A TEST OF OPTIMAL DEFENSE THEORY VS. THE GROWTH-DIFFERENTIATION BALANCE HYPOTHESIS AS PREDICTORS OF SEAWEED PALATABILITY AND DEFENSES

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A TEST OF OPTIMAL DEFENSE THEORY VS. THE GROWTH-DIFFERENTIATION BALANCE HYPOTHESIS AS PREDICTORS OF SEAWEED PALATABILITY AND DEFENSES

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SUMMARY

Because organisms have limited resources to allocate to multiple life history traits, the Optimal Defense Theory (ODT) and the Growth-Differentiation Balance Hypothesis (GDBH) were developed by terrestrial plant ecologists to predict intraindividual defense allocation based on the cost of defense and these life history trade-offs. However, these theories have garnered equivocal experimental support over the years and are rarely experimentally extended from predictions of plant physiology to the palatability of the tissues an herbivore experiences. We therefore examined tissue palatability, nutritional value, and defense mechanisms in multiple Dictyotalean seaweeds in two Caribbean locations, using two herbivores. Relative palatability of tissues varied greatly with algal species, grazer species, and location. Because older bases were not consistently defended, GDBH did not predict relative palatability. We could not reject ODT without intensive measures of tissue fitness value and herbivore risk, and this theory was therefore not useful in making broad predictions of tissue palatability. In testing the physiological predictions of these theories, we found the young, growing apices of these seaweeds to be generally more nutritionally valuable than the old, anchoring bases and found organic-rich apices to be more chemically deterrent, thus supporting ODT. However, the combined chemical, nutritional, and structural traits of these algae all influenced herbivore choice. As a result, these patterns of apical value and chemical defense reflected palatability of live tissues for only one of five algal species, which rendered ODT and GDBH poor predictors of relative palatability for most algae.

INTRODUCTION

Organisms have limited resources to allocate among critical processes such as defense, growth, and reproduction (Boudsocq et al 2011). Resource limitation can therefore lead to trade-offs between life history traits, with these trade-offs shaped by natural selection (Stearns 1989, Herms and Mattson 1992, Mole 1994, Reznick 1992, Sheldon and Verhulst 1997, Ricklefs and Wikelski 2002). Due to the prevalence of plant defenses and their likely physiological and ecological costs (Strauss et al. 2002), terrestrial plant ecologists have proposed a number of defense theories based on these allocation trade-offs (reviewed in Cronin 2001, Stamp 2003a). These theories predict plant defense type and quality, given different nutrient and consumer regimes across both ecological and evolutionary scales (Feeny 1976, Rhoades 1979, Bryant et al. 1983, Coley et al. 1985, Herms and Mattson 1992).

Of these plant defense theories, only the Growth-Differentiation Balance Hypothesis (GDBH; Herms and Mattson 1992) and Optimal Defense Theory (ODT; McKey 1974, 1979; Rhoades 1979) explicitly predict intraindividual variation in defense allocation. GDBH states plants should first allocate excess resources to growth rather than differentiation processes such as defense (Herms and Mattson 1992), thereby predicting older tissue should be more defended because it has finished growing and has surplus resources for defense (Herms and Mattson 1992; Cronin and Hay 1996). For example, defensive proteins in *Arabidopsis thaliana* were found to be more abundant in older leaves than in young leaves, despite young leaves contributing the most to plant fitness (Barto and Cipollini 2005). In marine systems, young apical tissues of the brown alga *Dictyota ciliolata* contain lower concentrations of defensive dictyols and are more susceptible to herbivory than are older, more basal tissues (Cronin and Hay 1996). However, few studies have directly tested the within-plant predictions of GDBH, despite widely citing it in

opposition to ODT. As a result, GDBH has found far more support at the population level than for intraindividual defense allocation (Stamp 2003a).

Many more studies have found support for ODT, which broadly states that within an individual, defense should be proportional to the fitness value of a tissue and its risk of attack, and inversely proportional to the cost of the defense (Rhoades 1979, Zangerl and Rutledge 1996, Stamp 2003a). This theory is often used to predict newer, growing tissues should be defended. For example, many terrestrial plants exhibit more defenses in budding versus older leaves (McKey 1974, McCall and Fordyce 2010). In one of the most widely-cited examples, constitutive xanthotoxins in the wild parsnip, *Pastinaca sativa*, are most concentrated in fruits, moderately concentrated in the leaves, and of low concentration in the roots, in direct proportion to the likelihood of attack (Zangerl and Rutledge 1996). ODT also reasonably predicts defense allocation in many marine organisms, where greater defenses are found in the outermost tissue of sponges (Becerro et al. 1998, Kubanek et al. 2002) and in apical portions of seaweeds (Steinberg 1984, Meyer and Paul 1992, Poore 1994, Van Alstyne et al. 1999). However, this pattern of increased allocation of defensive traits to the most apical or exposed tissues is not universal (Pavia et al. 2002, Iken and Amsler 2007, Freeman and Gleason 2010).

Although intraindividual defense allocation has been examined in numerous seaweeds and plants, neither ODT nor GDBH has been broadly affirmed or rejected (Stamp 2003a). Given that these theories are not newly developed, one has to ask why both are still debated (Stamp 2003b). First, these theories are not necessarily mutually exclusive. As noted by Herms and Mattson (1992), defenses exhibited in newly growing apices of vascular plants, as predicted in ODT, may in fact be produced by older tissues and translocated to the apices to minimize allocation costs in those tissues, as predicted by GDBH (McKey 1979). Two studies using

species without translocation systems found older tissues to be more defended, as predicted by GDBH (Cronin and Hay 1996, Barto and Cipollini 2006) despite higher fitness value of young tissue (Barto and Cippolini 2006). Examining defenses in plants and macroalgae with minimal transport tissues, like phloem, xylem, or sieve tubes thus removes some overlap between the defense patterns predicted by ODT and GDBH (Lobban and Wynne 1981, Barto and Cipollini 2006).

Second, defense allocation is often examined experimentally in only one species at a time and typically focuses on a single defense mechanism. While single-species experiments are useful for understanding allocation and physiology of each individual species (Zangerl and Rutledge 1996, Cronin and Hay 1996, Pavia et al. 2002), these studies effectively generate a sample size of one for testing ODT and GDBH. This tendency to extrapolate from individual patterns of defense allocation can be overcome by examining defense allocation in many species simultaneously (e.g. Tugwell and Branch 1989, Meyer and Paul 1992, Fairhead et al. 2005). However, many of these multi-species studies in terrestrial plants (McKey 1979, McCall and Fordyce 2010) and brown seaweeds (Van Alstyne et al. 1999, Iken and Amsler 2007) measure concentrations of putative deterrent chemicals, rather than actual palatability to grazers. Because known secondary metabolites do not necessarily govern overall palatability (Agrawal 2011, Carmona et al. 2011) and can interact with structural and nutritional properties to determine palatability (Hay et al. 1994, Cruz-Rivera and Hay 2003, Agrawal 2011), studies using feeding as a measure of defense are necessary to integrate plant defense traits. Fully evaluating ODT and GDBH thus requires evaluating not only multiple plant species, but also multiple, and often integrated and complex, defense mechanisms.

In addition to integrating defense traits, measures of palatability are crucial to understanding the selective pressures on plant defenses and the ecological consequences of defense allocation. Herbivores consume plant tissues, not isolated traits like chemical defense or protein content. While evolution can act on a single defensive or nutritional trait to decrease consumption (Berenbaum and Zangerl 1998, Lankau and Kliebenstein 2009), these traits are always presented to an herbivore in the context of the suite of traits governing palatability (Hay et al. 1994, Bullard and Hay 2002, Agrawal 2011, Carmona et al. 2011). For example, defenses must be increased not just to a concentration at which they are deterrent, but to a concentration at which their cost to the herbivore outweighs the nutritional benefit of consuming the tissue (Duffy and Paul 1992, Cruz-Rivera and Hay 2003). Furthermore, this interaction of traits and the herbivore's response must be considered in order to extend these defense theories from mere predictions of the physiological state of plants to hypotheses concerning the ecological interactions that stem from defense allocation. Palatability is thus the most integrative and comprehensive measure through which to expand both the physiological and ecological predictions of defense theories.

Dictyotalean seaweeds provide an opportunity to determine if predictions of either ODT or GDBH are broadly observed at both physiological and ecological scales. The Dictyotaleans all have apical growth and relatively similar planar morphologies, minimizing differences in structure and growth form that often plague studies of terrestrial plant defense allocation (Littler and Littler 2000, Carmona et al. 2011). Because they lack roots, all tissues are above-ground and available for consumption by the same suites of herbivores, unlike terrestrial plants (Cronin 2001). Additionally, they tend to lack rigid structural defenses that characterize woody plants and can suppress herbivore feeding on some tissues (Carmona et al. 2011). Furthermore, these

seaweeds have no specialized structures for translocation of defensive metabolites, so defenses are likely produced where they are found (Lobban and Wynne 1981, Cronin 2001, Dawes and Mathieson 2008). By spatially linking the value of the tissue, its risk of predation, and defense produced, the Dictyotales facilitate evaluation of the predictions of ODT and GDBH while minimizing overlap of the two theories and other confounding factors. For these reasons, defense theories were previously tested in *Dictyota ciliolata*, in which bases are more defended against amphipod grazing through higher allocation of defensive dictyols, in accordance with GDBH (Cronin and Hay 1996). We therefore chose to evaluate defense theories in Dictyotalean seaweeds to determine whether the pattern of defense allocation observed in *D. ciliolata* holds for other members of its family with similar structural properties.

We used Dictyotalean macroalgae to evaluate ODT and GDBH by asking the following questions: (1) Do apical and basal tissues differ in palatability within individual seaweeds, and are these differences consistent across species? (2) Do intra-individual palatability differences vary with geographic location? (3) Do patterns of intra-individual palatability vary between grazers? (4) What mechanisms drive within-individual differences in palatability: structural, chemical, or nutritional properties? (5) Are more nutritionally valuable tissues more defended?

METHODS

Collections

Using five genera of Dictyotalean algae from both Florida and Panama, we assessed whether bases and apices differed consistently in value and palatability and investigated which traits might produce such differences. In Florida, *Padina gymnospora*, *Dictyota mertensii*, and *Stypopodium zonale* were collected from rubble areas at 2-6 m depth behind Pickles Reef near Key Largo, FL (N 24°59.407' W 080°25.033'), while *Lobophora variegata* and *Dictyopteris jamaicensis* were collected at 8-10 m depths from *Diadema* Reef (N 24°59.172' W 080°26.108'). Collections were made on May 24-26 and June 7-13, 2009. Epiphytes and debris were gently removed from algae, which were then maintained in aerated seawater until used in experiments. Apical and basal tissues not used in live tissue assays were separated and frozen for further experiments. "Apices" contained meristems and young tissue, within 1-2 cm of the apical edge of the seaweed. "Bases" included holdfasts, stipes, and aged tissue within 1-2 cm of the holdfast.

In May-June 2009 and August 2010, *Mithrax sculptus* crabs were collected southeast of Rodriguez Key, FL (N 25°02.751' W 080°27.146') from branching coralline algae at 1m depth. *M. sculptus* are small generalists that feed well in captivity (Stachowicz and Hay 1996). Crabs were maintained in 11 L plastic containers holding aerated seawater. Crabs returned to Georgia Institute of Technology for further experiments were held in 38 L tanks of recirculating artificial seawater. Between experiments, crabs were fed live *Ulva* sp., artificial agar-based foods made from powdered *Ulva*, or commercial marine herbivore food.

To evaluate whether patterns of palatability varied with location due to altered herbivores, physical regimes, or other factors, we conducted similar collections and experiments in Bocas del Toro, Panama. *Padina gymnospora* (N 09°21.033' W 082°13.947') and *Dictyota ciliolata* (N 09°20.425' W082°13.761') were collected from Isla Caremero on June 16, 2009. On June 23, 2009, *Spatoglossum schroederii*, *Dictyopteris justii*, and *Lobophora variegata* were collected from Long Bay Point, Isla Colòn (N 09°23.450' W 082°14.400'). Seaweeds were separated and held in large flow-through seawater tanks until used in live-tissue assays or until tissues were separated and frozen as above.

Because *Mithrax sculptus* also occurs in Panama, it was used there to assess algal palatability while keeping grazer identity constant between locations. *M. sculptus* crabs were collected from crustose coralline algae-covered coral rubble at 1 m depth from Mangrove Point, Isla Colòn in July 2009 (N 09°54.776' W 082°15.441). We also used the sea urchin *Echinometra viridis* to determine if palatability differences varied between grazers. *E. viridis* were collected from Coco Point, Isla Cristobal in July 2009 (N 09°17.770' W 082°15.976') at depths of 1-6 m. Urchins were kept in 38 L flow-through aerated seawater tanks, while crabs were kept in shallow 19 L flow-through tanks. When not in use for assays, animals were fed *Acanthophora sp.* or artificial agar-based foods made with powdered *Ulva* sp.

Seaweed Density, Organic Content, and Protein Content

To create realistic artificial foods and evaluate the predictions of ODT, we first determined the natural dry mass per volume of each seaweed and tissue type by measuring 1-2 mL volumes (n=5) of each fresh seaweed tissue type as determined via displacement in a graduated cylinder, then dried each to a constant mass and calculated dry mass/mL.

The potential food value of each seaweed was estimated via their organic and protein content. To measure organic content, dried, powdered tissue from each species and tissue type was weighed into pre-ashed pans (100-700 mg, n=4), combusted at 460°C for 7-8 h, and ash-free dry mass determined. Protein content was measured using a microplate version of the Bradford assay (Bradford 1976). Dried pooled tissue (10 mg; n=6) was digested with 1 mL 1 M NaOH at 4°C for 24 h. A 100 μL aliquot was removed from centrifuged, digested samples and added to 900 μL DI. A standard curve was made with dilutions of bovine serum albumen. For each extraction replicate and point on the standard curve, three 40μL aliquots were plated onto a 96-well plate and 170 μL Bradford reagent was added. Absorbance was measured at 595 nm after 5 minutes. The three wells of each replicate were averaged within each plate to calculate an average per extraction replicate, and protein content calculated using the standard curves for each plate.

Palatability of Live Tissues

To assess their relative palatability, apical and basal tissues were removed from individual seaweeds, blotted dry on paper towels, weighed (0.040 ± 0.004 g, n=20), and placed haphazardly across from each other in a 240 mL plastic cup containing 75 mL seawater. Each replicate consisted of a treatment cup containing these algal pieces and a crab, and a control cup holding pieces of the same alga but lacking the crab to assess changes in algal mass unrelated to consumption. Crabs were removed from treatment cups after 50% of the total food had been consumed, after 80% of the other crabs had finished feeding, or after 28 h, whichever came first. Assays with urchins in Panama required 48 h for adequate feeding and used the same autogenic controls as simultaneously run *Mithrax sculptus* live tissue feeding assays. At the end of each

experiment, remaining algae were blotted dry and re-weighed. Using the autogenic controls, amount consumed was calculated as:

$$amount\ consumed = (T_i * C_f / C_i) - T_f$$

where T_i = initial treatment mass, T_f = final treatment mass, C_i = initial autogenic control mass, and C_f = final control mass (Stachowicz and Hay 1996).

Palatability of Artificial Foods

Palatability of live tissues could result from tissue properties such as structure, deterrent chemistry, or nutritional value. To elucidate the role of structural defenses for Florida samples, apical and basal tissues were pooled separately, dried, and ground to a fine powder with a mortar and pestle to destroy structural properties. These powdered foods were incorporated into gel-based foods that had the chemical and nutritional traits of the whole alga, but lacked the structural traits (Hay et al 1994).

We initially made agar-based foods to match the dry mass/volume of intact seaweeds, attempting to mimic the reward per bite of each tissue. However, the basal tissues of several seaweeds were too dense and the agar would not harden; therefore, all densities were halved to allow the agar to set (Bolser and Hay 1996). To separate the effects of dry mass/volume from other traits of these tissues (e.g., chemical or nutritional), we also conducted ground tissue assays with apical and basal tissues incorporated at equal dry mass/volume. For this assay, apical and basal foods were made at half of the natural dry mass/mL of the apices.

Agar-based foods were made by adding the ground dry mass from a 4.5 mL volume of fresh alga to 4 mL deionized H_2O, mixing this slurry with 5 mL of heated deionized H_2O holding 0.18 g agar, and molding this agar-based food onto window screens using a 2 mm x 1.3 cm x 24

cm lane in a formica mold (methods of Hay et al. 1994). Apical and basal foods for each seaweed were poured into adjacent lanes in the mold, so as to be connected by 2 cm of bare window screen when the food cooled and the mold was removed. The window screen and attached foods were then cut into strips, so that each strip (approximately 0.7 cm x 4 cm) contained equal amounts of both apical and basal foods on opposite ends of the strip (n=17-20). Each food covered a 7x7 area of window screen "rectangles" on each strip (approximately 0.7 cm x 1.3 cm). Feeding was measured as the number of window screen rectangles from which food had been removed.

Assays were run in the same containers described above, with *Mithrax sculptus* as the grazer. Controls for autogenic changes were not needed because screen coverage does not change unless a grazer is present (Hay et al. 1994). Crabs were removed when they had eaten 50% of their food, and the experiment was ended when 80% of the crabs had been removed or at 48 h, whichever occurred first. If a crab died before eating half of its food, it was removed, food and cup were thoroughly rinsed, and new water and a new crab were added (this occurred infrequently: 5 of 200 replicates for ground tissue assays, 1 of 140 for extract assays). The number of completely consumed window screen rectangles of food was counted under a dissecting microscope. Food that had been dislodged was realigned on the screen for scoring.

Palatability of Non-Polar Extracts

To determine whether lipid-soluble chemicals were responsible for observed differences in apical vs. basal palatability, we conducted feeding assays with non-polar extracts of the tissues. Frozen basal or apical tissues of each algal species from Florida were simultaneously extracted in enough methanol to cover the tissue. Each sample was extracted three times in the

freezer for a total of 12-24 h, after which they were filtered and dried on a rotary evaporator. Extracts were then were partitioned three times between equal parts deionized water and ethyl acetate, and these fractions were dried. We focused on the ethyl acetate fraction because herbivore deterrents in tropical seaweeds are most often non-polar (Bolser and Hay 1996) and because many polar compounds leach from assay foods placed in water, making it difficult to test their effects on herbivore feeding (Steinberg 1988). The lipophilic extract was suspended in diethyl ether and an aliquot equivalent to 1g of the dried algal tissue was removed. This aliquot was then coated onto 1 g of powdered *Ulva* (a palatable green alga) and the ether was removed via rotary evaporation.

This extract-coated *Ulva* was then made into an artificial food and molded onto window screen strips holding one food treated with extract from basal and one treated with extract from apical tissues (n=12-19 pairs). Crabs were offered these artificial foods as previously described, and assays were ended after 36 h. For *Dictyota mertensii*, extract-coated foods were also tested against a solvent-coated food control, to determine if extracts were palatable or unpalatable. Apical extract vs. control and basal extract vs. control assays were interspersed.

Statistical analyses

Results of feeding assays for live tissue, ground tissue, and extract-coated foods were compared with two-tailed, paired t-tests ($\alpha = 0.05$). Replicates where crabs ate less than 10% or more than 90% of the offered food were excluded because they provide poor data on relative preference. For assays testing palatability of extracts versus solvent controls, treatment and control consumption were first compared with paired, two-tailed t-tests as above. Subsequently, the difference in consumption of control and treatment foods in each replicate was calculated,

and these differences were compared between apical and basal extracts via a two-tailed t-test. Density, organic content, and protein content were compared within species with two-tailed t-tests.

Linear regressions were conducted to determine how feeding related to organic and protein contents. We first calculated *relative consumption difference = ((apical tissue eaten – basal tissue eaten)/total tissue eaten),* generating positive values if apices were eaten and negative values if bases were eaten. We also calculated organic or protein difference in the offered foods (e.g., apical organic content – basal organic content), generating positive values if apices had more organic content and negative values if the bases did. We then regressed mean relative consumption difference for each seaweed in a given assay against its mean organic or protein difference. We combined data from the ground tissue assays at natural and equal densities and performed the same analyses to observe how organic and protein contents drive feeding when structure is removed, and when densities of foods (and therefore relative difference in organics and protein) are altered. For lipophilic extract assays, we first calculated the apical deterrence (i.e., the opposite of relative consumption difference) of extract coated foods, using *apical deterrence = ((basal food eaten – apical food eaten)/total eaten),* generating positive values if basal extracts were more consumed (apices more deterrent) and negative values if apical extract foods were more consumed (bases more deterrent). We then calculated the amount of protein in the tissue that generated each extract used in the assay, and subtracted the apical protein value from the basal protein value, to obtain the protein difference that would have been present in the extracted tissues. Protein difference was positive if apices contained more protein, and negative if bases contained more. The same procedure was repeated for organic content. All statistical analyses were performed using GraphPad® statistical software.

RESULTS

Value of Tissues: Organic and Protein Content

To evaluate whether our seaweeds conformed to ODT, we first quantified organic and protein content of each tissue to obtain a rough measure of value of each tissue to the plant and to the herbivore. For 8 of the 10 contrasts, apical tissues contained significantly less dry mass per volume than bases (Table 1), but all had uniformly higher organic content ($P<0.017$ for all contrasts), indicating more inorganic structure in the bases. Apices also tended to have more protein per dry mass; this relationship was significant in two species in Florida and three in Panama ($P<0.040$), and 3 of the other 5 contrasts trended in the same direction. By these measures, apices appear to represent greater investment per mass by the plant, and this might make them more nutritionally valuable to herbivores.

Table 1. Algal tissue traits. Densities (n=5), ash-free dry mass (organic content; n=4), and protein content (n=6) of seaweed tissues. Bolded text indicates the trait value is significantly greater than in its paired tissue ($P<0.05$ two-tailed t-test).

Region	Alga	Basal Density (g dry mass /mL)	Apical Density (g dry mass /mL)	Basal Organic Content (% dry mass)	Apical Organic Content (% dry mass)	Basal protein (% dry mass)	Apical protein (% dry mass)
Florida	*Dictyopteris*	**0.328 ± 0.054**	0.099 ± 0.020	31.64 ± 0.49	**49.46 ± 0.32**	0.62 ± 0.14	**0.80 ± 0.12**
	Dictyota	**0.266 ± 0.052**	0.135 ± 0.009	41.54 ± 0.99	**62.83 ± 0.29**	0.46 ± 0.23	0.35 ± 0.12
	Padina	**0.335 ± 0.084**	0.155 ± 0.031	33.27 ± 0.41	**39.23 ± 0.15**	0.67 ± 0.30	0.98 ± 0.19
	Lobophora	**0.271 ± 0.078**	0.218 ± 0.030	44.54 ± 1.39	**54.48 ± 0.97**	0.97 ± 0.16	**1.57 ± 0.36**
	Stypopodium	**0.217 ± 0.038**	0.159 ± 0.015	58.06 ± 0.37	**75.01 ± 0.30**	0.50 ± 0.22	0.77 ± 0.23
Panama	*Dictyopteris*	0.164 ± 0.023	0.147 ± 0.012	33.79 ± 0.62	**57.66 ± 0.29**	0.76 ± 0.24	**1.99 ± 0.30**
	Dictyota	**0.206 ± 0.031**	0.118 ± 0.029	37.59 ± 0.64	**63.26 ± 0.41**	0.53 ± 0.18	0.42 ± 0.13
	Padina	**0.249 ± 0.028**	0.121 ± 0.008	37.77 ± 0.31	**50.71 ± 0.30**	0.63 ± 0.16	**1.19 ± 0.25**
	Lobophora	0.317 ± 0.033	0.275 ± 0.019	48.47 ± 1.69	**69.49 ± 0.28**	0.50 ± 0.13	0.83 ± 0.37
	Spatoglossum	**0.211 ± 0.049**	0.122 ± 0.004	40.75 ± 1.18	**56.40 ± 0.47**	1.19 ± 0.27	**2.05 ± 0.67**

Herbivore Choices between Live Apical and Basal Tissues

Herbivores did not consistently prefer either apices or bases across algal species, geographic regions or grazer species. In Florida, *Mithrax sculptus* preferred apices to bases of *Dictyopteris* (P=0.017) but significantly favored bases over apices of *Dictyota, Padina,* and *Lobophora* ($P \leq 0.021$ for all contrasts; Figure 1A). Crabs expressed no significant preference for bases or apices of *Stypopodium* (P=0.39), but consumed minimal amounts of either tissue. Feeding preferences in these live-tissue assays were not predicted by concentrations of protein (r^2=0.0234, P=0.81) or organic content (r^2=0.0153, P=0.83; data not shown. Similar to Florida, Panamanian *Mithrax sculptus* preferred apices to bases in *Dictyopteris* (P=0.004; Figure 2A), and tended to prefer bases to apices in *Dictyota* (P=0.19). However, unlike in Florida, Panamanian crabs did not differentiate between apices and bases of *Padina* (P=0.41) or *Lobophora* (P=0.35). In *Spatoglossum,* the additional species in Panama, apices were more palatable than bases ($P<0.001$). Crabs thus preferred bases in three species, apices in three, and made no choice among tissues for the remaining for species, and crab preferences for algal tissues varied between Panama and Florida (Figure 1, 2). The sea urchin *Echinometra viridis* did not differentiate between apical and basal tissues for any of the seaweeds tested ($P>0.12$ for all contrasts; Figure 2B), demonstrating that grazers are differentially affected by intraindividual variation in seaweed traits.

Effects of Structural Traits on Herbivore Choice

Structural traits govern relative palatability in two of the five algae from Florida. Herbivore preferences for apices of *Dictyopteris* and bases of *Padina* were lost when the structure of these algae was destroyed (P=0.13 and P=0.20; Figure 1B), indicating that

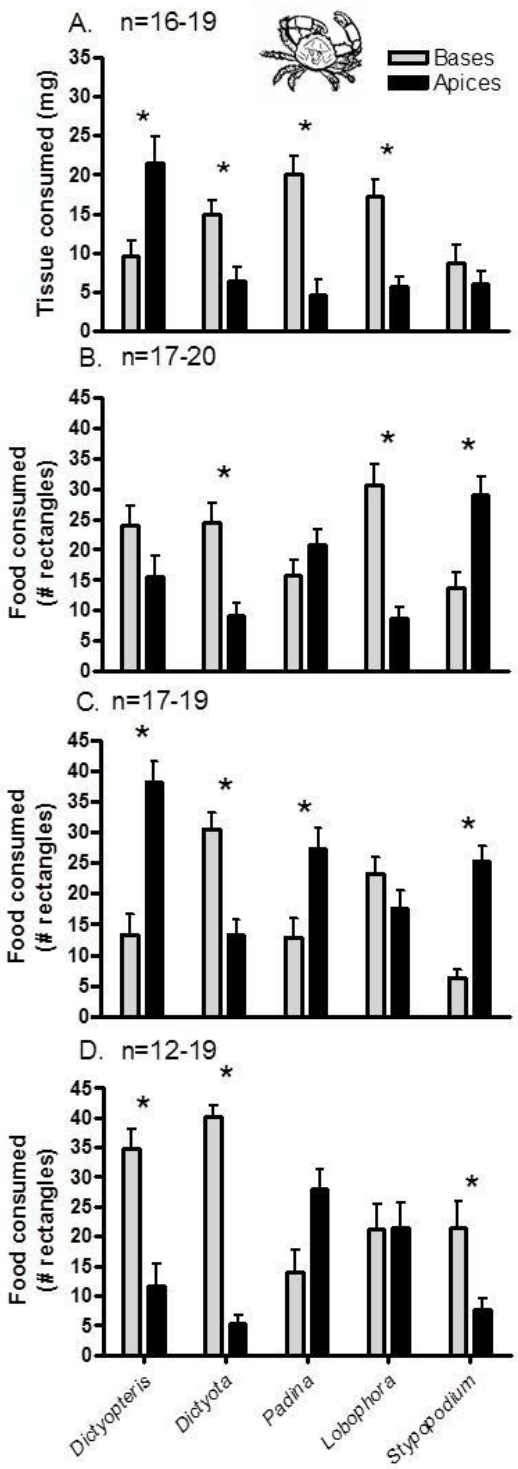

Figure 1: *Mithrax sculptus* preferences for live and artificial foods in Florida. A. Crab preferences for apical vs. basal live tissues **B.** Crab preferences for apical vs. basal artificial foods reconstituted at their natural density/volume **C.** Crab preferences for apical vs. basal artificial foods reconstituted at equal density/volume for both tissue choices **D.** Crab preferences for palatable artificial foods coated with apical vs. basal non-polar extracts. * indicates $P<0.05$ in a paired, two-tailed t-test.

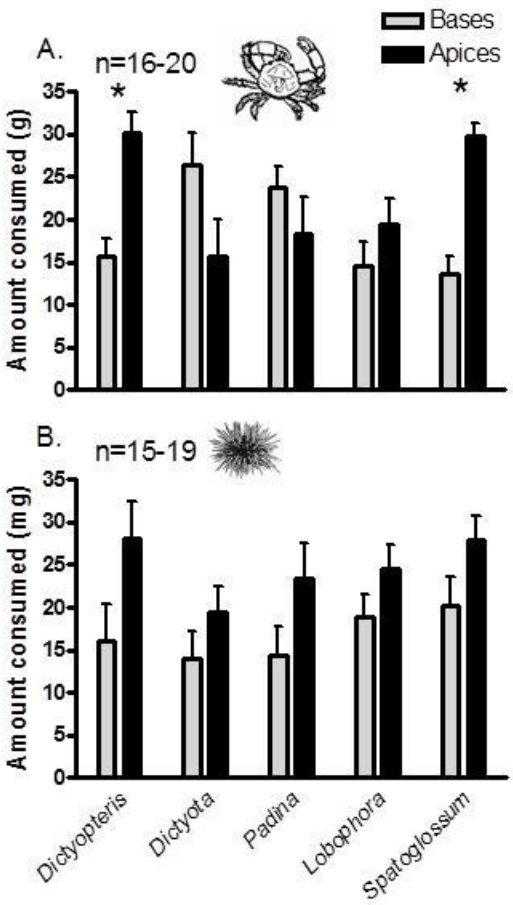

Figure 2: Herbivore preferences for live algal tissues in Panama. A. *Mithrax sculptus* preferences **B.** *Echinometra viridis* preferences. * indicates P<0.05 in a paired, two-tailed t-test.

Dictyopteris bases and *Padina* apices were structurally defended. However, *Mithrax sculptus* still preferred *Dictyota* and *Lobophora* bases to apices when structural traits were destroyed (P=0.002 and P<0.001; Figure 1B), suggesting that feeding preference was driven by other mechanisms for these seaweeds.

Effects of Nutritional Value on Herbivore Choice

After normalization of tissue densities to eliminate the possibility that preference could be confounded by differing dry mass to volume ratios among tissues, crab preferences for apical vs. basal foods changed in some, but not all, algal species. Because bases were denser than apices

but generally contained less organic and protein content, lowering the density of basal food usually decreased the nutritional value of basal food relative to apical food (Figure 3). Therefore, comparing herbivore preferences for artificial foods at natural vs. equal densities may demonstrate how nutritional value influences feeding. When tissue densities were made equal, *Padina* apices became more palatable than bases (P=0.006), opposite to that observed with live tissue. At equal densities, *Dictyopteris* bases were less palatable than apices, as with live tissues, (P<0.001), and *Lobophora* tissues no longer differed in palatability (P=0.24; Figure 1B,C). Taken together, these results suggest reward per bite influences herbivore choice because palatability of apices increased with decreasing density and reward per bite of the bases. Regardless of how we manipulated tissue density, crabs still significantly preferred *Dictyota* bases (P=0.001; Figure 1B,C), suggesting chemical deterrence of the apices or attractants in the

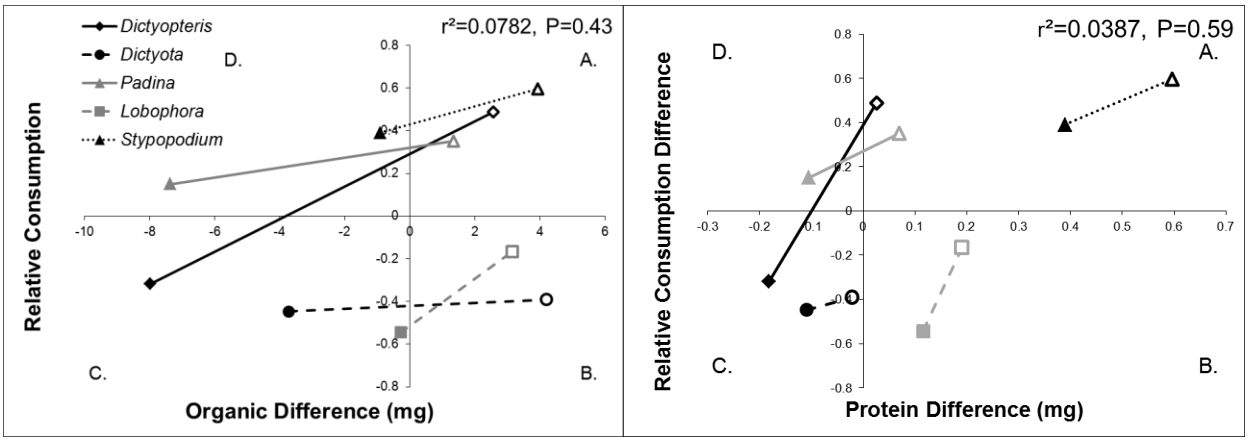

Figure 3. Relationships between herbivore choices of artificial foods and organic and protein content. Mean relative consumption differences for natural density and equal density artificial food assays (*relative consumption difference = (apical food eaten – basal food eaten)/total eaten*; solid data points represent natural density assays, open points represent equal density assays), versus the calculated trait difference between apical and basal foods. In each graph, quadrants correspond to (clockwise from upper right): a) apices more valuable, apices more consumed; b) apices more valuable, bases more consumed; c) bases more valuable, bases more consumed; d) bases more valuable, apices more consumed. **A.** Relative consumption difference vs. organic difference of artificial foods. **B.** Relative consumption difference vs. protein difference of artificial foods. R^2 and P-values from linear regression across all species.

bases. In contrast, *Stypopodium* apices were more palatable than bases regardless of tissue density (P<0.001 for both), indicating a chemical or nutritional driver of relative palatability in these foods that was not expressed in the whole tissue assay. When we plotted this relative consumption of apical vs. basal artificial foods against the organic and protein contents of those foods, preference for apices increased with their increasing nutritional value, but only within species (Figure 3). Across species, relative consumption did not correlate with either organic content (r^2=0.0782, P=0.43) or protein (r^2=0.0387, P=0.59). Relative nutritional content of tissues can thus influence herbivore preference, but is not the primary driver of choice even when confounding structural traits are removed.

Effects of Non-polar Chemistry on Herbivore Choice

Non-polar chemistry only explained one of five patterns of herbivore choice between live apical and basal tissues. For *Dictyota*, crabs consistently chose bases in live tissues, artificial foods lacking structure, and artificial foods laced with non-polar extracts of *Dictyota* (P<0.001 for the latter; Figure 1.) Basal extracts were marginally deterrent vs. a solvent control (P=0.053, Figure 4), apices were significantly deterrent (P<0.001), and the extract of apices was significantly more deterrent than the extract of bases (P<0.001; Figure 4). In short, apices of *Dictyota* are more chemically deterrent than bases, and it is this difference in non-polar chemistry that drives patterns of relative palatability within this seaweed. In the other four seaweeds, patterns of palatability in extract-coated foods were non-significant (*Lobophora* P=0.97), or were contrary to those observed in live tissue (*Dictyopteris* P=0.002; *Padina* P=0.053; *Stypopodium* P=0.021). Although *M. sculptus* can discriminate between foods based

on non-polar metabolites contained in these seaweeds, lipid-soluble chemistry is not the primary driver of live tissue palatability in species other than *Dictyota.*

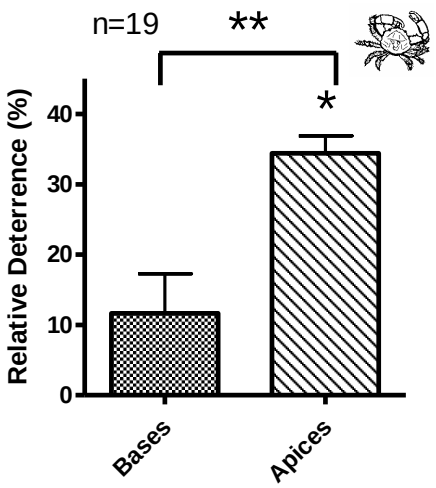

Figure 4. Deterrence of basal and apical extracts of *Dictyota mertensii* to *Mithrax sculptus*. Relative deterrence of artificial foods coated with non-polar extracts versus solvent-coated controls. Relative deterrence = (control eaten – treatment eaten)/(total eaten) * indicates P<0.05 in a two-tailed paired t-test between treatment and solvent control; ** indicates P<0.05 in an unpaired, two-tailed t-test between deterrence (i.e., control – treatment) of apices and bases.

Relationships between Non-Polar Chemical Deterrence and Tissue Value

To test whether chemical deterrence and possible food value of tissues interacted to influence within-plant preference by herbivores, we tested for an association between apical deterrence and difference in protein content and organic content of the extracted apical and basal tissues. Protein difference did not predict apical deterrence (r^2<.001, P=0.99). However, difference in organic content positively correlated with apical deterrence (r^2=0.991, P=0.004), indicating that more organic-rich tissue was better chemically defended (Figure 5).

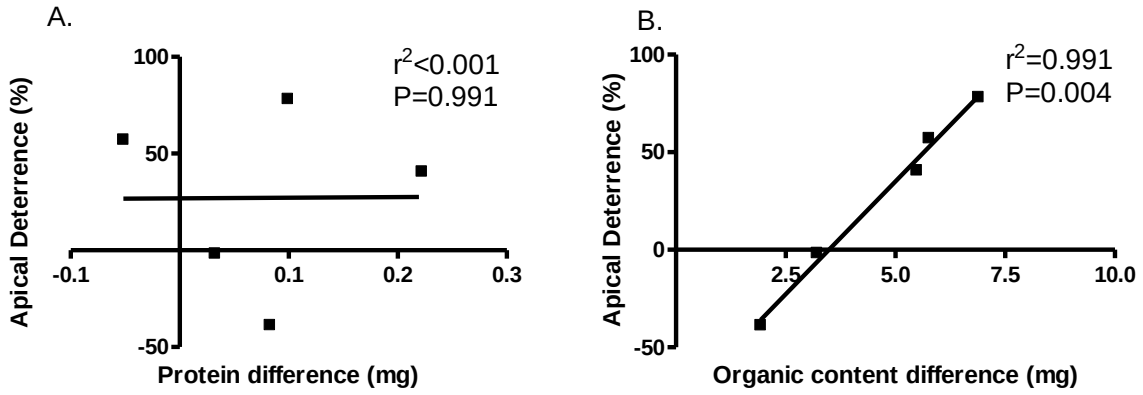

Figure 5. Relationships between crab avoidance of artificial foods coated with non-polar extracts and the nutritional value of that food. Mean herbivore choices between extract-coated foods are expressed as apical deterrence (100%*(basal food eaten – apical food eaten)/total eaten) versus the equivalent difference in **A.** protein (protein in extracted apical tissue – protein in extracted basal tissue) **B.** organic content (calculated as for protein). P- and r^2 values from linear regression.

DISCUSSION

By examining relative palatability of apical vs. basal tissues across several species of seaweeds from two locations using two grazer species, we tested whether theories of intraindividual defense allocation broadly predict defense allocation as perceived by herbivores. We can reject the Growth-Differentiation Balance Hypothesis (Herms and Mattson 1992, Cronin and Hay 1996) as a broad predictor of relative palatability of seaweed tissues because crabs avoided old, differentiated bases in favor of young, growing apices in only three of ten seaweeds across our experiments (Figure 1A, 2A). Although previous studies with *Arabidopsis thaliana* (Barto and Cipollini 2006) and *Dictyota ciliolata* (Cronin and Hay 1996) found these plants with few transport structures to contain defended older tissues, as predicted by GDBH (Herms and Mattson 1992), we find this pattern is not widespread in seaweeds (Figure 1A, 2A). This previously observed defense of bases in *D. ciliolata* (Cronin and Hay 1996) did not persist within even *Dictyota* spp. in our study: apices rather than bases of *D. ciliolata* tended to be deterrent, and *D. mertensii* apices were significantly so (Figure 1A, 2A). This striking contrast within the same species and genus highlights the importance of testing defense theories using multiple plant species in multiple regions, as single-species or single-location studies can easily lend support to a defense theory without testing its validity as broad and predictive. In addition, the Dictyotaleans we studied have minimal structures with which to transport defensive metabolites (Lobban and Wynne 1981, Dawes and Mathieson 2008), making it unlikely that defenses produced in the older, differentiated bases are then transported to the apices in the seven species where bases were not avoided (Figure 1A, 2A; Herms and Mattson 1992, Barto and Cipollini

2006). GDBH thus does not predict relative palatability of macroalgal tissues and can be rejected as a broad, encompassing theory of intraindividual defense variation.

Unlike GDBH, we cannot fully reject Optimal Defense Theory without rigorously testing all of its assumptions; however, it does not provide useful predictions of relative palatability of these macroalgae. Apices generally contained more organics and protein (Table 1), traits often used as a simplified measure of tissue attractiveness to the herbivore and investment value to the plant (Mattson 1980, Cronin and Hay 1996, Schupp et al. 1999, Pansch et al. 2008, Freeman and Gleason 2010). According to ODT, these more valuable and at-risk apices should be more defended. However, apices were less palatable than bases in only three of ten seaweeds (Figure 1A, 2A), indicating either that ODT does not predict palatability across species or that our measure of value and risk is insufficient. One of the downfalls of ODT as a predictive theory is that it is nearly impossible to comprehensively test optimal evolution of defenses and the assumptions underlying its predictions (Rhoades 1979, Stamp 2003a,b). Unless fitness value and herbivore risk of tissues are explicitly measured, ecologists can rationalize that ODT explains their data regardless of which tissues are most defended (Iken and Amsler 2007, Asplund et al. 2010). For example, it can be argued that marine macroalgae derive the greatest fitness benefit either from the new, growing, photosynthetic apices, or from the anchoring bases without which a seaweed floats away and dies. When fitness value has been measured, however, basal stipes (functionally equivalent to stems) have been found to be most valuable to future fitness and most defended, rather than apical or reproductive tissues (Pavia et al. 2002, Taylor et al. 2002). Herbivore risk to different tissues is tested even more rarely than fitness value because in most cases, defenses cannot be removed without killing the tissues (Asplund et al. 2010). However, measuring tissue risk with defenses present examines the integration of defense traits, nutritional

value, and tissue accessibility, rather than the herbivore's attraction to a tissue against which plants have evolved defenses (Asplund et al. 2010). The mixed patterns of herbivore choice we found thus either suggest that (1) if value and risk are constantly found in either apices or bases across these seaweeds, then ODT does not predict relative palatability, or (2) the identity of the most valuable and/or at-risk tissue varies greatly within this family of seaweeds.

Although we cannot accept ODT as a predictor of palatability, our results provide some support for the physiological aspects of this theory. The presence of enhanced lipophilic defenses in organic-rich apices in our seaweeds supports ODT's predictions of increased defense allocation in more valuable tissues (Figure 5B); however, this chemical defense of organic-rich apices does not coincide with herbivore preferences among most live tissues (Figure 1A,D, 3). Previous studies have found nutritional content and chemical deterrence to interact, such that chemicals that adequately defend low quality foods are inadequate at defending higher quality foods (Duffy and Paul 1992, Cruz-Rivera and Hay 2003). This same interaction may account for the inconsistency we observed between chemical deterrence of valuable tissues and herbivore feeding on live tissues (Figure 1A, D), pointing to the importance of evaluating the ecological predictions of defense theories with palatability rather than allocation of single traits. We thus find support for the physiological predictions of ODT because apices of higher relative organic content are more chemically defended (Figure 5B), although the interaction of these two traits, and possibly others, appears to mask this pattern of defended apices when we examine herbivore choices between live tissues.

While we used the crab *Mithrax sculptus* to evaluate palatability of seaweed tissues, it is important to note that marine macroalgae encounter more than one herbivore species (Hay et al. 1987, Hay 1997). Because most marine herbivores are roving generalists (Carpenter 1986, Sotka

2005), we have little idea against which herbivores seaweeds have evolved defense allocation patterns, nor do we know against which herbivores these defenses are effective (Strauss and Irwin 2004, Agrawal 2011). When we employed a different herbivore's perspective to account for these unknowns, our evaluation of defense allocation and palatability changed dramatically. *Echinometra viridis* urchins preferred neither apices nor bases in any of the tissues from Panama (Figure 2B). Insensitivity of urchins to within-individual variation in defense is consistent with previous studies (Cronin and Hay 1996). Had we only used *E. viridis*, we would have concluded that relative palatability does not vary within individual seaweeds, a strong contrast to our observations with *Mithrax sculptus*. Not only does allocation of plant traits influencing herbivore choice thus vary by algal species and region, but herbivore species are also differentially affected by these plant traits.

Most examinations of defense allocation focus only on chemical defenses (Tugwell and Branch 1989, Barto and Cipollini 2006, Zangerl and Rutledge 1996), partly because defense theories were historically framed around secondary metabolites (Rhoades 1979, Herms and Mattson 1992, Stamp 2003a). Despite such a focus on chemistry in theory and previous studies, chemical defenses drove within-species differences in palatability to *Mithrax sculptus* in only one seaweed, *Dictyota mertensii* (Figures 1, 3). *D. mertensii* bases were more palatable than apices regardless of how live or artificial foods were presented (Figure 1), because non-polar chemistry of the apices is much more deterrent than that of the bases (Figure 1D, 3). While in isolation the defense allocation in this alga supports ODT, in no other species did palatability of live tissues arise from differential allocation of chemical defenses (Figure 1A,D). This discrepancy emphasizes the importance of evaluating predictions of defense theory in multiple species. The focus of defense theory on chemical deterrence alone is also inappropriate for these

macroalgae and suggests defense theories must be expanded to include non-chemical defensive traits.

Our studies reveal that structure, nutritional value, and chemical defense all influence relative palatability, but that the dominance and interactions among these traits vary between species. A recent meta-analysis found that herbivore preferences between plants are influenced by structural traits more than by secondary metabolites (Carmona et al. 2011), but this is rarely studied in large surveys of within-plant variation in defense (Van Alstyne et al. 1999, Iken and Amsler 2007, McCall and Fordyce 2010; but see Fairhead et al. 2005). Structural traits drove crab choices between live tissues of two of five seaweeds we studied. (Figure 1A,B). *Dictyopteris* has tough and fibrous unpalatable bases, while unpalatable *Padina* apices have surface hairs (Littler and Littler 2000, Dawes and Mathieson 2008), which may have deterred crab feeding. Nutritional value of tissues also influences herbivore preference between plants (Carmona et al. 2011, Agrawal 2011). Altering the nutritional content of artificial foods in our study disrupted herbivore preferences for tissues within species (Figure 1B, C), even though preference did not correlate with organic or protein content across species (Figure 3). Nutritional quality thus appears to interact with other traits in these tissues and is one of several drivers of palatability. Previous experiments have shown structure, secondary metabolites, and nutritional quality to not only influence food choice individually, but to interact with one another, as may be the case here (Duffy and Paul 1992, Hay et al. 1994, Bullard and Hay 2002, Cruz-Rivera and Hay 2003). From the herbivore's perspective, ODT and GDBH thus do not predict patterns of relative palatability because many different defense mechanisms and their interactions drive these patterns of herbivore choice we observed.

When we examine multiple defensive traits across several species of seaweeds across two geographic locations with two grazers, we find that defense theories do not readily predict overall tissue palatability. We reject GDBH at both physiological and ecological scales because it does not predict palatability or chemical defense allocation. Although ODT predicted higher deterrence of organic-rich apices, it cannot be accepted or rejected as a predictor of palatability without incorporating additional measures of herbivore risk and tissue fitness. However, our studies thus demonstrate the inability of ODT to provide predictions of defense allocation that can be easily and rigorously evaluated across multiple species without making large assumptions of value and risk (Iken and Amsler 2007, McCall and Fordyce 2010). The complex interactions and varying influence of chemical defense, nutritional value, and structural defense in governing herbivore choice may have masked patterns of defense allocation that would have supported one of these theories. To extrapolate these defense theories from predictions of plant physiology to predictions of palatability, researchers thus must investigate the traits that drive food choices of multiple herbivores and how these traits interact. Admittedly, simultaneously undertaking such a complete investigation of value, risk, defensive traits, and herbivore preferences across multiple plant and herbivore species is a daunting task, and studies may have to be continued on a single-species basis to acquire this necessary depth. However, such single-species studies can only be used to test predicted patterns of palatability if considered in conjunction with the growing body of literature on defense allocation, rather than in isolation, as patterns of palatability are here shown to vary greatly with algal species, grazer species, and geographic location. Once sufficient data has been collected and considered in this way, existing theories will need to be expanded to include interactions of different defense types and their perception by herbivores in order to predict broader ecological outcomes of defense allocation.

REFERENCES

Agrawal, A. A. 2011. Current trends in the evolutionary ecology of plant defence. Functional Ecology **25**:420-432.

Asplund, J., K. A. Solhaug, and Y. Gauslaa. 2010. Optimal defense: snails avoid reproductive parts of the lichen Lobaria scrobiculata due to internal defense allocation. Ecology **91**:3100-3105.

Barto, E. K. and D. Cipollini. 2006. Testing the optimal defense theory and the growth-differentiation balance hypothesis in Arabidopsis thaliana. Oecologia **146**:169-178.

Becerro, M. A., V. J. Paul, and J. Starmer. 1998. Intracolonial variation in chemical defenses of the sponge *Cacospongia sp.* and its consequences on generalist fish predators and the specialist nudibranch *Glossidoris pallida*. Marine Ecology-Progress Series **168**:187-196.

Berenbaum, M. R. and A. R. Zangerl. 1998. Chemical phenotype matching between a plant and its insect herbivore. Proceedings of the National Academy of Sciences of the United States of America **95**:13743-13748.

Bolser, R. C. and M. E. Hay. 1996. Are tropical seaweeds better defended? Palatability and defenses of temperate vs. tropical seaweeds. Ecology **77**:2269-2286.

Boudsocq, S., S. Barot, and N. Loeuille. 2011. Evolution of nutrient acquisition: when adaptation fills the gap between contrasting ecological theories. Proceedings of the Royal Society B-Biological Sciences **278**:449-457.

Bradford, M. M. 1976. Rapid and sensitive method for quantitation of microgram quantities of protein using principle of protein-dye binding. Analytical Biochemistry **72**:248-254.

Bryant, J. P., F. S. Chapin, and D. R. Klein. 1983. Carbon/nutrient balance of boreal plants in relation to herbivory. Oikos **40**:357-368.

Bullard, S. B. and M. E. Hay. 2002. Palatability of marine macro-holoplankton: Nematocysts, nutritional quality, and chemistry as defenses against consumers. Limnology and Oceanography **47**:1456-1467.

Carmona, D., M. J. Lajeunesse, and M. T. J. Johnson. 2011. Plant traits that predict resistance to herbivores. Functional Ecology **25**:358-367.

Coley, P. D., J. P. Bryant, and F. S. Chapin. 1985. Resource availability and plant antiherbivore defense. Science **230**:895-899.

Cronin, G. 2001. Resource allocation in seaweeds and marine invertebrates: Chemical defense patterns in relation to defense theories. Pages 325-353 *in* J. B. McClintock and B. J. Baker, editors. Marine Chemical Ecology. CRC Press, Boca Raton, Florida.

Cronin, G. and M. E. Hay. 1996. Within plant variation in seaweed palatability and chemical defenses: Optimal defense theory versus the growth differentiation balance hypothesis. Oecologia **105**:361-368.

Cruz-Rivera, E. and M. E. Hay. 2003. Prey nutritional quality interacts with chemical defenses to affect consumer feeding and fitness. Ecological Monographs **73**:483-506.

Dawes, C. J. and A. C. Mathieson. 2008. The Seaweeds of Florida. University Press of Florida, Gainesville.

Duffy, J. E. and V. J. Paul. 1992. Prey nutritional quality and the effectiveness of chemical defenses against tropical reef fishes. Oecologia **90**:333-339.

Fairhead, V. A., C. D. Amsler, J. B. McClintock, and B. J. Baker. 2005. Within-thallus variation in chemical and physical defences in two species of ecologically dominant brown macroalgae from the Antarctic Peninsula. Journal of Experimental Marine Biology and Ecology **322**:1-12.

Feeny, P. 1976. Plant apparency and chemical defense. Pages 1-40 *in* J. W. Wallace and R. L. Mansell, editors. Recent Advances in Phytochemistry. Plenum, New York.

Freeman, C. J. and D. F. Gleason. 2010. Chemical defenses, nutritional quality, and structural components in three sponge species: Ircinia felix, I. campana, and Aplysina fulva. Marine Biology **157**:1083-1093.

Hay, M. E. 1997. The ecology and evolution of seaweed-herbivore interactions on coral reefs. Coral Reefs **16**:S67-S76.

Hay, M. E., J. E. Duffy, C. A. Pfister, and W. Fenical. 1987. Chemical defense against different marine herbivores: are amphipods insect equivalents? Ecology **68**:1567-1580.

Hay, M. E., Q. E. Kappel, and W. Fenical. 1994. Synergisms in plant defenses against herbivores: interactions of chemistry, calcification, and plant quality. Ecology **75**:1714-1726.

Herms, D. A. and W. J. Mattson. 1992. The dilemma of plants – to grow or defend. Quarterly Review of Biology **67**:283-335.

Iken, K., C. D. Amsler, J. M. Hubbard, J. B. McClintock, and B. J. Baker. 2007. Allocation patterns of phlorotannins in Antarctic brown algae. Phycologia **46**:386-395.

Kubanek, J., K. E. Whalen, S. Engel, S. R. Kelly, T. P. Henkel, W. Fenical, and J. R. Pawlik. 2002. Multiple defensive roles for triterpene glycosides from two Caribbean sponges. Oecologia **131**:125-136.

Lankau, R. A. and D. J. Kliebenstein. 2009. Competition, herbivory, and genetics interact to determine the accumulation and fitness consequences of a defence metabolite. Journal of Ecology **97**:78-88.

Littler, D. S. and M. M. Littler. 2000. Caribbean Reef Plants. Offshore Graphics, Inc., Washington, D. C.

Lobban, C. S. and M. J. Wynne. 1981. The Biology of Seaweeds. University of California Press, Berkeley.

McCall, A. C. and J. A. Fordyce. 2010. Can optimal defence theory be used to predict the distribution of plant chemical defences? Journal of Ecology **98**:985-992.

McKey, D. 1974. Adaptive patterns in alkaloid physiology. American Naturalist **108**:305-320.

McKey, D. 1979. The distribution of secondary compounds within plants. Pages 55-133 *in* G. A. Rosenthal and D. H. Janzen, editors. Herbivores: Their Interaction with Secondary Plant Metabolites. Academic Press, New York.

Meyer, K. D. and V. J. Paul. 1992. Intraplant variation in secondary metabolite concentration in three species of *Caulerpa* (Chlorophyta: Caulerpales) and its effects on herbivorous fishes. Marine Ecology-Progress Series **82**:249-257.

Pansch, C., I. Gomez, E. Rothausler, K. Veliz, and M. Thiel. 2008. Species-specific defense strategies of vegetative versus reproductive blades of the Pacific kelps Lessonia nigrescens and Macrocystis integrifolia. Marine Biology **155**:51-62.

Pavia, H., G. Toth, and P. Aberg. 2002. Optimal defense theory: Elasticity analysis as a tool to predict intraplant variation in defenses. Ecology **83**:891-897.

Poore, A. G. B., A. H. Campbell, and P. D. Steinberg. 2009. Natural densities of mesograzers fail to limit growth of macroalgae or their epiphytes in a temperate algal bed. Journal of Ecology.

Reznick, D. 1992. Measuring the costs of reproduction. Trends in Ecology & Evolution **7**:42-45.

Rhoades, D. F. 1979. Evolution of plant chemical defense against herbivores. Pages 1-55 *in* G. A. Rosenthal and D. H. Janzen, editors. Herbivores: Their Interaction with Secondary Plant Metabolites. Academic Press, New York.

Schupp, P., C. Eder, V. Paul, and P. Proksch. 1999. Distribution of secondary metabolites in the sponge Oceanapia sp and its ecological implications. Marine Biology **135**:573-580.

Sheldon, B. C. and S. Verhulst. 1996. Ecological immunology: Costly parasite defences and trade-offs in evolutionary ecology. Trends in Ecology & Evolution **11**:317-321.

Sotka, E. E. 2005. Local adaptation in host use among marine invertebrates. Ecology Letters **8**:448-459.

Sotka, E. E. and M. E. Hay. 2002. Geographic variation among herbivore populations in tolerance for a chemically rich seaweed. Ecology **83**:2721-2735.

Stachowicz, J. J. and M. E. Hay. 1996. Facultative mutualism between an herbivorous crab and a coralline alba: Advantages of eating noxious seaweeds. Oecologia **105**:377-387.

Stamp, N. 2003a. Out of the quagmire of plant defense hypotheses. Quarterly Review of Biology **78**:23-55.

Stamp, N. 2003b. Theory of plant defense level: example of process and pitfalls in development of ecological theory. Oikos **102**:672-678.

Steinberg, P. D. 1984. Algal chemical defense against herbivores – allocation of phenolic compounds in the kelp *Alaria marginata*. Science **223**:405-407.

Steinberg, P. D. 1988. Effects of quantitative and qualitative variation in phenolic compounds on feeding in 3 species of marine invertebrate herbivores. Journal of Experimental Marine Biology and Ecology **120**:221-237.

Strauss, S. Y., J. A. Rudgers, J. A. Lau, and R. E. Irwin. 2002. Direct and ecological costs of resistance to herbivory. Trends in Ecology & Evolution **17**:278-285.

Taylor, R. B., E. E. Sotka, and M. E. Hay. 2002. Tissue-specific induction of herbivore resistance: seaweed response to amphipod grazing. Oecologia **132**:68-76.

Van Alstyne, K., J. A. McCarthy III, C. L. Hustead, and L. J. Kearns. 1999. Phlorotannin allocation among tissues of northeastern Pacific rockweeds and kelps. Journal of Phycology **35**:483-492.

Zangerl, A. R. and C. E. Rutledge. 1996. The probability of attack and patterns of constitutive and induced defense: a test of optimal defense theory. The American Naturalist **147**:599-608.