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Pathways of arsenic uptake and incorporation in estuarine phytoplankton and the filter-feeding invertebrates *Eurytemora affinis*, *Balanus improvisus* and *Crassostrea virginica*

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Abstract

Arsenic uptake from water and from phytoplankton was followed in the copepod *Eurytemora affinis* and the barnacle *Balanus improvisus* collected from the Patuxent River estuary, Chesapeake Bay, eastern coast of the USA in 1987, and in the oyster *Crassostrea virginica* obtained from a hatchery on the shore of Chesapeake Bay in 1987. Dissolved arsenic was readily taken up by phytoplankton and by shell material of *B. improvisus* and *C. virginica*; however, no dissolved arsenic was incorporated into the invertebrate tissues. When *E. affinis*, *B. improvisus* and *C. virginica* were fed phytoplankton containing elevated arsenic contents, significant arsenic incorporation occurred. Juvenile *B. improvisus* incorporated relatively more arsenic than adults of all three species. Compared to the 100 to 200% increase in arsenic content by phytoplankton exposed to dissolved arsenic, the 25 to 50% increase in these invertebrate species via trophic transfer is relatively small. Even though the trophic pathway for arsenic transfer is the major one for higher trophic levels within an ecosystem, the potential for direct arsenic impact to trophic levels other than phytoplankton appears to be minimal.

Introduction

For most aquatic organisms, two pathways are most important for uptake of toxic substances: uptake from dissolved substances in water and assimilation of substances from food. Both pathways can be important to some organisms, but water is usually the principal source of most toxic substances to the greatest number of organisms, especially for primary producers. Uptake of contaminants from food is more important for herbivores and carnivores (Macek et al. 1979, Spacie and Hamelink 1985, Sanders and Riedel 1987a).

Arsenic is concentrated in the tissues of aquatic organisms. Arsenate, the principal form of arsenic in surface waters, is readily taken up and incorporated by aquatic plants because of its chemical similarity to phosphate, a necessary plant nutrient (Blum 1966, Sanders and Windom 1980). Arsenate can also undergo biological transformation after uptake, and be released into the water column as a reduced inorganic compound (arsenite) and as a variety of methylated arsenicals (Sanders 1980, Sanders and Windom 1980). Arsenic should be available to higher trophic levels in both forms: dissolved in water in a variety of chemical species (Sanders 1980, 1985) and incorporated into algal tissue. Because animals obtain the majority of their phosphate requirement from food rather than water (Valiela 1984), the ingestion of food should be an important pathway of arsenic uptake. The purpose of this study was to separate the two possible pathways for arsenic uptake and to determine their relative importance to various organisms and trophic levels. The study consisted of controlled experiments using single species and defined food sources, and a more complex, multi-trophic level experiment using natural estuarine populations.

Materials and methods

Experimental procedures and organisms

Experiments were conducted either in laboratory tanks or in outdoor, flow-through microcosms. All experiments were conducted using water from the Patuxent River estuary, a subestuary of the Chesapeake Bay, on the eastern coast of the United States. Organisms used included natural phytoplankton, the planktonic copepod *Eurytemora affinis*, the barnacle *Balanus improvisus*, and the oyster *Crassostrea virginica*. All except *C. virginica* were collected from the Patuxent River. *C. virginica* were obtained from a hatchery on the western shore of the Chesapeake Bay.

Phytoplankton feeding-cultures

Thalassiosira pseudonana (Clone SWAN1), *Dunaliella* sp. (DUN), and *Isochrysis galbana* (TISO), were originally obtained from the Provasoli-Guillard Center for the Culture of Marine Phytoplankton. *Rhizosolenia fragilissima* (RFRAG), and *Skeletonema costatum* (SCOST) were isolated from the Patuxent River. *Prorocentrum mariae-lebouriae* (PROML) was obtained from R. Rivkin, University of Maryland, Horn Point Environmental Laboratories. Algal cultures were maintained in a medium deficient in phosphate (*f*/5 with phosphate maintained at concentrations equivalent to *f*/10: Guillard and Ryther 1962). Arsenic (as sodium arsenate) was added to cultures as cells neared the end of logarithmic growth phase. Control cultures received no arsenic additions. Treatment cultures were incubated with arsenic for 48 to 96 h before being used as food. This procedure maximized the cellular arsenic content while minimizing variation (Sanders and Windom 1980, Sanders et al. 1988).

Studies with *Eurytemora affinis*

Adult *Eurytemora affinis* were collected from the Patuxent River in February 1986 (8 °C, 14‰ S) by net (202 μm mesh). *E. affinis* were separated from other plankton, fed on six species of phytoplankton (*Thalassiosira pseudonana, Dunaliella* sp., *Rhizosolenia fragilissima, Isochrysis galbana, Prorocentrum mariae-lebouriae,* and *Skeletonema costatum*) for 42 h, then rinsed free of algal cultures and kept without food for 24 h. The adults were allowed to graze for 24 h on unialgal cultures containing one of the six phytoplankton species above, both on control cultures and on cultures which had elevated arsenic concentrations. Other *E. affinis* were exposed to arsenic dissolved in water for 24 h. Approximately 5 000 individuals were placed in each culture, with culture densities equivalent to 5 μg wet weight of phytoplankton per individual. After grazing and exposure, individuals were removed from the culture, rinsed, and resuspended in filtered water for 1 h to allow them to clear their guts. After this period, several subsamples of each group of *E. affinis* were taken for enumeration, and the remainder were dried and prepared for arsenic analysis. Subsamples of the phytoplankton cultures were also taken for arsenic analysis.

Studies with *Balanus improvisus*

Balanus improvisus were collected on 115 cm^2 PVC panels in May, 1987. Approximately 200 panels were exposed in the lower Patuxent estuary 45 d prior to the beginning of the experiment. These panels were collected, cleaned of any debris, and all organisms other than *B. improvisus* were removed. After being cleaned, each panel was then assigned randomly to one of nine 80-liter environmental chambers. Chambers were then assigned randomly to one of three arsenic treatments. Treatments consisted of ambient (0.4 μg l^{-1}), 23 μg l^{-1}, and 55 μg l^{-1} added arsenic, as arsenate (actual measured concentrations). Arsenate concentrations were maintained in the environmental chambers using continuous-flow systems (Sanders et al. 1988). Panels were removed from the chambers every day, rinsed in clean, filtered water, and placed in feeding tanks for 3 to 4 h, after which they were rinsed and returned to the experimental chambers. The experiment lasted 22 d. At the conclusion, the panels were rinsed and blotted, and *B. improvisus* were removed, dried, and weighed prior to arsenic analysis.

To investigate arsenic uptake from food, a similar study was performed, except that feeding tanks contained known quantities of phytoplankton from control cultures and cultures containing elevated arsenic content. *Thalassiosira pseudonana* and *Dunaliella* sp. were used for feeding cultures.

Studies with *Crassostrea virginica*

Crassostrea virginica were maintained in a manner similar to *B. improvisus,* with individuals being randomly assigned to chambers and chambers assigned to treatments. Arsenic treatments were ambient, 13 μg l^{-1}, and 35 μg l^{-1} added arsenic (measured concentrations). As for *B. improvisus, C. virginica* were maintained under continuous flow, and removed to feeding chambers for 4 h per day. After 28 d, they were removed from the chambers, and tissues were removed from the shells, weighed, dried, and kept for arsenic analysis. Shell material was also retained for analysis.

Integrative studies using natural phytoplankton

A system of large-volume (500 liters), outdoor tanks was utilized. The tanks were submerged in a raceway through which running water was circulated to maintain the temperature in the tanks to within 1 C° of the ambient water temperature. The tanks were operated as continuous, flow-through phytoplankton cultures using the mesohaline river water without nutrient enrichment as the diluent (Sanders et al. 1981, Sanders and Cibik 1985). The tanks were initially filled during May 1987 (18 °C, 8.5‰ S) 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 continuously supplied to each tank at a nominal dilution rate of 50% per day (Sanders and Cibik 1985, D'Elia et al. 1986) and continuous infusions of arsenic were begun. The experiment lasted 28 d.

Three tanks received no arsenic additions; three received arsenic additions at a measured level of 8.12 ± 1.36 μg l^{-1}. Ambient arsenic concentrations averaged 0.32 ± 0.15 μg l^{-1}. After the experiment was begun, infusions of one control and one arsenic-dosed culture were inadvertently switched; 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 total cell density and phytoplankton species composition. Cell densities initially were 1×10^7 cells l^{-1} and increased slowly during the experiment, peaking at 4×10^7 cells l^{-1} after 25 d. The phytoplankton were dominated by centric diatoms (50 to 80% of total cell density), with *Thalassiosira pseudonana* the predominant species. Several other species were present in large numbers during the course of the experiment, including the diatoms *Rhizosolenia fragilissima* and *Cerataulina pelagica* and a number of small (< 10 µm), unidentified flagellate species (Sanders and Cibik 1988).

The overflow from each tank was split and was pumped into separate chambers containing either barnacles on PVC plates or adult oysters. These separate feeding cultures were started 5 d after arsenic flows had begun, allowing the phytoplankton assemblage time to respond to the arsenic.

At the completion of the experiment, phytoplankton, *Balanus improvisus* and *Crassostrea virginica* were sampled for analysis of arsenic content. Shell material was retained for analysis.

Arsenic analysis

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

Animal tissues were dried, pulverized, weighed, and ashed at 500 °C for 24 h in the presence of an ashing aid (Uthe et al. 1974) to prevent loss of arsenic. After ashing, the residue was dissolved in 1 *N* HCl and analyzed as above.

Phytoplankton were collected on acid-washed, glass-fiber filters, dried, weighed, and digested in teflon vials using redistilled, concentrated HNO_3 (Sanders and Windom 1980). Digest residues were redissolved in 1 *N* HNO_3 and analyzed as above.

In order to separate arsenic incorporated in *Balanus improvisus* tissue from that sorbed to shell, whole organisms were leached at room temperature in 1 *N* HCl overnight, then heated at 60 °C for 3 h to dissolve shell material and associated arsenic. The solute was analyzed as above. While this leach solubilizes some tissue, the complex arsenic-carbon compounds that form the majority of arsenic in animal tissue are not broken down and such compounds are not detected. Several standard-reference materials were subjected to the same leach. Arsenic yields were 1.8% for NBS 1 566 (oyster tissue), 0.6% for National Research Council of Canada DORM-1 (dogfish muscle), and 4.5% for NRCC TORT-1 (lobster hepatopancreas). In contrast, arsenic yields from a similarly leached plant material, NBS 1572 (citrus leaves), was 90%, demonstrating the greater acid lability of arsenic compounds in plant tissues. This estimate

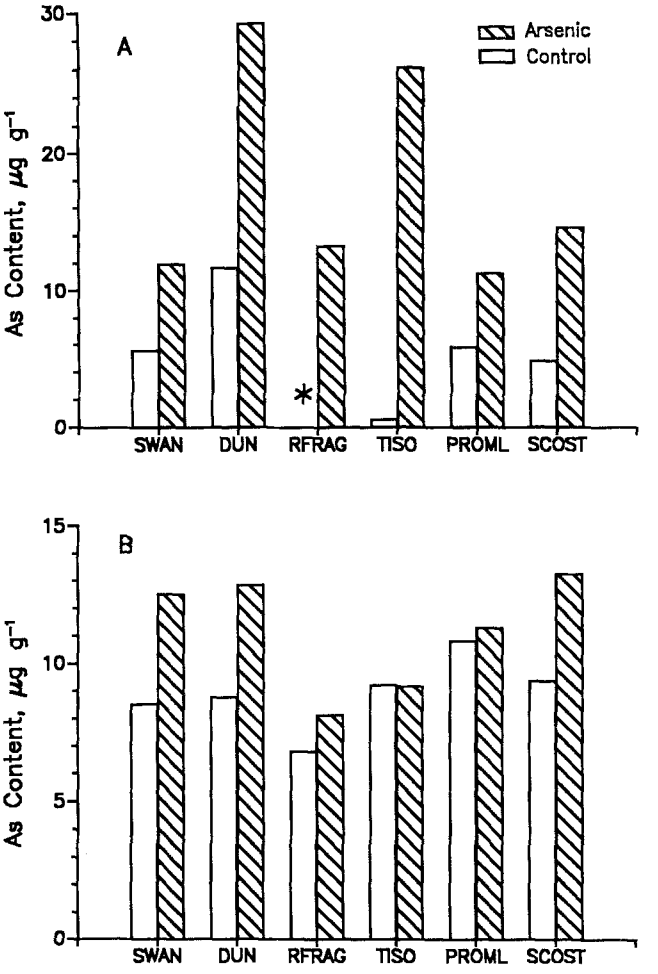

Fig. 1. *Eurytemora affinis.* Arsenic content of copepods fed control and arsenic-containing phytoplankton. (A) Arsenic content of phytoplankton feeding-cultures; (B) Arsenic content of *E. affinis.* Clonal abbreviations are as follows: SWAN, *Thalassiosira pseudonana* (Clone SWAN1); DUN, *Dunaliella* sp.; RFRAG, *Rhizosolenia fragilissima* (* no control sample available); TISO, *Isochrysis galbana*; PROML, *Prorocentrum mariae-lebouriae*; SCOST, *Skeletonema costatum*

of shell-associated arsenic was subtracted from our analyses of total arsenic to obtain an estimate of arsenic in tissues. Ground oyster shells were handled similarly.

Technique accuracy was assessed through the use of standard-reference materials, NBS #1 566 (oyster tissue) and NRC NASS-1 (a seawater standard). Recoveries of these materials averaged 95 and 91%, respectively. Standard materials were carried through each series of digestions to ensure sample integrity.

Results

Phytoplankton

Phytoplankton readily incorporated dissolved arsenic. In all experiments, regardless of algal type or culture technique, arsenic concentrations increased markedly. The average increase in arsenic content of six cultured species was 213%, and every species exhibited at least a doubling of arsenic

content (Fig. 1 A). Average arsenic contents for these species increased from 5.66 to 17.69 $\mu g\ g^{-1}$ when cultured at an arsenic concentration of 25 $\mu g\ l^{-1}$ (relative to ambient arsenic concentrations of 0.5 to 1.0 $\mu g\ l^{-1}$: Sanders 1985).

Eurytemora affinis experiment

Adult *Eurytemora affinis* exhibited no uptake of dissolved arsenic and a small increase when fed arsenic-contaminated phytoplankton. Overall, the arsenic content of *E. affinis* increased to an average of $11.18 \pm 0.86\ \mu g\ g^{-1}$ (mean $\pm$ SD) in organisms fed phytoplankton containing elevated arsenic concentrations, compared to an arsenic content of $8.91 \pm 0.53\ \mu g\ g^{-1}$ in organisms fed control phytoplankton. *E. affinis* feeding on four of the six algal species exhibited an increase in arsenic content that was greater than the error in analysis (5%; Fig. 1 B). The overall average increase, 25%, was less than the average increase in arsenic content of the phytoplankton, however.

Balanus improvisus experiment

Balanus improvisus exposed to dissolved arsenic in water did not incorporate arsenic in their tissues, but did increase the quantity of arsenic associated with shell material. While tissue concentrations remained constant at $0.88 \pm 0.37\ \mu g\ g^{-1}$ over a wide range of arsenic concentrations, shell content increased from 0.32 ± 0.04 to $2.01 \pm 0.52\ \mu g\ g^{-1}$ (Fig. 2).

Balanus improvisus fed arsenic-contaminated phytoplankton exhibited a large increase in total arsenic concentration over controls, from $0.34 \pm 0.11\ \mu g\ g^{-1}$ to $1.73 \pm 0.09\ \mu g\ g^{-1}$, with organisms feeding on individual phytoplankton species and combined phytoplankton species exhibiting similar arsenic incorporation (Fig. 3). This increase was much less than the increase in arsenic content of the phytoplankton on which they were fed. The small sample size available in this experiment (which was performed with young individuals) precluded a separate analysis for arsenic incorporated in tissue and shell material.

Balanus improvisus grown on the natural phytoplankton assemblage from the outdoor cultures exhibited a similar increase in arsenic content: an increase from $0.34 \pm 0.12\ \mu g\ g^{-1}$ in controls to $2.11 \pm 0.70\ \mu g\ g^{-1}$ in those fed phytoplankton with elevated arsenic concentrations (Fig. 4). Although the arsenic in the shell increased slightly, from 0.04 to 0.16 $\mu g\ g^{-1}$, the majority of the increase was in tissue content of arsenic, which increased from 0.30 $\mu g\ g^{-1}$ in controls to 1.95 $\mu g\ g^{-1}$ in organisms feeding on phytoplankton with elevated arsenic concentrations (Fig. 4).

Crassostrea virginica experiment

Crassostrea virginica exposed to dissolved arsenic did not incorporate significant quantities of arsenic within tissues. Average tissue concentrations ranged from 5.24 to 6.97 μg

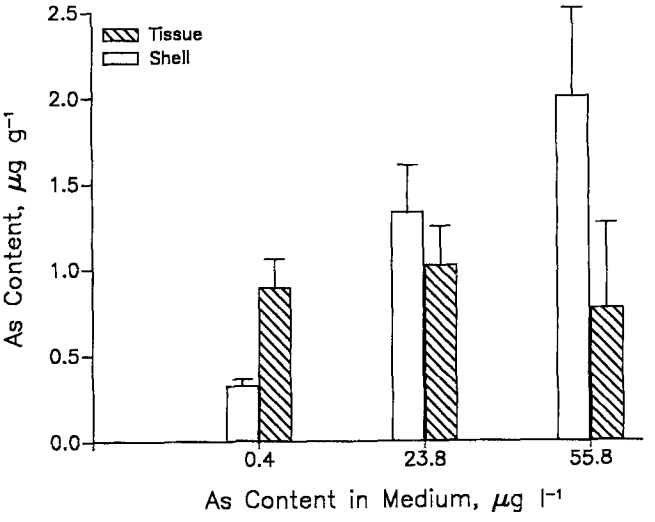

Fig. 2. *Balanus improvisus.* Arsenic content of tissue and shell of barnacles exposed to varying concentrations of dissolved arsenate for 22 d. Vertical bars indicate standard deviations

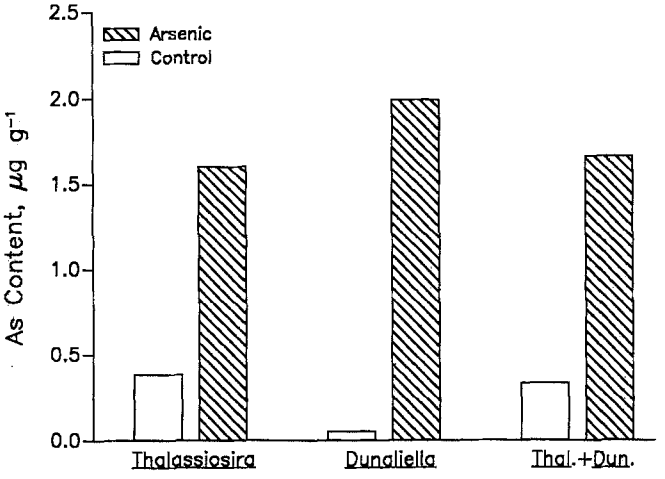

Fig. 3. *Balanus improvisus.* Arsenic content of barnacles fed control and arsenic-containing phytoplankton (*Thalassiosira pseudonana* and *Dunaliella* sp.)

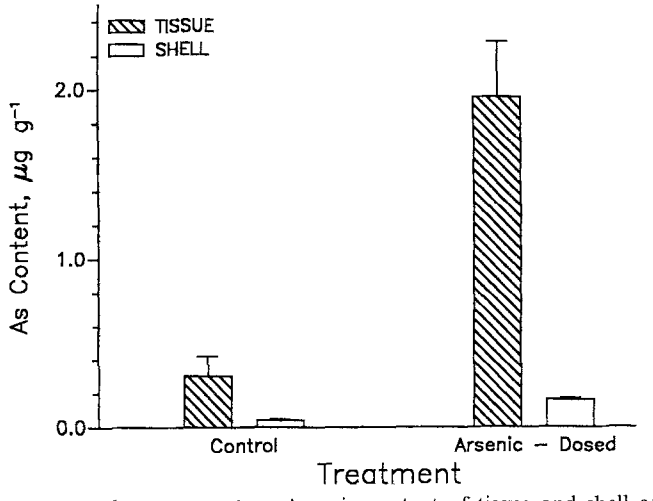

Fig. 4. *Balanus improvisus.* Arsenic content of tissue and shell of barnacles feeding on control and arsenic-containing phytoplankton for 28 d during the integrative experiment. Vertical bars indicate standard deviations

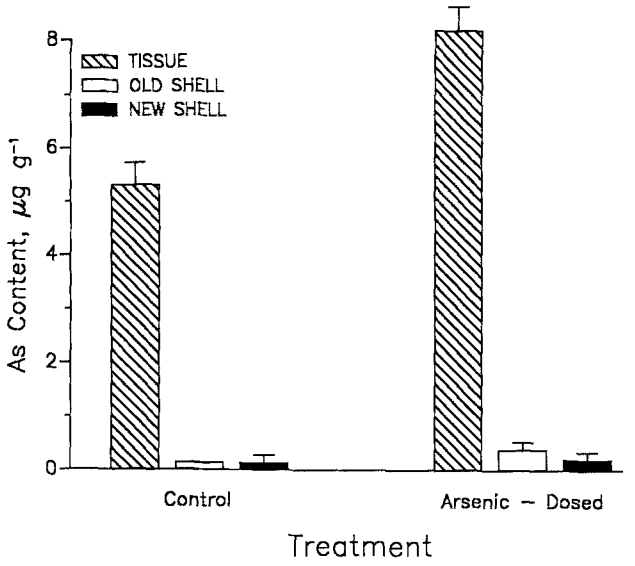

Fig. 5. *Crassostrea virginica.* Arsenic content of oysters (tissue, shell, new bill) feeding on control and arsenic-containing phytoplankton for 28 d during the integrative experiment. Vertical bars indicate standard deviations

g^{-1}, with no difference between treatments. Adults exposed to phytoplankton containing elevated concentrations exhibited significant increases in arsenic content of tissues, increasing from 5.30 ± 0.43 $\mu g\ g^{-1}$ in individuals fed control phytoplankton to 8.16 ± 0.44 $\mu g\ g^{-1}$ in individuals fed phytoplankton containing elevated arsenic concentrations (Fig. 5). Analysis of shell material indicated a small arsenic uptake: old and new shell material averaged 0.38 and 0.19 $\mu g\ g^{-1}$, respectively, in individuals fed phytoplankton containing elevated arsenic; and 0.13 and 0.12 $\mu g\ g^{-1}$, respectively, in control individuals (Fig. 5).

Discussion

Phytoplankton readily incorporate large quantities of arsenic and exhibit growth reductions at relatively low concentrations (5 to 10 $\mu g\ g^{-1}$) in estuarine and marine systems (Sanders and Cibik 1985, Sanders and Riedel 1987 b, Blanck and Wangberg 1988 a, b). While arsenic sensitivity is species-specific (Sanders and Vermersch 1982, Blanck and Wangberg 1988 b), it is tied to competition for uptake of the required phosphorus ion and resultant decreases in oxidative phosphorylation (DaCosta 1972), interference with phosphate metabolism (Planas and Healey 1978), and perhaps photosynthesis (Blanck and Wangberg 1988 a).

Estuarine invertebrates that have been studied are remarkably resistant to dissolved arsenic. Arsenic concentrations must exceed 100 to 1 000 $\mu g\ l^{-1}$ before significant impact occurs (Sanders 1986, Sanders et al. 1988). This resistance probably results from the relative unavailability of dissolved inorganic arsenic to invertebrates and vertebrates, as demonstrated in the present study on *Eurytemora affinis*, *Balanus improvisus* and *Crassostrea virginica* and similar studies with other estuarine organisms (species of *Diadumene*, Sanders et al. 1988; *Palaemonetes*, Lindsay and Sanders 1989; *Crassostrea*, Zaroogian and Hoffman 1982; whiting (*Sillago*), Edmonds and Francesconi 1987) and in the Baltic Sea in experimental microcosms containing a large variety of intertidal and subtidal organisms (Notini and Rosemarin 1986). In both the Baltic Sea experiments and the present study the only incorporation of dissolved arsenic was in calcareous shell material.

The high degree of arsenic incorporation by phytoplankton, however, allows a separate pathway of uptake for higher trophic levels that is important in the species studied here. Arsenic contained within food, unlike dissolved arsenic, was incorporated by the invertebrate species examined in the present study, but to a lesser extent than by phytoplankton. Similar results occurred in Baltic Sea experiments (Rosemarin et al. 1985).

The majority of the arsenic contained within tissues, both plant and animal, are organo-arsenic compounds (Lunde 1977, Sanders and Windom 1980, GESAMP 1986, Norin et al. 1987). A variety of arsenosugars are synthesized from arsenate by algae (Lunde 1973, Edmonds and Francesconi 1981, Wrench and Addison 1981, Norin et al. 1987) and, unlike, inorganic arsenic, these are assimilated by invertebrates (Klumpp 1980, Blanck et al. 1987, Hanaoka et al. 1987). However, the generally low degree of arsenic incorporation suggests that only some of the organo-arsenic compounds (the arsenosugars) are available. Arsenobetaine and other complex organoarsenic compounds found in animals are not available for assimilation and are eliminated unmetabolized (Crecelius 1977, Kaise et al. 1985, Norin et al. 1987, Lindsay and Sanders 1989). Thus, the potential for direct impact from arsenic ingested via food is not likely to be significant in most coastal systems. In the integrative experiment, less than 0.5% of the dissolved arsenate was incorporated into phytoplankton tissues. Of this, 7.2 to 10.2% was retained by the organisms feeding on the phytoplankton, yielding an overall retention of 0.04% of total arsenic within the system.

The low degree of incorporation and the apparent absence of food-chain magnification suggests that for many organisms arsenic-uptake rates will decline throughout an individual's development. For many suspension-feeders, the maximum size of food particles is positively correlated to the size of the individual. For example, newly settled *Balanus improvisus* feed principally on phytoplankton, causing high arsenic uptake rates. With increasing size and an increasing proportion of zooplankton in their diet, arsenic uptake rates in this and similar species should decline. If rapid increases in tissue biomass are accompanied by higher arsenic-uptake rates, arsenic uptake should also decline with age. For most invertebrates, relative growth rates are much higher for juveniles. Thus, lower adult growth rates may also contribute to a decline in uptake, as was exhibited in these experiments with *B. improvisus*.

Fig. 6 summarizes possible biotic interactions within these experiments and the potential for incorporation of arsenic. A comparison of the results of the individual and the integrative experiments suggests that the trophic path-

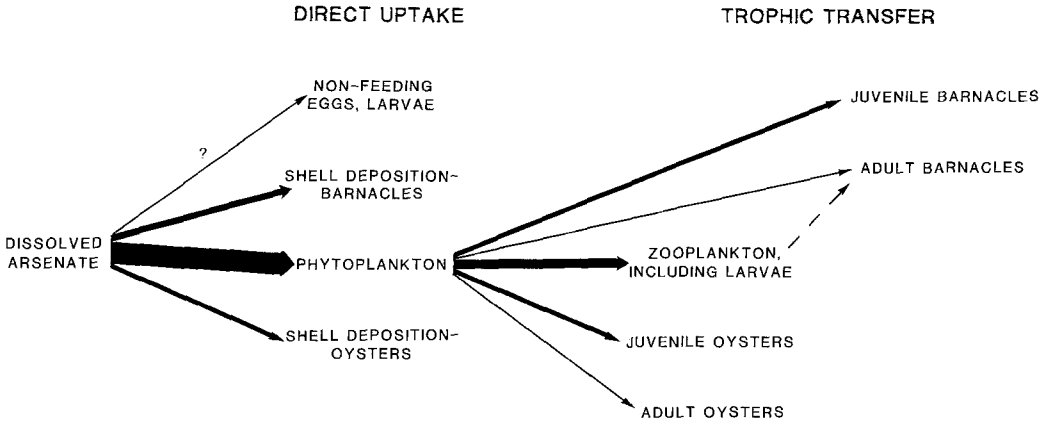

Fig. 6. Schematic representation of pathways of arsenic uptake in a simplified estuarine ecosystem through direct uptake and trophic transfer. Thickness of lines denote (relative) amount of arsenic incorporated; dashed line indicates no transfer of arsenic even though feeding occurs

way is the major one affecting higher levels of the ecosystem. Because of the relatively low efficiency of incorporation, however, potential direct impacts from elevated arsenic levels should not be important to trophic levels other than phytoplankton.

Other potential, indirect pathways for impact do exist, however, through alteration of species composition in the phytoplankton community and subsequent alteration of feeding patterns in herbivores (Sanders 1986, Sanders et al. 1988). Another type of indirect effect is the interaction of developmental, life-history, or reproductive changes with the trophic transport of arsenic. Arsenic can be incorporated into eggs and be passed onto the next generation. Under conditions of chronic exposure, the second and subsequent generations may accumulate increased concentrations, particularly in the youngest stages feeding on phytoplankton. For example, experiments with the copepod *Eurytemora affinis* (Sanders et al. 1988), showed a decreased ability of second-generation individuals to successfully develop to adults when cultured under conditions of chronic arsenic exposure. Thus, it is clear that bioaccumulation and the potential for impact, even in a relatively simple ecosystem, can be exceedingly complex.

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