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Adaptive behavior of euryhaline phytoplankton communities to arsenic stress

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ABSTRACT: Long-term experiments performed with large-volume continuous cultures of natural phytoplankton assemblages exposed to low levels of arsenate (1 to 10 × ambient concentrations) have shown that arsenate is differentially inhibiting to some phytoplankton species. This observed variance in sensitivity is sufficient to cause a marked change in species composition and succession of dominant species in arsenic-treated assemblages with a potential for impact upon carbon transfer between trophic levels, even though estimates of community biomass and productivity indicate no change.

INTRODUCTION

Arsenic is ubiquitous in nature, being present in water and soils and concentrated in a variety of ores. It is quite toxic to organisms. Presently, anthropogenic emissions of arsenic greatly exceed natural emissions (Ferguson & Gavis 1972, Mackenzie et al. 1979). Present and future increases have the potential to alter biological processes in marine systems.

Arsenic in oxidized aquatic systems is present primarily as an inorganic ion, arsenate (Andreae 1978, Waslenchuk 1978, Sanders 1980). Arsenate is chemically similar to phosphate and is taken up indiscriminately by phytoplankton (Sanders & Windom 1980). Once inside the cell, oxidative phosphorylation and growth are inhibited (Planas & Healey 1978, Sanders 1979). All species of phytoplankton are not equally sensitive to arsenate, however (Sanders & Vermersch 1982). Within the phytoplankton community, where rapid growth and species succession are normal occurrences, the impact of low levels of arsenate may be a shift in the dominant algal species toward arsenic-resistant forms, and not an overall decrease in primary productivity. These postulated shifts in species composition could have profound impacts upon the biota of the entire ecosystem.

In general, pollution-induced shifts in species dominance are toward smaller species, microflagellates, and/or pennate diatoms. These changes may result in an abundance of small grazers that can effectively feed on flagellates (tintinnids, rotifers) and a concomitant decrease in larger zooplankton (copepods) which cannot capture small cells efficiently (Parsons et al. 1969, Richman et al. 1977, Ryther & Sanders 1980). If this shift is extrapolated to the next trophic level, a possible prediction is that the same aquatic system will support a lower density of harvestable fish (Ryther 1969, Parsons 1976, Landry 1977, Steele & Frost 1977).

In order to evaluate changes in phytoplankton community structure due to anthropogenic stress, it is necessary to investigate the impact of trace substances upon phytoplankton assemblages under conditions that resemble a natural ecosystem as closely as possible and at levels of stress that are consistent with real-world concentrations. It is our contention that this type of research is necessary before we can make predictions as to fate and effect of chronic inputs of trace substances into an ecosystem. We have been working with this type of approach for several years and have developed a large-volume continuous culture system which is appropriate for this type of research (Sanders et al. 1981, Sanders & Vermersch 1982). We report here the results of several experiments designed to evaluate the impact of chronic doses of low concentrations of arsenate on estuarine phytoplankton.

METHODS

Natural phytoplankton from the Patuxent River (a sub-estuary of Chesapeake Bay, USA) were cultured in cylindrical fiberglass tanks 76 cm in diameter and 112 cm in height, containing a volume of approximately 500 l. The tanks are located outdoors, sub-

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merged in a raceway through which running water is circulated to control and maintain the temperature of the water within the tanks to within 1 C° of the ambient water temperature. The concept and objective was to operate the tanks as continuous, flow-through phytoplankton cultures using the mesohaline river water without nutrient enrichment as the diluent. The tanks were initially filled with raw water containing the natural phytoplankton assemblage from the Patuxent River (after the winter experiment, this water was passed through 35 µm nylon mesh to remove high densities of herbivores). After an initial period of stabilization (1 to 2 d), river water filtered to 1 µm was pumped to each tank at a nominal dilution rate of 50 % per day. A 2 d residence time was determined to be optimal for maintenance of proper cell densities and nutrient concentrations. Because assemblages were maintained as flow-through cultures, no adverse chemical changes associated with photosynthesis and respiration (low O_2, high NH_4^+ concentrations, wide variations in pH) occurred within the tanks.

Once filled, the tanks are immediately sampled and the phytoplankton assemblage enumerated. Once we determined that the assemblages within the tanks replicate within an acceptable limit of error using the measurements of species composition and biomass discussed below, daily dosing of tanks with arsenate began. Concentrated solutions of arsenate (added as the salt, Na_2HAsO_4) were added daily to each treatment tank in amounts sufficient to replace the arsenic lost during the previous 24 h by dilution. Initially, arsenate was pumped continuously into each tank, but numerous pump and line failures led us to daily dosing. This method of dosing meant that the actual concentration of arsenic to which the phytoplankton were exposed varied from approximately 0.5 to 1 times expected doses.

Arsenic was added to triplicate sets of culture tanks at 4 different levels: control, no arsenate added; low, 67 nM added; medium, 134 nM added; and high, 200 nM added. Background arsenic concentrations within the river varied between 9.5 and 23 nM during 1982, averaging 15 nM. The arsenic levels chosen are within the range of natural and moderately polluted river/estuarine systems (Andreae 1978, Andreae et al. 1983, Langston 1983).

Daily water samples were taken from each tank for phytoplankton enumeration. Of these, initially 3 wk^{-1} were examined; the remainder were archived for later examination if required. Samples were preserved with Utermohl's solution and the phytoplankton counted and identified to species using sedimentation technique (Utermöhl 1958). Similarity between morphological taxa and dominant species in the triplicate control and dosed assemblages was determined using 2-way analysis of variance. Assemblages were considered to be significantly different at a level of significance $p < 0.05$.

Samples were collected twice weekly from each tank for analysis of nitrate, nitrite, ammonium, phosphate, silicate, and particulate carbon and nitrogen.

Three experiments were performed, one each in winter, spring, and summer. Pump failures (freezing) during the winter experiment led to unnaturally high concentrations of arsenate in the cultures and subsequent inhibition of algal growth. The second and third experiments were performed as planned and are discussed below. Parameters for each experiment are detailed in Table 1.

RESULTS

Spring experiment

This experiment was conducted from April 29 to June 1. Water temperatures rose continually throughout the experiment, starting at 16.9 °C and ending at 24.6 °C. Dilution rate averaged 45.2 ± 4.5 % turnover per day.

Anticipated arsenic doses were 0 (control), 67 nM, 134 nM, and 200 nM, each in triplicate. Actual arsenic analyses (Table 2) indicate that doses to the lowest treatment, nominally 67 nM, were somewhat higher, averaging 96 nM. Other doses were close to expected values (Table 2). Background arsenic concentrations averaged 14 nM, with a range of 9.5 to 18 nM. The speciation, or chemical form, of arsenic did not change greatly during the experiment; arsenic was added as arsenate, and the majority remained in this form. Small amounts of arsenite and methylated arsenicals were present during the entire experiment.

Nutrient concentrations within the tanks were not

Table 1. Environmental and test parameters for each experiment

Season	Duration (d)	Dilution rate (% d^{-1} ± SD)	Arsenate doses (nM added)	Ambient temperatures (°C) Initial	Final
Spring	34	45 ± 4.5	67,134,200	16.9	24.6
Summer	23	48 ± 3.1	67,200	26.0	23.5

Table 2. Arsenic concentrations (nM) in spring and summer experiments in control and dosed tanks. Values are averages (± SD) for the duration of each experiment for triplicate tanks

Experiment	Treatment	Total As	Anticipated As dose	Measured As dose
Spring experiment	Control	14 ± 3.5	–	–
	Low	110 ± 14	67	96
	Medium	147 ± 19	134	133
	High	203 ± 23	200	189
Summer experiment	Control	17 ± 9.6	–	
	Low	99 ± 21	67	82
	High	204 ± 33	200	187

Table 3. Nutrient concentrations (μM) for the Patuxent River and experimental culture tanks. If unspecified, tank and river data were not significantly different, and are pooled (see text). Averages (± SD) are pooled data from the entire experiment for triplicate tanks

Experiment	Constituent	Average concentration
Spring experiment	Phosphate (culture tanks)	0.15 ± 0.02
	Phosphate (river)	0.74 ± 0.44
	Nitrate	2.6 ± 0.8
	Nitrite	0.15 ± 0.03
	Ammonium	4.9 ± 0.9
	Silicate	31 ±2.6
Summer experiment	Phosphate (culture tanks)	0.45 ± 0.08
	Phosphate (river)	2.2 ± 0.6
	Nitrate	3.4 ± 1.1
	Nitrite	0.69 ± 0.24
	Ammonium	6.3 ± 1.1
	Silicate (culture tanks)	52 ± 2.3
	Silicate (river)	73 ± 1.4

significantly different from one another, and with the exception of phosphate, were not significantly different from the Patuxent River source water. For this reason, with the exception of phosphate, the nutrient data are pooled in Table 3. Phosphate concentrations were very low. However, phytoplankton growth rates within the tanks remained remarkably constant for natural populations during spring months and cell densities, which ranged from 0.6 to 5.4 × 10^7 cells l^{-1} and averaged 1.9 × 10^7 cells l^{-1}, were comparable to river densities.

No reduction in biomass because of arsenic dosing occurred during this experiment. Total cell densities were unaffected by dosing and were essentially similar in all tanks. Particulate carbon concentrations and atomic C:N ratios were depressed slightly but not significantly during the latter half of the experiment in arsenic-treated tanks.

Species succession within the tanks varied considerably because of arsenic treatment; however, all arsenic treatments reacted similarly. There was no added effect as arsenic concentrations increased. While morphological groups such as pennate diatoms, centric diatoms, or dinoflagellates, did not show significant shifts due to arsenic treatment, a number of dominant species did exhibit significant differences in growth and dominance in control and treated tanks. This shift was probably most apparent in succession of centric diatoms. *Cerataulina pelagica* dominated assemblages for the entire experiment in control tanks, comprising an average of 15 to 40 % of total cell density. In all arsenic-treated tanks, regardless of dose, *C. pelagica* declined to insignificant densities within 5 days of the start of arsenic treatment (Fig. 1). Conversely, *Thalassiosira pseudonana* dominated arsenic-treated tanks to a much greater extent than control tanks, reaching densities approaching 50 % of total cells in treated assemblages *vs.* approximately 5 to 10 % of total cell

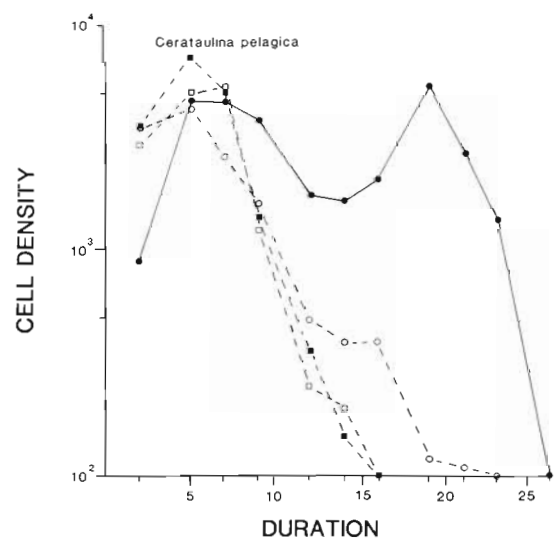

Fig. 1. Changes in density (cells ml^{-1}) over time (d) of the dominant species *Cerataulina pelagica* during spring experiment in control (——) and As-treated (– – –) large-volume cultures. Arsenate doses were: 67 nM (○), 134 nM (□), and 200 nM (■)

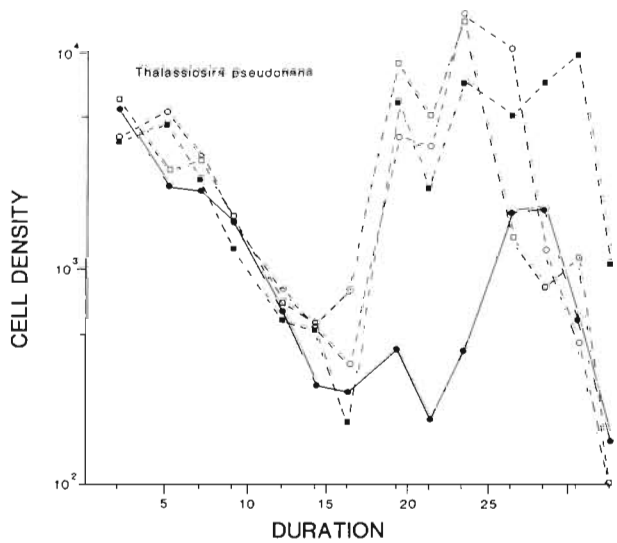

Fig. 2. Changes in density (cells ml^{-1}) over time (d) of the dominant species *Thalassiosira pseudonana* during spring experiment in control (——) and As-treated (– – –) large-volume cultures. Symbols as in Fig. 1

density in control assemblages (Fig. 2). The shift from *C. pelagica* to *T. pseudonana* is contrasted in control and treated assemblages in Fig. 3.

Some flagellates were sensitive to arsenic. Microflagellates as a whole were consistently (but not significantly) less abundant in treated tanks. *Pyramimonas* sp., a green flagellate, was significantly less abundant in treated tanks (Fig. 4), but was not a dominant species in either control or dosed tanks.

Summer experiment

This experiment was conducted from August 17 to September 9. Water temperatures remained relatively constant during the experiment, averaging 24.1 °C (range: 21.0 to 27.0 °C). Dilution rate averaged 48.4 ± 3.1 %.

Two arsenic doses (plus controls) were maintained in triplicate: 67 and 200 nM. Actual arsenic doses (Table 2) were similar to predicted values, with the low dose somewhat higher and the high dose somewhat lower than expected. Background arsenic concentrations averaged 17 nM, with a range of 9.5 to 23 nM.

Nutrient concentrations within the tanks did not vary significantly. With the exception of phosphate and silicate, Patuxent River concentrations were also similar (Table 3). Both phosphate and silicate were depleted within the culture tanks relative to river concentrations because of very high algal growth rates during this period. Cell densities varied between 1 and 5×10^7 cells l^{-1} during most of the experiment, with diatoms comprising 20 to 80 % of total cells.

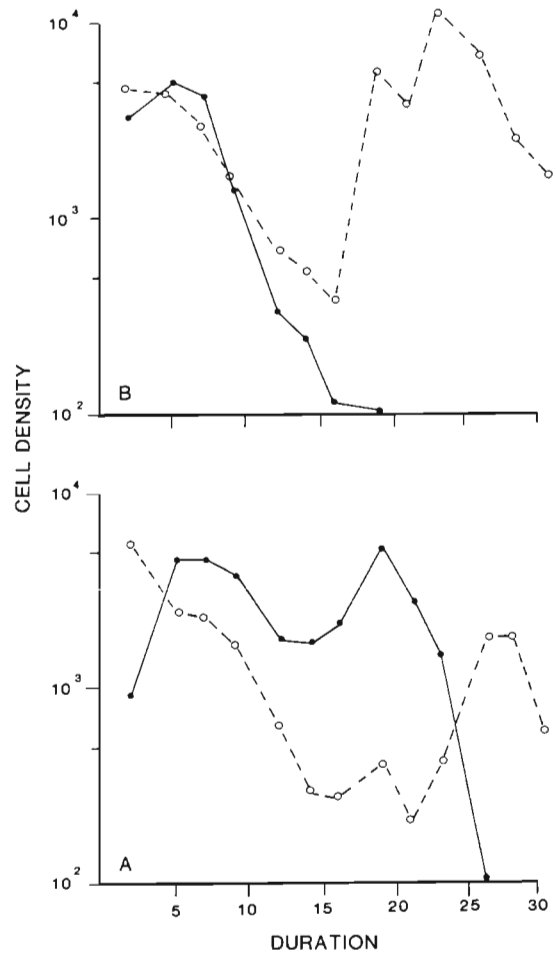

Fig. 3. Succession of *Cerataulina pelagica* and *Thalassiosira pseudonana* in spring experiment, in control (A) and As-treated (B) assemblages. Arsenic treatments were not significantly different (see text) so results are pooled here. Densities in cells ml^{-1}, duration in days. ● *C. pelagica*; ○ *T. pseudonana*

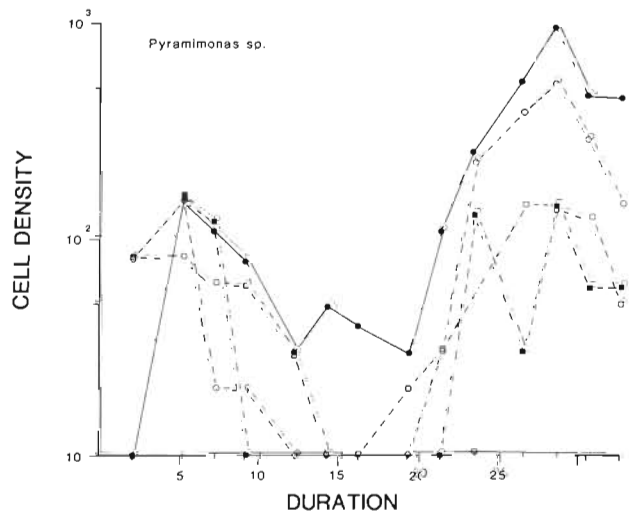

Fig. 4. Changes in density (cells ml^{-1}) over time (d) of *Pyramimonas* sp. during spring experiment in control (——) and As-treated (– – –) large-volume cultures. Symbols as in Fig. 1

Biomass estimates were not significantly affected by arsenic doses. Total cell densities were essentially similar in all treatments and particulate carbon concentrations were not significantly different. C:N ratios were depressed somewhat in the latter part of the experiment, but were not significantly lower.

Species succession within the tanks varied considerably. As in the spring experiment, both high and low arsenic doses caused similar changes, there was no additive effect of higher arsenic concentrations. Again, the shifts were most apparent in the diatom assemblages.

Two species of centric diatoms showed significant declines in growth rate and success resulting from arsenic dosing. *Chaetoceros subtile* was consistently less abundant in dosed tanks after dosing began; abundances in treated tanks were too low to count on several occasions (Fig. 5). Another species, *Rhizosolenia fragilissima*, bloomed dramatically in control tanks and the Patuxent River about mid-way through the experiment, but was unable to succeed in dosed tanks (Fig. 6).

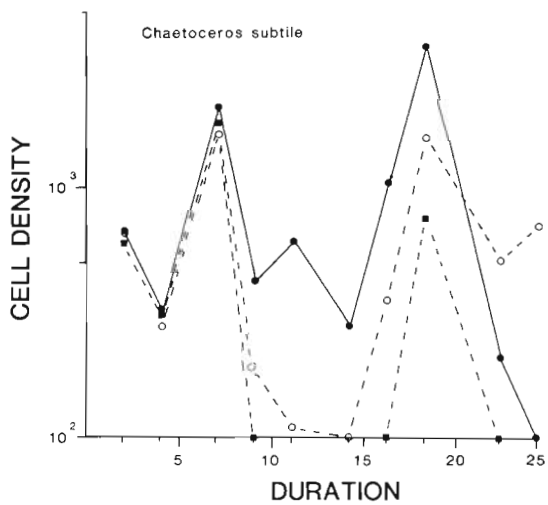

Fig. 5. Changes in density (cells ml^{-1}) over time (d) of *Chaetoceros subtile* during summer experiment in control (——) and As-treated (– – –) large-volume cultures. Symbols as in Fig. 1

As in the spring experiment, a species of *Thalassiosira, T. decipiens,* grew consistently better in treated tanks than in controls. Its success was not as dramatic as seen in the earlier experiment and did not match the decline due to sensitivity of *Chaetoceros subtile* and *Rhizosolenia fragilissima*. Therefore, centric diatoms as a group declined through time in treated tanks.

Flagellates as a group were not significantly affected by arsenic in this experiment. One small cryptophyte, *Chroomonas* sp., was significantly depressed by arsenic treatments, and grew consistently better in control assemblages (Fig. 7). However, flagellates were much less important overall in the summer experiment; as a group they comprised only 2 to 10 % of total cell densities.

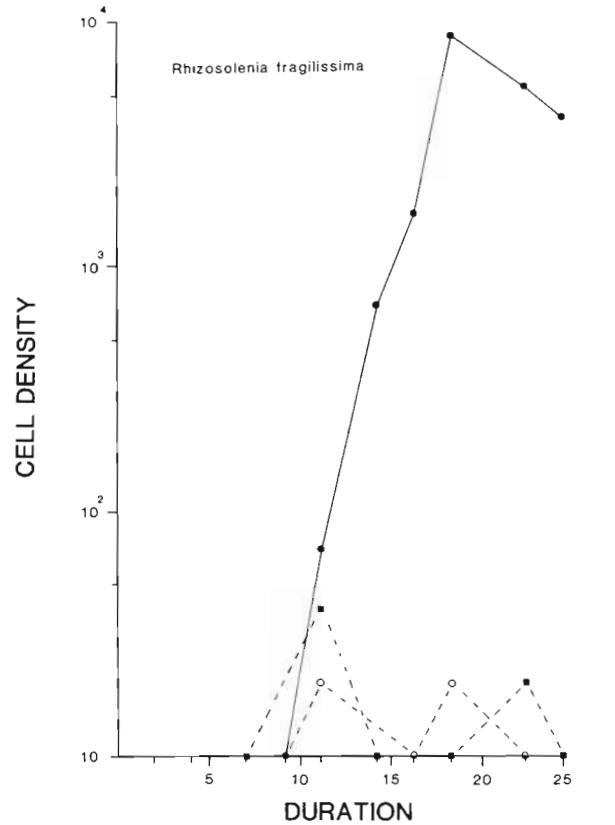

Fig. 6. Changes in density (cells ml^{-1}) over time (d) of the dominant species *Rhizosolenia fragilissima* during summer experiment in control (——) and As-treated (– – –) large-volume cultures. Symbols as in Fig. 1

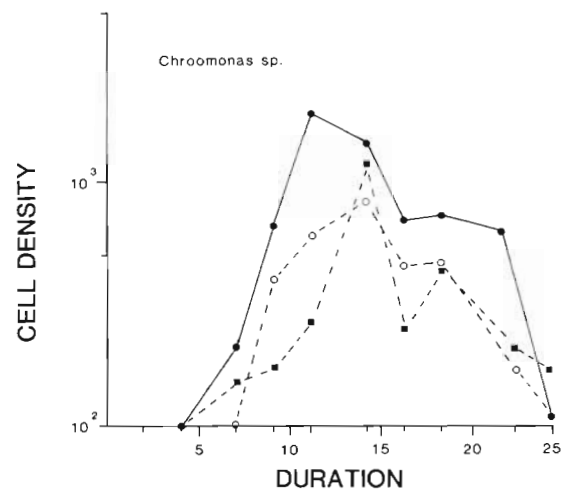

Fig. 7. Changes in density (cells ml^{-1}) over time (d) of *Chroomonas* sp. during summer experiment in control (——) and As-treated (– – –) large-volume cultures. Symbols as in Fig. 1

DISCUSSION

The response of phytoplankton species composition to arsenic was significant in both experiments. There was little, if any, effect of increased arsenate doses, however. In both instances, the lowest arsenate dose was sufficient to cause a significant deviation in growth rates of some species. Higher arsenate concentrations did not alter or increase this inhibition.

A common response to arsenic dose in these experiments was replacement of dominant algae by species that were taxonomically similar (i.e. replacement of one centric diatom by another). Although similar taxonomically, the replacement species varied greatly in size or shape from the original dominant. Consider, for example, the replacement of *Cerataulina pelagica* in the spring experiment and *Rhizosolenia fragilissima* in the summer experiment (both are 6 × 30 µm cylinders) by *Thalassiosira* species (5 to 10 µm pillboxes).

The replacement of relatively large centric diatoms by smaller species within a phytoplankton community can be of important ecological significance. The smaller species not only contain smaller quantities of C and N per cell, but also may be prey for different herbivores. Laboratory experiments with *Eurytemora affinis,* the dominant winter-spring copepod in the mesohaline region of the Chesapeake Bay, have shown drastically reduced survival and fecundity when this species is fed *Thalassiosira pseudonana* than when fed equal biomasses (as measured by C content, not cell density) of *Rhizosolenia fragilissima* (Sanders unpubl.). Therefore, this shift in algal speciation could lead to an increase in grazing by microzooplankton and perhaps altered trophic structure within the estuary. We are beginning a series of experiments designed to test the significance of such algal shifts on higher trophic levels.

Diatoms appeared either to be 'turned off' or 'turned on' in arsenic-dosed cultures. The decline of *Cerataulina pelagica* in treated assemblages during the spring experiment is consistent with physical washout of the species from the culture caused by no growth at all. In the summer experiment, *Rhizosolenia fragilissima* was unable to grow and remained at insignificant cell densities in dosed tanks, even though it was able to bloom in control tanks. Conversely, *Thalassiosira pseudonana* increased its growth rate nearly 5-fold in arsenic-dosed cultures relative to controls in the spring experiment, and *Thalassiosira decipiens* grew nearly 30 % faster in treated assemblages than in controls in the summer experiment. The *T. pseudonana* bloom began after *C. pelagica* had virtually disappeared from dosed tanks, suggesting a response to increased light or nutrient availability (Fig. 3). This type of indirect coupling between sensitive and resistant species is difficult to document in laboratory-based experiments, but is quite apparent in multispecies assemblages such as these.

Flagellates exhibited a somewhat different response. Flagellates that were affected by arsenate did not exhibit the abrupt declines in population densities seen in affected diatoms; rather the natural dips and surges in population abundance were mirrored in both control and dosed populations (e.g. Fig. 4 & 7). Thus, growth rates of these species were depressed somewhat but the species continued to react to environmental cues such as changing light intensities or nutrient concentrations.

Some algal species are very efficient at transforming arsenate after incorporation and releasing it in altered form; others are not (Andreae 1978, Andreae & Klumpp 1979, Sanders 1979, Sanders & Windom 1980, Nissen & Benson 1982). Indeed, unusual chemical forms of arsenic in natural systems have been correlated with single, dominant species of algae (Sanders 1983, 1985 and unpubl.). Because these transformations require energy, cell growth may be inhibited somewhat, but these efficient species can continue to exist within the community at lowered cell densities. The flagellate response seen in these experiments may be due to this process. *Chroomonas* sp., for example, was able to persist, at lowered cell densities, in arsenic-treated tanks in the summer experiment (Fig. 7). Interestingly, the occurrence of *Chroomonas* sp. as a dominant species in Chesapeake Bay during summer months is positively correlated with the appearance of an unusual chemical form of arsenic, methylarsonate (Sanders 1985 and unpubl.).

Responses of natural phytoplankton assemblages to low levels of trace substances are not apparent through examination of crude parameters such as carbon or chlorophyll *a* concentrations, primary production, or cell densities, but require a careful examination of the succession of dominant species. Such a succession can have significant impact on other trophic levels within the estuary or coastal ocean; the magnitude of this impact must still be investigated.

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