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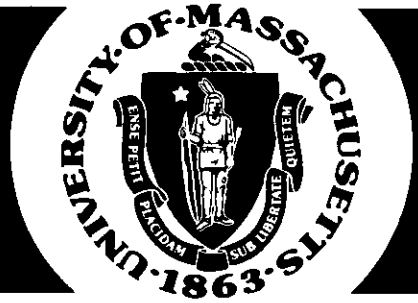

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Technical Report

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by

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Submitted to

Massachusetts Division of Water Pollution Control
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Executive Summary

A pilot algal assay monitoring laboratory was developed, capable of examining water and wastewater samples, using a modified version of the Algal Assay:Bottle Test (AA:BT) at a 1981 cost of about $30,000. The algal species, Selenastrum capricornutum was used to test lake, river, and wastewater treatment plant (WWTP) effluent samples for algal growth potential. Approximately 89.5 person hours were required to conduct a complete algal assay for one sample. Each additional sample, processed concurrently, required about 60 more person hours. Baseline data on selected sites in Massachusetts and Connecticut were collected. Of the 18 samples assayed, two were shown to be phosphorus limited, 11 were nitrogen limited, two were limited by nitrogen (N) and phosphorus (P), and three samples were either nitrogen or N + P co-limited. Results from this study and others demonstrated that N:P ratios ranging from 5:1 to 12:1 may correspond to waters that are co-limited by nitrogen and phosphorus. Another modified version of the test was developed to determine the effect of raw and alum-treated secondary wastewater treatment plant (WWTP) effluent on receiving waters. A linear relationship was observed between percent addition of raw or treated sewage to dilution water and algal growth response. This response was, in almost all instances, within ± 20 percent of the algal yields predicted by the nitrogen and phosphorus content. Neither the raw nor treated effluent was found to be toxic to the test alga. The N:P ratio was found to be a good first estimate for determining the limiting nutrient of a water sample. However, determination of nutrient limitation by algal assay technique was found to be more reliable, owing to the possible presence of algal toxicants, analytical errors in chemical determinations, and the range in N:P ratios for which co-limitation occurred. Algal assay corroberated nutrient limitation data predicted by N:P ratios in 13 out of 18 samples studied in this work. S. capricornutum was found to be a better test species than A. flos-aquae since the latter is difficult to enumerate, owing to its filamentous morphology, and because A. flos-aquae is able to fix atmospheric nitrogen. Guidelines were established for sampling stratified and unstratified lakes as well as streams and WWTP effluent. Seasonal variations in nutrient content and algal growth response in water samples emphasized the need for sampling a specific site at least four times per year. Guidelines for determining the concentrations of several water quality parameters both in situ and upon return to the laboratory were established.

The AA:BT was found to be an effective and reliable method for determining: a) the limiting nutrient of a water body, b) the presence of algal availabilility, and c) the sensitivity of a water to changes in its nutrient status attributable to nutrient additions from raw or treated wastewater. It is recommended that the AA:BT be used for regulatory purposes on a site-specific basis with only state operated or regulated laboratories performing the test to ensure honest and accurate results.

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INTRODUCTION

Discharge of anthropogenic wastes into the aquatic environment is of environmental concern because of resulting eutrophication or rapid aging of receiving waters. Efficient methods for predicting and assessing the impact of such wastes on a river or lake are necessary so that regulatory agencies can promulgate and enforce guidelines and requirements for such discharges and thereby delay, or prevent excessive nutrient enrichment of receiving waters.

Algae, being natural inhabitants of the aquatic environment, play an important role in establishing the trophic state of a river or lake. Algal photosynthesis, in addition to macrophytic photosynthesis and atmospheric reaeration, supplies oxygen into an aquatic ecosystem which is comprised of many organisms requiring this element in daily metabolic functions. Algae are also a critical component of the food web in the aquatic environment. However, when a river or a lake receives a surplus of phosphorus, nitrogen, and other nutrients, intensive algal blooms usually develop. The algal blooms may impart an unpleasant odor, liberate toxicants to the water, cause wide diurnal fluctuations in dissolved oxygen and pH levels and add considerably to the biochemical oxygen demand (BOD) of the sediment or, in the case of a lake, to the sediment and hypolimnion. Intensive algal respiration during the evening may result in considerable oxygen depletion. In grossly eutrophic waters, algal blooms may be followed by widespread algal death resulting in the resolubilization of the nutrients into the water, or sedimentation and accumulation of these components in the benthic environment. Dense populations of blue-green algae can additionally interfere with the recreational use of a water. Algal blooms have been associated with human health disorders, including contact dermatitis, symptoms of hay fever (Palmer, 1962), headaches, nausea, various gastrointestinal disorders, respiratory disorders, and eye inflammation (Mackenthan, 1973).

In order to control and regulate excessive nutrient discharges into natural waters, the Algal Assay:Bottle Test (AA:BT) has been suggested as a simple and reliable method to assess the impact of such loadings on the aquatic environment. Additionally, the high degree of correlation between the nitrogen and phosphorus content of natural waters and algal assay growth response has led investigators to interpret the N:P ratio as a useful preliminary assessment of algal growth limitation in natural waters. Placement into a nitrogen or phosphorus limitation category without actual assay analysis can be hazardous, however, owing to the possibility of other growth limiting constituents, the presence of inhibitory substances or the observed range in N:P ratios categorized by nitrogen and phosphorus co-limitation (Chiaudani and Vighi, 1976 and Weiss, 1976). Furthermore, the interpretation of

actual algal assay results and predicted yields based upon N:P ratios is highly dependent upon the reliability of the test procedure.

The AA:BT has been used by many investigators, such as those presented in a literature review by Leischman et al., (1979) to evaluate the nutritional status of a receiving water and to assess the need for, or the degree to which wastewaters must be controlled or treated to enhance water quality. The reliability of the AA:BT has been demonstrated by its repeated ability to accurately predict the effects of wastewater upon algal growth in natural waters and its ability to determine the primary limiting nutrient in receiving waters (Miller, et al. 1975; Miller, et al. 1974; Miller and Maloney, 1971; Maloney, Miller, and Blind, 1973; Greene, et al., 1975; Maloney, et al., 1971). One of the conclusions derived from these studies was that the assay is more appropriate for determining the availability of nutrients than the standard chemical analyses for nitrogen, phosphorus, and other growth promoting elements. The usefulness of the AA:BT has been recognized by several state governments, including Illinois, Pennsylvania and Virginia, who have either established state-run algal assay laboratories or have utilized U.S.E.P.A. laboratories for the assessment of the aquatic productivity using this approach.

The AA:BT is a biological analysis in which the green alga, Selenastrum capricornutum is grown in sample water plus various chemical additions, to determine the limiting nutrient of the water. The maximum cell biomass or maximum standing crop (MSC) of the alga, achieved after two to three weeks of incubation under defined conditions, is used in identifying the overall productivity of the water, and in defining the nutrient status of the sample. Growth rate is not used as the growth parameter in batch cultures because it is indirectly related to external nutrient concentrations. The MSC can also be compared to a growth value predicted from the analytically determined nutrient levels (phosphorus and nitrogen) in the samples. Such comparisons are used in evaluating the biological availability of nitrogen or phosphorus in test water, in determining the effects of inhibitory constituents in the water; and/or unreliable chemical analyses for orthophosphate and total soluble inorganic nitrogen (TSIN), as well as in identifying the presence of bioavailable organic nutrients. These effects are indicated when a test water fails to attain the expected correlation between the bioavailable nitrogen and phosphorus concentrations in the test water, with their chemically analyzed concentrations.

Algal cell biomass is the parameter used to describe the growth of the test alga, and is expressed as the dry weight of the alga per volume of culture solution. The MSC is obtained when the increase in cell biomass is less than five percent per day.

Several methods have been used in determining cell biomass including:

a. evaluation of mean cell volume, and cell numbers using a particle counter such as a ZBI Coulter Counter (Coulter Electronics Inc.);
b. cell enumeration using a hemecytometer;
c. direct gravimetric analysis;
d. determination of cell chlorophyll by fluorometric analysis; and
e. determination of spectrophotometric absorbance at a defined wavelength.

None of the latter alternatives are as accurate or expedient in determining the MSC of an algal culture as is the calculation of MSC from cell number and mean cell volume data determined by the Coulter Counter technique.

Selenastrum capricornutum is the algal species used in the AA:BT since it is easy to culture, ubiquitous in nature, and is a good indicator organism. The unicellular nature of S. capricornutum makes it conducive to cell enumeration and mean cell volume determination used in calculating the MSC of an algal culture.

Anabaena flos-aquae, a nuisance blue-green alga commonly found in eutrophic lakes in the summer time, has also been used in algal assays of water samples. However, the filamentous nature of this species makes biomass determination more difficult. As with S. capricornutum, a predicted MSC can be calculated based upon phosphorus limitation. Nitrogen limitation is not important, since this alga is able to fix atmospheric nitrogen. One advantage of using A. flos-aquae in addition to S. capricornutum as test alga in the AA:BT, is to observe varying levels of toxicity between the two algal species.

The protocol for the AA:BT involves the innoculation of S. capricornutum into water that has previously been autoclaved (to solubilize particulate nutrients and to destroy indigenous algae) and filtered through a 0.45 µm membrane filter to remove all particles including other algae. This is done to ensure that pure cultures of S. capricornutum are grown in order to avoid algal succession and accompanying die-off of individual species.

Nitrogen and phosphorus are taken up by S. capricornutum in a ratio of approximately 11.3:1. Miller, et al. (1978), defined a water to be nitrogen limiting for algal growth when the N:P ratio was less than 10:1, and phosphorus limiting for growth when the N:P ratio was greater than 12:1. Weiss (1976), however reported waters to be nitrogen limiting for algal growth when the N:P ratio was

less than 8:1, phosphorus limiting when N:P ratios were greater than 13:1, and phosphorus and nitrogen co-limiting when N:P ratios were between 9:1 and 12:1. Similarly, Chiaudani and Vighi (1976) found natural waters to be growth limiting by both nitrogen and phosphorus when N:P ratios were between 5:1 and 10:1, nitrogen limiting for growth below a ratio of 5:1, and phosphorus limiting for growth above a N:P ratio of 10:1. Given an excess of phosphorus and other nutrients and no toxicity, each mg N/L will support 38 mg ± 20 percent dry wt algae/L. A phosphorus limited system will result in 430 mg ± 20 percent dry wt algae/L per mg P/L. The values of 38 and 430 mg dry wt algae/L are referred to as the total soluble inorganic nitrogen and ortho-P yield factors, respectively. Therefore, by analyzing the components of inorganic nitrogen (NH_3^+, NO_3^- + NO_2^- = TSIN) and orthophosphate, one can predict the resulting MSC based upon the macronutrient limitation and these growth yield factors.

For example a sample having a N:P ratio of 2.0 would indicate nitrogen limitation. The expected standing crop of S. capricornutum could then be calculated using equation 1, assuming the absence of toxicants or micronutrient limitation.

$$\text{Predicted mass of algae in mg/L} = \text{TSIN (mg N/L)} \times 38 \frac{\text{mg dry wt algae/L}}{\text{mg N/L}} \pm 20\% \quad (1)$$

Similarly the expected standing crop of S. capricornutum could be calculated using equation 2, for waters that are phosphorus limiting.

$$\text{Predicted mass of algal in mg/L} = \text{orthophosphate concentration (mg P/L)} \times 430 \frac{\text{mg dry wt algae/L}}{\text{mg P/L}} \pm 20\% \quad (2)$$

The AA:BT test is used to further evaluate the nutrient limitation in natural waters by determining whether this limitation is attributable to nitrogen, phosphorus, or trace element deficiency. According to Liebig's Law of the Mininum, (Liebig, 1840) the essential nutrient in shortest supply relative to the needs of the algae, will limit growth when the concentration of that nutrient has been reduced to a level where it is no longer

utilizable by the algae. The MSC, then, is proportional to the concentration of the primary limiting nutrient, provided that no inhibitory chemicals are present.

Determination of nutrient limitation is accomplished by an experimental design which utilizes the growth response of S. capricornutum to singular and combined additions of nitrogen, phosphorus, micronutrients, and EDTA, to the test waters, to evaluate the interaction of nutrient dynamics with respect to aquatic productivity. The nutrients are added in amounts which drive the system to the secondary limiting macronutrient. For example, for a phosphorus limiting sample containing no toxicants, addition of excess phosphorus to the culture will stimulate algal growth and nitrogen will become the limiting nutrient.

The EDTA chelator is used for its ability to ensure trace element availability in the culture medium and to complex algal inhibitors which might suppress algal growth. For example, if an algal inhibitor is present at a toxic level in the test solution, then the EDTA addition will complex this component so that it will not exert its inhibitory effect on the algae. In waters having a low concentration of trace metals, EDTA complexation will enhance the availability of these elements, thereby making them more readily available to the algae.

The micronutrient addition is used to assess the possible cause for an increased growth response in the algal culture containing EDTA. Such a response could be attributable either to the presence of an algal inhibitor or the absence of some trace element. Algal cultures containing a micronutrient addition therefore will result in greater algal MSC values in samples where macronutrients (i.e. carbon, nitrogen, and phosphorus) are in plentiful supply, but trace elements are not. Comparison between the algal yields in water containing added micronutrients with those containing added EDTA, permits the investigator to distinguish between a trace element limitation and the presence of algal inhibitors.

Chemical analyses in conjunction with observed MSC values provide some additional information about the nutrient status of the test water. The biological availability of nitrogen and phosphorus in the test water can be calculated by dividing the MSC by either the total soluble inorganic nitrogen or ortho-P yield factors. The biological nitrogen availability is equal to the MSC obtained with the 0.05 mg P/L addition divided by 38 (equal to the TSIN yield factor). Alternatively, the biological phosphorus availability is equal to the MSC obtained with the 1.00 mg N/L addition divided by 430 (equal to the ortho-P yield factor). The bioavailable nitrogen and phosphorus may then be compared to concentrations of these nutrients determined by chemical analysis.

The fraction of inorganic phosphorus or nitrogen is another useful parameter in the interpretation of algal assay data. These are defined by equations 3 and 4:

$$\text{inorganic P fraction} = \frac{\text{ortho-P}}{\text{total-P}} \tag{3}$$

$$\text{inorganic N fraction} = \frac{NO_3^- + NO_2^- + NH_3}{\text{total organic nitrogen} + NO_2^- + NO_3^- + NH_3} \tag{4}$$

In summary, the AA:BT can be used to define and/or predict the nutrient availability in most natural waters, and can identify and/or predict the algal growth potential of natural waters. Such predictions are based upon both chemical analysis of the test water and the MSC data obtained by algal assay technique. These analyses provide such information as:

1. nitrogen to phosphorus ratio;
2. bioavailable phosphorus;
3. bioavailable nitrogen;
4. possible trace element limitation;
5. possible heavy metal toxicity;
6. possible presence of an algal growth inhibitor;
7. limiting macronutrient;
8. secondary limiting nutrient;
9. predicted MSC;
10. inorganic P fraction;
11. inorganic N fraction; and
12. possible utilization of organic nutrients.

OBJECTIVES

The overall objective of the study was to evaluate the AA:BT as a regulatory tool in promulgating and enforcing maximum nutrient discharges, contained in wastewater effluent, into natural waters. This included an evaluation of the AA:BT for its ability to determine the nutrient status of a water body, the receiving water's sensitivity to nutrient change resulting from the input of pollutional discharges, and the utility of the test in assessing the need for, and effectiveness of advanced wastewater treatment such as phosphorus removal using alum. Specific labor and cost requirements as well as the precision and accuracy of the test were also to be evaluated.

The specific objectives of the study were to:

1. develop practical laboratory techniques, using the AA:BT, for assessing the effects of a toxicant or nutrient input into natural waters;
2. develop an algal assay laboratory facility to be used for both research and monitoring purposes as part of the cooperative activities between the UMASS Civil Engineering Department and the Massachusetts Division of Water Pollution Control (MDWPC);
3. determine the costs, space, equipment and personnel requirements to perform routine algal assay analyses using the AA:BT;
4. determine sampling procedures and requirements for collecting river and lake water as well as WWTP effluent samples, including sampling type and frequency;
5. evaluate the AA:BT as a method for determining the limiting nutrient of a water body;
6. evaluate the AA:BT as a method for determining the presence of toxic or inhibitory chemicals in sample waters;
7. collect baseline data on selected sites in and near Massachusetts using the AA:BT;
8. evaluate the utility of the N:P ratio as a first estimate of the nutrient status of a water body;
9. evaluate the correlation between observed algal yields to predictions based upon the nitrogen and phosphorus content of the water;
10. identify those water quality parameters which influence the interpretation of algal assay data so that these parameters can be measured in the laboratory or at the time of sampling;
11. assess the ability of the AA:BT to determine the need for, and effectiveness of alum treatment, or other treatment methods, on reducing the phosphorus content of wastewater discharges;

12. examine the use of Anabaena flos-aquae as a test algal species; and
13. evaluate the AA:BT as a regulatory device to control nutrient discharges into natural waters.

ABSTRACTS OF LITERATURE REVIEWS RELATED TO ALGAL ASSAY TECHNIQUES AND RESEARCH

Two literature review papers were written by graduate students of the Environmental Engineering Program at UMASS/Amherst on subjects related to the objectives of the algal assay project. These reports (Marchaj, 1981: and Allain, 1981) were sent to the MDWPC in June of 1981 and are therefore not included, in their entirety, in this report. Copies of these papers can be obtained from the Environmental Engineering faculty at UMASS/Amherst. Additionally, a Master's project was completed by Austin (1982) on the Algal Growth Potential of Secondary Treated Wastewater Effluent, and was submitted to the MDWPC as a document, separate from this report. This project was an expansion of a topic submitted to the MDWPC previously, as a literature review in June, 1981. In view of the relevancy of these reports to the algal assay study, abstracts of the papers are presented here.

Exchange of Phosphorus in Lake Sediments with Emphasis on Chemical Effects

by

Dorothy Marchaj
June, 1981

The paper presents a review of literature concerned with the exchange of phosphorus between lake sediments and overlying water. Literature involved with the chemical parameters of the process is emphasized. Four research approaches to the study of exchange mechanisms are summarized: 1) predictions based on equilibrium calculations, 2) laboratory experimentation with actual sediments, 3) field studies, and 4) sediment fractionation procedures.

Phosphate exchange is controlled to a considerable extent by pH and oxidation conditions of the water. Substantial release of phosphorus can occur under both oxic and anoxic conditions depending on pH. The phosphate adsorption and precipitation reactions involve iron, aluminum, calcium and clay mineral constituents of the sediments. Evidence exists to indicate that the principal sediment constituent responsible for phosphate immobilization in the sediments is an amorphous iron oxide complex.

Different Approaches to Bio and Algal Assays Using Various Dilution Water

by

Mark Allain
June, 1981

The paper reviews, in tabular form, the methods used by various investigators in performing algal and bio assays. This abstract only includes the algal assay section of the paper. Various solutions have been used in studies involving the Algal Assay:Bottle Test. These include additions of inorganic nitrogen and phosphorus nutrients, EDTA, inorganic carbon, trace metals, singly and in combination, as well as varying additions of domestic, industrial or agricultural wastewater, or algal culture medium to either algal assay medium or environmental waters. A variety of water quality parameters have been monitored in conjunction with the AA:BT including: total carbon, total soluble inorganic nitrogen, total phosphorus, ortho-P, organic nitrogen, conductivity, soluble iron, zinc, silica, pH, temperature, organic carbon, calcium, magnesium, ammonia, trace metals, particulate

phosphorus, bacterial density and hardness. However nitrogen and phosphorus forms are the most commonly determined parameters. The studies involved the determination of one or more of the following subjects: nutrient limitation, algal growth stimulation by a wastewater, algal growth stimulation or inhibition by a specific chemical compound, varying algal growth potential above and below a wastewater outfall, presence of heavy metal algal toxicants, algal growth variability attributable to dilution water composition, algal species compatability, and the effects of varying pretreatment procedures for the dilution water on algal growth response. Several experimental procedures that have been used for determining the objectives described above are outlined. Algal test species include: Selenastrum capricornutum, Anabaena flos-aquae, Scenedesmus dimorphus, and Sphaerocystis. S. capricornutum is the most commonly used test alga.

Algal Growth Potential of Secondary Treated Wastewater Effluent

Master's Project by

Patricia E. Austin
January, 1982

The biostimulatory properties of secondary treated wastewater effluent collected from the Amherst, Massachusetts wastewater treatment plant were evaluated using an algal assay technique. Algal assays indicated that the algal growth potential of Amherst wastewater could be determined from the inorganic nitrogen and orthophosphate concentrations in the effluent. A chemical solution of the wastewater effluent, containing equivalent orthophosphate and inorganic nitrogen content, resulted in algal growth levels similar to those from direct effluent additions. Phosphorus removal by alum or lime treatment significantly decreased the algal growth in additions of both effluent and chemical equivalent solution to Mill River water.

METHODS AND MATERIALS

Algal assays were conducted using the general methods presented by Miller et al. (1978) with some modifications.

Sampling and Pretreatment

River samples were collected by grab sampling at either surface or mid-width, mid-depth locations, using either a standard type brass Kemerer water sampler (for depth sampling) or a plastic bucket (for surface sampling). Later sampling was conducted using a Van Dorn bottle sampler to preclude possible metal leaching (Cu) from the Kemerer sampler. Composite samples for lakes and impoundments were collected by combining water samples from specified depths or by submerging 0.64 cm tygon tubing (6.0 m) vertically through the water column and then lifting it carefully out after sealing the upper tubing opening to prevent water leakage. Wastewater treatment plant effluent was collected as a 24 hour time integrated (hourly) sample using an ISCO wastewater sampling device (Instrumentation Specialty Co., Lincoln, Nebraska). A description of the types of samples collected at the various field locations are shown in Table 1. All samples were transported in one gallon, pre-acid-washed glass bottles. Samples were placed on ice immediately after collection. Approximately three liters of water were collected at river and lake sites. The volume of WWTP effluent sampled varied with the needs of each experiment.

Upon return to the laboratory, river and lake water samples were transferred into two liter Erlenmyer flasks. Two and one-half liter, and 0.5 liter portions were autoclaved and left unautoclaved, respectively. Autoclaving was performed for 30 minutes at 121°C. The autoclaved samples were cooled and then purged with a one percent CO_2: 99 percent air mixture to bring the solutions to equilibrium with atmospheric pressure. Equilibrium was considered complete when a stable pH value was achieved. The pH of the solution was then adjusted to the in situ pH value of the lake or river using 0.5 N hydrochloric acid or 0.5 N sodium hydroxide.

Autoclaved and unautoclaved samples were filtered through Whatman glass fiber filters and then through 0.45 micron membrane filters to remove indigenous algae and other particles from the solution. *WWTP effluent samples were filtered but not autoclaved* since many organic compounds present in sewage are unstable at high temperature. Autoclaving might therefore have biased the algal growth response.

Chemical analyses were performed for all constituents usually within 48 hours after return to the lab. Samples not analyzed

Table 1
Description of Samples Collected at Various Field Locations

Site	Date	Water Body	Type of Sample	Total Depth of Water Body (meters)	Sampling Intervals
Spy Pond, Arlington, MA	4/3/81	Lake	composite	10	Sample taken with 6.0 m tygon tube
Flint Pond, Worcester, MA	4/3/81	Lake	composite	4.5	1 meter
Lake Quinsigamond I, Worcester, MA	4/3/81	Lake	composite	27	5 meters
Lake Quinsigamond II, Worcester, MA	4/3/81	Lake	composite	20.5	3 meters
Quaboag Pond, Brookfield-E. Brookfield, MA	5/8/81	Lake	composite	3.0	1 meter
Spencer WWTP Effluent, Spencer, MA	5/8/81	WWTP effluent	24 hr composite	NA*	24 hour
Housatonic River					
Bulls Bridge, Kent, CT	6/9/81	River	surface	1-1.5	
Bulls Bridge, Kent, CT	7/14/81	River	mid-depth	4.6	
Bulls Bridge, Kent, CT	8/11/81	River	mid-depth	4	
Andrus/Ranapo Rd. Sheffield, MA	6/8/81	River	surface	2	
Andrus/Ranapo Rd. Sheffield, MA	7/14/81	River	mid-depth	2.3	
Andrus/Ranapo Rd. Sheffield, MA	8/11/81	River	surface	1	
Lanesville Rd. Bridge, New Milford, CT	6/9/81	River	surface	11	

Table 1, continued

Site	Date	Water Body	Type of Sample	Total Depth of Water Body (meters)	Sampling Intervals
Lanesville Rd. Bridge, New Milford, CT	7/14/81	River	surface	8.5	
Lanesville Rd. Bridge, New Milford, CT	7/14/81	River	mid-depth	8.5	
Lanesville Rd. Bridge, New Milford, CT	8/11/81	River	mid-depth	7	
Holmes Rd. Bridge, Pittsfield, MA	11/3/81	River	mid-depth	--	
Pittsfield WWTP Effluent, Pittsfield, MA	11/2/81	WWTP	24-hr composite	NA*	

*Not applicable.

within this time period were stored in the dark at 4°C. All samples were analyzed within one week of sampling. The analytical methods used to measure the various water quality parameters are presented in Table 2.

Algal Assays

Algal assays were performed on the aqueous samples according to the objectives of the experimental design. Several different types of algal growth experiments were performed. These included: 1) determination of the specific weight of Selenastrum capricornutum, 2) determination of the limiting nutrient or presence of a toxicant, 3) WWTP effluent addition experiments, and 4) alum treatment of WWTP effluent. In all of these studies S. capricornutum was used to assess the growth potentials of various test solutions. Growth was considered complete when the MSC increase was less than five percent per day. This was usually observed after 14-18 days of growth. The last three experimental procedures were designed to address the utility of the algal assay as a method for assessing the nutrient status of a water body or resulting change in nutrient status following a pollutional input or chemical treatment method. The specific weight determination was a prerequisite step needed for converting cell counts to cell biomass.

Determination of the Specific Weight of Selenastrum capricornutum

Cell biomass, expressed as maximum standing crop (MSC) is the parameter used in all of the algal assay experiments to determine the growth of the test alga under specific test conditions. Cell biomass could have been determined for each sample by drying and weighing. However, this would have been extremely time consuming and tedious. A ZBI Coulter Counter was therefore employed. This instrument determines the total cell number and mean cell volume of algae in a given sample. These values can be directly converted to biomass if the specific weight coefficient, SWC, of the test alga is known, according to the equation:

$$\text{Total dry algal weight in mg/L} = \text{total cell number} \times \text{mean cell volume} \times \text{specific weight coefficient} \quad (5)$$

A procedure was developed to determine the SWC so that future Coulter Counter readings could be directly converted to cell biomass without actual drying and weighing.

Table 2

Methods for Determining Water Quality Parameters

Parameter	Method	Reference
Ammonia-N	Scaled down colorimetric determination using indophenol reaction	Ram, 1979
Total Organic Nitrogen	Micro/Kjeldahl nitrogen digestion of sample followed by indophenol colorimetric determination	Ram, 1979
Nitrate-N	Cadmium Reduction Method	EPA, 1979
Nitrite-N	Cadmium Reduction Method	EPA, 1979
Orthophosphate	Heteropoly Blue-Ascorbic Acid Spectrophotometric Method	Strickland and Parsons, 1972
Total Phosphorus	Potassium persulfate digestion followed by Heteropoly blue-ascorbic acid spectrophotometric determination	APHA, 1980 EPA, 1979 Strickland and Parsons, 1972
Dissolved Oxygen*	Azide modification of the Winkler Method	APHA, 1980
pH*	Hach Kit or pH meter	-

*Field Measurement

Four 500 ml Erlenmyer flasks each containing 250 ml of algal nutrient medium (Table 3) were innoculated from an S. capricornutum culture to give a final concentration equal to 1000 algal cells/ml, and incubated under continuous illumination with shaking. The algal innoculum was prepared from five to nine day old cultures of S. capricornutum, which had been centrifuged two times and resuspended in filtered (0.45 micron membrane filter) distilled water after each centrifugation. The suspended cells were diluted with filtered distilled water to achieve a concentration of approximately 51,000 cells/ml. Temperature was maintained at $24^{\circ}C \pm 2^{\circ}$. Lighting was kept constant at 400 ft-candles for 24 hours per day. The culture flasks were placed on a shaker table at 100 oscillations per minute to keep the cells in suspension and maintain equilibrium with atmospheric gases.

On days five through nine, 20 ml aliquots of algal culture were withdrawn from each flask using acid-washed, autoclaved, volumetric pipets. The aliquots were then filtered through 0.60 micron membrane polyvic filters (Millipore Co.). The weight of these filters had previously been determined after oven drying for at least two hours at $65^{\circ}C$, followed by placement in a dessicator for one hour. Following filtration, the filters were oven dried again, placed in a dessicator for an hour, and reweighed. The net weight gain was equal to the algal dry weight (in grams) per 20 ml of solution.

Samples were concurrently analyzed with the Coulter Counter to determine cell numbers and mean cell volume (MCV). The specific weight coefficient was then calculated by the equation:

$$\begin{array}{lcl} \text{Specific Weight} & & \\ \text{coefficient, SWC} & = & \text{dry weight} \times (\text{Cell count})^{-1} \\ (\text{mg}/\mu\text{m}^3) & & \text{of algae} \quad (\text{cells/ml}) \\ & & (\text{mg/L}) \end{array}$$

$$\times \frac{(\text{MCV})^{-1}}{(\mu\text{m}^3/\text{cell})} \times 10^{-3}\,\text{L/ml} \qquad (6)$$

Knowing the specific weight coefficient, the maximum standing crop in mg dry weight per liter could then be calculated using equation 1, for subsequent algal assay experiments, by simply determining cell number and MCV using the Coulter Counter.

Table 3

Components of Algal Growth Medium[1]

Compound	Concentration (µg/L)	Element	Element Concentration (µg/L)
$NaNO_3$	25,500	N	4,200
K_2HPO_4	1,044	P	186
$NaHCO_3$	15,000	C	11,001
$Na_2EDTA \cdot 2H_2O$	300.000	–	–
Micronutrients[2]			
$CaCl_2 \cdot 2H_2O$	4,410	Ca	1,202
H_3BO_3	185.520	B	32.460
$MnCl_2 \cdot 4H_2O$	415.610	Mn	115.374
$FeCl_3 \cdot 6H_2O$	160.000	Fe	33.051
$NaMoO_4 \cdot 2H_2O$	7.260	Mo	2.878
$ZnCl_2$	3.271	Zn	1.570
$CuCl_2$	0.012	Cu	0.004
$CoCl_2 \cdot 6H_2O$	1.428	Co	0.354
$MgSo_4 \cdot 7H_2O$	14,000	S	1,911
$MgCl_2 \cdot 6H_2O$	12.164	Mg	2,904

1. Taken from Miller et al., (1978)
2. Chemical components used in both algal growth medium and micronutrient additions.

Nutrient Limitation/Toxicity Assessment Studies.

Several studies were performed on surface water samples to evaluate algal assay monitoring as a method for determining the limiting nutrient (nutrient in relatively shortest supply that will limit growth) of a water body, and the water body's sensitivity to change in nutrient status. These determinations were made in order to evaluate the utility of the AA:BT in predicting the decreased algal growth attributable to nutrient removal by advanced wastewater treatment and the change in nutrient status resulting from such treatment. Nutrient limitation studies were therefore performed at a number of sites including: the Housatonic River, in Western Massachusetts and Connecticut, Quaboag Pond, Brookfield-East Brookfield, MA, Lake Quinsigamond and Flint Pond, Worcester, MA, and Spy Pond, Arlington, MA. Later studies examined the Housatonic River in greater detail to obtain a more complete evaluation of this river with respect to its nutrient limitation status.

Nutrient limitation studies were performed using autoclaved, filtered water. Autoclaving followed by filtration is the recommended pretreatment for nutrient limitation studies. Autoclaving is included to destroy indigenous algae and to solubilize some of the particulate matter which could become available to algal growth (Miller, et al., 1978). Such autoclaving may however volatilize or hydrolyze certain algal inhibitors if present in the water sample. Therefore, an algal assay was additionally performed on three replicates of unautoclaved samples containing no other additions, to observe these possible effects.

Fifty ml aliquots were transferred to 125 ml acid washed Erlenmyer flasks. The flasks had previously been rinsed with filtered distilled water and autoclaved. One ml portions of 2.55 mg P/L stock phosphorus solution, 51.00 mg N/L stock nitrate solution, 51.00 mg/L stock $Na_2EDTA \cdot 2H_2O$ solution and stock micronutrient solution were added singly or in combination to give the final concentrations shown in Table 4. The contents of the micronutrient solution were presented previously in Table 3. The one milliliter volumes were delivered using Eppendorf pipettes.

Algal cells were inoculated into the flasks according to the procedure described previously, and incubated under continuous illumination with shaking. Temperature and light illumination were maintained as discussed earlier.

The flasks were incubated for 14 to 21 days to reach the algal MSC. Achievement of the MSC was assumed when the algal specific growth rate was less than 5 percent per day. Cell

Table 4

Nutrient Additions Used in Determining Algal Assay Nutrient Limitation*

Control
Unautoclaved Control
Control + 0.05 mg P/L
Control + 1.00 mg N/L
Control + 1.00 mg N + .05 mg P/L
Control + 1.00 mg $Na_2EDTA \cdot 2H_2O$/L
Control + 1.00 mg $Na_2EDTA \cdot 2H_2O$/L + .05 mg P/L
Control + 1.00 mg $Na_2EDTA \cdot 2H_2O$/L + 1.00 mg N/L
Control + 1.00 mg $Na_2EDTA \cdot 2H_2O$/L + 1.00 mg N/L + 0.05 mg P/L
Control + trace metals

*Resultant concentration after 1 ml innoculum into 50 ml solution.

enumeration and mean cell volumes were determined using a Coulter Counter Model ZBI. Algal yield or MSC was then calculated.

Trace element or micronutrient limitation was determined using two approaches. 1.0 ml of the micronutrient solution comprised of the metals shown in Table 4 was added to three replicate flasks. If a trace element limitation existed, samples containing the trace element exhibited a greater MSC than the control.

Micronutrient limitation was also determined by 1.0 ml additions of 51.00 mg/L $Na_2EDTA \cdot 2H_2O$ to 50 ml water samples (final concentration equal to 1.00 mg $Na_2EDTA \cdot 2H_2O$/L). EDTA, at this concentration, complexes trace elements in solution thereby making them available for algal growth. Additionally, EDTA complexes toxic concentrations of algal inhibitors, and reduces the deleterious effect of such substances on algal growth. The presence of algal inhibitors or trace element limitation is indicated in samples containing EDTA which demonstrate a greater MSC of S. capricornutum than the control. Differentiation between the presence of algal inhibitors and trace element limitation can then be evaluated using Table 5.

Secondary Wastewater Treatment Plant Effluent Addition Experiments

The effect of WWTP effluent on the aquatic productivity of a receiving water was evaluated, using the Algal Assay:Bottle Test, by determining the response of S. capricornutum to varying dilutions of WWTP effluent. Additionally 1.00 mg $Na_2EDTA \cdot 2H_2O$/L was added to replicate sets of effluent additions to evaluate the possible presence of toxicants in the WWTP effluent. Unautoclaved, filtered effluent samples were used for such studies. Effluent additions ranging from 0 to 100 percent were used. The treatments of the replicate samples used in these studies are presented in Table 6.

The samples were innoculated with S. capricornutum cells and then incubated under continuous illumination according to the procedures described previously. MSC was determined after incubation for 14 to 21 days.

Alum Treatment of WWTP Effluent

Several studies were performed on alum ($Al_2(SO_4)_3 \cdot 18H_2O$) treated wastewater effluent in order to assess the effectiveness of such treatment on aquatic productivity. The response of S. capricornutum was used to monitor the decrease in algal productivity resulting from the precipitation and removal of

Table 5
Differentiation Between Algal Inhibitors and
Trace Metal Limitation

Treatment	Response	Interpretation
1 Control + EDTA	MSC > Control limitation	Algal inhibitor present or trace element
1a Control + Micronutrients	MSC > Control	Trace element limitation
1b Control + Micronutrients	MSC = Control	Algal inhibitor present

Table 6

Additions Used in WWTP Effluent Experiments

Control: dilution water only*
Control + varying percentage of untreated effluent
Control + effluent + 1.0 mg $Na_2EDTA \cdot 2H_2O$/L

*Dilution water was usually collected upstream from the wastewater outfall. Alternatively an unpolluted surface or ground water may be used.

phosphorus from effluent samples. Such assays could then be used to determine the need for, or the effectiveness of alum treatment as an advanced wastewater treatment procedure.

Alum was added to WWTP effluent in an Aluminum:Phosphorus molar ratio sufficient to reduce the phosphorus concentration to approximately 1.0 mg P/L. This is the phosphorus level frequently found in alum treated, secondary wastewater effluent. Typical alum dosage requirements for various levels of phosphorus removal have been reported and are shown in Table 7. The Al:P molar ratio needed to reduce the phosphorus level in the effluent to 1 mg/L is dependent upon a number of factors including pH, metal concentration, alkalinity, and hardness. This necessitates the use of a preliminary jar test to determine the Al:P application ratio needed to effect the desired degree of treatment.

Jar tests were therefore performed using 0, 0.5, 1.0, and 1.5 Al:P molar ratios of alum. One, two, or three mls of alum stock solution (9.72 g $Al_2(SO_4)_2 \cdot 18H_2O$/L) were added to 500 ml of WWTP effluent, mixed at 100 rpm for 30 seconds followed by a slow mix period of 20 minutes at 20 rpm (Martel, et al., 1974). The supernatant was then filtered through a 0.45 µm membrane filter followed by phosphorus determination. The residual phosphorus concentration was then determined and plotted against the Al:P molar ratio. The Al:P ratio resulting in a final phosphorus level of 1 mg/L was evaluated graphically.

The effect of alum treated effluent on the aquatic productivity of a dilution water was evaluated using the AA:BT by determining the response of S. capricornutum to varying dilutions of the treated effluent. The same procedure outlined in the previous section was followed. Phosphorus and nitrogen levels, both before and after treatment, were determined to evaluate the percent removal of these nutrients from the effluent attributable to the alum treatment. Additionally, 1.00 mg $Na_2EDTA \cdot 2H_2O$/L additions were added to replicate samples to evaluate the possible presence of algal inhibitors in the treated WWTP effluent. Both the treated and untreated effluent addition experiments were performed to determine:

1. the level of improvement in water quality resulting from reduction of WWTP effluent phosphorus loading;
2. the bioavailable phosphorus content in both the raw and treated wastewater;
3. possible shifts in nutrient limitation arising from the phosphorus and nitrogen levels in the receiving water relative to those in the treated and untreated WWTP effluent;
4. the percent contribution of nitrogen and phosphorus in the receiving water relative to the WWTP effluent

Table 7

Typical Alum Dosage Requirements
for Various Levels of Phosphorus Removal*

Phosphorus Reduction, %	Molar Ratio of Al:P Range	Typical
75	1.25:1-1.5:1	1.4:1
85	1.6:1-1.9:1	1.7:1
95	2.1:1-2.6:1	2.3:1

*Taken from Metcalf and Eddy,1979, Wastewater Engineering, p. 747.

5. the resultant eutrophic status of the receiving water after treatment; and
6. the effectiveness of established effluent guidelines in preventing nutrient enrichment, and aquatic weed proliferation in receiving waters.

EQUIPMENT AND PERSONNEL NEEDED FOR THE ALGAL ASSAY:BOTTLE TEST

Costs and personnel needs are an important consideration for using the AA:BT as a regulatory device and research tool. Tables 8 and 9 summarize the equipment and labor requirements, respectively, for preparing and conducting algal assays.

The equipment listed in Table 8 represents a minimal requirement for conducting reliable, efficient and accurate algal assays. Some substitutions can be made in certain instances in accordance with preference and equipment availability. Additionally, some alternative apparatus may improve the accuracy and efficiency of the AA:BT, but with added expense. For example, a specific ion auto analyzer with several electrodes may be purchased for approximately $2,000 (1981 cost). Specific ion electrodes are available for the analyses of orthophosphate, ammonia, and nitrate. Also a higher grade research spectrophotometer would increase the accuracy and precision of the various water quality analyses.

Personnel needs are difficult to estimate with any great precision since these needs will be dependent upon the capabilities of the personnel involved. Table 9 presents estimates of the person hours needed to perform a variety of water quality analyses, by graduate students in the Environmental Engineering Program at UMASS/Amherst. The students were relatively inexperienced prior to initiating this work. Their work proceeded at a non-rushed pace. The labor requirement for some of the analyses may be shortened somewhat using a specific ion auto analyzer. However, use of the specific ion auto analyzer on aqueous samples containing a large number of chemical constituents at elevated concentrations (such as in a WWTP effluent) is not recommended because of possible chemical interferences.

Table 8
Equipment Costs for Conducting the Printz
Algal Assay Bottle Test

Item	Specifications	Cost[1]	Vendor[2]
General Supplies and Apparatus			
Water sampler	non-metallic, Van Dorn bottle	300	WILECO
Sample bottles	glass, polypropylene or polyethylene capable of containing a total of 4 liters	100	Fisher Sci. Co.
CO_2-air tank and regulator	1% CO_2-99% Air	100	Merriam-Graves Corp.
filtering apparatus	for use with 142 mm glass fiber filters and 47 mm membrane filters	100	Millipore Corp.
filters	142 mm medium porosity glass fiber filters 47 mm diameter 0.6 µm polyvic filters	200/25 assays	Millipore Corp.
chemicals	certified ACS reagents for nutrient additions and analysis of NO_3^-, NH_3, TON, ortho-phosphate and total phosphorus	1,000	Fisher Sci. Co.
assorted glassware	10, 100 ml volumetric flasks 3,50 ml graduated cylinders		

Table 8, continued

Item	Specifications	Cost[1]	Vendor[2]
assorted glassware, cont.	4,50 ml centrifuge tubes 4 each of 0.5,1.0,2.0, 3.0,5.0,20.0 and 25.0 ml volumetric pipets 5, 50 ml beakers 60,50 ml test tubes with caps and rack 30,10 ml test tubes with caps and rack and 100, 125 ml Erlenmyer culture flasks	400	Fisher Sci. Co.
culture flask stoppers	foam plugs that permit gas exchange but prevents contamination	200/6 assays	VWR
lighting	must provide 400 ft-candles ± 10% (lighting to 3 shaker tables which hold a total of 360 flasks	80	
WWTP effluent sampler	samples one liter/hr/day	1,550	
Equipment			
centrifuge[3]	capable of 1000 RPM	330	Fisher Sci. Co.
balance[3]	capable of reading to fourth decimal place	2,600	Fisher Sci. Co.
constant temp. room[3]	must provide 24°C ± 2° constant temperature shaker bath or constant temperature room needed	-	Fisher Sci. Co.
shaker table	capable of 100 trips/ minute and able to carry 120, 125 ml Erlenmyer flasks	600	Fisher Sci. Co.

Table 8, continued

Item	Specifications	Cost[1]	Vendor[2]
pH meter[3]	range of 0-14 pH units ± 0.1 units	500	Fisher Sci. Co.
Coulter Counter with computer[4]	capable of enumerating algal cells and evaluating MSC[3]	12,000	Coulter Electronics
spectrophotometer[3]	must read in the range 400-900 nm	800	Fisher Sci. Co.
microscope[3]	for identification of algal cultures and use with hemecytometer	4,000	Fisher Sci. Co.
hemecytometer	used to determine cells/ml	50	Fisher Sci. Co.
autoclave[3]	used for sterilization and other lab procedures	3,000	Fisher Sci. Co.
5 repipets	volumes = 0.1,0.2,0.5, 10.0 and 50.0 mls (used for nutrient additions and dilution of algal cultures for Coulter Counter readings)	600	VWR
oven[3]	capable of 120°C	800	Fisher Sci. Co.
incubator[3]	capable of 65°C (used for dry polyvic filters)	600	Fisher Sci. Co.
refrigerator[3]	capable of 4°C (used for chemicals, culture stocks, and sample water)	200	Fisher Sci. Co.
dessicator[3]	used for oven dried filters and chemicals	100	Fisher Sci. Co.

Table 8, continued

Item	Specifications	Cost[1]	Vendor[2]
Light meter (GE 214)	used to measured 300-500 ft/candles	50	Fisher Sci. Co.
	TOTAL:.....	30,260[5]	

1. 1981 dollars.
2. See Appendix A for dealer addresses.
3. Apparatus and equipment owned by the Environmental Engineering laboratory at UMASS/Amherst prior to receiving funding from MDWPC Project Number 80-32.
4. A fluorometer for use in chlorophyll analyses may be substituted for the Coulter Counter. Although the cost of the fluorometer (~$2500) is substantially less than the Coulter Counter it estimates cell biomass less accurately and has greater labor requirements than does the Coulter Counter. Additionally it is not amenable to as wide a variety of research applications as the Coulter Counter.
5. Cost does not include: constant temperature room.

Table 9

Personnel Needs for Conducting the Printz Algal Assay:Bottle Test on One Water Sample from a River, Lake, or Wastewater Effluent[1]

Task	Person Hours
1. Sampling[2]	
Presurvey of site and planning	8
Sampling	8
2. Preparation	
Autoclaving	1
CO_2-air equilibration + pH adjustment	0.5
Filtering	2.5
Glassware washing and autoclaving	7.5
Dispensing 50 ml aliquots into culture flasks	1
Preparation of algal innoculum	2
Preparation of algal nutrient medium	1
Preparation of chemical additions	1.5
Addition of chemical spikes into culture flasks	1
3. Analyses and Calculations	
Orthophosphate	2.5
Total phosphorus	5.5
Nitrite-nitrogen	3
Nitrate-nitrogen	5.5
Ammonia-nitrogen	3
Coulter Counter (biomass determination for 30 flasks)	12
4. Results	
Data calculations and tabulation	20
Administrative and secretarial hours	4
TOTAL (excluding sampling)	73.5

1. Additional samples will increase the time requirements by about 60 percent per sample excluding sampling requirements.
2. Estimated time requirement. Amount may vary above or below this value as a function of location requirements, and distance from the lab.
3. Water quality parameter time estimates are based upon wet chemical methods. The use of a specific ion electrode would decrease these requirements to some extent.

RESULTS AND DISCUSSION

General

Water quality and meteorological data, collected at river and lakes sites (Figure 1), by the Massachusetts Division of Water Pollution Control (MDWPC) are presented in Table 10 and 11 respectively. Additional water quality and algal assay results determined upon return to the laboratory are summarized in Tables 12 and 13, respectively. It should be noted that the pH values of the samples prior to 11/2/81 were determined in the field using a Hach Kit Model 17N, and immediately upon return to the laboratory with an Accumet 140 pH meter. The pH determined in the laboratory was generally one to two pH units lower than that determined in the field (see Table 10). This was partially attributable to microbial respiratory CO_2 production. However, the discrepancy between the measured pH values was also attributable to the inaccuracy of the Hach pH determination in the field. A more accurate, electronic, portable pH meter is therefore recommended for future field determinations.

The pH values determined in the laboratory and shown in Table 10 were used for conducting the algal assay tests. The observed one to two pH unit discrepancy would not effect the assay results to a significant extent (Miller, et. al., 1978).

The Algal Assay:Bottle Test was used to define the nutrient limitation of water samples collected from several Massachusetts water resources including: Lake Quinsigamond, Worcester, MA, Flint Pond, Worcester, MA, Spy Pond, Arlington, MA, Quaboag Pond, Brookfield-E.Brookfield, MA, and the Housatonic River. The bioavailability of nutrients in wastewater effluent from the Spencer and Pittsfield WWTP facilities were also evaluated using this method. Locations of these sites are shown in Figure 1. As discussed previously the growth response of S. capricornutum arising from additions of nitrogen, phosphorus, micronutrient, and EDTA additions, singly and in combination, was used to ascertain the limiting nutrient(s) of the sample. Actual and predicted yields (mg dry wt S. capricornutum/L) for these additions to the 16 samples collected during this study are presented in Table 13.

A statistical approach was necessary to identify the algal growth response in a culture containing a certain chemical addition which is different, at some assigned statistical confidence level, from growth responses in algal cultures containing other chemical additions. Additionally, a statistical approach was used to determine the level of agreement between observed algal growth and that predicted by chemical analysis of the inorganic nutrient level of the test water. These statistical comparisons were determined using one of two approaches. The first method, taken from Miller et al. (1978) considered algal yields to be statistically equal to

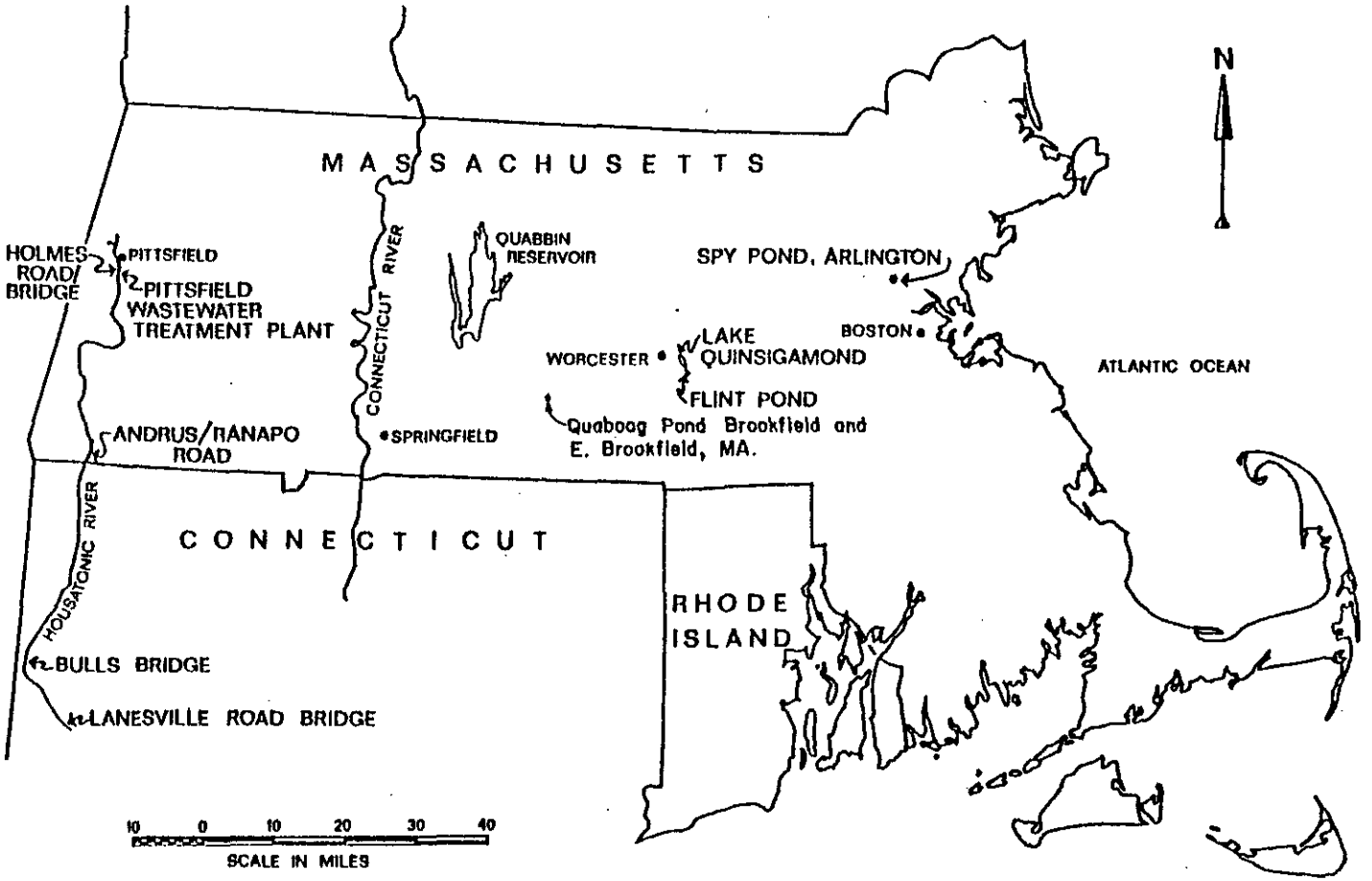

Figure 1. Map of sampling locations in Massachusetts and Connecticut.

Table 10
In situ Water Quality Data for Massachusetts and Connecticut Sampling Sites

Site	Date	Field pH[1]	Laboratory pH[2]	Dissolved Oxygen (mg/L)	Temperature (° C)
Site					
Spy Pond, Arlington, MA	4/3/81	8.0	-	10.4	9.5
Flint Pond, Worcester, MA	4/3/81	8.0	-	10.3	10.2
Lake Quinsigamond I, Worcester, MA	4/3/81	8.0	-	11.7	7.0
Lake Quinsigamond II, Worcester, MA	4/3/81	8.0	-	12.1	7.0
Quaboag Pond, Brookfield-E.Brookfield, MA	5/8/81	8.0-8.5	6.9	9.8	14.0
Spencer Secondary WWTP Effluent, Spencer, MA	5/8/81	-	-	0.0	15.0
Housatonic River					
Bulls Bridge, Kent, CT	6/9/81	9.5	8.2	7.3	21.0
Bulls Bridge, Kent, CT	7/14/81	9.5	8.1	7.4	25.0
Bulls Bridge, Kent, CT	8/11/81	10.1	9.05	9.7	24.0
Andrus/Ranapo Rd., Sheffield, MA	6/9/81	9.0	7.7	7.5	20.0
Andrus/Ranapo Rd., Sheffield, MA	7/14/81	9.5	8.45	9.6	23.9
Andrus/Ranapo Rd., Sheffield, MA	8/11/81	9.5	8.3	7.5	24.0

Table 10, continued

Site	Date	Field pH[1]	Laboratory pH[2]	Dissolved Oxygen (mg/L)	Temperature (° C)
Lanesville Road Bridge, New Milford, CT	6/9/81	9.5	8.15	9.5	21.0
Lanesville Road Bridge (surface), New Milford, CT	7/14/81	9.5	8.30	9.0	26.6
Lanesville Road Bridge (mid-depth), New Milford, CT	7/14/81	9.5	8.25	8.3	26.1
Lanesville Road Bridge, New Milford, CT	8/11/81	9.5	8.71	7.0	23.0
Holmes Road Bridge, Pittsfield, MA	11/3/81	7.7	7.7	-	7.8
Pittsfield Secondary WWTP, Pittsfield, MA	11/2/81	7.5[3]	7.5	-	-

1. pH determined in situ with Hach Kit Model 17N except as noted.
2. pH determined with a pH meter Accumet 140A immediately upon return to the laboratory.
3. pH determined at Pittsfield WWTP laboratory.

Table 11

Meterological Conditions at the Time of Sample Collection

Site	Date	Condition of Water Surface	Air Temperature (^{o}C)	Wind Speed (Km/hr)	Cloud Cover (%)
Spy Pond, Arlington, MA	4/3/81	Choppy	8.0	32	80-90
Flint Pond, Worcester, MA	4/3/81	Choppy	4.0	24-40	85
Lake Quinsigamond I, Worcester, MA	4/3/81	Choppy	6.0	24-40	85
Lake Quinsigamond II, Worcester, MA	4/3/81	Choppy	6.0	24-40	85
Quaboag Pond, Brookfield-E.Brookfield, MA	5/7/81	Choppy	7.0	24-40	0
Housatonic River					
Bulls Bridge, Kent, CT	6/8/81	Calm	-	0	100 (rain)
Bulls Bridge, Kent, CT	7/14/81	-	71	15	50
Bulls Bridge, Kent, CT	8/11/81	-	21	0	21 (hazy)
Andrus/Ranapo Road, Sheffield, MA	6/9/81	Rippled	-	10-15	100
Andrus/Ranapo Road, Sheffield, MA	7/14/81	Choppy	67	25	20

Table 11, continued

Site	Date	Condition of Water Surface	Air Temperature (^{o}C)	Wind Speed (Km/hr)	Cloud Cover (%)
Andrus/Ranapo Road Sheffield, MA	8/11/81	-	22	0	0 (hazy)
Lanesville Road Bridge, New Milford, CT	6/9/81	Ripples	-	<5	-
Lanesville Road Bridge, New Milford, CT	7/14/81	Calm	75	8	1
Lanesville Road Bridge, New Milford CT	8/11/81	-	19	0	0 (hazy)
Holmes Road Pittsfield, MA	11/8/81	-	-	-	-

Table 12

Water Quality Data (mg/L) for Massachusetts and Connecticut Sampling Sites

Site	Date	Type of Sample[2]	Parameter[1] NO_3^--N	NO_2^--N	NH_3-N	TSIN	TON	Ortho-P	Total-P	N:P
Spy Pond Arlington, MA	4/3/81	A	0.551	0.000	0.180	0.731	0.348	0.011	0.047	66.4
Flint Pond Worcester, MA	4/3/81	A	0.169	0.000	0.031	0.200	0.356	0.016	0.040	12.5
Lake Quinsigamond Station I, Worcester, MA	4/3/81	A	0.544	0.000	0.040	0.584	0.347	0.080	0.098	7.3
Lake Quinsigamond Station II, Worcester, MA	4/3/81	A	0.460	0.013	0.075	0.548	0.207	0.089	0.112	6.2
Quabog Pond Brookfield-E.Brookfield, MA	5/8/81	A	0.041	0.012	0.022	0.075	0.539	0.045	0.054	1.7
Spencer Secondary WWTP Effluent, Spencer, MA	5/8/81	B	1.827	0.019	3.969	5.815	1.323	3.018	3.056	1.9
Housatonic River										
Bulls Bridge, Kent, CT	6/9/81	C	1.320	0.000	0.083	1.403	0.456	0.041	0.109	33.4
Bulls Bridge, Kent, CT	7/14/81	D	0.001	0.006	0.009	0.016	0.639	0.027	0.066	0.6
Bulls Bridge, Kent, CT	8/11/81	C	0.119	0.000	0.016	0.135	0.375	0.017	0.040	7.9

Table 12. continued

Site	Type of Date	Sample[2]	Parameter[1] NO_3^--N	NO_2^--N	NH_3-N	TSIN	TON	Ortho-P	Total-P	N:P
Andrus/Ranapo Road, Sheffield, MA	6/9/81	C	0.830	0.010	0.060	0.900	0.602	0.082	0.171	11.0
Andrus/Ranapo Road, Sheffield, CT	7/14/81	D	0.005	0.004	0.016	0.025	0.676	0.047	0.104	0.5
Andrus/Ranapo Road, Sheffield, CT	8/11/81	C	0.820	0.013	0.007	0.840	0.472	0.093	0.135	9.0
Lanesville Road New Milford, CT	7/14/81	D	0.081	0.014	0.016	0.111	0.844	0.027	0.068	4.1
Lanesville Road New Milford, CT	7/11/81	D	0.331	0.027	0.062	0.430	0.313	0.024	0.053	17.5
Lanesville Road New Milford, CT	6/9/81	C	2.175	0.030	0.181	2.386	0.426	0.048	0.090	49.7
Lanesville Road New Milford, CT	7/14/81	C	0.087	0.014	0.013	0.124	0.389	0.015	0.069	5.0
Holmes Road Bridge Pittsfield, MA	11/3/81	D	0.030	0.006	0.124	0.434	0.549	0.037	0.052	11.8
Pittsfield Secondary WWTP Effluent, Pittsfield, MA	11/2/81	B	15.558	0.017	0.001	15.577	0.642	3.317	3.615	4.7

Table 12, continued

Site	Type of Date	Sample[2]	Parameter[1] NO_3^--N	NO_2^--N	NH_3-N	TSIN	TON	Ortho-P	Total-P	N:P
Alum-Treated Secondary WWTP Effluent, Pittsfield, MA	11/2/81	B	15.313	0.014	0	15.327	1.028	1.228	1.235	12.5

1. NO_3^--N = Nitrate; NO_2^--N = Nitrite; NH_3-N = Ammonia; TSIN = Total Soluble Inorganic Nitrogen; TON = Total Organic Nitrogen; Ortho-P = Orthophosphate: Total-P = Total Phosphorus; N:P = $(NO_3^- + NO_2^- + NH_3$.

2. A = composite sample: B = 24 hour composite sample: C = surface sample: D = mid-depth sample.

Table 13

Actual and Predicted Algal Yields (mg dry wt S. capricornutum/l) for Chemical Additions to 16 Samples Collected 4/3/81 - 11/3/81[5]

Sample Site	Date	Chemical Additions[4] Control	P	N	P+N	EDTA	P+EDTA	N+EDTA	P+N+ EDTA	Micro	CU
Spy Pond[1]	4/3/81	8.70	22.71	19.05	23.28	2.59	37.28	43.31	70.80	-	-
Arlington, MA		4.78	26.23	4.78	26.23	4.78	26.23	4.78	26.23		
Flint Pond[1]	4/3/81	7.51	12.16	17.16	39.92	-	15.92	48.81	65.27	-	-
Worcester, MA		6.88	7.60	6.88	28.38	6.88	7.60	6.88	28.78	-	-
Lake Quinsigamond Station I[1]	4/3/81	24.34	23.33	23.41	53.94	29.13	28.54	65.94	72.51	-	-
Worcester, MA		22.19	22.19	34.40	55.90	22.19	22.19	34.40	55.40	-	-
Lake Quinsigamond Station II[1]	4/3/81	24.98	27.81	33.53	35.41	31.83	32.69	66.57	73.28	-	-
Worcester, MA		20.82	20.82	38.27	59.77	20.82	20.82	38.27	59.77	-	-
Quaboag Pond[1]	5/8/81	3.28	3.72	8.30	39.72	3.59	3.82	9.36	40.40	-	-
Brookfield-E.Brookfield, MA		2.85	2.85	19.35	40.85	2.85	2.85	19.35	40.85	-	-
Housatonic River Bulls Bridge[2]	6/9/81	2.89	10.79	12.83	17.09	15.56	38.14	19.17	55.82	3.98	2.44
Kent, CT		18.15	39.65	18.15	39.65	18.15	39.65	13.15	39.65	18.15	17.20
Bulls Bridge[3]	7/14/81	1.56	0.84	7.55	34.63	0.72	0.68	7.86	34.30	0.76	1.01
Kent, CT		0.61	0.61	11.44	32.94	0.61	0.61	11.44	32.94	0.61	0.46
Bulls Bridge[3]		2.90	2.58	12.47	36.32	1.62	1.73	13.61	36.89	1.29	0.39
Kent, CT	8/11/81	5.13	5.13	7.31	28.81	5.13	5.13	7.31	28.81	5.13	1.72
Andrus/Ranapo Road[2]		29.87	29.65	24.20	26.04	36.45	39.60	47.83	41.36	15.90	13.11
Sheffield, MA	6/9/81	35.13	34.20	35.13	56.63	35.13	34.20	35.13	56.63	35.13	28.27
Andrus/Ranapo Road[3]	7/14/81	3.04	3.10	35.83	41.01	3.12	4.15	35.18	39.35	2.25	1.16
Sheffield, MA		0.94	0.94	20.21	41.71	0.94	0.94	20.21	41.71	0.94	0.74
Andrus/Ranapo Road[2]	8/11/81	32.67	33.39	65.35	66.15	32.17	32.49	66.18	70.30	27.66	30.46
Sheffield, MA		31.92	31.92	40.12	61.62	31.92	31.92	40.12	61.62	31.92	32.55
Lanesville Rd Bridge[2]	6/9/81	3.94	8.09	4.77	9.41	15.60	35.16	17.69	26.71	0.66	2.97
New Milford, CT		20.51	42.01	20.51	42.01	20.51	42.51	20.51	42.01	20.51	12.30
Lanesville Rd Bridge[2]	7/14/81	9.15	10.50	9.83	36.62	8.90	8.74	12.24	43.19	5.22	0.23
New Milford, CT		4.71	4.71	10.58	32.08	4.71	4.71	10.58	32.08	4.71	4.29
Lanesville Rd Bridge[2]	7/14/81	7.05	10.86	14.78	40.53	9.47	9.05	19.71	43.86	8.02	2.11
New Milford, CT		4.22	4.22	11.44	32.94	4.22	4.22	11.44	32.94	4.22	3.83
Lanesville Rd Bridge[2]	8/11/81	13.44	13.12	26.33	40.52	13.11	13.16	30.43	45.92	12.53	0.36
New Milford, CT		10.32	15.96	10.32	31.82	10.32	15.96	10.32	31.82	10.32	1.94
Holmes Road Bridge[3]	11/3/81	19.76	23.07	33.60	53.76	22.20	21.68	37.10	58.20	20.01	10.68
Pittsfield, MA		15.91	16.49	15.91	37.41	15.91	16.49	15.91	37.41	15.91	8.17

[1] Composite sample

[2] Surface Sample

[3] Mid-depth sample

[4] P = plus control; N = plus nitrogen; Micro = plus micronutrients; EDTA = plus EDTA; CU = Control, unautoclaved.

[5] Upper values = observed MSC; lower values = predicted MSC

predicted yields if the values were within ± 20 percent of each other. In the second method, statistical differences, at the 95 percent level, between observed algal growth yields in algal cultures receiving varying additions of phosphorus, nitrogen, micronutrients, and EDTA, singly and in combination, were determined using a two tailed t-test described by Meyer (1975). The equation used in calculating the t statistic for comparing two mean MSC values determined from the same number of replicate flasks was:

$$t = \frac{\bar{X}_1 - \bar{X}_2}{[\frac{1}{n}(S_1^2 + S_2^2)]^{1/2}} \qquad (7)$$

where: $\bar{X}_1$ = mean MSC of Set 1

$\bar{X}_2$ = mean MSC of Set 2

n = number of replicates in each set

S_1^2 = variance from first data set

S_2^2 = variance from second data set

The equation used in calculating the t statistic for comparing two mean MSC values determined from a different number of replicate flasks in each data set was:

$$t = \frac{\bar{X}_1 - \bar{X}_2}{[S_p^2 \ (\frac{1}{n_1} + \frac{1}{n_2})]^{1/2}} \qquad (8)$$

where: $\bar{X}_1$ or $\bar{X}_2$ = mean MSC of set 1 or 2, respectively;

n_1 or n_2 = number of replicates in set 1 or 2, respectively;

and

S_p^2 = pooled variance defined by the equation:

$$S_p^2 = \frac{(n_1 - 1)\ (S_1)^2 + (n_2 - 1)\ (S_2)^2}{n_1 + n_2 - 2} \qquad (8)$$

where S_1^2 = variance from first data set:

S_2^2 = variance from second data set.

A student t table was then used to determine if the two mean MSC values were statistically different from each other at the 95 percent level of confidence.

In addition to using observed MSC values to determine the limiting nutrient of the water and their agreement with predicted values, data obtained from cultures containing no chemical additions (controls) provided an indication of the productivity level of the particular lake or river sample. Categories of productivity, based upon MSC values, were reported by Miller, et al. (1975) and are presented in Table 14.

Specific Weight Coefficient (SWC) for S. capricornutum

Specific weight coefficient values for S. capricornutum were determined experimentally using equation 5, described previously in this report. Results are shown below in Table 15.

The mean specific weight coefficient was found to be equal to 3.6×10^{-7}, and was used with equation 5 to calculate all MSC data presented in this report.

Case Studies

Lake Quinsigamond, Station I, Worcester, MA

Lake Quinsigamond, Station I is located in the northern section of the lake just south of I-290 and north of Route 9. The 4/3/81 sample was taken as a composite over a 27 meter depth at

Table 14

Categories of Productivity Based Upon
Observed MSC values of S. capricornutum

Algal MSC Mg dry weight (S. capricornutum/L)	Productivity Level
0.00 - 0.10	low (oligotrophic)
0.11 - 0.80	moderate (oligo-mesoeutrophic)
0.81 - 6.00	moderately high (mesoeutrophic)
>6.00	high (eutrophic)

1. Taken from Miller, et. al., (1974).

Table 15

Specific Weight Coefficient (SWC^1) Values for
S. capricornutum

Replicate flask	SWC^1
1	3.49×10^{-7}
2	3.95×10^{-7}
3	3.61×10^{-7}
4	3.52×10^{-7}
mean	3.6×10^{-7}

1. Units for SWC = $\dfrac{(mg) \times 1000 \quad \frac{ml}{L}}{(\mu m^3)\ (cell)}$

five meter intervals (Table 1). Isothermal conditions equal to 7°C were found for the entire depth of the water column.

The maximum standing algal crop for the Lake Quinsigamond I sample was 24.34 mg dry weight/L indicating that the site was highly productive (Figure 2). The N:P ratio of 7.3:1 determined from chemical analysis of the water (Table 11) indicated that nitrogen was the primary limiting nutrient. Separate additions of 0.05 mg P/L and 1.0 mg N/L did not increase the MSC (23.33 and 23.41 mg dry wt/L respectively). However, additions of both nitrogen and phosphorus increased the algal MSC to 44.20 mg dry wt/L, which indicated nitrogen and phosphorus co-limitation. Samples with added EDTA did not verify this conclusion. Control water + EDTA resulted in an MSC equal to 29.13 mg dry wt/L. Phosphorus addition to the control + EDTA did not increase the MSC (28.54 mg dry wt/L). The control + N + EDTA, however, had an algal yield equal to 65.94 mg dry wt/L which indicated nitrogen limitation. The algal assay data clearly showed that phosphorus was not the limiting nutrient at the time of sampling.

There were statistically significant (95 percent confidence level) increases in all algal yields in samples containing added EDTA. Heavy metal toxicity or trace metal limitation was therefore likely.

The bioavailable nitrogen concentration of 0.614 mg N/L was nearly equal to the 0.584 mg N/L value determined by chemical analysis (Table 12). The bioavailable phosphorus concentration of 0.054 mg P/L was somewhat less the 0.080 mg P/L value determined by chemical analysis. The assay data therefore indicated that algal growth was either nitrogen limited or co-limited by both nitrogen and phosphorus at the time of sampling.

Lake Quinsigamond, Station II, Worcester, MA

The Lake Quinsigamond II station is located just south of Route 9 in Worcester, Massachusetts (Figure 1). A composite sample was taken over a 20.5 meter depth. The MSC for the site was equal to 24.98 mg dry wt/L which indicated that the water was highly productive. A N:P ratio of 6.2:1 indicated likely nitrogen limitation. Addition of 1.00 mg N/L significantly increased the algal yield (significant at the 95 percent level) to 33.53 mg dry wt/L (Figure 2). The control + 0.05 mg P/L resulted in an MSC value of only 27.81 mg dry wt/L (not statistically different at the 95 percent level). These data verified nitrogen limitation. The MSC value in the replicates with added N + EDTA was 66.57 mg dry wt/L compared to 31.83 and 32.69 mg dry wt/L for samples containing EDTA addition alone, and P + EDTA, respectively. These data further confirmed nitrogen limitation for this site at the time of sampling.

In all cases EDTA additions increased algal yields in comparison to samples without added EDTA. It was therefore likely that the water contained either an algal inhibitor, or was deficient in some essential trace metal at the time of sampling.

The biologically available nitrogen and phosphorus levels were both close to the values calculated from chemical data (Table 11). The bioavailable and orthophosphate concentrations were equal to 0.078 mg P/L, and 0.089 mg P/L, respectively. The TSIN concentration, equal to 0.548 mg N/L, was slightly less than the bioavailable nitrogen concentration of 0.732 mg N/L. This indicated that some organic nitrogen in the sample may have been utilized.

Flint Pond, Worcester, MA

Flint Pond, which is located in Worcester, Massachusetts, is connected to the southern most end of Lake Quinsigamond (Figure 2). One composite sample was taken from the pond, on April 3, 1981, over several depths during the spring turnover (Table 1).

The MSC for the Flint Pond sample, found to be 7.51 mg dry wt/L, indicated that the water was highly productive at the time of sampling. The N:P ratio of 12.5:1 indicated that a slight phosphorus limitation was likely. Addition of 0.05 mg P/L and 1.00 mg N/L to Flint Pond samples resulted in MSC values of 12.16 mg dry wt/L and 17.16 mg dry wt/L respectively (Figure 2). The increased algal yields were not statistically different from the control at the 95 percent level. Normally this would indicate co-limitation. However, addition of 0.05 mg P/L + 1.00 mg $Na_2EDTA \cdot 2H_2O$/L resulted in a MSC value of 15.91 mg dry wt/L while the addition of 1.00 mg N/L + 1.00 mg $Na_2EDTA \cdot 2H_2O$/L increased the algal yield to 48.81 mg dry wt/L. These data indicate that nitrogen was the likely limiting nutrient at the time of sampling. Algal growth was therefore either co-limited by nitrogen and phosphorus or nitrogen limited only.

EDTA additions substantially increased the MSC values in two out of three cases (Figure 2), indicating the possible presence of an algal inhibitor or micronutrient limitation at this site.

Actual algal yields were more than 20 percent greater than that predicted by equations 3 and 4 in all cases except for the control. The bioavailable nitrogen was 0.320 mg N/L as compared to the 0.200 mg N/L value determined from chemical data. The bioavailable phosphorus concentration of 0.040 mg P/L was equal to the total phosphorus concentration but greater than the ortho-P concentration of 0.016 mg P/L. These data indicated that more phosphorus and nitrogen were utilized by the algae than expected.

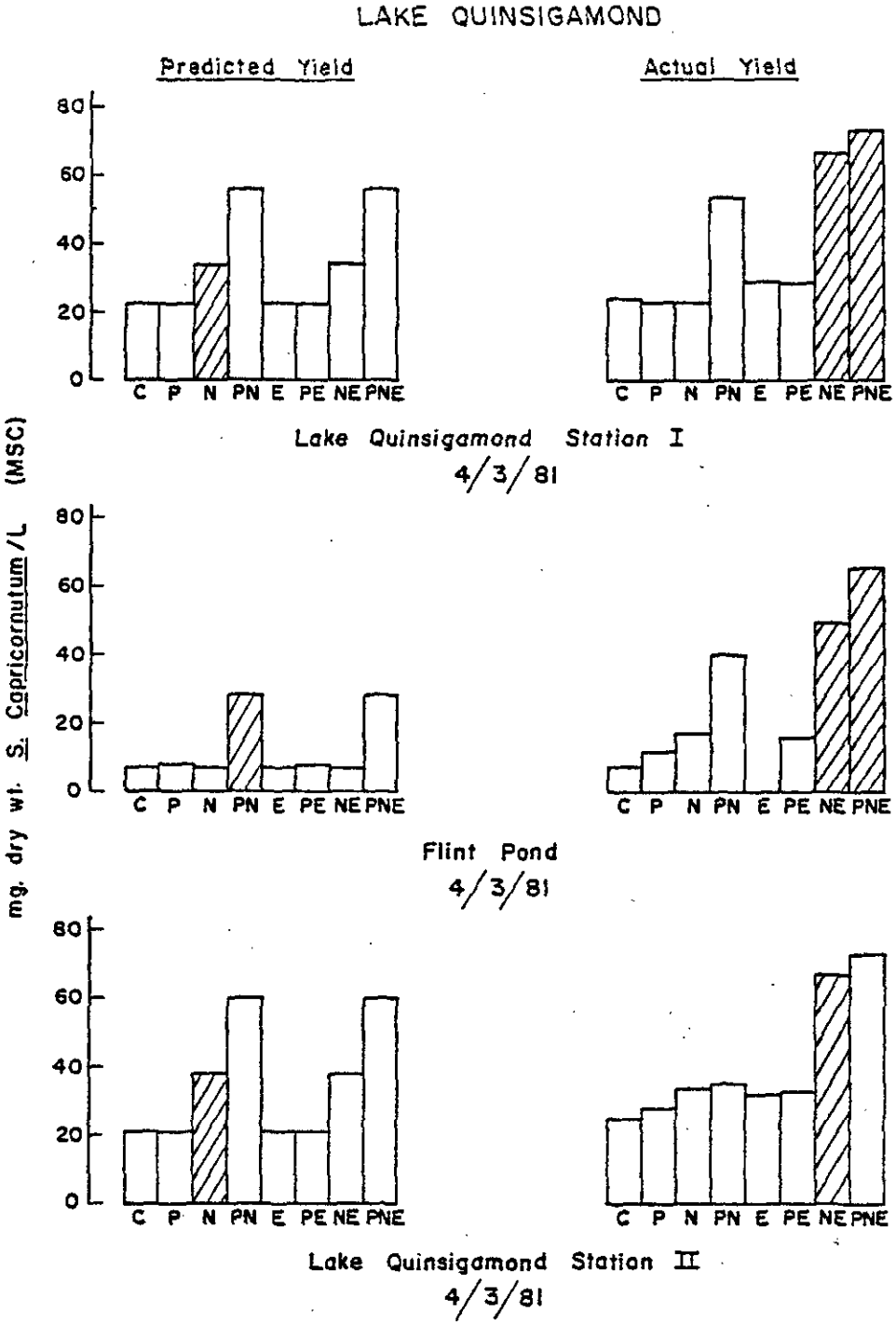

Figure 2. Predicted and actual yields (mg dry wt/L) of S. capricornutum grown in Lake Quinsigamond and Flint Pond Water[1].

[1]C = control
P = + phosphorus
N = + nitrogen
NP = + phosphorus + nitrogen
E = + EDTA
PE = + phosphorus + EDTA
NE = + nitrogen + EDTA
PNE = + phosphorus + nitrogen + EDTA

Crosshatching indicates nutrient limitation at the time of sampling and whether a postive response for micronutrient limitation or the presence of algal inhibitors was observed.

Spy Pond, Arlington, MA

One composite sample was taken from Spy Pond (Arlington, MA) during the spring turnover on April 3, 1981 (Figure 1). The MSC for the site was equal to 1.11 mg dry wt which indicated a moderately high productive water at the time of sampling (Figure 3). Chemical analyses of the site (Table 11) indicated a N:P ratio of 6.4:1 which indicated that the water was phosphorus limiting. Additions of 0.05 mg P/L and 1.00 mg N/L increased the algal yield to 22.71 mg dry wt/L and 19.05 mg dry wt/L respectively. An increased algal yield arising from both nitrogen and phosphorus additions is highly unusual. However, the algal yields from these two additions do indicate phosphorus and nitrogen co-limitation. The sample containing EDTA + 0.05 mg P/L had an MSC value of 37.28 mg dry wt/L while the sample with 1.00 mg N/L + EDTA had an MSC value of 43.31 mg dry wt/L. These results were both much greater than the 2.59 mg dry wt/L algal yield exhibited by the control + EDTA sample. The algal growth data indicated there was apparent co-limitation for this site. The data is somewhat atypical however since the observed algal yields, in most cases fell outside the usual 20 percent range of the predicted yield.

EDTA additions increased the algal yields substantially in all treatments but the control (significant at the 95 percent level). The MSC in the control + EDTA was equal to only 2.59 mg dry wt/L. It is therefore likely that this sample contained an algal inhibitor or was deficient in some essential trace metal at the time of sampling.

The biologically available phosphorus was equal to 0.44 mg P/L. This value was four times greater than the 0.11 mg P/L value obtained from chemical data and indicated that the orthophosphate analysis may have been in error. The bioavailable nitrogen concentration was 0.598 mg N/L or 82 percent of the 0.731 mg N/L value determined by chemical analysis.

Quaboag Pond, Brookfield and E. Brookfield, MA

An algal assay test was performed on a composite sample from Quaboag Pond, Brookfield and East Brookfield, MA (May 7, 1981). The MSC for the site was equal to 3.28 mg dry wt/L which indicated a moderately high level of productivity (Figure 3) at the time of sampling. A N:P ratio of 1.7:1 (Table 11) indicated that nitrogen limitation was likely. The MSC in the control + N was 8.30 mg dry wt/L and was significantly greater than the control (at the 95 percent confidence level). The MSC in the control + P was only 3.72 mg dry wt/L. These data confirmed that nitrogen was the limiting nutrient at this site. Neither the presence of algal inhibitors nor trace element limitation was observed in samples containing EDTA additions.

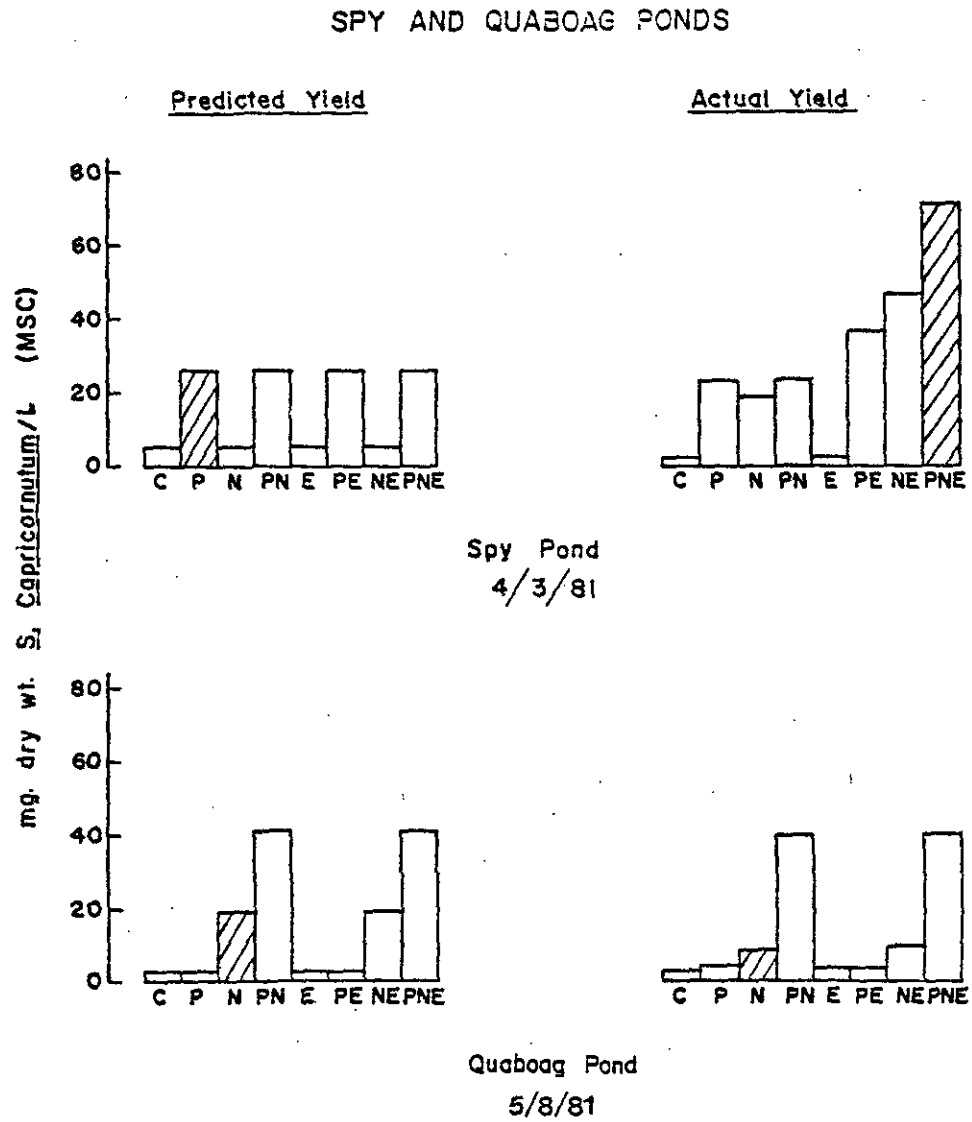

Figure 3. Predicted and actual yields (mg dry wt/L) for S. capricornutum *grown in Spy and Quaboag Ponds.*

[1]C = control
P = + phosphorus
N = + nitrogen
PN = + phosphorus + nitrogen
E = + EDTA
PE = + phosphorus + EDTA
NE = + nitrogen + EDTA
PNE = + phosphorus + nitrogen + EDTA

Crosshatching indicates nutrient limitation at the time of sampling and whether a positive response for micronutrient limitation or the presence of algal inhibitors was observed.

Good agreement between actual and predicted algal yields (within ± 20 percent) was found in most of the algal cultures containing chemical additions with the exception of the control + N and the control + N + EDTA samples, which displayed MSC values of about 45 percent less than that predicted from chemical data (Figure 3). The smaller algal yields may have been attributable to a lower bioavailable phosphorus concentration (0.019 mg P/L) as compared to the chemically measured orthophosphate concentration of 0.045 mg P/L. The chemically determined TSIN concentration of 0.075 mg N/L was in close agreement with the observed bioavailable value of 0.098 mg N/L.

Spencer Secondary Wastewater Treatment Plant Effluent, Spencer, MA

The Spencer Wastewater Treatment plant is located in Spencer, MA and discharges secondary treated sewage into Cranberry Brook which flows into the Seven Mile River which is located above Quaboag Pond. A 24 hour composite sample of the secondary WWTP effluent was collected on May 7 and 8, 1981. The average effluent flow for the sampling period was 2.89 L/min. The sample was dechlorinated using sodium thiosulfate and filtered through a 0.45 μm membrane filter prior to algal assay.

The high phosphorus concentration, equal to 3.018 mg P/L resulted in a N:P ratio of only 1.9:1 (Table 11). Various percent effluent concentrations were added to Quaboag Pond water in order to assess the ability of the WWTP effluent to stimulate algal growth. The N:P ratio of the Quaboag Pond water was also nitrogen limiting and was equal to 1.7:1. All predicted algal yields were therefore based upon nitrogen limitation (Table 16). Figure 4 shows the observed and predicted MSC values vs percent effluent addition. The observed values were all within 20 percent of the predicted levels further indicating that nitrogen was the limiting nutrient in all dilutions of the effluent and pond water.

The MSC values in the pond water with effluent additions containing EDTA were also in close agreement to both predicted values and observed MSC values in effluent additions without added EDTA (Figure 4). These data indicated the absence of organic toxicants, or trace element limitation.

Only nitrogen bioavailability was calculated since the effluent was nitrogen limiting. Based on the yield in the 100 percent effluent sample of 188.72 mg dry wt/L, the bioavailable nitrogen was found to be equal to 4.966 mg N/L. This value was 85 percent of the TSIN value of 5.815 mg N/L determined by chemical analysis.

Table 16

Algal Yield Data for Additions of Spencer Secondary WWTP Effluent to Quaboag Pond Water[1]

Percent Effluent[2]	N:P	Predicted ± 20%[3] Yield (mg dry wt/L)	Actual Yield (mg dry wt/L)
0	2:1	2.85 ± 0.57	3.28
0 + EDTA	2:1	2.85 ± 0.57	3.72
1	2:1	5.02 ± 1.00	6.05
1 + EDTA	2:1	5.02 ± 1.00	6.88
5	2:1	13.76 ± 2.75	13.89
5 + EDTA	2:1	13.76 ± 2.75	14.47
10	2:1	24.66 ± 4.93	22.42
10 ± EDTA	2:1	24.66 ± 4.93	25.40
30	2:1	68.29 ± 13.66	60.57
30 + EDTA	2:1	68.29 ± 13.66	64.51
50	2:1	111.91 ± 22.38	103.44
50 + EDTA	2:1	111.91 ± 22.38	102.85
70	2:1	155.53 ± 31.11	139.93
70 + EDTA	2:1	155.53 ± 31.11	146.67
100	2:1	220.97 ± 44.19	188.72
100 + EDTA	2:1	220.97 ± 44.19	204.17

1. WWTP effluent and Quaboag Pond dilution water were collected on 5/22/81.
2. EDTA was added to replicate aliquots to determine possible presence of algal toxicants or micronutrient limitation.
3. Actual yields are not considered statistically different from predicted yields if they fall within ± 20 percent of the predicted values determined by equation 3.

Table 17

Summary Table for Lake Sites

	Site and Date				
	4/3/81	4/3/81	4/3/81	4/3/81	5/8/81
Parameter	Quinsigamond I	Quinsigamond II	Flint Pond	Spy Pond	Quaboag Pond
Nitrogen to Phosphorus Ratio*	7.3:1	6.2:1	12.5:1	66.4:1	1.7:1
Predicted Limiting Nutrient	Nitrogen	Nitrogen	Phosphorus	Phosphorus	Nitrogen
Observed Algal Assay Limiting Nutrient Result	Nitrogen or Co-limited	Nitrogen	Nitrogen or Co-limited	Co-limited	Nitrogen
Possible Presence of an Algal Inhibitor	Yes	Yes	Yes	Yes	No

*A ratio below 10:1 indicates likely nitrogen limitation.

A ratio between 10:1 and 12:1 indicates likely co-limitation; however, co-limitation at values between 5:1 and 12:1 have been reported.

A ratio greater than 12:1 indicates likely phosphorus limitation.

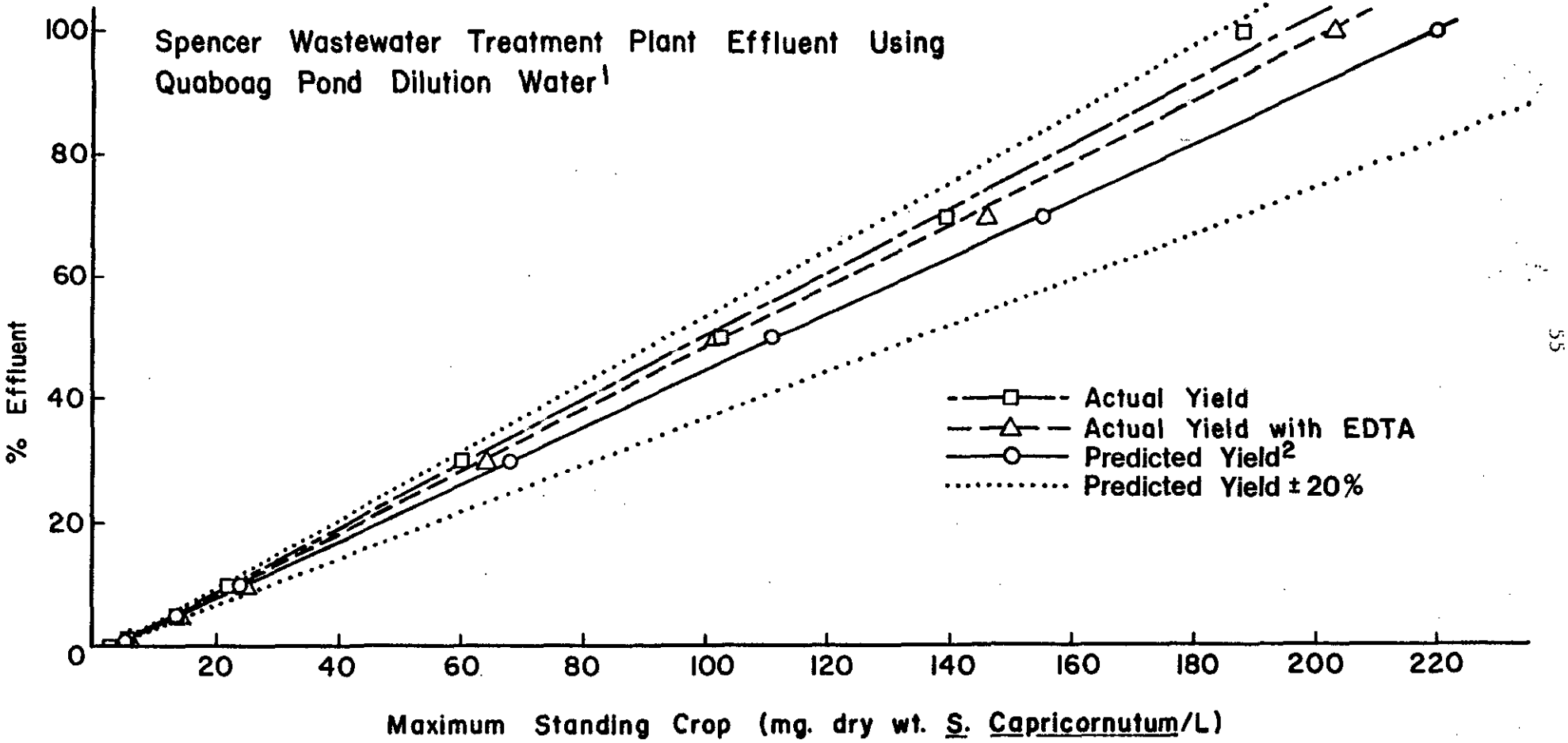

Figure 4. Predicted and actual (with and without EDTA) yields (mg dry wt/L) of S. capricornutum grown in Spencer secondary WWTP effluent and Quaboag Pond dilution water.

[1]Spencer WWTP effluent was composited over a 24 hour period from 5/7/81-5/8/81. Quaboag Pond water was sampled on 5/8/81.

[2]Predicted yield (mg dry wt/L) = 38 x TSIN (mg/L) ± 20 percent.

Summary of Data for Lake Sites

A summary of data for the lake sites is shown in Table 17.

Housatonic River Study

The University of Massachusetts/Amherst in cooperation with the Massachusetts Division of Water Pollution Control conducted a concentrated sampling program of the Housatonic River to evaluate the impact of phosphorus-containing discharges, located in Massachusetts, on the receiving water. The contributions of these point sources of phosphorus on observed eutrophication problems was investigated in both the free flowing and impounded reaches of the river. Particular concern has been raised about algal proliferation in three large impoundments of the River (Lake Lillanoah, Lake Zoar, and Lake Housatonic) located downstream in western Connecticut (see Figure 5).

The Housatonic River is located in Western Massachusetts and travels 131 miles through Connecticut to Long Island Sound (Figure 5). The total watershed area is 1650 square miles of which 500 square miles are in Massachusetts (MDWPC, 1978). The river is comprised of the main stem as well as east, west, and southwest branches and many tributaries. The discharge of the river varies considerably during the summer time and increases by about three fold from its northern Massachusetts headwaters before entering Connecticut. The river has 13 impoundments located in Massachusetts and is slightly turbid (mean NTU = 1.8) and colored (26.4 color units) (MDWPC, 1978).

Thirteen wastewaters are discharged into the river within Massachusetts. These include four paper mills, one manufacturing company and seven domestic wastewater discharges of which the largest, located in the upper reaches of the river, is from the city of Pittsfield.

A detailed study on the nutrient status of the river, and its sensitivity to change in this status resulting from various chemical inputs, was conducted for several samples collected at various locations along the river between 6/9/81 and 11/3/81. Additionally, effluent from the Pittsfield Wastewater Treatment Plant was studied using the algal assay bottle test, to determine the degree of potential algal growth stimulation caused by the discharge of this wastewater into the Housatonic River. Algal assays were conducted with wastewater effluent both before and after phosphorus removal by alum addition to assess the effectiveness of this process on decreasing nutrient enrichment of receiving waters. Sampling locations included: Bulls Bridge Station, Kent, CT: Lanesville Road Bridge Station, New Milford, CT: Andrus/Ranapo Road Station, Sheffield, MA: and the Holmes Road Bridge Station in Pittsfield, MA.

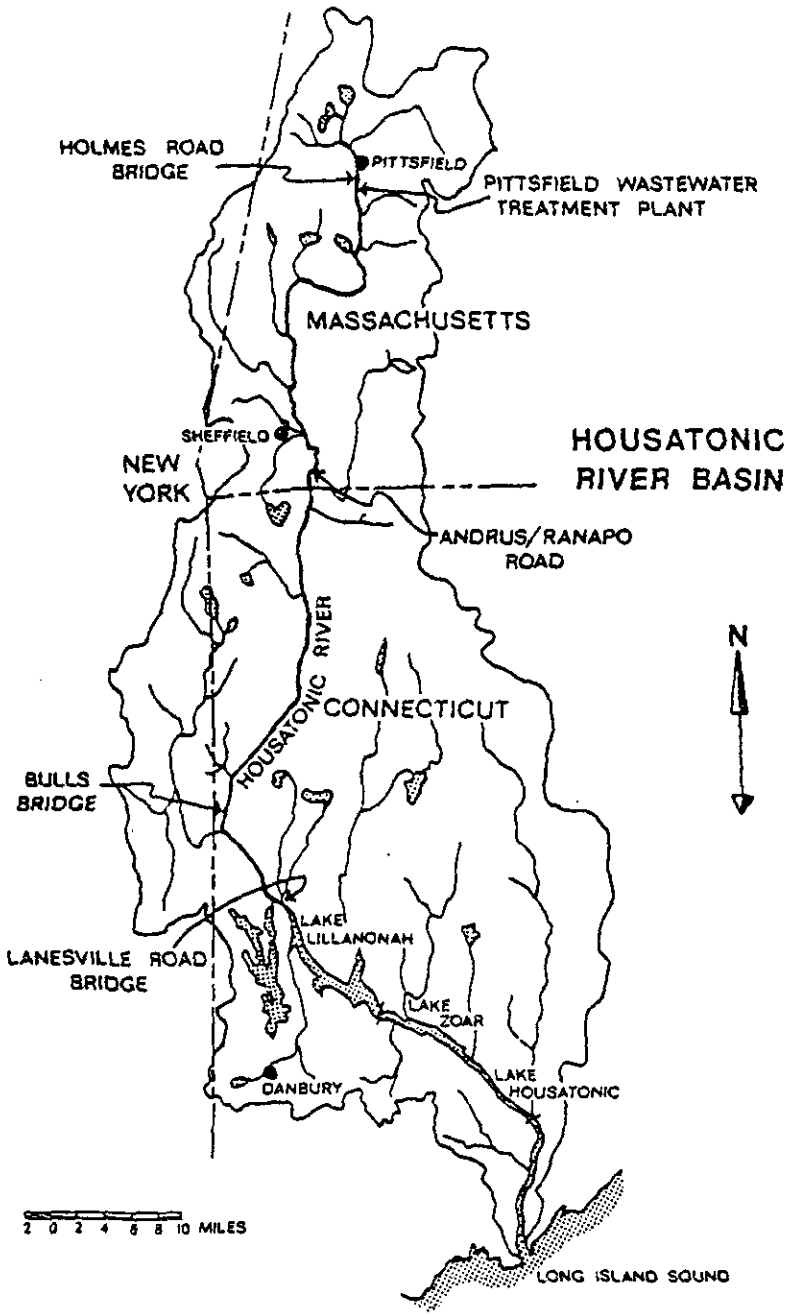

Figure 5. Map of the sampling locations for the Housatonic River Basin[1].

[1]The arrows indicate sampling locations.

1. Bulls Bridge Station, Kent, CT

The Bulls Bridge Station is located in Kent, CT (Figure 5). Samples for algal assay from this site were collected on 6/9/81, 7/14/81, and 8/11/81.

A. 6/9/81 Bulls Bridge Station Sampling

Table 11 presents water quality data for the 6/9/81 sampling of this site. A N:P ratio of 33:1 indicated that phosphorus limitation was likely at the time of sampling. The algal yield with no nutrient additions (control) was 2.89 mg dry wt/L and was only 16 percent of the predicted yield. Addition of 0.05 mg P/L or 1.00 mg/L nitrogen increased the algal yield to 10.79 mg dry wt/L and 12.83 mg dry wt/L, respectively (Figure 6). All of these responses were more than 20 percent below the predicted values and were therefore statistically different from the predicted growth response. The algal response in replicates with EDTA addition was therefore used to evaluate the limiting nutrient at this site.

Algal growth in samples containing EDTA was, in all instances, substantially greater than in samples without EDTA. The MSC in the control + EDTA and in the control + N samples were 15.56 and 19.17 mg dry wt/L, respectively. Both values were within 20 percent of the 18.15 mg dry wt/L predicted yield. The algal response in the sample containing 0.05 mg P/L + EDTA equal to 38.14 mg dry wt/L was substantially greater than the algal growth response in the control + EDTA sample and was statistically different from the control + EDTA sample at the 95 percent confidence level. These data verified that phosphorus was the limiting nutrient.

Additions of EDTA substantially increased the algal yields (Figure 6) in all treatments of the sample water. Micronutrient addition, however, only increased the algal yield from 2.89 mg dry wt/L to 3.98 mg dry wt/L. These results indicated the likelihood an algal toxicant was inhibiting algal production in samples without added EDTA.

The bioavailable phosphorus was slightly lower than the inorganic levels determined by chemical analysis. This lower response was attributable to heavy metal toxicity. Bioavailable phosphorus was equal to 0.030 mg P/L as compared to the chemically analyzed concentration of 0.042 mg P/L. Bioavailable nitrogen could not be calculated since the addition of 0.05 mg P/L did not cause the system to become nitrogen limited.

B. 7/14/81 Bulls Bridge Station Sampling

The July 14 Bulls Bridge Station water sample displayed an algal yield of only 0.92 mg dry wt/L and was therefore less

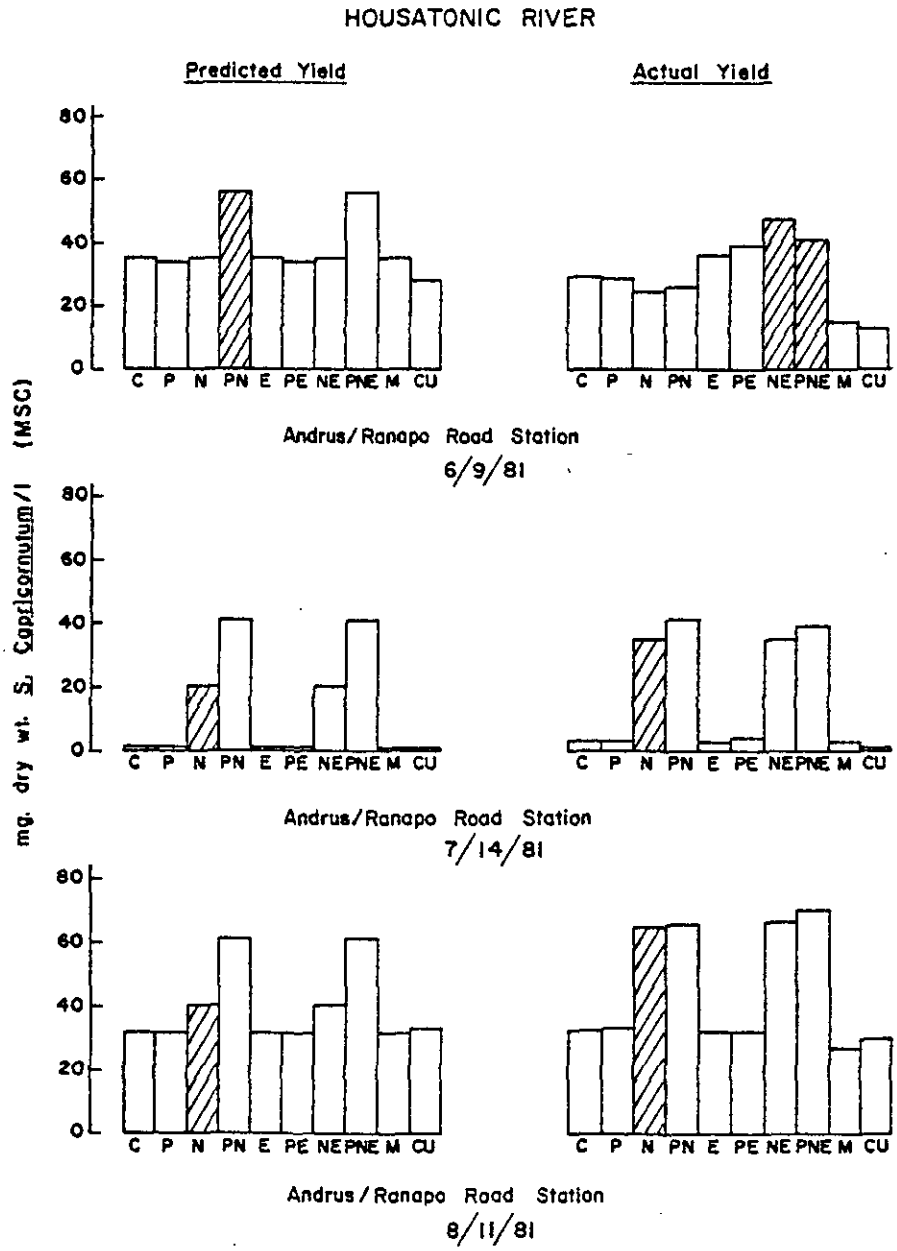

Figure 6. Predicted and actual yields (mg dry wt/L) of S. capricornutum grown in Housatonic River water, Bulls Bridge Station[1].

[1] C = control; P = + phosphorus; N = + nitrogen; PN = + phosphorus + nitrogen; E = + EDTA; PE = + phosphorus + EDTA; NE = + nitrogen + EDTA; PNE = + phosphorus + nitrogen + EDTA; M = + micronutrients; CU = control unautoclaved

Crosshatching indicates nutrient limitation at the time of sampling, and whether a positive response for micronutrient limitation or the presence of algal inhibitors was observed.

productive than the June sampling (Figure 6). Water quality data (Table 11) indicated that the site had become nitrogen limited (N:P ratio equal to 1:2). The 0.05 mg P/L addition did not increase the algal yield (0.84 mg dry wt/L). Addition of 1.00 mg N/L, however, increased algal growth dramatically to 7.55 mg dry wt/L. The control + N algal response was significantly different from the growth responses in the control (statistically significant at the 95 percent confidence level). This verified that nitrogen was the primary limiting nutrient at the time of sampling. There were no indications of either algal growth inhibitors or trace element limitation.

Biologically available nitrogen and phosphorus were close to the chemically determined concentrations. Bioavailable phosphorus and nitrogen were 0.018 mg P/L and 0.022 mg N/L respectively as compared to the chemically determined concentrations of 0.027 mg P/L and 0.016 mg N/L respectively (Table 10).

C. 8/11/81 Bulls Bridge Station Sampling

The MSC for water sampled at the Bulls Bridge Station on 8/11/81 was 1.90 mg dry wt/L which indicated that the water was moderately productive at the time of sampling. As in the July sample, chemical analysis indicated nitrogen limitation with a N:P ratio of 8:1. Phosphorus addition did not increase algal yield (2.58 mg dry wt/L), but the nitrogen addition substantially increased the MSC to 12.47 mg dry wt/L (Figure 6). The control + N sample was significantly different from the control at the 95 percent confidence level. These data confirmed that nitrogen was the limiting nutrient. No algal growth inhibitor or trace element limitation was indicated by changes in the MSC in samples containing EDTA or micronutrient additions.

Predicted MSC values for autoclaved and unautoclaved controls were more than 20 percent greater than actual yields. However, the MSC in cultures containing N + P or N alone were more than 20 percent greater than the predicted values. Therefore an organic inhibitor was probably not present.

Bioavailable phosphorus and nitrogen were equal to 0.029 mg P/L and 0.068 mg N/L, respectively as compared to chemically analyzed concentrations of 0.017 mg P/L and 0.135 mg N/L, respectively (Table 12).

2. Lanesville Road Bridge Station, New Milford, CT

The Lanesville Road Bridge Station is located in New Milford, Connecticut. Four samples were collected from this site on 6/9/81, 7/14/81 (two samples at different depths), and 8/11/81, and subjected to the Algal Assay:Bottle Test to assess the algal growth potential of these samples (Table 1).

A. 6/9/81 Lanesville Road Sampling

The surface sample was collected on 6/9/81. The total water depth was 11 meters. A high concentration of TSIN (2.386 mg N/L) relative to ortho-P (0.048 mg P/L) was determined, resulting in a N:P ratio of 49.7:1. An extreme phosphorus limitation at the time of sampling was therefore indicated. The MSC in the control was equal to 3.94 mg dry wt/L (Figure 7). Addition of 0.05 mg P/L increased the MSC value of 8.09 mg dry wt/L. This response was statistically different from the control at the 95 percent confidence level. The MSC in the control + N treatment was equal to 4.77 mg dry wt/L. These data verified that phosphorus was the limiting nutrient.

Addition of EDTA to the various samples increased the algal yields substantially. The micronutrient addition, however, did not increase algal yield. The presence of an algal growth inhibitor was therefore indicated.

Only bioavailable phosphorus was calculated for this sample since the N:P ratio of 49.7:1 indicated such extreme phosphorus limitation that the addition of 0.05 mg P/L to the sample water only decreased the N:P ratio to 24:1. The resultant ratio therefore still indicated phosphorus limitation. This addition, then, did not cause the secondary limiting nutrient, nitrogen, to become the primary limiting nutrient. Bioavailable phosphorus, equal to 0.011 mg P/L, was only 23 percent of the 0.048 mg P/L determined by chemical analysis. The lower bioavailable nitrogen response was attributable to the presence of an algal growth inhibitor.

B. 7/14/81 Lanesville Road Surface/Mid-Depth Water Samples

Two different samples were collected on 7/14/81: 1) a surface sample, and 2) a mid-depth sample collected at 4.2 meters. The samples were then algal assayed to discern if there was a change in nutrient status with depth. A N:P ratio of 5:1 was determined from chemical analyses for the surface sample which indicated nitrogen limitation. Algal yields, presented in Figure 7, however, suggested co-limitation since additions of phosphorus or nitrogen alone did not increase the algal yield beyond the levels which could have been attributed to experimental error. The MSC of 12.24 mg dry wt/L in the control + N + EDTA was slightly greater than the control + EDTA algal yield of 8.90 mg dry wt/L. Although the algal response in these two treatments were statistically different from each other at the 95 percent confidence level, the increased yield in the control + N + EDTA culture of about 37 percent may, in part, have been attributable to some experimental error. Overall, the data therefore indicated

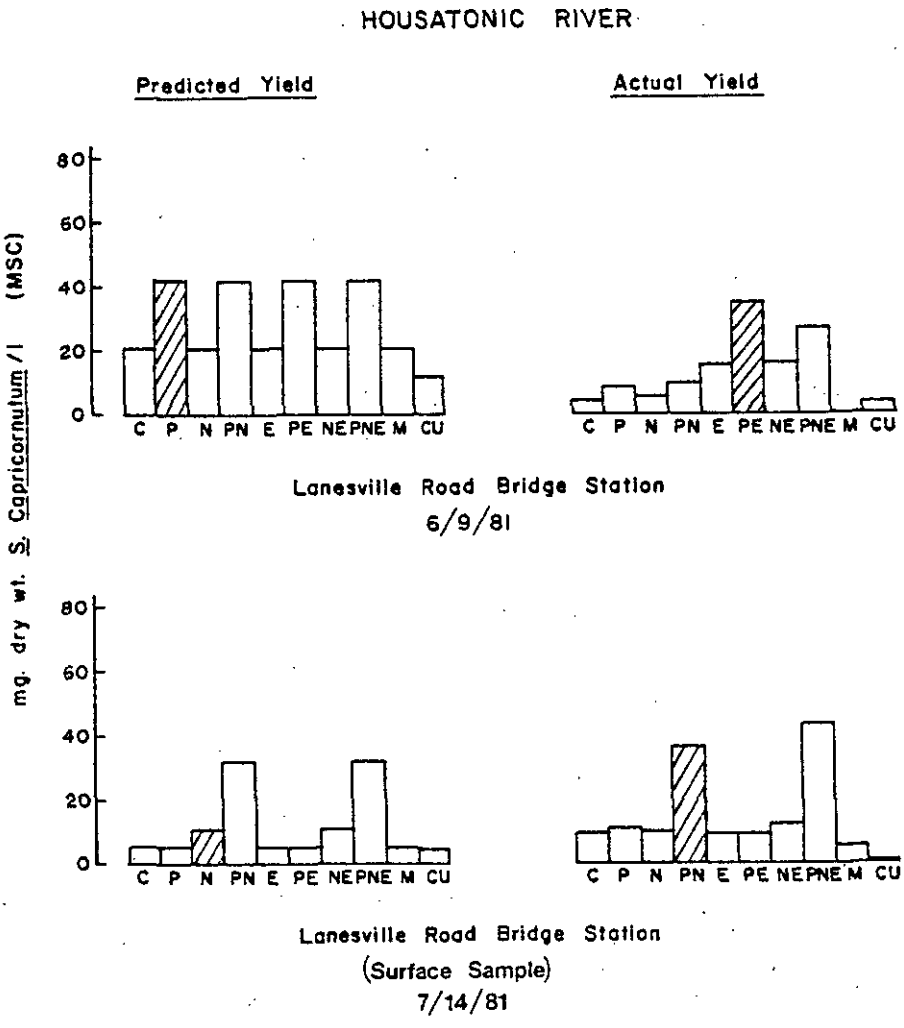

Figure 7. Predicted and actual yields (mg dry wt/L) of S. capricornutum grown in Housatonic River water, Lanesville Road Bridge Station[1].

[1]C = control
P = + phosphorus
N = + nitrogen
PN = + phosphorus + nitrogen
E = + EDTA
PE = + phosphorus + EDTA
NE = + nitrogen + EDTA
PNE = + phosphorus + nitrogen + EDTA
M = + micronutrients
CU = control unautoclaved

Crosshatching indicates nutrient limitation at the time of sampling, and whether a positive response for micronutrient limitation or the presence of algal inhibitors was observed.

co-limitation. Neither algal inhibitors nor trace element limitation were suggested by the data.

Bioavailable phosphorus was equal to 0.023 mg P/L and was very nearly equal to the chemically determined concentration of 0.025 mg P/L. The bioavailable nitrogen concentration of 0.276 mg N/L was greater than 0.124 mg N/L value determined by chemical analysis. This suggested that a portion of the organic nitrogen may have been utilized in algal growth thereby partially explaining the observed co-limitation of phosphorus and nitrogen.

The mid-depth sample had approximately the same algal productivity characteristics as the surface sample. The MSC in the control was equal to 7.05 mg dry wt/L. A N:P ratio of 4:1, calculated from chemical data, indicated nitrogen limitation as in the case of the surface sample.

Separate phosphorus or nitrogen additions (Figure 8) increased algal yields to 10.86 and 14.78 mg dry wt/L, respectively. These responses were statistically different from the control at the 95 percent confidence level. The algal growth data, however, indicated that the growth response in the control was unusually low. This conclusion is drawn from the observation that the MSC in the control + EDTA culture was equal to 9.47 mg dry wt/L, while N + EDTA, P + EDTA, or N + P + EDTA additions did not increase the algal yields above those observed in the same nutrient additions without EDTA. The MSC in the control + P + EDTA culture was not greater than the 9.05 mg dry wt/L algal yield in the control + P without EDTA treatment. The MSC in the control + N + EDTA treatment, however, was 19.71 mg dry wt/L, and was statistically different from the control + EDTA algal yield at the 95 percent confidence level. These data verified that nitrogen was the limiting nutrient at the time of sampling.

Although the surface sample was apparently co-limiting and the mid-depth sample was nitrogen limiting, the bioavailable phosphorus and nitrogen were about the same in both samples. The bioavailable nitrogen and phosphorus concentrations in the surface sample were 0.276 mg N/L and 0.023 mg P/L, respectively while the bioavailable nitrogen and phosphorus concentrations in the mid-depth sample were 0.286 mg N/L and 0.034 mg P/L, respectively.

C. 8/11/81 Lanesville Road Sampling

A fourth sample was collected on 8/11/81 at Lanesville Road at a mid-depth of 3.5 meters. The MSC for this site was equal to 13.44 mg dry wt/L which indicated a highly productive water (Figure 8) at the time of sampling. The N:P ratio was equal to 18:1 and indicated phosphorus limitation. The MSC in the control + P treatment however, equal to 13.12 mg dry wt/L, was not greater than in the control with no phosphorus addition. The nitrogen addition

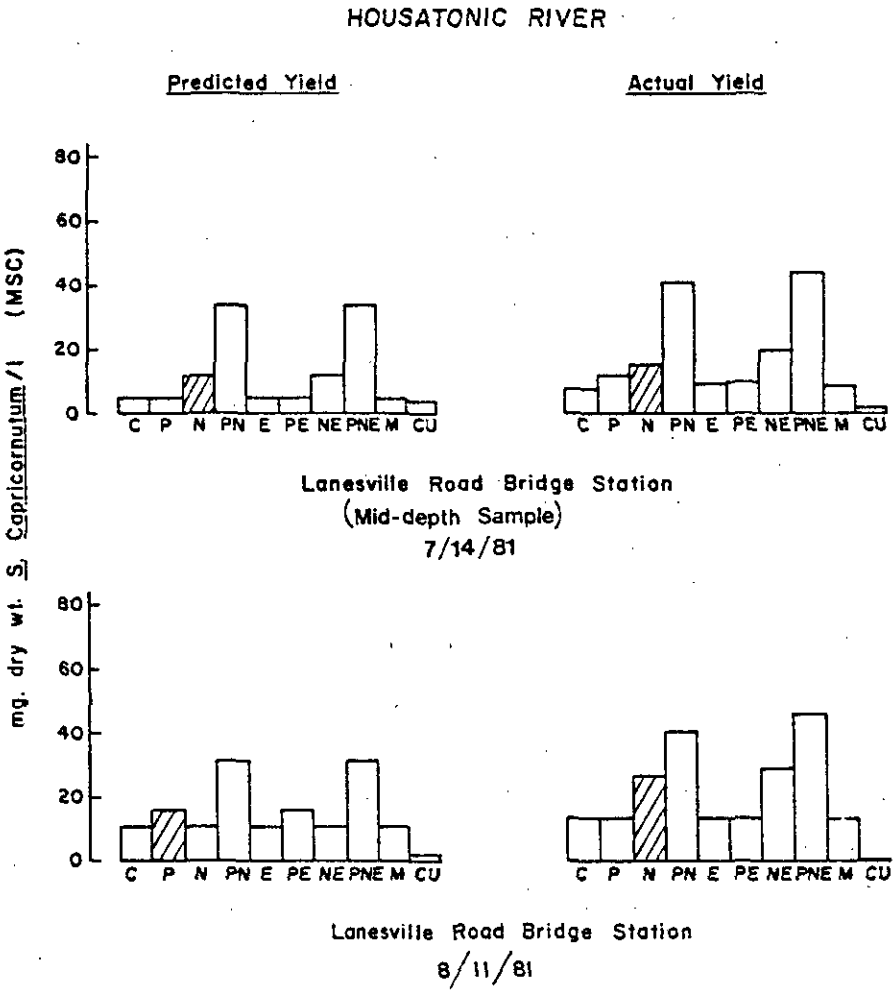

Figure 8. Predicted and actual yields (mg dry wt/L) of S. capricornutum grown in Housatonic River water, Lanesville Road Bridge Station.

[1]C = control
P = + phosphorus
N = + nitrogen
PN = + phosphorus + nitrogen
E = + EDTA
PE = + phosphorus + EDTA
NE = + nitrogen + EDTA
PNE = + phosphorus + nitrogen + EDTA
M = + micronutrients
CU = control unautoclaved

Crosshatching indicates nutrient limitation at the time of sample, and whether a positive response for micronutrient limitation or the presence of algal inhibitors was observed.

substantially increased the algal yield to 26.33 mg dry wt/L. This response was statistically greater than the control at the 95 percent confidence level. These data suggested that nitrogen rather than phosphorus was actually the primary limiting nutrient. Algal data indicated neither algal growth inhibition nor micronutrient limitation.

The bioavailable phosphorus concentration of 0.061 mg P/L was surprisingly high relative to the 0.024 mg P/L level determined by chemical analysis. The nitrogen bioavailable concentration was 0.345 mg N/L or 82 percent of the 0.430 mg N/L determined by chemical analysis.

3. Andrus/Ranapo Road, Sheffield, MA

Andrus/Ranapo Road is located in Sheffield, MA. This site was sampled on 6/9/81, 7/14/81, and 8/11/81. Samples collected on 6/9/81 and 8/11/81 were surface samples while the 7/14/81 sample was taken at mid-depth.

A. 6/9/81 Andrus/Ranapo Road Sampling

The MSC for a surface sample collected on 6/9/81 was equal to 29.87 mg dry wt/L and indicated that the water was highly productive (Figure 9) at the time of sampling. The 11.0:1 N:P ratio suggested co-limitation. There was no increased algal growth in cultures with added P or N, nor in additions of both N and P. All samples with EDTA additions showed an increase in the MSC in comparison to those without the added EDTA. The increased algal yield in the samples with EDTA were statistically different from those without EDTA at the 95 percent confidence level. No increase in the MSC for the micronutrient addition was observed. These data suggested that an algal growth inhibitor was present at this site. The MSC in the nitrogen + EDTA treatment was equal to 47.83 mg dry wt/L and was statistically greater than the 36.45 mg dry wt/L MSC in the control + EDTA at the 95 percent confidence level. This increase was not observed in the treatment containing P + EDTA which had a MSC value of 39.60 mg dry wt/L. The P + EDTA MSC value was not statistically different from the control. Slight nitrogen or co-limitation therefore was apparent.

Due to the toxicity, bioavailable phosphorus and nitrogen were lower than values determined by chemical analysis. The bioavailable concentrations were 0.56 mg P/L and 0.78 mg N/L. Inorganic nutrient content, determined by chemical analysis was 0.817 mg P/L and 0.900 mg N/L.

B. 7/14/81 Andrus/Ranapo Road Sampling

The 7/14/81 sample was collected from a mid-depth location, 1.2 meters below the surface. The N:P ratio of 2:1 suggested

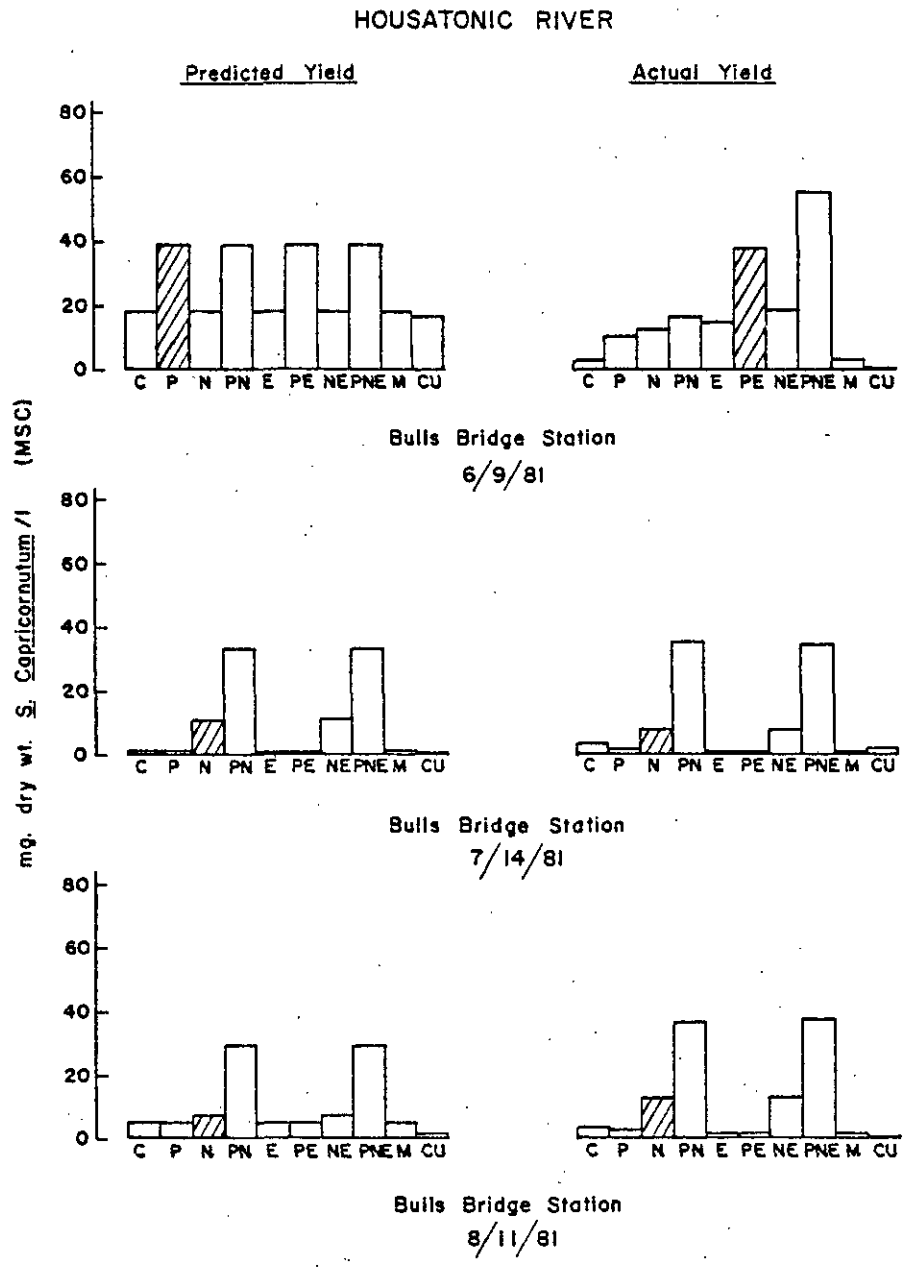

Figure 9. Predicted and actual yields (mg dry wt/L) of S. capricornutum grown in Housatonic River water, Andrus/Ranapo Station[1].

[1]C = control
P = + phosphorus
N = + nitrogen
PN = + phosphorus + nitrogen
E = + EDTA
PE = + phosphorus + EDTA
NE = + nitrogen + EDTA
PNE = + phosphorus + nitrogen + EDTA
M = + micronutrients
CU = control unautoclaved

Crosshatching indicates nutrient limitation at the time of sampling and whether a positive response for micronutrient limitation or the presence of algal inhibitors was observed.

nitrogen limitation (Table 12) at the time of sampling. The MSC in the control was equal to 3.04 mg dry wt/L. Addition of 0.05 mg P/L did not increase the MSC significantly. Addition of 1.00 mg N/L, however, increased the MSC by an order of magnitude (Figure 9). This increase was statistically greater than the MSC in the control at the 95 percent confidence level. These data verified nitrogen limitation. There was no indication of either algal inhibitors or trace element limitation.

Bioavailable nutrient concentrations were 0.083 mg N/L and 0.082 mg P/L. In organic nutrient levels determined by chemical analysis were 0.025 mg N/L and 0.047 mg P/L. Observed algal yields above that predicted by inorganic nutrient levels were probably attributable to algal utilization of organic nitrogen and phosphorus fractions.

C. 8/11/81 Andrus/Ranapo Road Sampling

The MSC value for the 8/11/81 Andrus/Ranapo Road Station sampling was equal to 32.67 mg dry wt/L reflecting a highly productive water (Figure 9) at the time of sampling. A slight nitrogen limitation was indicated based upon the 4:1 N:P ratio. Addition of 1.00 mg N/L resulted in a MSC of 65.35 mg dry wt/L which was statistically greater than the MSC of the control at the 95 percent confidence level. The MSC in the control + 0.05 mg P/L was equal to 33.39 mg dry wt/L. These values confirmed that the site was nitrogen limited as was found in the June and July samplings. No trace element limitation or toxicity was observed. The bioavailable phosphorus level, equal to 0.152 mg P/L, was greater than the 0.093 mg P/L data determined by chemical analysis. This could be attributable in part to error in the chemical analysis or to utilization of organic phosphorus fractions. The inorganic nitrogen level of 0.879 mg N/L was in close agreement with the calculated bioavailable concentration of 0.840 mg N/L.

4. 11/2/81 Pittsfield Secondary Wastewater Treatment Plant Effluent

A mid-depth river water sample was collected at the Holmes Road Bridge station, located one quarter of a mile above the Pittsfield Wastewater Treatment Plant outfall into the Housatonic River. An algal assay bottle test was performed using S. capricornutum to assess the nutrient status of the sample. A second algal assay test was additionally performed on varying percent additions of non-chlorinated Pittsfield WWTP effluent (24 hour composite sample) using dilution water from the Holmes Road Bridge Station. Portions of the effluent were treated with alum to reduce the phosphorus content to approximately 1.0 mg P/L. A third assay was performed using various percent additions of the treated Pittsfield wastewater and to water collected at Holmes Road.

The MSC in water samples collected at Holmes Road was equal to 19.76 mg dry wt/L which indicated that the water was potentially highly productive at the time of sampling even before receiving wastewater effluent (Figure 10). A N:P ratio of 11.8:1 was determined from chemical analyses and indicated that the water was likely to be co-limited by phosphorus and nitrogen. Addition of 1.00 mg N/L significantly increased the algal yield to 33.35 mg dry weight/L. This value was significantly greater than the control at the 95 percent confidence level. Addition of 0.05 mg P/L did not significantly effect algal yield. These data suggested that nitrogen was actually the limiting nutrient. No toxicity or trace element limitation was observed (Figure 10). Bioavailable phosphorus (equal to 0.078 mg P/L) and nitrogen (equal to 0.607 mg N/L) were somewhat greater than the 0.037 mg P/L and 0.434 mg N/L concentrations determined by chemical analyses.

Additions of varying percentages of untreated effluent to Holmes Road dilution water resulted in increased algal yields for all dilutions within $\pm$ 20 percent of predicted values, as shown in Figure 11. N:P ratios and algal yield values for each dilution are presented in Table 18. EDTA additions to each effluent dilution did not result in increased MSC values. The data indicated a predictable correlation between added effluent and algal yield limitation.

Only bioavailable nitrogen for the effluent was calculated since the effluent was nitrogen limiting. The algal yield for 100 percent effluent was needed to calculate this value. Since the highest percentage by volume of effluent tested was only 50 percent, a value was extrapolated using equation 10 from the 50 percent effluent culture to obtain a calculated value for 100 percent effluent.

$$\begin{array}{l}\text{Extrapolated MSC for algal culture containing 100 percent sewage}\end{array} = 2 \times \begin{array}{l}\text{MSC for algal culture containing 50 percent sewage + 50 percent Holmes Road dilution water}\end{array} - \begin{array}{l}\text{MSC for algal growth in Holmes Road dilution water alone}\end{array} \qquad (10)$$

The extrapolated algal yield was equal to 486.66 mg dry wt/L. The bioavailable nitrogen concentration of the effluent, equal to 12.81 mg N/L, was then calculated by dividing the 486.66 mg dry wt/L value by the nitrogen growth coefficient of 38. This value was about 82 percent of the 15.577 mg N/L inorganic nitrogen concentration determined by chemical analysis.

A third algal assay was performed using alum-treated effluent additions to Holmes Road dilution water. It is common practice in tertiary treatment of wastewater effluent to reduce the phosphorus

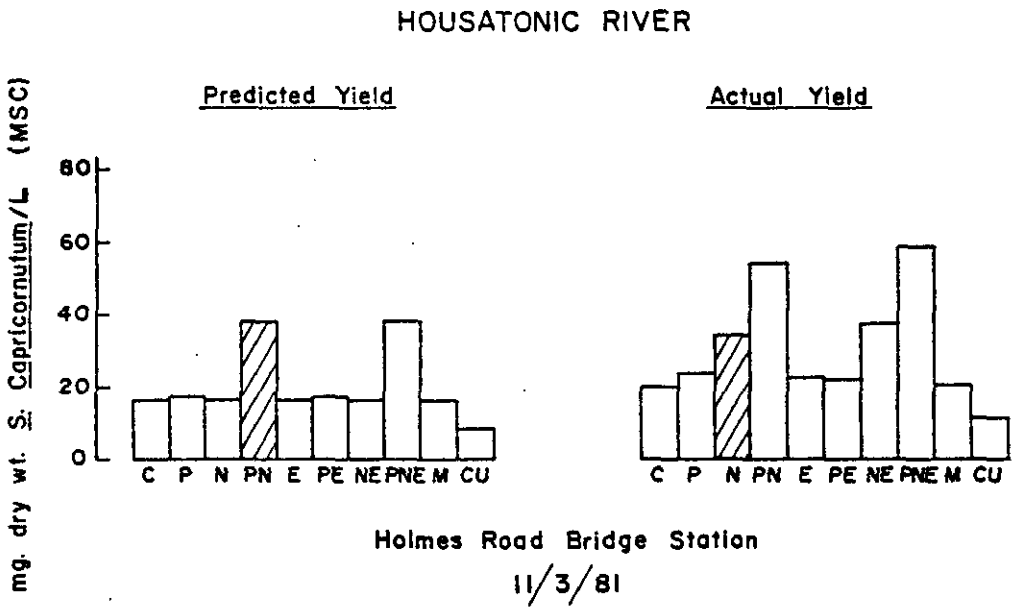

Figure 10. Predicted and actual yields (mg dry wt/L) of S. capricornutum grown in Housatonic River water, Holmes Road Bridge Station[1].

[1]C = control
P = + phosphorus
N = + nitrogen
PN = + phosphorus + nitrogen
E = + EDTA
PE = + phosphorus + EDTA
NE = + nitrogen + EDTA
PNE = + phosphorus + nitrogen + EDTA
M = + micronutrients
CU = control unautoclaved

Crosshatching indicates nutrient limitation at the time of sampling, and whether a positive response for micronutrient limitation or the presence of algal inhibitors was observed.

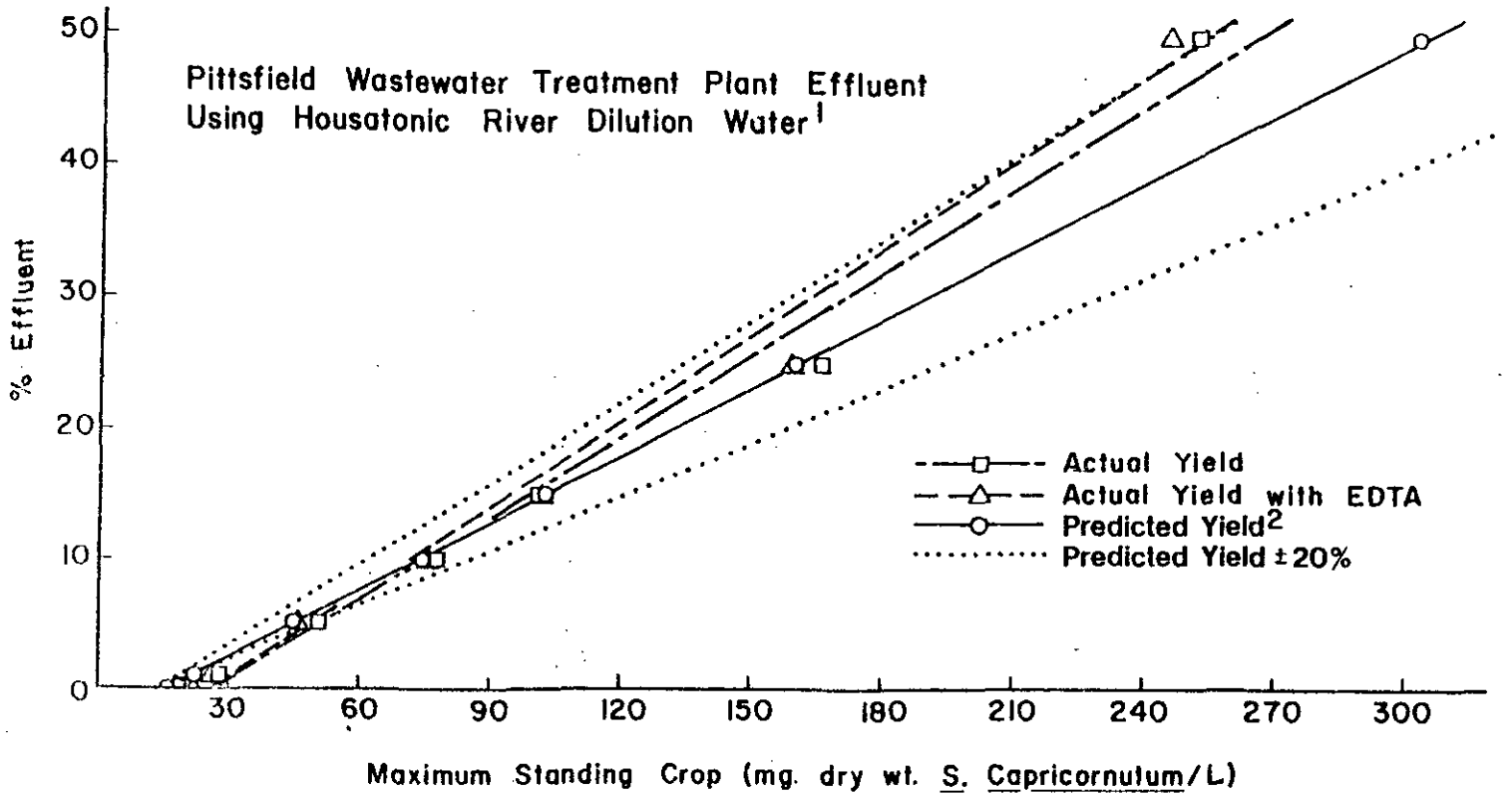

Figure 11. Predicted and actual (with and without EDTA) yields (mg dry wt/L) of S. capricornutum grown in Pittsfield secondary WWTP effluent and Housatonic River dilution water.

[1]Pittsfield sewage effluent was composited over 24 hours on 11/2/81-11/3/81. Housatonic River dilution water was sampled on 11/3/81.

[2]Predicted yield (mg dry wt/L) = 38 x TSIN (mg/L) ± 20 percent or 430 x ortho-P (mg/L) ± 20 percent depending on the N:P ratio.

Table 18

Algal Yield Data for Additions of Pittsfield Secondary WWTP Effluent to Housatonic River Water[1]

Percent Effluent[2]	N:P	Predicted ± 20%[3] Yield (mg dry wt/L)	Actual Yield (mg dry wt/L)
0	12:1	15.91 ± 3.18	19.76
0 + EDTA	12:1	15.91 ± 3.18	22.20
1%	8:1	22.25 ± 4.45	28.06
1% + EDTA	8:1	22.25 ± 4.45	25.28
5%	6:1	45.26 ± 9.05	49.66
5% + EDTA	6:1	45.26 ± 9.05	45.55
10%	5:1	74.04 ± 14.81	77.42
10% + EDTA	5:1	74.04 ± 14.81	74.95
15%	5:1	102.81 ± 20.56	101.39
15% + EDTA	5:1	102.81 ± 20.56	101.73
25%	5:1	160.35 ± 32.07	165.64
25% + EDTA	5:1	160.35 ± 32.75	160.02
50%	5:1	304.21 ± 60.84	253.21
50% + EDTA	5:1	304.21 ± 60.84	245.62

1. Dilution water was collected at the Holmes Road Bridge Station one mile above the sewage outfall on 11/3/81. The effluent was collected on 11/1/81.
2. Actual yields were not considered statistically different from the predicted yields if they fell within ± 20% of the predicted values determined by equations 1 or 2.
3. EDTA was added to replicate aliquots to determine the possible presence of algal toxicants or micronutrient limitation.

concentration of the effluent to 1.0 mg P/L using alum precipitation. The molar ratio of aluminum to phosphorus required to reduce the phosphorus level to 1.0 mg P/L depends on many factors including: alkalinity, pH, and concentration of cations. A preliminary jar test was therefore performed to determine the quantity of $Al_2(SO_4)_3 \cdot 18H_2O$ (alum) needed to reduce the effluent phosphorus level to 1 mg P/L. Orthophosphate was measured to evaluate removal efficiency.

Figure 12 illustrates an inverse relationship between residual phosphorus concentration and added alum. Using this figure, an Al:P molar ratio of 1.3:1 was chosen for the alum treatment algal assay study to reduce the effluent phosphorus level to 1 mg P/L. The addition of alum at this level did not significantly alter the pH (within 0.1 pH units) of the effluent.

Wastewater effluent was alum treated and filtered as described previously. Chemical analyses, after alum treatment, revealed ortho-P and total P concentrations of 1.228 mg P/L and 1.235 mg P/L, respectively (Table 11). The reduction in phosphorus increased the N:P ratio of the effluent from 4.7:1 to 12.5:1. This ratio indicated that the treated effluent was probably slightly phosphorus limited. Ratios of N:P resulting from the various volume dilutions of alum treated effluent and Housatonic River water are presented in Table 19.

Figure 13 illustrates the linear relationship between percent added effluent and algal yields. Actual yields in nearly all dilutions were within $\pm$ 20 percent of the predicted values. Predicted yield calculations were based upon phosphorus limitation.

Additions of EDTA did not increase algal growth, indicating that the alum was nontoxic to the algae, nor was there any trace element limitation (Figure 13).

The bioavailable phosphorus concentration of 1.235 mg dry wt/L was calculated using the extrapolated MSC for 100 percent effluent found from equation 10 (equal to 531.03 mg dry wt/L) divided by the phosphorus growth coefficient of 430. The bioavailable phosphorus concentration was very close to the inorganic phosphorus level of 1.228 mg P/L determined by chemical analysis.

The algal yields resulting from varying additions of either the alum treated or untreated effluent were nearly equal to each other (Tables 18 and 19). These data suggested that effective reduction in algal productivity resulting from discharge of the Pittsfield WWTP effluent into the Housatonic River can only be achieved by greater phosphorus removal than that used here or by

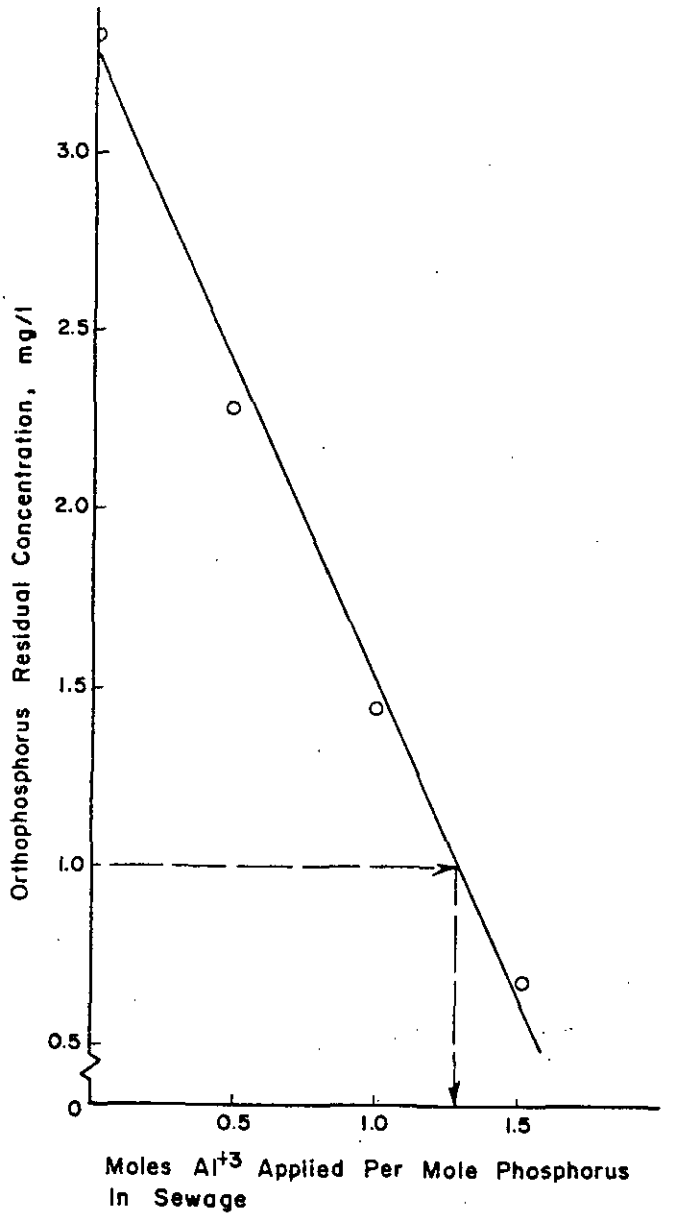

Figure 12. Effect of various alum additions upon the orthophosphate concentration in filtered Pittsfield secondary WWTP effluent.

Table 19

Algal Yield Data for Alum Treated Pittsfield Secondary WWTP Effluent Additions to Housatonic River Water[1]

Percent Effluent[2]	N:P	Predicted ± 20%[3] Yield (mg dry wt/L)	Acutal Yield (mg dry wt/L)
0	12:1	15.91 ± 3.18	17.21
0 + EDTA	12:1	15.91 ± 3.18	19.11
1%	12:1	21.03 ± 4.21	27.98
1% + EDTA	12:1	21.03 ± 4.21	26.79
5%	12:1	41.52 ± 8.30	51.58
5% + EDTA	12:1	41.52 ± 8.30	50.91
10%	12:1	67.12 ± 13.42	83.11
10% + EDTA	12:1	67.12 ± 13.42	85.37
15%	12:1	92.73 ± 18.55	113.43
15% + EDTA	12:1	92.73 ± 18.55	113.56
25%	12:1	143.94 ± 28.79	166.25
25% + EDTA	12:1	143.94 ± 28.79	165.27
50%	13:1	271.98 ± 54.46	275.41
50% + EDTA	13:1	271.98 ± 54.40	166.46

1. Dilution water was collected at the Holmes Road Bridge Station one mile above the WWTP outfall on 11/3/81. The effluent was collected on 11/11/81.
2. EDTA was added to replicate aliquots to determine the possible presence of algal toxicants or micronutrient limitation.
3. Actual yields are not considered statistically different from the predicted yields if they fall within ± 20% of the predicted values determined by equation 2.

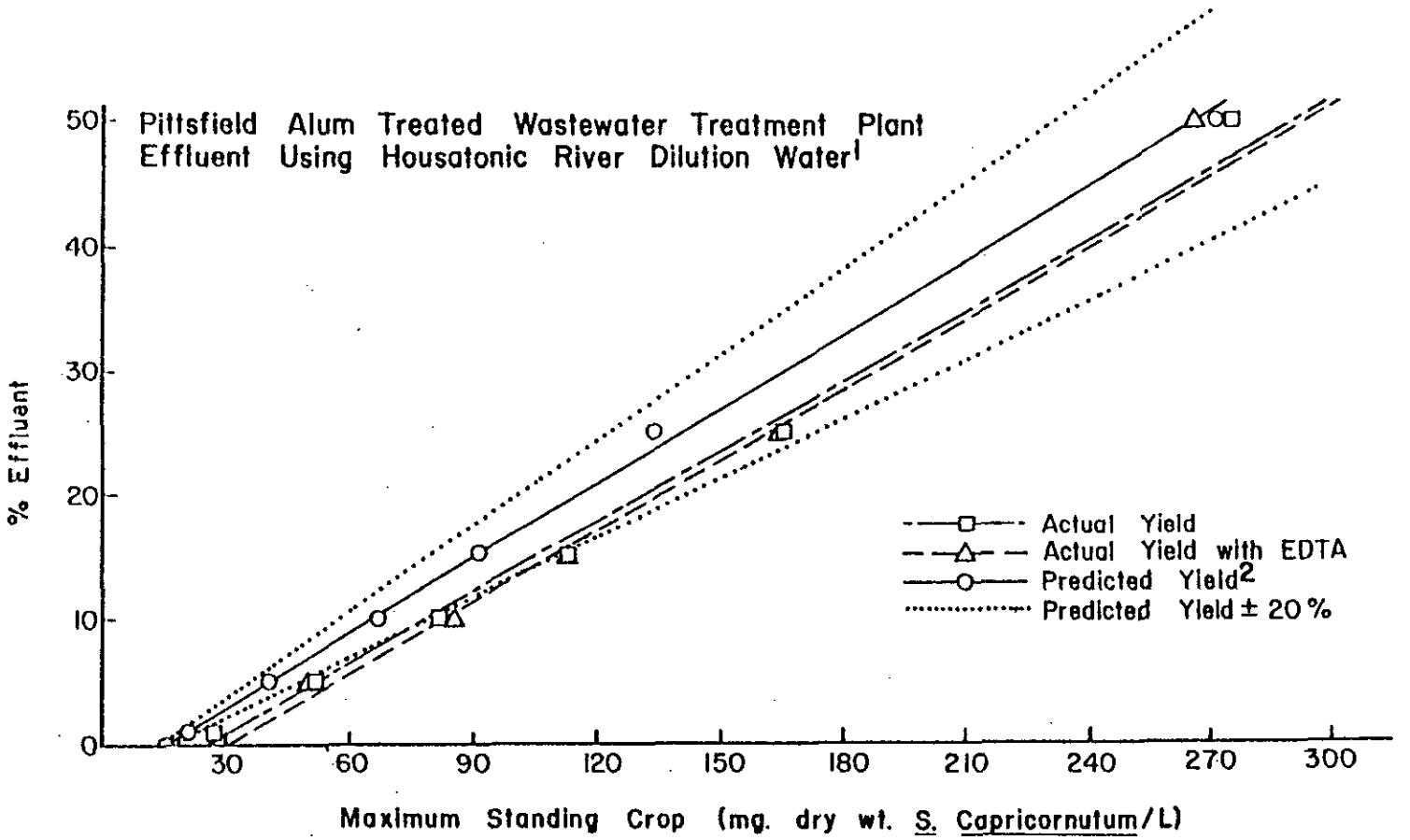

Figure 13. Predicted and actual (with and without EDTA) yields (mg dry wt/L) of S. capricornutum grown in alum treated Pittsfield secondary WWTP effluent and Housatonic River dilution water.

[1]Pittsfield alum treated wastewater was composited over 24 hours from 11/1/81 - 11/2/81. Housatonic River water, used as dilution water, was sampled at Holmes Road on 11/3/81

[2]Predicted yield (mg dry wt/L) = 430 x ortho-P (mg/L) ± 20 percent.

simultaneous reduction in both nitrogen and phosphorus levels in the effluent.

The N:P ratio of alum treated sewage was 12.5:1 reflecting slight phosphorus limitation or co-limitation by both nitrogen and phosphorus. Additional phosphorus removal, accomplished through greater alum addition, would further increase this ratio, reflecting a more extreme phosphorus limitation and would result in further reduction in the maximum standing crop of S. capricornutum.

Summary of Housatonic River Data

A general summary of the nutrient status of the Housatonic River for water samples collected from Andrus/Ranapo Road, Lanesville Road Bridge, Bulls Bridge, and Holmes Road stations is given below. Table 20 summarizes the chemical and algal assay data for the Housatonic River.

A. Nutrient limitation predicted by chemically measured N:P ratios indicated that water samples collected at Andrus/Ranapo Road were co-limiting or slightly nitrogen limiting on 6/9/81, but nitrogen limiting on 7/14/81 and 8/11/81. Samples from Lanesville Road Bridge were phosphorus limiting on 6/9/81 and 8/11/81 but nitrogen limiting on 7/14/81. Bulls Bridge samples were phosphorus limiting on 6/9/81 but nitrogen limiting on 7/13/81 and 8/11/81.

B. Algal assays of the above mentioned water samples confirmed the nutrient limitation predicted by N:P ratios in 8 out of 11 cases. Nutrient limitation determined by algal assay was different from that predicted by N:P ratios for the 7/14/81 and 8/11/81samplings of water from the Lanesville Road Bridge site,
collected from the surface and mid-depth, respectively. The surface sample displayed a 5:1 N:P ratio which normally indicates nitrogen limitation, but algal assay results indicated a N + P co-limitation. The N:P ratio for the surface sample collected on 8/11/81 was 18:1 which indicated phosphorus limitation. Algal assay results however, indicated that the sample was nitrogen limited. The former observation may be explained by a wider range in N:P ratios for which co-limitation is observed. Other investigators have confirmed co-limitation for N:P ratios ranging from 5:1 to 12:1. The latter observation is probably attributable to errors in chemical analyses or anomolous algal growth response. Nutrient limitation determined by algal assay was also different from that predicted by N:P ratios for the 11/3/81 sampling of the Holmes Road site. The sample displayed a N:P ratio of 12:1 which indicated

Table 20
Summary Table for the Housatonic River Study

	Site and Date												
	June 9, 1981				July 14, 1981				August 11, 1981				November 3, 1981
Parameter	Andrus/ Ranapo Rd	Lanesville Mid-depth	Lanesville Surface	Bulls Bridge	Andrus/ Ranapo Rd	Lanesville Mid-depth	Lanesville Surface	Bulls Bridge	Andrus/ Ranapo Rd	Lanesville Mid-depth	Lanesville Surface	Bulls Bridge	Holmes Road Bridge
Nitrogen* to Phosphorus Ratio	11:1	-	50:1	33:1	2:1	4:1	5:1	1:2	9:1	18:1	-	8:1	12:1
Predicted limiting nutrient	co-limitation	-	phosphorus	phos-phorus	nitrogen	nitrogen	nitrogen	nitrogen	nitrogen	phosphorus	-	nitrogen	co-limitation
Observed Algal Assay limiting nutrient result	nitrogen or co-limitation	-	phosphorus	phos-phorus	nitrogen	nitrogen	co-limitation	nitrogen	nitrogen	nitrogen	-	nitrogen	nitrogen
possible presence of algal inhibition	yes	-	yes	yes	no	no	no	no	no	no	-	no	no

*A ratio below 10:1 indicates likely nitrogen limitation
A ratio between 10:1 and 12:1 indicates likely co-limitation; however, co-limitation at values between 5:1 to 12:1 have been reported.
A ratio greater than 12:1 indicates likely phosphorus limitation.

co-limitation. Algal assay results, however, showed that the sample was nitrogen limiting. The results can be attributed to errors in chemical analyses or anamolous algal growth response.

C. Andrus/Ranapo Road samples, collected on 7/14/81 and 8/11/81, contained more inorganic and bioavailable nitrogen than did any other water sample collected from the Housatonic River during this study.

D. Micronutrient limitation was not observed in any of the Housatonic River samples.

E. Algal inhibition was observed in samples collected at all three sites of the Housatonic River on 6/9/81.

F. Effluent additions of treated or untreated effluent from the Pittsfield wastewater facility to Holmes Road dilution water displayed a linear relationship between increased algal growth response and percent effluent addition. Alum treatment of the effluent, however, did not reduce the phosphorus content sufficiently to shift the effluent to a phosphorus limiting state. Therefore no dramatic reduction in algal growth was observed for the treated effluent additions.

Evaluation of Anabaena flos-aquae as the Test Organism in the Algal Assay:Bottle Test

Most of the lakes and rivers sampled in this study were highly productive waters. In a eutrophic lacustrine ecosystem there is seasonal algal succession usually starting with diatoms in early spring, followed by green algal blooms in late spring, and blue-green algal blooms during the summer (Wetzel, 1975). Blue-green algal blooms are particularly noteworthy since they may impart noxious odor, liberate toxins harmful to other resident aquatic species, and cause wide diurnal fluctuations in dissolved oxygen and pH which are harmful to the in situ biota.

In response to a request from the MDWPC, this laboratory conducted an algal assay on a water sample taken from the Housatonic River using A. flos-aquae as the test organism. An experiment was performed, but the algal yield data proved to be unreliable owing to clumping of algal cells and an inability to disperse the clumps by sonification prior to cell enumeration. However, algal assays using A. flos-aquae have been successfully performed in previous studies (Shiroyama, et al., 1976, and Greene, et al., 1978). These studies reported an algal yield coefficient for A. flos-aquae based upon the phosphorus concentration in algal cultures, and have used this value in a manner similar to the algal yield coefficients of S. capricornutum for predicting algal productivity in solutions containing added nutrients. In applying the algal yield factor it is assumed that neither trace element limitation nor the presence of algal growth inhibitors exists in the algal culture. Nitrogen concentration can be discounted in

cultures of A. flos-aquae since this alga is capable of fixing atmospheric nitrogen. Thus, A. flos-aquae will only be phosphorus limited, and will, therefore, grow in direct proportion to the ambient phosphorus concentration in solution. Table 21 presents calculated algal growth yields for A. flos-aquae for the sampling sites studied in this investigation, using an algal growth factor of 450 (mg dry wt/L/mg P/L) reported in the literature. Values presented in Table 21 were calculated using the following equation:

$$\begin{array}{l}\text{Predicted yield of} \\ \underline{\text{Anabaena flos-aquae}} \\ \text{(MSC, mg dry wt/L)}\end{array} = \begin{array}{l}\text{orthophosphate} \\ \text{concentration} \\ \text{(mg P/L)}\end{array}$$

$$\times 450 \; \frac{\begin{array}{c}\text{mg dry wt} \\ \underline{\text{algae/L}}\end{array}}{\text{mg P/L}} \qquad (11)$$

It should be noted that actual in situ yields of A. flos-aquae would, most likely, be considerably less than the predicted values shown in Table 21 since other algal species would be competing for the phosphorus required for cell growth.

GENERAL DISCUSSION OF ALGAL ASSAY DATA

Nutrient Limitation Studies

The Algal Assay:Bottle Test was used in determining the limiting nutrient and bioavailable nutrient concentrations in water samples, as well as the presence of toxicants and the sensitivity of receiving water to wastewater nutrients. Two modifications were made to the test to expand its utility and increase the amount of information obtained. An addition of micronutrients to control water was used to assess the presence of trace element limitation in water samples. This modification proved to be of value in evaluating the nutrient status of samples collected from the Housatonic River on June 14, 1981. Addition of EDTA to these samples resulted in an increase in the maximum algal standing crop while addition of micronutrients did not increase growth. This indicated the presence of an algal inhibitor. The second modification to the AA:BT was the inclusion of unautoclaved controls in the test. This modification was used to determine the effect of autoclaving on the stability of certain complex organic compounds present in the water samples and on the solubilization of nutrients present as particulate matter. It was hypothesized that autoclaving might degrade some organic toxicants and thereby increase algal productivity in comparison to predicted values. Additionally, autoclaving could solubilize a portion of the particulate nutrients and thereby make them available to algal growth.

Table 21

Predicted Mean Standing Crop Values of Anabaena flos-aquae for the Sampling Sites Studied in this Investigation

Sample Site	Ortho-P Concentration (mg P/L)	Predicted Yield (mg dry wt/L^{1})
Spy Pond (4/31/81)	0.0111	4.95
Flint Pond (4/3/81)	0.016	7.20
Lake Quinsigamond I (4/3/81)	0.080	36.00
Quaboag Pond (5/8/81)	0.045	20.05
Housatonic River		
Bulls Bridge Station (6/9/81)	0.042	18.90
Bulls Bridge Station (7/14/81)	0.027	12.17
Bulls Bridge Station (8/11/81)	0.017	7.65
Andrus/Ranapo Road (6/9/81)	0.082	36.90
Andrus/Ranapo Road (7/14/81)	0.047	21.15
Andrus/Ranapo Road (8/11/81)	0.093	41.85
Lanesville Road Bridge (6/9/81)	0.048	21.60
Lanesville Road Bridge (7/14/81)	0.025	11.25
Lanesville Road Bridge (7/14/81)	0.027	12.15

Table 21, continued

Sample Site	Ortho-P Concentration (mg P/L)	Predicted Yield (mg dry wt/L[1])
Lanesville Road Bridge (8/11/81)	0.024	10.80
Holmes Road Bridge (11/3/81)	0.037	16.65

1. Predicted Yield of *Ababaena flos-aquae*, MSC in dry wt/L = Ortho-P concentration (mg P/L) x $\frac{\text{450 mg dry wt of algae/L}}{\text{mg P/L}}$

NOTE: Water displaying a *Selenastrum capricornutum* yield of greater than 6.0 mg dry wt. *S. capricornutum*/L is considered highly productive (Miller *et al.*, 1975)

No significant differences between the predicted and observed algal growth responses were observed in either autoclaved or unautoclaved samples, indicating the probable absence of labile organic toxicants in the samples studied. However, increased MSC values were observed in the autoclaved controls in comparison with unautoclaved portions indicating that this step did solubilize additional nutrients.

Miller, et al. (1978) recommends autoclaving followed by filtration as a pretreatment method for nutrient limitation studies. Cowen and Lee (1976) alternatively recommend autoclaving without filtration to obtain a more realistic estimate of the expected phosphorus and nitrogen availability in a given water sample. They found that dissolved reactive phosphorus was resorbed to the particulate matter after autoclaving and was then removed by filtration. Without filtering, however, use of the Coulter Counter for algal biomass determination could not be employed. It therefore seems appropriate to follow the recommendations of the U.S.E.P.A. (Miller, et al., 1978) by autoclaving and then filtering samples for algal assay. Inclusion of a non-autoclaved sample in the algal assay would provide additional information on the effect of autoclaving on the algal assay results.

Nitrogen and phosphorus analyses before and after autoclaving and filtration should be performed to provide additional information about the effects of these procedures on the nutrient content of the water sample. Analyses of sample water prior to autoclaving and filtration provide information about the concentrations of nutrients that potentially could become bioavailable to the algae. Predicted algal yields should be based upon chemical determinations of nitrogen and phosphorus compounds determined after autoclaving and filtration to include the effect of these procedures on the nitrogen and phosphorus constituents. Such a procedure will provide N:P ratios that more accurately predict the limiting macronutrient determined by the algal assay.

The AA:BT was found to be an effective and reliable method for determining the limiting macronutrient of a water sample as well as in determining trace element limitation or the presence of algal growth inhibitors. Such reliability was demonstrated by samples not displaying the presence of algal inhibitors or trace element limitation since similar algal growth responses were observed in aliquots of these samples, containing nutrient additions with and without EDTA spikes.

Standard errors of data taken from replicate flasks were usually much less than 12 and 25 percent for algal cultures having a mean standing crop greater, or less than about one mg dry wt/L, respectively. These values are well within the acceptable limits reported by Miller, et al. (1978).

Discounting toxic effects, observed algal yields were within 20 percent of the predicted values in about half of the samples with or without chemical additions. Actual yields not within the predicted range were attributed to: 1) an inability to assign a single limiting nutrient to the water sample because the N:P ratio was in the co-limitation range, 2) the presence of a heavy metal or organic toxicant, 3) trace metal limitation, 4) errors in chemical analyses, or 5) natural variability of algal growth response to nutrient additions in the AA:BT.

The AA:BT was used to determine the presence of algal inhibitors in several water samples (Table 11). In such samples, EDTA additions increased algal yields relative to those cultures containing nutrient spikes without EDTA additions. Similar algal growth response in cultures with or without micronutrient additions confirmed that the increased growth response in cultures containing EDTA was attributable to the presence of algal inhibitors.

Data from all sample sites in this study are summarized in Tables 18 and 19, and in the Summary of Data sections of this report. Of the 18 water samples tested, two were phosphorus limiting, 11 were nitrogen limiting, two were co-limiting and three were determined to be either nitrogen or P + N co-limiting.

N:P ratios were found to provide a good first estimate of nitrogen or phosphorus limitation or co-limitation. However, determination of nutrient limitation by algal assay technique was found to be a more reliable and accurate assessment of the nutrient status of a sample owing to the possible presence of algal toxicants, analytical errors in chemical determinations, and the range in N:P ratios for which co-limitation occurred. Algal assays corroberated nutrient limitation data predicted by N:P ratios in 13 out of 18 samples studied in this work. Occasional discrepancies between nutrient limitation predicted by N:P ratios vs actual algal assay data emphasizes the need for performing the AA:BT to accurately assess the nutrient status of a water sample. The range of nutrient co-limitation has been reported by Weiss (1976) and by Chiaudani and Vighi (1976) to be between 9:1 to 12:1, and 5:1 to 10:1, respectively. Co-limitation, determined by algal assay, was observed in this study for samples displaying N:P ratios ranging from 5:1 to 12.5.1. Thus the more defined limits for co-limitation of 10:1 to 12:1 presented by Miller, et al. (1978) should not be used as an absolute guideline for predicting the limiting nutrient of a water.

Algal Specific Weight Coefficient

An algal specific weight coefficient, SWC, equal to 3.6×10^{-7} was determined for use in equation 1 to calculate maximum standing crop from cell number and mean cell volume data. The SWC

found in this study was in close agreement to the value reported by Miller, et al. (1978).

Effluent Study

A reliable technique was developed to determine the effect of raw and alum-treated secondary WWTP effluent on a receiving water. Various percentages, by volume, of raw and alum-treated effluent were added to water collected from the streams or lakes actually receiving these wastes. One mg/L $Na_2EDTA \cdot 2H_2O$ was also added to a second set of the same dilutions to monitor possible toxicity in the samples. The effluent samples were not autoclaved to avoid possible degradation of complex organic compounds present in the WWTP effluent. The samples were filtered, however, to remove particulate matter and indiginous algae. Flasks containing the varying percent effluent additions were innoculated with S. capricornutum cells and incubated under continuous illumination for 14-21 days. The MSC was used to determine the algal growth stimulation by the effluent additions. Results showed a linear relationship between the percentage of added raw or treated effluent and resulting algal maximum standing crop. Algal yields were generally within 20 percent of predicted growth values. No additional growth was observed in EDTA treated samples. The linear response of the MSC to effluent additions demonstrated the sensitivity to change in nutrient status of the receiving water as determined by resulting algal productivity. Chemically analyzed and bioavailable nutrient concentrations were, however, undoubtedly lowered by the removal of organic particulates during filtration.

Alum treatment of effluent sampled from the Pittsfield wastewater treatment plant in Pittsfield, MA increased the N:P ratio in this sample from 5:1 to 12:1. Addition of alum at an Al:P molar ratio of 1.3 to 1 was used to achieve the corresponding 63 percent orthophosphate removal in the effluent sample. This treatment effectively decreased the phosphorus levels in the effluent, to an extent typically achieved in wastewater treatment facilities practicing such advanced treatment technology. The treated effluent, however, did not become phosphorus limiting. Consequently, algal productivity in dilution water receiving the treated effluent was not appreciably lower than for samples containing the untreated effluent. Greater phosphorus removal would therefore be required to shift the treated effluent to a phosphorus limiting state, in order to decrease its ability to promote algal growth. The feasibility of such increased phosphorus removal, however, may not be within Best Available Technology (BAT). Alum was found to be non-toxic to S. capricornutum at the concentrations used in this study.

The effect of secondary wastewater effluent on a receiving water cannot be completely predicted soley upon algal assay data.

Removal of organic particulates by filtration is required by the AA:BT protocol. This step may, however, result in an underestimation of algal growth response under field conditions owing to algal utilization of these organic particulates, either directly, or after solubilization. Alternatively, continuous shaking and dispersion of the algal cells during the AA:BT may overestimate actual growth in environmental waters where algal self shading mechanisms decrease algal proliferation. The extent of these two phenomena cannot really be assessed accurately by the AA:BT. However, the algal assay test does provide a good first estimate of the algal growth potential of nutrient-containing pollutant discharges into environmental waters.

Development of an Algal Assay Laboratory

A fully furnished algal assay laboratory was developed to analyze the samples in this study and provide facilities for future algal assay research and sample processing. A listing of equipment and supply requirements for such an algal assay laboratory as well as the associated costs are presented in Table 7. A total cost estimate for establishing an algal assay laboratory is $30,000 (1981 dollars). It was determined that approximately 89.5 person hours were required to perform the AA:BT for one sample including: planning, sampling, glassware preparation and clean-up, chemical analyses, performance of the AA:BT, data compilation and reporting. Additional samples, analyzed concurrently, would require an extra 60 hours each. A small algal assay laboratory capable of processing about eight water samples a month, would therefore require at least two laboratory technicians and a full time laboratory director, knowledgeable in algal assay technique and aquatic biology.

Sampling Guidelines

Guidelines were established for collecting lake, river, and WWTP effluent samples for algal assay, based upon previous studies reported in the literature. During lake turnover, depth integrated composite lake samples should be collected for the AA:BT (usually in the spring and fall in dimictic lakes). During stratified periods, a depth integrated sample taken from the epilimnion should be used since the water in this zone mixes separately from the metalimnion and hypolimnion, and contains most of the *in situ* indigenous algal population.

River water samples should be collected at a free-flowing area at mid-depth and mid-width. Depth variability in a river water sample was assessed in the July 14, 1981 sampling of the Housatonic River at the Lanesville Road Bridge Station. Both surface and mid-depth (4 meters) samples were collected at this site. Inorganic nutrient content as well as nutrient bioavailability for the two samples were about the same. The

surface sample appeared to be somewhat co-limiting, while the mid-depth sample was slightly nitrogen limiting. However, the actual MSC values for the nitrogen, phosphorus and N + P additions between the two samples were not appreciably different from each other. The data indicated that composite depth sampling of river sites (by depth and width) might provide a slightly more representative sample of water quality parameters, used for algal assay determinations, than either a surface or mid-depth sample alone. However, because of the labor and cost constraints involved in performing the AA:BT, a single sample collected from a free flowing, mid-depth, mid-width location is recommended for river sites.

WWTP effluent was collected as a 24 hour composite sample to account for diurnal variations in chemical composition. It should be cautioned, however, that while a composite sample does provide a solution containing an average nutrient content, actual discharge of nutrient levels into receiving waters may vary above and below this level in response to sewage content fluctuations.

Effluent samples were collected prior to chlorination at the Pittsfield plant to prevent algal toxicity by chlorine. Reduction of free chlorine by reaction with sodium thiosulfate for chlorinated samples is less than ideal since chloro-organic compounds or chloramines may not be removed by this reaction.

Data from this study suggests that samples should be collected at least four times per year (once per season) at each station in order to incorporate the effects of in situ water quality variability on algal assay results. For example, bioavailable phosphorus data varied considerably over time at the Andrus/Ranapo Road Station of the Housatonic River, ranging between 0.050, 0.083 and 0.152 mg P/L for the 6/9/81, 7/14/81, and 8/11/81 samplings, respectively. All inorganic nutrient levels varied at this site as well. Variations in the nutrient content of water samples taken at the Lanesville Road Bridge and Bulls Bridge Stations were also observed. Austin (1982) observed changes in both the N:P ratio and in the limiting nutrient of water sampled from the Mill River just north of the State Street Bridge, in Amherst, MA, over a several month period. Water samples should additionally be collected under dry weather conditions to minimize dilution effects from precipitation and nutrient loadings from land runoff.

Water Quality Parameters

Several in situ water quality parameters should be determined at the time of sampling including: dissolved oxygen, pH, and temperature. Dissolved oxygen and temperature provide general water quality information about the site. Determination of in situ

pH is needed for adjustment of sample aliquots to *in situ* pH values following autoclaving, prior to algal assay.

Chemical Analyses

Chemical analyses needed to properly interpret algal assay data include: total organic nitrogen, total soluble inorganic nitrogen (equal to $NO_3^- - N + NO_2^- - N + NH_3 - N$), orthophosphate, and total phosphorus. These analyses should be performed as soon as possible following return of water samples to the laboratory. Samples for chemical analysis should be stored in the dark at $4^{\circ}C$. Other investigators have shown the specific types of organic fractions present in a water sample may provide additional, useful information about the nutrient status of a water sample since *S. capricornutum* as well as other algal species may utilize such organic nutrient forms (Sachdev and Clesceri, 1978).

Algal Assays Using Anabaena flos-aquae

The blue-green alga, *Anabaena flos-aquae*, was examined as a test species for the AA:BT using samples collected from the Housatonic River. However, results were inconclusive because of difficulties in algal cell enumeration. Cell cultures tended to grow as clumps rather than as homogeneous algal dispersions. An accurate assessment of maximum standing crop could therefore not be made using Coulter Counter techniques, even with sonification. Spectrophotometric absorption was also examined as a surrogate parameter for algal population size. However, the same clumping phenomena precluded the use of this method as well as for measuring all biomass.

In addition to the limitations and problems encountered in determining the MSC for *A. flos-aquae*, the utility of this alga in determining nutrient limitation is limited since it is capable of fixing atmospheric nitrogen. Consequently *A. flos-aquae* can only be used to test for phosphorus or micronutrient limitation or to determine the presence of algal growth inhibitors in the water sample.

Predicted values of *A. flos-aquae* growth (Table 21) were calculated for the samples collected in this study, using a phosphorus growth coefficient of 450 reported in the literature (Shiroyama, *et al.*, 1976). These values are considered as upper growth values for this alga since competition for phosphorus by other indiginous algal species would reduce the actual maximum standing crop of *A. flos-aquae* under field conditions.

Biomonitoring Using the AA:BT

The data collected in this study support the use of the AA:BT as a reliable, and accurate biomonitoring method which could be incorporated into effluent standards and in state enforced regulations of pollutional inputs into environmental waters. The test provides effective, reproducible and accurate data about the limiting nutrient of a water sample, on nutrient bioavailability, the presence of algal inhibitors, and on the sensitivity of a water body to a change in nutrient concentration. These conclusions are based upon data presented here, as well as by the widespread use of the AA:BT in the literature. The costs and personnel needs for conducting algal assays are not prohibitive, although the two-to-three week period required for incubation of samples is somewhat inconvenient. The test requires laboratory technician personnel, as well as an overall director with expertise in aquatic biology.

SUMMARY

1. A version of the AA:BT, modified to include a micronutrient addition and an unautoclaved control, was successfully used to determine the limiting nutrient and sensitivity to change in nutrient status for river and lake waters. A technique to determine the effect of treated or raw WWTP effluent on receiving waters, using the AA:BT, was also developed. Use of the micronutrient modification is recommended to distinguish between trace element limitation and the presence of algal toxicants in water samples. Autoclaving and filtering is recommended as a pretreatment step for samples to be algal assayed.
2. A pilot algal assay monitoring laboratory was developed capable of examining water and WWTP effluent samples using the AA:BT in both routine sample assessment as well as in research efforts.
3. An estimated cost figure, for the year 1981, for fully furnishing an algal assay laboratory, including equipment and supplies (chemicals and glassware) was $30,000.
4. Approximately 89.5 person hours are required to conduct a complete algal assay for one sample. This time estimate includes: planning, sampling, glassware preparation and clean-up, all chemical analyses, performance of the AA:BT, data compilation, and reporting. Each additional sample, processed concurrently, would require about 60 more more person hours.
5. Water samples for algal assay, collected from unstratified lakes, should be taken as depth-integrated composite samples from the epilimnion, since algal growth occurs predominantly in this zone. Algal assays should be performed on lake sites both before and after overturn to assess the change in nutrient status attributable to this phenomenon. Representative river samples should be collected by grab

sampling at a free flowing area located at mid-depth and mid-width.
WWTP effluent should be collected, before the chorinator, as a 24 hour composite sample.

6. Samples should be collected, at each water resource under investigation, at least four times per year (one each season) to observe seasonal changes in the water's nutrient status. Such seasonal variations were observed in this study for samples collected from the Housatonic River and in a study by Austin (1982), on the Mill River. Samples should be collected under dry weather conditions to minimize dilution by precipitation.
7. The AA:BT was found to be an effective and reliable method for determining the limiting nutrient of a water body.
8. The AA:BT was found to be an effective and reliable method for determining the presence of algal growth inhibitors in water samples.
9. The AA:BT was found to be an effective and reliable method for determining trace element limitation in a water body.
10. The AA:BT was found to be an effective and reliable method for predicting the algal growth stimulatory potential of raw or alum-treated WWTP effluent on receiving waters, and in determining the effectiveness of phosphorus removal in decreasing nutrient enrichment and associated algal growth of receiving waters.
11. The N:P ratio is a good first estimate for determining the limiting nutrient of a water body. However, reliance on chemical analyses without performing an algal assay may lead to false conclusions owing to the wide range in N:P ratios (5:1 to 12:1) corresponding to nitrogen and phosphorus co-limitation, the presence of algal inhibitors, or trace element limitation in the water.
12. Baseline data on selected sites in Massachusetts and Connecticut are summarized in Tables 11 and 12 and in the Summary of Data section of this report.
13. Discounting toxic effects, about half of the observed algal yield data for the untreated samples and in aliquots containing chemical additions, singly or in combination, were within 20 percent of the values predicted by the inorganic nitrogen and phosphorus content (equations 1 and 2) of the sample. Observed algal yields falling beyond the 20 percent range of the predicted values may be attributable to:

    a. the presence of an algal growth inhibitory compound;
    b. micronutrient limitation;
    c. incorrect chemical analyses;
    d. difficulty in assigning predicted values based upon nitrogen or phosphorus limitation when the N:P ratio falls in the range of 5:1 to 12:1 possibly reflecting both nitrogen and phosphorus co-limitation; or

e. inherent variability in the algal assay test itself.

14. Temperature, dissolved oxygen and pH should be determined at the time of sampling. Appropriate samples should be autoclaved and all samples filtered immediately upon their return to the laboratory. TON, TSIN, NO_3^--N, NO_2^--N, ortho-P and total-P should then be determined at the earliest opportunity.

15. Alum treatment of WWTP effluent is an effective method to reduce algal growth in receiving waters because the phosphorus level of the effluent is considerably decreased by this process. The treatment, however, must be sufficient to decrease the phosphorus level to the point where the effluent is phosphorus, rather than nitrogen limiting. Aluminum, at levels measured in alum-treated wastewater, was not found to be toxic to algal growth.

16. An algal specific growth weight coefficient equal to 3.6×10^{-7} was determined so that algal biomass could be calculated directly from mean cell volume and cell number data using a Coulter Counter. The growth coefficient was found to be in good agreement with other values reported in the literature.

17. Although A. flos-aquae is a nuisance species, typical of fresh water summer time algal bloom populations, it is not a very good test species for the AA:BT because it is difficult to enumerate owing to its filamentous morphology. Additionally, it can only be used to test for phosphorus and/or micronutrient limitation because it is able to fix atmospheric nitrogen. Algal growth yield for A. flos-aquae can be predicted using a phosphorus growth coefficient equal to 450 reported in the literature. Simultaneous testing of A. flos-aquae and S. capricornutum may reveal a variable response of these algae to chemical inhibitors present in the water.

18. The AA:BT can be utilized as a regulatory tool since:

    a. the test has reasonable equipment, supply, and personnel requirements;
    b. the test is reliable, effective, and accurate;
    c. the test incorporates the interactiveness of water quality parameters within the sample, or, in the case of a WWTP effluent algal assay, the interactions between both effluent components and receiving water constituents;
    d. the test provides information on

        i. bioavailable nutrients;
        ii. nutrient or trace metal limitation;
        iii. the presence or absence of algal toxicants; and

iv. the sensitivity to change in nutrient status of the water sample;

e. the test can be performed within a reasonable period of time; and

f. there exists an abundance of literature supporting the validity of the AA:BT in assessing the proclivity for algal productivity in a water or effluent sample.

It is recommended that only state operated or state regulated algal assay laboratories perform the AA:BT for regulatory purposes to ensure honest and properly determined test results. It is also recommended that algal assay requirements for effluents be set on a site specific basis based upon the severity of nutrient enrichment from such wastes, because of the time and labor requirements for conducting such assays.

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Appendix A

Dealer Addresses

1. Coulter Electronics Incorporated
   Hialeah, Florida

2. Fisher Scientific
   461 Riverside Avenue
   PO Box 379
   Medford, MA 02155

3. General Electric Company
   Cleveland, Ohio

4. Hach Chemical Company
   PO Box 907
   Ames, Iowa 50010

5. Instrumentation Specialty Company
   Lincoln,Nebraska

6. Merriam-Graves Corporation
   1361 Union Street
   West Springfield, MA 01089

7. Millipore Corporation
   Bedford, MA 01730

8. VWR Scientific
   PO Box 232
   Boston, MA 02101

9. WILECO
   301 Cass Street
   Saganaw, Michigan 48602