# TISSUE TYPE MATTERS: SELECTIVE HERBIVORY ON DIFFERENT LIFE HISTORY STAGES OF AN ISOMORPHIC ALGA

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Abstract. Selective grazing by herbivores can have large effects on the population dynamics and community structure of primary producers. However, the ecological impacts of within-species herbivore preference for tissues of different phases (e.g., ploidy levels) or reproductive status remain relatively poorly known, especially among algae and other species with free-living haploid (gametophyte) and diploid (sporophyte) phases. We tested for herbivore selectivity among tissue types of the isomorphic (identical haploid and diploid freeliving stages) red alga Mazzaella flaccida. Laboratory feeding assays demonstrated that the snail Tegula funebralis exhibited more than a threefold preference for gametophyte reproductive tissue over other tissue types, due to morphological differences. In contrast, the urchin Strongylocentrotus purpuratus did not distinguish as clearly between gametophytes and sporophytes; but it did prefer sporophyte reproductive to nonreproductive tissue, due to differences in water-soluble chemicals. Field surveys of grazer damage on M. flaccida blades were consistent with these laboratory preferences, with more damage found on gametophytes than sporophytes and reproductive than nonreproductive tissues. Differential fecundity can contribute to a skew in relative frequencies of phases in the field, and our results suggest that differential grazing by snails may contribute to this pattern and thus play a role in algal population biology.

Key words: algae; gametophyte; herbivory; isomorphic life cycle; Mazzaella flaccida; population dynamics; sporophyte; Strongylocentrotus purpuratus; Tegula funebralis.

## INTRODUCTION

Interspecific variation in plant susceptibility to herbivores is widely recognized as a critical determinant of plant community structure and composition (Lubchenco and Gaines 1981, Crawley 1997). Mechanisms underlying herbivore preferences include nutritional content, noxious chemicals, and plant morphological structures such as wood, thorns, or trichomes (Hay and Fenical 1988, Strauss et al. 2002). However, as Dan Janzen famously pointed out: "herbivores do not eat Latin binomials" (Janzen 1973:203). A growing literature supports the existence of a tremendous amount of intraspecific variation in plant defensive traits that lead to intraspecific differences in plant susceptibility to herbivores (Tollrian and Harvell 1999, Wright et al. 2000. Van Alstvne et al. 2001). Much of this research has implicated spatial or temporal variation in herbivore pressure as the driver of these differences (Cronin and Hay 1996b). Fewer studies have examined how plant life history processes, such as diploidy vs. tetraploidy in allopolyploid species, or other intrinsic drivers may

Manuscript received 29 July 2005; revised 23 February 2006; accepted 2 March 2006. Corresponding Editor: P. D. Steinberg. <sup>3</sup> Present address: Department of Biological Sciences, University of Rhode Island, 100 Flagg Road, Kingston, Rhode Island 02881 USA. E-mail: thornber@uri.edu affect susceptibility to herbivores (Nuismer and Thompson 2001, Lou and Baldwin 2003).

A wide diversity of species (including fungi, ferns, and algae) have complex life cycles that involve multiple freeliving phases, each of which may differ in ploidy or morphology or both. Differential rates of herbivory have been documented within species that have heteromorphic life cycles (Lubchenco and Cubit 1980, Slocum 1980) in which the gametophyte and sporophyte phases appear morphologically distinct and occupy different ecological niches. Thus, there can be trade-offs in ecological characteristics among phases within a species, as well as the more widely appreciated interspecific ones.

When phases differ greatly in morphology, the mechanism underlying differential susceptibility to herbivores may be obvious. However, many algal species cycle between gametophyte and sporophyte phases that are morphologically indistinguishable (isomorphic) and also co-occur in space and time (Gaines 1985). Morphological similarities are commonly taken to imply ecological similarities, but ecological differences between isomorphic phases are relatively unstudied in these species (but see Carrington et al. 2001). However, isomorphy appears to be phylogenetically constrained, at least at the genus level, so selection for differentiation among phases, if present, may still occur and be manifested in non-morphological characters. Indeed, differentiation among phases is one reason posited for



FIG. 1. Life cycle of *Mazzaella flaccida*. Many red algae in the class Florideophyceae, including *M. flaccida*, have a life cycle that typically alternates between free-living independent, morphologically identical gametophytes and sporophytes (indicated here by ovals with stems). Sporophytes, via meiosis, produce haploid spores (n) that become gametophytes (either male or female). Gametophytes produce gametes; the female gamete remains on the female gametophyte, and the resulting fertilized "egg" develops into a very small but visible structure (carposporophyte) on the female blade. This structure is a mass of diploid spores (2n) which, when shed, grow into new free-living sporophytes. In this paper, "reproductive gametophytes" refers to female gametophytes with carposporophyte structures.

the maintenance of these complex life cycles. Unfortunately, to our knowledge, only a few studies directly address the relative susceptibility of isomorphic forms to herbivory (Luxoro and Santelices 1989, Cronin and Hay 1996*a*). Further complicating the matter, within many red algae, reproductive (diploid) sporophytes produce haploid spores on the entire surface of their blades, while reproductive female gametophytes (after gamete fertilization; see Lee [1999] for red algal life cycle details) contain diploid spores on the entire surface of their blades (Abbott and Hollenberg 1976). This can make it difficult to separate phase differences vs. ploidy differences.

Previous research has shown that the gametophyte and sporophyte phases of isomorphic species can have significantly different per capita demographic rates in the field (Engel et al. 2001, Thornber and Gaines 2004). For example, in the red alga *Mazzaella flaccida*, per capita sporophyte fecundity is twice as high as female gametophyte fecundity. These differing rates may impact the overall population structure of *M. flaccida* and balance between the two phases in the field, but the mechanisms underlying these differences are unclear. One possible mechanism is that of differential herbivory on phases. If herbivores preferentially graze one phase, this could contribute to differences in mortality rates (if grazing is lethal) and/or fecundity rates (if grazing is sublethal).

Here we examine the relative susceptibility of gametophyte and sporophyte phases of the isomorphic red alga *Mazzaella flaccida* to herbivory in the laboratory and field. Specifically, we ask: (1) Do individual herbivores (urchins and snails) distinguish between the isomorphic phases of *M. flaccida*? (2) If so, what are the morphological and/or chemical traits that underlie the choice? And (3) are individual grazer preferences reflected in damage levels of field-collected blades? We discuss the answers to these questions in the context of herbivore impacts on the population dynamics of species with complex life cycles.

#### MATERIALS AND METHODS

## Study organisms

The red macroalga Mazzaella flaccida (Setchell et Gardner) Fredericq is common on rocky intertidal shores in Oregon and California, USA (Abbott and Hollenberg 1976). This species has an isomorphic life cycle (Fig. 1) in which gametophytes and tetrasporophytes (henceforth referred to as sporophytes) are morphologically virtually identical but can be distinguished visually when reproductive and chemically when nonreproductive (Shaughnessy and De Wreede 1991). Thus, each blade may be assigned a phase designation (gametophyte/sporophyte) and a reproductive status designation (reproductive/nonreproductive). Logistically, because we needed to collect large amounts of tissue and run our experiments in a timely fashion, we used individuals that could be visually distinguished (reproductive tetrasporophytes and female gametophytes) and collected reproductive or nonreproductive blades from them. Mazzaella flaccida individuals typically each have several blades arising from a central holdfast, only some of which are reproductive at a given time. (Previous data have shown that all blades arising from one holdfast are either sporophytes or gametophytes [C. Thornber, *unpublished data*].) Reproductive blades used were wholly covered with reproductive structures on both sides of the blade.

There are several herbivore species present in this system; we selected two of the most abundant herbivores, the turban snail Tegula funebralis and the purple sea urchin Strongylocentrotus purpuratus (Gaines 1985) to assess variation in susceptibility to herbivory among plants that vary in phase and reproductive status. The two herbivores are distantly related and have very different feeding mechanisms (Brusca and Brusca 1990). Both species are frequently found grazing upon live M. flaccida in the field, including the two field sites we focused upon (repeated quadrat surveys in the M. flaccida zone found a mean of 1.9 T. funebralis/0.25 m<sup>2</sup> and 0.1 S. purpuratus/0.25 m<sup>2</sup> at Piedras and 0.04 T. funebralis/0.25 m<sup>2</sup> and 2.0 S. purpuratus/0.25 m<sup>2</sup> at Vandenberg), significantly higher than other grazers surveyed.

# Feeding preference assays

We first offered each grazer a simultaneous choice among all four living M. flaccida tissue types: gametophyte and sporophyte, reproductive and nonreproductive blades. Based upon these results, we then performed selected paired choice assays that would best test our hypotheses, out of the large number of all possible experimental combinations (see Results), to dissect the effects of phase vs. reproductive status on herbivore feeding patterns. Each piece of tissue was spun 40 times in a salad spinner to remove excess water prior to measuring its wet mass (initial wet masses were 2-3 g, approximate size  $6-9 \text{ cm}^2$ ). We calculated the difference between the initial and final wet masses, for each tissue sample, to assess grazer selectivity. An equal number of no-herbivore controls were used for each live-tissue assay; in all cases the changes in the mass of control pieces did not differ significantly from zero and are not considered further here. Herbivores were starved for 2-3 d prior to each experiment to standardize hunger levels among individuals. Pieces of algal tissue remained healthy in the absence of herbivores (did not lose mass) for only  $\sim 3$  d in the laboratory, and non-starved herbivores often consumed little algal tissue within that time frame (C. Thornber, personal observation). For each experiment, we placed one herbivore and the algal pieces in a container, with 20 replicates for each preference assay. We terminated individual replicates when approximately one-third of the total tissue biomass had been consumed; replicates in which herbivores did not consume any tissue after 3 d were dropped from the analysis because they provided no information on relative preferences.

Although isomorphic forms differ little in visual appearance to the human eye, there may be differences in blade structure or tissue toughness that alter herbivore preferences among phases. To test for this, we removed all effects of morphology of the tissue types by freeze-drying and grinding algae into a fine powder (Wiley Mill, mesh size 60); this process should not affect most chemical or nutritional differences among phases. Algae were subsequently reconstituted into squares of "artificial food" at natural wet: dry mass ratios by adding a heated deionized water: agar mixture (13 mL:0.18 g) to 2 g of dry powder (Bacto Agar, Becton, Dickinson and Company, Franklin Lakes, New Jersey, USA) dissolved in 5 mL of water. For the urchin feeding trials, we poured this slurry onto window screening, to which it adhered once cooled (Bolser and Hay 1996). We then counted the area (number of squares) of reconstituted algae of each tissue type eaten. For the snail feeding trials, we poured a very thin layer of the artificial food directly onto filter paper (T. funebralis feeds by removing tissue via radular scraping). We took digital photographs of each piece of artificial food before and after exposure to T. funebralis and calculated the area of algae eaten via image analysis (ImageJ, available on*line*).<sup>4</sup> Duration and design of feeding assays were as for the whole-tissue assays above.

To determine whether algal chemical differences were responsible for feeding preferences observed during the whole-tissue and fake-food trials, we extracted crude lipophilic and water-soluble chemical fractions from each of the *M. flaccida* tissue types (see Hay et al. [1994] for complete details). A known volume of tissue was ground in a blender and then extracted first with a 2:1 ratio of dichloromethane: methanol and subsequently with a 1:1 ratio of water : methanol for the water-soluble fraction (the volume of methanol was at least two times that of tissue for each extraction step). We removed the solids via filtration, partitioned the entire extraction between water and dichloromethane to separate the lipid- and water-soluble fractions, and removed the solvents via rotary evaporation (Hay et al. 1994). Chemical extracts were coated onto ground, freeze-dried tissue of a known palatable food (the kelp Macrocystis). Artificial food was then prepared as described above, such that chemical extracts were present in the same volumetric concentration, as they would be found in living M. flaccida tissue. These extract-treated foods were presented in pairs to urchins, as described previously for the whole-tissue assays. To determine whether a difference was due to a preference of one tissue type or an avoidance of another, we also constructed "solvent controls," Macrocystis powder prepared into fake food in the same manner (with solvents added) but without the addition of a chemical extract (Hay et al. 1994). The four-choice feeding assays were analyzed with the multivariate preference index (Lockwood 1998). Paired-choice feeding assays were analyzed via paired t tests or, where appropriate, Wilcoxon paired-sample tests.

<sup>&</sup>lt;sup>4</sup> (www.nih.gov)

#### Tissue analyses

For each tissue type, we measured tissue toughness of intact blades and total organic content to assess whether either of these variables correlated with feeding preferences. Tissue toughness tests were conducted with a tissue penetrometer (Duffy and Hay 1991), with five blades of each tissue type tested. Each blade was measured in 10 randomly selected locations, and results were averaged per blade. We measured organic content, expressed as a percentage of dry mass, by combusting six individuals of each tissue in a muffle furnace for 2 h at 500°C and subtracting the mass of remaining ash from the initial dry mass. All data were analyzed with twoway fixed-factor ANOVAs.

#### Field surveys

To determine whether M. flaccida blades of different phases or reproductive status were grazed equally by herbivores in the field, we surveyed grazer damage on blades collected from two central California sites. Pt. Piedras Blancas and Vandenberg Air Force Base (Vandenberg) during August 2000. We haphazardly placed two 10-m transects at each site in the M. flaccida zone, and we collected the closest clump of blades (henceforth referred to as an individual; some species of this genus have been shown to exhibit coalescence [Santelices 2003], but this is unknown but not likely for *M. flaccida*) to the transect tape at each 0.5-m interval, resulting in 40 individuals per site. We recorded the length, width, phase, reproductive status, and the number of interior holes, marginal bites, and grazing scars of each blade (we did not include the smaller, distinctive scars that indicate spore release). Tissue samples were preserved for resorcinol analysis from nonreproductive individuals. Because evidence of herbivore damage can change over time (for example, a grazing scar can become a hole; C. Thornber, personal observation), the total number of grazer marks per blade was summed. We calculated the perimeter of each blade with a previously established length: width to blade perimeter relationship (via image analysis using Image J) (Thornber and Gaines 2004). Two-way fixed-factor ANOVAs were conducted for each site to determine differences in grazer damage between phases and/or reproductive status.

## RESULTS

#### Laboratory assays

Grazers exhibited strong preferences among the tissue types in simultaneous multi-choice assays. *Tegula funebralis* consumed seven times more gametophyte reproductive living tissue than any of the other three choices (Fig. 2A, critical  $F_{3,8} = 72.75$ , Hotelling's  $T^2 =$ 132.05, P < 0.001, Lockwood (1998) multivariate preference index). Although the other choices did not differ significantly from one another, the snails did consume some sporophyte reproductive tissue but no nonreproductive tissue of either phase.

In the paired choice assays with live tissue, these trends became more evident. Tegula funebralis significantly preferred M. flaccida reproductive tissue of gametophytes over sporophytes (Fig. 2B;  $t_{11} = 4.732$ , P = 0.0006). For both phases, reproductive tissue was preferred over nonreproductive tissue (Fig. 2B; sporophyte,  $t_{13} = 2.952$ , P = 0.011; gametophyte,  $t_{13} = 3.40$ , P = 0.005). In all cases, the mean amount of the lesspreferred tissue consumed was not significantly different from zero. Paired feeding assays with nonreproductive gametophyte and sporophyte tissue yielded no results; the amount of tissue snails consumed of either tissue type was not significantly different from zero (this assay was performed multiple times, with identical results). When M. flaccida tissues were freeze-dried, homogenized, and reconstituted, all strong preferences disappeared (Fig. 2C; sporophyte,  $t_{16} = 2.057$ , P = 0.056; gametophyte,  $t_{19} = 0.008$ , P = 0.993; both reproductive tissues,  $t_{19} = 1.541$ , P = 0.140). The marginally nonsignificant effect of sporophyte reproductive status on consumption was in the opposite direction from the whole-tissue assays; thus, testing of the effects of tissue chemical extracts on grazing preferences was not performed.

Sea urchins also significantly distinguished among living M. flaccida tissue types (Fig. 3). In the four-tissue choice feeding assay, S. purpuratus preferred reproductive tissue to nonreproductive tissue, but did not distinguish between phases (Fig. 3A, critical  $F_{3,6}$  = 67.6,  $T^2 = 74.20$ , P < 0.0005, Lockwood multivariate preference index). In the paired assays, these trends were even more apparent. Urchins consumed eight times more M. flaccida sporophyte reproductive tissue than nonreproductive tissue (Fig. 3B;  $t_{13} = 4.382$ , P = 0.0007). Although urchins also ate twice as much gametophyte reproductive tissue as gametophyte nonreproductive or sporophyte reproductive tissue, these differences were not statistically significant (Fig. 3B; gametophyte,  $t_9 =$ 1.376, P = 0.202; both reproductive tissues,  $t_9 = 0.848$ , P = 0.418). Repeated assays for these tissue comparisons (gametophyte reproductive vs. nonreproductive, gametophyte reproductive vs. sporophyte reproductive, as well as gametophyte nonreproductive vs. sporophyte nonreproductive) all yielded nonsignificant results, suggesting that this was not due to a lack of statistical power.

When the two sporophyte tissues were freeze-dried, homogenized, and reconstituted, the urchins consumed five times more reproductive tissue than nonreproductive tissue (Fig. 3C; Wilcoxon two-tailed paired-sample test,  $t_{15} = 18$ , P = 0.02). Artificial foods containing the lipid-soluble fraction of the extracts of these two tissues did not differ in their attractiveness to herbivores (Fig. 3D;  $t_{16} = 0.169$ , P = 0.868). However, foods containing water-soluble extracts of sporophyte nonreproductive tissue were consumed 80% less than those containing



FIG. 2. Paired-choice laboratory feeding assays with *Tegula funcbralis* on *Mazzaella flaccida*: (A) multi-choice live-tissue comparison; (B) paired live-tissue comparisons; and (C) paired homogenized-tissue comparisons. Reproductive status abbreviations are: NR, nonreproductive; R, reproductive. An asterisk indicates a significant difference between tissue types (P < 0.05). All data are means  $\pm$  sE. Lowercase letters (a, b) indicate significant differences in herbivore consumption for the multi-choice feeding assays (Lockwood 1988).

their reproductive counterpart (Fig. 3D;  $t_{12} = 3.376$ , P = 0.006). To determine whether this difference for the water-soluble extracts was due to a chemical deterrent in the sporophyte nonreproductive tissue, we assayed grazing on the artificial food made of *Macrocystis* with the sporophyte nonreproductive extract vs. a solvent control (see *Methods*). Urchins significantly preferred consuming reconstituted *Macrocystis* (the solvent control) over *Macrocystis* with the extracts (42.3 ± 9.48 squares of artificial food vs. 9.9 ± 7.67 squares [means ± sE];  $t_{11} = 2.186$ , P = 0.05), indicating an avoidance of chemicals in sporophyte nonreproductive tissue.

#### Tissue analyses

We found no significant differences in *M. flaccida* tissue toughness among phases or reproductive status (phase,  $F_{1,16} = 0.646$ , P = 0.433; reproductive status,

 $F_{1,16} = 0.013$ , P = 0.911; phase × reproductive status,  $F_{1,16} = 3.186$ , P = 0.093). We did find significant differences among the percentage of organic content of tissue types, with reproductive gametophyte tissue the highest (Table 1; phase,  $F_{1,20} = 23.052$ , P = 0.0001; reproductive status,  $F_{1,20} = 5.217$ , P = 0.033; phase × reproductive status,  $F_{1,20} = 0.287$ , P = 0.598).

# Damage in the field

Herbivore preference for *M. flaccida* gametophyte blades and reproductive blades was also evident from field-collected individuals. At Piedras Blancas, gametophyte blades had 40% more grazer damage than sporophyte blades, and reproductive blades had nearly three times more grazer damage than their nonreproductive counterparts (Fig. 4A; phase,  $F_{1,148} = 4.456$ , P = 0.036; reproductive status,  $F_{1,148} = 47.635$ , P < 0.0001;



FIG. 3. Paired-choice laboratory feeding assays with *Strongylocentrotus purpuratus* on *Mazzaella flaccida*: (A) multi-choice livetissue comparison; (B) paired live-tissue comparisons; (C) paired homogenized-tissue comparison; and (D) paired chemical-extract comparisons for sporophyte NR (black) and sporophyte R (open). Extracts were divided into lipid-soluble and water-soluble fractions. Reproductive status abbreviations are: NR, nonreproductive; R, reproductive. An asterisk indicates a significant difference between tissue types (P < 0.05). All data are means  $\pm$  se. Lowercase letters (a, b) indicate significant differences in herbivore consumption for the multi-choice feeding assays (Lockwood 1988).

phase × reproductive status,  $F_{1,148} = 0.411$ , P = 0.522). When standardized by blade perimeter, the phase difference disappeared (Fig. 4B;  $F_{1,148} = 0.213$ , P = 0.645) but reproductive blades had ~50% more grazer damage per centimeter of perimeter than nonreproduc-

 
 TABLE 1. Percentage of organic content of Mazzaella flaccida tissue types.

	Organic content	
Tissue type	Mean	SE
Sporophyte nonreproductive Sporophyte reproductive Gametophyte nonreproductive Gametophyte reproductive	70.1 71.35 73.15 75.17	1.05 0.52 0.74 0.36

tive blades ( $F_{1,148} = 11.788$ , P = 0.0008) with a nonsignificant interaction ( $F_{1,148} = 0.239$ , P = 0.626). Patterns of grazer damage were similar for whole blades collected from Vandenberg (Fig. 4C; phase,  $F_{1,245} =$ 7.636, P = 0.006; reproductive status,  $F_{1,245} = 17.116$ , P< 0.0001; phase × reproductive status,  $F_{1,245} = 2.056$ , P= 0.153). Results for grazer damage were similar when standardized by blade perimeter (Fig. 4D; phase,  $F_{1,245} =$ 0.096, P = 0.027; reproductive status,  $F_{1,245} = 0.136$ , P= 0.0086; phase × reproductive status,  $F_{1,245} = 0.004$ , P =0.6622). Selectivity in grazer damage on *M. flaccida* blades was similar (gametophytes more heavily grazed than sporophytes, reproductive blades more heavily grazed than nonreproductive blades) for several other



FIG. 4. Grazer marks (mean  $\pm$  sE) at Piedras Blancas, California, (A) per blade and (B) per centimeter perimeter; and at Vandenberg, California, (C) per blade and (D) per centimeter perimeter. Grazer marks include interior holes, marginal bites, and grazing scars found on blades.

sites sampled across this species' geographical range (data not shown).

## DISCUSSION

We found strong evidence for significant grazer selectivity among Mazzaella flaccida's tissue types. The turban snail, Tegula funebralis, grazed selectively, based both upon tissue phase and reproductive status, and these differences appear to be morphologically based (Fig. 2). The purple urchin Strongvlocentrotus purpuratus did not distinguish significantly between the phases, although the preference trends are in the same direction as for T. funebralis. The urchins did prefer sporophyte reproductive to nonreproductive tissue; these differences appear to be chemically based (Fig. 3). Because reproductive individuals of either phase are not unambiguously haploid or diploid (e.g., sporophytes have diploid somatic tissue but the thallus is covered with haploid spores), our data suggest that some interaction between phase and reproductive status in M. flaccida affects levels of defenses. In particular, it is of note that, for urchins, changes in reproductive status only increased palatability for sporophytic thalli (Fig. 3), even though in general sporophytes were less palatable than gametophytes. Field surveys of grazer damage on Mazzaella blades were consistent with each of these laboratory preferences, with more damage found on gametophytes than on sporophytes and on reproductive than on nonreproductive tissues (Fig. 4).

Our findings provide support for the argument that significant ecological differences can exist among phases (or reproductive stages) within isomorphic algal species (Hughes and Otto 1999), exhibited in this case as differences in susceptibility to herbivores. Results of similar studies are mixed: the amphipod *Hyale media* did prefer reproductive gametophyte tissue of Mazzaella laminarioides over reproductive sporophyte tissue (Buschmann and Santelices 1987), but amphipods and sea urchins had no preference among phases for Dictyota ciliolata (Cronin and Hay 1996c). Tegula snails graze on Mazzaella flaccida by removing the surface tissue, which is where the spores are embedded (C. Thornber, personal observation). Female gametophyte reproductive structures (carposporophytes) are much larger and protrude out from the blade more than sporophyte reproductive structures. This may make them easier to consume, leading to snail preference for gametophyte over sporophyte reproductive tissue. Indeed, when we homogenized tissues, these preferences disappeared, consistent with the idea that preferences are driven by morphology rather than nutritional or chemical differences between phases. We suspect that reproductive structure morphology rather than overall tissue toughness was responsible for these differences as overall toughness did not differ among any of the tissue types. Although tissue homogenization could have diluted any differences among phases that were concentrated solely in the reproductive tissue, reproductive structures are macroscopic and densely cover the entire blade for both phases (Abbott and Hollenberg 1976), so we suspect that the strong preferences observed in whole-plant assays would have been preserved. Additionally, (homogenized) reproductive gametophyte tissue had the highest percentage of organic content, yet T. funebralis feeding trials with homogenized tissue indicated no significant preferences for a specific phase or tissue type, suggesting that at least this measure of nutritional value does not measurably influence herbivore preferences.

Although somewhat speculative, the differential rates of herbivory among phases that we observed in the field and laboratory could have broader impacts on the population dynamics of this species. In M. flaccida field populations, gametophyte blades have a lower spore density, leading to a lower per capita fecundity, than sporophytes (Thornber and Gaines 2004). Previous work has demonstrated that this differential fecundity of phases contributes to a skew in the relative frequency of the phases in the field (towards higher levels of gametophytes). There are many possible explanations for this skew, including differential allocation of resources to reproduction (Santelices and Martinez 1997), constraints on fertilization, or herbivore susceptibility (Buschmann and Bravo 1990). Our results are consistent with the idea that preferential grazing by snails may contribute to the observed fecundity differences between phases, as snails preferentially consume reproductive gametophyte tissue and thus may be effectively lowering the per capita fecundity of gametophyte tissue relative to that of sporophyte tissue. Indeed, at Piedras Blancas the density of snails was significantly higher than at Vandenberg  $(3.05 \pm 1.1 \text{ vs.})$  $0.025 \pm 0.05$  per 0.25 m<sup>2</sup> [mean  $\pm$  se]; C. Thornber, unpublished data) at the same time as the grazer damage surveys were conducted, which matches the higher overall levels of grazer damage at Piedras Blancas (Fig. 4). Although we cannot rule out the other mechanisms suggested above, our data do suggest that grazer preference may contribute to skewed gametophyte:sporophyte ratios in the field.

#### Chemical defenses

Chemical defenses against urchin herbivory clearly declined as individuals aged and became reproductive, but the magnitude of this effect depended on whether the thalli were sporophytes or gametophytes. The difference in the effect of water-soluble chemistry on urchin herbivory could also be interpreted as a stimulation of feeding by greater concentrations of water-soluble nutrients (carbohydrates, proteins) in the reproductive thalli. However, we suspect that the main reason is decreases in chemical defenses because: (1) water-soluble extracts from nonreproductive plants did deter feeding by urchins relative to palatable controls (Fig. 3D) and (2) gametophyte nonreproductive thalli had a higher mean percentage of organic content than did sporophyte reproductive thalli (Table 1), while the feeding preference experiments showed the reverse trend in tissue consumption (Fig. 3A).

Why might defenses decline with age? One possible reason for this is that younger tissues may have higher photosynthetic rates and may thus be more valuable and better defended than older tissues (Raupp and Denno 1983, Hay et al. 1988). In other algae and plants, specialized reproductive tissues can be more heavily chemically defended than vegetative structures (Van Alstyne et al. 2001, Strauss et al. 2002). However, for species such as *M. flaccida*, in which the entire thallus essentially becomes reproductive, this may not be an option. Instead, by morphologically and chemically deterring herbivores during the juvenile stage, *M. flaccida* may be increasing its likelihood of surviving to reproductive maturity, at which point resources are shifted from defense to reproduction (Herms and Mattson 1992).

Significant intraspecific variation in levels of chemical defenses has been documented for a variety of species at scales from within individuals (Meyer and Paul 1995, Van Alstyne et al. 1999) to among individuals within a population (Wright et al. 1997) to among geographically separated populations (Wright et al. 2000, Van Alstyne et al. 2001, Taylor et al. 2003). Many of these types of studies have focused upon species with heteromorphic life cycles, such as fucoids and kelps, but studies of isomorphic species are becoming more common (Barbosa et al. 2004, Pereira et al. 2004, Wright et al. 2004). Thus, our data, together with previous studies, indicate that differences in algal defenses and/or susceptibility to herbivory, among different phases and reproductive stages of isomorphic algae, pose additional challenges to studies on larger scale patterns of algal chemical defenses.

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