Marine Pollution Bulletin 128 (2018) 508–518

Contents lists available at ScienceDirect

Marine Pollution Bulletin

journal homepage: www.elsevier.com/locate/marpolbul

Determining light stress responses for a tropical multi-species seagrass assemblage

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ARTICLE INFO

Keywords:
Dredging
Light reduction
Tropical seagrass
Stress
Response
Seagrass assemblage

ABSTRACT

Existing mitigations to address deterioration in water clarity associated with human activities are based on responses from single seagrass species but may not be appropriate for diverse seagrass assemblages common to tropical waters. We present findings from a light experiment designed to determine the effects of magnitude and duration of low light on a mixed tropical seagrass assemblage. Mixed assemblages of three commonly co-occurring Indo-West Pacific seagrasses, *Cymodocea serrulata*, *Halodule uninervis* and *Halophila ovalis* were grown in climate-controlled tanks, where replicate pots were subjected to a gradient in light availability (0.9–21.6 mols PAR m^{-2} day^{-1}) for 12 weeks. Increased shading resulted in declines in growth and changes in cellular and photosynthesis responses for all species, although time-scale and magnitude of response were species-specific. Applying management criteria (e.g. thresholds) relevant to one species may under- or over-estimate potential for impact on other species and the meadow as a whole.

1. Introduction

In multi-species communities, co-existence of individual species is influenced by the interaction between environmental drivers and differences in response among species (Terrados et al., 1998; Ooi et al., 2014). The occurrence of multiple species of seagrass within meadows is common world-wide (Short et al., 2007), particularly in the tropical Indo-West Pacific, the region where this study was conducted (Ooi et al., 2011a; Ooi et al., 2011b). How species with different life history strategies in multi-species meadows respond to disturbances is poorly understood (Ooi et al., 2014; Kilminster et al., 2015)? This information would provide a strong basis for policy makers, industry, and scientists to make decisions about the sensitivity of multi-species meadows to disturbances.

Deterioration in water clarity can have profound impacts on seagrass health, distribution and abundance, often resulting in the absence or near absence of seagrasses (Short and Wyllie-Echeverria, 1996; Erftemeijer and Lewis, 2006; Orth et al., 2006; Waycott et al., 2009). Concerns about intensive use of coastal areas for commercial activities and deteriorating water clarity have become central issues in marine policy decision-making, and as a result, research in recent years has focused on trying to minimize impacts that cause light reduction to seagrass habitats, such as dredging, wastewater and nutrient discharge, coastal development, terrestrial run-off, and aquaculture (Cambridge and McComb, 1984; Kemp et al., 2005; Erftemeijer and Lewis, 2006). This focus has, in part, stemmed from an increasing awareness among coastal industry and regulatory bodies of the ecological and economic value of seagrass beds, reflected in many parts of the world in tighter control measures including appropriate impact assessments, strict water quality regulations, proper enforcement and monitoring, and mitigation strategies (Cardoso et al., 2008; Erftemeijer and Lewis, 2006; Greening and Janicki, 2006; Walker et al., 2007). In many cases, loss of seagrass is avoidable if early detection and immediate remediation processes are in place.

It is important to consider how individual species within multi-species assemblages will respond to changing light availability. Recent models have described in detail the diversity of seagrass responses to reduced light availability, ranging from physiological and plant-scale (sub-lethal) changes, through to longer term meadow-scale (lethal) losses (Waycott et al., 2005; Ralph et al., 2007; McMahon et al., 2013). The timescales of response can range from seconds to weeks for physiological and morphological changes, and weeks to months for meadow-scale impacts. A crucial factor in long-term survival and growth of seagrasses to declines in available light is how they balance photosynthetic carbon fixation and carbon consumption during respiration (Hemminga and Duarte, 2000). As such, optimisation of

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https://doi.org/10.1016/j.marpolbul.2018.01.060
Received 16 June 2017; Received in revised form 25 January 2018; Accepted 29 January 2018

growth and prolonged survival under reduced light conditions can be achieved through a number of physiological and morphological adjustments (Ralph et al., 2007). However, under severe or prolonged light reduction, the ability (rate and magnitude) to modify physiology and morphology and maintain a positive carbon balance differs between species (McMahon et al., 2013). Kilminster et al. (2015) proposed that colonising genera (e.g. *Halophila*) have a lower capacity to adjust and resist stress compared to opportunistic genera (e.g. *Cymodocea*) and persistent genera have the greatest capacity to resist stress. These differences in resistance to a stress could result in meadow-scale, species-specific losses and therefore loss of diversity.

For many sub-tropical and tropical multi-species seagrass assemblages, knowledge about light reduction effects has been described only through qualitative or correlative observations (e.g. Terrados et al., 1998; Longstaff et al., 1999; Livingston et al., 1998), affecting our ability to more accurately predict or manage impacts. Examples of quantitative experimental manipulations of sub-tropical and tropical seagrasses are almost exclusively single-species studies (e.g. Grice et al., 1996; Livingston et al., 1998; Longstaff et al., 1999; Collier et al., 2012; Chartrand et al., 2016). While single-species studies have progressed our understanding of light impacts and thresholds for some tropical species, the strict separation of species during experimentation and focus on individual species outcomes means these insights may not be representative of how a mixed species seagrass assemblage may respond. The advantage of using mixed-species experiments is that this more closely represents the diversity of seagrasses in tropical meadows in-situ. Clearly, to improve our understanding of impact predictions on tropical seagrasses, a multi-species approach is desired.

North-west Australia is within the Indo-West Pacific bioregion, globally the most species-rich (24 species total) (Short et al., 2007), and contains 11 species across 6 genera (Waycott et al., 2004). McMahon et al. (2015) identified three of these species (*Cymodocea serrulata* (R. Brown) Ascherson and Magnus, *Halodule uninervis* (Forsskål) Ascherson, and *Halophila ovalis* R. Brown) as being widespread. Furthermore, in most instances, these species co-occur, forming multi-species meadows, though the relative abundance of each species may vary throughout the year or at longer-timescales. The objective of this study was to determine the response to different magnitudes and durations of light reduction for a mixed-species tropical seagrass assemblage. Here, we examine the light stress:response pathway for three common and co-occurring north-west Australian tropical seagrasses, *C. serrulata* (*opportunitstic*), *H. uninervis* (*opportunistic/colonising*) and *H. ovalis* (*colonising*) grown together in large, climate-controlled tank systems. We hypothesized that the intensity and duration of light reduction will influence the time required and magnitude for plant responses to occur, and that the magnitude and timeframe of response will be species-specific.

2. Methods

2.1. Seagrass collection

On the 17th of April 2014, the seagrasses *Cymodocea serrulata* (R. Brown) Ascherson and Magnus, *Halodule uninervis* (Forsskål) Ascherson, and *Halophila ovalis* R. Brown were collected from Useless Loop, Shark Bay, Western Australia six and a half weeks prior to the beginning of the experiment. All species were collected by excavating fragments, or ramets. Ramets were then placed in aerated and insulated containers filled with seawater for transport to Fremantle, Perth, Western Australia (1000 km or 12 h travel time). Back at the Fremantle facility, ramets were prepared for planting. Ramets with at least one intact apical shoot and with at least three and up to six mature shoots were used. When a ramet had more than six mature shoots, additional shoots were removed using a razor blade, and if the apical shoot was damaged or missing, the ramet was discarded. On 20th April 2014, ramets were planted into circular pots (250 mm diameter × 250 mm deep). Because of the size differences (biomass and internode distance) between each species (*C. serrulata* > *H. ovalis* > *H. uninervis*) the number of ramets planted of each species in each pot was based on plant biomass (wet mass) and shoot densities found in the field. Subsequently, we planted one ramet of *C. serrulata*, two ramets of *H. ovalis* and three ramets of *H. uninervis* into each pot.

2.2. Tank system

The tank system consisted of 12, 1800 L rectangle plastic, fibreglass reinforced tanks. Each with their own illumination source provided by two 720 watt High Intensity Discharge (HID) lamps mounted 1.1 m above the sediment surface. Each HID lamp contained one metal halide globe (blue spectrum) and one high pressure sodium globe (red spectrum). This light source created a highly homogenous field of irradiance across the cross section of each tank (ca. 500 μmols PAR $m^{-2} s^{-1}$). Each 1800 L tank was a closed, recirculating system, with seawater circulating from a 600 L reservoir beneath each tank. Natural seawater was used to fill each tank system, with one quarter exchanged every two weeks throughout the experimental period. Seawater was circulated in each system using 8000 L h^{-1} submersible pumps, allowing complete replacement of water in each system 80 times per day. Within each tank, incoming seawater was spread through a diffuser (T-bar) to create homogenous movement of water. Each aquarium system had independent temperature control from a heater/chiller unit, which allowed water temperature to be controlled within 0.5 °C. Seawater quality was controlled through continuous chemical and mechanical filtration. Salinity levels were monitored daily and adjusted via addition of deionized freshwater.

2.3. Experimental design and setup

The objective of this study was to determine the response to different magnitudes and durations of light reduction for a mixed tropical seagrass assemblage. This was addressed in the following experimental design. We established twenty-five pots in each aquarium. Pots contained marine sediments from a dredged stockpile (Cockburn Sound, Perth, W.A.) with added organic matter (0.5% sediment dry weight) in the form of dried and ground (< 2 mm) seagrass leaves (Fraser et al., 2016; Statton et al., 2013). Prior to applying the experimental treatments, the plants were acclimated for 47 days, at a temperature of 27 °C, salinity of 37, and an irradiance of ca. 500 μmols PAR $m^{-2} s^{-1}$ measured 5 cm from the pot sediment surface (LiCor LI 1400 datalogger with a LI-192 underwater quantum sensor) on a 12 h:12 h light:dark cycle (i.e. 21.6 mols PAR m^{-2} day^{-1}). Plants were deemed acclimated to our aquarium conditions when we observed each species had produced at least one new shoot in the majority of pots. In fact, in most cases ramets of one or two species had expanded to overlap with adjacent species, reflecting a mixed species assemblage. After the acclimation period, six light shading treatments were applied on 6th June 2014 by placing shade screens of different density over the aquaria: the screens were supported by the rim of the aquaria and tensioned so as not to touch the water surface (to prevent any influence of algal attachment on light availability). Light treatments were pre-determined to cover a wide range of light availabilities, with greater resolution at lower light intensities, and to reflect light intensities reported near dredging operations (McMahon et al., 2015). Our shading levels consisted of controls or 100% Incident Photosynthetically Active Radiation, I_{PAR} (21.6 mols PAR m^{-2} day^{-1}), 60% I_{PAR} (13.1 mols PAR m^{-2} day^{-1}), 41% I_{PAR} (8.9 mols PAR m^{-2} day^{-1}), 23% I_{PAR} (5.0 mols PAR m^{-2} day^{-1}), 11% I_{PAR} (2.3 mols PAR m^{-2} day^{-1}) and 4% I_{PAR} (0.9 mols PAR m^{-2} day^{-1}). Two replicate aquaria were randomly assigned to each light treatment.

Physiological through to meadow level indicator measurements of seagrass status were measured throughout the experiment (Table S1). Plant harvesting was conducted at four times: 3 (27th June); 6 (18th