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Copper Transfer and Influence on a Marine Food Chain

M. Edding, F. Tala

Departamento de Biología Marina, Facultad de Ciencias del Mar, Universidad Católica del Norte, Casilla 117, Coquimbo, Chile

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Copper is an essential element, required for normal growth by all plants and animals; and a regular constituent in the environment (Lewis and Cave 1982; Lewis 1994). This heavy metal is an essential micronutrient that at higher concentrations can be deleterious to algae and other aquatic biota (Chang and Sibley 1993). Copper toxicity to algae depends upon the individual species, their physiological and environmental conditions, and the chemical forms of metal in the medium (Sunda and Guillard 1976). When copper is accumulated by phytoplankton it can be transferred and may produce toxic effects on zooplankton (Wikfors and Ukeles 1982).

Different species of microalgae present different capacities of resistance to copper. Cyanophyceae pre-cultured in a Cu-enriched medium (635 µgCu.·L^{-1}) showed an EC_{50} that could reach 318 µgCu·L^{-1} for *Plectonema radiosum* and 339 µgCu·L^{-1} in *Phormidium* sp. (Takamura et al. 1990). *Scenedesmus, Selenastrum* and *Chlorella* were reported able to accumulate copper and other metal ions with an efficiency of 67-98% (Brady et *al.* 1994). Also, *Dunaliella* resisted concentrations from 0.38 mgCu·L^{-1} (*D. minuta*) up to 50.8 mgCu·L^{-1} (*D. acidophila*), depending on the pH of the medium (Gimmler et al. 1991). Once the microalgae are copper-enriched, the copper that is part of the cell can be transferred to the surrounding water and to its predator producing uncertain effects. This study observed the effect of copper on the growth of *Dunaliella tertiolecta* and *Isochrysis galbana* that are currently used as food for hatchery-grown scallop larvae (*Argopecten purpuratus*). We observed the path of copper from the water column into the microalgal cell and the effect of copper-enriched food on the scallop larvae.

MATERIALS AND METHODS

Dunaliella tertiolecta was obtained from the "Unidad de Productión" algae collection at Universidad Católica del Norte and *Isochrysis galbana* var. *tahitiana* (CCAP 927-14, Scotland, U.K.) from Fundación Chile, Tongoy. Axenic microalgal stocks were cultured in artificial seawater (Starr and Zeikus 1993) and Provasoli medium, at 20 ± 1°C, under continuous fluorescent light (40W). Toxicity experiments were realized with algae in their exponential growth phase.

Correspondence to: M. Edding

Cultures were kept in 150-mL flasks. Copper effects were observed in 50 mL of sterile seawater by inoculation from axenic stock cultures. Inoculum concentrations were ca. $15 \cdot 10^4$ cells$\cdot mL^{-1}$ for both species. Cultures were shaken twice a day by hand. The source of copper used was $CuCl_2 \cdot 2H_2O$; *D. tertiolecta* received 0-312.5 mg $Cu \cdot L^{-1}$ and *I. galbana* received 0-25 mg $Cu \cdot L^{-1}$, in nominal concentrations. Both control and treatments were replicated 5 times. Algal growth was monitored daily by counting cells in a hematocytometer. Growth rate was determined at 24, 48 and 72 hr for each species. Copper toxicity expressed as EC_{50} was calculated by a simple regression of microalgae growth rate vs. log Cu concentration.

Copper accumulation was measured in *I. galbana* and *D. tertiolecta* during a 24-hr period when cultured at nominal concentrations of $8 \cdot 10^{-3}$ and 10 mg$Cu \cdot L^{-1}$, respectively. An initial algal density of $15 \cdot 10^4$ cells$\cdot ml^{-1}$ was used in both species. Total copper concentrations of algae and available copper (Cu^{++}) in the seawater media were determined every 6 hr after metal addition. Algae were separated from seawater by centrifugation at 10,000 rpm for 10 min. Later algae were washed with deionized water, resuspended, filtered through a 0.45µm membrane filter (Millipore) and dried in an oven at 60°C. The filter with algae was mineralized using an acid mixture $HNO_3/HCLO_4$ (3:1). Photolisis was utilized in seawater analysis, samples were exposed for 1-2 hr with $HCLO_4$ and H_2O_2 under UV light (1000 W). Copper was analyzed by square wave anodic stripping voltammetry (APHA 1992) and expressed as µg$Cu \cdot g^{-1}$ algae and µg$Cu \cdot mL^{-1}$ seawater. Control copper concentrations were 3.01 ± 0.54 µg$Cu \cdot g^{-1}$ dry wt for *I. galbana*, 3.69 ± 0.61 µg$Cu \cdot g^{-1}$ dry wt for *D.tertiolecta*, and $6.9 \cdot 10^{-3} \pm 4.24 \cdot 10^{-4}$ µg $Cu \cdot mL^{-1}$ in the seawater medium.

Transfer of copper to *Argopecten purpuratus* larvae was determined. Larvae (D stage) were fed with *I. galbana* and *D. tertiolecta* that had been grown for more than one week at $8 \cdot 10^{-3}$ and 10 mg $Cu \cdot L^{-1}$, respectively. Larvae were cultured in 150 mL at a density of 200 larvae$\cdot mL^{-1}$, and maintained at 20 ± 1 °C. The food (algae) was given as cell pellets, following centrifugation, with a final concentration of 40,000 cell$\cdot mL^{-1}$ Controls were fed with microalgae grown without copper. After 24 hr, larval survival was determined in each treatment. The samples, 2 in each treatment, were digested with HNO_3 (instr., J.T. Baker) in closed Teflon systems, at 150 ± 5°C for 1 hr. Copper was analyzed with a GBC 909 flame atomic absorption spectrophotometer (FAAS), using an atom enrichment system (atom trap) for the lowest concentrations. This method has a limit of detection for copper of 10 µg·L, with a sensitivity of 0.1 mg·L. Standards were prepared with a reference marine organism tissue (DORM-l) and standard seawater (CASS-2) obtained from National Research Council Canada, Division of Chemistry, Marine Analytical Chemistry Program. The analytical control quality of these standards gave a 100% recovery for DORM-.1 and 95% recovery for CASS-2 for 5 samples with a Coefficient of Variation of ±4.2% for DORM-1 and ±6,0% for CASS-2. The copper concentrations were expressed as µg$Cu \cdot g^{-1}$ dry weight larvae or algae and µg$Cu \cdot mL^{-1}$ seawater.

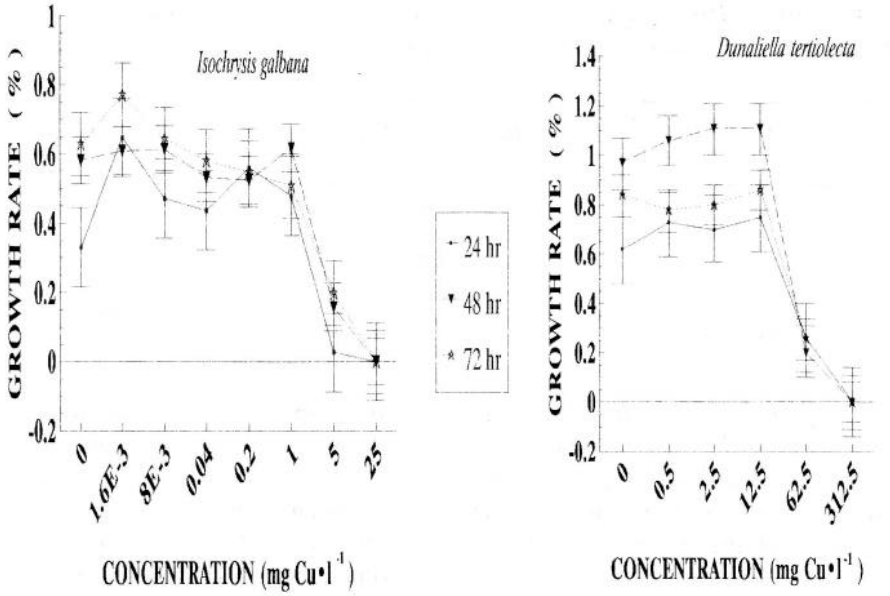

Figure 1. Growth rate in *I. galbana* and *D. tertiolecta* exposed to different Cu concentrations. Intervals are 95% confidence. Culture conditions: 20±1°C; pH 7.85 and 22‰ salinity.

Analysis of variance (ANOVA) was used to compare the algal growth rate, larval survival and metal concentration in algae, larvae, and seawater samples,

RESULTS AND DISCUSSION

The growth rate of *Isochrysis galbana* at < 1 mgCu·L^{-1} was similar to the control samples (Fig.1). Between 1 and 25 mgCu.·L^{-1}, growth rate was inhibited. The highest growth rate observed at 1.6 mgCu·L^{-1} differed significantly ($p>0.05$) from the control at 24 hr. At this time, *I. galbana* was more sensitive to the culture conditions and showed its lowest growth rate. There were no significant growth rate differences ($p>0.05$) after 48 and 72 hr (Fig. 1). The growth rate of *Dunaliella tertiolecta* in culture was not affected at values < 12.5 mg Cu·L^{-1} (Fig.1). The highest rates were detected at 48 hr in these treatments (Fig.1). It was observed that the growth rate decreased to 0 at the higher copper concentrations (Fig. 1). "No observed effect concentration" (NOEC) was 1 mgCu.·L^{-1} in *I. galbana* and 12.5 mgCu·L^{-1} in *D. tertiolecta* (Fig.1) after comparing the highest toxicant concentration with the control (Bonferroni test, Statgraph, Inc.). The EC_{50} values

Table 1. EC_{50} values ($mgCu \cdot L^{-1}$) of *Isochryris galbana* and *Dunaliella teriolecta* obtained from the linear regression of log concentration and daily growth rate.

Time (hr)	*Isochrysis galbana*	*Dunaliella tertiolecta*
24	5.88	58.93
48	2.38	44.76
72	1.58	38.80

were higher in *D. tertiolccta* than in *I. galbana* (Table 1). In both microalgae it was observed that the resistance to copper differed depending on the time of observation. *I. galbana* and *D. tertiolecta* growth rates showed the largest decrease after 72 hr (Table 1). Although *D. tertiolecta* has been shown to be more resistant to copper in the environment (Gimmler et al. 1991) *I. galbana* has a good resistance to available copper compared to other microalgae (Lewis and Cave 1982; Takamura et al. 1990).

At least three mechanisms have been proposed to explain the tolerance of microalgae to metal toxicity. Brown et al. (1988) observed physical exclusion of metal ions due to reduced cell membrane permeability, and internal immobilization by the formation of "copper" bodies and by the binding of metal ions to cell exudates (Xue and Sigg 1990). Most studies have demonstrated that *Dunaliella* cells exhibit a higher resistance to heavy metals than other algae, explained by several factors: a) lower uptake of heavy metals, b) better detoxification mechanisms, c) internal complexation by glycerol, and d) lower activity coefficients of polyvalent cations in saline and hypersaline media of comparable ionic strength (Gimmler et al. 1991).

Toxicity results for *1. galbana* and *D. tertiolecta* probably can be modified by increasing the test sensitivity by omitting the chelating agent ethylendiaminetetraacetic acid (EDTA) and reducing the micronutrient level in the assay medium (Wren and McCarroll 1990). In this way it is possible to predict lower EC_{50} values. Also, other genera may be more sensitive than *Isochrysis* and *Dunaliella.*

When copper was measured simultaneously through time in the water column and in *I. galbana* cells, it was observed that copper in algal cells was higher than in the culture medium, suggesting uptake of copper by *I. galbana* (Fig.2). Copper in the water increased after 6 hr and then decreased after 18 hr (Fig.2). Significant differences ($p>0.05$) in copper availability were not observed between 6, 12 and 24 hr. The growth rate during this experiment was 0.98, higher than the growth rate observed in Fig 1.

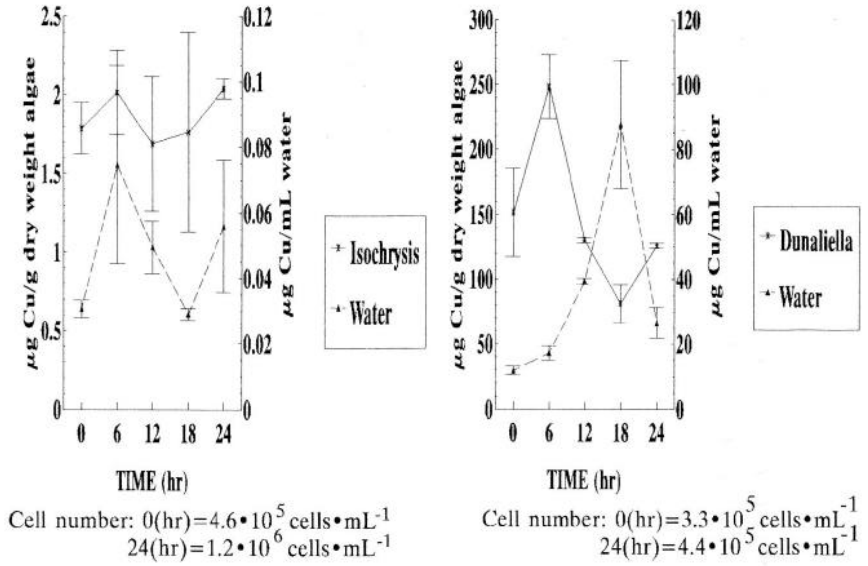

Figure 2. Total copper in *I. galbana*, *D. tertiolecta* and available Cu^{++} in water. Intervals are standard errors. Culture conditions: 20±1°C; pH 8.5; 22% salinity.

In the experiment with *D. tertiolecta* it was observed that copper fluctuated both in the water and in the cell (Fig.2). *D. tertiolecta* maximum copper content was observed after 6 hr, then decreased after 18 hr, increasing again after 24 hr (Fig.2). Water column copper (Cu^{++}) increased rapidly to a maximum available copper at 18 hr (90 µgCu·ml^{-1} water) and then decreased (Fig. 2). The fluctuation of available copper content in water and in the cells suggest an interaction of *D. tertiolecta* and the water, probably explained by utilization and detoxification (Twiss et al. 1993) of copper by the algae. During this experiment the growth rate of *D. tertiolecta* was 0.932, similar to the growth rate observed in Fig. 1. Brown et al. (1988) suggested that *D. tertiolecta* is a species that is able to exclude metals. The results of this study showed a probable different mechanism where copper can be taken up by the *D. tertiolecta* and then released as shown in Figure 2. Exclusion of copper from the cell interior has been suggested as the primary tolerance mechanism in *Scenedesmus acutus* (Twiss et al. 1993). Other processes such as internal detoxification might also be occurring (Brown et al. 1988).

Copper-enriched algae as food for *Argopecten purpuratus* larvae produced decreased larval survival in comparison to control treatments (Fig.3). The larvae showed the lowest survival when they were fed with *D. tertiolecta* grown at 10 mgCu·L^{-1}. This treatment showed also the highest copper concentrations in

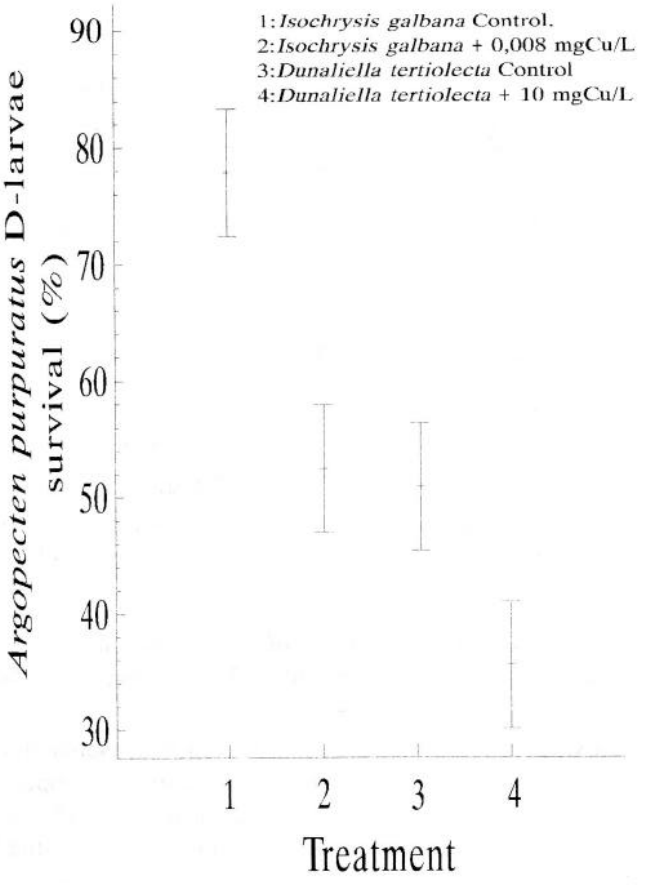

Figure 3. Comparison of *Argopecten purpuratus* larval survival exposed to copper for 24 hr. Intervals are 95% confidence. Culture conditions: 20±1°C; pH 8.18; 22‰ salinity.

seawater, microalgae and larvae (Table 2). Both *Isochrysis* treatments had similar copper concentrations in seawater and algae, but showed differences in concentrations in larvae (Table 2).

Food-chain transfer of copper has been observed in worms, oysters, snails and fish (Weis and Weis 1993) suggesting a decrease in predator growth rates following the consumption of copper-enriched prey. Also, Weeks (1993) found that Talitrid amphipods decreased their consumption rate when feeding on copper-enriched algae.

Table 2. Concentrations of available copper (Cu^{++}) in seawater medium, and total copper in microalgae and larvae after 24 hr.

Treatment	Seawater ($\mu g\ Cu \cdot mL^{-1}$) [1]	Microalgae ($\mu g\ Cu \cdot g^{-1}$) [2]	Larvae ($\mu gCu \cdot g^{-1}$) [3]
Isochrysis	0.07 ± 0.06	3.64 ± 0.66	26.49 ± 1.11
Isochrysis + 0.008 $\mu g\ Cu \cdot mL^{-1}$	0.08 ± 0.05	3.28 ± 0.95	18.28 ± 0.77
Dunaliella	0.14 ± 0.04	4.63 ± 0.71	7.72 ± 0.32
Dunaliella + 10 $\mu g\ Cu \cdot mL^{-1}$	8.65 ± 2.28	194.65 ± 63.40	43.57 ± 1.83
Control	0.06 ± 0.05	--------	23.70 ± 1.0

[1] F-ratio 18.107
[2] F-ratio 99.524
[3] F-ratio 410.903

In this study, copper magnification from seawater to microalgae and scallop larvae was observed. Also, a significant decrease in survival was observed for the scallop-D stage larvae when they were fed with copper-enriched food.

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