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Bioaccumulation of copper and zinc by the giant kelp *Macrocystis pyrifera*

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This study examined the bioaccumulation of the heavy metals copper (Cu) and zinc (Zn) by the giant kelp, *Macrocystis pyrifera*, by exposing meristematic kelp tissue to elevated metal concentrations in seawater within laboratory aquaria. Specifically, we carried out two different experiments. The first examined metal uptake under a single, ecologically-relevant elevation of each metal (30 ppb Cu and 100 ppb Zn), and the second examined the relationships between varying levels of the metals (i.e., 15, 39, 60, 120, 240, and 480 ppb Cu, and 50, 100, 200, 300, 500, and 600 ppb Zn). Both experiments were designed to contrast the uptake of the metals in isolation (i.e., when only one metal concentration was elevated) and in combination (i.e., when both metals' concentrations were elevated). Following three days of exposure to the elevated metal concentrations, we collected and analyzed the *M. pyrifera* tissues using inductively coupled plasma atomic emissions spectroscopy. Our results indicated that *M. pyrifera* bioaccumulated Cu in all treatments where Cu concentrations in the seawater were elevated, regardless of whether Zn concentrations were also elevated. Similarly, *M. pyrifera* bioaccumulated Zn in treatments where seawater Zn concentrations were elevated, but this occurred only when we increased Zn alone, and not when we simultaneously increased Cu concentrations. This suggests that elevated Cu concentrations inhibit Zn uptake, but not vice versa. Following this, our second experiment examined the relationships among varying seawater Cu and Zn concentrations and their bioaccumulation by *M. pyrifera*. Here, our results indicated that, as their concentrations in the seawater rise, Cu and Zn uptake by *M. pyrifera* tissue also rises. As with the first experiment, the presence of elevated Zn in the water did not appear to affect Cu uptake at any concentration examined. However, although it was not statistically significant, we observed that the presence of elevated Cu in seawater appeared to trend toward inhibiting Zn uptake, especially at higher levels of the metals. This study suggests that *M. pyrifera* may be useful as a bio-indicator species for monitoring heavy metal pollution in coastal environments.

Key Words: bioaccumulation; copper (Cu); heavy metal; *Macrocystis pyrifera*; zinc (Zn)

INTRODUCTION

Anthropogenic releases of heavy metals into the marine environment have become increasingly frequent in recent decades, causing public concern for the health of nearshore ecosystems. In particular, coastal ecosystems located near large metropolitan areas experience constant exposure to industrial effluents, urban and residential wastes, and recreational pollution, all of which add heavy metals to the ocean (Manahan 1991, Deheyn and Latz 2006, Kimbrough et al. 2008). In addition, excessive use of fertilizers and both organic and inorganic chemicals in urban environments also introduce heavy metals into the ocean, especially after rain events, when storm

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water runoff carries them to the sea (e.g., Kimbrough et al. 2008). Along the coastal environments of southern California, USA, heavy metal contamination primarily results from non-point sources, and it thereby becomes untraceable regarding its point of origin (Flegal and Sanudo-Wilhelmy 1993, Lenihan et al. 2003). For instance, San Diego Bay, CA is major source of Cu and Zn contamination, due to its high number of recreational and commercial boating marinas, shipyards, and US Naval facilities spreading throughout the bay (San Diego Port District 2009). The use of copper in anti-fouling paints on the hulls of boats (Blossom 2002), and zinc sacrificial anode in the marina's iron and steel structures (Bird et al. 1996) increase these metals' concentrations within the bay waters. As a consequence, tidal exchange can then transport this contaminated water out of the bay and into the adjacent Point Loma kelp forest, where the water's residence time can range from a few of days to a week (Jackson and Winant 1983), during which the metals can become available for uptake by the kelp forest organisms. Specifically, bay water can introduce Cu concentrations of 27 to 78 nmol L^{-1} km^{-1} and Zn concentrations of 120 to 200 nmol L^{-1} km^{-1} into the Point Loma kelp forest (Volpe and Esser 2002). In addition, the water entering the coastal environment from the adjacent, large metropolitan areas due to storm water runoff can contain heavy metal concentrations exceeding 30 ppb Cu and 100 ppb Zn (Schiff et al. 2001). Together, these can subject the Point Loma kelp forest to both chronic and acute heavy metals exposures that can last up to a week, affecting the ecosystem's health.

The term "heavy metal," as used in ecotoxicological studies, encompasses elements that industry commonly uses, that impact aerobic and anaerobic processes, and that researchers generally consider toxic when concentrated (Smith and Scott 1981, Duffus 2002). These metals can generally be categorized depending on how they affect organisms; "essential" metals are those needed, in trace amounts, for many physiological processes, and "non-essential" metals are those that are potentially toxic even at relatively low concentrations (Krzesłowska 2011). This study focused on copper (Cu) and zinc (Zn) and their uptake by the giant kelp, *Macrocystis pyrifera*. Cu and Zn occur in the world's oceans, usually in the form Cu (II) and Zn, free hydrated ions (Kimbrough et al. 2008). Cu is a toxin, and historically humans have used it as copper sulfate ($CuSO_4$) to prevent marine algae and invertebrates from settling and growing on structures (Rai et al. 1981). Cu and Zn are essential metals, as normal metabolic function requires them. For example, marine algae require Cu for the formation of plastocyanin, a protein functioning as the electron transfer agent between photosystem I and photosystem II (Graham and Wilcox 2000). Marine organisms need Zn for producing the enzyme alcohol dehydrogenase, which they use to break down alcohols and also use in many metalloenzymes helping to detoxify metal ions (Manahan 1991). Such organisms also use Cu and Zn in the Cu / Zn superoxide dismutase enzyme that allows superoxide to break down into oxygen and hydrogen peroxide, an important process for cellular antioxidant defense (Graham and Wilcox 2000). Consequently, marine organisms naturally accumulate metals from their surrounding environment through a variety of processes, of which bioaccumulation and biomagnification are the two most common (Rainbow and White 1989, Rainbow 1995, Gaudry et al. 2007). Bioaccumulation is an active process whereby metabolic activity removes metals from the surrounding environment (Davis et al. 2003), and it occurs when the organism's uptake rate from its environment exceeds the elimination rate from its body tissues (Naimo 1995). In the biomagnification process, organisms take up metals though their body surface or intestines (Ratte 1999), which generally results in a higher metals concentration within the organism than in its environment (Connell 1989, 1990, Rand et al. 1995, Gray 2002). This often occurs through predation and / or grazing transferring contaminants from lower to higher trophic levels, and, similarly, it can cause predators to have metal concentrations in their tissues that are higher than those in the prey's tissues (Reinfelder et al. 1998, Liu et al. 2002). Therefore, understanding how primary producers accumulate metals from the water, potentially making them available to other organisms through trophic interactions, is important.

Researchers have studied heavy metal bioaccumulation in plants and algae from terrestrial (e.g., Fu et al. 2008), tropic (e.g., Amado Filho et al. 1997), and temperate (e.g., Conti and Cecchetti 2003, Gaudry et al. 2007) marine environments. In terrestrial environments, bioaccumulation from contaminated soils may cause plant tissue heavy metal concentrations to exceed soil concentrations, making such plants toxic to consume (Food and Nutrition Board 2001). Likewise, in the marine environment, bioaccumulation from metal contaminated sites can cause marine algal tissue concentrations to exceed such concentrations from non-contaminated sites (e.g., Al-Homaidan 2007, Gaudry et al. 2007). This generally occurs in a two-step process: an initial, rapid, passive uptake, followed by a slower active uptake (Bates et al. 1982). During passive uptake, the cell surface absorbs metal ions within a few seconds to minutes. Dur-

ing active uptake, the cell membrane transports metal ions across and into the cytoplasm, via a metabolism-dependent route whereby heavy metals bind to intercellular compounds and exhibit intracellular precipitation (Kaduková and Virčíková 2004). In some cases, this can occur through passive diffusion, since metals increase the cell membrane's permeability (Gadd 1988, Mehta and Gaur 2005). These metals can then bind either to the cell membrane, one of its constituents, or intercellular molecules, such as metallothioneins, cytoplasmic ligands, and phytochelatins. For example, brown algae cell walls primarily comprise cellulose, for structural support, alginic acid (10-40% DW), in the intercellular matrix, and fucoidins (i.e., fucans, 5-20% DW sulfated polysaccharides) in the extracellular mucilage (Graham and Wilcox 2000, Mehta and Gaur 2005). Of these components, alginic acid and fucans have an affinity for binding metals, as they are abundant in carboxyl (-COOH) groups (Bryan 1971, Krzesłowska 2011). In addition, other functional groups within the cell wall, such as hydroxyl (-OH), amino ($-NH_2$) and sulfhydryl (-SH) increase the metals' abilities to bind, since they produce negative charges. In water, such metals usually appear in their cationic forms, which allows them to either absorb into, or bind to, the cell wall. Consequently, as metal concentrations increase in the organism's tissues, they have a greater toxicity potential (Rainbow 2002). For example, the bioaccumulation of metals in macroalgae can prevent the normal compound transport though the cell wall (Manahan 1991), inhibit growth (Amado Filho et al. 1997), prevent settlement (Bryan 1971), and, ultimately, result in mortality (Anderson et al. 1990, Huovinen et al. 2010).

In addition to their impacts on organism growth and survival, heavy metals can adversely affect human health as they build up in the marine organisms' tissues, through human consumption and utilization of contaminated species. Consequently, numerous US government agencies, such as National Oceanic and Atmospheric Administration (NOAA), US Geological Survey (USGS), and the Evironmental Protection Agency (EPA), have established programs to monitor trends in contaminates, such as heavy metals, within the coastal environment. Many of these monitoring programs rely on bioindicator species that sequester metals from the water column into their tissues, thus reflecting the degree of pollution over time (Martin and Coughtrey 1982). While many of these programs can identify longer-term (months to years) variations in metal concentrations, these programs often sample too infrequently to address short-term (days to weeks) variations. If exposures to elevated metals over these short time periods result in elevated heavy metal tissue concentrations in marine organisms, this can profoundly affect commercial and recreational fisheries. This may be especially important to the kelp forest ecosystems in southern California, where runoff from storms can result in Cu and Zn concentrations of 30-60 ppb Cu and 100-150 ppb Zn to enter the forests (Schiff et al. 2001), and where the residence times suggest such elevated concentrations can persist for several days to a week (Jackson and Winant 1983). Thus, understanding how marine algae take up heavy metals from the water column when exposed to these concentrations over a period of a few days may allow researchers to better understand their full impact on coastal ecosystems.

MATERIALS AND METHODS

Collection site and study species

We collected meristematic tissues of the giant kelp, *M. pyrifera*, from the center of the Point Loma kelp forest (23°69´ N, 117°26´ W), which lies adjacent to San Diego Bay, CA. San Diego Bay provides a variety of services, including naval, commercial, and recreational harbor activities, entertainment venues, and industrial shipyards, all of which introduce heavy metals into the water. As a result, San Diego Bay is ranked as one of the most polluted coastal areas in the United States. Along the larger San Diego coast, seawater Cu concentrations naturally range from 0.41 to 3.25 ppb, and seawater Zn concentrations range from 0.95 to 7.23 ppb. However, these concentrations can rise following rain events, when storm water runoff can produce local seawater concentrations reaching 30-60 ppb Cu and 100-150 ppb Zn for several days (Schiff et al. 2001). Studies on biosorption, the removal of heavy metals from an aqueous solution through their passive binding to a non-living biomass, have long used kelps (order, Laminariales) (Davis et al. 2003), but kelps have recently become more popular for use in toxicity assays (Anderson et al. 1990, Huovinen et al. 2010). Along the west coast of North America, *M. pyrifera* ranges from Alaska, USA to Baja California, Sur MEX and is the dominant forest-forming marine alga along much of the southern California coast. Its fronds can reach $\geq$ 40 meters in length and form dense surface canopies housing and protecting many important commercial and recreational species, and humans commercially harvest *M. pyrifera* for food and industrial uses (reviewed in Foster and Schiel 1985).

Experimental approach

Prior to running our experiments, we cleaned all glassware, tanks, and dissection materials using the following protocols: we first washed 10 L tanks with 7× cleaning solution for laboratory use, rinsed them with fresh water, and air dried them. Then we allowed the tanks to sit, filled with 2% HCl, for three days, rinsed them with double distilled water, and then air dried and covered them with plastic wrap, to prevent subsequent contamination. In addition, we soaked all glassware and dissection materials in 2% 7× clean solution for two days, rinsed them with fresh water, and placed them in a 1-2% HCl acid bath for two days. The materials were then rinsed with double distilled water, air dried, and wrapped in plastic wrap.

To prepare the *M. pyrifera* meristematic tissues for analysis, we used an acid digestion method modified from Warnau et al. (1995). Specifically, we oven dried tissue samples at 88°C for 24-72 h, weighed them, and placed them in glass tubes, adding 5 mL of HNO_3 per 0.5 g^{-1} dry tissue weight. The samples were then successively digested at 20, 40, 60, and 80°C for 24, 24, 12, and 12 h, respectively. Following this, we diluted the digest to 20 mL with double-distilled water and filtered it using What man 40 ashless filter paper (Whatman, Inc., LON, UK) The digests' Cu and Zn concentrations were analyzed using a Perkin-Elmer DV4300 (Norwalk, CT, USA) inductively coupled plasma atomic emissions spectrometer (ICP-AES) with a Perkin-Elmer AS-93 autosampler. The ICP-AES setup used a concentric nebulizer and a cyclonic spray chamber. We analyzed the axial reading on Zn at the 206.2 wavelength and on Cu at the 327.393 wavelength. Gas flow rate settings were as follows: plasma at 15 L min^{-1}, and the nebulizer at 0.8 L min^{-1}. The sample flow rate was 1.5 mL min^{-1}, and the ICP-AES setting was 1,450 W.

Background levels of copper and zinc

To characterize natural variabilities in Cu and Zn background levels in *M. pyrifera* tissues within the Point Loma kelp forest, we collected meristematic tissues from three haphazardly selected *M. pyrifera* on five occasions: May 14 and 28, July 7 and 29, and September 3, 2010. Samples were immediately placed in dark coolers and transferred to the laboratory, where they were placed in a -80°C freezer until they could be analyzed according to the methods described above.

Macrocystis pyrifera bioaccumulation

All laboratory aquaria experiments were performed at San Diego State University's Coastal Waters Laboratory San Diego, CA. We placed an array of 24 ten liter clean plastic aquaria (see above), 12 for assessing Cu uptake and 12, for Zn uptake, in a closed, temperature-controlled cold room and held them at 12°C. To circulate the water and aerate the tanks, we utilized four aquarium air pumps. Full-spectrum, 64-W fluorescent bulbs irradiated the room, producing 19-22 micromoles of photons above each tank.

Once the aquaria were set up, we prepared experimental treatments of elevated Zn and Cu from stock solutions. Specifically, we chose three treatments consisting of elevated Cu (30 ppb), elevated Zn (100 ppb), and both elevated Cu and Zn (30 ppb + 100 ppb, respectively), as they represent the expected levels following contamination events that storm water runoff might produce (Schiff et al. 2001). For Cu, we prepared a 1,000 ppm stock solution of copper sulfate ($CuSO_4$), and for Zn we prepared a 600 ppb solution of zinc sulfate ($ZnSO_4$) by dissolving analytical grade $CuSO_4$ and $ZnSO_4$ each into 1 L of double distilled water. Next, we weighed the salts for their respective elements, dissolved them in 1 mL of HCl, and diluted each result to 1 L with double-distilled water. Samples of the stock solutions were then sent to Enviromatrix Analytical Inc., San Diego, CA to validate their Cu and Zn concentrations prior to use. This revealed that the $CuSO_4$ stock solution contained 1,140 ppm Cu, and the $ZnSO_4$ stock solution contained 594 ppm Zn. Following this, we added 0.21 mL $CuSO_4$ stock solution to seawater to create the 30 ppb Cu treatment, 1.35 mL $ZnSO_4$ stock solution to seawater to create the 100 ppb Zn treatment, and used a combination of these two to make the 30 ppb Cu + 100 ppb Zn treatment. All solutions were added to 8 L of seawater collected from the SCRIPPS pier, La Jolla, CA (hereafter "clean" sea water), using a calibrated Pasteur pipette. In addition, we established a fourth, control treatment of clean seawater with no added metals.

After establishing the experimental aquaria, we examined *M. pyrifera*'s Cu and Zn bioaccumulation by collecting meristematic tissue from 48 haphazardly-selected individuals from the Point Loma kelp forest and transporting them to San Diego State University's Coastal Marine Institute Laboratory. At the lab, we randomly allocated two meristems to each of the four treatments, with three replicates each: 1) control (i.e., "clean" seawater), 2) 100 ppb Zn added to "clean" seawater, 3) 30 ppb Cu added to "clean" seawater, and 4) 100 ppb Zn plus 30

ppb Cu, both added to "clean" seawater. We then left *M. pyrifera* in the tanks for three days to accumulate metals from the water, after which we collected and froze them, in an -80°C freezer, until we could analyze them according to the methods described above. Separate analyses were performed for Cu and Zn uptake.

To determine whether *M. pyrifera*'s Cu and Zn accumulation varies when exposed to different concentrations of the metals in the water, we again collected 48 *M. pyrifera* meristems from the center of the Point Loma kelp forest and transported them to SDSU's CMIL. We allocated two meristems to each of 24 treatments with varying metals concentrations. Specifically, we established six treatments of increasing Cu concentrations, ranging from 15 to 240 ppb, without altering Zn concentrations and six treatments of increasing Zn concentrations, ranging from 50 to 600 ppb, without altering Cu concentrations. Finally, we established six treatments at the same Cu concentrations as the above, but adding 100 ppb Zn to each, and six treatments at the same Zn concentrations as the above, but adding 30 ppb Cu to each (see Table 1 for list of experimental treatment combinations). The *M. pyrifera* meristems were left in these tanks for three days, to accumulate metals from the water, after which they were collected and frozen in an -80°C freezer until they could be analyzed as described above. Separate analyses were performed for Cu and Zn uptake.

Statistical analysis

All data were analyzed using SYSTAT version 12 (Systat Inc., Chicago, IL, USA). To provide a value for each tank (replicate), we averaged the values of the two *M. pyrifera* meristems in each aquarium. Prior to testing, we examined the data for equal variances, using either *F*-tests or Bartlett's tests, and for normality, via graphical inspection of the residuals. We transformed and retested any data not meeting the required tests' assumptions, to correct any problems. This occurred only once, for Zn accumulation data. Consequently, we applied a square root transformation to these data, which corrected the problem.

Following the meristems' three-day exposures to elevated metal(s) concentrations and the assumptions testing, we compared *M. pyrifera* Cu and Zn tissue concentrations among the accumulative exposure treatments, using separate one-way analyses of variance (ANOVAs) for each metal. When we found significant variation among exposure treatments for one of the metals, we used Tukey's post hoc tests to assess which treatments differed from the others. Furthermore, we assessed Cu and Zn uptake under exposure to different water concentrations of each metal in the presence and / or absence of the other metal using separate analyses of covariance (ANCOVAs), with water concentration as the covariate and the presence / absence of the other metal as the categorical factor. For both metals, the interactions between the categorical factor and covariate were not significant (i.e., the slopes were homogeneous, see Results), and we therefore removed the interaction term from the linear models and retested the data.

RESULTS

Background levels of copper and zinc

The concentrations of Cu and Zn in naturally-occurring *M. pyrifera* meristematic tissues varied between May and November 2010. Specifically, Cu concentrations ranged from 0.6 to 4.4 ppb (mean = 2.6 ppb), while Zn

Table 1. Experimental metal concentrations used for the accumulation of Cu and Zn by *Macrocystis pyrifera*

Cu (ppb)	Zn (ppb)	30 ppb Cu + increasing Zn	100 ppb Zn + increasing Cu
15	50	+ 50 ppb Zn	+ 15 ppb Cu
30	100	+ 100 ppb Zn	+ 30 ppb Cu
60	200	+ 200 ppb Zn	+ 60 ppb Cu
120	300	+ 300 ppb Zn	+ 120 ppb Cu
240	500	+ 500 ppb Zn	+ 240 ppb Cu
480	600	+ 600 ppb Zn	+ 480 ppb Cu

The appropriate volume of stock solution of each metal was added to each tank containing 8 L of "clean" seawater to produce the desired final concentration.

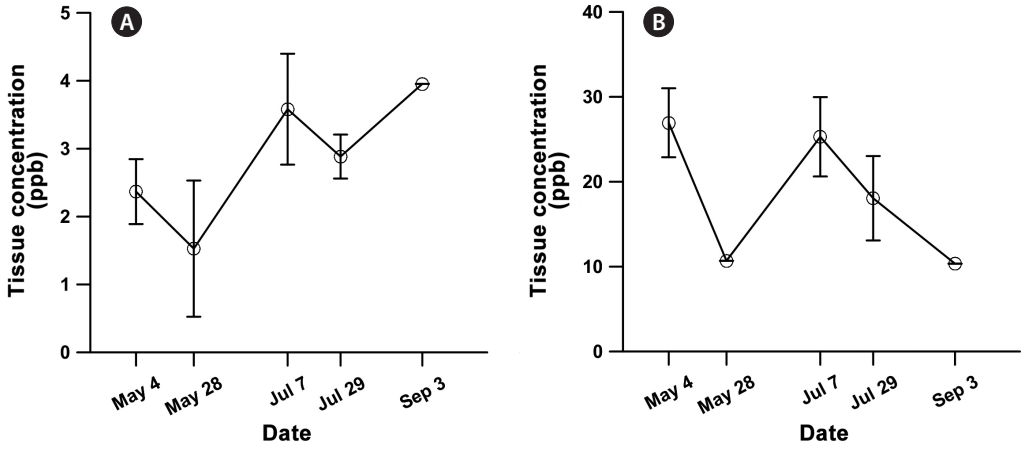

Fig. 1. Natural variations (mean ± standard error) in Cu (A) and Zn (B) concentrations measured within *Macrocystis pyrifera* meristematic tissues collected from the Point Loma kelp forest during 2010 (n = 3).

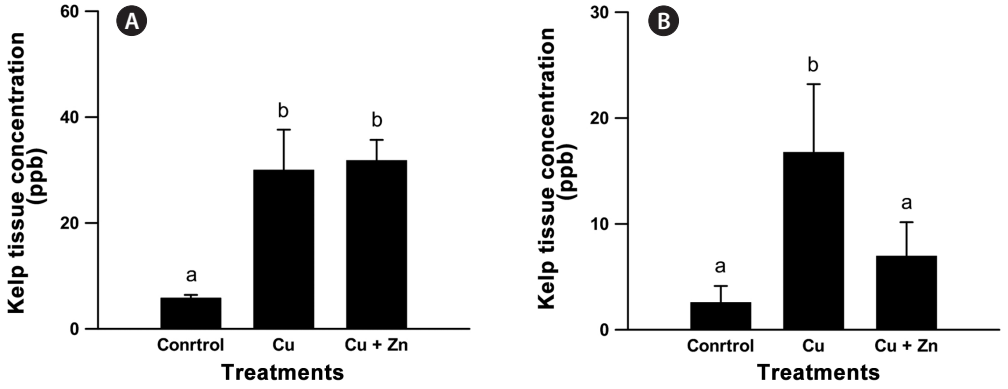

Fig. 2. *Macrocystis pyrifera* meristematic average tissue concentrations (± standard error) for Cu (A) and Zn (B), as measured in the experimental aquaria following three days' exposure to elevated concentrations in the seawater. The control tank held "clean" seawater, the Cu tank had 30 ppb $CuSO_4$ added to clean seawater, the Zn tank had 100 ppb $ZnSO_4$ added to clean seawater, and the Cu and Zn tank had 30 ppb $CuSO_4$ and 100 ppb $ZnSO_4$ added to clean seawater. Different letters represent statistically different treatments, as determined by Tukey's post hoc test following ANOVA (n = 3).

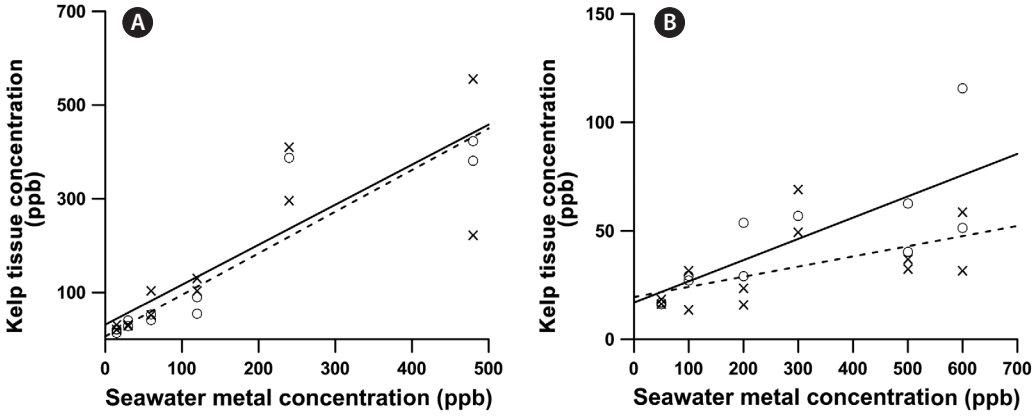

Fig. 3. Relationships among concentrations of Cu (A) and Zn (B) in seawater and in meristematic tissues of *Macrocystis pyrifra* following three days' exposure in the aquaria. For each graph, the two lines represent the relationships where we added only the metal of interest to the seawater (× symbol, solid lines) and where we also added the second metal (○ symbol, dashed lines).

concentrations ranged from 0 to 29.8 ppb (mean = 17.7 ppb, Fig. 1). Interestingly, the values of these two metals appeared to co-vary with each other, possibly reflecting contamination events in the water.

Macrocystis pyrifera bioaccumulation of Cu and Zn

After three days of exposure to elevated metal concentrations in seawater, metal concentrations in the *M. pyrifera* meristematic tissues varied significantly among the different treatments (ANOVAs, Cu $F_{2,15} = 20.440$, $p < 0.001$; Zn $F_{2,15} = 7.485$, $p = 0.006$). For Cu, the concentrations in the meristematic tissues in the control (i.e., "clean" seawater) aquaria were similar to, if not slightly lower than, those we observed in naturally occurring *M. pyrifera* from the Point Loma kelp forest (Figs 1A vs. 2A). This may reflect differences in water conditions between the Point Loma kelp forest and the SCRIPPS pier. However, with 30 ppb Cu added to the water, either alone or in combination with 100 ppb Zn, *M. pyrifera* bioaccumulated it into its tissues, producing significantly greater tissue concentrations than those in the control aquaria (Fig. 2A). We observed no differences between aquaria with only added Cu and those with added Cu and Zn, indicating Zn did not impact Cu uptake. Likewise, Zn concentrations in the control aquaria's *M. pyrifera* tissues resembled those in naturally occurring *M. pyrifera* tissues in the Point Loma kelp forest (Figs 1B vs. 2B). With 100 ppb Zn added to the water, *M. pyrifera* bioaccumulated Zn into its tissues, resulting in significantly greater tissue concentrations than those in the control aquaria (Fig. 2B). However, with 30 ppb Cu added to the water, *M. pyrifera* did not accumulate Zn into its tissues, yielding tissue concentrations significantly lower than tissue concentrations with added Zn alone, but not differing from those of the control aquaria (Fig. 2B). This indicates that Cu does inhibit *M. pyrifera*'s Zn uptake.

Cu and Zn uptake by *M. pyrifera* both varied as a function of their respective concentrations in the water. Specifically, Cu uptake increased as water concentrations increased (ANCOVA, water concentration $F_{1,21} = 36.33$, $p < 0.001$), with tissue concentrations approaching 500 ppb when water concentrations were 480 ppb (Fig. 3A). This reflected an increase of nearly two orders of magnitude above the values we observed in tissues from the aquaria with the lowest Cu concentrations or in naturally-occurring individuals in the Point Loma kelp forest. The presence of Zn did not affect Cu uptake, as we observed no differences in Cu tissue concentrations between aquaria with 100 ppb added Zn versus those with only Cu added, regardless of the Cu concentration (ANCOVA, Zn addition, $F_{1,21} = 0.121$, $p = 0.931$). Furthermore, the presence of Zn did not affect the relationship between Cu uptake and its concentration in the water (ANCOVA, concentration × Zn addition interaction, $F_{1,20} = 0.228$, $p = 0.638$), again indicating that Zn did not affect Cu accumulation (Fig. 3A). Similarly, Zn uptake also increased as water concentrations increased (ANCOVA, water concentration $F_{1,21} = 36.33$, $p < 0.001$), with tissue concentrations approaching 90 ppb when water concentrations were 600 ppb (Fig. 3B). This reflected an increase of an order of magnitude above the values observed in naturally-occurring individuals. Further, although the result did not reach significance (ANCOVA, Cu addition, $F_{1,20} = 0.271$, $p = 0.116$), overall Zn uptake generally trended higher in treatments without added Cu than in those with 30 ppb added Cu. Likewise, although the presence of Cu did not significantly affect the relationship between Zn concentration in the water and *M. pyrifera*'s Zn uptake (concentration × Cu addition interaction, $F_{1,19} = 2.061$, $p = 0.167$), we observed a general trend in which Zn uptake was greater without added Cu, and this appeared more pronounced at higher Zn concentrations (Fig. 3B). As with the previous experiment, this again suggested that Cu negatively affected Zn accumulation.

DISCUSSION

Anthropogenic actives along the coast of southern California, USA have produced higher heavy metals contamination in its coastal environments (Manahan 1991, Schiff et al. 2001, Deheyn and Latz 2006). Specific to this study, the numerous marinas, shipyards, and commercial activities in San Diego Bay, along with storm water runoff from the surrounding San Diego urban areas, have introduced elevated levels of Cu and Zn into the coastal waters (San Diego Port District 2009). While natural concentrations of these metals in the coastal zone of southern California are typically below 3 ppb Cu and 7 ppb Zn, they can temporarily increase to more than 30 ppb Cu and 100 ppb Zn following rain events, when storm water runoff carries pollutants from the surrounding urban areas into the bay and coastal waters (Schiff et al. 2001). This can cause the kelp forest organisms to accumulate these metals in their tissues, with possible negative impacts on this ecosystem's health. Our study indicates the giant kelp, *M. pyrifera*, can respond to such events by accumulating Cu and Zn from the water column, resulting in elevated tissue concentrations of these metals over the

course of only a few days.

Natural variation in *M. pyrifera* Cu and Zn tissue concentrations from the Point Loma kelp forest indicate that, while these metals varied over the course of this study, their values remained at approximately 1-4 ppb Cu and 5-30 ppb Zn. These levels are below those considered toxic for human consumption (Food and Nutrition Board 2001). Studies have thoroughly documented such variations in marine organisms' metal concentrations (e.g., Phillips 1976, Anderson et al. 1990, Amada Filho et al. 1997, Fytianos et al. 1999, Storelli et al. 2001). A number of factors can produce these variations. In particular, the bioavailability of Cu and Zn in seawater can vary, depending on the concentrations of their soluble or particulate forms (e.g., Phillips 1976) as well as seawater pH, salinity, nutrient concentration, and temperature (e.g., Rai et al. 1981, Rainbow 2007). In our experimental treatments, when Cu and Zn concentrations were higher, *M. pyrifera* accumulated both metals from the water, resulting in increased tissue concentrations. In contrast, tissue concentrations in *M. pyrifera* from the "clean" (control) tanks resembled those we observed in naturally occurring *M. pyrifera* in the Point Loma kelp forest. Moreover, as the metals' concentrations increased in the seawater, *M. pyrifera* also increased its uptake of these metals, resulting in an increase of one-to-two orders of magnitude when we exposed *M. pyrifera* to the highest levels tested, relative to the control tanks.

While these highest levels are well above those one would expect to occur naturally in the Point Loma kelp forest, they do suggest that, under extreme contamination events, such as might occur during sewage spills, industrial accidents, rain events, or re-suspension of metals from contaminated sediments, accumulation of these metals can produce *M. pyrifera* tissue levels exceeding 500 ppb Cu and 80 ppb Zn-toxic levels, for human consumption (Food and Nutrition Board 2001). This, however, varies depending on whether both metals' concentrations increased together, or only one metal's concentration increased. This is not surprising, given that researchers often regard macroalgae uptake of Cu and Zn as synergistic (Beckett and Davis 1978, Strömgren 1980, Pellegrini et al. 1993, Luo and Rimmer 1995, Rai et al. 2000). For example, while elevated Zn concentrations do not affect Cu accumulation in the brown algae *Laminaria digitata* and *Cystoseira barbata*, these algae show reduced Zn accumulation in the presence of elevated Cu concentrations (Bryan 1969, Pellegrini et al. 1993). This is likely because Cu is generally more toxic than Zn is to marine algae (Hamer 1986, Luo and Rimmer 1995, Hebel et al. 1997, Brown et al. 2004). Thus, we believe these differences are due to toxicity effects. In our study, the presence of elevated levels of Zn in the water did not affect *M. pyrifera*'s Cu uptake, but the presence of elevated Cu concentrations inhibited Zn uptake.

The different processes by which metals bind within the thalli of algae can allow some marine algae to develop tolerance to increased metal concentrations. Levitt (1980) suggests plant tolerance occurs when plants can neutralize metals inside their cells by removing them from the protoplast and / or neutralizing their toxic effects. Studies have shown brown algae, such as *M. pyrifera*, to be metal-tolerant because of both internal and external metal-complexing ligands. Specifically, phlorotannins can chelate metals and function as a detoxifying mechanism for metal contamination (Huovinen et al. 2010). In addition, alterations of cellular membrane structures and / or their permeability can also lead to metal tolerance (Verkleij and Schat 1989). For instance, Cu tolerance in the brown alga *Ectocarpus siliculosus* resulted in heavy metals acting as agents of directional selection, leading to the establishment of metal-tolerant ecotypes that can, in turn, alter the local flora (Reed and Gadd 1989). Organisms can also exhibit tolerance to several metals concurrently (i.e., multiple tolerances), and / or to one metal through exposure to another metal (i.e., co-tolerance) (Rai et al. 1981). Hall (1980) showed that the Cu-tolerant alga *Enteromorpha siliculosus* exhibited co-tolerance when exposed to Zn and Pb and concluded that tolerance to one of these metals results in tolerance to the other metal. Although we did not examine metal tolerance in this study, its importance seems likely, especially at high water concentrations, when metal uptake can produce tissue concentrations exceeding toxic levels. For example, we did not observe mortality in any of our treatments, though the *M. pyrifera* in the highest Cu concentration turned the tanks' water brown and appeared to begin senescence (Evans personal observation), again suggesting that the Cu may have had toxic effects.

Both macroalgae and marine invertebrates are well-documented as bio-indicator species for marine pollution (Bryan 1971, Phillips 1976, Ratte 1999, Rainbow 2007, Kimbrough et al. 2008). A bio-indicator species is one that can accumulate and integrate concentrations of several metals in seawater over relatively long intervals (Conti and Cecchetti 2003), reflecting pollution levels in their surrounding environment. Macroalgae generally have higher metal concentrations in their tissues than the surrounding seawater does (Mehta and Gaur 2005), but researchers still commonly use them as bio-indica-

tors. Some commonly-used macroalgae are species of green (e.g., *Ulva*), red (e.g., *Porphyra*), and brown (e.g., *Fucus*) algae. In addition, numerous government and private agencies have established monitoring programs to assess metal contamination. Along the California coast, these programs utilize invertebrate species, such as the mussels *Mytilus edulis* and *M. californianus*, that accumulate metals from the water column, using their tissue concentrations as proxies for water concentrations. However, such programs generally sample at frequencies too low to capture short-term variations in metal concentrations, such as might occur during the few days following rain events. We suggest *M. pyrifera* could be useful as a bio-indicator species in southern California, as it is readily available and ecologically important, its life history is well-known, and it exhibits rapid (within days) responses to elevated metal concentrations in the water. In addition, *M. pyrifera* is an important food source for many commercially and recreationally important species, and humans harvest it commercially for both human consumption and commercial use. If these metals transfer to grazers through trophic interactions (i.e., through biomagnification), an understanding of the heavy metal concentrations in it tissues could inform the management of recreational and commercial fisheries, as well as of commercial harvesters. As a result, we suggest that giant kelp, *M. pyrifera*, could be a useful bio-indicator species for monitoring heavy metal contamination in this coastal environment.

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