

Accepted Manuscript

Living at the margins – The response of deep-water seagrasses to light and temperature renders them susceptible to acute impacts

Kathryn M. Chartrand, Milán Szabó, Sutinee Sinutok, Michael A. Rasheed, Peter J. Ralph



PII: S0141-1136(17)30728-6

DOI: [10.1016/j.marenvres.2018.02.006](https://doi.org/10.1016/j.marenvres.2018.02.006)

Reference: MERE 4455

To appear in: *Marine Environmental Research*

Received Date: 30 November 2017

Revised Date: 5 February 2018

Accepted Date: 8 February 2018

Please cite this article as: Chartrand, K.M., Szabó, Milá., Sinutok, S., Rasheed, M.A., Ralph, P.J., Living at the margins – The response of deep-water seagrasses to light and temperature renders them susceptible to acute impacts, *Marine Environmental Research* (2018), doi: 10.1016/j.marenvres.2018.02.006.

This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

1 **Title:** Living at the margins – the response of deep-water seagrasses to light and temperature
2 renders them susceptible to acute impacts
3 (Old title: Living at the margins – the response of deep-water seagrasses to light and temperature
4 provides a framework for acute impact management)
5

6 **Authors:** Kathryn M. Chartrand^{*1,2}, Milán Szabó^{2,1}, Sutinee Sinutok^{2,II,III}, Michael A. Rasheed¹,
7 Peter J. Ralph²
8

9 **Institutional Affiliations:**

10 ¹ Centre for Tropical Water & Aquatic Ecosystem Research, James Cook University, Cairns, Queensland,
11 Australia.

12 ² Climate Change Cluster, University of Technology Sydney, Broadway, New South Wales, Australia.

13 ¹ Institute of Plant Biology, Biological Research Centre, Hungarian Academy of Sciences, Szeged, Hungary.

14 ^{II} Faculty of Environmental Management, Prince of Songkla University, Hat Yai, Thailand.

15 ^{III} Coastal Oceanography and Climate Change Research Center, Prince of Songkla University, Hat Yai,
16 Thailand.
17

18 * **Corresponding Author:** Kathryn M Chartrand

19 TropWATER, James Cook University

20 McGregor Road E01.16G

21 Cairns, QLD 4878 Australia

22 **Email:** Katie.Chartrand@jcu.edu.au

23 **Phone:** +61 7 4232 2027 / +61 409903113
24

25 **Running Head:** Impact of temp and light on deep-water seagrass
26
27
28

29

30

31

32

33 ABSTRACT

34 Seagrasses inhabit environments where light varies at different timescales, nonetheless are acutely sensitive
35 to reductions in light beyond some conditional bounds. Two tropical deep-water seagrasses, *Halophila*
36 *decipiens* and *Halophila spinulosa*, from the Great Barrier Reef were tested for their response to defined
37 light and temperature regimes to identify their growth requirements and potential thresholds of mortality.
38 Species were exposed to two light intensities, saturating ($75 \mu\text{mol photons m}^{-2} \text{s}^{-1}$) and limiting ($25 \mu\text{mol}$
39 $\text{photons m}^{-2} \text{s}^{-1}$) light and two temperature treatments (26°C and 30°C) over a four-week period. Wavelength-
40 specific parameters of PSII photochemistry were evaluated for seagrass leaves, as well as shoot density, gas
41 exchange, and pigment content. Both species were sustained under saturating light levels (3.2 mol photons
42 $\text{m}^{-2} \text{d}^{-1}$) while limiting light led to decreased shoot density for *H. decipiens* and *H. spinulosa* after two and
43 four weeks, respectively. Wavelength-specific photochemistry was also affected under light-limiting
44 treatments for both species while the functional absorption cross section was highly conserved.
45 Photoacclimation and physiological adjustments by either species was not adequate to compensate for
46 reduced irradiance suggesting these plants reside at the margins of their functional limits. As such, relatively
47 short periods of light attenuating events, like dredging or flood plumes, may be detrimental to deep-water
48 seagrass populations.

49

50 **Key words:** deep-water, seagrass, *Halophila decipiens*, *Halophila spinulosa*, light, temperature; PAM
51 fluorometry; wavelength-specific photochemistry; Great Barrier Reef

52

53 1. INTRODUCTION

54 It is widely accepted that seagrasses are critical to the health and ecosystem function of the coastal marine
55 environment. They provide key inter-habitat connectivity for migrating fauna, feeding grounds for globally
56 threatened turtles and dugong, habitat for commercially important fisheries, sediment trapping and
57 stabilisation, effective nutrient filtration from coastal inputs and carbon sequestration (Duarte et al., 2010;

58 Heck et al., 2008; Hemminga and Duarte, 2000; Jackson et al., 2001; Orth et al., 2006). Despite being highly
59 valued globally for their contribution to these ecosystem services, seagrass habitats are threatened by a range
60 of anthropogenic activities including coastal development and declining water quality from poor catchment
61 management practices (Costanza et al., 2014; Grech et al., 2012; Waycott et al., 2009), and compounded by
62 natural events such as severe storms and flooding that can accentuate seagrass decline (Rasheed et al., 2014).

63

64 The vast majority of seagrass species are located in relatively shallow water habitats with quiescent
65 conditions, favourable sediment chemistry, and where light is adequate to meet gross energy requirements
66 (Hemminga and Duarte, 2000; Koch, 2001). In the Great Barrier Reef World Heritage Area (GBRWHA),
67 research and monitoring programs have detailed the distribution, seasonality, environmental drivers, risks
68 and threats to these specialised plant communities (Bryant et al., 2013; Collier et al., 2012a; Grech et al.,
69 2011; McKenna et al., 2015). However, information on deep-water tropical seagrass communities —
70 generally classified as growing at depths >10-15 m — is limited (Carruthers et al., 2002; Fonseca et al.,
71 2008; Hammerstrom et al., 2006; Josselyn et al., 1986). These deeper meadows are primarily composed of
72 species from the genus *Halophila* (Hydrocharitaceae) and within the GBRWHA have been mapped down to
73 60 m and modelled to cover over 40,000 km² of the seafloor (Coles et al., 2009).

74

75 *Halophila* spp. have size-associated characteristics that are likely to play an important role in their
76 dominance of deep-water seagrass meadows. Small delicate leaves, oval or oblong in shape, occur in pairs
77 that attach directly to either a vertical stem or rhizome via a petiole. Their short canopy height may increase
78 risk of burial from sediment deposition; however, rapid leaf turnover and opportunistic growth can negate
79 this issue (Duarte et al. 1997, Terrados et al. 1998, Cabaço et al. 2008). With leaves only two-cells thick,
80 they have minimal lacunar space and contain densely packed chloroplasts in the epidermal layer (Roberts et
81 al. 1984, Josselyn et al. 1986, Kenworthy et al. 1989, Cambridge & Lambers 1998). Thin leaves allow for
82 quick and efficient gas exchange of evolved oxygen from saturated epidermal cells and reciprocal carbon
83 uptake for fixation (Larkum et al. 2006). Comparatively, seagrasses with high standing biomass (such as
84 *Posidonia* and *Zostera*) have higher diffusive boundary layers which could make living at depth with less
85 wave action and water movement a challenge for gas exchange and acquiring limited resources (Enríquez
86 and Rodríguez-Román, 2006). Minimal below-ground biomass also makes *Halophila* spp. well suited to

87 grow at greater depths and in shallow turbid areas with chronic low light (Kuo & Kirkman 1995, Durako et
88 al. 2003). Non-photosynthetic below-ground tissues can act as a respiratory burden which may ultimately
89 limit the compensation depth of structurally larger species (Fourqurean & Zieman 1991, Larkum et al. 2006).

90

91 The capacity to cope with both a quantitatively low-light environment and a narrowed spectral window of
92 light is critical to living at depth (Duarte, 1991; Ralph et al., 2007); yet little is known about the spectral
93 tuning of deep-water seagrasses. Deep-water seagrasses likely have a reduced threshold of tolerance to low-
94 light because they are growing under lower ambient light intensities, a smaller range of intensities, and a
95 significantly narrowed spectral window from which to harvest light energy (Larkum et al., 2006). Strategies
96 for deep-water seagrasses to maintain a positive carbon balance likely involve the same mechanisms
97 observed in their shallow water counterparts to cope under low-light conditions (Collier et al., 2012b):
98 modifying light harvesting capacity and the efficient use of light (Abal et al., 1994; Enriquez, 2005);
99 adjustments to rates of growth and plant turnover (Collier et al., 2012b); and drawing upon carbohydrate
100 reserves to maintain a positive carbon balance (Burke et al., 1996; Touchette and Burkholder, 2000a).
101 Temperature, which directly affects metabolic rates of carbon fixation and respiration in plants, influences
102 whether photophysiological adjustments to a low-light environment are sufficient to maintain a net positive
103 carbon balance, as opposed to a net negative; the latter of which would lead to plant- or meadow-scale losses
104 (Bulthuis, 1987; Lee et al., 2007).

105

106 Some deep-water *Halophila* populations are annual or ephemeral in their above-ground presence, likely in
107 response to seasonally unfavourable conditions to support positive growth and carbon balance (York et al.,
108 2015). In these circumstances the plants may rely on high investment in the production of seeds and a seed
109 bank on which recovery and population maintenance depend (Hovey et al., 2015; Rasheed et al., 2014).

110

111 Photoacclimation to low-light environments by seagrasses is similar to that seen in other higher plants (Ralph
112 et al., 2007; Smith, 1982). Changes in accessory pigment content can increase light capture efficiency and
113 its' relative use in the photochemical pathways (Falkowski and Raven, 2007). However, a point can be
114 reached beyond which the self-shading of pigments reduces the effectiveness of this strategy (i.e. the
115 package effect; Cummings and Zimmerman, 2003). While the capacity for photoacclimation to total light

116 reduction is well documented in higher plants including seagrasses, qualitative shifts in the spectral
117 distribution of available light at depth is largely undescribed for seagrass with an exception by Sharon et al.
118 (2011) on *H. stipulacea* growing at 48 m in the Red Sea. While the spectral distribution of light with depth
119 varies according to the absorption properties of the water, total spectral attenuation of light >600 nm occurs
120 in the GBRWHA at ≥ 10 m (Appendix Figure 1). Descriptions of seagrass pigment signatures is somewhat
121 typical of a green higher plant with chlorophyll and a suite of accessory pigments that absorb light largely in
122 the blue (400-500 nm) and red bands (650-680 nm) (Costa et al., 2014). How a spectrally-attenuated light
123 climate affects the photosynthetic properties, pigment composition, or light capture efficiency of *Halophila*
124 spp. growing in >10 m is largely undescribed.

125

126 Measuring photosynthetic capacity to understand plant condition under varying light intensities with pulse-
127 amplitude modulated (PAM) chlorophyll fluorometry is commonplace in recent years, whether in the
128 laboratory or field (Cosgrove and Borowitzka, 2010; Schreiber, 2004). A PAM fluorometer typically
129 measures variable chlorophyll fluorescence parameters, from which photochemical efficiency of PSII can be
130 calculated, energy transfer efficiency can be measured, and energy dissipation quantified. However, these
131 measurements do not account for variable absorption by PSII across the PAR spectrum which can vary
132 widely by wavelength depending on the pigment composition and its' ambient growing conditions (Schreiber
133 et al., 2012; Szabó et al., 2014).

134

135 In this study, we investigate the effects of light intensity and temperature on the two most prevalent deep-
136 water seagrasses in the GBRWHA (Coles et al., 2009), *Halophila decipiens* and *Halophila spinulosa*. *H.*
137 *decipiens* has a pan-tropical distribution and has a small stature in both above and below-ground tissues
138 (Waycott et al., 2004). *H. spinulosa* is limited to the Indo-Pacific region, has similar oblong leaf pairs, but
139 grows upright on a vertical stem, creating a much larger canopy-forming habitat.

140

141 Both species were expressly sourced from deep-water meadows, whereby depth creates unique inherent
142 challenges to the biology and physiology of the plant from that of a turbid shallow water habitat: 1) a unique
143 spectral signature in which light >600nm is absent; 2) lower variation in water quality related to tidal effects,
144 coastal runoff, and sediment re-suspension due to wind and wave activity; and 3) a unique pressure

145 environment which may impact leaf diffusion and plant physiology (Beer and Waisel, 1982). We assessed
146 morphological, optical and physiological adjustments to both plants when they were exposed to peak
147 growing season light (spectrally-adjusted) and temperature conditions (Chartrand in prep) versus reduced
148 light levels and elevated temperatures. We measured wavelength-dependent photochemical efficiencies,
149 oxygen production, pigment composition, carbohydrate reserves and shoot densities over a four-week period
150 to assess changes in the plants. The aim of this experiment was to i) describe the changes in optical,
151 photochemical, physiological, and physical characteristics used to cope with light and temperature stress
152 events, ii) evaluate wavelength-specific characteristics of light capture in response to the light/temperature
153 treatments, iii) identify the time to detect any such significant changes, and iv) establish an indicative
154 minimum light threshold for *H. decipiens* and *H. spinulosa* to help guide environmental management of
155 tropical deep-water seagrass communities. In addition, we aimed to describe potential differences in
156 physiological responses between two species from the *Halophila* genus.

157

158 2. METHODS

159 2.1 Sample Collection

160 *Halophila decipiens* Ostenfeld was collected adjacent to a long-term monitoring site off Green Island
161 (16°45.12354'S, 145°59.5494'E) at approximately 17 m depth below MSL. *H. spinulosa* (R. Brown)
162 Ascherson, was collected approximately 400 km to the south, at a location near Bowen at 10 m depth below
163 MSL (19° 54.4061 'S, 148° 11.0841'E). Plants were harvested in October and November 2013 on SCUBA
164 using a large metal scoop to place transplants and ~7 cm of sediment depth into 26 x 21 x 10 cm plastic tubs
165 in order to minimise disruption to their growing environment. Tubs were transferred overnight to the
166 University of Technology Sydney with enough water to keep shoots wet but not fully submerged, fastened
167 with lids, and kept in the dark during the approximately 18 hour transfer period. On arrival, tubs with
168 samples were maintained in 10-L aquaria with aerated natural seawater (26 °C, pH 8.1, 35 PSU) for one
169 month prior to the start of the experiment. Plants were illuminated using 150W four-channel LED lights
170 (Cidly, China) programmed to simulate an incident deep-water spectral profile with an intensity of 75 μmol
171 $\text{photons m}^{-2} \text{s}^{-1}$ over a diel light-dark cycle (ramping from 0 to 75 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ from 0500 h to 0700 h
172 and from 75 to 0 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ from 1800 h to 2000 h). The light, temperature, and salinity conditions
173 in the tanks were based on a two year record of water quality monitoring at the collection sites with in situ

174 loggers (Chartrand pers. obs.). The tank conditions mimic the mean maximum daily light intensity, mean
175 daily temperature, and salinity during the October/November period when plants were harvested.

176

177 2.2 Experimental design

178 Each tub of *H. decipiens* and *H. spinulosa* were randomly allocated to one of four treatments (4 tubs per
179 treatment) manipulating light intensity (*LI*) and temperature (*T*): (1) control (75 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$, 26 °C;
180 equivalent to mean daily irradiance at depth and mean ambient temperature at field sites), (2) elevated
181 temperature only (75 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$, 30 °C), (3) reduced light only (25 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$, 26 °C)
182 and (4) a combination of both reduced light and high temperature (25 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$, 30 °C). The
183 reduced *LI* and elevated *T* levels were chosen to reflect conditions beyond those recorded when plants were
184 actively growing but still found to occur at the collection site at times of the year when seagrasses were
185 absent (Chartrand pers. obs), and therefore realistic as a level of plant stress within their environment.
186 Temperature was controlled using submersible heaters (Aqua One, Australia). Water quality was the same as
187 the holding aquaria, with weekly 30% water changes to maintain salinity within 1 PSU and availability of
188 trace nutrients. Tubs were rotated every other day within tanks in order to remove an effect of location within
189 the tank in relation to the light source or water flow. The experiment was performed over 4 weeks.

190

191 The number of seagrass shoots (i.e. *H. decipiens* number of leaf pairs or *H. spinulosa* vertical shoots) in each
192 tub (0.05 m²) was recorded weekly during the study.

193

194 2.3 Oxygen determinations

195 Oxygen production and respiration rates of both species were measured at the start (T_0) and end (T_f) of the
196 experiment using oxygen optodes (OXSP5-OI, Pyroscience Germany) connected to a O₂ sensor unit
197 (FireStingO2 Fiber-Optic Oxygen Meter, Pyroscience, Germany). Samples were placed in 10 mL glass
198 bottles filled with 0.45 μm filtered seawater from treatment tanks with a magnetic stirrer and connected to an
199 oxygen sensor. Oxygen production and respiration were determined under treatment irradiance and darkness,
200 respectively and rates were calculated as oxygen differentials over the time of incubation and normalised to
201 leaf area.

202

203 2.4 Variable fluorescence measurements – wavelength-dependent parameters

204 The wavelength-dependent functional absorption cross-section of PSII, $\sigma_{II}(\lambda)$, was recorded according to
205 Schreiber and Klughammer (2013; and see Szabó et al., 2014) using a multi-colour pulse amplitude
206 modulated fluorometer (further referred as MC-PAM; Walz GmbH, Germany). Briefly, $\sigma_{II}(\lambda)$ calculations
207 are based on the so-called O-I₁ (or O-J, Strasser and Govindjee, 1992) fluorescence rise kinetics, which
208 corresponds to the photochemical phase of the polyphasic fluorescence rise upon the onset of strong actinic
209 illumination. These measurements were recorded using an automated measuring routine in PamWin v3.2
210 (Walz GmbH, Germany). At 500 μ s after the start of actinic illumination, i.e., before the secondary thermal
211 rise phases contribute significantly to the fluorescence rise, a saturating single-turnover flash (ST) was given
212 to estimate the I₁-level, which represents the fluorescence yield of the fully reduced primary electron
213 acceptor Q_A, with the PQ-pool being oxidized.

214

215 Consecutive measurements with the same leaf sample using 440, 480, 540, 590, and 625 nm measuring light
216 (ML) and actinic light (AL) were pre-programmed in a script-file with 10 s dark-time between
217 measurements. For each wavelength, ML intensity and gain settings were programmed to give approximately
218 equal F₀ values (designated as ‘O’ in the O-I₁ terminology). The AL and multiple turnover (MT) flash
219 intensity settings were programmed to obtain similar slopes of the O-I₁ curves for all wavelengths. When
220 kinetics of the O-I₁ fluorescence rise are identical among wavelengths, PAR is directly proportional to
221 changes in $\sigma_{II}(\lambda)$. Values of $\sigma_{II}(\lambda)$ were analysed using a dedicated fitting routine in the PamWin-3 software
222 to determine τ , the time-constant of light-driven Q_A⁻ reduction (ms) and used in the following equation:

$$223 \sigma_{II}(\lambda) = \frac{1}{\tau \cdot N_A \cdot E_d}$$

224 where τ is the time-constant of light-driven Q_A⁻ reduction (ms), N_A is Avogadro’s constant (3.03 · 10²³ mol
225 photons⁻¹) and E_d is the incident downwelling irradiance (see Schreiber et al. 2012 and Schreiber and
226 Klughammer 2013).

227

228 Wavelength-dependent chlorophyll fluorescence parameters, i.e. the effective quantum yield of PSII (Y(II)),
229 relative electron transport rate (rETR(II)) and non-photochemical quenching (NPQ) were determined by
230 using custom-made scripts to record steady-state light curves (SSLC) across the wavelengths provided by the

231 LEDs of the MC-PAM at a fixed intensity. Essentially, scripts were designed to start with the wavelength
232 with the lowest measured $\sigma_{II}(\lambda)$ then progressing towards greater theoretical wavelength-specific PSII
233 excitation pressure; this equated to a sequence order of 540, 590, 625, 480 and 440 nm. Two scripts were
234 used to determine the chlorophyll fluorescence parameters described above: a sub-saturating ($25 \mu\text{mol}$
235 $\text{photons m}^{-2} \text{s}^{-1}$) and a supra-saturating ($150 \mu\text{mol photons m}^{-2} \text{s}^{-1}$) AL at each wavelength. The F_v/F_M values
236 were calculated upon dark-adaptation for ~ 5 min, followed by a white saturating pulse (SP). At each of the
237 five wavelengths, AL was set for 3 min at the end of which a SP pulse was given with the same wavelength
238 as the AL to determine F_M' .

239

240 2.5 Pigment Characterisation

241 Seagrass leaves used in MC-PAM measurements were photographed and analysed by imaging software
242 (ImageJ) prior to the extraction and quantification of chlorophyll content. Each leaf sample was ground in 5
243 mL ice-cold 90% acetone using a mortar and pestle. Chlorophyll *a* (Chl *a*) and chlorophyll *b* (Chl *b*)
244 concentrations were determined spectrophotometrically using the equations and extinction coefficients of
245 Ritchie (2006) and normalised to surface area of the leaf.

246

247 2.6 Below ground carbohydrates

248 Additional samples of *H. spinulosa* only were collected at the beginning and end of the experiment for
249 below-ground carbohydrate analysis and were placed in liquid nitrogen to await further processing. Samples
250 were later defrosted, scraped clean and separated into below and above-ground structures. Root and rhizome
251 material was dried at 40°C for 48 hours when a constant dry weight was obtained. Dried material was ground
252 to a fine powder in a bead mill (Mini-Beadbeater, Biospec) for wet laboratory analysis. Prepared samples
253 were further processed at the University of Queensland where soluble and non-structural carbohydrates (i.e.
254 starch) were extracted and quantified relative to total sample dry weight according to Weir et al. (1977) and
255 Karkalas (1985). Briefly, carbohydrates were extracted by placing each sample in 80% ethanol in an 80°C
256 water bath for 5 min prior to centrifuging (3000 rpm for 5 min). The supernatant was diluted with 80%
257 ethanol to 25 mL and kept for soluble carbohydrate determination. The pellet remaining was dissolved in 10
258 mL of deionized water and placed in a 95°C water bath for a further 30 min while agitating samples at 10
259 min intervals to solubilize the starch. After coming to room temperature, samples were digested with

260 amylase and incubated at 55°C for 30 min prior to dissolving the extracted sample in 20% trichloroacetic
261 acid. Colorimetric determination of starch content was determined using a commercially available glucose
262 oxidase/peroxidase testing kit (GOPOD, Megazyme) with absorbance measured at 510 nm, and soluble
263 carbohydrates colorimetrically determined with a potassium ferricyanide reagent measured at 420 nm.

264

265 *H. decipiens* was not tested for below-ground carbohydrate concentrations due to extremely low below-
266 ground biomass insufficient to perform laboratory analyses.

267

268 2.7 Data analysis

269 Generalized linear mixed-effect models (GLMM) were used to examine the fixed effects of light intensity
270 (LI), temperature (T), and week (W) on shoot count (SC) and chlorophyll fluorescence parameters (F_v/F_m ,
271 $\Delta F/F_m'$, Q_m) for each species. Each factor was modelled as an additive term and as an interaction with other
272 factors. The factor tub was also included as a random effect in all models to eliminate potential bias resulting
273 from the non-independence of measurements taken from the same tub over the four-week study. Three-way
274 interactions between LI , T and W were considered in the analyses. The response variable SC was modelled
275 with a Poisson distribution with logit link function. To assess differences in response variables between
276 species, separate models were run on the effect of species (SPP) as an additive and interaction term to avoid
277 over-parameterization of the models.

278

279 To determine the optimal model, a global model was created for each response variable where all
280 explanatory variables up to 3-way interactions were considered. Sub-model sets of the global model were
281 then generated using the dredge function in the MuMIn package (Bartoń, 2013). The best-fit models were
282 considered to be those with the lowest Akaike's information criterion (AICc) and highest Akaike weight (w),
283 which by definition contain the best set of explanatory factors for adequately predicting each response
284 variable (Burnham and Anderson, 2002; Wagenmakers and Farrell, 2004). Models with AICc values within
285 2 of each other were considered strong models and are presented with the chosen model being the simplest of
286 this sub-set which was further used for multiple comparison analysis (Burnham and Anderson, 2002). All
287 models were validated by assessing Pearson residuals against fitted model values. The best model for *H.*
288 *decipiens* shoot count data did show some heterogeneity in the residuals versus fitted values. Two influential

289 tubs were identified using standardized measures of influential data for the point estimates of generalized
290 mixed effects models (Nieuwenhuis et al., 2012) and were removed, which greatly improved the residual
291 patterns while not changing the model selection or significance of the fixed effects in the model output.
292 Multiple comparison procedures using a Bonferroni adjustment were run on least square means when
293 significant overall effects were generated from all best fit models (lsmeans package, Lenth, 2016).

294

295 A similar GLMM approach was used to examine the fixed effects of light level (LI), temperature (T), and the
296 random effect of tub, on oxygen production, respiration rates, P:R ratios, pigment content and all
297 wavelength-dependent fluorescence measurements ($Y(II)$, F , F_m , $rETR$, NPQ , and $\sigma_{II}(\lambda)$), which were also
298 tested against wavelength (WV). Since these response variables were only measured at the start (T_0) and end
299 (T_F) of the study, a separate t-test was performed between T_0 and T_F “control” conditions to test whether the
300 tub conditions affected the status of leaves in addition to the treatment design. Model selection for
301 wavelength-dependent fluorescence measurements was also done using the dredge function, but with a
302 Gaussian or gamma distribution (with logit link) applied for continuous and positive data (Zuur et al., 2009).
303 Model selection for all other response variables (oxygen production, respiration rates, P:R ratios, pigment
304 content) was made using the drop1 command from the lmerTest package (Kuznetsova et al., 2015).
305 Validation steps were the same; Pearson residuals were assessed against fitted model values, and multiple
306 comparison procedures using a Bonferroni adjustment were run on least square means when significant
307 overall effects were generated from all best fit models.

308

309 All GLMMs were performed in R using lme, glmmADMB and mgcv packages (Bates et al., 2012; Fournier
310 et al., 2012; Wood, 2006).

311

312 3. RESULTS

313 3.1 Shoot density

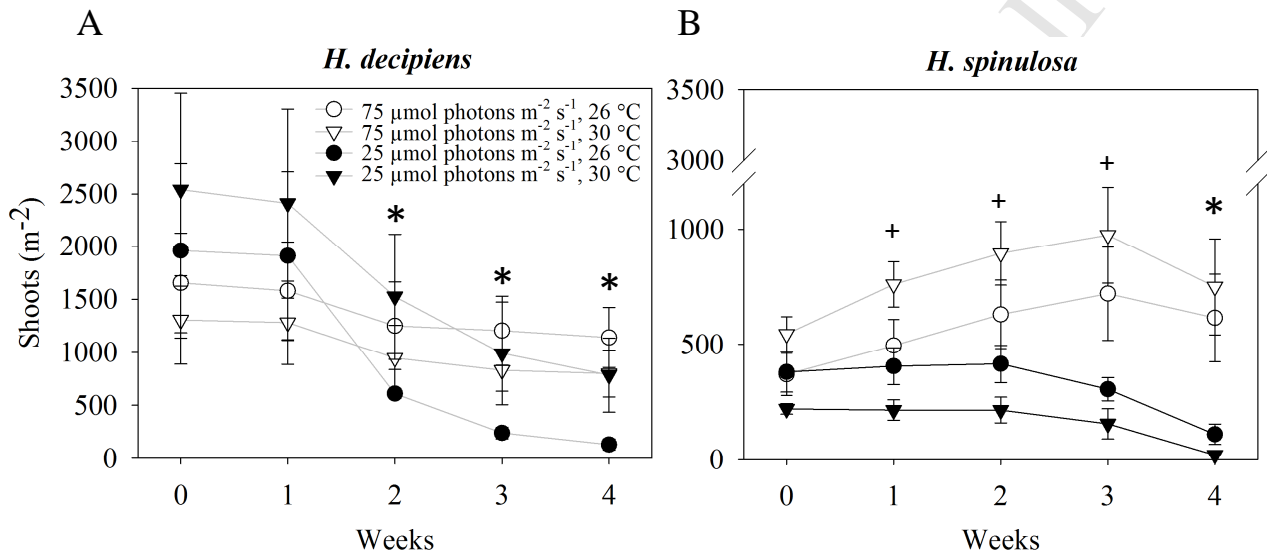
314 Light deprivation had a negative effect on *Halophila decipiens* and *Halophila spinulosa* shoot density after
315 two and four weeks, respectively (Figure 1). Change in shoot counts of *H. decipiens* were driven by LI and
316 W with no effect of T (Table 1). *H. decipiens* shoots under low LI were significantly lower by week 2 with
317 approximately 40% (26°C) and 60% (30°C) loss of total shoots and this declined further over the subsequent

318 two weeks of the study (Figure 1a). Overall, shoots under low *LI* decreased from 1960 ± 826 to 130 ± 52
 319 shoots m^{-2} and 2540 ± 915 to 785 ± 350 shoots m^{-2} from week 0 to week 4, for low and high temperature
 320 treatments, respectively.

321

322 Figure 1. Shoot density (shoots m^{-2}) for *H. decipiens* (a) and *H. spinulosa* (b) over a four-week study. *
 323 indicate significant declines in low *LI* treatments from *T0* while + indicate significant gains in high *LI* from
 324 *T0*. Data symbols and error bars represents mean \pm SE. (n = 4).

325



326

327

328 Shoots of *H. spinulosa* were also driven by *LI* and *W* with no effect of *T* (Table 1). In total, *H. spinulosa*
 329 shoots declined under low *LI* from 350 ± 88 to 110 ± 44 shoot m^{-2} (26 $^{\circ}\text{C}$) and 220 ± 23 to 20 ± 20 shoot m^{-2}
 330 (30 $^{\circ}\text{C}$) from week 0 to week 4. Shoot loss did not begin until after week 2 and was only significant at week 4
 331 (Figure 1b). Conversely, under high *LI* there was a significant gain in shoots from week 1 to week 3 (Figure
 332 2b).

333

334 3.2 Below-ground carbohydrates

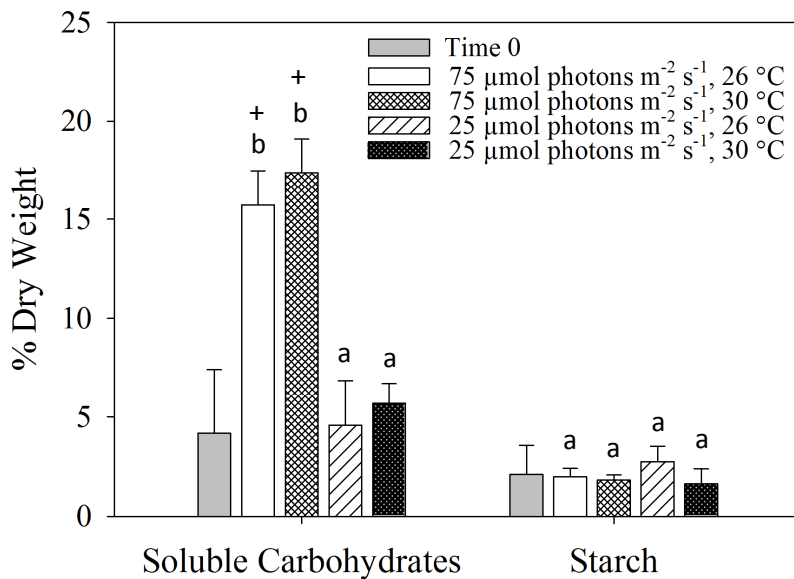
335 Below-ground tissues of *H. spinulosa* were significantly affected by *LI* (Table 1) with measurable increases
 336 of soluble sugar concentrations under high *LI* treatments compared to the start of the study and low *LI* tubs
 337 (Figure 2). In contrast, below-ground starch concentrations were unchanged over time and among treatments.

338 Table 1. *Halophila decipiens* and *H. spinulosa* parameter estimates where significant effects of covariates were found with generalized linear mixed-effects models
 339 (GLMM). Effects of light intensity (*LI*), temperature (*T*) and week (*W*) on shoot counts (*SC*), and wavelength-dependent fluorescence parameters are presented.
 340 Wavelength (*WV*) was also included in all models for wavelength-dependent fluorescence parameters. Models with interaction terms also include main effects.
 341 Shoot count was modelled with a negative binomial distribution, chlorophyll fluorescence parameters with a beta distribution and oxygen/respiration rates with a
 342 gamma distribution (all with logit link function). β_{tub} is the random effect of tub and ε is the error term.

<i>Halophila decipiens</i>					<i>Halophila spinulosa</i>				
Model	df	AIC _C	ΔAIC _C	w	Model	df	AIC _C	ΔAIC _C	w
Shoot Count (SC)					Shoot Count (SC)				
$LI * W + \beta_{tub} + \varepsilon$	12	745.3	0.00	0.703	$LI * W + LI * T + \beta_{tub} + \varepsilon$	13	511.8	0.00	0.472
					$LI * W + \beta_{tub} + \varepsilon$	11	512.2	0.34	0.398
$\sigma_{II}(\lambda)$					$\sigma_{II}(\lambda)$				
$T + WV + \beta_{tub} + \varepsilon$	8	71.6	0.00	0.288	$LI * T + WV + \beta_{tub} + \varepsilon$	8	239.6	0.00	0.386
$LI * T + WV + \beta_{tub} + \varepsilon$	9	71.8	0.22	0.259	$LI + T + WV + \beta_{tub} + \varepsilon$	9	240.1	0.50	0.300
$WV + \beta_{tub} + \varepsilon$	10	72.6	0.99	0.176	$WV + \beta_{tub} + \varepsilon$	7	240.9	1.31	0.200
$WV + \beta_{tub} + \varepsilon$	7	72.8	1.20	0.158					
YII _(LL)					YII _(LL)				
$LI + WV + \beta_{tub} + \varepsilon$	9	-194.8	0.00	0.956	$WV + \beta_{tub} + \varepsilon$	8	-290.2	0.00	0.773
YII _(HL)					YII _(HL)				
$WV + \beta_{tub} + \varepsilon$	8	-298.9	0.00	0.930	$LI + WV + \beta_{tub} + \varepsilon$	9	-434.9	0.00	0.418
					$LI * T + WV + \beta_{tub} + \varepsilon$	11	-433.7	1.19	0.231
					$LI + T + WV + \beta_{tub} + \varepsilon$	10	-433.0	1.92	0.160
rETR _{II(LL)}					rETR _{II(LL)}				
$LI + WV + \beta_{tub} + \varepsilon$	8	173.3	0.00	0.539	$LI + WV + \beta_{tub} + \varepsilon$	8	146.2	0.00	0.436
					$WV + \beta_{tub} + \varepsilon$	7	147.6	1.36	0.221
					$LI + T + WV + \beta_{tub} + \varepsilon$	9	148.2	1.99	0.161
rETR _{II(HL)}					rETR _{II(HL)}				
$WV + \beta_{tub} + \varepsilon$	7	211.7	0.00	0.477	$LI * WV * T + \beta_{tub} + \varepsilon$	22	277.3	0.00	0.250

$WV + T + \beta_{tub} + \varepsilon$	8	213.4	1.66	0.208	$LI * WV + LI * T + \beta_{tub} + \varepsilon$	14	277.7	0.32	0.213
$LI + WV + \beta_{tub} + \varepsilon$	8	213.4	1.72	0.202	$LI * WV + LI * T + LI * WV + \beta_{tub} + \varepsilon$	18	278.3	1.00	0.152
					$LI * WV + T + \beta_{tub} + \varepsilon$	13	278.5	1.18	0.138
					$LI * WV + \beta_{tub} + \varepsilon$	12	278.7	1.36	0.127
					$LI * T + WV + \beta_{tub} + \varepsilon$	17	278.8	1.46	0.120
NPQII _(LL)					NPQII _(LL)				
$WV + \beta_{tub} + \varepsilon$	7	-65.1	0.00	0.616	$WV + \beta_{tub} + \varepsilon$	7	-87.0	0.00	0.900
$T + WV + \beta_{tub} + \varepsilon$	8	-63.8	1.37	0.310					
NPQII _(HL)					NPQII _(HL)				
$LI + WV + \beta_{tub} + \varepsilon$	8	-95.8	0.00	0.599	$LI + WV + \beta_{tub} + \varepsilon$	8	-49.5	0.00	0.676
Below-ground Soluble Sugars					Below-ground Soluble Sugars				
–					LI	3	65.9	0.00	0.865
Below-ground Starch					Below-ground Starch				
–					$Null$	2	39.7	0.00	0.645

344 Figure 2. Percent soluble carbohydrates and percent starch in below ground roots and rhizomes at the start
 345 (Time 0) and end of the experiment (4 weeks) for *H. spinulosa* under each treatment. Differing letters
 346 indicate significant differences among treatments at the end of the study; + indicate difference from time 0
 347 measurements. Data symbols and error bars represents mean \pm S.E.M. (n = 3).



348

349

350 3.3 O_2 gas exchange determinations

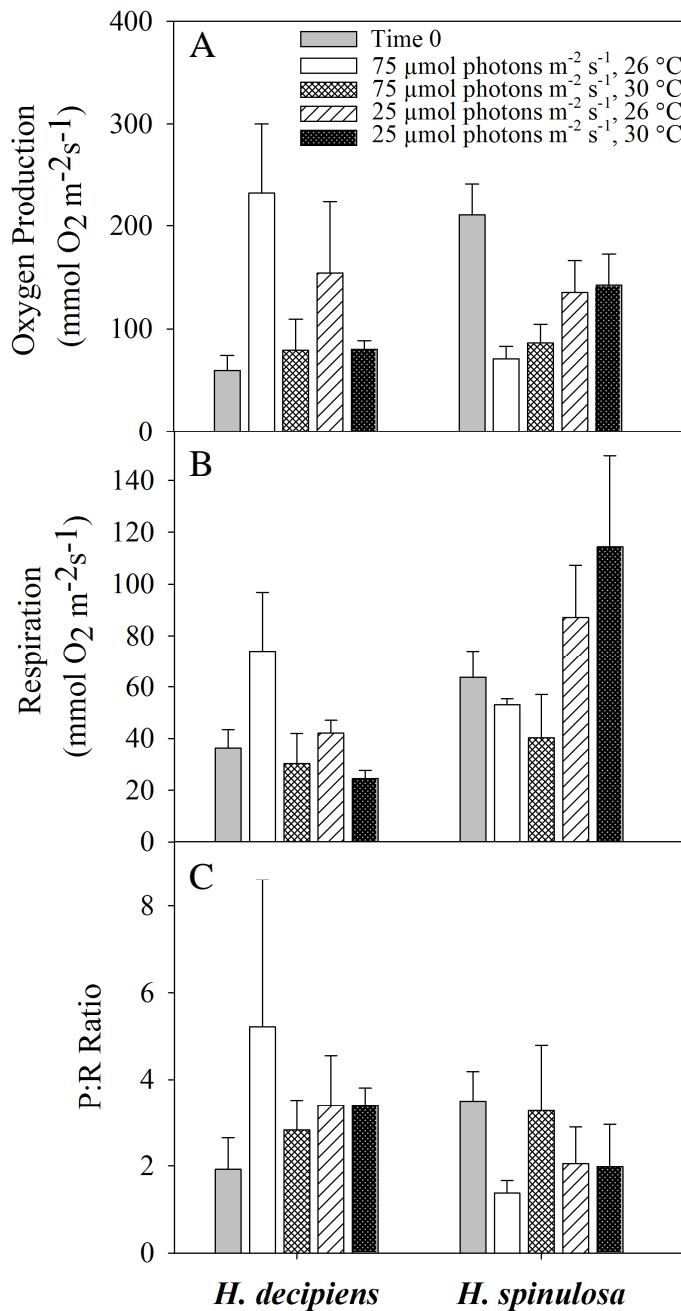
351 Overall, oxygen and respiration measurements of *H. decipiens* leaves were highly variable both within and
 352 among treatments (Figure 3). Gas exchange in leaves from the start to the end of the study was not
 353 statistically significant despite the appearance of a large increase in both oxygen production and respiration
 354 under control conditions at the end of the study due to the measured variance. For *H. decipiens* leaves,
 355 significant declines in oxygen production ($F = 6.8$, $p = 0.02$) and respiration ($F = 5.8$, $p = 0.03$) were related
 356 to high T alone, while there were no differences in the P:R ratios among treatments at the end of the study
 357 (Figure 3).

358

359 In *H. spinulosa* leaves, oxygen production and respiration were correlated with LI ; relatively higher oxygen
 360 ($F = 7.1$, $p = 0.02$) and respiration ($F = 6.56$, $p = 0.02$) under low LI with no measurable patterns in P:R
 361 measurements (Figure 3). Oxygen and P:R for *H. spinulosa* was significantly lower at the end of the study
 362 compared to the start in control treatments ($75 \mu\text{mol photons m}^{-2} \text{s}^{-1}$, 26 $^{\circ}\text{C}$; $F = 23.0$, $p = 0.005$ and $F = 10.1$,
 363 $p = 0.02$ respectively).

364

365 Figure 3. Oxygen production ($\mu\text{mol O}_2 \text{ m}^{-2} \text{ s}^{-1}$; A), respiration ($\mu\text{mol O}_2 \text{ m}^{-2} \text{ s}^{-1}$; B), and P:R ratios (C) of *H.*
 366 *decipiens* and *H. spinulosa* measured at the start (Time 0; $75 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$, 26°C) and the end of the
 367 experiment. Data symbols and error bars represents mean \pm S.E.M. (n = 4).



368

369

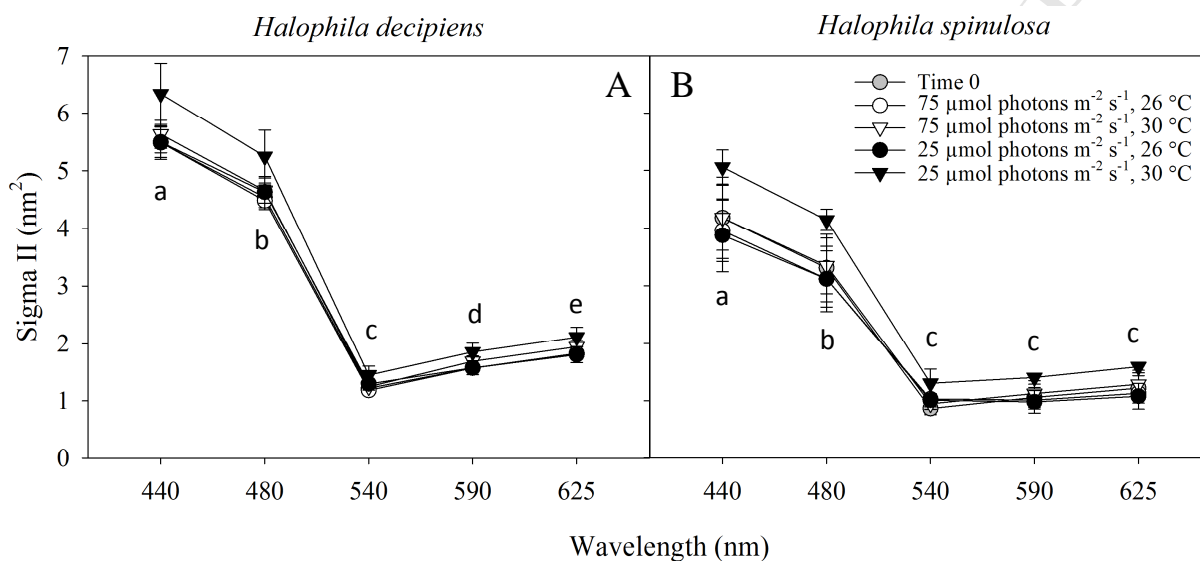
370 3.4 Wavelength-dependent variable chlorophyll fluorescence

371 Wavelength-dependent functional absorption cross-section of PSII ($\sigma_{II}(\lambda)$) of both *H. decipiens* and *H.*
 372 *spinulosa* significantly differed by *WV*, but *LI* and *T* had no effect on $\sigma_{II}(\lambda)$ for either species (Figure 4, Table
 373 1). Wavelength (*WV*) also significantly affected effective quantum yield ($Y(II)$), relative electron transport
 374 rate ($rETR(II)$), and non-photochemical quenching (NPQ) in both *H. decipiens* and *H. spinulosa* in all

375 treatments when exposed to sub- and supra-saturating AL (Figures 5-6; Table 1). These results are in
 376 agreement with the $\sigma_{II}(\lambda)$ spectrum (Figure 4); the largest differences among wavelengths were between
 377 those most absorbed (440 and 480 nm), whereas smaller differences were recorded between less absorbed
 378 wavelengths (540 and 590 nm) for both species.

379

380 Figure 4. $\Sigma II(\lambda)$ for *H. decipiens* (A) and *H. spinulosa* (B) measured across five wavelengths at the start
 381 (Time 0) and the end of the study. Data symbols and error bars represents mean \pm S.E.M (n = 4).



382

383

384 In addition to a clear separation among wavelengths, a pattern of low *LI* affected both species, but under
 385 opposing AL conditions and regardless of *T* (Table 1). For *H. decipiens*, leaves from low *LI* treatments had
 386 lower overall *Y*(II) and *rETR*(II) across all wavelengths under the sub-saturating AL, while *H. spinulosa*
 387 leaves did not have measurable differences under the same sub-saturating AL (Figure 5). Under the supra-
 388 saturating AL, leaves of *H. spinulosa* from low *LI* treatments had lower overall *Y*(II) and *rETR*(II) across all
 389 wavelengths, while *H. decipiens* did not measurably differ among treatments (Figure 6). NPQ for both
 390 species was significantly reduced under low *LI*, but only when exposed to supra-saturating AL (Figures 5
 391 and 6; Table 1).

392

393 The overall magnitude of wavelength-dependent photochemical parameters differed between the sub- and
 394 supra-saturating AL measurements (Figures 5 and 6). *Y*(II) values, as expected, were depressed under the

395 supra-saturating AL ranging from a mean of 0.05 – 0.2 (Figures 5) compared to 0.3 – 0.6 (Figures 6) under
 396 sub-saturating conditions. rETR(II) and NPQ concomitantly increased under the same conditions.

397

398 Y(II) and rETR(II) for *H. decipiens* and *H. spinulosa* under both sub- and supra-saturating AL measurements
 399 were significantly lower at 440 and 480 nm compared to all other wavelengths (Figures 5 and 6). The lowest
 400 quantum yields of PSII and relative electron transport rates were followed by measurements at 625 nm which
 401 were significantly lower than both 540 and 590 nm, the least absorbed wavelengths according to $\sigma_{II}(\lambda)$
 402 measurements. An inverse relationship found for non-photochemical quenching (NPQ), a measure of the
 403 plant's capacity to dissipate excess light energy, meant the highest values were recorded at 440 nm in *H.*
 404 *decipiens* and 440 and 480 nm for *H. spinulosa* where light was most absorbed and relative electron transport
 405 rates were lowest (Figures 5 and 6).

406

407 Overall species differences in wavelength-dependent parameters, irrespective of treatment, were found with
 408 both sub- and supra-saturating AL measurements (Table 2); however, stronger patterns were under supra-
 409 saturating AL. *H. decipiens* had higher overall $\sigma_{II}(\lambda)$ than *H. spinulosa* (Figure 4; Table 2) but lower Y(II),
 410 rETR(II), and NPQ than *H. spinulosa* irrespective of AL (Figures 5 and 6).

411

412

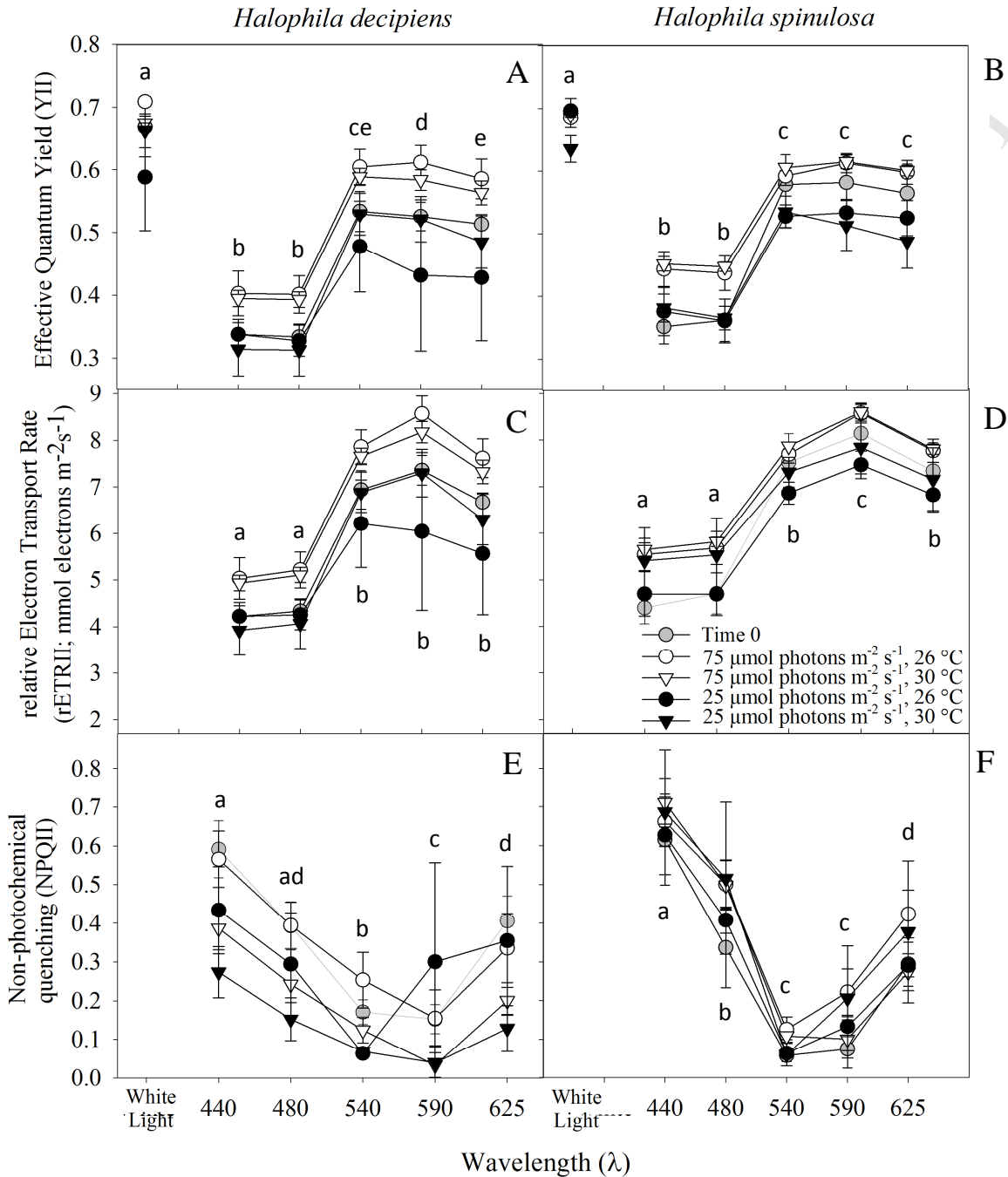
413 Table 2. GLMM model fit for species comparison (SPP) of wavelength-dependent variable fluorescence
 414 parameters. n = 4 ± SE. * p < 0.05, ** p < 0.01, *** p < 0.001

Model	MS	F	p
$\sigma_{II}(\lambda)$	55.98	19.30	***
YII _(LL)	0.04	4.60	*
YII _(HL)	0.07	23.59	***
rETR _(LL)	13.71	5.97	*
rETR _(HL)	396.88	23.05	***
NPQ _(LL)	0.51	10.87	**
NPQ _(HL)	2.18	42.48	***

415

416

417 Figure 5. Effective quantum yield (YII; A, B), relative electron transport rate (rETRII; C, D), and non-
 418 photochemical quenching (NPQII; E, F) for *H. decipiens* (A, C, E) and *H. spinulosa* (B, D, F) measured
 419 under sub-saturating AL at five wavelengths at start (Time 0) and the end of the experiment. Differing letters
 420 indicate significant differences among wavelengths at the end of the study based on a Bonferroni correction.
 421 Data symbols and error bars represents mean \pm S.E.M. (n = 4).



422

423

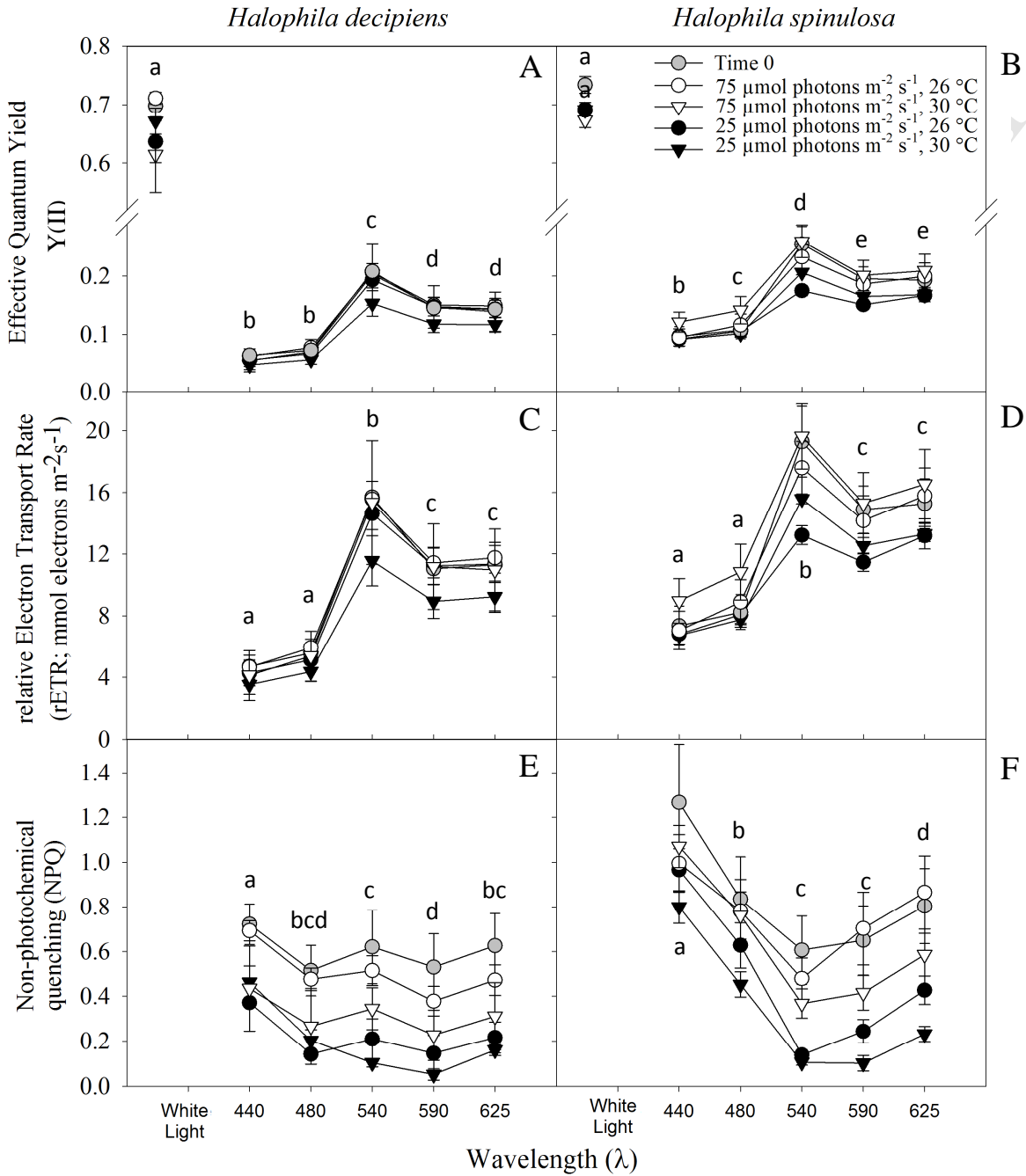
424

425

426

427

428 Figure 6. Effective quantum yield (Y(II); A, B), relative electron transport rate (rETR_{II}; C, D), and non-
 429 photochemical quenching (NPQ; E, F for *H. decipiens* (A, C, E) and *H. spinulosa* (B, D, F) measured under
 430 supra-saturating AL at five wavelengths at start (Time 0) and the end of the experiment. Differing letters
 431 indicate significant differences among wavelengths at the end of the study. Data symbols and error bars
 432 represents mean \pm S.E.M. (n = 4).



433

434

435

436

437

438

439 3.5 Pigment Characterisation

440 Effect of light and temperature treatments on chlorophyll content differed between the two species (Table 3).
441 *H. decipiens* total chlorophyll, Chl *a*, and Chl *b* were unaffected by treatment, while Chl *b* increased
442 somewhat under low *LI* ($F = 6.1$, $p = 0.02$). Chl *a:b* was significantly affected by *LI*, but dependent on *T* with
443 significantly lower Chl *a:b* only under low *LI* and low *T* ($F = 7.0$, $p = 0.01$; Table 3).

444

445 All measures of chlorophyll content relative to leaf area for *H. spinulosa* leaves were affected in some way
446 by *LI* and *T* treatments (Table 3). Total chlorophyll, Chl *a*, and Chl *b* were highest under low *LI* but with Chl
447 *a* only when under low *T* ($F = 5.65$, $p = 0.03$). Chl *b* was also significantly higher under high *LI* when
448 combined with high *T*. Chl *a:b* was significantly lower under high *T* irrespective of *LI* ($F = 16.2$, $p = 0.001$).

449

450 Table 3. Chlorophyll composition of MC-PAM leaves under two light treatments and two temperature treatments (n =4). Pigment concentrations units are $\mu\text{g cm}^{-2}$.
 451 Differing letters indicate significant differences among treatments for each species when the null model was rejected (Bonferroni correction method).

Treatment	<i>Halophila decipiens</i>				<i>Halophila spinulosa</i>			
	Total Chlorophyll	Chl <i>a</i>	Chl <i>b</i>	Chl <i>a:b</i>	Total Chlorophyll	Chl <i>a</i>	Chl <i>b</i>	Chl <i>a:b</i>
Time 0 (75 $\mu\text{mol m}^{-2} \text{s}^{-1}$; 26°C)	0.54 ± 0.04	0.34 ± 0.02	0.20 ± 0.01 ^a	1.68 ± 0.02 ^a	0.71 ± 0.05 ^a	0.47 ± 0.03 ^a	0.25 ± 0.02 ^a	1.89 ± 0.05 ^a
75 $\mu\text{mol m}^{-2} \text{s}^{-1}$; 26°C	0.53 ± 0.04	0.33 ± 0.02	0.20 ± 0.01 ^a	1.68 ± 0.06 ^a	0.64 ± 0.04 ^a	0.43 ± 0.03 ^a	0.22 ± 0.02 ^a	1.98 ± 0.03 ^a
75 $\mu\text{mol m}^{-2} \text{s}^{-1}$; 30°C	0.61 ± 0.05	0.36 ± 0.03	0.24 ± 0.02 ^a	1.52 ± 0.05 ^a	0.86 ± 0.08 ^a	0.55 ± 0.05 ^a	0.30 ± 0.02 ^b	1.81 ± 0.04 ^b
25 $\mu\text{mol m}^{-2} \text{s}^{-1}$; 26°C	0.58 ± 0.05	0.34 ± 0.03	0.24 ± 0.02 ^b	1.38 ± 0.05 ^b	0.98 ± 0.03 ^b	0.64 ± 0.02 ^b	0.34 ± 0.01 ^b	1.90 ± 0.01 ^a
25 $\mu\text{mol m}^{-2} \text{s}^{-1}$; 30°C	0.76 ± 0.08	0.46 ± 0.05	0.30 ± 0.03 ^b	1.55 ± 0.03 ^a	0.87 ± 0.06 ^b	0.54 ± 0.04 ^a	0.32 ± 0.02 ^b	1.67 ± 0.04 ^b

452 **4. DISCUSSION**

453 This is the first study to assess two deep-water seagrasses of which neither species' physiology, optical
454 characteristics or morphological response to light and temperature stress has been previously described.
455 Under light spectrally adjusted to mimic natural deep-water conditions, our study showed that a 66%
456 reduction in light availability from 75 to 25 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$ caused a decrease in shoots for both *H.*
457 *decipiens* and *H. spinulosa* after two and four weeks respectively. Surprisingly, temperature did not further
458 affect *H. decipiens* or *H. spinulosa* shoot density under low light. A reduction in light led to characteristic
459 optical changes in the leaves such as increases in Chl *b* concentrations and lower electron transport rates;
460 however, both species lacked the capacity to withstand shoot loss over the 4-week study. The effect of light
461 stress on both *H. decipiens* and *H. spinulosa* followed a characteristic response that has been well
462 documented in studies on other seagrass species (Chartrand et al., 2016; Collier et al., 2012b; Longstaff et
463 al., 1999).

464
465 Minimum light requirement and optimal temperature for growth and photosynthesis vary among species due
466 to unique physiological and morphological adaptation (Lee et al., 2007). Previous studies showed light
467 requirements for *H. decipiens* in Cuba and St. Croix, were 4.4 and 8.8% of surface irradiances, respectively
468 (Dennison et al., 1993). Additional work by Erftemeijer and Stapel (1999) off South Sulawesi, Indonesia on
469 similar deep-water *H. ovalis* beds found a light compensation point (i.e. when productivity equals respiration
470 and net carbon balance is zero) of 33 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$, equivalent to approximately 1.4 $\text{mol photons m}^{-2}$
471 d^{-1} . A contiguous deep-water *H. decipiens* meadow off the west coast of Florida was recorded growing year-
472 round at 20 m under light as low as 1.8 $\text{mol photons m}^{-2} \text{ d}^{-1}$ (Hammerstrom et al., 2006). Our study showed
473 that an average irradiance at 75 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$ (equal to 3.2 $\text{mol photons m}^{-2} \text{ d}^{-1}$) which is
474 approximately 4% of surface irradiance (based on a typical midday measurement of 2000 $\mu\text{mol photons m}^{-2}$
475 s^{-1} at the surface) is an adequate light regime for both *H. decipiens* and *H. spinulosa*. A 66% reduction in
476 light (25 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$, equal to 1.1 $\text{mol photons m}^{-2} \text{ d}^{-1}$) had a significant effect on shoot density and
477 optical properties of both *H. decipiens* and *H. spinulosa* within 4 weeks.

478
479 Elevated temperature had little overall effect on optical and physiological responses and no consequence to
480 shoot loss in either species. Seagrasses respond to light reduction in various ways e.g. including changes in

481 light absorption properties of the leaves, altering morphology and modifying carbon storage (Abal et al.,
482 1994; Campbell and Miller, 2002; Gordon et al., 1994; Ralph et al., 2007), however temperature is known to
483 have a direct effect on seagrass metabolism, nutrient uptake and enzyme activities (Lee et al., 2007; Short
484 and Neckles, 1999). The optimal temperature for seagrass growth is dependent upon irradiance (Bulthuis,
485 1987) and an increase in temperature to some optimal level promotes photosynthesis and higher growth rates
486 (Lee et al., 2007). If temperatures increase further without a concomitant increase in light levels to support
487 photosynthesis, metabolic demand will outstrip supply and seagrass condition will deteriorate (Collier et al.,
488 2011; Lee et al., 2007; Masini et al., 1995). In the current study, neither species appeared to be adversely
489 affected – metabolically nor physically — by elevated temperature under either light treatment. Seagrasses
490 are actively growing from July to October in waters of 24-28°C each year in the meadows where plants were
491 collected. Seagrass die-back occurs rapidly by the following January each austral summer when *in situ*
492 temperatures reach a maximum of 29°C (Chartrand pers. comm). The 30°C treatment in this study therefore
493 reflects a biologically-meaningful elevated condition for tropical deep-water seagrasses under a warming
494 climate. Our study showed no changes in P:R ratio in either species suggesting that the high temperature
495 (30°C) is not beyond some optima for these species and suggests both species are tolerant to minor
496 temperature increases irrespective of the light climate. Testing more extreme temperatures would likely
497 negatively affect plant metabolism and productivity and establish an upper thermal limit for these
498 populations as measured for other shallow water tropical seagrasses (Adams et al., 2017; Collier et al., 2011;
499 Lee et al., 2007; York et al., 2013).

500

501 The decrease in shoot density under low light that we measured is consistent with other studies (Collier et al.,
502 2012b; Longstaff et al., 1999), and yet the interactive effect with high temperature seen with strap-bladed
503 species did not occur (York et al., 2013). This may be a response of small-bodied opportunistic species that
504 are expected to exhibit fast growth to exploit resources under high light conditions and disappear when light
505 levels deteriorate (Kilminster et al., 2015; Ralph et al., 2007). Strap-bladed species may decrease leaf area
506 with loss of light which will reduce the respiratory demand of the shoot and reduce the photosynthetic
507 capacity of the leaves (Campbell and Miller, 2002). Instead of modifying leaf size, the reduction in shoot
508 density we observed may be a strategy used to restore carbon balance by reducing above-ground tissues,
509 which have higher respiratory demands than below-ground tissues (Alcoverro et al., 2001; York et al., 2013).

510

511 *Halophila* spp. have meager below-ground architecture compared to other morphologically large and long-
512 lived species which rely on below-ground reserves to compensate for poor water quality over short durations
513 (Collier et al., 2009; Collier et al., 2012b). A lack of below-ground tissues may further encourage a reduction
514 in shoot density as a quick strategy to restore an energy balance in the whole plant. While diminutive size is
515 an attribute of the *Halophila* family, some species do have greater structural complexity and size than others.
516 The disparity in morphological characteristics of *H. decipiens* and *H. spinulosa* may explain the time
517 differential to impact each species under low-light treatments in our study. We found *H. spinulosa*, the larger
518 of the two species, was able to increase its below-ground sugars if given saturating light (Figure 2). The
519 controlled mobilization and oxidation of stored carbohydrates can release free energy in the form of NADPH
520 and ATP (Touchette and Burkholder, 2000b). This energy supply can be used to support cellular and
521 metabolic processes in the absence of sufficient light (Touchette and Burkholder, 2000a). The larger physical
522 form of *H. spinulosa* may also provide energy to endure short durations of poor light, whereas the diminutive
523 form of *H. decipiens* has little capacity to draw from structural reserves. While we were not able to measure
524 below-ground carbohydrates in *H. decipiens*, the delayed decline in shoot density by 2 weeks under low light
525 in *H. spinulosa* compared to *H. decipiens* may be linked to relatively higher starting carbohydrate reserves in
526 the former species which allowed for it to thrive longer before losing shoots. The lack of appreciable below-
527 ground biomass to perform such tests in *H. decipiens* is in itself an indication of scarce carbohydrate
528 reserves.

529

530 Seagrasses, as with other higher plants, possess an array of optical strategies to enhance light harvesting and
531 photosynthetic efficiency, which include increasing chlorophyll content and decreasing Chl *a:b* ratio under
532 low light in order to enhance the capabilities of PSII reaction centres (Abal et al., 1994; Walters, 2005). We
533 found both *H. decipiens* and *H. spinulosa* modified their chlorophyll content somewhat in response to light;
534 Chl *b* was increased to a greater extent for both species under reduced light, but this did not always alter the
535 overall Chl *a:b*. As an important accessory pigment in the light harvesting complexes, Chl *b* is known to
536 enhance light absorption and capture via increased thylakoid grana stacking, particularly under low-light
537 growing conditions (Leong and Anderson, 1984; Voitsekhovskaja and Tyutereva, 2015). Although, the small

538 changes to Chl *a:b* and no significant effect on the functional absorption cross section by treatment suggests
539 that no net effect of enhanced light capture was observed.

540

541 Overall, both species had somewhat lower Chl *a:b* — independent of treatment — than found in other higher
542 plants and strap-bladed seagrasses which typically range in values from 2-3 (Abal et al., 1994; Czerny and
543 Dunton, 1995; Zivcak et al., 2014). Lower Chl *a:b* is consistent with other *Halophila* spp. studies (Longstaff
544 and Dennison, 1999; Ralph and Burchett, 1998; Williams and Dennison, 1990) and suggests an enriched Chl
545 *b* light-harvesting antenna independent of experimental conditions. *Halophila* spp. may have adapted their
546 photosynthetic machinery to capitalise on low-light habitats such as deep-water or turbid inshore areas where
547 their light harvesting capabilities maximise their chances of success in these extreme conditions to greater
548 extent than other shade-adapted land plants (Kitajima and Hogan, 2003).

549

550 The wavelength-dependent pattern of $\sigma_{II}(\lambda)$ measured in both seagrass species was similar to that measured
551 in other green phototrophs including *Chlorella* suspensions and terrestrial leaves (Schreiber and
552 Klughammer, 2013). However, overall $\sigma_{II}(\lambda)$ values measured on the adaxial surface of a dandelion leaf by
553 Schreiber and Klughammer (2013) were much lower than the algal suspensions. Authors of both studies
554 believe these reductions in overall $\sigma_{II}(\lambda)$ are likely due to the apparent gradient of light absorption within
555 optically-dense samples compared to those seen in optically thin algal suspensions (Evans, 2009; Schreiber
556 and Klughammer, 2013). Plant leaves are well known to have in-built light gradients affecting absorption by
557 different layers within the leaf tissue (Evans, 2009; Vogelmann and Evans, 2002), limiting the accuracy of
558 such intrinsic $\sigma_{II}(\lambda)$ measurements (Osmond et al., 2017; Schreiber and Klughammer, 2013). However, the
559 apparent differences in $\sigma_{II}(\lambda)$ can be assessed based on inherent properties of PSII and the assumption that
560 average leaf measurements are equivalent to average conditions within the leaf itself and therefore
561 acceptable to measure relative changes (Osmond et al., 2017). These relative changes in $\sigma_{II}(\lambda)$ have been
562 used in studies as a metric to assess the acclimation capacity of $\sigma_{II}(\lambda)$ in a number of genotypes, genetic
563 mutants, algae and higher plants (Osmond et al., 2017; Szabó et al., 2014; Ware et al., 2015). The optically
564 thin nature of *Halophila* leaves (2 cells-thick) and the location of chlorophyll pigments exclusively in the
565 outer epidermal layer of seagrass leaves (Ferreira et al., 2015; Kuo and Den Hartog, 2006) correspond with
566 the relatively high levels of $\sigma_{II}(\lambda)$ for a leaf sample in our study. Beyond $\sigma_{II}(\lambda)$, the morphological framework

567 of the seagrass blades allowed us to explore other spectrally-resolved measurements; wavelength-specific
568 electron transport rates (rETR(II)) and energy dissipation (NPQ).

569

570 Despite no effect of light or temperature on $\sigma_{II}(\lambda)$ in deep-water *Halophila* spp. there was a classic
571 wavelength-specific response of photochemical reactions of PSII. Both species absorbed the highest
572 proportion of light from the blue (440 and 480 nm) and secondarily from red wavebands (625 nm),
573 characteristic of a typical leaf (Schreiber and Klughammer, 2013; Vogelmann and Evans, 2002). The greater
574 absorption in the blue region in turn increases the potential of a photoinhibitory/damaging response. More
575 efficient photoprotective mechanisms are evolved and are evident in the higher NPQ measured at 440 and
576 480 nm (Figure 5, 6). Plants adapted to higher irradiance can regulate photosynthesis with a larger range of
577 NPQ, where-as 'shade-adapted' plants tend to have lower range and NPQ values. A 'shade-adapted' plant,
578 under low-light intensities has lower excitation pressure on its' light harvesting antennae and therefore NPQ
579 does not need to compete with energy delivery to the reaction centres (Ruban, 2014). On the other hand, a
580 'sun-adapted' plant effectively relies upon NPQ to cope with excess light above and beyond the state where
581 closed reaction centres have been saturated under high light. This effect was clear with NPQ measurements
582 in this study when plants were exposed to the supra-saturating light; HL treated plants had significantly
583 greater NPQ than those grown in LL treatments (Figure 6E, F). The wavelength-specific differences in NPQ
584 follow the same relationship; adaptation to greater absorption at 440, 480, and 625 nm correlate with greater
585 NPQ capacity to protect the productive light harvesting pigments at the corresponding wavebands. The
586 higher NPQ at 440 and 480 nm created the expected concomitant reduction in photochemical efficiency and
587 relative electron transport rates at these wavelengths for both *H. decipiens* and *H. spinulosa*.

588

589 Both seagrass species in this study grow at depths that create similar light challenges to that of a forest floor
590 where the niche is filled by shade-loving plants. *H. decipiens* has almost exclusively been described as
591 growing in turbid shallow waters or in deep-water habitats whereas *H. spinulosa* was mainly found in
592 subtidal and turbid water habitats (Coles et al., 2009; Kenworthy et al., 1989; Kuo and Kirkman, 1995;
593 Walker et al., 1988) where chronic low-light intensities would seem to reduce the need for high NPQ to
594 protect light harvesting and PSII reaction centre proteins from photodamage. Investigations by Dawes et al.
595 (1989) and a reciprocal transplant experiment by Durako et al. (2003) concluded *H. decipiens* is actually

596 intolerant of higher light intensities. Despite the lesser need for high light photoprotective processes in a
597 naturally low-light environment, the machinery and pathways are highly conserved across many higher
598 plants found in low-light habitats (Ruban, 2014). At what capacity or for how long photoprotection via NPQ
599 is sustained in both species under high light conditions, would be a valuable extension of research. It would
600 provide a better understanding of how flexible or rigid these plants are to acclimate to various light regimes
601 and by extension define the potential habitats each species could, hypothetically, inhabit. In particular, there
602 has been little research into the photobiology or metabolic tolerances of *H. spinulosa* outside of this study.

603

604 The wavelength-specific photochemistry did differ somewhat between species. For *H. decipiens*, the sub-
605 saturating AL measurements used to assess wavelength-specific parameters resulted in significantly higher
606 Y(II) and rETR(II) in high light treatment leaves, regardless of temperature, despite no measurable
607 difference in NPQ. Under supra-saturating AL, only wavelength-specific differences (no treatment effects)
608 were found in these parameters for *H. decipiens*. While NPQ was operational under supra-saturating AL for
609 both HL and LL treated plants, photodamage may have occurred in this species irrespective of growing
610 treatment conditions. Plants typically grown under lower light intensities produce greater amount of light
611 harvesting accessory pigments in the antennae, namely Chl *b*, than high light grown plants (Lichtenthaler et
612 al., 1981; Ruban, 2014). The larger antenna serves to increase light harvesting and therefore increased
613 photochemical reactions in shaded conditions. However, Ware et al. (2015) point out the enhanced antenna
614 size would actually increase excitation pressure unnecessarily under rare high light conditions, which is
615 suggested to be related to uncoupling of the antenna structure in LL grown plants having poor connectivity to
616 reaction centres. Their research found LL plants had high NPQ under high light intensities, but with poor
617 efficacy of dissipating excess energy and protecting PSII reaction centres. Therefore, measured NPQ was
618 accounting for both connected and disassociated antenna complexes and exaggerating the effect of NPQ on
619 bound antenna involved in the electron transport chain. This would explain the lack of treatment effect in our
620 study on Y(II) and rETR(II) measurements despite differences in NPQ between LL and HL treatments under
621 the supra-saturating light conditions for *H. decipiens*. It is also further photophysiological support that *H.*
622 *decipiens* is an obligate low-light adapted species compared to *H. spinulosa*, which is tolerant of a wider
623 range of irradiance. For *H. spinulosa*, the opposite was true. Higher Y(II) and rETR(II) values under supra-
624 saturating AL condition were measured in leaves from the high-light treatments, whereas only wavelength

625 was correlated with sub-saturating AL measurements. This outcome supports a greater inherent capacity to
626 maintain connectivity between antennae and reaction centres when exposed to supra-saturating conditions.

627

628 The pronounced wavelength-dependent patterns in these deep-water seagrasses are a reflection of
629 biochemical pathways used to maximise photochemical efficiencies. In order to integrate these patterns in
630 photo-physiology with the underlying molecular processes, techniques such as transcriptomics and
631 metabolomics would invaluablely enhance our understanding of the observations in this study. Efforts to
632 innovate and fuse classical ecological studies with molecular approaches has been established and used as an
633 important path forward to enhance multidisciplinary seagrass research (Macreadie et al., 2014; Mazzuca et
634 al., 2013).

635

636 Both species investigated did not show dramatic changes in photochemistry or metabolism due to light
637 stress, yet there was a significant decline in shoot density for *H. decipiens* and *H. spinulosa* over the four-
638 week period. The compensatory mechanisms and photoacclimation that did occur in low-light treated plants
639 are either not sufficient to counteract light limitation or the physiology is impacted downstream of
640 photochemistry in other metabolic pathways. An alternative explanation of shoot loss in our study is a
641 sacrificial approach whereby changes are made at the ramet scale instead of the leaf scale. This strategy
642 would place efforts on repartitioning resources away from new shoots and directing energy into a few
643 remaining leaves while sacrificing all others. While we were not able to reconcile this in the current study,
644 identifying regulatory pathways and resource allocation signalling through a gene expression and
645 bioinformatics approach could be correlated with threshold responses to light stress as we tested here.

646

647 Acute light stress to deep-water seagrasses has implications for the larger context of deep-water seagrass
648 meadow maintenance. Despite not directly investigating the effects of light on flowering and seed banks in
649 this study, the net effects of light stress on sexual reproductive effort by seagrasses has been described
650 (Cabaço and Santos, 2012). Seed production is likely vital in deep-water population in order to regenerate
651 annually or following natural disturbances such as storms or cyclones (Hammerstrom et al., 2006;
652 Kenworthy, 2000) and any impact on fruit production and seed recruitment into the sediment could have
653 significant impacts on the subsequent year's seedling recruitment. Ensuring suitable light levels to ensure

654 sufficient energy requirements to be put into reproductive output during key growing phases may be the most
655 important factor for long-term viability and deep-water seagrass' success.

656

657 5. CONCLUSIONS

658 This study has established a clear, negative effect of relatively small quantitative reductions in growing
659 season light on deep-water *Halophila decipiens* and *Halophila spinulosa* communities from the Great Barrier
660 Reef lagoon. It further highlights hitherto undetected differences of closely related species to light and
661 temperature conditions. This could have implications for making broad-based decisions using tools such as
662 form-function models (Kilminster et al., 2015; Walker et al., 1999) of seagrass to infer light requirements
663 and the associated response of seagrasses with limited understanding of their specific energetic needs. Such
664 approaches to curb seagrass loss could overlook the species-specific adaptations to the local environment and
665 lead to unintended negative outcomes for local seagrass communities. In spite of inter-species differences, *H.*
666 *decipiens* and *H. spinulosa* did show classic higher plant responses to low light and significant shoot loss as a
667 response to the same quantitative light levels over a short time-span. Some generalised decision tools to
668 mitigate impacts to deep-water *Halophila* spp. could therefore still foster suitable growing conditions. Some
669 generalised decision tools (Wu et al., 2017) to mitigate impacts to deep-water *Halophila* spp. could therefore
670 still facilitate best-practice management of these mixed species seagrass communities.

671

672 For the first time, wavelength-specific parameters of PSII photochemistry were evaluated for seagrass leaves.
673 While there was no effect of light or temperature on $\sigma_{II}(\lambda)$ in deep-water *Halophila* spp. in this study, there
674 was a wavelength-specific response of photochemical reactions of PSII. The effect of low-light acclimation
675 was apparent in non-photochemical quenching patterns including differences in tolerance between species to
676 supra-saturating intensities, which likely reflects their inherent adaptations to their natural light
677 environments. A valuable next step would be to integrate the measured patterns in photo-physiology with the
678 underlying biochemical processes through an interdisciplinary bioinformatics approach.

679

680 With measurable impacts to *H. decipiens* and *H. spinulosa* after 2 weeks and 4 weeks respectively, even
681 relatively short periods of increased light attenuation can affect key life history strategies used to ensure
682 long-term meadow maintenance such as flowering and seed bank generation.

683

684 **ACKNOWLEDGEMENTS**

685 The authors would like to thank the valuable work of the TropWATER research team who helped collect and
686 transport samples to UTS as well as Louisa Norman (UTS) for maintaining seagrass aquaria for the duration
687 of the study. This work was supported by funding from Queensland Gas Corporation Ltd.

688

689

690

691

692

693 **REFERENCES**

- 694 Abal, E.G., Loneragan, N., Bowen, P., Perry, C.J., Udy, J.W., Dennison, W.C., 1994. Physiological and
695 morphological responses of the seagrass *Zostera capricorni* Aschers, to light intensity. J. Exp. Mar.
696 Biol. Ecol. 178, 113-129.
- 697 Adams, M.P., Collier, C.J., Uthicke, S., Ow, Y.X., Langlois, L., O'Brien, K.R., 2017. Model fit versus biological
698 relevance: Evaluating photosynthesis-temperature models for three tropical seagrass species. Sci.
699 Rep. 7.
- 700 Alcoverro, T., Manzanera, M., Romero, J., 2001. Annual metabolic carbon balance of the seagrass *Posidonia*
701 *oceanica*: the importance of carbohydrate reserves. Mar. Ecol. Prog. Ser. 211, 105-116.
- 702 Bartoń, K., 2013. MuMIn: Multi-model inference. R package version 1.9. 13. The Comprehensive R Archive
703 Network (CRAN), Vienna, Austria.
- 704 Bates, D., Maechler, M., Bolker, B., 2012. lme4: Linear mixed-effects models using Eigen and
705 Eigen. J. Stat. Softw. 65, 1-68.
- 706 Beer, S., Waisel, Y., 1982. Effects of light and pressure on photosynthesis in two seagrasses. Aquat. Bot. 13,
707 331-337.
- 708 Bryant, C.V., Davies, J.D., Jarvis, J.C., Tol, S., Rasheed, M.A., 2013. Seagrasses in Port Curtis and Rodds Bay
709 2013: Annual Long Term Monitoring, Biannual Western Basin Surveys & Updated Baseline Survey.
710 Centre for Tropical Water & Aquatic Ecosystem Research (TropWATER), James Cook University
711 Cairns, p. 71.
- 711 Bulthuis, D.A., 1987. Effects of temperature on photosynthesis and growth of seagrasses. Aquat. Bot. 27,
712 27-40.
- 713 Burke, M., Dennison, W., Moore, K., 1996. Non-structural carbohydrate reserves of eelgrass *Zostera*
714 *marina*. Mar. Ecol. Prog. Ser. 137, 195-201.
- 715 Burnham, K., Anderson, D., 2002. Model Selection and Multimodal Inference: A Practical Information-
716 Theoretic Approach. 2nd edn Springer-Verlag. New York, NY.
- 717 Cabaço, S., Santos, R., 2012. Seagrass reproductive effort as an ecological indicator of disturbance. Ecol.
718 Indicators 23, 116-122.
- 719 Campbell, S.J., Miller, C.J., 2002. Shoot and abundance characteristics of the seagrass *Heterozostera*
720 *tasmanica* in Westernport estuary (south-eastern Australia). Aquat. Bot. 73, 33-46.
- 721 Carruthers, T.J.B., Dennison, W.C., Longstaff, B.J., Waycott, M., Abal, E.G., McKenzie, L.J., Long, W.J.L., 2002.
722 Seagrass habitats of Northeast Australia: Models of key processes and controls. Bull. Mar. Sci. 71,
723 1153-1169.
- 724 Chartrand, K.M., Bryant, C.V., Carter, A.B., Ralph, P.J., Rasheed, M.A., 2016. Light thresholds to prevent
725 dredging impacts on the Great Barrier Reef seagrass, *Zostera muelleri* ssp. *capricorni*. Front. Mar.
726 Sci. 3, 106.

- 727 Coles, R., McKenzie, L., De'ath, G., Roelofs, A.J., Lee Long, W., 2009. Spatial distribution of deepwater
728 seagrass in the inter-reef lagoon of the Great Barrier Reef World Heritage Area. *Mar. Ecol. Prog.*
729 *Ser.* 392, 57-68.
- 730 Collier, C., Waycott, M., McKenzie, L., 2012a. Light thresholds derived from seagrass loss in the coastal zone
731 of the northern Great Barrier Reef, Australia. *Ecol. Indicators* 23, 211-219.
- 732 Collier, C.J., Lavery, P.S., Ralph, P.J., Masini, R.J., 2009. Shade-induced response and recovery of the
733 seagrass *Posidonia sinuosa*. *J. Exp. Mar. Biol. Ecol.* 370, 89-103.
- 734 Collier, C.J., Uthicke, S., Waycott, M., 2011. Thermal tolerance of two seagrass species at contrasting light
735 levels: Implications for future distribution in the Great Barrier Reef. *Limnol. Oceanogr.* 56, 2200-
736 2210.
- 737 Collier, C.J., Waycott, M., Ospina, A.G., 2012b. Responses of four Indo-West Pacific seagrass species to
738 shading. *Mar. Pollut. Bull.* 65, 342-354.
- 739 Cosgrove, J., Borowitzka, M.A., 2010. Chlorophyll fluorescence terminology: an introduction, Chlorophyll a
740 fluorescence in aquatic sciences: methods and applications. Springer, pp. 1-17.
- 741 Costa, M.M., Barrote, I., Silva, J., Santos, R., 2014. Chapt. 3 Photosynthetic pigment content in seagrasses.
742 In: Effect of high CO₂ and ocean acidification on photosynthesis and response to oxidative stress in
743 seagrasses. , Ciências Biológicas. Universidade do Algarve, Portugal, p. 51.
- 744 Costanza, R., de Groot, R., Sutton, P., van der Ploeg, S., Anderson, S.J., Kubiszewski, I., Farber, S., Turner,
745 R.K., 2014. Changes in the global value of ecosystem services. *Global Environ. Change* 26, 152-158.
- 746 Cummings, M.E., Zimmerman, R.C., 2003. Light harvesting and the package effect in the seagrasses
747 *Thalassia testudinum* Banks ex König and *Zostera marina* L.: optical constraints on
748 photoacclimation. *Aquat. Bot.* 75, 261-274.
- 749 Czerny, A.B., Dunton, K.H., 1995. The effects of in situ light reduction on the growth of two subtropical
750 seagrasses, *Thalassia testudinum* and *Halodule wrightii*. *Estuaries and Coasts* 18, 418-427.
- 751 Dawes, C.J., Lobban, C.S., Tomasko, D.A., 1989. A comparison of the physiological ecology of the seagrasses
752 *Halophila decipiens* Ostenfeld and *H. Johnsonii* Eiseman from Florida. *Aquat. Bot.* 33, 149-154.
- 753 Dennison, W.C., Orth, R.J., Moore, K.A., Stevenson, J.C., Carter, V., Kollar, S., Bergstrom, P.W., Batiuk, R.A.,
754 1993. Assessing water quality with submersed aquatic vegetation. *Bioscience* 43, 86-94.
- 755 Duarte, C.M., 1991. Seagrass depth limits. *Aquat. Bot.* 40, 363-377.
- 756 Duarte, C.M., Marbà, N., Gacia, E., Fourqurean, J.W., Beggins, J., Barrón, C., Apostolaki, E.T., 2010. Seagrass
757 community metabolism: Assessing the carbon sink capacity of seagrass meadows. *Global*
758 *Biogeochem. Cycles* 24, GB4032.
- 759 Durako, M.J., Kunzelman, J.I., Kenworthy, W.J., Hammerstrom, K.K., 2003. Depth-related variability in the
760 photobiology of two populations of *Halophila johnsonii* and *Halophila decipiens*. *Mar. Biol.* 142,
761 1219-1228.
- 762 Enriquez, S., 2005. Light absorption efficiency and the package effect in the leaves of the seagrass *Thalassia*
763 *testudinum*. *Mar. Ecol. Prog. Ser.* 289, 141-150.
- 764 Enríquez, S., Rodríguez-Román, A., 2006. Effect of water flow on the photosynthesis of three marine
765 macrophytes from a fringing-reef lagoon. *Mar. Ecol. Prog. Ser.* 323, 119-132.
- 766 Erftemeijer, P.L.A., Stapel, J., 1999. Primary production of deep-water *Halophila ovalis* meadows. *Aquat.*
767 *Bot.* 65, 71-82.
- 768 Evans, J.R., 2009. Potential errors in electron transport rates calculated from chlorophyll fluorescence as
769 revealed by a multilayer leaf model. *Plant Cell Physiol.* 50, 698-706.
- 770 Falkowski, P.G., Raven, J.A., 2007. *Aquatic Photosynthesis*. Princeton University Press.
- 771 Ferreira, C., Horta, P.A., Almeida, G.M., Zitta, C.S., de M. Oliveira, E., Gueye, M.B.Y.B., Rodrigues, A.C., 2015.
772 Anatomical and ultrastructural adaptations of seagrass leaves: an evaluation of the southern
773 Atlantic groups. *Protoplasma* 252, 3-20.
- 774 Fonseca, M.S., Kenworthy, W.J., Griffith, E., Hall, M.O., Finkbeiner, M., Bell, S.S., 2008. Factors influencing
775 landscape pattern of the seagrass *Halophila decipiens* in an oceanic setting. *Estuar. Coast. Shelf Sci.*
776 76, 163-174.
- 777 Fournier, D.A., Skaug, H.J., Ancheta, J., Ianelli, J., Magnusson, A., Maunder, M.N., Nielsen, A., Sibert, J.,
778 2012. AD Model Builder: using automatic differentiation for statistical inference of highly
779 parameterized complex nonlinear models. *Optimization Methods and Software* 27, 233-249.

- 780 Gordon, D., Grey, K., Chase, S., Simpson, C., 1994. Changes to the structure and productivity of a *Posidonia*
781 *sinuosa* meadow during and after imposed shading. *Aquat. Bot.* 47, 265-275.
- 782 Grech, A., Chartrand-Miller, K., Erftemeijer, P., Fonseca, M., McKenzie, L., Rasheed, M., Taylor, H., Coles, R.,
783 2012. A comparison of threats, vulnerabilities and management approaches in global seagrass
784 bioregions. *Environ. Res. Lett.* 7, 024006.
- 785 Grech, A., Coles, R., Marsh, H., 2011. A broad-scale assessment of the risk to coastal seagrasses from
786 cumulative threats. *Mar. Policy* 35, 560-567.
- 787 Hammerstrom, K.K., Kenworthy, W.J., Fonseca, M.S., Whitfield, P.E., 2006. Seed bank, biomass, and
788 productivity of *Halophila decipiens*, a deep water seagrass on the west Florida continental shelf.
789 *Aquat. Bot.* 84, 110-120.
- 790 Heck, K.L.J., Carruthers, T.J., Duarte, C.M., Hughes, A.R., Kendrick, G., Orth, R.J., Williams, S.W., 2008.
791 Trophic transfers from seagrass meadows subsidize diverse marine and terrestrial consumers.
792 *Ecosystems* 11, 1198-1210.
- 793 Hemminga, M.A., Duarte, C.M., 2000. *Seagrass ecology*. Cambridge University Press.
- 794 Hovey, R.K., Statton, J., Fraser, M.W., Ruiz-Montoya, L., Zavala-Perez, A., Rees, M., Stoddart, J., Kendrick,
795 G.A., 2015. Strategy for assessing impacts in ephemeral tropical seagrasses. *Mar. Pollut. Bull.* 101,
796 594-599.
- 797 Jackson, E.L., Rowden, A.A., Attrill, M.J., Bossey, S., Jones, M., 2001. The importance of seagrass beds as a
798 habitat for fishery species. *Oceanogr. Mar. Biol.* 39, 269-304.
- 799 Josselyn, M., Fonseca, M., Niesen, T., Larson, R., 1986. Biomass, production and decomposition of a deep
800 water seagrass, *Halophila decipiens* Ostenfeld. *Aquat. Bot.* 25, 47-61.
- 801 Karkalas, J.J., 1985. An improved enzymatic method for the determination of native and modified starch. *J.*
802 *Sci. Food. Agric.* 36, 1019-1027.
- 803 Kenworthy, W., 2000. The Role of Sexual Reproduction in Maintaining Populations of *Halophila decipiens*:
804 Implications for the Biodiversity and Conservation of Tropical Seagrass Ecosystems. *Pac. Conserv.*
805 *Biol.* 5, 260.
- 806 Kenworthy, W.J., Currin, C.A., Fonseca, M.S., Smith, G., 1989. Production, decomposition, and
807 heterotrophic utilization of the seagrass *Halophila decipiens* in a submarine canyon. *Mar. Ecol.*
808 *Prog. Ser.* 51, 277-290.
- 809 Kilminster, K., McMahon, K., Waycott, M., Kendrick, G.A., Scanes, P., McKenzie, L., O'Brien, K.R., Lyons, M.,
810 Ferguson, A., Maxwell, P., 2015. Unravelling complexity in seagrass systems for management:
811 Australia as a microcosm. *Sci. Total Environ.*
- 812 Kitajima, K., Hogan, K., 2003. Increases of chlorophyll a/b ratios during acclimation of tropical woody
813 seedlings to nitrogen limitation and high light. *Plant, Cell Environ.* 26, 857-865.
- 814 Koch, E.W., 2001. Beyond light: physical, geological, and geochemical parameters as possible submersed
815 aquatic vegetation habitat requirements. *Estuaries* 24, 1-17.
- 816 Kuo, J., Den Hartog, C., 2006. Seagrass morphology, anatomy, and ultrastructure, *Seagrasses: Biology,*
817 *Ecology and Conservation*. Springer, pp. 51-87.
- 818 Kuo, J., Kirkman, H., 1995. *Halophila decipiens* Ostenfeld in estuaries of southwestern Australia. *Aquat. Bot.*
819 51, 335-340.
- 820 Kuznetsova, A., Brockhoff, P.B., Christensen, R.H.B., 2015. Package 'lmerTest'. R package version, 2.0-29.
- 821 Larkum, A.W., Drew, E.A., Ralph, P.J., 2006. Photosynthesis and metabolism in seagrasses at the cellular
822 level, *Seagrasses: Biology, Ecology and Conservation*. Springer.
- 823 Lee, K.-S., Park, S.R., Kim, Y.K., 2007. Effects of irradiance, temperature, and nutrients on growth dynamics
824 of seagrasses: A review. *J. Exp. Mar. Biol. Ecol.* 350, 144-175.
- 825 Lenth, R.V., 2016. Least-squares means: the R Package lsmeans. *J Stat Softw* 69, 1-33.
- 826 Leong, T.-Y., Anderson, J.M., 1984. Adaptation of the thylakoid membranes of pea chloroplasts to light
827 intensities. I. Study on the distribution of chlorophyll-protein complexes. *Photosynthesis Res.* 5,
828 105-115.
- 829 Lichtenthaler, H., Buschmann, C., Döll, M., Fietz, H.-J., Bach, T., Kozel, U., Meier, D., Rahmsdorf, U., 1981.
830 Photosynthetic activity, chloroplast ultrastructure, and leaf characteristics of high-light and low-
831 light plants and of sun and shade leaves. *Photosynthesis Res.* 2, 115-141.
- 832 Longstaff, B.J., Dennison, W.C., 1999. Seagrass survival during pulsed turbidity events: the effects of light
833 deprivation on the seagrasses *Halodule pinifolia* and *Halophila ovalis*. *Aquat. Bot.* 65, 105-121.

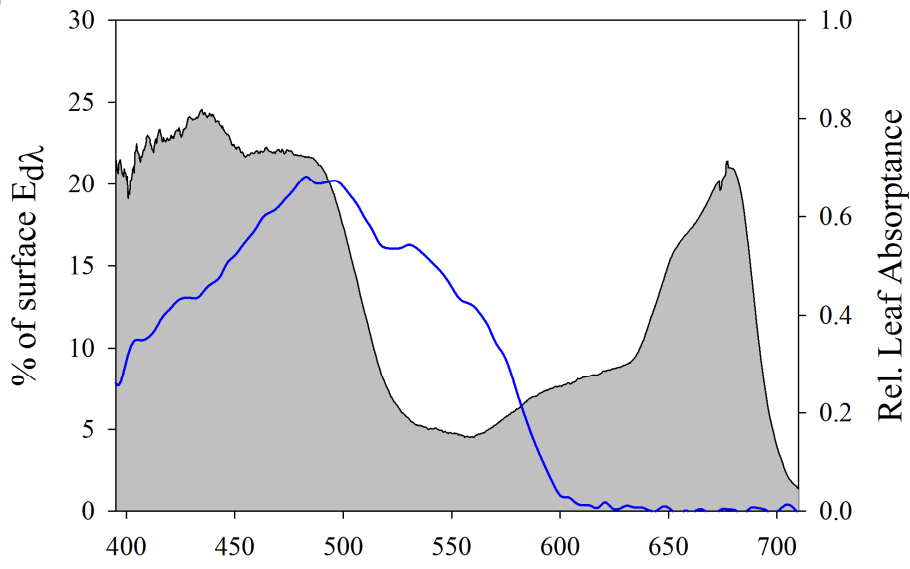
- 834 Longstaff, B.J., Loneragan, N.R., O'Donohue, M.J., Dennison, W.C., 1999. Effects of light deprivation on the
835 survival and recovery of the seagrass *Halophila ovalis* (R.Br.) Hook. J. Exp. Mar. Biol. Ecol. 234, 1-27.
- 836 Macreadie, P., Schliep, M., Rasheed, M., Chartrand, K., Ralph, P., 2014. Molecular indicators of chronic
837 seagrass stress: A new era in the management of seagrass ecosystems? Ecol. Indicators 38, 279-
838 281.
- 839 Masini, R., Cary, J., Simpson, C., McComb, A., 1995. Effects of light and temperature on the photosynthesis
840 of temperate meadow-forming seagrasses in Western Australia. Aquat. Bot. 49, 239-254.
- 841 Mazzuca, S., Bjork, M., Beer, S., Felisberto, P., Gobert, S., Procaccini, G., Runcie, J., Silva, J., Borges, A.,
842 Brunet, C., Buapet, P., Champenois, W., Costa, M., D'Esposito, D., Gullström, M., Lejeune, P.,
843 Lepoint, G., Olivé, I., Rasmunsson, L., Richir, J., Ruocco, M., Serra, I., Spadafora, A., Santos, R., 2013.
844 Establishing Research Strategies, Methodologies and Technologies to Link Genomics and
845 Proteomics to Seagrass Productivity, Community Metabolism, and Ecosystem Carbon Fluxes. Front.
846 Plant. Sci. 4.
- 847 McKenna, S., Jarvis, J., Sankey, T., Reason, C., Coles, R., Rasheed, M., 2015. Declines of seagrasses in a
848 tropical harbour, North Queensland, Australia, are not the result of a single event. J. Biosci.
849 (Bangalore) 40, 389-398.
- 850 Nieuwenhuis, R., te Grotenhuis, H., Pelzer, B., 2012. Influence. ME: tools for detecting influential data in
851 mixed effects models.
- 852 Orth, R.J., Harwell, M.C., Inglis, G.J., 2006. Ecology of seagrass seeds and seagrass dispersal processes,
853 Seagrasses: Biology, Ecology and Conservation. Springer.
- 854 Osmond, B., Chow, W.S., Wyber, R., Zavafer, A., Keller, B., Pogson, B.J., Robinson, S.A., 2017. Relative
855 functional and optical absorption cross-sections of PSII and other photosynthetic parameters
856 monitored *in situ*, at a distance with a time resolution of a few seconds, using a prototype light
857 induced fluorescence transient (LIFT) device. Funct. Plant Biol.
- 858 Ralph, P., Burchett, M., 1998. Photosynthetic response of *Halophila ovalis* to heavy metal stress. Environ.
859 Pollut. 103, 91-101.
- 860 Ralph, P.J., Durako, M.J., Enríquez, S., Collier, C.J., Doblin, M.A., 2007. Impact of light limitation on
861 seagrasses. J. Exp. Mar. Biol. Ecol. 350, 176-193.
- 862 Rasheed, M.A., McKenna, S.A., Carter, A.B., Coles, R.G., 2014. Contrasting recovery of shallow and deep
863 water seagrass communities following climate associated losses in tropical north Queensland,
864 Australia. Mar. Pollut. Bull. 83, 491-499.
- 865 Ritchie, R.J., 2006. Consistent sets of spectrophotometric chlorophyll equations for acetone, methanol and
866 ethanol solvents. Photosynthesis Res. 89, 27-41.
- 867 Ruban, A.V., 2014. Evolution under the sun: optimizing light harvesting in photosynthesis. J. Exp. Bot. 66, 7-
868 23.
- 869 Schreiber, U., 2004. Pulse-amplitude-modulation (PAM) fluorometry and saturation pulse method: an
870 overview, Chlorophyll a Fluorescence. Springer, pp. 279-319.
- 871 Schreiber, U., Klughammer, C., 2013. Wavelength-dependent photodamage to *Chlorella* investigated with a
872 new type of multi-color PAM chlorophyll fluorometer. Photosynthesis Res. 114, 165-177.
- 873 Schreiber, U., Klughammer, C., Kolbowski, J., 2012. Assessment of wavelength-dependent parameters of
874 photosynthetic electron transport with a new type of multi-color PAM chlorophyll fluorometer.
875 Photosynthesis Res. 113, 127-144.
- 876 Sharon, Y., Levitan, O., Spungin, D., Berman-Frank, B., Beer, S., 2011. Photoacclimation of the seagrass
877 *Halophila stipulacea* to the dim irradiance at its 48-meter depth limit. Limnol. Oceanogr. 56, 357.
- 878 Short, F.T., Neckles, H.A., 1999. The effects of global climate change on seagrasses. Aquat. Bot. 63, 169-196.
- 879 Smith, H., 1982. Light quality, photoperception, and plant strategy. Annu. Rev. Plant. Physiol. 33, 481-518.
- 880 Strasser, R., Govindjee, 1992. On the OJIP fluorescence transient in leaves and D1 mutants of
881 *Chlamydomonas-reinhardtii*, Photosynthesis Res. Kluwer Academic Publ pp. 135-135.
- 882 Szabó, M., Wangpraseurt, D., Tamburic, B., Larkum, A.W., Schreiber, U., Suggett, D.J., Kühl, M., Ralph, P.J.,
883 2014. Effective light absorption and absolute electron transport rates in the coral *Pocillopora*
884 *damicornis*. Plant Physiol. Biochem. 83, 159-167.
- 885 Touchette, B.W., Burkholder, J.M., 2000a. Overview of the physiological ecology of carbon metabolism in
886 seagrasses. J. Exp. Mar. Biol. Ecol. 250, 169-205.

- 887 Touchette, B.W., Burkholder, J.M., 2000b. Overview of the physiological ecology of carbon metabolism in
888 seagrasses. *J. Exp. Mar. Biol. Ecol.* 250, 169-205.
- 889 Vogelmann, T.C., Evans, J., 2002. Profiles of light absorption and chlorophyll within spinach leaves from
890 chlorophyll fluorescence. *Plant, Cell Environ.* 25, 1313-1323.
- 891 Voitsekhovskaja, O., Tyutereva, E., 2015. Chlorophyll b in angiosperms: functions in photosynthesis,
892 signaling and ontogenetic regulation. *J. Plant Physiol.* 189, 51-64.
- 893 Wagenmakers, E.-J., Farrell, S., 2004. AIC model selection using Akaike weights. *Psychon. Bull. Rev.* 11, 192-
894 196.
- 895 Walker, D., Dennison, W., Edgar, G., 1999. Status of Australian seagrass research and knowledge. Seagrass
896 in Australia. Strategic review and development of an R&D plan. CSIRO, Canberra Australia, 1-24.
- 897 Walker, D., Kendrick, G., McComb, A., 1988. The distribution of seagrass species in Shark Bay, Western
898 Australia, with notes on their ecology. *Aquat. Bot.* 30, 305-317.
- 899 Walters, R.G., 2005. Towards an understanding of photosynthetic acclimation. *J. Exp. Bot.* 56, 435-447.
- 900 Ware, M.A., Belgio, E., Ruban, A.V., 2015. Photoprotective capacity of non-photochemical quenching in
901 plants acclimated to different light intensities. *Photosynthesis Res.* 126, 261-274.
- 902 Waycott, M., Duarte, C.M., Carruthers, T.J., Orth, R.J., Dennison, W.C., Olyarnik, S., Calladine, A.,
903 Fourqurean, J.W., Heck, K.L., Hughes, A.R., 2009. Accelerating loss of seagrasses across the globe
904 threatens coastal ecosystems. *Proc. Natl. Acad. Sci. USA* 106, 12377-12381.
- 905 Waycott, M., McMahon, K.M., Mellors, J., Calladine, A., Kleine, D., 2004. A guide to tropical seagrasses in
906 the Indo-West Pacific. James Cook University Townsville.
- 907 Weir, K.L., Wilson, J.R., White, R.J., 1977. CSIRO Australian Division Tropical Paper No. 20 CSIRO, pp. 1-10.
- 908 Williams, S.L., Dennison, W.C., 1990. Light availability and diurnal growth of a green macroalga (*Caulerpa*
909 *cupressoides*) and a seagrass (*Halophila decipiens*). *Mar. Biol.* 106, 437-443.
- 910 Wood, S., 2006. Generalized additive models: an introduction with R. CRC press.
- 911 Wu, P.P.Y., McMahon, K., Rasheed, M.A., Kendrick, G.A., York, P.H., Chartrand, K., Caley, M.J., Mengersen,
912 K., 2017. Managing seagrass resilience under cumulative dredging affecting light: Predicting risk
913 using dynamic Bayesian networks. *J. Appl. Ecol.*
- 914 York, P., Carter, A., Chartrand, K.M., Sankey, T., Wells, L., Rasheed, M.A., 2015. Dynamics of a deep-water
915 seagrass population on the Great Barrier Reef: annual occurrence and response to a major dredging
916 program. *Sci. Rep.*
- 917 York, P.H., Gruber, R.K., Hill, R., Ralph, P.J., Booth, D.J., Macreadie, P.I., 2013. Physiological and
918 morphological responses of the temperate seagrass *Zostera muelleri* to multiple stressors:
919 investigating the interactive effects of light and temperature. *PLoS One* 8, e76377.
- 920 Zivcak, M., Brestic, M., Kalaji, H.M., Govindjee, 2014. Photosynthetic responses of sun- and shade-grown
921 barley leaves to high light: is the lower PSII connectivity in shade leaves associated with protection
922 against excess of light? *Photosynthesis Res.* 119, 339-354.
- 923 Zuur, A., Ieno, E., Walker, N., Saveliev, A., Smith, G., 2009. Mixed effects models and extensions in ecology
924 with R. New York: Springer.

925

926 **APPENDIX A**

927 Appendix Figure 1. Percent surface irradiance measured at the *H. decipiens* field collection site during the
928 peak growing season and relative leaf absorptance of a *H. decipiens* leaf from control tubs as measured with
929 an integrating sphere prior to MC-PAM measurements.



930

931

HIGHLIGHTS:

Living at the margins – the response of deep-water seagrasses to light and temperature renders them susceptible to acute impacts

- Deep-water seagrasses differ in scale and time of response to light and temperature
- Wavelength-specific parameters of leaf PSII photochemistry were evaluated
- Photoacclimation and physiological adjustments did not compensate for low light
- Acute turbidity plumes can drive rapid loss for seagrasses at the functional margins
- A light threshold is proposed to protect deep-water seagrasses from acute impacts