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Effects of copper and zinc on two planktonic ciliates*

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Abstract

The interactive effects of copper and zinc on two estuarine planktonic ciliates, *Favella* sp. and *Balanion* sp., were determined in seawater media in which the free metal ion activities were controlled by nitrilotriacetic acid (NTA) trace metal ion buffer systems. Cupric ion activities of 10^{-10} M caused abnormal motility in both ciliates in short-term (5 h) tests, and cupric ion activities as low as $10^{-12.8}$ M decreased the growth rates of both species in longer-term experiments. In the short-term tests, zinc ion activity by itself did not affect the motility of the ciliates, but there were significant interactions between copper and zinc. In the longer-term experiments, the growth of *Favella* sp. was optimal at the lowest cupric ion activity (10^{-13} M) and the two lowest zinc ion activities (10^{-12} and 10^{-11} M), and copper and zinc inhibited growth at activities above these values. By contrast, optimal growth rate of *Balanion* sp. occurred at the highest zinc ion activity (10^{-10} M) and the lowest cupric ion activities (10^{-13} to 10^{-12} M) and growth rate was reduced at zinc ion activities $\leqq 10^{-11}$ M. There was an antagonism between copper and zinc which was particularly pronounced in *Balanion* sp.

Introduction

Copper and zinc are essential for plant and animal growth, but both can be toxic at elevated concentrations. The availability of these and other trace metals to marine organisms has been shown to be controlled by free metal ion activity rather than the total metal concentration. This has been demonstrated for a number of metals and organisms, including copper toxicity to phytoplankton (Sunda and Guillard, 1976; Anderson and Morel, 1978; Jackson and Morgan, 1978), bacteria (Sunda and Gillespie, 1979), and copepods (Borgman, 1981); copper, iron, and manganese uptake by phytoplankton (Sunda and Guillard, 1976; Anderson and Morel, 1982; Sunda and Huntsman, 1985); copper uptake by oysters (Zamuda and Sunda, 1982) and zinc limitation in phytoplankton (Anderson *et al.,* 1978).

The toxicity of trace metals, such as copper and zinc, is often affected by the activities of other metals due to competition among metals for binding to cellular ligands; for example, in cases where a "toxic metal" interferes with the uptake and metabolism of essential nutrient metals (Sunda and Huntsman, 1983). Such competitive antagonisms have been identified in phytoplankton between copper and manganese (Sunda and Huntsman, 1983), copper and zinc (Price and Quigley, 1966; Rueter and Morel, 1981), and cadmium and iron (Harrison and Morel, 1983), and have been identified in amphipods between cadmium and zinc (Oakden *et al.,* 1984). As a result of competition among metals, metal ion ratios as well as individual metal ion activities are important in determining overall effects.

There is evidence that both zinc and copper may occur at levels in marine waters which are limiting or inhibitory to some organisms. There is no conclusive evidence for copper limitation in natural waters, but copper may at times be toxic to at least some phytoplankton species (reviewed in Huntsman and Sunda, 1980; Morel and Morel-Laurens, 1983). For zinc, there is evidence both for limitation by low free ion activities (Anderson *et al.,* 1978; Brand *et al.,* 1983) and for toxicity to phytoplankton in polluted waters (Davies and Sleep, 1979).

In general, much more is known about the effects of trace metals on phytoplankton than on the zooplankton in marine food webs. The ciliated protozoans (tintinnids and non-loricate forms) are thought to be important components of some planktonic food webs (Beers and Stewart, 1967, 1969, 1970, 1971; Heinbokel and Beers, 1979; Capriulo and Carpenter, 1983), but little is known about their

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sensitivity to trace metal perturbations. As part of the Controlled Ecosystem Pollution Experiment (CEPEX), planktonic populations in large enclosures were subjected to copper additions (free ion activities were not known). The tintinnids declined or disappeared in the 0.08 and 0.16 μM Cu treatments (5 and 10 $\mu g\ l^{-1}$). Some oligotrichs grew in the enclosures that received the 0.08 and 0.16 μM additions, but these species were different from those in the controls. It was not clear, however, whether the effects were due to the direct action of copper on ciliates or to changes in other trophic levels (food or predators of ciliates) that indirectly led to changes in the ciliate populations (Beers *et al.*, 1977).

In this study we examined the effects of free cupric and zinc ion activities on ciliates in a model food web consisting of a single phytoplankton species and two species of ciliates. As experimental organisms, we chose two planktonic ciliates which prey on the dinoflagellate *Heterocapsa triquetra* (Stoecker *et al.*, 1984). One of these ciliates, the tintinnid *Favella* sp. also preys on the smaller ciliate *Balanion* sp. (Stoecker and Evans, 1985). Because the availability or toxicity of trace metals depends on the free metal ion activities rather than on the total amount of metal in seawater, we used a synthetic chelator, nitrilotriacetic acid (NTA), in combination with added copper and zinc, to control free cupric and zinc ion activities in our experimental media. With this trace metal buffer system, the concentration of free cupric and zinc ions could be varied systematically using a single concentration of NTA (10^{-4} M), and different combinations of total dissolved copper and zinc.

Materials and methods

Culture of the dinoflagellates and the ciliates

Cultures of *Heterocapsa triquetra* (Stein) (Strain A984), *Favella* sp., and *Balanion* sp. were isolated from Perch Pond, a small estuary in Falmouth, Massachusetts, USA. The algae were maintained in enriched seawater medium f/2 (Guillard, 1975) without added silicate. They were grown on a 14 hL:10 hD cycle at 20 °C. *Favella* sp. was cultured at 15° or 20 °C as described in Stoecker *et al.* (1981), except that aged Sargasso seawater, diluted to a salinity of 32‰ S, was used to prepare the culture media. The isolation and culture methods for *Balanion* sp. were similar to those for *Favella* sp., except that *Balanion* sp. was fed approximately 1 000 cells ml^{-1} of *H. triquetra* (*Favella* sp. was fed less than 1 000 cells ml^{-1}) and was maintained at 15 °C.

Preparation of media

To minimize metal contamination, all filters, filtration apparati and storage bottles for seawater and experimental media were soaked in 2 M HCl for at least four days and then rinsed thoroughly with distilled water before use. Media were stored in polycarbonate, polypropylene, or polymethylpropylene bottles before use.

Table 1. Composition of the exposure medium

	Conc. (M)	
Major nutrients		
NO_3	3.5×10^{-4}	
PO_4	1.5×10^{-5}	
Metal salts		computed pM[a]
Mn	2.5×10^{-8}	8.5
Co	1.0×10^{-8}	11.1
Fe	1.0×10^{-6}	–
Mo	2.5×10^{-9}	–
Zn	2.7×10^{-8}	11.0
Cu	5.4×10^{-8}	13.0
Chelator		
NTA	5.0×10^{-4}	
NaOH	1.5×10^{-3}	

[a] pM = –log of the free metal ion activity

The basic exposure medium (Table 1) was made with Sargasso seawater, which had been collected in polyethylene carboys, stored for several months, and filtered through 0.2-μm polycarbonate membrane filters immediately before use. The seawater was diluted with 1 part distilled water to 5 parts seawater.

Metal ion activities in exposure media were determined from thermodynamic calculations described by Sunda (1975) and Sunda and Gillespie (1979) using stability constants listed in Martell and Smith (1974). Metal ion activities are expressed as pM, the negative logarithm of free metal ion activity. For example, pZn 11 corresponds to a free zinc ion activity of 10^{-11} M. Stock solutions for metal additions were prepared as 0.1 M $CuCl_2$ or $ZnCl_2$ with 0.1 M nitriloacetic acid (NTA) and 0.3 M NaOH (to neutralize the hydrogen ions liberated when NTA forms chelates with metals). When copper and zinc are added to the basic exposure medium with an equimolar quantity of neutralized NTA (Table 1), the calculated cupric ion activities are proportional to the total molar copper concentration:

$$\mathrm{pCu} = \mathrm{p}[\mathrm{Cu_{tot}}] + 5.73,$$

and the zinc ion activities are proportional to the total molar zinc concentration:

$$\mathrm{pZn} = \mathrm{p}[\mathrm{Zn_{tot}}] + 3.39.$$

In comparing trace metal ion activities, total metal concentrations were assumed to equal the concentrations added as metal salts.

Short-term experiments

Short-term experiments were conducted to determine the effects of combinations of cupric and zinc ion activities on the motility of *Favella* sp. and *Balanion* sp. In these experiments, ciliates were exposed for 5 h to a matrix of copper and zinc ion activities. *Favella* sp. were concentrated above a 41-μm mesh nitex screen, washed with the appropriate exposure medium, and added to exposure medium to achieve a density of 25 per ml. *Balanion* sp. are difficult to concentrate without injury to the cells, and thus, dense cultures of this ciliate were diluted with exposure medium (7–10 ml of culture to 100 ml of medium) to produce concentrations of about 15 *Balanion* sp. ml^{-1}. The *Favella* sp. or *Balanion* sp. were then incubated in 100 ml of medium in polycarbonate flasks for 5 h in the light at 15 °C.

Samples were withdrawn hourly and examined immediately under a dissecting microscope using darkfield illumination. A magnification of 15× was used for *Favella* sp. and 45× for *Balanion* sp. Individuals were considered abnormal if they were dead, non-motile, or if they were not swimming in the typical pattern for that species. Examples of abnormal motility in *Favella* sp. are swimming backwards or spinning, rather than swimming in a smooth forward, helical pattern. In *Balanion* sp., streaking or slow swimming, rather than swimming in tight helical coils, is abnormal. The percent of the ciliates that appeared normal was calculated for about 150 individuals for each hourly sample, beginning with time zero.

Long-term experiments

In the long-term experiments, the responses of cultures were observed over several days, which is longer than the normal generation times of these organisms. In preliminary experiments, changes in the *in-vivo* fluorescence of cultures of the dinoflagellate *Heterocapsa triquetra* were measured to obtain a general indication of the sensitivity of this food organism to copper and zinc. This information was essential in order to be able to grow the food at the same pCu and pZn combinations that the ciliates were to be exposed to. The algae used as food for *Favella* sp. were then grown in the appropriate exposure media for 10 to 14 d prior to use. To ensure that the ciliates in the different treatments were fed about the same total volume of dinoflagellates, the cell volumes of the algae were determined with a model TA II Coulter counter at the end of exponential growth.

In the long-term growth experiments with *Favella* sp., we examined the response of this ciliate to free cupric ion activities of 10^{-13} to 10^{-10} M and free zinc ion activities of 10^{-12} to 10^{-9} M. In each experiment (zinc series), a control treatment (pCu 13, pZn 11) was used to measure among-experiment variability. The medium we routinely use to culture ciliates has approximately 10^{-13} M free copper ion activity and 10^{-11} M free zinc ion activity, thus we chose these activities for our control treatment. Three replicates were used in all treatments.

Favella sp. were concentrated above a 41-μm mesh nitex screen and washed with the proper exposure medium. They were then added to polycarbonate flasks containing 200 ml of exposure medium to achieve densities of 2 *Favella* sp. ml^{-1}. Algae, which had been grown at the same copper and zinc ion activities, were added to the flasks to achieve densities of about 1 000 cells ml^{-1}. The flasks were incubated at 15 °C in dim light (53 μEm^{-2} s^{-1}) for 2 d. Ten-to fifty-ml volumes were withdrawn from the flasks initially and at 2 or 3 other times and ciliates and algae were counted using an inverted microscope (Hasle, 1978). A minimum of 100 ciliates were counted in each sample, except when this was not possible because of low ciliate density; in these cases, 50 ml of culture were counted. The algae were counted to ensure that low food levels were not responsible for decreases in ciliate division rates. Measurements of cell concentations were used to calculate division rates of *Favella* sp. Since *Favella* sp. does not vary much in size, the division rate for this species is a good approximation of growth rate.

The growth experiments with *Balanion* sp. were similar to those with *Favella* sp., except that the *Balanion* sp. were not concentrated or washed because this species is damaged by these procedures. Instead, dense *Balanion* sp. cultures were grown and used as inocula. Less than 3 ml of inoculum was added per 100 ml of exposure medium. Subsamples were taken initially and after 12, 24, 36 and 48 h.

Because the cell size of *Balanion* sp. can vary, the length and width of at least 100 individuals from each sample were measured using an ocular micrometer. Average volumes per cell were calculated based on a oblate spheroid approximation of the organism's geometry. Specific growth rates were calculated from data for total cell volume as computed from cell density and average cell volume.

Growth rate data were analysed by two-way analysis of variance (2-way ANOVA) (Sokal and Rohlf, 1981) in order to evaluate the effects of the main factors, i.e. copper and zinc ion activities, and the effects due to their interaction.

Results

Short-term effects

Effects on the motility of *Favella* sp. and *Balanion* sp. were evident after 1-h exposure to cupric ion activities of 10^{-11} M and 10^{-9} M, respectively. At higher cupric ion activities, motility generally continued to decrease between 1 and 5 h. As examples, the responses of *Favella* sp. and *Balanion* sp. to copper are shown in Fig. 1 at a zinc ion activity of 10^{-11} M.

The responses of the ciliates to different combinations of copper and zinc ion activities are compared, based on the percent of the ciliates with normal swimming behavior after a 2-h exposure. The effect of cupric ion activity on the motility of *Favella* sp. and *Balanion* sp. (Fig. 2) was dependent to some extent on zinc ion activity. At all zinc levels, the motility of *Favella* sp. was impaired by cupric ion activities $\geqq 10^{-10}$ M. At the highest free zinc ion

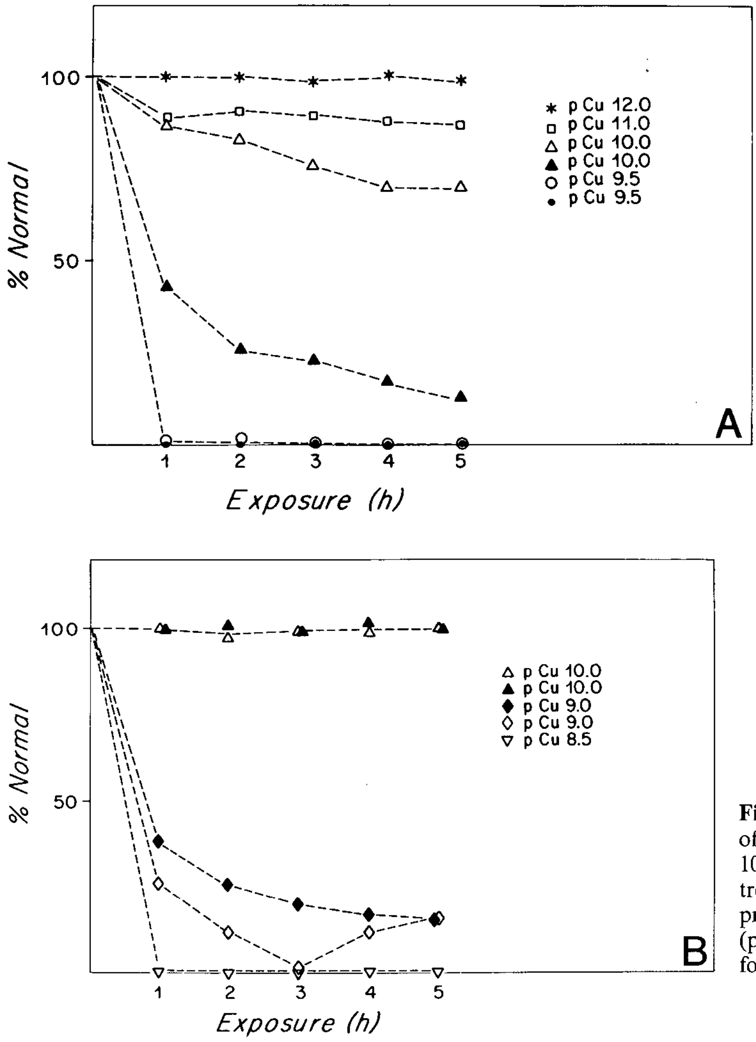

Fig. 1. Short-term effects of cupric ion activity on the motility of *Favella* sp. (A) and *Balanion* sp. (B) at a zinc ion activity of 10^{-11} M. Data are presented as the percent of individuals in a treatment with normal motility. Cupric ion activities are expressed as the negative logarithm of the free cupric ion activity (pCu). When two experiments were done at one pCu, the data for both are presented

activity, 10^{-9} M, at least 20% of the *Favella* sp. appeared normal at pCu 9.5 and 9, although none were normal at these pCu values at lower zinc ion activities (Fig. 2A). The motility of *Balanion* sp. was adversely affected by cupric ion activities of 10^{-10} M at pZn 12 and 10 but not at pZn 11 (Fig. 2B). At pCu 9, normal motility was decreased appreciably at all pZn levels. Thus, in short-term tests, the pattern of response of *Balanion* sp. appeared to differ somewhat from that of *Favella* sp.

The short-term effects on motility were reversible to some extent. At pCu 9.5 and pZn 11, no *Favella* sp. swam normally after a 2-h exposure, but when they were transferred to a new medium at pCu 13 and pZn 11, about 80% resumed normal swimming behavior after 19 h. Even after a 5-h exposure to pCu 9.5 medium, the effects on motility were partially reversible.

Long-term experiments

In-vivo fluorescence measurements indicated optimal growth of the food alga *Heterocapsa triquetra* at cupric ion activities of 10^{-13} to 10^{-12} M and zinc ion activities of 10^{-12} M to 10^{-10} M. Growth was reduced at cupric ion activities $\geqq 10^{-11}$ M and no growth occurred at 10^{-9} M. Growth was inhibited by zinc at a zinc ion activity of 10^{-9} M. Based on these data, we conducted the long-term experiments with ciliates at cupric ion activities between 10^{-13} and 10^{-10} M and zinc ion activities between 10^{-12} and 10^{-10} M (*Balanion* sp.) or 10^{-9} M (*Favella* sp.). The 10^{-} M zinc ion treatments were left out of the experiment with *Balanion* sp. because of difficulties in growing sufficient *H. triquetra* as food at this pZn.

Effects of copper on the division rate of *Favella* sp. and *Balanion* sp. were progressive with time and, in some cases, they were not apparent until after 24 h (Figs. 3 and 4). Because of this delayed effect, division rates were computed for the 24- to 48-h time interval for *Favella* sp., and the 24- to 34-h time interval for *Balanion* sp. Data at 48 h were not included in the rate computations for *Balanion* sp. because of inadequate food concentrations.

The control growth rate of *Favella* sp. in the pZn 9 series was significantly lower than in the other experiments; therefore, we did not include data from this series

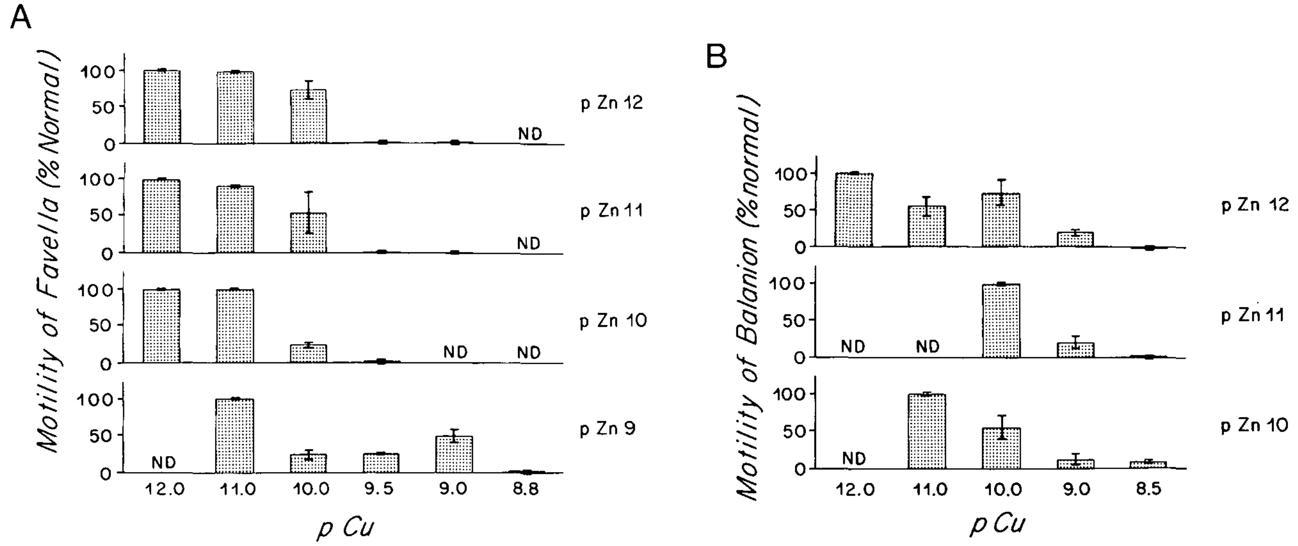

Fig. 2. The effects of exposure to a matrix of cupric and zinc ion activities on the motility of *Favella* sp. (A) and *Balanion* sp. (B) after 2 h. Data are presented as the mean percent (±SD) of individuals with normal motility. Metal ion activities are expressed as the negative logarithm of the free cupric ion activity (pCu) and free zinc ion activity (pZn). ND = no data.

(A) *Favella* sp. ANOVA (pCu 11–9.5, pZn 12–9; arcsine transformation):

Source of Variation	df	F	*p*
pCu	2	111.2130	<0.001
pZn	3	2.4016	>0.05
Interaction	6	3.8839	<0.05
Error	12		

(B) *Balanion* sp. ANOVA (pCu 10–8.5, pZn 12–10; arcsine transformation):

Source of Variation	df	F	*p*
pCu	2	81.0502	<0.001
pZn	2	3.0229	>0.05
Interaction	4	6.6513	<0.01
Error	9		

Table 2. The effects of exposure to a matrix of cupric and zinc ion activities on the division rate of *Favella* sp.

	Mean div. d^{-1} (SD) pCu					Control**
pZn	13	12.8	12	11	10	
12	0.81 (0.17)	0.31 (0.13)	−0.82 (0.13)	−0.33 (0.27)	–*	0.79 (0.06)
11	0.85 (0.09)	0.70 (0.02)	−0.26 (0.06)	−0.29 (0.26)	−2.90 (0.25)	–
10	0.41 (0.07)	0.38 (0.03)	0.10 (0.15)	−0.51 (0.11)	–*	0.97 (0.04)
9	0.56 (0.02)	0.68 (0.11)	0.28 (0.04)	−0.12 (0.16)	−2.68 (0.09)	0.69 (0.03)

ANOVA:***

Source of variation	df	MS	F	*p*
Cupric ion activity	3	2.6672	83.6113	<0.001
Zinc ion activity	2	0.2008	6.2947	<0.01
Interaction	6	0.2619	8.2100	<0.001
Error	24	0.0319		

* Dead by 24 h

** pCu 13, pZn 11 treatment (for pZn 11 series, this treatment is part of the experimental matrix)

*** Data for pCu 10, control, and pZn 9 series not included

in the ANOVA (Table 2). At the two lowest zinc ion activities, cupric ion activities as low as $10^{-12.8}$ M reduced the division rate and no reproduction occurred at a cupric ion activities $\geqq 10^{-12}$ M (Fig. 5a, Table 2). Increases in zinc ion activity, however, decreased the toxicity of copper, and at zinc ion activities of 10^{-10} and 10^{-9} M, copper reduced cell division rates only at cupric ion activities of 10^{-12} M and above. At a cupric ion activity of 10^{-13} M, zinc inhibited the division rate of *Favella* sp. at zinc ion activities of 10^{-10} M and above.

In contrast to *Favella* sp., maximum growth of *Balanion* sp. occurred at the highest zinc ion activity, 10^{-10} M, and the three lowest cupric ion activities, 10^{-13}, $10^{-12.8}$ and 10^{-12} M (Tables 3 and 4; Fig. 5B). Growth rates decreased at zinc ion activities $\leqq 10^{-11}$ M at all cupric ion activities. There was an antagonism between copper and zinc in

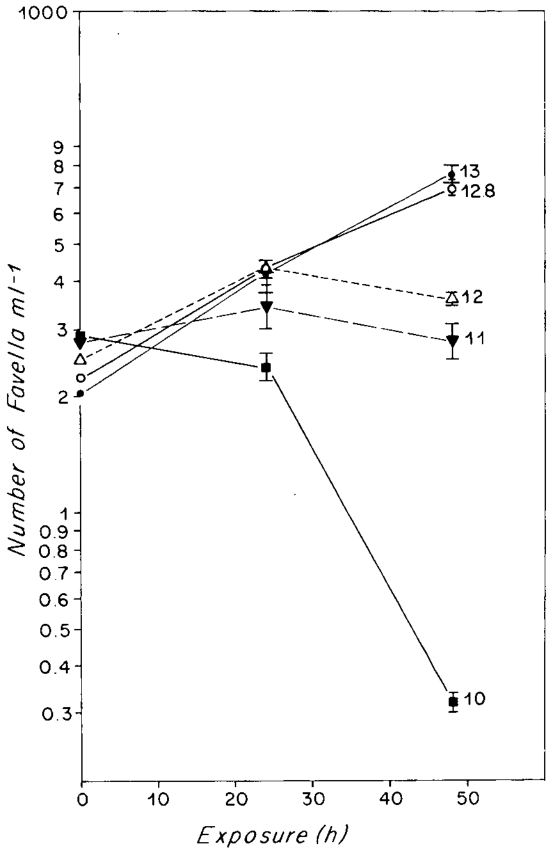

Fig. 3. Effect of cupric ion activity on increase in cell number of *Favella* sp. at a zinc ion activity of 10^{-11} M. Means (±SD) are shown. Cupric ion activities are expressed as the negative logarithm of the free cupric ion activity (pCu)

which increases in cupric ion activity greatly increased the adverse affect on growth of low zinc ion activity. For example, a decrease in zinc ion activity from 10^{-10} to 10^{-12} M caused only a 43% decrease in growth rate at a cupric ion activity of 10^{-13} M, but caused a total cessation of growth at cupric ion activites of 10^{-12} M and above.

Both copper and zinc affected growth rates and division rates differentially (Tables 3 and 4), and thus they affected the size of *Balanion* sp. Increases in the ratio of cupric ion activity to zinc ion activity decreased growth rate to a greater extent than cell division rate and consequently an increase in this ratio caused decreases in cell size.

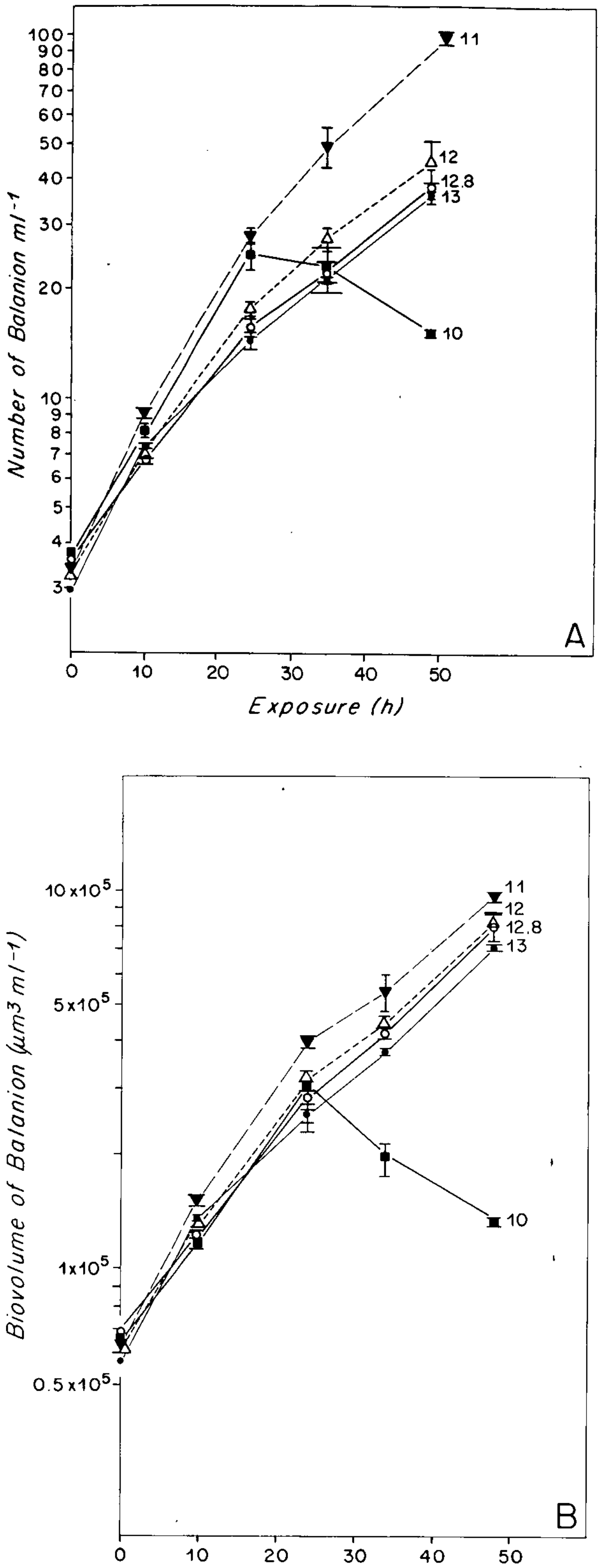

Fig. 4. Effect of cupric ion activity on increase in cell number (A) and total cellular volume (B) of *Balanion* sp. at a zinc ion activity of 10^{-11} M. Means (±SD) are shown. Cupric ion activities are expressed as the negative logarithm of the free cupric ion activity (pCu)

Discussion

The response of the two ciliates to the copper and zinc matrices are extremely complex, which perhaps is not surprising since both metals can act as either limiting nutrients or toxicants depending on their availability, and both metals can compete with one another for binding to metabolic sites. The reduced growth rate of *Balanion* sp. at zinc ion activities $\leqq 10^{-11}$ M (Fig. 5 B) is probably due to a zinc nutritional limitation and the strong antagonism

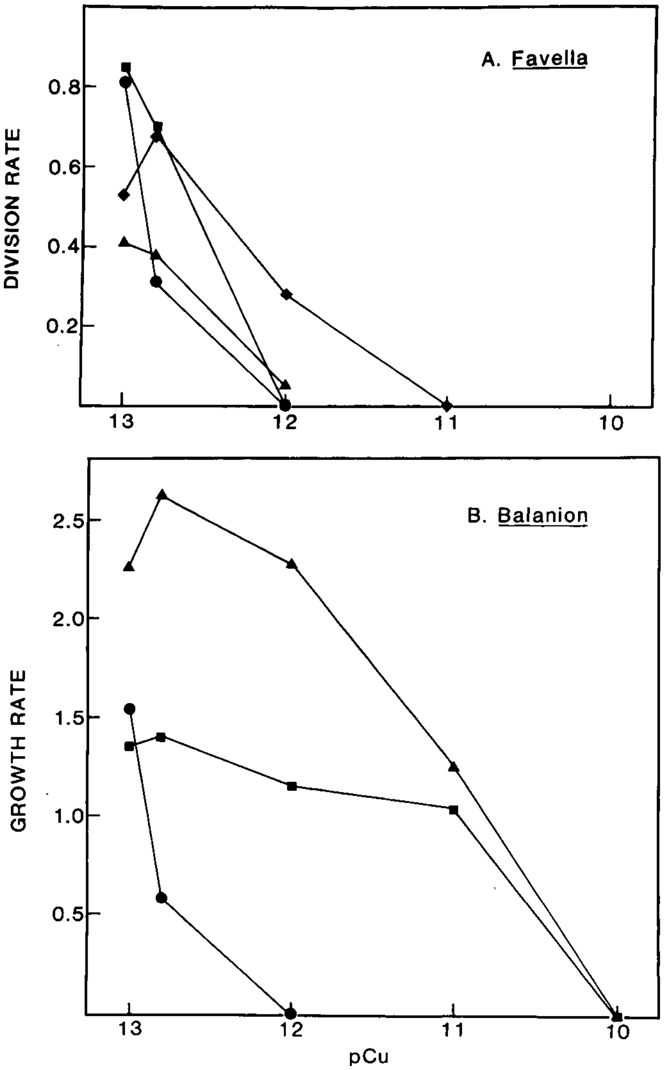

Fig. 5. Effect of cupric ion activity on the division rate (d^{-1}) of *Favella* sp. (A) and the growth rate (doublings d^{-1}) of *Balanion* sp. (B) at zinc ion activities of 10^{-12} M (●), 10^{-11} M (■), 10^{-10} M (▲), and 10^{-9} M (◆)

between copper and zinc in this species may reflect competition between the two metals for metal uptake sites or for internal metabolic sites such as zinc activitated enzymes. Competition for transport sites can have reciprocal effects in that each metal can competitively inhibit the cellular uptake of the other, as has been observed in the uptake of manganese and cadmium by *Chlorella pyrenoidosa* (Hart *et al.*, 1979). We have observed that copper reduces the uptake of zinc by several species of phytoplankton (unpublished data) and high levels of copper increase the cellular requirement for zinc in *Euglena* sp. (Price and Quigley, 1966).

Laboratory data on the sensitivity of planktonic ciliates to copper and zinc can be valuable in understanding the distribution of ciliates in marine waters and the effects of trace metal perturbations on food webs containing ciliates. Differential sensitivity to copper and zinc, as found with *Balanion* sp. and *Favella* sp., can lead to shifts in community composition under conditions of trace metal stress. To evaluate the significance of our results, it is useful to compare them to those obtained with other planktonic organisms and to estimated *in-situ* free metal ion activities.

Among phytoplankton, the cyanobacteria are the most sensitive group to copper, with the onset of inhibition at cupric ion activities of 10^{-12} M for the most sensitive species (Brand *et al.*, in press). The dinoflagellates and coccolithophores are intermediate in sensitivity and the diatoms are the least sensitive to copper toxicity (Brand *et al.*, in press). Because both *Favella* sp. and *Balanion* sp. are associated with dinoflagellate blooms and preferentially prey on algae of this taxon (Stoecker *et al.*, 1981; Stoecker *et al.*, in press), comparison of the ciliates' sensitivities to copper with that of the dinoflagellates is ecologically relevant. Brand *et al.* (in press) observed 50% inhibition of growth rate in nine dinoflagellate species at a mean pCu of 10.4 ± 0.4 (± SD). These results were obtained at pZn and pMn values of 10.6 and 8.3 in 30‰ media containing 10^{-4} M NTA and 10^{-6} M $FeCl_3$. Under

Table 3. The effects of exposure to a matrix of cupric and zinc ion activities on the division rate of *Balanion* sp.

	Mean div. d^{-1} (SD) pCu					Control**
pZn	13	12.8	12	11	10	
12	1.38 (0.04)	1.29 (0.19)	− 1.45 (0.44)	− 0.29 (0.29)	–*	1.56 (0.20)
11	1.41 (0.22)	1.20 (0.03)	1.49 (0.21)	1.91 (0.38)	− 0.31 (0.14)	–
10	2.41 (0.15)	2.59 (0.25)	2.85 (0.24)	1.87 (0.35)	2.38 (0.70)	1.68 (0.34)

ANOVA***

Source of variation	df	MS	F	*p*
Cupric ion activity	3	1.3297	12.8971	< 0.001
Zinc ion activity	2	14.5395	141.0233	< 0.001
Interaction	6	2.4955	24.2047	< 0.001
Error	24	0.1031		

* Dead by 24 h

** pCu 13, pZn 11 treatment (for pZn 11 series, this treatment is part of the experimental matrix)

*** Data for pCu 10 and control not included

Table 4. The effect of exposure to a matrix of cupric and zinc ion activities on the growth rate of *Balanion* sp.

	Mean doublings in volume d^{-1} (SD) pCu					Control**
pZn	13	12.8	12	11	10	
12	1.54 (0.03)	0.58 (0.21)	−0.82 (0.40)	0.27 (0.25)	–*	1.34 (0.18)
11	1.35 (0.23)	1.40 (0.03)	1.15 (0.21)	1.03 (0.39)	−1.61 (0.15)	–
10	2.26 (0.14)	2.62 (0.25)	2.27 (0.25)	1.25 (0.35)	−2.41 (0.71)	1.35 (0.35)

ANOVA:***

Source of variation	df	MS	F	*p*
Cupric ion activity	3	1.8158	18.4908	<0.001
Zinc ion activity	2	8.7538	89.1426	<0.001
Interaction	6	1.0724	10.9206	<0.001
Error	24	0.0982		

* Dead by 24 h

** pCu 13, pZn 11 treatment (for pZn 11 series, this treatment is part of the experimental matrix)

*** Data for pCu 10 and control not included

similar conditions in our experiments (i.e. at pZn 10 or 11), *Favella* sp. showed 50% inhibition of division rate at a cupric ion activity of ca $10^{-12.5}$ M, fully two orders of magnitude below the mean 50% value for dinoflagellates. *Balanion* sp. was considerably less sensitive under similar conditions (Fig. 5) and exhibited 50% inhibition of growth at a cupric ion activity of $10^{-10.9}$ to $10^{-10.7}$ M, still somewhat below the mean value for the dinoflagellates. The relative copper sensitivity of *Balanion* sp., however, was strongly influenced by zinc.

Anderson *et al.* (1984), using a bioassay for cupric ion activity, estimated that the maximum free cupric ion activity in the estuary from which we isolated the ciliates (Perch Pond, Falmouth, Massachusetts) was near 10^{-11} M during the spring dinoflagellate bloom. Cupric ion activities, however, were probably lower than this maximum (i.e. between 10^{-13} and 10^{-12} M) based on measurements from other coastal locations with more sensitive bioassays (Sunda and Ferguson, 1983) and with fixed potential amperometry (Hering *et al.*, in press) Thus, *in-situ* cupric ion activities may be sufficiently high to influence the growth of *Favella* sp., but are probably too low to inhibit the growth of *Balanion* sp., except perhaps at low, growth-limiting zinc ion activities.

Data are also available on the sensitivities of phytoplankton to free zinc ion activities. In general, neritic diatoms and coccolithophores are nutritionally limited by zinc ion activities less than about $10^{-11.5}$ M (Brand *et al.*, 1983; Anderson *et al.*, 1978). The onset of zinc toxicity occurs around 10^{-9} M for the coastal diatom *Thalassiosira weissiflogii* (Anderson *et al.*, 1978). In our preliminary experiments with *Heterocapsa triquetra*, this dinoflagellate grew optimally at zinc ion activities of 10^{-12} to 10^{-10} M, but was inhibited by a zinc ion activity of 10^{-9} M. *Favella* sp. showed no evidence for zinc limitation in our experiments, but appeared to be inhibited at zinc ion activities $\geqq 10^{-10}$ M. *Balanion* sp. on the other hand grew optimally only at the highest zinc ion activity tested (10^{-10} M), and had reduced growth rates at lower zinc ion activities (Fig. 5B). Thus, in contrast to *Favella* sp. and many phytoplankton, *Balanion* sp. appears to be generally well adapted to growth at high zinc ion activities.

Zinc ion activities are estimated to be in the range 10^{-12} to 10^{-11} M in the open ocean, as based on dissolved concentrations and inorganic speciation models (Brand *et al.*, 1983). Activities may be lower if there is significant organic complexation. By contrast, zinc ion activities in some polluted estuaries have been estimated to be as high as 10^{-7} M (Sunda, Tester and Huntsman, unpublished ms). Even in relatively uncontaminated estuaries, such as the lower Newport River estuary in North Carolina, zinc ion activities may be as high as $10^{-9.5}$ M (Willis and Sunda, 1984). These estimates suggest that zinc may be limiting to *Balanion* sp. and some neritic phytoplankters (Brand *et al.*, 1983) in oceanic waters, but may be inhibitory to *Favella* sp. and certain phytoplankters in some estuaries, especially those contaminated by trace metals.

The sensitivity of *Favella* sp. to copper and zinc toxicity and the growth limitation of *Balanion* sp. at low zinc ion activities suggest that free trace metal ion activities can be important niche parameters for marine planktonic ciliates. Based on estimates of the range of cupric and zinc free ion activities in coastal waters, it appears that both copper and zinc may influence the productivity and species composition of marine planktonic ciliate communities. Due to competition between metals, however, the ratios of cupric to zinc ions can be as important as the absolute activities of these metals in determining overall effects.

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