

BEING BULLATE IN THE INTERTIDAL:  
A NEW WRINKLE ON THE FUNCTIONAL MORPHOLOGY OF *SACCHARINA SESSILIS*

By

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in  
Biology

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4/27/2016

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**ABSTRACT**

*Saccharina sessilis*, a dominant kelp of rocky intertidal shores of the northeastern Pacific, has a variable morphology with two common forms: bullate and strap-like. Bullate thalli have upright, rugose lamina that grow in a compact, cabbage-like habit. Strap-like thalli have smooth and long lamina that lie prostrate on the rocks when emersed at low tide. Previously, researchers have observed that morphological variation in kelps is associated with variation in water flow or wave energy, yielding characteristic ‘wave-exposed’ and ‘wave-sheltered’ forms. They have also argued that the bullate morphology is an adaptive response to growing in wave protected shores where boundary layer formation can limit nutrient and carbon dioxide supply for strap-like individuals, while bullae disrupt boundary layers. For *Saccharina sessilis* we observed the two morphologies varied systematically with tidal height on wave-exposed shorelines, with bullate thalli dominating high on the shore. Furthermore, the bullate thalli appeared to retain small pools of water in the bullae on their blades and in the rosette at the base of the thallus during low tide. In addition, we observed some thalli had an intermediate morphology with lamina of both types, suggesting the trait might be plastic and driven by changing environmental conditions; we called these thalli semi-bullate. We hypothesized that the bullate morphology in intertidal kelps functions to ameliorate emersion stresses during low tide, and allows *Saccharina* to persist in higher intertidal habitats than would be possible for strap-like thalli. We monitored the density of the three morphotypes across a tidal height gradient over time at a site in Northern California. In the high zone bullate *Saccharina* were the dominate morphotype in spring and summer when extreme low tides occur more frequently in the morning and sun exposure during emersion is greatest, while straplike morphotypes were rare or absent. In the low zone straplike thalli were present during all surveys. During the fall when day time emersion times are shorter and extreme low tides are more frequent in the afternoon and evening, the straplike morphotype dominated and no bullate or semibullate morphotypes were observed. We characterized the rugosity of the lamina and found that the more rugose lamina, characteristic of the bullate morphology, retained more water on the thallus surface when emersed. Furthermore, the more upright habit of the bullate thalli resulted in reduced light interception, conferring a potential physiological benefit during sunny low tides. Using a pulse-amplitude-modulation (PAM) fluorometer to measure two metrics of photosynthetic performance: maximum quantum yield (MQY or  $F_v/F_m$ ) and electron transport rate (ETR) to make in situ field measurements we found that high zone, bullate thalli also had greater MQY and greater ETR than the low zone, strap-like thalli during a sunny morning low tide, despite longer emersion times. We also observed that the bullate morphotype desiccated at a slower rate than the straplike morphotype. These differences were less pronounced on days that were not sunny and dry during the morning low tide. These results challenge a prior paradigm about the function of variable morphology in

intertidal kelps, and contribute to our understanding of the physiological ecology *Saccharina sessilis*, and its scope for responding to climate change.

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## Introduction

Kelps are a major component of temperate and arctic coastal ecosystems. Subtidal kelp forests and intertidal beds have exceptionally high rates of primary production, alter water flow regimes and chemistry, create biogenic habitat, and provide sustenance for a diversity of marine organisms (Dayton 1972, Carr 1989). These productive and ecologically important marine macrophytes thrive in highly dynamic physical environments where seawater, temperature, light availability, nutrient supply, wave climate and currents vary substantially over time and space. All of these factors are well known to influence the productivity, growth, survivorship and fitness of seaweeds in general and kelps in particular. In addition to morphological and physiological adaptations many seaweeds exhibit a surprising degree of morphological plasticity. This plasticity was so great that taxonomists in the past were sometimes led to assign the distinctive morphotypes to different taxa. Here we examine the functional role of strong morphological variation in an intertidal kelp, and provide new insights, based on a combination of natural history observations, and morphological, physiological and functional measurements from the field and laboratory.

Morphological variation in intertidal and subtidal kelps is extensively documented in the scientific literature as being associated with variation in water flow or wave energy to yield characteristic 'wave-exposed' and 'wave-sheltered' forms (Widdowson 1965, Armstrong 1989, Hurd 2000, Blanchette et al. 2002, Hurd et al. 2007, Koehl et al. 2008, Miller et al. 2011). These two common kelp morphotypes have been associated with enhanced physiological or mechanical performance in their respective environments (Denny and Roberson 2002, Hurd 2000, Stewart and Carpenter 2003, Koehl et al. 2008).

Dynamic flow regimes create hydrodynamic stress and interact with thallus morphology to affect survivorship and tattering, as well as light, nutrient and carbon acquisition (Blanchette 1997, Armstrong 1998, Koehl 2008, Hurd 2011). For example, the blades of the bull kelp *Nereocystis luetkeana* are wide and undulated in slow flow environments while flat, strap-like blades are found in fast flow environments. The hydrodynamic stress of flow regimes is allayed by macroalgal size and shape. Koehl et al. (2008) found that wide, undulated blades of *Nereocystis luetkeana* remain spread out and flutter in moving water. This is thought to increase light interception but also increases drag on the thallus and the risk of breakage. Straplike blades form into stream-lined bundles under conditions of high water flow, reducing drag and light interception. In contrast, the intertidal alga *Fucus gardneri* gets tattered in wave-exposed habitats, resulting in a reduction in thallus surface area and thalli of smaller stature overall than in wave protected habitats (Blanchette 1997).

Nutrient uptake is influenced by flow regimes (Hurd 2000, Koehl 2008) and can limit seaweed growth (Nielsen 2001, 2003). In low flow environments ( $< 2\text{-}6 \text{ cm sec}^{-1}$ ) the transfer of dissolved nutrients to blades is decreased by the formation of diffusion boundary layers (DBLs) at the blade surface (Hurd 2000, 2011). As a result some researchers have argued that a rugose or undulated morphology, typical of wave protected environments, is a functional form that serves to disrupt DBLs. For example, *Macrocystis pyrifera* exhibits this characteristic variation in blade morphology between wave exposed and wave sheltered environments. Until recently the ruffled blades of wave protected thalli were thought to create small scale surface turbulence that would disturb the boundary layer at the blade surface in low flow conditions facilitating the delivery of

nutrients or carbon dioxide (Armstrong 1989, Roberson 2004, Hurd 2011). Interestingly, DBL's measured at the surface of the thalli surface in laboratory simulated laminar and high flow regimes showed that small surface corrugations, a feature characteristic of the wave exposed morphology of *M. pyrifera*, actually traps fluid. Comparison to measurement of the DBL's at the surface of edge undulations of wave sheltered morphotypes did not support the hypothesis that they increased surface turbulence in comparison to the wave exposed morphotype. Based on these results Hurd (2011) proposed the hypothesis that surface corrugations of wave exposed blades that trap fluid also trap extracellular enzymes such as carbonic anhydrase or other extracellular, bioactive molecules such as pheromones, enhancing their functionality under high flow conditions. While this hypothesis remains to be tested, it opens the door to more nuanced and varied consideration of the relationship between plasticity in an alga's form and its ecological functioning (Koehl 2008).

*Saccharina sessilis* (hereafter *Saccharina*; formerly *Hedophyllum sessile*) is a dominant kelp and ecosystem engineer of the low to mid intertidal zone from Alaska to central California. It is a stipe-less thallus composed of multiple large fronds that can create a canopy in the intertidal zone. *Saccharina* typically grows in dense aggregations and forms a conspicuous bed or zone just below the mussel bed. The canopy creates a microhabitat for a characteristic suite of invertebrates and seaweeds that would not occur in this zone otherwise (Dayton 1972, Dayton 1975, Duggins and Dethier 1985, Burnaford 2004, Burnaford, et al. 2014). *Saccharina* exhibits variable blade morphology. Two distinct morphotypes are bullate and straplike (Figures 1, 9) and have long been associated with differences in wave exposure (Widdowson 1965, Armstrong 1989).

Bullate thalli have a rugose surface with small, cup-like indentions called bullae and are commonly found in wave protected habitats. This morphotype grows in a compact, upright, rosette or cabbage-like habit typically on wave-protected shores. In contrast, the smooth, strap-like morphotype commonly observed at wave-exposed sites, is rarely observed at wave-protected sites, except on the vertical walls of channels. Strap-like thalli lamina are smooth and long and lay flat against the rock often in overlapping layers. In addition, some thalli have an intermediate morphology with lamina of both forms in one thallus, or vary within a single lamina (*pers. obs.*), suggesting the phenotype may be plastic; we refer to thalli with this intermediate morphology, semi-bullate (Figure 2).

Early studies by Widdowson (1965) suggested that wave action or exposure to sun might be responsible for the different morphotypes. However, subsequent work on the relationship between morphology and drag has shown that thallus size was more important to reducing drag forces than differences in morphology, because regardless of morphotype *Saccharina* streamlines quickly in flow (Armstrong 1989, Milligan and DeWreede 2004). Morphological acclimation and adaptation to flowing waters is important for sessile photosynthetic organisms, however water flow is only one of several potentially influential environmental factors that might drive morphological variation. Additionally, both morphotypes have been commonly observed at wave exposed, outer coast field sites in Northern California where we have worked for many years, providing additional evidence that wave exposure alone cannot be the primary driver of morphological variation in *Saccharina*. To our knowledge, no formal evaluation of the ‘sun exposure’ hypothesis has ever been pursued.

Our field observations of *Saccharina* over time, and the apparent plasticity of its morphology, suggest that the bullate morphology is instead a response to emersion stress. We hypothesize that the bullae mitigate emersion stress, allowing *Saccharina* to persist higher on the shore and in more wave protected environments than it would otherwise, probably because they retain water. Specifically, we hypothesize:

- (1) Bullate thalli retain more water and reduce planar exposure of thalli to direct sunlight relative to strap-like thalli.
- (2) Strap-like *Saccharina* will be more common where emersion stress is reduced (low on the shore, on vertical, shady surfaces, and where there is plentiful sea spray and splash), while bullate thalli will be more common where emersion stress is higher (high on the shore, on horizontal surfaces, and in wave-protected areas).
- (3) Strap-like thalli will be more common during cooler months or when daylight tidal emersion is least.
- (4) Photosynthetic performance of bullate thalli will be enhanced relative to strap-like thalli, especially under warm, sunny conditions when sea state is calm.

We tested these hypotheses using 1) laboratory measurements to quantify functional attributes of *Saccharina* morphology; 2) structured, quantitative field observations of the distribution and abundance of *Saccharina* morphotypes over time and space; and 3) *in situ* photosynthetic performance of the different morphotypes under a range of *in situ* environmental conditions.

## Methods

We made morphological, and physiological observations of a *Saccharina* population at wave-exposed site along the open coast in northern California. (Kibesillah Hill, 39.60047° N, 123.78927° W) (Figures 3, 4,). The intertidal zone at this site is typical of rocky areas in this region; the rocky benches have a mussel-dominated mid-zone and a macrophyte dominated low zone (Figure 5). The *Saccharina* bed at Kibesillah Hill has an average density of  $74 \pm 45$  (SD) per m<sup>2</sup> of mature thalli. The *Saccharina* bed on this large bench, bounded by steep surge channels, ranges from 2.5 (0.76) to 4.5 (1.37) ft. (m) above MLLW along approximately 140 m of shoreline.

### *Functional morphology (Hs1)*

We characterized two aspects of thallus morphology: the rugosity of the blades and the angle it formed with the substratum as it emerged from the holdfast. Thalli visually classified as bullate should have higher rugosity and blade angles than those classified as strap-like. Blades with reduced angles will intercept more direct sunlight when out of water than those with larger blade angles. More rugose blades should also retain more water.

To measure rugosity we adopted a method commonly used on coral reefs (Risk 1972). We placed a small metal chain along the blade surface following any surface undulations and compared its length to that of the straight line projection along the same dimension (Figure 6). The rugosity index is the chain length divided by projected length (RI = C/P), where larger values are more rugose. Rugosity measurements were made on the length and width of blades from specimens used in physiological field and lab studies.

Differences in rugosity between thalli visually classified as bullate or strap-like were formally assessed using a Student's T-test (JMP) (Figures 7, 8; Table 1).

To quantify a characteristic lamina angle for a thallus we photographed the profiles of thalli, secured to a flat rack by the holdfast and with the distal ends of 2-3 crown blades per thalli flagged with pin markers. Crown blades are located at the center of the rosette. The angles of the crown lamina from the plane of the rack were measured from the photographs using an angle tool in the Image J software package (Figure 9). Crown blades are the innermost lamina at the top of the thallus. We tested for differences in angle between bullate and strap-like thalli with a Student's T-test, as above (Figure 11, Table 1).

We measured water retention of bullate and strap-like *Saccharina* morphotypes in the laboratory. Thalli were secured upright, at the holdfast, to a metal rack, immersed into a bath of artificial sea water and removed (Fig. 9). After waiting 5 seconds to allow surface water to drain off the rack and the thallus (in an upright horizontal position), it was inverted over a container for 2 mins to collect water retained on the thallus. The procedure was repeated three times per thallus and then averaged to account for measurement error. The volume of water retained was measured in a graduated cylinder to the nearest milliliter. We compared water retention between morphotypes using a Student's T-test (JMP) (Figure 10, Table 1).

#### *Spatial and Temporal Distribution of Saccharina Morphotypes (Hs 2 & 3)*

To test our hypotheses that *Saccharina* morphotypes would vary predictably with tidal height and by season, we monitored their distribution and abundance over time at Kibesillah Hill (site described above). Densities of three *Saccharina* morphotypes

(bullate, semi-bullate, and straplike) were measured at the upper and lower levels of the *Saccharina* bed using belt transects (10 m long by 0.1 m wide) haphazardly placed, but without pre-conceived bias, parallel to the shore (n= 5 for each zone). The upper level of the zone was below the lower edge of the mussel bed and low zone transects were above the zone dominated by other low tidal kelps, mostly *Costaria costata* and *Lessoniopsis littoralis*. We repeated the surveys during morning low tides in June, August, and November in 2012 and April, May and July in 2013 (Figure 12). To investigate changes in density between zones and over time, we analyzed *Saccharina* density using a MANOVA with the density of each morphotype as response variables, and level and date as fixed factors (in SAS 9.4 Proc GLM). Data were log transformed prior to analysis to meet model assumptions (Table 2).

#### *Physiological Ecology (Hs 4)*

##### PAM Fluorometry

We used a diving pulse amplitude modulated (PAM) fluorometer (WALZ, Effeltrich, Germany) to investigate the physiological state of *Saccharina* during morning low tides in spring and summer months when the thalli were emersed. We used two measures of physiological state, photosynthetic potential as measured by maximum quantum yield of fluorescence (MQY) and the relative electron transport rate through PSII (rETR). Light energy absorbed by chlorophyll molecules has three potential fates: it can be used in photochemistry (photosynthesis); it can be dissipated as heat, or re-emitted as light in the form of chlorophyll fluorescence. These three pathways occur in competition so that an increase in one results in a decrease in the sum of the other two. By measuring chlorophyll fluorescence the physiological changes occurring in the

photochemistry of PSII can be understood (Genty et al. 1989, Maxwell et al. 2000). The effective quantum yield (EQY) is a fluorometric measure of the proportion of light absorbed by chlorophyll that is used by PSII in photochemistry ( $EQY = (F'_m - F_t) / F'_m$ , where  $F'_m$  is maximum fluorescence and  $F_t$  is steady state fluorescence in a light adapted state). The relative electron transport rate through PSII (rETR) is calculated as  $EQY \times PAR \times 0.5$ , where PAR is the photosynthetically active radiation incident on the thallus and 0.5 is a factor that accounts for the assumed partitioning of energy between PSII and PSI. Actual ETR is typically reduced further as a function of thallus absorbance, which can be used as scaling factor in the equation if there are substantial differences among populations under study (Nielsen et al. 2006). Photosynthetic potential, measured as the maximum quantum yield (MQY) in a dark-adaptation state ( $(F_m - F_o) / F_m$  where  $F_m$  is the maximum fluorescence and  $F_o$  the minimum fluorescence in the dark adapted state) is commonly used as a general screening indicator of physiological stress (Maxwell et al. 2000).

We measured MQY and EQY on emersed bullate and strap-like thalli during seven morning low tides in June and July of 2011 and 2012. EQY was measured using a WALZ universal sample holder to maintain a constant measurement geometry and MQY was measured after dark adaptation using WALZ leaf clips. We arrived just after daybreak and immediately placed light clips on *in situ* bullate and strap-like thalli in the upper and lower levels, respectively, of the *Saccharina* bed. Upper level bullate thalli had been out of water about 30-45 minutes longer than lower level strap-like thalli. Thalli were dark adapted for at least ten minutes before MQY was measured. We made repeated measurements on the same thalli up to three more times, conditions permitting, to capture

changes in MQY over the low tide period while thalli were out of water and before they had been splashed by the returing tide (Figure 13).

EQY measurements were made using the universal sample holder after each round of MQY measurements and included simultaneous measurement of PAR incident on the thallus immediately adjacent to the measurement of EQY. The irradiance sensor of the PAM fluorometer was calibrated against a Li-190 quantum sensor (LiCor, Lincoln, NE, USA) (Figure 17).

Because photosynthetic state is known to vary with hydration state (Nielsen et al. 2006, Burnaford et al. 2015) we also measured the relative water content (RWC) of thalli during a subset of our field physiological studies (June 20, July 1 & 4, 2011). Relative water content is defined as the ratio of thallus water content at a given time relative to its maximum water content. Following PAM measurements hole punches of thalli were collected using a cork borer of 5/8ths inch diameter and immediately stored in pre-weighed and numbered micro-centrifuge tubes and then transported to refrigeration in a cooler with ice. Field weights were measured and then punches were re-hydrated using artificial sea water (*Instant Ocean*) at 4 °C for 2 hours in a refrigerator and then re-weighed after damp-drying to remove surface water. Then the hole punches were dried to constant weight in a drying oven between 55-60 °C and weighed again. RWC was calculated as  $(\text{field weight} - \text{dry weight}) / (\text{hydrated weight} - \text{dry weight})$  (Figure 14).

Thallus absorptance of 10 high zone bullate thalli and 10 low zone strap-like thalli was measured in the field site in July 2012 (Figure 16). We used the universal sample holder with the PAM quantum sensor placed below the sample to measure the light transmitted to the sensor with ( $\text{PAR}_t$ ) and without ( $\text{PAR}_i$ ) the thallus present.

Absorptance was calculated as  $(PAR_i - PAR_t)/PAR_i$ . Measurements were made on crown blades. We used Student's t-test to determine if absorptance varied with morphotype (JMP) (Table 1).

#### Environmental Conditions

We collected air temperature, wind speed, and relative air humidity at 20 to 30 second intervals over the course of *in situ* studies using a Kestrel 4000NV Pocket Weather Tracker. Solar irradiance was recorded every ten minutes to a data logger from a Licor LI-190 PAR sensor installed at the site (Table 3, A1-A4).

#### Statistical Analysis of MQY, rETR and RWC

To see if the change in MQY over the morning low tide varied as a function of morphotype we used a mixed linear model with time of day and morphotype as fixed factors, and thallus and date as random effects to account for the repeated measurements on the same lamina of each thallus, and the variation on environmental conditions from day to day, respectively). If the bullate morphotype reduces emersion stress relative to the strap-like form, we would expect the decline of MQY over time to be less steep, resulting in a significant interaction term and a more negative slope for strap-like thalli. We used a similar approach to determine if morphotype influenced the relationship between rETR and PAR. rETR increases as a function of PAR, but the rETR should be higher at a given level of PAR for bullate thalli if the bullae function as hypothesized and alleviate emersion stress. Thus we also expected a significant interaction term between morphotype and PAR, with a larger, positive slope for bullate thalli than strap-like thalli. We also analyzed changes in RWC of *Saccharina* thalli over time under field conditions, using the same approach. A mixed linear model was fitted to the data with time of day

and morphotype as fixed factors, and date and thallus are random factors for the same reasons given above. We expected RWC to decline over time for all thalli during low tide, but to decline more rapidly for strap-like thalli. All analyses were done using SAS (proc mixed ver 9.4).

## Results

### *Functional Morphology*

Our overarching hypothesis was that the bullate form of *Saccharina* reduced emersion stress by both retaining more water upon emersion in the cup-like bullae and reducing incidence of light through the angle of its blades. We found substantial and measureable differentiation in form between thalli visually classified as either fully bullate or fully strap-like (Table 1). Mean blade width of bullate thalli was more than twice as wide as for strap-like thalli (t-test;  $t(239) = 9.37$ ,  $p\text{-value} < 0.0001$ ,  $n = 241$ ), but bullate blades were shorter (t-test;  $t(239) = 8.74$ ,  $p\text{-value} < 0.0001$ ) (Figure 7). Not surprisingly, rugosity was also greater for bullate than strap-like forms (Figure 8; t-tests: RI length,  $t(239) = -13.28$ ,  $p < 0.0001$ ; RI width,  $t(239) = -15.23$ ,  $p < 0.0001$ ). In addition, the angle of the bullate blades was substantially larger than those of strap-like thalli (Figure 11; t-test:  $t(16) = 25.46$ ,  $p < 0.0001$ ). Bullate thalli had double the mass-specific water retention capacity of strap-like thalli (Figure 10; t-test,  $t(16) = 4.77$ ,  $p = 0.0002$ ).

### *Spatial and Temporal Distribution of Saccharina Morphotypes*

The density of the three *Saccharina* morphotypes (bullate, semi-bullate and strap-like) varied between upper and lower levels and seasonally (Figure 12; Table 2). Strap-like thalli were virtually absent from the upper level of the *Saccharina* bed except in

November 2012. In contrast, bullate and semi-bullate thalli dominated the upper level in spring and summer months of 2012 and 2013, but disappeared in November 2012.

Morphological diversity at the lower edge of the bed in the spring and summer months was higher with all three morphotypes present. However, just as in the high zone, in November 2012 only the strap-like thalli were observed in the low zone. The abundance of bullate thalli was highest in May 2013 for both the upper ( $84 \text{ m}^{-2} \pm 7$  [SE]) and lower ( $35 \text{ m}^{-2} \pm 9$  [SE]) levels of the *Saccharina* zone. However, total density of *Saccharina* was greatest and did not differ between levels in November 2012 (upper:  $109 \text{ m}^{-2} \pm 13$  [SE] and lower  $107 \text{ m}^{-2} \pm 5$  [SE]).

### *Physiological Ecology*

Environmental conditions in the field during the seven days when fluorescence studies were conducted encompassed a typical range of conditions for spring and summer low tides (Table 3; Appendices A1-A4). PAR and air temperatures increased substantially over the morning as expected. Both were reduced and varied less on foggy and cloudy days. Coastal breeze typically increased over the morning, but wind speed did not substantially from day to day. Relative humidity remained very high, except on warm, sunny days. Of the days when MQY, rETR and RWC were all measured, 20 June 2011 was the hottest, least humid, sunniest and windiest day.

MQY and RWC both declined over the morning low tides (Figures.13, 14, 15; Tables 4, 5). However, the rates of decline in MQY and of RWC were twice as fast for strap-like thalli compared to bullate (Tables 4, 5). Inter thallus variation also increased substantially over time for both these responses. The MQY and RWC for strap-like thalli was greater than for bullate thalli in the early morning, most likely because they are

primarily found low on the shore during summer and thus emerge from the water later than the bullate thalli (*cf.* model estimates for the intercept (= strap-like thalli) and bullate thalli, Table 4). On the hottest day, when relative humidity was low, sea state was calm and it was windiest, MQY appeared to be strongly linked to RWC, with strap-like thalli losing the most water despite being out of the water for the less time, but this relationship was not as clear on days with more benign conditions (data not shown). Across all days and measurements not included in analysis with specimens analyzed for RWC, nine bullate thalli measurements of MQY out of 177 total measurements had MQY's below 0.5 by the end of low tide, compared with 24 of 169 strap-like thalli out (Figure 13).

Morphology also influenced the relationship between rETR and PAR (Figure 17). There was a more strongly linked relationship between the two variables for bullate thalli ( $R^2=0.87$ ) than for strap-like thalli ( $R^2=0.58$ ). There was no difference in rETR between the two morphotypes at the lowest light levels, but differences emerged as PAR levels increased to full sun (Table 6). In addition, except for a few strap-like thalli that performed well at high light, most strap-like thalli exhibited what appeared to be photo-inhibited rates of rETR at just moderately high levels of PAR, while bullate thalli did not show any evidence of photo-inhibition (note curvature in a portion of the data points for the strap-like thalli in.

## Discussion

In this study we are curious about morphological variation in seaweeds and the function of this heterogeneity beyond the commonly associated dynamic water regimes and wave activity. Here we describe our observations of a population of *Saccharina* morphotypes at a high wave action rocky shore where bullate, semibullate, and straplike

morphotype densities fluctuate over time as seasonal and zonal stresses vary. As daytime emersion times change the dominant morphotype of *Saccharina* shifts leading us to conclude that morphological variation and plasticity allow *Saccharina* to mitigate changing environmental stressors. Terrestrial plants are known to exhibit phenotypic plasticity in response to the environmental stress associated with seasonal change or in lab tested exposure to stress related hormones such as abscisic acid (Young et al. 1986). Sedentary photosynthetic organisms in marine and terrestrial environments rely on developmental plasticity to transform their morphology in response to dynamic and sometimes hostile environments; by employing their form they positively influence their function; they increase their ability to adapt and acclimate to a changing global atmosphere. What else could explain the dramatic changes in morphology over time for this population of *Saccharina*? Could seasonal shifts in nutrient availability effect the morphology of this population? In response to resource limitations terrestrial plants reduce biomass gain to modify the allocation of resources; this allows them to conserve in response to a changing environment. Root hair architecture changes in response to environmental signals, such as fluctuating nutrient availability (Lopez-Bucio et al. 2003). We did not measure nutrients at this site specifically, however it is possible that nutrients, light, and carbon themselves are not limited in their availability but the physiological potential for these for photosynthetic organisms to utilize these resources is compromised under stressful conditions.

Photosynthesis can be informative about physiological state with reductions in net photosynthetic rates as an indicator of stress, suggesting resources are being devoted to maintenance and repair, rather than growth and reproduction. For photosynthetic

organisms light, water, nutrients temperature and wind can directly influence net photosynthetic rate (NPR) All of these factors vary and emersion times can modulate the effects of these environmental factors (Dethier and Williams 2009). For example, it is often hypothesized that NPR should decline across the intertidal zone with tidal height, because organisms existing at the upper limit experience longer emersion times and greater environmental stress than those in the lower zones (Williams and Dethier 2005).

Williams and Dethier (2005) measured NPR in and out of water using gas exchange methods. Nielsen, et. al. (2006) studied the physiological and ecological performance of the intertidal kelp *Postelsia palmaeformis* using PAM fluorometry as part of their investigation into the factors that limit *Postelsia's* distribution at different tidal heights and different wave exposures. Electron transport rate (ETR) was found to increase over time for all tidal heights. This can initially be explained by photosynthetic processes influenced by diel cycles like the activation of Rubisco by light. ETR and MQY of fluorescence during sunny, morning low tides were lowest at the upper edges of *Postelsia's* distribution, where reproductive output, growth and survivorship were also lowest. ETR and MQY both increased when sea spray was used to relieve desiccation stress. Emersion stress such as desiccation influences the physiological ecology of the intertidal kelp *Postelsia palmaeformis* through reduced growth, survivorship, and reproductive output (Nielsen et al. 2006).

Our findings reinforce that there is not a predictable relationship between physiological response and increasing tidal height for kelps in the rocky intertidal (Williams and Dethier 2005, Dethier and Williams 2009). In this study morphology is a new factor influencing physiological performance during emersion. We found that

during summer morning low tides MQY declines over time for both straplike and bullate morphotypes however straplike *Saccharina* photosynthetic performance declines at a faster rate than the bullate morphotype. Bullate *Saccharina* have lower MQY's at the beginning of low tide physiological studies, we attribute this observation to high zone bullate thalli being emersed before low zone straplike morphotypes. Overall rETR increased with increasing light for both morphologies and a significant interaction was found between PAR and morphology; bullate thalli have a higher rETR at high light than low zone, strap-like thalli. We found absorptance did not vary between high zone bullate and low zone straplike *Saccharina* (Figure 16). Nielsen et. al. observed thallus absorptance declined with increasing tidal height in the intertidal kelp *Postelsia palmaeformis*. In response to increased exposure to emersion stress over tidal heights photosynthetic organisms can down regulate their capacity to absorb light to protect their photosynthetic machinery under potentially damaging conditions. Our observations suggest morphology is mediating the effects of increased emersion stress and the photosynthetic machinery does not have to compensate for the potentially damaging effects of solar radiation during emersion.

Laboratory experiments that mimic intertidal emersion report reduced net photosynthetic rate with increased temperature and desiccation (Davison and Pearson 1996, Skeene 2004). Williams & Dethier (2005) studied net photosynthetic rates of the common intertidal brown seaweed *Fucus gardneri* collected from two tidal heights. NPR was 2 orders of magnitude greater when the alga was in water compared to when it was emersed. Their results reinforce findings that desiccation has negative effects on photosynthesis. Interestingly their work did not support the paradigm that stress varies

predictably with tidal elevation. Their studies found that tidal height did not contribute significantly to the variation of NPR and that tidal heights alone may not always be an indicator of emersion stress. Desiccation has negative effects on photosynthesis and during summer net photosynthetic rates for *Fucus gardneri* reduce to 2/3 of their maximum rate in air. Here we asked if the bullate and straplike morphologies differ in their ability to withstand desiccation stress. High zone bullate thalli will remain hydrated longer than low zone strap-like thalli despite longer emersion times. We found the rate of desiccation for straplike *Saccharina* is twice the rate of the bullate morphotype during emersion (Table 5); we look to thallus morphology to further explain our observations.

There can be physiological costs associated with coping with stressful environments; thermotolerance increases with desiccation for *Endocladia muricata* but photosynthesis declines to a stop (Hunt 2008). How can an increase in thermo stress from direct sun exposure be limited and thallus hydration be maintained so that photosynthesis can continue when light resources are plentiful? Morphology can mitigate physiological costs in photosynthetic performance by ameliorating emersion stress and thereby strengthening fitness for *Saccharina*. Thallus morphology affects the ability of *Saccharina* to retain and utilize resources, such as water and solar radiation, during day time emersion. The photosynthetic potential of straplike morphotypes declines at a more rapid rate than for bullate thalli during day time emersion. Upright rugous blades in the rosette shape of the bullate morphotype enhance water retention and provide self-shading by reducing light interception during day time low tides. Straplike thalli blades lie flat and receive direct sun exposure or are completely shielded in the understory of blades that fall flat on top of each other.

Fox et al. (2007) employed molecular assays to examine vertical distributions of microscopic and macroscopic life-stages of *Saccharina* at 2 locations in the Pacific northwest. Their studies identified *Saccharina* as the kelp with a high tolerance to UV-B when compared to 6 kelp species. Their observations direct attention to the mechanisms that *Saccharina* employs to mitigate environmental stressors in the rocky intertidal. The population exploits its morphological plasticity to persist. Until recently morphological variation in kelps was thought to facilitate gas and nutrient exchange in fast and slow flow environments. We examine the role of morphology in ameliorating stress and facilitating resource acquisition and productivity during day time emersion. Osmotic stress is known to limit terrestrial plant productivity by altering leaf morphology and photosynthetic processes. In aquatic and semi-aquatic plants changes in water level influence the production of different leaf types (heterophylly) (Lin et al. 1999, Lin 2002). Submerged, floating, and aerial leaf forms are described and vary in size, shape, number of stomata and cuticle development. These morphologies are phenotypically plastic and change in response to seasonal fluctuations in water levels. Temperature, photoperiod, light intensity, osmotic stress, and carbon dioxide levels are all environmental factors that can promote the formation of one of the three different leaf morphologies. Leaf phenotypic plasticity is hypothesized to be an adaptive mechanism that promotes survival in changing environmental conditions.

Here we examine a new wave in understanding variation in kelp morphology and its role out of the water. Our observations challenge our view of water flow as the dominant determinant of kelp morphology and expand our view of the function of morphological variation in the ecophysiology of kelp. Characterization of the phenotype,

ecology and physiology of *Saccharina* morphotypes at a wave exposed rocky bench further our understanding of how this ecosystem engineer uses its morphology to interact with its environment during seasonal shifts in emersion patterns. *Saccharina* found at a wave exposed, fast flow environment exhibit morphological plasticity in response to changing environmental conditions overtime. The results of this investigation contribute to our understanding of the factors that influence the physiological ecology of this ecologically important intertidal kelp. Through our exploration of morphological plasticity as a response to environmental conditions we can better understand the potential impacts of global environmental changes that are driven by global natural cycles (ENSO) or anthropogenic change on this foundational species. Burnaford et al. (2014) have found emersion time, and subsequently exposure to stressful low tide conditions, to be a dominating influence of the canopy cover of a population of *Saccharina* on the San Juan Islands. They observed a connection between the canopy cover of *Saccharina* and the celestial driven 18.6 year cycle of emersion time. In the years with the least emersion time *Saccharina* cover was greatest, changed from >70% mean cover to a low of 21.5% mean cover. Emersion times varied from 344 hours of low tide during 1998 to 818 hours of low tide during 2007. As the population percent cover is changing is the population morphology being influenced over time? Our observations indicate yes.

Our results suggest a new paradigm in understanding variation in kelp morphology; not only does wave exposure influence blade type but the degree of emersion stress can drive morphological variation and influence the distribution and physiological performance of *Saccharina* in the intertidal zone. Intertidal zone kelps are vulnerable to projected changes in oceanographic and climatic conditions (due to a

combination of anthropogenic forces and natural cycles); how *Saccharina* morphologies adapt and acclimate over time in response to these changes will influence its role and presence in the rocky intertidal. Our ability to describe and characterize the distribution and functionality of morphological features in concert with physiological performance in photosynthetic organisms contributes to our ability to model and predict how these populations will change in our dynamic global environment.

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## Tables and Figures

### Tables

Table 1. Functional morphology measurements of bullate and strap-like *Saccharina* morphotypes.

All traits except water retention were made on individual blades (n = 76 for strap-like, n = 165 for bullate). Water retention was measured on 10 strap-like and 9 bullate thalli.

<b>Trait</b>	<b>Strap-like</b>	<b>SE</b>	<b>Bullate</b>	<b>SE</b>
Length (cm)	26.9	1.4	16	0.55
Width (cm)	23.2	1.0	9.0	0.51
Angle (degrees)	-3.8	0.5	56.6	2.7
Rugosity, length	1.0	0.01	1.2	0.01
Rugosity, width	1.1	0.01	1.5	0.02
Thallus water retention (mL/g)	0.24	0.03	0.48	0.05
Thallus absorptance	0.85	0.01	0.84	0.00

Table 2. MANOVA of three *Saccharina* morphotype.  
Morphotype densities are from belt transect surveys at upper and lower intertidal levels of a *Saccharina* bed over time.

<b>MANOVA</b>				
<b>Effect</b>	<b>DF<sub>num</sub></b>	<b>DF<sub>den</sub></b>	<b>F-value</b>	<b>p-value</b>
Level	3	38	31.36	<0.0001
<b>Time</b>	<b>12</b>	<b>100.83</b>	<b>43.66</b>	<b>&lt;0.0001</b>
<b>Level x Time</b>	<b>12</b>	<b>100.83</b>	<b>3.09</b>	<b>&lt;0.0001</b>

<b>ANOVAs (by morphotype)</b>				
<b><u>Bullate</u></b>				
<b>Effect</b>	<b>DF</b>	<b>Mean Square</b>	<b>F-value</b>	<b>p-value</b>
Level	1	64.90	48.73	<0.0001
Time	4	84.56	63.49	<0.0001
<b>Level x Time</b>	<b>4</b>	<b>4.41</b>	<b>3.31</b>	<b>0.0195</b>
Error	40	1.33		

<b><u>Semi-bullate</u></b>				
<b>Effect</b>	<b>DF</b>	<b>Mean Square</b>	<b>F-value</b>	<b>p-value</b>
Level	1	13.15	13.30	0.0008
Time	4	50.72	51.33	<0.0001
Level x Time	4	2.60	2.63	0.0485
Error	40	0.99		

<b><u>Strap-like</u></b>				
<b>Effect</b>	<b>DF</b>	<b>Mean Square</b>	<b>F-value</b>	<b>p-value</b>
Level	1	57.88	48.79	<0.0001
Time	4	150.85	127.15	<0.0001
Level x Time	4	6.63	5.59	0.0011
Error	40	1.19		

Notes: Data were square root transformed prior to analysis to conform to parametric assumptions. MANOVA F-statistics are reported for Wilks' Lambda.

Table 3. Environmental conditions during in situ fluorescence based measurements. Measurements of maximum quantum yield and relative electron transport rate and relative water content.

<b>Environmental Conditions</b>							
	<b>2011</b>					<b>2012</b>	
	<b>18-Jun</b>	<b>20-Jun</b>	<b>1-Jul</b>	<b>3-Jul</b>	<b>4-Jul</b>	<b>5-Jul</b>	<b>6-Jul</b>
<b><i>PAR (<math>\mu\text{moles m}^{-2} \text{s}^{-1}</math>)</i></b>							
<b>average</b>	1168	1200	517	-	831	869	418
<b>minimum</b>	890	946	55	-	107	115	165
<b>maximum</b>	1469	1388	1135	-	1385	1339	770
<b>SD</b>	168	158	367	-	389	402	181
<b>CV</b>	0.14	0.13	0.71	-	0.47	0.46	0.43
<b><i>Temperature (<math>^{\circ}\text{C}</math>)</i></b>							
<b>average</b>	-	13.7	11.1	11.5	10.1	10.9	11.5
<b>minimum</b>	-	10.3	8.6	9.7	8.4	9.0	11.2
<b>maximum</b>	-	20.7	17.6	18.3	12.9	12.6	12.1
<b>SD</b>	-	2.0	2.2	1.6	1.3	1.1	0.2
<b>CV</b>	-	0.15	0.20	0.14	0.13	0.10	0.02
<b><i>Wind Speed (<math>\text{m s}^{-1}</math>)</i></b>							
<b>average</b>	-	1.0	0.5	0.7	0.8	0.8	0.6
<b>minimum</b>	-	0.0	0.0	0.0	0.0	0.0	0.0
<b>maximum</b>	-	2.4	2.0	2.7	1.7	1.9	1.7
<b>SD</b>	-	0.7	0.6	0.6	0.3	0.5	0.4
<b>CV</b>	-	0.77	1.20	0.91	0.35	0.70	0.73
<b><i>Relative Humidity (%)</i></b>							
<b>average</b>	-	83	98	96	99	97	99
<b>minimum</b>	-	59	79	73	84	88	96
<b>maximum</b>	-	100	100	100	100	100	100
<b>SD</b>	-	9	5	5	2	3	1
<b>CV</b>	-	0.11	0.05	0.05	0.02	0.03	0.01

Table 4. Mixed linear model analysis of maximum quantum yield (MQY) of *Saccharina*. Analysis as a function of time of day and morphotype under field conditions.

Fixed effects				
Time of day	1	179	106.64	<0.0001
Morphotype	1	179	15.66	0.0001
Time x Morphotype	1	179	18.42	<0.0001
Covariance parameters		Estimate		
Thallus		0.001127		
Day		0.001516		
Residuals		0.006197		
Solution for fixed effects		Estimate	SE	DF p-value
Intercept		1.2296	0.06061	6 <0.0001
Time of day		-0.00002	0.000002	179 <0.0001
Bullate		-0.3040	0.07682	179 0.0001
Strap-like		0	-	- -
Time x bullate		0.000011	0.000003	179 <0.0001
Time x strap-like		0	-	- -

*Notes: Measurements were made over seven daytime low tides during June and July in 2011 and 2012. Day is treated as a random factor to account for variation in environmental conditions from day to day. Repeated measurements were made on same spot of each thallus within each day, thus thallus is treated as a random factor.*

Table 5. Mixed linear model analysis of *Saccharina* relative water content under field conditions as a function of time of day and morphotype.

<b>Mixed linear model analysis</b>				
<b>Fixed effects</b>	<b>DF<sub>num</sub></b>	<b>DF<sub>den</sub></b>	<b>F-value</b>	<b>p-value</b>
Time of day	1	63	17.82	<0.0001
Morphotype	1	63	8.27	0.0055
Time of day x Morphotype	1	63	8.20	0.0057
<b>Covariance parameters</b>		<b>Estimate</b>		
Thallus		8.7607		
Day		41.7671		
Residuals		79.9997		
<b>Solution for fixed effects</b>	<b>Estimate</b>	<b>SE</b>	<b>DF</b>	<b>p-value</b>
Intercept	143.20	11.4522	2	0.0063
Time of day	-0.00177	0.000366	63	<0.0001
Bullate	-39.5621	13.7586	63	0.0055
Strap-like	0	-	-	-
Time x bullate	0.001340	0.000468	63	0.0057
Time x strap-like	0	-	-	-

*Notes: Measurements were made over three daytime low tides during July in 2011. Day is treated as a random factor to account for variation in environmental conditions from day to day. Repeated measurements were made on same spot of each thallus within each day, thus thallus is treated as a random factor.*

Table 6. Mixed linear model analysis of *Saccharina* relative electron transport rate (rETR) as a function of incident PAR and morphotype under field conditions.

<b>Mixed linear model analysis</b>				
<b>Fixed effects</b>	<b>DF<sub>num</sub></b>	<b>DF<sub>den</sub></b>	<b>F-value</b>	<b>p-value</b>
PAR	1	192	650.90	<0.0001
Morphotype	1	192	0.04	0.8409
Time of day x Morphotype	1	192	22.20	<0.0001
<b>Covariance parameters</b>		<b>Estimate</b>		
Thallus		0.05601		
Day		1.8724		
Residuals		2.0268		
<b>Solution for fixed effects</b>	<b>Estimate</b>	<b>SE</b>	<b>DF</b>	<b>p-value</b>
Intercept	3.1479	0.6350	4	0.0077
PAR	0.008336	0.000567	192	<0.0001
Bullate	0.04374	0.2176	192	0.8409
Strap-like	0	-	-	-
PAR x bullate	0.003550	0.000753	63	0.0001
PAR x strap-like	0	-	-	-

*Notes: Measurements and analysis as in Table 3. Data were square root transformed prior to analysis.*

## Figures

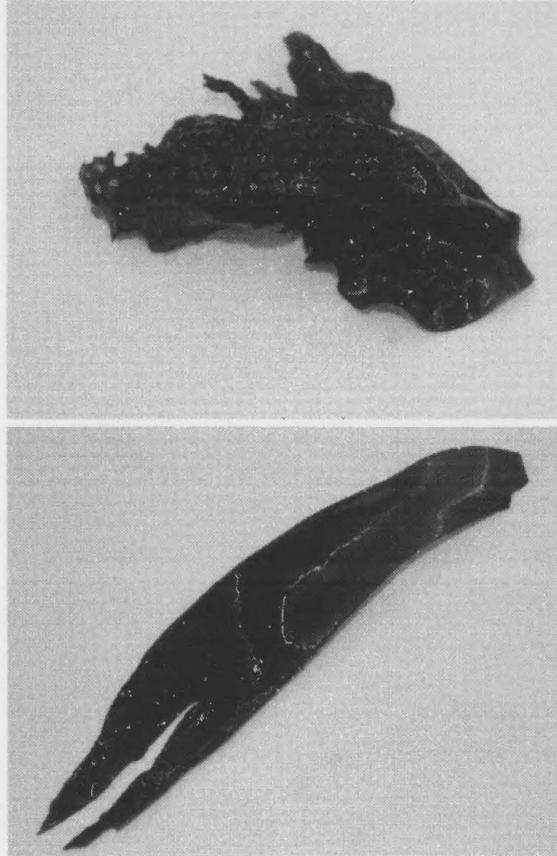


Figure 1. Characteristic lamina of the bullate (left) and strap-like (right) morphotypes of *Saccharina*.

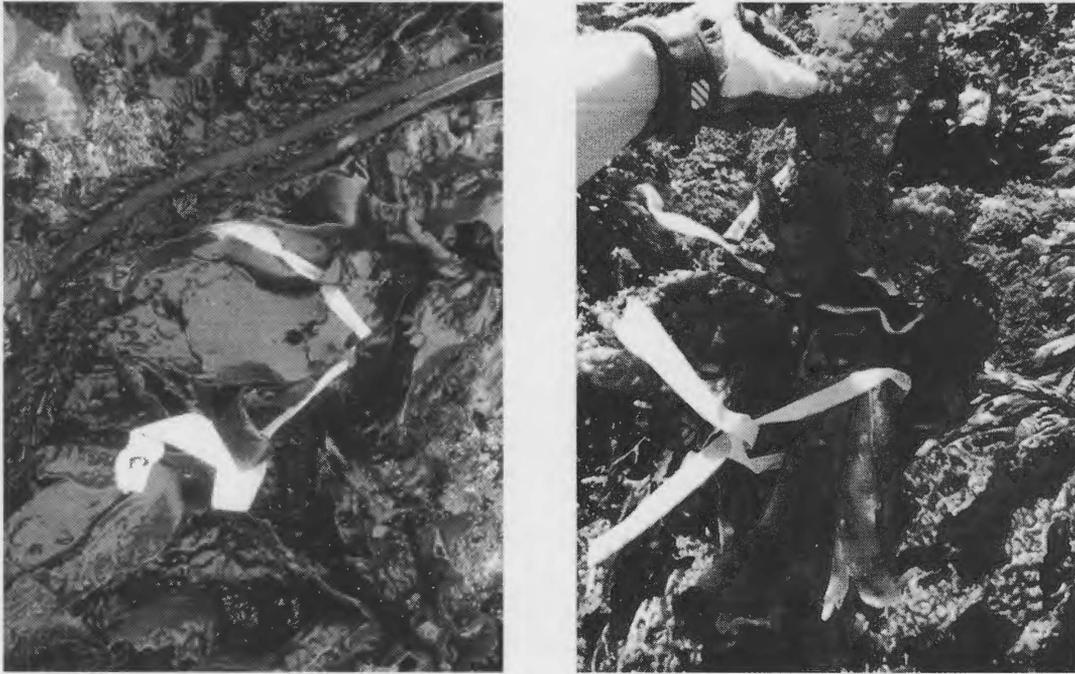


Figure 2. Semibullate morphotypes of *Saccharina*; within blade variation at Kibesillah Hill, California (left), and within thallus variation at Friday Harbor, Washington (right).



Figure 3. Location of Kibesillah Hill field site in northern California.

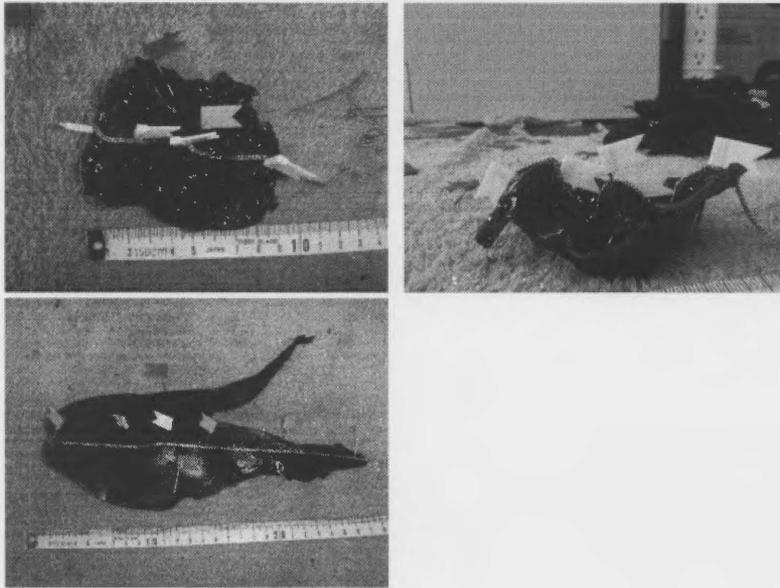


Figure 4. Aerial view of Kibesillah Hill field site in northern California (upper) and student research assistants collecting data in one of the extensive *Saccharina* beds on the wave exposed rocky benches (lower) typical of the region.



Figure 5: Kibesillah Hill northern California.

Figure 6. Rugosity index measurements of *Saccharina* lamina (blades); bullate top view (left), bullate side view (center), and straplike top view (right).



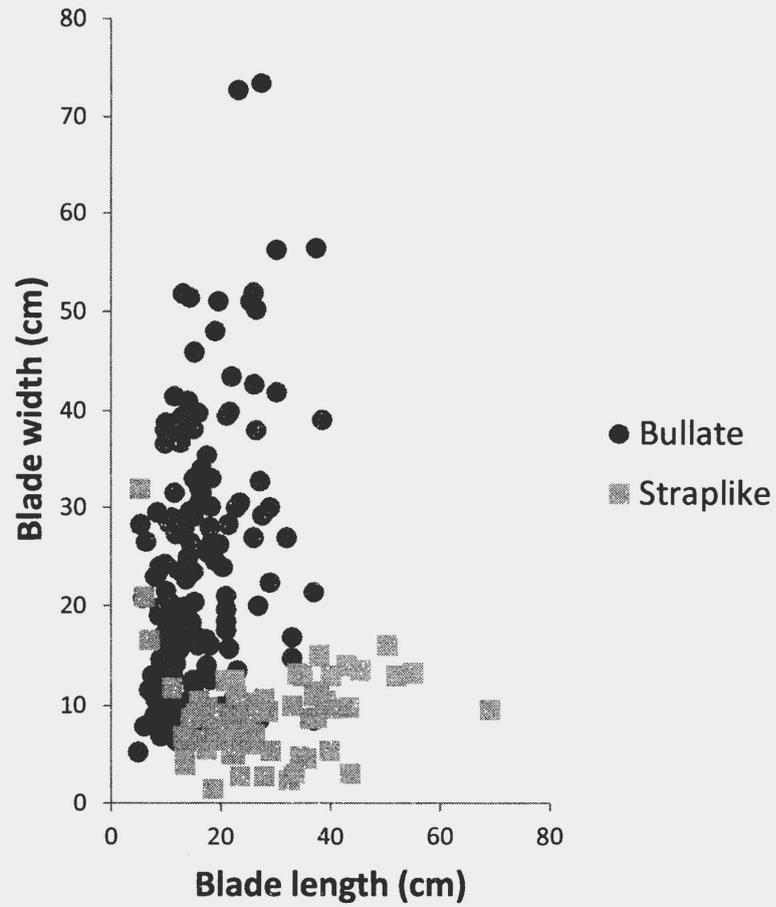


Figure 7. The relationship between *Saccharina* blade width and length for bullate and straplike morphotypes.

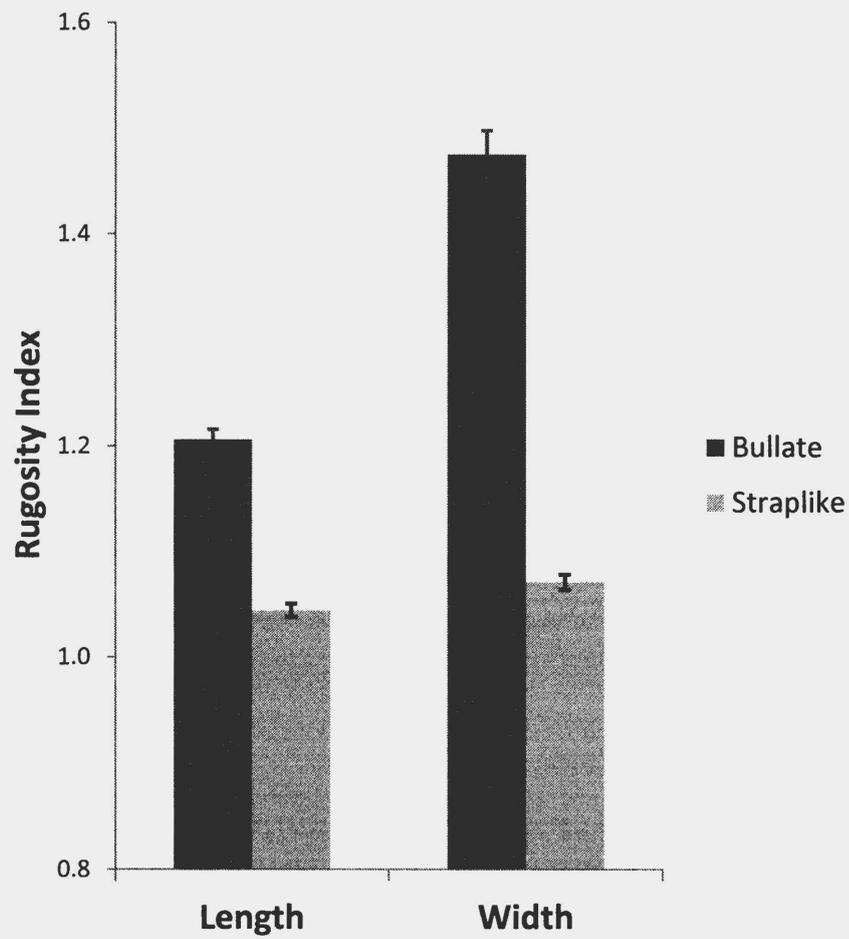


Figure 8. Rugosity of major (length) and minor (width) axes of bullate and straplike *Saccharina* morphotype blades (mean  $\pm$  S.E; n=165 for bullate and n=76 for straplike thalli).

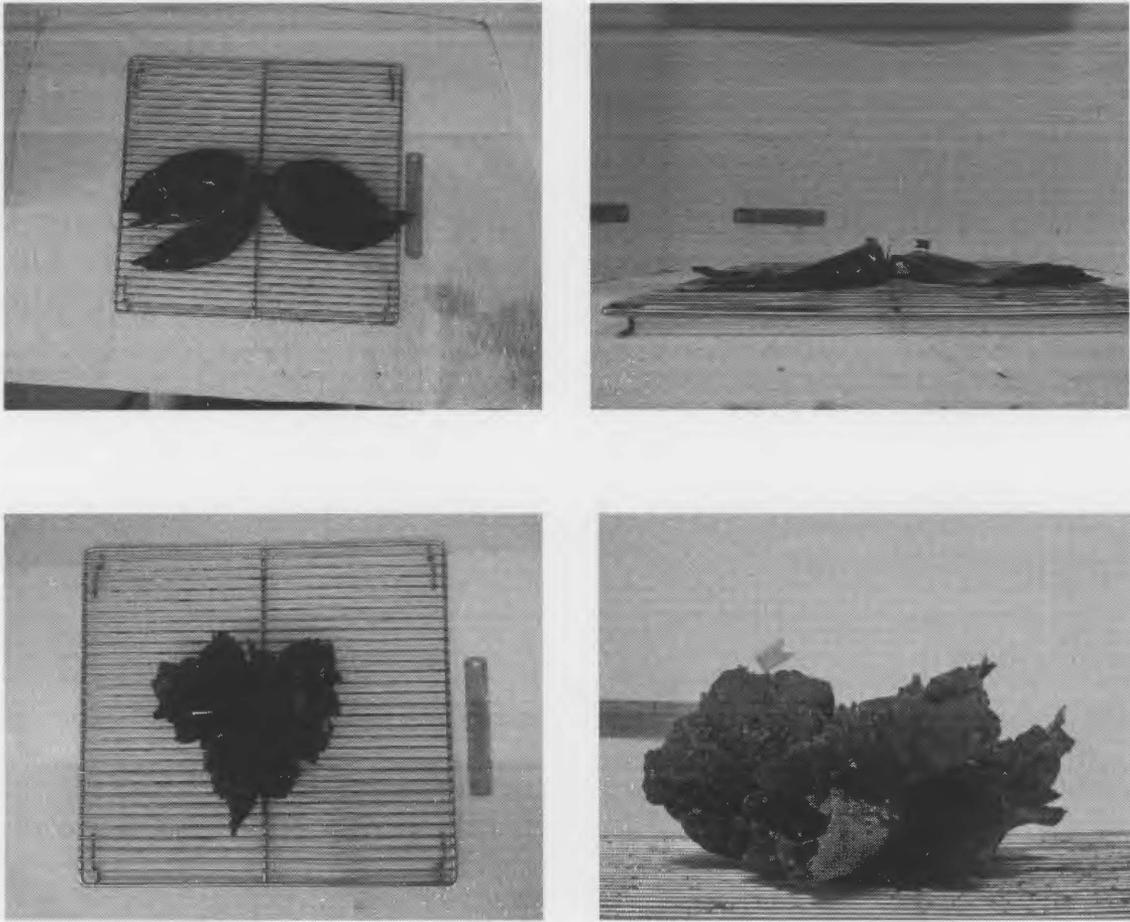


Figure 9. Straplike (top) and bullate thalli (bottom) specimens for measurement of the angle of the blade and water retention.

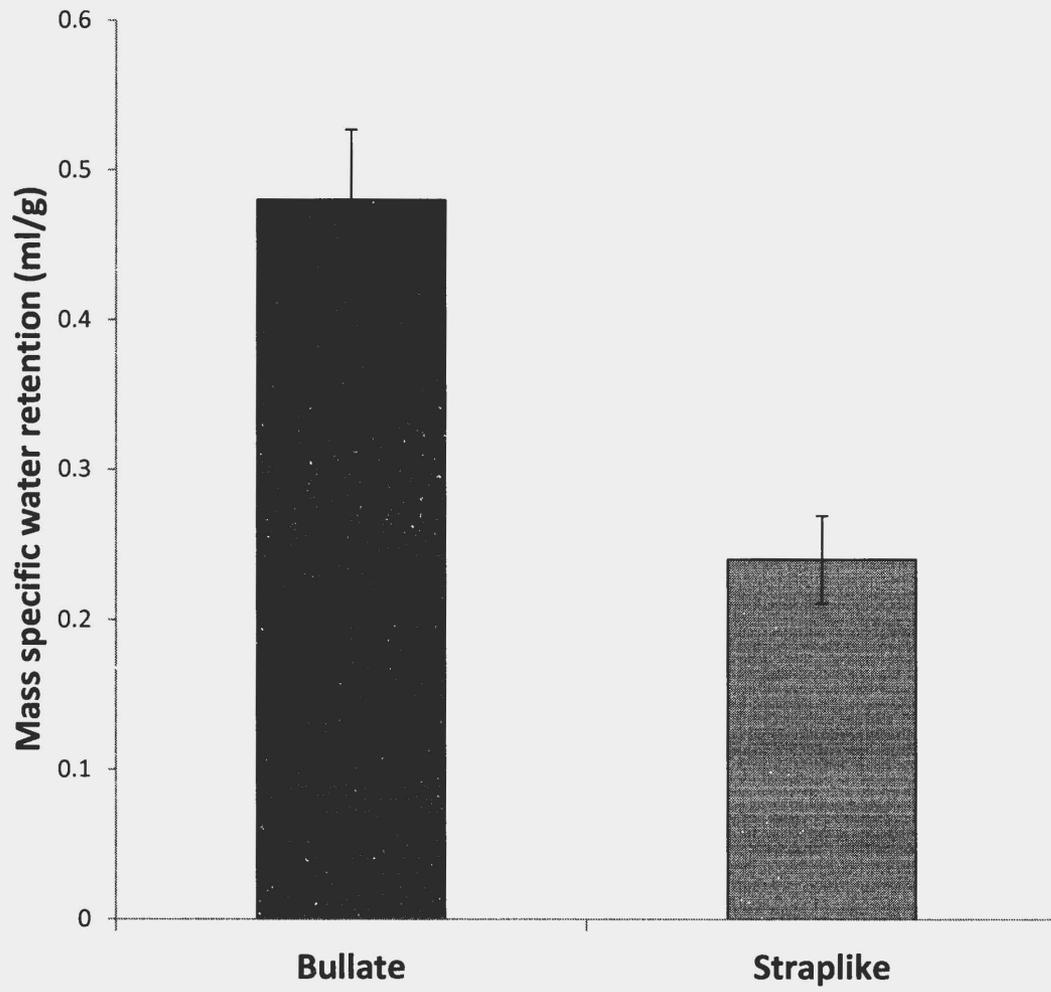


Figure 10. Mass specific water retention by bullate and straplike *Saccharina* thalli (mean  $\pm$  SE).

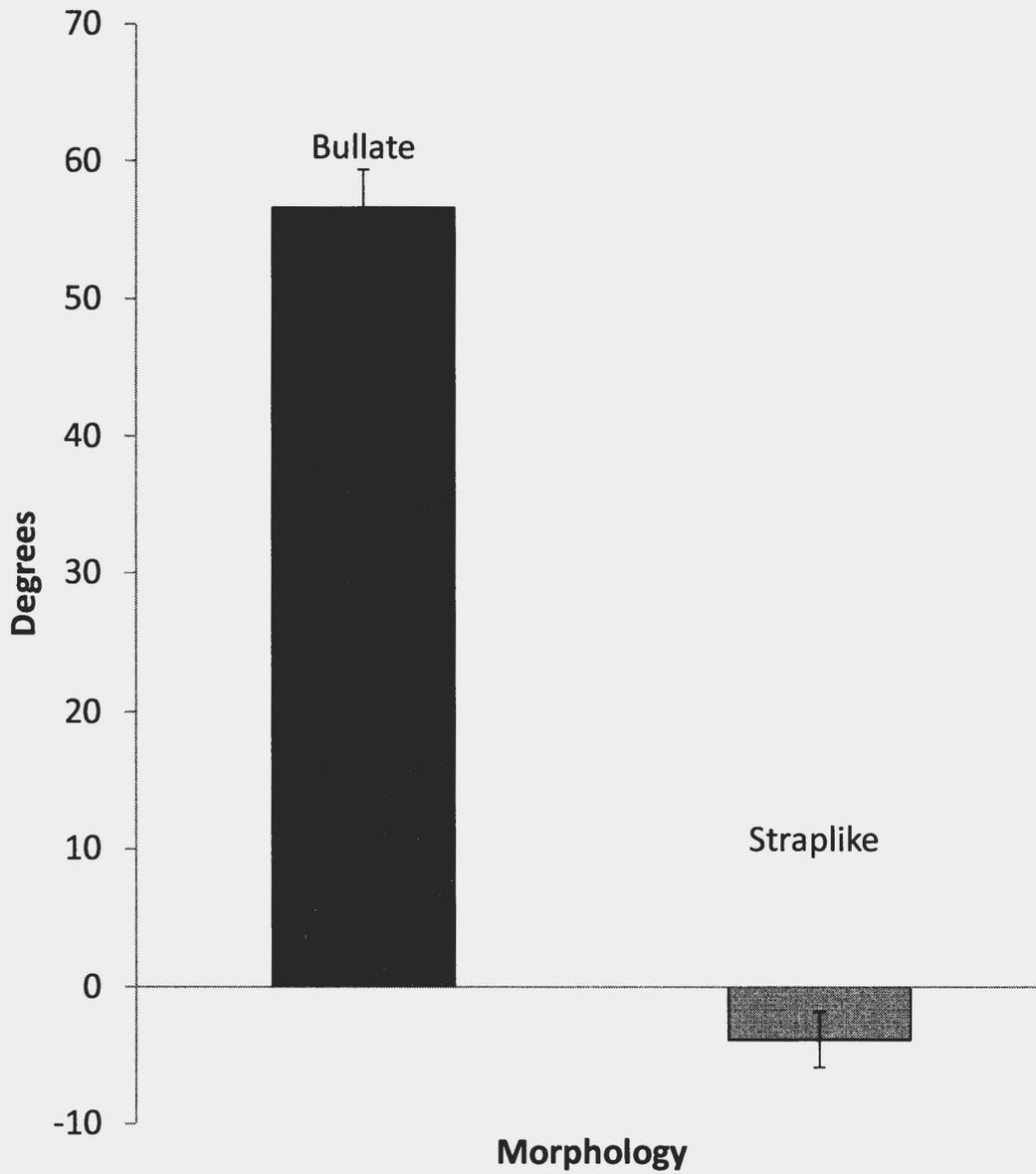


Figure 11. Mean angle ( $\pm$  SE) of blades for bullate and straplike morphotypes of *Saccharina*.

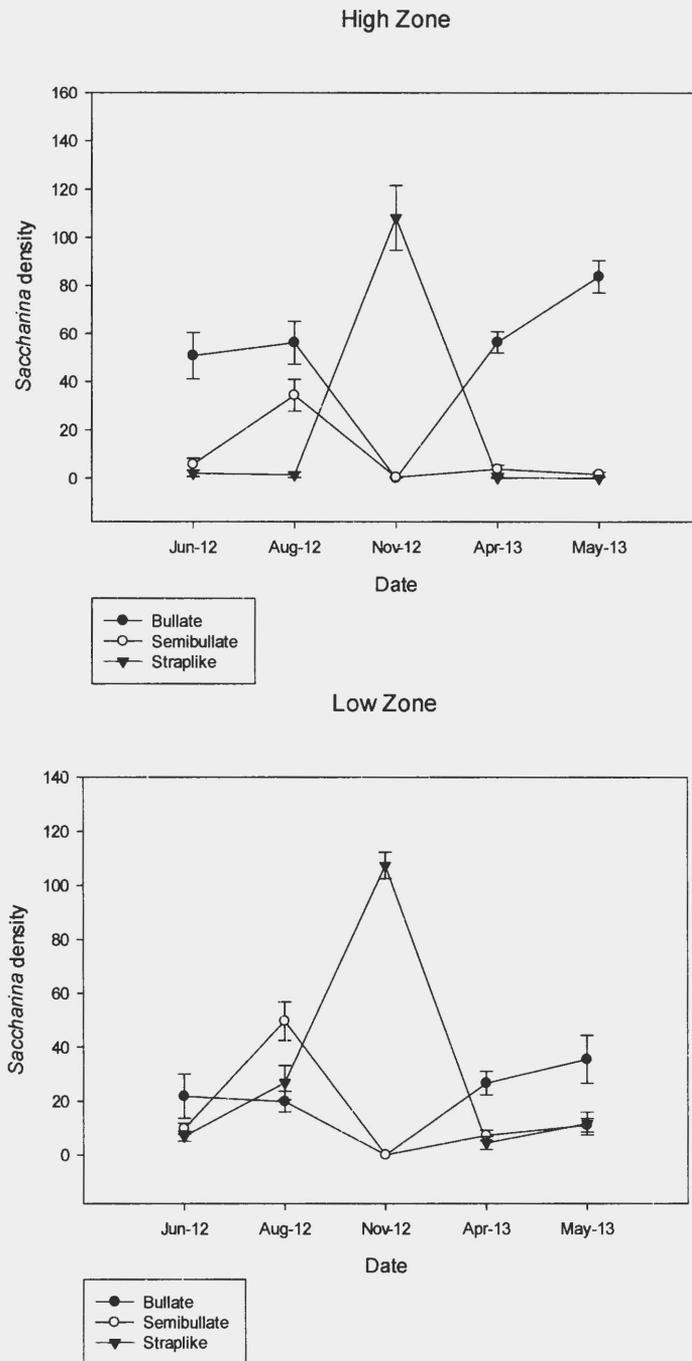


Figure 12. Variation in *Saccharina* bullate and straplike morphotypes over time in low and high intertidal zones. Mean density of *Saccharina* ( $\pm$  S.E.) of five belt transects 10.0 m x 0.1 m.

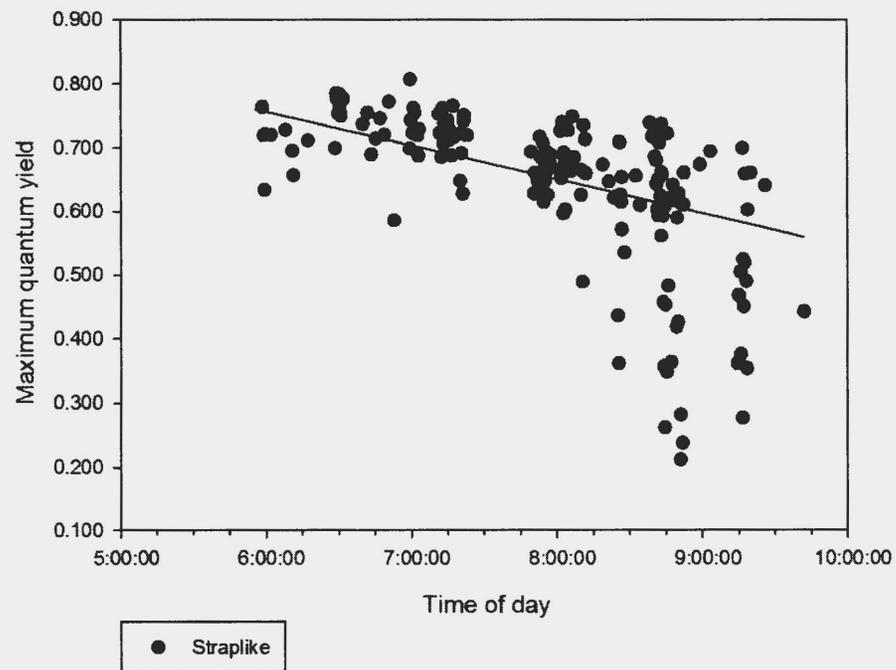
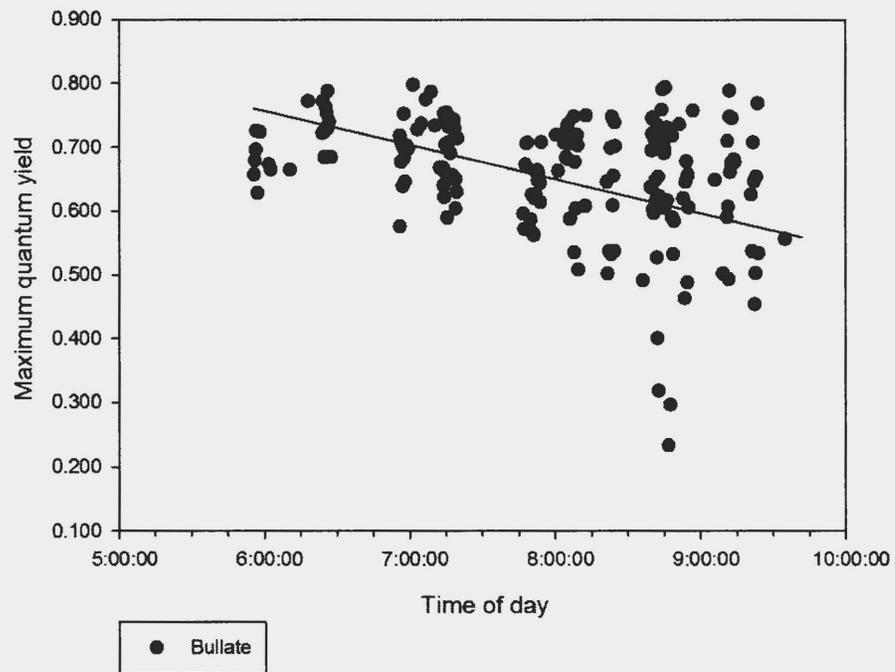


Figure 13. MQY in emersed *Saccharina* morphotypes, bullate and straplike, during morning low tides over seven days in June and July 2011 and July 2012 at Kibesillah Hill.

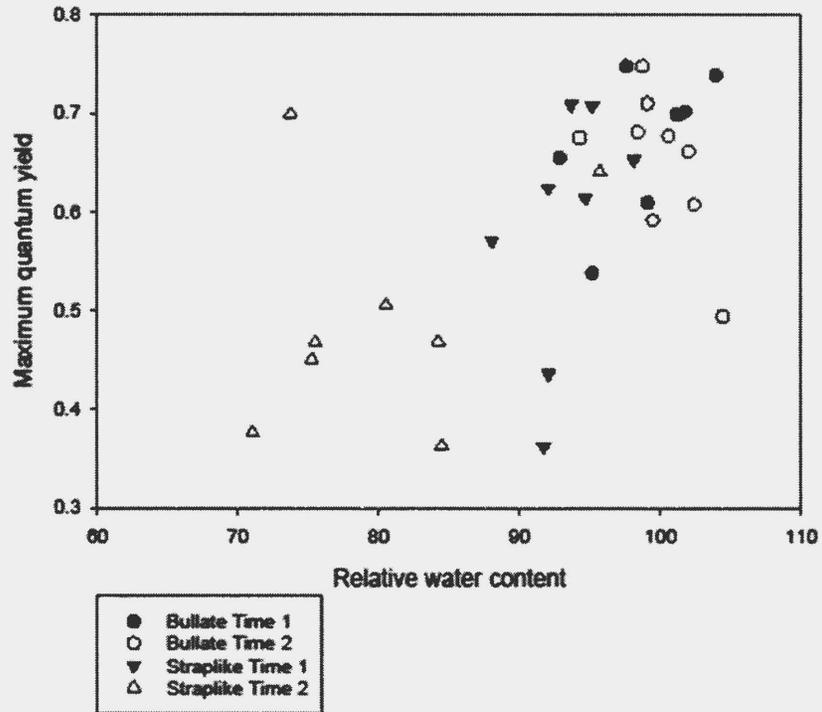


Figure 14. MQY of *Saccharina* as a function of relative water content and morphotype early and late during a low tide. This set of measurements was made on the warmest, least humid and windiest day (June 20, 2011) during this study period.

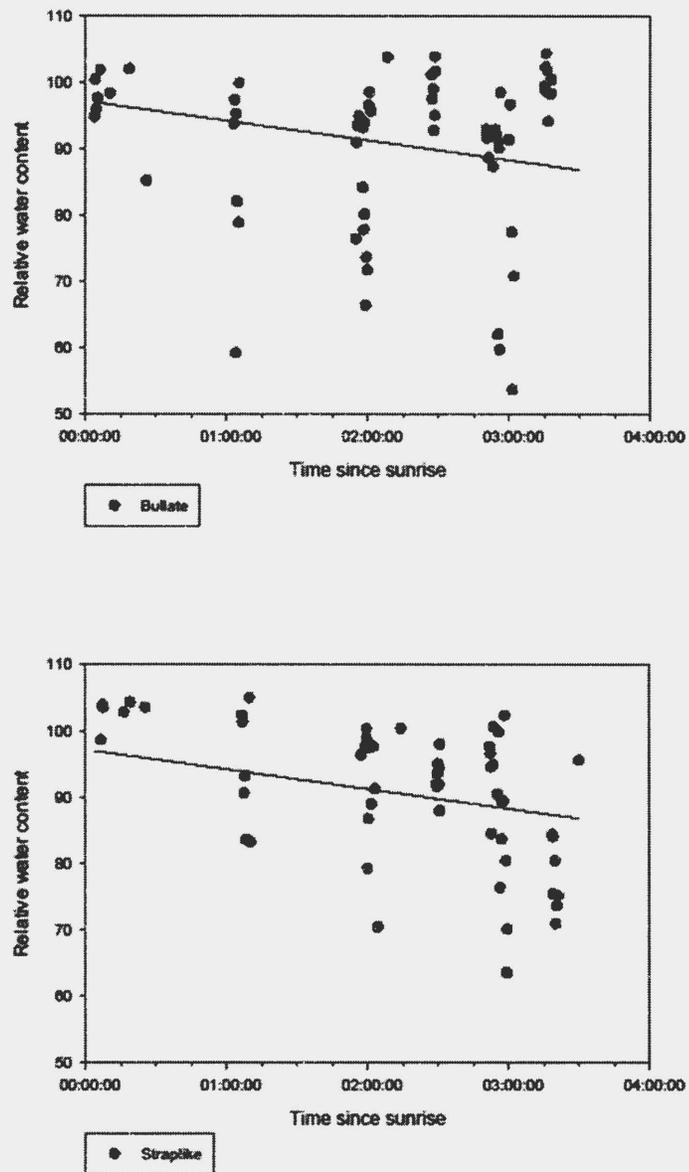


Figure 15. The relationship between relative water content and hours since sunrise during low tide emersion for bullate and straplike morphotypes during three morning low tides at Kibesillah Hill June 20, 2011, July 1, 2011, and July 4, 2011.

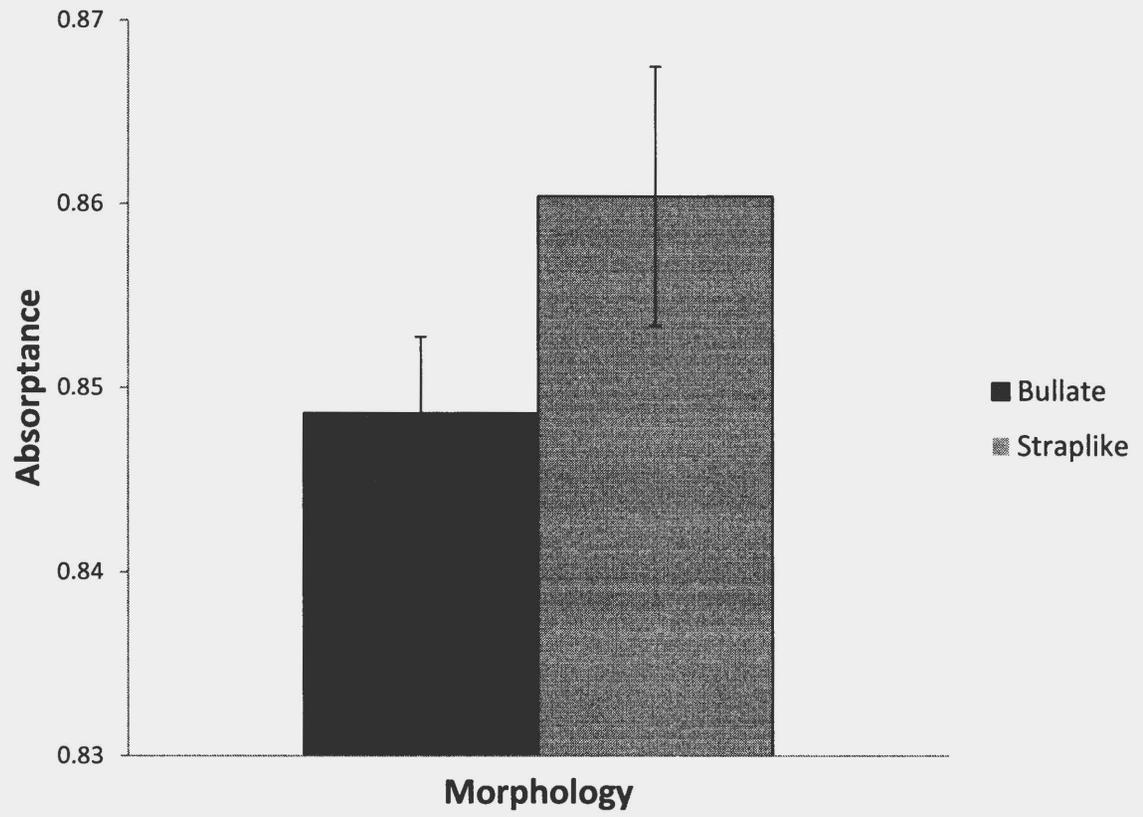


Figure 16. Absorbance of bullate and straplike *Saccharina* morphotypes (mean  $\pm$  SE).

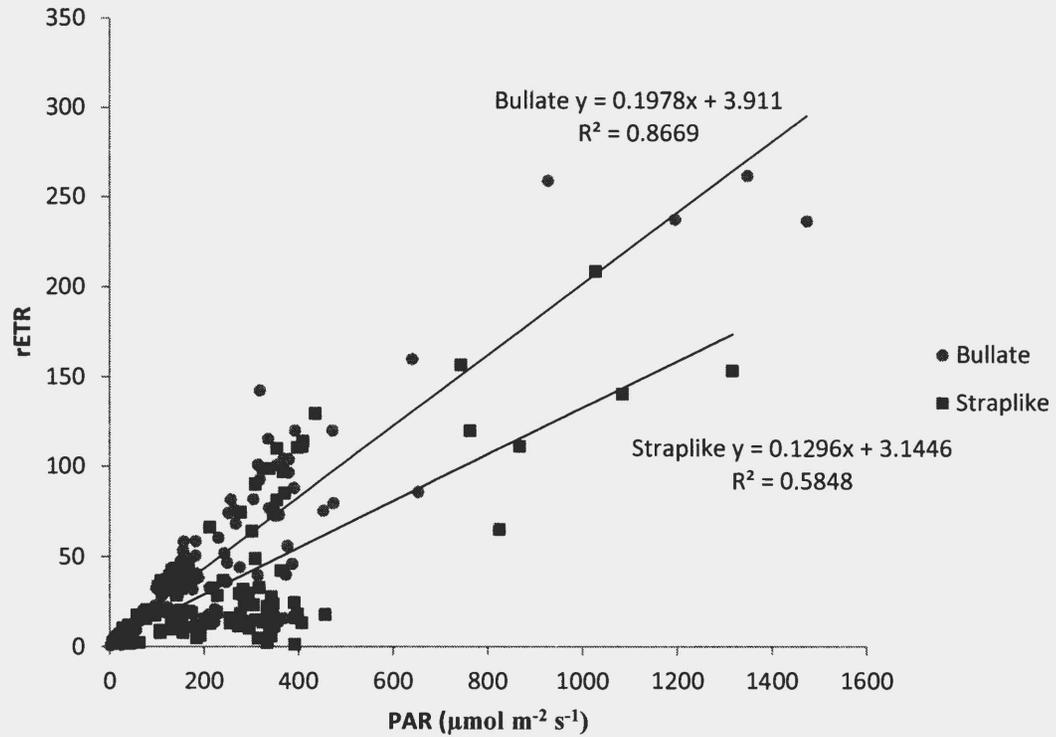


Figure 17. The relationship between rETR and solar irradiance (PAR) for bullate ( $n = 158$ ) and straplike ( $n = 155$ ) *Saccharina* morphotypes during six morning low tides at Kibesillah Hill in June and July 2011 and July 2012.

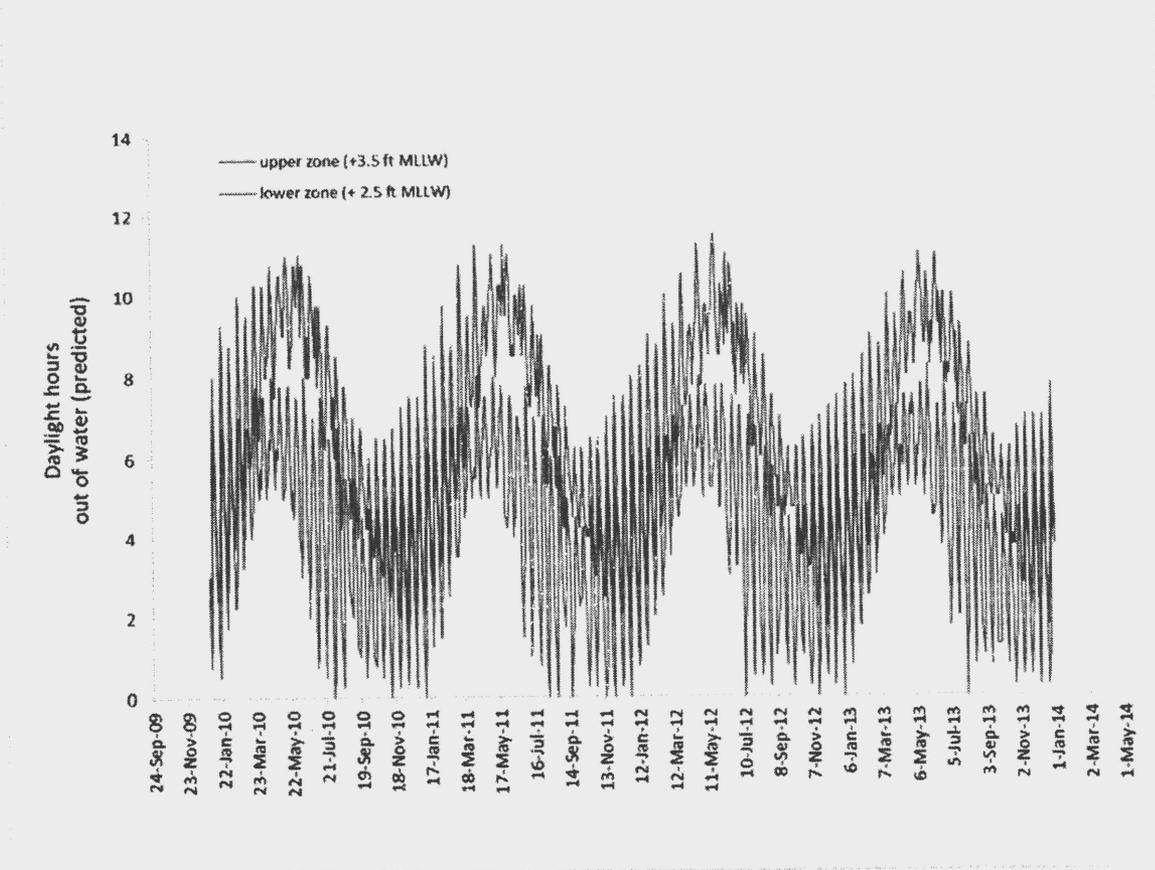


Figure 18. Predicted hours of emersion during daylight hours for the upper and lower levels of the *Saccharina* zone during the study period. Data acquired from the WWW Tide/Current Predictor web site (<http://tbone.biol.sc.edu/tide>, accessed on 2 April 2016).

## Appendix

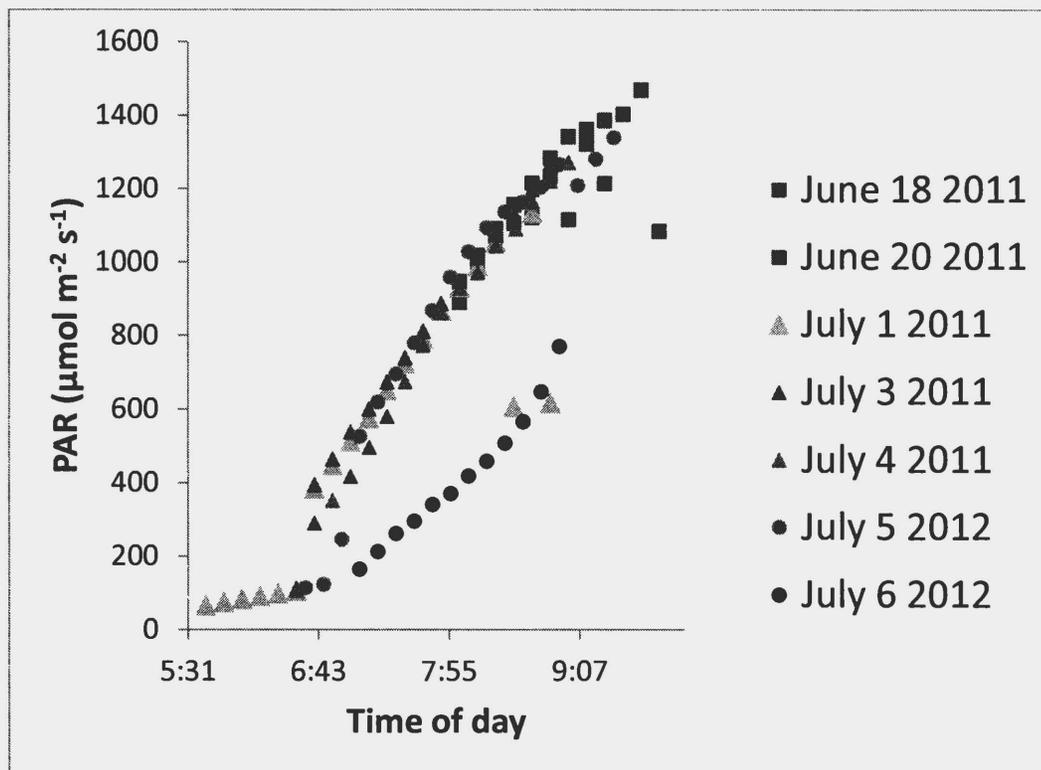


Figure A1. Solar irradiance (PAR) during physiological studies at Kibesillah Hill June and July 2011 and July 2012.

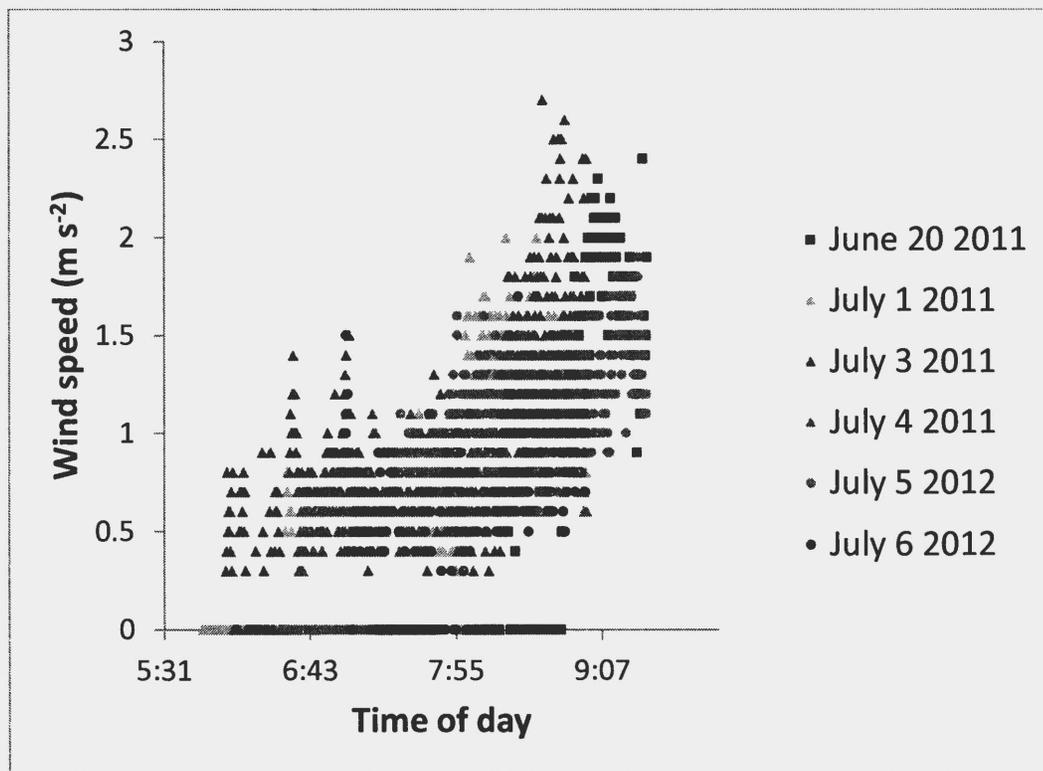


Figure A2. Wind speed during physiological studies at Kibesillah Hill during June and July 2011 and July 2012.

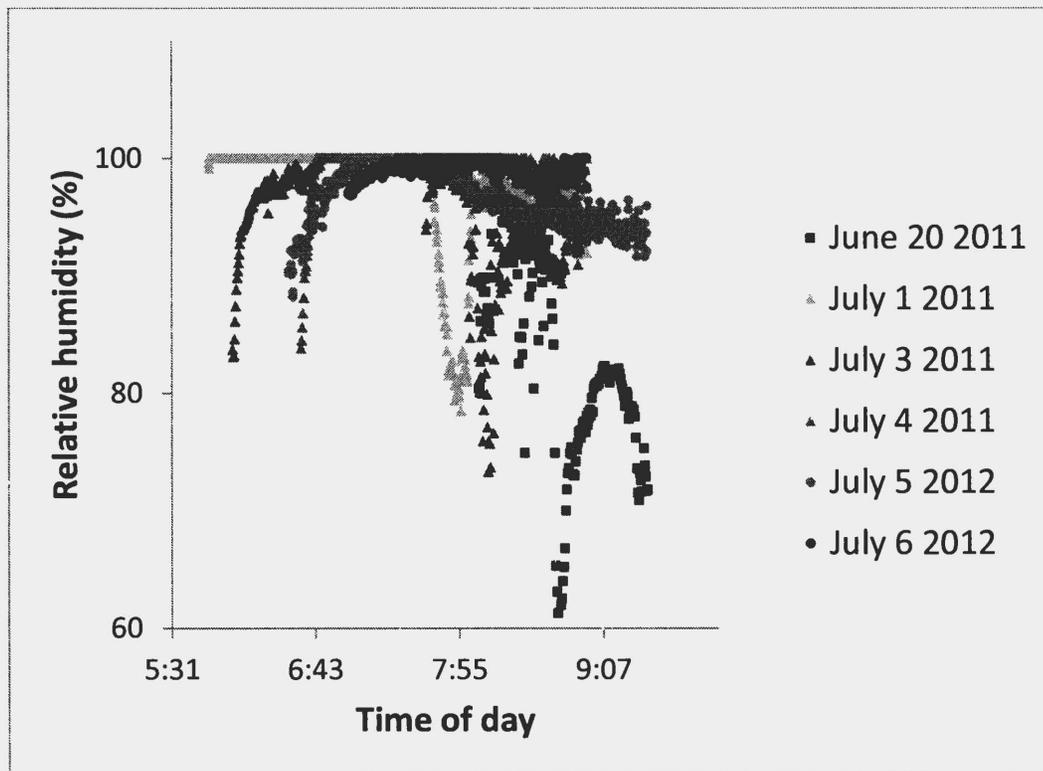


Figure A3. Relative humidity during physiological studies at Kibesillah Hill during June and July 2011 and July 2012.

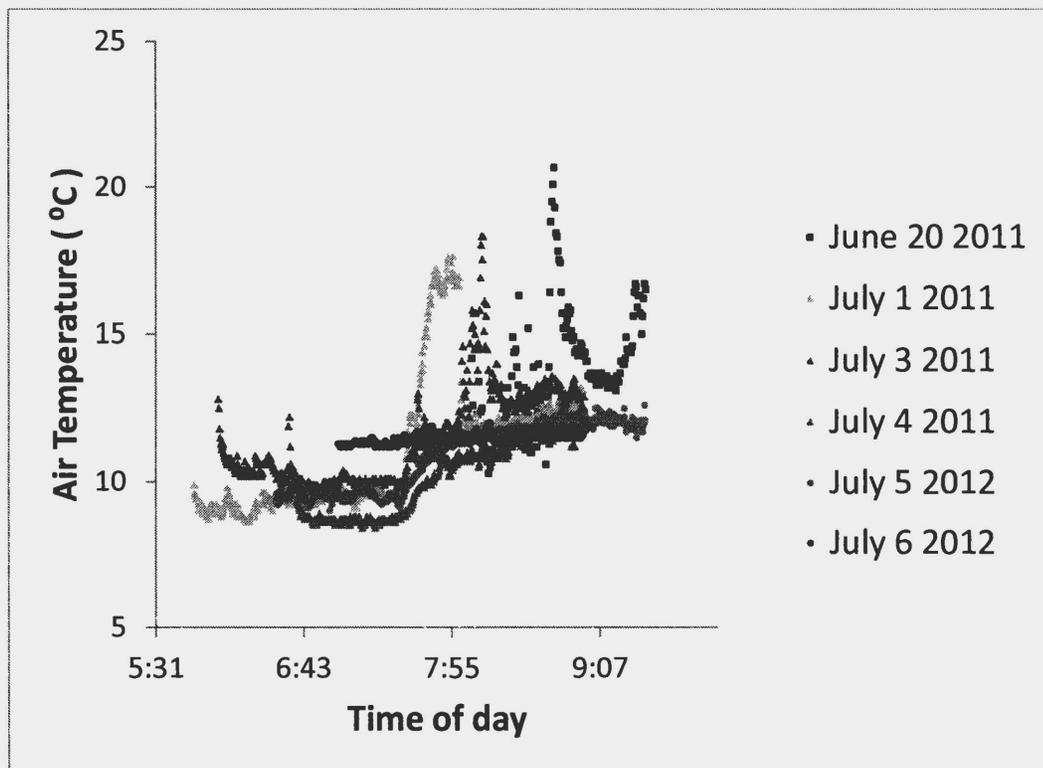


Figure A4. Air temperature during physiological studies at Kibesillah Hill during June and July 2011 and July 2012.