



REVIEW

# Combined impacts of photosystem II-inhibiting herbicides and light availability on seagrass and marine microalgae

Olivia C. King<sup>1,\*</sup>, Rachael A. Smith<sup>2</sup>, Michael St. J. Warne<sup>3,4,5</sup>,  
Jason P. van de Merwe<sup>1</sup>, Rod M. Connolly<sup>1</sup>, Christopher J. Brown<sup>6</sup>

<sup>1</sup>Australian Rivers Institute — Coast & Estuaries, School of Environment and Science, Griffith University, Gold Coast, Queensland 4222, Australia

<sup>2</sup>Office of the Great Barrier Reef, Department of Environment and Science, Queensland Government, Brisbane, Queensland 4000, Australia

<sup>3</sup>School of Earth and Environmental Sciences, University of Queensland, Brisbane, Queensland 4067, Australia

<sup>4</sup>Water Quality and Investigations, Queensland Department of Environment and Science, Brisbane, Queensland 4102, Australia

<sup>5</sup>Centre for Agroecology, Water and Resilience, Coventry University, West Midlands CV1 5FB, UK

<sup>6</sup>Australian Rivers Institute — Coast & Estuaries, School of Environment and Science, Griffith University, Nathan, Queensland 4111, Australia

**ABSTRACT:** The combined and interactive effects of multiple stressors threaten coastal ecosystems, yet most ecological risk assessments used to inform environmental management still treat stressors separately. For marine microalgae and seagrass — particularly those common to the Great Barrier Reef, Australia — key stressors include low light from increased turbidity and herbicide exposure that runs off agricultural land. Despite co-occurring in aquatic ecosystems, the effects of these stressors are often studied separately, meaning any combined or interactive effects are overlooked. Here, we aimed to develop a conceptual synthesis of the physiological responses of marine microalgae and seagrass when exposed to these key stressors. We reviewed marine microalgae and seagrass exposure studies to understand how herbicide and light stress is assessed and generated hypotheses for the combined effects. In particular, we predict that photo-physiological, biochemical and whole-organism responses of aquatic plants and algae will interact antagonistically, additively or synergistically depending on the level of light availability and the endpoint measured. We recommend that future multi-stressor exposure experiments study how specific physiological processes interact to impact the growth of important primary producers such as microalgae and seagrasses. This will enable management to accurately determine the ecological risk of multiple stressors to aquatic species and ecosystems.

**KEY WORDS:** Stressor interaction · Stressor combination · Coastal ecosystems · Seagrass · Microalgae · Photosystem II herbicides · Light availability

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## 1. INTRODUCTION

Inshore and coastal ecosystems are exposed to multiple stressors that act individually, but may also have interactive effects in combination (Lotze 2006, Brodie et al. 2013, Chapman 2017, Waterhouse et al.

2017). Stressors are influenced by local anthropogenic factors, global climate change and natural climate variability (Brown et al. 2014, Baird et al. 2016, Dafforn et al. 2016, Chapman 2017). Predicting the combined impacts of more than one stressor is complicated, and their interacting effects are not

\*Corresponding author: olivia.king@griffithuni.edu.au

routinely considered in risk assessments (Crain et al. 2008, Brown et al. 2014, Chariton et al. 2016, Côté et al. 2016, Dafforn et al. 2016). Ecological risk assessments (ERAs) are frequently used by management agencies to manage stressor inputs and inform decisions for ongoing monitoring and management. Incorporating multiple interacting stressors into ERAs is a high priority for environmental management (Halpern et al. 2008a, Van den Brink et al. 2016, Waterhouse et al. 2017), yet the vast majority of the most recent ERAs still treat environmental stressors separately (Brodie et al. 2013, Waterhouse et al. 2017) and as a consequence, the management of coastal ecosystems does not consider stressor interactions (Griffiths et al. 2019).

The problem with incorporating multiple-stressor research into risk assessments is that there are more combinations of stressors and potential interactions than can be empirically quantified by scientists (Côté et al. 2016); therefore, management relies on modelled predictions. To better predict stressor interactions in organisms and ecosystems, further research is needed to develop ecological process models that can evaluate the likely effects of any combination of stressors (Brown et al. 2014, Van den Brink et al. 2016). Models that draw upon information regarding mode of action may indicate whether responses to simultaneous stressors are independent, combined or interactive (Traas et al. 2002). For example, stressors that act on the same receptor (i.e. similar mode of action) could suggest similar types of interactions (Boyd & Brown 2015). Using models to predict the combined and interacting effects of stressors on ecosystems will allow ERAs to predict the environmental impacts of stressors that co-occur and enable management bodies to adapt decisions to cumulative impacts (Van den Brink et al. 2016, Chapman 2017, Bracewell et al. 2019). For example, the risk of photosystem II (PSII)-inhibiting herbicides to aquatic ecosystems may be severely underestimated because they are typically considered individually (Solomon et al. 2000, Van den Brink et al. 2006, Luo et al. 2011, Jesenska et al. 2013) but usually occur in mixtures with other pesticides and stressors (Lewis et al. 2012, Smith et al. 2012, Huggins et al. 2017, Warne et al. 2020).

Aquatic photosynthetic organisms are important primary producers; thus, identifying their physiological responses to natural and anthropogenic stressors is essential to understanding important connections and interactions in ecosystem dynamics. Primary producers include seagrasses, algal epiphytes (e.g. algae attached to seagrass leaves), mar-

ine microalgae (phytoplankton), benthic microalgae and macroalgae. Seagrass and marine microalgae, in particular, are important to coastal ecosystem functioning, nutrient cycling; they provide habitat and food for a variety of organisms as well as stabilizing substrate (Lee & Dunton 1996, Orth et al. 2006, Lee et al. 2007), and are likely to be affected by multiple stressors due to their proximity to anthropogenic stressors derived from terrestrial activities (Raven & Geider 2003, Halpern et al. 2008b, Collier et al. 2016, Wallace et al. 2016). Following periods of elevated rainfall, pollutants including excess suspended sediments, nutrients and pesticides are transported in runoff from agricultural land to the inshore marine environment (Huggins et al. 2017, Waterhouse et al. 2017) and have specifically been targeted as the 3 key pollutants contributing to poor water quality in the Great Barrier Reef (GBR) Marine Park, Queensland, Australia (Brodie et al. 2013, Waterhouse et al. 2017). However, excess nutrients as a result of anthropogenic activities are not considered to be a 'direct' stressor to phototrophic species, as low to moderate nutrient enrichment promotes rather than inhibits photosynthetic growth (Jaschinski et al. 2010). As such, nutrients as a stressor will not be considered further in this review. In contrast, increasing turbidity due to excess total suspended sediment (i.e. generated from anthropogenic activities) causes a decrease in the photosynthetically active radiation available to seagrass and microalgae (Fabricius 2011, Devlin et al. 2012). In addition, PSII-inhibiting herbicides inhibit photosynthesis, which can deplete energy reserves and reduce growth. Therefore, reduced light (from increased turbidity) alongside PSII-inhibiting herbicides has the potential for more than additive effects on phototrophic growth, productivity, abundance and distribution of seagrass and microalgae (Lee & Dunton 1996, Lee et al. 2007, Brodie et al. 2008, Collier et al. 2012, Negri et al. 2015). The impacts of these stressors can also make inshore ecosystems more vulnerable to other impacts of climate change and more intense and frequent weather events.

Here, we reviewed experiments that studied the effects of PSII-inhibiting herbicides and light reduction (via anthropogenic inputs of excess total suspended sediment and increased phytoplankton biomass) on the net photosynthetic efficiency and growth of seagrass and marine microalgae. We focused on these primary producers as they are two of the most abundant flora in the GBR lagoon. Seagrasses form nursery habitats for fish and invertebrates, sequester carbon and support the nutrition of herbivorous fish and macrograzers, such as sea tur-

tles and dugongs (Beck et al. 2001). Marine microalgae form the base of many food webs and contribute to nutrient cycling in marine waters (Arrigo 2005, Reynolds et al. 2014). The objectives of this research were to (1) quantitatively review the experimental literature to identify gaps in the geographies, stressor combinations and physiological endpoints that have been studied; (2) review the available information on the modes of action of how the stressors impact photosynthetic physiology; and (3) generate hypotheses of how these stressors will interact, based on the latest understanding of modes of action.

## 2. METHODS

### 2.1. Database acquisition

The ISI Web of Science (<https://apps.webofknowledge.com>) database was searched for both seagrasses and marine microalgae using the terms stated in Table 1. The latest searches were conducted in March 2020. The Web of Science database retrieves more citation results compared to the Google Scholar and Scopus databases, and thus has the most representative coverage of the literature (Adriaanse & Rensleigh 2013). Field terms for Web of Science are defined as follows: 'TS' finds records with terms in the abstract, title or keyword fields; 'ALL' finds records with terms anywhere in the full record; 'AND' acts as a Boolean function that requires multiple terms to be present together; and 'OR' acts as an operator that must include one or more of the terms.

The first line of the search term ensured that the bulk of the obtained literature related to seagrasses/microalgae. The second line of the search criteria was designed to capture alternative terms for 'photosystem II inhibition'. The third line was intended to target studies that tested the following stressors:

PSII-inhibiting herbicides and/or light reduction/increased shading and total suspended sediment. Additional papers that were relevant to the search criteria were obtained from other sources including co-authors, citations and reference lists, as well as from conducting further searches using the same terms in the 'simple search' function. The search was limited to peer-reviewed journal articles.

### 2.2. Database filtering and analysis

Papers were retained for analysis if they (1) used ecologically relevant or sub-cellular endpoints to assess the impact(s) of the stressor(s) and/or (2) identified the type of interaction that occurs as a result of multiple stressors in coastal ecosystems. Ecologically relevant and sub-cellular endpoints, where the effect is likely to occur based on the mode of action of the stressor(s) and the sites of impact (i.e. photosynthesis) were defined based on whether or not it negatively affected the ecological competitiveness of an organism (i.e. its ability to increase the frequency of its genes in subsequent generations) (Warne et al. 2018). For papers to be included in the data set, they needed to assess impacts of herbicide- and/or light-stressor on seagrasses and/or marine microalgae. Papers were removed if they were non-English, conducted prior to 1990 (to reflect the most up-to-date science), used polar or freshwater microalgae species, used communities of seagrass/microalgae rather than a specific species or examined the photosynthetic activity of seagrass seeds.

Meta-data from each individual exposure experiment within a paper was extracted and treated as an independent 'experimental' result. For example, if one paper tested 3 herbicides for 2 seagrass species, a total of 6 individual experiments were recorded in the database from that paper.

Table 1. Search terms used for seagrass and microalgae, with total citation count and the number of citations that were relevant for analyses. The number of experiments refers to relevant individual exposure experiments within a paper. See Section 2.1 for definition of search terms

	Seagrass	Microalgae
Search terms	(TS=(seagrass* OR macrophyte) AND ALL=(photosystem* OR PSII) AND TS=(herbicide OR light OR shad* OR sediment OR 'suspended sediment'))	(TS=(microalga* OR 'micro alga*') AND ALL=(photosystem* OR PSII) AND TS=(herbicide OR light OR shad* OR sediment OR 'suspended sediment'))
Total citations	401	585
Relevant citations (No. of experiments)	64 (142)	36 (176)

### 3. RESULTS

#### 3.1. Semi-quantitative synthesis of the literature

##### 3.1.1. Database overview

The search terms resulted in 401 seagrass papers and 585 microalgae papers. Of these, a total of 64 seagrass papers and 36 microalgae papers (Tables S1 & S2, respectively, in the Supplement at [www.int-res.com/articles/suppl/m668p215\\_supp.pdf](http://www.int-res.com/articles/suppl/m668p215_supp.pdf)) reported physiological responses to high and low light exposure and/or PSII-inhibiting herbicides. These papers were therefore deemed appropriate for inclusion in the analyses. This resulted in data from 142 individual seagrass experiments and 176 individual microalgae experiments.

The majority of the 142 seagrass experiments were conducted in tropical regions (73%) compared to the small proportion of experiments conducted in temperate regions (27%). The largest percentage of seagrass papers were from Australia (46%), followed by the USA (12%), China (9%), Mexico (5%) and 11 other countries that each contributed  $\leq 3\%$  of the remaining papers. Of the 30 Australian seagrass papers, more than half (53%) were from the GBR World Heritage Area. The remaining Australian papers originated from New South Wales (30%), Victoria (10%), South Australia (3.3%) and Western Australia (3.3%).

The majority of marine microalgae experiments were temperate (80%), with fewer tropical experiments (20%). The marine microalgae papers were more evenly spread across countries compared to that for seagrasses. The largest percentage of the microalgae studies were from Australia (19%), followed by China (14%), The Netherlands, Italy and Spain (each 11%), France and the USA (each 8%) and 6 other countries that each contributed  $<3\%$  of the remaining papers.

There were twice as many seagrass papers assessing the effects of high or low light exposure compared to those assessing the effects of herbicides (40 versus 20, respectively). Similarly, there were more than twice as many microalgae papers that assessed the

effects of light compared to those that assessed the effects of herbicides (26 versus 10, respectively).

Of the 142 individual seagrass experiments, the majority (62%) were conducted in a laboratory as opposed to those conducted *in situ* (23%) and in outdoor mesocosms (13%) (Fig. 1a). Three experiments (2%) did not state the type of study (i.e. lab, *in situ* or mesocosm). As the literature search excluded studies that used microalgae communities rather than a specific species, almost all of the 176 individual marine microalgae experiments were conducted in a laboratory (97%), with only 2% conducted *in situ* and 1% conducted in a mesocosm (Fig. 1b).

##### 3.1.2. Studies on stressors

Most of the seagrass and marine microalgae experiments (85% [122 experiments] and 81% [143 exper-

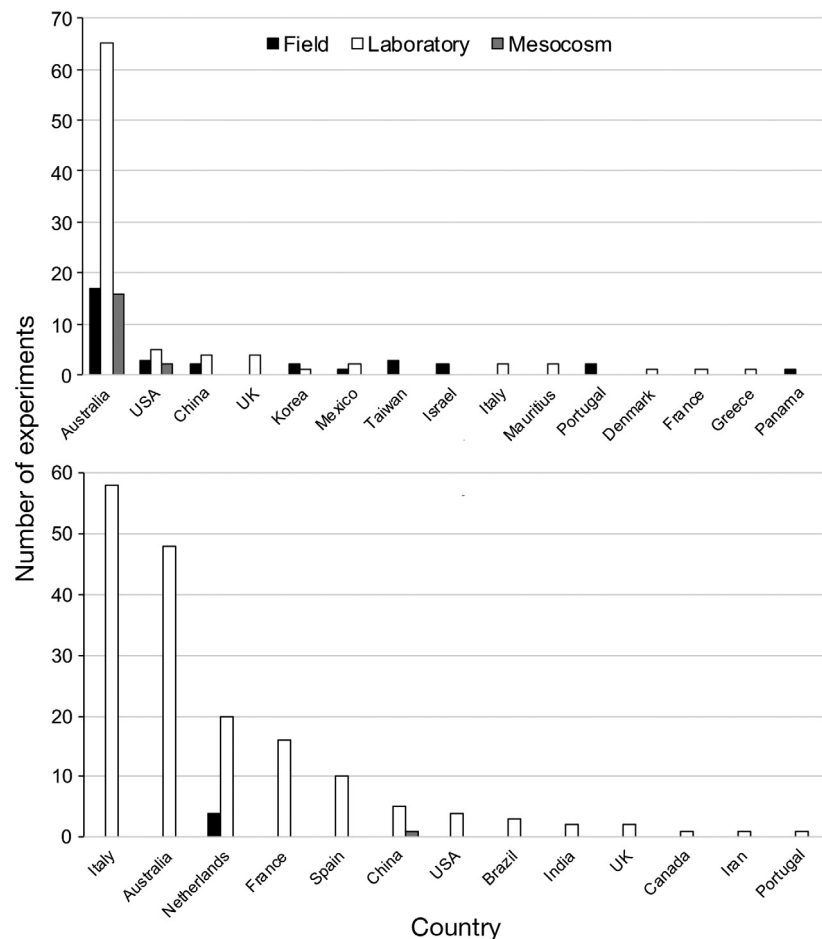


Fig. 1. Number of experiments (by country and study type) where (a) seagrass and (b) marine microalgae were exposed to light and photosystem II-inhibiting herbicides as stressors

iments], respectively) assessed the effects of an individual stressor, with only 16% (20 experiments) of seagrass experiments and 18% (33 experiments) of marine microalgae experiments assessing the effects of 2 or more stressors.

Some studies identified a combined effect between stressors and stated how one stressor altered the effects of the other. In this study, 'combined effects' were identified as being the additive responses, whereas 'interactive effects' were identified as being responses where one stressor directly influenced another and led to synergistic (more than the additive effects) or antagonistic (less than the additive effects) relationships. Of the 20 multiple-stressor experiments conducted on seagrass, only 60% (12 experiments) identified a combined and/or interactive effect (Table 2). There were 6 additive effects, 3 antagonistic and 2 synergistic. In addition, one experiment identified a statistically significant interactive effect of the stressors on seagrass but did not specify the type (Table 2). In comparison, 22 of the 33 multiple-stressor experiments (67%) conducted on marine microalgae identified a combined and/or interactive effect (Table 2). There were 13 additive effects and 6 synergistic. Additionally, 3 experiments identified a statistically significant effect for the stressors on marine microalgae but did not specify the type of response (Table 2).

### 3.1.3. Test endpoints used

A total of 21 endpoints were used to assess the impact of light and/or herbicides on seagrass, compared to 12 endpoints that were used to assess the impacts of

these stressors on marine microalgae. Endpoints were sorted into 9 broader categories as presented in Fig. 2; for example, fluorescence and chlorophyll a content were grouped into the 'photosynthesis' endpoint category. Photosynthetic and growth endpoints (59 and 34% of tests, respectively) were most commonly used for seagrass stressor effects, whilst photosynthetic endpoints alone (78% of tests) were most commonly used for marine microalgae, with oxidative stress and mortality contributing the remaining 22% of assessed endpoints. Photosynthetic endpoints, such as fluorescence, were the most commonly reported endpoints, which may indicate a propensity for using smaller-scale over larger-scale (such as growth) endpoints for stressors such as light and PSII-inhibiting herbicides. This may be because fluorescence is a specific and sensitive bioindicator of plant stress whilst being quick and easy to measure via technologies such as pulse amplitude modulated fluorometers (Seddon & Cheshire 2001, Küster & Altenburger 2007, Wilkinson et al. 2015). Endpoints of photosynthetic condition are highly responsive and biologically meaningful as they can have flow-on effects to other physiological responses and overall plant health (Negri et al. 2015). Photosynthetic endpoints are useful to study the responses of stress conditions to phototrophic species as they indicate a reduction in photosynthetic capacity and efficiency of the plant that is directly associated with growth and primary production (Magnusson et al. 2008, Malapascua et al. 2014, Negri et al. 2015). Even for non-PSII-inhibiting herbicides where the photosystem complex is not targeted (e.g. glyphosate, the active ingredient in Roundup®), similar metabolic pathways may be affected (Inderjit & Kaushik 2010, Qiu et al. 2013, Gomes & Juneau 2016) and are therefore relevant. The broader-scale effects, such as impacts on growth and the population density of species, can then have flow-on effects that negatively influence the ecological function (i.e. food, habitat) of primary producers. As a result, it is important to also assess responses on smaller scales alongside broader scales to obtain a better understanding of molecular, physiological and individual effects in 'real-time'. Overall, a greater selection of endpoints were used to assess the impacts of light on seagrass compared to herbicides, whereas the opposite was observed for marine microalgae with more endpoints being used to assess the impact of herbicides than the impact of light (Fig. 2).

Table 2. Number of seagrass and microalgae multiple-stressor experiments that identified additive (Add), synergistic (Syn) or antagonistic (Ant) effects, and number of experiments where the effect was found to be significantly different from the controls (Sig;  $p \leq 0.05$ ). HL: high light; LL: low light; PSII: photosystem II-inhibiting herbicide

Stressor combinations	Seagrass				Microalgae			
	Add	Syn	Ant	Sig	Add	Syn	Ant	Sig
HL + PSII	1		1			1		
LL + PSII								1 <sup>a</sup>
PSII + PSII	2	1			12	3		
PSII + other stressor	1 <sup>b</sup>	1 <sup>d</sup>	1 <sup>b</sup>					2 <sup>b</sup>
HL + other stressor			1 <sup>b</sup>		1 <sup>b</sup>	1 <sup>b</sup>		
LL + other stressor	2 <sup>b</sup>			1 <sup>b</sup>		1 <sup>c</sup>		
Total responses		12				22		

<sup>a</sup>Lower toxic response of PSII-inhibiting herbicides under low light conditions; <sup>b</sup>Elevated temperature; <sup>c</sup>Lowered temperature; <sup>d</sup>Copper

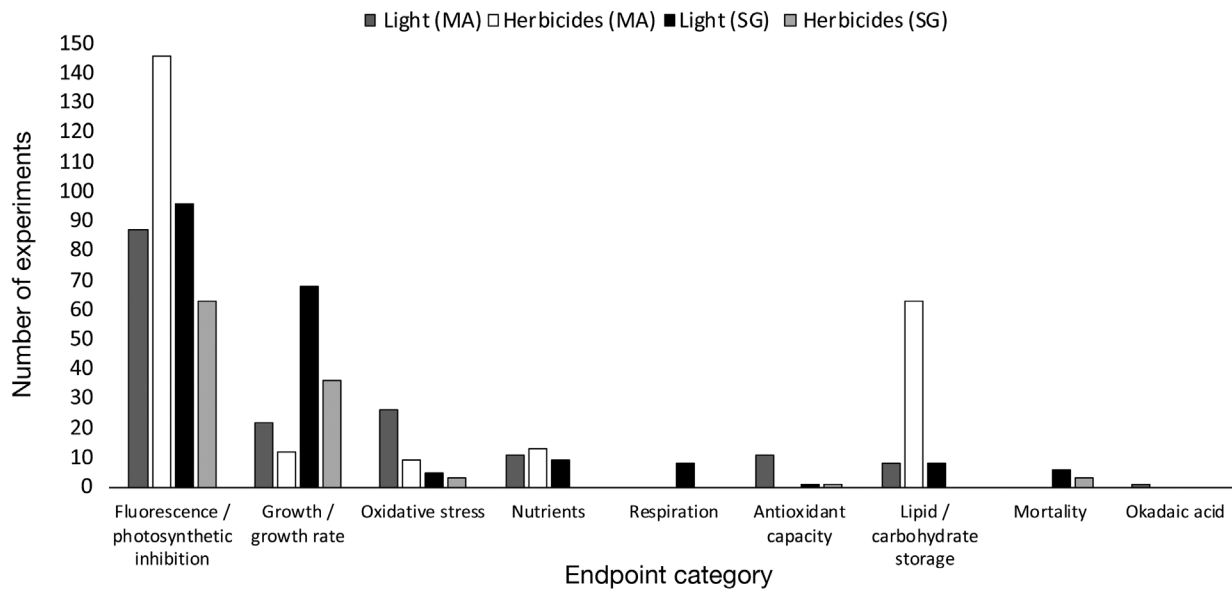


Fig. 2. Number of experiments (by endpoint category) where seagrass (SG) and marine microalgae (MA) were exposed to light and photosystem II-inhibiting herbicides as stressors

#### 3.1.4. Light stressor studies

For seagrass, a total of 66 experiments assessed the impact of light as a stressor in tropical (70%) and temperate (30%) regions. Of these, 30 experiments assessed the effects of light limitation such as shading stress either individually or in combination with another stressor. In comparison, 29 experiments assessed the effects of light saturation (i.e. higher than optimum) either individually or in combination with another stressor. There were 28 experiments that failed to identify if the light levels tested were considered saturating or limiting for the seagrass species used.

In comparison, a total of 50 marine microalgae experiments assessed the impact of light as a stressor on tropical (10%) and temperate (90%) species. Of these, 14 experiments assessed the effects of light limitation whilst 40 experiments assessed the effects of light saturation either individually or in combination with another stressor. There were 6 experiments that failed to identify if the light levels tested were considered saturating or limiting for the microalgae species used.

It is important to note that the counts of limiting- and saturating-light experiments do not equal the total number of experiments that assessed the effect of light on seagrass and marine microalgae, as various experiments consisted of multiple treatments that used both light levels.

#### 3.1.5. PSII-inhibiting herbicide stressor studies

Of the experiments that assessed the effects of herbicides, there were 83 seagrass experiments and 128 marine microalgae experiments that used a PSII-inhibiting herbicide. The most commonly tested PSII-inhibiting herbicides were diuron (~26%), atrazine (~17%), hexazinone (11%) and tebuthiuron (~10%). Only 5 seagrass experiments (5.7%) and no marine microalgae experiments used non-PSII-inhibiting herbicides in exposure combinations, which is likely an artefact of the search terms used.

PSII-inhibiting herbicides were used individually and in mixtures when testing on seagrass and marine microalgae. The majority of herbicide experiments (~82 and 81% of seagrass and marine microalgae experiments, respectively) used PSII-inhibiting herbicides individually (as a single stressor), with a small proportion testing mixtures of PSII-inhibiting herbicides (~9 and 16%, respectively), mixtures of PSII-inhibiting herbicides and non-PSII herbicides (~4 and 0%, respectively) and in mixtures with non-herbicide stressors (~5 and 3%, respectively) (Fig. 3).

#### 3.1.6. Light–PSII stressor interactions

Only two of the studies (Sjollema et al. 2014, Wilkinson et al. 2015) assessed the effects of both light and PSII-inhibiting herbicides in combination whilst also

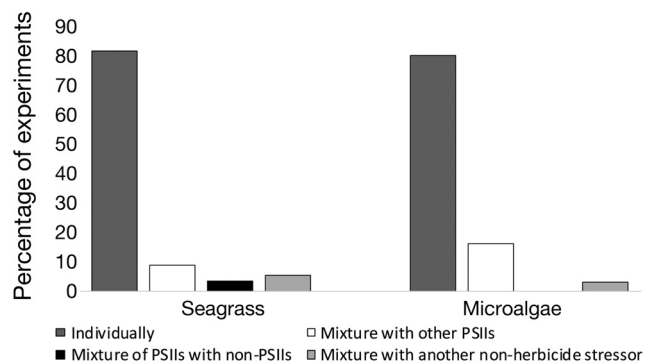


Fig. 3. Percentage of seagrass and marine microalgae experiments that assessed the effects of photosystem II (PSII)-inhibiting herbicides individually, in a mixture with other PSII-inhibiting herbicides (PSIIs), in mixtures of PSIIs and non-PSIIs and in mixtures with non-herbicide stressors

identifying a combined and/or interactive relationship. One seagrass study (Wilkinson et al. 2015) assessed the effects of a PSII-inhibiting herbicide (diuron) and light in combination. This test looked at moderate irradiance rather than light reduction and compared the measured photosynthetic efficiency of PSII to that predicted using the independent action (IA) model of joint action (Bliss 1939, Plackett & Hewlett 1952). Photosynthetic efficiency was measured, both in the light-adapted (photosynthetic yield,  $\Delta F/F_m'$ ) and in the dark-adapted (quantum yield,  $F_v/F_m$ ) state. A predominantly additive effect was identified after exposing *Halophila ovalis* to a combination of varied light levels (100, 200 and 400  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ ) and diuron concentrations (0–100  $\mu\text{g l}^{-1}$ ) for an acute exposure of 24 h. The photosynthetic yield (light-adapted state) response clearly showed an additive effect, whereas the quantum yield (dark-adapted state) response showed an antagonistic effect (Wilkinson et al. 2015).

One marine microalgae study (Sjollema et al. 2014) assessed the effects of diuron and irgarol (both PSII-inhibiting herbicides) with varying light intensities. That study looked at seasonal variation in irradiance, where *Dunaliella tertiolecta* was adapted to 2 different light intensities ( $\sim 244$  and  $\sim 929$   $\mu\text{mol photons m}^{-2} \text{s}^{-1}$  representative of autumn and spring, respectively), before being exposed to varying concentrations of the PSII-inhibiting herbicides. The experimental setup did not explicitly test for interactions between PSII-inhibiting herbicides and light stress but rather assessed the toxicity of herbicides after pre-adaptation to different natural light conditions. A higher toxic response was identified from the PSII-inhibiting herbicides in higher light conditions (spring), whereas the herbicides were less toxic in lower light conditions (autumn).

### 3.2. Synthesis of modes of action for stressors

This section explores the impacts of PSII-inhibiting herbicides and light availability on the physiology of seagrass and microalgae. A selection of endpoints was assessed to identify changes in aquatic plant productivity and health, via hierarchical links of cellular, individual and whole-organism effects following chronic exposure to PSII-inhibiting herbicides and low light availability (Table 3).

#### 3.2.1. Responses to PSII-inhibiting herbicide exposure

PSII-inhibiting herbicides exert their toxicity in aquatic phototrophs, such as seagrasses and marine microalgae, by blocking electron transport in the PSII complex—a key process of photosynthesis that occurs in the thylakoid membranes of chloroplasts (Ahrens 1994). PSII-inhibiting herbicides bind to the plastoquinone B ( $Q_B$ ) protein-binding site on the D1 protein in the PSII complex (Ahrens 1994). This in turn produces 2 adverse physiological responses. The first response prevents the transport of electrons to synthesise ATP (used for cellular metabolism) and NADPH in the chloroplast, used in converting  $\text{CO}_2$  to glucose. This limits the fixation of  $\text{CO}_2$  and the production of nutrients needed for the plant to grow and survive (Ahrens 1994, Wilson et al. 2000).

The second response is the increased formation of reactive oxygen species (ROS) (Halliwell 1991). These are highly reactive forms of oxygen that readily react with, and bind to, biomolecules including DNA and RNA. They are formed during photosynthesis when the absorbed light energy exceeds a plant's physiological ability to convert  $\text{CO}_2$  to organic molecules and leads to accumulating ROS (Chen et al. 2012). Normal concentrations of ROS are involved in a number of cellular processes (Chen et al. 2012). However, when a PSII-inhibiting herbicide blocks the electron transport chain (ETC), electron concentrations increase, causing the formation of excess ROS (Halliwell 1991). Prolonged exposure to elevated concentrations of ROS in plants, algae and cyanobacteria can cause irreversible cell damage and ultimately lead to cell death (Halliwell 1991).

Starvation via inhibited photosynthesis may not be the primary cause of PSII-inhibiting herbicide induced plant death. Rather, the second physiological process causing photo-oxidative damage through increased ROS may be the primary cause of mortality (Negri et al. 2015). This process initiates the damage

Table 3. Physiological responses of seagrass and marine microalgae to low light and the photosystem II (PSII)-inhibiting herbicide, diuron. Two sets of arrows are included in each response cell; black arrows: responses of seagrass (SG); grey arrows: responses of marine microalgae (MA). Solid arrows indicate the response has published evidence; dashed arrows or dashed dashes indicate an inferred response. Increased (upward arrow), decreased (downward arrow) or unaffected (dash) responses are compared to controlled conditions of optimal light and no exposure to diuron. Interactive responses of low light and diuron are untested in the literature; therefore, a predicted interactive response was inferred with an associated rationale (see Sections 3.2.1 and 3.2.2), based on the individual responses. C:N ratio: carbon:nitrogen ratio; ETC: electron transport chain; ROS: reactive oxygen species

Stressor(s)	Photophysiological responses		Biochemical responses		Whole-organism responses			
	Photosynthetic activity	Respiration	C:N ratio	Oxidative stress	Antioxidant activity	Energetic reserves	Growth	Mortality
Low light only (no diuron)	↑↑ SG: (Ralph 1999b, Ralph et al. 2007) MA: (Herzig & Dubinsky 1992, Dubinsky & Stambler 2009)	-/↓↓ SG: (Collier et al. 2011, Chartrand et al. 2018), (Ralph et al. 2007) MA: (Dubinsky & Stambler 2009)	↓↓ SG: (Collier et al. 2009, Flores et al. 2013) MA: (Popels et al. 2007)	↓↓ SG: (Jiang et al. 2013) MA: (Barros et al. 2003)	-/↑↓ SG: (Gavin & Durako 2012), (Silva et al. 2013) MA: (He et al. 2015), (Popels et al. 2007)	↓ <sup>a</sup> -/↓ <sup>a</sup> SG: (Flores et al. 2013) MA: (He et al. 2015), (Popels et al. 2007)	↓ SG: (Ralph 2000, Ralph et al. 2007, Flores et al. 2013) MA: (Popels et al. 2007)	↑↑ <sup>b</sup> SG: (Lee et al. 2007, Ralph et al. 2007, Yaakub et al. 2014) MA: Inferred from photo-physiology, bio-chemistry and growth responses
Diuron only (optimal light) <sup>e</sup>	↓↓ SG: (Ralph 2000, Gao et al. 2011) MA: (Magnusson et al. 2008, Thomas et al. 2020)	↓↓ SG: Inferred to decrease as less carbon is available. MA: (Pansook et al. 2019)	↓↓ SG: (Negri et al. 2015) MA: (Fiori et al. 2013) — <i>terbutylazine</i> , <i>not diuron</i>	↑↑ SG: (Silva et al. 2013) MA: (Jones 2005, Dupraz et al. 2016)	↓--- SG: (Kumar et al. 2010) MA: Antioxidant activity expected to remain constant	↓↓ SG: (Negri et al. 2015) MA: (Dupraz et al. 2016)	↓↓ SG: (Gao et al. 2011) MA: (Magnusson et al. 2008, Magnusson et al. 2010, Thomas et al. 2020)	↑↑ <sup>b</sup> SG: (Gao et al. 2011) MA: Inferred from photo-physiology, biochemistry and growth responses
Low light + diuron <sup>c</sup>	<b>Antagonism</b> Low light decreases the toxicity of diuron	<b>Additive or antagonism</b> Diuron inhibits photosynthesis, therefore decreasing respiration; respiration is not impacted by light availability	<b>Additive or synergism</b> Both diuron and low light decrease the photosynthetic assimilation of C relative to N	<b>Antagonism</b> Low light likely decreases ROS synthesis caused by diuron exposure via (1) compromising PSII-complex (less light saturated); (2) reducing the number of electrons in the ETC available to diuron; (3) increasing ROS scavenging	<b>Additive or antagonism</b> Diuron impairs photosynthesis, and therefore energetic reserves <sup>a</sup> . Low light may cause an aggregated effect on energy reserves	<b>Additive or synergism</b> Both diuron and low light reduce growth	<b>Additive or synergism</b> Diuron increases SG shoot mortality. Low light can affect initial health and resilience of seagrass and microalgae prior to diuron exposure	

<sup>a</sup>Energetic stores of MA (lipids and carbohydrates) are expected to deplete faster than SG (starch content)

<sup>b</sup>Mortality is not usually measured as standard endpoint for microalgae

<sup>c</sup>While these responses are based on reported effects of diuron, they should also apply to other PSII-inhibiting herbicides with the same mode of action (e.g. ametryn, atrazine, hexazinone, tebutyluron etc.)



of electron transport components and protein structure faster than plant starvation occurs (Vass 2011); therefore, under moderate to high light conditions, it is predicted that exposure to PSII-inhibiting herbicides is more likely to cause mortality via the generation of ROS rather than via the inhibition of photosynthesis (Jones 2005). In contrast, low light conditions result in less ROS being formed (Corbett et al. 1984); however, the effects of PSII-inhibiting herbicides may compound the effects of light limitation on growth (Sjollema et al. 2014).

### 3.2.2. Responses to light availability

Seagrass species have relatively high light requirements compared to other aquatic phototrophs such as algae (Collier et al. 2016). A reduction in light availability, either through decreased water clarity (increased turbidity from increased total suspended sediment) or shading by epiphytic algae, is a major driver of seagrass decline (Orth et al. 2006, Fabricius et al. 2014). Similar to the processes explained in Section 3.2.1, shading reduces the number of electrons transported along the ETC in the chloroplast thylakoid membranes of the PSII complex (Ahrens 1994). During photosynthesis, this electron transport synthesizes ATP and NADPH, allowing CO<sub>2</sub> fixation reactions to occur, which are necessary for cell function and metabolism (Ahrens 1994, Wilson et al. 2000). However, under sub-optimal and low light conditions, the rate of photosynthesis will either become equal to or less than the rate of respiration depending on the level of shading (whereas under optimal light, the rate of photosynthesis exceeds the rate of respiration). Therefore, when light availability is reduced, there is a net loss of ATP available to drive photosynthesis and, ultimately, plant growth.

Once the minimum light threshold of a plant is reached, only respiration occurs where inter-cellular O<sub>2</sub> concentrations decrease and CO<sub>2</sub> concentrations increase (i.e. a high CO<sub>2</sub>:O<sub>2</sub> ratio occurs) (Rasmusson et al. 2017). In contrast, high light intensities result in a low CO<sub>2</sub>:O<sub>2</sub> ratio, which initiates a pathway of photorespiration. However, the process of photorespiration generally only occurs in C<sub>3</sub> plants. As a result, photorespiration is absent in C<sub>4</sub> plants such as seagrasses and microalgae (a C<sub>3</sub> plant, but with C<sub>4</sub> pathways), meaning only respiration occurs (von Caemmerer & Furbank 2003).

Exposure to high intensity light (i.e. greater than the physiological saturation point of plants) can cause stress by elevating concentrations of ROS in

plant tissues (Asada 1999, Ralph & Gademann 2005, Janknegt et al. 2008, Waring et al. 2010). Primarily, ROS inactivate the repair of PSII when light saturated rather than damaging it directly (Nishiyama et al. 2011). In this way, increased light may cause significantly more inhibition to plant growth than the inhibition of photosynthesis via a PSII-inhibiting herbicide (Jones 2005).

The effects of low light stress on ROS accumulation are not yet clear, with studies finding increases in some situations and decreases in others. In general, plant stress is expected to increase ROS accumulation. Oxidative stress in the marine microalga *Dunaliella salina* increased slightly when exposed to low light conditions for 24 h (Madadkar Haghjou et al. 2006). However, these microalgae experiments were conducted at temperatures that were lower than optimum for the species (optimum temperature of 28°C; test temperature of 13°C). Therefore, the reported ROS responses could be the result of temperature stress alone or in combination with shading stress.

Several lines of evidence suggest that shading may reduce oxidative stress. One study concluded that shading may protect phytoplankton communities from oxidative damage (Barros et al. 2003). A detailed study of a cactus species, *Hylocereus undatus*, demonstrated that levels of ROS significantly decreased after 60 d of shading because ROS-scavenging enzymes were operating at a higher capacity (Wang et al. 2018). Similarly, a study of the seagrass *Thalassia hemprichii* also found increased ROS-scavenging under low light conditions after 10 d, resulting in a net decrease of ROS (Jiang et al. 2013). A 3 wk study conducted on the seagrass species *Cymodocea nodosa* also found that shading either did not increase ROS concentrations or there was adequate ROS-scavenging activity to regulate ROS (Silva et al. 2013). These studies suggest that cellular ROS concentrations are maintained or reduced via increased ROS-scavenging when shaded.

## 4. DISCUSSION

Only 16% of the seagrass stressor experiments and 18% of the marine microalgae stressor experiments assessed the combined effects of 2 or more stressors. The low proportion of studies testing interactions is consistent with similar reviews of experimental and field stressor studies (Brown et al. 2011, Wernberg et al. 2012, O'Brien et al. 2019, Stockbridge et al. 2020). Of the multiple-stressor studies we reviewed, only 2 tested for interactive effects of light and PSII-

inhibiting herbicides (see Section 3.1.6); however, both used moderate to high light. There were no studies that specifically assessed the combined effects of PSII-inhibiting herbicides and low light levels on seagrass or marine microalgae. The need to quantify the combined and/or interactive effects of multiple stressors for coastal ecosystems is well recognized, and this review has confirmed this knowledge gap for seagrass and marine microalgae systems.

The mode of action of PSII-inhibiting herbicides is intrinsically linked to light availability. For example, the toxic effects of PSII-inhibiting herbicides increase with increasing light intensity (O'Neal & Lembi 1983, Millie et al. 1992), whereas in the absence of light, these herbicides are ineffective (Gomes & Juneau 2017). PSII-inhibiting herbicides do not cause toxicity in complete darkness because photosynthesis is not occurring and ROS are not being formed (Corbett et al. 1984). Therefore, the response of PSII-inhibiting herbicides on photosynthetic organisms is heavily dependent on the prevailing light conditions (Sjollem et al. 2014).

We hypothesize that increased shading will help limit the formation of ROS that normally occurs from PSII-inhibiting herbicide exposure (Table 3). This is because less light is available for when the PSII-inhibiting herbicide blocks electron transport, resulting in reduced photodamage from ROS activity (Vass 2011). On the other hand, blocking the ETC by PSII-inhibiting herbicides under chronic low light conditions would add to the low light stress that results from reduced electron transport (Halliwell 1991, Ahrens 1994). In addition to the effects of oxidative stress, exposure to low light availability and PSII-inhibiting herbicides reduce photosynthetic efficiency and carbon fixation, which in turn decrease energy stores within the organism (rapidly for microalgae, slower for seagrass), and that ultimately causes starvation or reduced growth (Popels et al. 2007, Flores et al. 2013, He et al. 2015). Therefore, the overall impact on the organism will arise from many different physiological mechanisms ranging from cellular to whole-organism responses, which depend primarily on the level of light availability (Table 3).

The relationship between PSII-inhibiting herbicide exposure and light limitation is complex, as the stressors are in continual flux (Negri et al. 2015). When photosynthetic organisms are exposed to PSII-inhibiting herbicides and low light in combination, the interaction is likely to be additive or antagonistic, depending on the measured endpoint (Table 2). Often, direct measurements of smaller-scale end-

points such as fluorescence will not account for intracellular damage such as increased ROS; therefore, additive effects may be more prominent. However, higher-scale endpoints such as growth and mortality will account for secondary sites of impact that will more readily show antagonistic effects. Under low light and PSII-inhibiting herbicide exposure, the possibility of antagonistic effects is hypothesized due to a potential positive (beneficial) pathway of low light to growth as a result of decreased ROS production. The possibility of antagonistic effects aligns with literature findings, where one study reported a lower toxic response when marine microalgae were exposed to a mixture of PSII-inhibiting herbicides in lower light conditions compared to higher light conditions (Sjollem et al. 2014). Despite not explicitly testing for interactive effects, this is the only study from the preceding literature review that assessed the effects of PSII-inhibiting herbicides and low light on marine microalgae and/or seagrass. Therefore, further experimental studies are required to quantify individual physiological responses—specifically because of possible mitigative feedbacks via the ROS pathways.

PSII-inhibiting herbicides and light reduction cannot be considered directly interchangeable from a management perspective as the primary physiological effects are not identical (Table 3). This finding is important because earlier studies hypothesized that different stressors with similar modes of action will have similar types of interactive effects (Boyd & Brown 2015, Snell-Rood & Kobiela 2020). The present study indicates that this will not be true of PSII-inhibiting herbicides and low light because the physiological pathways involved in the stress responses are slightly different. However, the net impact they have on phototrophic organisms may be comparable. For example, an environmentally relevant concentration of the commonly used PSII-inhibiting herbicide diuron ( $0.4 \mu\text{g l}^{-1}$ ), has a potential shading equivalency of 10% (via suspended sediments and algae during flood plumes) based on photosynthetic efficiency (Negri et al. 2015). As a result, it might be possible, using ecological models, to predict and quantify the overall impacts of PSII-inhibiting herbicides and shading that occur together, provided those models account for interactions among physiological pathways—particularly the production of ROS.

We have identified 5 important knowledge gaps that need to be addressed so that ERAs can better accommodate the interactive effects of low light and PSII-inhibiting herbicides (Table 4). These knowledge

Table 4. Key knowledge gaps, possible ways to address the gaps and their likely outcomes. PSII: photosystem II; SG: seagrass; MA: marine microalgae

Knowledge gap	Potential way to address knowledge gap	Outcome
1 How responses at the sub-cellular (e.g. fluorescence endpoints) scale translate to responses at the whole-organism scale, e.g. does an additive fluorescence response to low light and PSII-inhibiting herbicides equal an additive growth response	<ul style="list-style-type: none"> <li>Quantify stressor responses at an individual level to identify</li> <li>(1) direct and indirect linkages between specific physiological processes; (2) the hierarchical links between exposure and whole-organism effects; and (3) how these individual responses coincide/differ for SG and MA</li> <li>Understand stressor responses across various levels of biological organisation (molecular, physiological, individual and ecosystem levels) for SG and MA</li> <li>Understand how additive/synergistic or antagonistic responses that are identified at the physiological level translate to whole-organism responses</li> <li>Conduct further experiments that expose SG and MA to multiple stressors</li> </ul>	<ul style="list-style-type: none"> <li>Increased knowledge of whether endpoints are early indicators of stress or are ecologically relevant, e.g. seagrass exposed to low PSII-herbicide concentrations may decrease photosynthetic efficiency; however, this may simply be an early indicator of stress at an individual level instead of an ecologically relevant response</li> <li>Allow researchers to use the most relevant endpoints or biomarkers (tailored to the stressor/organism) in future experiments</li> <li>Identify ecologically relevant, chronic-stress responses that have flow-on effects to whole-organism health and fitness</li> <li>Increase accuracy in overall reporting data of stressor responses</li> <li>Identify the level(s) of biological organisation likely to be most important to inform management, e.g. growth/mortality &gt; biochemistry &gt; physiology</li> </ul>
2 Empirical data of the various physiological responses of SG and MA to multiple stressors, particularly low light and PSII-herbicides	<ul style="list-style-type: none"> <li>Conduct further experiments that expose SG and MA to multiple stressors</li> </ul>	<ul style="list-style-type: none"> <li>Understand how each physiological process responds to stressors in combination</li> <li>Draw robust conclusions on the combined or interactive responses of seagrass and marine microalgae when exposed to multiple stressors</li> <li>Understand important connections between processes as well as identifying compensatory feedback loops or any mitigative processes</li> <li>Validate models of combined and/or interactive responses of multiple stressors</li> </ul>
3 Responses of a broader range of aquatic plants (e.g. sub-tropical, temperate and polar species) exposed to reduced light and PSII-herbicides such as diuron	<ul style="list-style-type: none"> <li>Conduct multi-stressor toxicity tests using sub-tropical, temperate and polar aquatic plants</li> </ul>	<ul style="list-style-type: none"> <li>Inform management more effectively depending on spatial specifications</li> </ul>
4 Gap between the parameters measured in experiments and the parameters needed for predictive models	<ul style="list-style-type: none"> <li>Conduct integrated experimental-modelling studies on the effects of multiple stressors on aquatic plants</li> </ul>	<ul style="list-style-type: none"> <li>A stronger link between observations and theoretical predictions for the interactive effects of stressors, resulting in better predictions of multi-stressor impacts</li> </ul>
5 Predictive models of the responses of seagrass and marine microalgae to PSII-inhibiting herbicides and reduced light availability	<ul style="list-style-type: none"> <li>Utilise the concentration addition and independent action models</li> <li>Develop joint action models further using empirical data on the physiological processes underlying stressor responses</li> <li>Utilise and develop more equivalency factors, e.g. a 20% reduction in photosynthetic efficiency by PSII-inhibiting herbicides may have a similar effect as 20% (of optimal light) shading—net effects could be comparable or possibly interchangeable</li> <li>Use species sensitivity distribution (SSD) models to combine light-limitation SSDs with PSII-inhibiting herbicides SSDs—as has been done for temperature/herbicides using joint-effect models</li> </ul>	<ul style="list-style-type: none"> <li>Obtain quantitative predictions of how primary producers respond to multiple stressors</li> </ul>

gaps were identified based on the literature review, with a final aim of developing predictive models that can inform management of interactive stressors. We suggest that translating experimental observations into predictive models requires (1) utilising ecologically relevant responses for both the organisms and stressors being tested; (2) measuring these responses; (3) testing for generalities across a broader range of aquatic plant species; (4) integrating experimental measurement studies with modelling studies to bridge the gap between what is measured and the parameters that are needed for models; and (5) developing predictive models of aquatic plant responses to multiple stressors. Addressing the knowledge gap of how each physiological process responds to stressors in combination (gap 2, Table 4) is crucial in understanding important connections and identifying compensatory feedback loops for predictive models. This will enable a better understanding of whether smaller-scale (physiology) endpoints translate to the same aggregative response at higher-scale (whole-organism) endpoints (gap 1, Table 4).

Whilst the combined and/or interactive effects of light availability and PSII-inhibiting herbicides were the focus for this review, other stressors such as nutrients (Howarth et al. 2000), chemical pollution (Shahidul Islam & Tanaka 2004, Wu et al. 2008), ocean acidification (Doney et al. 2009, Kroeker et al. 2010) and temperature (Somero 2002, Levitus et al. 2012) are also known to affect coastal ecosystems. For instance, during summer months, light availability and PSII-inhibiting herbicides also often coincide with periods of elevated surface water temperature (Berkelmans 2002, Massa et al. 2009, Rasheed & Unsworth 2011, Collier & Waycott 2014). For microalgae, the combined physiological effects between light availability and PSII-inhibiting herbicides can be strongly influenced by temperature (Gomes & Juneau 2017). These same interactive effects for seagrasses remain unclear in the literature; however, studies suggest that light reduction and high temperatures cause significant reductions in seagrass growth (Bulthuis 1987, Ralph 1999a, Collier et al. 2011, Collier & Waycott 2014). This is because high temperatures increase the rate of respiration, which means that higher light levels are required for the production of carbon to exceed the rate of respiration (Ralph et al. 2007, Collier et al. 2011, 2016). Therefore, during periods of low light the rate of photosynthesis decreases, which in turn increases the likelihood that the rate of respiration may exceed the rate of photosynthesis. This implies that low light and higher than optimum temperatures could have an

additive negative effect on seagrass growth. This additive effect has been confirmed in experimental studies (Hughes 2000), which suggests there are negligible benefits from reduced ROS production under low light stress.

This study did not consider ecological feedbacks, but these could also be an important cause of stressor interactions. For example, a common stressor that was not addressed in this study is the excess of nutrients discharged to coastal waters as a result of anthropogenic activities (e.g. fertilising). Often, excess nutrients are not considered to be a 'stressor', as low to moderate nutrient enrichment promotes photosynthetic growth rather than inhibiting it (Jaschinski et al. 2010). However, high inputs of nutrients (i.e. dissolved inorganic nitrogen such as  $\text{NO}_3^-$  and  $\text{NH}_4^+$ ) into aquatic environments can cause eutrophication and thus increased phytoplankton biomass (Martin-Jézéquel et al. 2015). Increased phytoplankton, or algal blooms, shade seagrass from prevailing light (Short et al. 1995, Fertig et al. 2013) and are a significant cause of seagrass decline globally (Waycott et al. 2005, McMahan et al. 2013). Thus, there is an important nutrient-driven connection between the 2 taxa reviewed here that warrants further study.

## 5. CONCLUSIONS

In Australia, including the GBR lagoon, excess sediments and nutrients as well as low levels of pesticides occur in flood plumes discharging from adjacent catchments. Flood plumes with high levels of fine sediment have been linked to declines in seagrass. Yet it is also known that phytoplankton blooms commonly occur after flood plumes, indicating that different phototrophic species respond to these anthropogenic stressors in different ways. Understanding the combined and/or interactive effects of these stressors is important for management to understand the full extent of impact from poor water quality, as current risk and management targets are only evaluated for individual pollutants (Brodie et al. 2017). We suggest that predictions of stressor interactions on seagrass and microalgae may be made based on the latest understanding of stressor modes of action. Understanding the physiological responses to stressors can generate hypotheses on the combined and/or interactive effects of water quality stressors. The overall combined response of low light and PSII-inhibiting herbicides is likely to be heavily dependent on the magnitude of each, as well as exposure time and ecological feedbacks. Therefore, it is imperative that potential mit-

igative physiological responses be investigated further through experimental studies that assess varying levels of PSII-inhibiting herbicides and light reduction over acute and chronic exposure periods.

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Editorial responsibility: Thomas Wernberg,  
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