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4 1 Recreating the shading effects of ship wake induced turbidity to test acclimation
5 2 responses in the seagrass *Thalassia hemprichii*.

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35 13 **Keywords:** Acclimation, sediment, light history, MLR, resuspension, Singapore
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62 **14 Abstract**
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65 15 Elevated sediment delivery and resuspension in coastal waters from human activities such as
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67 16 shipping can have detrimental effects on seagrass health by limiting light penetration.
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69 17 Managing seagrasses requires knowledge of their light acclamatory abilities so guidelines for
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71 18 coastal activities (e.g. ship movements) that influence sediment dynamics can be created.
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73 19 Guidelines typically focus on ensuring that seagrasses are able to meet their minimal light
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75 20 requirements (MLR). MLRs can be achieved by different light regimes, but it remains
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77
78 21 unknown whether a chronically low yet stable light regime is less or more detrimental than a
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80 22 highly variable regime with periods of extreme low to no light. To test this, we compared the
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82 23 physiological and morphological responses of *Thalassia hemprichii* among three light
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84 24 regimes: an open control (30-40% ambient light), a shaded control with (11-15% ambient
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86 25 light), and a fluctuating shade (4-30% ambient light). The MLR for the *T. hemprichii* we
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88 26 studied was lower (4-10% ambient light) than previous reports (mean = 18%) illustrating
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90 27 enhanced light acclimation in Singapore's chronically turbid waters. Seagrass shoots in the
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92 28 shaded control, however, exhibited significantly more morphological stress symptoms, with
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94 29 reduced shoot growth and lower below ground biomass. These data suggest that for seagrass
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96 30 exposed to periods of acute light stress, energetic costs associated with photo-acclimation to
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99 31 more variable light regimes can be offset if the plant can meet its daily light requirements
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101 32 during periods of high light. Management of seagrass beds should incorporate regular light
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103 33 monitoring and move towards an adaptive feedback-based approach to ensure the long-term
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105 34 viability of these vulnerable ecosystems.
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121 **36 1. Introduction**
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124 37 The environmental conditions in ports and harbours are often stressful for coastal marine
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126 38 organisms and systems. In particular, activities such as shipping, dredging and coastal
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128 39 modification result in increased rates of turbidity, leading to light attenuation and
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130 40 sedimentation, which both have serious consequences for sensitive marine ecosystems such
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132 41 seagrass habitats (Erftemeijer and Lewis III, 2006). Light is one of the primary limiting
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134 42 factors for seagrass growth (Ralph et al., 2007) and recent work has demonstrated the
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136 43 importance of the duration and intensity of light reduction (Lavery et al., 2009) as well as the
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138 44 interactive effects of light reduction with environmental factors such as water quality (Leoni
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140 45 et al., 2008; Michael Kemp et al., 2004). It is generally agreed that reduced light availability
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142 46 is a major threat to seagrass beds worldwide (Herzka and Dunton, 1997; Uy, 2001; Waycott
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144 47 et al., 2009).

148 48 As photosynthetic organisms, seagrasses harness light energy to meet their daily carbon
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150 49 budget (Kaldy et al., 2002). To maintain a positive carbon budget, gross photosynthetic yield
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152 50 (CO_2 taken up during photosynthesis) has to be equal or greater than the carbon expended
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154 51 during respiration and growth. Seagrasses have a relatively high respiratory demand due to
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156 52 the extensive non-photosynthetic tissues (e.g. roots and rhizomes) that the plant has to
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158 53 support, hence, they have developed several adaptations that range from physiological (e.g.
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160 54 increased photosynthetic efficiency) to morphological changes (e.g. leaf length and shoot
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162 55 density; Collier et al., 2012; Ralph et al., 2007; Yaakub et al., 2014). These adaptations,
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164 56 which include the capacity to rapidly acclimate to low light levels (Tuya et al., 2016), have
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166 57 improved the ability of seagrasses to meet daily carbon requirements. The results of these
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168 58 previous studies indicate that, as long as seagrasses receive the minimum light requirement
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170 59 (MLR) throughout the day, they can survive substantial periods of low to no light due to
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172 60 storage of carbon reserves.
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180 61 Sediment resuspension events contribute to the highly variable light conditions in shallow
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182 62 intertidal environments. The duration of a sediment resuspension event is largely dependent
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184 63 on sediment characteristics (which affects rate of settlement) and hydrodynamics (which
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186 64 affects the transport and movement of the resuspended particles; Madsen et al., 2001) and can
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188 65 last from minutes to hours. Prolonged resuspension events affect primary productivity of
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190 66 seagrass by reducing light availability and may impact the capacity of seagrass to acclimate
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192 67 to, and recover from, further disturbances in light availability (Yaakub et al., 2014). The
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194 68 physical effects of sediments, however, appear to be less detrimental and many common
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196 69 seagrass species are comparatively tolerant of scouring and settling sediments (Cabaço et al.,
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198 70 2008).

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202 71 Anthropogenically driven sediment resuspension can reduce light levels in shallow seagrass
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204 72 beds from 10-20% ambient photosynthetically active radiation (PAR) (Duarte, 1991;
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206 73 Kenworthy and Fonseca, 1996) to <5% (Browne et al., 2015; Larsen et al., 2017). These
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208 74 light conditions are considered to be below the MLR for most species (Duarte, 1991;
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210 75 Longstaff & Dennison, 1999), thereby impacting their daily carbon budget. However, it is
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212 76 possible that the daily carbon budget of the plant can be met during periods of low turbidity
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214 77 and higher light, such as those found in between pulsed turbidity events, for example. This
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216 78 could provide sufficient energy for seagrasses to meet their daily carbon requirement, but the
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218 79 physiological adjustment to these large fluctuations in light availability—and the required
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220 80 energetic expense of photo-acclimation at the expense of other processes (Major and Dunton,
221
222 81 2002)—may be too stressful for seagrasses and exceed their acclimation potential. Improved
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224 82 understanding of how seagrasses respond to large fluctuations in light availability will help
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226 83 determine whether short acute stress events are more detrimental than longer chronic stress
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228 84 events. This knowledge will aid in the management of activities such as dredging and
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239 85 dumping of sediments in marine waters, with the ultimate aim of preventing impacts to
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241 86 seagrass meadows.
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244 87 This study focuses on the sporadic loss of light associated with ship-wake induced sediment
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246 88 resuspension. This is an ongoing problem in Singapore, one of the world's busiest ports, due
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248 89 to layers of sediment on and near sensitive ecosystems such as coral reefs and seagrass beds
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250 90 being frequently disturbed by the hydrodynamic forces associated with ship-wakes and/or
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252 91 dredging activities (van Maren et al., 2014). We conducted a four week aquarium experiment
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254 92 to test the hypothesis that consistently low levels of light are more detrimental to seagrass
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256 93 than large fluctuations in light. Seagrass condition was compared between seagrasses that
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258 94 received constant low light levels and those that were exposed to large and variable
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260 95 fluctuations in light. Similar mean light levels between the two treatments were maintained
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262 96 to ensure that any difference in condition was due to the pattern of light availability and not
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264 97 total light levels. The experiment required the fabrication of novel apparatus whereby light
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266 98 conditions were pre-programmed to follow light levels measured within sediment
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268 99 resuspension zones in Singapore. These variable light levels were delivered by
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270 100 electronically-controlled shading apparatus.
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274 101 **2 Methods**

275 102 ***2.1 Site description***

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278 103 Approximately 250 ramets of *Thalassia hemprichii* were collected from the reef flat at
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280 104 Cyrene Reef (N 01°15'31, E 103°45'20), Singapore's largest patch reef system situated
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282 105 approximately 3 km south of the mainland (Yaakub et al., 2013). Cyrene Reef is surrounded
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284 106 by busy shipping channels with several hundred ships passing the reef daily (Chou, 2006;
285
286 107 Fig. 1) but, despite these pressures, the reef (1.4 × 0.4 km) hosts 37 coral genera and supports
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288 108 an abundant seagrass meadow comprising *Enhalus acoroides*, *Cymodocea rotundata*,
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298 109 *Cymodocea serrulata*, *Halodule uninervis*, *Halophila ovalis*, *Thalassia hemprichii* and
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300 110 *Syringodium isoetifolium* (Yaakub et al., 2013). *Thalassia hemprichii* was chosen for the
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302 111 present study as it is a climax species that is relatively widespread in Singapore and often
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304 112 dominates seagrass beds in the Indian Ocean and the western Pacific Ocean (Prathep, 2003;
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306 113 Tanaka and Kayanne, 2007).

309 114 **2.2 Experimental design**

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312 115 Seagrass ramets were transported within two hours of collection to the aquarium facilities at
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314 116 the Tropical Marine Science Institute (TMSI) on St. Johns Island. Seagrass ramets were then
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316 117 placed immediately into open-topped transparent acrylic chambers (20 cm diameter × 30 cm
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318 118 high) that had been filled with a 7 cm thick layer of sediment collected from Cyrene Reef. A
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320 119 total of 21 of these chambers were placed in a flow through seawater bath (1.5 × 1.6 m) in an
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322 120 outdoor aquarium (Fig. 2). The outdoor aquarium was covered in shading cloth that allowed
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324 121 ~30-40% of natural ambient sunlight through to represent light attenuation within 1-3 m
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326 122 water depth. Seawater was fed into each individual acrylic chamber from a central reservoir
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328 123 tank. Each chamber was also supplied with an air feed which pumped air directly into the
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330 124 sediments to reduce the build-up of anoxic conditions.

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333 125 The 21 chambers were distributed randomly among three treatments (n=7): Open Control
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335 126 (OC), Shaded Control (SC) and Fluctuating Shade (FS). Seagrass shoots in the open control
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337 127 received ~30-40% natural ambient sunlight while the shaded control treatment received ~11-
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339 128 15%. In contrast, seagrass shoots in the fluctuating shade were exposed to 8 to 12 rapid
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341 129 declines in light followed by a gradual increase in light availability (Rapaglia et al., 2011).
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343 130 These conditions closely followed field observations where sites close to heavy boat traffic
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345 131 receive 8 to 15 sizable ship-wakes (>0.4 m) during daylight hours (pers. Obs.). The severity
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347 132 of a sediment resuspension event following the ship wake is variable, hence the length of
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357 133 each suspension event (10 to 30 min) as well as the frequency of events, were varied daily in
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359 134 the experiment. In the fluctuating shade, light reduction events were executed by automated
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361 135 apparatus that created three light levels via a system of shading nets on motor-controlled
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364 136 rollers. At the start of an event, all three layers of shading net covered the fluctuating shade
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366 137 chambers, reducing light to 4% of natural light levels. Over the course of the next 10-30 min,
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368 138 light levels increased back to 30% as the other two shading layers were removed, thus
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370 139 mimicking the natural slow inclines in light levels following the rapid reduction during a
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372 140 sediment resuspension event.
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375 141 The amount of photosynthetically active radiation (PAR) received for the duration of the
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377 142 experiment was measured using Odyssey Photosynthetic Active Radiation Logger (Dataflow
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379 143 Systems Ltd, New Zealand). Odyssey loggers were calibrated with a high quality PAR sensor
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381 144 (LI-COR 192S) over a 24 h period in a range of light conditions. On a typical day, light
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383 145 levels ranged from 400 to 700 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ in the open control, from 100 to 300
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385 146 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ in the shaded control and from 50 to 450 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ in the
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387 147 fluctuating shade (Fig. 3). These values are comparable to light levels measured at clear and
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389 148 turbid water seagrass sites in Singapore (Yaakub et al., 2014). Over the course of the 4 week
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391 149 experiment, mean light levels in the fluctuating shade were ~13% of natural ambient light,
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393 150 comparable to mean light levels received by seagrass shoots in the shaded control (~11%).
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397 151 **2.3 Data collection**

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400 152 A total of 12 seagrass ramets were placed in each chamber with the rhizomes and roots
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402 153 covered with sediment. Following a one week acclimation period where all three treatments
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404 154 were exposed to ~30-40% ambient sunlight, baseline measurements on maximum quantum
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406 155 yield (F_v/F_m), number of shoots, average number of leaves per shoot, average leaf length, and
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408 156 below ground biomass were recorded for each chamber following the methods described in
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416 157 Duarte and Kirkman (2001). From 12 shoots per chamber, 8 were selected randomly for
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418 158 leaves per shoot count and five shoots for average leaf length measurements. The maximum
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420 159 photosynthetic yield was conducted on 20 min dark adapted shoots using the Diving-PAM
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422 160 Underwater Fluorometer (Walz, Germany). Three yield measurements were taken per
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424 161 chamber using randomly selected ramets. The below ground dry weight measurements were
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426 162 determined using one seagrass ramet per chamber, which was removed on a weekly basis.
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428 163 Care was taken to ensure that the rhizome length (1 cm either side of the shoot) was
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430 164 consistent between treatments and overtime. Each ramet was placed in a plastic sample bag
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432 165 and transported to the lab on ice packs in an opaque cooler. Shoots were then rinsed with
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434 166 filtered seawater to remove dead material and sediments, and kept at -20°C until processing.
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436 167 During seagrass processing, the frozen seagrass ramets were left to thaw in the dark on ice
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438 168 packs for three hours, after which, all epiphytes were removed from the blades using the edge
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440 169 of a glass slide. The ramets were then rinsed with deionised water to remove any remaining
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442 170 epiphytes, dead material or sediment left on the sample. The below ground fraction (stem,
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444 171 rhizome and roots) was dried at 60°C for 24 h. After drying, ramets were placed in a
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446 172 desiccating chamber for 20 min to cool to room temperature and weighed (+0.1 g; Short and
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448 173 Duarte, 2001). The above measurements were repeated weekly for one month. Light (PAR)
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450 174 levels were measured every 10 min over the four weeks using the Odyssey loggers.
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456 176 ***2.4 Statistical analysis***

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459 177 All statistical analyses were performed in RStudio (ver. 3.2.1). A Kruskal Wallis test was
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461 178 carried out to determine whether ambient light levels between 11 am to 3 pm varied
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463 179 significantly among weeks. A Linear mixed effects (LME) model using the lme4 package and
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465 180 normal distributions was used to analyse all variables (leaves per shoot, number of shoots,
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467 181 leaf length and photosynthetic yield). The model included fixed (treatment and week) and
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475 182 random variables (chamber). As the aim of the LME models was to determine whether
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477 183 treatment, week and treatment \times week had a significant influence on seagrass health,
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479 184 Likelihood Ratio Tests (LRTs) were used to determine whether a model including the
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482 185 treatment, week or interaction provided a significantly better fit than the alternative model
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484 186 (Stuard and Ord, 1994). The log-likelihood values for each model, calculated using the
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486 187 maximum likelihood procedure, were tested for significant difference in deviance. The χ^2
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488 188 output was compared against its probability distribution using the associated degrees of
489
490 189 freedom to determine the p-value. Pair-wise Tukey post hoc tests determined which
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492 190 treatments and/or weeks were significantly different. Model assumptions were tested by
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494 191 plotting the standardised residuals against the fitted values to ensure homoscedasticity, and a
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496 192 Q-Q plot of the residuals to ensure normality. Yield data were log₁₀ (K-X) transformed to
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498 193 meet model assumptions.
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504 195 **3. Results**

506 196 **3.1 Ambient light**

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508 197 Over the course of the four weeks, the mean midday (11 am to 3 pm) light level under the
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510 198 shade cloth (~30-40% of natural light) was $464 \pm 12 \mu\text{mol photons m}^{-2} \text{s}^{-1}$, although this
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512 199 varied significantly among weeks ($\chi^2 (3) = 83.9, p < 0.001$) with greater light levels recorded
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514 200 in week 1 (mean = 675 ± 25.6 , range = 170 to 1220 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$) and consistently
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516 201 low levels in week 2 (mean = 384 ± 12.9 , range = 20 to 675 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$). Weeks 3
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518 202 (mean = $454 \pm 25.3 \mu\text{mol photons m}^{-2} \text{s}^{-1}$) and 4 (mean = $361 \pm 21.7 \mu\text{mol photons m}^{-2} \text{s}^{-1}$)
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520 203 were characterised by variable light levels ranging from extremely low levels of <5 to 990
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522 204 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ (Fig. 4).
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3.2 Yield

The maximum photosynthetic yield varied significantly over the four weeks ($p < 0.001$; Table 1). Yields in week 3, when extreme low light levels were recorded, were significantly greater than all other weeks (Tukey post hoc test $p < 0.001$; Fig. 5a) and yields in week 1, when light levels were elevated, were significantly lower than all weeks (Tukey post hoc test $p < 0.01$). The removal of treatment from the model had no significant effect on the yield ($p = 0.363$), and the inclusion of the interaction between treatment and week did not improve the model confirming that yields among treatments were comparable over time (Table 1). Yields of *T. hemprichii* in the open control treatment during the two extreme weeks (week 1 and week 3) were lower than those measured for seagrasses in both the shaded control and fluctuating shade

3.3 Leaves per shoot

There was no significant difference in the number of leaves per shoot among treatments over the course of the experiment ($p = 0.117$, open control mean = 3.11 ± 0.08 , shaded control mean = 2.88 ± 0.05 , fluctuating shade mean = 2.84 ± 0.07). However, time had a significant influence on the model ($p = 0.002$) with significantly greater numbers of leaves (Tukey post hoc test $p = 0.05$) observed in week 4 compared to baseline levels (Fig. 5b). This increase over time was not consistent among treatments, illustrating that shoots were responding significantly differently among treatments over time. The best model, therefore included the interaction between treatment and time ($p = 0.003$; Table 1). The number of leaves in the shaded control were comparatively stable whereas more leaves per shoot were observed in the open control by week 1 and in the fluctuating shade chambers by week 3.

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593 **228 3.4 Number of shoots**
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596 229 Both treatment ($p=0.017$) and time ($p=0.046$) had a significant impact on the model for the
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598 230 mean number of shoots per chamber. However, the best model included the interaction
600 231 between treatment and time ($p=0.002$, Table 1) suggesting that the seagrass was responding
602 232 differently among treatments. The number of shoots observed in the open control steadily
604 233 increased during the four weeks whereas there was little change in both the shaded control
606 234 and fluctuating shade chambers (Fig. 5c).

609
610 **235 3.5 Leaf length**
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612 236 The best model for leaf length incorporated time only ($p<0.001$). There was a significant
614 237 decline in leaf length over the four weeks ($p<0.001$) with a reduction in mean leaf length
616 238 from 4.52 ± 0.13 cm in the baseline assessment to 2.98 ± 0.11 cm by the end of week 4 (Fig.
618 239 5d). This decline in leaf length was observed in all three treatments at comparable rates,
620 240 hence treatment and treatment \times time did not significantly improve the model ($p=0.126$ and
622 241 $p=0.868$; Table 1).

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626 **242 3.6 Below ground biomass**
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629 243 Below ground biomass differed significantly over time ($p=0.005$) and among treatments
630 244 ($p<0.001$, Table 1). The change in biomass over time was, however, comparable between
632 245 treatments, and hence the best model included treatment and time, but not the interaction.
634 246 Mass was significantly lower in weeks 2 and 3, before recovering in the final week (Fig. 5e).
636 247 The change in biomass was more variable in the open control and fluctuating shade, yet
638 248 biomass was significantly higher in these two treatments compared to the shaded control
640 249 (Tukey post hoc test $p<0.019$).

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644 **250 4. Discussion**
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652 251 *Thalassia hemprichii* displayed both physiological and morphological changes in response to
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654 252 variations in the light regime. Singapore's equatorial position results in high light levels
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656 253 ($>2000 \mu\text{mol photons m}^{-2} \text{s}^{-1}$), so even seagrasses growing in turbid waters may experience
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658 254 periods of excessive light and photo-inhibition, especially those growing in the intertidal
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660 255 zone, where they can be fully or partially exposed. However, reductions in light due to
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662 256 elevated sediment loads and sediment resuspension activities are potentially a greater threat
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664 257 to seagrass health. Minimum light requirements (MLR) vary within and between seagrass
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666 258 species (Lee et al., 2007) and have been shown to be dependent on their light history (Yaakub
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668 259 et al., 2014). Here we found that *T. hemprichii* at the equator can survive a few weeks at a
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670 260 lower MLR ($<30 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ or between 4-10% surface ambient) than previously
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672 261 reported (18%; Lee et al., 2007) suggesting that Singapore's seagrasses have acclimated to its
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674 262 turbid waters. However, it was the seagrass shoots in the chronically low light treatments, as
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676 263 opposed to the fluctuating light treatment (which included periods of extreme low light), that
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678 264 exhibited greater stress symptoms (reduced shoot growth and below ground biomass). We
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680 265 therefore postulate that a chronically low light level ($100\text{-}200 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ or 6-12%
681
682 266 ambient light) is more detrimental to seagrass health than acute stress events that result in
683
684 267 highly variable light levels (<10 to $400 \mu\text{mol photons m}^{-2} \text{s}^{-1}$) including periods of low to no
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686 268 light.

690
691 269 The maximum photosynthetic yield in all treatments was highly variable over time
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693 270 illustrating the capacity of *T. hemprichii* to change yield rapidly in response to differences in
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695 271 light. Maximum quantum yields were significantly lower during periods of high light and
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697 272 increased when light levels fell, a trend also observed for laboratory cultured *Halophila*
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699 273 *ovalis* (Ralph, 1999). Rapid acclimation of seagrass photosystems enables them to survive
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701 274 during periods of low light, whether due to natural daily and seasonal differences in ambient
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703 275 light or anthropogenic effects such as increased turbidity levels. In Singapore, coastal
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711 276 turbidity in <3 m water depth typically ranges from 5 to 50 mg l⁻¹ with peaks exceeding 100
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713 277 mg l⁻¹ following sediment resuspension events (Browne et al., 2015). Despite the lack of
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715 278 significant difference among treatments, yields in the shaded treatments were typically higher
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718 279 and more stable (F_v/F_m : 0.62 to 0.77) than in the open treatment (F_v/F_m : 0.56 to 0.73).
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720 280 Similar differences have also been observed in the field for two *Zostera* species, whose
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722 281 maximum yields were more variable at high light sites (F_v/F_m : 0.6 to 0.77) and more stable at
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724 282 turbid water sites (F_v/F_m : 0.7-0.8; Campbell et al., 2003). These data suggest that, although
725
726 283 rapid reductions in light (as observed during sediment resuspension events) will result in
727
728 284 increases in the photosynthetic yield of *T. hemprichii*, the daily and weekly fluctuations in
729
730 285 ambient light in Singapore waters have an important influence on seagrass and their survival
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732 286 in local turbid conditions.

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734
735 287 Light levels in both the shaded treatments ($\leq 13\%$) were well within the potential range of
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737 288 minimum light requirements (MLR) for seagrasses (Lee et al., 2007). The MLR varies
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739 289 between and within seagrass species as a result of species-specific differences in their
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742 290 photosynthetic capacity and previous light history regime (Chartrand et al., 2016). MLR
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744 291 usually falls between 2 to 37% of surface irradiance, with previous studies reporting 18% for
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746 292 *Thalassia* spp. (Lee et al., 2007). During week 2, ambient light levels were significantly
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748 293 lower and, hence, light reaching the seagrass dropped to 30-50 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ (10-13%
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750 294 ambient light) in the shaded control and 5-50 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ (4-13% ambient light) in
751
752 295 the fluctuating shade. Maximum quantum yield (F_v/F_m) remained at ~ 0.65 in the shaded
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754 296 control, but dropped to 0.58 in fluctuating shade for some seagrass shoots, signifying light
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756 297 stress. This indicates that *T. hemprichii* is able to tolerate low light levels of $< 30 \mu\text{mol}$
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758 298 $\text{photons m}^{-2} \text{s}^{-1}$ or between 4-10% ambient light: levels lower than previously reported
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760 299 averages for this genus in equatorial regions (Lee et al., 2007). *T. hemprichi* in Singapore
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762 300 may have acclimated to local turbid conditions with a widely fluctuating light regime,
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770 301 analogous to the findings of Yaakub et al. (2014) who reported an MLR of 11-18 μmol
771
772 302 photons $\text{m}^{-2} \text{s}^{-1}$ for *Halophila ovalis*. This highlights the lack of understanding regarding *in*
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774 303 *situ* light requirements for seagrass (Ralph et al., 2007) and supports the growing body of
775
776 304 evidence that MLRs have limited generality. It is clear that site-specific light regimes must
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778 305 be understood when managing seagrass meadows (Chartrand et al., 2016; Yaakub et al.,
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781 306 2014).

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783 307 Seagrasses in Singapore may also experience periods of photoinhibition despite high turbidity
784
785 308 due to the region's variable light regime. Singapore is located near the equator where light
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787 309 levels per square meter are maximised, but is also subject to a weather regime characterised
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789 310 by high cloud cover and storm events (Meteorological Service of Singapore, 2016). During
790
791 311 the four weeks of the experiment, ambient light levels typically ranged from <400 (week 2) to
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793 312 >2000 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ (week 1). It was during week 1 that the maximum quantum yield
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795 313 in the open control dropped to <0.57, suggesting that seagrass in the highest light treatment
796
797 314 were likely experiencing photoinhibition (Kitajima and Butler, 1975). Jiang et al., (2014)
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799 315 recorded photoinhibition in *T. hemprichii* when shoots were emerged at noon and light levels
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801 316 reached between 1000-1500 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$, comparable to levels recorded in the open
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803 317 control in week 1. In contrast, *T. hemprichii* shoots in the shaded treatments had yields >0.6,
804
805 318 demonstrating that during periods of high irradiance, shading from turbid waters may confer
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807 319 some benefits to seagrass as it has for other submersed macrophytes (Hanelt, 1992). The
808
809 320 potential benefits of shading by sediments for corals during bleaching events have recently
810
811 321 been described (Cacciapaglia and van Woesik, 2016) but comparable studies are lacking for
812
813 322 seagrass ecosystems. To date, most studies of photoinhibition have focused on exposure and
814
815 323 temperature stress (Campbell et al., 2006; Ralph and Burchett, 1995).

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817 324 The different morphological responses of *T. hemprichii* show that chronic low light levels
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819 325 potentially have more influence on seagrass growth patterns than a variable light regime that
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326 includes acute stress (very low light) events. Morphological responses under sufficient light
327 include increasing the number of leaves as well as the number of shoots (Abal et al., 1994;
328 Collier et al., 2012). Neither of these responses were observed in the shaded control where
329 light levels were consistently low. Previous studies have reported that when light becomes
330 limiting, decreasing and/or maintaining a low number of leaves can reduce self-shading
331 effects (Ralph et al., 2007). This morphological plasticity has the secondary benefit of
332 reducing the plant's respiratory demands, thereby maximising its carbon budget (Ruiz and
333 Romero, 2001). In contrast, high leaf production but not shoot production was observed in
334 the fluctuating shade. It is more energetically demanding to produce and maintain new
335 shoots (and associated rhizome extension and root formation) than new leaves, and in
336 environments where there are extreme fluctuations in light availability, the root/rhizome
337 systems can become a burden on the plant due to the high respiratory demand of these non-
338 photosynthetic parts (Hemminga, 1998). Hence, reduced shoot production is considered to
339 be an effective acclimation response to low light levels at the seagrass meadow scale
340 (Campbell and Fourqurean, 2013; Dennison and Alberte, 1985). The observed
341 morphological response suggests that the highly variable light regime in the fluctuating shade
342 was impacting seagrass growth in relation to the normal light levels in the open control, but
343 to a lesser extent than in the shaded control.

344 Seagrasses growing in light limited environments have also been reported to have wider
345 and/or longer leaves as a means of increasing surface area and light absorption efficiencies
346 (Lee and Dunton, 1997; Longstaff and Dennison, 1999; Olesen et al., 2002). Yet, we saw a
347 significant decline in leaf length in all three treatments during weeks 1-3, before leaf length
348 stabilised at 3 cm in week 4. Leaf size has been observed to decline during periods of
349 respiratory stress in order to reduce the respiratory demands of the plant (Campbell and
350 Miller, 2002). Given that there were no significant differences in leaf length among

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351 treatments, the decline in leaf length in the first three weeks was most likely due to additional
352 stress factors associated with aquarium experiments and not changes in the light regime. The
353 most likely explanation is the reduced water flow in the chambers, an artefact of conducting
354 experiments in a controlled and confined setting where the specific light environments could
355 be re-created. This may have further influenced the sea grass ability to effectively allocate
356 resources in this artificial environment. However, conducting such an experiment in situ
357 would be extremely difficult due to the inherently variable environmental conditions.

358 Fluctuations in the below ground biomass among treatments over the course of the
359 experiment were comparable, but total weight was consistently reduced in the shaded control.
360 Below ground biomass fell following low light levels in week 2 and it is possible that
361 seagrass shoots in all treatments were accessing energy reserves in their rhizomes to
362 compensate for reduced photosynthesis (Cabello-Pasini et al., 2002; Dawes and Lawrence,
363 1980; Lee and Dunton, 1996). Lee and Dunton (1997) also reported significant declines in
364 the tissue carbohydrate of *T. testudinum* due to declines in underwater light. For seagrass
365 shoots in the shaded control, the additional declines in below ground biomass may have been
366 due to anaerobic respiration caused by consistently low light, which leads to an increase in
367 carbon demand and a decrease in carbohydrate reserves (Lee and Dunton, 1997). Our results
368 suggest that the light regime in the shaded control caused the seagrass shoots to utilise more
369 of their energy reserves and this may have long-term detrimental effects on seagrass health
370 and survival.

371 This study shows that the temporary pattern of light levels can be used as an additional
372 component to the management of seagrasses. We showed that acute light reduction (such as
373 short term turbidity events) is less damaging to seagrass compared to chronic and sustained
374 light reduction. There is potential to apply this knowledge to manage anthropogenic activities
375 that result in short term turbidity pulses. For example, such operations could be performed

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947 376 during low tides only, where shallower water depths will militate against the amount of light
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949 377 reaching seagrass, ensuring sufficient light penetration and absorption to meet the plant's
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951 378 daily carbon budget. However, to implement such planning entails having sufficient
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953 379 knowledge of seagrass *in situ* light requirements and site-specific light patterns. It also
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955 380 highlights the need for regular monitoring and an adaptive management strategy that is able
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957 381 to react to the resultant data (e.g. Chartrand et al., 2016). Ensuring the adoption of these
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959 382 measures industry wide can be challenging, but having them incorporated into guidelines,
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961 383 such as those previously published by associations (e.g. PIANC - Permanent International
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963 384 Association of Navigation Congresses), can provide an avenue for widespread acceptance
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965 385 and adoption of these practices.
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973
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977
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981
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984 393 sample collection was carried out under the research permit NP/RP 12-007 and with
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986 394 permissions from the Singaporean Marine Port Authority (MPA).
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1006 **Figure legends**
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1009 Figure 1: Map of Singapore with seagrass meadows and major ship navigation channels
1010 highlighted. Cyrene Reef, where seagrass rametss were collected, is situated in between
1011 several major shipping channels.
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1016 Figure 2: Experimental setup demonstrating how the 21 acrylic chambers (circles) were
1017 positioned in the seawater bath, with seven chambers per treatment.
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1021 Figure 3: A comparison of light levels (PAR) between treatments (OC = open control, SC =
1022 shaded control, FS = fluctuating shade) on a typical day.
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1026 Figure 4: Light levels recorded for the open controls (OC) under the 30-40% shade cloth for
1027 the four weeks of the experiment.
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1031 Figure 5: *T.hemprichii* responses to the three treatments (OC = open control, SC = shaded
1032 control, FS = fluctuating shade) for A. maximum quantum yield, B. leaves per shoot, C.
1033 number of shoots, D. leaf length, and E. below ground biomass.
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1065 **Tables**
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1068 Table 1: Summary of the Linear Mixed Effects model for each parameter measured. The best model per parameter is highlighted in bold. Tukey
1069 post hoc results provided where necessary (BL = baseline).
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	Full Model	AIC	Alternative model	AIC	p-value of LRT	Post hoc
Yield	Yield~Treatment*Week + 1 Chamber	-198.5	Yield~Treatment+Week + 1 Chamber	-200.2	0.111	
	Yield~Treatment+Week + 1 Chamber	-200.2	Yield~Week + 1 Chamber	-202.1	0.363	
	Yield~Treatment+Week + 1 Chamber	-200.2	Yield~Treatment + 1 Chamber	-157.7	<u><0.001</u>	Week 3 >2,4 >1
Leaves per shoot	Density~Treatment*Week + 1 Chamber	79.7	Density~Treatment+Week + 1 Chamber	87.2	<u>0.003</u>	
	Density~Treatment+Week + 1 Chamber	87.2	Density~Week + 1 Chamber	87.5	0.117	
	Density~Treatment+Week + 1 Chamber	87.2	Density~Treatment + 1 Chamber	96.3	<u>0.002</u>	Week 4 > BL, 2
Shoots	Shoot~Treatment*Week + 1 Chamber	317.9	Shoot~Treatment+Week + 1 Chamber	327.3	<u>0.002</u>	
	Shoot~Treatment+Week + 1 Chamber	327.3	Shoot~Week + 1 Chamber	331.3	<u>0.017</u>	OC > SC
	Shoot~Treatment+Week + 1 Chamber	327.3	Shoot~Treatment + 1 Chamber	329.3	<u>0.046</u>	Week 4 > 1
Leaf length	Length~Treatment*Week + 1 Chamber	6924.4	Length~Treatment+Week + 1 Chamber	6912.2	0.868	
	Length~Treatment+Week + 1 Chamber	6912.2	Length~Week + 1 Chamber	6912.4	0.126	
	Length~Treatment+Week + 1 Chamber	6912.2	Length~Treatment + 1 Chamber	7024.5	<u><0.001</u>	BL > 1 > 2,3,4
Biomass	Biomass~Treatment*Week + 1 Chamber	-314.3	Biomass~Treatment+Week + 1 Chamber	-322.5	0.705	
	Biomass~Treatment+Week + 1 Chamber	-322.5	Biomass~Week + 1 Chamber	-304.7	<u><0.001</u>	OC , ST > SC
	Biomass~Treatment+Week + 1 Chamber	-322.5	Biomass~Treatment + 1 Chamber	-315.6	<u>0.005</u>	Week 1 > 2,3

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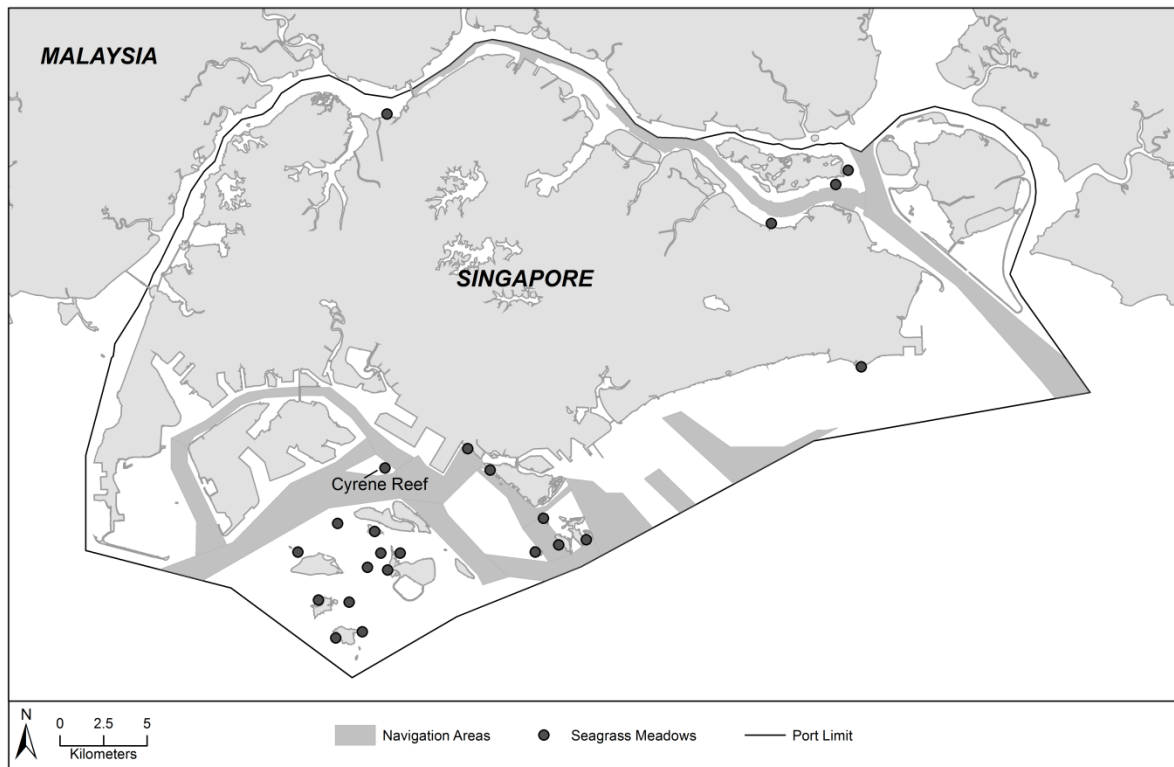
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1342 **Figures**
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1370 Figure 1: Map of Singapore with seagrass meadows and major ship navigation channels
1371 highlighted. Cyrene Reef, where seagrass ramets were collected, is situated in between
1372 several major shipping channels.
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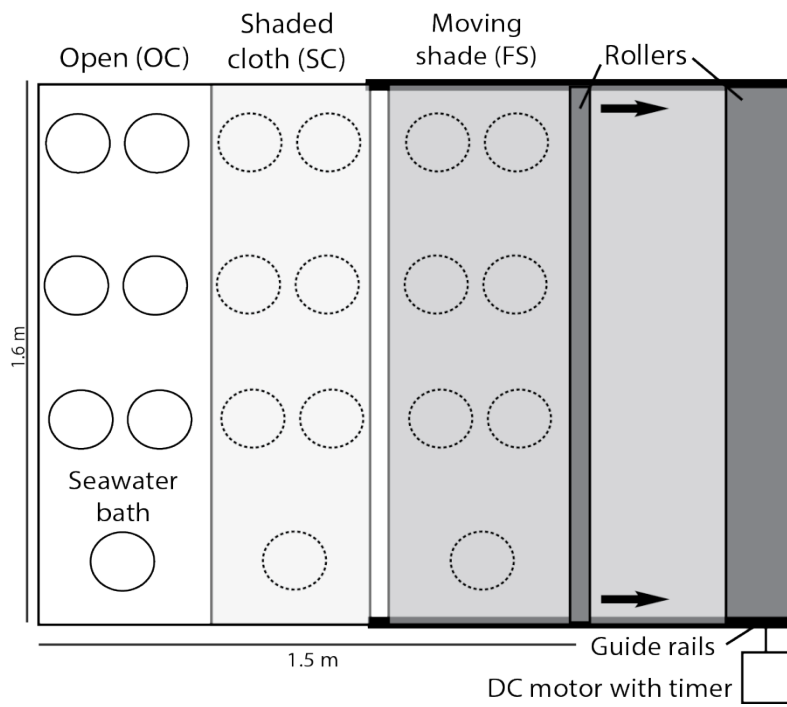


Figure 2: Experimental setup demonstrating how the 21 acrylic chambers (circles) were positioned in the seawater bath, with seven chambers per treatment.

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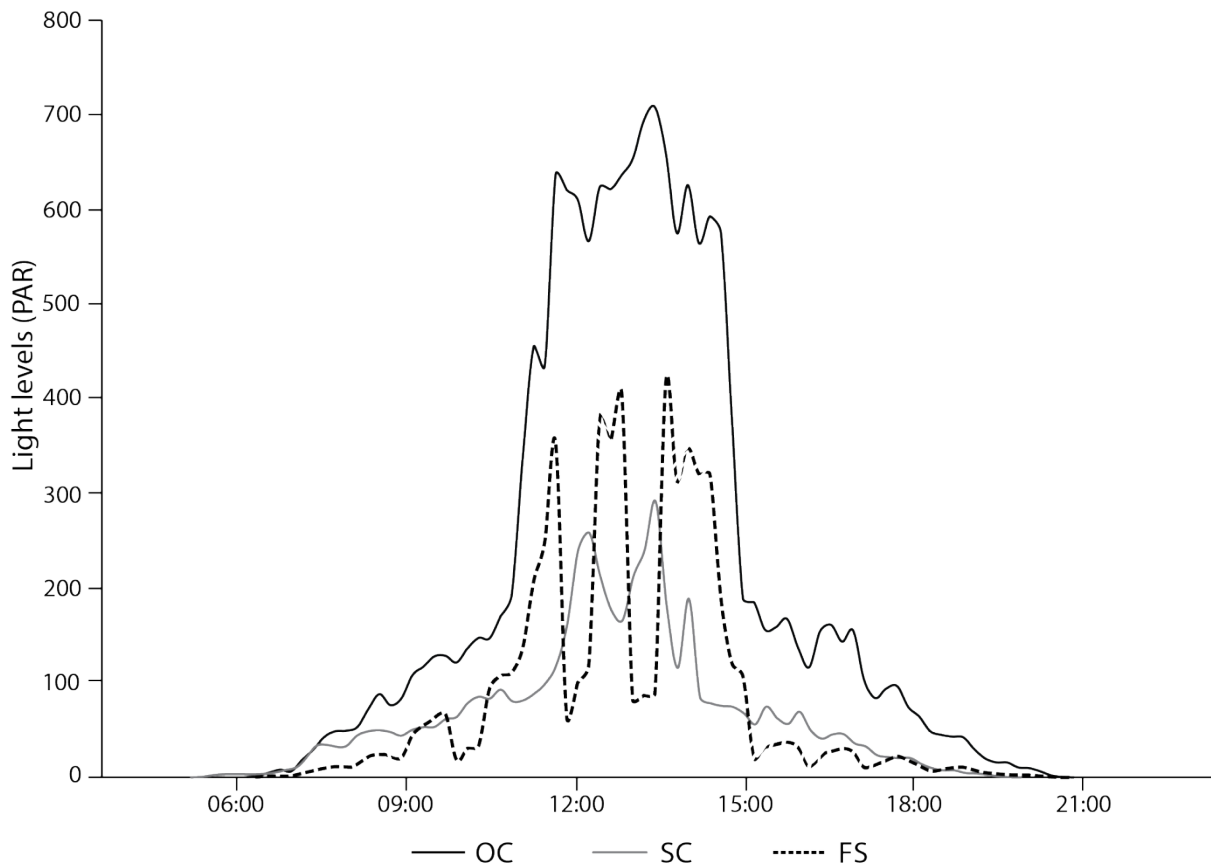


Figure 3: A comparison of light levels (PAR) between treatments (OC = open control, SC = shaded control, FS = fluctuating shade) on a typical day.

1 column

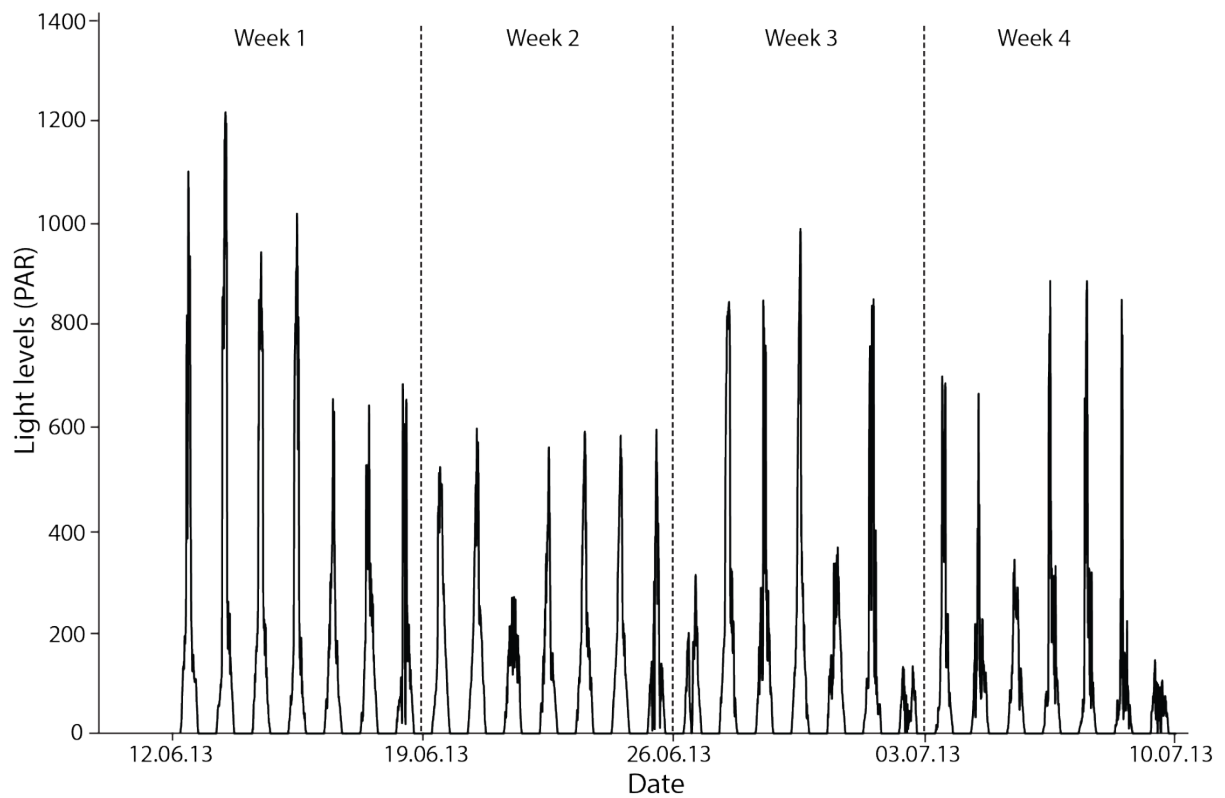


Figure 4: Light levels recorded for the open controls (OC) under the 30-40% shade cloth for the four weeks of the experiment.

1 column

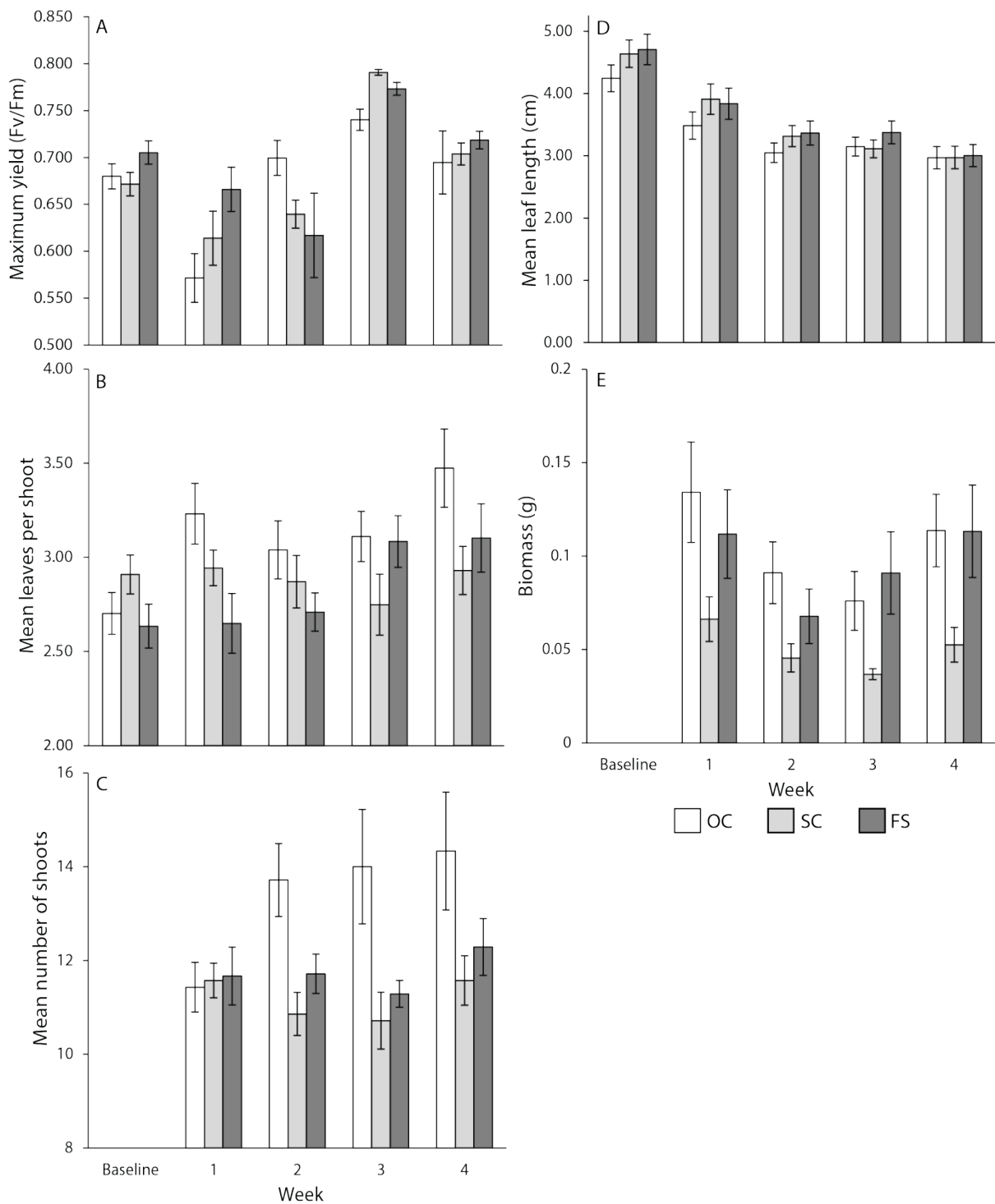


Figure 5: *T. hemprichii* responses to the three treatments (OC = open control, SC = shaded control, FS = fluctuating shade) for A. maximum quantum yield, B. leaves per shoot, C. number of shoots, D. leaf length, and E. below ground biomass.

2 columns