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Using Single Species & Whole Ecosystem Tests to Characterize the Toxicity of a Sewage Treatment Plant Effluent 53 pp

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Narragansett Bay Estuary Program

USING SINGLE-SPECIES AND WHOLE ECOSYSTEM TESTS TO CHARACTERIZE THE TOXICITY OF A SEWAGE TREATMENT PLANT EFFLUENT

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FOREWORD

The United States Congress created the National Estuary Program in 1984, citing its concern for the "health and ecological integrity" of the nation's estuaries and estuarine resources. Narragansett Bay was selected for inclusion in the National Estuary Program in 1984 and designated an "estuary of national significance" in 1988. The Narragansett Bay Project (NBP) was established in 1985. Under the joint sponsorship of the U.S. Environmental Protection Agency and the Rhode Island Department of Environmental Management, the NBP's mandate is to direct a five-year program of research and planning focussed on managing Narragansett Bay and its resources for future generations. The NBP will develop a comprehensive management plan by June 1991 , which will recommend actions to improve and protect the Bay and its natural resources.

The NBP has established the following seven priority issues for Narragansett Bay:

* management of fisheries
* nutrients and potential for eutrophication
* impacts of toxic contaminants
* health and abundance of living resources
* health risk to consumers of contaminated seafood
* land-based impacts on water quality
* recreational uses

The NBP is taking an ecosystem/watershed approach to address these problems and has funded research that will help to improve our understanding of various aspects of these priority problems. The Project is also working to expand and coordinate existing programs among state agencies, governmental institutions, and academic researchers in order to apply research findings to the practical needs of managing the Bay and improving the environmental quality of its watershed.

This report represents the technical results of an investigation performed for the Narragansett Bay Project. The information in this document has been funded wholly or in part by the United States Environmental Protection Agency under assistance agreement #CX812768 to the Rhode Island Department of Environmental Management. It has been subject to the Agency's and the Narragansett Bay Project's peer and administrative review and has been accepted for publication as a technical report by the Management Committee of the Narragansett Bay Project. The results and conclusions contained herein are those of the author(s), and do not necessarily represent the views or recommendations of the NBP. Final recommendations for management actions will be based upon the results of this and other investigations.

Previously published in *Aquatic Toxicology and Environmental Fate: Eleventh Volume,* ASTM STP 1007, G. W. Suter II and M. A. Lewis, Eds., American Society for Testing and Materials, Philadelphia, 1989, pp. 231-250. The interested reader is also encouraged to investigate a related report: " Transport, Fate, and Toxic Effects of a Sewage Treatment Plant Effluent in a Rhode Island Estuary, " by Edward Dettmann, John Paul, Jeffrey Rosen and Charles Strobel of the Environmental Protection Agency's Research Laboratory, 27 Tarzwell Dr., Narragansett, RI 02882-1198.

ABSTRACT

A four month experiment was conducted to evaluate the toxicity of a sewage effluent using both single-species and whole ecosystem tests. Fresh effluent from the East Greenwich, Rhode Island, sewage treatment plant was added daily to six experimental ecosystems (mesocosms). Sewage effluent additions were made at 0.1, 1.0 and 10 percent of the daily seawater input (960 l/d) from Narragansett Bay. Reagent grade, inorganic nutrients were added to control, 0.1 and 1.0 percent effluent treatments to normalize nutrient loadings in all treatments. The Arbacia punctulata sea urchin sperm cell test was conducted on effluent and mesocosm samples. In the mesocosms, measurements of responses at the population, community and ecosystem levels of biological organization were made.

The single-species toxicity test indicated the mean EC50 of the sewage effluent was 1.1 percent. Toxicity decayed rapidly over time, and was unrelated to carbon, nutrient, residual chlorine or metal concentrations. Toxicity in the mesocosms was variable due to short-term (4-5 h), incomplete mixing of the effluent. There was no evidence for a build-up of toxicity in the mesocosms. Mesocosm effluent additions decreased phytoplankton standing stock and produced an imbalance between total system production and respiration leading to hypoxia. At the 10 percent effluent loading, net system production was negative.

Results indicated both single-species and mesocosm approaches were useful to assess toxicity. The single-species test was best utilized to characterize the magnitude and persistence of toxicity, and the mesocosm experiments best employed to identify sensitive communities and processes.

Keywords: Toxicity testing, microcosms, mesocosms, ecosystem-level effects, system production, system respiration, dissolved oxygen, Arbacia punctulata.

INTRODUCTION

The assessment and management of hazardous waste disposal in the United States is the responsibility of the Environmental Protection Agency (EPA). This assessment includes efforts to predict the behavior of potential pollutants to define concentrations that will occur in the environment [1]. The use of large-scale, experimental ecosystems (mesocosms) [2-7] has been one approach used to study chemical behavior and fate as have field studies of planned and accidental discharges of pollutants [8,9]. Extensive models have been developed describing chemical fate and behavior [10,11]. These approaches have been used to refine predictions made from smaller-scale laboratory studies, and theoretical investigations of pollutant behavior.

Formerly, EPA has relied upon a chemical-specific approach to regulating discharges of potentially hazardous wastes [12]. In this approach, biological effects were predicted from extrapolating the results from single-species toxicity tests to the real world environment using models and empirical data to predict exposure concentrations [13-15]. However, because toxicological data are not (and can never be) available for the myriad compounds discharged, and because interactions between chemicals may ameliorate or magnify toxicity, the chemical-specific approach is being replaced by whole-effluent toxicity testing [12].

Single-species toxicity tests are by far the most commonly used approaches used to measure the toxicity of whole effluents. Inspection of past ASTM volumes related to toxicology demonstrates wider use of and more experience with fresh water test species than marine, although a variety of tests using estuarine and marine test species have been developed. Microcosm

tests, involving more complex systems with a greater level of biological organization, have not been conducted with whole effluents, but due to the past success of microcosm tests, studies using whole effluents can be expected. Single-species toxicity tests have been applied to the field to measure whole effluent toxicity [16]. However, field studies of all but the most dramatic of pollution events [17,18] often lead to ambiguous results, confounded by a range of uncontrolled environmental variables.

There exists a strong need to define how well single-species toxicity tests applied to whole effluents can be used to predict responses at higher levels of biological organization [19,21]. Further, the single-species, multiple community mesocosm, and the field measurement approaches to toxicity assessment have never been compared simultaneously for a discharge into a marine environment.

Such a comparison is one of the goals of a project jointly conducted by the Marine Ecosystems Research Laboratory (MERL) at the University of Rhode Island, and the EPA Environmental Research Laboratory in Narragansett, RI. The project is a part of the EPA Complex Effluent Toxicity Testing Program and involves laboratory, mesocosm and field assessments of the toxicity of sewage effluent from the East Greenwich, Rhode Island, sewage treatment plant.

This paper will present results from a mesocosm experiment conducted at MERL using the East Greenwich sewage effluent. The MERL mesocosms are functional analogs of shallow, unstratified coastal systems, (such as Narragansett Bay), and have become very useful tools with which to further understanding of ecosystem structure and function, and the behavior, fate and effects of pollutants (see [6,22-27] and references cited within).

The purpose of the mesocosm experiment was to to compare toxicity as measured by single-species tests to detrimental effects measured in the MERL mesocosm ecosystems. This paper presents mesocosm results demonstrating detrimental and toxic responses and the results from one single-species toxicity test, the Arbacia punctulata, sea urchin sperm cell test. We hope to demonstrate that the single-species test was most useful to characterize the magnitude and persistence of toxicity, whereas the mesocosms were most useful to identify sensitive ecosystem components and processes.

METHODS

Single-Species Tests

A variety of single-species tests were used to measure toxicity (Microtox, and tests utilizing Champia parvula, Laminaria saccharina, Mysidopsis bahia, Menidia beryllina, and sea urchin sperm cells), but only results from the sea urchin (Arbacia punctulata) test will be reported here. This test was consistently the most sensitive in this investigation and was easily and reliably repeated under a broad range of test conditions

The sea urchin sperm cell test was used to compare the toxicity of 13 Rhode Island sewage treatment plant effluents, set a range for the effluent concentrations to be used in the experiment, characterize the decay of effluent toxicity, and directly assess toxicity in the mesocosm water columns. The Arbacia punctulata sperm cell toxicity test has been previously described [28-30]. Briefly, sea urchin sperm were directly exposed to effluent dilutions. Following 1 h exposure, eggs were added, and incubated for an additional 20 min. Eggs were then fixed by the addition of buffered formalin and fertilization, defined as the presence of fertilization membranes, measured by microscopic observation. Results were expressed as the effluent concentration causing a 50 percent reduction in fertilization rates relative to Narragansett Bay, Rhode Island, seawater controls.

Description of MERL Mesocosms and Experiment

The experiment was conducted from 15 July to 14 November 1986 in eight mesocosms (Figure 1) containing sediment and seawater from Narragansett Bay. Sediments were collected from the northwest corner of East Greenwich Bay (Figure 2) using a 0.25 m^2 box corer and methods designed to preserve the

vertical structure of the sediments (see [31]). These sediments were chosen due to their proximity and expected similarity to sediments in Greenwich Cove, the receiving body of water for effluent from the East Greenwich, Rhode Island sewage treatment plant.

Two mesocosms were run as controls receiving no addition of sewage effluent. Daily additions of fresh East Greenwich sewage effluent were made to six mesocosms at three loading levels (Table 1). Each tank received 960 liters/d seawater (turnover time=volume/flow=13.5 d). Turnover time was selected to ensure development of water column communities and not to simulate turnover at the East Greenwich Cove receiving water body.

The highest effluent loading level corresponded to an input rate of 10 percent of the daily seawater flow to the mesocosms and amounted to 96 liters/d sewage effluent. Other effluent treatment levels corresponded to input rates of 1.0 percent (9.6 liters/d) and 0.1 percent (0.96 liters/d).

Mesocosm mixing was accomplished using a vertical plunger operating at 5 rpm in a 2 h on, 4 h off cycle. Seawater and sewage effluent additions were made four times daily. Seawater additions (8 liters/min) commenced 15 min after the start of mixing and lasted for 30 min. Effluent additions (0.24, 2.4 and 24 liters corresponding to the 0.1, 1.0 and 10 percent treatments) commenced 45 min later and were made in two equal pulses. Effluent additions ceased 14 min prior to the end of mixing. Effluent was refrigerated (4 ^{o}C) during storage between additions to retard the decay of toxicity. In most cases, effluent was stored no longer than 24 h since delivery was made daily. In no case was storage longer than 48 h.

All treatments received approximately the same nutrient loading to separate nutrient from effluent toxicity effects. Reagent grade inorganic nutrients (NH_4Cl, KH_2PO_4, $Na_2SiO_3 \cdot 9H_2O$) were added to control tanks and the low and medium effluent treatments once per day to match nutrient loadings expected at the high effluent treatment (Table 1). Reagent nutrient addition levels were held constant through the experiment. Effluent nutrient concentrations were determined before the start of the experiment. Nutrient concentrations were expressed as microgram-atoms (ug-at) where 1 ug-at/l = 1 ug/l divided by the atomic weight of N, P or Si depending upon the nutrient. Total nitrogen in these samples was 1117 (ug-at) N/liter sewage effluent with 73 percent (812 ug-at N/liter) present as dissolved, inorganic nitrogen (ammonia + nitrate + nitrite). Total phosphorus was 341 ug-at P/liter with almost 100 percent present as dissolved, inorganic phosphorus. The concentration of dissolved reactive silicate was 75 ug-at Si/liter. Nutrient additions to the mesocosms were made based upon the dissolved, inorganic nutrient concentrations since it was not known how biologically available other nutrient forms (particulate and dissolved organic) from sewage effluent would be.

Measurements were made to characterize daily batches of sewage effluent and to define ecosystem, community, and population level responses of the mesocosm ecosystems. Methods used have been previsouly described [32,33].

Effluent total suspended solids (TSS), particulate organic carbon (POC) and dissolved organic carbon (DOC) were sampled approximately daily. Daily samples were analyzed for TSS and POC. DOC samples from some weeks were pooled before analysis to reduce analytical time. Effluent nutrients were sampled five times per week during the first seven weeks of the experiment and once per week thereafter. All nutrient samples were analyzed using weekly

pooled samples. Selected metals in the effluent were measured periodically by EPA ERLN using standard methods. Effluent residual chlorine was measured as total residual chlorine by amperometric back titration.

In the mesocosms, DCMU fluorescence (a measure of phytoplankton biomass) was measured daily. Size fractionated and dissolved fluorescence, chlorophyll a concentration, nutrients and oxygen were measured weekly. Total system (total system=water column plus sediments) production and respiration were estimated from changes in oxygen concentration measured over a diel cycle once per week. Zooplankton biomass was measured once every two weeks. Zooplankton community composition and abundance were determined from separate samples taken at the same time. Sediment oxygen metabolism and inorganic nutrient fluxes across the sediment-seawater interface were measured approximately monthly. Sediment biota were collected once every three weeks. Preliminary results from the enumeration and identification of benthic macrofauna (organisms retained on a 300 um sieve) are reported.

Statistical Analyses

Statistical analyses and data management were completed using SAS software [34-36]. Data for each month of the experiment were analyzed separately using a two-way ANOVA. Factors were treatment with four levels (control, 0.1, 1.0, and 10 percent effluent) and replicate with two levels. Replicate mesocosms were nested within treatment. Student-Neuman-Kuels tests were used to separate treatment effects and statistical significance reported for $p<0.05$ unless otherwise stated.

RESULTS

Sewage Effluent Characterization

Sewage effluent from the East Greenwich sewage treatment plant was moderately toxic compared to effluents from twelve other municipal sewage treatment plants in Rhode Island (Table 2). This plant was built in 1928 and expanded to secondary treatment in 1956. The plant can efficiently treat 0.5 million gallons of sewage per day (MGD)(1514 m^3/d), but is currently discharging 0.7 MGD (2649 m^3/d). The plant is scheduled for enhanced secondary treatment within two years.

The composition of the East Greenwich effluent is given in Table 3. The toxicity of the sewage effluent was measured on 36 days during the experiment (Figure 3). Toxicity (EC50) ranged from 0.3 percent effluent to nontoxic (EC50>10 percent) and averaged 1.1 percent for all samples for which toxicity was detected. Toxicity was not significantly correlated with any measured parameters (Table 3). Additionally, toxicity could not be correlated with rainfall volume measured at T.F. Green Airport in Warwick, RI, approximately six miles north of the East Greenwich sewage treatment plant.

Toxicity decay was temperature dependent but the functional relationship between decay rate and temperature could not be defined with the data available. Laboratory experiments indicated decay was negligible at 4 ^{o}C but a toxicity half-life of 1.8 h was calculated at 25 ^{o}C. Toxicity decay was not related to residual chlorine concentrations.

Mesocosm Nutrient Additions, Temperature and Salinity

The nitrogen concentration of the sewage effluent added to the mesocosms was greater than that of the effluent samples taken prior to the start of the experiment. Total nitrogen was 1621 ug-at N/liter (CV=16 percent), with 64 percent (1032 ug-at N/liter) present as dissolved inorganic nitrogen. This was 27 percent higher than the concentrations of inorganic nitrogen selected for addition.

The total phosphorus concentration of the effluent added to the mesocosms was 382 ug-at P/liter (CV=21 percent), with 86 percent (329 ug-at P/liter; CV=19 percent) present as dissolved phosphorus. Dissolved inorganic phosphorus concentrations averaged 279 ug-at P/liter (CV=22 percent), 18 percent lower than concentrations of inorganic phosphorus selected for addition.

Silica concentrations in the effluent added to the mesocosms averaged 205 ug-at Si/liter (CV=13 percent), almost 3 times higher than initially determined concentrations. This large difference was traced to an error made in analyzing initial samples. Freezing water can convert reactive silicate to a nonreactive, polymeric form [37]. Initial silica samples were run immediately after thawing, thus causing erroneously low values. Subsequent samples were allowed to thaw for approximately one week prior to analysis thereby reversing the process. The bias introduced by freezing and thawing for one week is believed to be less than 5 percent [37].

Mesocosm water column temperatures during the experiment ranged from 7.8 to 22.2 ^{o}C. The salinity of the inflowing seawater from Narragansett Bay ranged from 30.3 to 32.0 ppt. Salinities in the control mesocosms were similar but due to effluent additions, salinities in the 10 percent treatment

ranged from 27.2 to 30.6 ppt and averaged 28.5 ppt. Single measurements made on the last day of the experiment indicated there was no significant salinity decrease compared to controls in the 0.1 percent and 1 percent treatments.

The temporal trend of salinity in the 10 percent treatment closely fit a predictive salinity model that assumed instantaneous mixing of seawater, then sewage effluent, each followed by the removal of an equivalent volume of well mixed water. The model included provisions for four daily additions of seawater and effluent. Measured salinities deviated from predicted salinities only slightly (ratio measured/predicted averaged 1.001, range 0.974 to 1.019, n=17). These results indicated that mixing over the 6 h mixing cycle was very good. Vertical salinity profiles taken after sewage effluent additions and during the 4 h no-mixing period indicated stratification, and therefore, incomplete mixing occurred during shorter time periods. Mixing occurred primarily when the oscillating plunger (Figure 1) was operating. Since this period continued for only 14 min after effluent addition, mixing was completed during the following mixing cycle, 4 h later.

Mesocosm Toxicity

Although the toxicity of whole water samples taken directly from the mesocosm systems was highly variable (Figure 4), surprisingly good replication occurred between mesocosms within each treatment. Treatment replication this good has been rarely observed in past mesocosm experiments wherein inorganic nutrient concentrations were usually the best replicated measured parameter.

Compared to Narragansett Bay seawater controls, significant toxicity was detected during most of the first 40 days of the experiment in the control mesocosms receiving no sewage effluent additions (Figure 4). Results were similar for the 0.1 and 1.0 percent sewage effluent treatment mesocosms,

although the 1.0 percent treatment was less toxic than the control mesocosms during this period. Toxicity in the 10 percent sewage treatment was similar to that measured in the mesocosm controls during the first part of the experiment, but remained high (low percent fertilization) during the remainder of the experiment when toxicity decreased in the controls (Figure 4). Vertical profiles of toxicity in the 10 percent effluent treatment indicated that toxicity was detected only in surface samples. Both the vertical profiles and the temporal trends of toxicity indicated that there was no residual component of toxicity that built-up in the mesocosms due to continued effluent additions. Toxicity measured in the mesocosms was not correlated with the EC50 of the sewage effluent.

Two experiments were conducted in the mesocosms during the study to investigate the persistence of toxicity. In one, toxicity persisted for greater than 3 h and in the other, no toxicity was measured after 1 h. These differences were traced to inadequate mixing of the most recently added sewage effluent at the time samples were taken.

The persistence of effluent toxicity was also assessed following termination of the mesocosm experiment by adding 1000 liters of fresh effluent, completely mixing the effluent, and measuring toxicity over time. At a temperature of 9 oC, the half-life of the toxicity was 12.4 h and no toxicity was detected after 99 h.

Mesocosm Responses

DCMU fluorescence was used as a daily measure of phytoplankton biomass (Figure 5). In this, and other studies [38-40], good correlations ($F=667$, $P<0.0001$, $R^2=0.685$) were been made between DCMU fluorescence (Figure 5) and

chlorophyll a concentrations, a more traditional measure of phytoplankton biomass.

Daily measurements of DCMU fluorescence (Figure 5) indicated phytoplankton biomass in the controls and all three sewage effluent treatments increased during the first month of the experiment. Increases were greatest in the control and 0.1 percent sewage effluent treatments ($p<0.1$), but less in the 1.0 and 10 percent treatments. Fluorescence decreased in all mesocosms after the initial phytoplankton blooms. After the first month, treatment differences were not significant. Direct measurements of chlorophyll indicated the same trends in phytoplankton biomass as the fluorescence measurements. Weekly size fractionated fluorescence measurements indicated there were no treatment differences in the proportion of total DCMU fluorescence less than 64 um (overall mean 94 percent) or in the proportion of total DCMU fluorescence less than 10 um (overall mean 83 percent).

Inorganic nutrient concentrations in the mesocosms generally showed treatment differences during the first half of the experiment corroborating the phytoplankton results. Ammonia concentrations (Figure 6), the dominant form of dissolved inorganic nitrogen, were greatest during the first month of the experiment in the 10 percent treatment (mean=28.92 ug-at N/liter) compared to other treatments and the controls (mean=12.92 ug-at N/liter). Similar treatment differences continued into the second month of the experiment (mean for the 10 percent treatment=51.33, other mesocosms 41.05 ug-at N/liter).

Silica concentrations in the mesocosms showed trends similar to that of nitrogen, with highest concentrations being in the 10 percent treatment mesocosms (Figure 7). Differences were significant only during the first month of the experiment.

Phosphate concentrations showed trends opposite to those of nitrogen and silica. During the first month concentrations were highest in the controls and the 0.1 treatment (mean=15.17 ug-at P/liter). Phosphate concentrations in the 1 percent treatment were significantly lower (mean=14.34 ug-at P/liter) and concentrations in the 10 percent treatment were still lower (mean=12.23 ug-at P/liter). Some treatment differences persisted into the second month when concentrations in the controls were higher than those in the 10 percent treatment, but no other differences could be discerned.

Dissolved oxygen concentrations demonstrated the most dramatic system response to sewage effluent additions. Oxygen concentrations, measured weekly over a diel cycle (dawn-dusk-dawn), were consistently lower throughout the experiment in the 10 percent treatment compared to other treatments (Figure 8). Oxygen concentrations reached as low as 1.15 mg O2/liter and were below 3 mg O2/liter (an upper limit often used to define hypoxia) for 22 percent of the weekly measurements in one mesocosm and 39 percent in the other of the 10 percent effluent mesocosms. The control, 0.1 percent and 1 percent treatments all had similar oxygen concentrations up until the last month, when the controls had the highest oxygen concentrations (mean=10.66 mg/liter), the 0.1 percent and 1 percent treatments were significantly lower (mean=8.80 mg/liter), and the 10 percent treatment was still lower (4.76 mg/liter).

Diel changes in water column oxygen concentrations were used as a measure of total system apparent daytime production and total system nighttime respiration. Total system metabolism is the net of all oxygen metabolism in the mesocosm ecosystem and includes phytoplankton production and respiration, zooplankton respiration, the benthic respiration of sedimented organics, and any respiration due to material on the mesocosm walls. Wall metabolism was

not measured during this experiment but generally is 10 percent of total system respiration [41].

There was only one significant treatment difference in total system production and respiration throughout the experiment. During the second month of the experiment, system respiration in the 10 percent treatment was significantly higher than the controls. No other treatment differences could be distinquished. There was a tendency for production to be smallest and respiration greatest in the 10 percent treatment, but the trends were not statistically significant (Table 4).

As a further measure of the metabolic behavior of the mesocosms the difference between production and respiration, (P-R) a measure of net system production, was computed. Strong treatment differences could be detected by the last two months of the experiment. Net system production was lower during month three in the 10 percent treatment compared with the controls and the 1 percent treatment. During month four, net system production in the 10 percent treatment was lower than any other treatment. For the entire experiment, net system production was negative in only the 10 percent treatment (Table 4).

Other parameters measured in the mesocosm ecosystem showed few significant treatment effects. Treatment effects that were present did not indicate toxicity due to sewage effluent additions. Rather, stimulatory effects were indicated.

Zooplankton biomass was similar in all treatments with treatment means for the entire experiment ranging from 71 to 81 mg dry wt/m^3. Enumeration of the zooplankton demonstrated few significant treatment effects for the numerically dominant species. The nauplii of Acartia sp., the dominant copepod species, were more abundant in the 10 percent effluent treatment

compared to other treatments, but only during the third month. During the fourth month, meroplanktonic polychaete larvae were nearly 10 times more abundance in the 10 percent effluent treatment (5635 individuals/m^3) compared to other treatments (537 individuals/m^3). This reflected the activity of benthic organisms. The spionids Polydora ligni and Streblospio benedicti and the amphipod Microdeutopus gryllotalpa were more abundant in the 10 percent treatment compared to other treatments. No other treatment differences were observed for the numerically dominant macrofaunal species using the data available. There were no significant treatment differences for benthic oxygen metabolism and inorganic nutrient fluxes across the sediment-seawater interface.

DISCUSSION

Sewage effluent additions caused prolonged and potentially detrimental effects in the mesocosm ecosystems. Effects were most dramatic in the 10 percent effluent treatment, but significant effects were also demonstrated at the 1 percent effluent treatment. Effects could be related to direct toxic responses, and to system responses to the organic carbon in the sewage effluent. The single-species tests and mesocosm measurements complimented each other, but contributed different types of information.

Toxicity Results

The sea urchin (Arbacia punctulata) sperm cell toxicity tests indicated that the East Greenwich sewage effluent was toxic with an EC50 averaging 1.1 percent. Toxicity was not detected in 2 of 36 samples. Daily additions to the mesocosms of up to 96 l sewage effluent per day (10 percent treatment) gave more variable results.

Three major factors influenced sewage effluent toxicity in the mesocosms: incomplete mixing, toxicity persistence, and the initial phytoplankton bloom in all treatments. Short term incomplete mixing contributed to variations in the mesocosm toxicity results. During most of the experiment, toxicity samples were taken directly after sewage effluent additions at the surface of the mesocosms where, due to the lack of complete mixing, effluent concentrations would be expected to be highest. When samples were taken at different depths, only surface samples showed significant toxicity. We believe that if samples were taken when mixing was complete (directly before sewage effluent additions), effluent concentrations in the toxicity samples would have been too low to cause toxicity in the sperm cell tests.

Both laboratory and mesocosm experiments demonstrated toxicity associated with the sewage effluent rapidly decayed with time. Toxicity in the mesocosms, as measured by the sperm cell test, did not increase over time. This indicated that there was no detectable fraction of the toxicity that persisted from one day to the next. We conclude that the rapid decay of effluent toxicity was most likely responsible for the absence of a build-up of toxicity in the mesocosms. However, the four month experiment may not have been long enough to detect such a build-up, and toxic fractions may have accumulated in the sediments. A build-up of toxicity might have been observed if the same experiment had been conducted at lower temperatures because the rate of toxicity decay would have been slower.

The third factor influencing toxicity in the mesocosms was the initial phytoplankton bloom in all treatments during the first month of the experiment. Toxicity was detected during this period in the control mesocosms, i.e., those receiving no additions of sewage effluent. Toxicity has been attributed to phytoplankton blooms in Narragansett Bay in the past [42,43]. Subsequent to this bloom, no toxicity was detected in the controls, and very little was detected in the 0.1 and 1.0 percent treatments. Toxicity in the 10 percent treatment continued to the end of the experiment, although toxicity was variable and attributed to the most recent addition of sewage effluent.

It is possible that the initial toxicity measured in all treatments at the start of the experiment was due to contaminants fluxing from the sediment to the water column. However, available mesocosm data for metals does not show evidence for this. Further, past experiments have demonstrated chemical fluxes from much more contaminated sediments were very small [25,31].

Mesocosm Responses

Evidence from the mesocosms suggests that sewage effluent additions limited some component of the phytoplankton. During the first month of the experiment, phytoplankton blooms occurred in the control and 0.1 percent sewage effluent treatments, but blooms were depressed in the 1.0 and 10 percent sewage effluent treatments.

Nutrient data corroborated the suppression of phytoplankton activity seen in the fluorescence and chlorophyll data. More nitrogen and silica were found in the 10 percent treatment than in other treatments and controls during this period. This can be linked to phytoplankton activity since phytoplankton primary production would have utilized and decreased nitrogen and silica concentrations. Nitrogen is thought to be the most limiting nutrient in marine systems [44,45], whereas silica is limiting only to those organisms, such as diatoms, that utilize silica in the formation of siliceous tests. Silica did not drop to near 0 ug-at Si/liter in the 10 percent treatment during the first month, as was seen in other treatments (Figure 7). This suggests that it was diatoms that were particularly sensitive to the sewage effluent additions.

Since reagent nitrogen and silica additions were lower and phosphorus additions higher than nutrients from sewage effluent additions, attributing the higher nitrogen and silica concentrations in the 10 percent treatment to reduced phytoplankton activity may be criticized. We do not believe this to be a valid criticism for the following reasons.

Past experiments [26,45] have repeatedly shown that increased nutrient loading causes a greater phytoplankton response, not the suppressed response observed. Additionally, if total nutrient loadings (sewage effluent plus reagent) in each treatment were significantly different, nutrient concentrations would have approached different steady-state concentrations in each treatment. This was not the case, as there were very few significant treatment differences for total dissolved nitrogen, phosphorus and silica after the first month of the experiment.

The mechanism whereby the early phytoplankton bloom was depressed in both the 10 and 1 percent treatments is not known. We believe that sewage effluent was directly toxic to some component of the phytoplankton, possibly diatoms. Previous studies have shown diatoms to be sensitive to sewage effluents [46]. Nutrient limitation is not a good explanation since there was an excess of all major nutrients. Light limitation was an unlikely factor since total suspended solids was similar in all treatments, averaging 25 mg/liter. Predation by zooplankton was probably not a controlling factor since the biomass of zooplankton was similar in all treatments and no zooplankton group or species increased abundance during the first month. Predation by micro-zooplankton is possible, but no measurements were made of these groups.

Carbon Loadings and System Metabolism

Sewage effluent additions constituted a significant carbon loading to the mesocosm ecosystem (Table 5). Approximately 249 g carbon per m^2 were added over 122 days to the 10 percent treatment mesocosms in the form of particulate and dissolved organic carbon. This is 93 percent of the mean annual primary productivity for Narragansett Bay [47]. Prior to the start of this experiment, we had thought this carbon would cause a considerable respiratory

demand, increasing system respiration and lowering oxygen concentrations. Although oxygen concentrations were indeed lower in the 10 percent treatments (Figure 8), total system respiration was only higher in this treatment during the second month of the experiment. We were not able to detect significant treatment differences in system respiration for any other time, nor were we able to detect differences in system production during the entire experiment. There was a trend for production to be smallest and respiration greatest in the 10 percent treatment. The cumulative effect of this was negative net system production (P-R) at the 10 percent sewage effluent treatment level.

A measure of the maximum amount of sewage effluent carbon metabolized during the experiment by the mesocosm system can be estimated by calculating the difference between net production in the controls and net production in the 10 percent treatment. This difference is 59 g c/m^2. The remainder of the sewage effluent carbon (249 g C/m^2 added - 59 g C/m^2 metabolised = 190 g C/m^2 remaining) can easily be accounted for by the loss due to water flow through the system. Assuming a completely mixed system, no deposition of sewage effluent carbon, and no biological utilization, nearly 90 percent of the added carbon could have been lost from the system simply due to flushing. This is obviously a maximum estimate since some particles were lost due to deposition and some carbon was respired. Nevertheless, since approximately 50 percent of the carbon was dissolved organic carbon, and 50 percent of the sewage effluent particulates were less than 5 um in size, a significant portion of the sewage effluent carbon could have been lost simply due to flushing.

The net effect of the addition of sewage effluent carbon and the decreased phytoplankton activity in the mesocosms was to bring about an imbalance of system production and respiration. This imbalance reduced oxygen concentrations in the 10 percent treatment. This was brought about mainly by metabolism in the water column since benthic oxygen fluxes were similar in all treatments. Hypoxia resulted, which under some conditions, could have progressed to total system anoxia.

CONCLUSIONS

The purpose of this experiment was to compare toxicity as measured by single-species tests, to those ecosystem level effects measured in mesocosm ecosystems. The single-species tests were useful in determining effluent concencentrations used in the experiment, and the magnitude and persistence of the effluent toxicity. Direct tests of effluent toxicity demonstrated toxicity at 1 percent effluent. However, few toxic effects were observed in this and the 0.1 percent mesocosm treatment levels. This lack of response was attributed to the rapid decay of effluent toxicity at summer temperatures. At the 10 percent effluent treatment level, where prior to this experiment massive toxic effects were expected, few toxic effects were directly observed. Greater toxicity might be expected during winter months when the decay of toxicity would be slower.

The mesocosm measurements were useful in showing effluent effects in simulated coastal marine systems. Effluent effects were attributed partially to a toxic response of the phytoplankton and partially to the carbon associated with the effluent. Toxicity to the phytoplankton was inferred from fluorescence, chlorophyll and nutrient data and these data indicated diatom production might have been reduced in the 10 percent effluent treatment. Other mesocosm communities in the 10 percent treatment were stimulated suggesting a lack of toxicity. Combined, the effects on the phytoplankton and the organic carbon loading caused an imbalance in production and respiration which lead to hypoxia. Results suggest carbon loadings may be as much a problem to coastal ecosystems as direct toxic effects when toxicity decay is rapid.

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FIGURE LEGENDS

Figure 1 A MERL Mesocosm. Seawater added at rate of 960 l/d. Sewage effluent added at surface. Descriptions of the behavior of the mesocosms and their fidelity to natural coastal systems may be found in [24,25,48-50].

Figure 2 Greenwich Bay, Rhode Island. East Greenwich sewage treatment plant discharges to East Greenwich Cove at 3 m depth. Effluent collected just prior to discharge into cove. Sediments collected south of Apponaug Cove, water depth 3 m. Depth contours in meters.

Figure 3 Toxicity of East Greenwich sewage treatment plant effluent. EC50 from the sea urchin sperm cell test, expressed as percent effluent. Samples represented by the filled-in circles were considered non-toxic (ED50 > 50%).

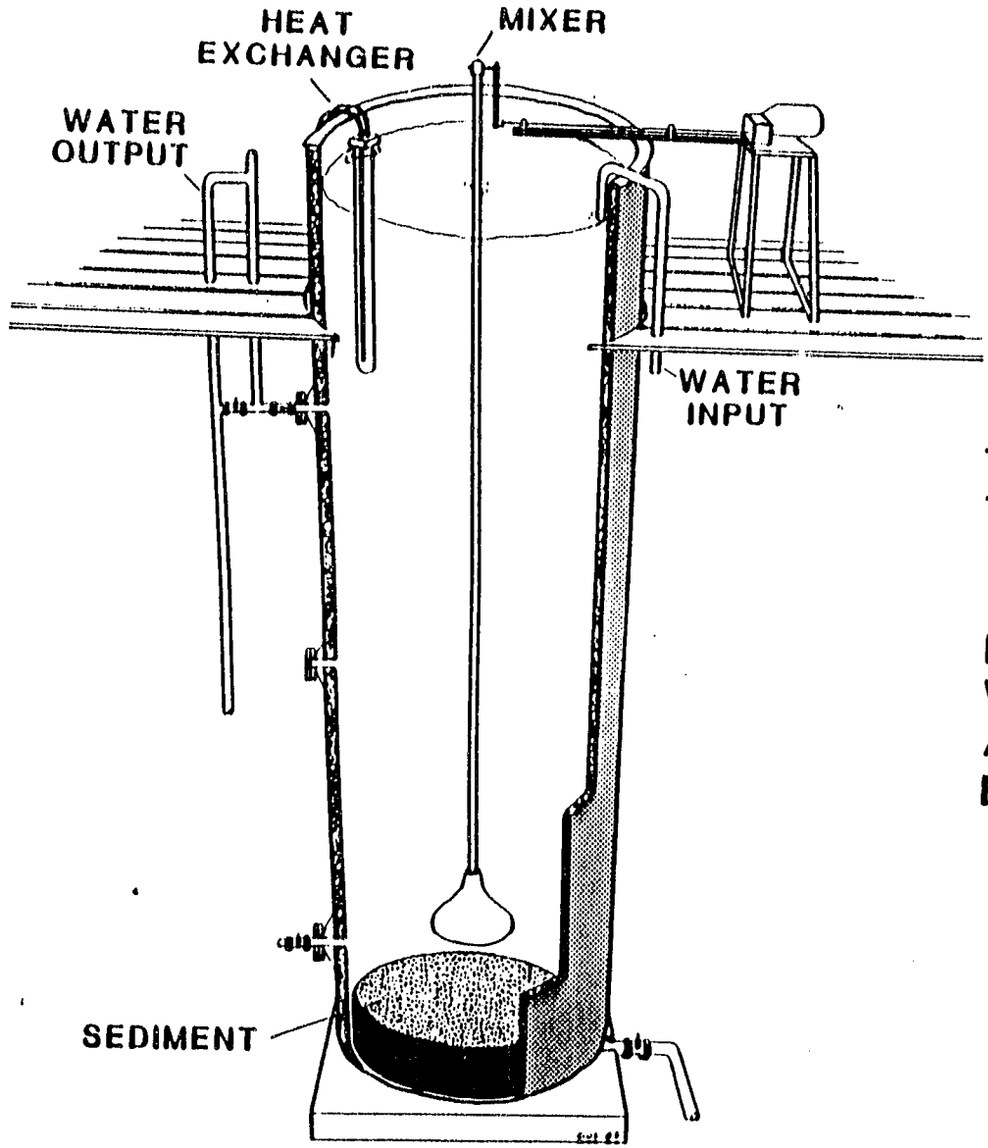
Figure 4 Toxicity of water from replicate mesocosms determined using the sea urchin sperm cell test and expressed as percent fertilization relative to Narragansett Bay seawater controls.

Figure 5 Total DCMU Fluorescence (fluorescence after addition of (3-(34-dichlorophenyl)-1-1-dimethylurea)) in replicate mesocosms.

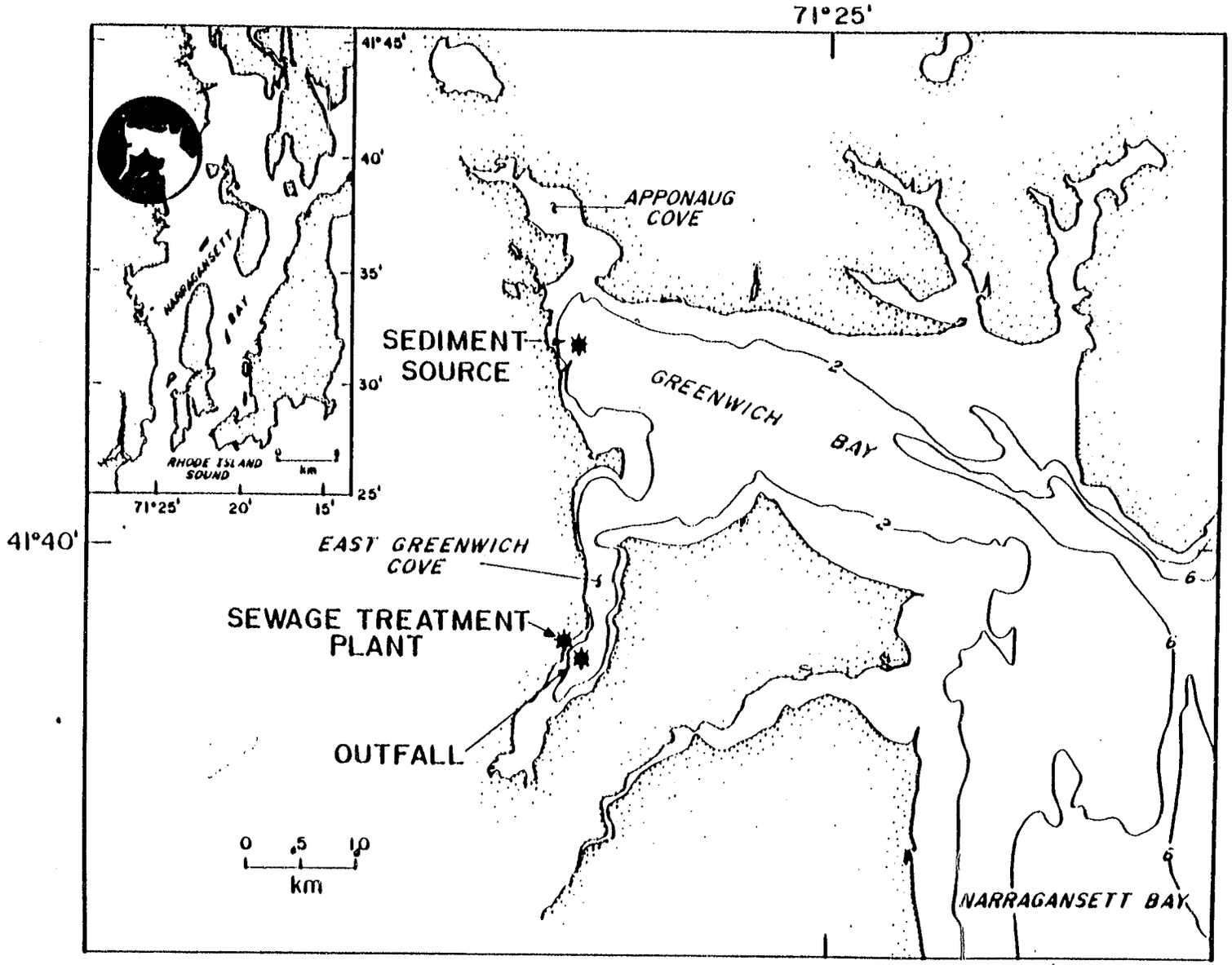
Figure 6 Ammonia concentrations in replicate mesocosms.

Figure 7 Silica Concentrations in replicate mesocosms.

Figure 8 Mean oxygen Concentrations in replicate mesocosms. Calculated as: (mean dawn values + mean dusk values)/2.



TANK DIAMETER	1.83m
TANK HEIGHT	5.49m
WATER SURFACE AREA	2.63m^2
DEPTH OF WATER	5.00m
VOLUME OF WATER	13.1m^3
AREA SEDIMENT	2.52m^2
DEPTH OF SEDIMENT	0.37m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SEWAGE TREATMENT PLANT
OUTFALL
NARRAGANSETT BAY
NARRAGANSETT BAY
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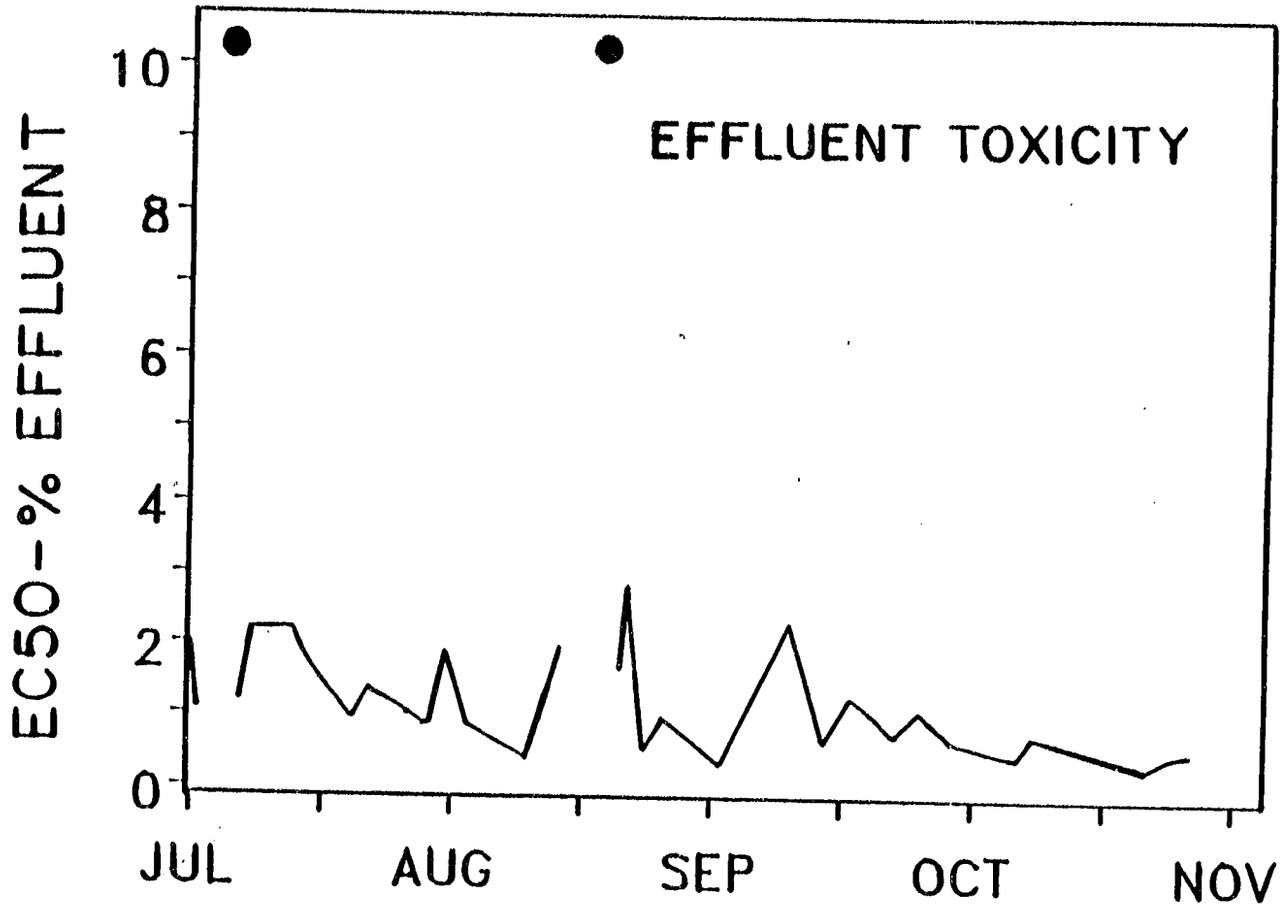
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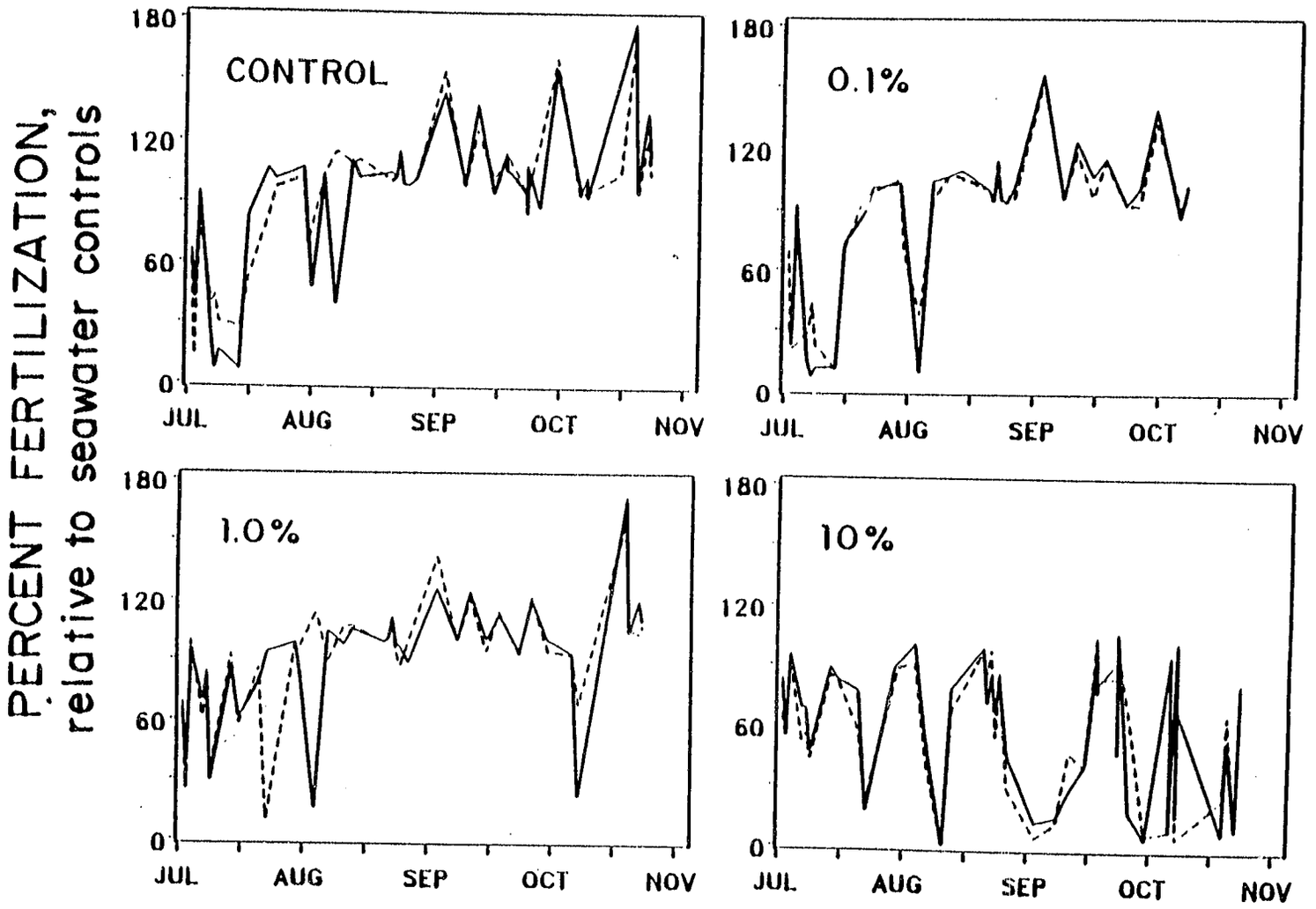

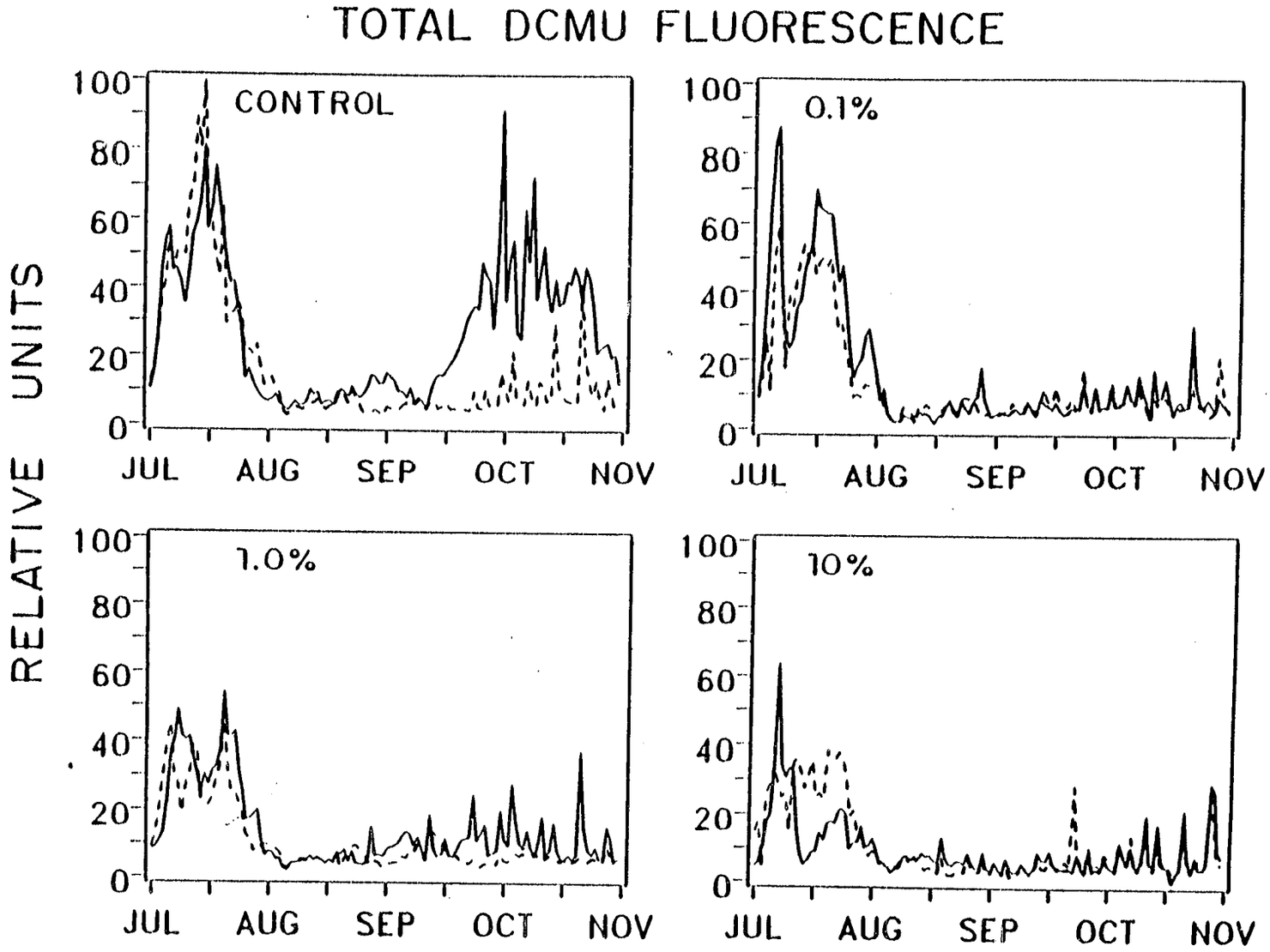
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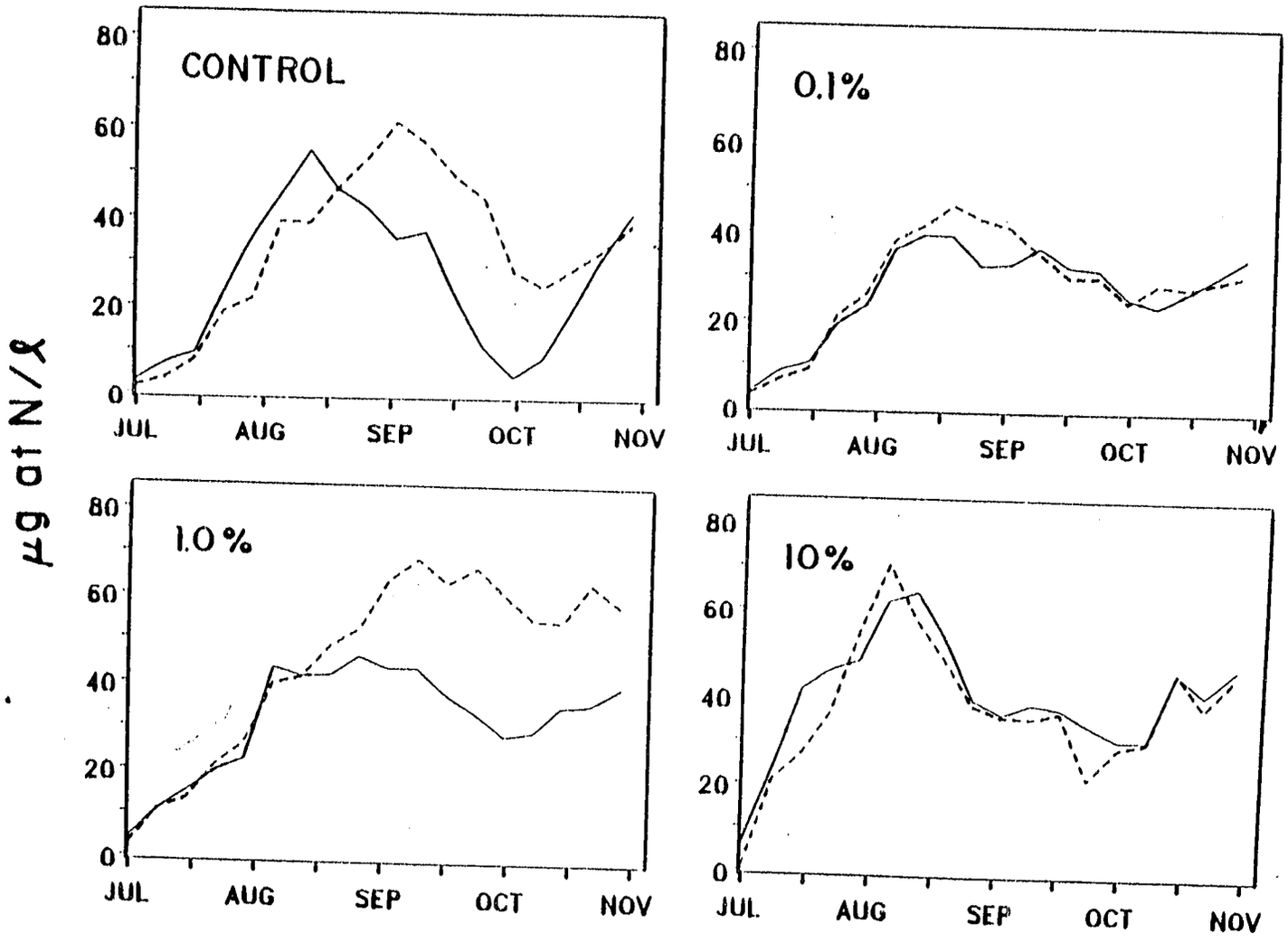

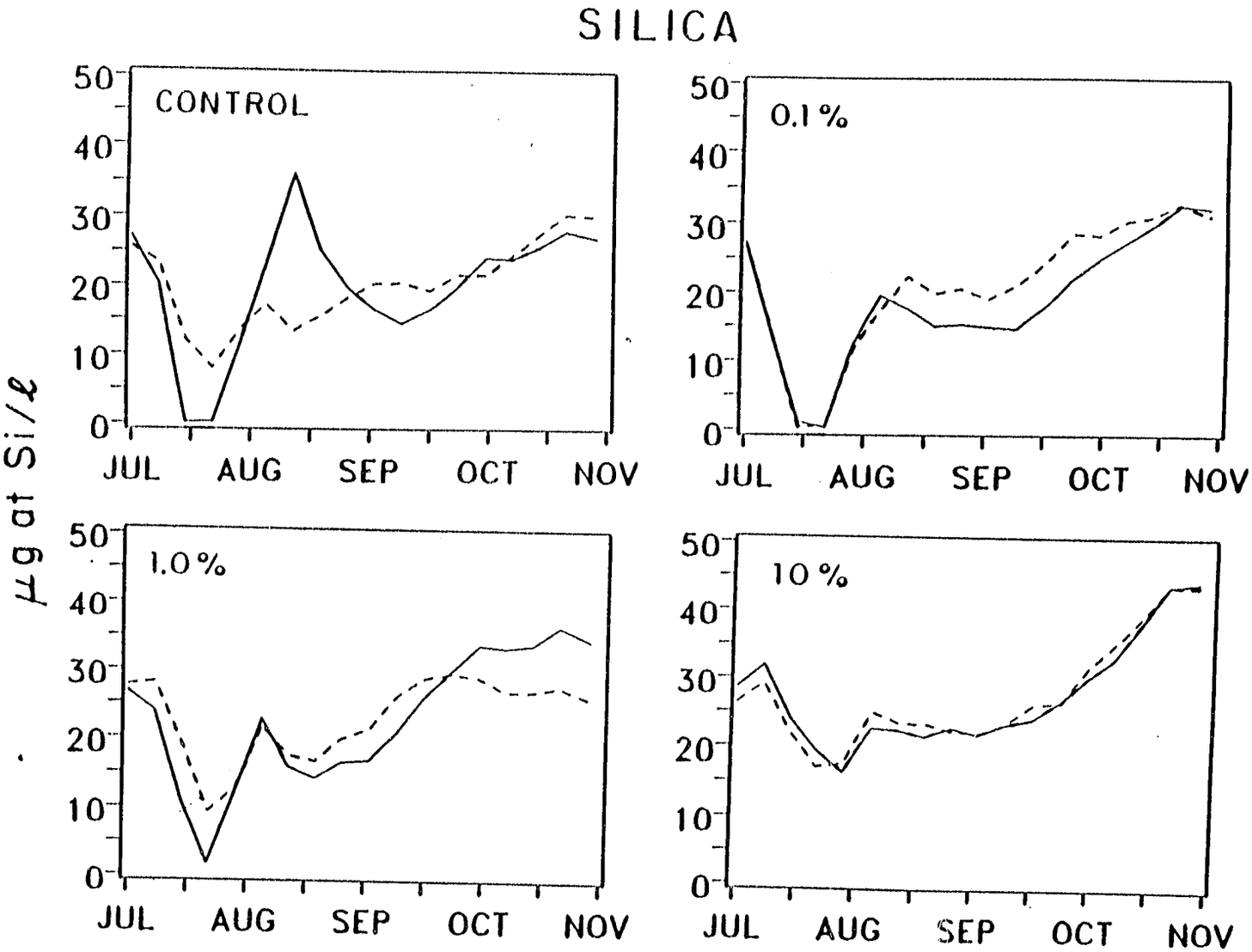
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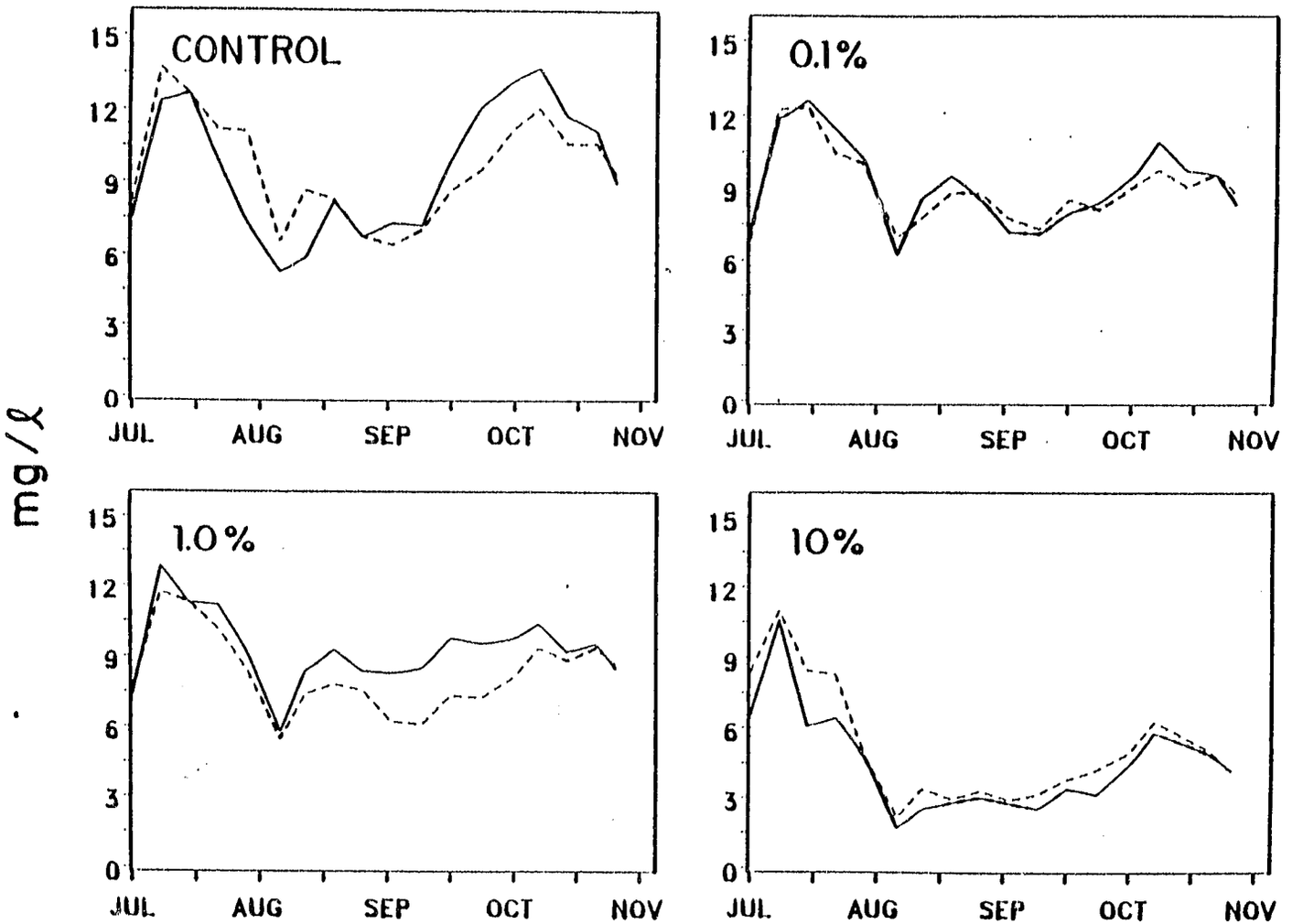

Table 1

Experimental Outline

mmoles / Mesocosm / day

Nutrient Source	Nitrogen SE*	Nitrogen RA**	Phosphorus SE	Phosphorus RA	Silicate SE	Silicate RA
TREATMENT						
10 Percent, Tanks 2 and 4 96 l effluent/day	77.9	0	32.	0	7.21	0
1.0 Percent, Tanks 5 and 6 9.6 l effluent/day	7.79	70.15	3.27	29.45	0.72	6.49
0.1 Percent, Tanks 1 and 8 0.96 l effluent/day	0.78	77.16	0.33	34.39	0.07	7.14
Controls Tanks 0 and 9 0 l effluent/day	0	77.94	0	32.72	0	7.21

* SE = Sewage Effluent
** RA = Reagent Additions

Table 2

Relative Toxicity of Rhode Island Sewage Treatment Plant Effluents

Based upon Arbacia punctulata sperm cell tests conducted on fresh samples

Municipal Treatment Plant	Treatment Type+	Flow(MGD) Design	Flow(MGD) Average*	EC50 percent Effluent
Bristol	3	3	2.1	0.15
New Shoreham	1	0.2	0.1	0.15
Blackledge Valley District	1	31	17.4	0.27
Providence	1	60	43.1	0.30
Warren	1	2	1.3	0.34
Quonset	3	2.3	0.6	0.36
Jamestown	1	0.7	0.4	0.42
East Greenwich	2	0.5	0.7	0.46
East Providence	1	10.4	5.3	0.62
Newport	3	5.8	8.1	0.99
Westerly	1	3.1	1.8	1.49
Narragansett	1	1.4	0.6	1.50
South Kingstown	1	4.1	2.7	1.63

+Treatment Type: 1 = Activated Sludge and chlorination
2 = Trickling filter and chlorination
3 = Primary treatment and chlorination

*Average for calendar year 1985 (R. Richardson, personal communication).

Table 3

Summary of East Greenwich Sewage Effluent Characteristics
July 15 - November 14, 1986

Parameter (units)	Mean	St.Dev.	C.V.%	Range
Sewage Treatment Plant Flow (MGD)	0.697	71	10	477 - 811
Total Suspended Solids (mg/l)	68.9	34.9	51	9.0 - 207.6
Particulate Organic Carbon (mg C/l)	26.5	15.2	57	7.5 - 117.8
Dissolved Organic Carbon (mg C/l)	29.6	15.3	52	11.6 - 154.0
Total Nitrogen (ug-at N/l)	1621	261	16	1200 - 2200
Total Dissolved Nitrogen (ug-at N/l)	1472	24	16	1100 - 2000
Total Dissolved Inorganic Nitrogen (ug-at N/l)	1032	166	16	806 - 1339
Ammonia (ug-at N/l)	915	195	21	613 - 1263
Nitrate+Nitrite (ug-at N/l)	117	78	67	12 - 290
Total Phosphorus (ug-at P/l)	382	74	19	200 - 490
Total Dissolved Phosphorus (ug-at P/l)	329	60	18	180 - 430
Total Dissolved Inorganic Phosphorus (ug-at P/l)	279	59	21	183 - 437
Silica (ug-at Si/l)	205	32	15	77 - 233
Chlorine - Residual (mg/l)	2.09	2.08	100	-3.00 - 7.97
Cadmium - Particulate (ug/kg)	0.44	0.38	86	0 - 2.01
Cadmium - Soluble (ug/kg)	0.59	1.06	178	0 - 7.62
Chromium - Particulate (ug/kg)	25.65	14.58	57	0.41 - 67.16
Chromium - Soluble (ug/kg)	9.41	9.29	99	0 - 65.58
Copper - Particulate (ug/kg)	72.25	57.77	80	2 - 339.31
Copper - Soluble (ug/kg)	101.90	38.15	37	0.66 - 189.74
Iron - Particulate (ug/kg)	4057.44	1795.56	44	466.21 - 10571.89
Iron - Soluble (ug/kg)	488.30	523.89	107	18.00 - 2523.69
Lead - Particulate (ug/kg)	54.70	56.74	104	3.21 - 446.52
Lead - Soluble (ug/kg)	7.84	3.85	100	0 - 43.95
Nickel - Particulate (ug/kg)	8.41	5.79	69	0 - 35.47
Nickel - Soluble (ug/kg)	22.58	7.10	31	12.00 - 58.00
Zinc - Particulate (ug/kg)	115.88	108.80	94	12.05 - 945.68
Zinc - Soluble (ug/kg)	65.39	81.84	125	22.00 - 768.77

Table 4

Total system Production and Respiration

Treatment	Production		Respiration		Difference	
	mg O_2/m^2	g C/m^2	mg O_2/m^2	g C/m^2	mg O_2/m^2	g C/m^2
Controls	477	179	413	155	64	24
0.1 percent	481	181	442	166	39	15
1.0 percent	501	188	439	165	62	23
10 percent	367	137	459	172	-92	-35

Oxygen production converted to carbon production by assuming a photosynthetic quotient (PQ) of 1.

Table 5

Total Carbon Loading Due To
Sewage Effluent
(July 15 to November 14)

Treatment	G C / m^2
Controls	0
0.1 %	2.5
1.0 %	25
10 %	249