



Trace Metal Control of Phytochelatin Production in Coastal Waters

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Trace metal control of phytochelatin production in coastal waters

Abstract—Concentrations of particulate phytochelatin, a metal-binding peptide produced by eukaryotic phytoplankton, ranged from 2 to 50 μmol (g Chl *a*) $^{-1}$ in samples collected from small harbors in southeastern New England. Although there was no obvious relationship between phytochelatin and total Cu concentrations, phytochelatin varied systematically with free Cu concentrations [Cu^{2+}]. Transects in which there was high [Cu^{2+}] revealed high phytochelatin concentrations with a general decrease seaward. In those where [Cu^{2+}] remained low and constant, phytochelatin levels also remained low and constant. Incubation experiments confirmed that Cu rather than Cd is likely responsible for the elevated concentrations of phytochelatin at our field sites, though Zn may also be important. Intracellular phytochelatin concentrations in laboratory cultures of *Skeletonema costatum*, a coastal diatom, increased with increasing concentrations of Cu and Zn in a metal-specific

dose–response relationship. Additions of 12 nM Zn' (inorganic Zn) to Cu-stressed cultures reduced phytochelatin production, suggesting that Zn competitively inhibits uptake of Cu. Antagonistic effects of metals in the field, as well as physiological differences between organisms growing in the field and in the laboratory, can probably explain why phytochelatin concentrations in *S. costatum* are higher than in particulate field samples at the same [Cu^{2+}].

The concentrations of many trace metals in coastal waters are elevated as a result of anthropogenic inputs. Direct inputs of both domestic and industrial wastes, as well as more subtle inputs such as leaching from pressure-treated wood pilings and antifouling boat paints, are examples of how metals enter the water column. Laboratory studies have demonstrat-

Table 1. Phytochelatin ($\mu\text{mol} [\text{g Chl } a]^{-1}$), Cu_T (nM), and Cu^{+2} measurements from Cape Cod and Providence Harbor sites. Samples were collected from 12 to 16 September 1993. Phytochelatin and Chl *a* concentrations are the average of duplicate samples. nd = not detected.

| Site | Sep | Phytochelatin | | $\Sigma \gamma\text{-glu-cys}$ μmol $(\text{g Chl } a)^{-1}$ | $\mu\text{g Chl } a$ liter^{-1} | $[\text{Cu}]_T$ | $\log[\text{Cu}^{+2}]$ |
|-------------------|-----|---------------|--------------|---|---|-----------------|------------------------|
| | | <i>n</i> = 2 | <i>n</i> = 3 | | | | |
| Cape Cod | | | | | | | |
| Eel Pond | 14 | 46 | 16 | 140 | 2.1 | 48 | -10 |
| Falmouth Harbor | 12 | 13 | 2.5 | 33 | 2.0 | | |
| | 14 | 16 | nd | 33 | 15 | 59 | -10.5 |
| Cuttyhunk Harbor | 13 | 2.4 | 0.9 | 7.5 | 3.5 | 11.5 | -11.7 |
| Buzzards Bay | 13 | 2.2 | 0.6 | 6.1 | 3.0 | 7.5 | <-13 |
| Waquoit Bay | 12 | 3.5 | 1.2 | 11 | 7.3 | 14 | -12.8 |
| Providence Harbor | | | | | | | |
| Shooters | 16 | 2.9 | nd | 5.9 | 9.3 | 52 | -12.9 |
| Hot Club | 16 | 3.7 | nd | 7.4 | 2.6 | 19 | <-13 |

ed that trace metals, particularly Cu, can be toxic to some phytoplankton species at concentrations present in coastal waters (Brand et al. 1986), but no evidence of metal toxicity in phytoplankton assemblages has been documented in situ. In fact, productivity is often higher where pollution is greatest, presumably due to the concomitant input of major nutrients such as nitrate and phosphate. It is possible, however, that the composition of these algal communities is altered by the high concentrations of metals such as copper (e.g. Moffett et al. 1997) or that the ambient organisms are under toxic metal stress.

One way to quantify trace metal stress in plants is to measure the concentration of phytochelatin (Schat and Kalff 1992)—intracellular metal-binding peptides produced by plants and algae to chelate heavy metals. Phytochelatin has the structure $(\gamma\text{-glu-cys})_n\text{-gly}$ where *n* = 2–11 (Grill et al. 1985) and are synthesized by the enzyme phytochelatin synthase from glutathione (Grill et al. 1989). This enzyme requires a metal ion for activity, and when the product, phytochelatin, is in sufficient quantity to chelate the metal, the enzyme is turned off (Loeffler et al. 1989).

Phytochelatin has recently been measured in marine phytoplankton cultured in conditions ranging from low to growth-inhibiting free ion concentrations of many heavy metals (Ahner et al. 1994, 1995; Ahner and Morel 1995). Phytochelatin concentrations increase with increasing metals well before other physiological parameters (such as growth rate) are affected. In a field study, particulate phytochelatin concentrations were highest in Boston Harbor, where toxic metal concentrations are known to be elevated and decreased toward Massachusetts Bay (Ahner et al. 1994). Subsequent laboratory studies indicated that variations in ionic Cd, Cu, and perhaps Zn should modulate intracellular phytochelatin concentrations in most coastal environments (Ahner and Morel 1995); however, because trace metal speciation data were not available, it was not known what metal or metals were responsible for the elevated levels of phytochelatin.

In surface seawater, Cu, Cd, and Zn are strongly complexed by organic ligands whose concentrations are typi-

cally in excess of those of the metals. These ligands have been quantified via electrochemical techniques (Bruland 1989, 1992; Moffett et al. 1990), and there is evidence that they are of biological origin (Moffett et al. 1990; Moffett and Brand 1996). The organic complexes typically buffer the free-metal ion concentrations orders of magnitude lower than the total metal concentrations in solution. Several laboratory studies that used synthetic trace metal chelators such as EDTA have demonstrated that biological uptake or availability is a function of the free or inorganic fraction of the metal in solution (e.g. Sunda and Guillard 1976; Anderson et al. 1978). The inorganic fraction (Me') includes kinetically labile species such as the hydroxide, chloride, and carbonate metal complexes as well as the hydrated metal ion concentration $[\text{Me}^{n+}]$. In a medium where the inorganic ligand concentrations and pH are constant (such as in seawater) Me' and $[\text{Me}^{n+}]$ are strictly proportional to each other.

In polluted harbors, total metal concentrations can approach and even exceed those of their organic-binding ligands, thus resulting in very high inorganic metal concentrations. For example, off the coast of Cape Cod total dissolved Cu increases by a factor of five to six from Vineyard Sound into Eel Pond (our primary study site; see below), but the Cu' , measured via electrochemical techniques, increases by at least four orders of magnitude (Moffett et al. 1997). The physiological and ecological implications of such gradients in Cu' are an important environmental question.

Here we present field measurements of phytochelatin and Cu speciation from several small harbors in SE New England. Sites were chosen initially to reflect a wide variety of pollution levels and in the following years we returned to four sites (two with high Cu and two relatively pristine) near Woods Hole, Massachusetts, for more detailed transects. In September 1993, duplicate samples were collected from each of the following locations: Eel Pond (Woods Hole), Falmouth Harbor, Cuttyhunk Harbor, Buzzards Bay, Waquoit Bay, and two sites in Providence Harbor (Rhode Island) (sites and sampling dates are summarized in Table 1). In

August 1994, samples were collected along transects into four harbors near Woods Hole: from Vineyard Sound into Eel Pond, Falmouth Harbor, and Great Pond, and from Nantucket Sound into Waquoit Bay (more fully described in Moffett et al. 1997). In July 1995, one transect was sampled from Vineyard Sound into Eel Pond. In 1993, samples were collected directly into acid-cleaned polycarbonate bottles. Subsequent sampling was done with a teflon bottle mounted on the end of a PVC-pole sampler (Boyle et al. 1981). Particulate samples were collected by gentle filtration on 25-mm GF/F filters for phytochelatin and Chl *a* analysis (Parsons et al. 1984). Particulate phytochelatin was analyzed as described by Ahner et al. (1995). Total Cu and Cu speciation were measured in each water sample by cathodic stripping voltammetry as described elsewhere (Moffett 1995; Moffett et al. 1997).

All phytochelatin concentrations have been normalized to total particulate Chl *a* throughout the paper unless otherwise indicated. In the field samples, a portion of the total Chl *a* was due to prokaryotic organisms that do not make phytochelatin. In the transect samples, prokaryotes accounted for no more than 10% of the total Chl *a* in any of the samples (on the basis of numbers of *Synechococcus* cells reported by Moffett et al. 1997), thus the normalized eukaryotic phytochelatin concentrations in the field are not influenced significantly.

Particulate phytochelatin concentrations in samples collected from the various harbors in September of 1993 ranged from 2 to 50 $\mu\text{mol (g Chl } a)^{-1}$ (Fig. 1, Table 1). The corresponding total Cu concentrations varied by less than an order of magnitude (Fig. 1, top) whereas the free Cu, modulated by organic complexation, spanned more than three orders of magnitude (Fig. 1, bottom). While there was no obvious relationship between phytochelatin and total Cu, phytochelatin concentrations varied systematically with free Cu concentrations: in three samples with similar total Cu of 48–59 nM, the free Cu increased with decreasing ligand concentration ($\text{pCu} = 12.9$ to 10) and the phytochelatin concentrations increased concomitantly from 2.6 to nearly 50 $\mu\text{mol (g Chl } a)^{-1}$.

For the August 1994 transects, phytochelatin and Cu were measured in samples taken from within each harbor and out into Vineyard Sound (Fig. 2A). Again, it is clear that elevated Cu correlated with increased phytochelatin concentration (Fig. 2B). The two transects, Great Pond and Waquoit Bay, in which the free Cu remained low and constant, exhibited low and constant particulate phytochelatin concentrations. In contrast, those harbors that have high Cu concentrations—Eel Pond and Falmouth Harbor—exhibit high phytochelatin concentrations in the harbor, generally decreasing seaward. There was considerable variability in Falmouth Harbor however; the phytochelatin concentration was lower at Sta. 1 and 3 than at Sta. 2 and 4. In the case of Eel Pond, the innermost sample (Sta. 1) also exhibited a low phytochelatin concentration, but a subsequent transect in July 1995 yielded a systematic decrease seaward. The Falmouth Harbor transect has also been plotted in moles of phytochelatin per liter, thus eliminating the normalization to Chl *a* (Fig. 2B). Values ranged from 10 to 120 μM .

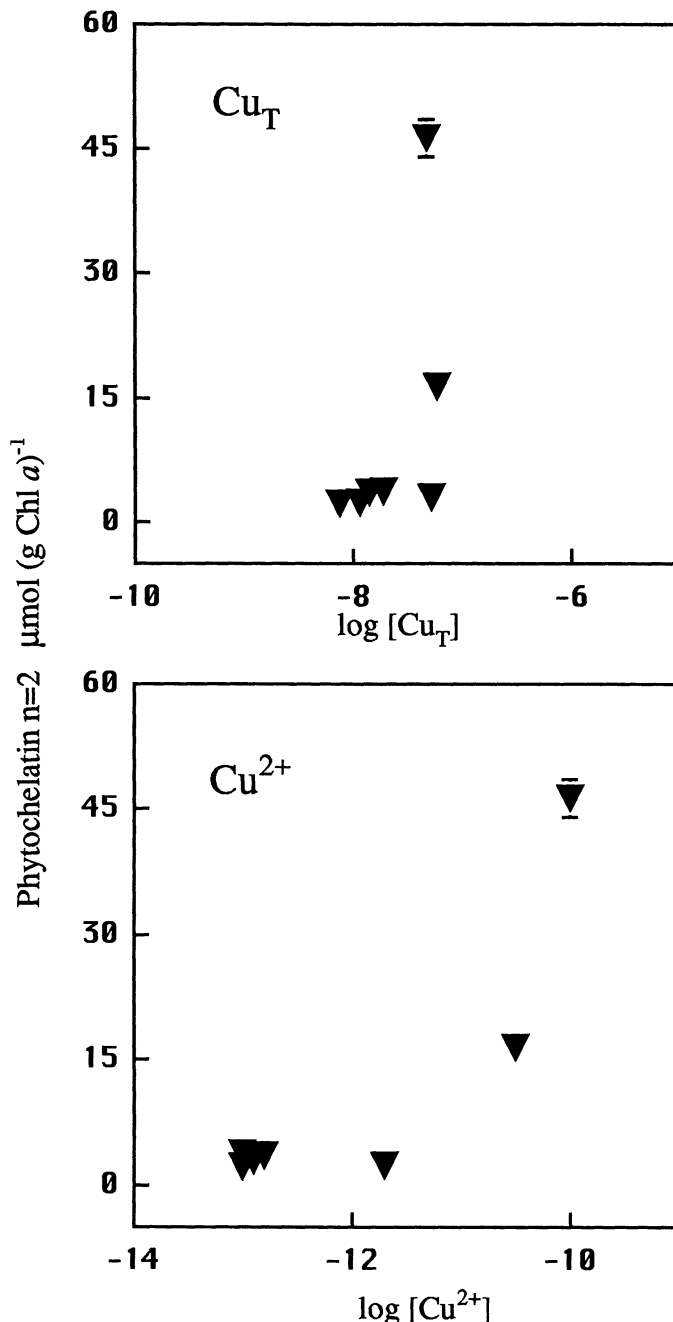


Fig. 1. Phytochelatin concentrations plotted as a function of total Cu (top) and free Cu concentrations (bottom). Samples were collected in September 1993 from various harbors in southeastern New England. Error bars represent the average of measurements made on two separate samples taken from the same bottle; for most of the data points the error bars are within the size of the symbol.

Incubation experiments, in which Cu was added to Vineyard Sound water at concentrations similar to those measured in Eel Pond, were performed to determine whether phytochelatin production could be stimulated by Cu. The first such experiment was performed in August

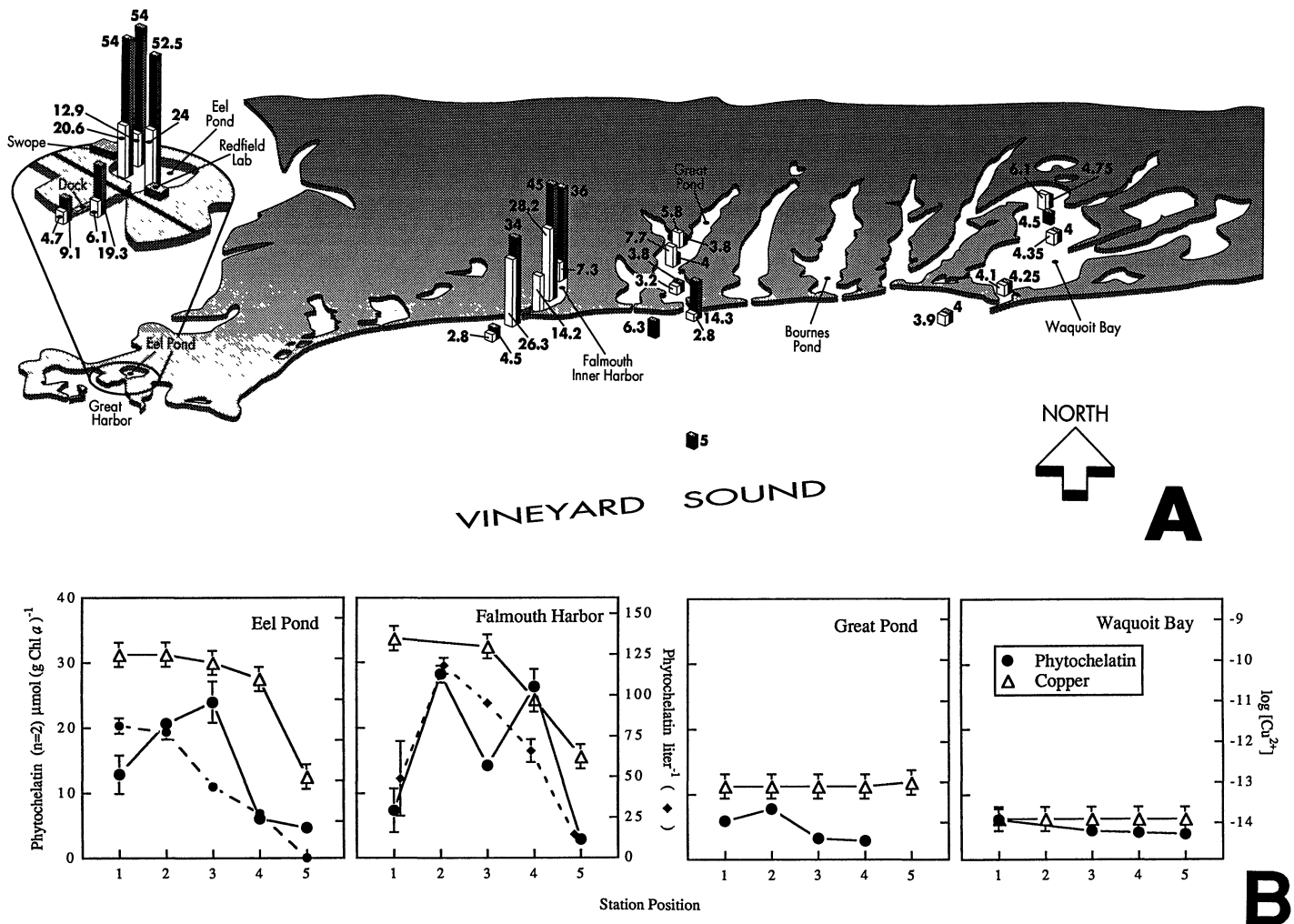


Fig. 2. A. Map of western Cape Cod showing ponds sampled in transects. Dark bars represent total Cu and light bars are the phytochelatin measurements. The Cu data are the same as reported in Moffett et al. (1997). B. Transects of phytochelatin and Cu concentrations in four harbors: Eel Pond, Falmouth Harbor, Great Pond, and Waquoit Bay. ●—Phytochelatin; Δ —Cu. The station numbers represent the relative spacing of samples taken in the harbors (low numbers are roughly inland and higher numbers are just outside harbor inlets; for exact station location see phytochelatin data on map). The small filled circles on Eel Pond panel are phytochelatin concentrations in samples collected in July 1995; corresponding Cu data were not available. The phytochelatin data for Falmouth Harbor have been replotted as phytochelatin liter⁻¹ (\blacklozenge).

1994. Water was collected in Vineyard Sound into four 20-liter acid-cleaned polycarbonate carboys; large carboys were used to minimize loss of Cu to container walls. Cu additions were made to two of the carboys ($\text{Cu}_T = 54 \text{ nM}$, $\text{pCu} = 10.3$); the remaining two were controls with no addition ($\text{Cu}_T = 4 \text{ nM}$, $\text{pCu} = 13.3$). Carboys were incubated in natural light bathed in a continuous flow of fresh seawater to maintain the temperature at 21°C. Duplicate samples were withdrawn from each carboy at $t = 3$ and $t = 5$ d. Samples were collected onto GF/F filters and analyzed for phytochelatin and Chl *a*. The consortium of algae present in Vineyard Sound responded to the increases in Cu by increasing phytochelatin concentrations roughly a factor of three above those measured in the control incubations (Fig. 3). The controls did increase somewhat, probably due to metal contamination.

The subsequent incubation experiment was done to determine whether Cd additions, comparable to concentrations measured in Eel Pond, would also stimulate phytochelatin concentrations to the same extent that Cu had. The total concentration of Cd in Eel Pond, determined by anodic stripping voltammetry (ASV) was 0.6 nM (P. Croot unpubl. data). In July 1995, an acid-cleaned polycarbonate carboy was filled with Vineyard Sound water, and ~2 liters was dispensed into each of eight acid-cleaned 2-liter polycarbonate bottles. Two samples (~500 ml each) were filtered for measurement of the initial phytochelatin concentration. Duplicate incubations of controls (nothing added), 1 nM Cd, 3.5 nM Cu, and 35 nM Cu were performed in a continuous light ($300 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$), 20°C incubation chamber for 2 d (35 nM Cu was at the low end of the range of total Cu concentrations measured in Eel Pond). Bottles were sampled at 24 h and

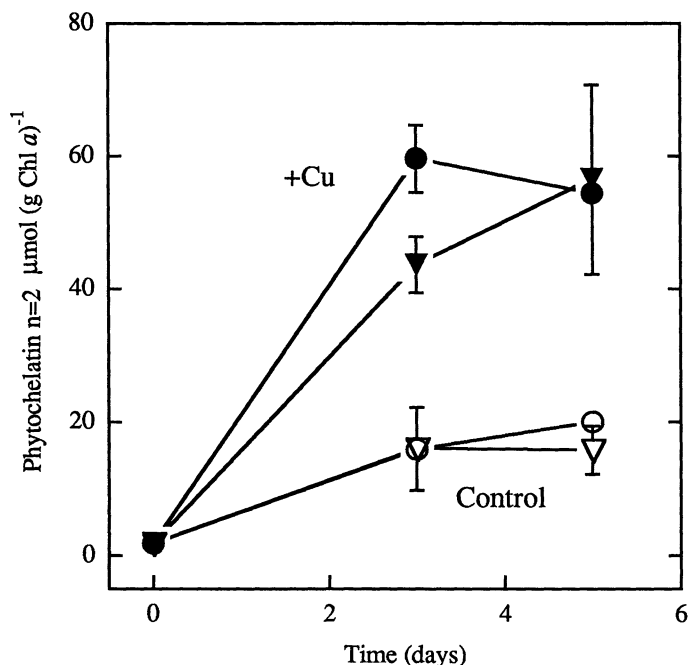


Fig. 3. Time evolution of phytochelatin concentrations in 20-liter carboy incubations. Filled symbols—Cu additions; open symbol—controls. Duplicate samples were withdrawn from each bottle at $t = 3$ d and $t = 5$ d. Each symbol represents a separate incubation bottle, and the error bars represent the average of measurements for two samples from each of the bottles.

again at 48 h. Phytochelatin was measured as described above and normalized to the total Chl a .

Bottles to which Cd had been added were not significantly different from controls to which no addition had been made (Fig. 4). Only the 35 nM Cu addition significantly increased phytochelatin concentrations above those in the controls. The induced concentrations were lower than the average measured in Eel Pond in July 1994, but the total Cu in the incubation bottle was also lower than in Eel Pond (53.5 ± 0.7 nM), and it is likely that some of the added Cu was lost to the container walls during the course of the incubation experiment. Again, the slight increase in the controls was probably due to contamination of bottles during sample handling (there was roughly 10 nM total Cu in the controls at the end of the experiment compared to 4.4 nM in the source water—see Fig. 4).

Notably, all phytochelatin production occurred after 1 d and there was no significant change in concentrations from days 3 to 5 in the first experiment and from days 1 to 2 in the second. Similar to what was observed in metal addition incubations with Boston Harbor water (Ahner et al. 1994), phytochelatin are produced rapidly in response to an increase in metal concentration.

Short-term Cu and Zn exposure experiments with *Skeletonema costatum* (A2896, isolated by L. Brand from Eel Pond where it is often a dominant species) were performed to examine the phytochelatin production under controlled laboratory culture conditions. *S. costatum* was cultured in the chemically defined seawater medium Aquil (Price et al.

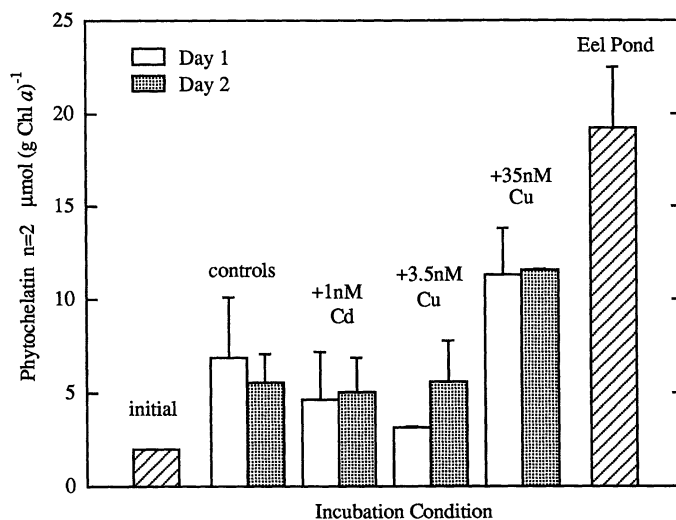


Fig. 4. Phytochelatin concentrations at two time points (1 d and 2 d after start of incubation) of the second incubation experiment. Error bars represent the average of measurements on duplicate samples withdrawn from each bottle. The initial phytochelatin concentration is the leftmost striped bar and the far right bar shows the average Eel Pond concentration (Sta. 1, 2, and 3) in July 1994. The average total Cu at these three stations was 53.5 ± 0.7 nM. At the end of the experiment, the total Cu concentrations measured in the controls and in the +3.5 nM Cu were 10.7, 12.4, 14.3, and 14.9 nM (± 1 nM), respectively.

1988/1989). The total EDTA was decreased to 10 μ M and the trace metal concentrations were decreased accordingly to achieve the following free ion concentrations: $p\text{Fe} = -\log[\text{Fe}^{3+}] = 18.2$, $p\text{Cu} = 13.8$, $p\text{Mn} = 8.3$, $p\text{Zn} = 10.9$, and $p\text{Co} = 10.9$. Additions of equimolar Cu-EDTA and Zn-EDTA were made when the cultures reached late exponential growth. The total Cu added and the corresponding calculated $p\text{Cu}$ were as follows: 1.2×10^{-7} M ($p\text{Cu} = 12$), 4×10^{-7} M ($p\text{Cu} = 11.5$), 1.2×10^{-6} M ($p\text{Cu} = 11$), 4×10^{-6} M ($p\text{Cu} = 10.5$), and 1.2×10^{-5} M ($p\text{Cu} = 10$). The total Zn added and the corresponding calculated Zn' (inorganic Zn) were as follows: 6×10^{-6} M [$\text{Zn}' = 12$ nM = Σ major inorganic species ($\text{Zn}^{2+} + \text{ZnSO}_4 + \text{ZnCl}^+$); $p\text{Zn} = 8$], 1.2×10^{-5} M ($\text{Zn}' = 24$ nM), 2.5×10^{-5} M ($\text{Zn}' = 50$ nM), 4×10^{-5} M ($\text{Zn}' = 80$ nM), 5×10^{-5} M ($\text{Zn}' = 100$ nM), 6×10^{-5} M ($\text{Zn}' = 120$ nM; $p\text{Zn} = 7$). Cultures were incubated in continuous light ($300 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$) at 20°C with the added metals for 2 d. Cells were harvested onto GF/F filters to determine total particulate phytochelatin and total Chl a .

Intracellular phytochelatin concentrations increased slightly from no added Cu to $p\text{Cu} = 12$ ($14\text{--}33 \mu\text{mol [g Chl } a]^{-1}$) and then increased sharply reaching concentrations of $>800 \mu\text{mol (g Chl } a)^{-1}$ at $p\text{Cu} = 10$ (Fig. 5). In contrast, Zn additions stimulated no phytochelatin production until $\text{Zn}' = 80$ nM ($p\text{Zn} = 7.2$) (Fig. 5), whereupon phytochelatin production dramatically increased to concentrations similar to those stimulated by Cu.

Incubations were also done with simultaneous addition of Cu and Zn to evaluate possible competitive inhibition

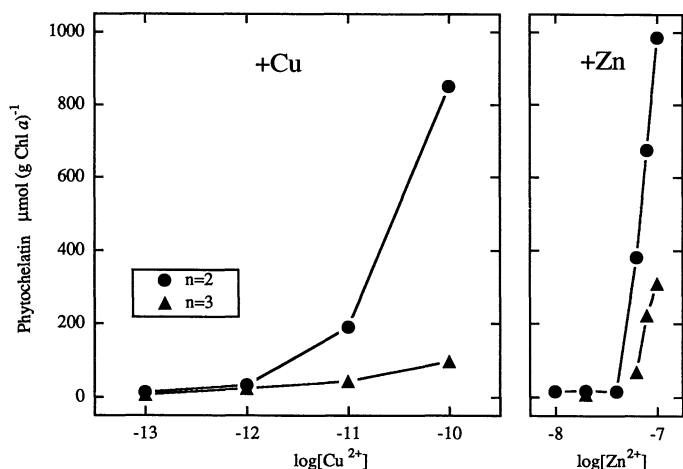


Fig. 5. Intracellular phytochelatin concentrations in cultures of *Skeletonema costatum* incubated for 2 d with additions of Cu and Zn. The chain lengths of phytochelatin are represented with different symbols. Duplicate cultures were prepared for the Cu addition experiments; error bars at each concentration are within the symbol. Note the change in x-axis scale in the two graphs.

between the two metals. Addition of 12 nM Zn' (pZn = 8) decreased phytochelatin concentrations slightly at pCu = 11.5, but at pCu = 10.5 phytochelatin production was suppressed by more than a factor of two (Fig. 6). However, the addition of the higher concentration of Zn' (120 nM, pZn = 7) to both Cu treatments stimulated phytochelatin production considerably. For pCu = 11.5 the phytochelatin concentration is not significantly higher than with Zn alone, but at pCu = 10.5 the effects of Zn and Cu appeared to be additive.

Our field measurements of phytochelatin and Cu, together with the incubation and laboratory data, help to further our understanding of the interactions between trace metals and phytoplankton in the field and serve to underscore the importance of organic chelators in natural waters. Phytochelatin concentrations are a biochemical parameter, mechanistically linked to trace metal uptake, that can be measured directly in natural algal assemblages and may allow us to quantitatively determine bioavailability of metals in the field.

Much laboratory work has demonstrated that organically complexed metals (usually hydrophilic) are not available for uptake by phytoplankton, hence the "free ion activity model" is the dominant paradigm of trace metal-biota interactions, with a few notable exceptions [see review by Campbell (1995)]. To date, however, there has been little direct field evidence supporting this model aside from a recent study of Cd bioavailability in freshwater by Hare and Tessier (1996). Demonstration in situ is necessary because the nature of the organic ligands in the field is unknown and it is quite possible that synthetic chelators, although useful tools, are not perfect analogues for natural ones. The data in Fig. 1 provide evidence that phytochelatin production in the field, a good proxy for trace metal uptake, is a function of free metal as opposed to the total metal concentration in solution.

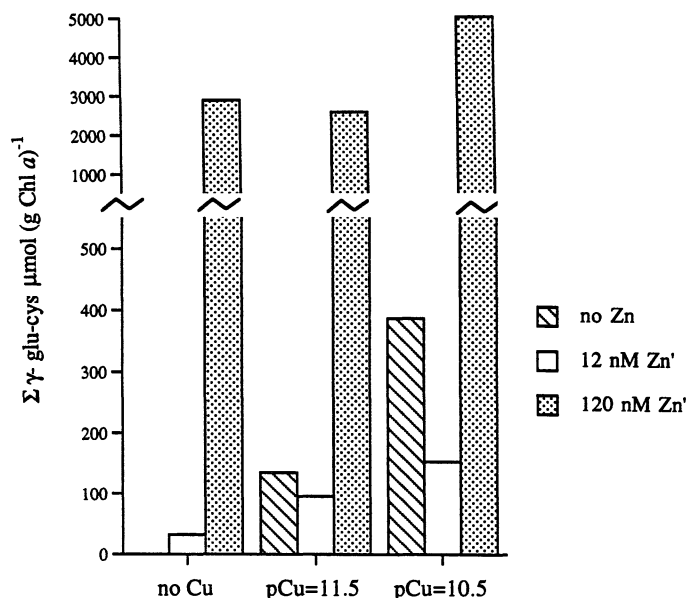


Fig. 6. Intracellular phytochelatin concentrations [reported as γ -glu-cys = $\Sigma 2 (n = 2) + 3 (n = 3)$] in cultures of *Skeletonema costatum* incubated for 2 d with simultaneous additions Cu and Zn.

The concentrations of phytochelatin measured in this study are very similar to those measured in the Massachusetts Bay study (Ahner et al. 1994). Although concentrations ranged from 2 to 20 $\mu\text{mol } n = 2 (\text{g Chl } a)^{-1}$ in that study, most of the measurements were < 10 . Values of ≥ 20 were often measured in Eel Pond and Falmouth Harbor, suggesting in fact that these two harbors have higher levels of bioavailable toxic metals than the much maligned Boston Harbor (Wallace et al. 1988). The trace metal chemistry in Boston Harbor may be similar to Providence Harbor where we measured high total dissolved Cu levels and low phytochelatin concentrations (Table 1). Free Cu was also very low in Providence Harbor, supporting the free ion activity model. Presumably $[\text{Cu}^{2+}]$ is low in these harbors because there are higher concentrations of organic ligands that form biologically unavailable Cu complexes. In Providence Harbor, such ligands could have been derived from terrestrial or riverine sources, because the salinity was only 22‰ (cf. > 30 ‰ at all Cape Cod stations).

The particulate phytochelatin concentrations per liter in Falmouth Harbor ranged from 10 to 120 pM (Fig. 2B). There has been some speculation as to whether phytochelatin may be one of the dissolved metal-binding organic ligands measured in surface seawater. It is unlikely that they could play a large role given their relatively small particulate concentration even if some phytoplankton are exporting the phytochelatin-metal complexes as reported recently by Lee et al. (1996). Estimating a turnover time of 1 d for the intracellular pool, released to the dissolved phase via export or cell breakage, the phytochelatin would have to persist several days in the ambient water for concentrations to reach 0.5 nM. We would not expect such a small peptide to persist for several days, given that the

average half-life of a much larger protein, Rubisco, in seawater is ~ 1.2 d (Taylor 1995).

Our incubation experiments show that Cu but not Cd is capable of modulating phytochelatin concentrations at our field site, but we cannot rule out the possible involvement of Zn. Recent ASV measurements of labile Zn in Eel Pond suggest that concentrations of inorganic Zn species (Zn') may be as high as 100 nM (Moffett et al. 1997). Although in coastal waters it is less certain what the ASV labile pool actually represents, if we assume that it is only inorganic Zn species and we compare phytochelatin production in *S. costatum* at 100 nM Zn' (Fig. 5) to production at high Cu, it is clear that Zn is as effective an inducer as Cu. Unfortunately, we did not do any incubation experiments with added Zn.

Concentrations of phytochelatin measured in the laboratory at the high Cu concentrations are much higher (over an order of magnitude) than those measured in the field. Although not precisely determined, *S. costatum* biomass constituted at least 10% of the total chlorophyll-containing biomass but was certainly $<50\%$ (L. Brand pers. comm.) during the September 1993 sampling when we measured roughly $50 \mu\text{mol } n = 2 \text{ (g Chl } a)^{-1}$ in Eel Pond. Concentrations of phytochelatin in *S. costatum* in the laboratory are more than an order of magnitude higher at the same Cu concentration ($pCu = 10$). If we assumed that *S. costatum* had contributed only 10% to the total Chl *a*, then no other Chl *a*-containing organism could have been synthesizing any phytochelatin—an improbable scenario.

Phytochelatin concentrations in laboratory incubations of *S. costatum* at $pCu = 10.5$ to which Zn ($Zn' = 12$ nM) had also been added were significantly lower than those to which Cu alone had been added (Fig. 6). This decrease is likely a result of competition between Zn and Cu for uptake sites on the cell surface. Increasing the Zn' to 120 nM, however, exceeds the competitive beneficial effect, and Zn becomes a toxicant. The competitive interactions of Cu and Zn have been particularly well documented (e.g. Rueter and Morel 1981; Sunda 1988/1989). If we assume Zn' to be actually less than what the ASV measurements suggest, then the high concentrations of Zn in Eel Pond may reduce Cu uptake by the cells and result in the lower phytochelatin concentrations measured in the field compared to those measured in the laboratory at the same pCu . Though a more thorough study at intermediate Zn concentrations is needed before we can be quantitative about this competitive effect (as well as a better understanding of the relationship between Zn' and ASV-labile Zn in these harbors), it is clearly an important factor to consider. The other competitive interaction that would be predicted to be important on the basis of laboratory studies is that of Mn and Cu (Sunda and Huntsman 1983). Mn concentrations are likely to be orders of magnitude higher in Eel Pond than in the Aquil culture medium (though no measurements were made).

Another explanation for the difference between the laboratory and field data may warrant further investigation. Light or nutrient limitation may restrict intracellular concentrations of glutathione, the precursor to phytochelatin, in the field, whereas in the laboratory cultures where con-

ditions are ideal, surplus amounts may be synthesized. Concentrations of glutathione in *Thalassiosira weissflogii* in the laboratory are roughly $750 \mu\text{mol (g Chl } a)^{-1}$ (Ahner and Morel 1995), significantly higher than the $40\text{--}200 \mu\text{mol (g Chl } a)^{-1}$ measured in the plankton off the coast of California (Matrai and Vetter 1988). Matrai and Vetter (1988) found that particulate glutathione increased or remained constant with higher than ambient light exposure in on-deck incubations and decreased in those incubated with lower than ambient light except when nitrate was high. These field measurements hint at what factors may alter glutathione production in marine algae, but for the most part changes in glutathione levels are not well understood and should be studied in a controlled laboratory setting.

We have demonstrated that elevated Cu (and perhaps Zn) is influencing the biochemistry of eukaryotic phytoplankton in a field setting, but can we assess whether these concentrations of phytochelatin actually reflect a significant stress caused by Cu on the organisms' physiological resources? One way to approach this question is to compare the concentration of phytochelatin to the concentration of its precursor glutathione. Glutathione is the principal thiol used by eukaryotic cells to maintain reducing conditions for proteins and amino acids as well as to protect against oxidative damage (Fahey et al. 1987). It is used by glutathione peroxidase to catalyze the destruction of hydrogen peroxide and by other enzymes to reduce organic hydroperoxides. Glutathione has also been shown to participate in the detoxification of xenobiotic organic compounds via the group of enzymes called glutathione-S-transferases (Sandermann 1992). As mentioned above, field concentrations of particulate glutathione measured by Matrai and Vetter (1988) ranged from 40 to $100 \mu\text{mol (g Chl } a)^{-1}$ in surface waters of Saanich Inlet and ~ 100 to $200 \mu\text{mol (g Chl } a)^{-1}$ in surface waters of the Southern California Bight (calculated from values plotted in graphs). If we assume that most of the glutathione is due to eukaryotic phytoplankton (a conservative assumption given that some bacteria and prokaryotic phytoplankton also produce glutathione (Sundquist and Fahey 1989), our high phytochelatin concentrations ($10\text{--}50 \mu\text{mol } n = 2 \text{ [g Chl } a]^{-1}$, corresponding to $20\text{--}100 \gamma\text{-glu-cys}$) rival these numbers. If our comparisons are to be trusted, then the phytochelatin measured in the field samples represents a significant biochemical cost. It is possible that eukaryotic cells in these mildly polluted ecosystems may have less than sufficient glutathione to combat oxidative damage. Future measurements of phytochelatin in the field should be accompanied by measurements of particulate glutathione in order to address this issue more fully.

In conclusion, phytochelatin measurements provide valuable information regarding the effect of elevated trace metals (and the fairly complex chemistry thereof in natural waters) on primary producers at the biochemical level. Phytochelatin concentrations in an organism provide a general measure of its overall toxic metal stress rather than a measure of the physiological effect of one particular metal. Because competitive interactions among metals are likely to be important in most field situations, mea-

asuring a biological indicator of stress rather than relying on direct measurements of a particular pollutant should help in assessing more accurately the resulting toxicity to the ambient biota.

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