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# INHIBITION OF DIGESTIVE ENZYME ACTIVITIES BY COPPER IN THE GUTS OF VARIOUS MARINE BENTHIC INVERTEBRATES

ZHEN CHEN,† LAWRENCE M. MAYER,*† DONALD P. WESTON,‡ MICHAEL J. BOCK,† and PETER A. JUMARS†
†Darling Marine Center, University of Maine, Walpole, Maine 04573, USA
‡Department of Integrative Biology, University of California, Berkeley, California 94720, USA

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**Abstract**—Digestive systems of deposit and suspension feeders can be exposed to high concentrations of copper (Cu) by ingestion of contaminated sediments. We assessed a potential impact of this Cu exposure on digestive enzyme activities in a wide range of benthic organisms by monitoring enzyme activities in their gut fluids during in vitro titrations with dissolved Cu, which mimics Cu solubilization from sediments. Increasing Cu inhibited digestive protease activities at threshold values, which varied widely among organisms, from 8 μM for an echinoderm to 0.4 M for an echiuran. More Cu was required to inhibit proteases in guts containing higher amino acid concentrations because strong Cu-binding sites on amino acids prevent Cu interaction with the enzymatically active sites. Threshold Cu concentrations were similar for proteases, esterases, lipases, and α- and β-glucosidases, suggesting the same inhibition mechanism. Copper was less effective at inhibiting enzymes at lower pH, suggesting that protons can compete with Cu ion for binding to enzymatically active sites or that enzyme conformation is less vulnerable to Cu inhibition at lower pH. These results lead to the counterintuitive conclusion that deposit feeders with low enzyme activity, low amino acid concentration, and high pH values are most vulnerable to harm from sedimentary Cu by this mechanism, although they solubilize less sedimentary Cu than their counterparts with high enzyme activity, high amino acid concentrations, and low gut pH. In general, digestive systems of echinoderms may therefore be more susceptible to Cu contamination than those of polychaetes, with various other phyla showing intermediate susceptibilities. If threshold Cu values are converted to solid-phase sedimentary Cu concentrations, the thresholds are at least consistent with Cu loadings that have been observed to lead to biological impacts in the field.

**Keywords**—Copper Digestive Enzyme Invertebrates

## INTRODUCTION

Copper is a common contaminant in coastal sediments, resulting from urban and industrial developments and shipping activities [1,2]. Laboratory and field studies indicated that Cu-contaminated sediments can cause bioaccumulation and toxic effects on marine benthic organisms [3–6] and result in mortality, reduction of hatching and growth rates, and alteration of community structure. Ingestion and digestion of metal-contaminated sediments have been demonstrated to be an important pathway leading to toxic effects [7,8].

A biomimetic method was developed to assess bioavailable Cu during digestion by incubation of Cu-contaminated sediments with gut fluids of deposit feeders [9]. Considerable fractions (10–30%) of sedimentary Cu can be solubilized by gut fluids, resulting in elevated Cu concentrations of 55 to 4,400 μM in gut fluids of the deposit-feeding lugworm *Arenicola marina* [9,10]. These results suggest that guts of deposit feeders could be exposed to high concentrations of dissolved Cu during in vivo digestive processes.

Copper can interfere with enzyme function [11–13], and the gut is a site of intense digestive enzyme activity [14]. To assess the threat from digestive Cu exposure, activities of extracellular proteases in gut fluids were monitored during in vitro incubation with Cu-contaminated sediments and during Cu titration experiments [10]. Inhibition of proteases occurred only after dissolved Cu concentration in gut fluid reached a threshold value, which was determined by the level of gut-dissolved amino acids. A subset of gut amino acids form strong complexes with Cu, preventing its inhibition of protease activity by interacting with the enzymatically active sites. Results from this type of experiment suggested that individual lugworms with higher gut amino acid concentrations are less vulnerable to Cu toxicity than others with lower amino acid concentrations.

Different species of macrobenthos contain different levels of gut amino acids [14], suggesting differential susceptibility among species to gut enzyme inhibition by Cu. The objectives of this paper are therefore to extend the previous work on lugworms to an interphyletic study by comparing the sensitivities (threshold Cu concentration) of digestive proteases among various deposit and suspension feeders and to examine effects of physiological conditions in the digestive system of different species, such as gut pH and amino acid concentrations, on the threshold Cu concentration. A Cu titration approach was used to mimic the solubilization of sedimentary Cu during digestion to determine the threshold Cu concentrations.

## MATERIALS AND METHODS

We used gut fluids of various deposit- and suspension-feeding organisms from previous studies that report on sites, methods of gut fluid extraction, and initial Cu and amino acid concentrations [15,16]. As reported previously, initial Cu concentrations were determined by graphite furnace atomic absorption spectroscopy and total amino acid concentrations determined after acid hydrolysis using fluorometric detection of orthophthaldialdehyde derivatives. One sample of gut fluid from each species was used for the current study (Table 1). The pH of each gut fluid was measured with pH electrodes

* To whom correspondence may be addressed (lmayer@maine.edu).

Table 1. Species used in this study, their feeding mode, collection site, gut pH, dissolved amino acid concentration (AA; mM), copper threshold concentration (as μM in gut fluid and as μg of bioavailable Cu per gram sediment after applying assumptions described in text), and maximum enhancement of protease activity during the Cu titration ($P_{max}/P_o$)

| Species[a] | Feeding strategy | Collection location (all USA) | pH | AA (mM) | Copper threshold μM | Copper threshold μg/g | $P_{max}/P_o$ |
|---|---|---|---|---|---|---|---|
| *Strongylocentrotus droebachiensis* (E) | Herbivore | Damariscotta Estuary, Maine | 7.92 | 16 | 8 | 1.0 | 1.2 |
| *Molpadia intermedia* (E) | Deposit feeder | Puget Sound, Washington | 8.29 | 2 | 46 | 5.9 | 1.7 |
| *Parastichopus californicus* (E) | Deposit feeder | Puget Sound, Washington | 7.56 | 10 | 76 | 9.7 | 1.3 |
| *Cucumaria frondosa* (E) | Suspension feeder | Damariscotta Estuary, Maine | 7.43 | 12 | 86 | 10.9 | 1.4 |
| *Brisaster latifrons* (E) | Deposit feeder | Puget Sound, Washington | 7.46 | 15 | 162 | 20.6 | 1.0 |
| *Mya arenaria* (M) | Suspension feeder | Damariscotta Estuary, Maine | 7.22 | 58 | 176 | 22.3 | 1.0 |
| *Crassostrea virginica* (M) | Suspension feeder | Damariscotta Estuary, Maine | 5.71 | 168 | 171 | 21.7 | 1.2 |
| *Siphonosoma ingens* (S) | Deposit feeder | Bodega Bay, California | 8.53 | 65 | 215 | 27.3 | 1.3 |
| *Echinarachnius parma* (E) | Deposit feeder | Damariscotta Estuary, Maine | 8.25 | 2 | 257 | 33 | 1.9 |
| *Chiridota laevis* (E) | Deposit feeder | Pemaquid Point, Maine | — | 40 | 262 | 33 | 1.5 |
| *Ensis directus* (M) | Suspension feeder | Damariscotta Estuary, Maine | 8.13 | 16 | 346 | 44 | 1.5 |
| *Chirdota* spp. (E) | Deposit feeder | Seldovia, Alaska | 6.61 | 14 | 379 | 48 | 1.8 |
| *Strongylocentrotus purpuratus* (E) | Herbivore | Princeton, California | 6.36 | 32 | 485 | 62 | 1.1 |
| *Eupentacta quinquesemita* (E) | Deposit feeder | Seldovia, Alaska | 6.52 | 13 | 500 | 64 | 1.4 |
| *Modiolus modiolus* (M) | Suspension feeder | Damariscotta Estuary, Maine | 6.61 | 44 | 544 | 69 | 1.1 |
| *Protoglossus graveolens* (H) | Deposit feeder | Damariscotta Estuary, Maine | 8.02 | 44 | 671 | 85 | 1.9 |
| *Saccoglossus bromophenolosus* (H) | Deposit feeder | Damariscotta Estuary, Maine | 7.38 | 149 | 686 | 87 | 1.1 |
| *Modiolus demissus* (M) | Suspension feeder | Narragansett Bay, Rhode Island | 5.61 | 216 | 868 | 110 | 1.1 |
| *Arenicola marina* (P) | Deposit feeder | Various sites, Maine | 7.05 | 342 | 2,355 | 299 | 1.2 |
| *Abarenicola pacifica* (P) | Deposit feeder | Puget Sound, Washington | 7.43 | 242 | 8,744 | 1,111 | 1.8 |
| *Travisia foetida* (P) | Deposit feeder | Puget Sound, Washington | 7.40 | 102 | 9,558 | 1,215 | 1.3 |
| *Nereis virens* (P) | Omnivore | Sheepscot Estuary, Maine | 8.28 | 215 | 10,844 | 1,378 | 1.3 |
| *Abarenicola vagabunda* (P) | Deposit feeder | Puget Sound, Washington | 7.65 | 351 | 13,308 | 1,691 | 1.9 |
| *Arenicola brasiliensis* (P) | Deposit feeder | San Francisco, California | 7.71 | 202 | 15,253 | 1,939 | 1.5 |
| *Amphitrite johnstoni* (P) | Deposit feeder | Sheepscot Estuary, Maine | 6.56 | 394 | 35,287 | 4,485 | 2.1 |
| *Nicolea venustula* (P) | Deposit feeder | Damariscotta Estuary, Maine | 5.64 | 202 | 55,958 | 7,112 | 6.1 |
| *Myxicola infundibulum* (P) | Suspension feeder | Damariscotta Estuary, Maine | 5.76 | 381 | 136,766 | 17,382 | 2.6 |
| *Urechis caupo* (U) | Suspension feeder | Various sites, California | 6.94 | 424 | 137,866 | 17,522 | 5.4 |
| *Clymenella torquata* (P) | Deposit feeder | Damariscotta Estuary, Maine | 6.34 | 169 | 148,483 | 18,871 | 22 |
| *Nereis diversicolor* (P) | Deposit feeder | Boothbay Harbor, Maine | 7.51 | 116 | 163,566 | 20,788 | 11 |
| *Eupolymnia heterobranchiata* (P) | Deposit feeder | Little Tutka Bay, Alaska | 7.33 | 436 | 191,105 | 24,288 | 8 |
| *Cirratulus cirratus* (P) | Deposit feeder | Damariscotta Estuary, Maine | 6.01 | 166 | 282,158 | 35,860 | 66 |
| *Spio setosa* (P) | Deposit feeder | Damariscotta Estuary, Maine | 6.32 | 430 | 346,474 | 44,034 | 5.6 |
| *Echiurus echiurus* (U) | Deposit feeder | Seldovia, Alaska | 8.42 | 156 | 388,110 | 49,326 | 21 |

[a] E = echinoderm, M = mollusk, S = sipunculan, H = hemichordate, P = polychaete, U = echiuran.

either immediately after the sampling or on samples stored frozen for approximately eight months at −80°C. No significant difference in pH values was found between fresh and frozen samples ($n = 9$).

Enzyme activities at various Cu concentrations were measured according to Mayer et al. [14] except a 0.01-M MOPS (3-[*N*-morpholino]propanesulfonic acid) or MES (2-[*N*-morpholino]ethanesulfonic acid) buffer system (in 0.01 M $NaNO_3$) was used in this study because of negligible Cu complexation by MOPS and MES under these conditions. For the cross-phyletic comparison, we used a constant pH = 7. We also tested the pH dependence of this reaction for digestive fluid from *A. marina*. Buffers for various pH were obtained by adding different amounts of 0.1-M potassium hydroxide solution into either MOPS ($pK_a = 6.9$, for pH 6.5–7.9) or MES ($pK_a = 6.1$, for pH 5.5–6.5). Both MOPS and MES are structurally similar, and no significant difference was observed in enzyme activities assayed in these two buffer systems at pH 6.5, indicating that the variation of enzyme activities measured in the varying buffers was due solely to pH. These buffers were tested and found to maintain pH during titration with acidic copper solutions.

To measure activities of various enzymes, gut fluids were diluted 1,000× with appropriate pH buffers (three replicates at each Cu concentration); then 25 μl of 100 mM fluorescent substrate solutions were added [14]. Protease activities were measured by adding alanine-methylcoumarinyl-amide (MCA). Esterase, lipase, and α- and β-glucosidase activities were measured by adding the various monomers (butyrate, palmitate, glucose, and glucose, respectively) attached through the appropriate bond (ester, ester, and α- and β-glucoside, respectively) to methyl-umbelliferone (MUF). Production of the MCA and MUF fluorophores was monitored fluorometrically at excitation/emission wavelengths of 340/445 nm with a Fluostar microplate reader (BMG, Durham NC, USA). Enzyme activities are expressed as nmol substrate hydrolyzed per minute per milliliter of gut fluid.

Copper titration experiments were performed in black 96-well microplates by adding incremental amounts of a $Cu^{2+}$ solution ($10^{-8}$–$10^{-2}$ M, as cupric nitrate) to a series of wells containing 230 μl of gut fluids in buffer. Copper was titrated up to a Cu:amino acid ratio of about 1:1, which, according to preliminary experiments, is sufficient to detect inhibition effects on enzymes. The $Cu^{2+}$ solutions were freshly made each day by diluting a 0.1-M $Cu(NO_3)_2$ standard solution (Orion, Beverly, MA, USA) with deionized water. The volume of all wells was then adjusted to 250 μl with deionized water before measuring enzyme activities with the microplate reader, so that liquid level of the wells had no effect on the fluorescence reading. To be consistent with previous studies, the Cu threshold is defined as the concentration of total dissolved Cu in gut fluid above which enzyme activity sharply drops below its

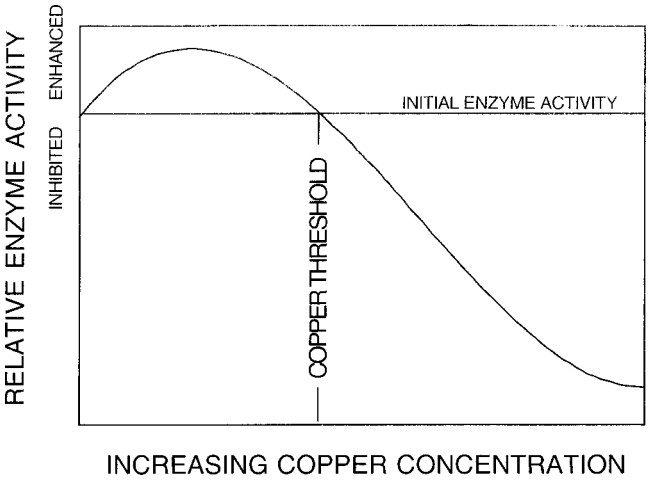


Fig. 1. Schematic of relative enzyme activity (compared to initial gut fluid without added Cu) during titration by ionic copper solution. The copper threshold concentration is the point at which enzyme activity falls below its initial value.

initial (pretitration) level. While Cu is not introduced to animal guts in vivo as an ionic cupric nitrate solution, the similar inhibition of enzyme activity by copper solubilized from contaminated sediments that we previously reported [10] gives confidence that cupric nitrate titrations adequately represent in vivo processes.

## RESULTS AND DISCUSSION

### *Copper concentration–response curve*

The response of protease activity to added Cu was found to be qualitatively similar to that reported for *A. marina* [10]. Adding Cu initially produced a rise in protease activity, followed a sharp decrease in activity on reaching a threshold concentration (schematically shown in Fig. 1). Our data using digestive fluids from *A. marina* in this study agreed with the previous report in that the initial rise in enzyme activity was relatively small: approximately 20%. However, for many polychaetes and echiurans, we found much more dramatic increases in protease activity, ranging up to 66-fold above the starting activity (Table 1). Only two species, *Brisaster latifrons* and *Mya arenaria*, showed no such increase in activity during the titration. This nearly ubiquitous pattern corroborates the previous single-species observation and suggests a potentially important role of solubilized gut metals in affecting enzyme activity in the positive as well as the negative direction. Such metal enhancement of digestive enzymes has been observed for pigs [17]. The mechanism of this Cu-enhanced activity is beyond the scope of this study, but it may involve conformational optimization of proteases or activation of ''apoproteases'' (dormant form of proteases) after Cu binding.

### *Effects of pH on Cu–response curve*

We tested the effect of gut pH on Cu inhibition of digestive proteases in *A. marina* by varying buffer pH in the assay (Fig. 2). The threshold Cu concentration peaked at pH 6.0 and then decreased markedly with increasing pH. This general decrease in threshold accompanies a rise in protease activity even in the absence of Cu due to the normal pH dependence of this enzyme. This decrease in threshold with increasing pH suggests that $H^+$ can compete with $Cu^{2+}$ for binding sites on the proteases or that protease conformation at lower pH was less

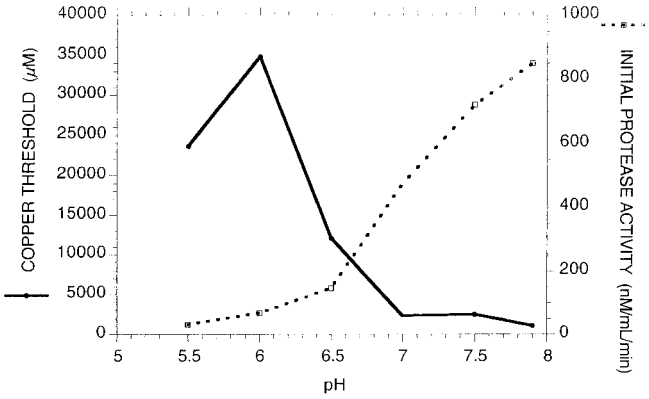


Fig. 2. The copper threshold concentration (μmol Cu/L gut fluid; solid line) in *Arenicola marina* gut fluid generally decreases with increasing pH, while the initial proteolytic activity (nmol MCA/ml gut fluid/min; dashed line) increases.

sensitive to Cu. Nevertheless, this pH-dependent behavior implies that animals with lower gut pH may be less vulnerable to Cu with respect to this enzyme inhibition mechanism.

Guts of many deposit feeders have relatively neutral pH, probably because of the difficulty of acidifying a large throughput of sediments [18]. However, pH values as low as 5.5 have been reported in some small polychaetes (M. Ahrens, personal communication), and a pH range of 5.6 to 8.5 was found for the species in this study. If the pH dependence found for *A. marina* is characteristic of that to be found for other species, then the threshold Cu concentrations for in vivo enzyme inhibition in other species, based on a determination at pH = 7, could be incorrect by as much as an order of magnitude.

### *Response of various enzyme types to Cu exposure*

Enzyme activities found in this study were similar to those reported in our previous cross-phyletic study [14]. All the enzyme types studied—protease, esterase, lipase, and α- and β-glucosidase—in digestive fluid from *A. marina* were inhibited by concentrations of Cu within a factor of two of one another (Fig. 3). Two important conclusions derive from this result. First, a variety of digestive processes besides the hydrolysis of proteinaceous food may be inhibited by Cu. Second, the relatively small range of threshold Cu concentrations suggests the same mechanism of inhibition, i.e. Cu inhibition of enzymes, occurs only after saturation of the Cu-binding capacity of strong binding sites not involved in the catalytic function of the enzymes.

Esterase seemed to differ from the other enzyme types in that it maintained significant activity after considerably exceeding the Cu threshold concentration. Its activity appeared to stabilize at approximately 50% of original activity over a wide range of Cu concentrations. Either esterase enzymes are less affected by Cu complexation than the other enzymes or multiple types of esterolytic enzymes exist that vary in their sensitivity, including at least one that is remarkably insensitive.

We previously found that contaminated sediments from the field could induce inhibition of digestive proteases of *A. marina* [10]. By extension, it follows that sufficient Cu levels can be solubilized from some contaminated sediments to inhibit different digestive enzymes from a wide variety of species.

### *Threshold Cu concentrations among species*

Threshold concentrations varied by more than four orders of magnitude among the species studied (Table 1) from a low

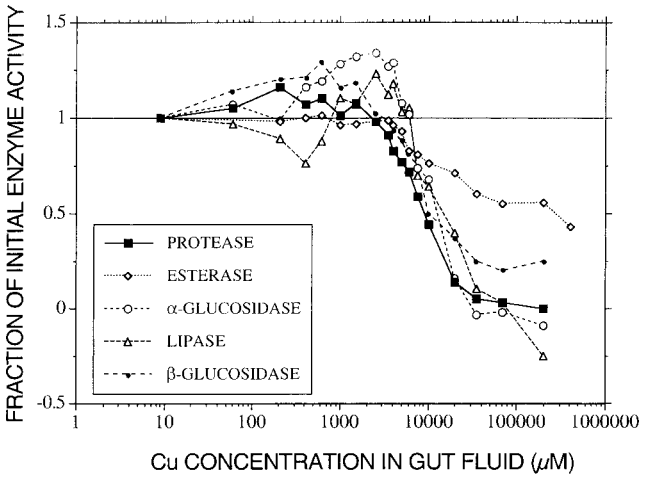


Fig. 3. Relative enzyme activity in *Arenicola marina* gut fluid (compared to gut fluid without any Cu added) versus concentration of added Cu (μmol Cu/L gut fluid) for protease, esterase, α- and β-glucosidase, and lipase.

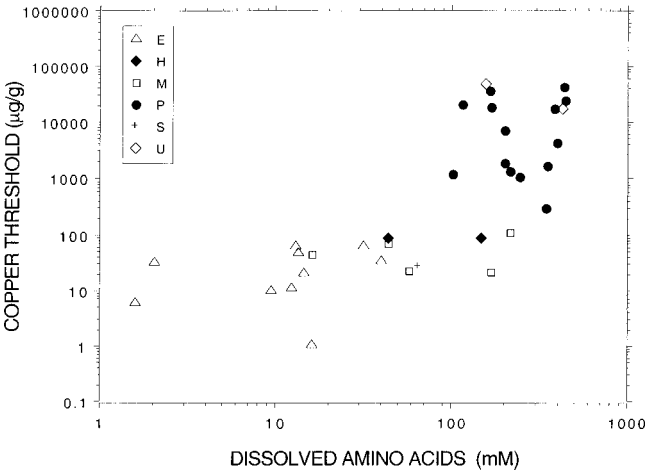


Fig. 4. Copper threshold concentration (μg/g sediment) versus dissolved amino acid concentration (mM) in various species, grouped according to major taxonomic group. E = echinoderm, H = hemichordate, M = mollusk, P = polychaete, S = sipunculan, and U = echiuran.

of 8 μM for an herbivorous echinoderm to a high of almost 0.4 M for a deposit-feeding echiuran. Digestive proteolytic activities of polychaetes were the among the most resilient to Cu additions, but their copper thresholds still ranged more than two orders of magnitude from 2.4 to 346 mM. Echinoderm proteolytic activities were least tolerant to Cu contamination (range = 8–500 μM), suggesting that this group of organisms could be affected relatively easily. Copper thresholds of other groups (hemichordates, sipuncula, and mollusks) generally ranked between polychaetes and echinoderms. This rank order among species may be disrupted if the factors not taken into account in our experiments (e.g., pH and particle selection) were capable of overcoming the >4 order of magnitude range. However, it seems unlikely that these other factors would change susceptibilities more than one to two orders of magnitude (e.g., Fig. 2), so that the distinction between polychaetes and echinoderms likely is valid. Within phyla, species differences in rank might well be affected by these other parameters.

We previously showed that different *A. marina* individuals had varying threshold concentrations, with greater concentrations of dissolved amino acids in the digestive fluid leading to higher threshold concentrations [10]. We therefore examined the role of amino acids in influencing the cross-phyletic results reported here (Fig. 4) and found a strong positive relationship between threshold Cu concentration and dissolved amino acids (Pearson product moment correlation: $p < 0.01$). Clearly, this relationship explains differences among phyla but not among species within phyla. Polychaetes and echiurans normally have the most intense digestive conditions, as evidenced by high enzyme activities and dissolved amino acid concentrations, while echinoderms typically have much less intense digestive conditions [14,16]. Despite the excellent correlation, clearly much variance remains, so that other factors must influence Cu inhibition of digestive protease activities. The pH of the gut fluids explains some of this variance; for example, among polychaetes alone, a significant inverse relationship was observed between threshold Cu concentration and pH. This inverse relationship follows the prediction of our experiment that varied pH only (Fig. 2). Other factors, such as varying composition of the amino acid pool or the presence of other protecting ligands, may also be important in determining the threshold Cu concentration.

Species with high amino acid concentrations in their gut fluids typically dissolve the greatest amounts of Cu from sediments [9,15,19,20]. The somewhat paradoxical implication is that those species that dissolve the most Cu might have their digestive enzymes least affected by it, depending on the relative amounts of Cu solubilized versus the relative Cu thresholds for inhibition. The solution to this apparent paradox lies in the conclusion that strong Cu-binding ligands accompany high dissolved amino acid concentrations but that Cu complexation to these strong ligands does not influence enzyme activity. Only when these strong ligands are saturated can the added Cu begin to bind to sites that affect enzyme activity. The cross-phyletic trends in complexation and enzyme inhibition are thus consistent with the binding–inhibition sequence observed in the titrations (Fig. 1).

### *Relevance to other contaminant impact data*

In the absence of in vivo experiments, our data cannot be used to assess the importance of digestive enzyme activity inhibition as a mechanism for toxic action in marine sediments. Inhibition of digestive enzyme activity has been observed under metal stress (e.g., for cladocerans [21]), though it is not clear if such stresses are due to direct metal–enzyme interactions as seen here or to more indirect effects on enzyme secretion.

We can assess the consistency of our findings with other types of studies by estimating the sedimentary Cu concentrations necessary to achieve the copper thresholds found in this study. This solid-phase threshold level was calculated by multiplying the dissolved copper threshold values by a typical gut fluid:sediment ratio of 1 ml gut fluid:0.5 g sediment (Table 1), common in deposit feeders and previously used in biomimetic assays [9,20]. These solid-phase concentrations represent the amount of Cu that can be solubilized from the sediment by the gut fluid and hence are equivalent to the Cu spike levels used in this study. Fluid:solid ratios may vary 16-fold among individuals and among species [22]; thus, errors may be introduced in the threshold calculations due to the assumption of constant fluid:solid ratio among species. Other factors, such as gut residence time and particle selection by animals from bulk sediment, also vary among animals and may significantly

affect the amount of Cu that can be solubilized from sediments by various animals. Thus, our calculations likely do not accurately represent the amounts of Cu necessary to inhibit digestive proteases in animals in the field. Nevertheless, these calculations have value in showing the potential for differential impacts among species.

The resultant concentrations (Table 1) range from values similar to those in uncontaminated sediments to those higher than typically encountered even in highly polluted environments [23]. For example, digestive protease activities of relatively sensitive species in this study show inhibition at threshold Cu concentrations between 10 (*Parastichopus*) and 87 (*Saccoglossus*) μg/g, which correspond to the 10th and 50th percentiles of the 34 species (Table 1). This 10- to 87-μg/g range is equivalent to 50 to 435 ppm of total sedimentary Cu, assuming that 20% of total Cu is bioavailable to gut fluid solubilization [9,20]. These values are of the same order of magnitude as other work showing biological impacts. For example, effects range–low ([ERL] concentration at which only 10% of studies show biotic impacts) and effects range–median ([ERM] concentration at which 50% of studies show biotic impacts) guidelines of 34 and 390 ppm were derived from an extensive database of sedimentary Cu toxicity effects [5]. In addition, major community structure changes have been observed at sedimentary Cu concentrations of approximately 150 to 200 ppm [4]. This coincidence should not be taken too strongly, as our assumption of 20% bioavailability is subject to considerable variation; for example, the fraction of bioavailable metal correlates positively with the amino acid concentration of the gut fluid [20]. Further, many of the ERL/ERM studies include short-term toxicity tests in which starvation due to digestive inhibition is unlikely to have been a cause for mortality.

Echinoderms, which are here shown to be especially sensitive to digestive inhibition, have been long recognized as a pollution-sensitive group, being less frequent than polychaetes in contaminated areas [4,24]. Tolerance to Cu contamination also varies greatly among polychaetes [6]. *Nereis diversicolor* [25] can live in sediments with more than 1,000 ppm Cu, while 110 ppm Cu has been found to be highly toxic to *A. marina* [26]. These trends are consistent with our data on *Arenicola* and *Nereis* species.

Changes in community structure result from many interactions between organisms and their environment. Digestive enzyme inhibition could be one of many stresses to benthic communities in Cu-contaminated areas. Our data indicate that these organisms can be subjected to stress when Cu concentrations are above these thresholds, but it does not imply that these species will not appear in sediments with higher-than-threshold Cu concentrations. Benthic invertebrates have mechanisms to regulate Cu in tissues, thus allowing them to thrive in heavily contaminated areas, and perhaps means exist to regulate Cu in digestive fluids as well.

## CONCLUSIONS

These results lead to the counterintuitive conclusion that deposit feeder taxa with low digestive intensities (low enzyme activity and low amino acid concentrations) and high pH are most vulnerable to sedimentary Cu inhibition by this mechanism, although they solubilize less sedimentary Cu than taxa with high digestive intensities and low gut pH. In general, echinoderms should therefore be more susceptible to digestive inhibition from Cu contamination than polychaetes. This differential response of benthos to Cu inhibition may be useful for explaining some patterns of stress of benthic organisms in Cu-contaminated areas. Varying gut amino acid concentrations among these organisms appear to be the major factor affecting the bioavailable Cu threshold, although other factors must also play a role. This short-term Cu toxicity affected all enzyme types at a similar bioavailable Cu threshold.

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