The Effects of Low Tide Exposure on the Photosynthetic Health of *Saccharina sessilis*

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Abstract

Intertidal seaweeds experience different environmental conditions during high and low tides. Low tide conditions may be stressful because desiccation and extreme light levels may impact their ability to photosynthesize. We examined the effect of low tide exposure on the photosynthetic health of the canopy-forming seaweed *Saccharina sessilis* in the field and lab. Field studies assessed the health of marked individuals on days with varying environmental conditions, while lab studies separated the effects of those different conditions. We hypothesized that the seaweed's photosynthetic health depended on weather. We predicted that health would decline over stressful low tides (high light, high wind, and high temperature), but not over benign low tides (low light, low wind, and moderate temperature). We assessed health in multiple ways. First, we measured dark-adapted Maximum Quantum Yield (MQY) via pulse-amplitude-modulated (PAM) fluorometry, which assesses the photosynthetic ability of Photosystem II. Second, because we observed that the seaweed changes color following intense stress, we evaluated tissue damage by quantifying the proportion of blade area in five color categories. Over the long term, damage from low tide exposure may limit growth and reproductive output. Understanding how individuals and populations respond to daily stresses will aid in understanding their response over longer time scales.

Introduction

The intertidal zone is an area that is covered by seawater at high tide and exposed to air (terrestrial conditions) during low tide. Intertidal organisms on the west coast of the United States are subjected to a mixed semi-diurnal tidal cycle, meaning that low intertidal organisms are exposed to terrestrial conditions once a day for multiple consecutive days during each tide series. During low tide, low intertidal kelps such as *Saccharina sessilis* may experience stressful low tide conditions. In the Pacific Northwest, winter emersion for low intertidal organisms occurs in the middle of the night, whereas in summer, emersion occurs early – morning to midday, coinciding with potentially high ambient temperature and light levels. Especially in the summer, repeated low tide exposure may be extremely stressful for intertidal seaweeds and invertebrates.

Intertidal seaweeds require specific levels of factors such as light in order to grow and reproduce. Excessive irradiance may lead to photoinhibition (Gévaert et al 2003) and inadequate irradiance will not allow the seaweeds to photosynthesize to their fullest potential. Low tide stresses for intertidal organisms include but are not limited to high light intensity, high temperature, and wind. A stressful low tide with high light intensity, high wind, low humidity, or a combination of those abiotic factors may lead to desiccation. Desiccation may in turn affect the physiological condition of an alga and reduce its photosynthetic rate (Williams and Dethier 2005, Hunt and Denny 2008).

Saccharina sessilis is a canopy–forming alga, which was the original "foundation species" (Dayton 1972). *Saccharina sessilis* has been shown to have strong positive non–trophic effects on the system's main herbivore *Katharina tunicata* (the black chiton), during periods of intense low tide stresses (Burnaford 2004). Researchers have also

shown that the abundance of *K. tunicata* dramatically decreases after the removal of *S. sessilis* during the summer when intertidal organisms are exposed during the hours of possible stress (Dethier and Duggins 1984, Burnaford 2004). Therefore, *S. sessilis* has an important role in the system because it modifies habitats and provides a shady refuge for *K. tunicata* and other marine organisms during intense abiotic stress (Burnaford 2001 and Burnaford 2004). In addition to the positive effects on intertidal animals, *S. sessilis* controls the algal diversity in the ecosystem (Dayton 1975, Duggins and Dethier 1985, Burnaford 2001). Removal of *S. sessilis* has been shown to increase growth of other small algae forms, partly because the kelp prevents sufficient sunlight from reaching the small algae (Dayton 1975, Duggins and Dethier 1985, Burnaford 2001).

Land plants and algae obtain energy for tissue construction, growth, and reproduction through photosynthesis. We utilized field and laboratory studies to investigate the effects of environmental factors and repeated low tide exposure on the health of *Saccharina sessilis*. We assessed health using three metrics: 1) measurements of dark – adapted Maximum Quantum Yield (MQY), 2) visual estimations of tissue damage and pigment loss, and 3) biomass loss.

A common method used to assess the photosynthetic health of algae is by measuring the dark – adapted Maximum Quantum Yield (MQY) via pulse – amplitude modulated (PAM) fluorometry (e.g. Gévaert et al 2002 and Gévaert et al 2003). PAM fluorometry assesses the photosynthetic ability of photosystem II, providing a quantitative measure of the alga's physiological condition (Falkowski and Raven 2007). When chlorophyll a absorbs light energy from the sun, electrons are excited and jump to a higher orbital state (Cosgrove and Borowitzka 2011). After electrons jump to a higher

orbital state, this energy has three possible fates: 1) the electrons get donated to the electron acceptor and proceed through the electron transport chain to make sugar via photosynthesis, 2) electrons return to their original energy state and the energy is released as fluorescence, 3) the electrons cannot be donated to the electron acceptor so the energy gets dissipated as heat. When Maximum Quantum Yield is measured, thalli are dark adapted to reset reaction centers so that they are all open, allowing for maximum fluorescence. A light of greater than saturating intensity is turned on the individual so that light is absorbed by chlorophyll a and electrons are donated to the electron acceptors, closing all the reaction centers. In a healthy individual, the excess light energy that is absorbed by the chlorophyll once the electron acceptors are full is then emitted back as fluorescence. High MQY indicates that the individual is healthy, and a low MQY reading suggests that the individual is damaged (Cosgrove and Borowitzka 2011).

After even a single stressful low tide, photosystem II may get damaged, leading to a decrease in the kelp's photosynthetic health. This damage could be seen first through a decrease in the alga's Maximum Quantum Yield values. Over time, damage to photosynthetic apparatus might become visible as a result of damage to photosynthetic pigments. If the damage is severe, the tissue may die. Consequently, damage from low tide exposure may result in the kelp getting shorter because damaged tissue sloughs off.

In addition to measuring MQY, we examined the linkage between MQY readings, visible tissue damage, and tissue loss. It is important to understand how the kelp's immediate physiological response to stressors (as indicated by MQY readings) links to longer – term (slower) responses such as tissue damage and tissue loss. Over the long

term, tissue damage and tissue loss may alter the kelp's energy investment for growth and maintenance, and possibly reproductive output.

We assessed how MQY readings change over the course of a single low tide and how MQY readings, tissue damage, and biomass changed over repeated low – tide exposures within and between tide series. We hypothesized that the health of the kelp would depend on weather conditions. In particular, we predicted that the health of *S. sessilis* thalli in the field would decline over the course of a low tide and throughout the tide series on stressful days but not on benign days. We predicted that stressful abiotic conditions (high light, high temperature, and high wind) would decrease the health of the kelp in laboratory experiments while kelps exposed to benign conditions would not show a decrease in health. Laboratory experiments focused on testing thalli under specific combinations of stresses such as high light and desiccation, high light with frequent hydration, and high light alone.

Materials and Methods

Study Site and Study Organism

All field experiments were carried out at Pile Point, on the west side of San Juan Island, WA (48° 28.9' N, 123° 05.7' W). Pile Point is characterized as a moderately wave – exposed site (Burnaford 2004). *Saccharina sessilis* is a stipeless perennial kelp that has multiple blades up to 30 cm in length arising directly from the holdfast (Duggins and Dethier 1985, Burnaford 2004). *Saccharina sessilis* is abundant in the low intertidal zone ranging from +1.9ft to below mean lower low water (MLLW); thus, this kelp is only exposed during the lowest low tide in each 24 hour period (Duggins and Dethier 1985).

Field Studies

To assess how low tide exposure affects the photosynthetic health of *Saccharina sessilis*, we chose 6 individuals at the highest edge of the *S. sessilis* zone at Pile Point (approximately +1.9ft tidal height) as the focal thalli for our study. We selected thalli that were isolated from other *Saccharina* individuals (so that they were not shaded by other thalli), on flat or only slightly sloping rock faces (so that they experienced similar light conditions), and not surrounded by any other canopy – forming algal species. We haphazardly chose our focal thalli from among the thalli at the site that met these criteria. Focal thalli were identified by markers placed on the rock surface near the thallus.

For MQY readings, we dark – adapted thalli using the standard PAM fluorometry procedure in which we placed a measurement clip over a portion of the blade for 10 – 17 minutes before MQY readings were taken. To characterize the physiological condition of the whole thallus, including blades that were exposed to the sun (canopy blades) and blades that were not exposed to the sun (understory blades) we took four MQY readings per thallus at each measurement time. We attempted to take these four readings in the following locations: at the tip of a top (canopy) blade, at the edge of a canopy blade, at the base of a canopy blade, and on a shaded understory blade. In cases where tissue loss prevented us from taking readings at the four locations, we took readings wherever there was enough tissue to do so.

On days that we measured MQY, we took our first measurement (= 4 readings per thallus) between 2 and 20 minutes post – emersion at the beginning of a low tide, attempting to measure thalli at the earliest possible moment where they were no longer washed by waves. We then took additional measurements at approximately hourly intervals until thalli were washed by waves or submerged. Thus our last reading was no

more than 1 hour and frequently less than 10 minutes before re-immersion at the end of the low tide. For one tide series (July 2 – 13, 2013) we took measurements for 9 out of 12 days on which the kelp were exposed. In addition, we took pre-dawn MQY readings of thalli on the first day of the following tide series (July 16, 2013).

Healthy *S. sessilis* individuals are a deep brown color, but when stressors such as high light or desiccation damage individuals, the color of the blade first changes to green, then tan, then white, then becomes transparent (T-V. Nguyen and J. Burnaford, personal observations). The change in color is likely due to the breakdown of pigments. On occasion, the color of the blade is obscured, as damaged blades can be covered with a layer of epiphytic diatoms (such layers are not found on healthy blades: J. Burnaford, personal communication). Holes can be created in a continuous blade if the damaged tissue sloughs off or by herbivores which are eating algal tissue (J. Burnaford, personal communication). We evaluated visible damage by quantifying the proportion of damaged tissue in six categories: brown, green, tan, white, transparent, diatom, and hole using the point – contact method with a 25 cm x 25 cm grid with points approximately 1 cm apart. The number of points per blade differed among blades because it depended on blade size.

We quantified biomass loss by measuring the two longest blades from the holdfast to the distal tip of the blades on each of the 6 marked thalli at the beginning, middle, and end of a tide series.

Because we predicted that weather conditions would affect the health of *Saccharina sessilis*, we measured environmental conditions during low tide. We measured light intensity ($\mu mol/m^2/s$) in the PAR wavelength range (400 – 700 nm) approximately every 60 minutes (or when time permitted) using a Li – 250A Light Meter

from Li-Cor and a Quantum Sensor. Light readings were taken on a level surface to ensure consistency among measurements. Air temperature (°C) was automatically recorded every 2 minutes by a HOBO Pendant® Temperature/Light Data Logger (UA-002-64, Onset Computer Corporation). Pendants were placed on open rock. Relative humidity (%), air temperature (°C) and wind speed (m/s) were logged every 20 seconds during low tide with a Kestrel 4500 Pocket Weather Meter placed on a rock near focal thalli. To characterize the conditions experienced by the organism during the low tide, we took the mean of each environmental parameter on each day.

Laboratory Studies

We used laboratory manipulations to examine the effects of light and desiccation on *Saccharina sessilis*. Blades (1 / individual) were collected at Pile Point, transported to Friday Harbor Laboratories (FHL), and trimmed to dimensions 10 cm x 7 cm. We selected blades with as little visible damage and epiphyte load as possible. Each blade was placed in a 1.9cm square mesh (Deer-X ® Deer Fencing) envelope that allowed water flow. Blades were maintained in an outdoor flowing seawater tanks with ambient water temperatures and light levels. Experimental blades were allowed to recover for 36 to 48 hours before the experiment began.

We simulated a 2 hour low tide exposure with the following treatments: no low tide (low light, no desiccation), cool low tide (low light, low desiccation), sun low tide (high light, ambient desiccation), sun + wind low tide (high light, accelerated desiccation), and sun + spray low tide (high light, reduced desiccation). Blades assigned to the sun, sun + wind, and sun + spray low tide treatments were all placed outside in ambient sunlight. Blades in the sun low tide treatment experienced ambient wind conditions. Each thallus

in the sun + wind treatment was placed 20 cm away from a fan with an airflow of ~ 1.0 m/s to augment natural wind conditions and accelerate desiccation. Blades in the sun + spray low tide treatment were sprayed approximately every 5 minutes with 1.60 – 1.83 g of seawater. In outdoor treatments, manipulated conditions (e.g. spray or wind) were applied independently to specific blades and blades were spaced so that they were not affected by the manipulation of conditions for blades in other treatments. Blades in the no low tide treatment were placed in a flowing seawater tank in the laboratory throughout the duration of the simulated low tide, while blades in the cool low tide treatment were placed on an inside lab bench. Indoor blades did receive low levels of sunlight (through laboratory windows).

We measured environmental conditions (light intensity, air temperature, relative humidity, and wind speed) for each treatment during the experiment. Light intensity ($\mu mol/m^2/s$) in the PAR wavelength range was measured using a Li – 250A Light Meter from Li – Cor and a Quantum Sensor approximately every 60 minutes in the laboratory near the cool low tide treatments and outside where the sun treatments were located. Air temperature (°C), relative humidity (%), and wind speed (m/s) were measured indoors and outdoors every 20 seconds using Kestrel 4500 Pocket Weather Meters. In addition, pendants from Onset Computer Corporation were used to measure temperature (°C) and light intensity (lumen per ft^2) of each treatment. One pendant was placed next to a randomly chosen replicate for each treatment so that the environmental conditions experienced by the specific treatments could be characterized. Days were characterized by taking the mean of each parameter during the low tide simulation.

Table 1 Environmental conditions in experimental treatments. Values of light intensity measurements, pendant temperature, wind speed, relative humidity, and air temperature are averages (±SD) of measurements taken over the two experimental low tides.

Treatment	Light intensity ($\mu mol/m^2/s$)	Light intensity ($lumen/ft^2$)	Wind speed (m/s)	Relative humidity (%)	Air temp (°C)	Pendant temp (°C)
No Low Tide	--	27.6 (±5.35 SD)	--	--	--	14.2 (±0.15 SD)
Cool Low Tide	11.1 (±0.275 SD)	66.9 (±14.3 SD)	0.00 (±0.05 SD)	58.4 (±2.8 SD)	23.2 (±0.57 SD)	24.9 (±4.5 SD)
Sun	1683.4 (±165.3 SD)	19978 (±954 SD)	0.92 (±0.6 SD)	47.3 (±3.7 SD)	25.2 (±1.3 SD)	45.9 (±2.8 SD)
Sun + Wind	1683.4 (±165.3 SD)	18766 (±2006 SD)	1.15 (±0.58 SD)	40.7 (±5.3 SD)	28.8 (±1.9 SD)	41.1 (±2.0 SD)
Sun + Spray	1683.4 (±165.3 SD)	18112 (±2473 SD)	--	--	--	40.5 (±2.4 SD)

One day prior to the simulated low tide experiment, each blade was randomly assigned to a treatment. Before the start of the experiment, we took MQY readings (3 – 4 readings per blade), evaluated tissue damage using point – contact methods, and measured the wet mass of each blade after blotting it with a damp paper towel to remove excess seawater.

On each experimental day, we tested 4 blades per treatment but we staggered the placement of blades into the experimental low tide treatments. One blade / treatment was placed into the experimental low tide every 10 minutes (starting at 1:40-2:20 pm for block 1; starting at 1:30 – 2:10pm for block 2). After one hour and again after two hours in the experimental treatment, we dark – adapted blades for 10 – 17 minutes before taking MQY readings. Following the final MQY reading at the end of the 2-hour low tide exposure, we weighed each blade to obtain its post- exposure wet mass. We then submerged blades in running seawater in outside tanks and allowed blades to recover for three days. On each recovery day, at approximately 24 hours intervals, we obtained MQY

readings, evaluated tissue damage, and measured wet mass of each blade to determine how the photosynthetic health, amount of visible tissue damage and biomass loss caused by low tide stress changed after a low tide exposure.

Statistical Analysis

We took 3 – 4 MQY readings per thallus at each measurement time in the field and lab. We averaged the MQY readings per thallus (field) or blade (lab) to get a single value for each individual to allow us to compare changes in MQY values over a course of a low tide, between low tides, and between tide series.

All statistical analyses were conducted using JMP v.10 statistical software (SAS Institute, Cary, NC). Data used in parametric tests were tested to determine whether they met test assumptions. The assumption of normality was tested with a Sharpio-Wilk W test comparing data to a continuous normal distribution. Minor departures from normality (p values > 0.04 for test statistics) were considered acceptable, as ANOVA and t-tests are relatively robust to violations of this assumption. The assumption of equal variances among groups was tested using Levene's test; no violations of this assumption were made (all p-values ≥ 0.05).

To determine whether any single environmental parameter showed an effect on MQY levels during low tide, we took the difference between the first measurement of the low tide and the last measurement of low tide for each day in the field and compared these values to the average wind speed, average relative humidity, average air temperature, and average light intensity using regression analysis. We used a paired t-test to determine if the thallus MQY values taken at the beginning of the first low tide of the first tide series were different from measurements taken at the beginning of the last

low tide of the first low tide series. In addition, we compared if those 'beginning MQY' values were different from the values taken at the beginning of the second low tide series. For the laboratory experiment, we compared the thallus MQY values among treatments at time 0 (pre-experiment) using an ANOVA on ln-transformed data. We compared thallus MQY values among treatments at the end of the simulated low tide and after 3 days of recovery using separate Kruskal-Wallis Tests with additional Wilcoxon Tests to compare among specific treatments.

For the assessment of damaged tissue, we calculated the percent of blade area which was brown (healthy) for each blade by dividing the number of recorded brown points by the total number of measured points for each blade. We followed the change in individuals in the field to see how tissue damage changes throughout a tide series. We compared the amount of healthy tissue (as % of total area which was brown) between treatments at the end of the experiment (after 3 days of recovery) using a Kruskal-Wallis test with Wilcoxon tests to examine differences among treatments.

In the field, we measured blade lengths of focal thalli to assess how biomass changed over time. We used a paired t-test to compare blade lengths of focal thalli on the first days of the two tide series. In the lab, we measured wet mass of each experimental blade at multiple time points and divided it by the starting (pre-exposure) mass to determine percent mass loss over time. We compared wet mass (as % of pre-exposure values) between treatments at the end of the experiment (after 3 days of recovery) using an ANOVA with Tukey-Kramer HSD tests to determine differences among treatments.

Results

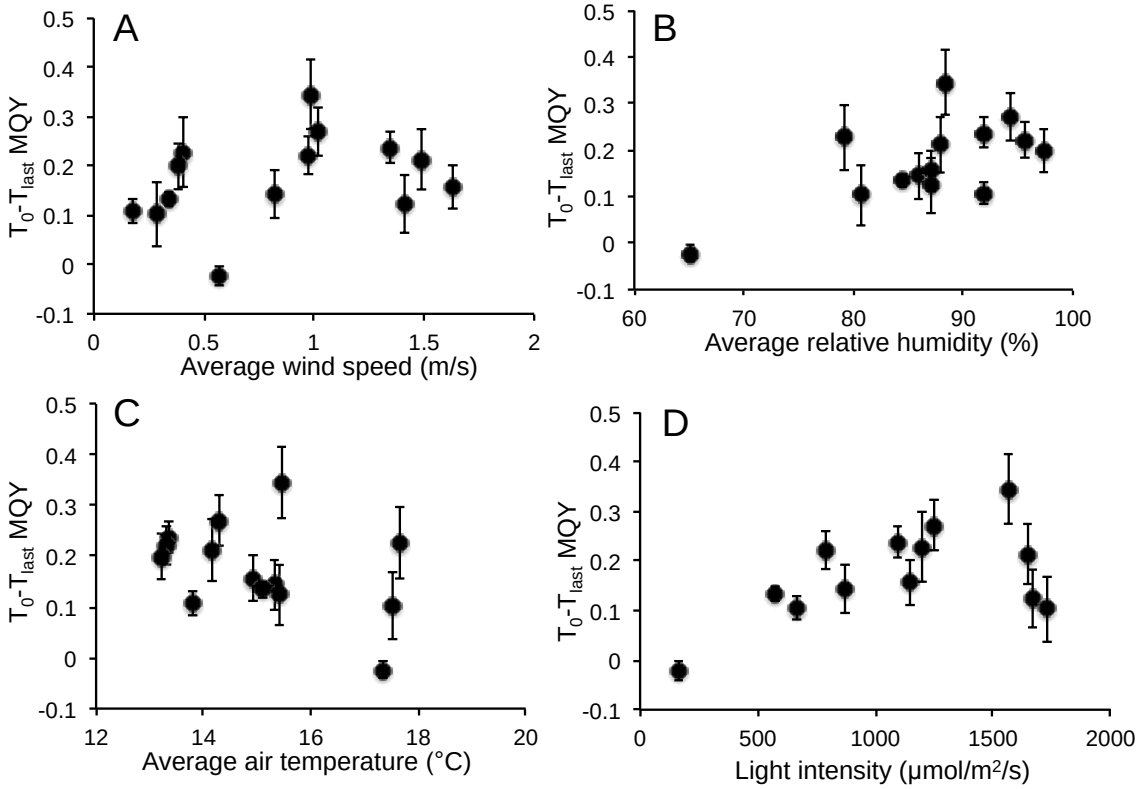

Figure 1. **Change in MQY over low tides with different environmental conditions.** A, B, C) Environmental conditions recorded every 20 seconds during low tide. D) Environmental conditions recorded ~ every 1 hour during low tide. Each point = difference in MQY between the first and last readings on that low tide. N=14 days in summer 2013.

To determine if specific environmental factors were related to change in MQY values in the field, we assessed the linear relationship between individual environmental factors and the change in thallus MQY values over single low tides. We measured environmental factors over several days (n = 14 days), which naturally varied in terms of wind speed, relative humidity, air temperature, and light intensity. There was no indication that the change in the thallus MQY values was related to the average wind speed (Figure 1A, R^2=0.10, p=0.26), air temperature (Figure 1C, $R^2$0.16, p=0.16), or light intensity (Figure 1D, R^2=0.26, p=0.07). Similarly, we did not see strong evidence that the

change in thallus MQY values was related to relative humidity (Figure 1B, with outlier R^2=0.41, p=0.01; without outlier R^2=0.09, p=0.30). No single environmental factor seems to be driving the change in MQY values over low tide. Given that the significant relationship between change in MQY values and average relative humidity is driven solely by a single outlier point (Figure 1), we assert that further investigation is needed to investigate the relationship between MQY and this environmental factor.

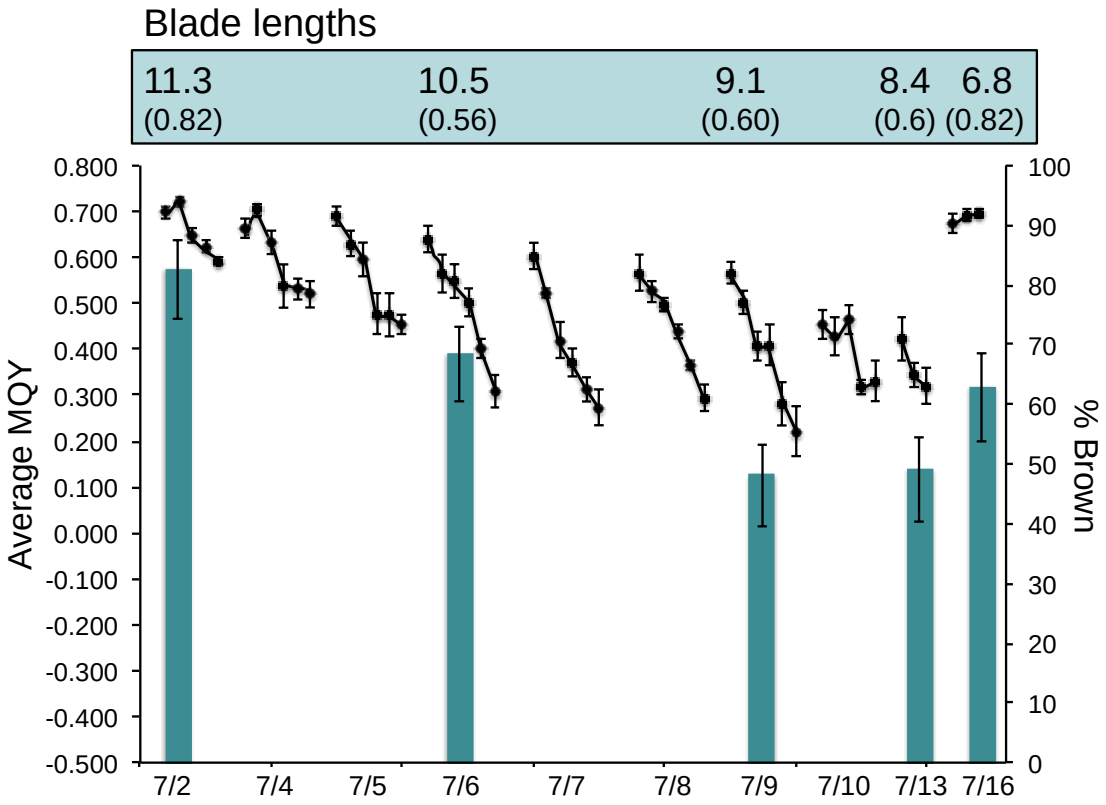

Figure 2 Effects of repeated low tide exposure on three metrics of health. Each circle represents the mean (± SE) MQY value of the focal thalli at a given measurement time in the field. Bars represent the mean (± SE) percent brown (healthy tissue) of the focal thalli. Numbers along the top are the mean (± SE) blade lengths (cm) of focal thalli. N=6 focal thalli for each measurement time, summer 2013.

We determined how MQY values change within a low tide, between days of a tide series, and between two tide series. On each day, average MQY values started high

and decreased throughout the course of a low tide (Figure 2). For example, the average MQY at the beginning of the low tide on July 2nd was 0.698 (±0.01 SE) and the average decreased to 0.592 (±0.01 SE) by the end of the low tide. Similarly, average MQY value on July 5th at the beginning of the low tide was 0.690 (±0.02 SE) and decreased to 0.453 (±0.02 SE) by the end of the low tide (Figure 2). Although the average MQY values decreased throughout each low tide, the magnitude of change was different for different days. The average change in MQY values on July 2nd was 0.107 (±0.02 SE), while the average MQY change on July 6th was 0.156 (±0.04 SE).

Over consecutive days, the first MQY reading of the low tide got lower and lower. For example, average MQY at the beginning of the low tide on July 6th was 0.639 (±0.03 SE), while the average MQY at the beginning of the low tide on July 7th was 0.602 (±0.03 SE) (Figure 2). Values decreased even further on July 8th as average MQY at the beginning of the low tide was 0.565 (±0.04 SE). First MQY readings on the last day of the tide series were significantly lower than the first readings on the first day of the tide series (Figure 2, $n = 6$ thalli, paired t-test, $t = -5.48$, $p=0.003$). As the low tide exposure time got longer throughout the tide series, average MQY values at the end of the low tide were also consistently lower than the ending values of the previous day.

Between the end of the first tide series and the beginning of the next, average MQY values increased. On average, MQY values at the beginning of the last day of the first tide series were 0.422 (±0.05 SE), lower than the average pre – dawn MQY values at the beginning of the first low tide of the second tide series, which was 0.674 (±0.02 SE). The increase in MQY values between tide series was so substantial that pre – dawn MQY readings for the first day of the second tide series were not different from the MQY

readings from the first day of the first tide series (Figure 2, N = 6 thalli, paired t – test, t = -1.7, p=0.15).

On the first day of the first tide series, focal thalli showed very little visible damage (mean % of blade area that was brown = 81% ± 6.5 SE, Figure 2). The amount of visibly healthy blade tissue declined over the course of the tide series: the mean percent of blade area that was brown had decreased to 67% (±6.2 SE) after 4 days and to 46% (±6.7 SE) after 7 consecutive days of low tides. Visibly healthy tissue increased to 61% (±7.3 SE) by the beginning of the second tide series.

Focal thalli also lost biomass over the course of a single tide series. Blade lengths averaged 11.3 cm (±0.82 SE) at the beginning of the first tide series and had decreased to 8.4 cm (±0.60 SE) by the end of the tide series (Figure 2). Unlike average MQY values and average % brown, average blade lengths did not recover by the beginning of the second tide series. Average blade length of the focal thalli at the beginning of the second tide series was 6.8 cm (±0.82 SE), lower than that of the end of the first tide series. By the beginning of the second tide series, average blade length was significantly smaller than the average blade length at the beginning of the first tide series (paired t – test, n = 6 thalli, t = -5.42, p=0.0028).

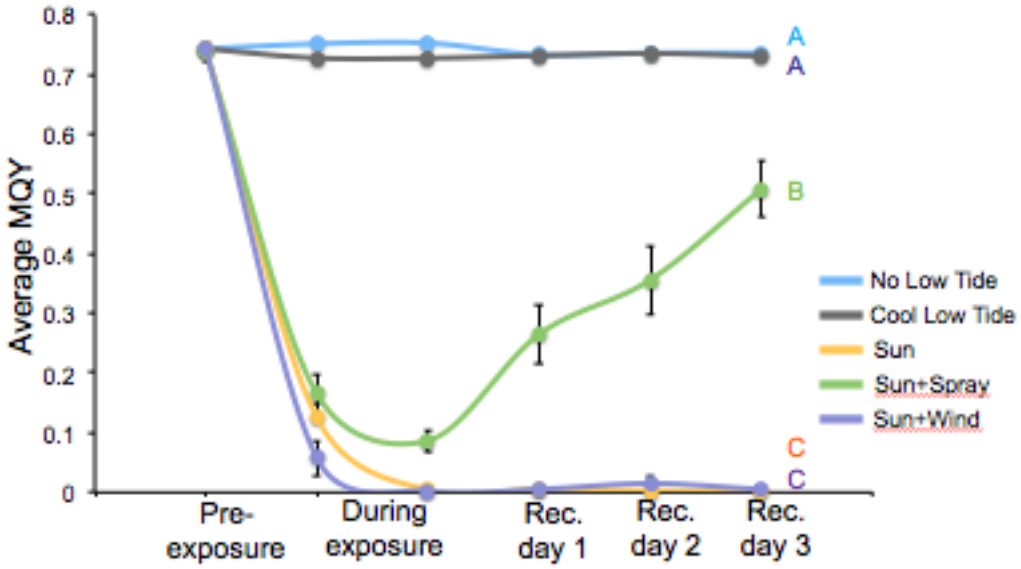

Figure 3. **Effect of low tide environmental conditions on Maximum Quantum Yield.** Each circle represents the mean (±SE) MQY of blades (N=8) in our experimental treatments. MQY values did not differ among treatments at the start of the experiment (ANOVA on LN-transformed data, F = 0.07, p=0.99) but did differ among treatments after low tide exposure (Kruskal Wallace Test, χ^2 = 35.4, DF = 4, p < 0.0001: different letters indicate treatments which are different based on multiple comparisons using the Wilcoxon method). After three days of recovery, MQY values still differed among treatments (Kruskal Wallace Test, χ^2= 34.19, DF = 4, p < 0.0001: different letters indicate treatments which are different based on multiple comparisons using the Wilcoxon method).

Laboratory Experiments

Prior to the simulated low tide exposure, average MQY values did not differ among blades that had been assigned to the different treatments (Figure 3, ANOVA on LN-transformed data, F = 0.07, p=0.99). Mean MQY values prior to low tide exposure in any treatment ranged from 0.740 (±0.003 SE) to 0.744 (±0.003 SE). However, after one hour of low tide exposure, average MQY values for blades in the sun, sun+spray, and sun+wind treatments dropped below 0.200 (Figure 3). During the second hour of low tide exposure, MQY values of the sun, sun+spray, and sun+wind treatments continued to decrease. However, the magnitude of decrease differed among the three outside sun

treatments, such that after the end of the 2-hour exposure, there were differences among treatments (Kruskal Wallace Test, χ^2= 35.4, DF = 4, $p < 0.0001$). After the low tide, blades in the three outdoor treatments had extremely low MQY values (Figure 3) but blades in the no low tide and cool low tide treatments had not decreased greatly from their pre-exposure average MQY readings (Figure 3). Blades from the no low tide treatment in fact changed very little with an average MQY value of 0.752 (±0.003 SE) after the first hour of the experiment and an average MQY value of 0.753 (±0.001 SE) after the second hour of the experiment.

Following 24 hours of immersion after low tide exposure, blades from the sun+spray treatments showed strong recovery, while the sun and sun+wind treatments showed no recovery and the two 'low stress' treatments had very high MQY values (Figure 3). MQY values for the sun+spray treatments increased from a mean of 0.087 (±0.02 SE) after the 2-hour low tide exposure to a mean of 0.265(±0.05) after 1 day of recovery. These blades from the sun+spray treatments continued to recover throughout the second and third day of recovery. By the third day of recovery, there were three distinct groups in terms of MQY values: sun+spray (average MQY value of 0.508 ±0.05 SE), sun and sun+wind treatments (average MQY values below 0.006) and the low stress treatments (average MQY values above 720; Kruskal Wallace Test, X2 = 34.19, DF = 4, $p < 0.0001$, Figure 3).

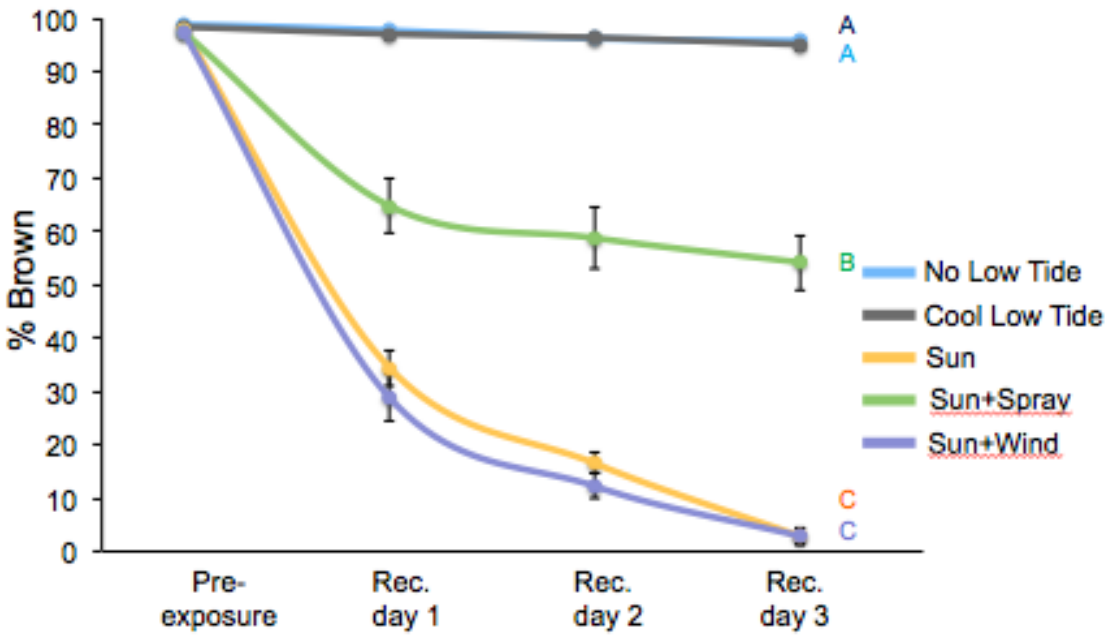

Figure 4. Effects of low tide environmental conditions on tissue damage. Each circle represents the average (±SE) percent brown of eight blades per experimental treatment. The amount of healthy tissue differed among treatments after 3 days of recovery from low tide simulations (Kruskal Wallace Test, $X2 = 34.18$, $DF = 4$, $p < 0.0001$: different letters indicate treatments which are different based on multiple comparisons using the Wilcoxon method).

At the start of the experiment, all thalli were visibly healthy, with more than 97% of blade area appearing brown. After the low tide exposure and through the first recovery day, the amount of healthy (brown) blade area decreased in the sun, sun+spray, and sun+wind treatments, while the cool low tide and no low tide treatments showed no noticeable reduction in the amount of visibly healthy tissue. The sun and sun+wind treatments showed a greater increase in visible damage than the sun+spray, no low tide, and cool low tide treatments (Figure 4). The amount of visibly damaged tissue in the sun, sun+wind, and sun+spray treatments continued to increase throughout recovery days 2 and 3, although the magnitude of change in percent brown was significantly different for different treatments (Figure 4, Kruskal Wallace Test, $X2 = 34.18$, $DF = 4$, $p < 0.0001$).

By the end of recovery day 3, on average, 56% of blade area in the sun+spray treatment appeared brown. However, for samples in the sun and sun+wind treatments, on average only 3% of blade area was brown. There was no major change in the average percent brown of the no low tide and cool low tide treatments among measurement times.

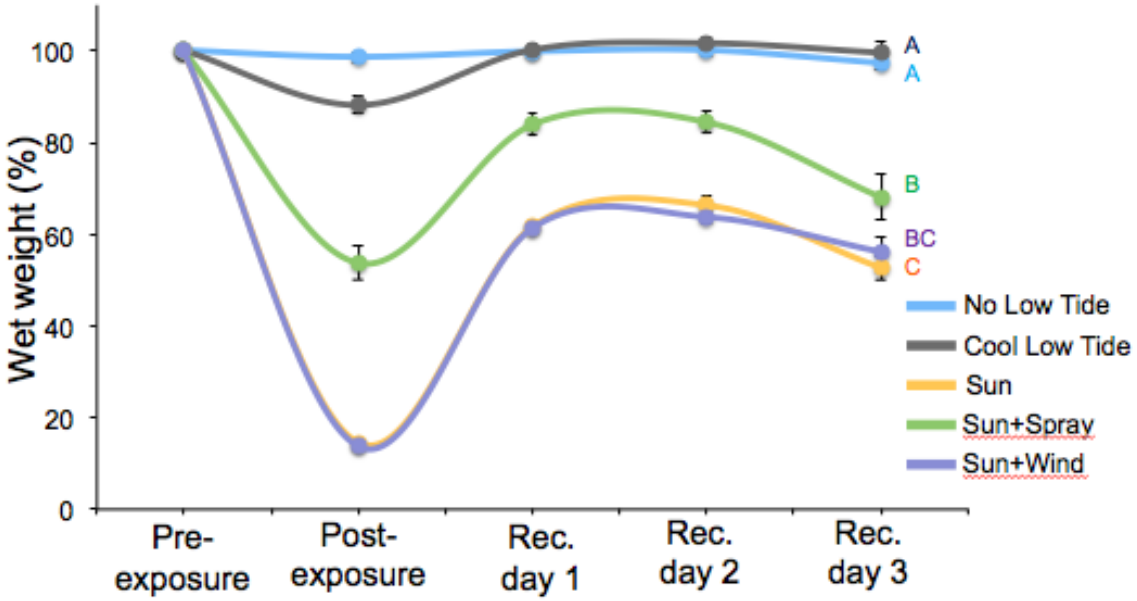

Figure 5. Effect of low tide environmental conditions on biomass. Each dot represents the average (±SE) of eight blades per experimental treatment . On the third day of recovery, wet mass was not the same among the three treatments (ANOVA $F = 51.79$, $p < 0.0001$). Groups with different letters are significantly different (Tukey-Kramer HSD Test).

Average pre – exposure weights of blades were used as their initial weight; subsequent weight measurements were divided by those pre-exposure values to calculate % of original wet weight. Wet weight for all treatments except for the no low tide treatment decreased over the course of the 2 hour low tide exposure (Figure 5). The magnitude of wet weight change was different for the different treatments. For example, the cool low tide treatment samples lost on average only 12% of initial weight while the sun+wind samples lost on average over 80% of initial weight (Figure 5). After 24 hours of immersion for recovery, all treatments increased their wet weight from the post –

exposure weights. Samples in the cool low tide treatment were able to recover to their initial weight, whereas samples in the sun, sun+spray, and sun+wind treatments were not able to recover to more than 85% of their initial weights on average (Figure 5). The wet weights of blades from the sun, sun+spray, and sun+wind treatments continued to decrease after the second and third day of recovery (Figure 5, ANOVA, $F = 51.79$, $DF = 4$, $p<0.0001$). At the end of the recovery period, the blades from the outside sun treatments had lost structure and were covered with a thick layer of mucous..

Discussion

Results show that with repeated stressful low tide exposure, the potential of *Saccharina sessilis* to conduct photosynthesis decreases. In some intertidal seaweeds, high light causes a reduction in the efficiency of Photosystem II (Gévaert et al 2002 and Lamote et al 2012) and desiccation, which can be caused by a combination of abiotic factors, also drastically reduces the potential of intertidal seaweeds to conduct photosynthesis (Lamote et al 2012). With intense repeated low tide exposure, the ability of *S. sessilis* to conduct photosynthesis is reduced probably due to damage to the photosynthetic apparatus. When individuals accumulate damage and have low MQY readings, it is usually due to the disruption in D1 proteins, the linkage between chlorophyll a and the electron receptor (Cosgrove and Borowitzka 2011). Repeated low tide stress not only reduces the potential for photosynthesis, but it also causes biomass loss. Our results show that blades become shorter as the damaged parts get sloughed off. Reductions in biomass may have many implications for the individual and other organisms. For example, since the blades get shorter, the ability of the canopy blade to shade the understory blades and the meristem is reduced; therefore, understory blades are

now exposed and can accumulate damage as the tide series progress. Over the long term, biomass loss may decrease the kelp's survivorship or reproductive output (Milligan 1998). Furthermore, shorter blades resulting from damage will provide less canopy cover for organisms that rely on the canopy cover for protection (Burnaford 2001, Burnaford 2004, and Dayton 1975). Low tide stress not only affects the individual, but it also affects other organisms in the community that rely on *Saccharina* for food and the amelioration of environmental stress.

There was no convincing evidence of a relationship between the change in MQY readings over any single low tide and any single environmental factor. Our lab experiment shows that high (but still ambient) light intensity causes temporary MQY decline in *S. sessilis* but recovery is possible if the individual is hydrated (as in the sun+spray treatments in this experiment). This indicates that in the field, it was combinations of stressful abiotic factors such as high light, low humidity, and wind that caused MQY to decline.

Laboratory experiments also suggest that desiccation lowers the photosynthetic potential in *S. sessilis*. This is supported by our field data, as desiccation is the result of many different combinations of environmental factors. With just a single 2-hour low tide exposure, ambient environmental conditions can permanently damage tissue to the point where it is not able to recover. This indicates that between one stressful low tide exposure and the next, intertidal seaweeds may not be able to fully recover as seen in other studies (i.e. Gévaert et al 2002), resulting in an accumulation of damage over time. Because there are known associations between *Saccharina sessilis* and other intertidal organisms,

understanding how climate change affects the health of the canopy kelp will aid in better understanding how the community composition may change over the long term.

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