

The relative strength of an herbivore-induced seaweed defense varies with herbivore species

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ABSTRACT: Although herbivores often interact indirectly via induced trait changes in food resources, ecologists are just beginning to understand the diversity of these interactions in multi-species communities. For instance, while herbivore identity is often important for eliciting trait changes, we know little about how the strength of induced responses varies with herbivore species. This variation is important, as it may affect interactions between herbivore species and, in turn, herbivore populations, community diversity, and the effectiveness of primary producer responses. To investigate herbivore-specific differences in the strength of induced defenses, we exposed the seaweed *Silvetia compressa* (hereafter *Silvetia*) to grazing by isopods (*Idotea wosnesenskii*, hereafter *Idotea*), snails (*Tegula funebris*, hereafter *Tegula*), or both herbivores together. After 2 wk, we compared relative tissue palatability by offering each herbivore a choice between conspecific-grazed tissues and either (1) non-grazed tissues, (2) heterospecific-grazed tissues, or (3) tissues grazed by both herbivores. Both species preferred non-grazed over conspecific-grazed tissues, confirming induced changes in palatability. When we directly compared tissues induced by isopods and snails, *Tegula*-grazed tissues were less palatable than *Idotea*-grazed tissues, suggesting that *Tegula* elicits stronger *Silvetia* responses than *Idotea*. When offered a choice between heterospecific-grazed and non-grazed tissues, both herbivores responded to heterospecific grazing. Thus, while both herbivores decrease *Silvetia* palatability, the strength of the response varies with grazer identity, which we would have not predicted had we only compared grazed and non-grazed tissues. Our results suggest that in communities where herbivores elicit defenses of different strength, interspecific herbivore interactions may be asymmetric, depending on which seaweed phenotypes have been induced.

KEY WORDS: Seaweed–herbivore interactions · Inducible defense · Macroalgae · Asymmetric competition

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INTRODUCTION

Herbivore-induced resistance, common across a variety of taxa, can alter primary producer chemistry, morphology, physiology, growth, and phenology (Karban & Baldwin 1997, Ohgushi 2005, Toth & Pavia 2007). In turn, these phenotypic changes can influence interactions among herbivores with shared food resources and shape herbivore populations and communities (Denno et al. 1995, Ohgushi 2005, Denno & Kaplan 2007, Ohgushi et al. 2007). Although it is well

established that the elicitation of resistant traits, and herbivore responses to prior grazing, are species-specific (e.g. Agrawal 2000, Walling 2000, Long et al. 2007, Molis et al. 2008, 2010, Yun et al. 2010), most comparisons between herbivore species are indirect, based on effect size differences between previously grazed and non-grazed tissues, rather than direct comparisons of grazed tissues. However, because herbivore preferences often depend on food choice, these indirect comparisons may not detect differences in the relative strength of traits induced by

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different herbivore species. Specifically, we lack experiments that use choice bioassays to assess preferences of tissues previously grazed by one herbivore species versus tissues previously grazed by another herbivore species. Thus, while herbivores in a community may induce primary producer traits that vary in type or quantity (e.g. Agrawal et al. 2014), it is unclear whether herbivores respond to this trait variation when choosing between more than one induced phenotype. These qualitative differences may be important, as they have the potential to alter how herbivores interact via these induced traits.

The relative strength of induced primary producer defenses may depend on the identities of the attacking herbivores for several reasons. First, the elicitation of induced traits can vary with the amount of herbivore damage (Karban & Baldwin 1997, Underwood 2000) or grazing mode (Ali & Agrawal 2012). For example, phloem-feeding insects induce a weaker overall response than chewing insects, likely because they cause less tissue damage (Ali & Agrawal 2012). In addition, herbivore-specific elicitation cues, such as oral secretions (Alborn et al. 1997, Coleman et al. 2007, Bonaventure et al. 2011, Tian et al. 2012), often trigger different signaling pathways (Voelckel & Baldwin 2004, Kessler & Halitschke 2007, Zarate et al. 2007), express different genes (Stam et al. 2014), and increase different secondary metabolites (Agrawal et al. 2014, Steele & Valentine 2015). Thus, although 2 herbivore species may both induce defenses, they may affect traits that alter palatability in different ways. Grazer-specific changes in palatability may not be obvious without direct comparisons of herbivore feeding preferences.

Understanding how herbivores differentially induce defenses is important, as interactions between herbivore species are commonly mediated by changes in primary producer traits. For example, palatability changes elicited by one herbivore species may indirectly affect the behavior, fitness, or distribution of sequentially feeding conspecific and heterospecific herbivores (reviewed by Denno & Kaplan 2007, Ohgushi et al. 2007, Ohgushi 2008). Although such interactions are generally believed to be asymmetric (i.e. one herbivore species more negatively impacts the other; Kaplan & Denno 2007), there is a lack of information regarding the mechanisms underlying this asymmetry, such as unequal changes in palatability. Such asymmetry of interactions may arise between 2 species when (1) one herbivore induces a primary producer response that affects the other but not vice versa (e.g. Denno et al. 2000, Viswanathan et al. 2005, Long et al. 2007) or (2) both herbivores elicit

responses that affect each other, but the strength of induced traits differs between herbivore species. The prevalence of this second mechanism is difficult to assess, given that most studies have examined the effects of induced defenses in relation to non-induced primary producers, as opposed to directly comparing the relative strength of defenses induced by different herbivores. Solely comparing induced and non-induced primary producers may provide limited information or be unrealistic for interactions occurring in diverse herbivore communities, where herbivores may encounter food sources previously attacked by conspecifics, heterospecifics, or both. By comparing defenses induced by different herbivores both directly and relative to non-grazed primary producers, we can determine whether variation in induced phenotypes contributes to asymmetric interactions between herbivore species.

By inducing defenses in response to herbivore attack, primary producers should increase their ability to successfully defend themselves against future damage (Agrawal & Karban 1999). However, variation in the strength of induced defenses may affect how effective these primary producer defenses are to different herbivores. For example, if herbivores induce responses of similar strength, then cross-resistance may occur when tissues have been grazed by both herbivores, reducing overall feeding (Kessler & Halitschke 2007). In contrast, if herbivores induce responses that differ in relative strength, primary producers grazed by the more weakly inducing herbivore may be susceptible to increased grazing even when induced, depending on herbivore food choices. Thus, a more thorough assessment of the relative palatability of induced tissues will provide a better understanding of the effectiveness of induced defenses in areas where seaweeds are attacked by multiple herbivores.

To better understand herbivore-specific differences in grazer-induced defenses and how this may affect herbivore–herbivore and seaweed–herbivore interactions, we compared the relative strength of an inducible seaweed defense using 3 co-occurring species from Northern California: the brown seaweed *Silvetia compressa*, the snail *Tegula funebralis*, and the isopod *Idotea wosnesenskii*. These 2 herbivore taxa represent different feeding guilds ('scrapers' and 'chewers', respectively), can interact asymmetrically via inducible seaweed defenses (Long et al. 2007, Yun et al. 2010), and can elicit the expression of different genes related to defense within the same seaweed species (Flöthe et al. 2014a,b). Based on these findings, we predicted that induced defenses might vary between these 2 herbivore taxa.

While prior studies have focused on detecting whether herbivores are able to elicit seaweed defenses and respond to defenses induced by conspecific and heterospecific herbivores, we sought to add to this knowledge by investigating differences in the relative strength of defenses induced by snails and isopods. However, testing the relative strength of induced defenses requires a system in which: (1) primary producers respond to grazing by more than one herbivore species and (2) herbivores respond to at least one of these seaweed responses. Therefore, we confirmed that snails and isopods elicited inducible seaweed responses that could be detected by both conspecific and heterospecific herbivores, while also assessing relative defense strength by directly comparing palatability of tissues grazed by both herbivore species. We also examined potential mechanisms for these differences in palatability.

MATERIALS AND METHODS

Study system

The brown seaweed *Silvetia compressa* (hereafter referred to by genus alone) is a common species in the mid to high intertidal zone along the California coast, where it provides food and habitat for a variety of herbivore species (Gunnill 1982, Sapper & Murray 2003, Jones 2016). In Northern California, 3 herbivores are commonly found on *Silvetia* in the field: the snails *Littorina* sp. and *Tegula funebris*, and the isopod *Idotea wosnesenskii* (E.J. pers. obs.) (hereafter referred to by genus alone). *Tegula* and *Idotea* regularly co-occur within the mid intertidal. At sites with high *Silvetia* cover, *Tegula* are far more common and abundant than *Idotea*, but isopods are often found with snails at low tide (E.J. pers. obs.). In surveys conducted at 3 sites during August 2015 (Appendix 1), snails occurred in 97% of plots, while isopods were only found in 13%. However, isopods were always found with snails.

In preliminary experiments, grazing by *Tegula* and *Idotea* decreased *Silvetia* palatability relative to non-grazed tissues, while grazing by *Littorina* had no effect (Appendix 2). These herbivore effects on palatability are due to herbivore-specific traits, as artificial scraping damage does not decrease palatability (E.J. unpubl. data). Together, the co-occurrence of *Tegula* and *Idotea* and the elicitation of *Silvetia* defenses by both species suggest that they are likely to interact via trait changes in food resources. This study builds on these prior observations by comparing the

relative strength of *Silvetia*'s induced defenses in response to grazing by *Tegula* and *Idotea*.

General induction experiment set-up

We conducted 3 induction experiments in flow-through, outdoor seawater tables at the UC Davis Bodega Marine Laboratory between July 2013 and August 2015. Each experiment consisted of 2 phases: a 14-d elicitation phase followed by a 5-d paired-choice feeding assay. For both phases, seaweeds were placed into 2.2 l mesocosms with or without herbivores. These containers had window screen mesh sides, which allowed for water movement and air exchange, and were layered with 3–5 cm pond pebbles, to provide refuge for herbivores during low tide. To mimic tidal immersion and emersion conditions found in the field, we controlled flow to the seawater tables based on a semidiurnal tide cycle, either manually or using a digital watering timer (DIG Model C002, DIG Corporation). Mesocosms were submerged at tide heights above 0.91 m above MLLW and exposed to low tide conditions below this height. This tide height is representative of locations where *Silvetia*, *Tegula* and *Idotea* co-occur in Northern California. We collected seaweeds from sites in Point Arena, CA, while herbivores were collected from Point Arena (all years) and Bodega Bay, CA (2013 and 2014).

Induction strength of conspecific-grazed tissues relative to heterospecific- and combination-grazed tissues

Our preliminary experiments suggested that *Silvetia* responds to grazing by both *Tegula* and *Idotea*. Therefore, the goals of our first experiment were to (1) confirm that both herbivores elicited responses in *Silvetia* that could be detected by conspecifics and (2) investigate differences in the strength of these induced defenses by directly comparing the palatability of tissues grazed by isopods and snails. To test this, we first exposed 37.5 ± 0.5 g *Silvetia* to 4 herbivore treatments during July–August 2013. Treatments were as follows: (1) 6 *Tegula* (n = 48), (2) 2 *Idotea* (n = 48), (3) a combination of 3 *Tegula* and 1 *Idotea* (n = 24), and (4) no herbivores (n = 24). We chose these herbivore abundances as we expected these treatments to have equivalent overall grazing rates, based on initial experiments, and they represent relevant densities found in the field (E.J. unpubl. data). After 14 d, we reweighed all *Silvetia* thalli to

measure changes in biomass, and calculated consumption using the equation $(H_i \times C_f/C_i) - H_f$, where H_i and H_f are the initial and final mass of the grazed seaweed and C_f and C_i are the final and initial mass of the non-grazed tissues.

Following the elicitation phase, we compared the palatability of conspecific-grazed (*Tegula* or *Idotea*) tissues to palatability of the other 3 treatments (non-grazed, heterospecific-grazed [*Tegula* or *Idotea*], or combination grazed) using paired-choice feeding preference assays with both snails and isopods. Comparing herbivore preferences between snail-grazed and isopod-grazed tissues allowed us to directly test the strength of the induced defenses, while a choice between conspecific-grazed and non-grazed tissues served as a positive control for *Silvetia* defenses being induced. Because herbivores may affect different seaweed traits, we also compared conspecific-grazed tissues to those grazed by both herbivores simultaneously. This allowed us to examine whether grazing by both herbivores together affected palatability differently than grazing by individual herbivore species. For these preference assays, we used new individual herbivores (not from the elicitation phase) that had been feeding on a mixed seaweed diet (e.g. *Pyropia*, *Mastocarpus*, and *Pelvetiopsis*), with 3 snails or 2 isopods per container. For each herbivore, we paired 7.5 ± 0.5 g of conspecific-grazed seaweed tissue ('*Tegula*-grazed' or '*Idotea*-grazed') with 7.5 ± 0.5 g tissue from either the heterospecific ('*Tegula*-grazed' or '*Idotea*-grazed'), combination ('*Tegula* + *Idotea*-grazed'), or no herbivore ('non-grazed') treatments. Paired seaweeds were placed into containers with and without herbivores (n = 12 each). Containers without herbivores controlled for changes in seaweed mass unrelated to herbivory. After 5 d, we measured final masses to determine the amount of seaweed consumed for each treatment, as described above.

Responses to heterospecific grazing relative to non-grazed tissues

Our first experiment found that palatability differed between *Tegula*-grazed and *Idotea*-grazed tissues, but it was unclear whether this was due to a difference in the strength of the induced defenses, or a lack of *Tegula* response to isopod grazing. To determine this, we conducted a second experiment during September–October 2014 to test whether snails and isopods responded to *Silvetia* defenses induced by heterospecific grazing. During the elicitation phase, we exposed 37.5 ± 0.5 g *Silvetia* to 3 treatments: 6

Tegula, 6 *Idotea*, or no herbivores (n = 16 each). We tripled the isopod abundance from the first experiment to increase the area of tissue grazed relative to snails. After 14 d, we reweighed all *Silvetia* thalli to measure changes in biomass and calculated consumption.

We examined whether snails and isopods responded to heterospecific grazing by offering both herbivores a choice between heterospecific-grazed ('*Tegula*-grazed' or '*Idotea*-grazed') tissues and non-grazed tissues (n = 8). We again tested the relative strength of induction, by offering herbivores a choice between *Tegula*-grazed and *Idotea*-grazed tissues (n = 8). As a positive control for *Silvetia* inducing defenses, we offered herbivores a choice between conspecific-grazed and non-grazed tissues (n = 8). We again used 7.5 ± 0.5 g of each tissue type, with 3 snails or 2 isopods per container and no herbivore controls. After 5 d, we measured final masses to determine the amount of seaweed consumed for each treatment, as described above.

This design also allowed us to investigate whether differences in palatability detected by direct comparisons of *Tegula*-grazed and *Idotea*-grazed tissues could be predicted by indirect comparisons between grazed and non-grazed tissues.

Relative strength of induction between grazed main-axis tissues

Although we offered herbivores whole branches of *Silvetia* in the first 2 experiments to represent realistic food choices, the majority of grazing damage for both herbivores occurred along the older tissue of the main axes and branches of the thalli, rather than at the younger distal tips and receptacles. Therefore, we ran a follow-up experiment in July 2015 to simplify tissue variability and further investigate whether the relative strength of *Silvetia* induced defenses differed between main axis tissues grazed by both herbivores. We cut main-axis *Silvetia* tissue into ~30 cm long pieces weighing 9.0 ± 0.5 g and placed 2 pieces in each container. We then exposed *Silvetia* to 3 *Tegula*, 3 *Idotea*, or no herbivores (n = 8 each). After 14 d, we reweighed the seaweed to measure changes in biomass and calculated consumption, as described above. Because we observed differences in the area of main-axis tissue grazed in the first experiment (despite similar consumption), we also estimated the percent area of tissue grazed by *Tegula* and *Idotea*. We did this by recording the presence or absence of grazing at 50 random points along each piece of grazed tissue.

We compared the palatability between *Tegula*-grazed and *Idotea*-grazed main-axis tissues by offering either 2 snails or 2 isopods a choice between 4–4.5 g *Tegula*-grazed and 4–4.5 g *Idotea*-grazed tissues. Containers without herbivores again controlled for autogenic changes in seaweed mass. After 5 d, we measured final masses to determine the amount of seaweed consumed for each treatment, as described above.

Relative strength of induction when removing morphological differences

The experiments outlined above showed that herbivores preferred *Idotea*-grazed over *Tegula*-grazed tissues. To test whether morphological (e.g. induced structures, changes in toughness, mechanical damage) differences between tissue types explained herbivore selectivity, we assayed palatability after homogenizing these tissues (Hay et al. 1998). To remove morphological differences between tissues, we first freeze-dried and ground each tissue type (i.e. *Tegula*- or *Idotea*-grazed) into a fine powder using a Wiley Mill (mesh size 60). We then reconstituted each tissue type into squares of artificial food by adding a heated, then cooled, deionized water–agar mixture to the dry seaweed powder dissolved in water. Although we attempted to match natural wet:dry mass ratios (~70% water and 30% seaweed), we altered this ratio so that foods solidified properly. The final ratio was 78% water, 19% seaweed, and 3% agar. We used 2 different 'fake food' methods because feeding mode differed between our herbivore species. For the snail assays, we spread a very thin layer (<1 mm high) of this mixture onto filter paper, which allowed snails to feed via radular scraping (Thorner et al. 2008). We then offered individual snails filter paper strips with ~4 cm² of both tissue types (n = 22). We determined the area of each tissue consumed by analyzing before and after images (ImageJ, www.nih.gov). Snail replicates were removed after 1–2 d, when ~1/3 to 1/2 the total food had been eaten. For the isopod trials, the agar mixtures were poured onto window screen and cut into strips. Unlike the fake foods for the snail trials, the isopod foods were raised ~2 mm from the window screen to facilitate isopod biting. We offered individual isopods one piece of window screen containing a ~1 cm² square of each tissue type (n = 22). These squares were smaller than the snail food due to the increased thickness. Although we planned to measure the area (number of squares) of each tissue consumed, isopod consumption was still very low after 7 d, with many isopods only eating the top layer of food (not ex-

posing full squares). Therefore, in addition to the number of squares eaten, we also recorded the presence or absence of grazing damage. We excluded 4 isopod replicates without any measurable grazing damage, as these did not provide any information on preference.

Statistical analyses

To determine whether differences in the strength of induction between treatments could be attributed to differences in the biomass consumed during the elicitation phase, we compared the total amount of control-corrected *Silvetia* consumed following each 2-wk elicitation experiment using a 1-way ANOVA or 2-tailed *t*-tests (JMP v. 11.0). For each feeding assay, we tested for differences in palatability by comparing the amount consumed between grazing treatments using paired *t*-tests (JMP v. 11.0). We did not adjust the critical value for multiple tests, in order to avoid increasing the probability of Type II errors and potentially obscuring ecologically significant effects (Cabin & Mitchell 2000, Moran 2003, Nakagawa 2004, Garamszegi 2006). For the isopod fake food assays, we also used McNemar's test for paired categorical data, to compare the proportion of isopods that preferred isopod-grazed tissues to the proportion of isopods that preferred snail-grazed tissues.

We also tested whether indirect predictions of *Tegula*- and *Idotea*-induced defense strength differed from our direct comparisons of palatability. First, we calculated the percent of grazed tissues consumed for each feeding assay pair in the second experiment. Then, we compared the difference between *Idotea*- and *Tegula*-grazed tissues in the presence of non-grazed tissue to the direct difference between *Idotea*- and *Tegula*-grazed tissues using a 2-way ANOVA, with responder (*Tegula* or *Idotea*) and comparison (direct or indirect) as factors.

RESULTS

Induction strength of conspecific-grazed tissues relative to heterospecific and combination-grazed tissues

Consumption during the 14-d elicitation phase was similar across herbivore treatments, with herbivores consuming 2.2 ± 0.19 g in the snail treatment (mean \pm SE), 1.8 ± 0.18 g in the isopod treatment, and 2.1 ± 0.31 g in the combination treatment ($F_{2,117} = 1.2$, $p = 0.29$). These masses represent 5.9%, 4.8%, and 5.6%

of the initial seaweed biomass, respectively. Thus, differences in palatability between herbivore treatments could not be attributed to herbivore-specific differences in feeding rate. In choice feeding assays, both herbivores responded to previous grazing by conspecifics, preferring non-grazed tissues to those grazed by conspecifics (snails, $t_{11} = 3.9$, $p = 0.0023$; isopods, $t_{11} = 3.0$, $p = 0.013$; Fig. 1A,D). This confirmed that both herbivores were inducing *Silvetia* defenses. However, when *Idotea*- and *Tegula*-grazed tissues were offered together, snails consumed 1.7 times more *Idotea*-grazed tissues ($t_{11} = 2.9$, $p = 0.014$; Fig. 1B). Isopods showed a similar pattern, consuming 1.6 times more *Idotea*-grazed tissues than *Tegula*-grazed tissues, although this was not significant ($t_{11} = -1.6$, $p = 0.13$; Fig. 1E). This suggested that *Tegula*-grazed tissues were less palatable than *Idotea*-grazed tissues, at least to conspecifics. Simultaneous grazing by both herbivores did not appear to have a different effect on palatability than grazing by either herbivore alone, as neither herbivore had a significant preference between conspecific-grazed tissues and tissues grazed by a combination of both herbivores (snails, $t_{11} = 0.98$, $p = 0.35$; isopods, $t_{11} = -1.43$, $p = 0.18$; Fig. 1C,F).

Responses to heterospecific grazing relative to non-grazed tissues

Although we tripled the number of isopods to increase grazing relative to snails, *Tegula* and *Idotea* still consumed the same amount of tissue during the elicitation phase (*Tegula*, 2.6 ± 0.21 g, 6.9% of initial biomass; *Idotea*, 2.5 ± 0.20 g, 6.7% of initial biomass; $t_{30} = 0.44$, $p = 0.66$). In choice feeding assays, we again found that both herbivores induced defenses in *Silvetia*, preferring non-grazed tissues to those grazed by conspecifics (snails, $t_7 = 7.8$, $p = 0.0001$, isopods, $t_7 = 4.5$, $p = 0.0026$; Fig. 2A,E). In addition, both herbivores responded to grazing by heterospecifics, consum-

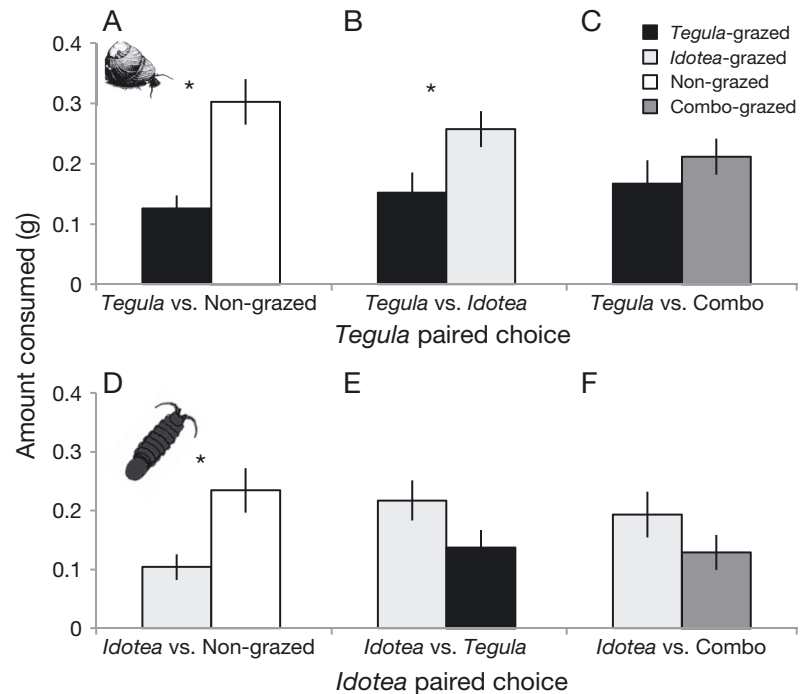


Fig. 1. Amount of *Silvetia compressa* consumed by snails, *Tegula funebris* (A,B,C), or isopods, *Idotea vosnesenskii* (D,E,F), in paired choice assays between conspecific-grazed tissues and non-grazed (control), heterospecific-grazed, or both herbivore (combination = 'combo') grazed tissues. Significant differences are marked by an asterisk. Means are presented \pm SE

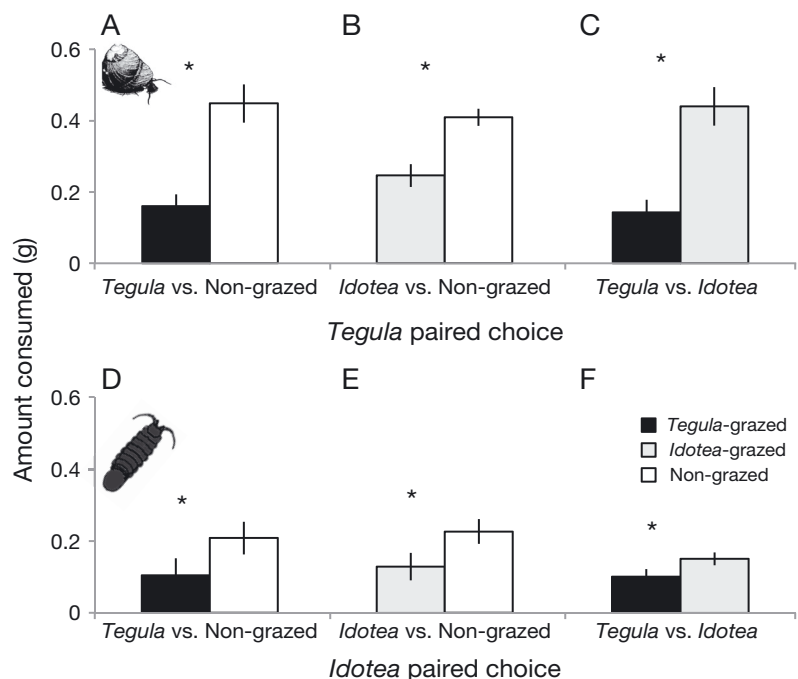


Fig. 2. Amount of *Silvetia compressa* consumed by snails, *Tegula funebris*, (A,B,C), or isopods, *Idotea vosnesenskii* (D,E,F), in paired choice assays between *Tegula*-grazed, *Idotea*-grazed, or non-grazed (control) treatments, as denoted on the x-axis. Significant differences are marked by an asterisk. Means are presented \pm SE

ing 1.7 and 2.0 times more non-grazed tissues than those grazed by *Idotea* or *Tegula* (snails $t_7 = 4.7$, $p = 0.0021$; isopods, $t_7 = 3.2$, $p = 0.016$, Fig. 2B,D). As in the experiment above (Fig. 1), both herbivores responded more strongly to *Tegula* grazing, preferring *Idotea*-grazed tissues to *Tegula*-grazed tissues (snails, $t_7 = 4.2$, $p = 0.0038$; isopods, $t_7 = 2.4$, $p = 0.048$, Fig. 2C,F). Because snails responded to heterospecific grazing when offered a choice between *Idotea*-grazed and non-grazed tissues (Fig. 2C), snail preference for *Idotea*-grazed tissues over *Tegula*-grazed tissues was not due to a lack of snail response to *Idotea* grazing.

When we assessed indirect versus direct comparisons of induction strength, we found that the comparative method mattered. The percent difference between *Idotea*-grazed and *Tegula*-grazed tissues was significantly greater for direct comparisons (37%) than indirect comparisons (0.089%) ($F_{1,28} = 9.6$, $p = 0.0045$). Thus, the difference in palatability between grazed tissues was greater than we would have predicted by only comparing grazed and non-grazed tissues. There was not a significant effect of responder ($F_{1,28} = 2.4$, $p = 0.13$) or interaction ($F_{1,28} = 1.5$, $p = 0.23$).

Relative strength of induction between grazed main axis tissues

When restricted to the main-axis tissue, isopods consumed less biomass and area than snails during the elicitation phase. Isopods consumed 0.75 ± 0.094 g and $40 \pm 5.6\%$ of the surface area, while snails consumed 1.2 ± 0.11 g and $79 \pm 3.5\%$ of the surface area. Although these consumption values were less than the first 2 experiments, the percentages of the initial biomass were greater (8.3% and 13%, respectively). When offered a choice between main-axis grazed tissues, we again found that both herbivores preferred *Idotea*-grazed tissues to *Tegula*-grazed tissues (snails, $t_7 = 4.6$, $p = 0.0025$; isopods, $t_7 = 3.9$, $p = 0.0057$; Fig. 3). Consumption of *Idotea*-grazed tissues was 2.4 to 3.8 times more than *Tegula*-grazed tissues (by snails and isopods, respectively).

Relative strength of induction when removing morphological differences

Similar to the whole-tissue feedings assays, snails consumed 1.7 times more fake foods made from *Idotea*-grazed than *Tegula*-grazed tissues ($t_{21} = -2.8$, $p = 0.010$, Fig. 4A). This suggests that differences in palatability are not simply due to morphological changes caused by the different grazers. Isopods displayed a similar pattern. Although consumption was low and only a portion of the replicates ($n = 12$ of 22) ate measurable amounts of food, isopods consumed 3.1 times more reconstituted food made from *Idotea*-grazed tissues than *Tegula*-grazed tissues ($t_{11} = -2.2$, $p = 0.054$; Fig. 4B). When we included all replicates with visible grazing damage and compared the presence or absence of grazing ($n = 18$), isopods preferred *Idotea*-grazed tissues ($\chi^2 = 5.8$, $p = 0.016$).

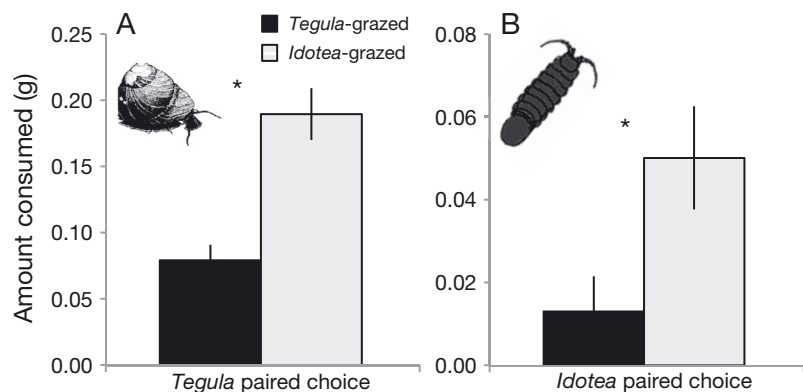


Fig. 3. Amount of *Tegula funebralis*-grazed and *Idotea wosnesenskii*-grazed *Silvetia compressa* consumed by (A) snails, *Tegula*, and (B) isopods, *Idotea*, when offered main-axis tissue only. Significant differences are marked by an asterisk. Means are presented \pm SE

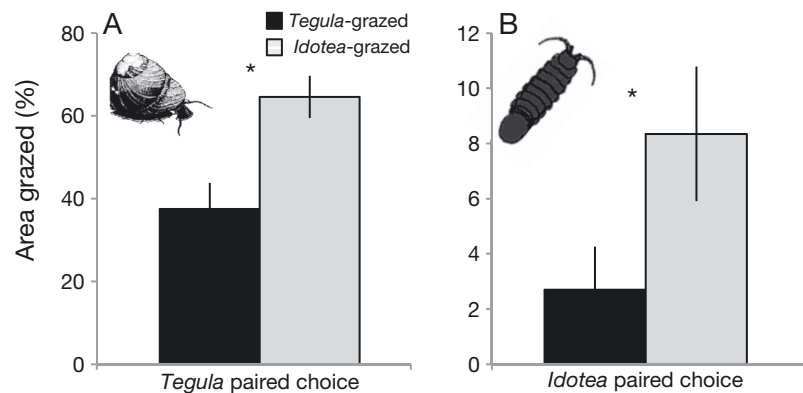


Fig. 4. Amount of *Silvetia compressa* consumed in fake food assays by (A) snails, *Tegula funebralis*, or (B) isopods, *Idotea wosnesenskii*, when offered a choice between *Tegula*-grazed and *Idotea*-grazed reconstituted tissues. Significant differences are marked by an asterisk. Means are presented \pm SE

DISCUSSION

Herbivores that disproportionately change primary producer traits relative to other species may impact herbivore selection of food resources. In this study, we found differences in the relative strength of an inducible seaweed defense elicited by 2 co-occurring herbivore species. Grazing by both snails (*Tegula*) and isopods (*Idotea*) decreased seaweed palatability, relative to non-grazed tissues. However, when compared directly, *Tegula*-grazed tissues were less palatable to both herbivores than *Idotea*-grazed tissues. Importantly, this large difference in palatability, would not have been apparent had we only compared the effect sizes between grazed and non-grazed tissues for each herbivore. For example, based on consumption relative to non-grazed tissues, we may have predicted *Idotea*-grazed and *Tegula*-grazed tissues to be similar in palatability. In contrast, direct comparisons show that *Idotea*-grazed tissues can be over 3 times more palatable than *Tegula*-grazed tissues (Fig. 2). Thus, using indirect comparisons would have caused us to draw incorrect conclusions about the relative defense strength.

Prior studies have found species-specific herbivore responses to seaweed defenses induced by different herbivores (Long et al. 2007, Yun et al. 2010). We additionally show that not only can the ability of herbivores to respond to different grazers vary, but also that herbivore responses can depend on both elicitor identity and food choice. In the presence of non-grazed tissues, prior grazing by both herbivores decreased herbivory by both conspecifics and heterospecifics. However, in the presence of grazed tissues only, both herbivores consumed more *Idotea*-grazed tissues than *Tegula*-grazed tissues, and did not distinguish between conspecific and combination-grazed tissues. This suggests that snails alone have a greater effect on seaweed palatability than isopods, even at similar feeding rates.

There are several mechanisms that may explain why the relative strength of the seaweed response differed with the identity of the attacking herbivore. First, inducible responses may depend upon the amount of grazing damage a primary producer experiences (Karban & Baldwin 1997, Underwood 2000). This is unlikely in our experiments, given that *Tegula* grazing elicited a stronger response, even when the grazing rates of the 2 herbivores were the same during the elicitation phase. A more likely explanation is that seaweed responses depend on mechanical or chemical differences in grazing, as snails feed via radular scraping, while isopods are chewers. For

example, Pavia & Toth (2000) found that concentrated, deeper grazing by snails elicited a seaweed defense, while superficial bite marks by isopods did not. In our experiments, snail grazing removed a large percentage of the outer tissue layer on *Silvetia*, while isopods caused patchy, but slightly deeper grazing marks. These differences may have contributed to the differential responses of seaweeds to these 2 herbivores, due to how or where the damage occurred. If the seaweed response is localized to where the damage occurs, snails could induce resistance across a larger portion of the seaweed than isopods, even when the amount consumed is the same. It is also possible that these different grazing modes or herbivore-specific secretions elicited different chemical pathways or expressed different genes, similar to vascular plants (Walling 2000, Thaler et al. 2012). Although far less is known about how different grazing guilds affect induced defenses in seaweeds, snails and isopods do express different numbers of genes related to defense in the confamilial seaweed *Fucus vesiculosus* (Flöthe et al. 2014a,b).

In addition to eliciting different seaweed responses, herbivore grazing can also alter primary producer structure or morphology (e.g. Fornoff & Gross 2014). For example, by removing outer tissue layers, snails might create a seaweed surface that is less suitable to isopod grazing. However, when we eliminated morphological differences and offered herbivores tissues reconstituted in agar, herbivore preferences for *Idotea*-grazed tissues persisted. This suggests that herbivore effects on palatability were not solely due to differences in morphology. Instead, snails and isopods may induce different concentrations or types of chemicals. Although some studies have found that herbivore grazing can increase phlorotannin concentrations in fucoids (e.g. Pavia & Toth 2000, Borell et al. 2004), we found that phlorotannin concentrations did not differ between *Tegula*-grazed and non-grazed tissues in other experiments (Jones 2016). Further research determining a more precise mechanism, such as the identity or concentration of chemicals and how defenses are elicited, will provide insight into how these herbivores contribute to phenotypic diversity and the evolution of these defenses.

Herbivore-induced changes in primary producer phenotypes can lead to several complex interactions among herbivore species that, in turn, structure communities (Denno et al. 1995, Denno & Kaplan 2007, Ohgushi et al. 2007, Ohgushi 2008). For example, numerous studies have shown that herbivore grazing can decrease palatability of food resources, impact-

ing the food choices and movement of other herbivores (e.g. Borell et al. 2004, Ohgushi 2005, Long et al. 2007). These induced changes in palatability that affect other herbivores may provide a mechanism for indirect competition or performance effects (e.g. Denno et al. 2000, Viswanathan et al. 2005, Long et al. 2007). Our findings suggest that competition between herbivore species may be asymmetric (Kaplan & Denno 2007, Long et al. 2007), but that this asymmetry is context dependent. In other words, while *Idotea* induces a weaker response than *Tegula*, it can still affect *Tegula* preferences in the absence of *Tegula*-grazed tissues. Therefore, the strength of interactions between herbivore species may depend on which induced or non-induced food choices are present in the field at a given time.

Although we confined our study to laboratory mesocosms, we suspect that asymmetric interactions between these herbivores are likely to occur in the field for several reasons. First, herbivores are more likely to encounter *Tegula*-grazed seaweeds than *Idotea*-grazed seaweeds, given that *Tegula* are far more abundant in *Silvetia* habitat than *Idotea* (Appendix 1). Second, while isopods are much less abundant on *Silvetia*, they are frequently found in patches with snails at low tide (Appendix 1). Therefore, isopods may be more likely to encounter snail-grazed tissues than vice versa. And third, because of their increased mobility when submerged (e.g. Pavia et al. 1999, Pavia & Toth 2000), isopods may more easily move away from seaweeds induced by snails. Thus, isopods may be better able to respond to snail-grazed tissues, such that snails have a larger effect on isopod feeding and movement than isopods do on snails. This snail effect on isopod movement may be particularly important, as snail-induced defenses in *Silvetia* can persist for up to 7 d (A. Warneke unpubl. data). Similarly, Yun et al. (2010) suggested that isopods might have been highly sensitive to snail grazing because their increased movement made them more likely to come in contact with tissues grazed by snails.

While variation in the relative strength of induced defenses may mediate interactions between herbivore species, it may also affect the ability of a primary producer to deter different herbivores. For induced defenses to be most effective, herbivores should respond to these trait changes and avoid defended individuals (Karban 2011). Due to their high abundance, *Tegula* may provide a more reliable risk cue and present a larger threat (Karban et al. 1999) to *Silvetia* than *Idotea*, causing a stronger seaweed response. Thus, the seaweed defenses induced toward

Tegula are likely to deter future grazing. However, if herbivores spend little time foraging and choose the most palatable food option, weaker defenses may be less effective. For instance, within our experiments, the total consumption during the feeding assays generally stayed the same across treatments. Thus, when choosing between grazed tissues, herbivores often ate greater amounts of the more weakly induced tissues than when choosing between grazed and non-grazed tissues. In contrast, if herbivores increase dispersal (Bergelson et al. 1986, van Dam et al. 2000, Borell et al. 2004) and spend more time searching for non-grazed tissues or other food sources, this variability in defenses may benefit the plant or seaweed by decreasing overall feeding (Roslin et al. 2008) and increasing herbivore foraging costs (Herrera 2009). Thus, there is a need to further study herbivore behavioral responses to induced defenses, especially in areas with variable primary producer quality.

Overall, these results suggest that snails and isopods can interact indirectly via seaweed-mediated interactions, with both species inducing seaweed defenses. These palatability changes may also be indicators of competition between herbivores, as they affect feeding preferences and consumption. However, by inducing defenses of different strength, these herbivores create gradients of palatability that may lead to asymmetric interactions between herbivore species, depending on available induced or non-induced phenotypes. These types of interactions are likely to occur in other systems, where inducing herbivores share food resources, but more work is needed to understand the diversity of induced primary producer phenotypes in the field and how herbivores respond to this variation. Future studies investigating the complexity of these primary producer-mediated interactions will thus improve our understanding of how they scale up to affect herbivore community composition and population dynamics.

Acknowledgements. We thank G. Leavitt, N. Kollars, M. Kardish, O. Rhoades, E. Oldach, K. Griffith, M. Pace, G. Greenberg-pines, J. Chow, G. Ha, and P. Sisommout for assisting with collections, field surveys, and laboratory experiments. We also thank the Stachowicz and Williams labs for providing lab space and equipment at the Bodega Marine Laboratory. Funding was provided to E. Jones from an Inamori Fellowship, a Susan and Stephen Weber Endowed Scholarship, a Harold and June Grant Memorial Scholarship, CSU COAST, a Phycological Society of America Grant-in-Aid of Research, a Lerner Gray Grant for Marine Research, CSUMB UROC, SDSU Graduate Student Travel Grants, and the SDSU JDPE. J. Stachowicz, S. Williams, J. Walker, and 2 anonymous reviewers provided

helpful comments on this manuscript. This is a contribution of Bodega Marine Laboratory, UC Davis and contribution no. 58 of the Coastal and Marine Institute, San Diego State University.

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Appendix 1. Field surveys of *Tegula* and *Idotea* abundances within the *Silvetia* zone

Methods

To better understand the co-occurrence of *Tegula funebris* and *Idotea wosnesenskii* in *Silvetia compressa* habitat, we performed field surveys at 3 sites during August 2015. At each site, we placed two 20 m transects at the upper, mid, and lower limit of the *Silvetia* zone. We then surveyed snail and isopod abundance every 2 m using 0.25 × 0.25 m quadrats (20 plots per tide height). We skipped plots without any *Silvetia* and added on additional meters as necessary. Within each quadrat, we

recorded the percent cover of *Silvetia* and the total number of snails and isopods.

Results

Average herbivore abundances across all sites were 60 ± 5.1 snails m^{-2} and 0.89 ± 0.21 isopods m^{-2} . Snails were found in 97% of surveyed plots (175/180) while isopods were only found in 13% of plots (24/180). All plots that contained isopods also contained snails.

Appendix 2. Testing for elicitation of inducible *Silvetia* defenses by 3 common herbivore species

Methods

During July 2012, we conducted 2 experiments to test for the elicitation of inducible defenses in *Silvetia* by 3 herbivore species. In the first experiment, we exposed 45 ± 0.5 g *Silvetia* to grazing by 7 *Tegula funebris* (n = 24) or no herbivores (n = 24). In the second experiment, we exposed 37.5 ± 0.5 g *Silvetia* to 2 *Idotea wosnesenskii* (n = 8), 4 *Littorina* sp., or no herbivores (n = 8 per herbivore treatment). After 14 d, we reweighed all *Silvetia* thalli to measure changes in biomass, and calculated consumption using the equation $(H_i \times C_f/C_i) - H_f$, where H_i and H_f are the initial and final mass of the grazed seaweed and C_i and C_f are the final and initial mass of the non-grazed tissues.

Following the elicitation phase, we compared palatability of conspecific-grazed and non-grazed tissues using paired-choice feeding preference assays. For each herbivore, we paired 7.5 ± 0.5 g conspecific-grazed seaweed tissue with

7.5 ± 0.5 g of non-grazed tissue. Paired seaweeds were placed into containers with and without herbivores (n = 24 for *Tegula*, n = 8 for *Idotea* and *Littorina*). Containers without herbivores controlled for changes in seaweed mass unrelated to herbivory. After 5 d, we measured final masses to determine the amount of seaweed consumed for each treatment, as described above.

Results

Both *Tegula* and *Idotea* preferred non-grazed tissues to tissues grazed by conspecifics (Fig. A1). *Tegula* consumed 1.8 times more non-grazed tissues than *Tegula*-grazed tissues ($t_{23} = 6.5$, $p < 0.0001$), while *Idotea* consumed 2.7 times more non-grazed tissues than *Idotea*-grazed tissues ($t_7 = 2.5$, $p = 0.040$). *Littorina* did not have a significant preference for either tissue type ($t_7 = -0.67$, $p = 0.52$).

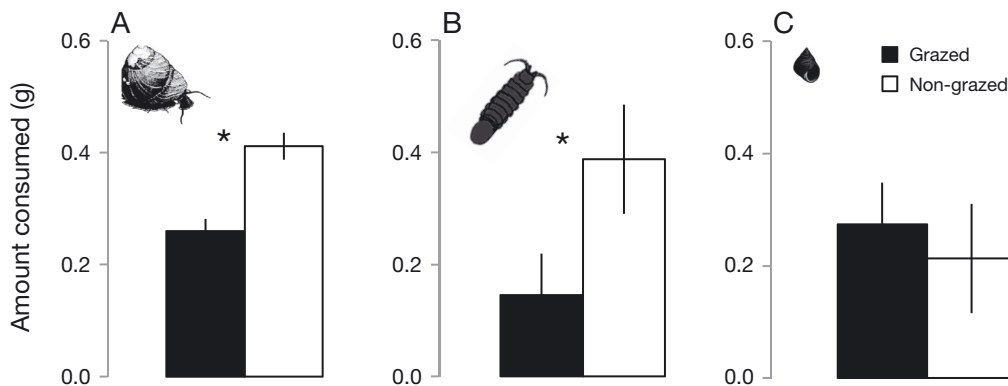


Fig. A1. Amount of *Silvetia compressa* consumed by (A) *Tegula funebris*, (B) *Idotea wosnesenskii*, or (C) *Littorina* sp. in paired choice assays between conspecific-grazed and non-grazed treatments. Significant differences are marked by an asterisk. Means are presented \pm SE