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Publication Date
2012-10-30
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Effects of tissue nitrogen and media nitrate on trace metal uptake and trophic transfer by *Ulva* spp.

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EFFECTS OF TISSUE NITROGEN AND MEDIA NITRATE ON TRACE METAL UPTAKE AND TROPHIC TRANSFER BY ULVA SPP.

A Thesis

Presented to

The Faculty of Moss Landing Marine Laboratories

San José State University

In Partial Fulfillment

of the Requirements for the Degree

Master of Science

by

Sonya M. Sankaran

August 2012
The Designated Thesis Committee Approves the Thesis Titled

EFFECTS OF TISSUE NITROGEN AND MEDIA NITRATE ON TRACE METAL UPTAKE AND TROPHIC TRANSFER BY *ULVA* SPP.

by

Sonya M. Sankaran

APPROVED FOR

MOSS LANDING MARINE LABORATORIES

SAN JOSÉ STATE UNIVERSITY

August 2012

Dr. Michael Graham  Moss Landing Marine Laboratories

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ABSTRACT

EFFECTS OF TISSUE NITROGEN AND MEDIA NITRATE ON TRACE METAL UPTAKE AND TROPHIC TRANSFER BY ULVA SPP.

by Sonya M. Sankaran

A general survey of trace metal content in Ulva spp. (Linnaeus) around Moss Landing, California was carried out. The next objective was to evaluate whether tissue nitrogen or media nitrate affects metal uptake (As, Pb, Mn, Zn) by Ulva spp. under eutrophic conditions. Additionally, the role of metal burden in the invertebrate Idotea resecata as a function of metal content in its diet was examined. Mean trace metal concentrations in Ulva differed significantly among sites in Moss Landing, California. Laboratory measurements using samples from 15 sites along the central California coast revealed a significant positive correlation between Ulva spp. tissue nitrogen and both arsenic and manganese uptake. No relationship was found between tissue nitrogen and lead or zinc, but a significant positive correlation with the relative change in manganese and lead was observed. Though statistically insignificant, a regression analysis revealed a logarithmic relationship between media nitrate and both tissue arsenic and manganese. Lead and zinc content in Ulva had no relationship with media nitrate. Metal in Idotea resecata was not significantly related to diet treatments; however, a bioaccumulation trend was observed for arsenic and manganese. Given the role of trace metals in the production of photosynthetic enzymes and proteins, variability in productivity may drive the uptake of essential and non-essential elements. Depending on the amount of Ulva consumed, elevated metal content in these macroalgae could pose a health risk to invertebrates and/or humans.
ACKNOWLEDGEMENTS

This research was made possible by funds from the San Jose State University Graduate Equity Fellowship, the Earl and Ethyl Meyers Oceanographic and Marine Biology Trust, the David and Lucille Packard Foundation, the COAST Travel Fund, and NOAA California Seagrant Program (#R/A – 125) awarded to Michael Graham. I would like to express sincere thanks to my advisor, Michael Graham, for accepting me into the MLML Phycology Laboratory and supporting my interests in land-sea interactions. His expertise and confidence in my work allowed me to explore an area of science that I had no experience in, but much curiosity about. I always walked out of Dr. Graham’s office with a sigh of relief; he is generous with his time, enthusiasm, and ability to clearly express the complexities of experimental design. Thanks to committee member Mark Clementz for aiding in the analysis of laboratory samples and for your invaluable input on my manuscript. I would also like to thank my committee member Kenneth Coale for his time and patience as I found my way through this tiny study in the cosmos of biogeochemistry. When I was in doubt, his passion, eloquence and kind words rejuvenated my curiosity and helped me maintain the momentum I needed to complete this work.

This thesis would not have been possible without the unconditional support from our extremely capable faculty and staff at Moss Landing Marine Laboratories. Thank you to the Trace Metals Lab (Mike Gordon, Sara Tanner, Craig Hunter, Sam Hulme and Kenneth Coale) for your friendship and extensive conversations on trace clean protocols. A very big thank you to the trace metals team at Norte (Mark Stephenson, Jon Goetzl,
Stephen Martenuk and Autumn Bonnema) for their time and support in sample analyses and data interpretation. Much appreciation goes out to the small boats program, with special thanks to John “JD” Douglas for the boating support that was required for this work. I am greatly indebted to William Watson, James and Billy Cochran and Ralph Dzuro who were always eager to offer their expertise and manpower in whatever work I needed done. Their friendship was equally unconditional and invaluable. To the numerous other students, staff, and faculty that helped along the way, thank you.

Thank you to the BEERPIGs (Benthic Ecology and Experimental Research, Phycology in General) group. This group encompasses phycology and ecology students and faculty and has supported me in developing and carrying out my thesis project, as well as in the preparation of posters and talks. They have committed hours upon hours to supporting me intellectually and personally for which I am extremely grateful.

Finally, I would like to thank my family for supporting me along the path that brought me to Moss Landing as well as through the last few years of my masters. I have been so fortunate to have a family that supports my interests and passions – this made it possible for me to even imagine completing this program. My sisters, Nandita and Shilpa have always shown interest in and admiration for my work, and have been the strongest pillars of emotional and intellectual support throughout my life. To my husband, Lucas Lippert, for his unwavering devotion to my pursuing my dreams, and for setting an inspiring example. My mother and father, Lalita and Narayan Sankaran, have always had the greatest confidence in my ability to achieve any goal I set my sights on, and now I understand how great a gift that is. Thank you.
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INTRODUCTION

Photoautotrophs perform numerous services for both marine and terrestrial systems, from oxygen production and carbon dioxide sequestration to inorganic nutrient cycling (Lobban and Harrison 1994; Raven 1997; Raven and Yin 1998). Their unique ability to use solar energy and inorganic nutrients to produce carbohydrates via photosynthesis naturally casts them in the role of primary producer at the base of all food webs. Thus, directly or indirectly, all higher trophic organisms depend on autotrophic productivity and the bulk of this is in the form of photoautotrophy. Kwak and Zedler (1997) explored food web dynamics in coastal wetlands and demonstrated the vital role of microalgae, macroalgae, and marsh plants to consumers such as invertebrates, fishes, and birds with the use of stable isotope techniques. Power (1990) revealed the reliance of fish on algivorous insects and fish fry and the indirect effects of fish abundance on algal biomass. While consumers certainly shape community composition, primary producers render basic inorganic building blocks, such as carbon and nitrogen, usable by higher trophic levels (Hunter and Price 1992; Power 1992).

It is well known that abiotic factors are integral in determining the magnitude of photoautotrophic productivity. Photosynthetic processes in aquatic systems are driven by various abiotic parameters such as light, nutrients, temperature, pH, and concentration of free carbon dioxide (Lobban and Harrison 1994). In most systems, light and nutrients are the limiting resources for photosynthesis. Assuming an adequate nutrient supply, light controls photosynthetic biomass production (King and Schramm 1976). However, in many systems nutrient supply is variable due to flux to and from adjacent systems.
Algae, abundant primary producers in many marine and aquatic systems, are frequently exposed to variable nutrient supply due to tidal or fluvial flux, seasonal upwelling, and terrigenous inputs. This variability has consequences for photosynthetic output, including concomitant sugar and protein synthesis. For the macroalga *Ulva* spp., gross photosynthesis and chlorophyll content are positively correlated with ambient nitrate concentrations (Cabello-Pasini and Figueroa 2005). Rosenberg and Ramus (1982) also found a positive correlation between pigment levels and soluble tissue nitrogen in *Gracilaria foliifera* and *Ulva* spp.

The central California coast is an exceptionally diverse environment in terms of geomorphology, marine life, and anthropogenic influences. Trace elements are naturally introduced to this region via upwelling (Bruland *et al.* 2001; Chase *et al.* 2005) and crustal weathering and subsequent erosion (Libes 1992; Luoma and Rainbow 2008). In addition, various anthropogenic sources such as harbor inputs, and agricultural, industrial and municipal effluents, lead to a complex mosaic of chemical conditions that is not easily characterized. Metals are introduced to nearshore systems from commercial harbors in Monterey, Moss Landing, Santa Cruz, and San Francisco and include zinc, copper, and lead as anti-corrosive, anti-fouling and anti-rust agents on ship hulls and dock pilings (Bird *et al.* 1996; Schiff *et al.* 2004; Warnken *et al.* 2004). Agricultural pesticides and herbicides, byproducts from industries such as mining and smelting, and municipal wastewater discharge all flow through rivers and culverts, eventually reaching coastal habitats. Trace metals, of natural and anthropogenic origin, are transferred through food chains with some bioaccumulating as they are passed on to higher trophic
levels (Wang 2002; Croteau et al. 2005). Thus, local biogeochemistry has far reaching ecosystem effects that often start with primary producers (Mason and Morel 1996).

Marine macroalgae passively and actively uptake various essential and non-essential elements. These elements can be adsorbed by cell wall polysaccharides or absorbed into algal cells. Essential elements, including trace metals, perform structural, and metabolic roles in protein and sugar synthesis (DeBoer 1981; Lobban and Harrison 1994). Some trace metals are essential micronutrients when incorporated in low concentrations but can become toxic at higher concentrations, such as manganese, copper, iron, selenium and zinc (Stauber and Florence 1986; Lobban and Harrison 1994; Rijstenbil et al. 1994). Non-essential elements can be benign or toxic to algae. Trace metals such as arsenic, cadmium, lead and mercury, have no nutritive value and are toxic at low concentrations (Rai et al. 1981; Lobban and Harrison 1994).

Some studies have linked trace metal uptake in algae to ambient nitrate concentrations. Lee and Wang (2001) demonstrated a positive relationship between ambient nitrate concentration and cadmium accumulation rate in *Ulva fasciata* (Linnaeus). Algal cells in nitrate enriched media can accumulate more copper, manganese, and zinc when compared to low nitrate media (Rijstenbil et al. 1998). Furthermore, studies by Rosenberg and Ramus (1982) revealed that nitrogen enrichment leads to an increase in photosynthetic pigment production and growth rate in *Gracilaria foliifera* and *Ulva* spp. An increase in nitrate concentration has also been shown to positively affect gross photosynthesis and chlorophyll content (Cabello-Pasini and Figueroa 2005). Given the role that trace metals play in the function and synthesis of
photosynthetic enzymes and proteins, variability in productivity could be affecting the uptake of essential and non-essential elements.

Trace metals such as zinc and manganese are essential for growth and photosynthesis in algae, and their uptake is driven in part by physiological requirements (Brand 1983; Sunda and Huntsman 1998). In nature, multiple factors influence algal growth rate aside from available ambient nutrient supply. Temperature and light also play a strong role in short term growth rates; therefore, nutrient history is an important parameter to examine in a broader context of productivity because maintenance of growth rates can depend greatly on nitrogen reserves.

Macroalgal species of the genus *Ulva* are distributed globally and thrive in intertidal and estuarine habitats. This genus is considered cosmopolitan and opportunistic, due to its early colonization of available substrate, rapid growth, and reproduction. *Ulva* spp. demonstrates higher stress tolerance, persisting despite varying levels of desiccation, salinity, and pollution (Abbott and Hollenberg 1976). *Ulva* spp. are common in nearshore, intertidal, and estuarine systems along the coastline of central California. This genus has a high nitrogen saturation point, making it possible for *Ulva* spp. to utilize nutrients toward increased growth, reproduction and/or productivity (Rosenberg and Ramus 1982). Additionally, *Ulva* spp. demonstrate relatively high metal accumulation potentials (Wang and Dei 1999).

The central California coast is naturally enriched with nutrients and trace elements as a result of annual coastal upwelling from March through August (Graham and Largier 1997). Additionally, this coast is exposed to significant amounts of
terrigeneous runoff, increasing in months with higher precipitation (November-April) (Caffrey et al. 2007). Natural terrigenous nutrients combine with significant anthropogenic inputs which discharge along the coast. Habitat degradation due to growing urban and agricultural development has led to increased soil erosion and nutrient runoff by destabilizing hillsides and waterway embankments, as well as increasing impervious surfaces (Peierls et al. 1991; Nixon 1995). This compromises natural mechanisms of runoff remediation such as riparian vegetation, marshes, wetlands, and adequate permeable surfaces, exacerbating the influence of terrigenous effluents in coastal systems.

Nutrient enrichment, whether caused by upwelling and/or anthropogenic activities, often affects ecosystems by supplying them with limiting compounds such as dissolved inorganic nitrogen. Shifts in productivity and even species dominance can result from shifts in nutrient availability (Valiela et al. 1997). Caffrey et al. (2007) presented evidence that the seasonal nutrient regime in Elkhorn Slough, an estuary adjacent to the Monterey Bay, corresponds to increased levels of gross marine photoautotrophic productivity.

Trace elements are transferred between trophic levels, becoming bioaccumulated (biomagnified, or increasing in concentration with each trophic step) or biopurified (decreasing in concentration with each trophic step) depending on the elemental species and particular pathway through the food web. Mercury, selenium, and arsenic have been shown to biomagnify under certain conditions (Ogle et al. 1988; Mason and Morel 1996; Barwick and Maher 2003), occurring in greater concentrations in higher trophic levels.
compared to primary producers and other lower trophic organisms. In contrast, other metals are biopurified as they are transferred up the trophic ladder. Trace element burden in herbivores that feed on *Ulva* spp. should therefore exhibit varying tissue element concentrations with varying algal metal concentrations. *Idotea* spp. is a common invertebrate genus in this region and is an example of an invertebrate genus that is known to eat *Ulva* (Kamermans *et al.* 2002) as well as reflect metal burden in toxicity studies (El-Nady and Atta 1996). It is a prey item for large inverts, omnivorous and carnivorous fish, and aquatic birds; therefore, *Idotea resecata* was used in this study to examine trophic transfer of metals by *Ulva* spp.

Exploring the fundamental physiological relationship between nutrient and metal uptake in algae is important in piecing together a biogeochemical picture of environments where nutrient and metal enrichment co-occur. Algae play an intrinsic role in the cycling of nutrients and metals by sequestering elements from their environment and transferring them down into sediments and up through food webs. Currently, the majority of global oceans are increasingly influenced by anthropogenic activities (Halpern *et al.* 2008) making the co-occurrence of metal and nutrient enrichment commonplace in coastal environments. Therefore, the goal of this study was to understand how nitrogen affects metal uptake in *Ulva* spp. under eutrophic conditions, and to examine if there exists a relationship between invertebrate tissue metal burden and the metal concentration in their diet. I conducted a series of experiments to assess the physiological relationship between 1) tissue nitrogen and metal uptake in *Ulva* spp., 2) media nitrate and metal uptake in *Ulva* spp. under eutrophic conditions, and 3) tissue metal burden of *I. resecata* and the
metal content in their diet. After conducting a general, local, spatial survey of metal content in *Ulva* spp., I focused specifically on arsenic, lead, manganese, and zinc for the remaining experiments. The first two of these metals are toxic and non-essential, and the latter two are essential to algal growth, yet toxic at high concentrations. To control for the great spatial and temporal variability in this region, most of my specimens were collected from Elkhorn Slough and surrounding areas.

**METHODS**

**Creation of an *Ulva* process reference material**

Thirteen kilograms (wet weight) of fresh *Ulva* spp. were collected from Elkhorn Slough (Fig. 1A) using sterile gloves and placed in polyethylene bags in a cooler for transport to the laboratory, where they were stored in flowing seawater for 48 hours. The likely species that were represented in this study were *U. expansa, U. lactuca, U. lobata* and *U. rigida*. The tissue was dried and homogenized for use as a process reference material for trace element analyses. A large glass jar was trace metal cleaned for storage of the homogenate: Soaked for 3 days in 1% micro and deionized water solution, rinsed 5 times with deionized water, rinsed 3 times with ultrapure water from a Milli-Q ion exchange system (Millipore Corporation), and soaked in the same overnight. The jar was then soaked in 5% trace metal grade HCl for 5 days, then rinsed 3 times with Milli-Q. One large sample was taken from one location in the glass jar and divided into five subsamples (“parsed”). Then, five more samples were taken from various parts of the container and run separately (“separate”). To test for its suitability for use as a process
reference material, trace metal analyses were performed. Six milliliters of concentrated Double Distilled HNO$_3$ (Seastar) were added to each sample and spike solutions were added for spike scans. Algal tissue was digested in a microwave digestor. Vials with 1 mL of digestate, 25 µL of internal standard and 10 mL of Milli-Q with 1% HNO$_3$ were analyzed using a Sciex Elan 6000 Inductively-Coupled Plasma Mass Spectrophotometer (Perkin-Elmer, Waltham MA, USA). An orchard leaf standard reference material (1571) was used to confirm accurate results. To test for trace metal homogeneity of the Ulva homogenate, a two sample $t$-test was performed comparing mean trace metal concentration of “parsed” samples to “separate” samples. The standard error and standard error as a percent of metal concentration were both reported as a measure of the variability in trace metals in the material.

Spatial variation in Ulva spp. trace metal burden in Moss Landing, California

To explore spatial variability in metal burden in *Ulva* spp., in October 2008, samples were collected from the following sites: the Old Salinas River Channel, Elkhorn Slough, Moss Landing Harbor, and the north Moss Landing jetty (Fig 1A). Samples were collected using polyethylene gloves and stored in polyethylene bags in a cooler for transport to the laboratory. Three to four blades from each site were homogenized and three subsamples used for analysis. Sub-samples were prepared by rinsing in Milli-Q and metals were analyzed using the protocols listed above, with one change: initial wet weight was recorded and $3.00 \pm 0.2$g of algal tissue was dried for 48 hours in an oven at 72 degrees Celsius. Final dry weights were recorded for moisture determination.
A. Collection sites for *Ulva*. Spatial variation in metal burden in *Ulva* spp.: a=Moss Landing north jetty, b=Moss Landing Harbor, c=Salinas Channel, d=Elkhorn Slough; Collection sites for the *Ulva* process reference material & trace metal uptake by *Ulva* spp. as a function of media nitrate=e.

The mean tissue concentration of each metal was calculated and a one-way ANOVA was used to determine if there were significant differences in metal concentrations among sampling sites. Crustal abundance tables (Korte 1999) were used to calculate the ratios of the metals of interest to crustal abundance relative to aluminum. The ratio of metal concentrations to aluminum concentration determined in this study was divided by the crustal ratio to compare relative metal concentrations.

**Trace element variability within and among blades**

To quantify the trace element variability both within and among *Ulva* blades, tissue samples and seawater were collected in accordance with trace metal clean protocols (Gordon *et al.* 1980) by using clean, polyethylene gloves, bags, bottles and carboys for collection and storage. All equipment that was in contact with tissue samples and seawater was cleaned in a trace metal clean laboratory according to methods described in U. S. Environmental Protection Agency Method 200.7 & 200.8 (1994; USEPA 1994). Ho (1993) indicated that *Ulva* thalli near the holdfast and marginal cells had slower growth rates and metabolism than the central thalli, therefore all tissue samples were taken from the central part of the blade and rinsed with seawater to remove organic matter and sediment. All samples were stored and transported in a cooler with ice packs. *Ulva* samples were cut with trace metal clean plastic implements, and swabbed with cotton that was soaked in 5% trace metal grade HCl for 1 hour, rinsed with Milli-Q, then soaked in clean seawater.
Five *Ulva* blades were collected from pilings in the Monterey Harbor (Fig. 1B) and eight 1.0 g tissue samples (wet weight) were taken from each blade. Four of the eight tissue samples were homogenized together and reparsed into four separate samples, while the other four were analyzed separately. An orchard leaf standard reference material (1571) was used to assure accuracy in the analysis and the *Ulva* substandard from the previous experiment was used as a process control standard. Metals were analyzed using the same methods as described above. Trace metal values were recorded in parts per million dry weight. A two-way ANOVA was run on the data using the five blades (8 subsamples each) as a random factor and the two tissue preparations (homogenized and separate) as a fixed factor, to test the hypothesis that metal concentrations do not significantly vary within or among *Ulva* blades collected from the same site.

**Trace metal uptake by *Ulva* spp. as a function of tissue nitrogen**

Experiments were used to test the hypothesis that increased tissue nitrogen results in increased metal uptake by *Ulva*. Seawater used in these experiments was collected 5 miles offshore of Moss Landing (N 36° 50.134, W 121° 54.129) using trace metal clean methods and filtered with a Millipore Millipak 0.22 μm filter, rendering it comparable to open ocean water in terms of trace metal content (Table 1). Seawater analyses were performed using a Thermo Finnigan Element 2 high-resolution ICP-MS (Hulme *et al.* 2008). A 10% dilution was used with standards in 10% low-metal surface seawater from
the Southern Pacific Ocean collected during the SoFeX cruise (Coale et al. 2004). Metal-spiked seawater was also analyzed for metal content.

**Table 1.** Trace metal content in filtered seawater used for *Ulva* tissue nitrogen and eutrophication experiments in ng/mL. 10% SW Blank and Blank1 refer to water collected during the SoFex cruise in 2004 (Coale et al. 2004). 1643e is a National Institute of Standards and Technology (NIST) standard reference material. HNO$_3$ blank is a standard nitric acid blank required for quality control. Exp 1 and 2 SW1 and SW2 refer to filtered seawater used in experiments. “Exp1 - spiked” refers to seawater after metals (Pb, Mn, Zn, As) were added. DL = detection limit.

<table>
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<th></th>
<th>Al</th>
<th>Ag</th>
<th>Cd</th>
<th>Pb</th>
<th>Cr</th>
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<td>Exp2 SW2</td>
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<td>0.1</td>
<td>0.1</td>
<td>0.3</td>
<td>&lt;DL</td>
<td>&lt;DL</td>
<td>&lt;DL</td>
<td>0.2</td>
<td>0.7</td>
<td>0.4</td>
<td>0.0</td>
</tr>
<tr>
<td>DL</td>
<td>0.45</td>
<td>0.02</td>
<td>0.16</td>
<td>0.16</td>
<td>0.21</td>
<td>0.23</td>
<td>0.11</td>
<td>0.16</td>
<td>0.33</td>
<td>0.66</td>
<td>0.27</td>
</tr>
</tbody>
</table>

A range of *Ulva* tissue nitrogen (2.5% - 4.7%) was created by sampling *Ulva* from 15 sites along the central California coast (Fig. 1B): 1) Big Creek Marine Reserve; 2) Soberanes Point; 3) Stillwater Cove; 4) South Point Pinos; 5) North Point Pinos; 6) San Carlos Beach; 7) Monterey Harbor; 8) Elkhorn Slough; 9) Moss Landing Harbor; 10) Moss Landing Jetty; 11) Moss Landing Yacht Club; 12) Kirby Park; 13) Santa Cruz Harbor; 14) Santa Cruz Jetty; 15) Davenport Beach. A power analysis (G*Power 3.1.3) was used to determine the sample size (ANOVA: Multiple regression, omnibus; $\alpha = 0.05$, power = 0.95), which was the minimum to detect an $R^2$ of 0.5 (Faul et al. 2009).
Two *Ulva* blades were collected from each site, and each blade was split into three 1.0 g pieces for: (1) analysis of initial trace metal concentration, (2) spiking with additional metal, and (3) tissue nitrogen analysis. Samples were stored and transported to a clean laboratory according to protocols described above. For nitrogen analysis, 0.5 gram wet *Ulva* was dried and homogenized for each sample. Samples (~150 µg) were loaded in 3.5x5 mm tin capsules and analyzed with a CE Elantech 2500 CHN Elemental Analyzer at the University of Wyoming Stable Isotope Facility, Laramie, Wyoming. Although cutting the algae into pieces may stimulate the release of chemicals (Webster and Gadd 2004), no evidence has indicated that metal accumulation is significantly affected by this treatment (Ho 1993).

To test for tissue nitrogen effects on metal uptake, thirty 500 mL, clear, trace metal cleaned polycarbonate bottles were filled with clean, filtered seawater and spiked with four metals: arsenic \((30 \times 10^3 \text{ nmol/kg})\), lead \((150 \text{ nmol/kg})\), manganese \((7.3 \times 10^3 \text{ nmol/kg})\) and zinc \((5000 \text{ nmol/kg})\) (Claritas brand 1000 uM metal standards dissolved in 3% HNO\(_3\)). The nitric acid in the metal solutions brought the baseline nitrate level in the media to 1000uM, and the pH to 6.4. These conditions are similar to conditions observed at sites in the Salinas Channel and Elkhorn Slough, California: local, eutrophic water bodies where *Ulva* is common (Monterey Bay Aquarium Research Institute’s Land/Ocean Biogeochemical Observatory (LOBO) network; J. Plant pers. comm.). The relatively low pH may lower adsorption to bottles and *Ulva* thalli; therefore, the reported results likely represent mostly the incorporation of metals by algae. Pieces of *Ulva* (1.0 g wet weight) from each site were placed in separate bottles, and attached to an acrylic rotating carousel (plankton wheel) in
order to circulate seawater and minimize the diffusion boundary layer. Experiments were carried out in a temperature controlled room at 12°C and were incubated for 24 hours. All samples were exposed to a full spectrum light source (96 W, 6700 K) that provided 50 PAR at the bottom of the rotating wheel, and 500 PAR when the bottles moved to the top of the wheel, closer to the light source (~2.22 rpm). Tissue was then removed and blade surfaces were cleaned with cotton swabs and rinsed in clean seawater. Trace element analysis was carried out as described.

For each metal, a linear regression analysis was carried out to determine the relationship between tissue nitrogen and metal uptake in the *Ulva* tissue. Uptake was calculated by subtracting initial metal concentration from final metal concentration for each sample. An additional regression analysis was performed comparing the percent change for each metal (final concentration minus initial concentration divided by the initial concentration) to the tissue nitrogen. Enrichment factors were calculated by dividing the average of final *Ulva* metal concentrations (multiplied by 0.17 to convert to ppm wet weight) by spiked seawater metal concentrations.

**Trace metal uptake by *Ulva* spp. as a function of media nitrate**

Experiments were used to test the hypothesis that, under eutrophic conditions, *Ulva* metal uptake increases with 0-20% additions of nitrate. As in the previous experiment, nitric acid in the metal solutions brought the baseline nitrate level in the media to 1000 µM and the pH to 6.4. Fifteen *Ulva* blades were collected on one date from one site in Elkhorn Slough (Fig. 1A) and randomly assigned to 15 nitrate
treatments: 0, 1, 2, 4, 6, 8, 10, 15, 20, 30, 40, 50, 75, 100, and 200 µM nitrate. Trace metal-clean 500 mL polycarbonate bottles were filled with clean seawater. Nitrate treatment was administered using a potassium nitrate standard, and all bottles were spiked with arsenic (30*10³ nmol/kg), lead (150 nmol/kg), manganese (7.3*10³ nmol/kg) and zinc (5000 nmol/kg) (Claritas brand 1000 uM metal standards dissolved in 3% HNO₃). Pieces of Ulva (1.0 g wet weight) were placed in the bottles, and then incubated for 24 h in identical conditions to those described above. Trace metal analysis was carried out as described above, and a regression analysis was used to evaluate the relationship between media nitrate and final trace metal concentration.

**Trace metal trophic transfer to Idotea resecata**

A laboratory feeding experiment was used to test the hypothesis that tissue metal burden in the isopod *I. resecata* would increase when fed *Ulva* with a higher than normal tissue metal burden. *Ulva* was collected from one site in Elkhorn Slough, Moss Landing (Fig. 1A) and placed in an aquarium with flowing seawater for 10 days. Four diets were created: control, low, medium, and high metal treatments. The low batch was treated with seawater enriched with arsenic (0.1 ppm), manganese (0.065 ppm), lead (0.0075 ppm), and zinc (0.05 ppm). The medium treatment was placed in seawater spiked with arsenic (0.5 ppm), manganese (0.325 ppm), lead (0.0375 ppm) and zinc (0.25 ppm). The high treatment was placed in seawater enriched with arsenic (1.0 ppm), manganese (0.65 ppm), lead (0.075 ppm) and zinc (0.5 ppm). These batches of algae were left to incubate
at 12°C for 36 hours with air bubblers and a 200 PAR light source with a spring photoperiod of 12 hours of light and 12 hours of dark.

One hundred and fifty individual *I. resecata* between 2-3 centimeters length were collected from kelp beds near Point Pinos, Pacific Grove, California (Fig. 1B). For an estimate of baseline metal burden in *I. resecata*, 28 individuals were split into 4 groups (baseline A-D). Individuals from each group were diced finely with a clean scalpel and a 1.0 g sample was used for metal analysis. The other 122 were kept in flowing seawater and fed clean, “control” *Ulva* for 7 days and then starved for one week. At the end of the week, they were separated into 16 mesocosms with identical air bubblers and placed in an aquarium with running seawater at 13°C. A mesh opening in the top of each mesocosm allowed seawater to freely flow in and out of the containers. They were split into 4 replicates of 4 treatments (about 6-9 individuals, 2.5-3.0 total grams per replicate) and administered the corresponding diet ad libidum for 7 days. On the seventh day, 3 individuals (~1.0 grams wet) were removed and starved for 5 days in order to purge undigested *Ulva* from their digestive tract. They were then frozen at -20°C. The remaining individuals were fed unspiked *Ulva* that had been in flowing seawater to investigate their ability to recover from the contaminated food. After 7 days of this “clean” diet, they also were starved for 5 days and frozen. Individuals from each group were homogenized with a clean scalpel and a 1.0 g sample was used for metal analysis.

One-way ANOVA analyses were used to determine if treatments (control, low, medium, and high metal-dosed *Ulva* diets) caused a change in *I. resecata* tissue metal burden (As, Mn, Pb, Zn), and to determine the effects of the “recovery” diet. A Tukey’s
post-hoc test was used to detect differences among treatments (SPSS 16.0.1, \( \alpha = 0.05 \)).

Homogeneity of variance was tested using Levene’s test and normality with a Kolmogorov-Smirnov test.
RESULTS

Creation of an *Ulva* process reference material

A two-sample *t*-test comparing “parsed” samples to “separate” samples revealed no significant differences in arsenic, manganese, lead, or zinc concentrations, confirming that the *Ulva* process reference material was adequately homogenized (Fig. 2, Table 2A). Additionally, for every metal, agreement between the sample concentrations was within the standard error, demonstrating the sufficient precision of the *Ulva* process reference material to detect differences in treatments for the following experiments (Table 2B).

![Figure 2: Mean metal concentrations (As, Mn, Pb* & Zn) in samples of *Ulva* process reference material in ppm dry weight. Pb* = Pb*10. Error bars represent ± SE. “Parsed” samples were taken from one location in a container of homogenate and compared to “separate” samples, taken from different places in the container. No significant differences between metal concentrations were detected, demonstrating the adequate homogeneity of *Ulva* process reference material.](image)
Establishing a reliable, accurate process reference material, of the same tissue used in this study, was integral for the quality control of metal analyses. Verifying that the variability within the stock *Ulva* homogenate was insignificant provided the necessary confidence for its use as a process reference material for all experiments in this study.

<table>
<thead>
<tr>
<th>Metal</th>
<th>t</th>
<th>df</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>As</td>
<td>-1.202</td>
<td>8</td>
<td>0.264</td>
</tr>
<tr>
<td>Mn</td>
<td>-0.047</td>
<td>8</td>
<td>0.964</td>
</tr>
<tr>
<td>Pb</td>
<td>0.886</td>
<td>8</td>
<td>0.401</td>
</tr>
<tr>
<td>Zn</td>
<td>-1.460</td>
<td>8</td>
<td>0.182</td>
</tr>
</tbody>
</table>

TABLE 2A. Results of an independent samples *t*-test comparing 1 parsed sample (5 subsamples) to 5 separate samples of *Ulva* process reference material. No difference was found between samples.

<table>
<thead>
<tr>
<th>Sample / Metal</th>
<th>Average (ppm) ± SE</th>
<th>Standard error (% of avg)</th>
<th>Coefficient of variation</th>
</tr>
</thead>
<tbody>
<tr>
<td>“Parsed” / As</td>
<td>7.51 ± 0.04</td>
<td>0.55</td>
<td>1.22</td>
</tr>
<tr>
<td>“Parsed” / Mn</td>
<td>32.6 ± 0.17</td>
<td>0.51</td>
<td>1.14</td>
</tr>
<tr>
<td>“Parsed” / Pb</td>
<td>17.1 ± 0.16</td>
<td>0.96</td>
<td>2.15</td>
</tr>
<tr>
<td>“Parsed” / Zn</td>
<td>18.25 ± 0.07</td>
<td>0.36</td>
<td>0.81</td>
</tr>
<tr>
<td>“Separate” / As</td>
<td>7.59 ± 0.05</td>
<td>0.70</td>
<td>1.56</td>
</tr>
<tr>
<td>“Separate” / Mn</td>
<td>32.64 ± 0.39</td>
<td>1.20</td>
<td>2.69</td>
</tr>
<tr>
<td>“Separate” / Pb</td>
<td>16.81 ± 0.28</td>
<td>1.66</td>
<td>3.70</td>
</tr>
<tr>
<td>“Separate” / Zn</td>
<td>18.55 ± 0.18</td>
<td>0.99</td>
<td>2.20</td>
</tr>
</tbody>
</table>

TABLE 2B. Average metal concentration, standard error and coefficient of variation in *Ulva* process reference material.
Spatial variation in *Ulva* spp. trace metal burden in Moss Landing, California

Mean trace metal concentrations in *Ulva* differed significantly among sites (Fig. 3, Table 3). Samples from the Salinas River Channel exhibited significantly higher trace metal concentrations than the other sites for chromium, nickel, lead and manganese. The *Ulva* from the Salinas Channel and Moss Landing Harbor had significantly greater arsenic, copper and zinc concentrations than the *Ulva* from the other two sites.
Fig. 3. Mean concentrations of trace metals in *Ulva* tissues (ppm dry weight) collected from four sites in Moss Landing, CA: NJ= north jetty at Moss Landing, ES= Elkhorn Slough, MLH= Moss Landing Harbor, SC= Salinas Channel. Fig. 3A. Mean As, Cd (*) = Cd*10), Cr, Cu, Ni, Pb, Se & Zn (*) = Zn/10). Fig. 3B. Mean Mn. Error bars represent ± SE. Metal concentrations differed significantly for all metals but selenium (Table 2).
TABLE 3. Results of one-way ANOVA comparing mean algal tissue metal concentrations among sites. Those below the critical p-value of 0.05 in bold. Tissue metal content in *Ulva* differed significantly among sites for all metals tested except selenium.

<table>
<thead>
<tr>
<th>Source</th>
<th>Sum of Squares</th>
<th>df</th>
<th>Mean Squares</th>
<th>F-ratio</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>As</td>
<td>Between Groups</td>
<td>39.252</td>
<td>3</td>
<td>13.084</td>
<td>26.862</td>
</tr>
<tr>
<td></td>
<td>Within Groups</td>
<td>3.897</td>
<td>8</td>
<td>0.487</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>43.149</td>
<td>11</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cd</td>
<td>Between Groups</td>
<td>0.203</td>
<td>3</td>
<td>0.068</td>
<td>19.603</td>
</tr>
<tr>
<td></td>
<td>Within Groups</td>
<td>0.028</td>
<td>8</td>
<td>0.003</td>
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</tr>
<tr>
<td></td>
<td>Total</td>
<td>0.230</td>
<td>11</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cr</td>
<td>Between Groups</td>
<td>303.980</td>
<td>3</td>
<td>101.327</td>
<td>6.058</td>
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<td></td>
<td>Within Groups</td>
<td>160.263</td>
<td>8</td>
<td>20.033</td>
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</tr>
<tr>
<td></td>
<td>Total</td>
<td>464.243</td>
<td>11</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cu</td>
<td>Between Groups</td>
<td>447.678</td>
<td>3</td>
<td>149.226</td>
<td>13.546</td>
</tr>
<tr>
<td></td>
<td>Within Groups</td>
<td>88.132</td>
<td>8</td>
<td>11.017</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>535.810</td>
<td>11</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ni</td>
<td>Between Groups</td>
<td>233.874</td>
<td>3</td>
<td>77.958</td>
<td>4.832</td>
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<td></td>
<td>Within Groups</td>
<td>129.076</td>
<td>8</td>
<td>16.135</td>
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</tr>
<tr>
<td></td>
<td>Total</td>
<td>362.950</td>
<td>11</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pb</td>
<td>Between Groups</td>
<td>37.938</td>
<td>3</td>
<td>12.646</td>
<td>5.281</td>
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<tr>
<td></td>
<td>Within Groups</td>
<td>19.156</td>
<td>8</td>
<td>2.395</td>
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<tr>
<td></td>
<td>Total</td>
<td>57.094</td>
<td>11</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Se</td>
<td>Between Groups</td>
<td>20.775</td>
<td>3</td>
<td>6.925</td>
<td>2.905</td>
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<tr>
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<td>Within Groups</td>
<td>19.069</td>
<td>8</td>
<td>2.384</td>
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<tr>
<td></td>
<td>Total</td>
<td>39.843</td>
<td>11</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Zn</td>
<td>Between Groups</td>
<td>1211.929</td>
<td>3</td>
<td>403.976</td>
<td>6.799</td>
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<td></td>
<td>Within Groups</td>
<td>475.353</td>
<td>8</td>
<td>59.419</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>1687.283</td>
<td>11</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mn</td>
<td>Between Groups</td>
<td>161739.967</td>
<td>3</td>
<td>53913.322</td>
<td>7.538</td>
</tr>
<tr>
<td></td>
<td>Within Groups</td>
<td>57214.240</td>
<td>8</td>
<td>7151.780</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>218954.207</td>
<td>11</td>
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<td></td>
</tr>
</tbody>
</table>
The Moss Landing north jetty site showed the largest discrepancies between measured algal metal concentrations and crustal abundances (Table 4). Relative to crustal abundances, arsenic, cadmium, copper, nickel, lead, selenium and zinc were elevated at all sites.

Table 4. Relationship between ratio of sample metal concentrations to sample aluminum concentrations and ratio of crustal metal abundances to crustal aluminum abundance. ES = Elkhorn Slough, MLH = Moss Landing Harbor, NJ = North Jetty, SC = Salinas Channel. As, Cd, Cu, Se and Zn were more than 3 times their natural crustal abundance at 2 or more sites.

<table>
<thead>
<tr>
<th></th>
<th>As</th>
<th>Cd</th>
<th>Cr</th>
<th>Cu</th>
<th>Mn</th>
<th>Ni</th>
<th>Pb</th>
<th>Se</th>
<th>Zn</th>
</tr>
</thead>
<tbody>
<tr>
<td>ES</td>
<td>29.08</td>
<td>28.96</td>
<td>1.64</td>
<td>2.59</td>
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<td>2.68</td>
<td>1.80</td>
<td>2459.00</td>
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</tr>
<tr>
<td>MLH</td>
<td>50.58</td>
<td>7.18</td>
<td>1.03</td>
<td>4.59</td>
<td>0.58</td>
<td>1.16</td>
<td>1.24</td>
<td>1778.55</td>
<td>5.95</td>
</tr>
<tr>
<td>NJ</td>
<td>87.87</td>
<td>95.70</td>
<td>1.64</td>
<td>3.74</td>
<td>0.61</td>
<td>2.11</td>
<td>3.51</td>
<td>7163.60</td>
<td>9.59</td>
</tr>
<tr>
<td>SC</td>
<td>20.52</td>
<td>6.24</td>
<td>0.98</td>
<td>1.76</td>
<td>1.74</td>
<td>1.17</td>
<td>2.26</td>
<td>1004.39</td>
<td>3.03</td>
</tr>
</tbody>
</table>

Trace element variability within and among blades

Neither the method of preparation (subsamples homogenized and reparsed or run separately) nor sampling from different blades had a significant effect on metal concentration in *Ulva* (Tables 5A-D). For arsenic and lead there was a significant interaction between sample preparation and blade.
TABLE 5. Results from 2-way ANOVAs determining trace metal variability (A. As; B. Mn; C. Pb; D. Zn) within and among *Ulva* spp. blades. Analyzed with a critical p-value of 0.05. Those below the adjusted critical value for 4 comparisons of 0.0125 in bold.

A. Test of Between-Subjects Effects

<table>
<thead>
<tr>
<th>Source</th>
<th>Sum of Squares</th>
<th>df</th>
<th>Mean Square</th>
<th>F</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Preparation</td>
<td>0.406</td>
<td>1</td>
<td>0.406</td>
<td>0.107</td>
<td>0.760</td>
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<tr>
<td>Blade</td>
<td>45.713</td>
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<td>11.428</td>
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<td>0.155</td>
</tr>
<tr>
<td>Preparation * Blade</td>
<td>15.164</td>
<td>4</td>
<td>3.791</td>
<td>5.989</td>
<td><strong>0.001</strong></td>
</tr>
<tr>
<td>Error</td>
<td>18.988</td>
<td>30</td>
<td>0.633</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

B. Test of Between-Subjects Effects

<table>
<thead>
<tr>
<th>Source</th>
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<th>df</th>
<th>Mean Square</th>
<th>F</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Preparation</td>
<td>0.710</td>
<td>1</td>
<td>0.710</td>
<td>0.315</td>
<td>0.605</td>
</tr>
<tr>
<td>Blade</td>
<td>63.282</td>
<td>4</td>
<td>15.820</td>
<td>7.012</td>
<td>0.043</td>
</tr>
<tr>
<td>Preparation * Blade</td>
<td>9.025</td>
<td>4</td>
<td>2.256</td>
<td>0.510</td>
<td>0.729</td>
</tr>
<tr>
<td>Error</td>
<td>132.804</td>
<td>30</td>
<td>4.427</td>
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<td></td>
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</table>

C. Test of Between-Subjects Effects

<table>
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<th>Source</th>
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<th>df</th>
<th>Mean Square</th>
<th>F</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Preparation</td>
<td>1.612</td>
<td>1</td>
<td>1.612</td>
<td>0.925</td>
<td>0.391</td>
</tr>
<tr>
<td>Blade</td>
<td>6.323</td>
<td>4</td>
<td>1.581</td>
<td>0.907</td>
<td>0.537</td>
</tr>
<tr>
<td>Preparation * Blade</td>
<td>6.971</td>
<td>4</td>
<td>1.743</td>
<td>10.869</td>
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<td>Error</td>
<td>4.811</td>
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D. Test of Between-Subjects Effects

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<th>Mean Square</th>
<th>F</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Preparation</td>
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<td>3591.025</td>
<td>1.022</td>
<td>0.369</td>
</tr>
<tr>
<td>Blade</td>
<td>66721.850</td>
<td>4</td>
<td>16680.462</td>
<td>4.746</td>
<td>0.080</td>
</tr>
<tr>
<td>Preparation * Blade</td>
<td>14057.350</td>
<td>4</td>
<td>3514.337</td>
<td>0.971</td>
<td>0.438</td>
</tr>
<tr>
<td>Error</td>
<td>108530.750</td>
<td>30</td>
<td>3617.692</td>
<td></td>
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</tr>
</tbody>
</table>
Trace metal uptake by *Ulva* spp. as a function of tissue nitrogen

Arsenic exhibited a significant positive relationship with tissue nitrogen ($F=4.215$, df=29, $p=0.050$, $r^2=0.135$), which explained approximately 14% of the observed variability in arsenic uptake (Fig. 4A). Tissue nitrogen was also positively correlated with manganese uptake ($F=10.005$, df=29, $p=0.004$, $r^2=0.272$), accounting for approximately 27% of the manganese uptake observed (Fig. 4C).

**A.**  
\[
\Delta \text{As} = 131.41(\pm63.21)\%N - 217.12(\pm226.17)
\]

**B.**  
\[
\Delta \text{Pb} = -1.09(\pm5.78)\%N + 38.7(\pm20.69)
\]

**C.**  
\[
\Delta \text{Mn} = 117.62(\pm36.69)\%N - 226.39(\pm131.26)
\]

**D.**  
\[
\Delta \text{Zn} = 31.82(\pm31.56)\%N + 129.07(\pm112.34)
\]

**FIG. 4.** Results of a linear regression analysis to evaluate the effect of tissue nitrogen on metal uptake in *Ulva* (n = 30) over 24 hours variability (A. As; B. Pb; C. Mn; D. Zn). Tissue nitrogen was significantly correlated with arsenic ($4.215_{1}, 28(0.05), 0.050, 0.135$) and manganese uptake ($10.005_{1}, 28(0.05), 0.004, 0.272$), but not with lead ($0.050_{1}, 28(0.05), 0.825, 0.001$) or zinc ($0.992_{1}, 28(0.05), 0.330, 0.044$).
Neither lead nor zinc was significantly correlated with tissue nitrogen (Fig. 4B & 4D), although for zinc the lowest metal concentration occurred at the lowest percent tissue nitrogen and the *Ulva* with the highest percent tissue nitrogen had the highest zinc concentration.

Tissue nitrogen did not affect the percent change in arsenic concentrations in *Ulva* (Fig. 5A). However, tissue nitrogen was significantly correlated with the percent change in manganese (F=5.663, df=29, p=0.024, $r^2=0.161$) (Fig. 5B) and lead (4.078, df=29, p=0.053, $r^2=0.121$) (Fig. 5C), contributing to 16% and 12% of the positive trend in percent uptake of those metals, respectively.
FIG. 5. Results of a linear regression analysis to evaluate the effect of tissue nitrogen on percent change in metal concentration in *Ulva* (*n* = 30) over 24 hours (A. As; B. Pb; C. Mn; D. Zn). Tissue nitrogen was significantly correlated with percent change in manganese (5.663, 28(0.05), 0.024, 0.161) and lead (4.078, 28(0.05), 0.053, 0.121), but not with arsenic (0.093, 28(0.05), 0.763, 0.003) or zinc (3.112, 28(0.05), 0.092, 0.119).

The enrichment factor (EF) of an organism represents the degree to which an element is concentrated (Libes 1992). Lower trophic organisms generally concentrate more metals in their tissues than those higher on the trophic ladder. However, the concentration of some metals, such as mercury, increases as they are passed to higher trophic levels. When the EF of an organism for a particular element is less than one, it is said to “biopurify” that element. An EF greater than one indicates that the organism is “bioaccumulating” that particular element. *Ulva* spp. in this study demonstrated
bioaccumulation for all metals of interest, with lead exhibiting the greatest enrichment factor (Table 6).

**TABLE 6. Average enrichment factors for *Ulva* spp for As, Mn, Pb and Zn. Positive values for all metals tested demonstrates bioaccumulation in *Ulva*, with the greatest bioaccumulation occurring for lead.**

<table>
<thead>
<tr>
<th></th>
<th>As</th>
<th>Mn</th>
<th>Pb</th>
<th>Zn</th>
</tr>
</thead>
<tbody>
<tr>
<td>EF</td>
<td>21.89</td>
<td>127.19</td>
<td>330.01</td>
<td>307.25</td>
</tr>
</tbody>
</table>

Trace metal uptake by *Ulva* spp. as a function of media nitrate

Both arsenic and manganese uptake increased, then seemed to saturate with increasing media nitrate (F=4.219, df=13, p = 0.062, $r^2=0.260$; F=2.862, df=13, p = 0.119, $r^2=0.191$ respectively; Fig. 6A & 6B). Lead and zinc demonstrated no relationship with media nitrate (F=0.103, df=13, p = 0.754, $r^2=0.008$; F=0.191, df=13, p = 0.670, $r^2=0.016$ respectively; Fig. 6C & 6D). Metal concentrations at the 200 $\mu$M nitrate level were excluded from analyses because they clearly did not fit the pattern exhibited by the rest of the data. This could have been an artifact of the experimental process, possibly reflecting the adsorption of metals to the vessel walls or an anomaly in the *Ulva* blade itself.

The samples for this experiment were collected from the same site in Elkhorn Slough as the *Ulva* SRM tissue. Since among blade trace metal variability was shown to be insignificant (Table 5A-D), the *Ulva* SRM values were used to represent initial metal concentrations for this experiment (dashed lines below).
Fig. 6. Results of logarithmic regression analyses to test the effect of media nitrate on metal uptake (n=14) (A. As; B. Pb; C. Mn; D. Zn). Dashed line denotes Ulva SRM metal concentration. Red star indicates metal concentration at 200 µM media nitrate (excluded from analysis). Arsenic and manganese were correlated with media nitrate (4.219, 12(0.05), 0.062, 0.260; 2.862, 12(0.05), 0.119, 0.191). Lead and zinc were not related to media nitrate (0.103, 12(0.05), 0.754, 0.009; 0.191, 12(0.05), 0.670, 0.016).

Trace metal trophic transfer to *I. resecata*

Average values for baseline arsenic, manganese, lead and zinc concentrations in *I. resecata* tissue were 11.0, 2.4, 0.88, and 47.0 ppm dry weight respectively (Fig. 8).

Average baseline metal concentrations in *I. resecata* were notably different than metal concentrations in individuals in both “contaminated” and “recovery” diet trials (Tables 7 & 8, Appendices A & B). All baseline metal concentrations were lower in baseline individuals than treatment individuals except arsenic, which was higher.
A.

Fig. 7. Effect of treatments in mesocosm experiments in which *Idotea resecata* individuals were fed diets with low, medium or high concentrations of metals. Zn* = Zn/10. Bars represent average As, Mn, Pb, & Zn ± SE. Fig. 7A. Mean trace metal concentration in *I. resecata* after 7 days of “contaminated” *Ulva* diet and Fig. 7B. after additional 7 days of “recovery” diet.
No significant differences among treatments were found based neither on “contaminated” diets nor on “recovery” diet (Fig. 7A & 7B, Tables 7A & B). However, a slight positive trend can be seen for arsenic and manganese, which increased with metal contamination in their diet (Fig 7A).

**TABLE 7A.** Results of one-way ANOVAs for *I. resecata* tissue metal concentrations (As, Mn, Pb, Zn) across control, low, medium, and high metal diet treatments.

<table>
<thead>
<tr>
<th>Source</th>
<th>Sum of Squares</th>
<th>df</th>
<th>Mean Squares</th>
<th>F-ratio</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>As</td>
<td>Treatment</td>
<td>2.815</td>
<td>3</td>
<td>0.938</td>
<td>1.663</td>
</tr>
<tr>
<td></td>
<td>Error</td>
<td>6.770</td>
<td>12</td>
<td>0.564</td>
<td></td>
</tr>
<tr>
<td>Mn</td>
<td>Treatment</td>
<td>9.083</td>
<td>3</td>
<td>3.028</td>
<td>2.396</td>
</tr>
<tr>
<td></td>
<td>Error</td>
<td>15.161</td>
<td>12</td>
<td>1.263</td>
<td></td>
</tr>
<tr>
<td>Pb</td>
<td>Treatment</td>
<td>2480.603</td>
<td>3</td>
<td>826.868</td>
<td>1.883</td>
</tr>
<tr>
<td></td>
<td>Error</td>
<td>5270.797</td>
<td>12</td>
<td>439.233</td>
<td></td>
</tr>
<tr>
<td>Zn</td>
<td>Treatment</td>
<td>422.122</td>
<td>3</td>
<td>140.707</td>
<td>1.231</td>
</tr>
<tr>
<td></td>
<td>Error</td>
<td>1371.238</td>
<td>12</td>
<td>114.270</td>
<td></td>
</tr>
</tbody>
</table>

**TABLE 7B.** Results of one-way ANOVAs for *I. resecata* tissue metal concentrations (As, Mn, Pb, Zn) across control, low, medium, and high metal diet treatments after 1 week of “recovery” diet.

<table>
<thead>
<tr>
<th>Source</th>
<th>Sum of Squares</th>
<th>df</th>
<th>Mean Squares</th>
<th>F-ratio</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>As</td>
<td>Treatment</td>
<td>1.172</td>
<td>3</td>
<td>0.391</td>
<td>2.121</td>
</tr>
<tr>
<td></td>
<td>Error</td>
<td>1.289</td>
<td>7</td>
<td>0.184</td>
<td></td>
</tr>
<tr>
<td>Mn</td>
<td>Treatment</td>
<td>7.462</td>
<td>3</td>
<td>2.487</td>
<td>0.786</td>
</tr>
<tr>
<td></td>
<td>Error</td>
<td>22.160</td>
<td>7</td>
<td>3.166</td>
<td></td>
</tr>
<tr>
<td>Pb</td>
<td>Treatment</td>
<td>569.036</td>
<td>3</td>
<td>189.679</td>
<td>0.722</td>
</tr>
<tr>
<td></td>
<td>Error</td>
<td>1838.971</td>
<td>7</td>
<td>262.710</td>
<td></td>
</tr>
<tr>
<td>Zn</td>
<td>Treatment</td>
<td>828.596</td>
<td>3</td>
<td>276.199</td>
<td>1.285</td>
</tr>
<tr>
<td></td>
<td>Error</td>
<td>1504.840</td>
<td>7</td>
<td>214.977</td>
<td></td>
</tr>
</tbody>
</table>
Mean arsenic concentration in *I. resecata* tissue decreased when comparing the low “contaminated” diet individuals to those that were fed a “recovery” diet (Table 8). No other metal significantly changed in *I. resecata* tissues after being fed a “recovery” diet.

**TABLE 8.** Results of an independent samples *t*-tests comparing average metal burden in *Idotea resecata* fed with “contaminated” diets and after “recovery” diet.

<table>
<thead>
<tr>
<th>Metal</th>
<th>Treatment</th>
<th>t</th>
<th>df</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>As</td>
<td>Low</td>
<td>-2.166</td>
<td>5</td>
<td>0.083</td>
</tr>
<tr>
<td></td>
<td>Medium</td>
<td>-1.011</td>
<td>5</td>
<td>0.358</td>
</tr>
<tr>
<td></td>
<td>High</td>
<td>1.343</td>
<td>5</td>
<td>0.237</td>
</tr>
<tr>
<td>Mn</td>
<td>Low</td>
<td>-0.574</td>
<td>5</td>
<td>0.591</td>
</tr>
<tr>
<td></td>
<td>Medium</td>
<td>-0.010</td>
<td>5</td>
<td>0.993</td>
</tr>
<tr>
<td></td>
<td>High</td>
<td>-1.192</td>
<td>5</td>
<td>0.287</td>
</tr>
<tr>
<td>Pb</td>
<td>Low</td>
<td>-1.013</td>
<td>5</td>
<td>0.358</td>
</tr>
<tr>
<td></td>
<td>Medium</td>
<td>0.401</td>
<td>5</td>
<td>0.705</td>
</tr>
<tr>
<td></td>
<td>High</td>
<td>-0.381</td>
<td>5</td>
<td>0.719</td>
</tr>
<tr>
<td>Zn</td>
<td>Low</td>
<td>-0.273</td>
<td>5</td>
<td>0.796</td>
</tr>
<tr>
<td></td>
<td>Medium</td>
<td>-2.018</td>
<td>5</td>
<td>0.100</td>
</tr>
<tr>
<td></td>
<td>High</td>
<td>-0.278</td>
<td>5</td>
<td>0.792</td>
</tr>
</tbody>
</table>

**DISCUSSION**

**Spatial variation in Ulva spp. trace metal burden between sites in Moss Landing, California**

Spatial variability in metal concentrations in *Ulva* spp. has been observed in previous studies, which can be due to a variety of factors. Brown *et al.* (1999) concluded that variation in copper and zinc concentrations in *Ulva* spp. from Otago Harbor, New Zealand, reflected variability in soluble metal concentrations in surface waters. The
authors cited two other studies that reported surface water copper and zinc concentrations (i.e. Dickson and Hunter 1981; Hunter and Tyler 1987) and found that anthropogenic, riverine, and atmospheric inputs could not account for the magnitude of metal content in that water body. They concluded that dredging activities and resuspension of sediments due to storms caused the release of particle-bound metal from the substrate. In another study, Ho (1990) demonstrated spatial variability in metal concentrations in *Ulva lactuca* from intertidal sites around the island of Hong Kong. The author found that *U. lactuca* collected from polluted industrial and urban centers reflected 1.8 to 4.6 times the mean metal burden of comparatively unpolluted, rural sites for Mn, Fe, Ni, Cu, Zn and Pb.

A spatial snapshot of metal distribution in *Ulva* in the Moss Landing area revealed elevated arsenic, cadmium, copper, nickel, lead, selenium, and zinc concentrations relative to crustal abundances (Korte 1999) at all sites in my study area. Samples from the Salinas River Channel exhibited significantly higher chromium, manganese, and lead concentrations relative to the other three sites (Fig. 2A & B). *Ulva* from the Salinas Channel and Moss Landing Harbor both had elevated arsenic, copper, and zinc relative to the other two sites. The elevated zinc and copper are likely artifacts of sacrificial anodes and anti-fouling paint on boat hulls and/or pilings in Moss Landing Harbor.

Though the sites in this study were all within connected water bodies, there were distinct chemical, physical, and oceanographic differences between sites. The average dissolved nitrate values reported at the Salinas Channel site are regularly orders of magnitude higher than all other sites sampled (Monterey Bay Aquarium Research
Institute’s Land/Ocean Biogeochemical Observatory (LOBO) network). Differences in ambient nitrate concentrations between sites may play a role in metal uptake by *Ulva*; elevated metal burden in samples from the Salinas River could be due to higher rates of productivity resulting from extreme nitrate loading. It is also possible that there were differences in the amount of dissolved trace metals in surface waters between sites. The Salinas Channel is a terminal segment for the Salinas River and Tembladero Slough, which carries sediments from upstream mountains and agricultural fields. The damming and degradation of riparian and wetland habitats has resulted in erosion-prone embankments along the Salinas River, greatly increasing the agricultural effluent and sediment discharge downstream in the Salinas Channel (Farnsworth and Milliman 2003).

The water of the Tembladero Slough originates from an agricultural reclamation ditch and creeks that, together, form the Gabilan Watershed. This watershed is significantly influenced by agricultural activities in the Salinas Valley. The Salinas Channel is a shallow, muddy basin with anoxic pore waters, which lead to a chemically reducing environment. This may lead to the release of particle-bound metals, which could diffuse up through sediments, making them more readily incorporated into and/or adsorbed by *Ulva* spp.

The biogeochemical and oceanographic complexity of this nearshore system undoubtedly has an impact on resident foodwebs and is, in part, reflected in the metal composition of one of the predominant primary producers: *Ulva* spp. Further studies are needed to explore the cause/s of the spatial variability found in this study, as well as the potential impacts on resident invertebrate and fish populations. Understanding the
natural variability in *Ulva* metal content in this local system, as well as within and among blades, informs the interpretation of induced variability in laboratory experiments.

**Trace metal uptake by *Ulva* spp. as a function of tissue nitrogen and media nitrate**

In a study by Rosenberg and Ramus (1982), an increase in inorganic nitrogen in the thallus of *Ulva* spp. was accompanied by an increase in soluble organic nitrogen, a portion of which was in the form of proteins and possibly free amino acids. These nitrogen reserves were depleted in proportion to algal growth rate. With further protein analysis, the authors demonstrated the role of soluble tissue nitrogen in the production of photosynthetic pigments. Hanisak (1983) explained that nitrogen is an important component of proteins, nucleic acids, and cell components such as carboxylating enzymes and photosynthetic pigments. Thus, nitrogen availability can impact metabolism by influencing photosynthetic capacity.

Interestingly, although these experiments were carried out under eutrophic conditions, the results suggest an effect of nitrogen on metal uptake by *Ulva*. A broad range of “saturating” media nitrate has been reported for *Ulva* spp., from 20 to 85 µmol N (g DM)^-1 h^-1, suggesting Michaelis-Menten uptake kinetics (Lavery and McComb 1991; Pedersen and Borum 1997; Naldi and Viaroli 2002). However, a study by Cabello-Pasini (2005) demonstrated an unabated increase in both electron transport rate and gross photosynthesis in *Ulva rigida* as media nitrogen is increased from 0 to 50 µM. It is unknown to what concentration this trend continues. A study by Rivera and Graham (unpub. data) reported no apparent saturation point for nitrogen in *Ulva*. The saturation
points and uptake kinetics for *Ulva* spp. in extremely eutrophic systems is relatively unknown. The results of this study show increasing arsenic and manganese uptake with an increase in both tissue and media nitrate, suggesting that *Ulva* continues to respond to changes in nitrogen, even in replete environments.

When examining the effects of tissue nitrogen and media nitrate on arsenic uptake by *Ulva* spp. in this study, a weakly significant positive relationship was revealed. Arsenic is a phosphorous analogue, and could possibly be behaving as a nutrient in both situations (Crane 1953; Wolfe-Simon et al. 2009; Wolfe-Simon et al. 2011). When tissue nitrogen or ambient nitrate levels are high, growth rates and photosynthesis increase; for balanced growth of marine primary producers, this demands the uptake of nutrients in accordance with the Redfield Ratio. According to this stoichiometric ratio, nitrogen and phosphorus occur in marine organisms in a proportion of 16:1. It is possible that in the absence of phosphate enrichment to match the nitrogen reserves in the case of the first experiment, and the nitrate additions in the second, the algae absorbed arsenic in place of phosphorus.

Tissue nitrogen and media nitrate related positively with manganese uptake in *Ulva* in this study. The percent change in manganese was also significantly directly correlated with tissue nitrogen. Manganese is an important element in photosynthetic oxygen evolution (Rai et al. 1981), and it is a part of the Kreb’s cycle enzymes malic dehydrogenase and oxalosuccinic decarboxylase. Manganese atoms are the core of water-oxidizing centers in in photosystem II, thus playing an integral role in electron transport (Debus 1992). It has also exhibited influence in the maintenance of chloroplast
structure (DeBoer 1981). Superoxide dismutase is a protein that is associated with metals such as manganese, and is vital in removing toxic superoxide radicals that are produced during photosynthetic oxygen evolution (Burnell 1988). In this study, manganese uptake could be reflecting an increase in growth and/or photosynthesis concomitant with nitrogen reserves and media nitrate supply.

Zinc uptake was greatest in samples with the highest tissue nitrogen. Though these results were statistically insignificant, zinc has been shown to maintain the structure of ribosomes and play a role in photosynthesis as a component of carbonic anhydrase (DeBoer 1981; Rai et al. 1981). This might explain higher zinc uptake in tissues with greater nitrogen reserves, which likely have a higher photosynthetic capacity than those Ulva tissues with lower tissue nitrogen. No effect of tissue nitrogen was observed for lead or zinc uptake, nor was there a relationship between media nitrate and lead or zinc in Ulva. Lead is a non-essential metal, and plays no physiological role in algae, possibly explaining its uptake being independent of any change in nutrients. A study by Lee and Wang (2001) lead to a similar result for zinc when testing the effects of media nitrate on metal accumulation in Ulva fasciata.

Enrichment factors for Ulva spp. for all metals of interest were positive, indicating bioaccumulation, with lead and zinc being accumulated to the greatest degree. Alginic acid in brown algal cell walls has an exceptionally high affinity for lead (Haug 1961) relative to other metals; it is possible that sulfated polysaccharides in the cell walls of Ulva passively adsorb lead to a similar extent. Zinc, however, is known to be actively
taken up against large intracellular concentration gradients (Eide et al. 1980); therefore some other mechanism must be responsible for its relatively high enrichment factor.

Many coastal environments are exposed to both nitrogen and metal enrichments due to anthropogenic and natural, terrigenous inputs. The chemical interaction/s between these inputs have varying consequences for sediments, water quality and marine and estuarine organisms. If tissue nitrogen or media nitrate were enhancing metal uptake in a globally dominant primary producer such as Ulva, many coastal marine foodwebs could be impacted. No previous studies have explored the relationship between metal accumulation in macroalgae and tissue nitrogen; this study revealed a positive relationship for both tissue nitrogen and media nitrate and arsenic and manganese uptake by Ulva spp.

**Trace metal transfer to Idotea resecata**

Baseline I. resecata individuals reflected a significantly different metal burden than those in both “contaminated” and “recovery” diet studies. Interestingly, arsenic decreased from baseline values. It is apparent that they are exposed to a level of arsenic in the water or in their natural diet of Macrocystis pyrifera (Point Pinos, California) sufficient for them to bioaccumulate to such a high level. The subsequent decrease could be due to the starvation period that all individuals underwent to purge their digestive tracts before treatments were applied. It is possible that the I. resecata were able to detoxify their tissues of arsenic before they received the “contaminated” Ulva diet.
Manganese and zinc were significantly elevated in *I. resecata* from all treatments when compared to baseline samples. Though insignificant, lead in the *I. resecata* fed a “contaminated” diet also seemed to increase from baseline values. This trend demonstrates accumulation of metals by these invertebrates from their environment. In a study by Radenac *et al.* (2001) the bioaccumulation and toxicity of zinc and lead in their dissolved form was demonstrated in sea urchin embryos (*Paroecentrotus lividus*). Norwood *et al.* (2006) found arsenic and manganese to bioaccumulate with increasing dissolved metal concentrations in the benthic amphipod, *Hyalella azteca*. Though these studies did not explore dietary uptake and accumulation, their results support the observed bioaccumulation of arsenic, manganese, lead and zinc by invertebrates. These trends could apply to the dietary uptake observed by *I. resecata* in this study.

No significant difference in *I. resecata* tissue metal concentration was exhibited among control, low, medium or high metal diet treatments in the “contaminated” or “recovery” diet trials. However, in the “contaminated” diet study, a slight positive trend was observed for arsenic and manganese that implied bioaccumulation. Studies have shown both arsenic and manganese to bioaccumulate in algae (Sanders *et al.* 1989), invertebrates (Barwick and Maher 2003; Meador *et al.* 2004) and fish (Patrick and Loutit 1978; Barwick and Maher 2003). It is possible that arsenic and manganese were being accumulated in this study, and would be passed on and accumulated in predators of *I. resecata* in a natural system. Though insignificant, *I. resecata* showed a weak depuration of arsenic when fed a “recovery” diet (p=0.083); this was consistent with the decrease in arsenic seen from baseline individuals to those fed with the “contaminated” diet, which
possibly had lower arsenic content than their natural diet of *M. pyrifera*. Manganese concentrations did not change from “contaminated” to “recovery” diets in this study. Norwood *et al.* (2006) found that manganese elimination rates decreased with total body concentration of the metal in the brackish, benthic amphipod *Hyalella azteca*. The authors proposed that metal uptake and elimination rates could be saturating based on total body concentration of the metal, perhaps independent of the availability of binding sites on/in the organism. It is possible that the dietary concentration of manganese presented to *I. resecata* in this study was high enough to saturate its capacity for elimination, resulting in a lack of recovery upon receiving an uncontaminated food source.

Lead and zinc concentrations in *I. resecata* did not differ among treatments nor between “contaminated” and “recovery” diets. Lead concentrations in *I. resecata* increased from baseline numbers demonstrating dietary accumulation; however, the high variability among the contaminated diet treatments and comparison to recovery means were difficult to interpret. High variability may have masked a treatment effect, but additional studies are needed to clarify these results. In regards to zinc, Ahsanullah and Williams (1991) found that, in the marine amphipod *Allorchestes compressa*, zinc did not accumulate linearly with dietary metal content but rather with dissolved metal content in the media. This is presumably due to internal metabolic regulation of essential metals (White and Rainbow 1984). In *Idotea baltica*, zinc accumulates in the hepatopancreas at levels that are independent of the external concentrations (Gambardella *et al.* 1998). In this study, *I. resecata* may have similarly been able to regulate the zinc concentration in
their tissues across treatments, thus eliminating any effect of variable dietary zinc content.

The spike concentrations used in this study were large relative to most natural systems, yet less than metal concentrations of sediments in Elkhorn Slough, where the Ulva was collected (Nelson 2011). Nonetheless, 41% of ocean systems are now significantly and negatively impacted by anthropogenic activity (Halpern et al. 2008) and perturbed metal and nutrient values are becoming the norm in coastal systems. Manganese (0.065 – 0.65 ppm) concentrations in this study are relevant when compared to 0.255 ppm in San Francisco Bay (SFEI, n.d.). Lead (0.0075 – 0.075ppm) and zinc (0.05 – 0.5ppm) concentrations are comparable to concentrations in urban stormwater runoff reported by Gobel et al. (2007) diluted to 10% (Pb: 0.0525 ppm; Zn: 0.2 ppm), which is a relevant stormwater dilution in offshore surface waters (Bay et al. 2003). The arsenic spike in this study was higher than naturally occurring concentrations. A future study with a greater spread of dietary metal concentrations, starting at lower values, could clarify trends in the uptake of these metals by I. resecata. Repeating this experiment over a longer time span and sampling the molts of these invertebrates could elucidate the rate of bioaccumulation and possible purification mechanisms. Pourang et al. (2004) found that most metals accumulate to some degree in the exoskeleton of shrimp species of the genus Penaeus. The authors did not examine arsenic, but for lead, zinc and manganese, it is possible that some portion of these trace metals accumulated in the exoskeleton of Idotea individuals in this study, and were subsequently removed through molting. This mechanism may have complicated the results of this study. Additionally, conducting
metal analyses on the algal tissue after the spikes are administered would result in a more accurate picture of bioaccumulation and/or magnification trends.

CONCLUSION

Both macro and micronutrients play complex and inter-related physiological roles in algae. This picture becomes more complex with environmental enrichments, whether natural or anthropogenic, toxic or non-toxic. In parts of Elkhorn Slough and the Salinas Channel, as is typical in eutrophic estuaries globally, nitrogen enrichments from agriculture are orders of magnitude greater than any natural influence, leading to extensive seasonal algal blooms of *Ulva* spp. (Caffrey *et al.* 2007). Aside from its prolific nature, *Ulva* is particularly morphologically and physiologically well-suited for nitrogen uptake, with a high surface area to volume ratio (Rosenberg and Ramus 1984) and exceptionally high capacity for nitrogen uptake and storage (Naldi and Viaroli 2002), making it one of the most efficient genera for nitrogen metabolism among algae. Studies on saturation kinetics of nitrogen in *Ulva* may lead one to believe that differences in tissue nitrogen or relatively small additions of nitrate in such a nutrient rich scenario would not result in any chemical consequences for this abundant primary producer. However, despite an environment replete with media nitrate, both tissue nitrogen and minor additions of media nitrate seem to partially drive arsenic and manganese uptake in *Ulva* spp.

Studies have revealed that arsenic and lead, both non-essential metals, begin to have toxic effects in *Ulva* at dissolved concentrations of 3.75 and 5.0 ppm respectively,
inhibiting photosynthesis, competing with nutrients such as phosphorus, inhibiting the flow of electrons in mitochondria and reducing chlorophyll $\alpha$ (Rai et al. 1981; Knauer et al. 1999). Zinc and manganese can have toxic consequences at 10 and 30 ppm, respectively, with zinc inhibiting manganese uptake, compromising the integrity of plasma membranes, and manganese causing asymmetric cell division, and inhibiting gamete formation and algal growth (Rai et al. 1981).

More importantly, however, is the toxic effect that these incorporated metals would have on higher trophic levels. *Ulva* can absorb high concentrations of both essential and non-essential metals making those elements available in their tissues. As is common in many eutrophic systems, the fate of those tissues after a bloom is to become particulate organic matter in sediments. Inverts that graze directly on *Ulva* such as *Idotea* spp., and a variety of amphipods (Kamermans et al. 2002), and those that feed in sediments (such as polychaete worms) could be impacted by high metal concentrations in their diet. Unfortunately, very few studies have been done to examine the toxicity of these metals to invertebrates through dietary sources. In addition, a variety of fish and birds graze directly on *Ulva* spp., and could also be vulnerable indirectly when preying upon organisms that feed on *Ulva* (Ramer et al. 1991; Barry 1993; Barry et al. 1996).

Overall, results of this study reveal that nitrogen continues to have an effect on arsenic and manganese uptake, even under nutrient rich conditions, and in turn, these could be bioaccumulating in an important invertebrate grazer. This study could prove useful from a bioremediation perspective, both for metal and nitrogen remediation. *Ulva* spp. is an opportunistic genus that proliferates in nearshore and estuarine ecosystems
where chemical influences from watersheds, municipal outfalls, atmospheric deposition, road and highway runoff, and industrial, municipal and agricultural wastes converge. The interaction and ultimate fate of these inputs are largely determined by the biogeochemical processes that occur in these systems. Whether *Ulva* is a step along the path of the biopurification or biomagnification of these metals is yet to be known. Future studies, which explore the influence of nitrogen on metal uptake in *Ulva* using a wider range of dissolved metal exposure, and test the impacts of dietary uptake by organisms from various trophic levels, will be vital in completing this picture.
LITERATURE CITED

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