

**COPPER CONTAMINATION INFLUENCES THE EFFECTIVENESS OF
SEAWEED INDUCIBLE DEFENSES**

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San Diego State University

In Partial Fulfillment

of the Requirements for the Degree

Master of Science in Biology

with a Concentration in

Ecology

by

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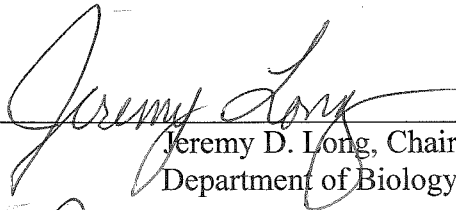
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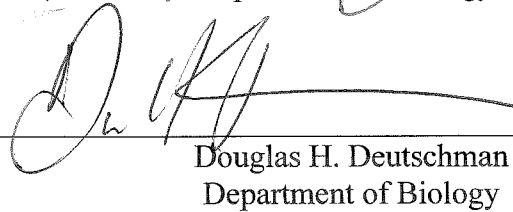
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Copper Contamination Influences the Effectiveness of Seaweed Inducible

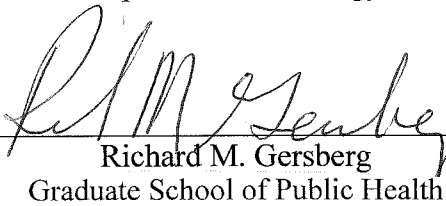
Defenses



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ABSTRACT OF THE THESIS

Copper Contamination Influences the Effectiveness of Seaweed
Inducible Defenses

by

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Master of Science in Biology with a Concentration in Ecology
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Seaweed-herbivore interactions are often mediated by environmental conditions, yet the roles of emerging anthropogenic stressors on these interactions are poorly understood. For example, chemical contaminants have unknown consequences on seaweed inducible resistance and herbivore response to these defenses despite known deleterious effects of contaminants on animal inducible defenses. Here, we investigated the effect of copper contamination on the effectiveness of inducible resistance in the brown seaweed, *Silvetia compressa*, to a snail herbivore, *Tegula funebris*. We examined the effectiveness of inducible resistance for organisms exposed to copper at two time points, either during induction or after herbivores had already induced seaweed. Under ambient conditions, non-grazed tissues were more palatable than grazed tissues. However, copper additions negated the preference for non-grazed tissues regardless of the timing of copper exposure, suggesting that copper decreased the effectiveness of inducible defense. This effect of copper was only evident during five day exposures, and was largely a result of copper influencing snail behavior. Thus, contaminants can stress communities by reducing the effectiveness of seaweed inducible defenses. Given the ubiquity of seaweed inducible resistance and their potential influence on herbivore performance and populations, we hypothesize that reduced effectiveness of seaweed inducible defenses in the presence of copper contamination can have cascading effects on marine communities.

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INTRODUCTION

Environmental stressors, occurring together or in sequence, may create surprising, interactive effects on communities (Paine et al. 1998, Crain et al. 2008). Unfortunately, our ability to predict these ecological surprises (Paine et al. 1998) is limited due to a historic bias for studies examining the effects of individual stressors. For example, the grazing history of seaweed individuals, especially brown algae, often influences their susceptibility to future herbivory via induced resistance (Pavia and Toth 2000, Taylor et al. 2002, Toth and Pavia 2007). Likewise, abiotic stressors such as depleted and enriched nutrient levels (Yates and Peckol 1993, Pavia and Brock 2000, Weidner et al. 2004), desiccation (Renaud et al. 1990, Cronin and Hay 1996), UV irradiance (Cronin and Hay 1996, Pavia and Brock 2000), temperature (Dethier et al. 2005), and light availability (Kubicek et al. 2011, Weinberger et al. 2011) can directly and indirectly influence herbivory on seaweeds. However, the outcomes of induced seaweed resistance are uncertain given that most studies tested for their presence in the absence of abiotic stress. This is particularly true for emergent stressors that have unknown impacts on inducible defenses but might commonly influence their effectiveness via information disruption (Lürling and Scheffer 2007, Lürling 2012).

Chemical contaminants are emergent stressors to aquatic habitats that may influence plant-herbivore interactions via changes in inducible resistance. Although studies examining impacts of contaminants on plant resistance are rare, several studies demonstrated negative impacts of contaminants on induced defenses of animals. For example, contaminants reduced the expression of defensive structures of *Daphnia* (Hanazato and Dodson 1993, Barry 1998, Hunter and Pyle 2004, Mirza and Pyle 2009) and prevented expression of defensive behaviors in juvenile salmon and trout (Scott et al. 2003, McIntyre et al. 2012). Importantly, contaminant-mediated weakening of responses to alarm cues increased salmon susceptibility to trout predators (McIntyre et al. 2012). Similar effects of contaminants on inducible resistance of aquatic plants and seaweeds might occur via at least three pathways. First, contaminants can decrease grazing rates (Hassell and Rushton 1982, Weeks 1993, Martens and Boyd 2002) and subsequently weaken the cue that initiates these defenses. Second,

contaminants acting as stressors can directly affect plant traits (Renaud et al. 1990, Yates and Peckol 1993, Cronin and Hay 1996), some of which may be related to these defenses. Third, contaminants may interfere with chemoreception and thereby prevent herbivores from recognizing inducible defenses of macrophytes (Lürding and Scheffer 2007, Lürding 2012).

In the only known test of the effects of chemical contaminants on inducible seaweed defenses, copper exposure did not alter phlorotannin production in the seaweed *Ascophyllum nodosum* (Toth and Pavia 2000b). Previous studies by these authors demonstrated that phlorotannins, a class of polyphenolic compounds, became elevated after herbivore attack as part of an inducible response (Pavia and Toth 2000, Toth and Pavia 2000a). However, Toth and Pavia (2000b) only examined the direct effect of copper on phlorotannin production as opposed to the indirect effect of copper on phlorotannins via changes in herbivore behavior. Given that many of the pathways by which contaminants affect inducible resistance are mediated by changes in herbivore behavior (see above), we need studies that test for these effects.

We investigated the influence of copper contamination on the effectiveness of seaweed inducible defenses. While copper plays a key role in the metabolism of aquatic invertebrates (Rainbow 2007), human activities can elevate copper within coastal embayments to levels that negatively affect marine organisms (Schiff et al. 2005). Using a seaweed that displays inducible resistance to herbivores, we examined the separate impact of copper contamination on the elicitation of these defenses and on their effectiveness towards subsequent herbivory.

MATERIALS AND METHODS

STUDY ORGANISMS AND EXPERIMENTAL MAINTENANCE

To examine the influence of chemical contamination on the effectiveness of seaweed inducible defenses, we selected a model seaweed (*Silvetia compressa*) that reliably becomes less palatable to snails (*Tegula funebris*) in uncontaminated scenarios (Jones and Long, *unpublished data*). Contaminant effects on seaweed inducible resistance might be particularly common in brown algae (Phaeophyceae) because these algae (1) commonly display inducible resistance (Toth and Pavia 2007) and (2) contain large amounts of polysaccharides with high affinities for heavy metal pollutants (Davis et al. 2003, Evans and Edwards 2011).

We collected all organisms from the high intertidal zone of La Jolla, California, USA (32°48'39" N, 117°16'10" W) and transported them to San Diego State University's Coastal and Marine Institute Laboratory. Prior to experiments, we maintained organisms for 72 h in an outdoor, flow-through seawater system with tanks that drained and flooded to mimic semi-diurnal tidal conditions. Snails were fed *Silvetia* ad libitum and then starved for 24 h leading up to experimentation to ensure even satiation across all treatments (Borell et al. 2004).

We created copper solutions by diluting a stock solution (1 mg/L copper chloride, CuCl₂) with filtered seawater collected from the pier at Scripps Institution of Oceanography. Stock solutions were created with analytical grade copper chloride (CuCl₂, Sigma-Aldrich Co.). We verified the concentration of these solutions through an independent laboratory (Enviromatrix Analytical Inc., San Diego, CA). Stock solutions were stored at 12°C to minimize reduction of chemical ions in solution. All containers and materials used for chemical exposure were acid washed using a 10% Nitric Acid solution and thoroughly rinsed with deionized water between each experimental run (USEPA 2002). All experimental seawater was passed through a 0.45-micron filter to remove particulates that could decrease copper bioavailability via precipitation. Since collected seawater may have contained low

levels of copper, we refer to our controls as “Ambient” as opposed to “No Copper” treatments. Our “Elevated” treatments increased copper above these background levels.

Seaweed induction experiments followed the standard approach for assessing inducible defenses, whereby defenses were elicited during an induction phase and the effectiveness of these defenses were measured during a separate bioassay phase (Pavia and Toth 2000, Taylor et al. 2002, Long et al. 2007, Flöthe et al. 2014). The induction phase lasted for 14 d to facilitate comparisons with other studies of inducible seaweed defenses (Toth and Pavia 2000a, Taylor et al. 2002, Long and Trussell 2007). Because copper effects may depend upon the timing of exposure relative to the timing of induction, we examined the consequences of copper pollution during both the induction phase and bioassay phase.

EFFECTS OF CONTAMINATION DURING INDUCTION

To investigate the influence of copper on the elicitation of inducible defenses in *Silvetia*, we conducted the induction phase under Ambient or Elevated copper levels. During this phase, *Silvetia compressa* was grown individually in the presence or absence of realistic densities of *Tegula funebris* (8 *Tegula* per 20 ± 0.2 g *Silvetia*, E. Jones, *personal communication*) for 14 d. Individual seaweeds were placed in independent replicate containers (1 L volume; 190 x 165 x 91 mm) and no snails were added to Non-grazed controls (n=20). Plastic window screen mesh (6 mm openings) covered openings on all containers to prevent snail escape. Treatments were assigned randomly with proper interspersal to minimize any potential positional effects. All replicates were maintained in a larger recirculating seawater system (19 ± 1.0 °C) where water was replenished daily to minimize nutrient and copper loss, and prevent waste buildup. Experimental mesocosms were illuminated by fluorescent tubes (85.6 ± 5 $\mu\text{mol photons} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ (PAR); Philips Natural Light 40W) placed on a 12:12 hr light/dark cycle.

Copper was manipulated in our experiments with the addition of copper chloride (CuCl_2) solutions to Elevated copper treatments (Cu concentration=50 $\mu\text{g/L}$). This concentration is representative of high levels that herbivores may encounter in coastal systems. For example, in San Diego marinas, dissolved copper concentrations typically range between 0-21 $\mu\text{g/L}$ (Schiff et al. 2005) but can reach 66.0 $\mu\text{g/L}$ in storm water (Eisler 1998, Schiff et al. 2001).

Following the 14 d induction phase, *Silvetia* were removed from Ambient and Elevated treatments, and offered to another set of *Tegula* (3 *Tegula* per 3 ± 0.1 g *Silvetia*) in a paired choice bioassay for 5 d (Grazed vs. Non-grazed). Grazed and Non-grazed pieces were marked with colored rubber bands and paired within either Ambient or Elevated treatments. We measured the initial wet mass of each seaweed (T_i). After 5 d of grazing, we measured the final wet mass of each seaweed (T_f). Grazing rates were corrected for growth in an equivalent number of snail-free controls using the formula $T_i(U_f/U_i) - T_f$ where U_i and U_f represented the initial and final masses of Non-grazed tissue from controls, respectively (see Peterson and Renaud 1989).

EFFECTS OF CONTAMINATION AFTER INDUCTION

To examine the influence of copper contamination occurring after induction on the effectiveness of these defenses, we repeated the above experiments with two major changes. First, the induction phase was only conducted in Ambient conditions. Second, organisms were exposed to Ambient or Elevated copper levels during the bioassay phase.

Silvetia was collected at the end of a new 14 d uncontaminated induction phase and subsequently offered to another set of *Tegula* (3 *Tegula* per 3 ± 0.1 g *Silvetia*) in a choice assay. During this 5 d bioassay phase, Grazed and Non-grazed tissues were offered as pairs to *T. funebris* in Ambient or Elevated copper exposures (50 $\mu\text{g/L}$). Under Ambient conditions, *Silvetia* displaying induced resistance after growing in the presence of snails during the induction phase should be less palatable than seaweeds that grew in the absence of snails (Jones and Long, *unpublished data*). To prevent the release of copper into our seawater system, all bioassays ran for 5 d in a closed seawater system within temperature- and light-controlled growth chambers (19 ± 1.0 °C, 12:12 hr light/dark cycle). We replaced the water in these containers daily to maintain nutrient and copper levels (*sensu* Toth and Pavia 2000a).

DECOUPLING ORGANISMAL EXPOSURE AND INFLUENCE OF EXPOSURE DURATION

Because copper reduced the effectiveness of seaweed inducible defenses, we conducted experiments to identify the mechanisms underlying this change. In particular, we studied the role of exposure duration to contaminants and the impact of only exposing snails to contaminants. We grew *Silvetia* with and without herbivores for 14 d during an

uncontaminated induction phase (8 *Tegula* per 20 ± 0.2 g *Silvetia*). Grazed and Non-grazed seaweeds from this induction phase were paired during choice bioassays (3 *Tegula* per 3 ± 0.1 g *Silvetia*) conducted under four different copper exposures (n=20). As before, the Ambient treatment consisted of filtered seawater without copper additions. We expected herbivores to prefer Non-grazed seaweeds in this Ambient treatment. The three remaining treatments (Acute, Chronic, Chronically exposed snails) exposed at least one organism to copper. The Acute treatment exposed both snails and seaweed to elevated copper (50 $\mu\text{g/L}$) for one day, and then measured snail feeding preference for Grazed and Non-grazed tissues in uncontaminated conditions for the duration (4 d) of the bioassay phase. In contrast, the Chronic treatment exposed both snails and seaweeds to copper for the entire duration (5 d) of the bioassay phase.

Our fourth treatment, Chronically exposed snails, allowed us to examine the influence of chronic exposure of snails independent of effects on seaweeds. We created a stock of contaminated snails in a separate tank containing *Silvetia* and seawater with 50 $\mu\text{g/L}$ CuCl_2 . For the duration of the 5 d bioassay phase, contaminated snails (3 *Tegula* per day) were then placed into uncontaminated bioassay treatments containing paired Grazed and Non-grazed *Silvetia*. Snails were removed from experimental mesocosms during daily water changes and new sets of snails were rotated in from the contaminated stock to maintain consistent levels of herbivore contamination. Snails were not re-used in the bioassay on multiple days.

STATISTICAL ANALYSIS

Adjusted masses of Grazed and Non-grazed *Silvetia* tissues were tested for meeting the assumptions of normality and homogeneity of variances. We examined the influence of copper on snail feeding rates by comparing corrected consumption rates in Ambient versus Elevated treatments during the induction phase of the experiment examining effects of contamination on elicitation. These feeding rates were compared with a two-tailed, two-sample t-test. We used separate repeated measures ANOVAs to examine the effect of copper pollution on feeding preference for Grazed and Non-grazed tissues during each of our three major experiments. The repeated measures analysis was necessary due to lack of independence between tissue types during the paired feeding assays. This statistical method has been increasingly utilized when choice assays are used in more complex designs (i.e.

those with multiple factors; Dixon et al. 2014, Flöthe et al. 2014). These repeated measures ANOVAs examined the impact of Copper Contamination Status (between-subjects factor; Ambient, Elevated) and Induction Status (within-subject factor; Grazed, Non-grazed) on the amount consumed during the bioassay. To better understand interactions between Copper Contamination and Induction Status, we conducted post-hoc comparisons with t-tests. We conducted separate paired t-tests at each level of Copper Contamination to identify if snails responded to the two tissue types (Grazed, Non-grazed) differently. Because we were also interested in how copper exposure affected feeding on the two tissue types (i.e. does copper decrease feeding on Grazed, Non-grazed, or both?), we also conducted two-tailed t-tests at each level of tissue type (Grazed, Non-grazed). For the last repeated measures analysis comparing Ambient, exposure duration (Acute, Chronic) and decoupled organismal exposure (Chronically exposed snails), paired t-tests were used as post-hoc analyses to compare Grazed vs. Non-grazed seaweeds within each of the four treatments.

RESULTS

Elevated copper concentration decreased snail feeding by approximately 66% (Figure 1, $t_{38}=4.010$, $p<0.001$). Snail feeding preferences during the bioassay phase were also influenced by previous exposure of snails and seaweeds to copper during the induction phase (Figure 2, Table 1). Specifically, copper contamination interacted with induction status to influence snail feeding. Consistent with preliminary observations (Jones and Long, unpublished data), snails preferred Non-grazed seaweeds in treatments that had not been exposed to copper in the induction phase (Ambient treatment, $t_{19}=7.127$, $p<0.001$). In contrast, snails did not distinguish between Grazed and Non-grazed seaweeds when previous grazing during the induction phase occurred in the presence of elevated copper levels (Elevated treatment, $t_{19}=0.803$, $p=0.432$). Thus, elevated copper during the induction phase influenced the effectiveness of seaweed inducible defenses. Interestingly, previous copper exposure during the induction phase appeared to act primarily by decreasing feeding on Non-grazed seaweeds ($t_{38}=6.221$, $p<0.001$). Previous exposure did not influence feeding on Grazed seaweeds ($t_{38}=1.616$, $p=0.114$).

Copper exposure after induction (i.e. during the bioassay phase) interacted with grazing history of the seaweed (Grazed vs. Non-Grazed) to determine herbivore preference (Figure 3, Table 2). Consistent with the previous experiment, snails preferred Non-grazed seaweeds in the Ambient copper treatment ($t_{19}=3.213$, $p=0.005$). In contrast, snails did not distinguish between Grazed and Non-grazed seaweeds during bioassays with Elevated copper concentrations ($t_{19}=0.698$, $p=0.494$). Similar to the previous experiment, copper contamination decreased herbivore feeding on Non-grazed seaweeds ($t_{38}=4.248$, $p<0.001$) but not Grazed seaweeds ($t_{38}=0.999$, $p=0.324$) in the bioassay phase.

In the third experiment that examined possible mechanisms by which copper influenced these species, contamination treatment interacted with grazing status to influence feeding rates (Figure 4, Table 3). Post-hoc analyses revealed that snails preferred Non-grazed seaweeds in both the Ambient ($t_{19}=7.538$, $p=0.000$) and Acute exposure treatments ($t_{19}=3.909$, $p=0.001$). However, this preference was eliminated by exposing both snails and

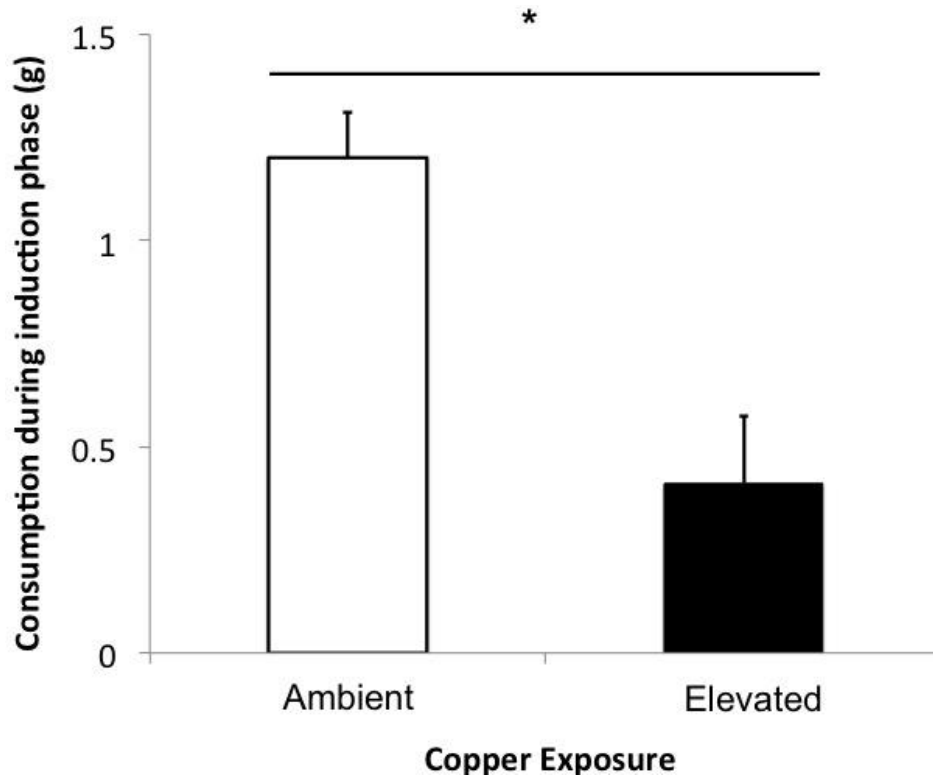


Figure 1. Mean consumption ($\pm$ SE) of *Silvetia compressa* by *Tegula funebralis* under Ambient and Elevated copper exposures. For elevated treatments, both herbivores and seaweeds were exposed to 50 μ g/L Cu for 14 d. Asterisk (*) designates statistical difference of two-sample t-test ($p < 0.05$).

seaweeds (Chronic treatment, $t_{19}=0.931$, $p=0.363$) or just snails (Chronically exposed snails treatment, $t_{19}=0.338$, $p=0.739$) to copper during the bioassay phase.

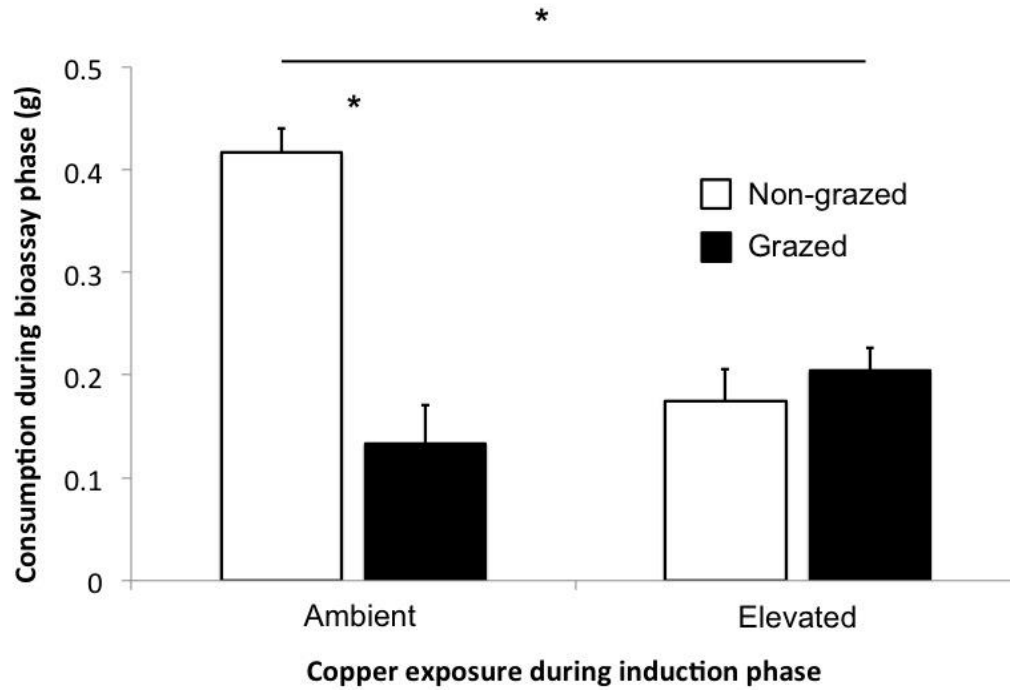


Figure 2. Mean consumption ($\pm$ SE) of grazed and non-grazed *Silvetia compressa* by *Tegula funebralis* during uncontaminated choice bioassays using seaweeds that were previously induced with snails under ambient and elevated copper exposures. Grazed seaweeds are denoted by black bars and non-grazed seaweeds by white bars. Asterisks (*) above horizontal bars designate statistical difference of repeated measures test ($p < 0.05$). Asterisks (*) found above paired Ambient bars designate statistical difference between grazed and non-grazed tissues ($p < 0.05$).

Table 1. Repeated Measures ANOVA for Mean Consumption of Seaweeds (Grazed, Non-grazed) Contaminated in the Induction Phase (Elevated, Ambient) and Placed in a Subsequent Uncontaminated Paired Choice Assay

Source of Variation	SS	df	MS	F	P
Between subjects					
Contamination status	0.145	1	0.145	7.295	0.010
Error	0.756	38	0.02		
Within subjects					
Grazing status	0.324	1	0.324	22.556	0.000
Contamination status	0.487	1	0.487	33.948	0.000
•Grazing status					
Error	0.545	38	0.014		

Note. This analysis had a between-subjects factor (Contamination status) and a within-subject factor (Grazed status). Bold values indicate significance ($p < 0.05$).

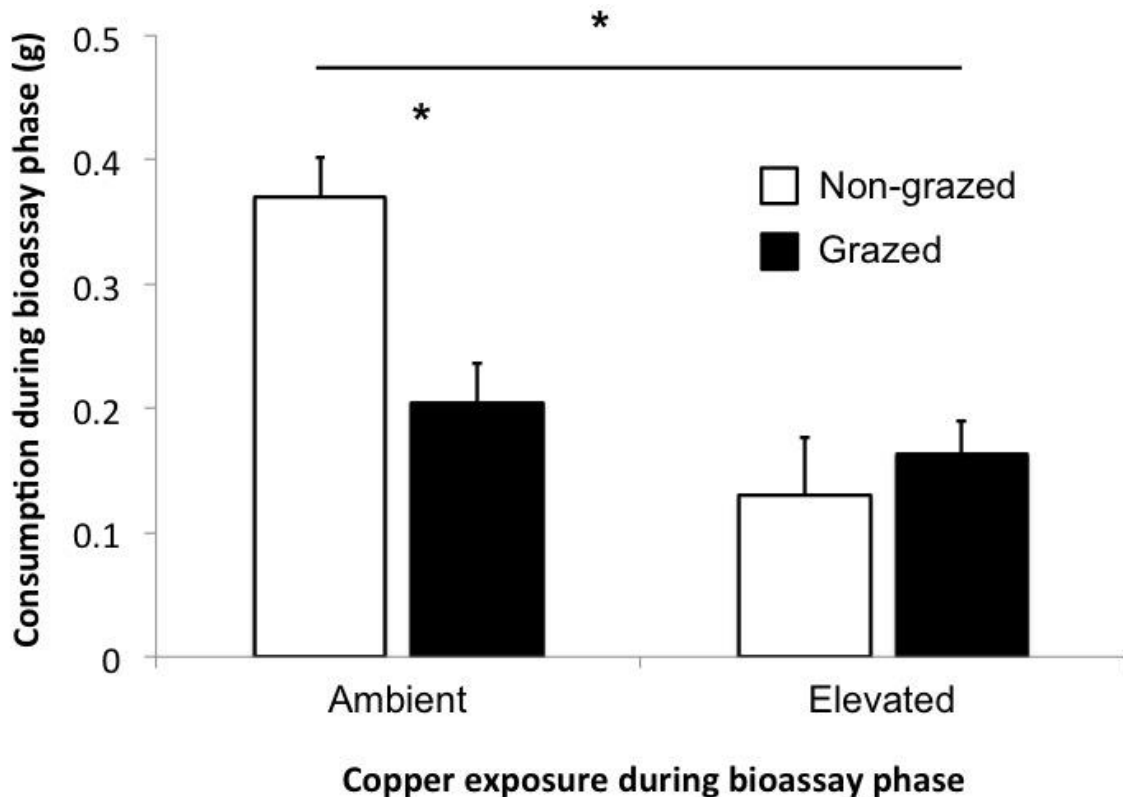


Figure 3. Mean consumption ($\pm$ SE) of grazed and non-grazed *Silvetia compressa* by *Tegula funebris* in choice bioassays under ambient and elevated copper exposures. Induction occurred in the absence of elevated copper levels. Grazed seaweeds are denoted by black bars and non-grazed seaweeds by white. Asterisks (*) above horizontal bars designate statistical difference of repeated measures test ($p < 0.05$). Asterisks (*) found above paired ambient bars designate statistical difference between grazed and non-grazed tissues ($p < 0.05$).

Table 2. Repeated Measures ANOVA for Mean Consumption of Uncontaminated Seaweeds (Grazed, Non-grazed) Placed in a Subsequent Contaminated Choice Bioassay (Elevated, Ambient)

Source of Variation	SS	df	MS	F	<i>P</i>
Between subjects					
Contamination status	0.396	1	0.396	16.555	0.000
Error	0.910	38	0.024		
Within subjects					
Grazing status	0.086	1	0.086	3.445	0.071
Contamination status	0.199	1	0.199	7.923	0.008
•Grazing status					
Error	0.952	38	0.025		

Note. This analysis had a between-subjects factor (Contamination status) and a within-subject factor (Grazed status). Bold values indicate significance ($p < 0.05$).

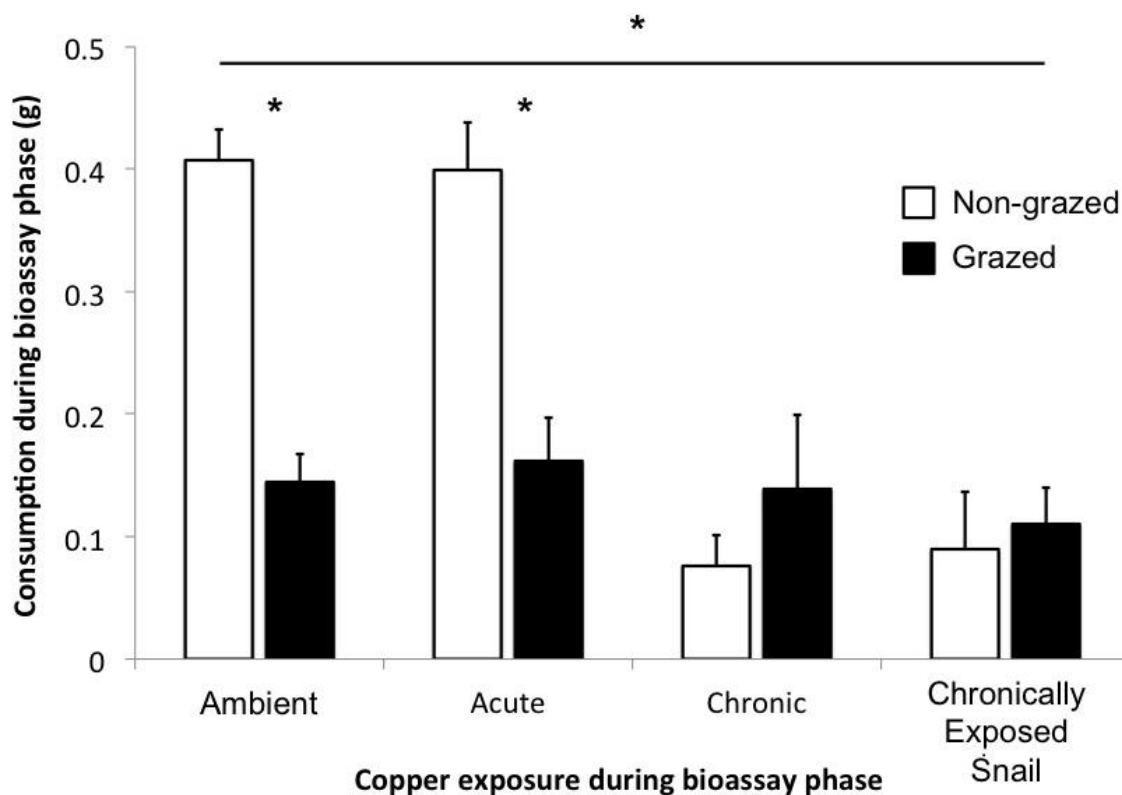


Figure 4. Mean consumption ($\pm$ SE) of uncontaminated grazed and non-grazed *Silvetia compressa* by *Tegula funebralis* in four contaminated choice assays treatments. Exposure treatments in the bioassay phase were as follows: no exposure, acute (exposed for only 1 day), chronic (exposed for 5 days), and a chronically exposed snail treatment whereby uncontaminated seaweed was grazed by chronically contaminated herbivores. Grazed seaweeds are denoted by black bars and non-grazed seaweeds by white. Asterisks (*) above horizontal bars designate statistical difference of repeated measures test ($p < 0.05$). Asterisks (*) found above paired Ambient bars designate statistical difference between grazed and non-grazed tissues ($p < 0.05$).

Table 3. Repeated Measures ANOVA for Mean Consumption of Uncontaminated Seaweeds (Grazed, Non-grazed) Placed in a Subsequent Contaminated Choice Bioassay with Four Treatments (Ambient, Acute, Chronic, Chronically Exposed Snail Only Exposure)

Source of Variation	SS	df	MS	F	P
Between subjects					
Contamination status	1.225	3	0.408	17.476	0.000
Error	1.775	76	0.023		
Within subjects					
Grazing status	0.436	1	0.436	13.25	0.000
Contamination status	0.863	3	0.288	8.748	0.000
•Grazing status					
Error	2.500	76	0.033		

Note. This analysis had a between-subjects factor (Contamination status) and a within-subject factor (Grazed status). Bold values indicate significance ($p < 0.05$).

DISCUSSION

Environmental stressors can change species interactions in surprising ways. In our study, copper contamination reduced the effectiveness of inducible defenses in the brown seaweed, *Silvetia compressa*. Previous grazing reduced *Silvetia* palatability to snails in controls without added copper. However, snail preference for non-grazed tissues disappeared when copper was added during the induction of defenses or during the assessment of these induced defenses in feeding bioassays. This behavioral shift could be caused simply by exposing snails to copper for a chronic, five day period.

The consequences of these findings should be intimately connected to the timing of copper exposure relative to the induction status of seaweeds. If copper exposure occurs while defenses are being induced (or while induced defenses are being maintained), the strength or frequency of induced defenses should weaken because copper decreased overall grazing rates (Figures 1-4). Reduced grazing can lead to weaker inducible defenses because grazing intensity can determine defense strength (Bolser and Hay 1996). Alternatively, if copper exposure occurs after defenses are induced, herbivores may consume a relatively greater proportion of defended tissues (Figures 3-4). Though relatively well understood in terrestrial systems (Karban and Baldwin 1997, Nykänen and Koricheva 2004), the consequences of this diet shift are difficult to predict in aquatic systems given the limited number of studies examining how inducible seaweed defenses affect herbivore performance (Toth and Pavia 2007). However, a single study found that the snail, *Littorina obtusata*, produced less viable eggs when restricted to seaweeds that were previously grazed (Toth et al. 2005). Given that previously grazed *Silvetia* can reduce snail growth and gamete production (Long, *unpublished data*), copper may negatively affect snail populations by preventing snails from avoiding these defended tissues.

Copper may have influenced these species interactions via at least four mechanisms. First, copper contamination may have a general negative effect on the elicitation of inducible plant resistance because copper suppresses grazing – the cue that elicits these responses. For example, copper and zinc decreased feeding rates of amphipods on the brown seaweed

Laminaria digitata (Weeks 1993) and copper-contaminated *Betula pendula* leaves induced starvation in the terrestrial isopod *Porcellio scaber* (Hassell and Rushton 1982). Roberts et al. (2006) found that reductions in feeding of the amphipod *Perampithoe parmerong* on contaminated *Sargassum* habitat reduced overall herbivore survivorship. Similarly, we found that copper decreased snail feeding by ~66%, and this reduced consumption was associated with a loss of seaweed inducible resistance.

Second, copper may prevent herbivores from choosing non-grazed tissues versus grazed tissues because of impaired chemosensory abilities. A growing body of evidence for “information disruption” demonstrates that sub-lethal chemical contamination can act to disturb critical ecological interactions through interferences in predator perception (Lürling and Scheffer 2007). For example, rainbow trout exposed to cadmium fail to respond to alarm chemicals (Scott et al. 2003). More recent studies demonstrated that larval fish and foraging sharks exposed to future ocean acidification scenarios display impaired olfactory and homing abilities (Munday et al. 2009, Dixon et al. 2014). Similarly, our study suggests that copper impaired the ability of snails to choose previously preferred tissues. Interesting to note, copper exposure did not alter herbivore grazing on previously grazed tissues. This response may have stemmed from a threshold level of snail grazing necessary to maintain biological function regardless of stress elicited by both induction and copper exposure.

Third, copper may impair *Silvetia*’s ability to produce inducible defenses. Abiotic stressors can alter seaweed susceptibility to herbivory by modifying seaweed defensive traits (Renaud et al. 1990, Yates and Peckol 1993, Cronin and Hay 1996). In the only potential test of the effect of chemical pollution on seaweed inducible defenses, Toth and Pavia (2000b) discovered that copper exposure did not elicit the production of phlorotannins in *Ascophyllum nodosum*. Although more research is needed, direct negative effects of heavy metals on seaweed defense traits may be unlikely given the well-known ability of brown seaweeds to hyper-accumulate heavy metals without immediate detrimental effects (Roberts et al. 2008).

Fourth, copper accumulation in seaweeds may overwhelm herbivore preference (i.e. an elemental defense *sensu*, Boyd 2010). Some terrestrial plants can experience reduced herbivory after opportunistically accumulating metals (Hay et al. 1994, Martens and Boyd 2002, Jhee et al. 2006, Boyd 2010). In support for this hypothesis, we found that *Silvetia* can

hyper-accumulate copper from contaminated waters ($9.744 \pm 2.485 \mu\text{g/g Cu}$, Warneke, *unpublished data*). This hyper-accumulation of copper in seaweed tissues may have resulted in the continued snail deterrence we observed when contaminated seaweed was placed into a clean water environment.

Environmental stressors can create surprising ecological effects on communities (Paine et al. 1998, Crain et al. 2008). For example, we found that copper contamination removed the effectiveness of seaweed inducible defenses. Copper contamination can disrupt these pathways via potential alterations in herbivore grazing rates, how herbivores perceive defenses, and the elicitation of the actual seaweed defensive mechanism. Although the generality of our research remains to be tested, stressor-mediated disruption of seaweed-herbivore interactions could shape impacted aquatic communities. For example, contamination reduced epifaunal grazing, survivorship, and colonization rates on *Sargassum* in the field (Roberts et al. 2006). Release of herbivore control, facilitated by contaminant-mediated behavioral modifications, could reduce the effectiveness of top-down control by herbivores. In a review of the current literature, Fleeger et al. (2003) found that trophic cascades, resultant of contaminant interruption of grazer control, were found in approximately 60% of manipulative studies. We provide continued evidence of the indirect effects of contaminants on species interactions. However, our results further elude that contaminant-mediated shifts in community composition are inherently more complex. Within the lens of other stressors, such as plant inducible defenses, outcomes of seaweed herbivore interactions influenced by contaminants are heavily reliant on timing of exposure. If contamination occurs during defense induction, herbivore grazing will be suppressed resulting in lower expression of seaweed defenses. Though this might equate to higher seaweed density in the short-term, the magnitude of herbivore grazing will likely increase upon dispersal of the contaminate. This interaction will be nuanced depending on the contaminant depuration rate of the seaweed in question. Conversely, if exposure occurs post-induction, grazer release will result in seaweed proliferation due to loss of herbivore preference. Herbivore fitness may also be impacted with the consumption of a relatively higher proportion of grazed tissue, perpetuating the influence of contaminants through multiple generations (Toth et al. 2005). Understanding how these interactions are differentially impacted within the context of chemical contamination and other

environmental stressors will offer us better insight and predictive capability on how these communities will shift in response to rapidly changing aquatic environments.

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