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Short communication

Comparative phosphate acquisition in giant-celled rhizophytic algae (Bryopsidales, Chlorophyta): Fleshy vs. calcified forms

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ABSTRACT

Phosphate uptake through above-ground thalli vs. subterranean rhizoids has been compared in siphonaceous rhizophytic green algal species from five globally distributed tropical genera: *Avrainvillea nigricans* Decaisne, *Caulerpa lanuginosa* J. Agardh, *Halimeda incrassata* (J. Ellis) J.V. Lamouroux, *Penicillus capitatus* Lamarck, and *Udotea flabellum* (J. Ellis & Solander) M. Howe. Plants were collected, acclimated to lab conditions for 3 days, and then incubated for 8 h at saturating light intensity with 30 μM PO_4^{3-} added to their above-ground thallus or below-ground rhizoids. Percent tissue phosphorus was then compared to control specimens, which were run simultaneously in the absence of phosphate. The two fleshy species, *A. nigricans* and *C. lanuginosa*, showed no significant differences in tissue nutrient status, and displayed much larger variation among controls than the three calcified species. Calcified species showed greater phosphorus content after being exposed to either above- or below-ground thallus portions, indicating that these seaweeds can respond to short term increases in nutrient availability and have a more regulated nutrient acquisition mechanism. Results suggest that calcification may play an important role in phosphorus absorption.

1. Introduction

Tropical macroalgae are faced with two strong selective pressures: nutrient limitation and herbivory (see review in Littler and Littler, 2007). While the extent to which these top-down vs. bottom-up factors regulate reef macroalgal communities varies greatly (Burkepile and Hay, 2006), both appear to be evolutionarily significant, given the number of herbivore-defense and nutrient-uptake adaptations (Padilla, 1989; Williams, 1984). Calcification, or the formation of calcium carbonate crystals in or on the algal cell wall, is one of the most well studied anti-herbivory defenses among macroalgae (Paul and Hay, 1986). Additionally, calcification is thought to facilitate nutrient absorption through the generation of protons (McConnaughey and Whelan, 1997), although this hypothesis requires further investigation in most calcareous macroalgae. If calcification provides relief from both of these selective pressures, this may explain the remarkable abundance of calcareous seaweeds in the tropics (Littler, 1976).

Rhizophytic algae of the order Bryopsidales employ an extensive rhizoidal system that anchors them to sandy substrates and allows for nutrient absorption and translocation from nutrient-rich sediment pore waters (Williams, 1984). This adaptation may well have adaptive benefit to rhizophytes in tropical and subtropical coastal waters where water-column nutrient levels are often below detection limits (Lapointe et al., 1992; Littler and Littler, 1990). However, since the documentation of this phenomenon (Williams, 1984), the relative importance of above-ground thalli and below-ground rhizoids in nutrient acquisition has remained poorly understood.

The detailed studies of Williams (1984) and Williams and Fisher (1985) suggest that the rhizoids of *Caulerpa cupressoides* (Vahl) C. Agardh are likely its dominant source of nitrogen acquisition. We set out to augment these two single-species studies with a broader comparative analysis of phosphorus assimilation in tropical macroalgae. We focused on phosphate because it is thought to be the limiting nutrient in the tropical carbonate sediments inhabited by rhizophytic algae (Lapointe et al., 1992; Lapointe, 1987). Unlike nitrogen, phosphate is known to bind to carbonate compounds (Lin and Singer, 2006), potentially causing differences in phosphate acquisition in fleshy vs. calcified species. We investigated the effects of calcification on phosphate absorption

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by asking (1) Do fleshy and calcified rhizophytic algae show differences in tissue phosphate incorporation after short term phosphate exposure? (2) Is phosphate acquisition the same in above-ground thalli and subterranean rhizoids of calcified vs. fleshy members of the Bryopsidales?

2. Methods

2.1. Field sites and maintenance

Harry Harris Park in Tavernier, Florida (25°01′30.52″N, 80°29′35.18″W) is characterized by a large *Thalassia testudinum* Banks ex König bed and a diverse flora of rhizophytic, drift, and epilithic seaweeds in ambient seawater with a salinity of 36–37 ppt and temperature between 29 and 31 °C during the study. Fleshy *Caulerpa lanuginosa* J. Agardh and *Avrainvillea nigricans* Decaisne and calcified *Penicillus capitatus* Lamarck were collected from this site. The remaining two calcified species, *Halimeda incrassata* (J. Ellis) J.V. Lamouroux and *Udotea flabellum* (J. Ellis & Solander) M. Howe, were collected from Jupiter Inlet, Florida across from Coral Cove Park (26°57′46.06″N, 80°04′47.38″W), with a seawater temperature of 20–21 °C and salinity values of 34–35 ppt at the times of collection. The latter site is characterized by a mixed-species seagrass bed, including *T. testudinum*, *Halophila decipiens* Ostenfeld, *Syringodium filiforme* Kützing in Hohenacker, and *Halodule wrightii* Ascherson, with considerably lower algal diversity than Harry Harris Park.

Seaweeds of average size for each species were collected at both sites by hand from approximately 0.5 m depth while snorkeling, with special attention given to the careful removal of the rhizoidal masses from the sediment. Individuals were then transported in seawater from the collection site back to the Smithsonian Marine Station in Fort Pierce, Florida, where they were placed in aquaria with re-circulating InstantOcean© seawater mixed to 35 ppt for 3–4 days to allow for stabilization to the lab conditions prior to the nutrient acquisition experiments.

2.2. Nutrient incubation trials

Chambers constructed of PVC were used to separate the above-ground thallus portions from the below-ground rhizoidal masses in nutrient incubation trials (adapted from McRoy and Barsdata, 1970). PVC was 15 cm in diameter with an 18 cm × 18 cm PVC sheet attached at the base. The top chamber (23 cm high) was connected to the bottom chamber (8 cm high) with a 3 cm-wide PVC coupler. A plastic plate of 14 cm diameter with a 3.5 cm hole in the center was placed between the two chambers. The bottom chambers held 1.5 L and were added to the 3 L-top chambers. Intact algal thalli were then placed in the apparatus with rhizoids sealed within the bottom chamber, while their above-ground thallus was completely submerged in the top chamber. Non-toxic adhesive putty was used to seal the hole and keep the algae upright.

Seaweeds were incubated for 8 h in the aforementioned isolated chambers in three different phosphate treatments with four replicates per treatment per species: controls (no phosphate added), above-ground thallus exposure (phosphate added to the top chamber only), and rhizoidal exposure (phosphate added only to the bottom chambers). To demonstrate the complete lack of flow between the two chambers, Allura Red AC, E129 food coloring was added to the water in either the top or bottom of the chamber during preliminary trial runs. After 12 h, visual evidence of interchange was not observed. Each chamber was filled with the same artificial seawater used in the aquaria, with additional nutrient spikes for each treatment. Monohydrous NaH_2PO_4 was added to provide 30 μM PO_4^{3-}, a concentration chosen to ensure saturation of nutrient absorption, i.e. much higher than normally reported in the water-column and sediments around these algae (Lapointe et al., 1992; Lapointe, 1987). Using supersaturating phosphate levels substantially improves the chances of seeing differences in tissue phosphorus levels after only a couple of hours. However it should be noted that lower phosphate levels might have caused different results.

To accommodate 12 samples per species over only 8 experimental chambers, two species were run over a 3-day period; one trial containing eight samples of each species separated by a trial containing four samples of both species simultaneously, with treatments randomly assigned. Light reaching the seaweeds in each of the eight upper chambers was measured using a Li-Cor 4π sensor and averaged 311 ± 34 μM photons m^{-2} s^{-1}. Although this value represents only one fourth of the maximum light reaching the seaweeds at the collection sites, it is well above the photosynthetic saturation point found for related species in other studies (Littler et al., 1988). The plastic separator between the two chambers inhibited light from entering the bottom chambers. While light may affect nutrient absorption, rhizoids are normally buried in sediments and would not naturally be exposed to light. The chambers were placed in a water bath in an air-conditioned wet lab and water temperature remained within 23–24 °C throughout the experiments, representing an intermediate temperature value for both field sites.

2.3. Tissue processing

Immediately after the incubation trial, seaweeds were rinsed with distilled water and the two thallus portions were separated with a razor blade, which was rinsed and wiped after each incision. The above-ground thallus portions were placed directly into 20 mL plastic scintillation vials and dried for 48 h at 65 °C. The tissue was then homogenized by adding two clean steel ball bearings to each ball-mill tube and placed in a mill for three minutes. Tissue samples were sent to the University of Maryland Chesapeake Biological Laboratory where a modified version of the method developed by Aspila et al. (1976) was used to determine total phosphorus content of samples.

2.4. Statistical analyses

One-way analysis of variance (ANOVA) was used to test the effects of treatment on percent tissue phosphorus for each species (Levene's test was used to check homogeneity of variance). Tukey post hoc groupings by treatments were used to test for significant differences among treatments. All statistical analyses were performed using SYSTAT 12 and were considered significant with $p < 0.05$.

3. Results

Analysis of variance found no trends in percent thallus tissue phosphorus by treatment for either *C. lanuginosa* or *A. nigricans*, likely due to the high variation in starting (control) percent tissue phosphorus values (Fig. 1). In contrast, a significant effect of treatment was detected for two calcified species, *H. incrassata* and *P. capitatus*. In *H. incrassata*, Tukey's post hoc groupings showed a significant increase in thallus phosphorus content after exposure of phosphate to either thallus portion as well as an increase in thallus phosphorus content when phosphate was exposed to the above-ground thallus compared to below-ground rhizoids. For *P. capitatus* post hoc comparisons showed a significant effect of phosphate exposure on percent tissue phosphorus and no effect of thallus exposure site. In the third calcified species, *U. flabellum*, ANOVA did not find a significant effect of treatment.

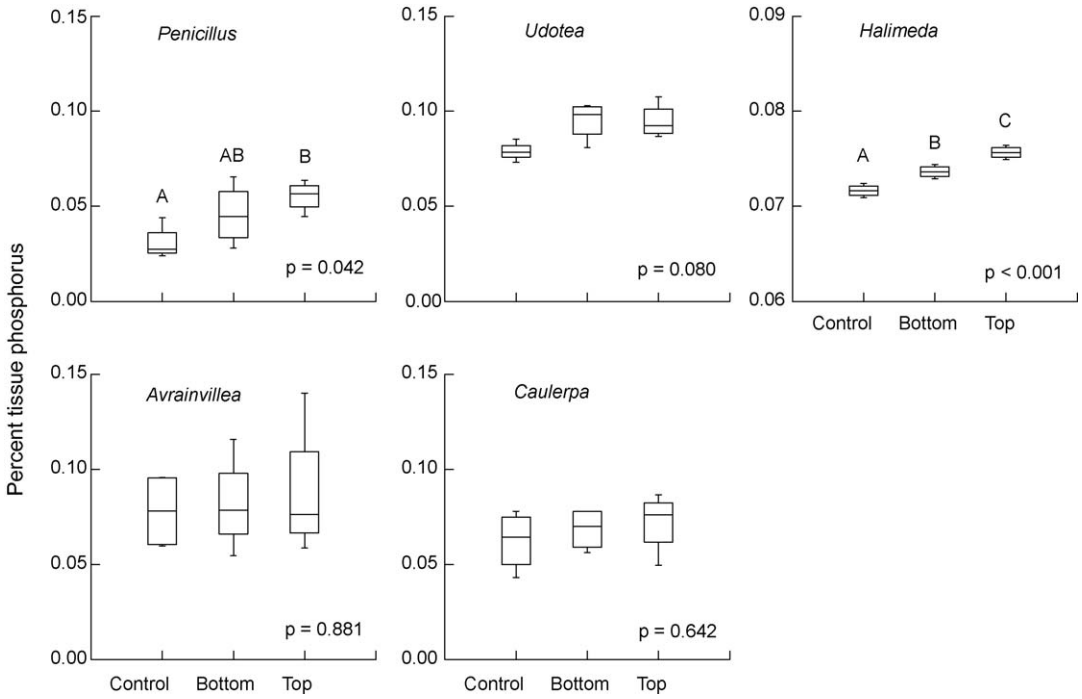

Fig. 1. Percent tissue phosphorus after incubation per treatment grouped by species. Values are mean ± SE and 95% C.I. *P*-values for one-way ANOVAs are included. Note the difference in scale for *Halimeda*.

4. Discussion

Fleshy and calcified algae responded differently to phosphate exposure, such that an increase in tissue phosphorus content was observed for two calcified species but not for fleshy species. Calcified *H. incrassata* was the only species to show differences in above-ground vs. below-ground phosphate acquisition. The observed increase in phosphorus content after exposure of phosphate to the above-ground thallus vs. rhizoids may represent either a lag in nutrient acquisition due to translocation or a decreased capability of the rhizoids to acquire phosphate relative to the above-ground thallus and warrants further investigation.

While significantly higher percent tissue phosphorus values for calcified species implies that these seaweeds respond rapidly to nutrient pulsing, e.g. on the order of half a day, the lack of effect of treatment for fleshy algae should not be taken as an inability to respond to short term nutrient pulsing (Williams and Fisher, 1985) especially in light of the high variability of controls. The large variation in nutrient status of controls for fleshy algae species might suggest superfluous uptake and "luxury" storage of phosphorus from the field sites, whereas the low variation among calcified species may indicate a more regulated nutrient acquisition strategy.

The striking difference in variation of percent tissue phosphorus between calcified and fleshy algae suggests differing nutrient acquisition strategies by functional group. This could be explained by findings that protons generated by the calcification process may serve an important role in nutrient acquisition (McConnaughey and Whelan, 1997). While a mechanism for phosphate acquisition would be beneficial in low phosphate environments, such as tropical carbonate reefs, it would likely not be as important in phosphate-rich environments (i.e. subtropical siliciclastic sediments and temperate systems).

Since nutrient acquisition often limits seaweed growth and productivity (Lapointe et al., 1987; Larned, 1998), strategies and mechanisms for acquiring nutrients may explain distributional patterns. It is therefore not surprising that calcified vs. fleshy rhizophytic algae dominate in differing nutrient regimes. For instance, abundances of calcified rhizophytic algae (Collado-Vides et al., 2005) appear to be lower in areas with increasing phosphate levels in Florida Bay (Fourqurean and Zieman, 2002). Additional support that calcified green algae are adapted to phosphate-limiting conditions comes from the finding that elevated levels of phosphate inhibit calcification and adversely affect growth of *P. dumetosus* (Delgado and Lapointe, 1994) and *H. incrassata* (Demes et al., 2009). Furthermore, eutrophication of coral-reef habitats has been shown to cause blooms of the fleshy bryopsidalean seaweeds *Caulerpa* and *Codium* (Lapointe et al., 2005). It is also interesting to note that the two families in the Bryopsidales with calcified species (Udoteaceae and Halimedaceae) are found only in tropical to subtropical environments, whereas fleshy relatives (Caulerpaceae, Bryopsidaceae, and Codiaceae) are common in oligotrophic tropical to nutrient-rich temperate waters.

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