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How are seagrass processes, genetics and chemical composition monitored?

Growth rates, recruitment and mortality rates, chemical and isotopic composition, genetic diversity all provide different indications about the status, susceptibility and change within a seagrass community. These indicators all require relatively much man power and some of them, in addition, require access to advanced and expensive equipment. Hence, they should only be applied where important specific questions have been clearly defined.

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There are many natural and anthropogenic causes that can contribute to the deterioration of seagrass meadows but it is the overall effect of any impact that is the most critical parameter to determine. However, the consequence of any impact on the seagrass ecosystem is more difficult to assess because it depends, in part, on the ability of the meadow to withstand any or further change. This aspect has to be addressed because seagrass restoration is both costly and problematic and so we must have accurate knowledge about the status of seagrass meadows and indicators of the nature and rate of any seagrass decline. Thus process indicators, which point to potential or actual differences over the course of time, are an important addition to any monitoring strategy.

In this chapter we provide an illustration of some of the process indicators that are available, to indicate the growth and demographic history of the meadow, to detect incipient or on-going change and to evaluate the ability of a population to adapt to a changing environment. Ecological, chemical and genetic indicators are used. Each technique varies in terms of type of information it provides, the facilities required, their accessibility and cost. All of these factors must be taken into account and must have consequences for their use.

Process indicators

Recruitment and mortality of shoots

The persistence of seagrass meadows depends on the flux of shoots in the population, which results from the rates of shoot recruitment and mortality, both processes highly sensitive to anthropogenic disturbance. The difference between shoot recruitment and shoot mortality rates is a direct estimate of net population growth rate, hence revealing if the meadow is expanding, declining or in steady-state, as well as providing the rate of change.

Response pattern: Seagrass shoot recruitment and mortality rates vary about 2 orders of magnitude across seagrass flora, due, to a large extent, to the large differences in clonal growth rates across species. Moreover, seagrass shoot recruitment and mortality rates vary seasonally, achieving, in *Zostera marina* beds, maximum rates during summer. Shoot mortality rates tend to increase with increasing water depth in *Cymodocea nodosa*, suggesting that it responds to light availability. Seagrass shoot mortality rate increases in response to burial. However, seagrasses are expected to respond to sediment accretion by increasing shoot recruitment, since shoots surviving burial, increase both vertical and horizontal branching rate. Seagrass shoot recruitment and mortality rates are also sensitive to sediment deterioration. For instance, shoot recruitment of *Posidonia oceanica* decreases and shoot mortality increases in carbonate sediments

with reducing conditions derived from sulphide re-oxidation.

The magnitude of the response and the sensitivity of shoot recruitment and mortality rates to environmental change also depend on the species involved (e.g. response to burial).

Because of the higher recruitment and mortality rates experienced by small, relative to large European seagrasses, meadows of small species exhibit larger changes in population abundance, and hence are more vulnerable, than those of large ones for similar relative population growth changes. Hence, seagrass population decline is easier to be detected in meadows of large, slow-growing species than in those of small, fast-growing ones. Indeed, *P. oceanica* shoot population may be in decline for decades before the meadow is lost, whereas shoot demography would be hardly able to detect declining *Zostera spp.* meadows because the changes in net population growth experienced would have already led to large-scale seagrass loss.

Method description: Seagrass shoot recruitment, mortality and net population growth rates can be assessed using retrospective techniques and repeated shoot census. The selection of the methodological approach depends on the species involved in the study, and the time scale at which shoot population dynamics is examined.

Demographic parameters can be estimated retrospectively from the age distribution of shoots. This technique, described in detail in Duarte et al. (1994), consists of harvesting and ageing at least 200 intact living shoots (i.e. clusters of standing leaves plus the attached vertical rhizome since its insertion in the horizontal rhizome) per population. For *P. oceanica* and *C. nodosa*, shoot age (in yr) is estimated by counting the number of leaf scars on the vertical rhizome plus the number of standing leaves, and then dividing the number of leaves produced by the shoot during its life by the average number of leaves produced annually by a shoot. The number of leaves annually produced by a shoot is estimated retrospectively from the seasonal changes in vertical internodal length imprinted along vertical rhizomes. Seagrasses with vertical rhizomes produce the longest vertical internodes in spring-summer, whereas the length of the internodes is the shortest in those internodes produced during winter. Because seagrasses produce one internode for each leaf produced, the number of vertical internodes (i.e. leaves) in between two consecutive maxima (or minima) in vertical internodal length equals the number of leaves produced per shoot per year. The annual shoot recruitment rate is calculated as

the natural logarithm of the total number of aged shoots in the sample minus the natural logarithm of the number of shoots older than 1 yr. Shoot mortality rate is derived from the exponential decline in the abundance of living shoots with time, assuming constant mortality over shoot age classes and years.

Shoot population dynamics can also be assessed through direct shoot census in permanent plots with marked shoots. The size of the plots depends on the number of shoots expected to recruit and die in between consecutive visits. At the beginning of the study, all shoots present inside the plots are counted and tagged with a plastic cable tie placed around their vertical (for *P. oceanica* and *C. nodosa*) or horizontal (for *Zostera* species) rhizomes. During the following visits to the plots, the number of surviving shoots (i.e. tagged shoots) and newly recruited shoots (i.e. young, untagged shoots) are counted. The repeated shoot census allows direct estimates of shoot density, absolute (shoots $\text{m}^{-2} \text{yr}^{-1}$) and specific (yr^{-1}) rates of shoot mortality and recruitment and net population growth rates.

The specific net population growth rate (yr^{-1}) using both approaches is estimated as specific recruitment rate minus specific mortality rate. Similarly, absolute recruitment rate minus absolute mortality rate provides an estimate of absolute net population growth.

Method evaluation: Retrospective approach has been used to assess shoot demography of *Posidonia oceanica*, *Cymodocea nodosa*, *Zostera marina*, and, to some extent, *Z. noltii*. Retrospective analyses, however, provide average demographic estimates during the life span of shoot population, which, across seagrass flora, ranges from several weeks (*Z. noltii*) to decades (*P. oceanica*). Hence, assessment of seagrass shoot demography at annual scales requires to sample shoot populations several times per year in species with an average shoot life-span shorter than 1 yr. Conversely, retrospective techniques allow evaluation of the average shoot population dynamics occurred for the last 1-2 decades in meadows of long-living species such as *P. oceanica* from a single visit. Yet, retrospective examination of shoot demography of long-living species provides little information about the present shoot dynamics in the seagrass bed.

Direct shoot census is particularly useful to examine the recent shoot population dynamics in seagrass beds of long-lived species, such as *Posidonia oceanica*. For this species, an inventory of permanent plots may be made at annual or bi-

annual time intervals. Direct shoot census approach is difficult to use in *Zostera* sp. meadows, as a consequence of high shoot density, fast shoot turnover rate, and rhizome fragility.

Indicative potential: Shoot recruitment and mortality rates, assessed retrospectively in species with shoots with intermediate life-span and by direct shoot census in long-living species, allow evaluation of the present status of seagrass meadows, and detection of on-going population decline. The use of direct shoot census is particularly useful to assess recent population dynamics of slow-growing seagrass species, such as *P. oceanica*, where other approaches, such as shoot density monitoring using random quadrats, are ineffective. Therefore, the incorporation of assessment of shoot mortality and recruitment rates to monitoring programmes will help them provide the early-warning signals necessary to support management decisions to conserve seagrass meadows.

Where and when it is useful: Retrospective examination of shoot demography is useful to assess seagrass population status across broad spatial scales and that of remote meadows. Direct shoot census requires much greater diving and man-power effort than retrospective assessments, restricting their use to smaller spatial scales, unless involving participation of larger number of personnel in the study. Direct shoot census is useful to detect on-going meadow decline of slow-growing seagrass species.

Use of the indicator for forecasting: Net population growth rate allows forecasting the expansion or declining rate of the meadows when assuming that shoot mortality and recruitment rates experienced by the meadows during the study, and, hence, environmental conditions leading to present population change, will be maintained in the future. If these assumptions do not hold the indicator cannot be used for forecasting.

Leaf elongation

European seagrasses are characterized by strap-like leaves that are produced continuously by a leaf meristem ("leaf-replacing"). Leaf production is a major component of seagrass productivity, and the growth rate of the leaves of "leaf-replacing" species can be easily estimated by measuring their rate of elongation. Leaf elongation is an integrative indicator of how favourable environmental conditions are for seagrass growth.

Response pattern: Water temperature, light, and nutrient availability are the main environmental

factors determining seagrass growth (Chapter 4). The first two variables have a strong seasonality in European coastal waters which drives a strong seasonal component of leaf elongation rates, with minimum values during winter time, and maximum values in summer. Additional variance in elongation rates among locations is set by differences in nutrient availability. Hypoxia and sulphide also reduce the elongation of seagrass leaves. Human-associated activities in the coastal zone can modify light and nutrient availability, and promote hypoxia in the water column and exposure of seagrasses to sulphide, environmental changes that will affect leaf elongation of seagrasses. The response of leaf elongation rates of seagrasses to environmental change will depend on the relative effects of the different components of change on seagrass growth.

Method description: Leaf elongation is estimated using leaf-marking techniques. First it is necessary to estimate the time interval between the generation of two consecutive leaves in shoot, the leaf plastochrone interval. At time 0 all leaves present in a seagrass shoot are marked by making two holes using a syringe needle or pin in the lower part of the outer leaf sheath. At time 't' the marked shoot is retrieved and the number of "new" leaves (those unmarked and longer than the distance between the base of the sheath and the height at which the pair of mark holes were made) is counted. A minimum of 15-20 shoots should be marked. The leaf plastochrone interval is calculated as the number of days since marking divided by total number of new leaves produced. The length (cm) of the youngest mature leaf of each marked shoot is measured from the leaf meristem at the base of the shoot to the leaf tip (only leaves with unbroken tips should be used). Leaf elongation ($\text{cm shoot}^{-1} \text{day}^{-1}$) is calculated as leaf length divided by the leaf plastochrone interval.

Accurate measurements require that the duration of the marking interval be longer than leaf plastochrone interval, which duration varies depending on the species (7 to 51 days for European seagrasses). If it is not possible to set a marking interval longer than the leaf plastochrone interval, leaf elongation can be also estimated by measuring the length of the new part of the leaves present at the time of marking and that of the new leaves. Marking holes are located in each leaf and the distance between them and the height corresponding to the holes marked in the sheath of the oldest leaf in the shoot (reference height) are measured. The length of the new leaves (i.e., the distance between the tip of each unmarked

leaf and the reference height) is also measured, added to the new length of the marked leaves, and divided by the marking interval (in days) to calculate leaf elongation ($\text{cm shoot}^{-1} \text{day}^{-1}$).

Method evaluation: Leaf marking will be difficult in locations where the sediment is silty, because resuspended sediment will decrease water transparency and when the width of the leaves is very small (some *Zostera noltii*). Except for scuba diving equipment (not essential for intertidal species if shoot marking and retrieval are performed during low tide), materials needed are rather inexpensive and easy to acquire.

Indicative potential: Several factors affect leaf growth which makes it difficult to explain differences of leaf elongation rates among locations unless the estimates are obtained simultaneously and information of environmental conditions (water temperature, light availability, and others) in each location is available/collected.

The potential of leaf elongation to evaluate growth conditions for seagrasses is higher when the locations compared are spatially close. The effect of point sources of disturbance (i.e., sewage emissaries, aquaculture facilities) can be effectively evaluated by estimating leaf elongation along the generated environmental gradients.

Nutrient and carbohydrate storage in seagrass rhizomes, and translocation between the shoots connected by the rhizome may uncouple leaf elongation rates from local environmental conditions for short (days) to medium (weeks) periods of time.

Where and when it is useful: This indicator can always be used.

Use of the indicator for forecasting: The forecasting power of this indicator is poor.

Vertical rhizome growth

Some seagrass species present vertical rhizomes in addition to horizontal ones. Vertical rhizomes keep the leaves above the sediment surface. *Posidonia oceanica* and *Cymodocea nodosa* are the only European seagrass species with vertical rhizomes. The meristem of vertical rhizomes is located at the junction between new leaves and vertical rhizome, and vertical rhizomes elongate simultaneously with production of new leaves. The maximum life span of European seagrass vertical rhizomes varies from 5-7 years in *C. nodosa* to 4 decades in *P. oceanica*.

Response pattern: The growth of seagrass vertical rhizomes is sensitive to sediment

accretion and environmental conditions such as sediment quality. Seagrass vertical rhizomes respond to sediment burial by enhancing their growth, whereas they grow at minimum rates when sediment is eroded. Growth of vertical rhizomes, particularly of *P. oceanica*, declines in response to deterioration of sediment quality (e.g. enrichment of organic matter, increase of sulphate reduction rate). In addition, variability in vertical rhizome growth exhibits an important seasonal component, the longest internodes being produced in late spring-summer and the shortest ones in winter.

Method description: Seagrass vertical growth can be estimated retrospectively from the sequence of internodal lengths along vertical rhizomes. Summarizing, the sequence of vertical internodal length is measured under a dissecting microscope along 5-10 old vertical rhizomes. The seasonality imprinted on the length of vertical internodes allows calculation of annual vertical rhizome elongation, as the sum of internodal lengths between consecutive maxima or minima, and identification of annual vertical rhizome elongation across the life span of the rhizome.

Method evaluation: The method is destructive. However, one sampling visit provides estimates of seagrass vertical rhizome elongation for particular years during shoot life span (i.e., 5-7 yr for *C. nodosa*, 40 yr for *P. oceanica*). Conversely, this method does not allow elucidation of seagrass vertical growth responses at shorter than 1 year time scales, because of the seasonality in vertical rhizome internode production.

Indicative potential: Examination of the pattern (e.g. cyclical, sustained trend, discontinuities) and magnitude of interannual variability in vertical rhizome elongation allows identification of the chronological time when seagrasses have been disturbed, and provides insights on disturbance forcing. For instance, cyclical fluctuations in vertical rhizome growth reveal the passage of natural disturbances across seagrass beds often associated with sediment coastal dynamics (e.g. subaqueous dune migration, climatic change). Conversely, sustained or abrupt declines in seagrass vertical rhizome growth indicate the presence of anthropogenic effects (e.g. sediment erosion, deterioration of sediment quality) disturbing plant growth.

Where and when it is useful: This method is useful to reconstruct coastal sediment dynamics occurring at time scales within 1 yr and 7 yr if the area is colonised by *C. nodosa*, and within 1 yr and 40 yr if the area is colonised by *P. oceanica*. In addition, temporal changes in vertical growth,

particularly in *P. oceanica*, reveal the existence of anthropogenic impacts on sediment quality, and, thus, they allow detection of coastal sediment deterioration.

This method has been useful to quantify the migration velocity of subaqueous dunes across a shallow *Cymodocea nodosa* bed, to examine the relative importance of climatic vs anthropogenic disturbances along the Spanish Mediterranean coast, and to assess the impact of fish farm activities on *P. oceanica* meadows.

Use of the indicator for forecasting: Rates of vertical rhizome elongation close to minimum values are indicative of growth cease (due to erosive conditions and/or sediment quality deterioration), and, hence, a possible increase of shoot mortality. Similarly, extremely high vertical growth rates are indicative of intense sand burial, and eventual shoot mortality. However, examination of the temporal trend of vertical rhizome growth variability will help (1) to elucidate the type, and natural/anthropogenic origin of the disturbance deteriorating the plants (and possibly the coastal zone), and (2) to evaluate the capacity of plant growth to recover based on past growth dynamics, both contributing to forecast the future of the meadow.

Chemical and isotopic composition indicators

Nutrient content of seagrass leaves

Leaf tissue contents of the macro-nutrients nitrogen (N) and phosphorous (P) are indicators of the nutrient status of seagrasses determined by nutrient availability at the locality, which in turn is, at least partly, a function of anthropogenic loading.

Response pattern: The contents of N and P in seagrass leaves are functions of the nutrient availability in sediments and water column at the growth site. Hence, leaf nutrient contents reflect the nutrient richness of the site, which in turn is determined by the background nutrient status (natural nutrient richness) and any anthropogenic nutrient loading. At low nutrient contents seagrass growth may be nutrient-limited, and at high nutrient contents the tissues accumulate nutrient stores reflecting that nutrient availability have exceeded plant nutrient requirements for growth possibly due to anthropogenic nutrient enrichment. Excess nutrient content in seagrass leaves most often has no negative impact by itself on seagrass performance.

Method description: Comparison of nutrient status is best done during the growth season (e.g. late summer when nutrient contents are at a minimum) and samples must be collected at the same time of the year and at the same depth at the different localities.

Nutrient contents are determined on dried leaf material. Standardised leaf samples (e.g. the four youngest leaves of each shoot) are collected at the selected localities. Epiphytes are scraped off the leaves with a scalpel. The leaves are dried for 24-48 hours at 60°C and homogenised by grinding. A minimum of 3 replicates is required.

Nitrogen can be measured automatically in a CHN elemental analyser or by standard manual methods for determining nitrogen contents in organic matter. Phosphorous can likewise be determined by standard manual methods. Modified techniques can be found by searching the seagrass literature.

Method evaluation: The method is a destructive and not awfully sensitive but repeatable and feasible technique to assess seagrass nutrient status of all four European species and approximate nutrient richness of sampling sites.

Apart from being dependent on environmental nutrient richness, however, tissue nutrient contents are also highly dependent on seagrass nutrient requirements for growth determined by species, season and depth. Therefore, comparisons of nutrient contents among species, season and depth must be interpreted with caution.

The analysis of nitrogen is relatively costly in terms of consumables if conducted on an elemental analyser. Manual analysis of both N and P is time consuming but in the order of 20 samples can be analysed by one person per day. To this comes handling time for sampling, cleaning and drying the leaf material.

Indicative potential: Due to the low sensitivity and high seasonal and spatial variability, the seagrass nutrient contents in leaves cannot be recommended as routine parameters within monitoring programmes.

Where and when it is useful: The indicators are useful for assessing whether low nutrient availability may limit seagrass colonisation and growth in a specific area. Nutrient limitation may occur at nitrogen contents below 1.8 % of dry weight and at phosphorus contents below 0.2 % of dry weight. However, severe nutrient limitation is rarely seen because seagrasses are relatively

slow-growing plants adapted to nutrient poor environments.

Use of the indicator for forecasting: The indicator can be applied to examine areas for differences in nutrient richness as a feasible scanning technique because seagrass nutrient contents are integrating parameters of nutrient richness. However the forecasting power of this indicator is poor.

Stable isotope composition of nitrogen and sulphur in seagrass tissues

Seagrasses are linked closely to the environmental quality of their habitat through nutrient exchange with the surrounding water. Seagrass plants can record perturbations in environmental quality in the chemical composition of their tissues with respect to nutrient elements. Nitrogen and sulphur are essential nutrients for growth, and are taken up by the plants in the form of their dissolved inorganic ions present in the aquatic environment. Nitrogen and sulphur occur naturally in different atomic forms, which are known as isotopes. The abundance of the stable isotopes of sulphur and nitrogen in seagrass tissues can be measured, providing the potential for exploratory information about the environmental status of seagrass meadows, specifically:

- in certain cases, detection of anthropogenic nutrient load in the habitat, and
- detection of sulphide penetration events into the plants through their root and rhizome tissues.

The above cases are linked because nitrogen that is derived from anthropogenic sources may have a characteristic stable isotope ratio that will be reflected in that of the aquatic plants, and may be linked to eutrophication of the water column in seagrass habitats. Prolonged eutrophication may cause insufficient oxygen supply to the benthic environment to counteract the toxic sulphide produced in the underlying sediments. This will eventually stress seagrass plants and cause irreversible decline and eventually loss.

Response pattern: The ratio of the stable isotopes of nitrogen in seagrass tissues can mirror that of the nitrogen source to seagrass habitats because of the low standing stock of dissolved inorganic nitrogen in coastal waters and its seasonal availability to aquatic plants.

The main source of sulphur to seagrasses is the pool of dissolved sulphate ions in seawater, with a relatively invariant stable sulphur isotope ratio of

about +20‰. The stable sulphur isotope ratio of seagrass tissues can be expected to be close to this value within $\pm 2\%$. Dissolved molecular oxygen is essential for the maintenance of well oxygenated roots. Insufficient root oxygenation due to low oxygen availability in seagrass habitats receiving large organic inputs can lead to development of diurnal events of root anoxia. Under such circumstances, the concentration of dissolved sulphide produced in the sediments builds up in the rhizosphere and can invade the plant through the roots. Sulphide is a potent phytotoxin, and its invasion into plant tissue is often associated with die-off of seagrasses. Sedimentary sulphide has a much lower stable sulphur isotopic composition than seawater sulphate (see above) which can extend to highly negative values, allowing detection of its presence in seagrass tissues. The stable sulphur isotope ratio of seagrass tissues, which have been affected by sulphide penetration, is lower than $+20 \pm 2\%$ and can be as low as -15% .

Method description: For the determination of the ratio of the stable nitrogen isotopes in seagrasses, intact shoots ($n = 5$) should be collected randomly from the meadow. Careful removal of epiphytes from leaves using a scalpel blade is critical to obtaining representative measurements. Analysis can be done in approximately 30 – 40 mg of finely ground material following standard isotope analytical procedures. No significant difference can be expected in the stable nitrogen isotope ratio of above- and below-ground seagrass tissues. However, for consistency, routine measurements for monitoring purposes should be done on the same part of the plant, preferably the leaves, as this method of sample collection is potentially the least destructive. Seasonal differences do occur, and sample collection may, therefore, take place twice a year, in winter and in summer, or should be consistently done within a season, e.g., in the summer when standing biomass is at its maximum. It is also suggested that, at any one time, five shoots be removed randomly from a site, and the youngest leaf analysed, resulting in one person week per sampling interval per site.

For the determination of the ratio of stable sulphur isotopes in seagrasses, intact shoots ($n = 5$) should be collected randomly from the meadow. Careful removal of epiphytes from leaves using a scalpel blade and thorough removal of sediment particles are critical to obtaining representative results. The ratio of stable sulphur isotopes in the roots and rhizomes can additionally be determined as these tissues are more sensitive indicators of sulphide ingress, however complete

removal of sediment particles is problematic. Analysis should be done of roots, rhizomes and leaves separately in approximately 5 to 10 mg of finely ground material following standard isotope analytical procedures.

Method evaluations: The reproducibility of measurements of the ratio of the stable nitrogen isotopes in seagrass tissue can be very high on small spatial scales up to meadow size. The variability of these measurements begins to increase on large temporal (annual) and spatial scales. Spatial variability depends on the frequency of sources and the rate of their dilution by local waters. In areas where spatial eutrophication gradients have been established (e.g. estuaries, fjords), widely different values can be found along the gradient due to different nitrogen sources and or gradual mixing with other water sources of very different signature. In these situations, the stable isotope ratio of nitrogen in seagrass tissues can still be distinguishable at different points along the gradient.

The timeframe from sample collection to analysis for the stable sulphur isotope measurements is similar to that for the stable nitrogen isotope measurements, but the analyst should beware of sulphide mineral contamination of seagrass tissues and of the need for a higher sampling frequency. This is because the within site, between sites and temporal (annual) variabilities of the stable sulphur isotope measurements can be high and comparable (up to 6‰), hence seasonal sampling will only give a measure of sulphide stress (if any) at the time of sampling.

Where and when they are useful: Measurement of the ratio of stable nitrogen isotopes in seagrass tissues has the potential to help elucidate the origin of nitrogen that enters the estuarine and coastal zone. In multi-source cases, the prerequisite is that nitrogen sources are isotopically distinct from each other. For example, open ocean, land, airborne and anthropogenic nitrogen contribute significantly to the nitrogen load in coastal waters. These sources of nitrogen can have a distinct stable nitrogen isotope ratio, such as +5 to +6‰ for deep ocean nitrate, +2 to +8‰ in groundwater influenced only by atmospheric deposition, -3 to +3‰ in fertilisers, and +10 to +20‰ in human and animal waste (sewage). Despite the complex natural cycle of nitrogen isotopes, in areas where cultural eutrophication conditions have been long standing, the ratio of stable nitrogen isotopes in seagrass plants can extend over a much wider range of values than in pristine coastal areas. However, it is pointed out that interpretation in

such cases is qualitative and not quantitative, i.e., the values of the ratio of nitrogen isotopes are not related to the actual nitrogen load of the habitat.

The stable isotope ratio of sulphur in seagrass tissues can provide a proxy for sulphide invasion into seagrass plants and, hence, a detection tool of conditions potentially detrimental to the seagrass habitat.

Use of the indicators for forecasting: The stable isotope ratio of nitrogen in seagrass plants can serve only as an evaluator of nitrogen sources to the habitat and not as a forecasting tool of its status and fate. The stable isotope ratio of sulphur in seagrass tissues offers information primarily about the source of this nutrient to the seagrass plants. Shifts in the stable isotope ratio of sulphur in seagrass tissues are linked directly to processes that can be detrimental to the health status of seagrass meadows (i.e., toxic sulphide invasion into the plants). Sulphur isotopic measurements can therefore contribute to forecasting the health status and fate of seagrass habitat.

Diversity-indicators

Genetic diversity

Genetic diversity is a fundamental component of biodiversity, forming the basis of species and ecosystem diversity. Since species are composed of populations that exist somewhat independently of each other, genetic diversity exists both within and among populations of each species. Seagrass habitat degradation may cause loss of genetic diversity, consequently lowering the potential for populations to survive and to adapt to changing environmental conditions.

Response pattern: Measurement of genetic diversity with molecular markers that are not under selection can be used to understand not only the genetic composition of each population but also at what scales there is dispersal of seeds and pollen, and what is a management unit (population genetically differentiated from others). Characterisation of the geographic differentiation of populations is critical for understanding whether meadow recovery can rely on the neighbouring populations as sources for recovery, or to choose source populations for restoration. A recent reduction in genetic diversity or in gene flow between populations can be indicative of deteriorating environmental conditions.

Method descriptions: Sample selection: A pilot study is necessary to be able to estimate the

number of samples needed for a representative sample of the population but it is often suggested that 50 samples per population are sufficient to give a reliable estimate of the genotypic diversity.

From the samples DNA is extracted. From every sample genetic markers are used to determine the genotype of the sample at each locus (i.e., determining the multilocus genotype). The genetic markers yielding the best resolution to identify distinct clones on the basis of their multilocus genotypes are usually microsatellite loci, because they are hypervariable markers (i.e. show more distinct alleles in the population than other markers). Having this data, various population genetic parameters are estimated (genetic diversity, population differentiation and gene flow at various scales), for which a variety of software packages are available.

Since seagrasses are clonal organisms, low resolution of genetic markers can lead to the erroneous conclusion that the population is highly dependent on clonal propagation rather than sexual reproduction, just because the markers cannot distinguish genotypes. So it is essential to choose markers that are capable of distinguishing different genotypes as opposed to clonal repeats.

Evaluation of the method: Microsatellite markers are very sensitive for determination of genotypic diversity and its spatial and temporal variability. However this requires a large number of polymorphic microsatellite loci to be used, and therefore high costs. The feasibility of the method is limited by the requirement of rather specialised expertise and equipment. It is the most repeatable PCR-based technique for nuclear DNA genetic studies. The method is destructive in the sense that cells must be obtained (and therefore destroyed) from the organism, but it is non-destructive for the whole organism, because only a small portion of material is sufficient for the analysis of the genotype.

- Development of microsatellites is necessary for each species, and it is expensive. However microsatellites have already been developed for all four European seagrass species and primers are available in the literature. The method can be used for all habitats (as long as sampling of seagrass material is possible).

Indicative potential: This indicator provides an assessment of the current status of genetic diversity of any seagrass species from any habitat, but this current status is the consequence of long term past events and dynamics. Its interpretation is difficult because it is influenced by

so many factors. It is not a general early warning indicator, but under certain conditions changes can take place in this indicator before other warning signs can be detected, such as restriction to gene flow due to habitat fragmentation. Due to the high cost in equipment, consumables and man-power, and the high level of specialised training that this indicator requires, it is not recommended as a general use indicator, but only when more thorough population studies are needed/possible.

Where and when it is useful: This indicator is useful in any meadow at any time, depending on what population parameters are required to be understood. For example, within-population genetic diversity (e.g., if it has been reduced or increased following some perturbation), or scales of gene flow (e.g., if barriers to dispersal have been created).

Use of the indicator for forecasting: The forecasting power of the indicator is poor in terms of short term predictions, because the genetic composition reflects events that took place over a long time scale, and the factors shaping it may have changed considerably for the time period for which one is attempting to make predictions. It is more an indicator of the past history. However, understanding past history can certainly be helpful in predicting what will happen if the same factors/events that took place in the past will continue to occur.

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