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# Canopy-scale modifications of the seagrass *Amphibolis griffithii* in response to and recovery from light reduction

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ABSTRACT

A manipulative experiment using *Amphibolis griffithii* seagrass, a clonal plant with a complex canopy, tested within-canopy responses to and recovery from light reduction. There were consistent patterns in the distribution and arrangement of leaves, growth rates and resources within the seagrass canopy, and they were modified under reduced light and following recovery from this impact. Under light reduction, plants responded by: reducing leaves in the mid-canopy where the maximum biomass was located; maintaining meristems throughout the canopy; and re-allocating nitrogen to the top of the canopy. During recovery plants increased the number of leaves throughout the canopy and enhanced growth in the part of the canopy that had lost most biomass. These responses have the potential to enhance light capture and recovery of the meadow. There is a clear evolutionary advantage for these submerged plants to be able to modify traits within the canopy, which increase the chance of survival under light stress.



## 1. Introduction

Light is a key regulator of plant growth and the responses of plants that allow them to persist in heterogeneous or reduced light environments are reasonably well documented (Lee et al., 2007; Ralph et al., 2007; Schurr et al., 2006). If the light environment changes, plants respond in ways that enhance light capture and carbon gain. There may be physiological changes in photosynthesis, nutrient uptake, growth and productivity or resource allocation, or structural changes in leaf size or leaf density (Evans and Poorter, 2001; Lambers et al., 2008; Ralph et al., 2007). These can all interact at different time-scales and different levels of plant organisation, constituting plant functional dynamics (Schurr et al., 2006).

In plants with complex canopies, these dynamic processes can vary in different parts of the canopy in response to local variations in light and other conditions (Carruthers and Walker, 1997; Schurr et al., 2006), or because of physiological controls in the plant (Dodd et al., 2005). Most studies on the within-canopy variation in responses to light have been carried out in terrestrial systems. These studies have shown a clear evolutionary advantage to be able to acclimate to variation in the light climate. As light is attenuated through canopies (Carruthers and Walker, 1997) and the amount of attenuation is strongly influenced by the canopy structure (Hedley and Enriquez, 2010) we propose that within-canopy variation in the structure of submerged plants canopies to light reduction would aid their survival.

Seagrasses are a polyphyletic group of clonal marine plants that form extensive and highly productive meadows across the globe and strongly influence the physical, chemical and biological environment in coastal waters. They provide key ecological services to the marine environment including coastal protection, carbon storage and nutrient cycling, and they support marine food webs and fisheries (Hemminga and Duarte, 2000). As in terrestrial plants, light is a key regulator of seagrass distribution and plant dynamics, and seagrasses are extremely sensitive to light reduction because of high light requirements (Ralph et al., 2007).

This study examines the impact of light reduction through the canopy of one species of seagrass, *Amphibolis griffithii* (J.M. Black) den Hartog, in the family Cymodoceaceae (den Hartog and Kuo, 2006). *A. griffithii* is an ideal seagrass to examine canopy-scale response to light reduction as it has a complex canopy compared to most other seagrass species, with vertical, branching stems bearing terminal leaf clusters (Cambridge, 1999). Stems range from 30 to 100 cm high and generally persist for 2–3 years (den Hartog, 1970), whereas leaves are shorter-lived, generally lasting about 90 days (Marba and Walker, 1999). These features, particularly the placement of meristems throughout the canopy with leaves that have a relatively fast turnover, have the potential to strongly influence the distribution of resource in time and space. Secondly, *A. griffithii* is one of the dominant seagrasses in southern Australia (Holmes et al., 2007). These meadows support exceptional levels of biodiversity, productivity and habitat complexity, with *A. griffithii* the keystone species (Gartner et al., 2010; Jernakoff et al., 1996).

Previous studies have shown that with three months of light reduction there are significant changes to the biomass, morphology and resources of an *A. griffithii* meadow but no changes in canopy position,

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implying that at this time-scale, these changes are due to with-in canopy modification (Lavery et al., 2009; Mackey et al., 2007). Thus the aim of this study is, by a manipulative experiment, to improve the understanding of how the biomass, structure, morphology, growth and resource allocation vary through an *A. griffithii* canopy under natural light conditions, and how this canopy structuring responds to and recovers from ecologically relevant levels of light reduction.

## 2. Materials and methods

We conducted a field experiment at Jurien Bay, Western Australia, in a monospecific meadow of *A. griffithii* at 5 m depth (30° 18′ 34″ S, 115° 00′ 26″ E; WGS84 datum). The experiment used the treatments described by Lavery et al. (2009) and McMahon et al. (2011) but collected additional information to determine the response through the canopy. In summary, the experiment used neutral density shading screens to produce two levels of light reduction (moderate and heavy, n = 5 plots/treatment) over a period of three months, from the end of summer to early winter (10th March–14th June 2005). We measured a range of parameters to describe the impact of light reduction during the shading period. To follow recovery of the meadow, the shaded plots were re-sampled 3 and 10 months after shading was removed (7th Oct 2005 and 18th April 2006, respectively). Light was continuously monitored throughout the experiment just above the seagrass canopy as described in Lavery et al. (2009). Light-reduction treatments resulted in plots receiving 16% (moderate) and 5% (heavy) of ambient light (PAR), equivalent to a total irradiance (mol $m^{-2}$) over the treatment of 1942 (control), 317 (moderate) and 95 (heavy) or an average daily irradiance (mol $m^{-2}$ $day^{-1}$) of 19 (control), 3.1 (moderate) and 0.9 (heavy) (Lavery et al., 2009). During the recovery period average daily irradiance was 16.1 mol $m^{-2}$ $day^{-1}$ for the first 3 months and then 41.2 over the remaining 7 months (McMahon et al., 2011).

The method used to establish the light-reduction treatments is described by Lavery et al. (2009). Each plot measured 4.5 m × 3 m, and was constructed from six, 2 m long cement reinforcing bars (12 mm diameter) driven into the sediment with pole drivers. A plastic frame (32 mm diameter) was attached to the bar at a height of ~1.2 m above the sediment. The light-reduction treatments were created with a woven shade cloth (moderate — 50% shade cloth; heavy — 80% shade cloth), which was attached to the frame and replaced every 3–6 weeks. An effective sampling area of 3 m × 1.5 m (4.5 $m^2$) was chosen to avoid the effects of stray light which encroached into the edges of the plots over a belt approximately 750 mm wide (Mackey et al., 2007).

### 2.1. Light

Canopy transmission was measured 30 times in control plots through the course of the experiment with an instantaneous light meter calibrated to a standard light source at the top and base of the canopy, and similarly in three 'moderate' and 'heavy' plots at the end of each period of treatment or recovery. All measures were taken between 10 am to 12 noon to minimise effects of canopy attenuation due to changes in the sun angle.

### 2.2. Field collection

Samples of *A. griffithii* were collected to measure biomass, density, morphology, growth and chemical composition as described by Lavery et al. (2009) but separated into 10 cm height categories from the base of the stem. Above-ground samples for leaf biomass, density, morphology and area, and internode length were pooled from five randomly selected 10 × 10 cm units within a 50 × 50 cm quadrat (total sample area of 0.05 $m^2$). Leaf growth was estimated by tagging all leaf clusters on 6 stems using the leaf-punch methodology (Short and Duarte, 2001). Leaves were punched 1–2 weeks before the end of the treatment, and then these stems were collected at the end of the treatment, at the same time as the aboveground biomass samples. Six stems were collected separately from within the plot for chemical composition measures.

#### 2.2.1. Biomass, density, morphology and growth

The numbers of clusters and leaves from each aboveground sample were counted to estimate cluster density and number of leaves per cluster in each height category. A cluster was defined as a group of leaves separated from the next cluster by visible stem. A leaf was counted if it had emerged from the sheath. One stem was randomly selected from the aboveground biomass sample for additional measures of leaf area and internode length. The lengths of the five internodes behind each cluster (most recently produced internodes) were measured. Leaves were separated from stems, algal epiphytes removed by razor blade and then dried at 60 °C for 24 h and weighed. Growth of the clusters that had been tagged was expressed as leaf extension rate (the sum of all leaves that grew in a cluster — mm leaf $cluster^{-1}$ $day^{-1}$). During the recovery period, leaf growth was only measured once, 3 months after shade was removed, due to logistic constraints at the time of the 10-month recovery sampling.

#### 2.2.2. Chemical composition

Living leaf material was selected from the upper (40–50 cm) and lower (20–30 cm) canopy. Samples were scraped free of epiphytes, dried and ground in a mill grinder. Samples were analysed in a continuous-flow isotope-ratio mass spectrometer (20–20 IRMS, Europa, Crewe, United Kingdom) for nitrogen (% DW). Leaf nitrogen was expressed as leaf nitrogen per leaf area (g N $m^{-2}$ of leaf) by converting as follows: (% N DW / 100) × leaf dry weight (g) / leaf area ($m^{-2}$). Soluble sugars (% DW) were determined colourimetrically (420 nm) (Yemm and Willis, 1954).

### 2.3. Statistical analysis

To analyse the impact of light-reduction treatments on the response of seagrass variables at different points in the canopy, and how this varied over time a Repeated Measures two-way nested ANOVA was used with the canopy position nested within the light treatment. Mauchly's test of sphericity was used to test the variance–covariance matrices, and if the assumptions are not met, the Greenhouse–Geisser epsilon adjustment was applied to the degrees of freedom. ANOVA results are presented as between subject effects (Light and Canopy position nested with light treatment, as it was assumed that the values from each height category were not independent) and within-subject effects (over time). Each time period (impact; 3 months of recovery; and 10 months of recovery) was considered a repeated measure as samples were taken from the same plot. Data were tested for normality using the Kolmogorov–Smirnov goodness of fit test (Zar, 1999) and heterogeneity using Cochran's Test (Cochran, 1951), and transformed if necessary. If after transformations the data were not normally distributed and were unimodal, it was assumed that, in view of the large number of samples, the analysis would be robust to deviations from normality (Underwood, 1997). Where variances were heterogeneous after transformation, there was an increased risk of a Type 1 error but the balanced experimental design makes ANOVA robust to this departure (Underwood, 1997). Nonetheless, the significance level was set to 0.01 in these circumstances as a precaution. Fisher's LSD post-hoc tests were carried out if there were significant effects in the ANOVA. Where more than one measure was taken for a variable in each plot i.e. the number of leaves per cluster was measured on all clusters collected in the plot, an average was generated for each replicate plot and used in the ANOVA. Not all height categories were available for all variables, since there was insufficient plant material at some heights.