Capacity of a temperate intertidal seagrass species to tolerate changing environmental conditions: Significance of light and tidal exposure

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ABSTRACT

Seagrass meadows perform several important ecological roles in coastal areas. However, with multiple stressors threatening these aquatic plants, their current rate of decline is likely to increase, so understanding their ability to acclimate to a range of environments may be key to their survival. Light availability is critical for seagrass health, with loss of water clarity likely to cause decline. This study assessed the ability of the temperate intertidal species, *Zostera muelleri*, to compensate for variations in light in its natural environment. The impacts of light during inundation (high tide) and exposure (low tide) were examined along a vertical gradient from the high to low intertidal at two sites with differing light histories. Photo-acclimation was evaluated through morphological and physiological characteristics over two tidal cycles. Results were consistent with seagrasses having optimized their photosynthetic capacity, with physiological acclimations being site specific. Longer-term morphological changes were also noted, suggesting a dissimilar light history between sites for an extensive period. Interestingly, at the site with the greatest light range, but the lowest light penetration at depth, a significant reduction in photosynthetic activity during air exposure was observed. This suggests that *Z. muelleri* is capable of tolerating light stress through photo-acclimation, allowing for the more efficient harvesting of light at low levels. Overall, this study demonstrates that *Z. muelleri* has the ability to adjust both physiologically and morphologically to changing environmental conditions, a key aspect to survival and persistence in temperate intertidal zones.

1. Introduction

Marine embayments are often comprised of soft sediment habitats dominated by seagrass meadows. These high-value ecosystems are worth an estimated US$1.9 trillion to global markets each year in terms of nutrient cycling, with their support for commercial fisheries worth as much as US$3500/ha per year (Waycott et al., 2009). Seagrass habitats play a vital role in supporting ecosystems as they oxygenate water, regulate nutrients, stabilize sediments, provide nursery grounds for recreationally important fisheries, and are an essential food source for dugongs and turtles (Collier et al., 2012; Connolly, 2009). Unfortunately, multiple stressors (e.g. environmental, biological and climatological) are threatening these aquatic plants in many temperate and tropical ecosystems (Collier and Waycott, 2009). Seagrass meadows have been declining since 1990 at a rate of 7% per annum (Waycott et al., 2009), with up to 35% of worldwide seagrass beds significantly impacted (Nagelkerken, 2009).

Fluctuations in light, temperature, nutrients and substrate suitability severely impact primary production and cause seagrass decline (Connolly, 2009), particularly reductions in light availability (Collier et al., 2011). In addition to minimum light requirements for most seagrass species being within the range of 2–37% of surface irradiance (Lee et al., 2007), seagrasses that grow within the intertidal zone are often subjected to oversaturating irradiances which can also cause seagrass decline through thermal stress, desiccation and photo-inhibition (Petrou et al., 2013). As such, seagrass survival depends on their ability to acclimate to site-specific conditions (Silva and Santos, 2003), with an understanding of light thresholds crucial for effective management (York et al., 2013).

To ensure a constant adjustment to current light conditions, the

physiological responses of seagrasses (such as maximal quantum yield (F_v/F_m), maximum electron transport rate (ETR_{max}) and photosynthetic light harvesting efficiency (α) can be altered within minutes or even seconds (Ralph and Gademann, 2005). Over the past decade, studies have provided evidence of adjustment through physiological (e.g. Campbell et al., 2003; Collier et al., 2009; Larkum et al., 2006; Silva and Santos, 2003) and morphological (e.g. Collier et al., 2007, 2009; Longstaff and Dennison, 1999; Olive et al., 2013) characteristics. Photosynthetic processes of seagrasses are highly responsive, with chlorophyll fluorescence techniques (e.g. Pulse Amplitude Modulated (PAM) fluorometry) allowing for measurements of real-time changes (Campbell et al., 2007). As chlorophyll fluorescence is inversely correlated with photosynthetic efficiency, such measurements are a useful tool for detecting physiological stress (Krause and Weis, 1991). Additionally, the overall photosynthetic performance of a leaf and its capacity to tolerate short-term changes in light can be determined using rapid light curves (RLCs) with associated derived parameters used (α and $rETR_{max}$) (Ralph and Gademann, 2005). These measurable responses are often the first evidence of acclimation (Maxwell et al., 2014), a form of plasticity that involves the modification of morphological and/or physiological characteristics in relation to changes in the environment (Angilletta, 2009).

To date, the majority of seagrass research has focused on the effects of low light on growth and photosynthesis, with few studies examining the impact of high light levels (Schubert et al., 2015). Of those investigations conducted, responses to higher irradiances demonstrate a reduction in photosynthetic capacity and significant photo-inhibition (e.g. Petrou et al., 2013; Ralph and Burchett, 1995; Unsworth et al., 2012). However, with light regimes fluctuating as a function of time of day, tidal schedule and vertical distribution, it is difficult to confidently predict the response of a population within a site. Therefore, this study compared the acclimation abilities of an intertidal seagrass species growing within two ecological niches (light regimes), located within the same temperate embayment.

The objective of this study was to investigate the capacity of an intertidal species to tolerate changing environmental conditions, by quantifying the influence of light and tidal exposure on physiological and morphological characteristics. Specifically, we measured photosynthetic characteristics, elemental ratios, isotope composition, genetic diversity and changes in seagrass cover and morphology. We focused on the seagrass species, *Zostera muelleri*, the dominant intertidal species in temperate Australia (Green and Short, 2003). With several climate models suggesting future increases in sea level rise and air temperatures (CSIRO, 2015), understanding how this species tolerates changing environmental conditions, such as light, is critical to predicting the effects of climate change and developing effective management strategies.

2. Methods

This study was conducted during summer at two sites on opposite sides of Western Port, a large embayment in southeastern Australia (Crib Point (38°22′26.6″ S, 145°13′22.9″ E) and Coronet Bay (38°27′57.0″ S, 145°25′29.6″ E)). Within the bay *Z. muelleri* plants are present from the high tide mark to the low tide mark. Western Port was chosen as a study site due to its previous history of extensive seagrass loss. In the early 1980's losses of up to 75% were reported (Melbourne Water, 2011), with increased anthropogenic sediment inputs and associated changes in water quality a likely, but unconfirmed cause. Seagrass recovery was limited in many areas of the bay, with those of poor water quality still showing minimal signs of recovery (Melbourne Water, 2011). Current research and management-focused approaches are needed to not only prevent further declines, but to also assist in recovery.

At each site (Crib Point and Coronet Bay) three zones along a vertical gradient of the intertidal zone were established (high intertidal (HI), mid-intertidal (MI) and low intertidal (LI) − Fig. 1). The physiological and morphological characteristics of *Z. muelleri* was recorded at both sites. Physiological measurements were conducted on four separate days (two at low tide and two at high tide). Sampling days were chosen to coincide with the low/high tide between 11:00 and 13:00 when photosynthesis is expected to be at a maximum (Ralph et al., 1998). Morphological measurements were conducted once at the start of the experiment during low tide to allow for easy access.

2.1. Photosynthetically active radiation (PAR)

Photosynthetically Active Radiation (PAR) (400–700 nm) was measured prior to and during, the experiment to give an indication of the availability of light for seagrass photosynthesis. PAR was measured using 2π light loggers (Submersible Odyssey Photosynthetic Irradiance Recording System, Dataflow Systems Pty Ltd, New Zealand) placed in the high intertidal zone at seagrass canopy height. PAR was recorded every 30 min, with benthic daily averages per month calculated and examined using *t*-tests. Loggers were calibrated against a reference sensor (LI-COR LI-190 quantum sensor, Licor Corp, USA). From February to May 2015 light as a function of depth was also measured within the high intertidal zone, with single measurements recorded every 30 mins. The maximal tidal height at Crib Point was 2.1 m (0.73 m below reference point at Stony Point) and at Coronet Bay 1.6 m (1.23 m below reference). A *t*-test was then used to compare light measurements above 0.1 m and below 1.6 m between sites.

2.2. Physiological measurements

2.2.1. Chlorophyll fluorescence − maximal quantum yield (F_v/F_m)

During both low and high tide, three randomly selected leaves (total of 6 replicates per cycle) from each zone were examined for photosynthetic performance and efficiency. Water depth made *in situ* measurements difficult at high tide, so samples were collected by snorkelling and immediately wrapped in aluminium foil before a 15-min dark adaptation period. To ensure no effect of sampling, samples at low tide were also collected and dark-adapted. Chlorophyll fluorescence using a pulse amplitude modulated (PAM) fluorometer (Diving-PAM, Walz, Effeltrich, Germany) was used to determine the fluorescence parameters, F_o (initial chlorophyll fluorescence after dark adaptation), F_m (maximal chlorophyll fluorescence after dark adaptation) and F_v/F_m (maximal quantum yield, where $F_v = (F_m\text{-}F_o)$ is variable fluorescence). Differences in maximal quantum yield between tidal cycles within each site and zone were then analysed with a three-way analysis of variance (ANOVA), using site, tidal state and zone as the three fixed factors.

2.2.2. Rapid light curves

Relative maximum ETR ($rETR_{max}$) and Photosynthetic light harvesting efficiency (α)

Rapid light curves (RLCs) of rETR (relative electron transport rate through Photosystem II) versus irradiance were obtained by exposing leaves to a range of light intensities (nine steps) from 0 to 1200 μmol m^{-2} s^{-1} for 10 s each. Relative electron transport rates (rETR) were calculated from the RLC data as rETR = yield x irradiance x 0.5 x AF (Beer et al., 2001). The maximum rETR ($rETR_{max}$) and photosynthetic light harvesting efficiency (the initial slope of the RLC, α) were derived by fitting the RLCs to the equation of Eilers and Peeters (1988) using the least square method (SYSTAT version 7.0). A series of three-way ANOVAs was then used to compare between tidal cycles, sites and zones (Prism V7.0). The absorption factor (AF) of *Z. muelleri*, necessary to determine absolute rates of electron transport, was not measured due to logistical reasons (width of leaves); therefore, the in-built absorption factor of 0.84 was used. As a result, we can only report our data as rETR and we accept that this poses limitations as plants can adjust their AF in response to the light environment. However, such changes would manifest, for example, in increases in α under low light even if $rETR_{max}$ is underestimated.