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7. Photographs were available from the U.S. Geological Survey, the U.S. Department of Agriculture Soil Conservation Service, the National Oceanic and Atmospheric Administration, the Virginia Department of Highways, and the National Aeronautics and Space Administration. The earliest photographs were taken in 1937 by the Soil Conservation Service.
8. We surveyed numerous watermen, hunters, fishermen, and owners of homes on the bay's shoreline who were familiar with the submerged vegetation. Many of their remarks confirmed trends noted in more quantitative surveys.
9. Vegetation in this part of the Potomac was *Z. marina*. Intensive surveys by the U.S. Geological Survey revealed localized but small stands of vegetation in portions of the river up to Washington, D.C., in the late 1970's [G. M. Haramis and V. Carter, *Aquat. Bot.* **15**, 65 (1983)].
10. J. Davis and B. Laird, in *The Effects of Tropical Storm Agnes on the Chesapeake Bay Estuarine System*, J. Davis and B. Laird, Eds. (Johns Hopkins Univ. Press, Baltimore, 1977), pp. 1–29.
11. We have monitored the north shore of the lower York River since 1974 for changes in both existing beds of *Z. marina* and denuded areas. This species has increased in abundance in several sections, apparently from germination and growth of seeds transported from adjacent beds. Transplantation of *Z. marina* into denuded areas has been attempted with the greatest long-term success in areas closest to existing vegetation [R. J. Orth and K. A. Moore, Final Report, Environmental Protection Agency grant R805953 (1982)].
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13. We contacted scientists and resource managers in states from North Carolina to Maine. There were no reports of declines of submerged vegetation. A. C. Churchill (personal communication) reported increases in Long Island Sound during the period when the bay was experiencing the decline.
14. "Submerged aquatic vegetation" (synthesis paper summary), in *Chesapeake Bay Program Technical Studies: A Synthesis* (Final Report, Environmental Protection Agency, Washington, D.C., 1982), pp. 633–635. Herbicides were initially implicated in the decline, but final results indicated that they were not the single cause.
15. Examination of long-term data bases (up to 30 years) showed increases in phosphorus, nitrogen, and chlorophyll *a* in many regions of the bay [D. R. Heinle *et al.*, Final Report, Environmental Protection Agency grant R806189 (1980)].
16. J. van Montfrans, R. J. Orth, S. Vay, *Aquat. Bot.* **14**, 75 (1982).
17. M. C. Perry *et al.*, in *Transactions of the 46th North American Wildlife and Natural Resources Conference* (Wildlife Management Institute, Washington, D.C., 1981), p. 299.
18. The commercial catch of hard blue crabs in the bay during the 1970's was below average. Speculation has centered on the decline of vegetation as a possible cause. Commercial crabbers have had problems harvesting peeler crabs in areas where vegetation was once abundant (W. Conley and R. C. V. Seafood, personal communication).
19. Our work was supported by grants R805951, X003201, and X003246 from the Environmental Protection Agency Chesapeake Bay Program to the Virginia Institute of Marine Science. We thank A. Evans, J. van Montfrans, and K. Webb for critical reviews of this manuscript. This is contribution No. 1124 from the Virginia Institute of Marine Science.

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Free Cupric Ion Activity in Seawater: Effects on Metallothionein and Growth in Crab Larvae

Abstract. *Crab zoeae (*Rhithropanopeus harrisii*) were exposed during their development to a range of free cupric ion activities regulated in seawater by use of a copper chelate buffer system. Most cytosolic copper was found to be associated with metallothionein. Copper-thionein could be related to free cupric ion activity, and a shift in copper-thionein accumulation was correlated with inhibition of larval growth. These data reveal predictable relations between cupric ion activity in seawater and processes at the cellular and organismic levels.*

The biological impact of increases in trace metal concentrations in the oceans has become a major concern (*1*). Trace metals such as copper at nanomolar concentrations similar to those in natural seawater inhibit nutrient uptake in both phytoplankton (*2*) and bacteria (*3*). However, numerous chemical species of copper are present in natural seawater (*4*), and chemical speciation often varies considerably between samples (*3*). The biological availability and toxicity of copper appear to be related to free cupric ion activity, $\{Cu^{2+}\}$, rather than to total copper concentration or the concentration of copper complexes (*3, 5*). Since most copper toxicity studies have related cellular or organismic responses to total copper added to seawater (*6*), the biological availability of the metal, even on a relative scale, is usually unknown.

The cysteine-rich metal-binding protein metallothionein serves as a major intracellular metal-binding ligand whose synthesis can be induced by metals, including copper, cadmium, zinc, and mercury (*7*). Metallothioneins are widely distributed and have been isolated from various vertebrates, invertebrates, and higher plants (*8, 9*). These proteins have been associated with metal uptake, metabolism, and detoxification (*7*). The primary structure of crab thionein is homologous to both mammalian and fungal thioneins, and its synthesis is induced by copper, zinc, and cadmium (*9, 10*).

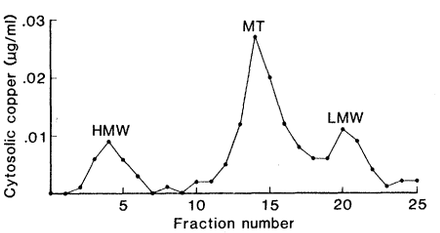

Fig. 1. Cytosolic distribution of copper in crab larvae (*R. harrisii*) exposed to free cupric ions in seawater (*13*). *HMW, MT,* and *LMW* represent high molecular weight, metallothionein, and low molecular weight pools, respectively.

Research on the mechanisms of copper toxicity has focused on either biochemical responses (for example, metallothionein synthesis) or physiological effects at the population level (for example, growth rate), but not at both biological levels simultaneously (*6*). As a consequence, the relations between metal exposures, metallothionein synthesis, and population effect remain unclear. Correlating the amount of biologically available copper in seawater with cellular and molecular data and with the impact on organisms and populations is even more difficult. However, predictions of the ecological consequences of increased copper in seawater and of subsequent copper accumulation and subcellular distribution will be possible only if they can be related to population effects. In this study we have used a copper–nitrotriacetic acid (NTA) buffer system (*11*) to control free cupric ion activity, and have examined the relations between $\{Cu^{2+}\}$ in seawater, cytosolic copper, copper-thionein accumulation, and growth in crab larvae. Our data indicate that copper-thionein can be related to $\{Cu^{2+}\}$ in seawater and that a shift in copper-thionein accumulation is correlated with inhibition of larval growth.

Newly hatched larvae of the mud crab *Rhithropanopeus harrisii* were exposed to a range of $\{Cu^{2+}\}$ values for the duration of zoeal development (*12*). The larvae were sampled immediately after they had molted to the megalopa stage. Survival, time to megalopa, and dry weights

were determined for each value of $\{Cu^{2+}\}$. Replicate samples were pooled, homogenized, and centrifuged, and the resultant cytosol was fractionated by high-performance liquid chromatography (HPLC). Cytosolic copper accumulation and subcellular distribution were subsequently determined by atomic absorption spectrophotometry (*13*).

Three major peaks of copper binding were observed upon fractionation of the cytosol (Fig. 1): (i) a high molecular weight peak, which eluted just behind the void volume and contained most of the soluble enzymes (molecular weight $\gg$ 20,000); (ii) a metallothionein peak, which had an apparent molecular weight of 10,000 to 12,000 (*14*); and (iii) a low molecular weight peak, which included all molecules too small to be resolved by the column (molecular weight $<$ 5000).

Most (47 to 82 percent) of the cytosolic copper was associated with the metallothionein pool over the entire range of cupric ion exposures, with copper-thionein most prominent (72 to 82 percent) at lower $\{Cu^{2+}\}$ (Figs. 1 and 2). Both copper-thionein and cytosolic copper were relatively independent of external free cupric ion activity at low $\{Cu^{2+}\}$, increased above this range, and approached saturation at high $\{Cu^{2+}\}$. These data suggest that crab larvae can regulate cytosolic copper accumulation over several orders of magnitude of environmental $\{Cu^{2+}\}$. Although the biological significance is still unclear, the relation of $\{Cu^{2+}\}$ in seawater to copper-thionein and cytosolic copper can be described by a hyperbolic equation (Fig. 2). Copper-thionein accumulation reflects the processes of net copper transport across membranes, induction of metallothionein synthesis, metallothionein degradation, and the net binding of copper to metallothionein. The finite capacity for copper-thionein accumulation may be the result of limiting steps in any of these processes.

To determine the physiological impact of total copper-thionein and cytosolic copper accumulation, we examined the relations between cupric ion activity, survival, larval duration, and growth. Neither survival nor larval duration changed significantly over the entire range of cupric ion exposures (*15*). However, significant differences in the dry weight of larvae at megalopa were observed (Fig. 2). Increased megalopa weight was observed for larvae exposed to a $\{Cu^{2+}\}$ of $5 \times 10^{-12}M$. This phenomenon, termed hormesis, is thought to result from an overcompensation of homeostatically regulated processes in response to stress (*16*). Hormesis has been observed in *R. harrisii* larval growth in response to petroleum hydrocarbons and a cyclic temperature regime (*17*). Megalopa weight decreased rapidly at higher $\{Cu^{2+}\}$ and was significantly lower at activities greater than approximately $2 \times 10^{-11}M$. These activities are similar to those at which algal growth is inhibited (*2*) and approach estimated ranges of $\{Cu^{2+}\}$ in seawater [$10^{-11}M$ (*3*)]. Furthermore, we found a quantifiable relation between accumulation of copper-thionein and larval growth; the hyperbolic equation used to describe the accumulation data also provides a reference point, the half-saturation constant, k, beyond which $\{Cu^{2+}\}$ in seawater adversely affects growth (Fig. 2).

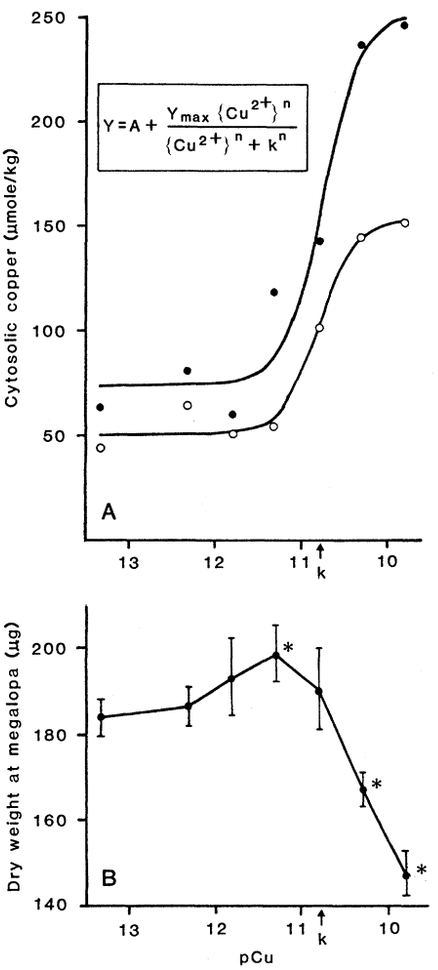

Fig. 2. (A) Cytosolic distribution of copper expressed in micromoles per kilogram (wet weight) of tissue (*13*) in mud crab *R. harrisii* megalopa exposed to a range of values of $\{Cu^{2+}\}$ (free cupric ion activity) throughout the duration of larval development (pCu = $-\log \{Cu^{2+}\}$). Curves for total cytosolic copper (●) and copper-thionein (○) are modeled by means of the inset hyperbolic equation (*18*). This equation describes the concentration of either total cytosolic copper or copper-thionein, Y, as a function of $\{Cu^{2+}\}$; Y_{max}, the maximum increase in Y; A, the minimum Y at low $\{Cu^{2+}\}$; k, the half-saturation constant, at $\frac{1}{2}$ Y_{max}. (B) Dry weights of mud crab megalopa described above. Vertical lines are 1 standard error of the mean for five replicates. Asterisks delineate data points that are significantly different from the other points by analysis of variance and the Student-Newman-Keuls multiple range test (F = 7.6, d.f. = 6, 28; P = 0.05).

Our data relate $\{Cu^{2+}\}$ in seawater to cellular responses and to processes that affect the population. These data indicate that metallothionein is the major cytosolic copper–binding ligand and is involved in cytosolic copper accumulation in crab larvae. Copper-thionein concentration can be related to the $\{Cu^{2+}\}$ in the seawater by a saturation equation. This equation provides a quantitative representation of shifts in copper metabolism that are correlated with adverse effects on growth.

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11. The Cu-NTA buffer system was made up in 35 parts per thousand seawater diluted with distilled water to 20 parts per thousand and contained $10^{-4}M$ NTA, $1.1 \times 10^{-8}M$ $ZnCl_2$, $6.0 \times 10^{-9}M$ $CoCl_2$, $2.5 \times 10^{-9}M$ $MnCl_2$, and $2.5 \times 10^{-8}M$ $FeCl_3$. NaOH was added at $4 \times 10^{-4}M$ to adjust the pH to 8.0 ± 0.1, and copper was added as Cu-NTA to achieve concentrations of $9 \times 10^{-9}M$ to $2 \times 10^{-5}M$. Free

ion activities of Cu^{2+}, Zn^{2+}, Co^{2+}, and Mn^{2+} were computed from metal ions–NTA equilibriums as described by W. G. Sunda, D. W. Engel, and R. M. Thuottle [*Environ. Sci. Technol.* **12**, 409 (1978)]. Computed free ion activities based on added metal concentrations are: Cu, $10^{-13.3}M$ to $10^{-9.8}M$; Zn, $10^{-10.3}M$; Co, $10^{-10.3}M$; and Mn, $10^{-9.5}M$.

12. Gravid females of the mud crab were collected near Beaufort, North Carolina. They were kept in tanks of running seawater and transferred to culture bowls when their eggs were ready to hatch. Newly hatched larvae were then reared at 27.5°C in 8-cm (diameter) culture bowls containing 50 ml of metal-buffered seawater with ten zoeae per bowl. There were five replicates for each $\{Cu^{2+}\}$. Larvae were transferred to clean bowls with fresh media and fed newly hatched *Artemia* nauplii daily.
13. Lyophilized *Rhithropanopeus harrisii* larvae were rehydrated and homogenized in 0.2*M* tris-HCl, *p*H 7.4, with an acid-washed Teflon pestle tissue grinder. The homogenate was centrifuged at 100,000*g*, and the resulting supernatant was filtered by centrifugation through a 0.2-μm nylon filter. A 100-μl portion of the filtered cytosol was chromatographed on a HPLC gel permeation column (Toyo Soda TSK SW 3000) at 1 ml/min with 0.25*M* tris-HCl, *p*H 7.4. Fractions (1 ml) were collected, and copper concentrations were determined by atomic absorption spectrophotometry (Perkin-Elmer 5000) with a graphite furnace (HGA 500). The coefficient of variation was 7.56 percent for copper-thionein.
14. This metal-binding ligand (i) bound zinc, copper, and cadmium; (ii) was induced by elevated $\{Cu^{2+}\}$; (iii) had a low absorption at 280 nm, suggesting a lack of aromatic amino acids; (iv) co-migrated (HPLC) with a scorpion-fish metallothionein that had previously been characterized by amino acid analysis (unpublished data); and (v) had a DEAE elution profile similar to that of metallothioneins that have been characterized in other species of crab (*10*). Final confirmation of this ligand as metallothionein, however, awaits amino acid analysis.
15. Survival [mean ± standard error (S.E.)] for five replicates each at the seven $\{Cu^{2+}\}$ data points: x = 72.3 ± 8.9 percent; F = 1.18; d.f. = 6,28; P = 0.25. Duration: x = 10.6 ± 0.16; F = 1.25; d.f. = 6,28; P = 0.25.
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18. Our seven data points were fitted by a derivative-free nonlinear regression program, in *BMDP Statistical Software*, W. J. Dixon, Ed. (Univ. of California Press, Los Angeles, 1981), p. 305. This program also calculates asymptotic standard deviations of parameter estimates. For copper-thionein: Y_{max} = 101.4 ± 7.4 μmole/kg; A = 50.8 ± 4.2 μmole/kg; $k = 1.6 \times 10^{-11} \pm 9.83 \times 10^{-12}M$; n = 2.12; r^2 (coefficient of determination) = 0.9804. For cytosolic copper: Y_{max} = 176.8 ± 18.4 μmole/kg; A = 74.6 ± 10.1 μmole/kg; $k = 1.78 \times 10^{-11} \pm 1.267 \times 10^{-11}M$; N = 2; r^2 = 0.9613.
19. Supported by the Office of Health and Environmental Research, Ecological Division of the Department of Energy and the Ocean Assessment Division, National Ocean Services, NOAA. We thank D. Brown, S. George, D. Manahan, and G. Roesijadi for reviewing earlier drafts of this manuscript; R. Burri and T. Marshall for technical assistance; and D. Bradley for statistical assistance.

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Catch a Falling Star: Meteorites and Old Ice

Abstract. *A model for the process of meteorite concentration in blue ice regions of the Antarctic ice sheet is proposed based on data from near the Allan Hills and the assumptions that both meteorite influx and glacial flow have been constant. The meteorite influx is calculated to be 60 × 10^{-6} kilogram per square kilometer per year, and the age of the exposed ice to be 0 to 600,000 years, varying with distance from the Allan Hills. These results are in line with other estimates of influx rate and with measurements of the terrestrial ages of the meteorites, providing support for the assumption of steady flow and meteorite influx. This may be the oldest sequence of ice in stratigraphic order yet discovered, and the results imply that this part of the east Antarctic ice sheet has been approximately steady during this time interval.*

There are places on the Antarctic ice sheet where meteorites are found in large numbers. Ordinarily meteorites become buried in the snow, incorporated in the ice, carried to the edge of the continent (*1*), and discharged into the sea. In special places, however, the ice does not reach the sea but evaporates or otherwise ablates at the surface, and the meteorites are exposed. These collect at the ablating surface where they are joined by direct falls. Compressive ice flow, characteristic of ablation zones, further concentrates meteorites.

Steady-state meteorite fall rate and steady-state glacial flow are assumed. Meteorites that fall onto the accumulation zone (Fig. 1) attain a concentration inside the ice given by $\gamma = f/A_c$, where f represents the meteorite infall rate (mass per unit area per unit time) and A_c the snow accumulation rate expressed in meters of ice equivalent per year. This concentration does not depend on depth or age since we take both f and A_c as constant in both time and position. Thus the meteorites are uniformly distributed inside the ice sheet along the flow line leading to the ablation zone.

Three mechanisms concentrate meteorites. Those that are buried in the accumulation zone reappear in the ablation zone (*2*) at a rate γA_b, where A_b represents the rate of ice loss—in the case of the Allan Hills region, loss is mainly by evaporative sublimation. These meteorites are joined by direct falls at rate f. Finally, ablation zones usually show compressive flow, such that the area between three or more points on the ice surface becomes smaller with time. This affects the meteorite concentration (M), which is expressed in mass of meteorites per unit area. Let $\dot{\varepsilon}_s$ represent the sum of the two horizontal strain rates at the surface; then by adding the three effects, the time-rate of surface concentration is

$$\frac{dM}{dt} = \gamma A_b + f - \dot{\varepsilon}_s M \qquad (1)$$

Since the strain rate $\dot{\varepsilon}_s$ is on average negative, all three terms contribute to increasing M. For ice flow, expressions are obtained for $\dot{\varepsilon}_s(x)$ and $t(x)$, where x is horizontal position along the flow line. Then this equation is solved for $M(x)$, our objective.

Assume that the glacier has been steady—that is, its geometry and velocity have not changed with time. Then the shape of the glacier, the rates of accumulation or ablation at the surface, and ice movement are related to one another by continuity. In our case we need consider only the ablation zone

$$Z\,\overline{\frac{dx}{dt}} = -\int_0^x (Z\bar{\dot{\varepsilon}}_y + A_b)\,dx \qquad (2)$$

where x represents distance from the lower end of the glacier (the snout), $Z(x)$ ice thickness, and $\bar{\dot{\varepsilon}}_y$ the strain rate for flow-line spreading (positive) or convergence (negative) in map view. Both the velocity, $\overline{dx/dt}$, and the lateral spreading, $\bar{\dot{\varepsilon}}_y$, are expressed as means through the ice thickness, but Eq. 1 calls for surface values. The surface velocity, dx/dt_s, and surface spreading, $\dot{\varepsilon}_{ys}$, are larger

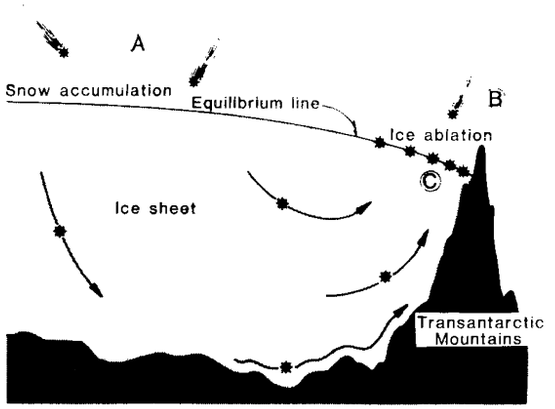

Fig. 1. Profile of an ice sheet and mechanisms for concentration of meteorites. (A) Meteorites fall into the snow accumulation zone and are transported to the ablation zone by ice flow; (B) meteorites fall directly onto ablation zone; and (C) compressive ice flow "crowds" meteorites together. Vertical exaggeration, approximately ×50.