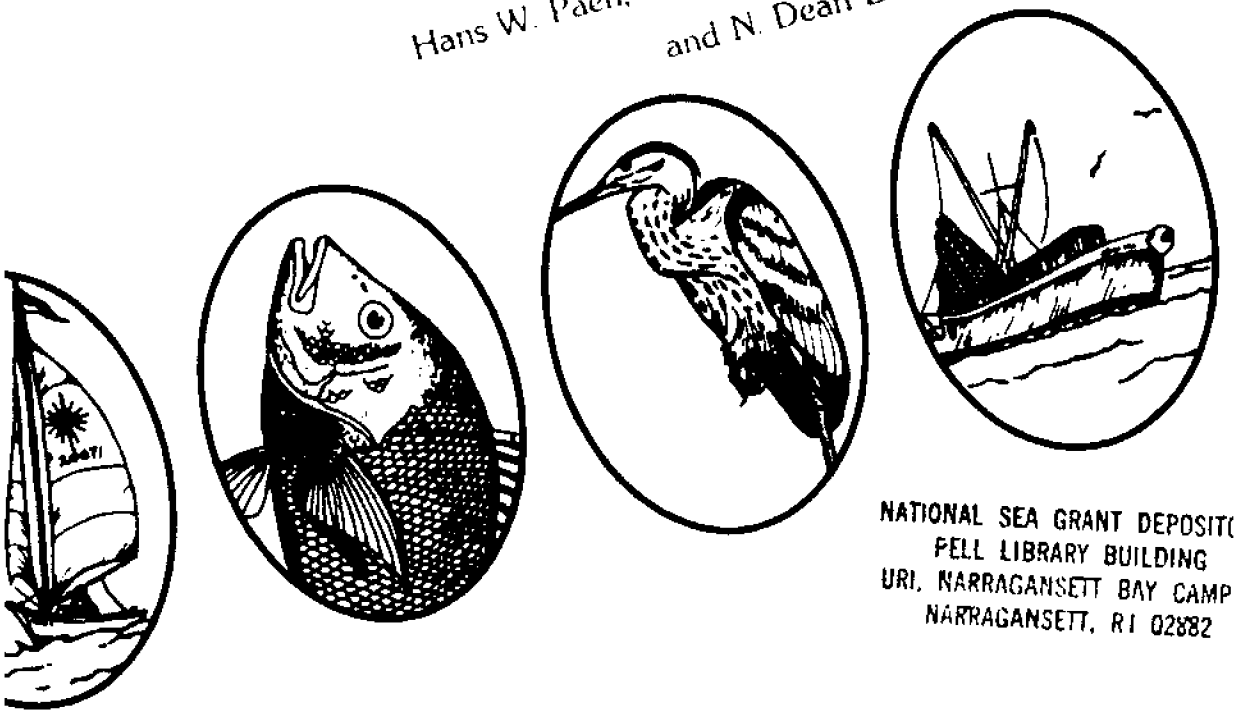

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Statement of Problem and Research Objective:

Water quality in the lower Neuse River, particularly downstream from Kinston, N.C., has rapidly degraded over the past 8 years. Accelerated eutrophication, culminating in nuisance blooms of the blue-green alga Microcystis aeruginosa, has been a river feature over the past 5 years. Recent field and laboratory studies by Paerl (1983), Stanley (1983) and the N.C. Department of Natural Resources and Community Development (Tedder et al. 1979 NCDNRCD Working Paper: Neuse River Investigation 1979), have implicated increased nutrient loading in the river basin as the most likely cause of water quality degradation. A study of aquatic physical and chemical factors has revealed the lower Neuse River to be particularly susceptible to the most troublesome manifestation of bloom activity, the formation of surface scums (Paerl, 1983). A combination of low turbulence (absence of vertical mixing), warm sunny weather, low dissolved inorganic carbon concentrations and nutrient sufficiency initiates surface blooms. Excess nutrient (nitrogen and phosphorus) loading throughout much of the year aggravates and ultimately perpetuates this problem.

Since blue-green algal biomass is thought to be an undesirable, and at times, unpalatable food source for zooplankton grazers in freshwater and estuarine food chains (Bogatova 1965; Porter 1977; Porter and Orcutt 1980), the proliferation of blue-green algal blooms in the nursery and spawning areas of the oligohaline Neuse River Estuary between Vanceboro and Cherry Point, N.C. could potentially alter food availability to both herbivorous fish and shellfish species as well as carnivorous consumers of grazing zooplankton in this region. Prior to the initiation of research reported here, the potential for intrusion of a blue-green algal bloom into the estuarine environment was largely unknown. Accordingly, the principal objectives of the work described below were directed at; a) gaining

an understanding of general primary production rates and patterns of nutrient-enriched waters entering the oligohaline (0-5 ppt salinity) areas of the Neuse River Estuary (downstream from the freshwater riverine segment of the Neuse River) (Paerl et al. in preparation), b) examining the potential for blue-green algal bloom intrusions into the oligohaline segments of the Estuary (Paerl et al. in preparation) and, c) establishing salinity tolerance levels in response to specific ionic components of seawater for major bloom-forming nuisance species of blue-green algae entering the Estuary from freshwater habitats. Information obtained from this three-prong study is of future use in predicting both the degree to which blue-green algal blooms could be expected to proliferate and survive in the oligohaline environment, and secondly, whether salinity or other environmental constraints (nutrient deficiencies, high degrees of turbulence, increased inorganic carbon content) dictate the ability of blue-green algal blooms to penetrate the Neuse River Estuary.

As outlined in the original Sea Grant proposal, the study was divided into two components: 1) Field studies were conducted on a monthly basis to quantify in situ primary production (phytoplankton growth) over a transect of locations in the oligohaline region of the Estuary between New Bern and Cherry Point, N.C. During each sampling and incubation period phytoplankton growth nutrients (nitrate, ammonia, phosphate), total dissolved inorganic carbon (DIC), salinity, oxygen, temperature and photosynthetically active radiation (PAR) were determined over depth, at each location. The main objective of this study was to identify the magnitudes and patterns of primary productivity in relation to relevant environmental parameters (i.e. nutrient availability, salinity, PAR transparency, etc.). 2) Secondly, a set of laboratory and field studies was designed and executed throughout both the blue-green algal bloom period (May-September) as well as in the laboratory on a relevant blue-green algal species isolated from

nature, examining growth responses to specific ionic (cations and anions) components comprising the salinity of seawater. The following manuscript summarizes major research findings relevant to the second research question.

Introduction

The Neuse River is, commercially and recreationally, one of North Carolina's most valuable river-estuarine systems. The river drainage basin measures 5,710 square miles or about 12 percent of the land area of North Carolina. Rapidly expanding agricultural, industrial and municipal development in this basin during the last 20 years have resulted in enhanced water use and nutrient inputs (Harned 1980). Accelerated eutrophication and resultant changes in the trophic status of the river have accompanied recent urban and rural development in the basin. A particularly disturbing consequence of such eutrophication has been the appearance of blue-green algal blooms during the past five years.

The worst bloom on record in the river occurred during July and August of 1980. This bloom was dominated by the scum-forming, non N_2-fixing genus, Microcystis. Possible consequences of scum-forming blooms include deoxygenation of underlying waters, fish kills, foul odors and tastes, toxicity, and the physical and chemical alteration of sediments as a substrate and nutrient source/sink (Reynolds and Walsby 1975). This general deterioration in water quality threatens the use of the river as a source of water for municipal, agricultural, industrial and recreational purposes. Since 1978, blooms have been common between Kinston and New Bern. Perhaps the most serious threat from blooms is their potential proliferation into estuarine and marine environments.

Pamlico Sound, North Carolina's largest coastal sound, is one of the most important commercial shrimping and fishing areas in the state. The extensive shallow areas and salt marshes along the sound's fringes serve as nurseries for a variety of important commercial and recreational marine species, including

shrimp, oysters, clams, scallops, blue crabs, spot, striped bass, croaker and flounder (Giese et al. 1979). The Neuse River and its estuary are among the major freshwater sources supplying soluble nutrients and particulate matter to the Pamlico Sound.

A better understanding of the potential for bloom proliferation in the saline portions of the Neuse River Estuary is urgently needed in order to recognize and understand the potential ecological and commercial impacts which this form of water-quality deterioration might have on the neighboring marine environment. The lower Neuse River is a dynamic system with respect to salinity regimes and gradients. Depending on freshwater runoff, winds, tide and weather conditions, the extent of saltwater intrusion into the river varies greatly (Paerl et al. in preparation). Salt wedges are known to intrude as far as six km upstream from New Bern, where severe bloom activity is commonplace during mid-summer. Conversely, following periods of precipitation and elevated runoff, salt wedges are pushed downstream of New Bern. Nuisance blooms have been observed during both sets of events (Tedder et al. 1980; Paerl 1983). The nuisance bloom genera thus far detected are of freshwater origin. Little is known about their saltwater tolerance, and hence potential ability to survive and proliferate in the marine environment.

Methods

Maintenance of *Microcystis* cultures

During initial field and laboratory experiments (July-August 1981), large surface scums of *Microcystis* were present in the river. Concentrated scums were collected in 4-l acid-cleaned polypropylene bottles. If the algae were not used for a field experiment the bottles were placed in a cooler and transported to the Institute of Marine Sciences Laboratory (IMS) located in Morehead City, N.C. for further experimentation. The surface scums disappeared shortly after

the August experiments. All other experiments were conducted with Microcystis populations isolated from the Neuse River. We attempted to culture Microcystis collected from the scums in July and August, but were not successful. On two other occasions, however, we were successful in isolating viable cultures from the river. During the winter of 1981 we collected mud and river water (approximately 50:50 w/v) in 1-l polypropylene widemouth bottles. The tops of the bottles were loosened and the bottles were left standing in the laboratory. Within one week, Microcystis colonies were visible in the bottles (Time of appearance varied from two days to one week; if no colonies were visible in two weeks, the sample was discarded). These colonies were transferred to 125 ml Pyrex, cotton-plugged flasks containing approximately 75 mls of ASM-J medium. ASM-J medium is a modification and combination of ASM-1 (Gorham et al. 1964) and Jansen (J) (Corbett and Parker 1976) media. Its preparation is outlined in Table 1. The flasks were illuminated from above with approximately 60 $\mu E/m^2/sec$ photosynthetically active radiation (PAR 400-700 μm) supplied by cool white and gro-lux fluorescent lamps. Non-axenic cultures were maintained on this medium in a 27-degree incubator and transferred weekly or biweekly. Half of the flasks were left standing and swirled by hand twice a week. The other half of the flasks were maintained on rotary shakers. The cultures on the shakers, however, tended to form one or two very large colonies, and therefore were very difficult to disperse uniformly. The cultures left standing tended to grow smaller colonies, facilitating uniform dispersal. Accordingly, they were used for experimentation.

Field Experiments

On July 20, 1981, we conducted our initial salinity experiment. The area sampled ranged from an upstream station at the Streets Ferry bridge to a downstream station at M19 (Fig. 1). At these two stations and at M52A (approximately halfway between the two stations), we routinely determined salinity, dissolved oxygen,

and temperature at 0.5 meter intervals. At the bridge, M74, M64, M38, M24 and M19, we measured salinity, dissolved oxygen, and PAR transmittance on water specifically collected for lab experiments. Salinity and temperature were measured with a YSI model 33 salinity-conductivity-temperature meter. Oxygen and temperature were measured with a YSI Model 54 A oxygen meter. PAR transmittance in the water column was determined with a Li-Cor Model 185A Quantum/Radiometer/Photometer, having a Li-192 S underwater sensor. Upon returning to the lab, we measured pH, salinity, DIC and humics (measured by the absorbance at 456 nm in a 5-centimeter cell). The water was first filtered through a GF/C and then through a GF/F filter to remove naturally-occurring phytoplankton. The filtered water was transferred in 140-millimeter aliquots (in quadruplicate) to 250-milliliter polycarbonate flasks. For testing the impacts of an NaCl salinity gradient, appropriate dry weights of NaCl were added to each treatment. Ten milliliters of the Microcystis suspension was then added. The flasks were incubated in the laboratory on rotary shakers under continuous illumination for 14 hours. At the end of the incubation, samples were assayed for photosynthetic $^{14}CO_2$ incorporation and ^{14}C dissolved organic carbon (DOC) excretion. $^{14}CO_2$ incorporation was measured as follows: Flasks were injected with 0.5 ml of a ^{14}C bicarbonate solution ($NaH^{14}CO_3$ at 58 $mCi \cdot mmole^{-1}$ specific activity, obtained from Amersham Searle Corp.), containing 2.2 µCi of ^{14}C. The technique used for incubation and assessment of primary productivity was essentially that used by Steeman-Nielsen (1952) as modified by Goldman (1963). Twenty five milliliters of each flask were filtered at a gentle vacuum of 200 Torr on a 25 millimeter diameter Whatman GFF filter. Filters were then air dried, fumed in an HCl atmosphere, vented and placed in 7-ml scintillation vials containing 5 ml of Fisher Scintilene cocktail. Incorporation of ^{14}C was detected with a Beckman LS 7000 microprocessor-controlled liquid scintillation counter. Dissolved inorganic carbon (DIC) content

of samples taken was determined by infra-red analyses. A 0.25 ml water sample was injected into a 4-ml reservoir of 50% H_2SO_4, which was continually purged with CO_2-free argon. The argon was then passed through a Beckman 864 infrared analyzer for DIC measurements. All DIC standards were made up of reagent grade Na_2CO_3 dissolved in deionized water (Paerl 1983).

Primary productivity was determined using the following equation:

$$^{12}C \text{ fixed} = \frac{^{14}C \text{ fixed (I)}}{^{14}C \text{ available}} \times A \times B \times C$$

where: A = volume correction for the aliquot filtered

B = correction for incubation time to an hourly rate

C = conversion factor to µg/l or mg/m³

(I) = isotope effect = 1.06

^{14}C-DOC excretions were determined by collecting five millimeters of filtrate from $^{14}CO_2$ uptake filtrations (GFF filters having a porosity of 0.45µm). Filtrate samples received approximately four drops of 0.5 N HCl in order to reduce the pH to 3. This was monitored with a Fisher Accumet 750 pH meter. Samples were then bubbled with air for 30 minutes, thereby expelling the remaining ^{14}C labeled inorganic carbon. Samples were then neutralized (pH 7-8) by dropwise (4-5 drops) addition of 0.5 N NaOH. The remaining ^{14}C-labeled DOC was quantified by liquid scintillation counting in 10 milliliters of Fisher Scintiverse water-soluble cocktail. Counting efficiencies ranging from 85-92 percent were determined using an internal ^{14}C-hexadecane standard obtained from New England Nuclear Corporation.

On August 3, 1981, we conducted a similar experiment, sampling at the bridge, M74, M68, M58, M52A, M38, M24 and M19 (Fig. 1). Three hour surface incubations were conducted in 125-milliliter Pyrex reagent bottles fitted with ground glass stoppers. For each station, three incubation bottles were filled with filtered

water from that station and 10 milliliters of the collected Microcystis suspension. Two clear bottles and one opaque bottle were filled from the bridge, M74, M68, M58, M52A and M19 to assess the incorporation of $^{14}CO_2$ by the natural phytoplankton at those stations. The incubations were conducted in situon a surface-incubation rig. At the end of the three-hour incubation period, the bottles were collected, immediately placed in a light-tight box, brought back to IMS and filtered as described for the experiment conducted July 20, 1981.

Additional water was brought back to the lab to conduct a time-course response experiment in response to NaCl additions. The water was filtered as described for the July-20 experiment. To the 250-milliliter polycarbonate screw-top flasks (in quadruplicate) we added 190 milliliters of filtered water, 10 milliliters of Microcystis suspension, given NaCl concentrations, if applicable, and 0.5 millilites of ^{14}C-$NaHCO_3$. Five different treatments were examined, bridge water, M19, bridge:M19 (50:50), bridge + 3.5 ppt NaCl, bridge + 7 ppt NaCl. At half-hour intervals up to four hours, as well as five-, six-, and 24-hour intervals, we assayed for ^{14}C incorporation as outlined previously.

Laboratory Experiments

All laboratory experiments were conducted in quadruplicate in 250-milliliter Corning, polycarbonate, screw-top flasks. Each incubation contained 130 milliliters of fresh ASM-J medium (at the desired salinity), 5 milliliters of the Microcystis inoculum, and 0.3 milliliters of ^{14}C-$NaHCO_3$. For the experiment with natural sea salts, the medium was prepared with diluted seawater to give the desired salinity (2.5 or 5 ppt). In all other experiments, stock solutions were prepared as stated below such that a ten-fold dilution of the stock in the final medium would give the desired salinity. The experimental medium also contained 7 mgC/l (as $NaHCO_3$) to prevent inorganic carbon depletion during the experiment.

The incubations were conducted on rotary shaker tables under 60 $\mu E/m^2/sec$ PAR illumination. The incubations were terminated by filtration through Whatman GF/C filters and the incorporation of ^{14}C assayed as stated earlier.

Preparation of Artificial Salt Mixture

In order to prepare one liter of a stock solution, the appropriate weight of each respective dry salt was added to approximately 900 milliliters of deionized water. The solution was then diluted to the desired salinity as measured with an American Optical Corporation Model 10402 Refractometer or a YSI Model 33 Salinity-Conductivity-Temperature meter. The preparation of the artificial salt mixture was based on a commercial sea-salt preparation obtained from the Dayno Sales Company. We used the five major salts NaCl, $MgCl_2$, $MgSO_4$, $CaCl_2$, and KCl in the same relative concentrations as the commercial Dayno preparation to prepare our mixture (Table 2).

Results

In July and August 1981, we conducted two sets of initial field and laboratory experiments. During this time the level of salinity intrusion was relatively high and large surface scums of viable Microcystis were present in the Neuse River. The presence of this scum facilitated collection of a very high concentration of nuisance algae suitable for experimental treatments. In both sets of experiments, our sampling stations ranged from an upstream station at the Streets Ferry bridge to a downstream station at M19.

The field data collected on July 20, 1981 for the vertical profiles at the upstream station (bridge), the downstream station (M19) and approximately midway between the two extreme stations (M52A), are presented in Table 3. No measurable salinity was found at the bridge. At M52A, however, the salt wedge was quite evident and salinity increased rapidly with depth. At M19 the water column revealed a more uniform distribution of salinity. All three of the stations

were heavily stratified with respect to oxygen and were approaching anoxic conditions in the near-bottom waters. Table 4 shows surface measurements of salinity, pH, dissolved inorganic carbon (DIC), and humics at all of the sampled stations. The DIC and humic levels at M74 are much higher than at the other stations. This is also the site of the Weyerhaeuser Paper Mill effluent input near Vanceboro, N.C.

In the laboratory we examined the uptake of $^{14}CO_2$ and excretion of ^{14}C-labeled organic carbon by Microcystis in waters having different salinities. We incubated Microcystis in filtered bridge water to which we had added 0.5, 1, 5, 10, 15, 20, 30 ppt NaCl. We also incubated Microcystis in filtered water from each of six stations routinely sampled in the river (ranging from marker 52A to Cowpens-near Vanceboro) in order to approximate a natural salinity gradient. The results are shown in Table 5 and Figure 2. A large decrease in inorganic carbon uptake and an increase in organic carbon excretion was observed with increasing concentrations of NaCl. This trend was not, however, clearly displayed among primary productivity samples incubated (in situ) in the natural salinity gradient. Although all of the natural salinity gradient incubations exhibited lower inorganic carbon uptake than the control (bridge water) and excretion levels of approximately twice that of the control, no clear pattern evolved and salinity did not necessarily appear to be the factor dominating inorganic carbon fixation. The minimal rates of inorganic carbon uptake and maximum organic carbon excretion rates were found in M74 water, while the inorganic carbon uptake at M24 (9 ppt) was higher than that at M38 (6 ppt).

In the second set of field experiments (Aug. 4, 1981), we repeated the Microcystis-transfer experiments. This time however we incubated samples in the field. We found a definite trend towards decreasing inorganic carbon uptake with increasing salinity in the natural salinity gradient. This trend did not,

however, hold true for the natural phytoplankton assemblage (Fig. 3). Although no data were obtained from the mid salinity regimes, the highest salinity showed the highest productivity (among natural phytoplankton assemblages) of any of the stations.

Using river water and Microcystis collected in the field, we conducted a time-course experiment in the laboratory examining the highest and lowest salinity waters, a 50:50 v/v mixture of the two, as well as low salinity water + 3.5 ppt NaCl and + 7 ppt NaCl. We sampled every half hour up to four hours, and then at five, six, and 24 hours. Between the six- and 24-hour sampling periods, however, all of the inorganic carbon had been removed from the water, so only the first six hours could be validly assessed. Due to a high degree of variability, little could be interpreted from the first several hours. After six hours, the 50:50 treatment gave results intermediate between the M19 and bridge water, but M19 water showed the highest uptake of inorganic carbon. This is opposite of what was found in the field. The NaCl treatments showed inhibition when compared to natural water treatments; 7 ppt showed greater inhibition than 3.5 ppt NaCl.

In subsequent lab experiments we compared the effect of NaCl and natural sea salts on inorganic carbon uptake in Microcystis. The NaCl treatments resulted in a much higher level of inhibition than the natural sea salts. Although it appeared that NaCl is not suitable as a substitute for the natural sea salts, we preferred to use an artificial salt source (as opposed to sea water) for the laboratory experiments, because humic components and non-nutrient substances in seawater may have interferred with growth responses. In additon, an artificial salt mixture would allow us to achieve a higher and more practical salt concentration for the stock solution and a lower dilution of experimental media than the naturally occurring 35 ppt. The artifical mixture also reduces the

chance of contamination by naturally occurring saltwater phytoplankton. We compared the effect of 2.5 and 5 ppt of natural sea salts, our artificial mixture and NaCl on inorganic carbon uptake. The inhibitory effect of our artificial mixture was comparable to the effect of natural sea salts at similar concentrations (Fig. 4). NaCl, however, showed much greater inhibition than was found with natural sea salts.

As a result of the wide difference between the effect of our artificial mixture and NaCl on carbon uptake, we examined the effect of the different major salts in seawater on the uptake of inorganic carbon. At five ppt, NaCl, $MgCl_2$, KCl, $MgSO_4$, and $NaSO_4$ ($CaCl_2$ was not examined due to its limited solubility characteristics) all inhibited carbon uptake significantly greater than the artificial mixture at five ppt (Figure 5). At five ppt, KCl exhibited greater inhibition than any of the other salts tested. However, as a component of the salt mixture, KCl represents less than 0.2 percent of the total salt content. Figure 6, although somewhat difficult to interpret due to the differing salinities of each treatment, shows the effect of the total salt mixture, the total salt mixture minus one constituent, NaCl and KCl individually, and NaCl and KCl in combination at the levels found in the salt mixture. NaCl, even at 3.9 ppt, had at least as great an inhibitory effect on inorganic carbon uptake as five ppt of the mixture. The absence of NaCl from the mixture removed any observable inhibition over a 24-hour period. KCl alone, at the very low levels found in the mixture, had no observable effect; however, its removal from the mixture appeared to reduce inhibition.

We compared the effect of a range of salinities over three- and 24-hour periods (Fig. 7). Although the pattern was similar, the inhibition after 24 hours was much more pronounced than after three hours. There appeared to be some inhibition at one and two ppt after 24 hours and a large decrease in inorganic carbon uptake resulted in response to salinity levels between two and

five ppt. In all experiments, we found a statistically significant ($p<0.01$) inhibitory effect (usually between 20 and 30 percent inhibition) at five ppt. Among the lower salinities, however, especially at one ppt, any observed inhibition was rarely significant; on several occasions the lower salinities even showed a trend towards very slight stimulation of inorganic carbon uptake.

Discussion

We have assessed the potential for blue-green algal blooms currently plaguing the upper regions of the Neuse River to proliferate in the estuarine and marine environment. The nuisance bloom genera involved are of freshwater origin, and it is not known if these blooms can survive as they are transported into a saline environment by the natural flow of the river. Thus far, serious problems from these blooms have not been detected below New Bern. These blooms, however, are a relatively recent occurrence and all of their ecological impacts have yet to be realized.

The coincidental occurrence of the Microcystis surface scums and a high level of salinity intrusion in the river during July and August of 1981 enabled us to examine the effect of salinity on algae from an actual bloom. The NaCl treatments clearly inhibited the uptake of inorganic carbon by Microcystis. Inhibition was observed at NaCl concentrations as low as 0.5 ppt, while higher salinities consistently caused severe inhibition of inorganic carbon uptake (Fig. 2). The same treatments resulted in extremely high values for percent of organic carbon excreted. This, however, was probably due more to a decrease in organic carbon uptake by this blue-green algae than to an increase in excretion, as the absolute values observed of organic carbon excretion did not increase as dramatically as the values of inorganic carbon uptake decreased.

The transfer of Microcystis to river water of various salinities, however, did not result in such a clear pattern of inhibition. Incubations in water

from M38 (6 ppt) and M19 (10 ppt) showed inhibition over the control, but the relatively low inhibition in M24 (9 ppt) water suggests that the salinity-growth interactions in the natural environment are more complicated than suggested by the NaCl data alone (Fig. 2). The unexpected inhibition observed at M74 (no detectable salinity) is perhaps due to the high levels of humic substances in the water at this station (Table 4). (M74 is the site of Weyerhaeuser's paper mill discharge).

During the August 4, 1981 experiment we found an inverse relationship between salinity and inorganic carbon uptake. These incubations were similar to those conducted in July, except that the incubations were conducted in the field. Our simultaneous examination of inorganic carbon uptake by the naturally occurring phytoplankton bring out an important point. Although we found the lowest inorganic carbon uptake by Microcystis at M19 (7.5 ppt), the naturally occurring phytoplankton at this station showed a greater uptake of inorganic carbon than any of the lower salinity stations (Fig. 3). In a related report (Paerl et al. in preparation) maximum primary productivity and chlorophyll a values occurred in medium salinity (2-4 ppt) waters of the Neuse River Estuary, further confirmation that increased salinity does not necessarily inhibit natural phytoplankton photosynthesis. Apparently, oligohaline-adapted phytoplankton communities thrive and form an important primary production component of the Neuse River. In summary, more saline areas of the upper Neuse Estuary are quite capable of supporting high levels of primary production, even though the Microcystis transferred from up-stream does not appear to thrive in such waters.

It is unfortunate that the inorganic carbon levels in the water for our time course experiment on August 5 were not sufficient to support the growth of Microcystis for 24 hours. The shorter incubations may not allow sufficient time to see the full effect of the salinity of the alga. At the other extreme,

Microcystis may be capable of adapting to elevated salt concentrations during a longer incubation. After six hours, the NaCl additions lead to the lowest level of inorganic carbon uptake (as also observed in the last experiment). However, the results of the incubations in natural salinity waters were surprising. The high salinity water resulted in the highest level of inorganic carbon uptake, exactly opposite of what we observed in the field incubations with the same water and Microcystis a day earlier. This may be a result of different conditions between the two incubations (e.g., light intensity or length of incubation) or a difference in the condition of the Microcystis on the two different days.

We found that NaCl, individually, inhibited inorganic carbon uptake much more severely than natural sea salts, but that our artificial salt mix (Table 2) was a good substitute for the natural sea salts (Fig. 4). Nearly all of the inhibition seen in response to our artifical salt mixture was due to NaCl, since its absence from the mixture completly eliminated inhibition (Fig. 6). KCl actually inhibited carbon uptake more severely than NaCl at similar concentrations, but the amount of KCl in natural sea salts is very low relative to NaCl. The mechanism of inhibition by NaCl appeared to involve an imbalance of ions. Although the extent of inhibition of inorganic carbon uptake by the salt mixture was dependent on concentration (Fig. 7), 2.5 ppt of NaCl inhibited inorganic carbon uptake to a greater extent than five ppt of the salt mixture, which is comprised of 70 percent NaCl (Fig. 4 and Table 2). Hence, NaCl appears to be the most active inhibitory agent in sea salt, and the presence of the other salts actually reduces some of the inhibition attributed to the imbalance of ions caused by NaCl.

Salinity concentrations above 5 ppt severely inhibited inorganic carbon uptake by Microcystis. Below 2 ppt, however, this inhibition was not as pronounced, and on several occasions we observed what appeared to be slight stimulation

of inorganic carbon uptake in Microcystis at the lower salt concentrations. We suspect that this may be a result of increased photosynthetic production of low molecular weight compounds by blue-green algae as a response to osmotic stress (Blumwald and Tel-or 1982). We are presently investigating this possibility by examining the partitioning of recently fixed $^{14}CO_2$ into different cell constituents (Blackwell et al. in preparation).

Our salt mixture inhibited uptake of inorganic carbon by Microcystis approximately twice as much over a 24-hour period as over a three-hour period (Fig. 7). Continuation of this trend past 24 hours would eventually lead to death of the alga. Longer incubations should reveal whether this blue-green alga does in fact die or whether the trend is reversed as the alga adapts and possibly recovers. Preliminary indications (Blackwell et al. in preparation) are that recovery is unlikely and death follows extensive residence in waters supporting at least 2 ppt salinity. Incubations longer than 24 hours would allow chlorophyll a determinations to be used as another parameter in assessing the effect of salinity on Microcystis. Changes in chlorophyll a in our 24-hour incubations were seldom significant except in the very high salinity treatments. This was largely due to the relatively low sensitivity of techniques available for detecting changes in cellular chlorophyll a levels (relative to C-14 uptake techniques) and extensive time required for chlorophyll a degradation to phaeopigments. Incubations longer than 24 hours were not conclusive however, largely due to inorganic carbon limitation overshadowing differential inhibitory effects of the salinity treatments. Only through excessive inorganic carbon enrichment (to unnaturally high levels) could we extend incubations for a significant length of time. Furthermore, we were unable to culture Microcystis at low level (0.5 ppt) salinities. Natural isolates of Microcystis, however, are generally difficult to maintain in the lab under optimum conditions, even though the organism flourishes in the river.

In the natural environment, the movement from fresh to saline water is gradual, which could potentially allow for adaptation to the salinity by the phytoplankton, including Microcystis. We have observed large colonies of Microcystis as far down the Neuse River as the mouth of the Trent River. The level of salinity in the Neuse River undergoes large fluctuations and it is possible that Microcystis would not encounter a lethal salinity level until well down the river, especially along the surface of the river. Although our data suggests that Microcystis could not survive transfer into the saline environment, serious consequences could still result from the transfer of large masses of blue-green algae into the Neuse River oligohaline estuary. It has been shown that Microcystis, as well as other bloom-forming, blue-green algae, are avoided as food sources by grazing zooplankton and larval fish (Porter 1977). Hence, potential disruption of estuarine food chains could result. Furthermore the death and decay of such large masses of organisms could result in serious consequences, including oxygen depletion, deterioration of water quality and fish kills (Reynolds and Walsby 1975).

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Table 1. ASM-J Medium Preparation (for one liter) (a modification and combination of ASM-1 (Gorham et al. 1964) and Jansen (J) (Corbett and Parker 1976).

Compound	g/l
Stock solutions for solution A	
$NaCO_3$	170.00
$Na_2HPO_4(7H_2O)$	26.79
K_2HPO_2	17.40
$Na_2SiO_3(9H_2O)$	56.80
Stock solutions for solution B	
$MgSO_4(7H_2O)$	49.27
$MgCl_2(6H_2O)$	40.57
$CaCl_2(6H_2O)$	43.78
$Na_2EDTA(2H_2O)/FeCl_3(6H_2O)$	7.35/1.08
G9 trace element solution (modified)	
H_3BO_3	3.25
$MnSo_4(H_2O)$	1.80
$ZnSO_4(H_2O)$	0.30
$(NH_4)_6Mo_7O_2(4H_2O)$	0.08
$CuSO_4(5H_2O)$	0.05
$CoCl_2(6H_2O)$	0.12
$AlCl_3(6H_2O)$	0.47
$NiSO_4$	0.10
KI	0.09

Solution A: To a final volume of 500 mls, and 25 mg of Tricene buffer and one ml of each of the stock solutions. Adjust the pH of the solution to 8.2.

Solution B: To a final volume of 500 mls, and one ml of each of the stock solutions and one ml of the G9 trace element solution.

Autoclave the two solutions separately and combine.

Table 2. Artificial salt mixture preparation (salinity = 35 ppt)

Additive	g/l
NaCl	27.52
$MgCl_2(6H_2O)$	5.79
$MgSO_4(7H_2O)$	14.17
$CaCl_2(6H_2O)$	2.72
KCl	0.73

Table 3. Vertical profiles for three stations on the Neuse River sampled on July 20, 1981.

Depth (m)	Bridge *Sal (ppt)	Bridge **D.O. (ppm)	Bridge T °C	M52A *Sal (ppt)	M52A **D.O. (ppm)	M52A T °C	M19 *Sal (ppt)	M19 **D.O. (ppm)	M19 T °C
0.00	0	7.9	30.7	1.1	8.0	28.8	11.5	11.5	28.5
0.5	0	6.2	28.0	---	8.0	28.5	---	11.0	28.5
1.0	0	5.4	27.8	2.2	7.0	27.5	12.6	8.8	28.0
1.5	0	4.9	27.3	---	4.7	27.3	---	1.7	27.0
2.0	0	4.1	27.1	3.3	2.6	27.0	13.1	1.1	27.0
2.5	0	3.9	26.9	6.6	1.3	26.8	---	0.5	26.3
3.0	0	3.8	26.9	8.8	0.4	26.5	16.4	0.5	26.0
3.5	0	3.6	26.9	13.1	0.2	22.0	---	0.4	25.8
4.0	0	3.6	26.8	13.1	0.2	25.9	---	---	---
4.5	0	3.1	26.6	---	---	---	---	---	---
4.6	0	0.2	26.6	---	---	---	---	---	---

* Salinity measurements in ppt (parts per thousand)
** Dissolved oxygen measurements in ppm (parts per million)

Table 4. Surface measurements of salinity, dissolved inorganic carbon (DIC), humics (absorbance at 465 nm in 5 cm cell), and pH at field stations on July 20, 1981.

Station	salinity ppt	DIC mgC/l	humics	pH
bridge	0	3.8	0.092	7.70
M74	0	21.4	0.740	7.75
M64	0	6.9	0.202	7.75
M38	6	10.9	0.065	7.85
M24	9	14.0	0.042	8.10
M19	10	12.0	0.051	7.60

Table 5. CO_2 uptake, dissolved organic carbon (DOC) excretion and percent excretion for lab incubations conducted July 21, 1981 with filtered water and *Microcystis*. Standard deviation values are given for each set of replicates.

Treatment	CO_2 Uptake ($mg\ C\ m^{-3}\ hr^{-1}$)	DOC excretion ($mg\ C\ m^{-3}\ hr^{-1}$)	% excretion
bridge (control)	244 ± 10.0	5 ± 2.0	2
bridge + 0.5 ppt NaCl	192 ± 29.0	4 ± 1.0	2
bridge + 1.0 ppt NaCl	190 ± 31.0	4 ± 0.5	2
bridge + 5.0 ppt NaCl	125 ± 52.0	5 ± 1.0	4
bridge + 10.0 ppt NaCl	49 ± 7.0	5 ± 1.0	10
bridge + 15.0 ppt NaCl	40 ± 8.0	8 ± 1.0	20
bridge + 20.0 ppt NaCl	9 ± 0.5	5 ± 1.0	56
bridge + 30.0 ppt NaCl	4 ± 1.0	2 ± 0.5	50
M74	118 ± 39.0	10 ± 1.0	8
M64	197 ± 20.0	5 ± 0.1	3
M38	188 ± 4.0	7 ± 0.5	4
M24	222 ± 25.0	8 ± 0.2	4
M19	141 ± 19.0	7 ± 0.5	5

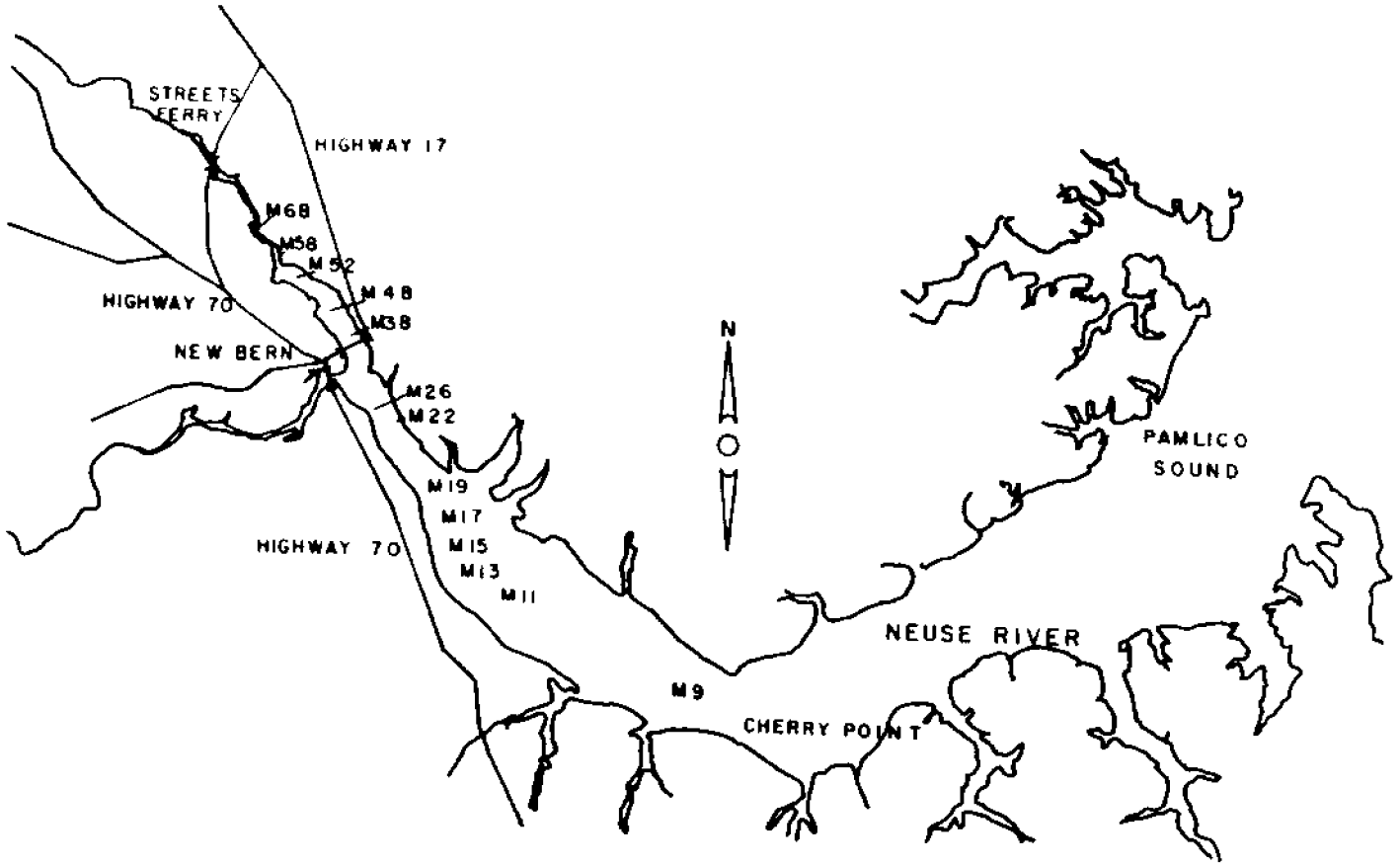

Figure 1. Map showing oligohaline portions of the Neuse River Estuary where research was conducted. Shown are locations and numbering sequences of channel markers, which were used as sampling and incubation locations during this study. The extent of sampling ranged from 10 km above New Bern (Streets Ferry) to Cherry Point.

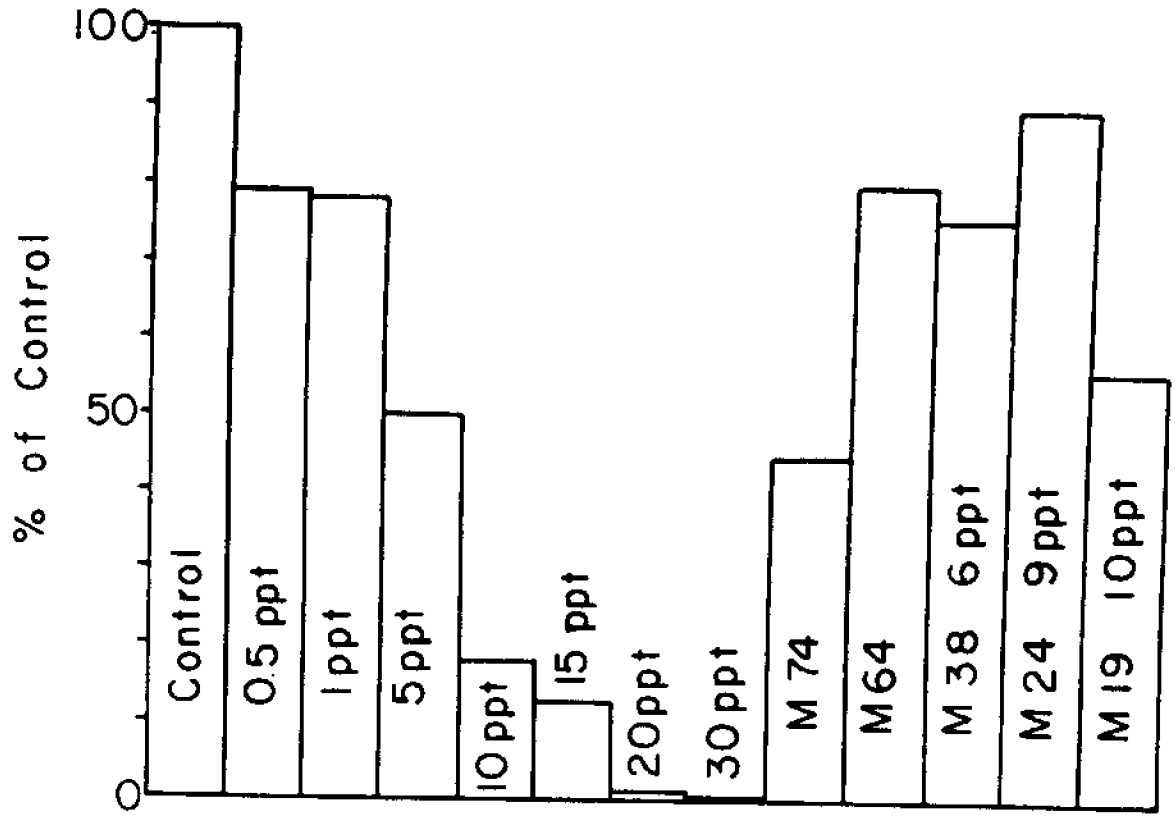

Figure 2. Percent of control ^{14}C incorporation by Microcystis incubated in filtered river water in artificial (NaCl) and natural salinity gradients. Water for control and NaCl additions was collected at the Streets Ferry Bridge (salinity = 0 ppt). Remaining water (M74-M19) was collected at Neuse River Navigational Markers as indicated.

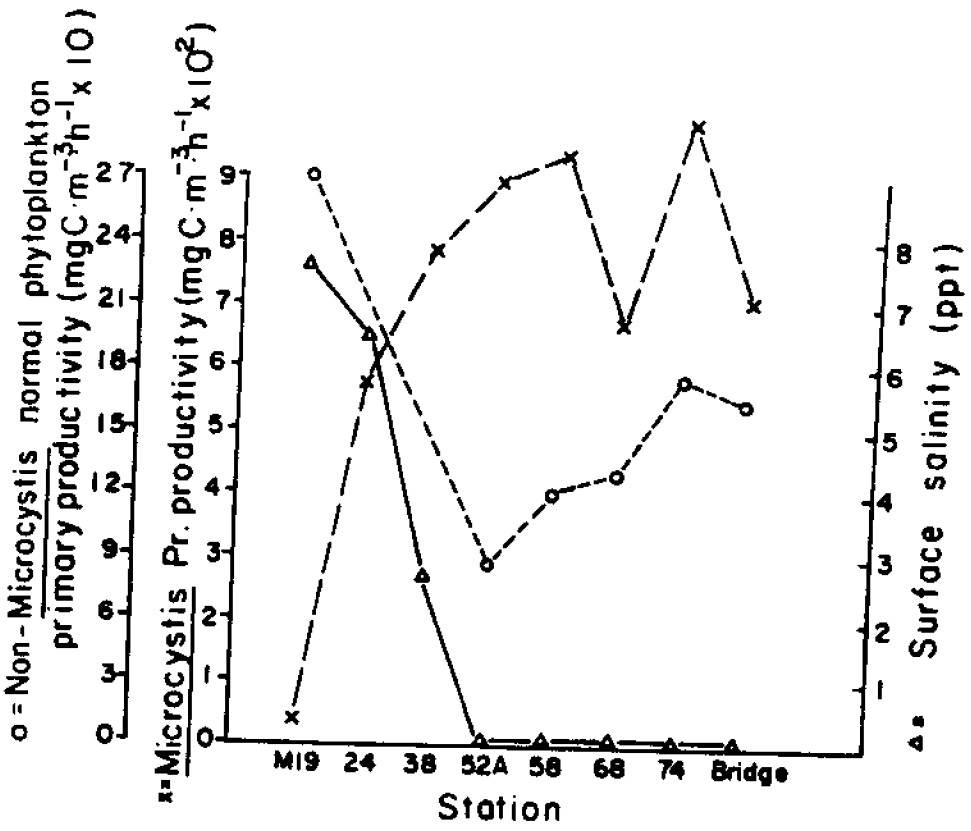

Figure 3. Results of an experiment conducted on August 3, 1981: A transect comparing primary productivity of natural phytoplankton communities to productivity of Microcystis exposed to filtrates from the same samples is shown. The salinity values of all transect (surface) samples are also given. All samples were incubated in situ at marker 52A. These results show the inhibitory effect of high salinity filtrate on natural Microcystis populations, while non-Microcystis communities readily show adaptation to increasing salinity.

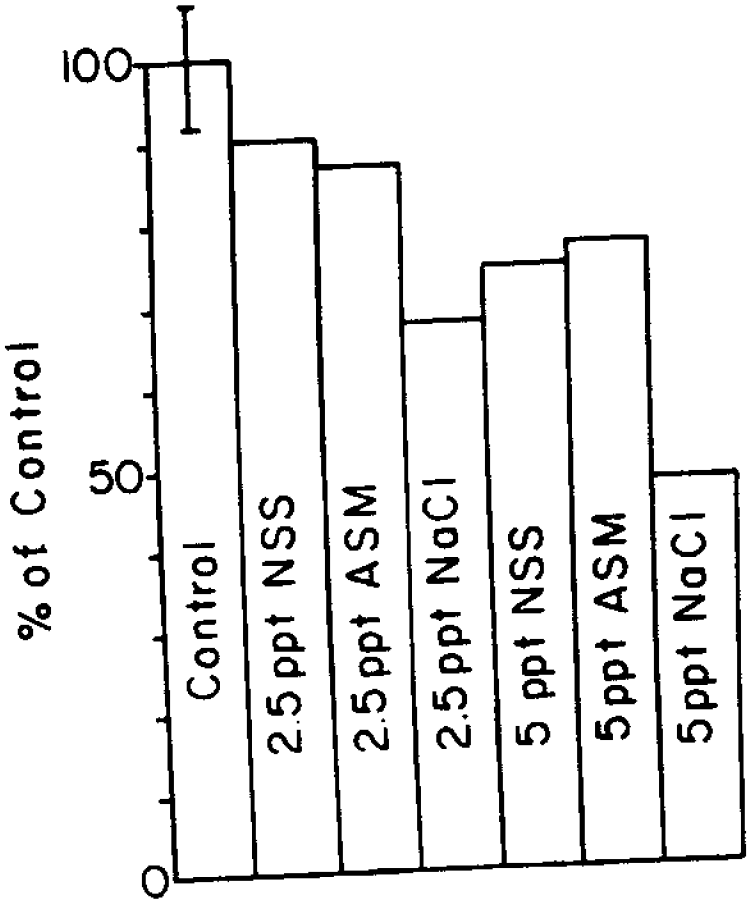

Figure 4. Differential effects of natural sea salts (NSS), artificial salt mixture (ASM) and NaCl on growth in Microcystis maintained in culture on ASM-J nutrient medium. The culture was exposed to various salt additions for 24 h. Error bar represents 95 percent confidence interval.

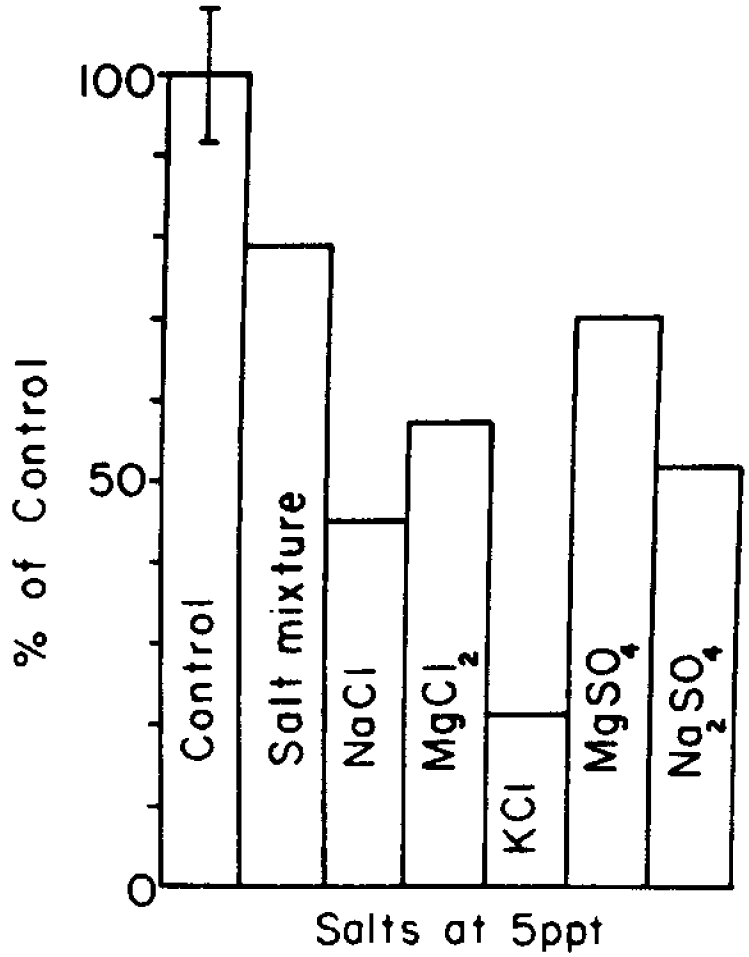

Figure 5. Differential effects of an artificial salt mixture and individual salt components, all at five ppt concentration, on Microcystis growth, using a ^{14}C bioassay conducted in the laboratory. Microcystis was originally cultured on ASM-J medium. Growth response following 24 hours of exposure to various salt additions is shown.

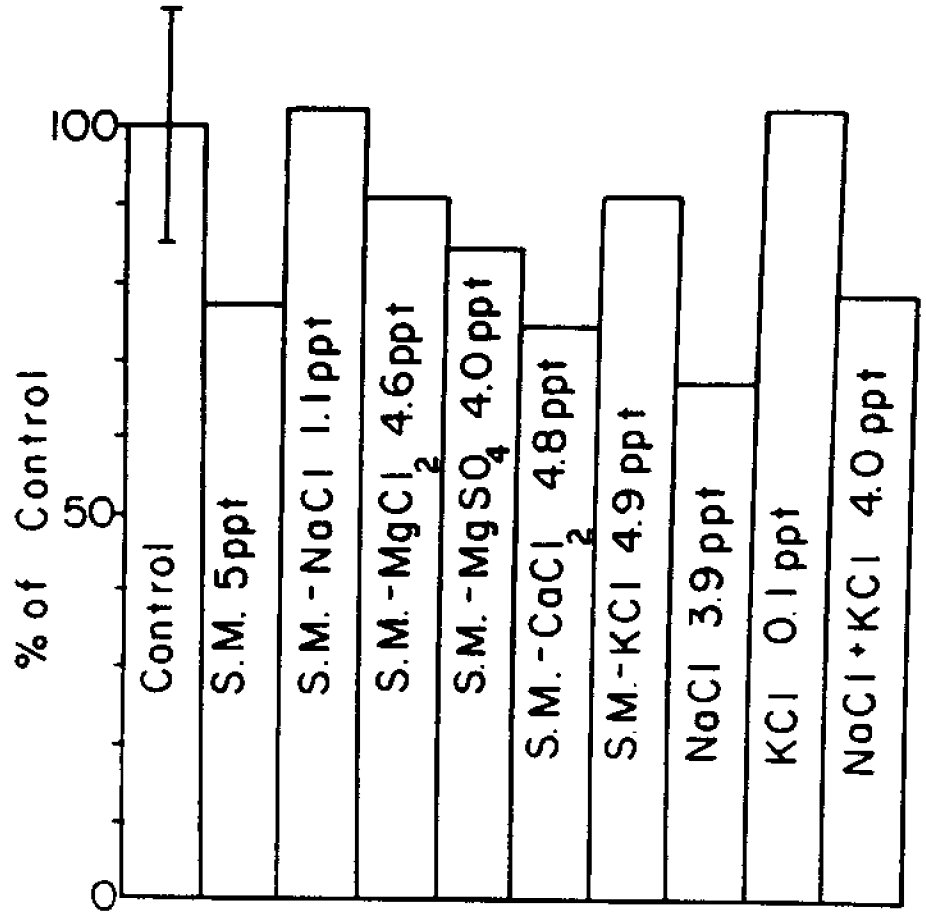

Figure 6. Growth response (using ^{14}C bioassay techniques) to diverse salt components of an artificial salt mixture in a laboratory culture of Microcystis. Controls received no salt additions. The error bar represents the 95 percent confidence interval.

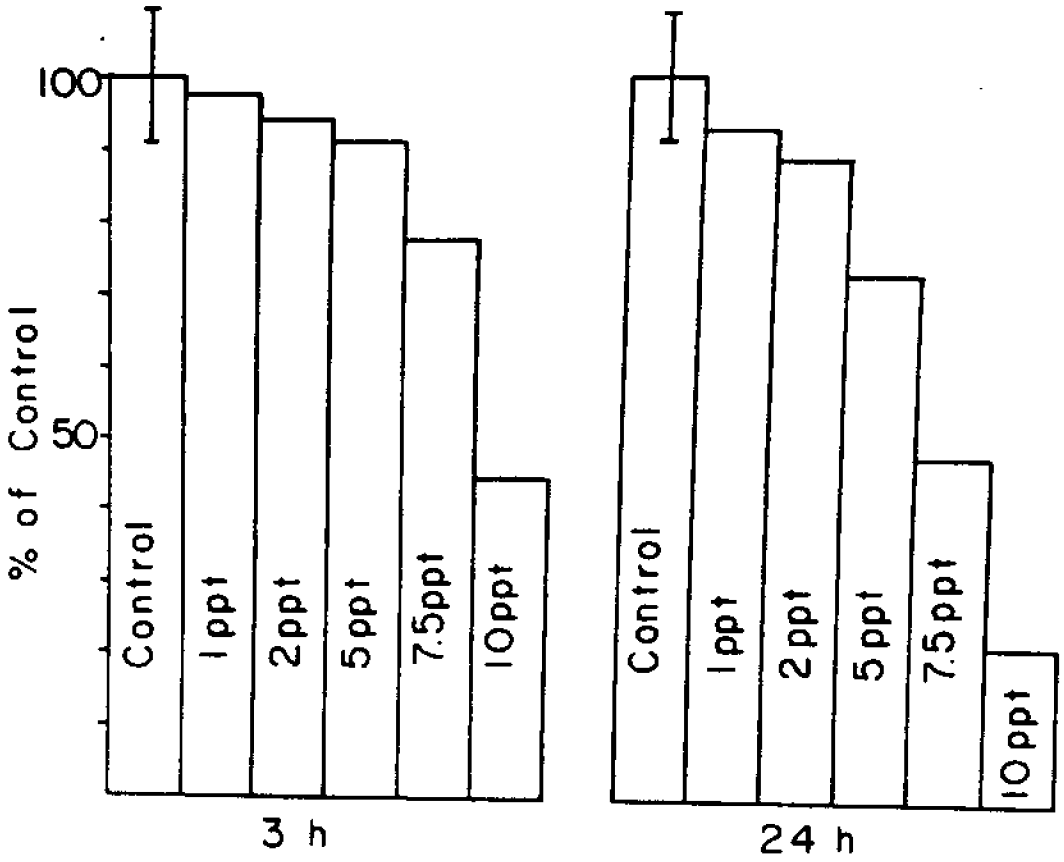

Figure 7. A comparison of growth responses at three and 24 hours, following the addition of various concentrations of artificial salt mixture. The 95 percent confidence intervals are given at each incubation period. Microcystis, which was cultured on ASM-J medium, was once again the test organism.