Response of seagrass indicators to shifts in environmental stressors: a global review and management synthesis

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Abstract

Although seagrass-based indicators are widely used to assess coastal ecosystem status, there is little universality in their application. Matching the plethora of available indicators to specific management objectives requires a detailed knowledge of their species-specific sensitivities and their response time to environmental stressors. We conducted an extensive survey of experimental studies to determine the sensitivity and response time of seagrass indicators to ecosystem degradation and recovery. We identified seagrass size and indicator type (i.e. level of biological organization of the measure) as the main factors affecting indicator sensitivity and response time to degradation and recovery. While structural and demographic parameters (e.g. shoot density, biomass) show a high and unspecific sensitivity, biochemical/physiological indicators present more stressor-specific responses and are the most sensitive detecting early phases of environmental improvement. Based on these results we present a simple decision tree to assist ecosystem managers to match adequate and reliable indicators to specific management goals.

1. Introduction

The global decline of critical ecosystems to human pressures makes it increasingly urgent to effectively track ecosystem status, in order to detect, halt, and, where possible, reverse these losses (Millennium Ecosystem Assessment, 2005). Seagrass meadows are among the most threatened ecosystems, declining at an estimated 7% per year globally (Waycott et al., 2009). This is being driven by a range of anthropogenic disturbances related to eutrophication (e.g. organic matter and nutrient increases), shading, siltation from deforestation, shoreline modification, and physical removal by trawling and anchoring (Duarte, 2002). Because many seagrass species are also particularly sensitive to disturbance, they are ideal systems to assess environmental change (Marbà et al., 2012). Tracking changes to environmental quality and the ecosystem itself have become increasingly important mandates for ecosystem managers and scientists (Montefalcone, 2009). As a result, there has been a recent burgeoning of monitoring programmes based either directly or indirectly on seagrass responses to environmental change (Martínez-Crego et al., 2008).

In general, monitoring programs have evolved in response to three principal management goals: tracking general trends in ecosystem status, assessing environmental quality, and evaluating impacts of development projects or effectiveness of management actions. Monitoring of ecosystem status is typically linked to habitat management (for instance within Marine Protected Areas), where it primarily serves as an early-warning of change in response to a wide variety of potential stressors. In contrast, monitoring environmental quality (e.g. the European Water Framework Directive) aims at detecting if changes – degradation or amelioration – in water quality are reflected in ecosystem status. Impact assessment focuses instead on detecting if a set of specific, known pressures, associated with a particular action (a coastal development or a management intervention for instance), are affecting the ecosystem. Each of these management objectives places a very different set of requirements in terms of the specificity and expected response time of the indicators used. It is unlikely that a universal set of indicators can be developed to suit all needs, and a more bespoke solution will require a careful matching of management goals with the characteristics of available indicators. These can vary strongly between target seagrass species, the time scale of disturbance and post-disturbance processes, and the sensitivity of the chosen indicators to the stressors of interest. One approach has been to develop multi-metric indices which provide a synthetic measure of environmental or ecological quality based on a combination of parameters (García-Marín et al., 2013; Gobert et al., 2009; Lopez y Royo et al., 2010; Romero et al., 2007). While certainly powerful, there are currently insufficient data to test these composite indices perform in terms of response or recovery time when exposed to known events of environmental disturbance or recovery. As a result, we have explicitly excluded multi-metric indices from this review.

In this review, we adopt the relatively broad definition of indicators proposed by Heink and Kowarik (2010). By their definition, an indicator in ecology and environmental planning is something used to depict or evaluate environmental conditions or changes or to set environmental goals, where this something can be either a component or a measure of environmentally relevant phenomena. This definition is useful since it reflects the wide diversity of contexts within which indicators have been used. A large number of indicators have been developed, based on different seagrass species, and encompassing a broad spectrum of biochemical, physiological, organismal, population and community level traits (Marbà et al., 2012; Martínez-Crego et al., 2008; Rees et al., 2008). Choosing adequate sets of indicators from this plethora to meet management objectives can be challenging. Indicators are not universally sensitive to changes in ecosystem status or environmental conditions, and there are few objective means to evaluate their appropriateness to specific mandates. Understanding how sensitivity and response time vary between seagrass indicators is essential to rationalising the choice of indicators and to designing monitoring and impact assessment programmes.

Response time is the time an indicator takes to register changes (degradation or recovery) in ecosystem (or coastal) health (Contamin and Ellison, 2009), and helps determine its potential either as an early warning indicator (sensitive to degradation) or an improvement indicator (sensitive to recovery). Response times and sensitivity to stressors of environmental change may vary with the type of indicator (biochemical, physiological, growth, morphological, structural, community, etc), and intrinsic species traits that constrain organism and population dynamics (e.g. size or growth and demographic dynamics) (Collier et al., 2009). In fact McMahon et al. (2013) in a recent review found important differences in the response time of indicators between those responding to light stress. Moreover, response times may also differ during degradation and recovery since ecosystem responses often display hysteresis, tracking very different trajectories during decline and recovery phases (Andersen et al., 2009; Duarte et al., 2013; Heide et al., 2007).

The relative sensitivity of indicators to specific stressors is also critical in the assessment of seagrass indicators. Non-specific seagrass indicators that integrate ecosystem health such as shoot density or cover, may be best suited to detect unanticipated environmental or ecosystem changes not linked to a specific impact (e.g.

monitoring climate change or general environmental quality). More stressor-specific indicators may be more appropriate when a clearly identified stressor, such as light availability, excess of organic matter or nitrogen, is being monitored (McMahon et al., 2013; Pérez et al., 2008; van Lent et al., 1995). Stress-specific indicators are best suited to evaluating the effectiveness of mitigatory management actions (Roca et al., 2015). As a rule of thumb, indicator specificity tends to decrease with the level of biological organisation (*sensu,* Whitham et al., 2006), from more integrative, structural metrics to specific physiological and molecular indicators (Adams and Greeley, 2000). How this general rule holds between seagrass species is completely unknown.

We evaluate the utility of the most common seagrass-based indicators to objective-specific management. We identify a wide set of indicators currently employed in seagrass monitoring programs and, where possible, assess their sensitivity (percent of response) to increased/decreased stressors and their response time to degradation and recovery. We test how universal these responses are between species, level of biological organisation and type of stressors. We do this by conducting a comprehensive survey of published and unpublished experimental studies that report the time-response of seagrass parameters currently being used as indicators to a variety of stressors. We use this to develop a simple decision tree to help managers choose a set of seagrass indicators best suited to their specific mandate, be it monitoring general trends in ecosystem health, assessing environmental quality or evaluating the consequences of a known impact or mitigation measure.

2. Materials and Methods

2.1. Identifying and selecting relevant studies

We compiled an extensive database on the likelihood of response to increased or reduced stressors and the response time to degradation and recovery of different seagrass indicators from experimental, mesocosm or field studies (Table 1). Our approach in compiling this database was to focus on a suite of parameters that have been employed by indicator studies across the world, starting with a list initial reviewed by Marbà et al. (2012) and extending it based on more updated reviews (see Table 2). For this shortlist of parameters, we looked for studies that specifically tested their responses to gradients (or levels) of stressors, regardless of whether these studies were specifically designed to test the efficacy of these parameters as indicators. For the purposes of this review, we refer to these chosen parameters as indicators. The data was extracted from scientific reports of experiments from the laboratory, mesocosms or the field. The database was compiled by conducting an exhaustive literature survey on seagrass experiments published before March 2013 using the "Scopus" search engine. We used the search terms ("seagrassses" OR "eelgrass" OR "*Posidonia*" OR "*Zostera*" OR ...(i.e. all seagrass genera)) AND ("response" OR "recovery") AND ("light" OR "shade" OR "shading" OR "dredge" OR "dredging" OR "sediment" OR "burial" OR "organic matter" OR "salinity" OR "hypersalinity" OR "brine" OR "nutrients" OR "N" OR "P" OR "eutrophy" OR "mechanical removal" OR "anchoring"). In addition, to account for older references that may not have been available through "Scopus", the reference lists of each article was also scanned and the bibliographic sources checked for relevant additions to the database. We also updated the dataset with our own unpublished data from recent experiments. Decisions to include or exclude particular studies can have a large impact on the results of meta-analyses, particularly if the number of studies is small (Englund et al., 1999; Gates, 2002; Hughes et al., 2004). To avoid bias in the selection of studies we attempted to be as consistent as possible, only extracting information from those experiments in which indicator responses were estimated under clearly defined possible stressors (organic matter, nutrients, shading, mechanical removal, burial, hypersalinity). For instance, we avoided all studies that examined the effect of multiple stressors acting together since we would be unable to attribute responses to a single stressor. In addition, we separated between three principal factors associated with eutrophication (light, nutrient and organic matter) as they do not always co-occur (Erftemeijer and Robin Lewis III, 2006; Roca et al., 2014). A *study* was defined as every individual publication or experiment. A *case* was defined as every single measurement of responses to increased/decreased stressors or response time to degradation/recovery of a particular indicator taken from each study, carried out in a particular *site*, for a single *species* under a certain *stressor* recorded and measured *indicator*. Seagrass response to increased/decreased level of stressors as well as the response time to degradation/recovery was recorded for each *case*.

The response time of each indicator to increased stress (henceforth, “indicator response time to degradation”) was identified as the time taken for the indicator to register a statistically significant change when exposed to a specific stressor (e.g. increased nutrient level, increased shading). Similarly, the response time of the indicator to the removal of the stress (henceforth, “indicator response time to recovery”) was identified as the time before a statistically significant change was detected after the removal of the stressor. Therefore, “degradation” and “recovery” refer to environmental quality and do not necessarily imply seagrass degradation or recovery. This estimate is conservative since significant effects could perhaps have been registered over a shorter time span and we did not take into account variations in the responses of indicators to different stressor intensities; there is no consistent way to compare stressor intensities between studies and experiments, which are often also conducted in different seasons. In both cases, if no significant change was registered, we recorded this as "no degradation/no recovery". The time intervals between sampling events can strongly influence the precision of the estimates of indicator responses. We, therefore, discarded studies using long sampling intervals, established as at least 1.5 times longer than the minimum response time observed for the same indicator, stress and species in all the data sets, to avoid biasing our estimates of indicator response time.

Indicators were classified into three broad types based on the level of biological organization they addressed: physiological and biochemical, growth and morphological, and structural and demographic (Fig. 1, Table 2). Physiological and biochemical indicators included metabolic processes and chemical constituents of the plant. Growth and morphological indicators included descriptors related to shoot/leaf morphometry or production. Finally, structural and demographic indicators included parameters that characterise the configuration of meadows such as cover, as well as population parameters such as shoot density. We ignored indicators that employed meadow community composition from the analysis because these indicators ranged widely in the level of biological organisation or the species on which they relied. We additionally classified indicators according to the environmental stressor their response was tested against (shading, nutrients, burial, organic matter and hypersalinity). Finally, we also classified seagrass species based on their rhizome diameter, considered one of the best proxies of seagrass size (Duarte, 1991). We grouped seagrasses into small (rhizome diameter ≤ 3.5mm) and large (rhizome diameter >3.5mm) species (Marbà and Duarte, 1998).

2.2. Data analysis

Indicator response to increased stressor levels

We used generalized linear mixed effect models (glmm) to examine the relationship between the two principal dependent variables, *Indicator response time to degradation* (in weeks) or *Indicator response to increased stressor* (yes/no) observed and the type of stressor, the plant size and level of biological organisation of the indicator. In the two models, seagrass size (rhizome diameter), level of organisation (structural/demographic, growth/morphological, physiological/biochemical) and stressor type (organic matter, nutrients, shading, burial, hypersalinity) were treated as fixed factors. The interaction between "study" and "species" was treated as a random factor to account for the influence of data from different indicators belonging to the same study (sample dependence). The variable *Response to increased stressor* was analysed using a binomial distribution due to the dichotomic nature of the data (response yes or no, i.e. a statistically significant change vs. no response in the absence of such changes). We used a Poisson distribution to model the variable *Indicator response time to degradation*. In addition, we used the same *Indicator response time to degradation* model with indicators instead of level of biological organisation to check the variance due to differences in response time among individual indicators. All models were performed using the Lme4 package in the statistical software, R (Bates, 2008, 2005; R core Team, 2013). To avoid the influence of stressors that cause immediate responses, the pressure 'mechanical removal' was extracted from the analysis because this stress involves, by definition, plant removal, and the response of structural indicators is self-evident. We used Tukey's HSD post-hoc comparisons to check for differences between indicator types and stressors in both models using the MULTCOMP R package. In addition, we examined correlations of the variable *Indicator response time to degradation* with log-rhizome diameter for each level of biological organisation.

An indicator was considered robust when it showed a clear response (statistically significant change) to the stressor in question in at least 66% of independent cases. For most stressors, we evaluated robustness only for those indicators that had 5 or more independent assessments of response. For indicators with fewer than 5 independent assessments, we considered it to be potentially robust when it showed a consistent response in more than 75% of reported studies, highlighting that further assessments are needed to confirm its utility. We determined the specificity/generality of each indicator to an increased stressor level by assessing the proportion of studies that showed responses. Indicators were classified as general indicators when they responded to three or more stressors while specific indicators were those that responded to one independent stressor or two related stressors.

Indicator response to decreased stressor levels

Indicator response to decreased stressor levels (yes/no) and *Indicator response time to recovery* were tested using models similar to those described above. The dataset to test responses to decreased stressor levels (24 studies) was much smaller and less balanced than for responses to increased stressor levels (74 studies). In order to avoid potential biases due to this reduced sample, analyses of *Indicator response to decreased stressor levels* and *Indicator response time to recovery* were simplified to focus on three separate, more balanced models. To test the variable *Indicator response to decreased stressor levels* (yes/no) we first ran an analysis with the whole dataset to test for effects of the level of biological organisation and species size (fixed factors). As the factor "size" appeared to introduce some potentially confounding variability, we ran two separate analyses for large seagrass species (12 studies, 42 cases) and small species (10 studies, 57 cases) to identify size-dependent differences among indicator types. All three models were fitted to a binomial distribution. To test the variable *Indicator response time to recovery* we included the effects of level of biological organisation and species size (as fixed factors). The number of studies was relatively small for this model (19 studies). Due to the lack of significant random effects, we ran response and time response to decreased stressor levels models without random effects using the *glm* function in the R stats package (R core Team, 2013).

3. Results

The compiled dataset included 25 of the 60 existing species of seagrasses (Green and Short, 2003), with *Zostera marina, Posidonia oceanica*, *Cymodocea nodosa* and *Thalassia testudinum* accounting for the highest records (Table 1). Most studies used indicators to assess responses to environmental degradation (n=74) with far fewer studies assessing recovery after the cessation of stress (n=24, Table 1). The studies covered a wide geographic extent, including coastal areas in Australasia (Australia 10), Asia (Korea 1, Philippines 1, India 1, Malaysia 1, Indonesia 1), Europe (Denmark 4, Italy 1, Netherlands 5, Germany 1, Portugal 2, Spain 23, France 1, Italy 1), North America (USA 16), Central America (Puerto Rico 1), South America (Brazil 1). In total we identified 85 distinct indicators (Table 2). The vast majority were physiological and biochemical indicators (61 unique measures), while growth/morphological and structural/demographic indicators were much less common (13 and 10 respectively).

Response to increased and decreased stressor levels

The likelihood of responses to increased levels of stressors (n=668) differed significantly between physiological/biochemical indicators (58%) and the other two groups belonging to higher levels of biological organisation (Fig. 1 and Table 3). Structural and demographic indicators showed the highest percentage of significant responses (75%) followed by growth and morphological indicators (70%).While most indicators recorded a high percentage of response to increased stressor levels (see Table 4), a few showed no significant response (C content in epiphytes, $\delta^{13}C$ in rhizomes, and $\delta\ ^{34}S$ in leaves). However, the number of cases for these indicators was too low to adequately evaluate their responses (n=1 or 2).

While structural and demographic indicators were very effective in detecting degradation, they were not as effective in signalling the cessation of stressing agents as other indicators at experimental time-scales. They showed responses in 60 % of recorded cases, whereas physiological/biochemical and growth/morphological indicators detected recovery processes in around 80% of the cases (Fig. 1). The proportion of responses to decreased stressor levels among indicators belonging to different biological organisation levels showed a mild difference between small and large species, although it was not significant (interaction between seagrass size and level of biological organisation, $p = 0.09$, Table 5). Indeed, the response of indicators to decreased stressors differed significantly between level of biological organisation in large species but not in small ones (Table 5).

Response time of indicators to degradation and recovery

The response time of seagrass indicators to degradation was dependent on seagrass size interacting with the level of biological organisation and showed a mild, though non-significant difference between stressors (Table 6). In fact, the response time of structural/demographic, growth/morphological and physiological/biochemical indicators to degradation increased with seagrass size (Fig. 2 and 4, Table 4), with structural/demographic parameters showing significantly longer response times for seagrasses with larger rhizome diameters (Seagrass size: level of biological organisation, $p= 0.01$) (Fig. 2, Table 6).

In general, indicators took longer to respond to recovery processes than to degradation conditions for all levels of biological organisation (Fig. 3). This was particularly true for structural indicators that did not recover within the experimental time frame of the studies (Fig. 3). Unfortunately, the data from available studies were insufficient to explore how recovery response times of indicators differed between stressors.

General versus specific indicators

Two structural parameters (density and aboveground biomass), one morphological indicator (leaf growth) and one physiological indicator (sucrose concentration in rhizomes) were found to be general indicators of a wide range of stressors for both small and large seagrasses (>60% response, responding to at least 3 stressors) (Table 4). Nitrogen concentration in leaves responded consistently across species, increasing with shading and increasing nutrient availability. Likewise, decreased photosynthetic rates responded to increased loads of organic matter inputs and hypersalinity. The structural indicators shoot mortality and belowground biomass each showed robust responses to two types of stressors; shoot density decreased in response to burial and hypersalinity while below-ground biomass decreased in response to burial and nutrients (Table 4). In contrast, most indicators were much more specific, responding to a single stressor. Physiological/biochemical indicators were particularly good in detecting single stressors, with more than 60% of positive responses. This was true for $\delta^{13}C$ in leaves, $\delta^{15}N$ in leaves, and S concentrations in roots and rhizomes, which appeared to be clearly stressor-specific (Table 4). However, while $\delta^{13}C$ decreased with shading, the time-scale of response was longer than other physiological and biochemical indicators (see Fig. 4). Nutrient addition in small plants resulted in decreased levels of $\delta^{15}N$ in leaves. An important caveat, however, is that $\delta^{15}N$ response is not unidirectional and depends on the $\delta^{15}N$ signal of the source. While the S content in roots and rhizomes of large seagrass species was also a potentially robust indicator – its concentration increased with organic matter loading – it requires independent validation from more studies before it can be fully trusted (Table 4, Fig. 4).

Although chlorophyll content, tissue C/N ratios, necrosis in leaf tissues and dark respiration rates showed higher percentages of response (>60%) for one stressor, they cannot be considered stressor-specific since they also responded to other stressors with lower percentages of positive responses (Table 4). Thus, chlorophyll content and tissue C/N ratios while mainly decreasing with nutrient additions also responded to changes in shading. Similarly, necrosis and dark respiration showed potential as indicators of hypersalinity, increasing and decreasing with high salinity, respectively. However, necrosis also increased with nutrient additions, whereas for dark respiration, there were far too few cases available to assess its specificity (Table 4, Fig. 4).

4. Discussion

As developmental pressures increase in the coastal ocean, the need to keep track of this change is becoming increasingly acute (Agardy et al., 2005; Carpenter et al., 2009; Erftemeijer and Robin Lewis III, 2006; Martínez-Crego et al., 2008). Our review reflects this growing urgency to document decline, with the vast majority of seagrass indicators developed to measure ecosystem and environmental degradation rather than improving conditions. This bias is perhaps also due to the difficulty of tracking seagrass recovery after the removal of stresses, since recovery responses may take place over considerably longer time scales than most studies allow (e.g. Heide *et al.* 2007; Duarte *et al.* 2009, 2013, this study). Nonetheless, we were able to assess the performance of 34 indicators in relation to six of the most common and important drivers of seagrass decline (shading, increased nutrient and organic inputs, burial and hypersaline effluents, see Waycott *et al.* 2009). These are among the stressors of most concern for seagrass managers. Indicators ranged from physiological and biochemical parameters to ecosystem-level measures and included 25 species of seagrass from across the globe. Indicators clearly varied widely in their sensitivity, specificity and response time while tracking degradation and recovery.

Our meta-analysis shows that most indicators clearly differed in their ability to detect degradation and recovery processes. Thus while more integrative structural and demographic parameters (like shoot density or biomass) were very responsive to degradation from multiple stressors, they were not as effective in reflecting improvements at short management time-scales when these stressors reduced. In contrast, physiological and biochemical indicators were much more effective in documenting recovery processes, particularly for large seagrass species. The underlying ecological processes of degradation and recovery are likely very different. Seagrasses respond predictably to a range of stressors, often with noticeable declines in meadow structure. However, the capacity for seagrasses to recover these structural losses when conditions improve is driven by species-specific demographic rates, largely dependent on plant size (Marbà and Duarte, 1998). It is therefore unsurprising that structural indicators may be ineffective in tracking recovery of environmental conditions (particularly for larger, slow-growing species), since it may often take several decades before these changes are reflected at the level of the meadow (Badalamenti et al., 2011; Meehan and West, 2002) (see later).

In tracking degradation, physiological/biochemical indicators showed considerable variability in their response, due, at least in part, to their higher stressor specificity. Thus, while highly integrative variables like seagrass shoot density and biomass responded to increased stressor levels across the spectrum of examined stressors, physiological/biochemical parameters like $\delta^{13}C$, $\delta^{15}N$ and S were linked to changes in few or single stressing agents (shading, nutrients or organic matter inputs respectively) (Table 4).

Most parameters in our review were very reliable indicators of generic or specific stressors. For instance, robust indicators to light disturbances found here were quite consistent with those previously identified by McMahon et al. (2013), with the exception of some physiological and morphological measures, which we attribute to differences in the studies reviewed. However, some measures showed rather limited response for the stressors we tested here. For example, C content in epiphytes or $\delta^{34}S$ in leaf tissue showed no significant responses to shading, nutrients, burial, organic matter or hypersalinity. Though they may not be useful as indicators of these stressors, they may still respond to stressors not included in our study. For instance, $\delta^{34}S$ in leaf tissue has been shown, experimentally and in the field, to respond to warming (García et al., 2013), and % inorganic carbon in epiphytes may be a useful indicator of ocean acidification (Campbell and Fourqurean, 2014; Fabricius et al., 2011). As CO_2 emissions rise, warming and acidification are likely to increase, making seagrasses and their epiphytes potentially important sentinels of future climate change (Duarte, 2002; Koch et al., 2013).

The time scale of responses differed greatly between indicators, varying with level of biological organisation and plant size. Physiological/biochemical and growth/morphological indicators were generally able to detect degradation responses much faster than structural/demographic indicators, especially for large, slow-growing, seagrass species. This contrast likely reflects the strong hysteresis that operates in many coastal ecosystems as the mechanisms controlling the recovery of indicators differ from those controlling degradation (see Fig. 5- Heide et al. 2007; Duarte et al. 2009, 2013). This is particularly true for structural and demographic indicators in long-lived seagrass meadows (e.g., shoot density, above- and belowground biomass). These meadows are often characterised by positive feedbacks that buffer the structure of the habitat against even relatively high levels of environmental stress. For instance, larger plants have greater reserves, making them better able to resist short-term adverse conditions. Once a particular threshold is breached however, the effects of degradation can accrue very rapidly as the structural integrity of the meadow unravels. Recovery from this point can be protracted, with recovery rates often almost four or five times slower than degradation (Backman and Barilotti, 1976; Collier et al., 2009). As discussed earlier, there is an important size-dependence in seagrass growth, tissue turnover and demographic dynamics (Duarte, 1991) which determines response time of indicators. The time lags imposed by species-specific intrinsic growth rates are further compounded by shifts in ecosystem baselines that further impede or slow down natural recovery (Duarte et al., 2009). In habitats dominated by large, slow-growing species like *Posidonia oceanica*, this recovery may require several decades, if not centuries (Duarte, 2002; González-Correa et al., 2005).

The natural hysteresis that characterises seagrass ecosystems has important implications when choosing indicators to monitor ecosystem status. Structural and morphological indicators, while responsive to a range of stressors, may, especially for large species, detect impacts much too late for effective action to be taken (van Katwijk *et al.* 2010, this study). Physiological and biochemical parameters are less influenced by hysteretic properties, making them much better early-warning candidates to detect changes (both decline and recovery) in environmental conditions over time-scales relevant for management. However, these indicators, since their response is highly stress-specific, need to be used as part of a set and may not be appropriate to be used on their own.

Designing Fit-for-Purpose Seagrass Monitoring Programs

From the discussion above, it is clear that no single indicator can satisfy every management objective. The array of available indicators represents a valuable toolbox from which to choose a set of indicators to match specific management goals. Given the number of indicators available and their differences in specificity, sensitivity and response times, it is unsurprising that selecting the appropriate set of indicators can be perplexing. We provide a generic decision tree to assist this process, following the potential life cycle of a monitoring programme, when there is no change with respect to reference conditions, and under conditions of change whose source is either known (in some cases even planned) or unknown (Figure 6). Each condition requires a design that employs a contingent set of indicators best suited to the task. In general, the scheme is designed to ensure that the resulting programme (i) provides early warning responses to degradation (Generic ecosystem monitoring strategy), (ii) can attribute changes in indicators to specific pressures (Stress screening strategy), and (iii), detect the onset of ecosystem recovery (Assessment strategy). We suggest sets of potential indicators to match these monitoring strategies used together as a multi-metric index or separately. These sets of indicators serve merely as a general heuristic that will require context-specific tailoring based on management goals, environmental conditions and the seagrass species present. While the objectives of management can vary widely, the figure indicates how this scheme could be employed for typical management scenarios: (i) assessing general trends in ecosystem health, (ii) assessing environmental quality and (iii) assessing impacts or remediation measures. The decision tree allows entry and exit at any point based on needs and circumstances.

Generic ecosystem monitoring strategy Tracking ecosystem health under normal conditions is important to detect unforeseen changes in overall condition and their causes, so that remedial actions can be taken to stop the decline. This is often an essential management mandate and chosen indicators need to be both generic, to detect responses from a wide variety of stresses, and respond rapidly, to serve as an early warning. Structural and demographic indicators have a large integrative capacity and are linked most directly to ecosystem structure and function, making them ideal generic indicators. Indicators such as shoot density, seagrass cover or meadow depth limit are widely used in monitoring programmes (Marbà et al., 2012), and have proven excellent in detecting generalized degradation responses, mostly linked to eutrophication (Martínez-Crego et al., 2008). However, most of these variables respond very slowly. With some exceptions, such as mechanical removal (which directly modifies structure and demographics) changes in structural indicators are the result of changes in the environment first reflected in plant physiology, which modifies seagrass growth and morphology, finally triggering changes in meadow structure and demography (Fig. 5) (Collier et al., 2012), and it can be fairly long before these changes are detectable. As a result, ecosystem monitoring strategy benefit from incorporating early-warning indicators together with these structural measures, especially for large species. Some physiological/biochemical indicators such as sucrose or N respond to a range of stressors and their inclusion can serve as early warnings of eutrophication processes such as shading, nutrients, and organic matter.

Stress screening strategy: Often, when change is registered, for example through a generic ecosystem monitoring, the drivers/stressors for these changes are difficult to establish. Screening strategies help in identifying these drivers using stressor-specific indicators. Many physiological and biochemical parameters are particularly useful here, since they respond reliably to changes in single or few drivers. For instance, $\delta^{13}C$ responds only to changes in light availability (Serrano et al., 2011), and S content in roots and rhizomes responds to intrusion of H_2S under organic inputs (although this needs independent confirmation, but see Frederiksen et al., 2008, 2006; Pérez et al., 2007) (Table 4, Fig. 4). While $\delta^{15}N$ mostly responds to variations in nitrogen inputs (Christianen et al., 2012), it may also be influenced by changes in light conditions (Lavery et al., 2009), and while it is a useful stress screening indicator, it needs to be interpreted with caution. In addition, the elemental contents of rhizomes are very reliable indicators of detecting metal variations (Fe, Cd, Pb, Ni, Cu) in the environment (Richir et al., 2013; Roca et al., 2014). Because several of these measures respond predictably to both increasing and decreasing drivers, they are also useful in monitoring improvements in environmental quality. For instance, specific elemental indicators can effectively track reductions in inputs of silver or lead into coastal waters, linked to the advent of digital photography or unleaded fuel, respectively (Tovar-Sánchez et al., 2010). While stressor-specific indicators are generally good at identifying drivers of change, it is useful to include structural and demographic parameters in the monitoring program; used together, these indicators can provide a more accurate assessment of ecosystem function.

In addition, since stress specific indicators can respond to more than one driver (e.g. $\delta^{15}N$ to nutrients and light (Lavery et al., 2009), it is advisable to include more than one indicator that responds to the same driver in order to increase the reliability of identifying the relevant stressor.

Assessment strategy: Assessment strategies are employed when the nature of the stressors is well understood, and the interest of management is to assess impacts or the efficacy of remedial actions. For instance, managers may want to test if stress-reducing interventions are actually working (e.g. reducing nutrients from urban sewage), or may need to evaluate the impact of coastal development projects such as harbour constructions or beach replenishments. In order to detect these effects as early as possible (within weeks or months), monitoring needs to be based on physiological/biochemical indicators that respond rapidly and specifically to the drivers in question (a subset of the screening set, see Fig. 6). These indicators are thus a valuable tool in evidence-based management and can also help managers quickly adapt their interventions based on measured efficacy. As with all strategies, these assessments must also include the more integrative structural/demographic drivers to track potential ecosystem-level effects.

In attempting to address these different needs, researchers have developed a suite of synthetic and integrative multi-metric indices to measure ecological status or water quality (García-Marín et al., 2013; Gobert et al., 2009; Lopez y Royo et al., 2010; Romero et al., 2007). While very useful in summarizing ecosystem status, these multi-metric indices still depend eventually on the behaviour and response of their individual constituent indicators. Analysed individually, the detection of indicator trends in environmental or ecological status may be less integrative, but allows for far greater precision than multi-metric indices.

5. Summary and conclusions

Indicators based on seagrass parameters provide robust measures of change, which explains their proliferation and use in monitoring programmes in recent decades. The analyses performed here showed that the 34 indicators we evaluated ranged widely in their responsiveness, relative specificity and response time, dependent largely on the size of the plant and the level of biological organisation of the measured indicator. Taken together, these indicators serve as an invaluable toolbox to address a range of monitoring needs. Employing purpose-specific indicators to match management goals enables the detection of change within weeks to months, allows managers to ascertain the cause of these changes, and provides a means to evaluate recovery after the particular stressor has been reduced. This review establishes objective criteria by which the perplexingly large number of available indicators can be critically assessed and used to monitor and manage globally threatened seagrass ecosystems.

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Figure and table footnotes.

Fig.1. Percentage of reviewed studies that documented significant responses of indicators to degradation (increased stressor levels) and recovery (decreased stressor levels), classified by indicator type (physiological/biochemical, growth/morphological or structural/demographic). Post-hoc comparisons highlight significantly different indicator types (a and b).

Fig.2. The relationship between response time to increased stress and rhizome diameter for different indicator types (physiological/biochemical, growth/morphological or structural/demographic). Solid lines represent the fitted log-log regression equations for structural and demographic indicators (R^2 = 0.225, P= $4*10^{-9}$), dashed lines represent growth and morphological indicators (R^2 =0.028, P= 0.041) and dotted line represents physiological and biochemical indicators (R^2 =0.142, P= $5*10^{-7}$).

Fig.3. Mean indicator response time to increased stressor levels and recovery (decreased stressor levels) for each level of biological organization. Error bars represent standard errors. The asterisk indicates significant differences based on model results. Refer to Methods and Results for details on datasets employed and model specifications.

Fig.4. Indicator response times of small and large seagrass species to common stressors. Dots indicate mean response times and bars represent the minimum and maximum observed response times reported in the literature. Black dots represent a negative relationship (an increase in stressor levels results in decreased indicator values), white dots represent a positive relationship (an increase in stressor levels result in increased indicator values) and black and white dots represent situations when both positive and

negative relationships were reported. Rhiz suc = Sucrose in rhizomes, A. biomass = Aboveground biomass, B. biomass = Belowground biomass, Dark resp = Dark respiration, Photosyn rate = Photosynthetic rates.

Fig. 5. Degradation and recovery pathways in response to variations in environmental stress. (a) Responses of structural and demographic indicators; small seagrass species (blue dashed line) respond faster to environmental improvements than large species (blue solid line). (b) Physiological and biochemical indicators are more quick to respond to degradation and improvement of environmental conditions and show less hysteresis than structural and demographic indicators.

Fig. 6. Designing a fit-for-purpose seagrass monitoring program. Above: Decision tree to help choose monitoring strategies based on three common management objectives. Below: Sets of suggested indicators corresponding to each management objective in the decision tree above. A single asterisk (*) represents indicators not tested in our study but widely used and accepted, a double asterisk (**) represents stressor-specific indicators that require further testing. A. biomass = Aboveground biomass, B. biomass = Belowground biomass, EIA: Environmental Impact Assessment.

Table 1. Number of cases (Nº cases) and sources for indicator response time to degradation (increased stress levels) and recovery (decreased stress levels) for different species. See table references.

Table 2. The 85 indicators compiled in the study classified in three different levels of biological organization. N: number of cases. APA: Alkaline phosphatase, Ek= Light

saturation, Etr= Electron Transport Rate, Max and min fluorescence, Above.= aboveground, Below.= belowground, Fv/Fm: chlorophyll fluorescence measurement, LAI= leaf area index.

Table 3. Results of analyses of variance (Type III tests) of percentage of responses (%) to increased stressor levels of seagrass indicators in relation to seagrass size (as reflected by rhizome diameter). Biological organisation refers to either structural and demographic, growth and morphological, or physiological and biochemical indicators. Seagrass size:level of biological organization = Interaction between rhizome diameter and level of biological organization. The percent response (%) was fitted to a binomial distribution. DF (degrees of freedom), DenDF (denominator DF). For further details, refer to Methods.

Table 4. List of robust and potentially robust indicators to degradation. Number of cases, percentage of indicator response to increased stressor levels and associated indicator response time (weeks) are shown only for the most robust indicators (% response >60) and potential indicators for each driver. For example, we recorded 5 cases of Leaf N measured in shading experiments, of these 100% (all 5 cases) responded with changes in Leaf N. In subsequent columns we indicate the minimum and maximum response time recorded in these experiments for large and small seagrass species. Level = level of biological organization, Physiological = physiological and biochemical, Morphological = growth and morphological, Structural = structural and demographic, A. Biomass = Aboveground biomass, B. Biomass = Belowground biomass, References = references used in each line (see table1). Indicators marked with an asterisk (*) are potentially robust indicators, but have limited sample cases.

Table 5. Results of analyses of variance (Type III tests) of indicator recovery response (%) in relation to level of biological organization (structural and demographic, growth and morphological, physiological and biochemical) for all species together, large species and small species. All three models are fitted to a binomial distribution. The analysis of all species also includes the effect of seagrass size (as reflected by rhizome diameter). DF (degrees of freedom), LR Chi (likelihood ratio Chi squared test). For further details, refer to Methods.

Table 6. Results of analyses of variance (Type III tests) on indicator response time (top) and recovery time (bottom) in relation to seagrass size (as reflected by rhizome diameter), level of biological organization (structural and demographic, growth and morphological, physiological and biochemical) and type of environmental stressor. Seagrass size: level of biological organization = Interaction between rhizome diameter and level of biological organization. Response time was fitted to a Poisson distribution and recovery time to a quasi-Poisson distribution with an overdispersion parameter taken to be 29.3). DF (degrees of freedom), LR Chi (likelihood ratio Chi squared test). For further details, refer to Methods.

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Table 1. Number of cases (Nº cases) and sources for indicator response time to degradation (increased stress levels) and recovery (decreased stress levels) for different species. (Size. rhiz. diam, cm): size of rhizome diameter in centimetres. See table references.

		Indicator degradation response		Indicator recovery response	
Species	**Size (rhiz. diam, cm)**	**Nº cases**	**References**	**Nº cases**	**References**
Amphibolis griffithi	2	28	1	31	1
Cymodocea nodosa	3	92	2, 3, 4, 5, 6, 7, 8	7	6, 5, 2
Cymodocea rotundata	2.4	3	9	0	
Cymodocea serrulata	2	17	10, 11, 12	0	
Enhalus acoroides	14.1	9	9	0	
Halophila engelmani	-	1	13		
Halophila johnsonii	-	3	14	0	
Halophila ovalis	1.5	43	12, 15, 16,17	15	15, 16
Halophila pinnifolia	1.5	6	18	0	
Halophila spinulosa	1	2	10	0	
Halophila tasmanica	1.74	4	19	0	
Halodule uninervis	1.4	39	10, 11, 20, 12	2	16
Halodule wrightii	1.6	9	21, 22, 23	2	23
Posidonia australis	7.2	5	24, 25, 26	2	25, 26
Posidonia oceanica	9.7	133	27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40	16	29, 41, 30, 42, 27, 39
Posidonia sinuosa	5.5	26	43, 44, 25, 45	12	43, 25, 45
Ruppia maritima	-	7	21, 22	0	
Syringodium isoetifolium	1.7	12	10, 46, 12	1	46
Thalassia hemprichii	3.6	17	9, 11	0	
Thalassia testudinum	6	53	47, 48, 49, 50, 51, 52-54	4	47
Zostera capricorni	1.4	10	10, 55	0	
Zostera japonica	1	2	56	2	56
Zostera marina	3.5	98	57, 58, 41 21, 59, 60, 61, 62, 63, 64, 65, 66, 67	6	41, 64, 65
Zostera muelleri	2	10	11, 68	0	
Zostera noltii	1.6	49	69, 70, 2, 71, 72, 73	3	72, 73

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Table 2. The 85 indicators compiled in the study classified in three different levels of biological organization. N: number of cases. APA: Alkaline phosphatase, Ek= Light saturation index, Etr= Electron Transport Rate, Max and min fluorescence, Above.= aboveground, Below.= belowground, Fv/Fm: chlorophyll fluorescence measurement, LAI= leaf area index.

Physiological and biochemical						Morphological and growth		Structural and demographic	
	N		N		N		N		N
Amino acid content	2	Dark respiration	4	P roots	1	Internode distance	2	Above. biomass	41
APA leaf	1	Ek	4	P total	1	LAI	4	Below. biomass	30
C rhizomes	1	Etr	2	Na	1	Leaf growth	72	Cover	10
C leaf	14	Fe rhizomes	2	Pb rhizomes	1	Leaf length	18	Depth limit	6
C/N aboveground	6	Fe leaf	2	Phenolics	1	Leaf necrosis	7	Leaf biomass	17
C/N belowground	11	Fv/Fm	3	Photosynthesis rates	16	Leaf number	23	Leaf density	78
C/N_leaf	6	K content	1	Quantum yield	2	Leaf thickness	4	Mortality	13
Ca	2	Max fluorescence	2	S leaf	2	Leaf width	15	Rhizome biomass	4
Carotenoids	2	Min fluorescence	2	S rhizomes	4	Mean canopy height		Root biomass	4
Cd rhizomes	1	Mg rhizomes	1	S roots	4	Plastochrone interval	1	Shoot biomass	14
Chlorophyla a	18	Mn rhizomes	1	Starch leaf	6	Rhizome elongation	1		
Chloroplast density	1	N leaf	23	Starch rhizomes	11	Root length	1		
Cu rhizomes	1	N rhizomes	18	Starch roots	6	Root/shoot ratio	1		
$\delta^{13}C$ leaf	12	N roots	2	Sucrose leaf	6	Shoot size			
$\delta^{13}C$ rhizomes	6	N total	1	Sucrose rhizomes	11				
$\delta^{13}C$ shoots	6	N/P aboveground	6	Sucrose roots	9				
$\delta^{15}N$ leaf	4	N/P belowground	3	Total carbohydrates	2				
$\delta^{15}N$ rhizomes	10	Ni rhizomes	1	Zn leaf	2				
$\delta^{34}S$ leaf	5	P rhizomes	2	Zn rhizomes	2				
$\delta^{34}S$ rhizomes	4	P leaf	13						
$\delta^{34}S$ roots	2	P rhizomes	6						

Table 3. Results of analyses of variance (Type III tests) of percentage of responses (%) to increased stressor levels of seagrass indicators in relation to seagrass size (as reflected by rhizome diameter). Biological organisation refers to either structural and demographic, growth and morphological, or physiological and biochemical indicators. Seagrass size:level of biological organization = Interaction between rhizome diameter and level of biological organization. The percent response (%) was fitted to a binomial distribution. DF (degrees of freedom), DenDF (denominator DF). For further details, refer to Methods.

Response %	DF	DenDF	F.value	P.value	
Level of biological organization	2	630	5.29	0.005	**
Stressor	4	93	0.79	0.537	
Seagrass size	1	1	68.2	0.23	
Seagrass size : Level of biological organization	2	630	1.20	0.303	

Significance level: '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Stressor	Level	Robust indicators	N	% Response	Indicator response time (weeks) Large species Min	Max	Small species Min	Max	References
Shading	Physiological	Leaf N	5	100	8	24	2	8	29,44,48,55,63
	Physiological	Rhizome N	7	85	-	-	2	12	1,29,45,71
	Physiological	Rhizome sucrose	10	88	3	15	0.5	2	1,15,29,45,71
	Physiological	Leaf $\delta^{13}C$	7	100	28	28	4	11	10,18,32,45,55,
	Growth	Leaf growth	30	76	1	20	1	8	1,11,15,19,26,29,32, 43,45,48,55, 63,71
	Structural	Density	27	85	4	36	2	11	11,15,18,19,26,29,32, 43,44,45,48,55,58,63, 64
	Structural	A. biomass	17	88	6	29	1	15	1,11,15,45,48,58,
Nutrients	Physiological	Leaf N	16	75	4	24	1.4	14	4,9,20,21,37,38,48, 52,59,60,62
	Physiological	Rhizome N	7	85	32	32	8	20	9,20,21,34
	Physiological	Chlorophyll a	5	80	5	20	20	20	9,61,62
	Physiological	C/N	5	80	3	12	-	-	21,48
	Physiological	Rhizome sucrose*	4	100	14	24	-	-	21,34
	Physiological	Leaf $\delta^{15}N$ *	1	100	-	-	8	8	20
	Growth	Leaf growth	18	78	1	20	2	14	4,9,21,33,34,48,49, 50,59,60
	Structural	Density	15	73	5	12	4	24	4,9, 21,22,48,50, 61,62
	Structural	A. biomass	12	58	6	48	8	24	9,20,48, 50,52,58
	Structural	B. biomass*	4	100	6	48	8	8	20,48,52,58
Burial	Structural	Mortality	10	100	-	-	3	4	69,70
	Structural	Density	20	65	1	36	2	5	8,12,27,28,30,69
	Structural	A. biomass	13	85	-	-	4	15	4,5
	Structural	B. biomass	13	77	-	-	4	4	8,12
OM	Physiological	Rhizome sucrose	10	60	2	12	-	-	35,57
	Physiological	Photosyntesis*	3	100	1	1	-	-	57,66
	Physiological	Roots S*	2	100	12	12	-	-	35
	Physiological	Rhizome S*	2	100	12	12	-	-	35
	Growth	Leaf growth*	4	75	2	2	-	-	50,53,57
	Structural	A. biomass*	3	67	24	24	-	-	50
	Structural	Density*	3	67	12	12	-	-	35,50
Hypersalinity	Physiological	Photosyntesis rate	5	100	7	12	0.14	7	3,7,14,17,39,67
	Physiological	Dark respiration*	3	66	7	7	7	7	7,39
	Growth	Leaf growth	6	100	4	12	1	2	2,3,14,31,39,40, 54
	Growth	Necrosis*	3	66	7	7	-	-	7,39,40,67
	Structural	Mortality	7	71	12	12	1	2	2,3,7,14,39,40,67
	Structural	Density*	2	100	5	8	-	-	31,62

Table 4. List of robust and potentially robust indicators to increased stressor levels. Number of cases, percentage of indicator response to degradation and associated indicator response time (weeks) are shown only for the most robust indicators (% response >60) and potential indicators for each driver. For example, we recorded 5 cases of Leaf N measured in shading experiments, of these 100% (all 5 cases) responded with changes in Leaf N. In subsequent

columns we indicate the minimum and maximum response time recorded in these experiments for large and small seagrass species. Level = level of biological organization, Physiological = physiological and biochemical, Morphological = growth and morphological, Structural = structural and demographic, A. Biomass = Aboveground biomass, B. Biomass = Belowground biomass, References = references used in each line (see table1). Indicators marked with an asterisk (*) are potentially robust indicators, but have limited sample cases.

Table 5. Results of analyses of variance (Type III tests) of indicator recovery response (%) in relation to level of biological organization (structural and demographic, growth and morphological, physiological and biochemical) for all species together, large species and small species. All three models are fitted to a binomial distribution. The analysis of all species also includes the effect of seagrass size (as reflected by rhizome diameter). DF (degrees of freedom), LR Chi (likelihood ratio Chi squared test). For further details, refer to Methods.

Recovery % (all species)	LR Chi	DF	P.value	
Level of biological organization	0.1738	2	0.91676	
Seagrass size	0.5283	1	0.46733	.
Seagrass size: Level of biological organization	4.6562	2	0.09748	

Recovery % (large species)	DF	Deviance	P.value	
Level of biological organization	2	7.6594	0.021	*
Residuals	39	47.088		

Recovery % (small species)	DF	Deviance	P.value
Level of biological organization	2	1.98	0.371
Residuals	54	56.69	

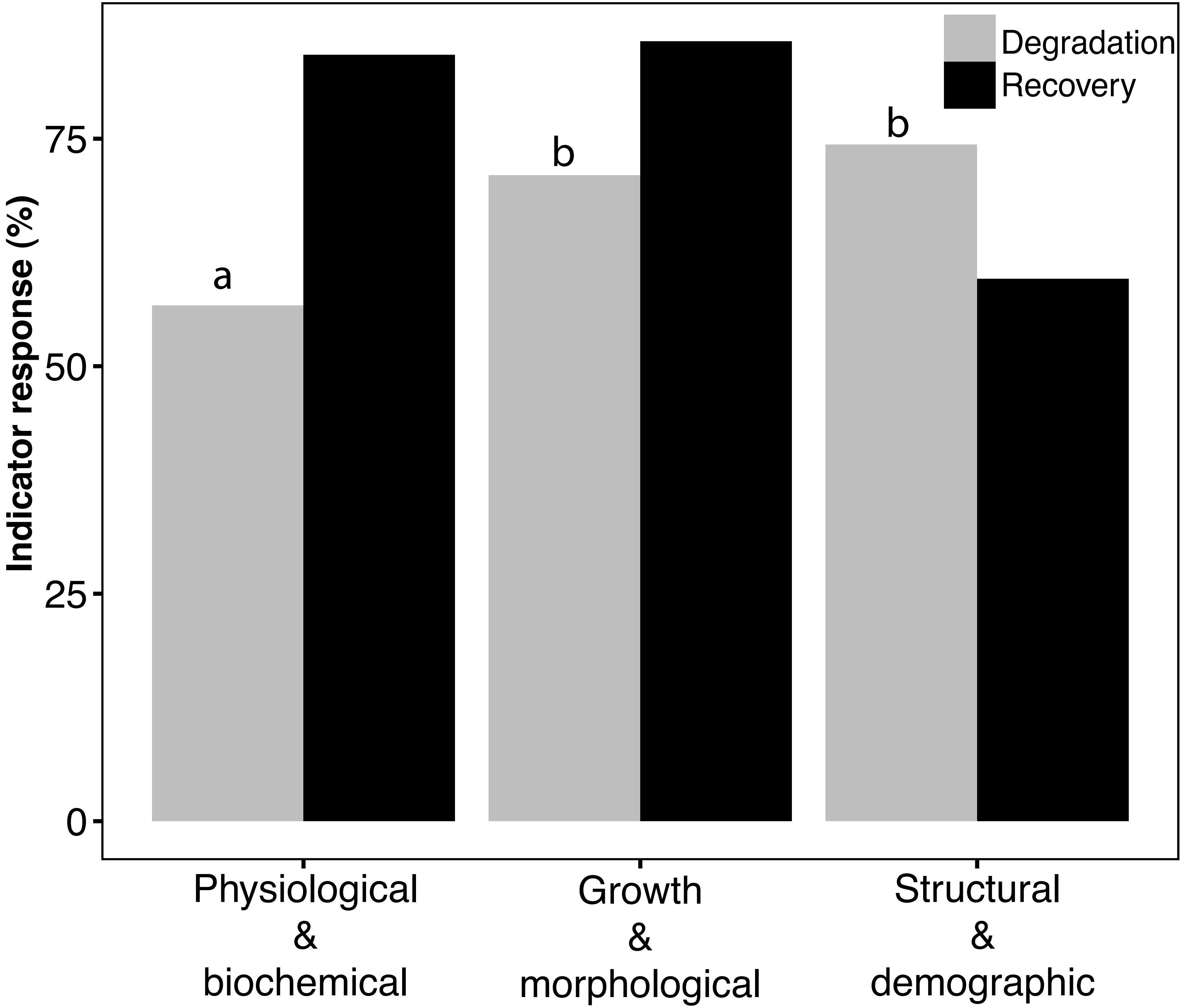
Significance level : ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Table 6. Results of analyses of variance (Type III tests) on indicator response time (top) and recovery time (bottom) in relation to seagrass size (as reflected by rhizome diameter), level of biological organization (structural and demographic, growth and morphological, physiological and biochemical) and type of environmental stressor. Seagrass size: level of biological organization = Interaction between rhizome diameter and level of biological organization. Response time was fitted to a Poisson distribution and recovery time to a quasi-Poisson distribution with an overdispersion parameter taken to be 29.3). DF (degrees of freedom), LR Chi (likelihood ratio Chi squared test). For further details, refer to Methods.

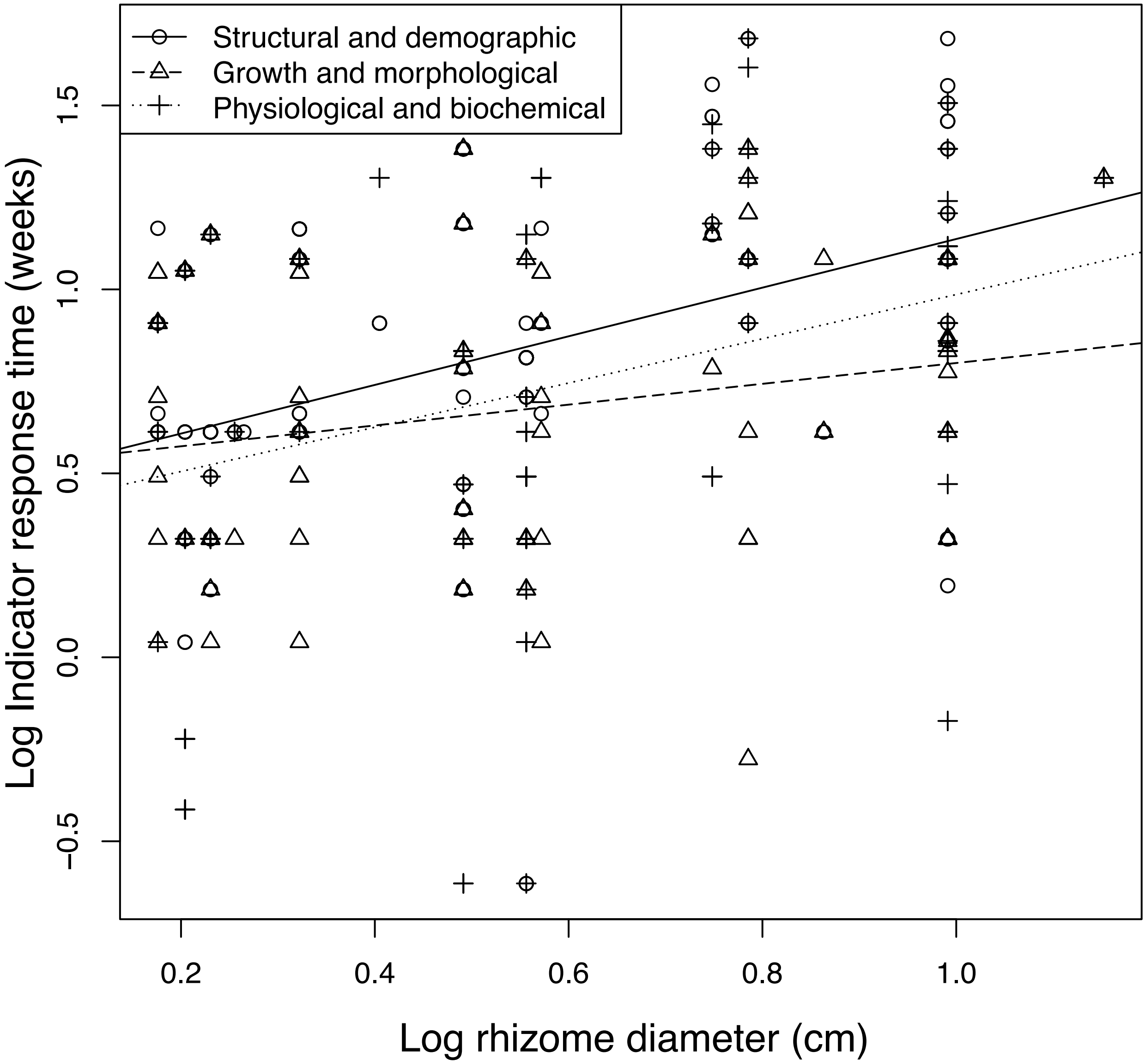
Response time	DF	DenDF	F.value	P.value	
Level of biological organization	2	346.7	0.09	0.91	
Stressor	4	80.7	2.36	0.06	.
Seagrass size	1	56.2	18.91	1.00E-04	***
Seagrass size : Level of biological organization	2	346.9	4.57	0.01	*

Recovery time	LR Chisq	Df	P.value	
Level of biological organization	16.8057	2	0.0002242	***
Seagrass size	2.2123	1	0.1369122	
Seagrass size: Level of biological organization	1.8116	2	0.4042195	

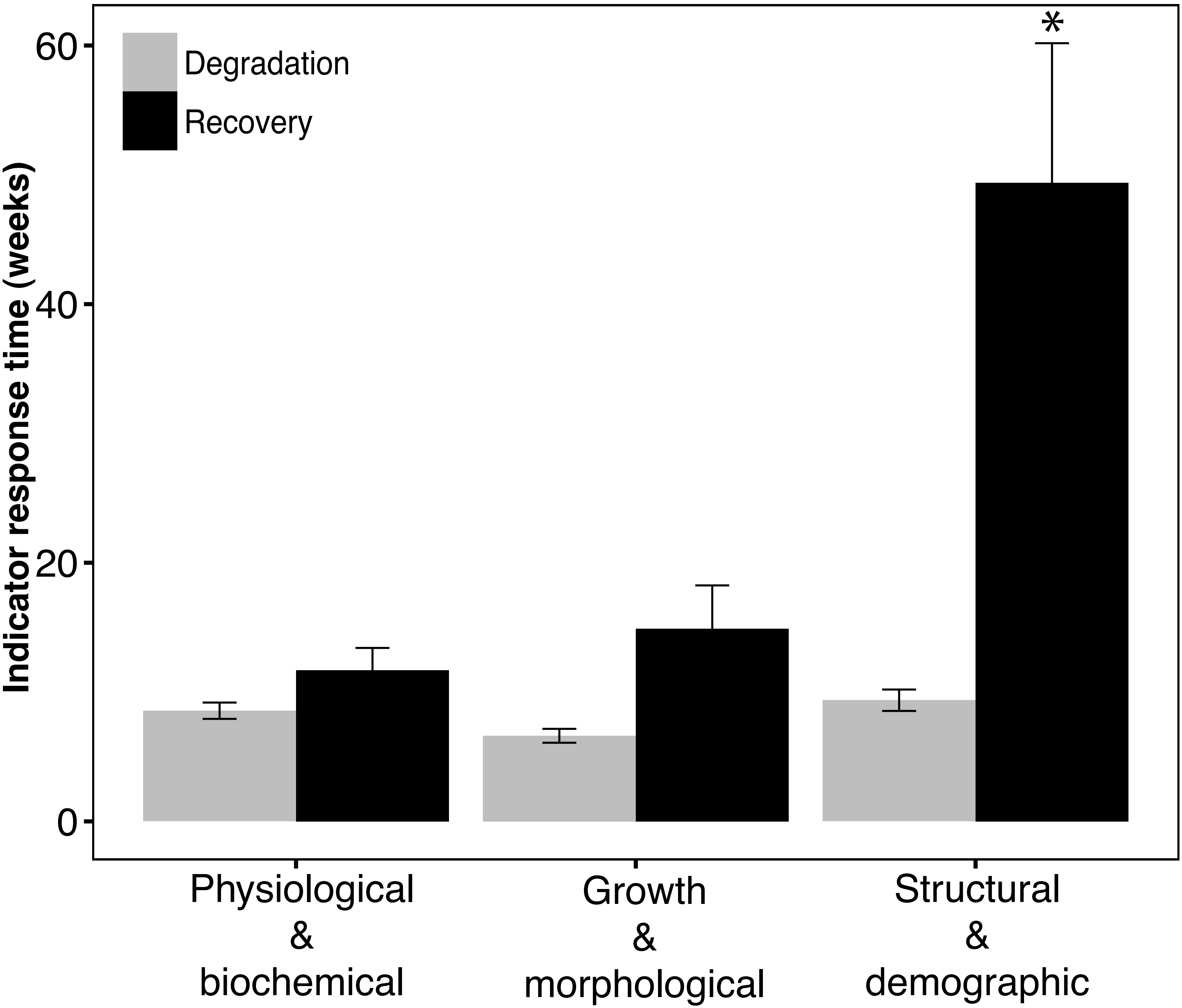
Significance level: ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1



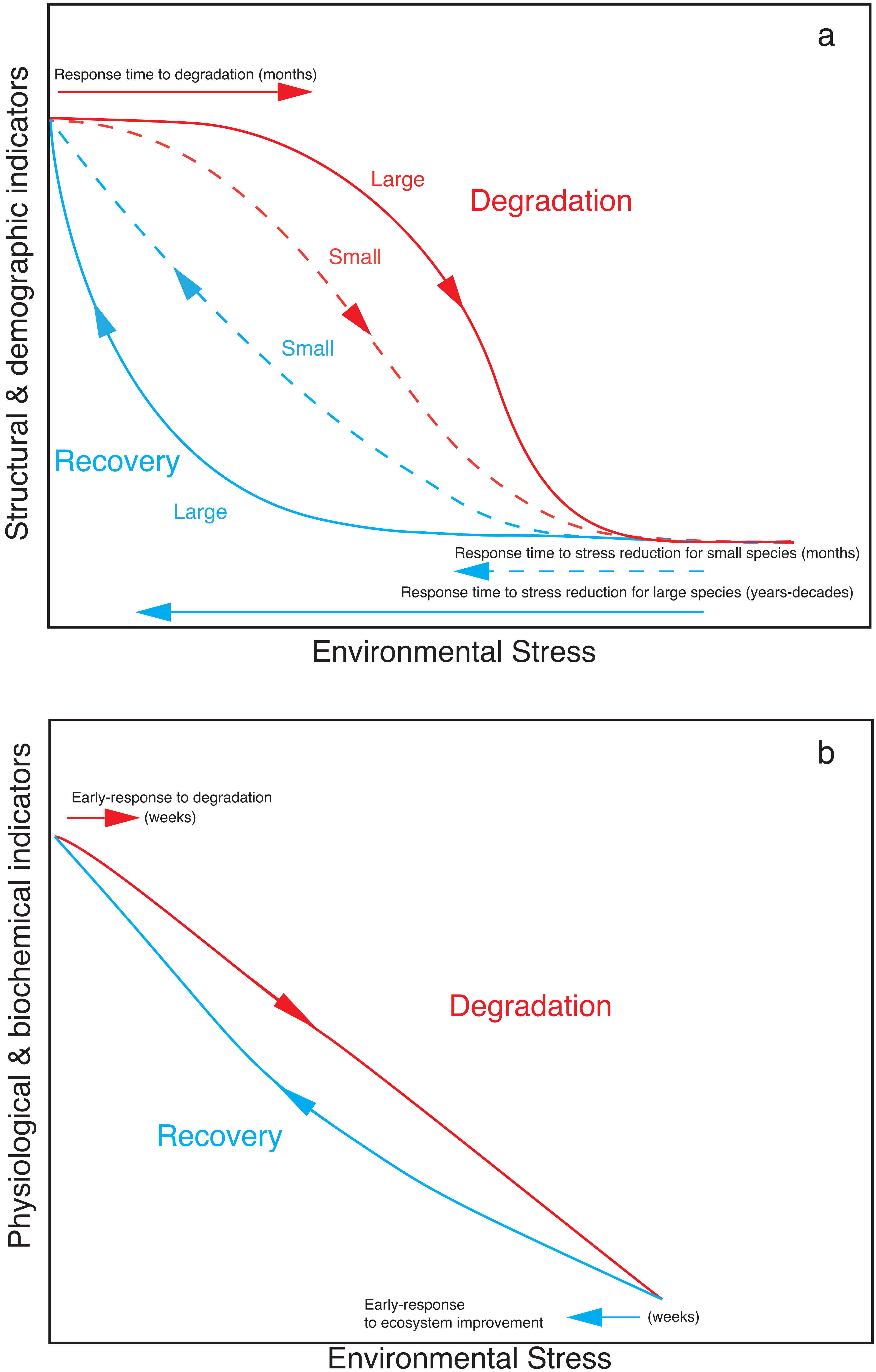
Degradation
Recovery
Indicator response (%)
75
50
25
0
a
b
b
Physiological & biochemical
Growth & morphological
Structural & demographic

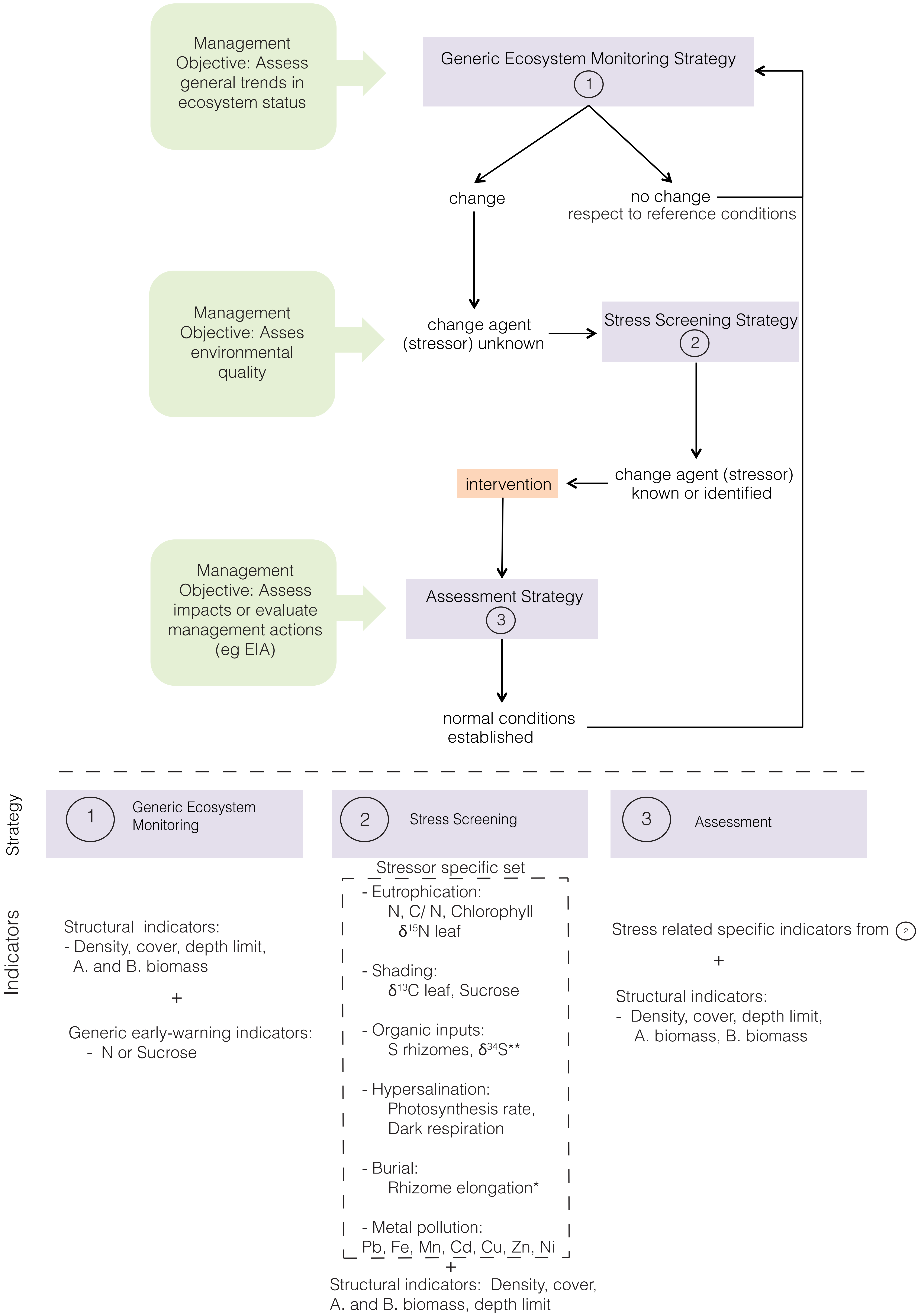


Structural and demographic
Growth and morphological
Physiological and biochemical
Log Indicator response time (weeks)
−0.5
0.0
0.5
1.0
1.5
0.2
0.4
0.6
0.8
1.0
Log rhizome diameter (cm)


Degradation
Recovery
*
60
40
20
0
Indicator response time (weeks)
Physiological
&
biochemical
Growth
&
morphological
Structural
&
demographic

Indicator response time to stressors
Shading
Nutrients
Burial
Organic Matter
Hypersalinity
Small
Large
Leaf N
Rhiz N
Rhiz suc
Leaf δ13C
Leaf growth
Density
A. biomass
C/N
Chlorophyla
Leaf δ15 N
B. biomass
Photosyn rate
Dark resp
Necrosis
Mortality
Rhiz Pb
Rhiz Fe
Rhiz Mn
Rhiz S
Roots S
0 10 20 30 40
0 10 20 30 40 50
Response time (weeks)
Positive relationship
Negative relationship
Positive and negative relationships


a
Response time to degradation (months)
Large
Degradation
Small
Small
Recovery
Large
Response time to stress reduction for small species (months)
Response time to stress reduction for large species (years-decades)
Structural & demographic indicators
Environmental Stress
b
Early-response to degradation
(weeks)
Degradation
Recovery
Early-response
to ecosystem improvement
(weeks)
Physiological & biochemical indicators
Environmental Stress



Management Objective: Assess general trends in ecosystem status
Generic Ecosystem Monitoring Strategy 1
change
no change respect to reference conditions
Management Objective: Asses environmental quality
change agent (stressor) unknown
Stress Screening Strategy 2
change agent (stressor) known or identified
intervention
Management Objective: Assess impacts or evaluate management actions (eg EIA)
Assessment Strategy 3
normal conditions established
Strategy
1 Generic Ecosystem Monitoring
2 Stress Screening
3 Assessment
Indicators
Structural indicators:
- Density, cover, depth limit, A. and B. biomass
+
Generic early-warning indicators:
- N or Sucrose
Stressor specific set
- Eutrophication: N, C/ N, Chlorophyll $\delta^{15}N$ leaf
- Shading: $\delta^{13}C$ leaf, Sucrose
- Organic inputs: S rhizomes, $\delta^{34}S$**
- Hypersalination: Photosynthesis rate, Dark respiration
- Burial: Rhizome elongation*
- Metal pollution: Pb, Fe, Mn, Cd, Cu, Zn, Ni
+
Structural indicators: Density, cover, A. and B. biomass, depth limit
Stress related specific indicators from 2
+
Structural indicators:
- Density, cover, depth limit, A. biomass, B. biomass