

**Biotic and Abiotic Transformations of Arsenic
in the Upper Mystic Lake**

by

Henry Matthew Spliethoff

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Signature of the Author

Department of Civil and Environmental Engineering
February 3, 1995

Certified by _____

Harold F. Hemond
Professor of Civil and Environmental Engineering
Thesis Advisor

Accepted by _____

Joseph M. Sussman
Departmental Committee on Graduate Studies

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Abstract

The increase in hypolimnetic arsenic concentration in the Upper Mystic Lake during fall anoxia was found to be an order of magnitude greater in 1993 than in the previous year. Iron and manganese measurements indicated that the elevated arsenic concentration was associated with the release of iron from the bottom and suspended sediments. In 1993, the presence of both arsenite and sulfide, neither of which was detected at significant concentrations in 1992, suggested that the reduction potential of the bottom waters was significantly lower in 1993.

Mass balance calculations indicated that there was *in situ* production of arsenite in the epilimnion during the summer of 1994. Monthly average epilimnetic total phosphorus concentrations correlated with arsenate concentrations. There was also a temporal correlation between arsenite concentration and bacterial numbers. Culture studies with lake water samples demonstrated the capacity of natural assemblages of microorganisms to reduce added arsenate to arsenite. Approximately 40% of this total reductive capacity was found in water samples which had been filtered through 1.0 μm pore size filters; water samples treated with antibacterial inhibitors had significantly decreased rates of reduction. A culture of heterotrophic bacteria isolated from the lake was shown to reduce arsenate at rates comparable to those measured for the mixed cultures.

Thesis Advisor: Professor Harold F. Hemond
Title: Professor of Civil and Environmental Engineering

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I. INTRODUCTION

In western culture, arsenic, perhaps more than any other element, has been equated with "poison." The mention of "arsenic" conjures up visions of treachery and intrigue, and justifiably so, because historically, poisoning by arsenic was a favorite means of eradicating one's enemies. In this century, the toxicity of arsenic has been capitalized on through the development of agents of chemical warfare, such as the toxic gas lewisite (chlorovinyldichlorarsine, ClCH=CHAsCl₂) (1). In addition to being acutely toxic, arsenic has been shown to be carcinogenic and mutagenic, causing lung and skin cancer in humans (2), and mutations in exposed hamster cells (3). Yet, in the past, arsenic was used for medicinal purposes (4), and, more recently, it has been proposed as an essential human micronutrient (5).

I.1 The Environmental Chemistry of Arsenic

Arsenic is ubiquitous in the aquatic environment, although in some locations, it is found at particularly high concentrations. Arsenic in contaminated surface waters can have natural origins, as is true for rivers and lakes in proximity to geothermal hot springs (6), or anthropogenic origins, as is true for water bodies downstream for mining operations (7). Other common anthropogenic sources of arsenic to the environment

include metal smelting and pesticide manufacturing plants.

The behavior of arsenic in aquatic systems is a function of its chemistry. It is a group VA element, and is chemically similar to phosphorus. Arsenic has four oxidation states: -III, 0, III, and V, although As(0) is rare in the natural environment. Arsenate (H_3AsO_4), the thermodynamically favored species in oxic waters, is a triprotic acid ($pK_{a1}=3.6$, $pK_{a2}=7.26$, and $pK_{a3}=12.47$) and is typically present as an anion in solution (8). In reducing environments, Arsenic (III) is often found as arsenite ($HAsO_2^-$), which usually is fully protonated and has a neutral charge. Other naturally occurring inorganic arsenic compounds include orpiment (As_2S_3), realgar (As_2S_2), and arsenopyrite ($FeAsS$), which are only slightly soluble. A variety of organic arsenicals, such as arsenolipids and arsenosugars, have been identified in the aquatic environment, and many more are thought to exist (9), although the most commonly detected organic arsenic compounds are monomethylarsonate ($CH_3AsO_2OH^-$) and dimethylarsinate ($(CH_3)_2AsOO^-$).

I.2 *The Upper Mystic Lake*

The Upper Mystic Lake drains the industrial and residential Aberjona watershed north of Boston, Massachusetts. The Aberjona watershed has a long history of anthropogenic disturbance, and contains numerous hazardous waste sites (10,11). The watershed has been, and is currently, the subject of an interdisciplinary

research effort with the goal of identifying the fate and transport of chemicals contained within the watershed, and elucidating possible past and present health threats posed by these chemicals.

One of these chemicals, arsenic, is present in substantial quantities in the soils (12) and sediments (10,13) of the watershed. The migration of arsenic via the surface waters of the Aberjona watershed (13,14), has raised questions about the fate of this metalloid in the recreationally-used Upper Mystic Lake. A previous study has shown that arsenic undergoes a seasonal biogeochemical cycle in the lake, with arsenite predominating in the epilimnion in summer (15). The present study was undertaken to further characterize this cycle, and to determine the origin of arsenite in the epilimnion.

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II. Interannual Variability in the Concentration and Speciation of Arsenic in the Hypolimnion

II.1 Introduction

Recent studies (1-5) have demonstrated that the speciation and distribution of dissolved arsenic (As) in natural waters is often far from thermodynamic equilibrium, with arsenite (As(III)) predominating in oxic surface waters, and arsenate (As(V)) persisting in anoxic and even highly sulfidic environments (2,3,6,7). Reduction of arsenate in surface waters by microorganisms (8-10), differential scavenging of arsenite and arsenate by iron and manganese (hydr)oxides (11-14), and relatively slow reduction of arsenate in anoxic waters (3,14,15) are often quoted reasons. The recently reported investigations of 1991/92 (2), which focussed on the dimictic Upper Mystic Lake - a lake within the historically contaminated Aberjona watershed (16,17) - presented a picture of arsenic cycling and speciation that generally paralleled results found elsewhere (3-5). We report here additional data collected in 1993 that provide a striking example of how sensitive arsenic speciation and concentrations can be to subtle changes in redox conditions. In 1992, there was little evidence of arsenite in the hypolimnetic waters, even after a prolonged period of low oxygen (no sulfide present) (2). In contrast, in October 1993, when sulfide was

present in the lake bottom waters, arsenite and total dissolved arsenic concentrations were more than an order of magnitude higher than those found in October 1991 or 1992 (although far lower than the solubility limits (18), and lower than arsenic concentrations found in permanently anoxic waters (2,5)).

These results raise important questions about the role of atypical seasonal redox cycles in the potential seasonal contamination of the water column with arsenic. Not only were the 1993 arsenic concentrations in the hypolimnetic waters of the Upper Mystic Lake greatly elevated with respect to previous years, but a study of the mixing regime of the lake in the fall of 1993 (19), using microstructure profiling techniques, demonstrated that the hypolimnetic waters of the lake can be mixed into the surface layer by thermocline seiching induced by axial winds. This mixing enhances the potential for direct human contact and food chain contamination in this urban lake, which is used extensively for swimming, fishing, sailing and other recreational activities.

II.2 Methods

The Upper Mystic Lake is the first of two lakes receiving drainage from the Aberjona watershed. The sediments of this 58-ha eutrophic lake contain a large inventory of arsenic and other toxic metals, as a result of historic contamination of the watershed (20). The lake stratifies in summer, with oxygen

depletion of the bottom waters typically beginning in May and persisting into late fall (November). Mixing in November and December leads to an isothermal water column and oxygenation of the bottom waters (2).

Dissolved oxygen concentrations and temperature were measured using a portable oxygen/temperature meter, and water samples were collected in acid-washed bottles using a peristaltic pump and acid-washed tubing. Samples, stored cold, were analyzed for arsenic speciation within 24 hours of collection. Dissolved arsenic speciation and concentration were measured using hydride generation techniques with detection by atomic absorption (2). Total acid-soluble iron and manganese concentrations were determined in acidified samples by ICP emission spectroscopy (Thermo Jarrell Ash, Atomscan 25). Filterable dissolved iron and manganese concentrations were measured by ICP in samples after filtration through 0.45 μm Millipore HA filters, while particulate fractions were estimated by difference between acid-soluble total and filterable dissolved concentrations. Samples for sulfide analysis were collected in BOD bottles, and were analyzed for sulfide with the methylene blue method (21).

II.3 Results

The results obtained in 1991 and 1992 were described in detail in *ES&T* by Aurilio et al. (2). Concentrations of arsenate increased in the hypolimnion throughout the fall of 1992, and

reached a maximum (20 nM) in October at the deepest depth sampled. By November, concentrations of arsenate had decreased, but were still elevated relative to concentrations in mid-summer. Similar arsenate concentrations (> 10 nM in the bottom waters) had been measured in October and November 1991.

III.2.1 1993 Arsenic Species Profiles

In 1993, the arsenic concentration profiles for the spring and summer were similar to those found in 1992 (Figure II.1). The arsenic concentration and speciation measured in the fall of 1993 (Figure II.2), however, were substantially different from those in either 1991 or 1992. Much higher concentrations of inorganic arsenic were found in the hypolimnion, with a maximum concentration (380 nM) occurring in October. In addition, the arsenic speciation in the bottom waters changed dramatically in 1993, relative to the speciation in 1991 and 1992. In 1991/92, arsenite was consistently a small fraction of the total dissolved inorganic arsenic (<10%) in the bottom waters. This was also the case in the spring and summer of 1993 (Figure II.1). However, in September 1993, the arsenite concentration had increased sharply in the bottom waters, and arsenate and arsenite concentrations had become similar; total inorganic arsenic concentration (80 nM) was a factor of 4 higher than the maximum concentration found in 1992. By October, the speciation had shifted further toward the

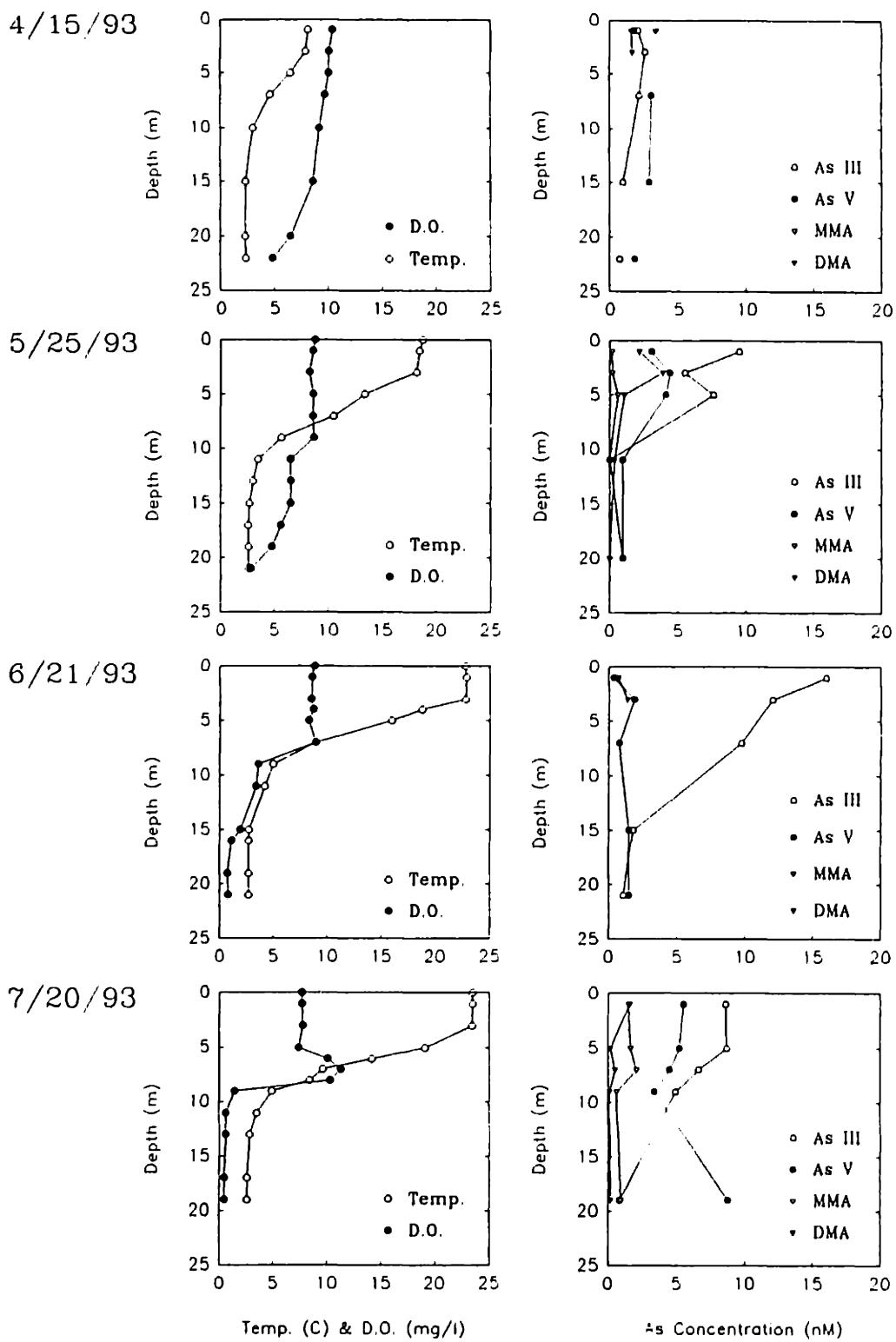


Figure II.1 Summer Depth Profiles of Dissolved Oxygen, Temperature and Arsenic Species

reduced form, with the arsenite concentration (225 nM) at 58% of the total inorganic arsenic concentration (380 nM). In November, the concentration of arsenic in the bottom waters had begun to decrease, and the speciation had reverted to a predominance of arsenate. Total concentrations continued to decrease in December.

II.3.2 Dissolved and Particulate Iron and Manganese

Iron (Fe) and manganese (Mn) concentrations were also measured throughout the water column in the fall of 1993. Concentrations of dissolved (filterable) and particulate iron and manganese were low in the surface waters, but higher in the hypolimnetic waters (Figure II.2.b). Dissolved iron concentrations were highest in October and November, and represented >90% of the total iron. Between November and December the total iron concentration decreased in concert with the proportion of dissolved to total iron. By December, only 40% of the total iron was dissolved. Total manganese concentrations remained constant throughout this period, with dissolved manganese being predominant.

II.3.3 Sulfide and Dissolved Oxygen

The increase in concentration and change in speciation of arsenic coincided with the appearance of sulfide in the bottom

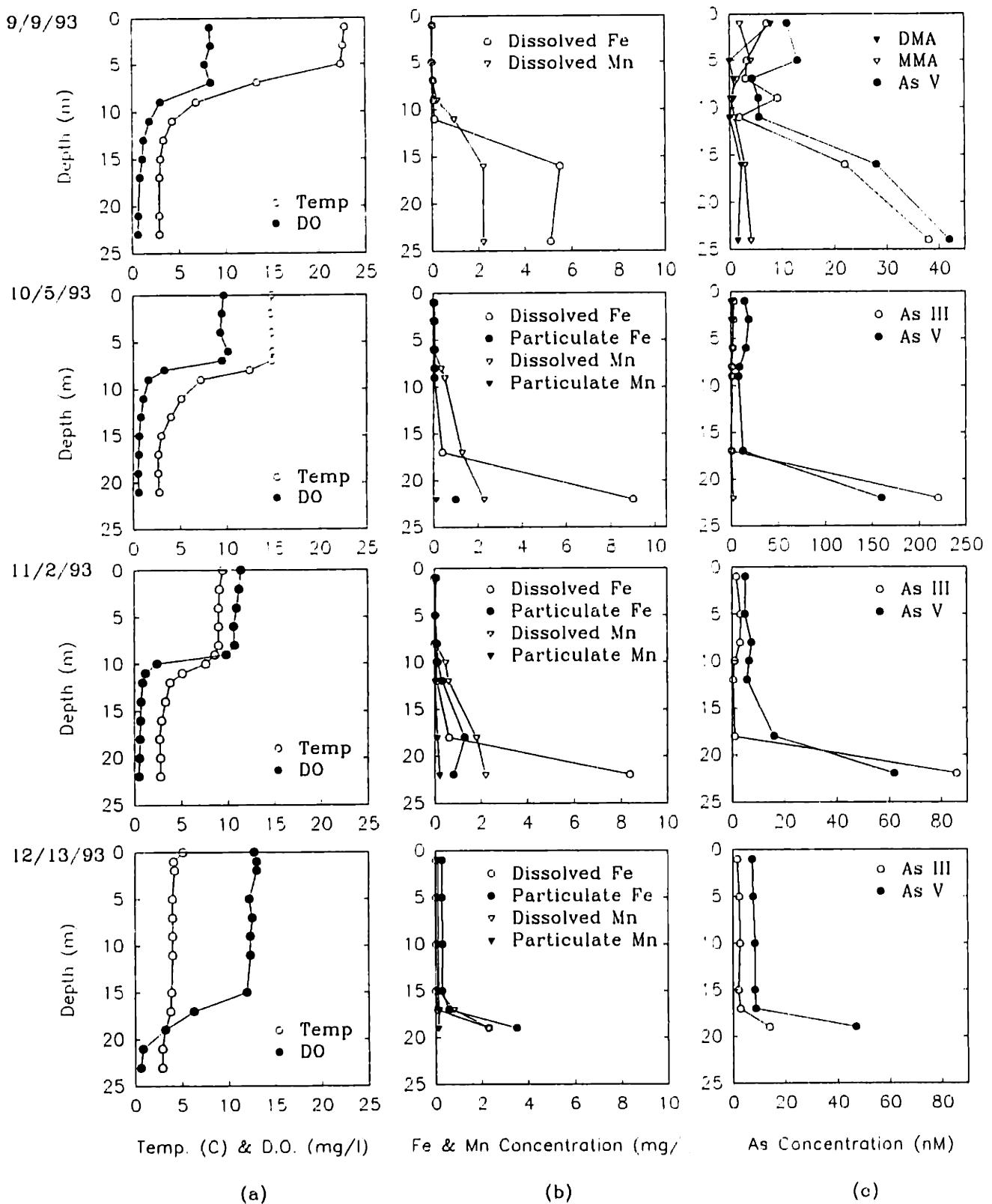


Figure II.2 Fall Depth Profiles of Dissolved Oxygen and Temperature (a), Iron and Manganese (b), and Arsenic Species (c).

waters of the lake. There was no evidence of sulfide (by odor) in the deep waters in the summer/fall of 1992. In September 1993 the odor of sulfide was detected in the deepest water during sampling, but the sulfide had decreased to below the detection limit ($1 \mu\text{M}$) by the time the samples were analyzed (6 hours later). In October, up to $25 \mu\text{M}$ sulfide was measured in the deeper samples (Figure II.2-b). In November, there was no evidence of sulfide in the deeper samples. The dissolved oxygen profiles were consistent with those of iron, manganese and sulfide. There was little change in the dissolved oxygen profiles between September and October (Figure II.2-a), but there was a deepening of the oxycline (the region of greatest oxygen concentration change) between October and November, with a more rapid oxygenation of the bottom waters occurring between November and December. This suggests that October was the month when the bottom waters were the most reducing, as was indicated by the sulfide concentration.

II.4 Discussion

The cycling of manganese, iron and sulfide in this lake thus appears similar to that found in other freshwater and marine systems. As low oxygen conditions developed, manganese oxides were reduced before iron oxides, releasing manganese into solution (20,22). By September, essentially all the manganese was reduced (Figure II.2-b), while the concentration of dissolved

iron reached a maximum in October/November. Sulfide, which has a lower reduction potential than either iron or manganese, was first detected in September, reached a maximum in October, and then declined in concentration (before that of iron or manganese). Iron oxidation occurred between November and December, as indicated by the increase in particulate iron. Little oxidation of manganese occurred, however.

The water column in this lake is very stable because of both low temperature and elevated salinity of the bottom waters (19). Because of the salinity gradient, a strong temperature gradient is not required to maintain stability of the deeper water column, as was demonstrated by the persistence of low oxygen conditions even after the water column became essentially isothermal in December (Figure II.2.a). The higher iron and manganese concentrations in the epilimnion in December suggest that while there was mixing of oxygen into the hypolimnetic waters during this period (20), there was a concurrent injection of bottom waters rich in dissolved iron and manganese into the epilimnion.

II.4.1 Sources of Arsenic and Iron

Calculations of the scavenging of arsenic by iron hydroxides (FeOx), using the Dzomback and Morel model (24), suggested that in the surface waters of the Upper Mystic Lake, where total iron concentrations are typically 10-50 $\mu\text{g/L}$ (assumed 100% FeOx), 40-60% of the arsenate should be absorbed to FeOx at equilibrium,

while none of the arsenite should be complexed. In the bottom waters, however, at the height of anoxia, assuming 10% of total iron as FeOx, the dissolved arsenate concentration is estimated as 75-95% of the total arsenate. Thus, dissolved arsenate and iron could possibly have been released from both sinking particulate matter and from surficial bottom sediments during periods of anoxia. In December, the concentrations of particulate iron were high throughout the water column and, as a result, arsenate was being actively scavenged from solution (the equilibrium prediction is only 5-20% dissolved arsenate for a particulate iron concentration of 0.2-1 mg/L). Thus, the concentration of arsenate in the bottom waters of this lake is expected to be largely controlled by the redox-driven dissolution-precipitation of iron. Manganese oxides (MnOx) did not appear to play a direct role in the scavenging/release of arsenate; based on Scott (13), arsenate would not be absorbed to any extent onto the "neutral" MnOx surfaces.

A mass balance was calculated for iron, assuming 10 mg/L total particulate in the river input to the lake and 15% FeOx in particulate (20). The net input of iron into the lake was estimated at 3×10^{10} mg/yr, or about 20 g m⁻² month⁻¹. This estimate is a maximum value as a substantial fraction of the particulate material in the river is deposited in the forebasins of the lake (see Appendix III.A). During the anoxic period, the maximum monthly increase in iron in the hypolimnion was equivalent to about 50 g m⁻²; about two and a half times the total

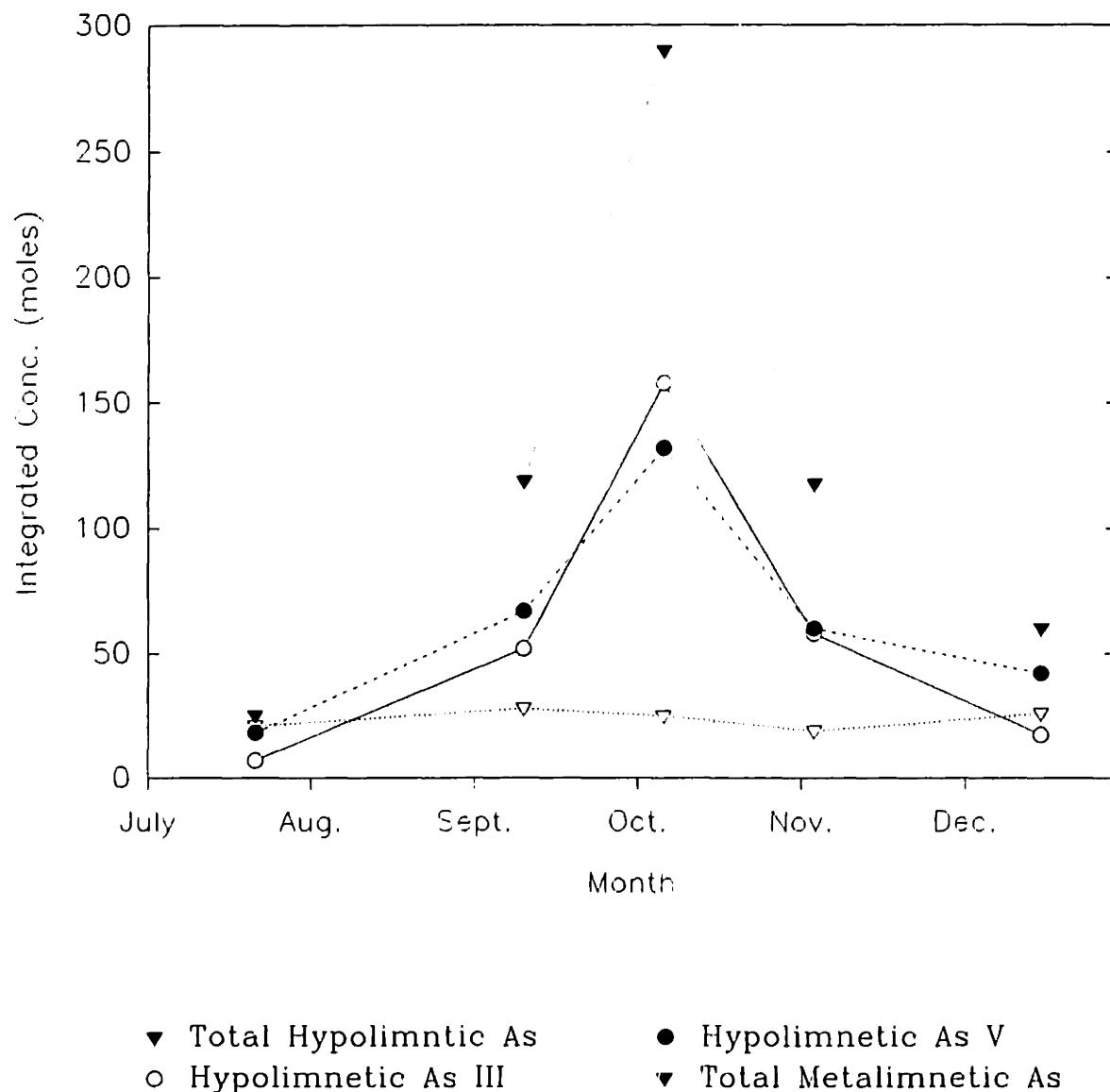


Figure II.3 Seasonal Trend in Integrated Hypolimnetic and Metalimnetic Dissolved Arsenic Concentrations.

estimated monthly input from the Aberjona River. This calculation suggests that, in addition to dissolution of settling particulate matter, iron is being released from the bottom sediments. The increase in arsenic in the hypolimnion between September and October of 1993 was estimated to be 180 moles; between October and November, a decrease of 170 moles occurred (Figure II.3). The maximum net input to the lake was estimated previously to be 400 moles yr⁻¹ (33 moles month⁻¹) (2), a factor of five less than the estimated September-October increase. These results suggest that arsenic is being released from the sediments during anoxia.

The amount of arsenic in the hypolimnion increased dramatically between September and October, and decreased as rapidly within the next month (Figure II.3). Although the iron concentration increased from September to October, it remained relatively constant from October to November (Figure II.2-c); i.e. between October and November, the temporal trends in arsenic and iron in the hypolimnion were decoupled. Considering the water column profiles (Figure II.2), the increases in both arsenate and arsenite are most closely correlated to that of sulfide. In the absence of sulfide, there was no arsenite buildup in the hypolimnion (2). As arsenite is not strongly bound to either iron or manganese (hydr)oxides under the conditions found in the lake, there is little transport of arsenite from the surface waters via scavenging by iron and/or manganese hydr(oxides), therefore arsenite formation must occur

within the hypolimnion or the bottom sediments. The dramatic increase in arsenic concentration in October 1993 was most likely due to the increased release of arsenate from solubilizing FeOx, during sulfidic conditions, with the subsequent reduction of arsenate to arsenite in the hypolimnion and sediment porewaters. It is likely that the release of dissolved iron from the sediments in October was significantly masked by the formation (and subsequent settling out) of iron sulfide (FeS) (22). When sulfide production stopped, no further arsenate was released, and both arsenic species concentrations decreased, as arsenite was oxidized, and arsenate was scavenged from solution onto particles and at the sediment-water interface.

Kuhn and Sigg (4) suggested that abiotic reduction by sulfide in the water column was the primary source of the arsenite found in Lake Greifen, Switzerland. We propose, instead, that biotic reduction of arsenate in the bottom sediments could be an alternative source for the arsenite found in the hypolimnion in 1993. Sulfate-reducing bacteria, isolated from the Aberjona watershed - upstream of the Upper Mystic Lake - are able to reduce arsenate to arsenite, both in culture and in natural samples (23). It is possible that similar organisms exist in the bottom sediments of the Upper Mystic Lake and that these organisms are reducing arsenate.

II.5 Conclusions

Overall, these results provide a consistent explanation for the interannual variability found between 1991/92 and 1993. The variability was not due to differences in inputs to the lake, but was the result of differences in the redox state of the hypolimnion and the bottom sediments, which accounts for the increased release of arsenic into the water column during the heightened anoxia of 1993. It is probable that, under 1992 conditions, even though the hypolimnion had low oxygen (<0.1 mg/L), the surface sediment maintained a sufficiently oxidizing layer to prevent - by oxidizing arsenite and scavenging arsenate - the release of a significant amount of arsenic into the water column from the sediments. The mechanism accounting for the oxidation of arsenite could involve manganese oxides (13), or other oxidants in the water column. Arsenite predominates only in the deepest part of the lake and arsenate is typically > 75% of the total inorganic arsenic at the 15-17 m sampling depths throughout the fall.

This research, when coupled with the arsenic data from other sites within the Aberjona watershed (2), and elsewhere (3,12,15), demonstrates that elevated concentrations of arsenic, and predominance of the more toxic arsenite, occur in reducing environments where sulfide persists. Although redox interfaces and other boundaries would often limit the movement of this arsenic into surface waters, it is possible that situations could exist where enhanced human exposure is possible. Due to the variability in annual redox cycles of some systems, limited

temporal characterization of the concentration and distribution of arsenic (and other redox-controlled contaminants) in a lake might not provide sufficient information to accurately evaluate the potential impact of these contaminants on these systems.

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III Biological Reduction of Arsenate in Epilimnion

III.1 Introduction

Despite thermodynamic predictions (1), arsenite (As III) is often found at measurable concentrations and is sometimes the dominant dissolved arsenic species in the oxygenated epilimnia of lakes (2,3), rivers (4), and throughout the marine photic zone (5,6). Kinetically slow abiotic arsenite oxidation (7,8) explains the persistence of arsenite in aquatic systems, but does not explain its source. The presence of methylated arsenic compounds, which are not known to form in nature abiotically, in aquatic systems (9,10,5) suggests the role of biota in arsenic cycling.

The ability of microorganisms to transform arsenic species has been well documented. Pure and mixed culture experiments have demonstrated that bacteria are able to reduce arsenic (11), to oxidize it (12,13) and to methylate it (14,15). The production of arsenite and methylated arsenic has also been demonstrated in yeast (16), fungi (16) and algae (17,18).

It is now generally believed that arsenite and methylated arsenic are produced in lake and ocean surface waters by the microorganisms living in these waters. Aquatic microorganisms - marine heterotrophic bacteria (19,20) and diatoms (17,21), and freshwater cyanobacteria (22) and green algae (23,24) - have been

found to carry out reduction and methylation of arsenate (As V) in culture. However, there is little direct evidence of *in situ* microbially mediated production of these arsenic species. Attempts at obtaining this evidence have been limited to the comparison of temporal and spatial distributions of arsenite or methylated arsenic concentrations with indicators of primary productivity (5,25,6,9), and, because arsenate is known to be transported into cells as a phosphate analog (26), nutrient depletion (2,25). The results of these investigations have been mixed. The temporal trend in arsenate concentration was found to correlate well with that of phosphate in the eutrophic Lake Greifen in Switzerland (2), implying that the former was being taken up at rates proportional to productivity. The ratio of arsenate concentration to phosphate concentration correlated spatially with dimethylarsinate (DMA) concentration in depth profiles off the coast of Southern California (25). Chlorophyll *a* and C-14 uptake rates were found by the same author to correlate well with methylated and reduced arsenic concentration at some of the sites investigated, while at other sites a correlation was found only with C-14 uptake rates, and, in the case of arsenite, no correlation was found at all (5,25). An inverse spatial (horizontal) correlation was found between chlorophyll *a* and methylated arsenic species in the surface waters of the Baltic Sea, (6), and no temporal correlation was found between chlorophyll *a* and DMA concentrations in Davis Creek Reservoir in Northern California (9).

The reports of positive correlations between phosphate and arsenate, or between the arsenate/phosphate ratio and arsenite or methylated arsenic levels, provide evidence for a relationship between biological productivity and arsenic transformation in aquatic systems. Correlations of indicators of primary productivity with reduced or methylated arsenic suggest that phytoplankton may be responsible for arsenic reduction or methylation within some of these systems. Because of these findings, phytoplankton have been cited as the probable source of reduced and methylated arsenic measured in the oxygenated water column, even with little or no site-specific data to that effect (2,3). However, based on laboratory culture experiments (19,16,20), it is possible that microorganism groups other than the chlorophyll α -containing primary producers may contribute substantially to arsenic transformations in the oxygenated water column. No studies have yet attempted to determine the *in situ* contribution of these other organism groups to arsenate reduction or methylation in surface waters.

In this study, we document the *in situ* production of arsenite in the epilimnion of Upper Mystic Lake, located north of Boston, Massachusetts. This eutrophic lake, which receives drainage from the Aberjona watershed, undergoes annual chemical and biological cycling which controls the concentration and speciation of arsenic in the water column. In the upper waters, elevated arsenite and DMA concentrations have been shown to occur in the summer and fall (27,28). The present study was undertaken

to characterize the organism groups responsible for this increase.

III.2 Methods and Materials

III.2.1 Study Area

Upper Mystic Lake has a surface area of 58 hectares, a total volume of 6.8 million cubic meters, and a maximum depth of 24 meters. It is separated into three distinct sub-basins: the shallow upper forebay, which receives the flow of the Aberjona River, the lower forebay, and the main basin (Figure III.1). Stratification develops in the main basin in early summer, resulting in hypolimnetic anoxia (27,28). In the spring and fall, surface water diatom blooms have been observed, while green algae and cyanobacteria have been shown to dominate the phytoplankton population through the summer (29). Upper Mystic Lake drains the historically contaminated Aberjona Watershed (30,31), which contains an EPA superfund site harboring a large inventory of arsenic and other toxic metals (32). The lake receives from the Aberjona River an annual input of approximately 100 Kg of arsenic, of which there are nearly equal amounts in the dissolved and particulate ($>0.45 \mu\text{m}$) phases (33). The historical deposition of arsenic and other toxic metals is recorded in the sediment column of the main basin, where a maximum concentration of nearly 2000 mg/kg total arsenic was measured 60 cm beneath the sediment surface (30).

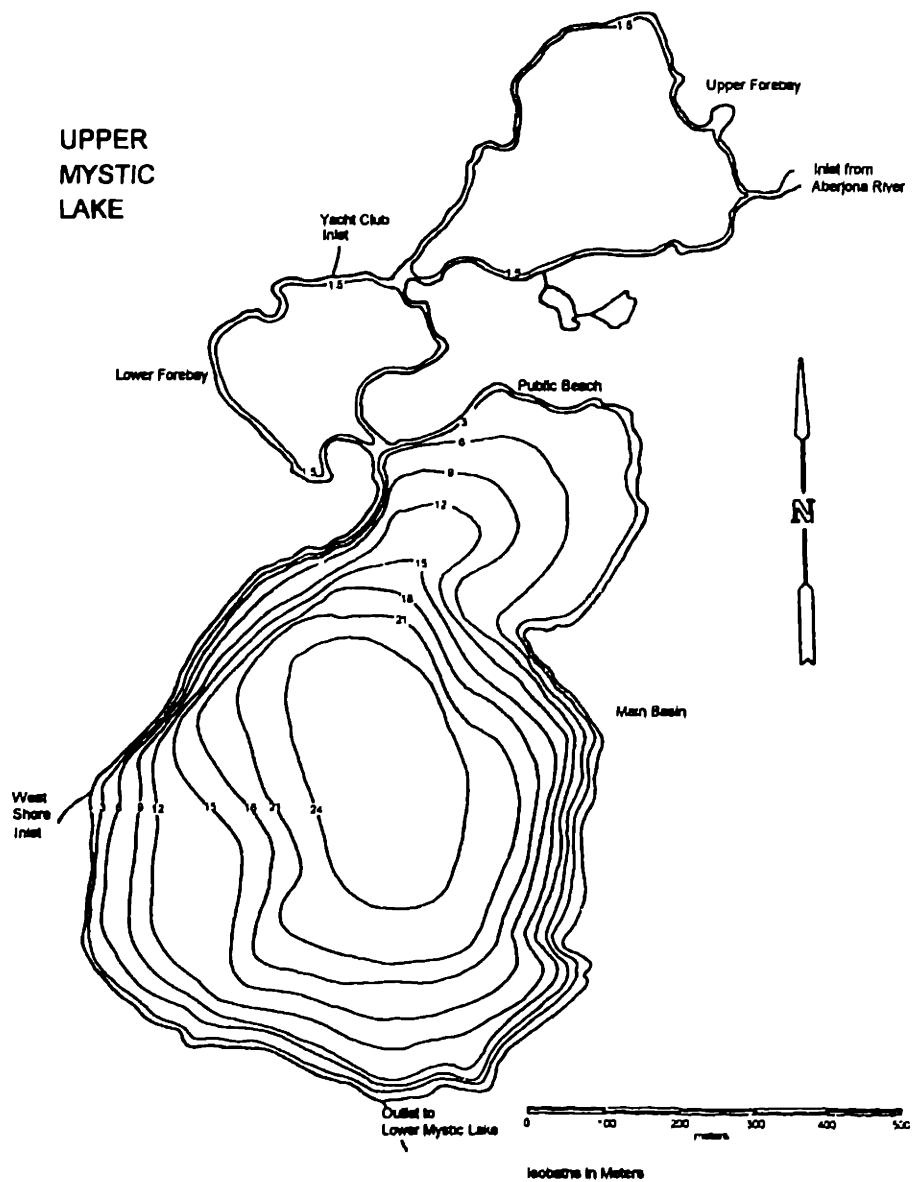


Figure III.1 Bathymetric Map of Upper Mystic Lake.
 Adapted from map by Office of Planning and Program
 Management; Department of Environmental Quality
 Engineering, Massachusetts. Data from DWPC (1981).

III.2.2 Sample Collection and Preparation

Surface water samples were collected from the center of the Upper Mystic Lake at least monthly from April to December in 1993 and 1994, and more frequently during the summer months of 1994. The sampling vessels were 4-L opaque polypropylene bottles, which were pre-sterilized in a microwave oven on the sampling day. Samples were transported to the lab, initially stored at 5° C, then divided into subsamples for incubation experiments, bacteria counts, and analysis of arsenic speciation, chlorophyll a and total phosphorus. To provide data for mass balance calculations, samples for arsenic speciation analysis were collected from the shore of the upper forebay at the mouth of the Aberjona River, between the lower forebay and the main lake basin, and at the outlet to Lower Mystic Lake (Figure III.1). Additionally, samples for arsenic speciation at different depths in the lake were collected in June and July of 1994 according to methods described elsewhere (27, 28).

III.2.3 Analytical Techniques

Analysis of dissolved arsenic speciation was completed within 24 hours of sampling using batch hydride generation with an atomic absorption detector (27), or automated flow-cell hydride generation with an atomic fluorescence detector (PSA, Excaliber), the latter having been modified for detection of As III and total dissolved arsenic. Both methods had a minimum detection limit of approximately 0.2 nM. The fraction of total

acid-soluble arsenic in particulate form was determined for some samples by difference, using filtered (0.45 μm) and unfiltered aliquots which were acidified prior to analysis. Bacterial cells were counted within a few hours of sampling by DAPI staining and epifluorescence microscopy (34). At least 20 grids or 200 cells were counted for each sample. For chlorophyll *a*, subsamples of 500 ml were passed through GF/F filters; the filters were subsequently stored at -20° C prior to extraction in acetone and spectrophotometric (Beckman, Model DU-640) analysis following the method of Parsons *et al* (35). Subsamples of 75 ml (in duplicate) were removed for total phosphorus analysis and kept refrigerated for no more than thirty days prior to digestion (persulfate oxidation) in the sample tubes and analysis with the molybdate-blue spectrophotometric method (36).

III.2.4 Lake Water Incubation Experiments

Arsenate was added to freshly collected lake water in pre-sterilized polystyrene culture tubes, which were then incubated at room temperature (20°C) under ambient laboratory light levels. Reduction rates were determined by measuring the increase in arsenite concentration in the incubated lake water over time. Initial incubation experiments indicated that the added arsenate was continuously reduced for approximately 30 to 40 hours; beyond this time, arsenite concentration decreased (see Appendix, Tables III.E.10-12), perhaps as a result of ecological succession of arsenate reducers to arsenate oxidizers within the incubated

microcosm, as other investigators have suggested (37). Results of experiments reported in this paper are for incubation periods of less than 25 hours. Abiotic control samples were prepared by microwaving lake water to boiling (6 minutes/liter) prior to arsenate spiking. The effect of light intensity on arsenate reduction rates was determined by incubating some samples in the dark, and at controlled light levels of $150 \mu\text{Einstein m}^{-2} \text{ s}^{-1}$ (38). An incubation experiment was also conducted at 5°C to determine the effect of temperature on reduction rates. The sizes of organisms responsible for arsenate reduction were characterized by filter-fractionating lake water with Nuclepore polycarbonate membrane filters of 0.2, 0.8, 1.0, 3.0 and 5.0 μm pore size, and conducting incubation experiments on the filtrates. The relative contributions of prokaryotic and photosynthetic organisms to arsenate reduction were characterized by the application of the antibiotics chloramphenicol and tetracycline (each at 30 mg/l) and the photosynthesis-inhibiting compound DCMU (3-(3,4-dichlorophenyl)-1,1-dimethylurea) (10 mg/l) (39).

A bacterium was isolated from the lake water by plating on PDYG solid medium (a general medium for aerobic heterotrophs (40)), and subsequently cultured in filter-sterilized (0.2 μm) and autoclaved lake water. Cell growth in the lake water medium was monitored by direct microscopic counts using the DAPI method. Arsenate was added to the culture during log phase growth, and arsenite concentration was determined within five hours.

III.3 Results

III.3.1 Surface Water Arsenic Speciation

In both 1993 and 1994, the concentration of arsenite in the surface water of Upper Mystic Lake underwent a rapid increase in late spring with a maximum in mid-summer, followed by a more gradual decline through the fall (Figure III.2-a). The arsenite concentration maximum in 1994 occurred in mid-July, a month later than the maximum in 1993, and the concentration decline was somewhat more gradual in the fall of 1994, than it was in 1993. Furthermore, the early spring concentrations of 1994 were significantly higher than those measured in 1993. Otherwise the temporal pattern of arsenite concentration remained remarkably similar during the two years of field sampling; the midsummer maxima in 1993 and 1994 were nearly identical (16.0 nM and 17.2 nM respectively). Arsenate concentration generally followed the opposite pattern, with minimum concentrations being reached in mid-summer (Figure III.2-b). Monomethylarsonate (MMA) and dimethylarsinate (DMA) remained at relatively low concentrations (< 3 nM) throughout the sampling period with the exception of September 1993, when the concentration of DMA reached 6.4 nM, nearly 25% of the total dissolved arsenic (Figure III.2-c). The sum of the four measured arsenic species, referred to here as total dissolved arsenic, followed a similar temporal pattern in

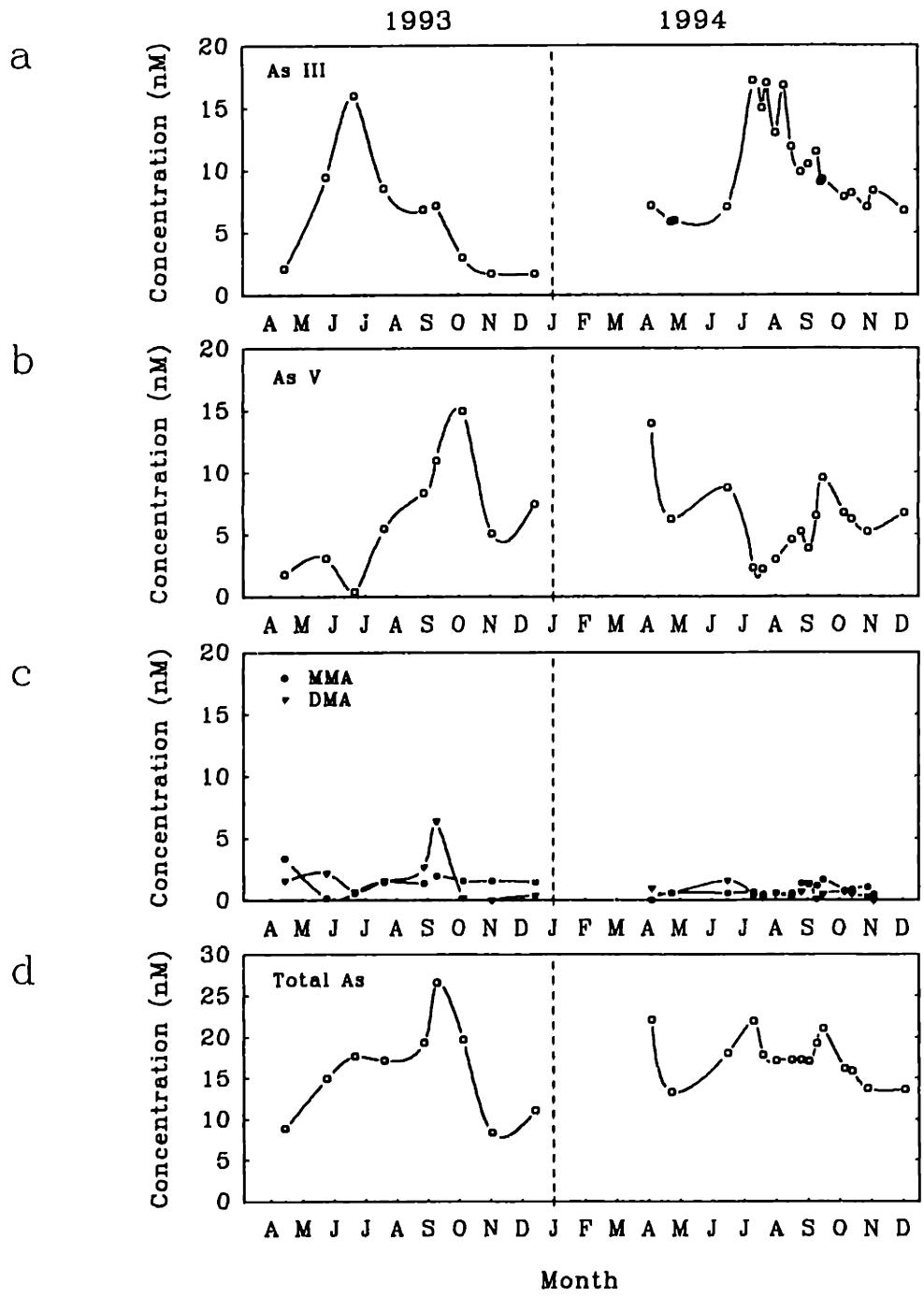


Figure III.2 Temporal Trends in Surface Water Arsenic Species Concentrations.

both sampling years, with an initial rise corresponding to the late spring increase in arsenite, followed by a period of nearly constant concentration, a further increase to a maximum in September, and then a decline. Although the temporal patterns of arsenic species concentrations varied little from 1993 to 1994, they differed more substantially from those found in 1991 and 1992. Aurilio et al (27) measured two maxima in arsenite concentrations in the Upper Mystic Lake epilimnion, one in mid-summer, and another greater maximum in October, the latter concurrent with the maximum in total dissolved arsenic. In fall of 1993 and 1994, when total dissolved arsenic was reaching its maximum concentrations, only minor peaks in arsenite concentration were evident, although, in 1993, there was a September DMA maximum (Figure III.2-c).

III.3.2 Chlorophyll, Total Phosphorus and Bacteria

The temporal variation in chlorophyll *a* concentration in the surface water of the Upper Mystic Lake (Figure III.3-a) reflected the succession of dominant phytoplankton. Early in the summer, a bloom of diatoms, primarily *Fragilaria* and *Asterionella*, occurred, and the end of this bloom is reflected in the first chlorophyll measurement. Later in the summer, green and blue-green algae were evident, but in smaller numbers, as reported in an earlier investigation (29). No significant second bloom of phytoplankton was observed in the fall, although chlorophyll *a* levels were elevated. Total phosphorus concentration decreased

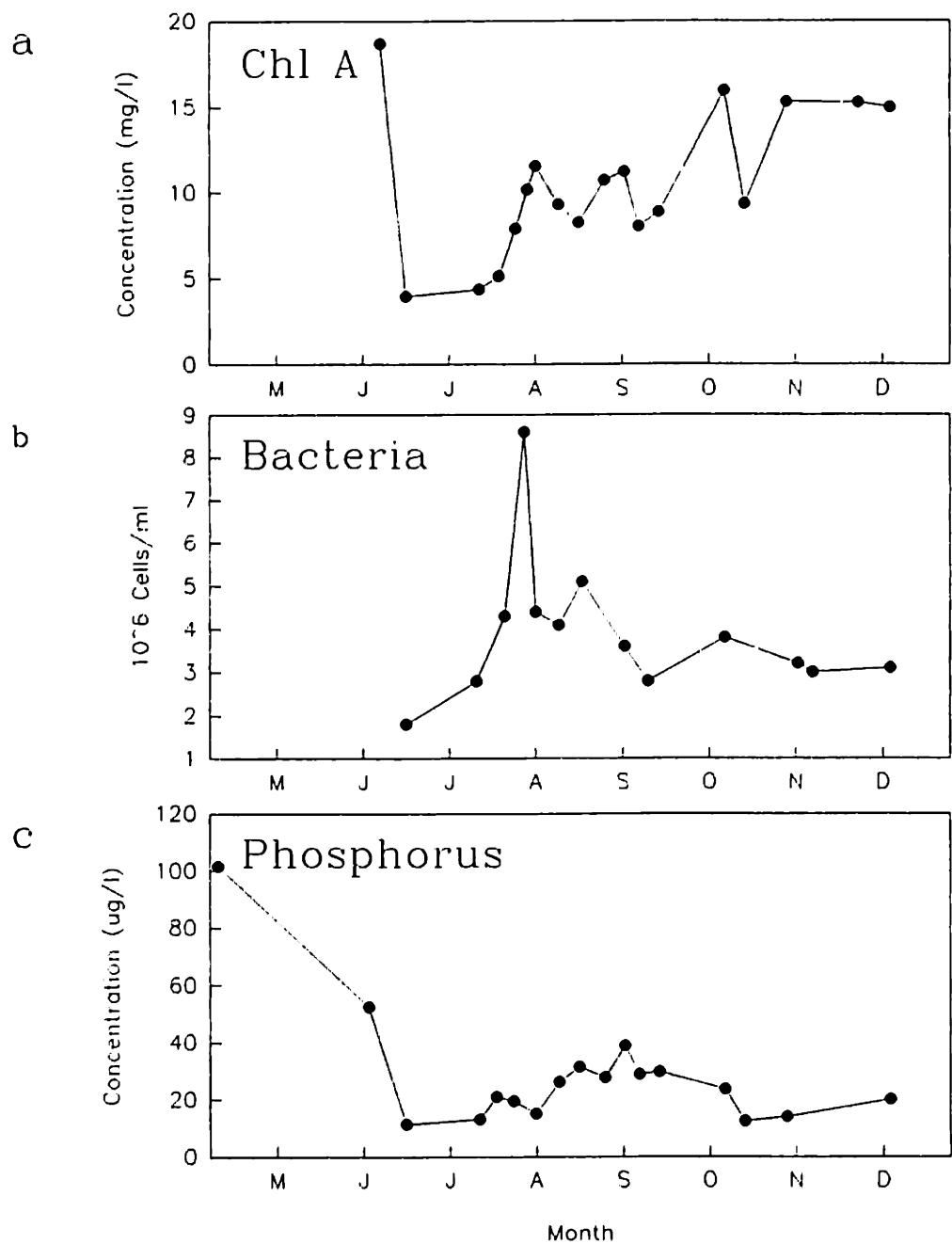


Figure III.3 Temporal Trends in Surface Water Chlorophyll, Phosphorus, and Bacteria.

early in the summer, and reached a second maximum of lesser magnitude in September (Figure III.3-c). Counts of bacterial cells ranged from 2×10^6 to 9×10^6 cells/ml throughout the summer, with a maximum near the end of July (Figure III.3-b).

III.3.3 Reduction Rates

Arsenite was produced at significant rates over a period of hours in lake water spiked with arsenate, while little or no increase in arsenite was measured in killed (microwaved) controls (see Appendix, Table III.E.9). Arsenate reduction rates, measured over a range of arsenate spike concentrations, were found to approximately follow Michaelis-Menten enzyme kinetics (Figure III.4). Because measured arsenate concentrations in the Upper Mystic Lake were substantially lower than the incubation experiment spiking concentrations, *in situ* arsenate reduction was treated as a first order process. Pseudo-first order arsenate reduction rate constants (hr^{-1}) were determined from laboratory incubation experiments with spiking concentrations up to 267 nM. Arsenate reduction was observed in all of these incubation experiments, hence the estimated rate constants were all positive. The temporal pattern of these estimated first order rate constants rate constants in 1994 was found to reach a single maximum, at the end of July (Figure III.5).

Because the 0-order range of arsenate concentrations resulted in maximum rates of arsenite production and minimal analytical error in arsenite measurement, experiments

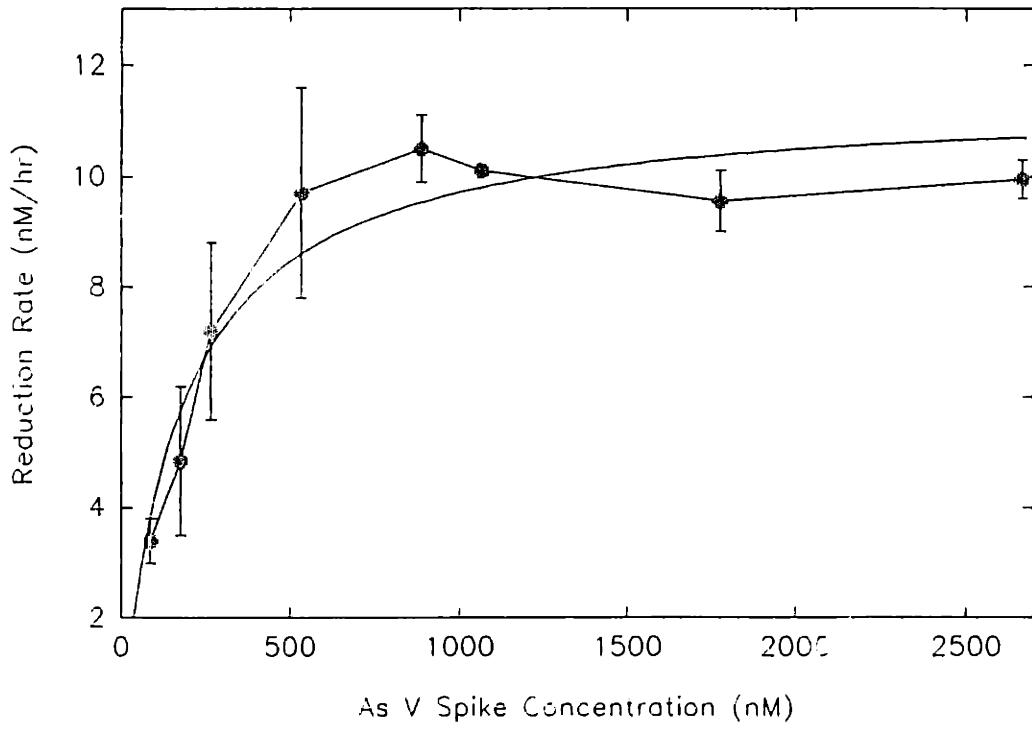


Figure III.4 Reduction Rates over a Range of Arsenate Concentrations.

Michaelis-Menten kinetics curve fit to data. Error bars represent standard deviation of duplicate incubations.

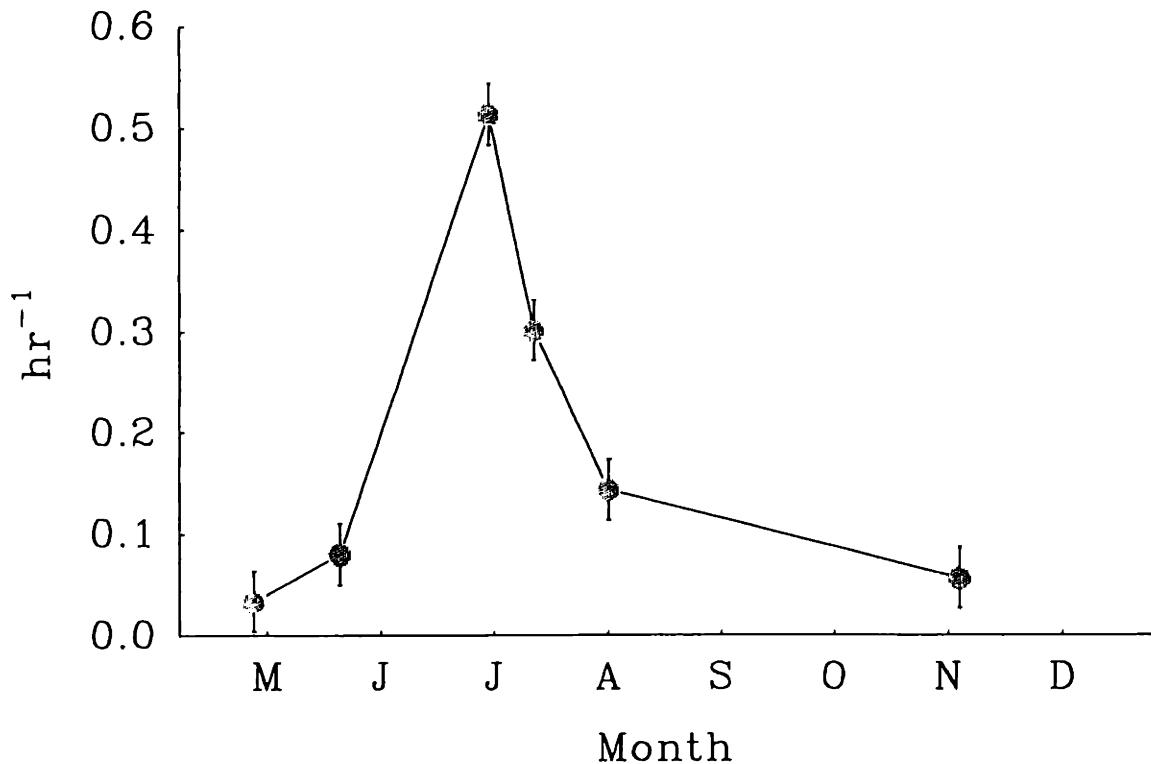


Figure III.5 Temporal Trend in First Order Reduction Rates.

characterizing the origin of the reductive capacity were based on arsenate spike concentrations of 533 nM or 1067 nM, and results are reported as 0-order reduction rates (nM/hr). Filtration experiments typically resulted in a fraction of the reductive capacity being removed with each filter of sequentially smaller pore size (Figure III.6). The extent to which a given size fraction appeared responsible for arsenate reduction varied throughout the summer. A significant fraction of the reductive capacity was consistently removed by the 5.0 μm filter, and a significant fraction remained after filtration with the 3.0 μm filter (see Appendix, Table III.E.2).

The inhibitor experiments invariably resulted in a substantial portion of the reductive capacity being lost with the application of the prokaryotic inhibitors chloramphenicol and tetracycline (Figure III.7). In contrast, reduction rates were not significantly diminished by the addition of the photosynthetic inhibitor DCMU (Figure III.8); occasionally, significant increases in the reduction rate were observed (see Appendix, Table III.E.2). When antibiotics were added to the various size fractions, the absolute loss in reductive capacity produced by the antibiotic addition was nearly the same for all the size fractions, except the smallest ones (Figure III.9). This indicates that the organisms inhibited by the antibiotics were also the organisms filtered out only by the small pore size filters (0.2 and 0.8 μm). A culture of heterotrophic bacteria (3 $\mu\text{m} \times 1 \mu\text{m}$ rods) isolated from the lake water and then

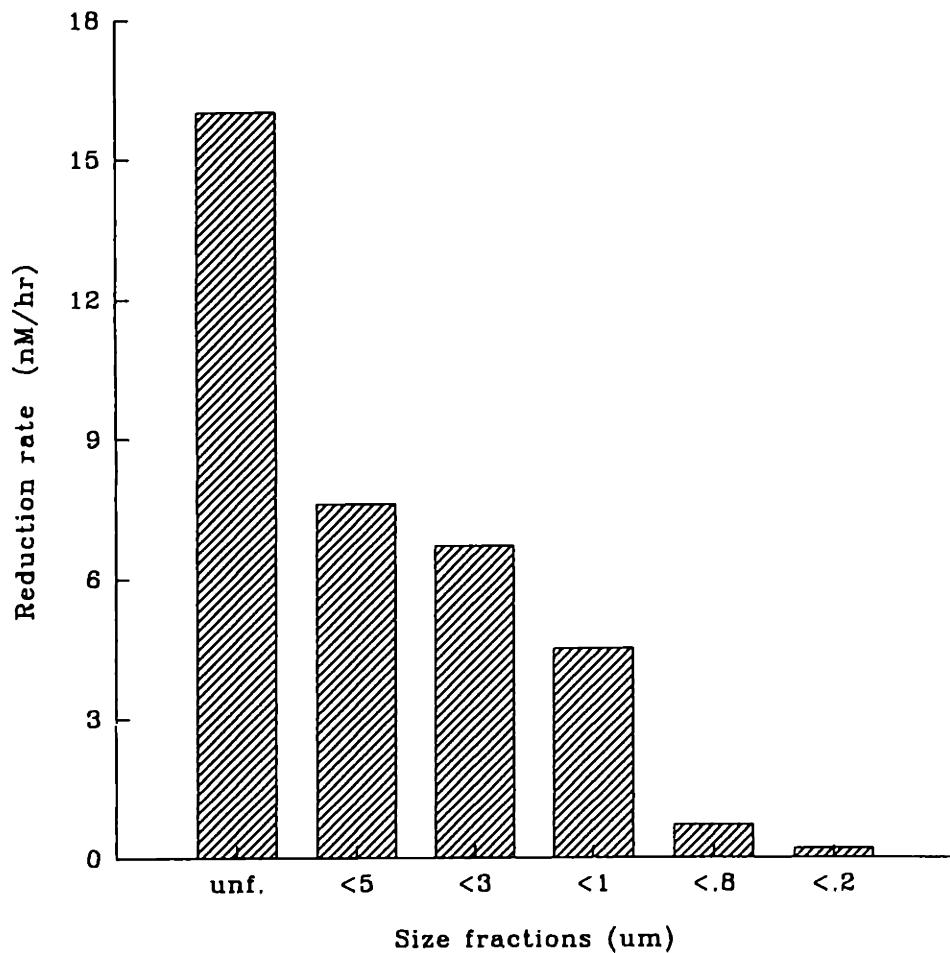


Figure III.6 Reduction Rates for Size Fractions.

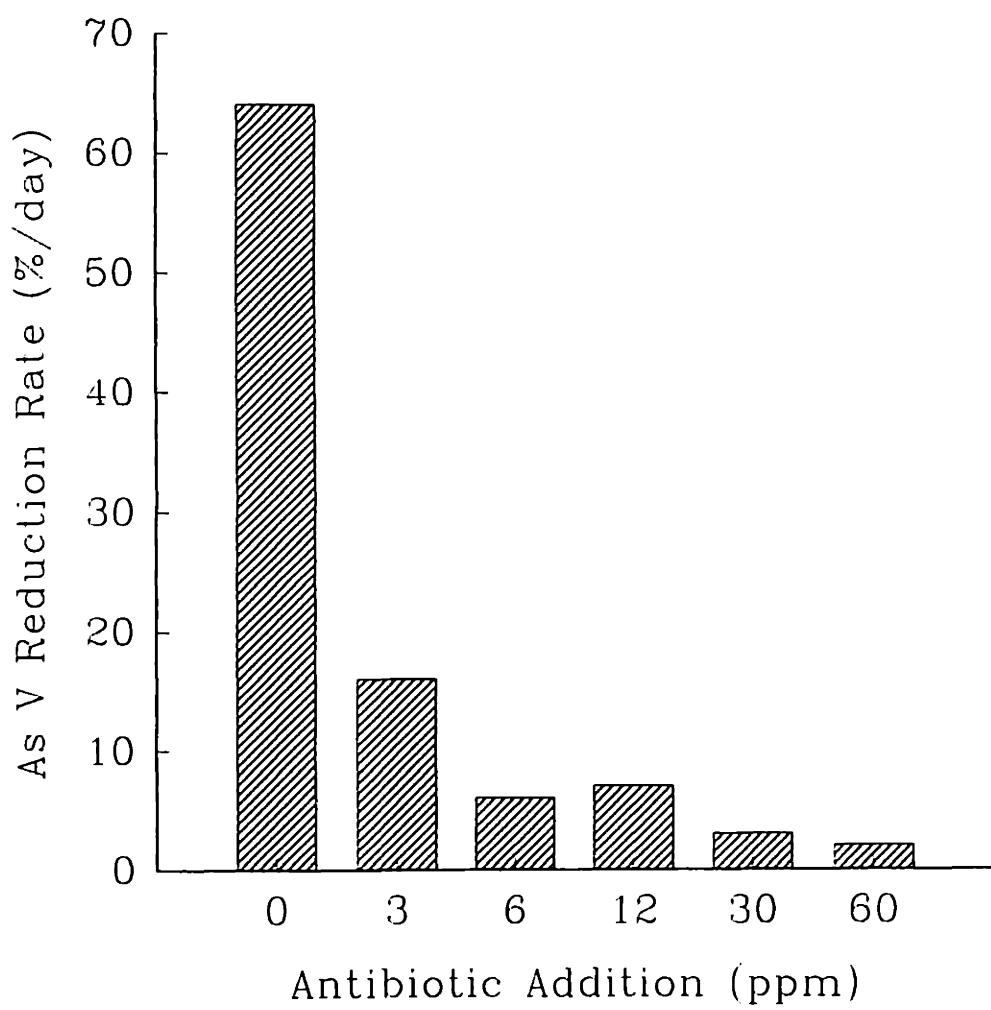


Figure III.7 The Effect of Antibiotics on Reduction Rate

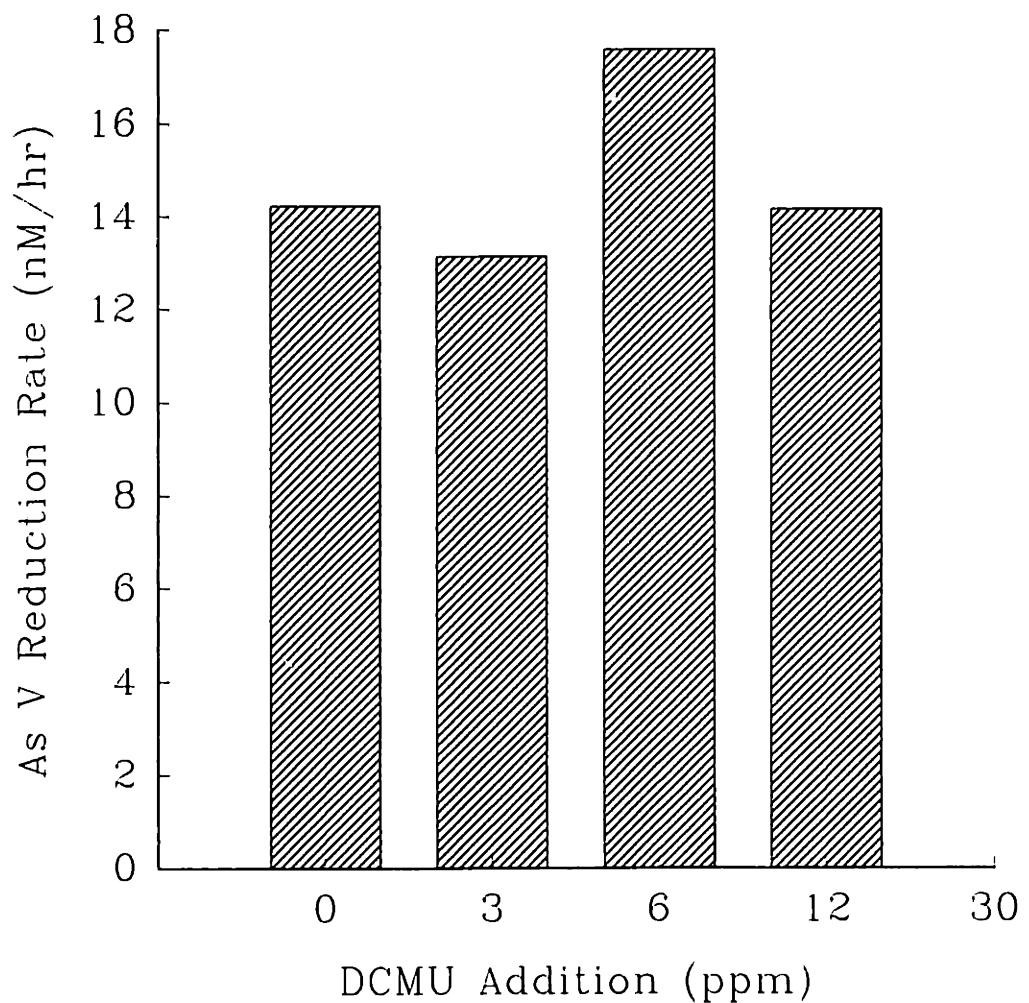


Figure III.8 The Effect of DCMU on Reduction Rate.

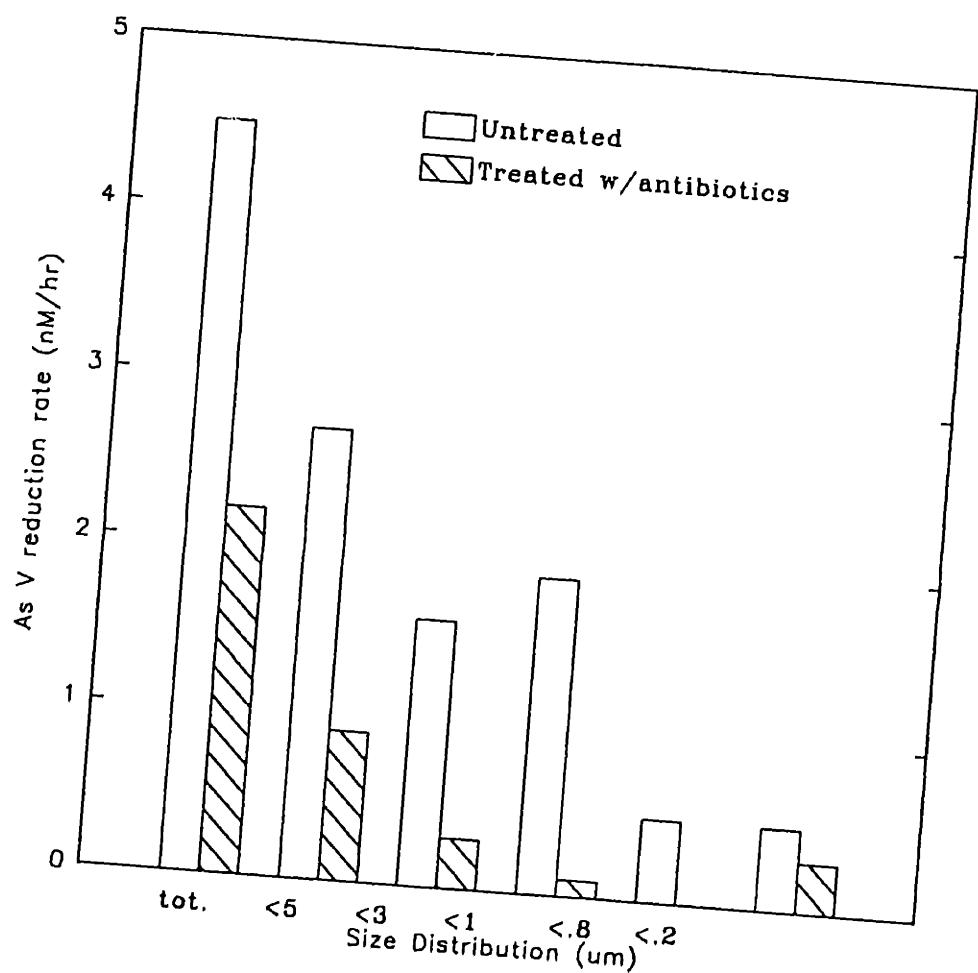


Figure III.9 Filtration Experiment with Antibiotic Additions.

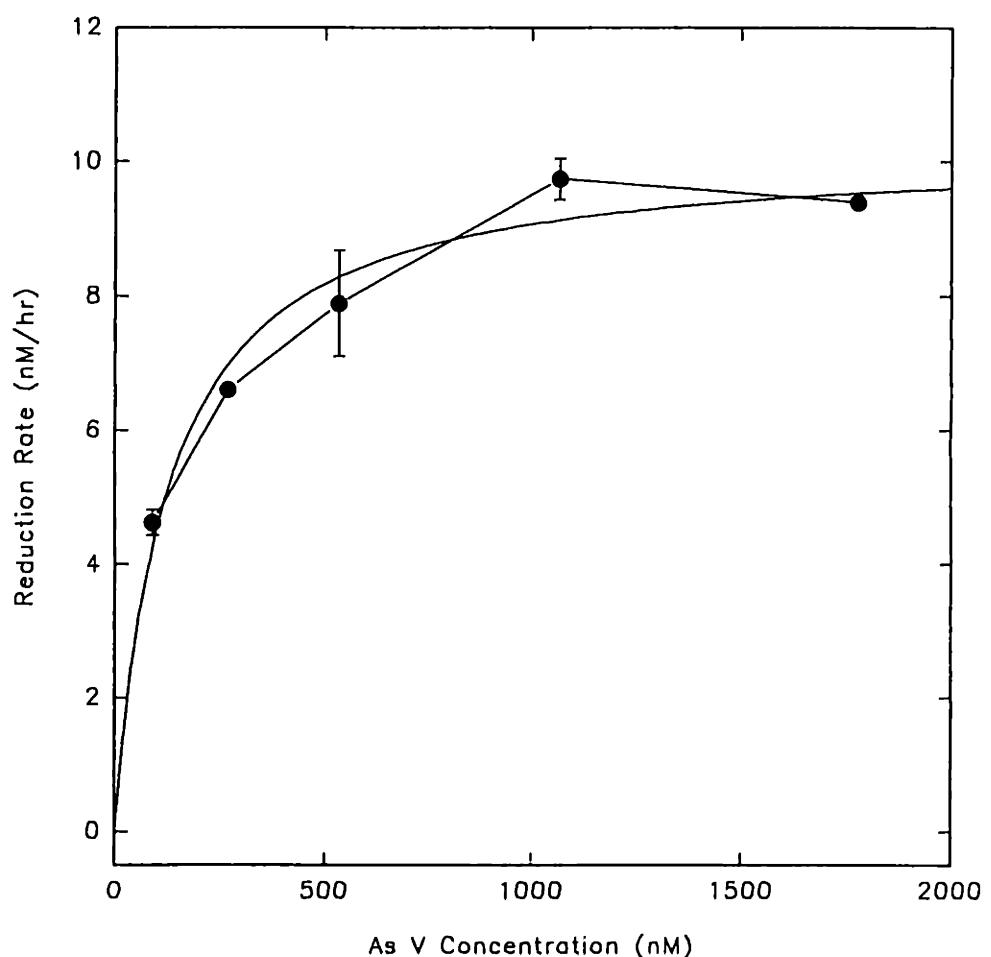


Figure III.10 Bacteria Culture Reduction Rates with Michaelis-Menten Curve Fit.

reintroduced to sterilized lake water rapidly entered log phase growth, and, at a cell density of approximately 4×10^6 cells/ml, were found to reduce arsenate at rates comparable to those calculated for the mixed cultures.

III.4 Discussion

III.4.1 Mass Balance

A mass balance for arsenite in the Upper Mystic Lake was calculated for a portion of the period of increasing epilimnetic arsenite concentration (6/16/94 through 7/11/94). The data used in these calculations are tabulated in Appendix III.C. Total moles of arsenite in the lake were estimated by dividing the lake into discrete depth intervals of 3 meters each, and summing the products of the mean arsenite concentration measured at each interval and the corresponding interval volume (as determined from bathymetric data - see Figure III.1). The mass of arsenite in Upper Mystic Lake increased from 22.62 moles on 6/16/94 to 52.70 moles on 7/11/94, or, assuming a linear rate of increase, at 50.1 umoles/hr. Of this increase, 40.6 umoles/hr, or 81%, was accounted for in the epilimnion (above 6 m).

The flux of arsenite into the lake over this time period was estimated from measured concentrations of arsenite at the inlet to the upper forebay, and the mean of measured hourly river flow rates during the summer of 1993 (33) (with the assumption that flow rates were similar in the summer of 1994). Significant

variations in arsenic concentrations at the inlet to the upper forebay of the Upper Mystic Lake occurred throughout the year, and perhaps even on an hourly basis as predicted by the Solo (33). Concurrently, variations occurred in the flow of the Aberjona River at its inlet to the upper forebay (in 1993 (33), and presumably, in 1994), with the result that the arsenite flux into the lake during the period of epilimnetic arsenite increase is difficult to accurately assess. However, based on the 1993 mean summer flow rate (901,000 L/hr), and the 6/16/94 upper forebay inlet arsenite concentration (10.9 nM), we can estimate a flux into the lake of 10 $\mu\text{mole}/\text{hr}$. Similarly, the flux out of the lake for this flow rate (taking into account a 5% increase in flow contributed by two other minor lake inlets) can be estimated from the 6/16/94 Lower Mystic Lake inlet arsenite concentration (4.1 nM) as 3.88 $\mu\text{mole}/\text{hr}$. The difference between flux in and flux out (5.95 $\mu\text{mole}/\text{hr}$) can only account for 12% of the increase in arsenite in the lake between June 16 and July 11 of 1994.

The mass balance calculations indicate that arsenite was being produced in the epilimnion of the Upper Mystic Lake *in situ*. Similar calculations indicate the magnitude of arsenate loss from the system was nearly the same as that of arsenite increase. However, the mass balance for arsenate is more complicated, because there are additional source/sink terms for this species. Much of the total river arsenic concentration was lost as arsenate to the sediments of the upper and middle forebays, and perhaps also to the littoral sediments of the lake

(see Appendix III.B). Concurrently, as anoxia developed in the hypolimnion, dissolved arsenate was released from the bottom (and suspended) sediments to the water column (28).

III.4.2 Biological and Chemical Parameters

The trend in total phosphorus concentration (Figure III.3) resembles that of arsenate, suggesting that both parameters may reflect uptake by biota. Because samples for total phosphorus and arsenic species were not always collected on the same days, a direct correlation cannot be calculated. However, there was a correlation between monthly average total phosphorus and arsenate concentrations ($r=0.69, n=7$).

No correlation was found between chlorophyll a and either arsenite concentration or the concentration ratio of arsenite to arsenate. The maximum recorded chlorophyll a concentration occurred in the beginning of June, reflecting the end of a major bloom of the diatoms *Asterionella* and *Fragillaria* which had begun earlier in the spring. The bloom resulted in no significant increase in arsenite concentration in the lake epilimnion (Figure III.3-a), nor in an increase in the arsenite/arsenate ratio. It was after the bloom, during the period of minimum chlorophyll a concentration (and depleted total phosphorus), that arsenite began to increase. In mid-July, soon after arsenite had attained its maximum concentrations, chlorophyll a increased and remained at higher concentrations throughout the rest of the summer and fall, corresponding to observations of increased numbers of green

and blue-green algae. Although a second bloom of diatoms in the fall was reported for Upper Mystic Lake in a previous year (29), no second bloom was observed in 1994. Because chlorophyll *a* has been found in some studies to conflict with other measures of primary productivity such as C-14 uptake (5), it cannot be accepted as an unequivocal indicator of primary productivity. However, in the absence of any other such measures, these results suggest that primary producers were not the dominant agents of arsenate reduction in the Upper Mystic Lake epilimnion in 1994.

Bacterial numbers increased from mid-June through the end of July (Figure III.3-b), with a concurrent increase in arsenite concentration, although the latter reached its maximum concentration two weeks earlier. A positive correlation was found between monthly averages of bacterial numbers and both arsenite concentration ($r=0.85, n=7$) and the arsenite/arsenate ratio ($r=0.81, n=6$). This suggests that bacteria may have played a role in arsenate reduction in the Upper Mystic Lake. This inference is not altogether unlikely, in the light of findings that marine bacteria reduce arsenate (19,20). It is also interesting to note that heterotrophic bacteria are frequently found to be more abundant (in terms of carbon-biomass) in some aquatic systems than both photosynthetic eukaryots and prokaryots (41).

III.4.3 Arsenate Reduction: Laboratory Findings

The finding that arsenite was produced at significant rates

only in lake water that had not been sterilized by microwave, demonstrates the capacity of Upper Mystic Lake biota for arsenate reduction, and suggests that the production of arsenite in the lake epilimnion is a biologically mediated process. Furthermore, the ability to monitor arsenate reduction in laboratory microcosms containing natural assemblages of microorganisms, provides an opportunity to make inferences concerning both the nature of the organisms responsible for arsenate reduction, and the temporal trend in the biological reductive capacity of the lake epilimnion.

The results of four size fractionation experiments in the summer and fall of 1994 demonstrated that significant portions of the reductive capacity of the lake water resided in both the >5.0 μm and the <1.0 μm size fractions (each averaging 41% of the unfiltered reduction rates) (Figure III.6; see also Appendix, Table III.E.4). The intermediate (1.0 μm to 5.0 μm) and smaller (<0.8 μm) size fractions, were responsible for less of the arsenate reduction (19% and 9%, respectively), implying that there were fewer arsenate reducers, and/or there were less active arsenate reducers, present in these size fractions. Upon microscopic inspection of the 5.0 μm filtrates, it was evident that the chain-forming algae, most of the diatoms, and many of the larger green and blue-green single-cell algae had been removed by filtration. It was also apparent that some clumped bacteria, and those attached to particles, were filtered out by the 5.0 μm filter. In the 5.0 μm filtrates, there was a variety

of bacteria and chlorophyll-containing cells, while in the 1.0 μm filtrates, there were primarily bacteria. Few bacteria and no chlorophyll-containing cells were found in the 0.2 μm filtrates, although it was observed that the bacteria that did pass through the filter grew rapidly, as found by Li and Dickie (31), and significantly increased the measured arsenate reduction rate if the incubation experiments were allowed to continue for more than one or two days. Overall, the results of the filtration experiments suggest that both the larger ($>5.0 \mu\text{m}$) algae and the bacteria significantly contributed to the reduction of arsenate in the incubated lake water.

It was determined that the application of tetracycline and chloramphenicol considerably diminished the reductive capacity of the lake water (Figure III.7); a mean decrease in reduction rate of 59% was measured for seven incubation experiments throughout the summer and fall. Because both antibiotics are broad-spectrum inhibitors of prokaryotic organisms, and have little or no effect on eukaryotes (42), this result implies that prokaryotes were responsible for a large portion of the arsenate reduction in the incubated lake water samples, and suggests that prokaryotes also may be important arsenate reducers in the lake epilimnion. During the late summer, an experiment demonstrated that the extent to which the antibiotics diminished the reductive capacity of several size fractions of the lake water was relatively constant over the larger size fractions (Figure III.6), suggesting that the size of organisms affected by the antibiotics

was small ($<1 \mu\text{m}$). This is evidence that the organisms affected by the antibiotics were not larger chain-forming cyanobacteria, which may have been abundant at this time of year (43). The photosynthetic inhibitor DCMU was not found to diminish rates of arsenate reduction in culture experiments with Upper Mystic Lake water. Because of the results of the experiments already discussed, showing that larger organisms not inhibited by antibiotics were significantly responsible for arsenate reduction, and because of previous reports of arsenate reduction by photosynthetic organisms in culture (e.g., 17), it seems unlikely that photosynthetic organisms did not reduce arsenate in these experiments. Rather, it appears that, over the short term, phytoplankton do not have to be actively photosynthesizing to reduce arsenate.

Arsenate reduction in the incubated lake water over a range of spiking concentrations was found to follow Michaelis-Menten kinetics, as other researchers have noted for pure cultures of microorganisms (17,18), suggesting that arsenate reduction in the lake water was an enzymatic process. This established a working range of incubation arsenate concentrations (the pseudo first order range) in which inferences concerning the *in situ* epilimnetic arsenate reduction rate could be made.

The first-order rate constants calculated for incubation experiments throughout the summer of 1994 (Figure III.5) imply positive arsenate reduction rates for Upper Mystic Lake throughout the summer and fall, although significant arsenite

production was only observed in the lake in June and July. The maximum reduction rate constant (0.51 hr^{-1}) was measured in the laboratory at the end of June, during the period when arsenite was increasing in the lake at an estimated rate constant of only 0.0029 hr^{-1} . Several explanations are possible for this overestimation of the field reduction rate by the lab reduction rate.

The arsenate available for biological uptake in the lake may have been less than the total dissolved arsenate measured by the hydride system. If a significant portion of the arsenate in the lake was either in colloidal phase or otherwise complexed, it might not have been readily available for uptake by organisms, although it may have been measured as dissolved arsenate. In this case, first order reduction rates based on low concentrations of available arsenate would have been much higher than those estimated from the measured total dissolved arsenate in the lake. However, although there are reports of organic arsenical compounds which are refractory to hydride analysis (44), complexation of arsenate with cations present in solution may not significantly alter its bioavailability. In 1994, arsenate in the Upper Mystic Lake was found be primarily in the dissolved phase ($<0.45 \mu\text{m}$), although in the fall, high concentrations of particulate arsenic (up to 48% of total arsenic) were measured in the epilimnion (45). On one occasion, also in the fall, 11% of the total arsenic was measured in the 0.02 to $0.45 \mu\text{m}$ size fraction, implying that colloidal arsenate

was present in the "dissolved" size fraction ($<0.45\text{ }\mu\text{m}$) . However, a much greater colloidal fraction of arsenate would have been necessary to significantly diminish reduction rates in the lake. Hence it seems unlikely that a significant amount of the arsenate measured in the Upper Mystic Lake epilimnion was unavailable for uptake by microorganisms.

It seems more likely that the disagreement between reduction rates measured in the laboratory and those observed in the field reflected a change in the net *in situ* arsenate reduction rate induced by some aspect of the sampling/incubation process, which may have either enhanced the gross reduction rate or diminished the gross oxidation rate. During the cooler months of the year, the temperature difference between the lab and the lake was found to dramatically increase the measured reduction rate in the lab, as illustrated by the comparison of 0-order reduction rates for samples taken in early December and incubated at 5°C (1.9 nM/hr) and 20°C (21.6 nM/hr). However, this effect could not explain the two order of magnitude difference between lab and field reduction rates during the mid summer months when field and lab temperatures were similar. Differences in light levels between the lab and the field were shown to have little effect when samples were incubated in the lab under different light conditions (complete darkness and $150\text{ }\mu\text{Einstein m}^{-2}\text{ s}^{-1}$ (38)) (see Appendix, Table III.E.6).

One possible explanation that deserves further investigation is that the disagreement in reduction rates could have resulted

from the increased growth of heterotrophic organisms fostered by the internal surfaces of the sample and/or incubation vessels, a phenomenon reported by other investigators. Because the net reduction rate in the lake epilimnion may have reflected the difference between much larger gross rates of reduction and oxidation, a relatively small enhancement of the gross reduction rate could result in a large increase in the measured net reduction rate. Such an enhancement could have been due to the growth of arsenate reducers induced by the surfaces of sample/incubation vessels.

Another explanation is that the organisms may activate a mechanism to reduce arsenate only when arsenate is found above a threshold concentration; at UML arsenate concentrations, the mechanism is not activated, and the reduction rates are effectively lower than predicted by lab incubation experiments. However, preliminary experiments seemed to indicate that even for arsenate spikes near ambient concentrations (5.3 to 26.7 nM), reduction rates constants were not much different than those for higher spiking concentrations (see Appendix, Table III.E.1).

Although the disagreement between reduction rates measured in the laboratory and those observed in the field cannot be adequately explained at this time, it is evident that reduction rates measured in the lab reflected a biological reductive capacity inherent to the lake water which changed throughout the sampling year, and reached a maximum which corresponded to the initial portion of the period of increasing arsenite

concentration in the epilimnion of Upper Mystic Lake. Even if a minor enhancement of the gross reduction rate resulted from the sampling/incubation process, inferences concerning the nature of organisms responsible for arsenate reduction would remain unchanged.

Although it is likely that aerobic free-floating bacteria found in lakes are able to reduce arsenate, no previous studies have demonstrated this. In this study we have shown that a culture of heterotrophic bacteria isolated from the epilimnion of Upper Mystic Lake and grown in sterile lake water was able to reduce arsenate at rates (Figure III.10) comparable (during log-phase growth at 4×10^6 cells/ml) to reduction rates determined for the natural consortia of organisms present in the lake water (Figure III.4).

III.5 Conclusions

Mass balance calculations indicated that arsenite was produced *in situ* in the epilimnion of the Upper Mystic Lake between the middle of June through the middle of July 1994. No temporal correlation was found between either arsenite concentration or the concentration ratio of arsenite/arsenate and chlorophyll a. There was, however, a correlation between bacterial numbers and arsenite concentration and the arsenite/arsenate ratio. Laboratory incubations of lake water with added arsenate demonstrated the ability of the natural

consortia of organisms present in the lake to reduce arsenate. Filter fractionation experiments indicated that the organisms responsible for arsenate reduction varied in size, and may, therefore, have included both larger photosynthetic phytoplankton and smaller heterotrophic microbes. Further evidence that both phytoplankton and bacteria contributed to arsenate reduction was provided by the finding that antibiotics significantly diminished arsenate reduction rates in the incubated lake water. The ability of heterotrophic bacteria isolated from the lake to reduce arsenate was demonstrated. Previous studies of arsenic cycling in lakes (2,3) have cited only phytoplankton as probable sources of arsenite in the oxygenated water column. Our results suggest that both phytoplankton and bacteria may play important roles in the production of arsenite in the surface waters of aquatic systems.

III.6 Literature Cited

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APPENDICES

Appendix II.A Arsenic Water Column Depth Profiles

Note: ND = none detected

DEPTH (m)	As III (nM)	As V (nM)	MMA (nM)	DMA (nM)
1	2.1	1.8	3.4	1.6
3	2.6	-	-	1.7
7	2.2	3.1	ND	ND
15	1.0	2.9	ND	ND
22	0.8	1.9	ND	ND

Table II.A-1 April 15, 1993; Water Column Arsenic Species Concentrations.

DEPTH (m)	As III (nM)	As V (nM)	MMA (nM)	DMA (nM)
1	9.5	3.1	0.2	2.2
3	5.5	4.4	0.2	3.9
5	7.6	4.1	0.6	1.1
11	0.6	1.0	ND	0.4
20	1.0	1.0	ND	ND

Table II.A-2 May 25, 1993; water column arsenic species concentrations.

DEPTH (m)	As III (nM)	As V (nM)	MMA (nM)	DMA (nM)
1	16.	0.4	0.6	0.7
3	12.	1.9	ND	1.4
7	9.7	0.8	ND	ND
15	1.8	1.5	ND	ND
21	1.1	1.5	ND	ND

Table II.A-3 June 21, 1993; water column arsenic species concentrations.

DEPTH (m)	As III (nM)	As V (nM)	MMA (nM)	DMA (nM)
1	8.6	5.5	1.5	1.6
5	8.7	5.2	1.7	0.2
7	6.6	4.5	2.1	0.5
9	4.9	3.4	0.6	0.1
19	0.9	8.8	1.0	0.2

Table II.A-4 July 20, 1993; water column arsenic species concentrations.

DEPTH (m)	As III (nM)	As V (nM)	MMA (nM)	DMA (nM)
1	7.2	11.	2.0	7.9
5	3.4	13.	4.1	ND
7	3.1	4.3	1.4	0.8
9	9.2	5.5	0.2	0.7
11	1.8	5.7	1.0	ND
16	22.	28.	2.9	2.2
24	38.	42.	4.0	1.5

Table II.A-5 September 9, 1993; water column arsenic species concentrations.

DEPTH (m)	As III (nM)	As V (nM)	MMA (nM)	DMA (nM)
1	3.0	15.	1.6	0.2
3	2.9	19.	2.0	0.4
6	1.5	16.	2.2	ND
8	1.2	9.0	0.5	ND
9	1.2	8.1	1.1	ND
17	0.8	13.	0.7	ND
22	220	160	2.4	ND

Table II.A-6 October 5, 1993; water column arsenic species concentrations.

DEPTH (m)	As III (nM)	As V (nM)	MMA (nM)	DMA (nM)
1	1.7	5.1	-	-
5	3.3	4.9	-	-
8	3.0	7.4	-	-
10	0.9	6.6	-	-
12	0.4	5.7	-	-
18	0.9	16.	-	-
22	86.	62.	-	-

Table II.A-7 November 2, 1993; water column arsenic species concentrations.

DEPTH (m)	As III (nM)	As V (nM)	MMA (nM)	DMA (nM)
1	1.7	7.5	-	-
5	2.5	7.9	-	-
10	2.6	8.5	-	-
15	2.2	8.5	-	-
17	2.8	8.9	-	-
19	14.	47.	-	-

Table II.A-8 December 14, 1993; water column arsenic species concentrations.

APPENDIX II.B WATER COLUMN IRON AND MANGANESE DEPTH PROFILES

Note: all concentrations in mg/l

DEPTH (M)	DISS. Fe	TOTAL Fe	DISS. Mn	TOTAL Mn
1	0.01	-	ND	-
5	ND	-	ND	-
7	0.04	-	0.02	-
9	0.06	-	0.21	-
11	0.09	-	0.94	-
16	5.5	-	2.2	-
24	5.1	-	2.2	-

Table II.B-1 September 9, 1993; Dissolved and total iron and manganese concentrations.

DEPTH (m)	DISS. Fe	TOTAL Fe	DISS. Mn	TOTAL Mn
1	ND	0.07	ND	0.02
3	ND	0.05	0.01	0.02
6	ND	0.06	ND	0.01
8	0.04	0.09	0.34	0.26
9	0.03	0.07	0.51	0.4
17	0.4	1.3	-	-
22	9	10	2.3	2.4

Table II.B-2 October 5, 1993; Dissolved and total iron and manganese concentrations.

DEPTH (m)	DISS. Fe	TOTAL Fe	DISS. Mn	TOTAL Mn
1	ND	0.07	ND	0.04
5	0.02	0.03	ND	0.04
8	ND	0.09	0.01	0.04
10	0.07	0.2	0.46	0.52
12	0.07	0.39	0.58	0.59
18	0.62	1.9	1.8	1.9
22	8.4	9.2	2.2	2.4

Table II.B-3 November 2, 1993; Dissolved and total iron and manganese concentrations.

DEPTH (m)	DISS. Fe	TOTAL Fe	DISS. Mn	TOTAL Mn
1	0.01	0.28	0.11	0.20
5	0.02	0.28	0.11	0.20
10	0.01	0.30	0.10	0.21
15	ND	0.26	0.12	0.20
17	0.07	0.63	0.78	0.92
19	2.3	5.8	2.3	2.4

Table II.B-4 December 14, 1993; Dissolved and total iron and manganese concentrations.

APPENDIX II.C WATER COLUMN DISSOLVED OXYGEN AND TEMPERATURE DEPTH PROFILES

DEPTH (m)	TEMP. (°C)	D.O. (mg/l)
1	8.1	10.4
3	7.9	10.1
5	6.5	10.1
7	4.6	9.7
10	3.0	9.2
15	2.3	8.6
20	2.3	6.5
22	2.4	4.9

Table II.C-1 April 15, 1993; Dissolved oxygen and temperature profiles.

DEPTH (m)	TEMP. (°C)	D.O. (mg/l)
0	18.8	8.8
1	18.5	8.6
3	18.2	8.3
5	13.4	8.6
7	10.5	8.6
9	5.7	8.7
11	3.5	6.5
13	3	6.5
15	2.7	6.5
17	2.6	5.6
19	2.6	4.8
21	2.6	2.8

Table II.C-2 May 25, 1993; Dissolved oxygen and temperature profiles.

DEPTH (m)	TEMP. (°C)	D.O. (mg/l)
0	22.8	8.8
1	22.9	8.6
3	22.8	8.5
4	18.8	8.7
5	16.0	8.3
7	8.9	8.9
9	5.0	3.6
11	4.2	3.4
15	2.7	1.9
16	2.7	1.1
19	2.7	0.7
21	2.7	0.8

Table II.C-3 June 21, 1993; Dissolved oxygen and temperature profiles.

DEPTH (m)	TEMP. (°C)	D.O. (mg/l)
0	23.5	7.7
1	23.5	7.7
3	23.5	7.8
5	19.1	7.4
6	14.2	10.1
7	9.6	11.3
8	8.4	10.3
9	4.9	1.4
11	3.5	0.64
13	2.8	0.58
17	2.6	0.46
19	2.6	0.43

Table II.C-4 July 20, 1993; Dissolved oxygen and temperature profiles.

DEPTH (m)	TEMP. (°C)	D.O. (mg/l)
1	22.8	8.3
3	22.6	8.4
5	22.4	7.8
7	13.3	8.4
9	6.8	3.0
11	4.3	1.8
13	3.3	1.2
15	3.0	1.1
17	2.9	0.81
21	2.9	0.66
23	2.9	0.60

Table II.C-5 September 9, 1993; Dissolved oxygen and temperature profiles.

DEPTH (m)	TEMP. (°C)	D.O. (mg/l)
0	14.8	9.7
2	14.7	9.5
4	14.8	9.3
6	14.9	10.1
8	12.4	3.3
7	14.8	9.5
9	7.2	1.6
11	5.1	1.1
13	4.0	0.80
15	3.0	0.66
17	2.7	0.59
19	2.7	0.55
21	2.8	0.60

Table II.C-6 October 5, 1993; Dissolved oxygen and temperature profiles.

DEPTH (m)	TEMP. (°C)	D.O. (mg/l)
0	9.5	11.4
2	9.1	11.2
4	9.0	10.9
6	9.0	10.6
8	9.0	10.7
9	8.6	9.8
10	7.6	2.4
11	5.1	1.2
12	3.8	0.89
14	3.3	0.70
16	2.9	0.65
18	2.7	0.58
20	2.8	0.54
22	2.8	0.48

Table II.C-7 November 2, 1993; Dissolved oxygen and temperature profiles.

DEPTH (m)	TEMP. (°C)	D.O. (mg/l)
0	5.1	12.7
1	4.1	13.0
2	4.2	13.0
5	4.0	12.2
7	4.0	12.5
9	4.0	12.3
11	4.0	12.3
15	3.9	11.9
17	3.8	6.30
19	3.2	3.20
21	2.9	0.83
23	2.9	0.61

Table II.C-8 December 14, 1993; Dissolved oxygen and temperature profiles.

APPENDIX II.D DATA USED IN MASS BALANCE CALCULATIONS

	7/20/93	9/9/93	10/5/93	11/2/93	12/14/93
DISS. Fe (0-24 m)	-	5.1	9.0	8.4	2.3
TOTAL Fe (0-24 m)	-	-	10.	9.2	5.8
DISS. Mn (0-24 m)	-	2.1	2.1	2.2	2.1
TOTAL Mn (0-24 m)	-	-	2.3	2.4	2.3
As III (13-24 m)	7.0	52.	158	58.	18.
As V (13-24 m)	18.	67.	132	60.	42.
DISS. As (7-12 m)	21.	28.	2.5	19.	26.
DISS. As (13-24 m)	25.	119	290	118	60.

Table II.D-1 Integrated Concentration (moles) of iron, manganese and arsenic (1993).

APPENDIX III.A Inlet Dissolved Arsenic Species Concentrations

Note: All concentrations in nM.

UFB = upperforebay inlet; MAIN = main basin inlet;

LML = Lower Mystic Lake inlet; ND = none detected;

TOTAL = total dissolved arsenic

DATE	UFB AsIII	UFB AsV	UFB MMA	UFB DMA	UFB TOTAL	MAIN AsIII	MAIN AsV	MAIN MMA	MAIN DMA	MAIN TOTAL	LML AsIII	LML AsV	LML MMA	LML DMA	LML TOTAL
7/20/93	7.5	14.0	1.0	0.2	22.7	-	-	-	-	-	-	-	-	-	-
11/2/93	6.1	14.5	1.1	0.3	22.0	-	-	-	-	-	-	-	-	-	-
12/14/93	9.2	22.0	ND	1.2	32.4	-	-	-	-	-	-	-	-	-	-
4/5/94	14	24.0	ND	2.4	40.4	-	-	-	-	-	-	-	-	-	-
4/19/94	7.4	15.0	2.1	2.7	27.7	7.7	8.9	0.8	1.5	18.9	4.7	9.0	0.5	1.4	15.6
4/24/94	7.9	14.4	-	-	-	-	-	-	-	-	9.3	3.7	ND	ND	13.0
6/16/94	10.9	-	-	-	43.9	7.3	-	-	-	31.5	4.1	-	-	-	18.3
8/1/94	4.6	24.0	0.4	0.2	29.2	10.0	9.0	0.2	1.2	20.4	14.0	4.7	0.8	1.5	21.0
8/16/94	15.3	18.7	0.3	0.1	34.4	9.5	-	-	-	-	-	-	-	-	-
9/15/94	4.7	12.9	0.2	0.2	18.0	7.0	6.5	0.9	0.2	14.6	8.9	5.4	1.7	1.4	16.4
10/13/94	8.2	17.0	0.4	0.3	25.9	8.8	6.2	1.0	0.4	16.4	8.9	8.9	0.9	0.7	18.3
10/28/94	9.0	10.7	0.4	0.2	20.3	6.6	5.6	0.9	0.1	13.2	5.5	6?	1.2	0.5	13.4
MEAN	8.7	17.0	0.6	0.8	27.1	8.1	7.2	0.8	0.7	16.8	7.8	6.3	0.9	0.8	15.8

Table III.A.1 Inlet Dissolved Arsenic Species Concentrations

APPENDIX III.B Lake Surface and Inlet Dissolved and Particulate Arsenic Arsenic, Iron, and Manganese Concentrations

DATE	UFB DISS.	UFB PART.	MAIN DISS.	MAIN PART.	LML DISS.	LML PART.	SURFACE DISS.	SURFACE PART.
12/14/93	32.4	8.4	-	-	-	-	11.1	3.1
9/15/94	18.0	16.8	14.6	5.6	16.4	1.7	-	-
10/13/94	25.9	33.0	16.4	2.0	18.3	-1.2	-	-
10/28/94	20.3	11.6	13.2	5.1	13.4	3.8	13.7	2.6
MEAN	24.2	17.5	14.7	4.2	16.0	1.4	12.4	2.9

Table III.B.1 Dissolved and Particulate Arsenic

Note: All concentrations in nM

DATE	UFB DISS.	UFB TOTAL	MAIN DISS.	MAIN TOTAL	LML DISS.	LML TOTAL	SURFACE DISS.	SURFACE TOTAL
12/14/93	0.28/0.11	0.97/0.14	-	-	-	-	0.01/0.11	0.28/0.20
9/15/94	-	0.60/0.24	-	0.21/0.13	-	0.02/0.03	-	-
10/13/94	-	0.73/0.24	-	0.09/0.03	-	0.12/0.03	-	-
10/28/94	-	0.49/0.18	-	0.05/0.04	-	0.01/0.02	-	ND/0.02
MEAN	0.28/0.11	0.70/0.20	-	0.12/0.07	-	0.05/0.03	0.01/0.11	0.14/0.11

Table III.B.2 Lake Surface and Inlet Total and Dissolved Iron/Manganese Concentrations

Note: All concentrations in mg/L
ND = none detected

APPENDIX III.C DATA USED IN MASS BALANCE CALCULATIONS

Depth (m)	[As III] 6/16/94	[As III] 7/11/94
0	-	17.2
1	7.1	-
2	7.2	16.5
3	8.0	-
4	4.9	16.3
5	3.6	-
6	2.4	5.8
7	1.0	-
8	1.3	2.5
9	0.2	-
10	0.7	2.2
11	1.4	-
12	1.7	2.2
13	1.4	-
14	1.9	3.1
15	2.1	-
16	1.8	2.2
17	1.3	-
18	1.9	3.3
19	2.1	-
20	2.2	3.2
21	2.8	-
22	1.6	4.1
23	2.7	-

Table III.C-1 Upper Mystic Lake Arsenite Concentration (nM) Depth Profiles

Depth Interval (m)	Interval Area (10^5 m 2)	Interval Volume (10^6 m 3)	6/16/94 [As III] (nM)	6/16/94 As III (moles)	7/11/94 [AS III] (nM)	7/11/94 As III (moles)
0-3	5.83	1.53	7.28	11.14	16.73	25.60
3-6	4.19	1.20	4.57	5.48	12.80	15.36
6-9	3.67	1.04	1.20	1.25	3.60	3.74
9-12	3.19	0.90	1.02	0.92	2.20	1.98
12-15	2.70	0.73	1.73	1.27	2.80	2.04
15-18	2.06	0.56	1.70	0.95	2.57	1.44
18-21	1.62	0.43	2.22	0.95	3.23	1.39
21-24	1.20	0.28	2.35	0.66	4.10	1.15

Table III.C-2 Integrated Arsenite (moles)

APPENDIX III.D TEMPORAL TRENDS IN SURFACE WATER PARAMETERS

DATE	[As III]	[As V]	MMA	DMA	TOTAL
4/15/93	2.1	1.8	3.4	1.6	8.9
5/25/93	9.5	3.1	0.2	2.2	15.0
6/21/93	16.0	0.4	0.6	0.7	17.7
7/20/93	8.6	5.5	1.5	1.6	17.2
8/28/93	6.9	8.4	1.4	2.7	19.4
9/9/93	7.2	11.0	2.0	6.4	26.6
10/5/93	3.0	15.0	1.6	0.2	19.8
11/2/93	1.7	5.1	1.6	ND	8.4
12/14/93	1.7	7.5	1.5	0.4	11.1
4/5/94	7.2	14.0	ND	1.0	22.2
4/24/94	5.9	6.3	0.6	0.6	13.4
4/28/94	6.0	-	-	-	-
6/16/94	7.1	3.0	0.6	1.6	12.3
7/11/94	17.2	2.3	0.7	0.3	20.5
7/20/94	15.0	2.2	0.5	0.2	17.9
7/25/94	17.0	-	-	-	-
8/1/94	13.0	3.0	0.6	0.6	17.2
8/9/94	16.8	-	-	-	-
8/16/94	11.9	4.6	0.6	0.2	17.3
8/25/94	9.9	5.3	1.4	0.7	17.3
9/1/94	10.5	3.9	1.3	1.4	17.1
9/6/94	9.1	13.0	1.6	1.1	24.8
9/9/94	11.5	6.5	1.2	0.1	19.3
9/13/94	9.1	-	-	-	-
9/15/94	9.3	9.6	1.7	0.5	21.1
10/6/94	7.9	6.8	0.8	0.7	16.2
10/13/94	8.2	6.3	0.9	0.5	15.9
10/28/94	7.1	5.3	1.1	0.3	13.8
11/3/94	8.4	-	-	-	-
11/22/94	10.2	-	-	-	-
12/3/94	6.8	6.4	0.5	ND	13.7

Table III.D-1 Surface Water Arsenic Speciation

DATE	Chlorophyll a (mg/L)	Phosphorous (μ g/L)	Bacteria (10^6 cells/ml)
4/11/94	-	101.6	-
6/3/94	-	52.5	-
6/7/94	18.7	-	-
6/16/94	4.0	11.4	1.8
7/11/94	-	-	2.8
7/12/94	4.4	13.0	-
7/19/94	5.1	20.9	-
7/22/94	-	-	4.3
7/25/94	7.9	19.4	-
7/29/94	10.2	-	8.6
8/1/94	11.5	15.1	4.4
8/9/94	9.3	26.2	4.1
8/16/94	8.2	31.4	5.1
8/25/94	10.7	27.8	-
9/1/94	11.2	38.8	3.6
9/6/94	8.0	28.8	-
9/9/94	-	-	2.8
9/13/94	8.8	29.7	-
10/6/94	15.9	23.5	3.8
10/13/94	9.3	12.5	-
10/28/94	15.3	14.0	-
11/1/94	-	-	3.2
11/6/94	-	-	3.0
11/22/94	15.3	-	-
12/3/94	15.0	20.0	3.1

Table III.D-2 Chlorophyll, Total Phosphorous, and Bacteria

MONTH	As III (nM)	As V (nM)	As III/ V ¹⁾	BACTERIA (10 ⁶ cells/ml)	PHOSPHOROUS (ug/L)
April	6.4	10.2	0.7	-	101.6
June	7.1	8.8	0.8	1.8	32.0
July	16.4	2.3	7.2	5.2	17.8
August	14.9	4.3	2.7	4.5	22.6
September	9.9	8.3	1.2	3.2	32.4
October	7.7	6.1	1.3	3.8	16.7
November	8.4	-	-	3.1	-
December	6.8	6.4	1.1	3.1	20.0

Table III.D-3 1994 Monthly Averages⁽¹⁾: As III, As V, As III/V ratio, Bacteria, Total Phosphorous

Notes:

- 1) All monthly means are calculated from data in Tables III.D-1 and III.D-2.
- 2) The As III/V ratio is the monthly average of As III/V ratios calculated only from samples for which both species were analyzed, and therefore is not equivalent to the ratio of the monthly average As III and As V concentrations, which may include results from samples only analyzed for one species.

APPENDIX III.E INCUBATION EXPERIMENTS

Note: "TIME (HRS)" refers to experiment duration
 "CONTROL" refers to sample with added arsenate, but no additional treatment.

DATE	[As V] (nM)	As III (nM/hr)	V _{max} (nM/hr)
9/30/93	53	1.2	10.
	267	4.0	
	533	8.1	
	800	8.5	
	2667	9.0	
11/2/93	53	1.38	8.4
	267	4.8	
	533	5.38	
	800	6.38	
	1067	7.13	
4/28/94	267	8.13	11.
	533	9.26	
5/21/94	133	11.50	14.
	533	13.10	
6/30/94	67	12.29	18.
	133	13.64	
	533	16.38	
	1067	17.38	
7/12/94	53	11.6	22.
	533	18.5	
	2667	22.7	
	5333	21.4	
8/1/94	53	4.8	19.
	267	8.3	
	533	15.3	

DATE	[As V] (nM)	As III (nM/hr)	V _{max} (nM/hr)
	1067	15.3	
11/3/94	88	3.4	11.
	178	4.85	
	267	7.2	
	533	9.7	
	888	10.5	
	1067	10.1	
	1778	9.55	
	2667	9.95	
12/20/94	0	0.22	18.
	5.3	0.71	
	10.6	0.72	
	26.67	1.17	
	53.3	1.12	
	267	10.42	
	533	11.85	
	1067	12.8	
	1600	15.95	

Table III.E-1 Effect of As V concentration on reduction rates.

Note: V_{max} estimated with a curve fit to data following Michaelis-Menten Kinetics,

$$V = V_{\text{max}} * [\text{As V}] / ([\text{As V}] * K_m)$$

where V = reduction rate (nM/hr)
 V_{max} = maximum rate of reduction (nM/hr)
 [As V] = arsenate spiking concentration
 K_m = half saturation constant (nM)

DATE	TIME (hrs)	CONTROL (nM/hr)	DCMU (nM/hr)	AMPI- CILLIN (nM/hr)	CHLORA- AMPHEN. (nM/hr)	TETRA- CYCLINE (nM/hr)	CHLOR. & TETRA. (nM/hr)
7/27/93	49	1.9	1.4	2.9	-	-	-
11/2/93	43	6.4	7.1	4.9	-	-	-
12/14/93	17	4.8	6.1	3.1	-	-	-
12/14/93	43	2.9	10.6	1.2	-	-	-
4/8/94	20	20.1	17.6	-	-	-	1.3
4/25/94	22	14.2	13.1	-	-	-	0.7
8/3/94	30	21.4	11.9	10.9	12.3	11.3	11.9
8/27/94	26	5.9	8.2	3.3	-	-	1.9
9/1/94	7	7.4	7.5	7.9	-	-	5.6
9/13/94	19	4.5	-	-	-	-	2.2
11/3/94	8	10.1	-	-	9.7	5.3	6.1

Table III.E-2 Summary of inhibitor experiments

DCMU CONC. (ppm)	REDUCTION RATE (nM/hr)	CHLOR. & TETRA. CONC. (ppm)	REDUCTION RATE (nM/hr)
0	14.2	0	14.2
10	13.1	3	3.6
20	17.6	6	1.3
40	14.1	12	1.6
-	-	30	0.7
-	-	60	0.4

Table III.E-3 Dependence of reduction rate on DCMU and antibiotic (chloramphenicol and tetracycline) concentration (4/25/94).

DATE	TIME (hrs)	CONTROL (nM/hr)	<5.0 μm (nM/hr)	<3.0 μm (nM/hr)	<1.0 μm (nM/hr)	<0.8 μm (nM/hr)	<0.2 μm (nM/hr)
6/29/93	51	4.9	-	2.9	3.8	-	0.4
7/27/93	49	1.9	-	0.5	1.7	-	3.6
9/9/93	65	4.0	-	0.5	1.5	-	5.9
11/2/93	46	6.4	-	5.2	7.6	-	-
12/14/93	19	4.8	-	4.3	2.9	-	-
7/28/94	6	16.0	7.6	6.7	4.5	0.7	0.2
8/1/94	8	15.3	11.4	8.7	7.3	0.5	0.3
9/9/94	5	4.8	1.8	1.1	0.4	0.2	0.0
9/13/94	20	2.3	1.8	1.3	1.8	0.5	0.2

Table III.E-4 Summary of Filtration Experiments

	TIME (hrs)	CONTROL (nM/hr)	<5.0 μm (nM/hr)	<3.0 μm (nM/hr)	<1.0 μm (nM/hr)	<0.8 μm (nM/hr)	<0.2 μm (nM/hr)
CONTROL	20	4.5	2.7	1.6	1.9	0.5	0.5
ANTIBIOT.	20	2.2	0.9	0.3	0.1	0.0	0.3

Table III.E-5 Filtration experiment (9/13/94) with antibiotic (chloramphenicol and tetracycline) addition.

DATE	TIME (hrs)	HIGH LIGHT (nM/hr)	NO LIGHT (nM/hr)
7/20/93	72	1.0	0.3
7/27/93	49	1.9	0.4
8/28/93	43	2.2	2.4
8/28/93	43	2.4	2.0
8/28/93	43	2.7	2.0
12/14/93	19	4.8	6.2
12/14/93	19	4.8	5.0
4/8/94	21	21.	15.
8/3/94	7	19.	10.
8/27/94	28	7.2	5.7

Table III.E-6 The response of reduction rate to light levels.

DATE	TIME (hrs)	CONTROL (nM/hr)	SHAKEN (nM/hr)
8/3/94	7	14.	21.
8/27/94	28	4.5	5.9

Table III.E-7 The response of reduction rates to sample agitation.

DATE	TIME (hrs)	20° C (nM/hr)	5° C (nM/hr)
12/4/94	14	21.1	2.1
12/4/94	14	22.9	2.4

Table III.E-8 The response of reduction rate to incubation temperature.

DATE	TIME (hrs)	CONTROL (nM/hr)	MICROWAVED (nM/hr)
6/29/93	51	4.9	-0.3
7/20/93	72	1.0	0.1
7/27/93	49	1.9	0.1
9/9/93	64	3.9	0.2
9/9/93	64	4.1	0.3
12/14/93	17	4.8	0.1
11/3/94	8	10.	0.0
11/3/94	8	10.	-0.2

Table III.E-9 Comparison of microwaved samples with controls.

533 nM (dup. 1)		533 nM (dup. 2)	
TIME (hrs)	[As III] (nM)	TIME (hrs)	[As III] (nM)
0	1.7	0	1.7
16.58	89.	17.00	80.
40.00	270	40.33	260
46.50	250	46.67	220
66.33	100	66.58	89.

Table III.E-10 Time series (11/2/93)

533 nM SPIKE (DUP. 1)		533 nM SPIKE (DUP. 2)	
TIME (hrs)	[As III] (nM)	TIME (hrs)	[As III] (nM)
0.0	1.7	0.0	1.7
16.0	73.	17.	84.
37.5	120	42.0	120
64.0	47.	64.0	62.

Table III.E-11 Time series (12/14/93).

533 nM SPIKE		267 nM SPIKE	
TIME (hrs)	[As III] (nM)	TIME (hrs)	[As III] (nM)
0.00	6.0	0.00	6.0
0.63	11.	0.40	8.5
1.55	12.	0.87	12.
2.45	28.	1.73	21.
3.28	43.	2.65	24.
4.12	36.	3.48	25.
4.75	45.	4.32	34.
5.13	49.	4.93	44.
6.47	48.	5.30	43.
22.03	200	6.67	52.
25.27	240	24.55	180
25.82	240	25.50	230
46.51	70.	46.18	130

Table III.E-12 Time series (4/28/94) .

533 nM SPIKE		133 nM SPIKE	
TIME (hrs)	[As III] (nM)	TIME (hrs)	[As III] (nM)
0.00	11.	0.00	11.
1.60	28.	1.23	22.
2.35	41.	1.98	31.
3.10	52.	2.72	41.
3.85	56.	3.47	49.
4.60	72.	4.22	58.

Table III.E-13 Time series (5/21/94) .

1067 nM SPIKE		533 nM SPIKE		133 nM SPIKE		67 nM SPIKE	
TIME (hrs)	[As III] (nM)						
0.83	38.	0.72	35.	0.58	28.	0.46	20.
1.45	53.	1.33	59.	1.08	33.	0.97	28.
2.08	64.	1.95	54.	1.83	52.	1.70	45.
2.58	75.	2.45	63.	2.95	68.	2.20	48.
3.20	84.	3.07	79.	5.33	93.	2.83	54.

Table III.E-14 Time series (6/30/94).

DATE	SPIKE CONC. (nM)	REDUCTION RATE (nM/hr)	RATE CONSTANT (hr ⁻¹)
4/28/94	267	8.1	0.03
5/21/94	133	10.	0.08
6/30/94	67	17.	0.51
7/12/94	53	12.	0.30
8/1/94	53	4.8	0.14
11/3/94	88	3.4	0.06

Table III.E-15 Seasonal variations in first-order reduction rate constants.

88 nM	267 nM	533 nM	1067 nM	1778 nM	MICROWAVED (1067 nM)
4.6	6.6	7.9	9.7	9.4	1.5

Table III.E-16 Reduction rates for bacterial culture.