Are the Lichens Likin' Salt?:

Salinity Tolerance and Resilience of Littoral Lichen Species

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Abstract:

A limited number of lichen species live on seaside rocks, and little has been studied for these species on the California coast. The salt tolerance of these organisms is interesting – it is unclear whether they have adapted to survive with salt exposure, or if some species depend upon it. Three species of littoral lichens, including *Caloplaca coralloides*, were collected along the rocky intertidal zone of Monterey Bay and subjected to treatments of varying salinity levels. Photosynthetic activity was assessed based on the Electron Transport Rate (ETR) measured by a miniature Pulse-Amplified Modulated . No significant effect of treatment type was found for any of the species, but there was an effect of the number of days since start of treatment, and no interaction was found between these variables. Based on the results of this preliminary experiment, I propose a follow-up experiment involving the investigation of another possible factor influencing littoral lichen distribution: inundation time. My preliminary work serves to inform how lichens fare in laboratory settings, and how and my persisting inquiries will serve to improve our understanding of their physiology, as well as their role in ecosystem functioning.

Introduction:

Algae are key components of marine ecosystems, and they generally require these aquatic environments to grow and survive. A special mutualism with certain types of fungi, however, allows some species of algae and cyano-bacteria to survive in harsh conditions. This composite organism is called a lichen. Lichen symbiosis involves a fungus, or mycobiont, which depends on a photobiont, either an alga or a cyanobacterium. The photobiont produces sugars through photosynthesis, and in return, the mycobiont provides a tough thallus as protection for the photobiont (Dobson 2010).

Though lichens are dependent on high moisture environments for survival, once they reach maximum rates of photosynthesis, they can maintain that level of production until they face extreme desiccation (Gasulla et al. 2012). This results in an overall net gain and sequestration of carbon. Lichens can also be indicators of air quality because of their sensitivity to pollutants absorbed through their thalli (Matos et al. 2019). Though the majority of lichen species inhabit inland terrestrial ecosystems, a limited selection of lichen species are able to withstand the harsh conditions of marine rocky intertidal zones (Dobson 2010) . Vast inquiry has been made into the flora and fauna hugging the shores, but marine, or littoral lichens are rarely included in these studies, and those that do have largely been based on European coasts. Little is established in the literature for littoral lichens in California.

Littoral lichen species have a wide range of tolerances to salinity and water exposure depending on their position along intertidal zones (Nash and Lange 1988) . Inland halophilic lichens receive little saltwater exposure, while lichens in lower zones experience increasingly harsh wet/dry periods down to the eulittoral zone, where they can be found along the shells of barnacles and limpets (Dobson 2010). Few lichens can withstand total immersion in water, but all lichens, both terrestrial and marine, depend on a balance of wetting and drying for survival (Hawksworth 2000). Lichens have the unique ability to maintain a 10-20% water composition when desiccated, and can return to peak photosynthetic activity in as few as 15 minutes from rehydration (Gasulla et al. 2012). On the other end of the spectrum, total submersion of lichens in water can prevent the mycobiont from receiving carbon from the photobiont (Gasulla et al. 2012). This is likely the reason lichens appear primarily above zones of total submersion, but perhaps there is more involved in determining their placement on rocky shores.

Salt tolerance has been explored on a species level and across zones, but it is unclear whether salt tolerance is a physiological adaptation to withstand intertidal conditions, or if some lichen species in the lower zones are in fact salt dependent. After a study of the lichen species *Ramalina menziesii*, inhabiting the rocky headland of Point Lobos in Monterey County, indicated that salinity was not responsible for variations in photosynthetic rates for that species (Matthes-Sears et al. 1987), more investigation was needed to determine why so few lichen species inhabit marine environments. Nash and Lange (1988) conducted an experiment testing the effect of salinity on lichen photosynthetic productivity using measures of gas exchange within a chamber to assess photosynthetic activity. Their experiment compared terrestrial and littoral lichens, and unsurprisingly they found that the lichens from marine environments were more salt tolerant than those from terrestrial ecosystems, suffering little decline in productivity after being submerged in salt water for extended periods. They also found that for certain species, the osmotic effect of sea salt coating explained over half of the net photosynthetic loss. This is likely related to the fact that photosynthetic rate in lichens depends on moisture

In this proposal, I will explore the ambiguity between salt tolerance and dependence in littoral lichens. Through field observation and lab experimentation, I have tested how salt exposure affects littoral lichens. After presenting my results, I will propose a follow-up study to answer some of the many questions my exploration has opened up. Studying these complex organisms, which are so underrepresented in the literature, will elevate our understanding of their physiology and their distribution along the California coast.

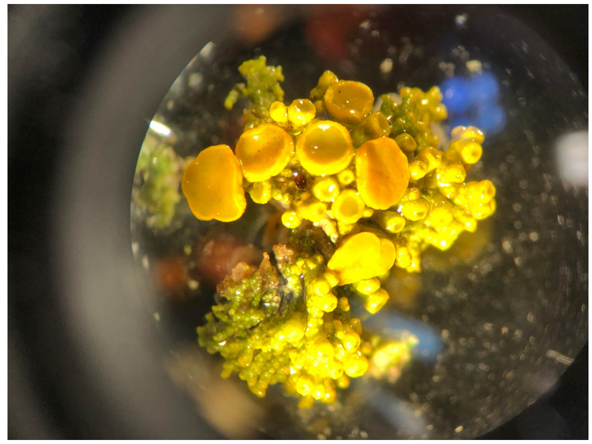
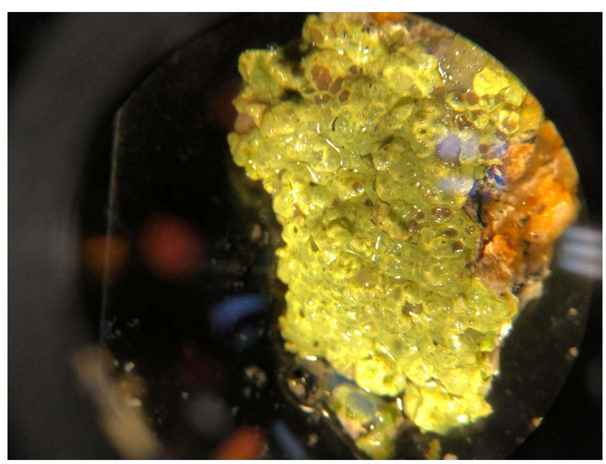
Preliminary Data:

Methods

I collected three species of littoral lichen on the rocky intertidal zone of Monterey bay (Table 1). *Caloplaca coralloides*, included in the previous salinity study (Nash & Lange 1988), is a bright yellow lichen with a dwarf fruticose thallus. The second species is another *Caloplaca* species, potentially *C. marina*, though I cannot confirm its identity without further input. The third species is a crustose green lichen, also unidentified. I have reached out to lichenologists, Dr. Jesse Miller, a Biology lecturer at Stanford, and Tom Carlberg, the president of the California Lichen Society, to get a better identification, though the identification of species using morphological features can be difficult.

I collected 9 samples of each lichen species from a small set of rocks in the high intertidal zone with a chisel and hammer, keeping the substrate intact. In the lab, I set up trays with glued-on aluminum cups to mimic the vertical position of lichen on the sides of rocks. For each lichen species, I had two treatment groups and one control. All lichens were placed in a safe location with access to sunlight, and they were watered once a day either with 1) alternating fresh and saltwater, 2) freshwater, or 3) saltwater. Samples were watered with a mister, ten sprays per sample per day, to avoid total submersion and to replicate typical wetting and drying periods

Table 1: Three littoral lichens with different morphological features.

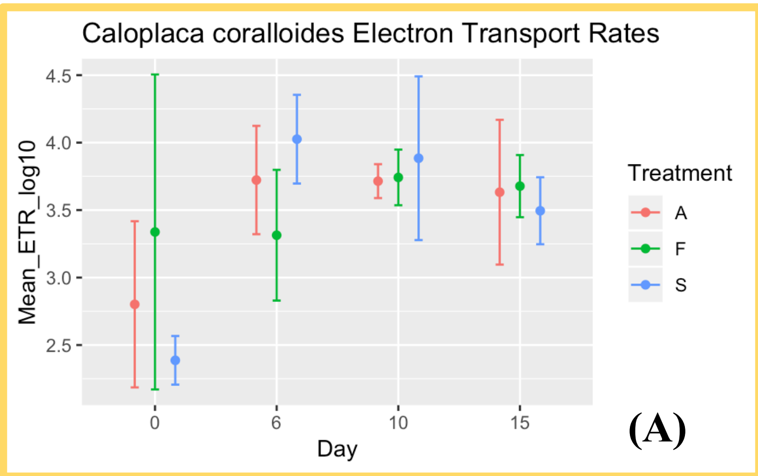
Caloplaca coralloides Dwarf fruticose thallus with terminal apothecia and branches up to 2-3 mm; grows on vertical surface of seaside rocks (McCune, p. 128)	
Caloplaca sp. Reddish-orange apothecia only, no branching or crust. Likely *C. marina* or *C. bolacina*.	
Green, crustose lichen with perithecia. Using dichotomous key, I suspect it may be *Cliostomum flavidulum* or *Mycoblastus alpinus*, but requires chemical testing and further analysis to confirm.	

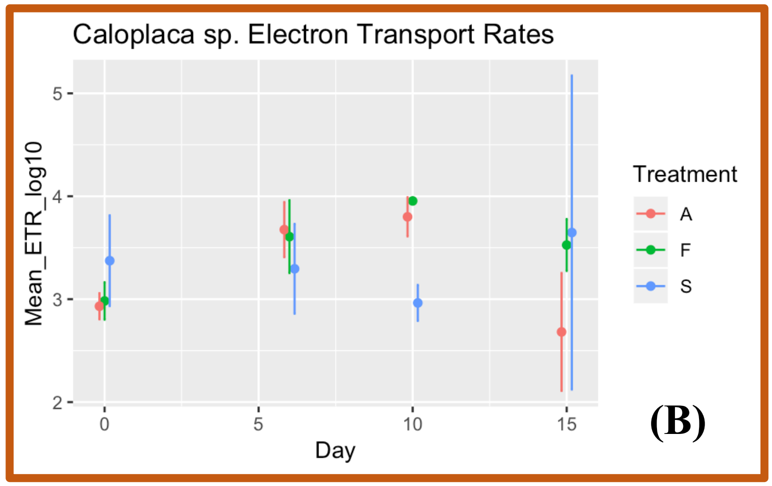
along the shore. In total, the experiment included 27 samples, with three replicates for each treatment within each lichen type. About every five days, the photosynthetic productivity of the lichens was measured using a miniature Pulse-Amplified Modulated chlorophyll fluorometer (mini-PAM), and the Electron Transport Rates (ETR) for the first and second regressions of the measured light curve were recorded.

I hoped to compare the productivity of the lichens primarily across the different treatments, but also across the different species if I could key them out confidently. Because my lichen samples were from the intertidal zone, I expected them to be somewhat salt tolerant, but I hypothesized that the lichens treated with saltwater would face a reduction in ETR as a result of the osmotic effect and the ionic effect of the salt, as well as potentially due to a restriction of exposed surface area to preventing access to sunlight. I also hypothesized that the alternating treatment would sustain a higher ETR than the other two treatments if these lichens were in fact salt-dependent, as the freshwater days would reduce the osmotic effect by clearing the salt crystals off the thalli.

Results

I looked at each species' salt tolerance individually over 15 days. For *C. coralloides* (Figure 1A), the ANOVA revealed no significant effect of treatment on the average Electron Transport Rates, but there was a significant effect of day on average ETR ($p \ll 0.01$), with an overall increase across the days of treatment. No interaction was found between the effects of





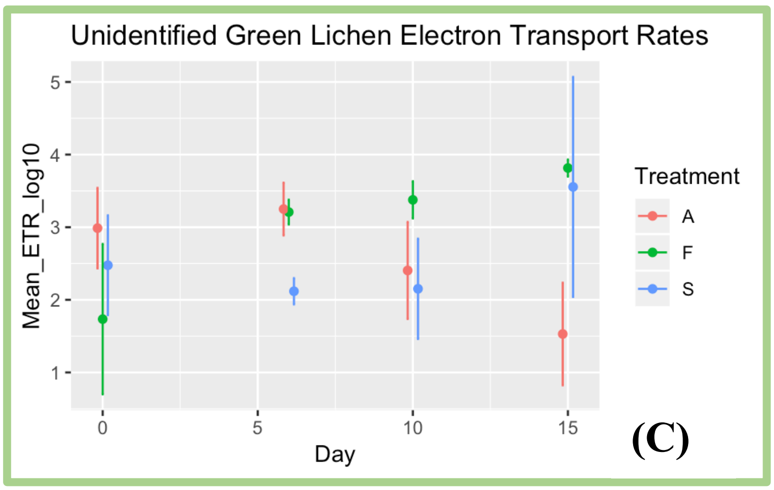


Figure 1: Average ETR, scaled logarithmically, over the course of treatment days for each species (*C. coralloides* in (A), *C. sp.* in (B), and green lichen in (C)). Treatments included Freshwater, ("F"), Saltwater, ("S"), and Alternating ("A").

treatment and day. The same result was found for *C. sp.,* with no effect of treatment type, but a significant effect of day (p = 0.012). For the green crustose lichen, no effect was found for either variable. Particularly for *C. coralloides*, but to some extent for all three species, there was a positive correlation between days passed and ETR.

In my observations, salt crystals were found coating the saltwater treated lichens as early as the third day of treatment. The apothecia visibly began to shrivel or breakdown entirely on many of the *Caloplaca* samples. By the end of the experiment, the reddish-orange *C. sp.* samples had in most cases lost their apothecia, and a coating of bright green algae remained clinging to the substrate.

The positive trend in ETR was a surprising result, as an increased ETR tends to have a positive linear relationship with photosynthetic rate (Haimeirong, et al., 2019) and I had expected the lichens to lose productivity at least for the saltwater treatments, if not for all three treatments given the disturbance in their environment. I expected my second treatment, with the alternating salt and freshwater, to result in the highest mean ETRs. I did not see a significant difference in the data from the effect of treatment type, but their resilience was fascinating; these lichens are somehow able to become more productive despite being uprooted and sprayed each day. It appears they acclimate well to living in the lab environment, which is an important advantage to know about for any scientific inquiry surrounding lichen. My sample size was relatively low, and the readings of the mini-PAM were not always consistent, so it is worth noting that there may be confounding variables in the data, but my results have brought me to a proposal for further investigation.

Proposed Work:

The aim of this study was to examine the environmental factors that influence lichen distribution on the rocky intertidal zone of Monterey Bay. My preliminary results were somewhat inconclusive, largely due to limited time and resources, but they have revealed that perhaps for these littoral lichen species, salinity is not much of a hindrance to their productivity, supporting the conclusions of Nash & Lange (1988). Since there has been evidence in the literature to suggest that the osmotic effect of salinity does not impact the dark respiration system of lichen species (Nash et al. 1990), there is little explanation of why these salt-tolerant littoral lichens remain above the intertidal zones that receive partial or full inundation at high tide. To further investigate this question, I propose an experiment to test the effects of other possible factors on lichen productivity.

For this proposed experiment, I will focus on the species *Caloplaca corraloides* and an inland lichen species, *Evernia prunastri* (Nash & Lange 1988), confirming their identity by conducting a series of microchemical tests (McCune Vol. 1 2017), which I did not have the resources for during my preliminary work. There will be four racks, fashioned in the same manner as the methods for my preliminary work, of lichens receiving different saltwater exposure treatments. Each treatment will mimic the inundation conditions experienced at four general intertidal zones: low, mid, high, and splash zones (Figure 2).

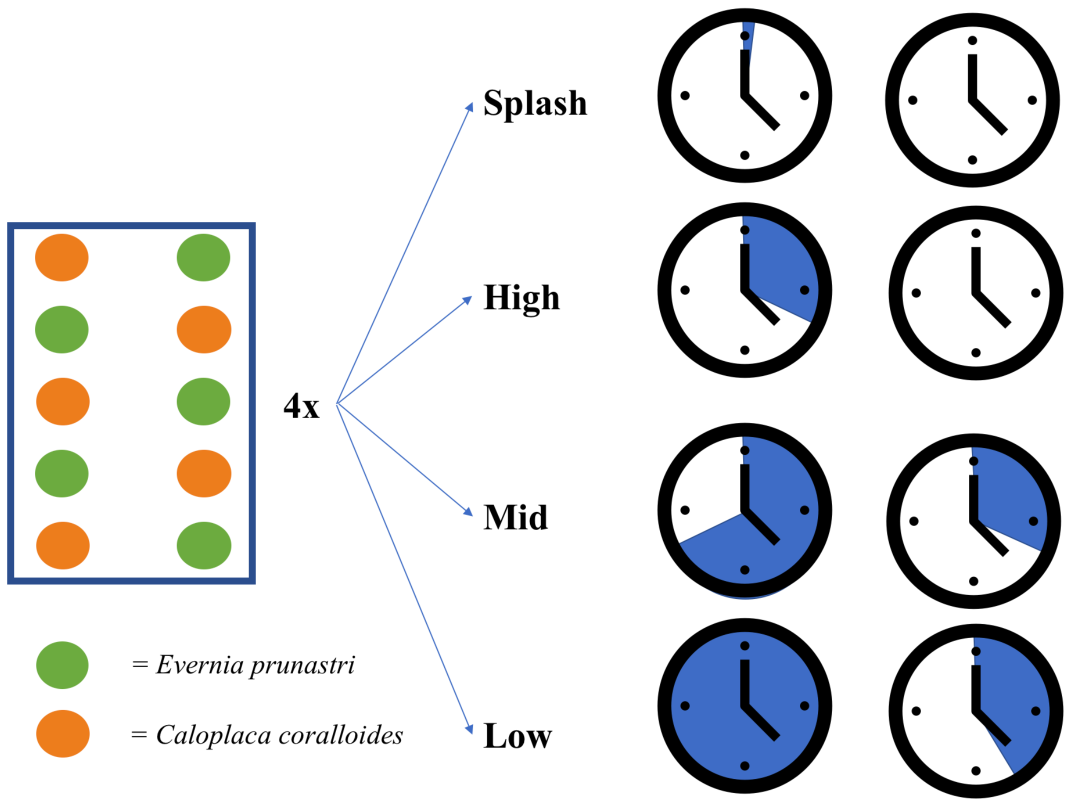


Figure 2: Four racks will be set up with 10 lichen samples, 5 of each species. The first rack, with the "Splash Zone" treatment, will get misted with a spray bottle ten times each day. The second, with the "High intertidal" treatment will be submerged in a saltwater tank for 4 hours once a day. The third, with "Mid intertidal" treatment, will be submerged for 8 hours overnight, then for an additional 4 hours in the afternoon. Finally, the "Low intertidal treatment" group will be submerged for 18 hours each day.

When racks are being submerged in saltwater, mesh coverings will be placed over the foil cups so as not to lose the lichen samples. When they are removed from the saltwater, racks will be placed in a vertical position facing a light source, which while be controlled on schedule such that each each rack receives equivalent light exposure. This experiment will be carried out over the course of 30 days. I will replicate my methods for the mini-PAM, but I will ensure that each lichen sample is measured exactly 15 minutes after water treatment to ensure peak photosynthetic activity (Gasulla et al. 2012). I also propose using an assimilation chamber to monitor gas exchange, for a potentially more reliable measure of photosynthetic productivity. In my analyses of these data, I will compare photosynthetic rates across treatments and between species.

Perhaps lichen do not appear to play a critical role in ecosystem functioning much beyond their abilities to store carbon and react to changes in air quality, but with how little research has been conducted on these species, there is always the possibility that they are more important to their ecosystems, we are simply lacking the understanding of their physiology. By conducting experiments to investigate the factors driving lichen distribution, we can learn more about which parts of their environment they interact with, and about the niche that they fill in those environments.

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