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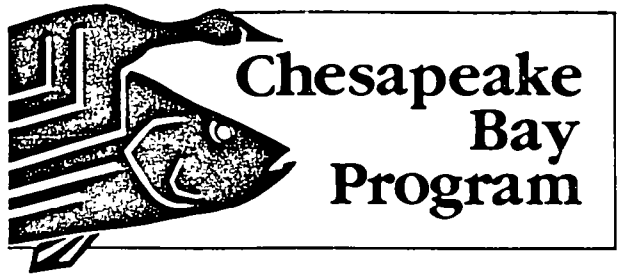
Arsenic Transport and Impact in Chesapeake Bay Food Webs



ATTACHMENT A

June, 1987

ARSENIC IMPACT ON GROWTH, FECUNDITY, SPECIES COMPOSITION AND SUBSEQUENT TRANSPORT OF ARSENIC IN ESTUARINE FOOD WEBS

by

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ABSTRACT

Estuarine organisms respond differently when exposed to dissolved arsenic. Phytoplankton, particularly centric diatoms, exhibit large changes in growth rate of dominant species, leading to changes in the species composition of the phytoplankton community. Zooplankton and benthic organisms, on the other hand, were quite tolerant of high concentrations of arsenic. Only the barnacle, *Balanus improvisus*, was significantly affected by concentrations of arsenate as high as 56 $\mu g \cdot l^{-1}$. *Balanus* was also the only organism to incorporate significant concentrations of arsenic. However, this incorporation may have been in shell material and not in animal tissue. Other organisms did not incorporate significant quantitites of arsenic. The concentrations of arsenic used in these experiments, 1-56 $\mu g \cdot l^{-1}$, effectively bracket concentrations in estuaries, even those receiving considerable impact from man. Thus, the lack of direct response to arsenic seen in these experiments, except within the phytoplankton community, can be considered to be relevant to arsenic impacts to estuarine and coastal marine systems.

However, there are other pathways for impact of a toxic substance within an aquatic food web. Arsenic, because of its large impact upon phytoplankton species composition and community structure, is a prime candidate for such indirect impacts. Even though direct effects of arsenic are limited to phytoplankton and direct impacts to other trophic levels are minor, potential exists for indirect effects associated with changes in trophic structure or the ingestion of arsenic through food. Our experiments have shown that changes in dominant species can drastically alter an herbivore's ability to procure enough food to successfully reproduce. In addition, although arsenic dissolved in the water may be unavailable and nontoxic to higher trophic levels, arsenic incorporated in their food may be quite toxic.

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INTRODUCTION

The pollution of an estuary is often the initial and greatest impact borne by the marine system as a whole. Estuaries serve as principal conduits for man's inputs to the oceans. Many industrial and municipal activities, such as power generating stations, wastewater treatment facilities, and industrial plants of all types, are located on estuaries. These activities generate liquid and solid wastes, some of which deliberately or accidentally are discharged into the nearby water. In addition, the concentration of boat traffic in these same waterways results in many small spills and leakages which add pollutants. Therefore, estuaries are necessary places to investigate potential impacts of toxic compounds.

Zero discharge of toxic wastes into marine and estuarine environments, although an enviable goal, may never be a realistic alternative for modern man. Even if deliberate introduction of toxic compounds does not occur, the oceans will always face the inevitable, accidental discharge of these materials. Many compounds within these discharges are of extreme interest to environmental planners because:

1. they are greatly elevated in affected waters relative to natural water concentrations,
2. they are toxic to biota, and
3. they are actively accumulated by biota in aquatic systems.

We do not know to what extent chronic or acute discharges of industrial wastes will alter aquatic ecosystems. The literature suggests that the trace materials likely to be contained within these wastes are toxic to some organisms. However, the results from these single-species bioassays are difficult to extrapolate to estuaries which have complex hydrography and food webs. Sessile organisms in the vicinity of an effluent have the greatest potential for harm, but plankton contained within the

effluent plume also should be affected. Additionally, if the compound is bioactive, uptake and transformation by organisms will greatly affect its toxicity and transfer to other organisms within the estuary.

Arsenic is an example of this type of compound. It is a classic poison, with its primary commercial use as a pesticide (Mackenzie et al., 1979). It is considered a "priority pollutant" by EPA, and was present in 48% of industrial effluents tested by the Agency (Keith & Telliard, 1979). Arsenic is present in all aquatic systems, principally in the form of an inorganic ion, arsenate (Waslenchuk, 1978; Sanders, 1980). Reduced arsenic (arsenite) is generally considered more toxic and is used as a pesticide (Peoples, 1975). However, at low levels, arsenate is likely to have the greatest impact on aquatic primary producers. It is chemically similar to phosphate, a necessary nutrient for plant growth, and it is readily taken up by phytoplankton (Sanders & Windom, 1980). Arsenate uptake can be followed by accumulation within the cell, or it can be chemically altered (reduced to arsenite and methylated) and released into the surrounding water. As much as 80% of the dissolved arsenic present in Chesapeake Bay is taken up and released in this fashion (Sanders, 1980, 1983, 1985).

Arsenic concentrations within aquatic ecosystems vary widely. Fresh waters free from anthropogenic contamination generally contain 1 to 10 $\mu g\ l^{-1}$ (Andreae, 1978; Forstner & Wittmann, 1983) with a world-wide, average concentration of 1.7 $\mu g\ l^{-1}$ (Martin & Whitfield, 1983). Oceans are much less variable, ranging between 1.0 and 1.5 $\mu g \cdot l^{-1}$ (Andreae, 1978; Waslenchuk, 1978; Sanders, 1980). Estuaries fall somewhere in between. In the Chesapeake Bay, concentrations are lowest at the head of the Bay, with concentrations less than 0.5$\mu g \cdot l^{-1}$. Average concentrations peak in the mid-Bay region around an average of 1.5-2.0 $\mu g \cdot l^{-1}$, falling slowly offshore (Sanders, 1985). Higher concentrations in the mid reaches of the Bay imply inputs from man's activities, with annual additions recently estimated at 36 metric

tons (Sanders, 1985). Occasionally, very high concentrations can be found, with the highest known concentration, 60 $\mu g \cdot l^{-1}$, being found in the Nanticoke River near the location of an abandoned fly ash pile (Sanders, unpublished data). Presumably, other "hotspots" occur.

All organisms do not react similarly to a given pollutant. Although phytoplankton are most affected by arsenate, the least toxic form, invertebrates are more susceptible to arsenite and methylated forms (Peoples, 1975). Therefore, they could be harmed by the biogeochemical alterations discussed above. Even within a particular taxon, all species will not react to the same levels of arsenic. For example, all phytoplankton species are not equally sensitive to arsenate; some are inhibited at levels just exceeding ambient concentrations of arsenate in the oceans (1.0-1.5 $\mu g \cdot l^{-1}$), while others are resistant to arsenate concentrations two orders of magnitude higher (Sanders & Vermersch, 1982). Within the phytoplankton community, where rapid growth and species succession are normal occurrences, arsenic can cause a shift in the dominant species toward resistant forms and a concurrent decrease in productivity (Sanders & Vermersch, 1982; Sanders & Cibik, 1985). Therefore, the addition of arsenic to the water column not only can have a direct toxic effect, but also can result in dramatic shifts in both the dominance within the phytoplankton community and possibly the flux of carbon and nitrogen between trophic levels. Coupled with this is the alteration of chemical form, which will affect (generally increase) arsenic toxicity to higher trophic levels.

Beyond the single trophic level, the effects of a toxic substance (i.e., arsenic) on an ecosystem such as the Chesapeake Bay are potentially quite complex. Besides the direct effects postulated above, individuals of heterotrophic species can be influenced by either changes in the abundances and sizes of prey species or the contamination of these food organisms. For example, an arsenic-induced shift in phytoplankton composition to small flagellates or small centric diatoms could reduce the ingestion

of phytoplankton by copepods and therefore their fecundity. Small cells are not captured as efficiently as larger diatoms (e.g., Nival & Nival, 1976; Bartram, 1980). Thus changes in phytoplankton size may ultimately result in an abundance of small, noncrustacean grazers (ciliates, rotifers) that can effectively feed on flagellates and a concomitant decrease in larger zooplankton. This shift, extrapolated to the next trophic level, predicts that the same aquatic system will support a lowered density of harvestable fish (see Ryther, 1969; Parsons, 1976; Landry, 1977; Hendrickson et al., 1982).

Little is known about how such primary and secondary effects of a toxin combine to change a community or ecosystem. Except for simple systems with two or three species or communities dominated by a "keystone species" (e.g., Paine, 1969; Dayton, 1975), we have no models, empirical or theoretical, which could be used to predict how (or if) changes to a single species might alter the whole system. Therefore, it is important that the contributions of the various pathways to an overall toxin-effect be determined for aquatic ecosystems.

Previous research has documented that toxic compounds, including arsenic, are present in the Chesapeake Bay (Bieri et al., 1982; Sanders, 1985, 1986; Sanders & Riedel, 1987a), perhaps in quantities large enough to adversely affect water quality, sediment quality, and the biota of the Chesapeake Bay. The annual loadings of toxic substances will increase in coming years, even though immediate control of some point sources may mitigate contamination in specific sub-estuaries. In order to implement the most effective management practices and to evaluate their success, we must be able to predict not only which compounds are likely to cause serious impacts to various biota but also how these impacts affect the dynamics of the ecosystem.

In an earlier study funded by the U.S. Environmental Protection Agency's Office of Research and Development (Sanders et al., 1987; Attachment A), we

examined the effects of arsenic on three trophic levels within a representative estuarine food web. Our purpose was to distinguish and quantify those changes in species abundance, mortality, growth rates, and reproduction that are caused directly by the dissolved arsenic and those that could be a function of trophic relationships. The three trophic levels we investigated were the phytoplankton assemblage, zooplanktonic herbivores, and benthic suspension feeders. We chose this food web because 1) trophic relationships were fairly simple and not confounded by such factors as the complex behavior of highly motile species or the biological, physical, and chemical variability found in sedimentary environments, 2) all species could be easily manipulated for experimentation, and 3) many species were economically important within the Chesapeake Bay (e.g., the oyster, *Crassostrea virginica*) or were important in estuarine food webs (e.g., phytoplankton and zooplankton).

Estuarine organisms responded differently when exposed to dissolved arsenate (Sanders et al., 1987). Phytoplankton, particularly centric diatoms, exhibited large changes in growth rate of dominant species, leading to changes in the species composition of the phytoplankton community. Zooplankton and benthic organisms, on the other hand, were quite tolerant of high concentrations of arsenic. Over a range of arsenate concentrations of 1 to 56 $\mu g\ l^{-1}$, only the barnacle, *Balanus improvisus*, was affected by arsenate which significantly reduced the barnacle's growth rate by 6 %. Although a statistically significant result, such a small decrease is unlikely to affect the organism in a natural system. *Balanus* was also the only organism to incorporate significant concentrations of arsenic. However, this incorporation may have been in shell material and not in animal tissue. Other organisms did not incorporate significant quantities of arsenic. The concentrations of arsenic used in these experiments were chosen to effectively bracket concentrations in estuaries, even those receiving considerable impact from man.

Thus, the lack of direct response to arsenic seen in these experiments, except within the phytoplankton community, indicates that at present levels of arsenic input, this pathway will have little impact on estuarine and coastal marine systems.

However, there are other pathways for impact of a toxic substance within an aquatic food web. Arsenic, because of its large impact upon phytoplankton species composition and community structure, is a prime candidate for such indirect impacts. Even though direct effects of arsenic are limited to phytoplankton and direct impacts to other trophic levels are minor, potential exists for indirect effects associated with changes in trophic structure or the ingestion of arsenic through food. Experiments performed during the first phase of the study indicated that changes in dominant species can drastically alter an herbivore's ability to procure enough food to successfully reproduce (Sanders, 1986; Sanders et al., 1987). In addition, although arsenic dissolved in the water may be unavailable and nontoxic to higher trophic levels, arsenic incorporated in their food may be quite toxic. This research program was designed to follow up on the earlier study, and has investigated the effects of arsenic-induced changes in phytoplankton dominance on higher trophic levels and the impact of ingestion of arsenic-contaminated food items. We have compared the growth, mortality, and fecundity of dominant zooplankton species and important benthic species when fed arsenic-sensitive, arsenic-resistant, and arsenic-contaminated foods. These experiments were conducted both in the laboratory, using cultured species, and under natural conditions utilizing natural phytoplankton assemblages that had been exposed to arsenic. In the latter experiments, the response of natural microzooplankton assemblages to arsenic and arsenic-induced changes in phytoplankton composition were also investigated.

Many different kinds of test systems have been developed or promoted for use in pollutant assessment at the community level. In addition, several volumes have been released recently which detail and contrast many systems (e.g.,White, 1984;

Cairns, 1986). Perhaps one of the most persuasive approaches has been the development of microcosms, or miniature ecosystems. Early attempts at enclosing oceanic plankton communities met with limited success (Strickland & Terhune, 1961; McAllister et al., 1961; Antia et al., 1963) but captured the immediate attention and interest of aquatic scientists. Since then, the use and sophistication of such enclosures have increased steadily (Menzel & Case, 1977; Grice & Reeve, 1982). Microcosms generally are defined as confined pieces of a natural ecosystem maintained under controlled (or known) environmental conditions, which are representative of the portion of the ecosystem from which they were taken, and which cannot be readily duplicated in the laboratory by the assembling of component parts (e.g., Pritchard & Bourquin, 1984). They are not, however, perfect simulations of ecosystems, nor do they contain all of the important biogeochemical processes that control the ecosystem.

Enclosures offer many advantages:

1. Two or more trophic levels, with their representative species, can be maintained for relatively long (weeks) periods of time.

2. The same populations can be repeatedly sampled.

3. Communities can be manipulated.

4. The systems are amenable to mathematical modeling (Grice, 1984).

There are disadvantages as well; Grice (1984) lists 4 major ones:

1. Horizontal and vertical mixing are reduced or eliminated.

2. Wall effects can become severe.

3. The structures can be fragile and difficult to maintain.

4. They can be relatively expensive.

However, they are well-suited and perhaps the best way for us to experiment with whole ecosystems, to allow the field of ecotoxicology to move beyond the descriptive stage (Oviatt, 1984; Cairns, 1986).

For this study, we have developed a series of separate and linked experiments using cultured and natural communities that combine the control and flexibility of laboratory experimentation with the realism of microcosm studies. Our approach has not been to enclose and manipulate the complex components of an actual ecosystem. Rather than attempting to model completely such a system we chose to work with a small subset of species and examine discrete processes (e.g., trophic transport of a pollutant) that would be common to most systems. An added advantage to this approach is that it is less expensive to large-scale microcosm studies. Our design is described in more detail in the following sections.

METHODS

EXPERIMENTAL PROCEDURES

Experiments were conducted either in closed-system laboratory tanks or in outdoor flow-through microcosms. The outdoor systems were designed for studies utilizing natural phytoplankton. Laboratory studies were designed to mimic the changes in phytoplankton community structure that occur when natural Chesapeake Bay communities are exposed to arsenic.

Natural phytoplankton and zooplankton communities were sampled locally from the Patuxent River estuary, a subestuary of the Chesapeake Bay (Figure 1). Zooplankton were collected using hand-held nets from the laboratory pier; dominant species (*Acartia tonsa*, *Eurytemora affinis*) were separated from the remaining organisms and cultured in the laboratory using standard techniques. *Balanus* was collected by exposing artificial substrates on which larvae attached (fouling panels similar to those used by Osman, 1977, 1982). *Crassostrea* larvae and juveniles were obtained from an oyster hatchery on the eastern shore of Maryland (Flo-Max Industries, Crisfield). The experimental designs are described below in detail.

Arsenic levels in these experiments ranged from ambient (0.5-1.0 $\mu g \cdot l^{-1}$) to 25 $\mu g \cdot l^{-1}$. Treatments receiving arsenic inputs (10-25 $\mu g \cdot l^{-1}$) were designed to contain enough arsenic to greatly increase the cellular arsenic content of cultured phytoplankton or to significantly alter the species composition and succession of dominant species within natural phytoplankton assemblages without harming any of the invertebrate species studied. Earlier work (Sanders, 1986; Sanders et al., 1987) had shown that this range would achieve this result.

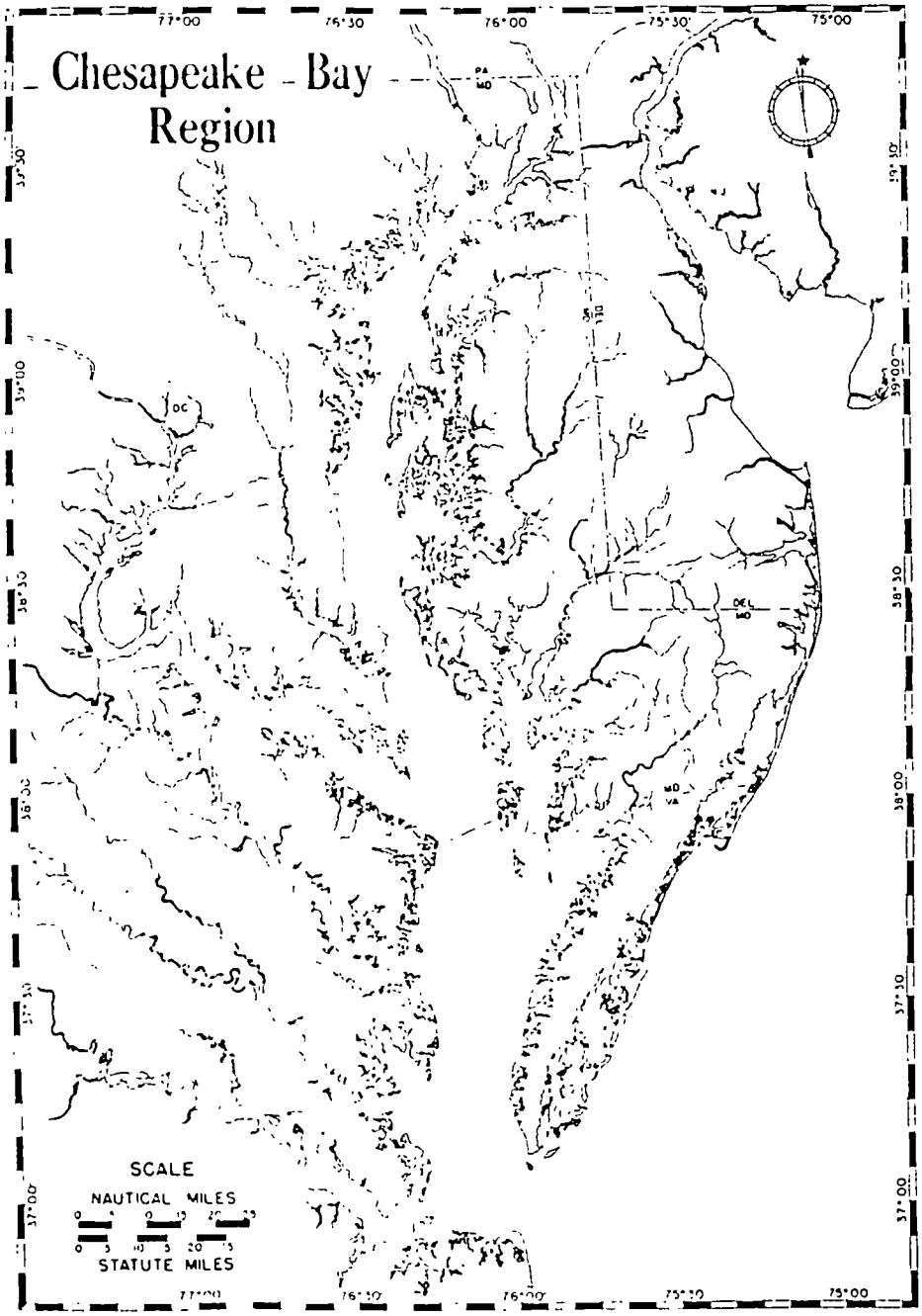


Figure 1. The Chesapeake Bay region.

Benthic Experiments

Two different benthic species were chosen for study: the oyster, *Crassostrea virginica*, and the barnacle, *Balanus improvisus*. These organisms were selected as representative and important members of the filter feeding community of Chesapeake Bay. Separate experiments were performed with each.

The general experimental design utilized three feeding treatments for each test species: 1) algal species sensitive to arsenic, 2) algal species resistant to arsenic, and 3) resistant species contaminated by arsenic.

Using this design we hypothesized:

1. If the uptake and transformation of arsenic by a food organism had a deleterious effect on the benthic species, this would result in a significant difference in growth and/or survivorship between resistant species treatments with and without arsenic.

2. If the replacement of a normally dominant sensitive species by a resistant species affected the benthic herbivore, then a significant difference in growth or survivorship between the feeding treatments (without arsenic) would result.

3. If the processes in 1 and 2 above interacted then the greatest differences should be found between individuals fed sensitive species and individuals in the treatment with resistant species and arsenic.

The first experiment was conducted with newly attached oysters (*Crassostrea virginica*). Cultured oyster larvae were exposed to sixty 115 cm^2 PVC panels in an oyster hatchery on the eastern shore of Maryland. The panels with attached juveniles were placed in coolers filled with Bay water and transported to the laboratory. At the laboratory panels were sorted into three groups based on oyster density, and equal numbers from each of these groups were randomly assigned to one of twelve 80 liter treatment tanks. In the tanks, panels were placed in racks which separated them and held them vertically. Six tanks were then assigned randomly to

each of the 2 arsenic treatments (0, 22 μg l^{-1}). As in previous experiments, a continuous flow design was used and panels were removed to 12 separate, 40 liter feeding chambers for 4 h per day. Feeding chambers were aerated and filled with static cultures of either *Isochrysis* sp. (sensitive species) or *Thalassiosira pseudonana* (resistant species). Half of the tanks within each of the 2 arsenic treatments were randomly assigned to the *Isochrysis* feeding treatment and the other half were assigned to the *Thalassiosira* treatment. Algal concentrations ranged between 1 x 10^4 and 2 x 10^4 cells·ml^{-1} during the course of the experiment. These densities were based on estimates of oyster nutritional requirements (Kennedy & Breisch, 1981) and estimates of algal carbon content.

The experiment was continued for 4 weeks and each experimental panel was photographed at 0, 2, and 4 weeks. The area covered by each individual oyster was used as a measure of its size and growth. These measurements were made by analyzing the photographs using the laboratory's image analysis system. Oysters showing no growth (75% of all oysters, with no significant differences between treatments) were considered unsuccessful recruits and were assumed to have died before the beginning of the experiment. They were not included in any growth rate analyses.

The second experiment was performed with newly settled barnacles. PVC panels (115 cm^2) were placed in the Patuxent River at Solomons, Maryland for 2 wks; the result was a heavy set of new individuals with little recruitment of competing species. All panels were returned to the laboratory and carefully inspected. The density of barnacle settlement varied greatly among the panels and 60 with approximately equal densities were chosen from the 180 panels originally exposed. These panels were gently washed, photographed and 5 panels were randomly chosen and assigned to each of twelve 20 liter chambers. These chambers were then assigned to 1 of 6 algal food treatments combined with 2 arsenic

treatments. Table 1 shows the overall design. Two species of algae sensitive to arsenic (*Cerataulina pelagica* and *Isochrysis* sp.) and two resistant species (*Thalassiosira pseudonana* and *Dunaliella* sp.) were used. Regardless of treatment, each tank received an equivalent amount of food (7.8 mg $C \cdot d^{-1}$). This amount was based on estimates of barnacle biomass and nutritional requirements. Observations indicated that preferred algal species were cleared in 4-6 hours but that nonpreferred species remained even after the 24 h feeding cycle. In each of 4 treatment tanks, barnacles were fed one of the four algal species. In addition, each of two other tanks received one of the resistant species and 25 $\mu g \cdot l^{-1}$ arsenic per day. The remaining 6 tanks were assigned randomly to one of three treatments: both sensitive species (*Cerataulina pelagica* and *Isochrysis* sp.), both resistant species (*Thalassiosira pseudonana* and *Dunaliella* sp.), and both resistant species + dissolved arsenic. In each of these treatments, equal amounts of each of the two species were used with the total amount fed equivalent to the single species treatments.

All chambers were aerated and maintained as static cultures. Water and algae were completely replaced every 24 hours. All panels were photographed after 2 weeks and at the end of the experiment (4 weeks). Photographs were then analyzed using the laboratory's image analysis system. This analysis consisted of measuring the surface area covered by individual barnacles and recording whether they were alive or dead. The majority of panels had more than 1000 barnacles, all of which were of similar age and size. Because of this similarity in size, a sample size of 100 barnacles per panel was judged to be more than sufficient for estimating growth. To eliminate any bias in choosing those barnacles measured, panels were divided into 7 equal-sized sectors and one sector was chosen randomly for analysis. If <100 barnacles were found in the sector, additional sectors were analyzed until this

Table 1. Experimental design for the barnacle experiment. Shown are the species of algae fed to barnacles in each type of treatment, the number of experimental chambers used for each treatment, and whether the algae were cultured with or without arsenic. Algal species: *Cerataulina pelagica* (CPEL), *Isochrysis galbana* (TISO), *Thalassiosira pseudonana* (SWAN1), *Dunaliella tertiolecta* (DUN).

TREATMENT	NO. OF CHAMBERS	ARSENIC	SPECIES OF ALGAE USED FOR FEEDING			
			CPEL	TISO	SWAN1	DUN
1	1	-	×			
2	1	-		×		
3	1	-			×	
4	1	-				×
5	2	-	×	×		
6	2	-			×	×
7	1	+			×	
8	1	+				×
9	2	+			×	×

minimum was reached. In order to measure growth unbiased by sampling different individuals, the same sectors were analyzed at the different sampling times.

At the end of the experiment, barnacles were removed from the panels for arsenic analyses (see below).

Zooplankton Experiments

The effects of arsenic on trophic relationships between the dominant copepod in the Chesapeake Bay, *Acartia tonsa*, and phytoplankton species were investigated. Both the direct effect of ingesting arsenic-laden phytoplankton and the indirect effect of changes in food types on the fecundity and development of this important zooplankton species were studied. In a previous phase of these studies, dissolved arsenic had a negligible effect on copepods below a concentration of 100 $\mu g \cdot l^{-1}$ (Sanders, 1986).

As in the barnacle experiment, the same two phytoplankton species known to be sensitive to arsenic (*Cerataulina pelagica and Isochrysis* sp.) and another pair known to be resistant to arsenic (*Thalassiosira pseudonana and Dunaliella* sp.) were used as food for *Acartia tonsa*. The resistant algae were offered with and without incorporated arsenic. Arsenic loading of the phytoplankton cells was accomplished by incubating the cells in 25 $\mu g \cdot l^{-1}$ arsenate for at least 2 days and not more than 4 days before offering it to the copepods, a procedure designed to maximize the cellular arsenic content. Thus, three treatments, arsenic-sensitive algae, arsenic-resistant algae and resistant algae contaminated with arsenic were run in triplicate for both the fecundity and development studies described below. Initially and at each transfer, cell densities were set to provide a surplus of food (greater than 0.67 mg $C \cdot l^{-1}$). The algae were kept in suspension by gentle swirling of the dishes at least once per day. The concentrations of arsenic carried over in the culture medium and in the arsenic loaded cells were measured in each of the experiments (see below).

All experiments were conducted in acid-washed 500 ml polymethylpentene containers (11 cm diameter, 7 cm depth) incubated at 25° C using a light:dark cycle of 12:12 hr. For the experiments, Patuxent River water (11-13‰) was filtered (1 µm nominal pore size) and autoclaved (121° C, 15 PSI, 3 min). 300 ml of this water was used in each experimental vessel. *Acartia tonsa* used in the experiments were cultured at 25° C on a mix of *Thalassiosira weissflogii* and *Isochrysis* sp. on a 12:12 hr light:dark cycle. Several days prior to the experiments, the copepods were switched to a diet that contained a combination of all four of the experimental algae.

Fecundity--Adult female copepods were isolated from healthy stock cultures and 20 were placed in each experimental vessel. Algae were added according to the three treatments described above. Every two days, the adult females were transferred using large-bore pipettes to clean chambers to which the prescribed concentrations of algae were added. The remaining eggs and nauplii were removed using a 20 µm mesh net and preserved in buffered formaldehyde (2% final concentration) for future counting. Males were not added for the following reasons: 1) it was assumed that the females had been fertilized in the stock cultures, 2) males would increase grazing pressure on eggs and nauplii, and 3) Parrish & Wilson (1978) found that egg laying in *Acartia tonsa*, following a general increase over the first 2-3 days after fertilization and removal of males, showed a relatively high daily egg production for the next 10 to 14 days.

Development--One hundred early stage (N1 or N2) nauplii were added to each experimental vessel and the appropriate algae added for each treatment. The early stage nauplii were obtained by isolating adult females in fresh food for 24 hours then removing them and allowing the eggs to incubate for another 24 hours. The nauplii produced were then transferred to glass depressions, inspected microscopically for eggs (which were removed), and then rinsed into the experimental chambers. At 2 and 4 days into the experiment, the copepods were gently collected on a 54 µm screen

and transferred to clean dishes with fresh food. On the 6th day, the experiment was terminated and the copepods were preserved in buffered formaldehyde for later counting and determination of developmental stage.

Arsenic Uptake--Adult *Eurytemora affinis* were collected from the Patuxent River in large numbers by towing a zooplankton net (202 µm mesh) along the laboratory pier. The animals were preconditioned on 6 species of phytoplankton, *Thalassiosira pseudonana*, *Dunaliella* sp., *Rhizosolenia fragilissima*, *Isochrysis galbana*, *Prorocentrum mariae-lebouriae*, and *Skeletonema costatum*, for 42 hr, then rinsed free of algal cultures and kept without food for an additional 24 hr. The adults were then exposed to unialgal cultures containing one of the six phytoplankton species above, both clean cultures and cultures which had elevated arsenic concentrations in their cells, and allowed to graze for 24 hr. Approximately 5,000 individuals were placed in each culture, with culture densities equivalent to 5 µg wet weight of algae per individual. After grazing, individuals were removed from the culture, rinsed, and resuspended in filtered water for 1 hr to allow them to clear their guts. After this period, several subsamples of each group of *Eurytemora* were taken for enumeration, and the remainder were dried and prepared for arsenic analysis.

Subsamples of the algal cultures were also taken for arsenic analysis.

Integrative Experiment

A final experiment was performed that combined all direct arsenic and indirect trophic response effects. The natural phytoplankton assemblage was exposed to elevated arsenic concentrations, then was used as food for a variety of filter-feeding organisms. A system of large-volume, outdoor tanks was utilized for this experiment. Natural phytoplankton from the Patuxent River were cultured in cylindrical fiberglass tanks 76 cm in diameter and 112 cm in height, containing a volume of 500 l. The tanks are submerged in a raceway through which running water was circulated to control and maintain the temperature of the water in 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 Patuxent River water containing the natural phytoplankton assemblage after passage through 35-μm nylon mesh to remove large herbivores. This initial screening did not remove a significant fraction of the phytoplankton. After filling, each tank was sampled and the phytoplankton species composition and abundance compared between tanks as a measure of tank-to-tank variability (Sanders et al., 1981). Filtered (1-μm) water was then continuously supplied to each tank at a nominal dilution rate of 50% per day (Sanders & Cibik, 1985; D'Elia et al., 1986) and continuous infusions of the toxic substances were begun. Because assemblages were maintained as flow-through cultures, adverse chemical changes associated with photosynthesis and respiration (low O_2, high NH_4^+ concentrations, wide variations in pH) were minimized within the tanks.

Three tanks received no arsenic additions, three received arsenic additions. The cultures were maintained for a period of 4 weeks. Arsenic was added to the cultures at one level, 10 $\mu g \cdot l^{-1}$, chosen after earlier experimentation had shown that this concentration was sufficient to inhibit the growth of several dominant species without affecting overall biomass levels or productivity (Sanders & Vermersch, 1982; Sanders & Cibik, 1985). After the experiment was begun, doses of two cultures were inadvertently altered (one control, one arsenic-dosed); thus results of these two cultures were not included in this analysis.

The phytoplankton assemblage in each tank was monitored daily for *in vivo* fluorescence, a rapid measurement of plant biomass (D'Elia et al., 1986), and was sampled every other day for phytoplankton species composition and total cell density (Sanders & Cibik, 1985; Sanders et al., 1987).

Microzooplankton were sampled from the tanks every 2-3 days from initiation to termination of the experiment. Whole water samples (500 ml) were preserved by pouring them onto 12.5 ml of formalin (2% formaldehyde final concentration). Subsamples, 25 ml for the initial time period and 50 ml for all other time periods, were settled and enumerated using the Utermohl technique (Lund et al., 1958). Taxonomic identification was made to the lowest taxonomic level possible using the above techniques.

The overflow from each tank was split into three parts and was pumped into separate chambers containing either barnacles on plates, adult oysters, or *Acartia* individuals. In each chamber organisms fed on the natural phytoplankton that were present in the overflow. The separate feeding tanks were started 5 d after arsenic flows began thus allowing the phytoplankton assemblage a period of time to respond to the arsenic stress.

Feeding Cultures - Water from each of the control and arsenic-treated microcosms was continuously pumped (50 ml·min^{-1}, flushing rate = 1.9 times per day) into eighteen 38 liter aquaria which were located in a separate raceway. The aquaria used for zooplankton experiments contained two plexiglass enclosures (11.5 x 11.5 x 35 cm) with four circular windows (5.7 cm diameter, 25.5 cm^2), two each on opposing sides, covered with 35 µm mesh netting. The volume of the enclosures, determined by the cross-sectional dimensions of the chambers and the height of the aquaria (30 cm), was 3.9 liters. The experimental organisms, *A. tonsa* adults for the fecundity study and nauplii for the development study, could thus be contained within the enclosures and exposed to the phytoplankton populations which developed in the individual microcosms.

Six days after the start of the experiment, organisms were added to the aquaria. In each of the 6 zooplankton aquaria, 20 adult, female copepods were added to one enclosure. Adult survival was very poor after two days so this first attempt

was aborted. The aquaria and enclosures were set up again on day 11. Thereafter, every two days for six days total, the enclosures were gently drained and the contents rinsed into a 500 ml polymethylpentene container. The contents of the containers were scanned using a dissecting microscope and the viable adults enumerated and transferred to 35 µm filtered water from the appropriate chamber. Care was taken not to transfer any eggs or nauplii with the adults. The remaining concentrate from the enclosure which contained the eggs, nauplii and expired adults was poured into a net cup fitted with 20µm mesh nytex netting. The contents of the cup were then rinsed into a sample jar and preserved with formaldehyde (2% final concentration). The number of eggs, nauplii and adults in the samples were enumerated in a Wildco plexiglass sorting wheel using a dissecting microscope.

The development study was initiated on the seventh day of the integrative experiment. The second enclosure in each aquaria received 150 nauplii (stages 1 & 2) which were obtained as in the laboratory study described above. After eight days of incubation in the enclosures, the entire contents of the chambers were gently drained and rinsed into sample bottles and fixed with formaldehyde as above. The total number of eggs, nauplii, copepodites, and adults in the sample were determined microscopically as well as the developmental stage (i.e. six naupliar, 5 copepodite, and adult males and females). Subsampling was necessary for some of the samples which had very high densities of eggs and nauplii.

Each of the aquaria with oysters contained 10 individuals placed haphazardly on the bottom. Oysters were cleaned, and each had an edge of its shell filed straight to create a reference point for measurements. Using calipers, each oyster was measured perpendicular to the filed edge and then photographed. After two weeks and at the end of the experiment, oysters were remeasured to determine the extent of shell growth, and rephotographed for image analysis.

Each of the remaining 6 aquaria were assigned 5 panels with attached barnacles. Panels were held on a rack which oriented them perpendicular to the bottom. As in the earlier barnacle experiment, individual plates were photographed before and after the growth period, and individual growth rates were determined using image analysis.

Because of their small size, enough zooplankton could not be collected for analysis of arsenic content. Tissues of both barnacles and oysters were sampled for arsenic analysis. Some barnacles were too small to allow for separation of tissues from shell material. For oysters tissues, newly formed shell and old shell were analyzed separately for arsenic content. Phytoplankton samples were also taken for arsenic content.

ARSENIC ANALYSIS

The concentration and chemical form of arsenic within each experiment were monitored within the water column and organisms. Water samples were collected in rigorously cleaned (Boyle & Huestedt, 1983) plastic bottles and analyzed by hydride generation (Braman et al., 1977). This method of analysis permits determination of the total concentration of arsenic and also its chemical form. Limits of detection in our laboratory are about 20 ng l^{-1}.

Solids were dried, weighed, and ashed at 500°C for 24 hr in the presence of an ashing aid [$Mg(NO_3)$ and MgO] to prevent loss of arsenic (Uthe et al., 1974). After ashing, the residue was dissolved in 1N HCl and analyzed as above.

Technique accuracy was assessed through the use of standard reference materials, NBS #1566, oyster tissue, and NRC NASS-1, a seawater standard. Recoveries of these materials averaged 95% and 91%, respectively.

RESULTS

BENTHIC EXPERIMENTS

Juvenile Oysters

This experiment employed a nested design and was analyzed using nested analysis of variance. Oysters were nested on panels which were nested within replicate treatment tanks which, in turn, were nested within the 4 treatments. This analysis allowed us to identify any potential differences between panels within chambers and chambers within treatments that may have contributed to observed differences.

At the beginning of the experiment over 20,000 newly settled oysters were found on the experimental panels. Of these more than 5,500 increased in size over the course of the experiment and were judged to have successfully recruited prior to the study. The sizes of these recruits after 4 weeks were quite variable (Figure 2), but no significant differences were found between any of the treatments (Table 2). In fact, the combined effects of arsenic and algal species explained less than 4% of the variation in oyster size.

Because it was not possible to distinguish living and dead oysters on the photographs, much of the size variation found among the oysters probably resulted from mortality during the study. Small individuals were likely to be ones that had died early in the experiment. To examine whether growth differences existed among individuals that survived the length of the experiment, we reanalyzed growth using only individuals that reached sizes larger than 10 mm^2. This analysis assumed that those individuals that had attained this arbitrary size ($< 10\%$ of successful recruits) were still alive (they had clearly grown) and examined whether differences existed among treatments for this group of oysters. As in the previous analysis, there was no significant effect resulting from arsenic or algal food (Table 3).

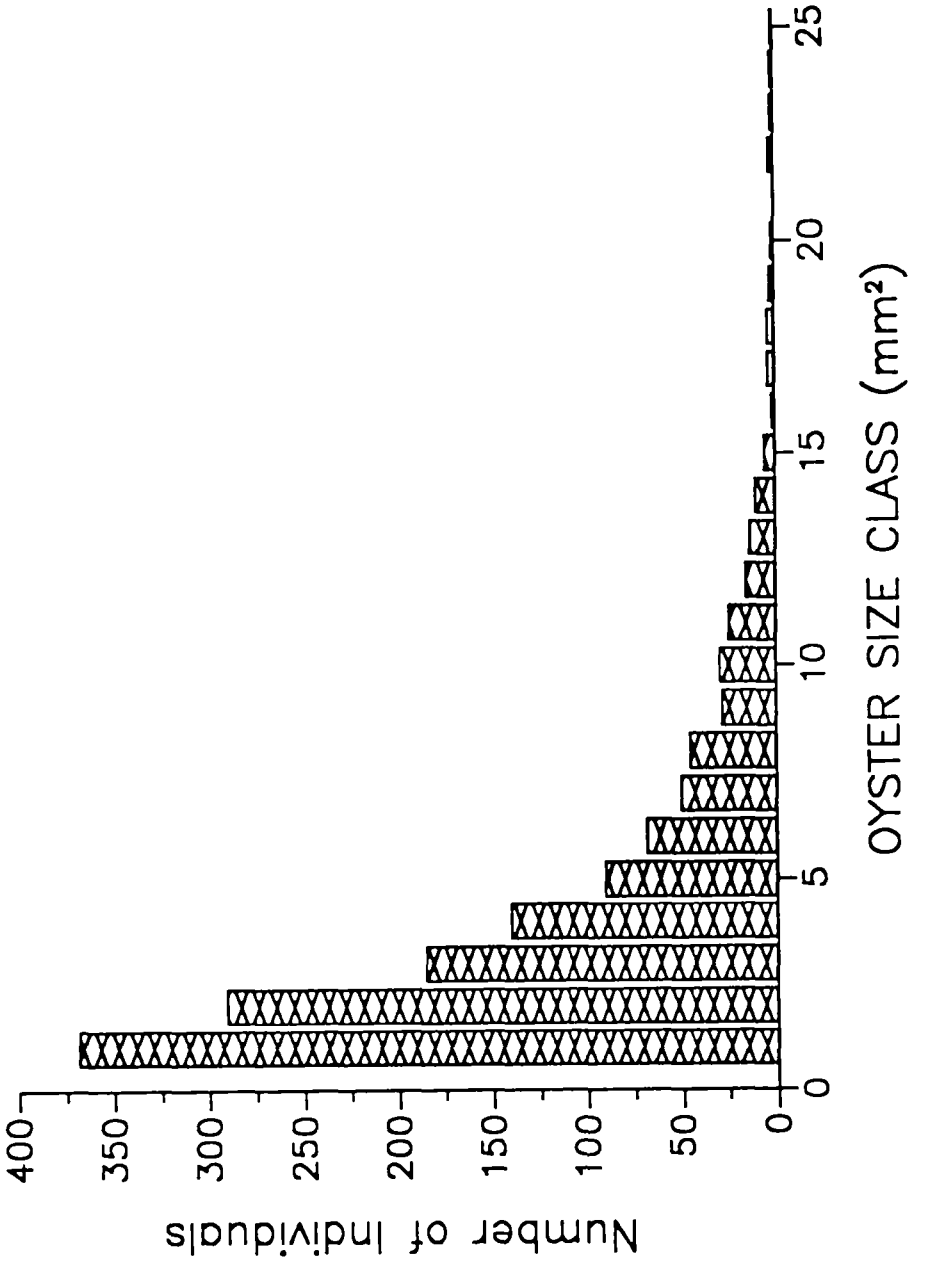


Figure 2. Variability in size of juvenile oysters at the end of the feeding experiment.

Table 2. Nested Analysis of Variance of Juvenile Oyster growth data. Data were analyzed at the end of the experiment. *MS for Chamber used as the error term.

	Juvenile Oysters			
Source	SS	DF	F	p
Arsenic	1.1887	1	0.04*	0.8419
Algae	98 2979	1	3.51*	0.0979
Arsenic*Algae	3 5919	1	0 22	0 6364
Chamber in Treatment	224 0476	8	1 74	0.0835
Panel in Chamber	3223.3201	46	4.36	0.0001
Error	87901.3163	5471		

Table 3. Nested Analysis of Variance of Juvenile Oyster growth data. Data were analyzed at the end of the experiment. Only individuals larger than 10 mm^2 were analyzed. *MS for Chamber used as the error term.

	Juvenile Oysters			
Source	SS	DF	F	p
Arsenic	216.0456	1	2.49*	0.1781
Algae	37.7710	1	0.44*	0.5417
Arsenic*Algae	1.1508	1	0.05	0.8259
Chamber in Treatment	694.7792	8	3.65	0.0003
Error	36309.4393	1528		

Again, assuming that size and length of survivorship had a positive relationship, size distributions were used to determine whether survivorship differed among treatments. Figure 3 compares the number of individuals that attained successively larger size classes. These "survivorship" curves obviously do not differ between treatments.

Given the large number of juvenile oysters measured, the results of this experiment are unambiguous. Even though we might expect these juvenile life stages to be fairly intolerant of stress, they did not demonstrate any reductions in growth or survivorship that could be related to the experimental treatments.

Juvenile Barnacles

Both the growth and survivorship of barnacles were analyzed using analysis of variance and treatments were compared using either Duncan's or Bonferoni's (when sample sizes were unequal) *a posteriori* tests. The percent survivorship was computed for each panel and transformed for analysis using an arcsine square root transformation. A one-way ANOVA was used with treatment tank as the main effect. Growth was analyzed using a nested ANOVA model with panels nested within tank and tanks nested with treatment.

As can be seen in Table 4, survivorship was clearly affected by species of algal food, but not by the presence of arsenic. Barnacle survivorship was reduced by as much as 30% when fed *Dunaliella* (a species resistant to arsenic) and 25% when fed *Isochrysis* (a sensitive species). The survivorship of barnacles in chambers fed a mixture of *Dunaliella* and *Thalassiosira* (both resistant species) was also lower than other treatments, but not significantly ($p > 0.05$) different.

An analysis of barnacle sizes at the beginning of the experiment showed no significant differences between those assigned to the different treatments. More than 90% of the barnacles in each treatment were < 0.5 mm^2. After 4 weeks there

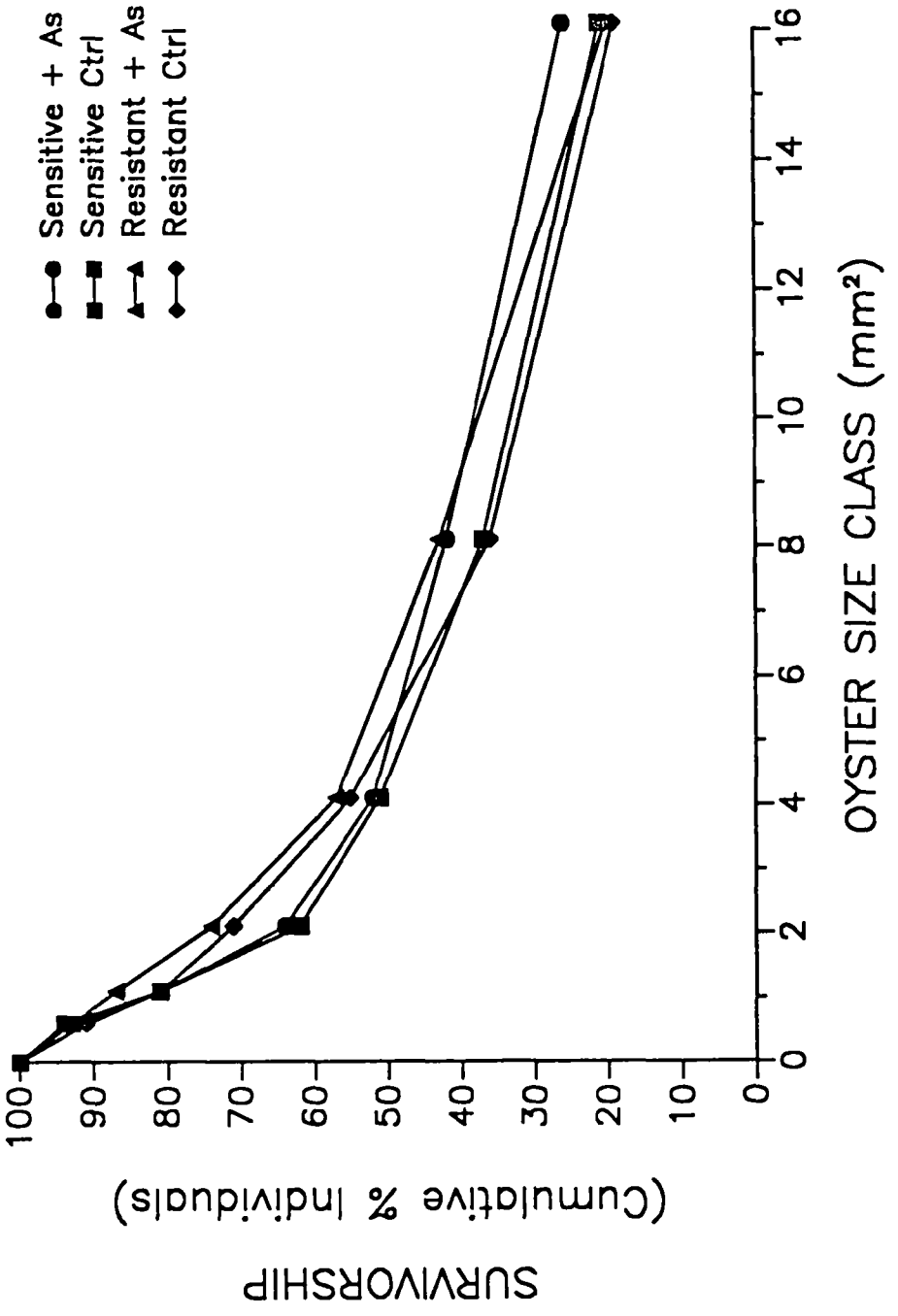


Figure 3. Survivorship of oysters in the various feeding treatments.

Table 4. A one-way analysis of barnacle survivorship. Data were transformed using an arcsine square root transformation and means show percent survivorship based on that transformation. Lines connect means with no significant differences (based on Duncan's *a posteriori* test).

Source	DF	SS	F	p
Treatment	11	1.5002	4.05	0.0003
Error	48	1.6149		

			TREATMENT	
DUNCAN	GROUPING	MEAN	ALGAE	ARSENIC
		97.2	SWAN1	-
		96.9	CPEL	-
		96.9	CPEL+TISO	-
		95.6	CPEL+TISO	-
		94.9	SWAN1+DUN	+
		93.5	SWAN1	+
		90.5	SWAN1+DUN	-
		89.9	SWAN1+DUN	-
		86.9	SWAN1+DUN	+
		72.3	TISO	-
		68.3	DUN	+
		67.8	DUN	-

were significant differences in the mean size of barnacles within the various treatments (Table 5) despite the large variability in the growth (size) of barnacles within treatments (Figure 4). This variability can be seen in the significant differences found in mean barnacle size between tanks within treatments and panels within the same tank. Nevertheless, several patterns can be seen in the *a posteriori* comparisons:

1. Barnacles fed the sensitive species *Cerataulina* grew to a significantly larger mean size (12.3 mm^2) than those in other treatments.

2. Barnacles fed the resistant species *Thalassiosira* grew to a mean size significantly smaller (10.1 mm^2) than those fed *Cerataulina*, but significantly larger than those in all other treatments.

3. Barnacles fed the second sensitive species *Isochrysis* grew to a larger size (8.9 mm^2) than those fed the resistant species *Dunaliella* (7.8 mm^2), but less than those fed the other two species.

4. Because barnacles grew well when fed 1 of the 2 sensitive species and when fed 1 of the 2 resistant species, mixed cultures produced intermediate growth, not different from one another.

5. Barnacles fed *Thalassiosira* with arsenic grew significantly more slowly (8.5 mm^2) than those fed clean *Thalassiosira* (10.1 mm^2).

6. Finally, as was found with *Thalassiosira*, the mixed culture of the resistant species, *Thalassiosira* and *Dunaliella*, with arsenic resulted in significantly lower barnacle growth than the same treatment without arsenic.

Table 5. A nested ANOVA of barnacle growth. Growth was measured as area covered (mm^2) by each individual after 4 weeks. Lines connect means with no significant differences (based on Bonferoni's *a posteriori* test).

Source	DF	SS	F	p
Main Variable				
Treatment	8	13872	37.31	<0.0001
Nested Variables				
Tank in Treatment	3	3535	28.98	0.0001
Panel in Tank	48	24681	13.70	<0.0001
Error	7370	326387		

		TREATMENT	
BONFERONI GROUPS	MEAN	ALGAE	ARSENIC
I	12.27	CPEL	-
I	10.08	SWAN1	-
I	8.93	TISO	-
I	8.88	SWAN1 + DUN	-
I	8.84	CPEL + TISO	-
I	8.53	DUN	-
I	8.50	DUN	+
I	7.96	SWAN1	+
I	7.83	SWAN1 + DUN	+

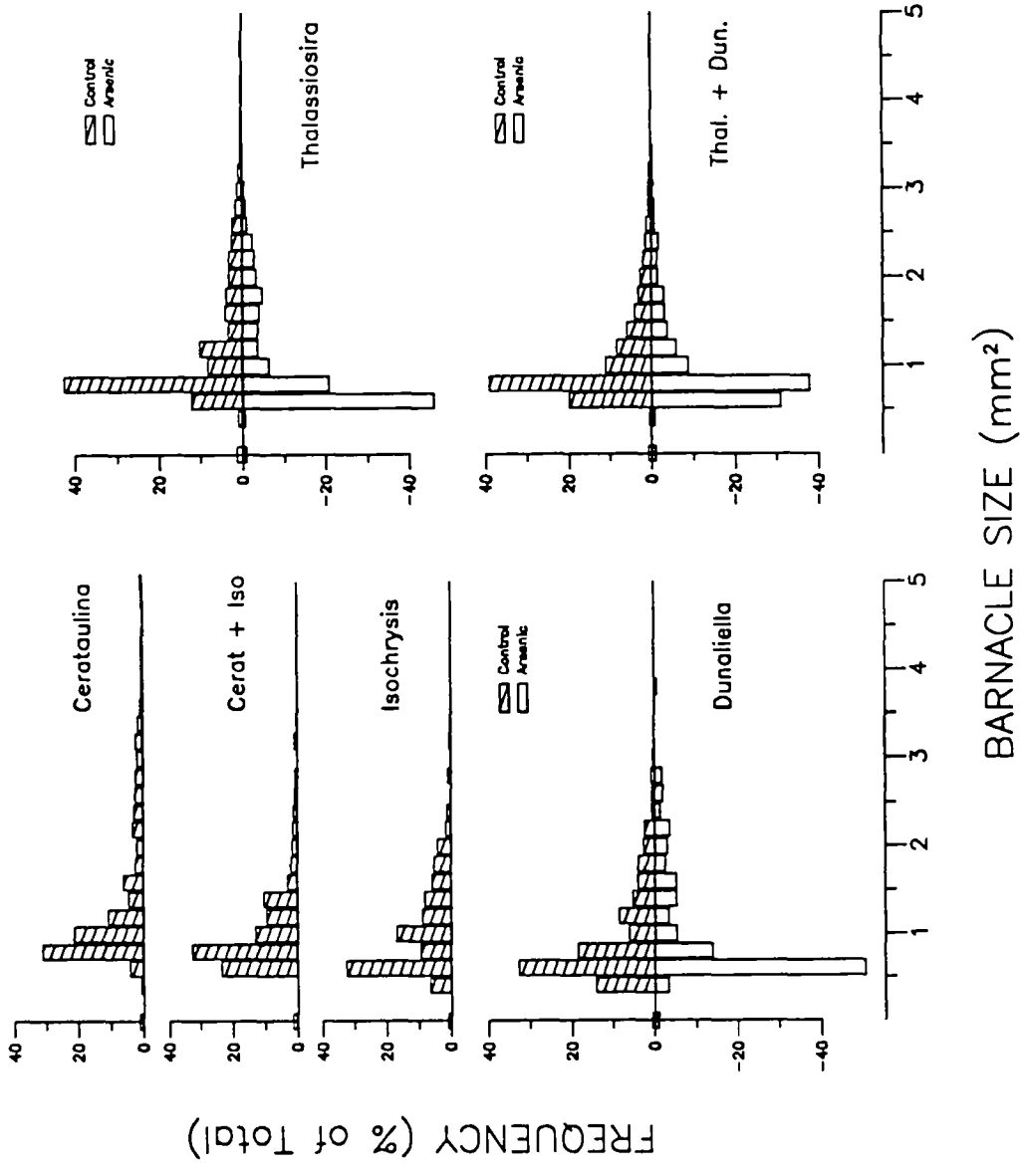


Figure 4. Size distributions of barnacles in each of the 9 treatments used in the feeding experiment. Shown are the distributions at the end of the experiment. Size categories are in 0.5 mm^2 intervals. Feeding treatments with and without arsenic are displayed together.

ZOOPLANKTON EXPERIMENTS

Fecundity

The results of the fecundity study are given in Table 6 and Figure 5. The results indicate inhibitory effects of both ingestion of arsenic-laden food and algal species composition.

After two days, the copepods fed sensitive algae had produced approximately the same numbers of offspring (eggs + nauplii) as those fed the resistant algae without arsenic loading (Table 6). However, those fed the resistant algae with arsenic produced significantly fewer offspring, approximately 25% of control production. Production in both treatments fed resistant algae (both with and without incorporated arsenic) continued to decline relative to controls as the experiment progressed. By the end of the experiment, copepods fed either the resistant algae with or without arsenic loading produced less than one offspring copepod^{-1} d^{-1} while those fed the sensitive algae had continued to produce 26.9 offspring·copepod^{-1}·d^{-1} (Table 6).

Development

The results of the development study are presented in Figure 6. As with the fecundity study, both the ingestion of arsenic-laden phytoplankton and the change in species composition had an inhibitory effect. Though survival was low (8-40% overall), differences between treatments could be seen in percent survival and in the advancement of juveniles though the developmental stages. The low survival may have been caused by handling of the copepods during transfers to clean culture vessels and fresh food or perhaps to overcrowding. Heinle (1966) found decreased development to adults at concentrations above 40 copepods·l^{-1}. Our experiments were performed with 333 nauplii·l^{-1}, as this density was within the range of natural densities encountered in Chesapeake Bay (up to 500·l^{-1}; Brownlee & Jacobs, 1987).

Table 6. Summary of *Acartia tonsa* fecundity study. Egg and naupliar production by females fed sensitive, resistant, or resistant, arsenic-laden phytoplankton.

	Production, eggs and nauplii·female $^{-1}$·d^{-1} ($\bar{x} \pm SE$)			
Algal Type	Day 0-2	Day 2-4	Day 4-6	Overall
Sensitive	34.8 ± 1.4	26.8 ± 1.9	26.9 ± 6.3	29.5 ± 2.3
Resistant	32.6 ± 3.9	8.2 ± 4.9	0.4 ± 0.05	13.7 ± 5.2
Resistant, arsenic laden	8.8 ± 0.7	0.4 ± 0.3	0.4 ± 0.3	3.2 ± 1.4

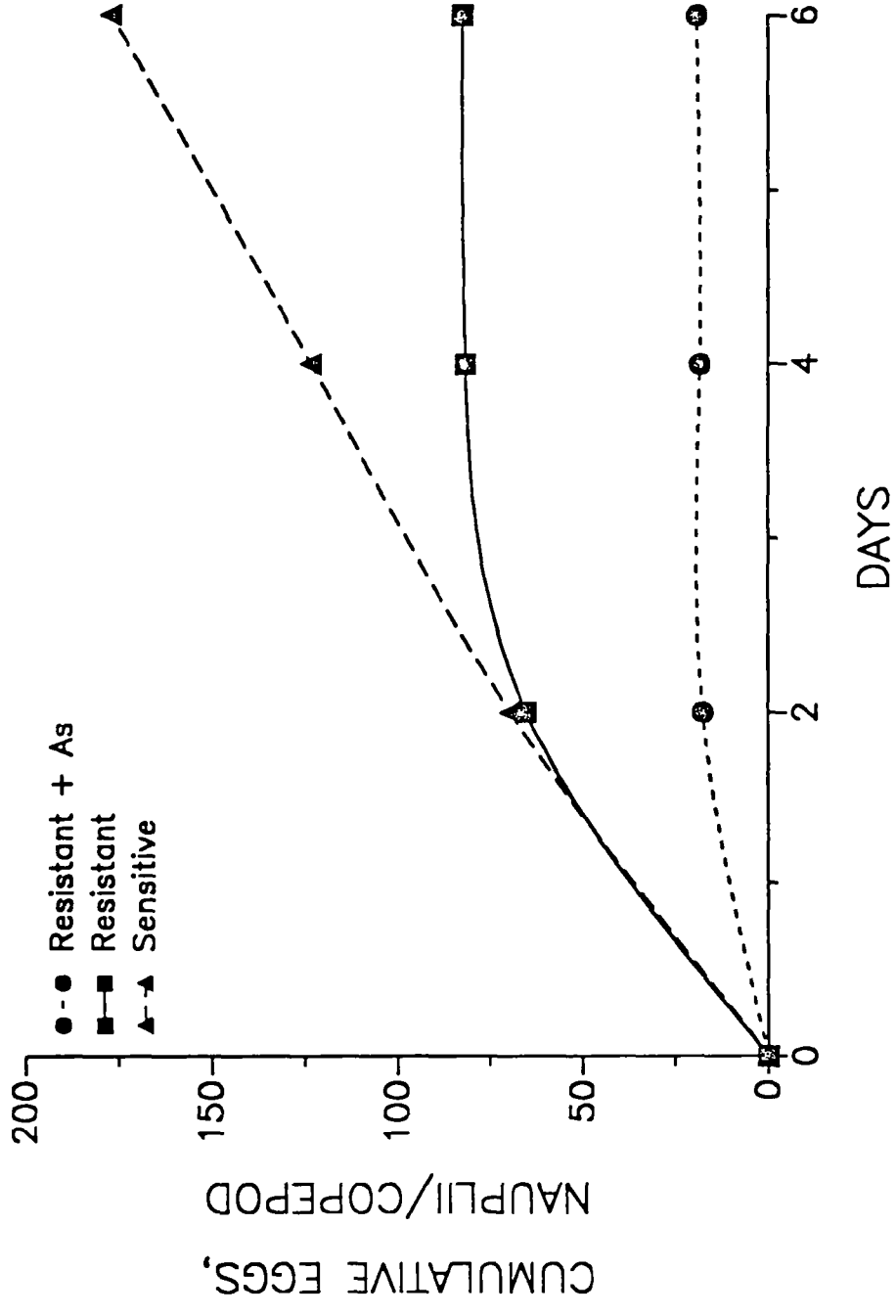


Figure 5. Response of *Acartia tonsa* fecundity to phytoplankton species composition and arsenic laden phytoplankton. Treatments: arsenic-sensitive algae, arsenic-resistant algae without arsenic loading of the phytoplankton and arsenic-resistant algae with arsenic loading. See text for species composition of algae.

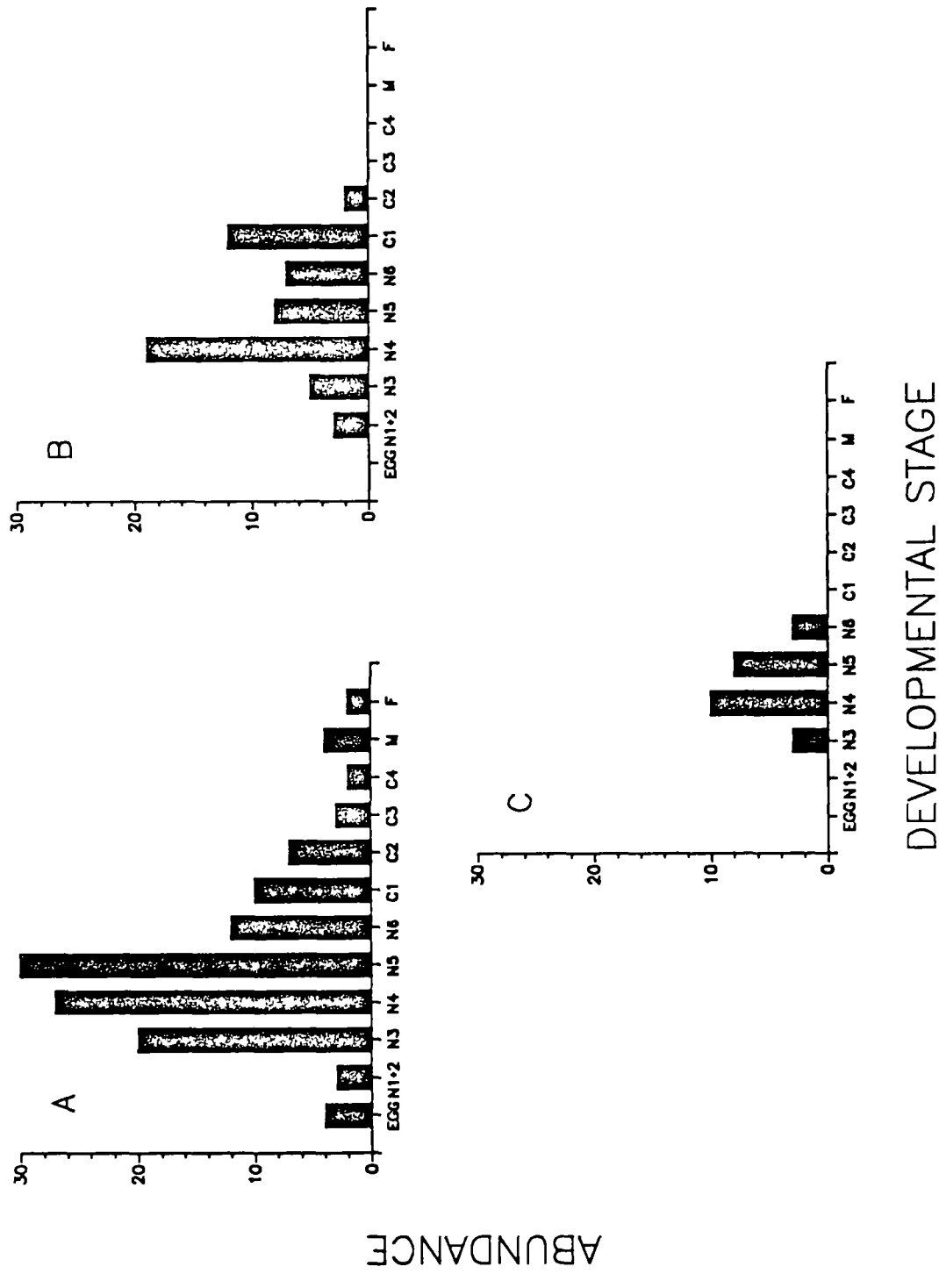


Figure 6. Development of *Acartia tonsa* over a six-day period in response to phytoplankton species composition and arsenic-laden phytoplankton. Number of occurrences of each developmental stage totaled for the replicates. N1-N6, copepod naupliar stages 1-6; C1-C4, copepod copepodite stages 1-4; C5-M, copepodites stage 5 males; A-M, adult males; C5 + A-F, copepodite stage 5 females and adult females. A. Arsenic-sensitive algae, B. Arsenic-resistant algae without arsenic loading of the phytoplankton, and C. Arsenic-resistant algae with arsenic loading. See text for species composition of algae.

The average survival over the six day period was 40, 19 and 8% for those copepods fed sensitive algae, resistant algae without arsenic, and resistant algae with arsenic, respectively. In the sensitive algae treatment, many of the nauplii had developed into early and late stage copepodites and a few had reached maturity with some eggs having been produced. In the treatment with resistant algae without arsenic, development never proceeded beyond the early copepodite stages (C1 and C2) and in the arsenic treatment development was restricted to the naupliar stages.

In both experiments, arsenic in the water carried over with the phytoplankton offered as food was considered insignificant. Sanders (1986) found that levels in water must be greater than 100 $\mu g\ l^{-1}$ to affect the survival of adult and juvenile *Eurytemora affinis*, another important copepod in the Chesapeake Bay. Arsenic analyses showed that $<7\ \mu g\ l^{-1}$ arsenic was present in the water of the experimental vessels containing the arsenic-laden phytoplankton.

The egg laying rates (fecundity) obtained with the sensitive algae throughout the experiments and with the resistant algae without arsenic during the first 2 day period were within the normal ranges reported for *Acartia tonsa* by Parrish & Wilson (1978, means between 26 and 38 eggs $copepod^{-1} \cdot d^{-1}$). The six day time period for development from N1-N2 stage nauplii to adult, which was found for at least a few individuals fed the sensitive algae, agrees well with the results of Heinle (1966). Thus, the sensitive algae treatment was considered to reflect reasonably natural conditions.

INTEGRATIVE EXPERIMENT

Changes in Phytoplankton Species Composition

The natural phytoplankton assemblage responded rapidly to arsenic additions. Species composition and cell densities underwent very large changes in response to arsenate. Cell densities rapidly increased in arsenic-dosed cultures, peaking at

levels greater than 4 times that of controls (Figure 7). This increase was largely attributable to an increase in the growth of one small centric diatom, *Thalassiosira pseudonana*. By day 9, average densities of *T. pseudonana* in arsenic-dosed tanks exceeded 9 times the density in controls. During this time, its growth rate averaged 1.1 div·d^{-1} in arsenic-dosed tanks and 0.7 div·d^{-1} in controls. After this period, this species did not exhibit differences in growth rate between treatments. The species persisted in arsenic-dosed tanks 2 d longer than in controls, then experienced a precipitous drop in density (Figure 8).

After the *T. pseudonana* bloom, arsenic-dosed tanks were dominated by small flagellates, a succession which was not reflected in control tanks. These changes to dominant species in response to arsenic stress resulted in markedly altered assemblages. This trend continued in that the growth rate of another important large diatom, *Cerataulina pelagica*, was reduced 33% by arsenic and this species did not succeed in arsenic-dosed tanks as it did in controls during the latter part of the experiment.

In contrast, phytoplankton biomass was not altered by arsenic dosing; all tanks exhibited similar levels of biomass throughout the experiment (Figure 9). The biomass of the assemblages remained relatively constant because, as a consequence of the increase in *Thalassiosira* sp. within arsenic-dosed tanks, there was corresponding decline in the growth rate of larger centric diatoms such as *Cerataulina pelagica* and to a lesser extent, *Rhizosolenia fragilissima*, relative to controls.

The large increase in *Thalassiosira* and small flagellates in the first half of the experiment caused large differences in the taxonomic composition of the two phytoplankton arsenic assemblages on a numerical basis; however, after the initial bloom declined, there was little difference between treatments (Figure 10).

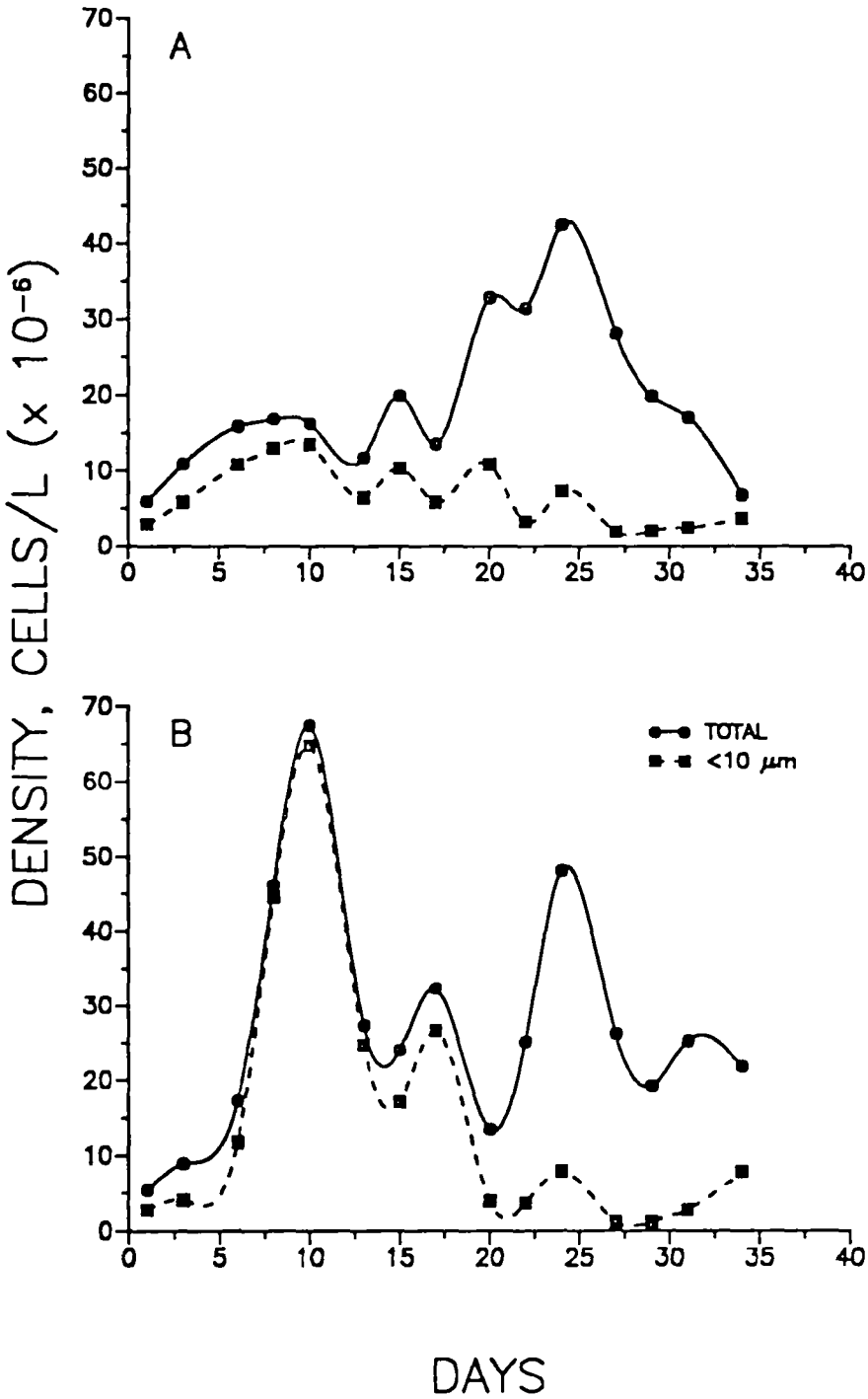


Figure 7. Phytoplankton total cell densities and densities of cells < 10μm in size in control and arsenic-dosed cultures during the Integrative experiment. A. Control tanks, B. arsenic-dosed tanks.

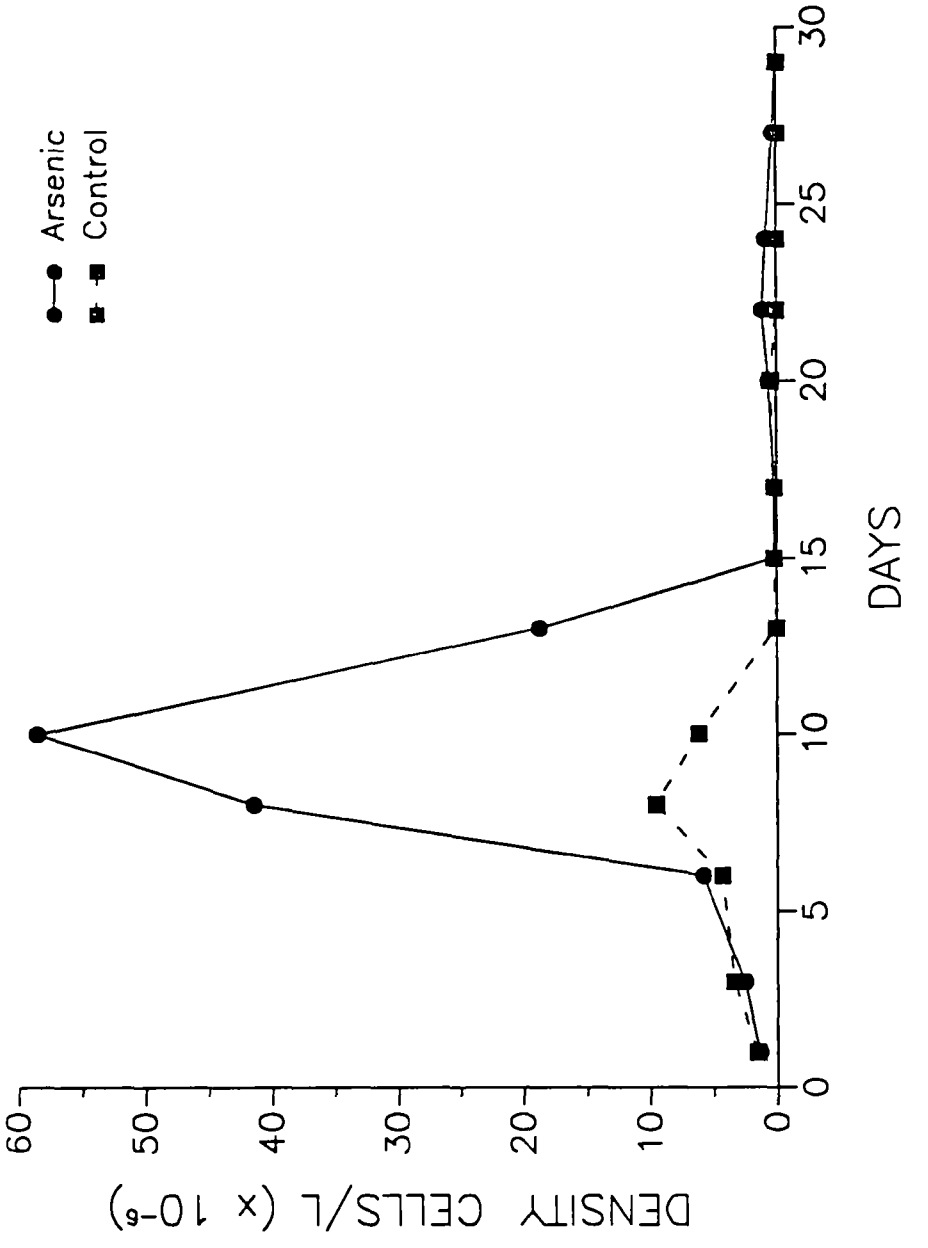


Figure 8. Densities of *Thalassiosira* sp. densities during the Integrative experiment.

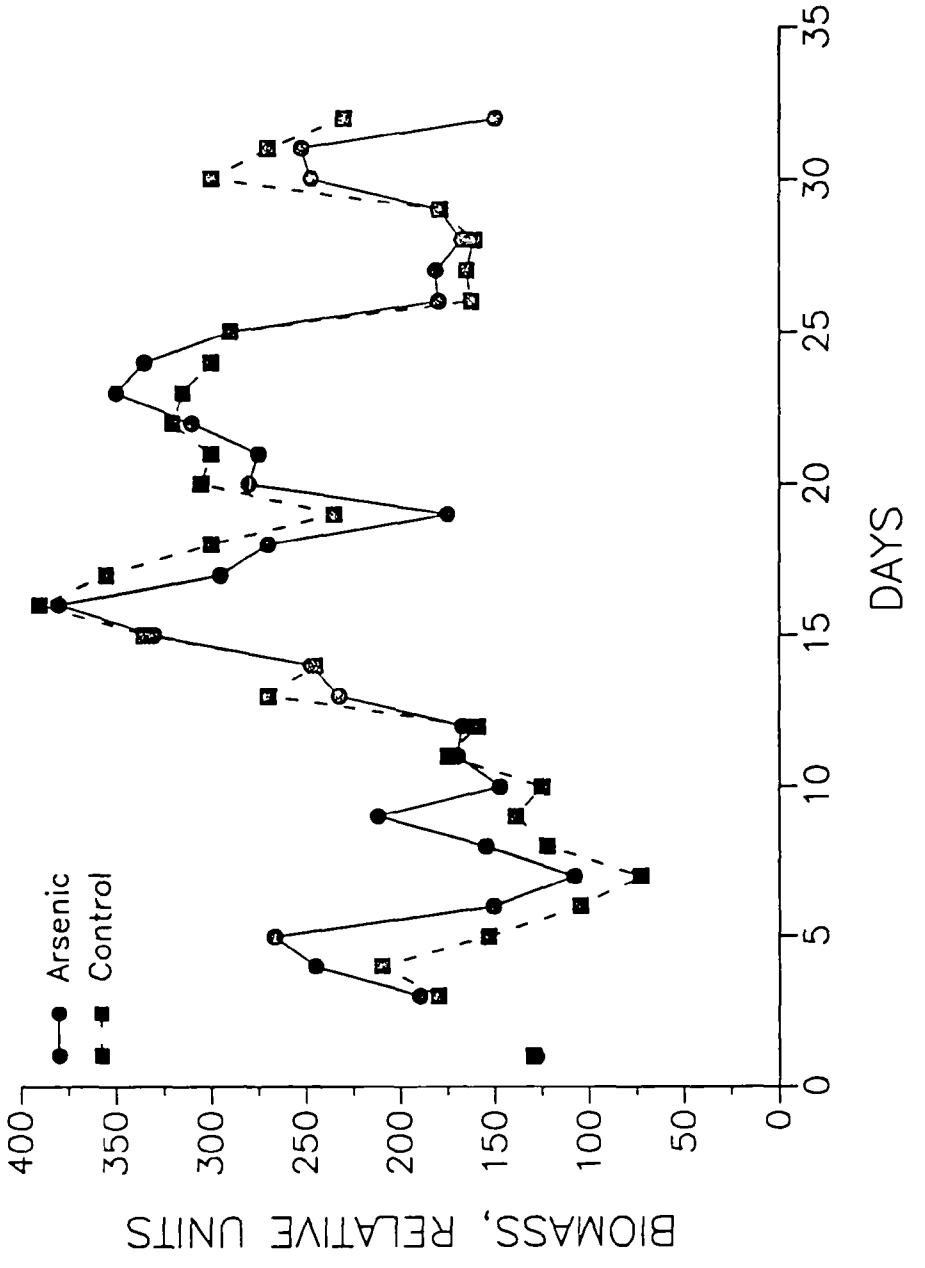


Figure 9. Phytoplankton biomass (as measured by *in vivo* fluorescence) during the Integrative experiment.

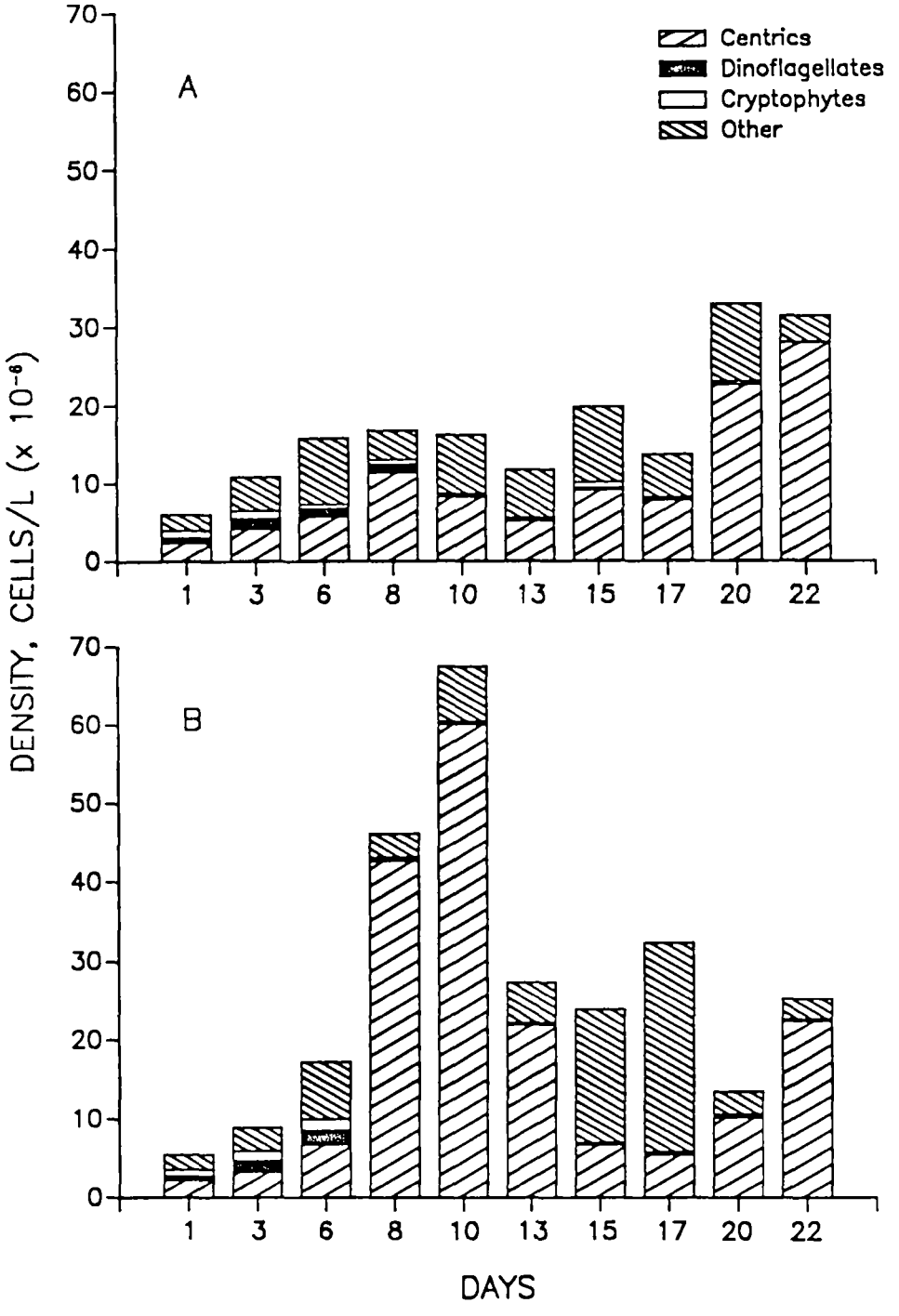


Figure 10. Composition of phytoplankton during the Integrative experiment. A. Control tanks. B. Arsenic-dosed tanks.

There was also considerable difference in the size structure of the community. The success of *Thalassiosira* sp. and the decline in growth of larger diatoms led to a large increase in the proportion of the population that was <10μm in size (Figure 7).

Microzooplankton Species Composition

On the average over all time periods, microzooplankton densities were higher in the arsenic (mean = 6800 l^{-1}) than in the control (mean = 4200 l^{-1}) culture tanks (Table 7). Average total phytoplankton density (Figure 7) also showed the same pattern. Analysis of the phytoplankton by size (< or >10 μm) indicated that the difference between the two treatments was due to an increase in the <10 μm size category in the arsenic-dosed cultures. As discussed in the Introduction, and as hypothesized, the arsenic stress led to an increase in the numbers of small phytoplankton (Figure 7) in the treatment tanks which correlates with the relative increase in microzooplankton densities.

The microzooplankton tended to respond more to the changes in size distribution and species composition of the phytoplankton than to the levels of arsenic in the tanks. Total microzooplankton (Figure 11) in the control cultures, maintained moderate (900-4000 l^{-1}) abundances during the first 17 days of the experiment. The control populations peaked on day 24 reaching about 15,000 organisms l^{-1} and returned to about 1,000 l^{-1} at the end of the experiment. Between culture variability of total microzooplankton abundance for the two controls was low (mean CV over all time periods = 10%) relative to that of the arsenic treatments (mean CV over all time periods = 46%). Phytoplankton variability was also greater in the arsenic treatments with the mean CV over all time periods equal to 10 and 16% for the controls and arsenic treated populations, respectively.

In both arsenic-dosed cultures abundance maxima occurred on day 10 reaching between 7,000 and 9,000 l^{-1}. By day 17, microzooplankton numbers had decreased to 230 and 2600 l^{-1} for the arsenic-dosed tanks 1 and 2, respectively. By the 24th day,

Table 7. Microzooplankton densities (individuals·l^{-1}) during the integrative experiment.

	Control			Arsenic-dosed		
Day	Tank 3	Tank 4	Mean	Tank 1	Tank 2	Mean
1	1260	2226	1743	1344	2520	1932
5	3129	3696	3413	4368	2877	3623
10	2730	2226	2478	8883	7140	8012
17	903	1029	966	231	2625	1428
24	15204	14763	14984	49098	1239	25169
33	1302	1407	1355	420	840	630
Average	4088	4225	4156	10724	2874	6799

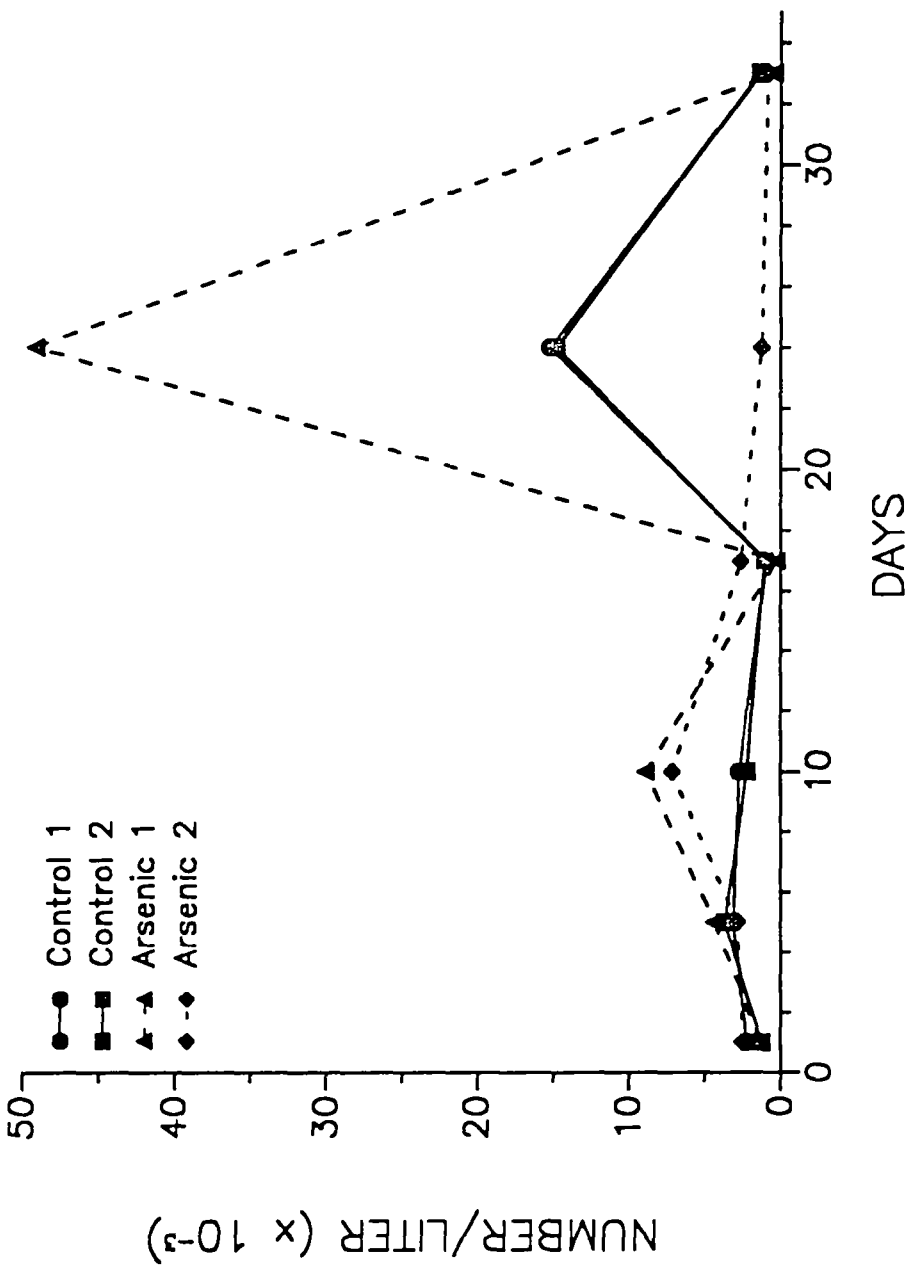


Figure 11. Microzooplankton densities during the Integrative experiment.

microzooplankton densities in tank 1 had increased dramatically to 49,000 l^{-1}, while the densities in tank 2 had decreased to 1,000 l^{-1}. Just preceding and during this period of peak abundance in tank 1, the phytoplankton populations were different in abundance and species composition. For example, on the 17th day of the experiment when microzooplankton densities in the arsenic tanks were relatively low, tank 1 had high densities of phytoplankton mostly (83%) composed of small (<10 µm) forms. Though small phytoplankters were also dominant (81%) in tank 2 at this time period, the total densities were less than half those in tank 1.

The microzooplankton were composed of ciliated protozoa, rotifers, sarcodinids and copepod nauplii. Those species which obtained abundances greater than 1,000 l^{-1} were the oligotrich ciliates *Lohmaniella* sp. and *Laboea* sp, the tintinnine ciliate, *Eutintinnus* sp., and the rotifer *Synchaeta* sp. In the first half of the experiment, *Synchaeta* sp. densities tracked very closely the distribution of *T. pseudonana*. During the second half of the experiment, ciliates (mostly *Lohmaniella* and *Eutintinnus* sp. dominated the microzooplankton in the controls and arsenic tank 1 and appeared to be associated with relatively high abundances of the <10 µm phytoplankton. Arsenic tank 2, which had the lowest microzooplankton densities, also had the lowest concentrations of small phytoplankton.

Feeding Cultures

Zooplankton Fecundity - Adult survival in the fecundity enclosures was low in the arsenic tanks relative to the controls (Figure 12). Average survival was 56% for the controls, and 38% for the arsenic treatments over the three time periods. Conversely, fecundity (eggs + nauplii) on a per female per day basis was higher for the copepods exposed to arsenic, especially during the last two time periods (Figure 13, Table 8). Mean offspring per female per day averaged 27 for controls as compared to 50 in the arsenic treatments over the same time periods as above. By the end of

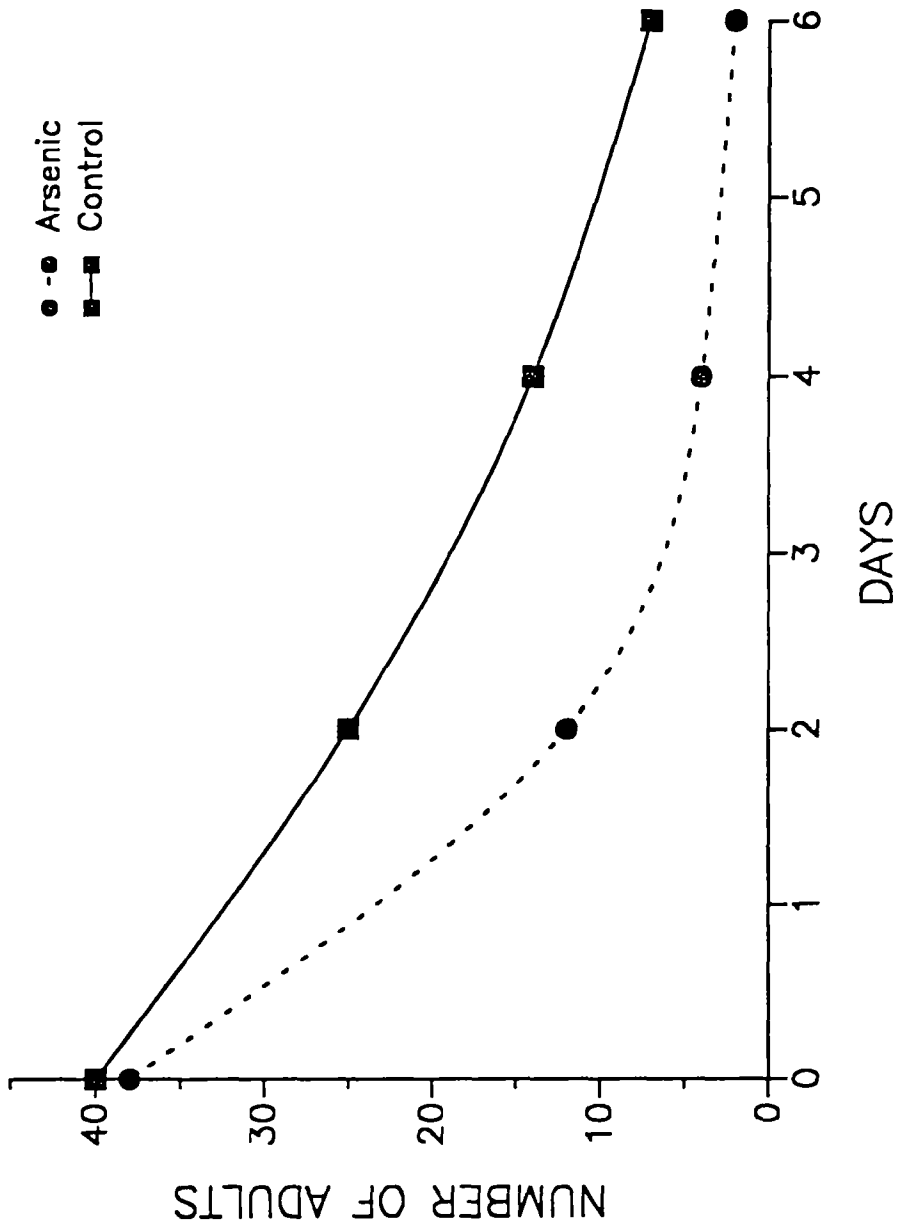


Figure 12. Adult *Acartia tonsa* survival during the fecundity study.

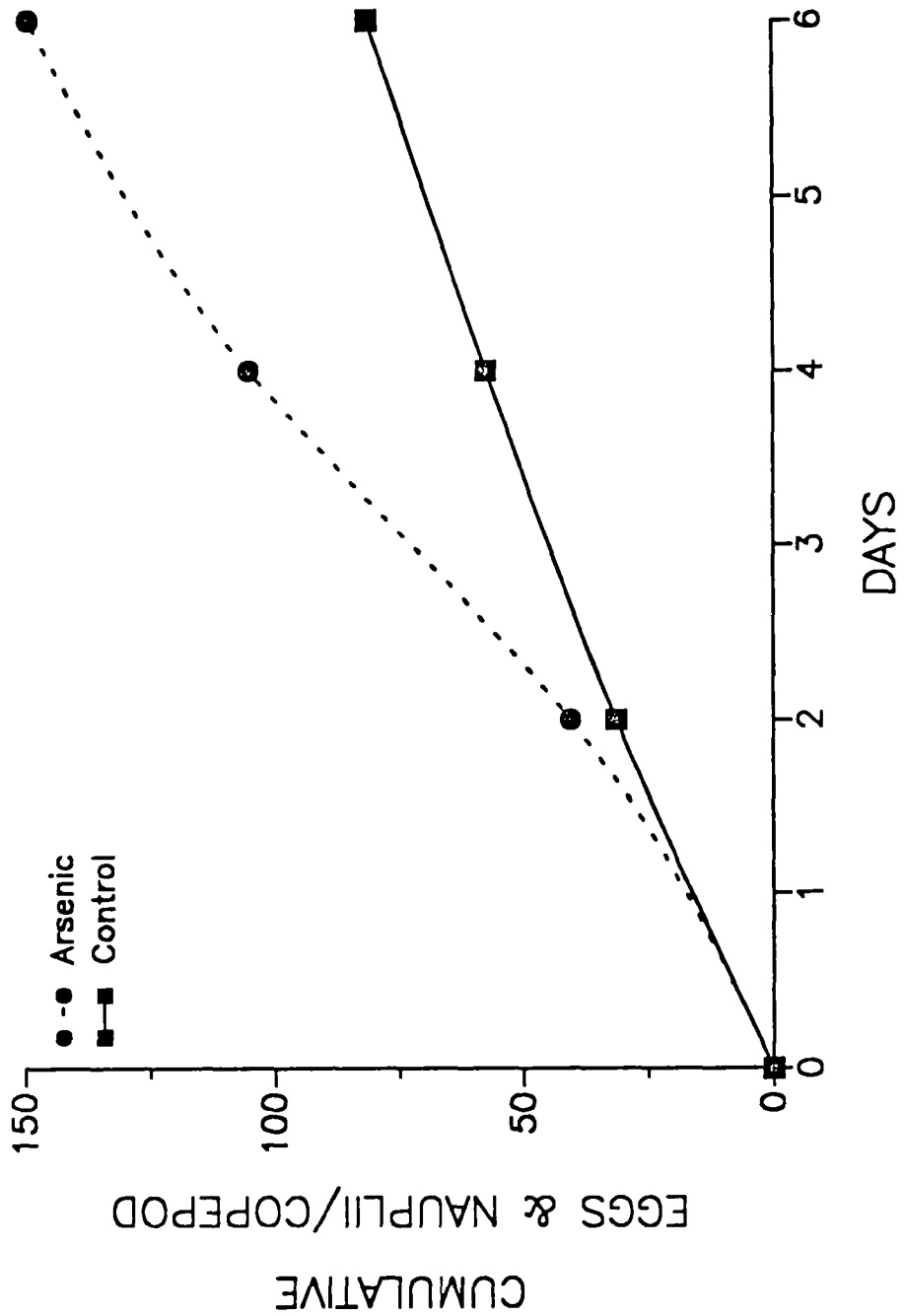


Figure 13. Fecundity (cumulative eggs + nauplii·copepod^{-1}·d^{-1}) of *Acartia tonsa*.

Table 8. Summary of *Acartia tonsa* fecundity. Egg and naupliar production by females fed from control and arsenic-dosed phytoplankton cultures.

Treatment	Production, eggs and nauplii·female $^{-1}$·d^{-1}			
	Day 0-2	Day 2-4	Day 4-6	Overall
Control				
Eggs	12.4	8 1	5.8	8.8
Nauplii	19 0	17.9	18.0	18.3
Arsenic-dosed				
Eggs	10.4	21.1	13.8	15.1
Nauplii	30.2	43.4	30.2	34.6

the fecundity study, the accumulative number of offspring per female copepod per day was 149 for the arsenic treatments and 81 for the controls.

During the fecundity study, phytoplankton biomass was similar for both the control and arsenic treatments. However, the species composition and dominance of the phytoplankton differed between the two treatments with the arsenic treatment dominated by <10 µm phytoplankton and the controls dominated by >10 µm phytoplankton. As adult copepods prefer cells >10 µm in diameter (Nival & Nival, 1976; Berman & Heinle, 1980; Ryther & Sanders 1980), this could explain the poor survival of the adult copepods in the arsenic treated cultures. Based on the same argument, reduced fecundity in the arsenic treatments would be expected but the opposite was found. Average microzooplankton densities were over 2 $\frac{1}{2}$ times higher in the arsenic relative to control tanks. As microzooplankton are within the preferred size range for copepod feeding and have been shown to be prey for copepods (Berk et al., 1977; Robertson, 1983; Stoecker & Sanders, 1985; Stoecker & Egloff, 1987), their greater presence might explain the higher fecundity in the arsenic treatments, but would also imply that adult survival rates should also be higher. It has been argued that microzooplankton are a better quality food than phytoplankton and it has been shown that their inclusion in the diet of *Acartia tonsa* results in enhanced egg production (Stoecker & Egloff, 1987). In an attempt to explain the discrepancy between the adult survival results and fecundity, it is possible that the higher quality food provided by the microzooplankton could have resulted in greater fecundity but perhaps lacked something essential for adult survival.

Zooplankton Development - Copepod development was rapid in the integrative experiment relative to the laboratory studies described earlier. Very few adults were produced in the laboratory study after six days. The integrative experiment was thus allowed to continue for eight days. The results (Table 9, Figure 14) suggest that most of the naupliar stages were second generation nauplii,.i.e. produced from

Table 9. Summary of *Acartia tonsa* development after 8 days when fed from control or arsenic-dosed phytoplankton cultures.

	Treatment	
Developmental Stage	Control	Arsenic-dosed
Adult-female	10.5	5
Adult-male	17	9
CV-female	0.5	1.5
CV-male	0.5	1.5
CIV	0	0
CIII	0	0 5
CII	1	0.5
CI	0	0
NVI	0	0
NV	74.6	4.3
NIV	146.7	23.0
NIII	427 4	123.9
NI&II	811.3	667.7
Eggs	1034	357.5
Eggs·female^{-1}	94.4	57.9

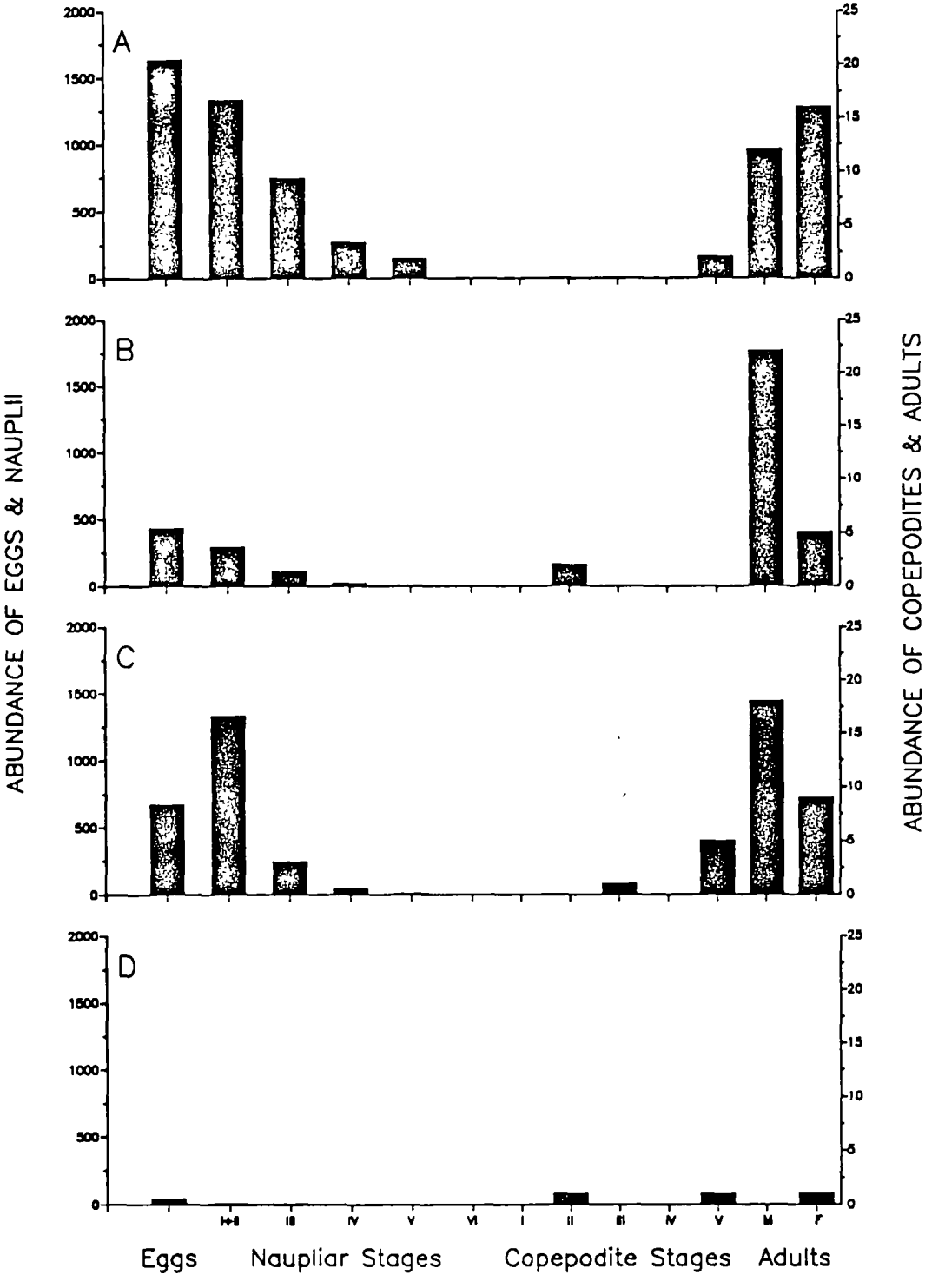


Figure 14. *Acartia tonsa* development in the two arsenic-dosed tanks and the two control tanks. A. Control tank 3. B. Control tank 4. C. Arsenic tank 2. D. Arsenic tank 1.

adults which had developed from the original nauplii. This suggestion is supported by the greater than 10 fold increase in the number of nauplii in two of the treatments, the paucity of copepodites, and the number of adults present in most treatments.

The average number of eggs per adult female copepod was depressed in the arsenic enclosures (58) relative to the controls (94). However, for the other life history stages, the copepods receiving effluent from control tanks and those receiving effluent from arsenic tank 2 developed well, whereas the populations receiving effluent from arsenic tank 1 developed poorly. *Thalassiosira pseudonana*, one of the arsenic resistant species which resulted in poor fecundity and development when offered as food to copepods in the laboratory studies, dominated the arsenic treated assemblages. The high densities of this species, particularly in arsenic tank 1, could explain the poor development found for this treatment.

Oyster Growth - Oyster growth was analyzed in terms of shell size and measured as the change in length perpendicular to the marked edge and as a change in shell area measured from photographs. Growth was analyzed separately for the first and second halves of the experiment and for the whole experiment.

Although there were some differences in results based on linear and areal estimates of growth, the major patterns were the same. Differences probably resulted from the more variable and less accurate linear measurements. The results of these analyses based on area are shown in Table 10, and several patterns can be seen:

1. No differences were found in mean growth between oysters feeding on effluent from the control tanks. This was true over all time periods and using both types of growth estimates.

Table 10. One-way ANOVA of oyster growth with growth measured as percent change in size (based on shell area measured from photographs). Data were transformed (arcsine square root) for analysis and Bonferoni *a posteriori* tests were used to compare means.

Sampling Period	Source	DF	SS	F	p
Weeks 0 - 2	Chamber	3	0.1493	5.09	0.0051
	Error	34	0 3323		
Weeks 2 - 4	Chamber	3	0 0878	6.97	0.0008
	Error	36	0 0126		
Weeks 0- 4	Chamber	3	0.0129	0.90	0.4489
	Error	34	0.0143		

Weeks 0 - 2

Treatment	Mean	Grp	
Arsenic 2	7.35		
Control 2	4.77		
Control 1	1.98		
Arsenic 1	1.29		

Weeks 2 - 4

Treatment	Mean	Grp	
Arsenic 1	12.0		
Control 1	10.4		
Control 2	9.7		
Arsenic 2	2.2		

Weeks 0 - 4

Treatment	Mean	Grp
Control 2	14.1	
Arsenic 1	13.9	
Control 1	12.2	
Arsenic 2	9.1	

2. Oyster feeding on effluent from tanks treated with arsenic exhibited significant differences between tanks and from control tanks during both sampling periods. These differences resulted principally from the effects of the effluent from arsenic tank 2. During the first half of the experiment oysters fed from this tank grew at the highest rate. However, during the second half of the experiment these same oysters grew at a rate significantly less than those in all other tanks.

3. Because growth rates switched between the two time periods, no significant differences were found between any of the treatments over the course of the whole experiment.

Barnacle Growth - The results for barnacle growth were similar to those found for oysters. As in the barnacle feeding experiment, barnacle growth was analyzed at the end of the experiment as a difference in mean area covered. A nested ANOVA was used with tanks nested within treatments.

As can be seen in Table 11 the mean growth of barnacles was reduced when fed the effluent from the arsenic-dosed cultures. However, as we found for the oysters, the reduced growth resulted mostly from the strong effects in arsenic tank 2 (Figure 15). Growth was severely reduced to approximately 30% of that found for barnacles feeding from other tanks.

It is interesting to note that oyster and barnacle growth, and densities of microzooplankton and the < 10 µm phytoplankton were lowest in arsenic tank 2 during the second half of the integrated experiment. The low numbers of small phytoplankton might explain the poor growth of all the other organisms examined. Alternatively, if either the oysters or barnacles were depending on the microzooplankton for an important part of their daily ration, then the low densities of microzooplankton might explain the lower growth in these benthic filter feeders.

Table 11. Nested ANOVA of barnacle growth in the integrative experiment. Bonferoni's *a posteriori* test was used to compare mean growth in mm^2 among treatments and among chambers.

Source	DF	SS	F	p
Main Variable				
Live/Dead at Wk 4	1	11172	63.19	0.0001
Treatment	1	5584	31.58	0.0001
Nested Variables				
Tank in Treatment	2	20375	57 67	0.0001
Error	1234	218188		

By Treatment

Source	Mean	Grp
Control	14.74	|
Arsenic	9.97	|

By Chamber

Source	Mean	Grp
Arsenic 1	16.83	|
Control 2	16.23	| |
Control 1	13.80	|
Arsenic 2	5.01	|

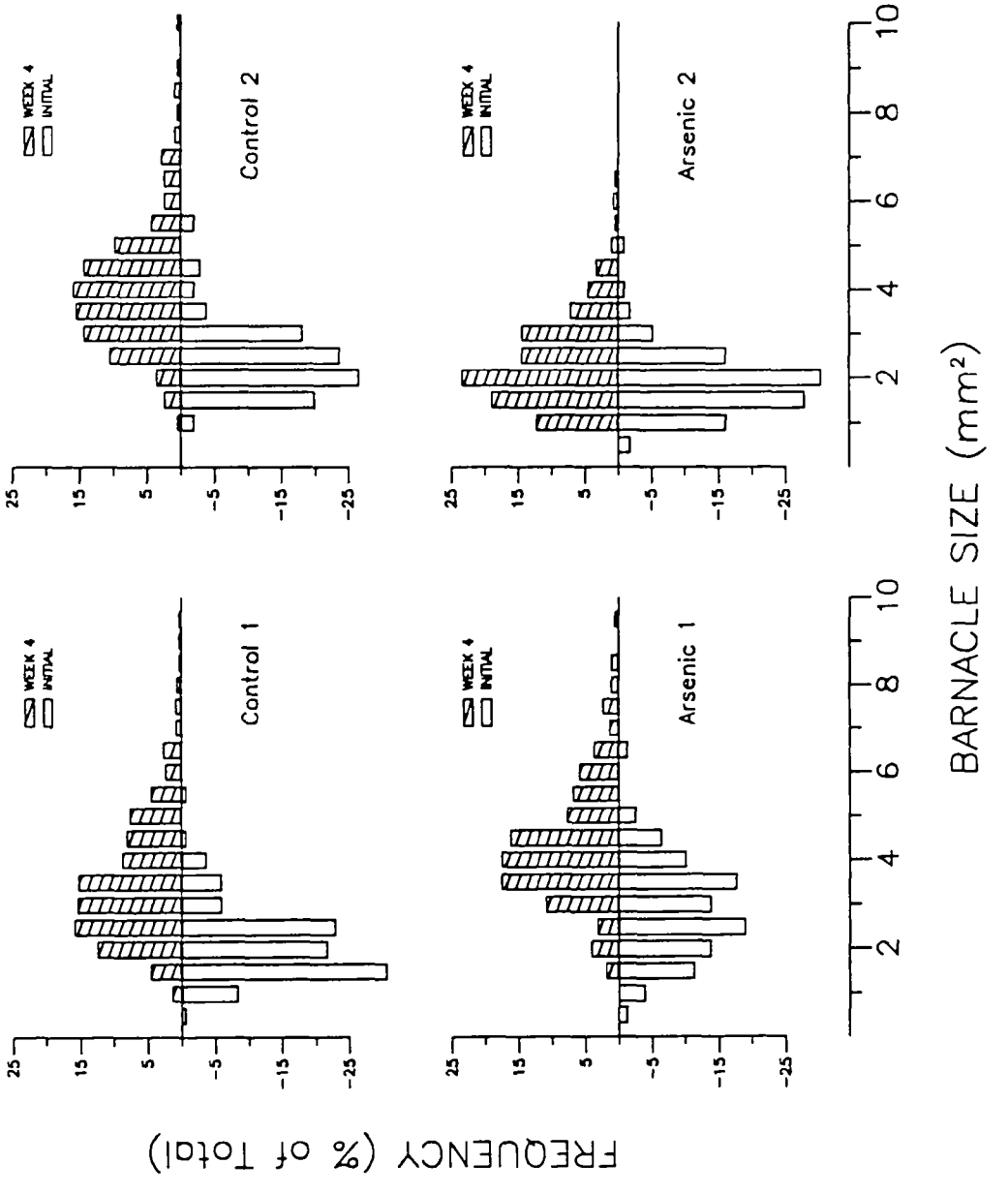


Figure 15. Initial and final size distributions of barnacles in the Integrative experiment. Data for each treatment tank shown separately. Size categories are in 0.5 mm^2 intervals.

ARSENIC INCORPORATION AND TRANSFER

As noted in previous research (Sanders et al., 1987), there was little uptake of dissolved arsenic by trophic levels other than phytoplankton; however, the potential exists for arsenic uptake through trophic relationships. The arsenic content of the biota in the barnacle and adult oyster experiments was determined at the end of each experiment. In addition, the potential for arsenic transfer during zooplankton feeding was also examined.

Zooplankton

Adult *E. affinis* showed a small incorporation of arsenic when fed arsenic-contaminated phytoplankton. Overall, the arsenic content of *E. affinis* significantly increased to an average of 11.18 $\pm$ 0.86 $\mu g \cdot g^{-1}$ in organisms fed contaminated food, as compared to an arsenic content of 8.91 $\pm$ 0.53 $\mu g \cdot g^{-1}$ in organisms fed control algae. In all but one instance (those fed *Isochrysis* sp.), an increase was noted (Figure 16). The average increase, 25%, was far less than the average increase in arsenic content of the phytoplankton, an increase of 213%, from 5.66 to 17.69 $\mu g\ g^{-1}$. All species of phytoplankton exhibited an increase of at least 93% (Figure 16).

Barnacles

Barnacles also exhibited significant increases in arsenic content when fed arsenic-contaminated phytoplankton, larger in magnitude than the increases noted when they were exposed to elevated dissolved arsenic concentrations (Sanders et al., 1987). It was noted in this previous work that barnacle analyses were performed on the total organism, shell and tissue; thus, it was not possible to separate tissue incorporation from adsorption to shell. Additional samples from an earlier experiment with adult barnacles (Sanders et al., 1987) were analyzed using both an acid digestion to remove shell-associated arsenic and a dry ash technique to determine total arsenic. When these additional analyses were performed, it was clear that the arsenic uptake that occurred as a result of elevated dissolved arsenic

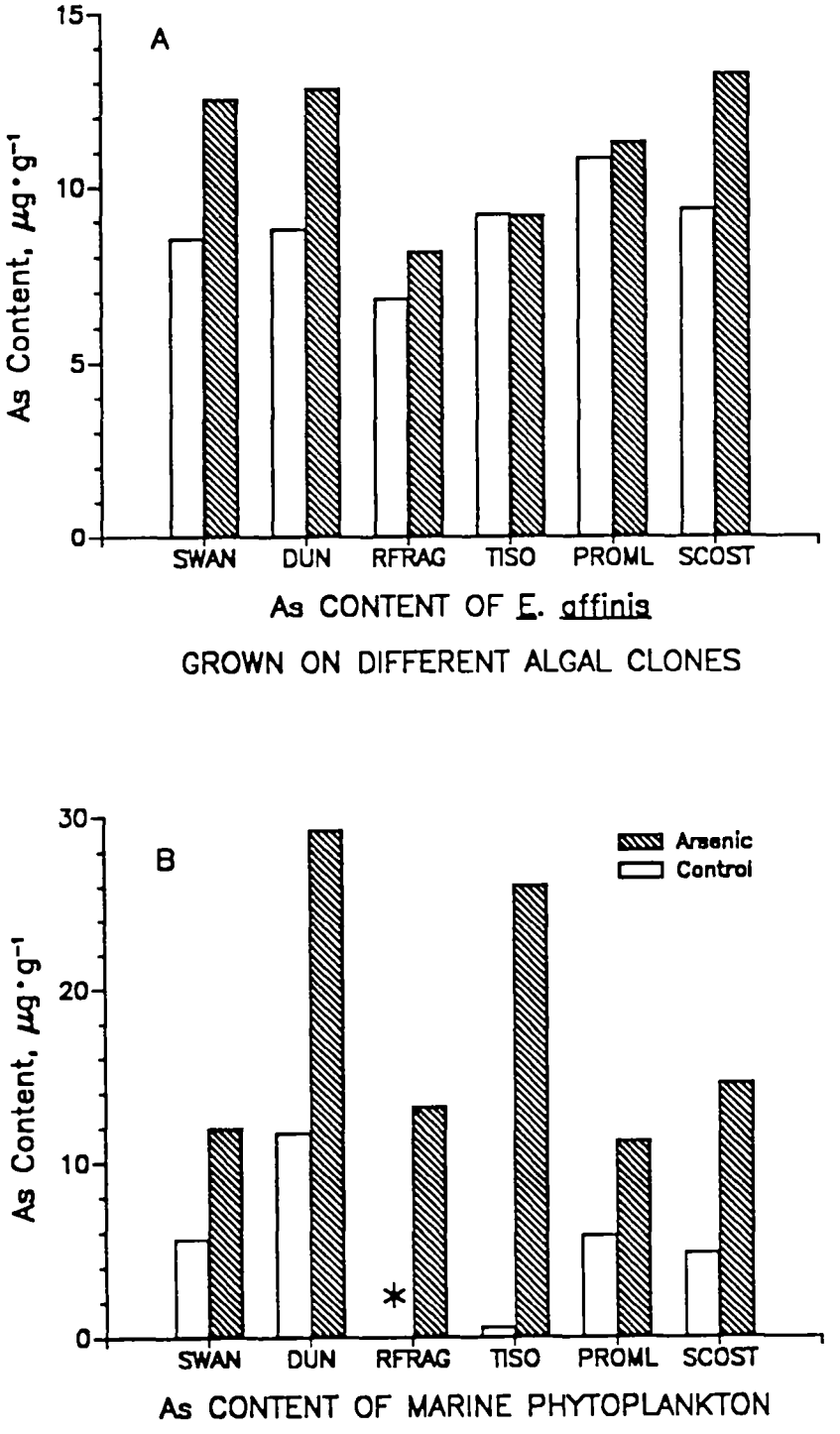


Figure 16. Arsenic content of zooplankton fed control and arsenic-contaminated phytoplankton. A. Zooplankton B. Arsenic content of phytoplankton.

concentrations was associated with shell material; no arsenic was incorporated in tissue (Figure 17).

The young barnacles fed clean and arsenic-contaminated phytoplankton exhibited a large increase in arsenic concentration, from 0.34 ± 0.11 $\mu g \cdot g^{-1}$ to 1.73 ± 0.09 $\mu g \cdot g^{-1}$, with barnacles feeding on individual species and combined algal species showing similar arsenic incorporation (Figure 18). As noted in zooplankton uptake studies, this increase was much less than the increase in arsenic content noted in the two species of phytoplankton on which they were fed (Figure 18). Because of the very small sample size, we were not able to distinguish between incorporation in tissue or shell material; concentrations shown are for the total organism.

Barnacles grown on effluent from the outdoor phytoplankton cultures also exhibited a similar increase in arsenic content: an increase from 0.34 ± 0.12 $\mu g \cdot g^{-1}$ in controls to 2.11 ± 0.7 $\mu g\ g^{-1}$ in those fed arsenic-contaminated foods (Figure 19). In this experiment, we were able to separate arsenic incorporated in tissue from that associated with shell material. Although the arsenic in the shell increased slightly, from 0.04 to 0.16 $\mu g \cdot g^{-1}$, most of the increase was in tissue content of arsenic, which increased from 0.30 $\mu g \cdot g^{-1}$ in controls to 1.95 $\mu g \cdot g^{-1}$ in barnacles feeding on arsenic-dosed effluent (Figure 19).

Oysters

Adult oysters exposed to arsenic-contaminated food also exhibited significant increases in arsenic content of tissues from 5.30 ± 0.43 $\mu g\ g^{-1}$ in oysters fed control phytoplankton to 8.16 ± 0.44 $\mu g \cdot g^{-1}$ in oysters fed contaminated phytoplankton (Figure 20). Analysis of shell material did not indicate large arsenic uptake. Old and new shell material averaged 0.38 and 0.19 $\mu g\ g^{-1}$, respectively, in arsenic-dosed tanks and 0.13 and 0.12 $\mu g \cdot g^{-1}$, respectively, in control tanks. Although slightly higher in arsenic-dosed tanks, the variability of arsenic in all samples was quite high and the samples were near the limit of detection of the method.

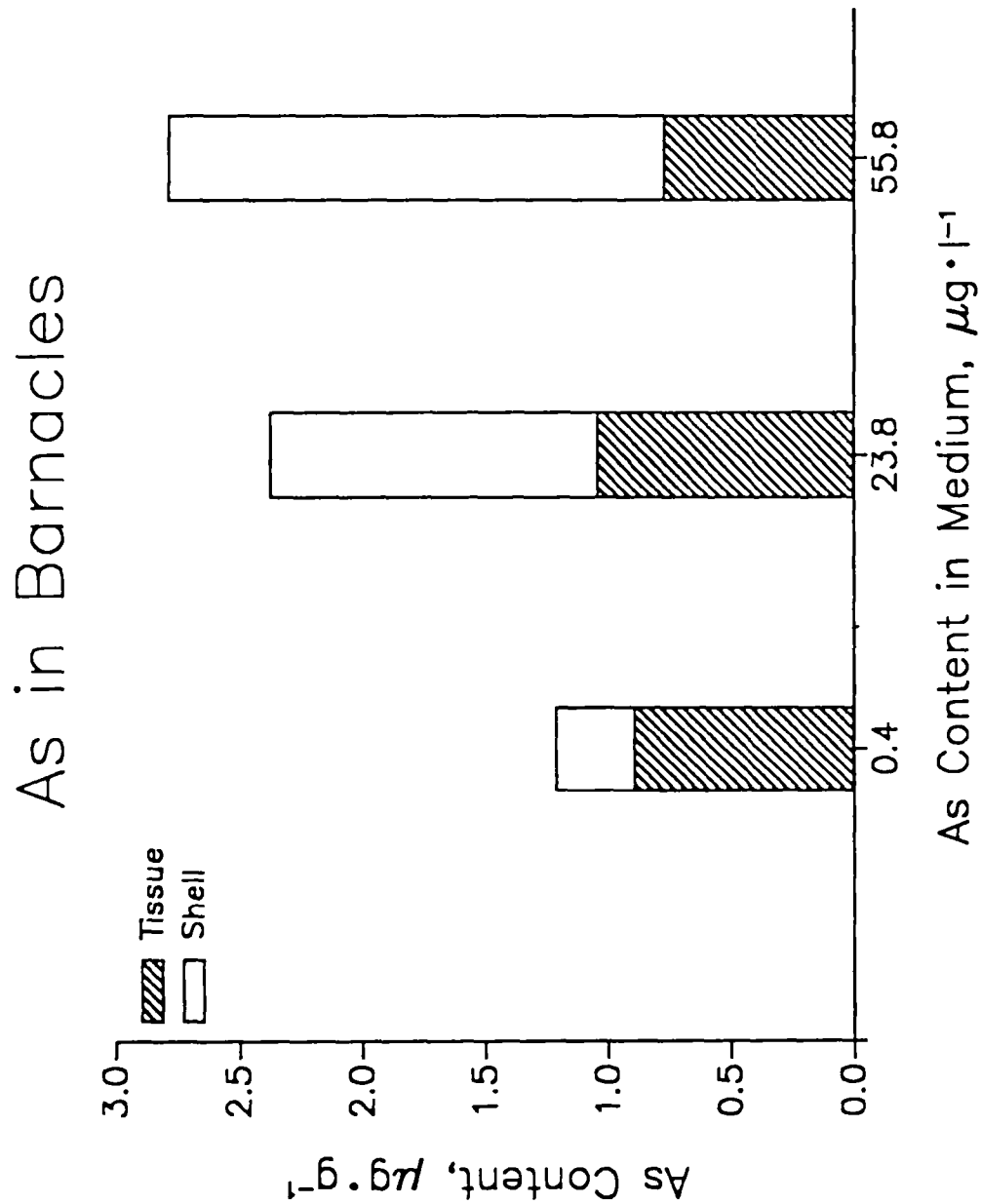


Figure 17. Arsenic content of the shell and tissue of barnacles exposed to various dissolved arsenic concentrations.

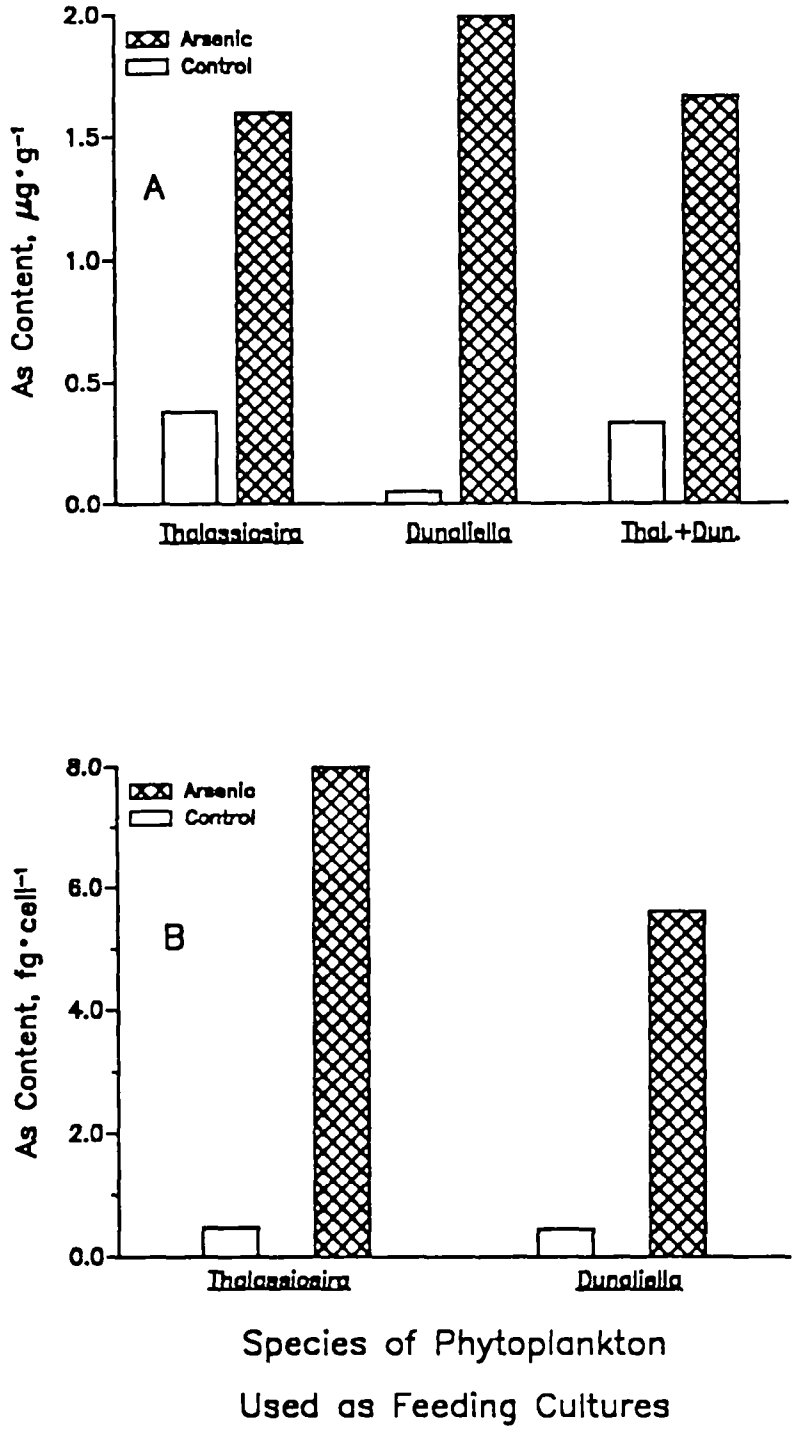


Figure 18. Arsenic content of barnacles fed control and arsenic-contaminated phytoplankton. A. Arsenic content of barnacles. B. Arsenic content of phytoplankton.

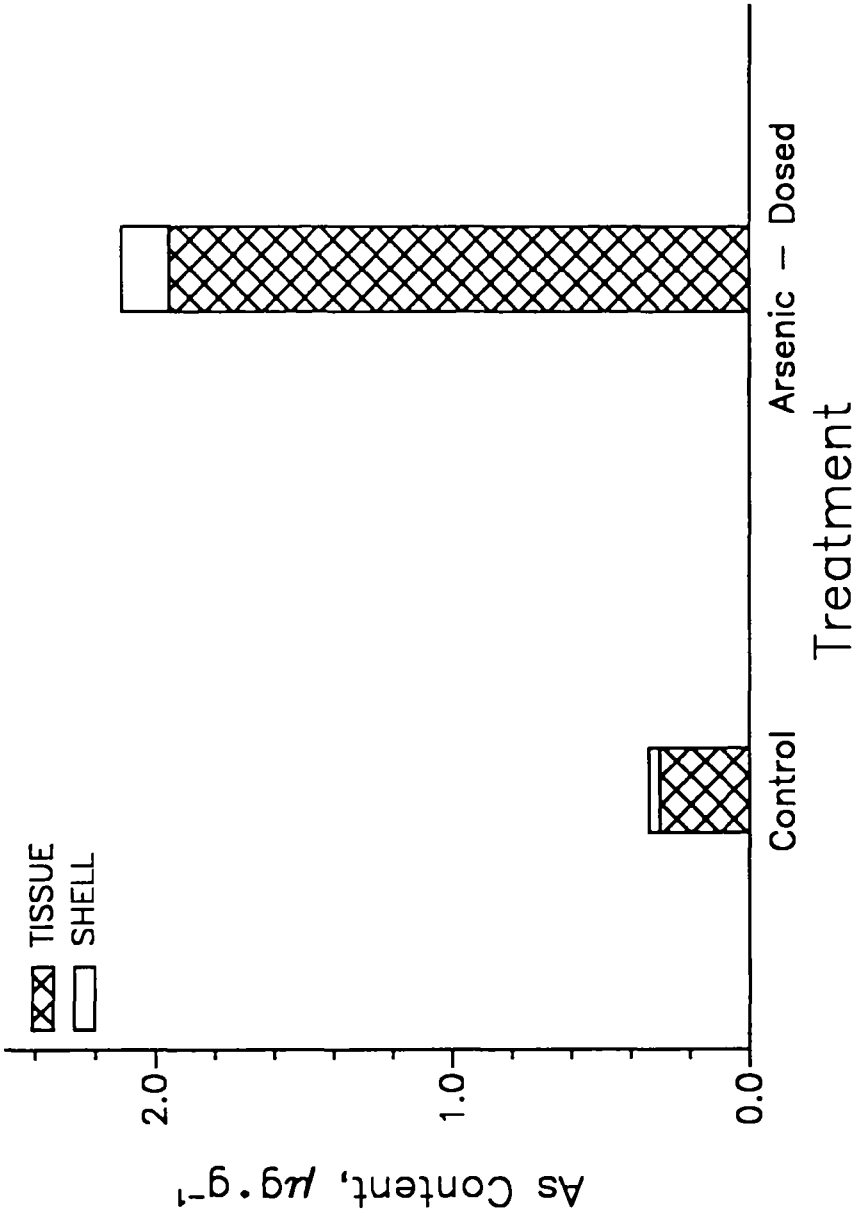


Figure 19. Arsenic incorporation by barnacles feeding on control and arsenic-dosed phytoplankton during the Integrative experiment.

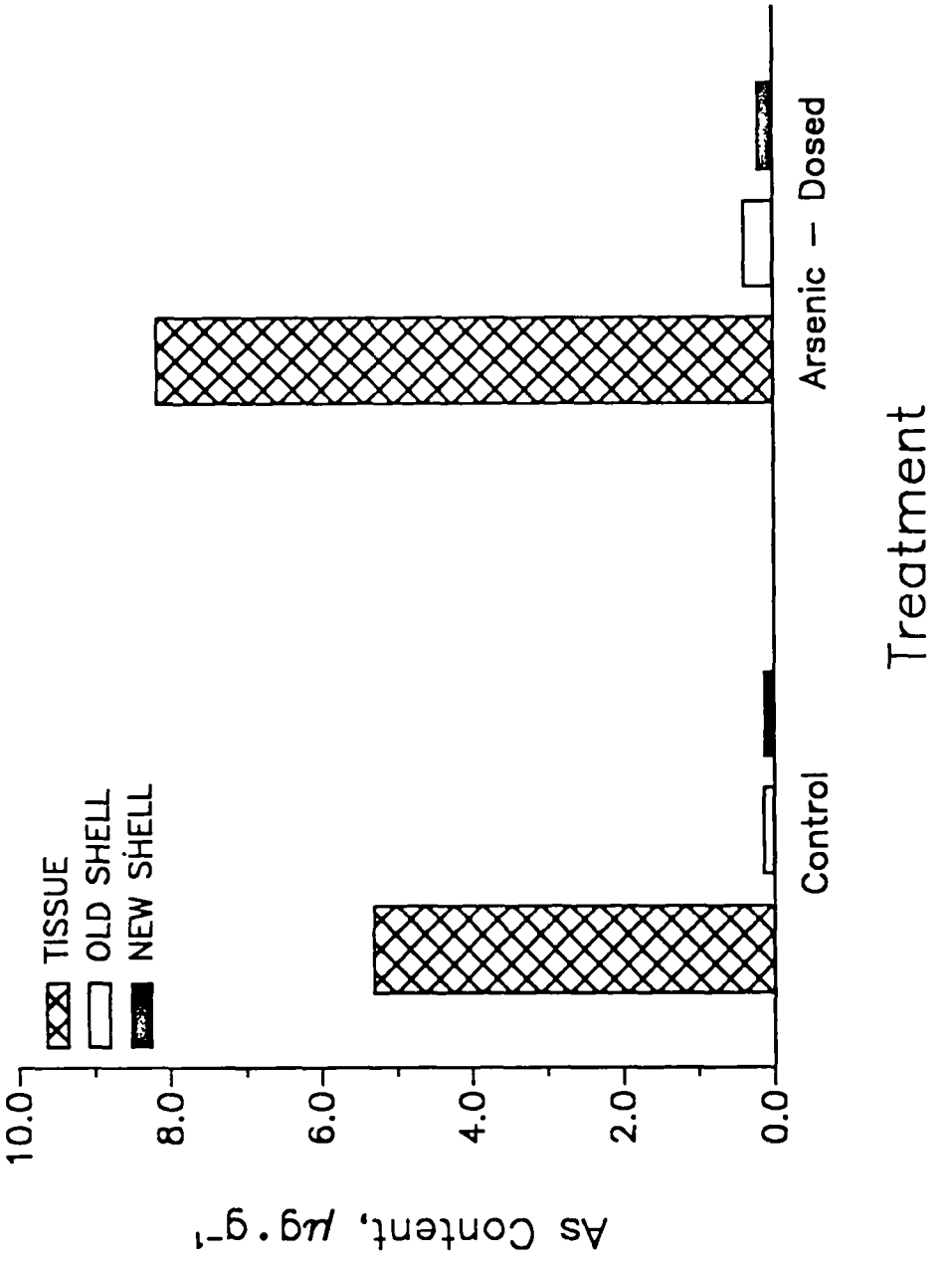


Figure 20. Arsenic incorporation by oysters (tissue, shell, new bill) feeding on control and arsenic-dosed phytoplankton during the Integrative experiment.

DISCUSSION

Throughout this study and its predecessor (Sanders et al., 1987), the response of estuarine organisms has been largely as originally hypothesized. Phytoplankton, particularly centric diatoms, exhibited declines in growth rate upon exposure to arsenic, leading to changes in the species composition of the assemblage. In different experiments, different centric diatoms were sensitive to arsenate: *Cerataulina pelagica*, *Chaetoceros debile*, and *Rhizosolenia fragilissima* are most notable. In all cases, however, the growth of one small centric, *Thalassiosira* sp., has been greatly accelerated by arsenate dosing. The increase in growth rate and strong dominance exhibited by *Thalassiosira* sp. is not likely to be a reaction to the arsenic itself, but rather a reaction to reduced competition with other, inhibited dominant species. This particular pattern of replacement of large centric diatoms by the small *Thalassiosira* sp. has been observed throughout experiments with arsenate and Chesapeake Bay phytoplankton (Sanders & Cibik, 1985; Sanders, 1986; Sanders et al., 1987). In the experiment discussed here and in previous studies, the increase in *Thalassiosira* sp. did not lead to elevated biomass levels (Figure 9); rather the species replaced other dominants.

Other trophic levels, however, exhibited relative resistance to dissolved arsenic. Only the barnacle, *Balanus improvisus*, exhibited any sensitivity to dissolved arsenic in earlier studies, and although significant, the reduction in growth rate was extremely small, less than 6%. Such a reduction is unlikely to have ecological significance.

Arsenic incorporation by the various trophic levels followed a similar pattern: with the exception of the phytoplankton there was no incorporation of dissolved arsenic by the tissues of estuarine organisms. Again, these findings were strengthened by earlier work in the United States with *Crassostrea* (Zaroogian &

Hoffman, 1982) and in the Baltic Sea in experimental microcosms containing a large variety of intertidal and subtidal organisms (Notini & Rosemarin, 1986). In both the Baltic Sea experiments and in our studies, the only arsenic incorporation was sorption to calcareous shell material. Our conclusion at the end of the previous study was that direct exposure of animals to dissolved arsenic, even at high levels sometimes found in heavily impacted areas, was not likely to cause harm; only the plants within the estuary are at risk (Sanders et al., 1987). The results of this study support this conclusion. However, as we theorized, there are other, less direct pathways for impact within the estuarine ecosystem. We hypothesized that, because of the high degree of arsenic incorporation within the phytoplankton and the potential for significant shifts in phytoplankton species composition, estuarine animals may be affected considerably, either from ingestion of arsenic indirectly from their food and/or from alteration of feeding relationships. The results of this study support this hypothesis, but, at the same time, underscore the considerable complexity of the system (and make us realize how little we really understand). Arsenic contained within food, unlike dissolved arsenic, appeared to be incorporated by all organisms. Although not concentrated to the extent seen in marine algae (Sanders, 1978; 1979; 1980), all species tested had elevated arsenic concentrations. Such a result has been seen in an earlier, integrated study of arsenic impact in the intertidal Baltic Sea (Rosemarin et al., 1985). In those experiments, organisms feeding on arsenic-contaminated macroscopic algae (*Fucus*) had elevated arsenic content of similar magnitude to our results. The levels of arsenic incorporation are not large, however. Only the juvenile barnacles exhibited high levels of arsenic incorporation; other species increased their arsenic content by only 25-50%.

In addition to arsenic incorporation, we have shown that some estuarine organisms can be inhibited by arsenic within the food chain. Zooplankton showed strong reductions in survival and fecundity when fed an altered phytoplankton

assemblage and even larger reductions when fed an altered, arsenic-contaminated assemblage. Barnacles showed a similar reduction in growth when fed arsenic-contaminated algae; however, their response to altered species composition was considerably more complex and less well understood. Indeed, it appears that zooplankton and barnacles react quite differently to the same dominant alga, *Thalassiosira* sp.: zooplankton were strongly inhibited, barnacles did quite well. Clearly, we do not yet understand feeding relationships for these important species.

In contrast, oysters were not at all affected in these experiments. Oysters, although they incorporated significant quantities of arsenic contained in food species, showed no change in growth, either in response to altered diet or to arsenic-contaminated food.

The large differences in response of the various organisms is quite interesting. It is our belief that the varying response is caused by the variable ability of these organisms to select food. The mechanisms, degree, and importance of particle selection by oysters are still poorly understood and under debate (Kennedy & Breisch, 1981). It appears that oysters can grow successfully on a wide variety of different diets (assuming that concentrations are sufficient), being able to collect and assimilate small phytoplankton species; perhaps down to 3 μm in size (Roger Newell, University of Maryland, personal communication), as well as microzooplankton over 100 μm in size (Krsinic, 1987). In this sense, then, oysters may be relatively non-selective feeders. Copepods, on the other hand, have been shown to select prey based upon both size (Parsons et al.,1969; Berman & Heinle, 1980; Ryther & Sanders, 1980) and shape or species composition (Provasoli, 1977). In addition, they appear to be much less efficient at capturing small phytoplankton less than 7-10 μm in size (Nival & Nival, 1976; Berman & Heinle, 1980; Ryther & Sanders, 1980). Thus, arsenic-induced changes in phytoplankton species composition might be expected to affect *Acartia* to a greater extent than *Crassostrea*.

Microzooplankton densities and species composition also appeared to be affected more by changes in phytoplankton size distribution and species composition than by the presence of arsenic in the culture tank experiments. Most microzooplankton prefer small (<10 µm) phytoplankton cells (Heinbokel, 1978; Capriulo, 1982; Rassoulzadegan, 1982), and thus responded in general with increased numbers in the arsenic treatments which contained increased numbers of small phytoplankton. In fact, the large discrepancy between microzooplankton densities in the two arsenic tanks during the second half of the experiment might be explained by the difference in abundance of small phytoplankton, because one arsenic tank contained elevated phytoplankton densities as well.

Balanus remains somewhat of an enigma. Barnacles, as oysters, can capture and ingest a wide range of particle sizes. Although Crisp & Southward (1961) found that 30 µm particles were the smallest that could be retained by barnacle cirral nets, fine particles as small as 1-2 µm can be captured and ingested (Southward 1955a, b; Barnes, 1959). On the other hand, barnacles are capable of capturing larger particles such as nauplii and are likely to expand the size range of particles they capture as they grow. This potential shift in preferred food makes the effects of arsenic-induced changes in algal species composition quite complex for this species. However, we used newly attached *Balanus* in our experiments, and our results suggest that these newly recruited individuals can ingest and may even prefer small diatoms over flagellate species of any size. In addition, the high densities of microzooplankton, a potential food item, in one arsenic treatment tank help explain the unusual barnacle results. It is interesting that arsenic tank 1 contained higher densities of small phytoplankton which may have stimulated microzooplankton growth, leading to elevated microzooplankton densities. These elevated densities, in turn, may have stimulated barnacle growth in this tank.

The potential complexity of the effects of a pollutant within even a simple trophic system, is demonstrated by the integrative experiment. The arsenic-induced bloom of *Thalassiosira* during the first two weeks resulted in increased oyster growth, increased microzooplankton densities, but reduced zooplankton survivorship and fecundity. In the second two weeks, no differences in phytoplankton species were obvious but microzooplankton densities clearly diverged in the two arsenic tanks while the controls remained intermediate. Both barnacles and oysters showed similar reduced growth in arsenic tank 2. Additionally, as with the microzooplankton, barnacles exhibited increased growth in arsenic tank 1. Thus, it would appear that the benthic suspension feeders were responding to arsenic-induced changes in the phytoplankton and the microzooplankton which were responding in part to changes in the phytoplankton. However, changes were not simple and the two arsenic-dosed trophic systems did not respond in the same way, but within each system the changes were consistent between trophic levels.

A number of investigators have been studying the intertidal, *Fucus vesiculosus*-based ecosystem of the Baltic Sea (Rosemarin et al., 1985; Notini & Rosemarin, 1986; Notini et al., 1988; Rosemarin & Notini, 1988). These mesocosm studies demonstrated that *Fucus*, as with phytoplankton, is greatly inhibited by low levels of arsenic (7 $\mu g \cdot l^{-1}$) and completely eliminated by higher levels (75 $\mu g \cdot l^{-1}$). Inhibition and elimination of the *Fucus* caused major structural shifts in algal and invertebrate communities and dramatically reduced net ecosystem production.

In contrast to our studies of a plankton-based ecosystem in which trophic pathways are quite complex, the Baltic Sea ecosystem is a straight-forward food chain, based upon the macroalga, *Fucus*. Thus, the large, significant effects seen in such a system are predictable. In our experiments, the effects at the primary trophic level were exactly as in the Baltic: loss of sensitive species and replacement by less desirable species. However, at secondary levels, the response in our studies was less

clear-cut, presumably caused by higher tropic levels switching between a variety of food types. The mechanisms of trophic transfer and carbon cycling and feeding relationships is an area receiving a great amount of attention at present (see Verity, 1987; Lessard et al., 1987 for examples) in the Chesapeake Bay and in other coastal ecosystems. As our understanding of such feeding relationships improves in coming years, our ability to predict indirect toxic effects involving food web alterations will improve as well.

Clearly, alteration of trophic pathways is potentially a very important component of pollution. The arsenic-induced shifts in the abundances of dominant phytoplankton species had significant effects on higher trophic levels, as did the ingestion of arsenic-contaminated food. That these effects were very complex and differed greatly between the species investigated is important. Equally important is the potential ability to predict these effects from existing knowledge of the biology and feeding relationships of important species. In the case of the three tested species, their relative abilities to select and utilize different algal species would seem to be the principal cause of the observed differences in the effects of arsenic. If such an event can be generalized to other species, to the extent that we understand the above selectivity, then we can model and predict arsenic impact. However, the results of the integrative experiment underscore both the complexity and variability of the natural system: seemingly small changes in phytoplankton species composition may result in larger fluctuations in microzooplankton densities, leading to large differences in impact in larger animals. We are still not at the point of confident predictions at the ecosystem level. However, the model systems tested here provide an important first step toward such predictions. We were able to perform only one integrative experiment because of fiscal constraints, yet we were able to predict many of the results. With refinements, and as our understanding of carbon transfer within the Chesapeake Bay improves, our predictions will improve.

In addition, the use of such systems points out clearly where further information about the ecosystem needs to be gathered. This aspect is of great value in identifying future research needs.

The use of regulated, model systems such as these should be continued. The type of experiments performed here, using natural phytoplankton assemblages maintained under realistic environmental conditions, can provide community-level information. However, it is important that experiments such as these are carried out over the annual cycle because of seasonal variability of phytoplankton communities and variability in environmental characteristics of the estuary. In addition, other contaminants will behave differently from arsenic. Arsenic was chosen as a model contaminant for this research because, as a nutrient analogue, it reacts with the lowest trophic levels. Other elements and organic compounds will have differing geochemistries and necessarily different biological reactivity (Sanders & Riedel, 1987b). However, the approach and methods that are used here are applicable to virtually any dissolved contaminant. We recommend that several more experiments be performed using the same techniques as the integrative experiment. These experiments would build on the knowledge and understanding gained within our first experiment. At least one experiment should be performed with arsenic; others should be performed with other contaminants for which we have adequate background information (e.g., copper).

In addition, we need to continue to develop new ways to examine how communities and ecosystems respond to chemical stress. Another method for determining disturbance in an algal community may be a concept termed the pollution induced community tolerance (PICT) (Blanck et al., 1987). This suggests that a phytoplankton community, if disturbed by a pollutant, will respond by developing an increased tolerance to that pollutant through the replacement of sensitive species by more resistant ones. This can result either through selection for

tolerant genotypes of the same species or a shift in species composition toward resistant forms of other taxa. By itself, this concept allows simple, inexpensive determinations of community disturbance and may be very useful in future assessment of pollutant impact. Used in conjunction with the methods employed in this study, PICT may provide important resolution into the mechanisms of community shifts in the phytoplankton.

These two approaches by no means are the sole mechanisms available for determining pollution-induced stress. There are others, and more will be introduced with time. However, these techniques cannot stand alone. After a determination of disturbance has been made, it is critical that we evaluate its effects and consider whether in the context of the ecosystem the disturbance is likely to have a negative impact. The application of new and innovative research tools permits such evaluations to rely more on scientific judgement and less on guesswork.

REFERENCES

Andreae, M. O. 1978. Distribution and speciation of arsenic in natural waters and some marine algae. Deep-Sea Res. 25:391-402.

Antia, N.J., C.D. McAllister, T.R. Parsons, K. Stevens and J.D.H. Strickland. 1963. Further measurements on primary production using a large volume plastic sphere. Limnol. Oceanogr. 8:166-184.

Barnes, H. 1959. Stomach content and micro-feeding of some common cirripedes. Can. J. Zool.: 37:231-236.

Bartram, W. C. 1980. Experimental development of a model for the feeding of neritic copepods on phytoplankton. J. Plankton Res. 3:25-51.

Berk, S.G., D.C. Brownlee, D.R. Heinle, H.J. Kling and R.R. Colwell. 1977. Ciliates as a food source for marine planktonic copepods. Microb. Ecol. 4:27-40.

Berman, M. S. and D. R. Heinle. 1980. Modifications of the feeding behavior of marine copepods by sub-lethal concentrations of water-accommodated fuel oil. Mar. Biol. 56:59-64.

Bieri, R., O. Bricker, R. Byrne, R. Diaz, G. Helz, J. Hill, R. Huggett, R. Kerhin, M. Nichols, E. Reinharz, L. Schaffner, D. Wildung and C. Strobel. 1982. Toxic substances, p. 263-375. *In* E. G. Macalaster, D. A. Barker, and M. Kasper (eds.), Chesapeake Bay Program technical studies: a synthesis. EPA, Washington, D.C.

Blanck, H., S-A. Wangberg, and S. Molander. 1987. Pollution-induced community tolerance (PICT) - a new ecotoxicological tool. *In* Tenth symposium on aquatic toxicology, ASTM, in press.

Boyle, E. A. and S. Huestedt. 1983. Aspects of the surface distributions of copper,

nickel, cadmium, and lead in the North Atlantic and North Pacific, p. 379-394. *In* C. S. Wong, E. Boyle, K. W. Bruland, J. D. Burton, and E. D. Goldberg (eds.). Trace metals in sea water. Plenum Press, New York.

Braman, R. S., D. L. Johnson, C. C. Foreback, J. M. Ammons and J. L. Bricker. 1977. Separation and determination of nanogram amounts of inorganic arsenic and methylarsenic compounds. Anal. Chem. 49:621-625.

Brownlee, D.C. and F. Jacobs. 1987. Mesozooplankton and microzooplankton in the Chesapeake Bay, p. 217-269. *In* S.K. Majumdar, L.W. Hall, Jr., and H.M. Austin (eds.), Contaminant problems and management of living Chesapeake Bay resources. Pennsylvania Acad. Sci., Easton, PA.

Cairns, J. 1986. Community toxicity testing. ASTM publication 920, Philadelphia, PA.

Capriulo, G. M. 1982. Feeding of field collected tintinnid microzooplankton on natural food. Mar. Biol. 71:73-86.

Crisp, D. J. and A. J. Southward. 1961. Different types of cirral activity of barnacles. Phil. Trans. Roy. Soc. B 243:271-308.

Dayton, P. K. 1975. Experimental evaluation of ecological dominance in a rocky intertidal algal community. Ecol. Monog. 45:137-159.

D'Elia, C. F., J. G. Sanders, and W. R. Boynton. 1986. Nutrient enrichment studies in a coastal plain estuary: phytoplankton growth in large-scale, continuous cultures. Can. J. Fish. Aquat. Sci. 43:397-406.

Förstner, U. and G.T.W. Wittmann. 1983. Metal pollution in the aquatic environment. Springer-Verlag, Berlin.

Grice, G.D. 1984. Use of enclosures in studying stress on plankton communities, p. 563-574. *In* H.H. White (ed.), Concepts in marine pollution measurements. MD Sea Grant publication UM-SG-TS-84-03.

Grice, G. D. and M. R. Reeve. 1982. Introduction and description of experimental

ecosystems, p. 1-10. *In* G.D. Grice and M.R. Reeve (eds.), Marine mesocosms. Springer-Verlag, New York.

Heinbokel, J. R. 1978. Studies on the functional role of tintinnids in the Southern California Bight. I. Grazing and growth rate in laboratory cultures. Mar. Biol. 47: 191-197.

Heinle, D. R. 1966. Production of a calanoid copepod, *Acartia tonsa*, in the Patuxent River estuary. Chesapeake Sci. 7:59-74.

Hendrickson, P., K. G. Sellner, B. Rojas de Mendiola, N. Ochoa and R. Zimmermann. 1982. The composition of particulate organic matter and biomass in the Peruvian upwelling region during ICANE 1977 (Nov. 14-Dec. 2). J. Plankton Res. 4:163-186.

Keith, L. H. and W. A. Telliard. 1979. Priority pollutants. I--A perspective view. Environ. Sci. Technol. 13:416-423.

Kennedy, V. S. and L. L. Breisch. 1981. Maryland's oysters: research and management. Univ. of Maryland Sea Grant Publication TS-81-04. 286 pp.

Krsinic, F. 1987. On the ecology of tinitnnines in the Bay of Mali Ston (eastern Adriatic). Est. Coast. Shelf Sci. 24:401-418.

Landry, M. R. 1977. A review of important concepts in the trophic organization of pelagic ecosystems. Helgol. wiss. Meeres. 30:8-17.

Lessard, E.J., D.A. Caron, K. Ho and M.A. Voytek. 1987. Grazing impact and food selection of nano-,micro-, and macrozooplankton in natural estuarine communities. Trans. Amer. Geophys. Union 68:1782.

Lund, J. W. G., C. Kipling, and E. D. LeCren. 1958. The inverted microscope method of estimating algal numbers and the statistical basis of estimations by counting. Hydrobiologia 11:143-170.

McAllister, C.D., T.R. Parsons, K. Stevens and J.D.H. Strickland. 1961.

Measurements of primary production in coastal sea water using a large volume plastic sphere. Limnol. Oceanogr. 6:237-259.

Mackenzie, F. T., R. J. Lantzy, and V. Paterson. 1979. Global trace cycles and predictions. Math. Geol. 11:99-144.

Martin, J-M. and M. Whitfield. 1983. The significance of the river input of chemical elements to the ocean, p. 265-296. *In* C. S. Wong, E. Boyle, K. W. Bruland, J. D. Burton, and E. D. Goldberg (eds.). Trace metals in sea water. Plenum Press, New York.

Menzel, D.W. and J. Case. 1977. Concept and design: controlled ecosystem pollution experiment. Bull. Mar. Sci. 27:1-7.

Nival, P. and S. Nival. 1976. Particle retention efficiencies of an herbivorous copepod, *Acartia clausi* (adult and copepodite stages): Effects on grazing. Limnol. Oceanogr. 21:24-28.

Notini, M. and A. Rosemarin. 1986. Fate and effects of arsenic in a mesoscale model ecosystem. Status report prepared for the ESTHER Project, Swedish Environmental Research Group, Karlskrona, Sweden, January.

Notini, M., K. Holmgren and A. Rosemarin. 1988. Long-term fate and effects of low-levels of arsenate on the Baltic Sea *Fucus vesiculosus* ecosystem. *In* Proceedings, 21st european marine biology symposium, in press.

Osman, R. W. 1977. The establishment and development of a marine epifaunal community. Ecol. Monog. 47:37-63.

Osman, R. W. 1982. Artificial substrates as ecological islands, pp. 71-114. *In* J. Cairns (ed.), Artificial Substrates. Ann Arbor Science Publ., Massachusetts.

Oviatt, C.A. 1984. Introduction: ecology as an experimental science and management tool, p. 539-548. *In* H. H. White (ed.), Concepts in marine pollution measurements. MD Sea Grant publication UM-SG-TS-84-03.

Paine, R. T. 1969. A note on trophic complexity and community stability. Am. Nat.

100:65-75.

Parrish, K. K. and D. F. Wilson. 1978. Fecundity studies on *Acartia tonsa* (Copepoda:Calanoida) in standardized culture. Mar. Biol. 46:65-81.

Parsons, T. R. 1976. The structure of life in the sea, pp. 81-97. *In* D. H. Cushing and J. J. Walsh (eds.), The ecology of the seas. W. B. Saunders Co., Philadelphia.

Parsons, T. R., R. J. LeBrasseur, J. D. Fulton, and O. D. Kennedy. 1969. Production studies in the Straits of Georgia. II. Secondary production under the Fraser River plume, February to May, 1967. J. Exp. Mar. Biol. Ecol. 3:39-50.

Peoples, S. A. 1975. Review of arsenical pesticides, p. 1-12. *In* E. A. Woolson (ed.), Arsenical pesticides. ACS Symp. Ser. 7, American Chemical Society.

Pritchard, P.H. and A.W. Bourquin. 1984. A perspective on the role of microcosms in environmental fate and effects assessments, p. 117-138. *In* H.H. White (ed.), Concepts in marine pollution measurements. MD Sea Grant publication UM-SG-TS-84-03.

Provasoli, L. 1977. Cultivation of animals, pp. 1295-1319. *In* Kinne, O. (ed.) Marine ecology, Vol.III, Cultivation, Part 3. Wiley, London.

Rassoulzadegan, F. 1982. Dependence of grazing rate, gross growth efficiency and food size range on temperature in a pelagic oligotrichous ciliate *Lohmanniella spiralis* Leeg., fed on naturally occurring particulate matter. Ann. Inst. Oceanogr. 58(2):177-184.

Robertson, J.R. 1983. Predation by estuarine zooplankton on tintinnid ciliates. Est. Coast. Shelf Sci. 16:27-36.

Rosemarin, A. and M. Notini. 1988. Structural and functional effects of low levels of arsenic in a Baltic Sea littoral model ecosystem. Unpublished manuscript.

Rosemarin, A., M. Notini, and K. Holmgren. 1985. The fate of arsenic in the Baltic Sea *Fucus vesiculosus* ecosystem. Ambio 14:342-345.

Ryther, J. H. 1969. Photosynthesis and fish production in the sea. Science 166:72-76.

Ryther, J. H. and J. G. Sanders. 1980. Experimental evidence of zooplankton control of the species composition and size distribution of marine phytoplankton. Mar. Ecol. Prog. Ser. 3:279-283.

Sanders, J. G. 1978. Interactions between arsenic and marine algae. Ph.D. Dissertation, University of North Carolina, Chapel Hill, 84 p.

Sanders. J. G. 1979. The concentration and speciation of arsenic in marine macroalgae.Est. Coast. Mar. Sci. 9:95-99.

Sanders, J. G. 1980. Arsenic cycling in marine systems. Mar. Environ. Res. 3:257-266.

Sanders, J. G. 1983. Role of marine phytoplankton in determining the chemical speciation and biogeochemical cycling of arsenic. Can. J. Fish. Aquat. Sci. 40:192-196.

Sanders, J. G. 1985. Arsenic geochemistry in Chesapeake Bay: dependence upon anthropogenic inputs and phytoplankton species composition. Mar. Chem. 17:329-340.

Sanders, J. G. 1986. Direct and indirect effects of arsenic on the survival and fecundity of estuarine zooplankton. Can. J. Fish. Aquat. Sci. 43:694-699.

Sanders, J.G. and S.J. Cibik. 1985. Adaptive behavior of euryhaline phytoplankton communities to arsenic stress. Mar. Ecol. Prog. Ser. 22:199-205.

Sanders, J. G. and G. F. Riedel. 1987a. Control of trace element toxicity by phytoplankton, p. 131-149. *In* J. A. Saunders, L. Kosak-Channing, and E. E. Conn (eds.), Recent advances in phytochemistry, vol 21. Plenum Press.

Sanders, J.G. and G.F. Riedel. 1987b. Chemical and physical processes influencing

bioavailability of toxics in estuaries, p. 87-106. *In* M.P. Lynch and E.C. Krome (eds.), Perspectives on the Chesapeake Bay: advances in estuarine sciences. Chesapeake Research Consortium Publ. No. 127.

Sanders, J. G. and P. S. Vermersch. 1982. Response of marine phytoplankton to low levels of arsenate. J. Plankton Res. 4:881-893.

Sanders, J. G. and H. L. Windom. 1980. The uptake and reduction of arsenic species by marine algae. Est. Coast. Mar. Sci. 10:555-567.

Sanders, J. G., J. H. Ryther, and J. H. Batchelder. 1981. Effects of copper, chlorine, and thermal addition on the species composition of marine phytoplankton. J. Exp. Mar. Biol. Ecol. 49:81-102.

Sanders, J. G., R. W. Osman, and K. G. Sellner. 1987. Arsenic impact on growth, fecundity, species composition and subsequent transport of arsenic in estuarine food webs. Final report to EPA Office of Research and Development under grant R810680-01. April 1987.

Southward, A. J. 1955a. Feeding of barnacles. Nature 175:1124.

Southward, A. J. 1955b. On the behaviour of barnacles. I. The relation of cirral and other activities to temperature. J. Mar. Biol. Assoc. U. K. 34:403-422.

Stoecker, D.K. and D.A. Egloff. 1987. Predation by *Acartia tonsa* Dana on planktonic ciliates and rotifers. J. Exp. Mar. Biol. Ecol. 110:53-68.

Stoecker, D.K. and N.K. Sanders. 1985. Differential grazing by *Acartia tonsa* on a dinoflagellate and a tintinnid. J. Plank. Res. 7:85-100.

Strickland, J.D.H. and L.D.B. Terhune. 1961. The study of in situ marine photosynthesis using a large plastic bag. Limnol. Oceanogr. 6:93-96.

Uthe, J. F., H. C. Freeman, J. R. Johnston, and P. Michalik. 1974. Comparison of wet ashing and dry ashing for the determination of arsenic in marine organisms, using methylated arsenicals for standards. J. Assoc. Off. Anal. Chem. 57:1363-1365.

Verity, P.G. 1987. Factors driving changes in the pelagic trophic structure of estuaries, with implications for the Chesapeake Bay, p. 35-56. *In* M.P. Lynch and E.C. Krome (eds.), Perspectives on the Chesapeake Bay: advances in estuarine sciences. Chesapeake Research Consortium Publ. No. 127.

Waslenchuk, D. G. 1978. The budget and geochemistry of arsenic in a continental shelf environment. Mar. Chem. 7:39-52.

White, H. H. 1984. Concepts in marine pollution measurements. MD Sea Grant publication UM-SG-TS-84-03, College Park, MD.

Zaroogian, G. E. and G. L. Hoffman. 1982. Arsenic uptake and loss in the American oyster, *Crassostrea virginica*. Environ. Monit. Assess. 1:345-358.

INTRODUCTION

Estuaries serve as principal conduits for man's inputs to the oceans. Many industrial and municipal activities, such as power generating stations, wastewater treatment facilities, and industrial plants of all types, are located on estuaries. These activities generate liquid and solid wastes, some of which deliberately or accidentally are discharged into the nearby water. In addition, the concentration of boat traffic in these same waterways results in many small spills and leakages which add pollutants. Consequently, pollution of an estuary is often the initial and greatest impact borne by the marine system as a whole. Therefore, estuaries are necessary places to investigate potential impacts of toxic compounds.

Zero discharge of toxic wastes into marine and estuarine environments, although an enviable goal, may never be a realistic alternative for modern man. Even if deliberate introduction of toxic compounds does not occur, the oceans will always face the inevitable, accidental discharge of these materials. Many compounds within these discharges are of extreme interest to environmental planners because:

1. they are greatly elevated in affected waters relative to natural water concentrations,
2. they are toxic to biota,
3. they are actively accumulated by biota in aquatic systems.

We do not know to what extent chronic or acute discharges of industrial wastes will alter aquatic ecosystems. The literature suggests that the trace materials likely to be contained within these wastes are toxic to some organisms. However, the results from these single-species bioassays are difficult to extrapolate to estuaries which have complex hydrography and food webs. Sessile organisms in the vicinity of

an effluent have the greatest potential for harm, but plankton contained within the effluent plume also should be affected. Additionally, if the compound is bioactive, uptake and transformation by organisms will greatly affect its toxicity and transfer to other organisms within the estuary.

Arsenic is an example of this type of compound. It is a classic poison, with its primary commercial use as a pesticide (Mackenzie et al., 1979). It is considered a "priority pollutant" by EPA, and was present in 48% of industrial effluents tested by the Agency (Keith & Telliard, 1979). Arsenic is present in all aquatic systems, principally in the form of an inorganic ion, arsenate (Waslenchuk, 1978; Sanders, 1980). Reduced arsenic (arsenite) is generally considered more toxic and is used as a pesticide (Peoples, 1975). However, at low levels, arsenate is likely to have the greatest impact on aquatic primary producers. It is chemically similar to phosphate, a necessary nutrient for plant growth, and it is readily taken up by phytoplankton (Sanders & Windom, 1980). Arsenate uptake can be followed by accumulation within the cell, or it can be chemically altered (reduced to arsenite and methylated) and released into the surrounding water. As much as 80% of the dissolved arsenic present in coastal marine systems is taken up and released in this fashion (Sanders, 1980, 1983, 1985).

All organisms do not react similarly to a given pollutant. Although phytoplankton are most affected by arsenate, the least toxic form, invertebrates are more susceptible to arsenite and methylated forms (Peoples, 1975). Therefore, they could be harmed by the biogeochemical alterations discussed above. Even within a particular taxon, all species will not react to the same levels of arsenic. For example, all phytoplankton species are not equally sensitive to arsenate; some are inhibited at levels just exceeding ambient concentrations of arsenate in the oceans (1.0-1.5 $\mu g \cdot l^{-1}$; Andreae, 1978; Waslenchuk, 1978; Sanders, 1980), while others are resistant to arsenate concentrations two orders of magnitude higher (Sanders and Vermersch,

1982). Within the phytoplankton community, where rapid growth and species succession are normal occurrences, arsenic can cause a shift in the dominant species toward resistant forms and a concurrent decrease in productivity (Sanders and Vermersch, 1982; Sanders and Cibik, 1985). Therefore, the addition of arsenic to the water column not only can have a direct toxic effect, but also can result in dramatic shifts in both the dominance within the phytoplankton community and possibly the flux of carbon and nitrogen between trophic levels. Coupled with this is the alteration of chemical form, which will affect (generally increase) arsenic toxicity to higher trophic levels.

Beyond the single trophic level, the effects of a toxic substance (i.e., arsenic) on an ecosystem are potentially quite complex. Besides the direct effects postulated above, individuals of heterotrophic species can be influenced by either changes in the abundances and sizes of prey species or the contamination of these food organisms. For example, an arsenic-induced shift in phytoplankton composition to small flagellates or small centric diatoms could reduce the ingestion of phytoplankton by copepods and therefore their fecundity. Small cells are not captured as efficiently as larger diatoms (e.g., Nival and Nival, 1976; Bartram, 1980). Thus changes in phytoplankton size may ultimately result in an abundance of small, noncrustacean grazers (tintinnids, rotifers) that can effectively feed on flagellates and a concomitant decrease in larger zooplankton. This shift, extrapolated to the next trophic level, predicts that the same aquatic system will support a lowered density of harvestable fish (see Ryther, 1969; Parsons, 1976; Landry, 1977; Hendrikson et al., 1982).

Little is known about how such primary and secondary effects of a toxin combine to change a community or ecosystem. Except for simple systems with two or three species or communities dominated by a "keystone species" (e.g., Paine, 1969; Dayton, 1975), we have no models, empirical or theoretical, which could be used to

predict how (or if) changes to a single species might alter the whole system. Therefore, it is important that the contributions of the various pathways to an overall toxin-effect be determined for aquatic ecosystems.

We have examined the effects of arsenic on three trophic levels within a representative estuarine food web. Our purpose was to distinguish and quantify those changes in species abundance, mortality, growth rates, and reproduction that are caused directly by the dissolved arsenic and those that could be a function of trophic relationships. The three trophic levels we investigated were the phytoplankton assemblage, zooplanktonic herbivores, and benthic supension-feeders ingesting phytoplankton and/or zooplankton. We have chosen this food web because 1) trophic relationships are straight-forward, 2) all species can be easily manipulated for experimentation, and 3) many species are economically important within the estuary (e.g., the oyster, *Crassostrea virginica*) or are important in many estuarine food webs (e.g., phytoplankton and zooplankton, Sellner, 1983, 1987).

METHODS

EXPERIMENTAL PROCEDURES

Experiments were conducted either in closed-system laboratory tanks or in outdoor flow-through microcosms. The outdoor systems were ideal for studies utilizing natural phytoplankton. Experiments with representative species were performed indoors in continuous flow systems. All treatments in each experiment (including controls) were replicated three times.

Natural phytoplankton and zooplankton communities were sampled locally from the Patuxent River estuary, a subestuary of the Chesapeake Bay. Benthic species were either harvested from local populations or collected by exposing artificial substrates on which larvae of the chosen species attached (fouling panels similar to those used by Osman, 1977, 1982). The three trophic levels and experimental design are described below in detail.

Phytoplankton Experiments

The phytoplankton are dominated by diatoms and a variety of flagellates at different times of year. By timing experiments to this temporal change in species composition, we studied the reaction of diatom and microflagellate dominated communities. Algal densities in the Patuxent River are high, approximately 10^6 - 10^7 cells·l^{-1}, similar to densities found in other mesohaline regions of the Chesapeake Bay (e.g., Van Valkenburg et al., 1978; Sanders, 1985; Sellner, 1987).

Natural assemblages from the river were cultured outdoors in large-volume microscosms under ambient nutrient concentrations and natural light and temperature conditions. The tanks were operated as flow-through cultures (see Sanders et al., 1981; Sanders and Cibik, 1985 for details of design). Under these conditions, the natural assemblage can be maintained for weeks at cell densities

similar to those in the river (Sanders et al., 1981; Sanders and Vermersch, 1982). The advantage of this approach was that we could use actual multi-species assemblages (rather than laboratory clones), with replication, that had not been exposed to high or artificial light and nutrient conditions.

Zooplankton Experiments

The zooplankton community of the Patuxent River is dominated by high densities of copepods, with *Acartia tonsa* forming >70% of total numbers in mesohaline waters in spring-summer-fall and *Eurytemora affinis* the principal winter copepod (Heinle, 1966; Heinle and Flemer, 1975; ANS, 1981; Sellner and Horwitz, 1982; Brownlee and Jacobs, 1987). Maximum densities and highest growth rates for *A. tonsa* occur in July and August (Heinle, 1966; ANS, 1981; Sellner and Horwitz, 1982) while *E. affinis* densities in the lower estuary peak in March (Heinle, 1969). Although copepods form the largest component of the zooplankton biomass, rotifers, ciliates, and bivalve larvae also contribute significantly to the community, the latter group in late spring (Sellner and Horwitz, 1982; Brownlee and Jacobs, 1987).

Two sets of experiments (one with *Acartia*, one with *Eurytemora*) were conducted with the initiation of each dependent on seasonal abundances of the two species. The direct effects of sublethal concentrations of dissolved arsenic on these copepods were determined in a series of experiments using long-term culture procedures and short-term grazing techniques. Culture experiments were undertaken to determine the effects of dissolved inorganic arsenic on copepod survival and egg and nauplii production, e.g, whether exposure over the life of adult copepods resulted in significant alterations in longevity or reproductive potential. Grazing experiments were conducted to determine whether chronic, low-level arsenic exposures resulted in decreased feeding ability of the copepod. If arsenic

reduced feeding and carbon intake, energy reserves possibly used for reproduction could decline resulting in lower fecundity and eventually population mortality.

Eurytemora--Laboratory cultures of *E. affinis* were acclimated to 15°C and 11-14‰ salinity. Cultures were fed saturating levels of *Thalassiosira weisflogii* and *Isochrysis* sp. After isolation of egg-bearing females, all nauplii produced over a 2-d period were collected and sorted into 4 groups, a group for arsenic levels of 0, 15, 35 and 50 μg l^{-1}. Approximately 200 nauplii were placed in individual 1.5 liter flasks containing phytoplankton noted above and after acclimation overnight, >63 μm nauplii were transferred to control and arsenic solutions without phytoplankton. Approximately 6 h later, animals were returned to solutions containing equal portions of *T. weisflogii* and *Isochrysis* sp.

Direct effects of arsenic on the copepod populations were determined using a diel feeding and exposure schedule. Thirty-five adult females and 7 adult males (F_0 generation) were placed in individual 500 ml containers in a water bath. Populations were transferred to solutions containing saturating mixtures of the two phytoplankton species for overnight feeding in darkness. Each morning, the populations were removed from each container and gently rinsed free of cells and placed in solutions free of phytoplankton but containing the four concentrations of dissolved arsenic. The solutions from the overnight feeding period were passed through 53 μm mesh; trapped material (nauplii and eggs) was washed into small vials and preserved with buffered formalin. Numbers of dead adults were noted and the adults were transferred to the vials. *In vivo* fluorescence measurements were made on the phytoplankton cultures from the overnight feeding period and after addition of fresh phytoplankton mixtures to initial prefeeding saturating levels. Copepods in phytoplankton-free arsenic solutions were placed in a constant temperature water bath under low light for the day. In the evening, copepods were returned to phytoplankton cultures without arsenic with the nauplii and eggs

produced in the day added to the vials containing preserved nauplii and eggs from the morning.

In order to estimate long-term effects on reproductive potential of *Eurytemora*, nauplii from adults reared in arsenic were transferred to four arsenic levels and cultured for 19 d. At the end of this period, these F_1 populations were sacrificed and preserved with buffered formalin. Numbers of females (egg-bearing and total), adult males and immature stages were determined. In addition, offspring from this population were hatched (F_2 generation) and reared for 17 d to determine numbers of mature and immature individuals.

Grazing estimates for the initial populations (F_0 generation) were estimated using two techniques. Daily changes in *in vivo* fluorescence were determined from the differences between initial and final fluorescence values for each nocturnal feeding period (see above). Radioisotopes were also employed where phytoplankton were pre-labelled with ^{14}C-bicarbonate and offered to replicate subsample populations from each of the arsenic concentrations 4 d after exposure to arsenic. Adult *Eurytemora* were allowed to feed for 2.9-3.1 h, screened, held over fuming, concentrated HCl, and transferred to scintillation vials. Following digestion, activities of phytoplankton and copepods were estimated using liquid scintillation counting procedures (Sellner and Olson, 1985).

Acartia--Experiments with *Acartia tonsa* were conducted in a similar but simpler manner. Populations were collected from Chesapeake Bay in August, 1985 and returned to the laboratory. Nauplii were collected from the populations and transferred as 4 distinct groups to 25°C, 15 ‰ solutions containing 0, 15, 35 and 50 $\mu g\ l^{-1}$ of arsenate. Feeding was undertaken as described above for *Eurytemora*. After 1-10 d, three groups of 35 females and 7 males were isolated from each arsenic concentration and transferred to fresh solutions. Using the protocols for long-term culture experiments for estimating nauplii produced per day described above,

nauplii numbers, fluorescence changes and grazing rates were determined over a 3-week period.

The effects of arsenic concentrations and day on each of these parameters were determined using ANOVA procedures.

Benthic Experiments

Four species of benthic suspension-feeding invertebrates were used in the study. Species were chosen to represent both a broad range of feeding types and a diversity of taxonomic groups. Each of the four species can be locally dominant, several are classified as representative important species, and one, the oyster, *Crassostrea virginica*, is economically important.

The experiments with benthic invertebrates were designed to examine the direct effects of dissolved arsenic on a variety of common estuarine species. A general experimental design was used in all experiments with this design modified to accommodate differences between the species examined.

In general, two sets of environmental chambers were used in each experiment. These were experimental chambers in which the organisms were kept for most of an experiment and exposed to a particular level of arsenic and the feeding chambers in which the organisms were placed to feed in isolation from the experimental conditions. Using this design, the organisms were exposed to dissolved arsenic only, and could not assimilate arsenic by ingestion of contaminated food organisms. In each experiment, the experimental animals were rinsed in flowing filtered water before being placed in either the experimental or feeding chambers.

Four species were used in a total of six experiments. Three experiments were conducted with the oyster *Crassostrea virginica*. Separate experiments were conducted with larval oysters, newly settled juvenile oysters, and adults. These experiments were designed to examine whether this common and important species

was sensitive in a particular life-history stage. In the remaining three experiments the barnacle *Balanus improvisus*, the colonial bryozoan *Victorella pavida*, and the anemone *Diadumene leucolena* were investigated. All four species are very common within the Chesapeake Bay and together are representative of the phylogenetic, morphologic, behavioral, and trophic groups common to estuaries. The species were all from very different phyla; the bryozoan was colonial while the remaining species were solitary, *Diadumene* had limited motility compared to the others which were permanently attached to substrates, and the oysters and *Victorella* fed exclusively on phytoplankton, *Diadumene* fed on zooplankton, and *Balanus* was omnivorous.

Barnacles--Experimental populations of the barnacle, *Balanus improvisus*, were collected on 115 cm^2 PVC panels. Approximately 200 panels were exposed in the lower Patuxent River estuary 45 days prior to the beginning of the experiment. These panels were collected, cleaned of any debris, and all organisms other than *Balanus* were removed. In particular, the predatory flatworm *Stylochus ellipticus* was carefully removed from all panels. After being cleaned, each panel was blotted dry, numbered, and weighed. Twenty randomly chosen panels were then assigned to one of nine 80 l environmental chambers. Chambers were then assigned randomly to one of three arsenic treatments, resulting in 3 replicate chambers per treatment.

Treatments consisted of control, low, and high concentrations of arsenate (0, 12, and 42 $\mu g \cdot l^{-1}$, respectively). Arsenate levels were maintained using the continuous flow design discussed previously. The experiment continued for 22 days during which time the water temperature fluctuated between 10 and 12°C and salinity was 8-9‰. For 3-4 h each day panels were placed in the feeding chambers. These chambers were supplied with a continuous flow of raw (unfiltered) Patuxent River water.

As a control for the abbreviated feeding time, 6 panels were kept in a continuous flow of raw water for the duration of the experiment. Also another 6 panels were selected haphazardly at the beginning of the experiment and frozen for later analysis.

After 22 d of exposure to arsenic, panels were placed in continuously-flowing seawater for an additional 10 d. This allowed us to test for any residual effects of exposure after this short recovery period. At the end of this 10 d period, all panels were dried and weighed. All barnacles on each panel were sampled and frozen for later analysis.

Bryozoans--The methods used for the bryozoan experiment were very similar to those used for the barnacle experiment. Two hundred previously numbered PVC panels were suspended in the Patuxent River for 45 days prior to the experiment. After this time each panel was colonized and completely covered by the bryozoan, *Victorella pavida*. Panels were recovered two days before the beginning of the experiment. *Victorella* was completely removed from one surface of each panel and the remaining surface was designated the test surface. A border 1-2 cm wide was cleared of all *Victorella*, leaving a central area of approximately 50-75 cm^2 covered by the bryozoan.

After these manipulations each panel was photographed to record the abundance of *Victorella* in terms of area covered. At the end of the experiment each panel was again photographed to record the change in cover during the experiment. The clean border and back surface of the panel were designed to supply sufficient space for colony growth.

As in the barnacle experiment, 20 panels were randomly assigned to one of 9 environmental chambers. Three replicate chambers were assigned to each of three arsenic treatments (0, 13, and 36 $\mu g \cdot l^{-1}$). Panels were kept in the experimental

chambers for 20 h per day. For 4 h each day panels were removed and placed in the feeding chambers. In these chambers *Victorella* colonies were exposed to unfiltered river water, allowing them to feed on their normal diet of phytoplankton.

Anemones--The anemone, *Diadumene leucolena*, was collected from oyster reefs in the Patuxent River and Chesapeake Bay. Individual anemones were carefully removed from the oyster shell. Each individual was placed on a separate 115 cm^2 PVC panel. Panels were held in flowing water in a laboratory seatable. After approximately 24 h most anemones had reattached to the experimental substrates. Anemones were held for 2 weeks in the laboratory seatable and any unhealthy or damaged individuals were removed. Each individual was fed daily with an abundance of nauplii of cultured brine shrimp, *Artemia* sp.

At the beginning of the experiment, panels with attached anemones were photographed, blotted dry, and weighed. Panels were then assigned randomly to one of 9 environmental chambers resulting in 6 - 7 panels in each chamber. As in the previous experiments the 9 chambers were randomly assigned to one of three arsenic treatments (0, 13, and 36 $\mu g \cdot l^{-1}$).

Panels were kept in the experimental chambers for 20 h per day. During the remaining 4 h panels were placed in feeding chambers, each with an equal volume of water. The feeding chambers were filled with filtered river water immediately before the anemones were added and were constantly aerated during the feeding period. An equal volume of *Artemia* nauplii were added to each chamber with this volume dependent on the productivity of laboratory cultures.

At the end of the experiment each panel was photographed, blotted dry, and weighed. Anemones were then removed for arsenic analyses and the panel was reweighed to determine its weight alone. The growth of the anemones over the 30 day experiment was determined both as a change in wet weight and as a change in

area of the basal disc. Areas were measured on photographs using the laboratory's image analysis system.

Adult Oysters--Approximately 400 cultured oysters were obtained from a local hatchery and acclimated to the Patuxent River for two weeks prior to the beginning of the experiment. Each oyster was cleaned and 180 were numbered and weighed at the beginning of the experiment. Weights were measured using the technique of Andrews (1961). Twenty numbered and twenty unnumbered oysters were randomly assigned to each of the 9 treatment chambers and chambers were then randomly assigned to one of 3 arsenic treatments (0, 13, and 35 $\mu g \cdot l^{-1}$). An additional 20 oysters were sacrificed at the beginning of the experiment to measure initial tissue levels of arsenic. Tissue was carefully removed, weighed, and dried for analysis. Finally, 10 oysters were maintained in constantly flowing river water for the duration of the experiment. These were used to evaluate the reductions in growth resulting from the limited feeding schedule used during the experiment.

The experiment ran for a period of four weeks. Oysters were kept in the experimental chambers for 20 h per day and moved to the feeding chambers for the remaining 4 h. In the feeding chambers the oysters were exposed to a continuous flow of unfiltered river water. After 2 weeks 10 of the unnumbered oysters were chosen haphazardly, and the tissue removed and dried for analysis. At the end of the experiment the numbered oysters were weighed and the tissue of all oysters was collected and prepared for analysis.

Larval Oysters--In this experiment, conducted simultaneously with the Juvenile Oyster Experiment, 3 replicate chambers were used for each treatment. Oyster larvae, approximately 4 d old were obtained from a hatchery on the eastern shore of Maryland. After transport to the laboratory an equal volume of larvae were

added to each of 6 environmental chambers. Random counts indicated that each chamber contained a mean of 187 ± 20 larvae·ml^{-1}. Chambers were randomly assigned to one of two treatments (0, 25 µg l^{-1}).

As in previous experiments, larvae were fed in isolation from dissolved arsenic. However, to prevent stress and injury resulting from daily transport between chambers, separate feeding chambers were not used and larvae were fed in the experimental chambers. Prior to feeding water was gently siphoned from the chambers using plankton net filters to prevent removal of larvae. After removal of the treatment water, *Isochrysis* sp. was added to each chamber to bring cell densities to 10^4 cells·ml^{-1}. Cell counts were made daily to ensure that densities were the same in all treatments. These experiments were designed to continue for 10 - 15 days. The number of larvae successfully settling on substrates in each chamber were used to estimate the effects of each treatment.

Juvenile Oysters--This experiment was conducted using newly attached oysters. Cultured oyster larvae were exposed to 60 115 cm^2 panels in an oyster hatchery on the eastern shore of Maryland. The panels with attached larvae were then transported to the laboratory, placed in one of three groups depending on the density of oysters, and an equal number from each of these groups was randomly assigned to one of 12 treatment tanks. Of the 12 chambers, only 6 were relevant to the present study. These were assigned randomly to one of two arsenic treatments (0, 25 µg·l^{-1}). As in previous experiments, the continuous flow design was used and panels were removed to separate feeding chambers for 4 h per day. Feeding chambers were aerated and filled with static cultures of *Isochrysis* sp. Algal concentrations ranged between 1×10^4 and 2×10^4 cells·ml^{-1} during the course of the experiment.

The experiment was continued for 4 weeks and each experimental panel was photographed at 0, 2, and 4 weeks. The area covered by each individual oyster was used as a measure of its size and growth. These measurements were made by analyzing the photographs using the laboratory's image analysis system. Oysters showing no growth were considered unsuccessful recruits and were assumed to have died before the beginning of the experiment. They were not included in any growth rate analyses.

Arsenic Analyses

The concentration and chemical form of arsenic within each experiment were monitored within the water column and organisms. Water samples were collected in rigorously cleaned (Boyle and Huested, 1983) plastic bottles and analyzed by hydride generation and detection of specific arsenic hydrides using atomic absorption spectrometry (Braman et al., 1977). This method of analysis permits determination of the total concentration of arsenic and also its chemical form. This technique is very sensitive; limits of detection in our laboratory are about 20 $ng \cdot l^{-1}$. Solids were dried, weighed, and ashed at 500°C for 24 hr in the presence of an ashing aid [$Mg(NO_3)$ and MgO] to prevent loss of arsenic (Uthe et al., 1974). After ashing, the residue was dissolved in 1N HCl and analyzed as above.

Technique accuracy was assessed through the use of standard reference materials, NBS #1566, oyster tissue, and NRC NASS-1, a seawater standard. Recoveries of these materials averaged 95% and 91% for the oyster tissue and seawater, respectively.

RESULTS

PHYTOPLANKTON

Two experiments were performed, timed to coincide with different dominant algal forms within the phytoplankton community: one during spring, to coincide with the diatom/dinoflagellate bloom, and one during late summer, a time of microflagellate dominance.

Spring Experiment--The spring experiment was conducted during 18 April and 17 May. Water temperatures rose during the experiment, and ranged between 16-25°C. Salinity varied slightly, ranging between 11.0-13.5‰. Arsenate was added continuously at three estimated concentrations: 3, 6, and 21 $\mu g\ l^{-1}$, plus controls. Each concentration of arsenate was added to triplicate sets of tanks. Actual measured arsenate concentrations were very close to predicted values (Table 1) with the low and medium treatments receiving approximately 30% more arsenic than predicted. Arsenic concentrations in control tanks and the Patuxent River (source of seawater for the experiments) were similar, approximately 0.3 $\mu g\ l^{-1}$ (Table 1).

Arsenate had little effect on the overall growth rate or total cell densities of the assemblages, even at the highest concentration. In fact, tanks exposed to the various levels of arsenate contained slightly higher cell densities after the first 12 d, resulting from the elevated growth of a small centric diatom, *Thalassiosira* sp. (see below). However, the higher densities were not significantly different from control densities (Figure 1A).

Diversity also remained constant between the controls and various treatments. Number of species, and measurements of species richness (Margalef, 1951) and

Table 1. Dissolved arsenic concentration in phytoplankton experiments. Values shown are mean ± S.E., in $\mu g \cdot l^{-1}$. All arsenic present was in the form of arsenate, no reduced or methylated forms were present.

	Added As, $\mu g \cdot l^{-1}$	Actual As, $\mu g \cdot l^{-1}$ ± SE
Spring Experiment	0 (source)	0.32 ± 0.06
	0 (control tanks)	0.34 ± 0.03
	3	4.25 ± 0.29
	6	8.60 ± 0.62
	21	19.8 ± 0.79
Summer Experiment	0 (source)	1.22 ± 0.13
	0 (control tanks)	1.29 ± 0.15
	3	11.0 ± 1.36
	6	22.3 ± 2.43
	21	62.0 ± 9.14

Figure 1A. Changes in total cell density at various arsenate concentrations through time, in the spring experiment. ●——● = control (range indicates 2 SE), ■——■ = 3 µg·l-1, ▲——▲ = 6 µg·l-1, ◆——◆ = 21 µg·l-1.

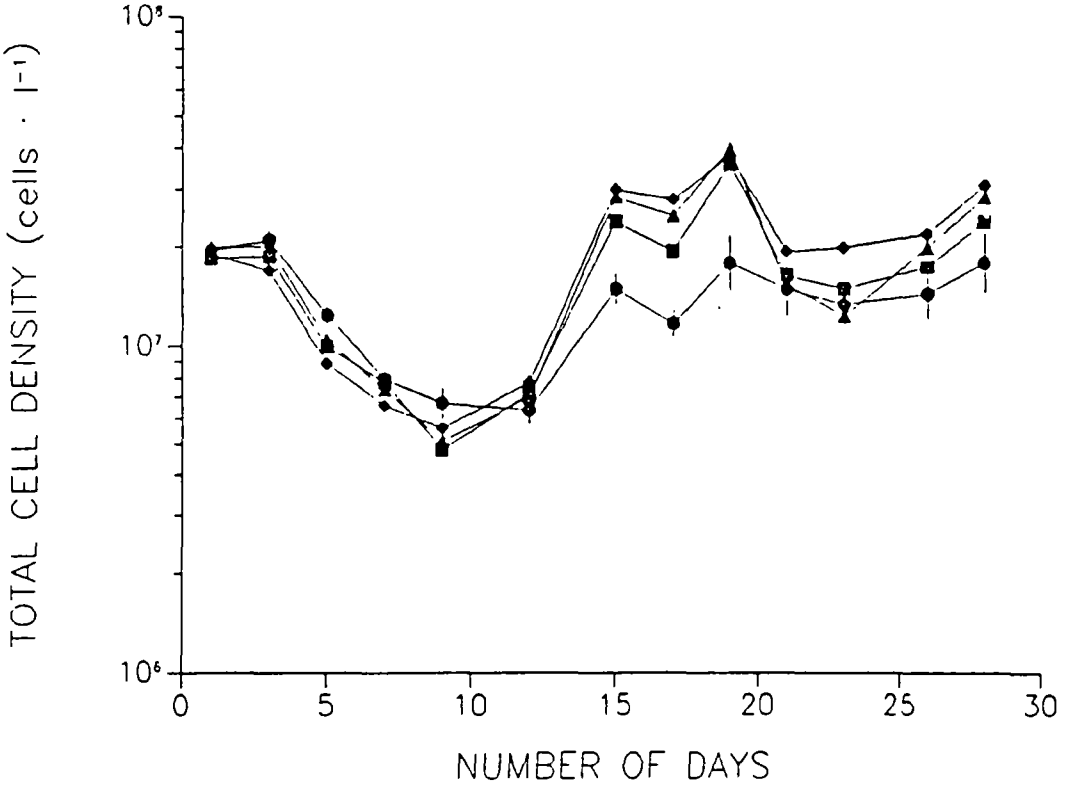


Figure 1B. Changes in density of *Cerataulina pelagica* as a % of total cell density at various arsenate concentrations through time, in the spring experiment. ●——● = control, ■——■ = 3 µg l-1, ▲——▲ = 6 µg·l-1, ◆——◆ = 21 µg·l-1.

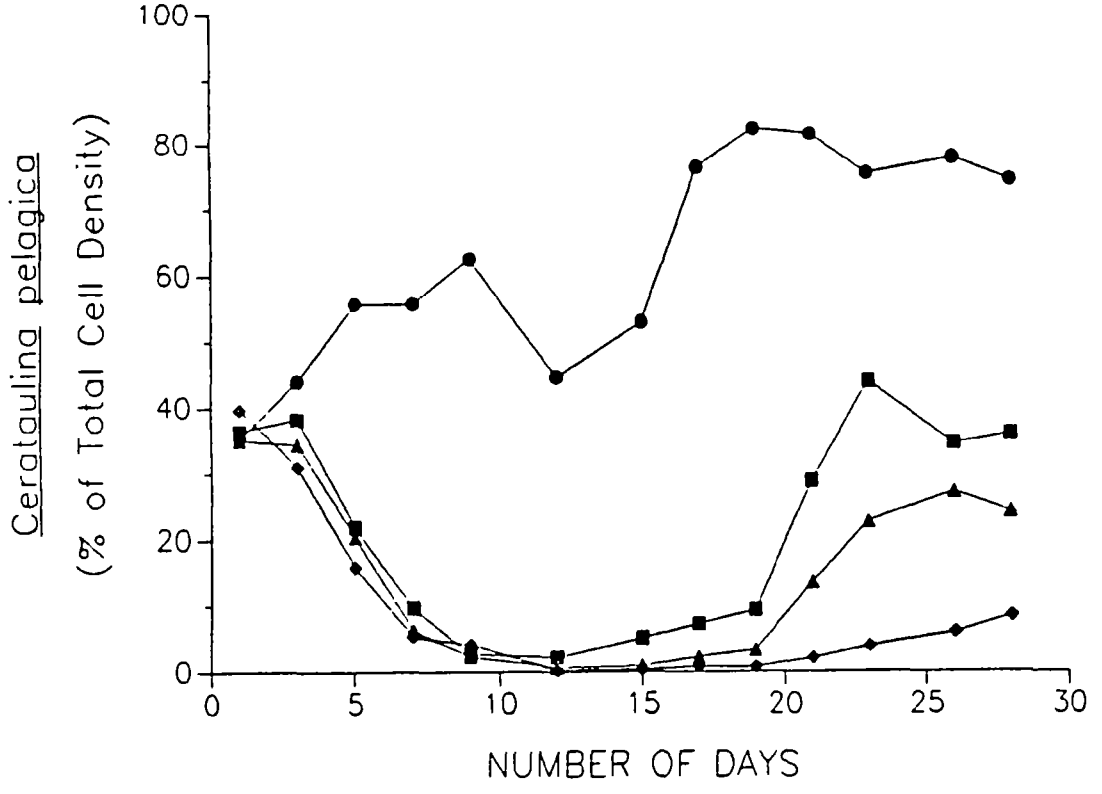
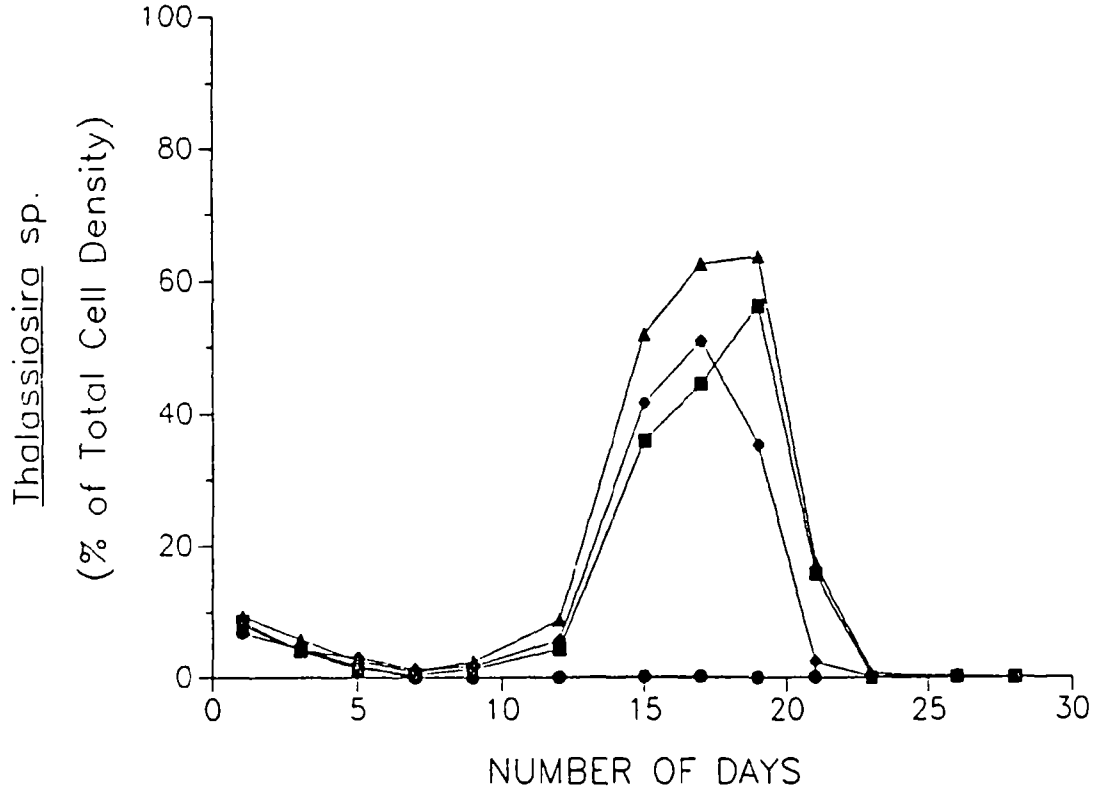


Figure 1C. Changes in density of *Thalassiosira* sp. as a % of total cell density at various arsenate concentrations through time, in the spring experiment. ●——● = control, ■——■ = 3 µg·l-1, ▲——▲ = 6 µg·l-1, ◆——◆ = 21 µg·l-1.



species evenness (Pielou, 1966) showed no significant differences between treatments and controls (ANCOVA, $p > 0.05$).

A number of dominant species, however, were greatly affected by arsenate. All three arsenate levels significantly inhibited growth of the most dominant algal species, *Corataulina pelagica*, a relatively large centric diatom. This species comprised 40-80% of the total cell densities in control tanks throughout the experiment. It rapidly disappeared, however, in arsenate-treated tanks; after only 7 d, it was present at densities less than 5% of total (Figure 1B). After 18 d, cell densities of this species rose slightly in treated tanks, but never approached the densities seen in control tanks.

On the other hand, another important centric diatom, *Thalassiosira* sp., was not affected by arsenate treatment; its growth was accelerated in treated tanks. *Thalassiosira* sp. bloomed only in arsenate-treated tanks; densities of this species in control tanks were quite low (Figure 1C). Another diatom, *Rhizosolenia fragilissima*, was not affected by arsenate: The combined density of these two species more than offset the loss of *C. pelagica* in control assemblages; therefore, the total number of centric diatoms remained essentially unchanged due to arsenate, even though the most important dominant was almost eliminated in treatment tanks.

Other species, of lesser importance numerically, were similar in both controls and treatments. The relative abundance of dinoflagellates and small, unidentified flagellates increased slightly in arsenate-treated assemblages. Other species showed no response to arsenate.

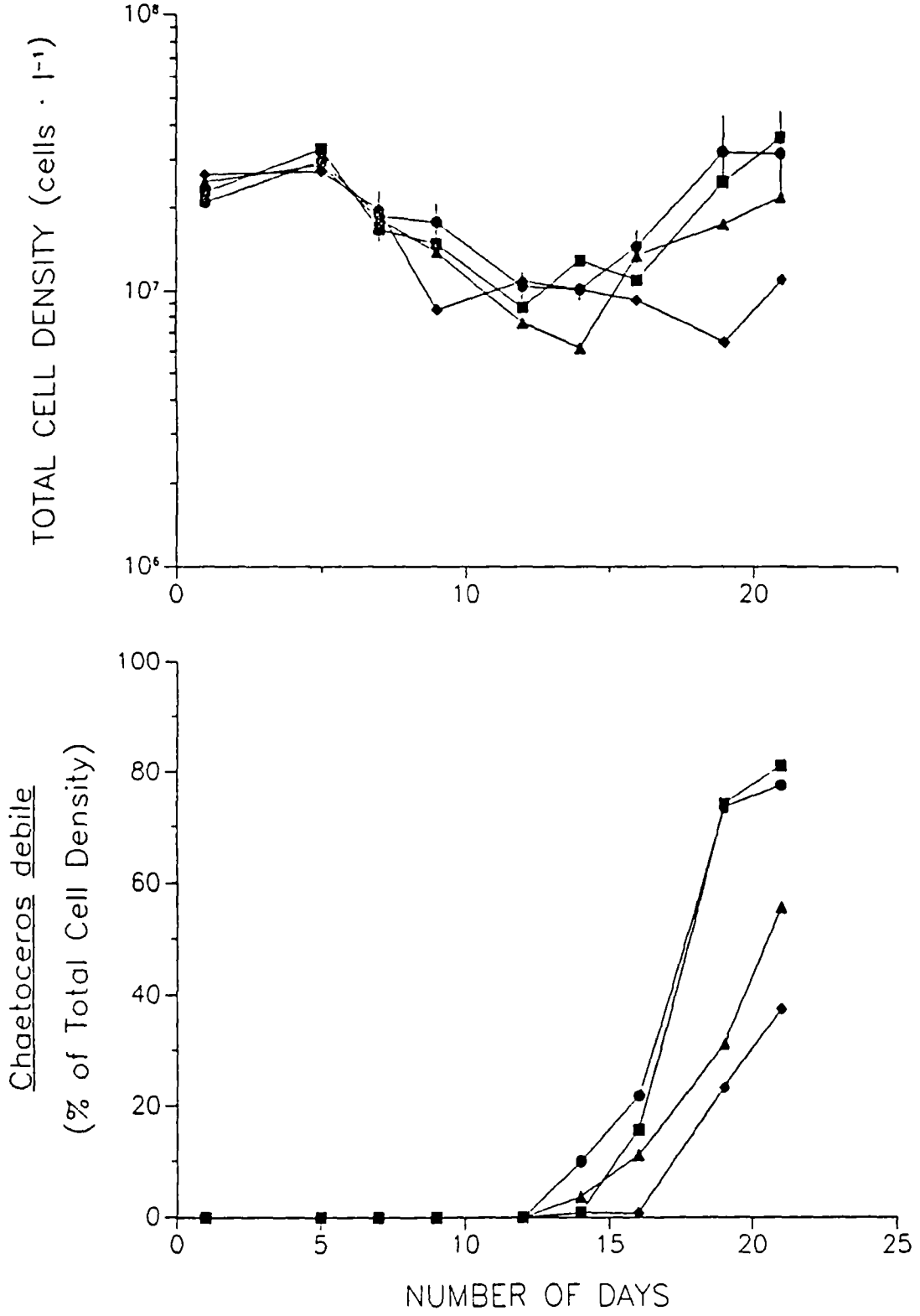
Late Summer Experiment--This experiment was performed between 5 and 29 September. Water temperatures were high at the beginning and fell slowly throughout the experiment, ranging between 29.0-21.0°C. Salinities varied between 11.5-14.5‰. Arsenate was added at three concentrations: 3, 6, and 21 $\mu g\ l^{-1}$. Actual

arsenate concentrations, however, were approximately three times these levels (Table 1).

As in the spring, arsenate had little effect on the overall growth rate of the assemblage or the total cell densities, at least for the first 15 d (Figure 2A). After this period, cell densities declined somewhat relative to controls in the tanks receiving the highest arsenic concentration. At the end of the experiment, cell densities in these tanks were only 33% of those in controls (Figure 2A). This decline was due solely to the decline of one sensitive diatom species (discussed below). As in the earlier experiment, species diversity in the various treatments did not vary significantly (ANCOVA, $p > 0.05$).

Species composition during this experiment was quite different than during the spring experiment. The spring experiment was dominated by centric diatoms, which comprised an average of 79.6% of total cell density. In the summer experiment, diatoms were much less important, averaging 42.1%. The assemblages in the summer experiment were dominated by a number of small, flagellated species, particularly cryptophytes such as *Chroomonas* sp., and other small, unidentified flagellates. During the last 7 d of the experiment, a centric diatom, *Chaetoceros debile*, bloomed, dominating until the end of the experiment. *C. debile* exhibited the greatest response to arsenate. Its growth rate was reduced from 1.44 div·d^{-1} in control assemblages to 1.28 div d^{-1} in the 6 µg·l^{-1} arsenate treatment, and finally to 1.10 div·d^{-1} in the 21 µg l^{-1} arsenate treatment, a 24% reduction in growth rate from controls to the highest arsenic concentration (Figure 2B). Because this species strongly dominated the phytoplankton for the last 10 d of the experiment, the overall assemblage showed a significant reduction in total cell density, as discussed above (Figure 2).

Figure 2. Changes in total cell density and density of *Chaetoceros debile* as a % of total cell density at various arsenate concentrations through time in the summer experiment. ●——● = control, ■——■ = 3 µg·l-1, ▲——▲ = 6 µg·l-1, ◆——◆ = 21 µg·l-1.



Other dominant species showed little, if any, response to arsenate. Growth of cryptophytes was slightly reduced by arsenate; small, unidentified flagellates grew slightly better in arsenate treatments; both differences were slight.

ZOOPLANKTON

Eurytemora affinis--Eurytemora were maintained at four estimated arsenic concentrations, 0, 15, 35, and 50 $\mu g\ l^{-1}$. Actual arsenic concentrations were quite similar to predicted levels; the two highest concentrations were slightly lower than predicted (Table 2). Data collected from replicate cultures of *Eurytemora affinis* indicated that dissolved arsenic concentrations ranging from 0-38 $\mu g\ l^{-1}$ had no demonstrable effect on the copepod, even over the long term. Average daily reproduction rates, measured as the number of nauplii produced per female, for an 8-day period were similar ($F = 0.66$, $p > 0.5$) at 25.5, 27.3, 24.6 and 26.6, respectively, for populations maintained in 0, 13, 30 and 38 $\mu g\ l^{-1}$ (Figure 3). Grazing rates were also similar over the four arsenic concentrations. Copepod feeding resulted in average *in vivo* fluorescence decreases of 4.30-5.05 relative fluorescence units $copepod^{-1}\ h^{-1}$ over 10 days, indicating similar feeding rates for control and arsenic-exposed populations ($F = 1.56$, $p > 0.25$) (Figure 4). Carbon incorporation rates calculated from experiments with ^{14}C-labeled phytoplankton were also identical ($F = 3.11$, $p > 0.1$) and averaged 0.018-0.026 ml of culture cleared $copepod^{-1}\ h^{-1}$ (Figure 5).

Data were also collected for survivorship and development in the three generations. The results are qualitative due to funding limitations for collecting, preserving and counting additional samples. Over an 11-d period, 86%, 77%, 81% and 74% of initial female densities in the F_0 generation were still viable in the four arsenic levels (0, 15, 35 and 50 $\mu g\ l^{-1}$).

Table 2. Dissolved arsenic concentration in zooplankton experiments. Values shown are mean ± S.E., in $\mu g \cdot l^{-1}$. All arsenic present was in the form of arsenate.

Organism	Added As, $\mu g \cdot l^{-1}$	Actual As, $\mu g \cdot l^{-1}$ ± SE
Eurytemora affinis	0	0.15 ± 0.02
	15	13.4 ± 0.69
	35	29.7 ± 3.03
	50	38.4 ± 4.73
Acartia tonsa	0	1.01 ± 0.37
	15	15.6 ± 1.09
	35	30.8 ± 1.92
	50	42.8 ± 3.70

Figure 3. Nauplii production by female *Eurytemora affinis*, in nauplii·d^{-1}, at various arsenate concentrations. Values shown are mean ± SE.

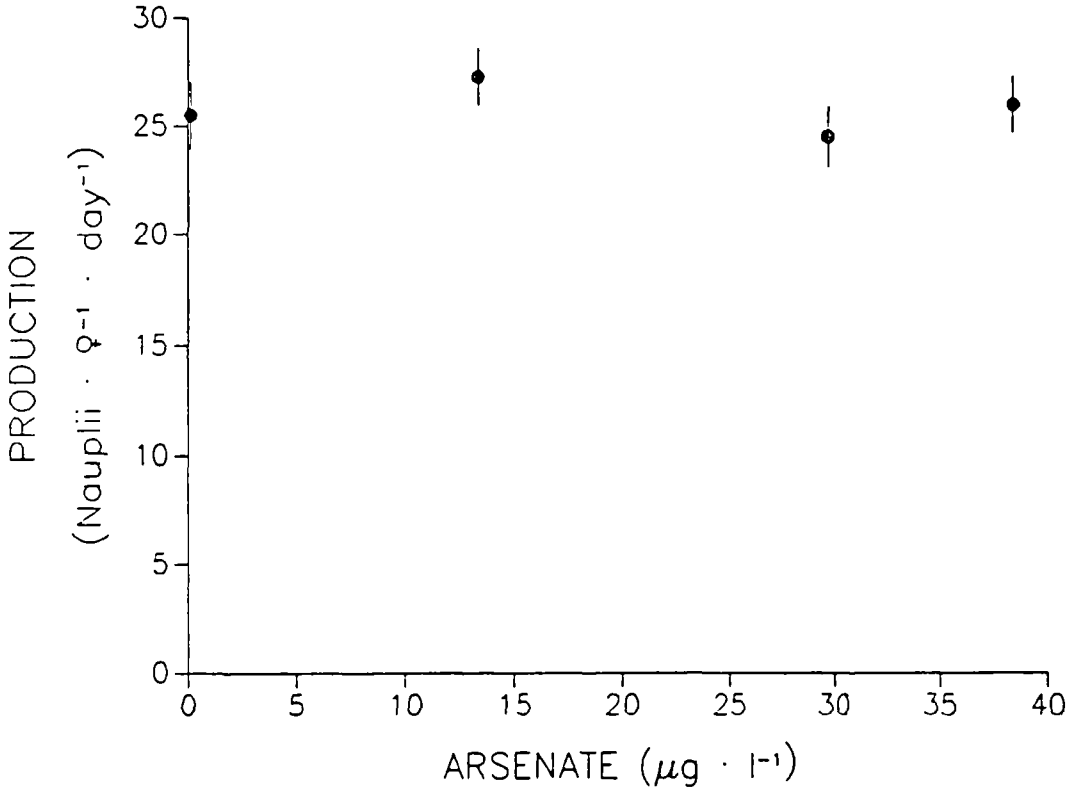


Figure 4. Grazing by *Eurytemora affinis*, measured as change (Δ) in relative fluorescence units·copepod^{-1}·h^{-1}, at various arsenic concentrations. Values shown are mean ± SE.

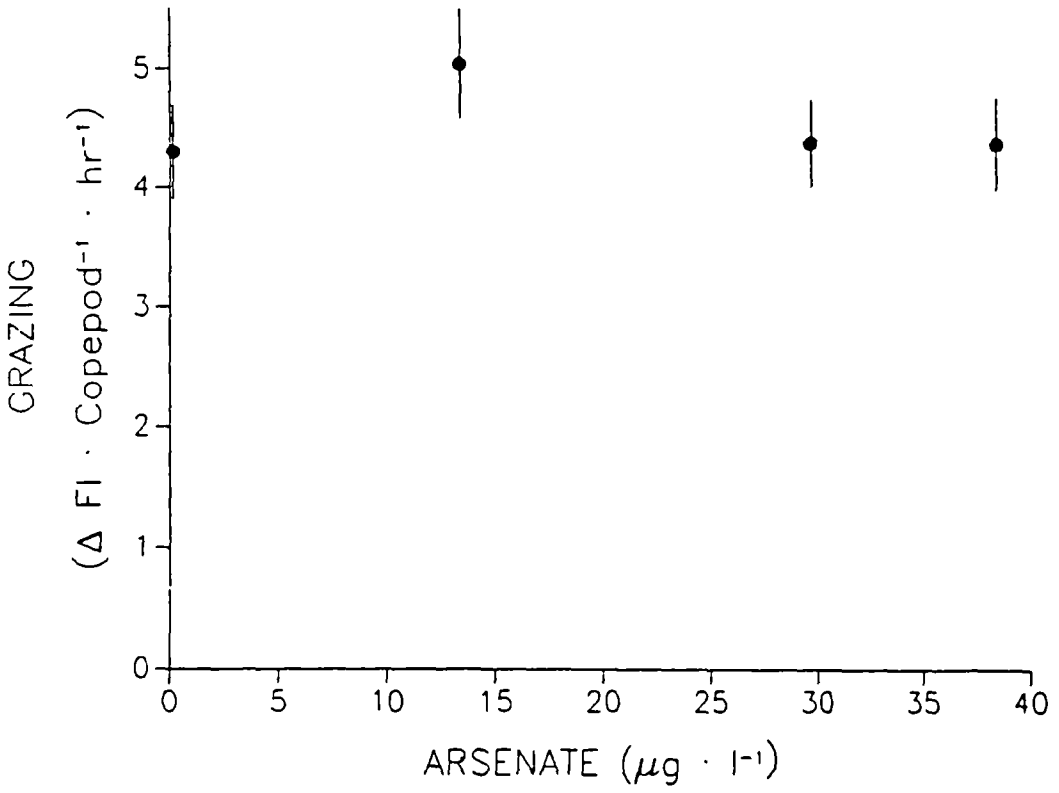
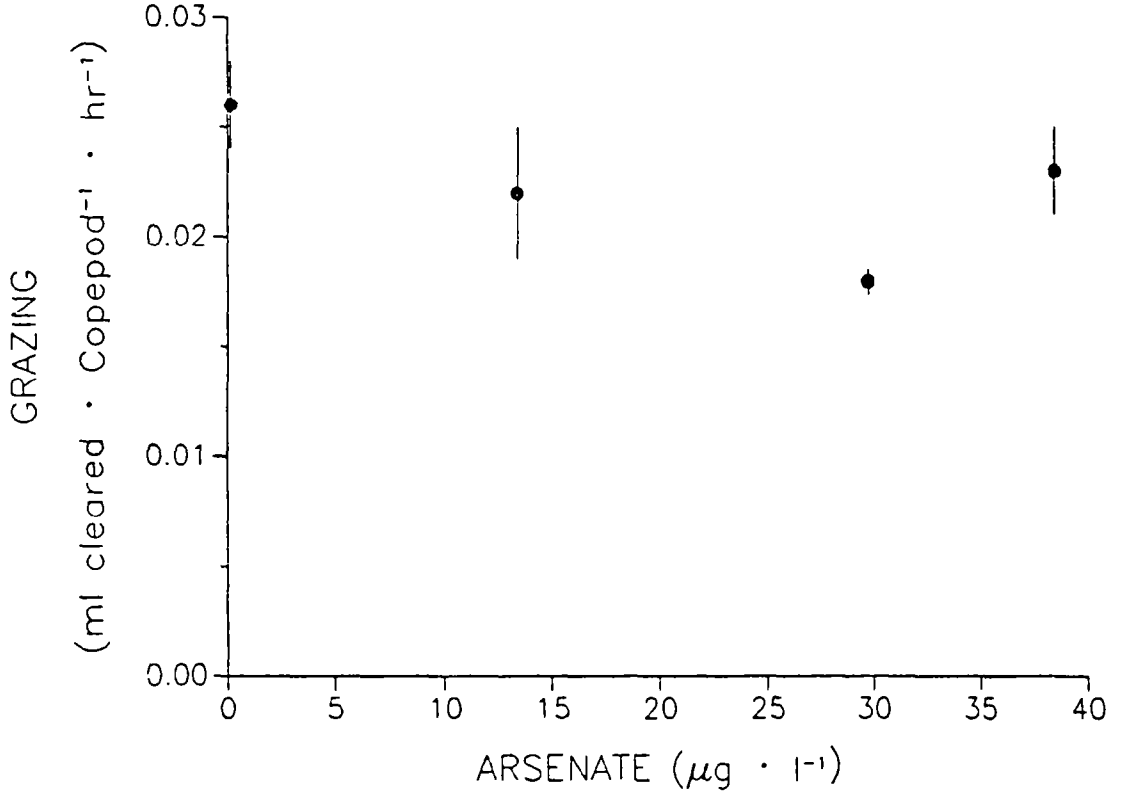


Figure 5. Grazing rate of *Eurytemora affinis* measured with ^{14}C-labelled phytoplankton (ml cleared·copepod^{-1}·h^{-1}) at various arsenic concentrations. Values shown are means ± SE.



In contrast to other results, the distributions of sexually immature CIV (fourth copepodite stage) copepodites and mature adults in F_1 and F_2 generations indicate that sublethal arsenic levels have detrimental effects on population success for copepods. The relative abundances of copepods reaching maturity were higher in control cultures versus cultures containing 50 $\mu g\ l^{-1}$ of arsenate; in the F_1 generation, 92% and 49% of individuals reached maturity in control and arsenic solutions, respectively (Table 3). In the F_2 populations, mature individuals reached 72% and 41% of total numbers in the two solutions (Table 3). The female:male ratio fluctuated widely in cultures dosed with arsenate; in contrast, both generations of *E. affinis* grown under control conditions exhibited a very stable ratio of 1.5-1.7 (F:M). In both treatments, the number of egg-bearing females declined with each successive generation, albeit more rapidly in arsenate-treated cultures.

Other qualitative information also supports the concept of long-term arsenic exposure reducing population success in *Eurytemora*. Daily nauplii production was estimated for 22 additional days beyond the data presented above and data generated from these collections suggest that egg production was significantly lower in high arsenic solutions than in control or low arsenic levels. Unfortunately, these results are equivocal because of copepod contamination of the phytoplankton cultures (*T. weisfloggii* or *Isochrysis*) used as food in the study. Assuming random transfer of contaminating copepods and offspring from the food into the experimental containers, significantly lower egg production was observed in the highest arsenic solutions over the study period ($F = 8.77$, $p < 0.05$).

Acartia tonsa--*Acartia* were maintained at the same estimated arsenic concentrations as were *Eurytemora*. Actual arsenic concentrations were quite similar to predicted (Table 2). As noted in *Eurytemora*, there was no detectable effect of increasing arsenic levels on nauplii production in *Acartia tonsa* over a 10-d period.

Table 3. Number (percent) of juvenile copepods that reach maturity and breakdown by sex in successive generations of *Eurytemora affinis* exposed to 0 (control) and 50 µg.l-1 added arsenic.

	Control			50 µg.l-1		
Generation	Male (%)	Female (%)	Immature (%)	Male (%)	Female (%)	Immature (%)
F_1	31(36%)	48(56%)	7(8%)	53(39%)	14(10%)	70(51%)
F_2	63(27%)	106(45%)	67(28%)	19(9%)	69(32%)	126(59%)

Similar nauplii production rates ($F = 0.24$, $p = 0.87$) were obtained for the four arsenic concentrations, with 17.8, 19.0, 18.1 and 20.5 nauplii produced female^{-1} d^{-1}, respectively (Figure 6).

Grazing rates for *Acartia* ranged from 1.19-1.53 relative fluorescence units copepod^{-1} h^{-1} with highest rates observed in cultures containing 50 µg l^{-1} of arsenate; however, there were no significant differences between the rates. Rates were 1.33, 1.19, 1.26 and 1.53 relative units copepod^{-1} h^{-1} suggesting grazing rate fluctuations independent of arsenate level (Figure 7).

BENTHIC ORGANISMS

The results of the benthic invertebrate experiments were fairly consistent. Although a diversity of taxa and trophic groups were examined, different life-history stages used, and a variety of measurements made, only barnacles seem to have been affected significantly by the presence of arsenate in the water.

Each experiment employed a standard nested design. Individual organisms or groups of organisms (i.e. panels) were nested or grouped within experimental chambers. The experimental chambers were then grouped by treatment. This design allowed us to identify any potential differences between chambers within treatments that may have contributed to observed differences. Because of this standard design, all experiments were analyzed using the same analysis model: nested analysis of variance with the observed variable nested within chamber and chamber nested within arsenic treatment.

Barnacles--Barnacles were assigned to estimated arsenic concentrations of 0 (control), 12, and 42 µg·l^{-1}. Measured arsenic concentrations were higher; concentrations averaged 23.8 and 55.8 µg·l^{-1} in the two arsenic treatments (Table 4). The barnacles on each panel were considered to be a small discrete population and

Figure 6. Nauplii production by *Acartia tonsa* females, as nauplii d^{-1}, at various arsenate concentrations. Values shown are mean ± SE.

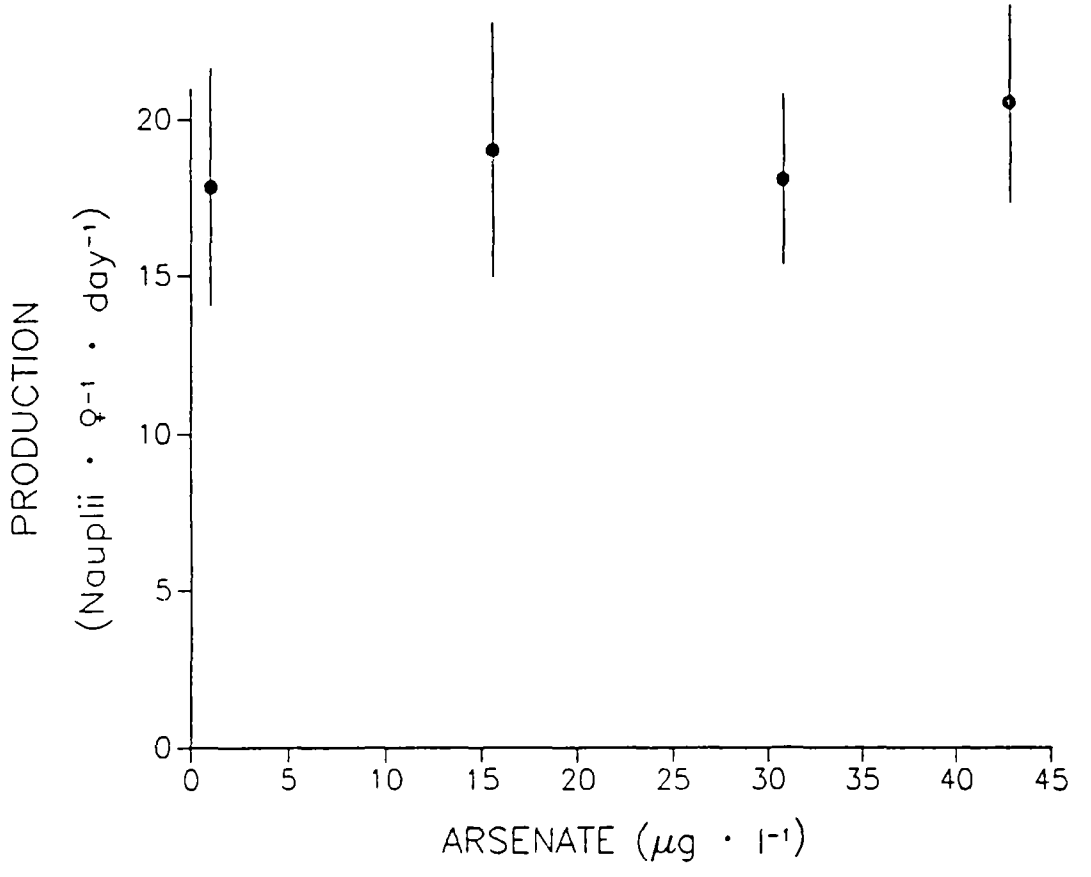


Figure 7. Algal ingestion by *Acartia tonsa* measured as change (Δ) in relative fluorescence units·copepod^{-1}·hr^{-1}, at various arsenic concentrations. Values shown are means ± SE.

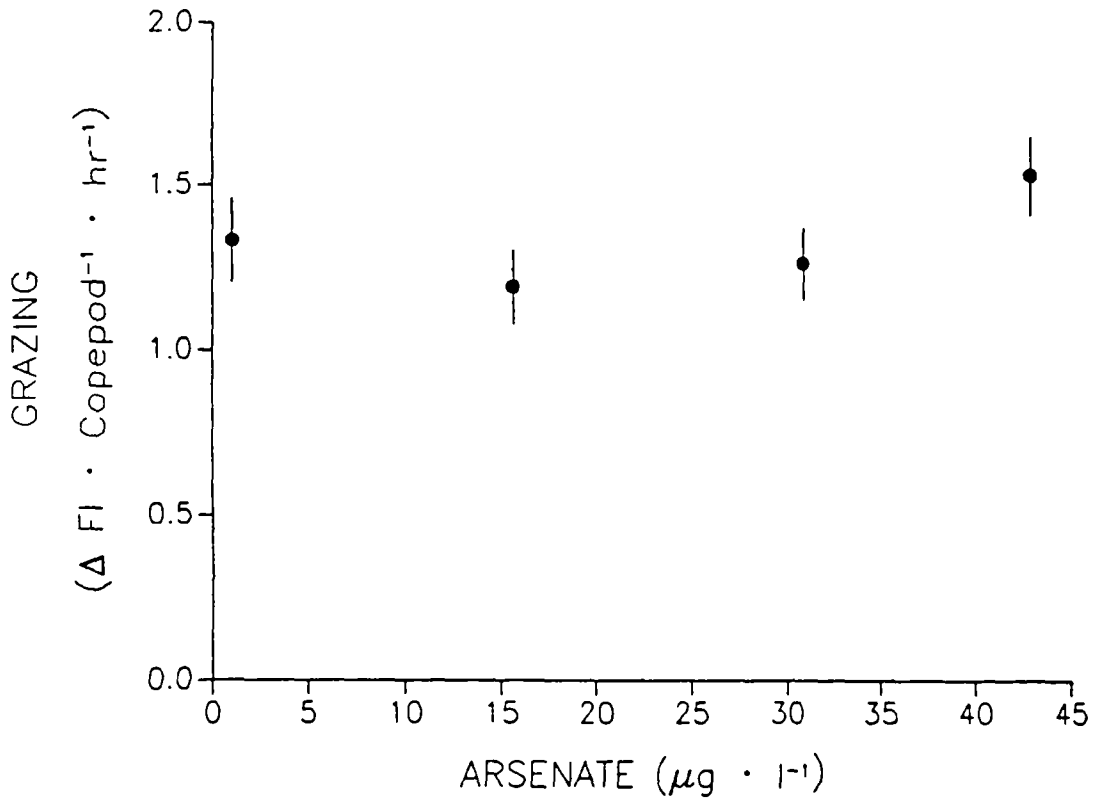


Table 4. Dissolved arsenic concentration in benthic experiments. Values shown are mean ± S.E., in $\mu g \cdot l^{-1}$. All arsenic present was in the form of arsenate.

Organism	Added As, $\mu g \cdot l^{-1}$	Actual As, $\mu g \cdot l^{-1}$ ± SE
Barnacles	0 (source)	0.27 ± 0.05
	0 (control tanks)	0.38 ± 0.04
	12	23.8 ± 2.99
	42	55.8 ± 11.66
Bryozoans	0 (source)	0.70
	0 (control tanks)	0.66 ± 0.21
	13	7.06 ± 0.51
	36	28.1 ± 4.95
Anemones	0 (source)	1.07 ± 0.11
	0 (control tanks)	1.03 ± 0.07
	13	12.9 ± 1.29
	36	35.5 ± 2.06
Oysters (adult)	0 (source)	0.44 ± 0.11
	0 (control tanks)	0.51 ± 0.05
	12	8.97 ± 0.89
	35	31.4 ± 2.23
Oysters (juvenile)	0	0.17 ± 0.09
	25	23.1 ± 3.28

the growth of these populations was measured as a change in wet weight. Panel weights were measured after 2 weeks and at the end of the experiment. The change in weight of the barnacle populations was compared over both time periods. The original weight of each panel was also included in the analysis model as a covariate to correct for initial differences among panels in the sizes or numbers of barnacles.

Table 5 shows the results of these analyses. After 2 weeks neither the high nor the low arsenic treatments had any measurable effect on the barnacles. However, by the end of the experiment there was a small but significant reduction in the growth rates in both arsenic treatments relative to growth in the control chambers (Figure 8). Both analyses also indicate that there were no significant differences between chambers within treatments, indicating that conditions not controlled (e.g. chamber location) had no observable effect.

Growth rates of barnacles in the experimental treatments were also compared to those kept in constantly flowing raw seawater (Table 5). It is clear that the reduction in feeding time associated with the experiment had a much greater effect on barnacle growth rates than the presence of arsenic in the water. However, the significant reduction in growth in arsenic treatments during the second half of the experiment (which included 10 d of no arsenic exposure and continuous feeding) indicated a residual effect of arsenic exposure on growth.

Bryozoans--Bryozoans were maintained in estimated arsenic concentrations of 0 (control, 13, and 36 $\mu g\ l^{-1}$. Measured concentrations were somewhat lower, averaging 7.06 and 28.1 $\mu g \cdot l^{-1}$ (Table 4). As in the barnacle experiment, population growth was used to estimate the effect of arsenic on *Victorella* colonies. However, growth was measured as the change in area occupied rather than a change in weight.

When observed after 1 week, it was clear that *Victorella* was growing onto clean panel surfaces but generally at a slow rate. However, when observed and

Table 5. Nested analysis of variance (ANOVA) of barnacle growth data. Data were analyzed after two weeks and at the end of the experiment. A posteriori tests used Duncan's Multiple Range test. Means are mean panel weight in grams. The second a posteriori test for the two-week data was based on a similar analysis that included the data for panels kept in a continuous flow of raw water. *MS for Chamber used as the error term.

Balanus - Growth after 2 Weeks

Source	SS	DF	F	p
As Treatment	21.2952	2	4.65*	0.0646
Chamber in Treatment	13.7249	6	0.62	0.7138
Weight in Chamber	137.4066	9	4.14	0.0001
Error	597.3182	162		

A POSTERIORI TESTS:

Main Experiment

Low	Control	High
2.63	2.36	1.86

Main Experiment Compared to Continuous Flow Raw Water

Raw	Low	Control	High
11.62	2.63	2.36	1.86

Balanus - Growth through End of Experiment

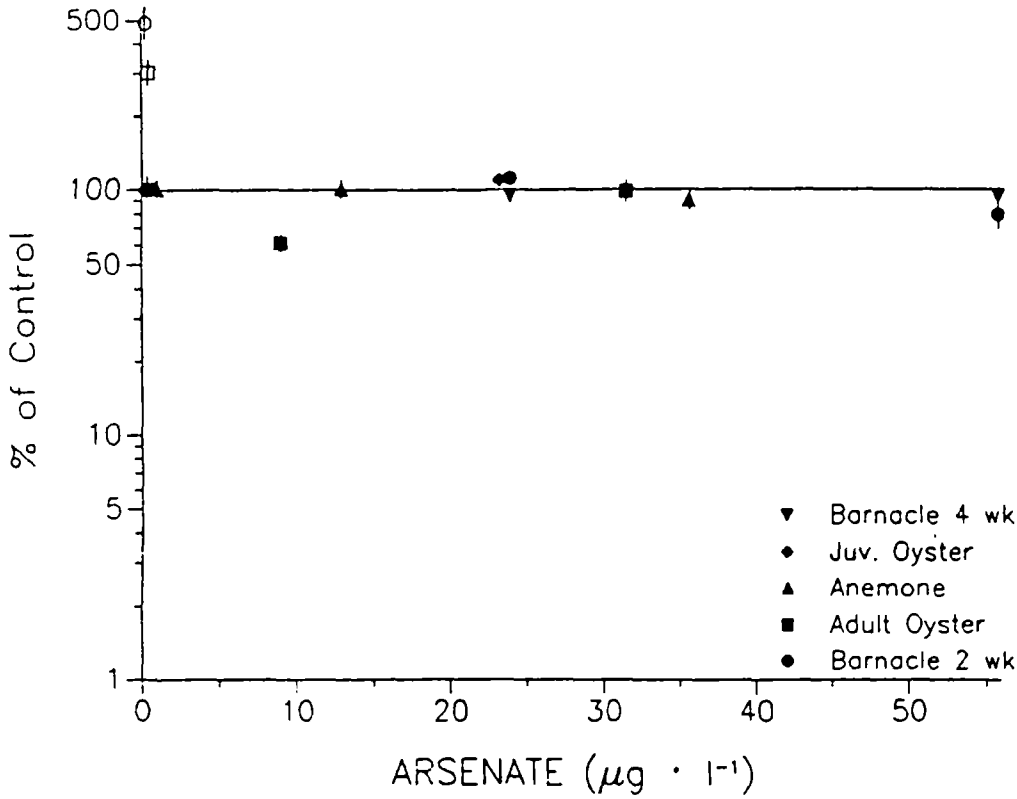
Source	SS	DF	F	p
As Treatment	41.6794	2	11.25*	0.0095
Chamber in Treatment	11.0492	6	0.32	0.9256
Weight in Chamber	95.5604	9	1.83	0.0766
Error	416.8746	72		

A POSTERIORI TEST:

Main Experiment

Control	Low	High
6.01	5.69	5.66

Figure 8. Growth rates, expressed as % of control growth, of *Balanus*, *Diadumene*, and *Crassostrea* exposed to various concentrations of arsenate. Open symbols indicate growth rate of populations maintained in flowing, unfiltered seawater.



photographed after 2 weeks, populations had declined markedly in all tanks. Detailed observation of randomly selected panels did not reveal anything abnormal. Colonies continued to feed when placed in the feeding chambers, but abundances declined greatly and by the end of the experiment the area covered on panels was 10 - 25% of that observed at the start of the experiment. Because these reductions occurred in all chambers and all treatments, no significant effect was found between treatments.

The reductions appear to have resulted from predation by species such as the polychaete worm, *Nereis succinea*. These worms were able to colonize panels when they were exposed to unfiltered water in the feeding chambers. With little other food available within the experimental chamber, bryozoans became their principal food. Therefore, if arsenic had an effect on bryozoan growth it was not detectable in the presence of such extreme predation pressure.

Sea Anemones--Anemones were maintained in similar arsenic concentrations. Arsenic averaged 12.9 and 35.5 $\mu g\ l^{-1}$ in the two treatments, essentially as predicted (Table 4). Unlike the previous 2 experiments, individual anemones rather than populations were measured. The two growth measures, basal area and wet weight are compared in Table 6. As in the barnacle experiment, no differences were found between experimental chambers within treatments. Also it is clear that significant differences in *Diadumene* growth were not found among the three treatments. Although the anemones exhibited rapid and significant growth, their growth rate was not measurably affected by the presence of dissolved arsenic (Figure 8).

Adult Oysters-- Arsenic concentrations in the treatments averaged 8.97 and 31.4 $\mu g \cdot l^{-1}$ (Table 4). In terms of shell weight, no significant differences were found among the three arsenic treatments (Figure 8; Table 7). Also, as in the previous

Table 6. Nested analysis of variance (ANOVA) of sea anemone growth data. Data were collected at the end of the experiment. A posteriori tests used Duncan's Multiple Range test. Means are for individual anemones and are in square cm or grams. *MS for Chamber used as the error term.

	Diadumene - Basal Area			
Source	SS	DF	F	p
As Treatment	0.0736	2	0.48*	0.6387
Chamber in Treatment	0.4568	6	0.79	0.5824
Error	4.6278	48		

A POSTERIORI TEST:

Low	Control	High
0.71	0.71	0.64

	Diadumene - Weight			
Source	SS	DF	F	p
As Treatment	0.0367	2	0.27*	0.7744
Chamber in Treatment	0.4128	6	1.72	0.1373
Error	1.9231	48		

A POSTERIORI TEST:

High	Low	Control
0.36	0.35	0.32

Table 7. Nested analysis of variance (ANOVA) of oyster growth data. Data were analyzed at the end of the experiment. A posteriori tests used Duncan's Multiple Range test. Means are mean weight in grams. The second a posteriori test was based on a similar analysis that included the data for oysters kept in a continuous flow of raw water. *MS for Chamber used as the error term.

Source	Adult Oysters			
	SS	DF	F	p
As Treatment	0.3765	2	1.57*	0.2985
Chamber in Treatment	0.7173	6	1.38	0.2255
Weight in Chamber	0.6803	9	0.87	0.5510
Error	14.0323	162		

A POSTERIORI TESTS:

Main Experiment

Control	High	Low
0.51	0.50	0.31

Main Experiment Compared to Continuous Flow Raw Water

Raw	Control	High	Low
1.54	0.51	0.50	0.31

experiments no effects attributable to environmental chamber were found. In general the oysters added 0.3 - 0.6 g of shell during the experiment. Increases in shell mass were not influenced by dissolved arsenic. As might be expected growth rates were proportional to the size of the oyster. However, because individual oysters were of similar size, no significant differences in weight within chambers were found (Table 7).

Because some oysters were maintained in flowing, unfiltered seawater during the experiment, the effect of the experimental reductions in feeding times were also measured. As can be seen in Table 7, oyster growth was significantly reduced in the experimental treatments and was approximately 30% of that observed for those individuals exposed continuously to food.

Larval Oysters--When panels in the larval oyster chambers were examined after 10 days, no successful recruits were found. Many empty larval shells were found adhering to the panel surface, but these individuals appeared to have died as larvae and fell to the bottom of the chamber. Water samples indicated that no larvae remained in the water column.

The cause for the complete mortality of larvae in all treatments has not been determined. However, a calculation error resulted in much lower quantities of algal food being used than planned in the experimental design. The amounts available may have been insufficient for growth and development.

Juvenile Oysters--Measured arsenic levels in this experiment were very close to predicted (Table 4). At the beginning of the experiment over 10,000 newly settled oysters were found on the experimental panels. Of these more than 2,500 increased in size over the course of the experiment and were judged to have successfully recruited prior to the study. As in the previous experiments the growth of these

juveniles was not affected by arsenic (Table 8). The analysis did demonstrate that the juvenile oysters were quite variable in their growth rates and that within chambers growth rates were significantly different among panels. However, there were no differences among chambers within treatments in mean growth rate and no differences among treatments.

Given the large number of juvenile oysters measured, the results of this experiment are unambiguous. Even though we might expect these much smaller and younger life stages to be less tolerant of stress than adults, the results were the same as observed in the adult experiment.

ARSENIC INCORPORATION BY BENTHIC ORGANISMS

Arsenic concentrations within tissues was measured at the completion of experiments with barnacles, anemones, and adult oysters. Barnacles exhibited significantly higher ($p < 0.01$) body burdens of arsenic that were proportional to the concentration of arsenic in the treatment (Table 9; Figure 9). Barnacles exposed to 23.8 $\mu g \cdot l^{-1}$ contained approximately twice the amount of arsenic, on a weight basis, as controls; barnacles exposed to 55.8 $\mu g\ l^{-1}$ contained over 3 times the arsenic.

Other benthic organisms did not incorporate significant quantities of arsenic. Tissue levels remained relatively constant, regardless of treatment arsenic concentration (Table 9; Figure 9).

Table 8. Nested analysis of variance (ANOVA) of juvenile oyster growth data. Data were analyzed at the end of the experiment. A posteriori test used Duncan's Multiple Range test. Means are mean area covered by an oyster in square mm. *MS for Chamber used as the error term. **MS for Panel used as the error term.

Source	Juvenile Oysters			
	SS	DF	F	p
As Treatment	0.3305	1	0.01*	0.9354
Chamber in Treatment	177.5131	4	0.49**	0.6984
Panel in Chamber	2070.8024	23	5.71	0.0001
Error	41059.7918	2603		

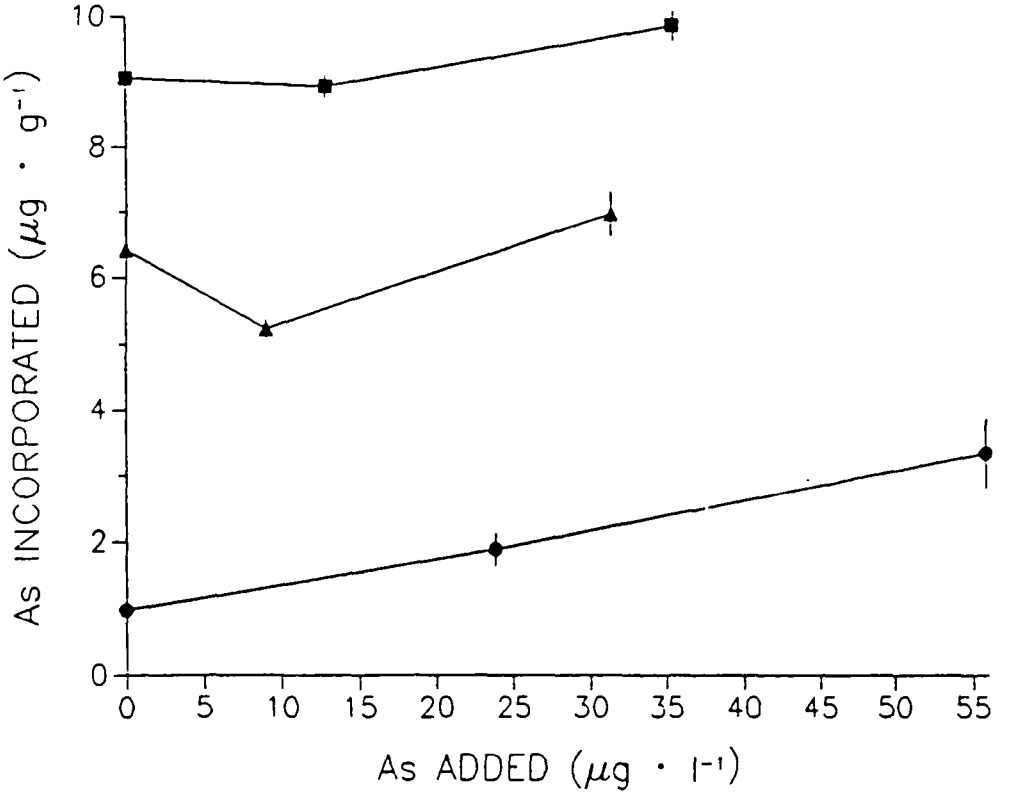
A POSTERIORI TEST:

Arsenic	Control
4.23	3.46

Table 9. Arsenic incorporation by benthic organisms. Values are in $\mu g \cdot g^{-1}$ dry weight. *Balanus* values include shell and tissue concentrations, others include only tissue concentrations.

Organism	As added $\mu g \cdot l^{-1}$	As incorporation, $\mu g \cdot g^{-1} \pm SE$
Balanus sp.	0	0.98 ± 0.09
	23.8	1.89 ± 0.24
	55.8	3.33 ± 0.52
Anemones	0	9.07 ± 0.38
	12.9	8.93 ± 0.18
	35.5	9.84 ± 0.23
Oysters	0	6.42 ± 0.38
	9.0	5.24 ± 0.14
	31.4	6.97 ± 0.34

Figure 9. Arsenic content, in $\mu g \cdot g^{-1}$, of benthic organisms maintained in various arsenic concentrations. Values shown are means ± SE. ● = Barnacles, ■ = anemones, ▲ = adult oysters.



DISCUSSION

The response of estuarine organisms to arsenate was largely as hypothesized. Phytoplankton, particularly centric diatoms, exhibited large changes in growth rate of dominant species upon exposure to arsenic, leading to changes in the species composition of the assemblage. In each phytoplankton experiment, different centric diatoms were sensitive to arsenate, *Cerataulina pelagica* during the late spring, and *Chaetoceros debile* during the summer. In the spring experiment, *Thalassiosira* sp.was able to fluorish in the absence of *C. pelagica*; it was not present in large numbers in the summer experiment. Similar changes have been observed in earlier experiments with arsenate and Chesapeake Bay phytoplankton (Sanders and Cibik, 1985; Sanders, 1986). In every experiment in which *Thalassiosira* sp. was a dominant, its growth rate in arsenic-treated assemblages exceeded its growth in controls. This increase in arsenic treatments is probably not triggered by the arsenic itself; rather, this species likely is responding to increased availability of light or nutrients caused by the decline of another centric diatom, in this case, *Cerataulina pelagica*. Note that *Thalassiosira* sp. did not bloom until *C. pelagica* had essentially disappeared from arsenic-treated tanks (Figures 1B, 1C).

The flagellate-dominated assemblage in the summer experiment was not greatly affected by arsenate. Only the diatom, *Chaetoceros debile*, exhibited significant growth inhibition (Figure 2B). Therefore, arsenate impacts to phytoplankton communities in temperate ecosystems are likely to be seasonal, causing alteration to spring and fall diatom blooms and perhaps having little effect during summer months.

Earlier work had indicated that zooplankton are quite resistant to arsenic (Biesinger and Christensen, 1972; Passino and Novak, 1984); initial studies with *Eurytemora affinis* had yielded similar results (Sanders, 1986). Therefore, we

designed a series of experiments to study the impact of arsenate to all life stages of the copepods, *E. affinis* and *Acartia tonsa*, and to follow the success of the population through several generations. Our long term studies largely upheld earlier results; there was no effect of arsenate on algal incorporation by either species, nor did arsenic impair production of nauplii. Thus, relatively high arsenic concentrations appear to be necessary to alter crustacean metabolic activities, as suggested by earlier studies. There was, however, an apparent impact of chronic arsenic exposure on the ability of nauplii in the F_1 and F_2 generations to mature to adult; in each generation, far fewer nauplii matured to adulthood when exposed to arsenic (Table 3). In addition, female to male ratios varied considerably in arsenate-dosed generations while ratios in control populations remained constant. These results may be important to long-term success of the population if copepod populations are exposed to chronic elevation of arsenic concentrations. Residence times of effluents as well as dilution within the system become increasingly important factors in the assessment of a chronic effect. However, the importance of lower population success over two to three generations, as observed here, may be masked by the relatively large potential for indirect effects from the ingestion and assimilation of arsenic-rich phytoplankton species or the food web effects resulting from arsenic-induced shifts in phytoplankton species or sizes (e.g., Sanders, 1986).

Benthic organisms also exhibited general tolerance to arsenic. With the exception of *Balanus*, none of the organisms tested showed reduced growth rates in response to arsenic treatments. With *Balanus*, there was a small, nonsignificant reduction in growth rate over the first two weeks of the experiment in response to the highest arsenic treatment, approximately 56 $\mu g\ l^{-1}$. By the end of the experiment, both of the arsenic-treated populations had significantly reduced growth, but the reduction was small, approximately 6% (Table 5). The importance of such a small decrease to the overall population is unknown.

Balanus was also the only organism to incorporate significant concentrations of arsenic during the experiments (Figure 9, Table 9), doubling and tripling its arsenic content at the two levels of arsenic treatment. However, because of its small size, *Balanus* was also the only organism in which shell material was tested for arsenic content along with muscle tissue; thus, the increased arsenic content could be caused simply by adsorption of arsenate to the carbonate shell matrix. Studies of arsenic flux through Baltic Sea microcosms have indicated that shells of organisms (*Lymnea peregra, Mytilus edulis, Cardium* sp.) exhibited high uptake of arsenic (1.7-4.6 times control) when exposed to 7.5 $\mu g\ l^{-1}$ arsenate (Rosemarin et al., 1985). Therefore, further analyses need to be performed to determine whether barnacles actually incorporated arsenic into body tissues.

A similar study of arsenic incorporation by *Crassostrea virginica* also demonstrated that chronic exposure to low arsenic concentrations did not lead to significant incorporation of arsenic (Zaroogian and Hoffman, 1982). In addition, studies of estuarine organisms (*Lymnea peregra, Gammarus oceanicus, Idotea baltica*) have indicated low to insignificant uptake from exposure to elevated arsenic concentrations in water (Notini and Rosemarin, 1986).

Other benthic organisms exhibited little response to arsenate. Although experiments with larval oysters were not successful, experiments with newly-settled juveniles demonstrated that even these fragile organisms were not affected by the arsenic concentrations presented. In addition, none of the other tested species incorporated significant quantities of arsenic during the 4-week test periods, further evidence that arsenate does not cause direct harm to trophic levels above phytoplankton.

The concentrations of arsenate used in these experiments were designed to assess direct potential impact at the highest possible concentrations that are environmentally realistic. Arsenic concentrations within the open ocean average

1.0-1.5 $\mu g \cdot l^{-1}$ (Andreae, 1978; Waslenchuk, 1978; Sanders, 1980). Within estuaries, the arsenic concentration is much more variable; however, the usual range is between 0.1-5 $\mu g \cdot l^{-1}$ (Andreae, 1978; Martin and Whitfield, 1983). Even in impacted estuaries, arsenic concentrations rarely rise above 10 $\mu g \cdot l^{-1}$. The highest concentration that we have measured in the Chesapeake Bay was in the vicinity of an abandoned fly ash dump in the Nanticoke River; concentrations ranged between 4.15 - 60.6 $\mu g \cdot l^{-1}$ in a localized area around the dump (Sanders, unpublished data). To our knowledge, the concentrations used in this study cover the range of possible concentrations in natural systems, even those receiving considerable impact from man. Therefore, the lack of direct response to arsenic seen in these experiments, except with the phytoplankton community, can be considered to be relevant to arsenic impacts to estuarine and coastal marine systems.

However, as outlined in the Introduction, there are other pathways for impact of a toxic substance within an aquatic food web. Arsenic, because of its large impact upon phytoplankton species composition and community structure, is a prime candidate for such indirect impacts. We hypothesized that direct effects of arsenic would be limited to phytoplankton and that direct impacts to other trophic levels would be minor relative to potential indirect effects associated changes in trophic structure or the ingestion of arsenic through food. Our experiments have shown that changes in dominant species can drastically alter an herbivore's ability to procure enough food to successfully reproduce (Sanders, 1986). In addition, although arsenic dissolved in the water may be unavailable and nontoxic to higher trophic levels, arsenic incorporated in their food may be quite toxic.

For example, a shift to smaller phytoplankton taxa could lead to reductions in preferred phytoplankton food items and favor prduction of smaller microzooplankton species. Enhancement of the microbial loop theoretically reduces carbon transfer to highest trophic levels, thereby reducing production in fish and shellfish stocks.

Transfer of arsenic incorporated in resistant phytoplankton to planktonic and benthic suspension feeders could also prove more of an immediate danger for the food web than exposures to dilute dissolved arsenic levels. Ingestion and subsequent assimilation of arsenic in phytoplankton might conceivably result in accumulation to levels that might alter normal cellular metabolism in metazoans, potentially reducing viability, diversion of energy to reproduction and/or offspring or slower growth.

Another potential indirect effect is the chemical transformation of arsenate to arsenite and methylarsonate after algal uptake. This transformation, which readily occurs in productive ecosystems (Sanders, 1985, 1986), effectively increases the potential for direct arsenic toxicity, for the transformed arsenic species are far more toxic to organisms than arsenate (Nissen and Benson, 1982).

Such studies of the indirect impacts of arsenic and other toxics largely remain to be done. Although this research project originally included these studies, funding reductions precluded their completion. We are currently proceeding with a limited set of experiments through funding from another branch of EPA (the Chesapeake Bay Program); if those funds continue, we will be able to complete the entire project.

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REFERENCES

Andreae, M. O. 1978. Distribution and speciation of arsenic in natural waters and some marine algae. Deep-Sea Res. 25:391-402.

Andrews, J. D. 1961. Measurement of shell growth in oysters by weighing in water. Proc. Nat. Shellfisheries Assoc. 52:1-11.

ANS (Academy of Natural Sciences, Philadelphia). 1981. Zooplankton, pp. V-H.1 to V-H.117. *In* Acad, Nat. Sci. Phila., ed. Vol. III: Chalk Point 316 demonstration of thermal entrainment and impingement impacts on the Patuxent River in accordance with COMAR 08.05.04.13. Prepared for the Potomac Electric Power Company by The Academy of Natural Sciences. Acad. Nat. Sci. Phila. Report No. 81-5.

Bartram, W. C. 1980. Experimental development of a model for the feeding of neritic copepods on phytoplankton. J. Plankton Res. 3:25-51.

Biesinger, K. E. and G. M. Christensen. 1972. Effects of various metals on survival, growth, reproduction, and metabolism of *Daphnia magna*. J. Fish. Res. Bd. Canada 29:1691-1700.

Boyle, E. A. and S. Huestedt. 1983. Aspects of the surface distributions of copper, nickel, cadmium, and lead in the North Atlantic and North Pacific, p. 379-394. *In*: C. S. Wong, E. Boyle, K. W. Bruland, J. D. Burton, and E. D. Goldberg (eds.). Trace metals in sea water. Plenum Press, New York.

Braman, R. S., D. L. Johnson, C. C. Foreback, J. M. Ammons and J. L. Bricker. 1977. Separation and determination of nanogram amounts of inorganic arsenic and methylarsenic compounds. Anal. Chem. 49:621-625.

Brownlee, D. C. and F. Jacobs. 1987. Mesozooplankton and microzooplankton in the Chesapeake Bay. *In*: S. K. Majumdar, L. W. Hall, Jr., and H. M. Austin (eds.), Contaminant problems and management of living Chesapeake Bay resources. Pa. Academy Sci., Philadelphia, in press.

Dayton, P. K. 1975. Experimental evaluation of ecological dominance in a rocky intertidal algal community. Ecol. Monog. 45:137-159.

Heinle, D. R. 1966. Production of a calanoid copepod, *Acartia tonsa*, in the Patuxent River estuary. Chesapeake Sci. 7:59-74.

Heinle, D. R. 1969. Temperature and zooplankton. Chesapeake Sci. 10:186-209.

Heinle, D. R. and D. A. Flemer. 1975. Carbon requirements of a population of the estuarine copepod *Eurytemora affinis*. Mar. Biol. 31:235-247.

Hendrickson, P., K. G. Sellner, B. Rojas de Mendiola, N. Ochoa and R. Zimmermann. 1982. The composition of particulate organic matter and biomass in the Peruvian upwelling region during ICANE 1977 (Nov. 14-Dec. 2). J. Plankton Res. 4:163-186.

Keith, L. H. and W. A. Telliard. 1979. Priority pollutants. I--A perspective view. Environ. Sci. Technol. 13:416-423.

Landry, M. R. 1977. A review of important concepts in the trophic organization of pelagic ecosystems. Helgol. wiss. Meeres. 30:8-17.

Mackenzie, F. T., R. J. Lantzy, and V. Paterson. 1979. Global trace cycles and predictions. Math. Geol. 11:99-144.

Margalef, R. 1951. Diversidad de especies en les communidades naturales. Publnes. Inst. Biol. apl. Barcelona 9:5-27.

Martin, J-M. and M. Whitfield. 1983. The significance of the river input of chemical elements to the ocean, p. 265-296. *In*: C. S. Wong, E. Boyle, K. W. Bruland, J. D. Burton, and E. D. Goldberg (eds.), Trace metals in sea water. Plenum Press, NY.

Nissen, P. and A. A. Benson. 1982. Arsenic metabolism in freshwater and terrestrial plants. Physiol. Plant. 54:446-450.

Nival, P. and S. Nival. 1976. Particle retention efficiencies of an herbivorous copepod, *Acartia clausi* (adult and copepodite stages): Effects on grazing. Limnol. Oceanogr. 21:24-28.

Notini, M. and A. Rosemarin. 1986. Fate and effects of arsenic in a mesoscale model ecosystem. Status Report prepared for the ESTHER Project, Swedish Environmental Research Group, Karlskrona, Sweden, January.

Osman, R. W. 1977. The establishment and development of a marine epifaunal community. Ecol. Monog. 47:37-63.

Osman, R. W. 1982. Artificial substrates as ecological islands, pp. 71-114. *In:* J. Cairns (ed.), Artificial substrates. Ann Arbor Science Publ., Massachusetts.

Paine, R. T. 1969. A note on trophic complexity and community stability. Am. Nat. 100:65-75.

Parsons, T. R. 1976. The structure of life in the sea, pp. 81-97. *In:* D. H. Cushing and J. J. Walsh (eds.), The ecology of the seas. W. B. Saunders Co., Philadelphia.

Passino, D. R. M. and A. J. Novak. 1984. Toxicity of arsenate and DDT to the cladoceran *Bosmina longirostris*. Bull. Environ. Contam. Toxicol. 33:325-329.

Peoples, S. A. 1975. Review of arsenical pesticides, p. 1-12. *In:* E. A. Woolson (ed.), Arsenical pesticides. ACS Symp. Ser. 7, American Chemical Society, Washington, D.C.

Pielou, E. C. 1966. The measurement of diversity in different types of biological collections. J. Theor. Biol. 13:131-144.

Rosemarin, A., M. Notini, and K. Holmgren. 1985. The fate of arsenic in the Baltic Sea *Fucus vesiculosus* ecosystem. Ambio 14:342-345.

Ryther, J. H. 1969. Photosynthesis and fish production in the sea. Science 166:72-76.

Sanders, J. G. 1980. Arsenic cycling in marine systems. Mar. Environ. Res. 3:257-266.

Sanders, J. G. 1983. Role of marine phytoplankton in determining the chemical speciation and biogeochemical cycling of arsenic. Can. J. Fish. Aquat. Sci. 40:192-196.

Sanders, J. G. 1985. Arsenic geochemistry in Chesapeake Bay: dependence upon anthropogenic inputs and phytoplankton species composition. Mar. Chem. 17:329-340.

Sanders, J. G. 1986. Direct and indirect effects of arsenic on the survival and fecundity of estuarine zooplankton. Can. J. Fish. Aquat. Sci. 43:694-699.

Sanders, J. G. and S. J. Cibik. 1985. Adaptive behavior of euryhaline phytoplankton communities to arsenic stress. Mar. Ecol. Prog. Ser. 22:199-205.

Sanders, J. G. and P. S. Vermersch. 1982. Response of marine phytoplankton to low levels of arsenate. J. Plankton Res. 4:881-893.

Sanders, J. G. and H. L. Windom. 1980. The uptake and reduction of arsenic species by marine algae. Estuarine Coastal Mar. Sci. 10:555-567.

Sanders, J. G., J. H. Ryther, and J. H. Batchelder. 1981. Effects of copper, chlorine, and thermal addition on the species composition of marine phytoplankton. J. Exp. Mar. Biol. Ecol. 49:81-102.

Sellner, K. G. 1983. Plankton productivity and biomass in a tributary of the upper Chesapeake Bay. I. Importance of size-fractionated phytoplankton productivity, biomass and species composition in carbon export. Est. Coastal Shelf Sci., 17:197-206.

Sellner, K. G. 1987. Phytoplankton in Chesapeake Bay: role in carbon, oxygen and

nutrient dynamics. *In*: S. K. Majumdar, L. W. Hall, Jr., and H. M. Austin (eds.), Contaminant problems and management of living Chesapeake Bay resources. Pa. Academy Sci., Philadelphia, in press.

Sellner, K. G. and R. J. Horwitz. 1982. Plankton interactions in the Patuxent River estuary: Field studies of community composition and density, with a deterministic model of the effects of zooplankton grazing on phytoplankton carbon, production of fecal matter, sediment oxygen demand and nutrient regeneration. Acad. Nat. Sci., Philadelphia, PA. Rept. No. 82-14D. 111 pp.

Sellner, K. G. and M. M. Olson. 1985. Copepod grazing on red tides of Chesapeake Bay, pp 245-250. *In*: D. M. Anderson, A. W. White and D. G. Baden (eds.), Toxic dinoflagellates. Elsevier, NY.

Uthe, J. F., H. C. Freeman, J. R. Johnston, and P. Michalik. 1974. Comparison of wet ashing and dry ashing for the determination of arsenic in marine organisms, using methylated arsenicals for standards. J. Assoc. Off. Anal. Chem. 57:1363-1365.

Van Valkenburg, S. D., J. K. Jones, and D. R. Heinle. 1978. A comparison by size and volume of detritus versus phytoplankton in Chesapeake Bay. Est. Coastal Mar. Sci. 6:569-582.

Waslenchuk, D. G. 1978. The budget and geochemistry of arsenic in a continental shelf environment. Mar. Chem. 7:39-52.

Zaroogian, G. E. and G. L. Hoffman. 1982. Arsenic uptake and loss in the American oyster, *Crassotrea virginica*. Environ. Monit. Assess. 1:345-358.