Identifying robust bioindicators of light stress in seagrasses: A meta-analysis

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ABSTRACT

Bioindicators are used to monitor responses to environmental pressures. They should reflect important ecological values, be scientifically defensible, respond in a predictable manner and be easy to measure and interpret. Seagrasses are significant marine habitat, which globally are under threat and are considered "sentinels" of coastal degradation. Light reduction via (for example) eutrophication, dredging and turbid terrestrial run-off is a key anthropogenic pressure impacting seagrasses. Consequently, seagrasses are regularly included in monitoring programs, both to protect them and for their value as indicators of change in light availability. This paper assessed published literature on seagrass responses to light reduction to identify which seagrass characteristics provide the most robust bioindicators of light reduction. ISI Web of Science was searched in July 2011 to retrieve refereed publications that documented the response of seagrasses to light reduction. Only studies with a control were included, giving confidence that the response was due to light reduction and not other, unexplained factors. This yielded a dataset of 58 published studies, covering eight of 11 seagrass genera and 18 species, with a wide geographic range. In each study, the response of each variable to light reduction was categorised into no effect, reduce or increase. Where studies tested the intensity and durations of light reduction, the consistency of responses at these different levels was also assessed. A set of consistent and robust bioindicators is proposed that respond to the pressure of light reduction and can indicate different timescales and levels of pressure. These include: those that respond early and reflect sub-lethal changes at the scale of the plant, such as rhizome sugars, shoot C:N, leaf growth and the number of leaves per shoot; and those that respond later, reflecting changes at the meadow-scale, such as shoot density or above-ground biomass. We recommend these variables for monitoring programs with the goal of detecting significant light reduction and indicating the severity and duration of impact.

1. Introduction

Bioindicators are used to monitor biotic responses to environmental pressures and are applicable to individual species or assemblages (e.g. Markert and Wünschmann, 2011). Many monitoring programs incorporate measures of both environmental pressures and bioindicators. The advantage of measuring biota is that they integrate a temporal component, reflecting both the past and current environmental condition, related to the life-span or residence time of the particular organism in a system, whereas environmental measures usually reflect a single point in time. Good bioindicators should be scientifically defensible, such that the cause–effect pathway that relates the state of the biota to the pressure is reasonably understood, respond in a predictable manner to the pressure of concern across different locations and times and in proportion to the degree of pressure, be repeatable, that is, they can be measured on more than one occasion over space and time, cost effective, easy to measure and provide outputs that are easy to interpret (ANZECC, 2000; EPA, 2008; Niemi and McDonald, 2004). There is a plethora of potential bioindicators but ecological health assessments need to be based on simple yet scientifically sound methodologies (Borja et al., 2008). An integral component of bioindicator development is to pause and review the suitability of the many potential indicators on the basis of the above criteria.

Coastal zones are highly valued for their ecosystem services as well as their socio-economic benefits. Yet, they are exposed to multiple pressures, including eutrophication, construction works for ports and marinas, increased sediment runoff, fisheries activities and aquaculture (Costanza et al., 1997; Gladstone, 2010) and degradation of the coastal zone continues at an increasing rate (Duarte, 2009; Waycott et al., 2009). Effective monitoring, detection of change and management of these localised impacts is growing in importance as global climate changes create further pressures on coastal ecosystems (Hughes et al., 2003).

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Seagrass meadows are considered "sentinels" of coastal degradation (Orth et al., 2006) and, as such, they are frequently incorporated into assessments of estuarine and coastal integrity (e.g. Borja et al., 2008; Fourqurean et al., 1997; Romero et al., 2007). They are a dominant habitat of most coastal environments providing important ecosystem services, globally valued at approximately US$19,000 ha^{-1} yr^{-1} (Costanza et al., 1997). This means that, in addition to being good bioindicators of impacts to the coastal zone, changes in the health or abundance of seagrasses indicate likely flow-on effects to the broader ecological and economic systems. One of the key causes of seagrass decline is light reduction (Waycott et al., 2009). They have high light requirements but often occur in shallow estuarine or coastal regions, which are readily impacted by human activities. Monitoring of seagrass condition and health is a key priority in many coastal monitoring programs, and in environmental impact assessment and management, particularly related to dredging e.g. (EnviCom-Working-Group-108, 2010; EPA, 2011; Fourqurean et al., 2003; McKenzie et al., 2010).

Seagrass responses to light reductions have been reasonably well documented (Fig. 1). Plants initially respond to stress through physiological adjustments and later, if the stress continues or increases in intensity, through morphological adjustments (Waycott et al., 2005). Consequently, a set of sub-lethal effects occur, where the plant modifies its physiological processes, resource allocation or structure (Lee et al., 2007; Ralph et al., 2007), in order to maintain a positive carbon balance (Collier et al., 2009; Touchette and Burkholder, 2000). In the face of ongoing pressure, declines in spatial extent or density of seagrass meadows will then occur (Backman and Barilotti, 1976). These responses can be easily explained through a cause–effect pathway of reduced light interception through to meadow-scale changes (Fig. 1).

1.1. The need for this review

Due to the relatively long history of research on responses of seagrasses to light related stress, there is a reasonable mechanistic understanding of the plant responses to light reduction (Fig. 1). However, even in experimental studies where conditions can be tightly controlled, there are inconsistencies among studies as to whether potential indicators do respond to changing light levels. Effective monitoring programs need to be part of a broader management framework that requires responses to the monitoring data. Typically, this is in the form of predetermined criteria or thresholds, which, if exceeded, trigger a management response. Given the often significant implications in triggering management actions, it is important that there is confidence in the choice of indicators on which monitoring is based. It is timely to assess what are the most robust seagrass bioindicators of light reduction while considering the following criteria:

relevance and appropriateness – they respond to light reduction;

consistency – respond in the same manner (increase or decrease) with increasing intensity or duration of stress or at a particular point along the stress-response pathway;

reproducibility & repeatability– responds across the range of locations and times that light reduction is imposed; and

easy-to-measure and cost-effective.

This paper reviews the published literature on seagrass responses to experimental light reduction in order to identify the most robust bioindicators. A sub-set of bioindicators of light reduction in seagrasses is then proposed taking into account the above criteria.

2. Materials and methods

ISI Web of Science was searched in July 2011 to retrieve refereed publications that documented the response of seagrasses to light reduction. *Ruppia*, a genus which is not universally recognised as a seagrass, was not included. Two sets of keywords were used. The first set included words associated with seagrasses (seagrass or eelgrass or SAV or all the seagrass genera names (e.g. *Halophila*, *Posidonia*, etc. and including old genera names such as *Heterozostera*). The second set of keywords contained words associated with light reduction (light or shade/shading or dredge/dredging or irradiance). Each word in the first set was searched in combination with each word from the second set. In addition, to take into account older references that may not be available through ISI Web of Science, the reference list in each article was also scanned for any other relevant publications. This process generated 184 refereed publications. Only those studies that included a control were included to account for any seasonal changes in the seagrass variables that may be related to factors other than the light reduction (in aquaria studies control was 100% surface light or the maximum light intensity treatment, in *in situ* experiments the control was typically ambient light within the seagrass meadow). There were a total of 58 publications on experimental manipulation of light, once those without control treatments were removed which are listed and numbered in Appendix A. This yielded 104 independent studies, considered observations, as many publications included more than one experiment and multiple species. Data was extracted from each of these publications to generate the summary statistics presented in this paper as described below.

From each publication the following information was extracted: country and location where experiment was conducted; experimental set-up (in situ, mesocosms); genus and species studied; start, end and duration of study; season of study; light reduction treatments; and response variables ($n = 119$).

Each response variable measured in each study was assessed to determine if, and how, it responded to a particular level of light reduction relative to the control (increase, decrease or no effect). 'No effect' was defined as not statistically significantly different to the control (typically $p > 0.05$), 'Increase' was defined as significantly ($p < 0.05$) greater than the control and 'Decrease' as significantly lower than the control. These allocations were further subdivided into the time-step at which the response was observed as follows: hours (where the treatment was imposed for <24 h), days (<8 d), weeks (<4 weeks) and months (>4 weeks). If there was more than one light reduction treatment, we recorded whether the response varied among the treatments at different time-steps. In some cases, it was not possible to categorise a particular response variable into the three main categories (increase, decrease, no effect). For example, there may have been more than one light reduction treatment and a variable responded in different directions in the different treatments (e.g. under extreme light reduction, responses differed to those under mild light reduction). If an observation could not be assigned into the three categories, it was deemed inconclusive and not included.

Where variables measured similar plant responses, or where there were only a few observations, they were pooled together with functionally similar measurements. These included: leaf growth (g t^{-1}) with growth per meristem (g meristem^{-1} t^{-1}); and cluster and shoot mortality, cluster and shoot density and leaves per shoot and leaves per cluster, as a cluster is analogous to a shoot. Leaf biomass per leaf, shoot and cm^{-2} were also combined. After this consolidation, only variables that were recorded in three or more different studies or species were included in the analysis of seagrass responses to light reduction, a total of 56 response variables (Appendix B). These response variables were categorised into five groups: those associated with photosynthesis, other
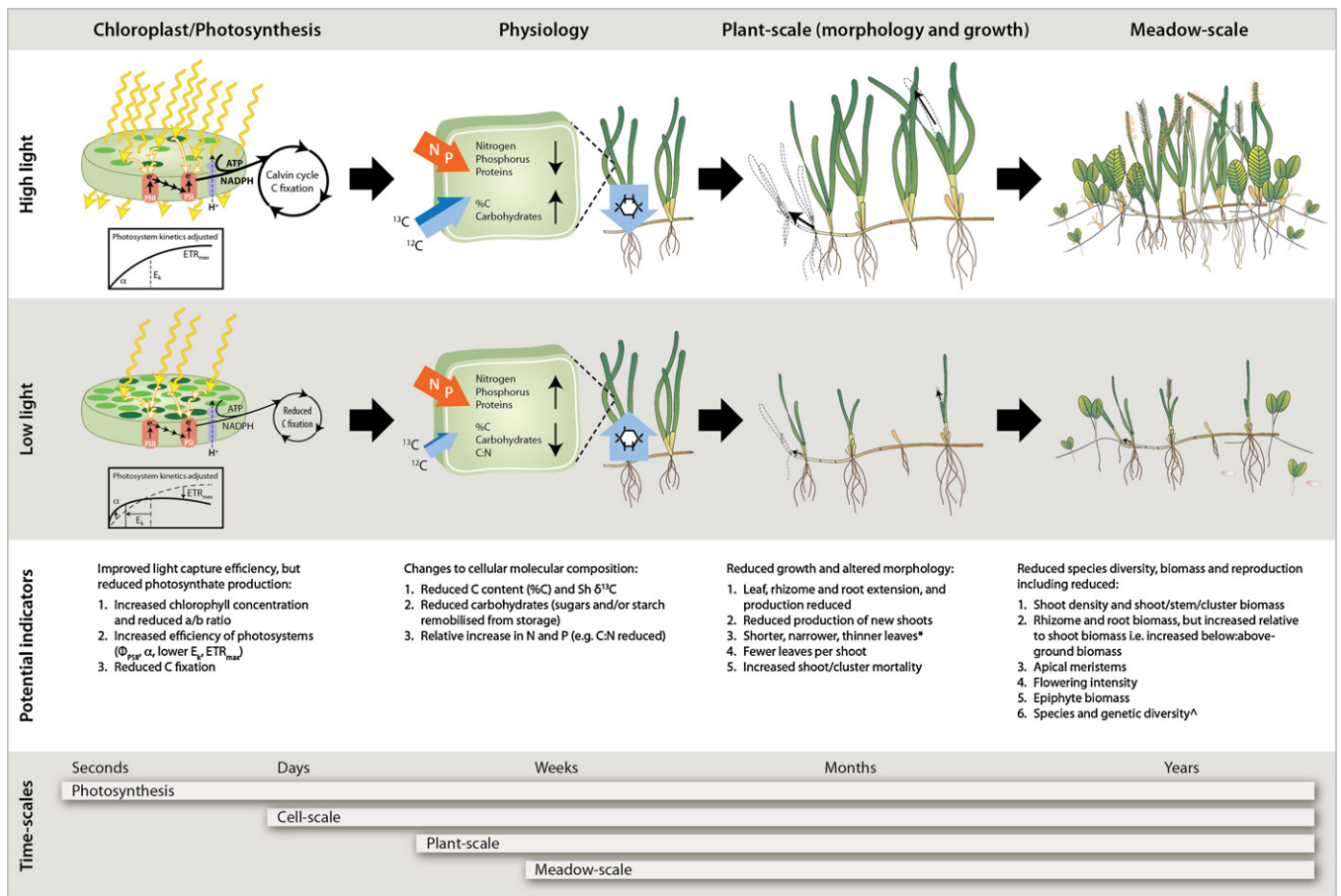
Fig. 1. Conceptual diagram of the current understanding of the of seagrass response pathway under low light conditions separated by photosynthetic, other physiological, plant-scale (growth and morphology) and meadow-scale variables. The timescales at which the responses to light reduction generally occur are indicated at the base of the diagram. Potential bioindicators are highlighted.

physiological measures, growth, morphology and meadow-scale responses.

We first focused our analysis on seagrasses as a pooled group and, in a separate analysis, we then explored differences in responses among key genera. From this data set, three key questions were asked for all seagrass species pooled together:

Across all studies, which variables responded most consistently to light reduction?

Consistency was defined as responding in the same direction and was quantified as the percentage of observations showing each response (no effect, increase, decrease), categorised as follows: (1) those that responded in the same manner (increased or decreased) in 90% or more of the observations; (2) in 80–89% of observations; (3) in 70–79% of observations; and (4) in 50–69% of the observations.

Was the response consistent with intensity or duration of light reduction?

We assessed this question using the subset of most consistent variables (>50% of observations responded in the same direction). The consistency of the response with increasing intensity of light reduction could be determined for studies with more than one light reduction treatment (i.e. one control plus two or more light reduction treatments). Once again, the percentage of observations that were consistent at different intensities of light reduction was calculated. This analysis was repeated with duration, and only conducted on the observations where the study measured the response to light reduction over different periods of time. For some response variables there were no data to assess the consistency of the response

with intensity or duration of light reduction. Only those response variables that had three or more observations were assessed for consistency.

Over what timescale were responses observed?

This information is useful in developing early warning (sub-lethal) indicators of light reduction. The timescales were categorised into days, weeks and months as defined above; however, there were insufficient observations to assess the time-step 'hours'. For the subset of consistent variables (>50% of observations responded in the same direction), the percentage of observations for each variable in each time period category was tallied for the responses, no effect, reduced or increased. The time period at which 50% or more of the observations for a particular variable responded in a particular direction was defined as the time-step at which a response was detected. If in the successive time period there was a decrease in the percent of observations that responded to less than 50%, then it was considered that the particular variable did not respond at that time-step. However, if the percent of observations at the successive time step was remained above 50% then it was considered that the response of this variable occurred over a longer time-period. If there were no observations in the time-step before the time the first response was observed this was also noted, as it is possible that responses could have occurred earlier.

The above set of analyses was repeated on the data for each seagrass genus. Only those genera with five or more studies were included (Hydrocharitaceae: *Halophila*, *Thalassia*; Cymodoceaceae: *Halodule*; Posidoniaceae: *Posidonia*; Zosteraceae: *Zostera*). These include at least one representative from each seagrass family (Les

et al., 1997) and a range of growth forms from the smaller, faster growing to largest and slower growing genera (*Halophila*, *Halodule*, *Zostera*, *Thalassia* and *Posidonia* (Walker et al., 1999)). Three genera were excluded from the analysis due to insufficient studies: *Amphibolis* ($n = 2$), *Cymodocea* ($n = 4$) and *Syringodium* ($n = 3$). Only variables that had three or more observations in a genus were included, or variables that were recorded in all genera regardless of the number of observations. A consistent response was defined as 50% or more of the observations responding in the same direction. There was not enough data to assess the timescales at which responses occurred.

3. Results

The 58 publications which met the criteria for inclusion in the review covered 8 of the 11 seagrass genera (Hydrocharitaceae: *Halophila*, *Thalassia*; Cymodoceaceae: *Halodule*, *Cymodocea*, *Amphibolis*, *Syringodium*; Posidoniaceae: *Posidonia*; Zosteraceae: *Zostera*), and 18 species. There were no publications about the effects of light reduction on the genera *Thalassodendron* and *Enhalus*. The publications covered a wide geographic range, including Australasia (Australia), Europe (Denmark, Italy, Netherlands, Portugal, Spain), North America (USA), Central America (Mexico), Indian Ocean Islands (Mauritius) and Pacific Islands (Philippines).

3.1. All seagrass species

3.1.1. Consistency of direction of response

Growth (7/9 variables responded in >50% of observations in the same direction), morphology (7/8 variables) and meadow-scale variables (10/11) responded in a more consistent manner to light reduction than did photosynthetic (3/10) and other physiological variables (7/17, Fig. 2). All of the responsive variables were lower in low light, except for shoot mortality, which was higher. Those growth, morphology and meadow-scale variables that responded most consistently (>90% observations responded in the same direction) were shoot production, root extension, lacunal area, flowering intensity and leaf area index (Table 1, Fig. 2). The next most consistent set of variables (80–89% of observations) were leaf growth (g), leaf density, shoot density, above-ground biomass and algal epiphyte biomass (Table 1, Fig. 2). The variables that responded in the same direction in 70–79% of observations were leaf and rhizome extension (mm), shoot mortality, leaves per shoot, leaf thickness, root biomass and percent cover (Table 1, Fig. 2).

The photosynthetic variables that responded most consistently (declined with light reduction, >90% observations) were E_k and ETR_{max} (Table 1, Fig. 2). The only other physiological variables to show a reasonable consistency of response to light reduction were shoot C:N ratio (80–89% observations) and rhizome sugars (70–79% observations), both declining in response to light reduction.

A number of variables consistently showed no response (i.e. no effect in >50% of observations) to light reduction, including nine of the 17 physiological variables (Fig. 2). Only one out of nine growth response variables consistently had no response to light reduction (leaf plastochrone interval, Fig. 2).

#### 3.1.2. Consistent response with increasing intensity and duration of light reduction

All of the 28 growth, morphology and meadow-scale variables, and 19/22 physiological variables, responded consistently with increasing intensity of light reduction (i.e. the same response in >50% of the observations) and were included in this analysis (Table 1, Appendix C). The variables that had responded most consistently to light reduction (Table 1) also responded consistently to increasing intensity of light reduction. Root extension and lacunal area showed the least consistent response to increasing intensity of light reduction (around 60%, Table 1, Appendix C).

Table 1
Response variables summarised by key research questions (1) observations that responded in the same direction (by percentage), (2) observations that were consistent with increasing amount and duration of light reduction (by percentage, nd: no data for this measure, <3: not enough observations to assess the consistency), and (3) the timescale at which the response was observed (ˆ time-steps before this were measured, a range indicates the response was observed at multiple timescales). For consistency columns asterisks indicates percentage of cases consistent, *70–79%, **80–89%, ***>90%. Numbers indicate the number of studies contributing to that statistic. References are listed and numbered in Appendix 1.

| Question Consistency	1 Overall	2 Intensity	Duration	3 Response timescale	References
Physiological					
E_k	***14	***11	nd	Weeks	9,19,20,45,47,55
ETR_{max}	***12	***12	<3	Week	9,19,45,47,48,52,53,55
Shoot C:N	**9	*8	nd	Weeks	19,32,34,50
Rhizome sugars	*34	*9	6	Days–weeks	2,10,11,12,16,19,26,36,38,39,40,41,42,48,50,55
Growth					
Shoot prod. (t^{-1})	***8	**7	<3	Weeks	1,2,8,24,47,48
Root ext. (mm t^{-1})	***3	3	nd	Weeks	12,24,50
Leaf growth (g t^{-1})	55	**46	***6	Days–months	1,8,10,11,12,17,19,20,21,24,25,27,2931,32,34,39,40,41,44,48,49,50,55,56
Leaf ext. (mm t^{-1})	36	**35	*8	Weeks	2,3,7,12,14,18,21,23,25,28,32,33,36,38,42,50
Shoot mort. (t^{-1})↑	*23	**20	3	Weeks–months	1,6,7,12,18,24,38,47,50,51,55
Rhiz. ext. (mm t^{-1})	*9	*7	nd	Weeks–months	1,8,12,24,47,48,49,50
Morphology					
Lacunal area (%)	***6	5	nd	Months	1,32
Leaves per shoot	*41	***29	9	Weeks–months	6,8,17,19,20,21,27,31,33,34,38,42,46,54,55
Leaf thickness (mm)	*8	***7	nd	Months	18,19,21,24
Meadow					
LAI (m^2 m^{-2})	***8	***8	***4	Weeks	21
Flowering intensity	***6	*4	nd	Months	4,7,47,58
Leaf density (m^{-2})	**9	***8	<3	Months	31,38,46
Algal epi. bio (g m^{-2})	**14	**18	3	Months	19,21,27,34,38,44,54
Above-gr. bio (g m^{-2})	**59	***49	***12	Monthsˆ	1,2,3,6,8,14,15,19,20,21,25,27,33,34,38,39,4041,42,44,45,46,51,56,57,58
Shoot density (m^{-2})	**15	**46	*11	Weeks–months	4,5,14,15,16,17,19,21,23,26,27,28,31,33,34,38,39,40, 41,42,45,46,47,55,56,57
Root biomass (g m^{-2})	*12	***10	<3	Weeks–months	1,2,32,39,41,42
Cover (%)	*7	***6	***6	Months	5,57
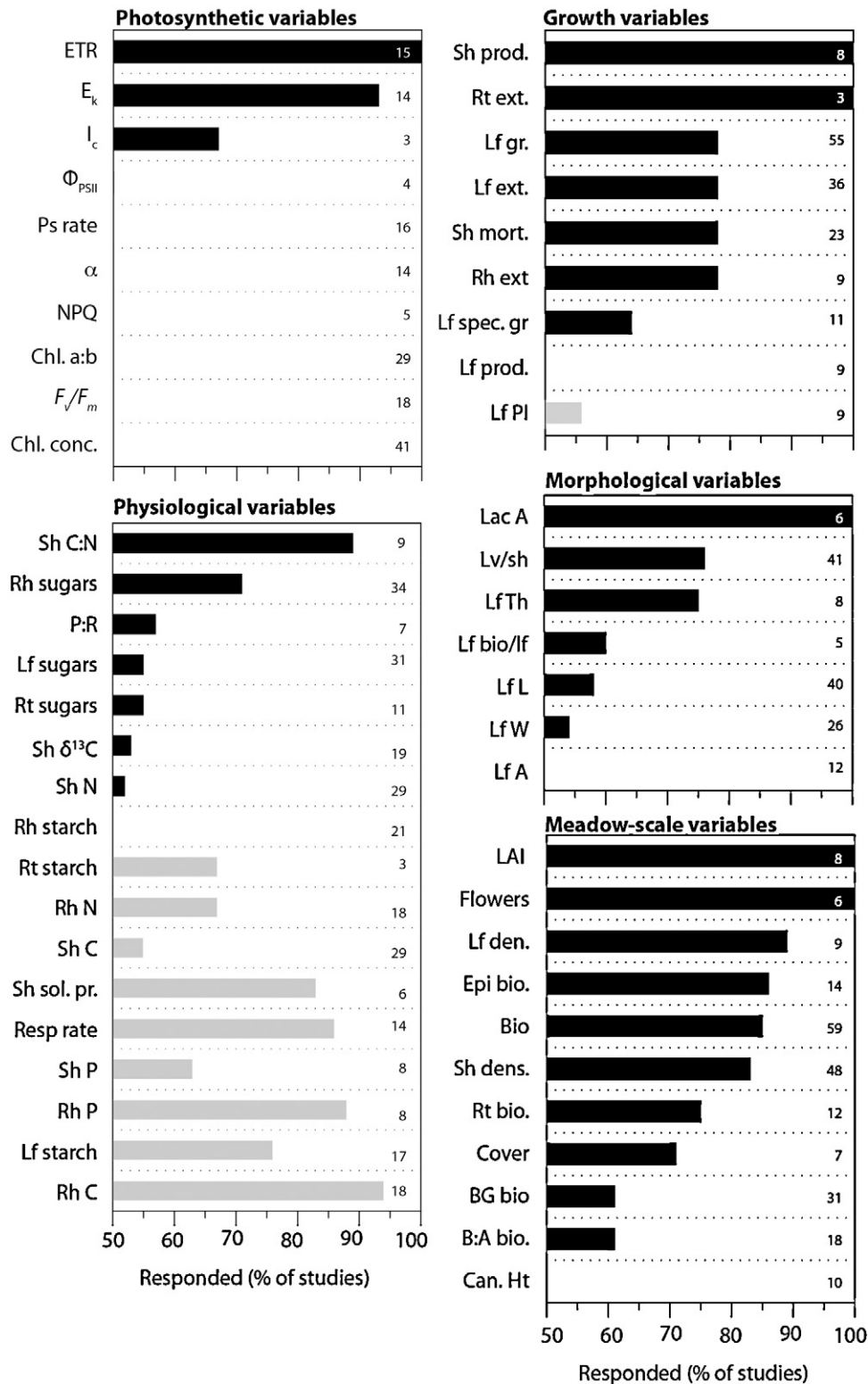
Fig. 2. Consistency of response (*x*-axis) of photosynthetic, other physiological, growth, morphological and meadow-scale variables (*y*-axis) to light reduction. In each graph variables are ordered from most consistent to least responsive (no effect). Bars indicate the percent of observations: black, declined; grey, no effect; and absence of bars, variable. Numbers on the right hand-side of graph are total number of observations. An upward arrow indicates the few cases where a variable increased.

Fewer studies assessed seagrass responses in relation to duration of light reduction. Consequently, only 11 growth, morphology and meadow-scale variables and seven physiological variables had three or more observations (Appendix D). Leaves per shoot was the only variable out of those identified as responding most consistently to light reduction (Table 1), that did not respond consistently

with increasing duration of light stress, initially it decreased and then with longer durations, there was no effect.

3.1.3. Timescales of response

Most of the variables responded at the shortest timescale at which they were measured. The only exception was above-ground
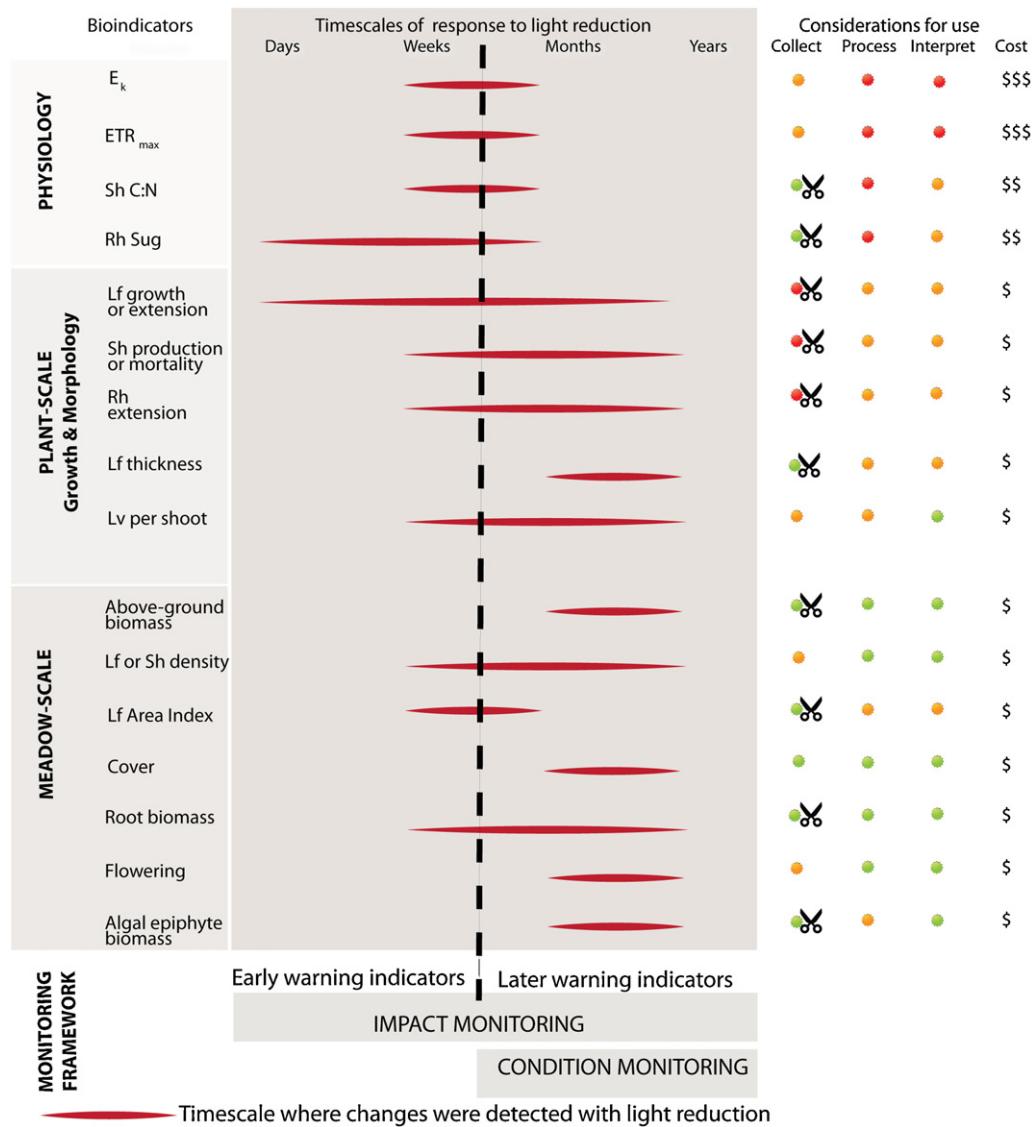
Fig. 3. A summary of the key bioindicators of light reduction identified from this review, the type of monitoring that they would be suited for (impact assessment or condition monitoring) and the ease of collecting, processing and interpreting the measure, as well as a relative cost.

biomass, which responded at the timescale of months but had observations at timescales less than this which did not consistently show a decline (Table 1). Rhizome sugars and leaf growth responded within days, and continued to decline over weeks–months. Leaf extension, shoot production, root extension and LAI responded over timescales of weeks, while shoot mortality, rhizome extension, leaves per shoot, shoot density and root biomass responded over the timescale of weeks and continued to decline over months. Flowering, lacunal area, leaf thickness, algal epiphyte biomass, leaf density and percent cover responses were observed at the timescale of months (Table 1).

3.2. Genera-specific summary

The entire data-set was re-analysed by genera, and 28 variables responded consistently (>50% of observations in one direction) to reductions in light. These included some variables that responded consistently in some genera, but which had not responded consistently in the overall analysis. Chlorophyll *a:b* ratio responded consistently but only in *Posidonia*. Shoot δ^{13}C consistently declined in the *Halodule*, *Zostera* and *Posidonia*. For *Zostera*, the growth

measures shoot production and mortality responded more consistently to light reduction than leaf growth or extension and, unlike other genera, there was a decline in leaf starch (>90% of observations).

4. Discussion

This synthesis is the first of its kind that has derived robust bioindicators of light stress in seagrasses through an analysis of all peer-reviewed, controlled light studies of seagrasses, which were found according to specific search criteria. This approach provides increased confidence that responses are due to light reduction, as pooling results across 104 independent observations and eight of 11 seagrass genera increases sample size, and takes into account species, geographic and temporal variability. Furthermore, all studies had appropriate and justifiable control conditions such that the clear factor being tested was light reduction.

The variables that responded most consistently across space, time and seagrass species were growth and meadow-scale characteristics. Physiological variables, in particular, did not respond as consistently. The patterns observed in the variables that

consistently responded to light reduction are congruent with the current understanding of strategies plants use to cope with reduced light (Fig. 1). This supports their use as bioindicators, as they are relevant, appropriate, consistent, reproducible and repeatable, key requirements of bioindicators.

Notwithstanding this, this review is inherently limited by the designs of the studies reviewed. For example, in some studies, variables may not have responded to light reduction as the intensity or duration of the reductions was insufficient to cause an effect at that particular time. This would largely result in a nil response. A nil response in one or a few studies does not necessarily work against proposing a particular variable as a bioindicator, as long as the responses in the other studies (>50%) were consistent. Also, if there was a different direction of response but a valid explanation, then this also still allows the variable to be considered as a potential bioindicator. A further limitation is that we cannot consider local modifiers of responses to light reduction in a quantitative sense, e.g. nutrient availability. Finally, there is a bias in the species and genera covered, with some more intensively studied than others.

Based on this review, the 56 variables that were assessed can be grouped into those that:

should not be considered as bioindicators as they did not respond at all, or were variable in response;

could potentially be considered as bioindicators, but only in some genera, and following validation; and

should be considered as bioindicators of light reduction as they are relevant, appropriate, reproducible and respond consistently, but need to be assessed for ease of use and cost-effectiveness in monitoring programs.

4.1. Non- robust bioindicators

Many variables, particularly those reflecting physiological processes, did not respond consistently to light reduction despite a reasonable, theoretical expectation that they would (Fig. 1). Consequently, they would not make robust bioindicators. Other measures had a highly variable response to light reduction, i.e. did not respond consistently in any direction and, similarly, should not be considered as routine bioindicators unless additional studies can elucidate the confounding factors (see Appendix B for complete list). Physiological processes respond to many different environmental parameters, often over very short timescales and are very sensitive, which may explain why so few responded consistently to light reduction in this synthesis.

4.2. Potential bioindicators requiring further validation

Some response variables, such as shoot $\delta^{13}C$ and leaf starch, showed potential for use as bioindicators in some, but not all genera. There is a reasonable explanation as to why shoot $\delta^{13}C$ should change: as light becomes limiting, carbon becomes less limiting to photosynthesis and so the lighter isotope is preferentially assimilated, reducing the $\delta^{13}C$ value (Cooper and DeNiro, 1989; Ralph et al., 2007). However, to detect this requires significant tissue replacement, as much of the carbon in seagrass leaves is structural, and if the plant reallocates stored carbohydrates to assist growth, then the shoot $\delta^{13}C$ signal will reflect what was laid down under previous light conditions. Since changes in shoot $\delta^{13}C$ would likely occur after a long exposure to low light it is unlikely to be a useful early warning indicator of reduced light. Starch, not sugars, can form the dominant energy storage compound in the leaves of some genera, including *Zostera* (Brun et al., 2003) and, for this genus, starch may be a useful bioindicator. However, starch utilisation may be inhibited under anaerobic conditions, which may occur under severe light reduction (Longstaff et al., 1999),

potentially confounding its use as a bioindicator, and requiring further validation of its usefulness.

4.3. Robust bioindicators

This review identified 21 response variables which clearly and consistently responded to light reduction and should be robust bioindicators (Table 1). Theoretically, these could be applied immediately in suitably designed monitoring programs as there is a good body of evidence to indicate their responsiveness to light reduction. However, we would not recommend to use root extension ($n = 3$) and lacunal area ($n = 6$) at this time, as they both had a small number of observations and did not respond consistently with increasing intensity of stress.

The remaining 19 bioindicators in this category follow theoretical predictions of how plants respond to light reduction. However, not all observations for each variable responded in the same direction. In a few studies, the variables rhizome sugars, leaf extension and the number of leaves per shoot (or cluster) showed an initial decline and then no further effect or an increase (Bulthius, 1983; Collier et al., 2009). This can be explained by physiological integration within the plant: leaf loss reduces self-shading and increases the light reaching the remaining leaves so that these variables are no longer negatively affected, even if the reduced light conditions persist. In addition, there were also different responses depending on the time of year. For example light reduction in autumn had no effect on leaf extension, whereas in spring or summer there was a reduction (Czerny and Dunton, 1995; Holmer and Laursen, 2002; Lavery et al., 2009). This may be due to seasonal patterns in the growth, structure and biomass allocation of the plant.

4.4. Incorporating bioindicators into a monitoring context

When applying bioindicators in a monitoring context, a range of environmental factors that may influence their response to light reductions needs to be considered. A key consideration is the inherent variation in the bioindicator and the timescale over which this variation occurs. There are daily, seasonal and inter-annual cycles in physiological and growth processes, patterns of resource allocation, and the structure and biomass of plants and meadows. For example, photosynthetic processes vary greatly over daily cycles in response to variations in light (Ralph and Gademann, 2005), all variables change over seasonal cycles due to drivers such as light, temperature and nutrients, and they can vary between years depending on climatic conditions or other external factors (e.g. grazing pressure). The magnitude of the response of any bioindicator must be sufficiently large to separate impacts from inherent background variation (daily, seasonal, inter-annual). Alternatively, the changes in the indicator must be interpreted in the context of this natural variation, through comparison to appropriate reference sites. Therefore, the timing and design of monitoring, and an understanding of the background variability in bioindicators is critical to detecting and interpreting effects.

Further factors to consider when selecting bioindicators are the interaction among bioindicators and the timescales over which they respond. For example, physiological adjustments to photosynthesis can occur in seconds (Enriquez, 2005; Ralph et al., 2002) whereas morphological adjustments can occur from days to weeks (Collier et al., 2009; Longstaff et al., 1999). Depending on the aims of the monitoring, it may be inappropriate to react to a short-term change in photosynthetic parameters if this does not result in a subsequent morphological response. Timescales of response were examined in this synthesis but yielded little insight, as high-resolution temporal studies are rare and our understanding of the progression of plant responses comes from just a few detailed studies. This highlights that a suite of bioindicators should be measured

and their responses interpreted in the context of our understanding of the plant's pressure–response pathways.

The ease of collection, processing of samples and interpretation of responses, as well as cost-effectiveness should also influence bioindicator selection for monitoring programs. Some preliminary indications of these are outlined in Fig. 3. Measuring some bioindicators, such as saturating irradiance for photosynthesis (E_k) and maximum electron transport rate (ETR_{max}), requires specialist equipment and expertise, which for that reason is considered costly. In contrast, shoot C:N and rhizome sugars are relatively easy to collect but require specialist equipment and expertise to analyse, resulting in a moderate cost. Leaves per shoot or shoot density are relatively easy to collect but time consuming to process in the laboratory, so their expense will depend on labour costs. Finally some bioindicators can only be measured with destructive sampling (indicated by the scissor icon in Fig. 3), and these should be avoided if non-destructive techniques are required.

Based on the above considerations, an ideal suite of bioindicators would include: (1) those that respond very early to light reduction, such as photosynthetic variables (E_k, ETR_{max}) which reflect changes at the photosynthesis scale; (2) those that respond over longer time-scales and reflect sub-lethal changes at the scale of the plant, such as rhizome sugars, shoot C:N, leaf growth and leaves per shoot; and (3) those that reflect changes at the meadow-scale such as shoot density or above-ground biomass. The bioindicators that respond later along the cause–effect pathway, i.e. meadow-scale variables, would be more appropriate for condition or health monitoring, whereas the bioindicators that respond earlier on the pathway would be more appropriate for impact assessment monitoring.

Finally, this analysis has targeted low light as an environmental concern, which is relevant in a number of cases where high turbidity is the primary impact (Collier et al., 2012; Erftemeijer and Lewis, 2006). However, there are many situations when multiple anthropogenic impacts occur simultaneously (e.g. nutrient enrichment and contaminants). These additional environmental parameters could confound interpretability of some variables recommended here (e.g. shoot C:N) and additional robust indicators will be required for these other environmental impacts. Therefore, indicators need to be developed to reflect the suite of anthropogenic impacts of concern (e.g. Martinez-Crego et al., 2008) and/or to build indices that reflect specific management goals (e.g. Madden et al., 2009).

5. Conclusion

Monitoring of seagrass condition and health is a key priority in many coastal monitoring programs, and in environmental impact assessment and management and light reduction is one of the main pressures associated with seagrass decline. This meta-analysis is the first of its kind to assess the robustness of different bioindicators of light stress in seagrass systems. We conclude that, across a broad range of seagrass species, there are at least 19 robust bio indicators of light stress that can be confidently incorporated into monitoring programs, out of 119 variables that have been previously used to measure seagrass responses to light reduction. Their consistency across the range of species studied provides confidence that they could be applied to less-studied species, at least until further data become available for those species. However, when incorporating these into monitoring programs, the design to ensure responses to light reduction can be detected against natural variation is critical. There is a need for additional research to evaluate the usefulness of a new set of seagrass characteristics becoming available through new techniques, such as gene expression. More research is required to improve our understanding of the timescales over which some

variables are likely to respond, so that this can inform the selection of early-warning indicators in monitoring programs. Finally, there is a need for finer resolution of light reduction treatments as a way to be able to effectively develop thresholds of seagrass response to light reduction.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ecolind.2013.01.030.

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