Desiccation in *Mastocarpus papillatus* and *Porphyra perforata*

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Abstract

For algae in the upper intertidal zone, desiccation is one major stress that determines species fitness (Celia M. Smith & Berry, 1986). Here, I used lab experiments to examine desiccation in upper intertidal red alga species *Mastocarpus papillatus* and *Porphyra perforata*. Preliminary data gathered was inconclusive regarding the impact of collection site and species on changes in photosynthetic activity as measured by electron transport rate (ETR). As such, further research is proposed to take into account the small sample size of this initial dataset as well as other potential variables of interest, such as heat stress, wave action stress, vertical distribution across intertidal zones, and geographic distribution across latitudes.

Introduction

To thrive in the rocky intertidal zone, organisms must overcome many environmental stressors, including desiccation during low tide, variable heat, and the tremendous force of wave action. From the hard shells of limpets to the soft bodies of sea anemones, organisms employ a wide variety of responses to these challenges. One such response of sessile organisms in this ecosystem is zonation; desiccation-tolerant organisms occupy the upper zones, while desiccation-sensitive organisms occupy lower zones (Celia M. Smith & Berry, 1986). Red macroalgae in particular respond to desiccation stress in this way. Upper zone-occupying species correlate strongly with longer periods of emersion (air exposure) and desiccation (Dring, 1909), so higher-zoned species are more desiccation-tolerant than lower-zoned species.

While many studies have compared multiple species across different intertidal zones (Skene, 2004), less research has been done focused on comparing two species within the same

zone. The goal of this study is to compare the desiccation tolerance between *Mastocarpus papillatus* and *Porphyra perforata* (henceforth referred to as *M. papillatus and P. perforata*), two of the most common higher intertidal zone red algae in Monterey Bay (Hunt & Denny, 2008). This tolerance will be expressed as the difference in electron transport rate (ETR) from each sample's hydrated state to a desiccated state. ETR measures photosynthesis rates and therefore can be used to demonstrate healthiness of a photosynthetic organism, with higher ETR slope and higher maximum ETR values corresponding with higher photosynthesis levels and greater overall health. As with the previous literature cited, this study reveals which of the study species has greater tolerance to a stress factor (in this case, desiccation). However, with the context that the two species compete for the same physical niche, this study considers tolerance a competitive factor between the two species as long-term desiccation tolerance is key to survival in the upper intertidal zone (Lipkin et al., 1993).

M. papillatus, a foliose red brown algae, occupies the mid- to high intertidal zones (1-1.5m). *M. papillatus* was chosen for this study as it is the dominant algae species in the upper intertidal zone of Monterey Bay according to SEANET, the Stanford University catalogue of nearshore plants and animals of Monterey Bay. *P. perforata* is found in similar mid- to high intertidal ranges but differs from *Mastocarpus* and other foliose thallic red-green algae because its blades are monostromatic, or comprised of a single layer of cells, and folded.

Preliminary observation conducted while setting up this study demonstrated that *P. perforata* and *M. papillatus* displayed different responses to desiccation from air exposure during low tides. *M. papillatus* displays no visible structural change between its wet and dry states. However, hydrated *P. perforata* is loose, flexible, and ruffled, whereas dry *P. perforata* is compactly crumpled and inflexible. The differing responses to desiccation indicate a difference

in adaptation to long periods of air exposure both species face in the upper intertidal zone, and potentially that desiccation resistance could play a role in determining relative species fitness within zones.

The findings of this study are particularly timely as California becomes hotter and drier as a result of climate change (Barboza & Fox, 2018), indicating that desiccation stress may be a factor that increasingly determines competition of macroalgae species within their respective intertidal zones. As a consequence, this changing stress may even lead to zonal reorganization of some communities within the intertidal community. Therefore, it is critical to understand respective tolerances of specific inter-competitive species to gain a clue as to how species interactions may begin to shift as the result of climactic shifts.

Materials

Mastocarpus papillatus and *Porphyra perforata* were collected from Hopkins Marine Station in Pacific Grove, California from three collection sites (5 samples of each species from three collection sites; 15 total samples). Both species were maintained in seawater tanks in the Fisher Lab building on the Hopkins Marine Station campus over the course of two weeks. To account for previous desiccation stress experienced in the intertidal zone, algae samples were fully rehydrated for 24 hours before experimenting, and after that, the algae t=did not experience desiccation until experimentation occurred.

Methods

To answer the question of relative desiccation tolerance, three things needed to be measured: level of desiccation (relative water content, or RWC), electron transport rate (ETR) as a measurement of photosynthetic activity, and statistical significance of the difference of ETR from hydrated to desiccated samples.

All samples were fully hydrated via submersion for at least 24 hours, then thoroughly surface-dried with a towel and weighed on a Fisher Scientific Model S-3000 scale to find "fresh" weight. An initial ETR test was run on the Junior PAM chlorophyll fluorometer, yielding ETR-alpha (initial slope of the ETR curve) and maximum ETR (measured in $\frac{\mu mol * electrons}{(m^2 * s)}$). These results are noted in the data table (Figure 1). After this test, each sample was desiccated under a box fan for two hours, weighed, and another ETR test was run. Samples were subsequently rehydrated for two hours, weighed, and a final ETR test was run. Lastly, each sample was fully dried in a drying oven at 60C for 24 hours, yielding dry weight. Relative water content (RWC) was then determined as a way to check for consistency of water loss by the equation:

$$RWC = \frac{desiccated\ weight - dry\ weight}{fresh\ weight - dry\ weight} * 100\% = 100 - percent\ desiccation$$

The two particular measurements of interest were 1) the change between ETR from the samples' fresh state to desiccated state and 2) from the desiccated state to the rehydrated state. The first measurement indicated desiccation impact on photosynthetic rate, with a smaller difference between the two indicating a higher degree of desiccation sensitivity. Similarly, the second measurement denoted resilience after desiccation, with a more negative value indicating a greater regain of photosynthetic activity after rehydration. Both these measurements of interest were recorded for the maximum ETR values and ETR alpha values, resulting in a total of four combinations: ETR alpha change from fresh samples to desiccated samples, maximum ETR change from fresh samples to desiccated samples, ETR alpha change from desiccated samples to rehydrated samples, and maximum ETR change from desiccated samples to rehydrated samples.

Data Analysis

Data from each collection site was recorded in Microsoft Excel (example for Site 1 in Figure 1), then imported into RStudio, where the ANOVA statistical analyses were run.

Interspecies crossed ANOVA statistical analysis of the sampled populations determined if the two species demonstrated significant difference in their ability to recover photosynthetic activity after desiccation and rehydration (i.e., that species was the main influencing factor over site).

Results and Analysis

Figures 2 through 5 show the mean differences of the five individuals within each test site, with error bars denoting standard error. From an examination of the charts, there was no apparent trend in the data considering species as the main effect of interest, and it was difficult from the data to determine if site or species acted as a main effect at all. As such, it was necessary to refer to the results from the crossed ANOVA statistical analyses. However, none of the ANOVA tests yielded statistically significant results for the effect of species on ETR (p-values were all greater than 0.1). Thus, there was no demonstrated significant effect of species on differences in ETR rate or maximum value. However, it would still be too early to declare with certainty that the site or the interactive effect of site and species has no a main effect of interest on the difference of both ETR measurements. For the difference between fresh and desiccated samples for maximum ETR values, site had a pr(>F) of 0.016, which indicates a significance of 0.05. That particular trial showed that site was a main effect of interest. However, because none of the other comparisons showed significance across sites, the results of this preliminary data collection thus are inconclusive and require further research.

Future Work

While the inconclusive result of the preliminary data does not lend itself to a conclusive direction for further study, it does illuminate the need for some refinement of the experimental design described in the methods section. With the hopes of producing results demonstrating consistent significant effects of either site or species, I propose a study that replicates my

methods with five sample sites and ten samples per site. This modification to the experimental design would increase the randomness of sampling, decrease potential skewing effect of individual outliers within sample sites, and decrease any potential skewing effect of anomalous sample sites among the samples. This study would test specifically for three sets of hypotheses:

1. **H0:** Sample site has no impact on mean changes in ETR measurements from fresh to desiccated and desiccated to rehydrated samples.

   HA: Sample site had a significant effect on individual samples' changes in ETR measurements from fresh to desiccated and desiccated to rehydrated samples.
2. **H0:** Species has no impact on mean changes in ETR measurements from fresh to desiccated and desiccated to rehydrated samples.

   HA: Species had a significant impact on mean changes in ETR measurements from fresh to desiccated and desiccated to rehydrated samples.
3. **H0:** The combined effect of sample site and species has no impact on mean changes in ETR measurements from fresh to desiccated and desiccated to rehydrated samples.

   HA: The combined effect of sample site and species has a significant impact on mean changes in ETR measurements from fresh to desiccated and desiccated to rehydrated samples.

If these studies accept the second or third alternative hypotheses, this could show that desiccation tolerance does play a role in determining relative species fitness within intertidal zone niches. In these cases, the suppositions laid out in the introduction of this paper would be supported. If the second and third alternative hypotheses are not accepted, then this would indicate that while inter-zone distribution is dependent on desiccation tolerance (as supported by Dring (1909)), interspecific competition within zones is not dependent on desiccation tolerance.

If this is the case, it would be reasonable to explore the possibility of the first alternative hypothesis, wherein site plays a more important role in determining desiccation tolerance than species. This result would indicate the possibility of acclimatization as a driver of adaptation to stressors.

While the conclusions drawn from accepting/rejecting the above hypotheses create different contexts for following research, other factors that may be of interest in studying *M. papillatus* and *P. perforata* include tolerance to heat stress and wave action stress, range of vertical distribution across intertidal zones, and latitudinal distribution as determining factors in interspecific competition. These finding could help contribute to a body of literature assisting in more complex understanding of how species distributions and interactions change on the most fundamental levels of an ecosystem as climactic conditions change. A more developed understanding of these factors will be critical as humans increasingly try to intervene and mitigate in conserving coastal ecosystems in the face of climate crisis.

Works Cited

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Figures

Site	Spp	Sample	Wt_F	ETR_F_A	ETR_F_max	Wt_DES	ETR_DES_A	ETR_DES_max	Wt_F2	ETR_F2_A	ETR_F2_max	Wt_DRY
1	M	1	9.704	0.135	66.586	3.489	0.021	11.261	9.687	0.125	60.031	2.696
1	M	2	4.111	0.185	53.268	1.829	0.004	50.72	4.245	0.16	71.355	1.587
1	M	3	2.172	0.176	32.666	0.977	0.02	16.83	1.996	0.107	69.243	0.582
1	M	4	4.25	0.106	41.521	1.475	0.023	18.435	4.021	0.142	76.153	1.243
1	M	5	2.741	0.13	63.219	0.694	0.031	21.808	2.54	0.147	60.774	0.528
1	P	1	2.063	0.161	72.523	0.651	0.011	2.775	2.062	0.17	71.581	0.506
1	P	2	1.233	0.176	52.948	0.458	0.015	6.306	1.04	0.138	83.108	0.307
1	P	3	0.72	0.138	39.644	0.227	0.015	16.975	0.705	0.125	34.756	0.187
1	P	4	0.533	0.147	74.34	0.179	0.02	10	0.492	0.151	64.235	0.141
1	P	5	0.787	0.161	67.472	0.245	0.03	19.782	0.759	0.121	48.679	0.2

Figure 1. The dataset for the first collection site. The Spp column denotes if the sample is M. papillatus or P. perforata (M or P). Wt indicates a weight measurement, ETR_xxx_A indicates ETR alpha, ETR_xxx_max indicates maximum ETR value. F in columns 4-6 indicates fresh samples, DES indicates desiccated samples, F2 indicates rehydrated samples, and DRY indicates the oven-dried samples.

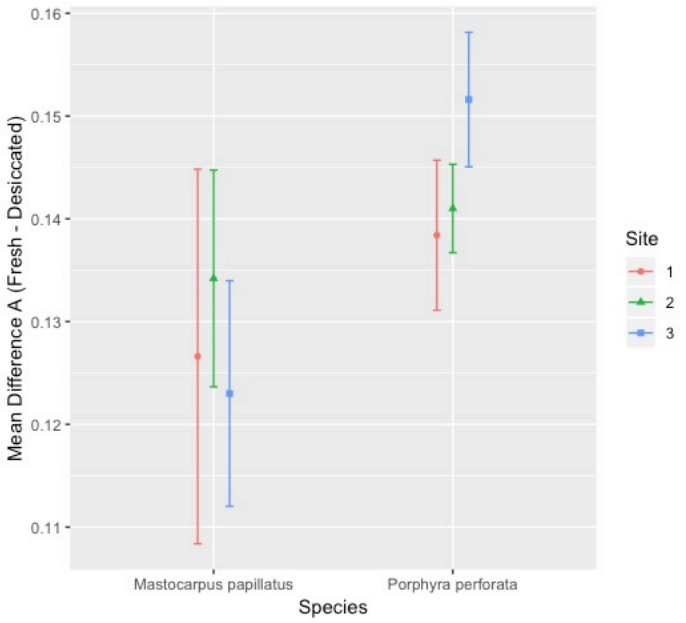

Figure 2.

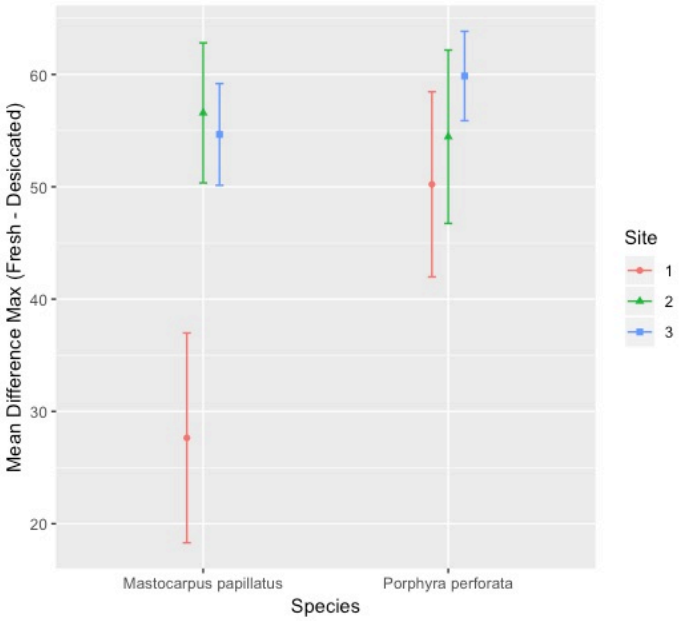

Figure 3.

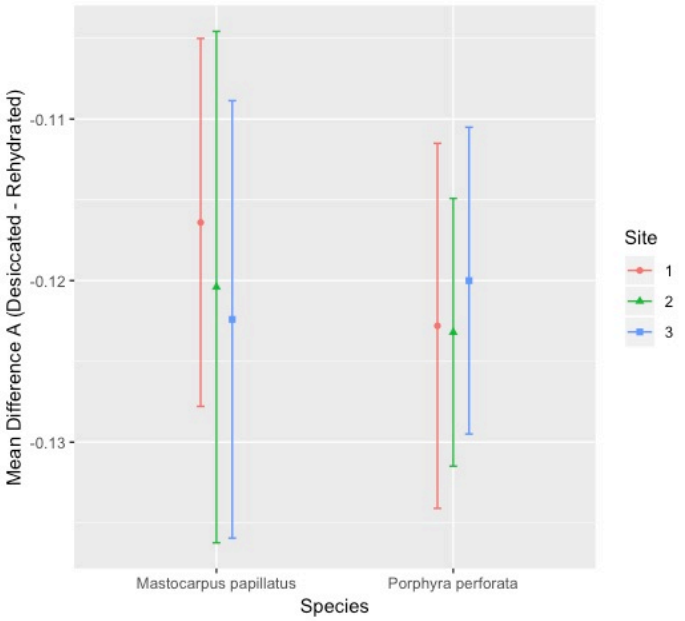

Figure 4.

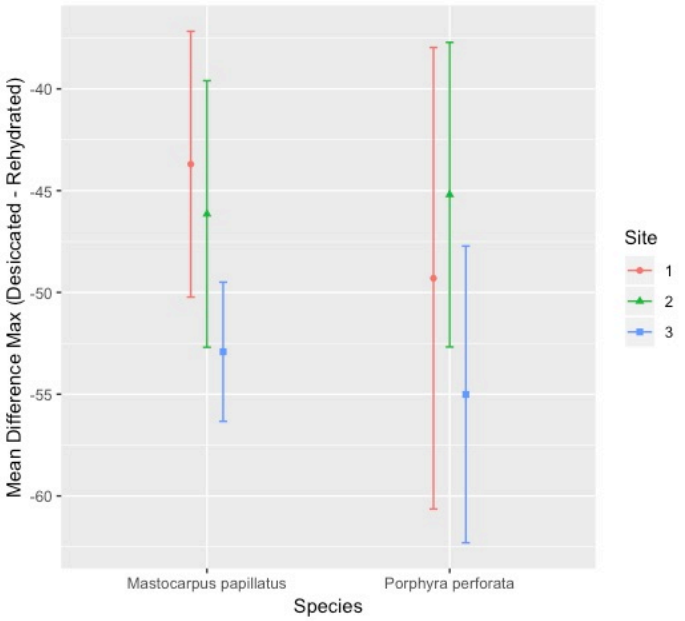
Mean Difference Max (Desiccated - Rehydrated)
-40
-45
-50
-55
-60
Mastocarpus papillatus
Porphyra perforata
Species
Site
1
2
3

Figure 5.