Cyfoeth Naturiol Cymru
Natural Resources Wales

Guidance note

Benthic habitat assessment guidance for marine developments and activities

A guide to characterising and monitoring seagrass beds

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1. Introduction and summary

This guidance document is one of a series of Benthic Habitat Assessment Chapters developed by Natural Resources Wales (NRW) for key habitats of conservation importance around Wales. It has been prepared by NRW with the initial document prepared under contract by Ocean Ecology Limited.

The guidance aims to assist developers in designing and undertaking robust benthic habitat characterisation surveys and monitoring of these habitats in the context of Ecological Impact Assessment, thereby helping streamline the regulatory review and consultation process.

This chapter will be relevant if you already have seashore/seabed habitat data and know that intertidal or subtidal seagrass beds are present, and you need to carry out habitat characterisation and/or monitoring of these.

If you are unsure about the habitats present, you should:

- for intertidal areas, consult existing information (see section 4.1) and/or you may need to carry out a Phase 1 intertidal survey (see section 5.1 in chapters GN030a and GN030b) to determine the habitats present before undertaking more focussed characterisation surveys
- for subtidal areas, refer to chapter GN030g[1] for guidance on characterisation of subtidal habitats

This habitat chapter (GN030f) is not intended to be used alone and should always be used in conjunction with the NRW Guidance Note GN030 and the Introductory chapter (GN030-intro).

1.1. What are seagrass beds and where are they found in Wales?

Seagrass beds are biogenic habitats formed by flowering plants adapted to saline conditions. These plants produce shoots that grow above the substrate, forming expansive 'meadows'. The two recognised species found in Wales are generally, but not always, restricted to either the intertidal (*Zostera noltei*) or subtidal (*Zostera marina*). Widgeon grass (*Ruppia* sp.) is also found around Wales and, whilst not strictly considered as part of the traditional seagrass assemblage it is commonly grouped with *Zostera* spp. as it can occupy a similar niche.

The majority of known Welsh seagrass beds are found in more sheltered bays, inlets and estuaries around the coast (see section 2.3 for more details).

1.2. The conservation importance of seagrass beds

Seagrass beds create a 3-dimensional structure in what would otherwise be a far less complex seabed habitat and they provide a range of ecosystem services. They help to stabilise the sediment and influence local environmental conditions such that a wider variety of species are generally able to exist than would be present if the seagrass bed was not there. The seagrass itself provides a habitat for other species including protection

[1] This document is currently in preparation

for invertebrates (such as burrowing anemones, molluscs and urchins) living in the stabilised sediment at the base of the plant.

Seagrass beds are highly productive systems, rapidly turning over large amounts of organic carbon as leaf material which is often exported to other ecosystems. Particulate matter in the water becomes trapped amongst the seagrass plants and sequestered into the sediments within the bed. Seagrass beds provide a nursery habitat for fish species with studies from the UK providing evidence that his includes a number of commercially important species.

Seagrass beds provide a habitat for species of conservation importance in the UK and Wales such as seahorses and stalked jellyfish. The seagrass roots and shoots can also provide an important food source for some species of wildfowl.

More information is provided in section 2.4.

1.3. What kind of developments and activities might affect seagrass beds?

Developments or activities that could potentially affect seagrass beds during construction and/or operation phases include those involving actions that could result in:

- changes to temperature and salinity
- changes to emergence regime, water flow and wave exposure
- nutrient and organic enrichment
- introduction or release of chemical pollutants
- changes to, removal and disturbance of substrate surface and subsurface
- changes to sediment transport dynamics, erosion/accretion regime, sedimentology and geomorphology
- changes in suspended solids (water clarity)
- siltation rates changes (smothering)
- introduction or reduction of light
- introduction or spread of invasive non-native species (INNS)
- introduction of microbial pathogens
- extraction of other species

Further detail relating to potential pressures from developments and activities on seagrass beds is provided in section 2.5.

1.4. Existing data and guidance for surveying and monitoring seagrass beds

A brief summary of available information is provided in section 3. Key sources of existing data and guidance for surveying and seagrass beds are:

- Joint Nature Conservation Committee (JNCC): recent JNCC guidance for the monitoring of marine benthic habitats (Noble-James *et al.*, 2017)
- Common Standards Monitoring: developed for site monitoring and assessment of protected sites. Habitat guidance relevant to seagrass beds: Inshore Sublittoral Sediment Habitats (JNCC, 2004a), Littoral Sediment Habitats (JNCC, 2004b), Estuaries (JNCC, 2004c), and Inlets & Bays (JNCC, 2004d)
- Marine Monitoring Handbook (Davies *et al.*, 2001)
- Phase I intertidal habitat mapping handbook (Wyn *et al.*, 2006)
- feature condition monitoring reports from work in Wales and the rest of the UK (references provided in sections of the document)

- Water Framework Directive (WFD) Monitoring approaches for Transitional and Coastal (TraC) waterbody assessment to assess the ecological health of the biological quality element 'angiosperms – intertidal seagrass' (UKTAG, 2014). This uses a multimetric tool composed of:
    - seagrass shoot density
    - taxonomic composition (of seagrass taxa)
    - total bed extent
- Mapping European Seabed Habitats (MESH) and MESH Atlantic recommended operating guidelines, including those for:
    - swath bathymetry (Hopkins, 2007)
    - side scan sonar (Henriques *et al.*, 2012)
    - single beam echo sounder (Populus & Perrot, 2007)
- North-East Atlantic Marine Biological Analytical Quality Control (NMBAQC):
    - Remote monitoring of epibiota using digital imagery (Hitchin *et al.*, 2015)
    - Analysis of remote underwater video footage and still images (Turner *et al.*, 2016)
- Seagrass-specific survey and monitoring guidance produced by individuals and as part of collaborative projects and initiatives such as: Seagrass Watch, SeagrassNet, Borum *et al.* (2004), Jackson *et al.* (20130 and Jones & Unsworth (2016)
- NRW Guidance GN006: Marine Ecology Datasets for marine developments and activities (Natural Resources Wales, 2019). Identifies data sources for subtidal habitat maps and provides information on the marine ecology data sets we hold and routinely use and how you can access them.

1.5. Survey and monitoring design

The requirements for habitat characterisation survey and monitoring design are covered in section 4. The following provides a brief summary of key points:

- the aim of the habitat characterisation survey is to collate data to describe the seagrass beds within the survey area, identify any other habitats and/or species of conservation importance and provide an up-to-date ecological appraisal to inform Ecological Impact Assessment (EcIA)
- the aims of any monitoring required for a proposed development or activity will depend on the potential impacts as identified through the EcIA and any conditions set by the regulator
- a comprehensive desk-based review of all available existing data should be conducted prior to designing any habitat characterisation or monitoring programmes. This will help determine the scope of survey that may be required
- if there is little or no existing seashore or seabed habitat data or it is out of date or of poor quality, you may: for intertidal areas, need to undertake a Phase 1 intertidal survey or, for subtidal areas, undertake a general benthic habitat survey to determine the seashore / seabed habitats present and their distribution and extent in order to target habitat characterisation and monitoring surveys
- Common Standards Monitoring, Water Framework Directive and OSPAR guidance suggest that surveys should be undertaken during the period of peak growth of seagrass between June and September. However, due to the large seasonal variations in bed extent that can occur, it is suggested that monitoring of Welsh seagrass beds should be conducted during periods of peak biomass (August – September). Within a monitoring programme, all beds should be surveyed during

the same month of the year, and repeat surveys should be in the same month as the baseline survey

- relevant ecological parameters for survey and monitoring need to be selected. The parameters for a particular development or activity should be selected based on assessment of the predicted impacts. The main ecological parameters/indicators are:
    - ‘bed-scale’ indicators (indicators of long-term, change), such as:
        - bed extent and fragmentation
        - percentage seagrass cover
        - shoot density
        - epiphytic cover
    - ‘plant-scale’ morphological indicators (indicators of short-term change):
        - Leaf length and width
        - Shoot biomass (above ground)
    - ‘plant-scale’ physiological indicators (indicators of short-term change): ratios of shoot carbon, nitrogen and phosphorous
    - environmental parameters: light and temperature in particular
    - other indicators, such as: seed bank and viability, sediment composition and dynamics, associated species composition and diversity
- the aims of the habitat characterisation survey and monitoring need to be clearly stated and the survey programmes tailored to deliver these requirements. This includes defining hypotheses and trigger levels for monitoring.
- generally, habitat characterisation surveys will involve a single sampling event to characterise all the beds in the area of interest. If suitable existing data are not available, mapping the extent of the beds is an essential first step that provides a framework to inform any more detail sampling required for characterisation. Triangular grid patterns are advised for sample stations to reduce the chance of bias.
- monitoring programme design will be influenced by the specific hypotheses to be tested and the indicators to be measured, and these need to be determined on a project-by-project basis. An ‘investigative’ monitoring approach is often the most appropriate for seagrass beds. However, ‘sentinel’ and/or ‘operational’ approaches may need to be considered when the requirement for complex sampling designs makes ‘investigative’ monitoring unfeasible.
- the ‘beyond-BACI’ sample design is considered as best practice for designing seagrass bed monitoring programmes. This requires a minimum of two control beds which must be selected carefully. If control beds are not available, a Before-After-Control-Impact Paired Series design should be considered.
- sampling stations for monitoring should be located on a systematic grid across impact and control beds. If environmental indicators such as light and temperature need to be monitored, they will not need to be measured at every sampling station.
- Seagrass beds are sensitive to certain physical impacts and care should be taken to ensure that the methods used for characterisation and monitoring have as little impact as possible.
- other parameters of the wider environment that influence seagrass beds may need to be characterised and monitored; this will depend on the nature and location of a proposed development or activity and the associated pressures arising from this. This could include parameters such as: patterns of sediment transport and the hydrodynamic regime and water quality.

1.6. Survey and monitoring methods and analysis

A range of survey methods can be appropriate for survey and monitoring of seagrass bed parameters/indicators (section 5). The main options include:

- Phase I walkover survey and habitat mapping (intertidal beds)
- Aerial surveys / Unmanned Aerial Vehicle (UAV) (intertidal and subtidal beds)
- manta-tow / systematic sampling / diver swims (subtidal beds)
- acoustic survey (such as side scan sonar, sediment imaging sonar, single beam and multibeam echosounders) for habitat mapping (subtidal beds)
- quantitative sampling (for example, quadrats) (intertidal and subtidal beds)
- underwater image survey (such as drop-down video and still images) (subtidal beds
- dive survey for quantitative and semi-quantitative sampling (subtidal beds).
- other sampling for fine scale or environmental parameters/indicators (intertidal and subtidal beds)

Quality control measures for the field methods need to be clearly defined and implemented by field staff undertaking the survey work.

Not all methods will be required for a particular development or activity and proposed methods need to be defined on a project-specific basis. The JNCC Marine Monitoring Method Finder, a web-based information hub, has been developed to provide a single point of access to the numerous guidance documents and tools generated both within and outside the UK. It can be used in conjunction with this document to ensure a consistent approach to data collection and analysis.

2. Habitat Introduction

2.1. Overview

Seagrass beds are biogenic habitats formed by flowering plants (angiosperms) adapted to saline conditions. These plants have stems (rhizomes) that spread horizontally below the sediment surface, and shoots that grow above the surface forming expansive 'meadows' in both the intertidal and shallow subtidal zones. In contrast to other marine vegetation (such as macroalgae), seagrasses flower, develop fruit and produce seeds like terrestrial plants. They also have roots and a vascular system that transports gases and nutrients around the plant.

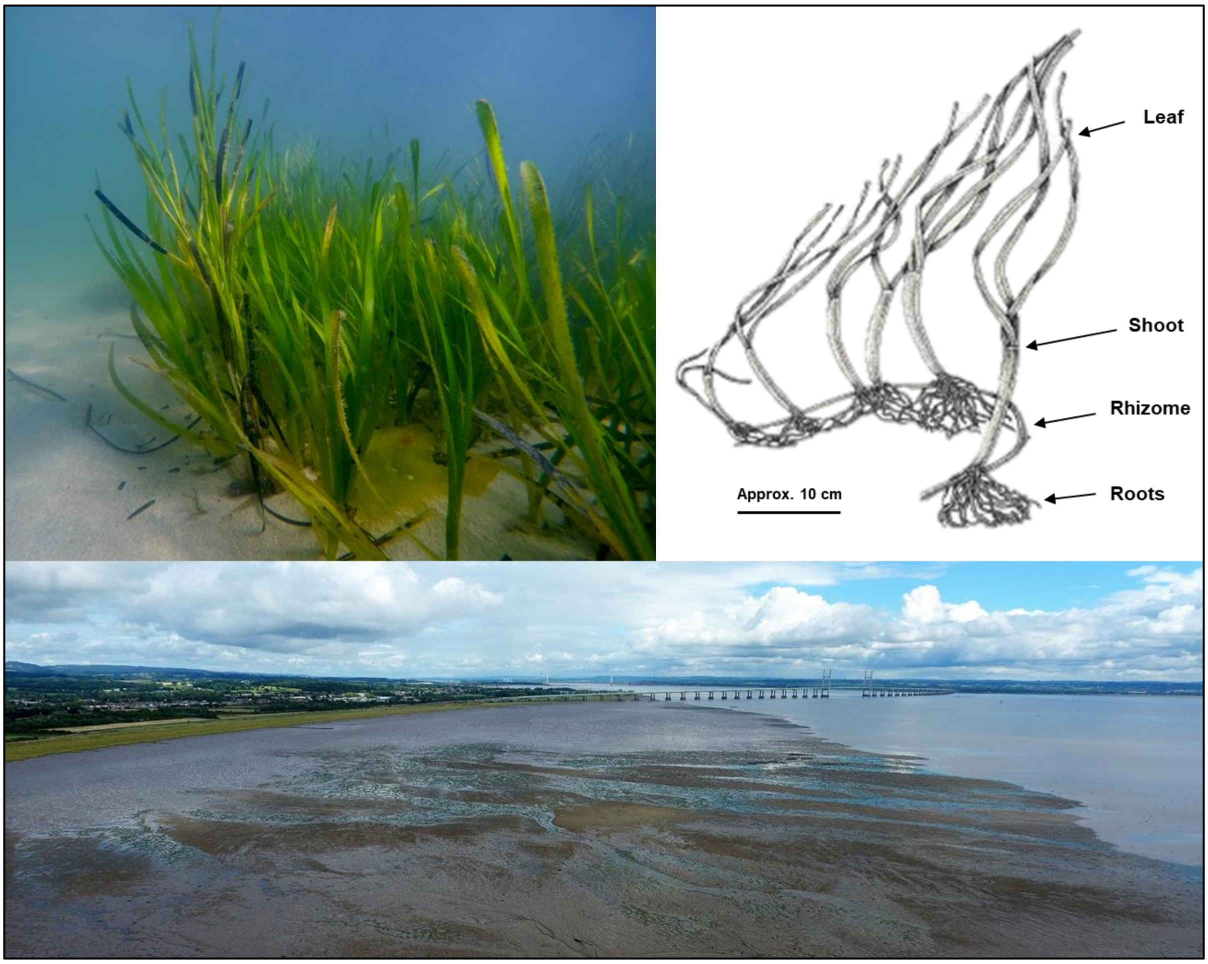

Figure 1. *Zostera marina* at Porthdinllaen (left) © R.K.F Unsworth. Schematic of *Zostera marina* plant (Dawes, 1998) (right). Intertidal *Z. noltei* and *Z. marina* bed in the Severn Estuary, South Wales (bottom) © Ocean Ecology Limited.

In the UK, there are two recognised species of seagrass: eelgrass (*Zostera marina*) and dwarf eelgrass (*Z. noltei*[2]). A third species, narrow-leaved eelgrass (*Z. angustifolia*), continues to be recorded in the UK (chiefly during Water Framework Directive (WFD) monitoring – see section 3.2) despite genetic evidence indicating that it is likely to be an ecotype of *Z. marina* (Becheler *et al.*, 2010). *Z. angustifolia* is not recognised by the

[2] For the purpose of this guidance dwarf eelgrass is referred to as *Z. noltei* . The alternative name that is common in the UK scientific literature, *Z. noltii*, is not accepted by the World Register of Marine Species (WoRMS) nomenclature. WoRMS suggests that it should be recorded as *Zostera (Zosterella) noltei.* There is also current discussion as to whether dwarf eelgrass may in fact be of a separate genus, *Nanozostera noltii* (Coyer *et al.*, 2013).

international seagrass scientific community and does not appear in major publications about global or European seagrass (Borum *et al.*, 2004; Short *et al.*, 2011).

Z. marina is an intertidal to sublittoral species found in shallow, fully marine conditions on muddy to relatively coarse sediment. When observed intertidally, it can sometimes be interspersed with *Z. noltei* in between the mid- and low-tide mark. In these cases, it is usually recorded as *Z. marina var. angustifolia*, preferring poorly drained muddy sediments, particularly pools, creeks and wet sand ripples that are unlikely to entirely dry out during low tide. *Z. noltei* occurs higher on the shore to the high-tide mark, on mud, sand and muddy sands and, being more tolerant of desiccation, will inhabit areas that entirely dry out at low tide.

Widgeon grass (*Ruppia* spp.) is a genus of aquatic freshwater plants found in the UK including Wales, that have similar environmental preferences to *Zostera* spp., i.e. temporarily to permanently flooded mesohaline-hyperhaline estuarine wetlands (Kantrud, 1991), brackish waters of lagoonal habitats, lochs and estuaries. The two species of widgeon grass found in the UK (beaked tasselweed, *R. maritima* and spiral tasselweed, *R. cirrhosa*) are not strictly considered as part of the traditional seagrass arrangement (Kuo & Den Hartog, 2001), but they are commonly grouped with *Zostera* spp. as they can occupy a similar niche due to their pronounced salinity tolerance (Zieman, 1982). For ease, *Ruppia* spp. are treated as seagrasses throughout this guidance document.

2.2. Sub-habitat types

A variety of physical, biochemical and biological factors regulate the colonisation, growth and health of seagrasses. Physical factors include light, substratum and wave exposure. Biological factors include the associated grazing community (controlled through top-down processes of predation) and the connectivity of the bed with other beds within a wider seascape. It is the balance of these factors that govern the distribution of the seagrass bed sub-habitat types which include both mono-specific and two-species stands.

The majority of seagrass beds around Wales are thought to be representative of two intertidal and two subtidal biotopes listed in the European Nature Information System (EUNIS)[3] habitat classification system (Table 1). These mostly occur on muddy sand sediments, although some beds have also been recorded on mixed sediments (for example the 'Welsh Grounds' bed in the Severn Estuary) which may warrant addition of further mixed sediment biotopes (Ocean Ecology Limited, 2016a).

Biotope mosaics also exist where two or more of the listed biotopes occur over small spatial scales (<25 m^2). The most common seagrass mosaic biotope occurs on the lower shore where the lower portions of *Z. noltei* beds merge with the upper portions of *Z. marina* beds, or where semi-permanent channels run down the shore. This is represented as either biotope A2.6111 / A5.5331 or A5.5331 / A2.6111 depending on the predominant biotope (see Parry, 2015).

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[3] EUNIS is a pan-European habitat classification system developed by the European Environment Agency to provide a comprehensive habitat classification for Europe. The Marine Habitat Classification for Britain and Ireland has been incorporated into the EUNIS classification. There is more information about these habitat classifications in the introductory chapter of the guidance (GN030-intro section 3.2.4.)

Table 1. EUNIS biotopes listed for seagrass beds. Those thought to represent the majority of Welsh seagrass beds are displayed in bold. The inclusion of *Z. angustifolia* within this classification remains but requires revision to reflect its taxonomic status as an ecotype of *Z. marina*

EUNIS Code	JNCC Code[4]	Biotope Description
A2.6	LS.LMp	Littoral sediments dominated by aquatic angiosperms
A2.61	LS.LMp.LSgr	Seagrass beds on littoral sediments
A2.611	-	Mainland Atlantic [*Zostera noltii*] or [*Zostera angustifolia*] meadows
A2.6111	LS.LMp.LSgr.Znol	[*Zostera noltii*] beds in littoral muddy sand
A2.614	-	[*Ruppia maritima*] on lower shore sediment
A5.53	SS.SMp.SSgr	Sublittoral seagrass beds
A5.533	-	[*Zostera*] beds in infralittoral sediments
A5.5331	SS.SMp.SSgr.Zmar	[*Zostera marina*]/[*angustifolia*] beds on lower shore or infralittoral clean or muddy sand
A5.5343	SS.SMp.SSgr.Rup	[*Ruppia maritima*] in reduced salinity infralittoral muddy sand

For Water Framework Directive monitoring purposes, intertidal seagrass beds are further subdivided into sub-habitats representing >5 % coverage and <5 % coverage, the latter commonly associated with the periphery of the bed (UKTAG, 2014).

2.3. Extent and distribution in Wales

2.3.1. *Zostera* beds

The distribution of subtidal *Zostera* beds around Wales is limited to 10 known major locations, whereas intertidal beds are found at just eight locations (Figure 2). These can occur as isolated intertidal or subtidal beds (such as the Welsh Grounds bed in the Severn Estuary), or as one continuous bed where the intertidal and sublittoral stands merge (for example, at Porthdinllaen on the north Llŷn coast). Other small isolated patches of both species have also been recorded.

2.3.2. *Ruppia* beds

Records for *Ruppia* spp. are far sparser but are mostly represented by isolated swards in brackish pools located within areas of saltmarsh (for example, Carmarthen Bay).

2.3.3. Additional beds and records

Despite the relatively specific environmental requirements of seagrasses, habitat suitability modelling conducted for the Welsh coastline, Brown (2015) has determined that many additional locations throughout Wales may provide suitable conditions for supporting seagrass beds. Such areas may only support isolated clusters of seagrass plants or discrete patches which, due to their limited extent, do not necessarily qualify as beds[5]. These occurrences have been largely under-recorded around Wales. However, the recent development of mobile mapping applications such as SeagrassSpotter has led to seagrass plants being documented in previously unrecorded locations around Wales.

[4] This is the biotope code from the Marine Habitat Classification for Britain and Ireland (Conner *et al.*, 2004)

[5] OSPAR seagrass bed definition: a seagrass meadow is defined when seagrass cover is a bigger area than 2 x 2 m. When patchy it is still a meadow if it is less than 10 meters between the patches, if bigger than 10 m between patches it should be counted as a new meadow (MARBIPP, 2006) .

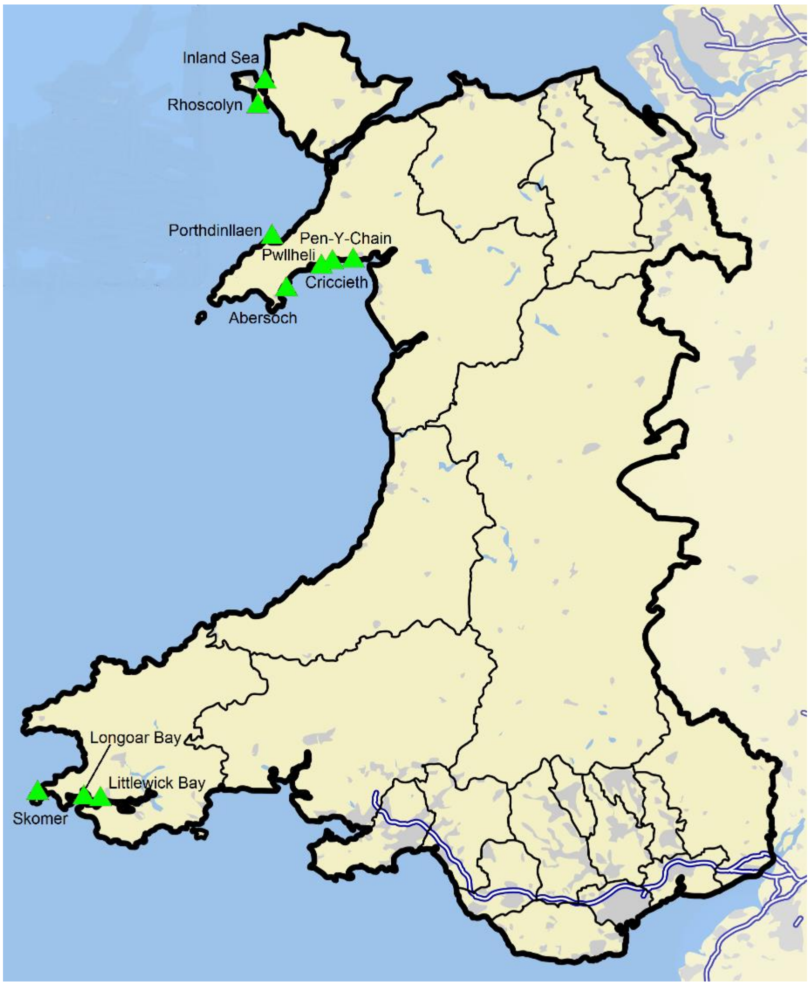

Figure 2. Locations of known seagrass beds in Wales.

2.4. Conservation importance

As bioengineers, seagrasses play an important role by establishing positive feedbacks that lead to the local environment becoming more conducive for both their own productivity and that of associated flora and fauna (Maxwell *et al.*, 2016). This trait ultimately leads to seagrass beds being highly valued for the ecosystem services they provide (Jackson *et al.*, 2013; Mtwana Nordlund *et al.,* 2016). By developing a 3-dimensional structure in an otherwise barren seascape, seagrasses slow the speed of passing water, resulting in filtration of the water, trapping suspended sediments and particles (Borum *et al.*, 2004), and reducing nutrients (McGlathery *et al.*, 2007), bacteria and viruses (Lamb *et al.,* 2017). Unfortunately, to date, no such assessments of these roles have occurred in the UK. However, these processes have mostly been demonstrated for the seagrass species that occur in the UK, indicating a high likelihood that these services hold true for Welsh seagrass beds.

Seagrasses are highly productive, rapidly turning over large amounts of organic carbon as leaf material. This organic carbon is often exported to other ecosystems, subsequently becoming trapped in sediments below the bed or stimulating other food webs. In addition, the trapping of suspended particulate matter also leads to additional allochthonous carbon becoming sequestered in sediments underlying seagrass beds. Due to the refractory nature of seagrass tissues, these processes can result in the build-up of long-term carbon deposits (Fourqurean *et al.*, 2012). Although there has been limited study of seagrass carbon deposits in the UK, recent spatially restricted samples (Unsworth unpublished data) from seagrass in Porthdinllaen (North Wales) reveal that the rates of carbon storage (7 to 45 $tC.ha^{-1}$) are similar to those observed in other parts of Northern Europe (Röhr *et al.,* 2016). The complex mat of roots and rhizomes created by seagrasses means they are thought to play an important role in stabilising sediments in the UK (Wilkie, 2011). This potentially helps maintain the presence of fine sand on beaches and reduces coastal erosion (Christianen *et al.,* 2013).

In addition to these regulatory and provisioning functions, seagrass beds also provide nursery habitat for fish species (Cullen-Unsworth & Unsworth, 2013). There is now increasing evidence that this role is pronounced in UK seagrass beds, with a range of studies confirming that they play such a role for numerous species of commercial importance (for example, plaice, bass, cod) (Bertelli & Unsworth, 2014; Lilley & Unsworth, 2014; Peters *et al.*, 2015). Seagrass beds also constitute permanent habitats for species of principal importance for conservation such as stalked jellyfish and seahorses (Hiscock, *et al.*, 2005). Although no quantitative scientific data exist for this role in Wales, significant anecdotal evidence has been found in Porthdinllaen and in Milford Haven (R. Unsworth pers. obser). As well as being an important habitat for fish and invertebrate species, the roots and shoots provide important food for wildfowl such as Brent geese (Ganter, 2000).

In addition to the more tangible benefits afforded by seagrass beds in Wales, seagrass meadows also play a significant cultural role on a local scale in such places as Porthdinllaen where communities and individuals gain well-being from their use of seagrass beds for recreation, such as prawn fishing (Unsworth & Cullen-Unsworth, 2015).

Conservation legislation and policies relevant to *Zostera* spp. seagrass beds

In recognition of their ecological and economic importance, seagrass beds are afforded protection through a variety of conservation legislation and polices. The Introductory Chapter (GN030-intro, section 3.2.2) provides more general information on conservation policies and legislation, but key aspects relevant to seagrass beds are highlighted below.

2.4.1. Habitats Directive

The Habitats Directive lists habitats and species of interest in Annex I and Annex II respectively. *Z. noltei* and *Z. marina* seagrass beds are encompassed by the following Annex I habitats:

- Sandbanks (code 1110[6]) (*Zostera marina* only)
- Estuaries (code 1130)
- Mudflats and sandflats not covered by seawater at low tide (code 1140)
- Large shallow inlets and bays (code 1160)

Special Areas of Conservation (SACs) are protected sites designated under the Habitats Directive. In Wales, seagrass beds are part of Annex I features in a number of SACs as shown in Table 2.

[6] The code assigned to the Annex I features is the Natura 2000 code which is a four digit code given in the Natura 2000 standard data-entry form. Natura 2000 is a network of nature protection sites in the territory of the European Union. It is made up of Special Areas of Conservation and Special Protection Areas.

Table 2. MPAs providing protection for seagrass beds around Wales

SAC	Relevant SAC Feature	Seagrass present	Examples of relevant studies
Carmarthen Bay and Estuaries SAC	• Mudflats and sandflats	Intertidal *Z. noltei*	Howson (2012); CCW Phase I mapping
Menai Strait & Conwy Bay SAC	• Mudflats and sandflats	Intertidal *Z. noltei*	Boyes *et al.* (2009); CCW Phase I mapping
Pen Llŷn a'r Sarnau SAC	• Mudflats and sandflats • Large shallow inlet and bay • Estuary	Intertidal and subtidal *Z. marina*	Egerton (2011); Unsworth & Cullen-Unsworth (2015)
Pembrokeshire Marine SAC	• Mudflats and sandflats • Large shallow inlet and bay • Estuary	Intertidal *Z. noltei* Subtidal *Z. marina*	Duggan-Edwards & Brazier (2015); CCW Phase I mapping
Severn Estuary SAC	• Estuary	Intertidal *Z. noltei* and *Z. marina*	CCW Phase I mapping

2.4.2. Birds Directive

This Directive aims to protect all European wild birds and the habitats of listed species, in particular through the designation of Special Protection Areas (SPAs), including all the most suitable territories for these species. Seagrass beds can provide an food resource for some SPA bird features.

2.4.3. Ramsar Convention on Wetlands

The adoption of the Ramsar Convention on Wetlands of International Importance in 1971 committed the UK to conserve and sustainably use intertidal mudflats and salt marshes. Seagrass beds, as a habitat present on mudflats and sandflats and in estuaries can be part of supporting habitats for birds within Ramsar sites and can also be part of the designated features of a Ramsar site.

2.4.4. Water Framework Directive

Seagrass beds on intertidal sediment habitats are one of several indicators used for Water Framework Directive (WFD) monitoring in transitional and coastal (TraC) waterbodies. They represent a sub-element (along with saltmarsh) of the angiosperm Biological Quality Element (BQE) which is one of five BQEs used to classify the ecological status of waterbodies (see section 3.2).

'Intertidal seagrass' is identified as one of several higher sensitivity habitats that specifically need to be considered if a proposed development or activity needs to be subject to a WFD assessment (see the Guidance Note GN030 section 2.2).

2.4.5. Marine Strategy Framework Directive

Two of the 11 high level descriptors of Good Environmental Stats (GES) in Annex I of the Directive (Defra, 2014) relate directly to sedimentary benthic habitats (D1 Biodiversity and D6 Seafloor integrity), with others relating to aspects of benthic ecology (e.g. food webs and commercial fishing).

2.4.6. OSPAR list of threatened and/or declining species and habitats

'*Zostera* beds' are on the OSPAR list of threatened and/or declining species and habitats.

2.4.7. Environment (Wales) Act 2016 Section 7 list of habitats/species of principal importance (previously NERC S42 lists)

Seagrass beds are included under 'intertidal sediments' on the list of Section 7 habitats.

2.4.8. The Wildlife and Countryside Act 1981 (amended by the Countryside and Rights of Way (CROW) Act 2000)

The Act provides for the designation of Sites of Special Scientific Interest (SSSIs). There are more than 1,000 SSSIs in Wales, covering about 12% of the country. The seaward limit of SSSIs in Wales does not extend into the subtidal but does encompass intertidal areas. Intertidal *Zostera noltei* and *Zostera marina* beds are a designated feature of a number of SSSIs in Wales. In SACs, SPAs and Ramsar sites, SSSI designations also underpin the terrestrial and intertidal components of these sites.

2.4.9. Marine and Coastal Access Act 2009

The Act enables Marine Conservation Zones (MCZs) to be designated to conserve 'nationally important' features including marine flora, fauna, habitats and geological or geomorphological structures. Seagrass beds can be MCZ features; a *Zostera marina* bed is one of the features of Skomer MCZ, currently the only MCZ designated in Wales.

The Act also established the requirement for marine licences for developments and activities in the marine environment.

2.4.10. Welsh Marine Protected Area Network

Zostera spp. seagrass beds are considered within the Marine Protected Area network feature list for Wales (Carr *et al.*, 2016).

2.5. Key potential pressures

The key potential pressures of marine developments and activities on seagrass beds vary in relation to factors such as, the nature of the development or activity, construction methods, mode of operation and scale of the project. In order to assess the significance of the effect of a given pressure on a specific receptor (such as a seagrass bed), you will need to identify the factors and pressures associated with your proposed development or activity. You will need to consider these, along with the conservation value and sensitivity of the habitat/species present and the magnitude of effect, as part of the Ecological Impact Assessment (EcIA) (CIEEM, 2018). The main potential pressures include, but are not restricted to, those indicated in Table 3.

Table 3. Key potential pressures of marine developments and activities on seagrass beds

Pressure	Examples
Changes to temperature, salinity and water flow	Cooling water discharges, freshwater inputs or construction of coastal structures (lagoons, ports etc.) resulting in changes in coastal processes.
Water flow (tidal current) changes; changes to emergence regime and wave exposure	Construction and operation of coastal structures (ports, pilings, jetties, coastal defences, tidal lagoons etc.); coastal defences (e.g. managed realignment); Extraction industry.
Nutrient and organic enrichment; introduction / release of pollutants	Sewage effluent, agricultural runoff, marinas, aquaculture; Spillage of contaminants during development construction/operation.
Loss of habitat in development footprint; Changes to, removal and disturbance of substrate surface and subsurface (including scour and sediment compaction)	Bait digging, dredging, trawling, anchoring/mooring, vehicle use, construction and operation of coastal structures/developments; Coastal defences (e.g. managed realignment); Extraction industry; Recreation.
Changes to sediment transport and erosion/accretion regime; Changes in suspended solids (water clarity); Changes to intertidal habitat structure /sedimentology/geomorphology	Dredging, construction and operation of coastal structures/developments; Coastal defences (e.g. managed realignment); Extraction industry.
Siltation rates changes (smothering)	Dredging, managed realignment, construction and operation of coastal structures.
Introduction or reduction of light	Construction of coastal structures.
Introduction or spread of invasive non-native species (INNS)	Vessel activity, anchoring/mooring, marinas, aquaculture, construction and operation of coastal structures/developments.
Introduction of microbial pathogens	Vessel activity, marinas, aquaculture, construction activities.
Removal of target and non-target species	Trawling, bait digging.

2.6. Sensitivity (resistance/resilience to pressures)

For any species or habitat found in the Zone of Influence (ZoI)[7] of a development or activity, it is important to understand their sensitivity to each of the specific associated pressures arising from the proposed works.

D'Avack *et al.* (2014) provides a detailed account of the sensitivity of each of the seagrass bed biotopes listed in Table 1 to various pressures and established the levels of resistance and resilience to each of these. In summary, this assessment concluded that seagrass beds have a medium to high sensitivity to the defined intensities of pressures (benchmarks) set for the majority of hydrological, chemical, physical and biological pressures assessed. Key examples are provided in Table 3. These are fully described in

[7] Zone of Influence (ZoI) - the area of the seabed or foreshore that could be affected by the proposed development or activity, during both construction and/or operation.

the sensitivity review provided on the Marine Life Information Network (MarLIN) website, which should be used when establishing the sensitivity of specific seagrass bed[8].

It is important that you read the further information and considerations related to MarLIN assessments in the Introductory Chapter (GN030-intro, section 3.2.6.). It is also important to consider the sensitivities and traits of species found within these benthic habitats. These are discussed by Tillin & Tyler-Walters (2014) and incorporated into MarLIN and its Biological Traits Information Catalogue (BIOTIC) resource, with further information in the wider scientific literature.

In general terms, the resilience of seagrass beds to external pressures in the coastal waters of the UK is seemingly low. This is illustrated in Wales by the limited recovery of seagrass from losses over the last 100 years (Kay, 1998). This is further evidenced by the limited wider scale recovery from the 1930s 'wasting disease' epidemic (Muehlstein, 1989) - which was caused by the protist *Labyrinthula zosterae*[9] (Short *et al.,* 1987), and more recent localised die-offs (McKone & Tanner, 2009).

Seagrass resilience is largely dependent on the balance of both the biological features of the meadow (and its associated fauna) and a number of critical bio-physical features, including moderate temperatures (lacking temperature anomalies or extremes) and good water quality (low turbidity and low-moderate nutrients) (see Unsworth *et al.*, 2015). Human-induced changes to these critical features can result in declines in the health of seagrass beds inducing a negative feedback loop, making them increasingly sensitive to future impacts (Unsworth, *et al.,* 2015). Evidence indicates that some Welsh seagrass beds (particularly those in Milford Haven) may be in a poor state of health (Jones & Unsworth, 2016), suggesting that they may therefore be particularly sensitive to any future developments and activities.

Owing to their high sensitivity to changes in environmental conditions, seagrasses are frequently used as indicators of wider ecosystem quality and health (Dennison, *et al.,* 1997).

[8] It is important to note that the MarLIN method assesses sensitivity against a set benchmark. Therefore, it is important that the scale of effect from any proposed development is considered in relation to the MarLIN benchmark. For example, when assessing sensitivity to smothering and siltation rate changes the MarLIN benchmark is "deposition of up to 5 cm (light) or 30 cm (heavy) of fine material added to the habitat in a single, discrete event". If a proposed development/activity may result in deposition of sediment greatly in excess of this, a habitat assessed as having low sensitivity may actually have a higher sensitivity to the development or activity.

[9] Parasitic slime mould that causes black spot disease on seagrass.

3. Existing guidance and data

This section identifies information and guidance that may be useful in the context of survey and monitoring of seagrass beds. Whilst some of the guidance is primarily for statutory monitoring work undertaken by ourselves and others, the documents and references may still provide useful contextual information and guidance on methods.

The JNCC has recently produced specific guidance for the monitoring of marine benthic habitats (Noble-James *et al.*, 2017) which is a useful reference document for many aspects of monitoring.

3.1. Marine Protected Area monitoring

Seagrass beds are designated as protected features of a number of Marine Protected Areas (MPAs) around the Welsh Coast (section 2.4). As a result, a series of historic monitoring surveys have been undertaken extending back almost 20 years in some cases (for example, at Skomer MCZ (formerly Skomer Marine Nature Reserve)). The methods, sampling design and indicators used vary markedly between MPAs and between monitoring surveys within the same MPAs, largely due to advances in sampling technologies and in the understanding of seagrass ecology.

In general, these methods have been developed based on the high-level guidance provided in the relevant Common Standards Monitoring (CSM) Guidance (for example, CSM guidance for Inshore Sublittoral Sediment Habitats (JNCC, 2004a), Littoral Sediment Habitats (JNCC, 2004b), Estuaries (JNCC, 2004c), and Inlets & Bays (JNCC, 2004d)). The CSM documents provide broad guidance for feature-specific monitoring indicating the background, targets and monitoring techniques for feature attributes. In terms of survey methods, the CSM guidance primarily directs the reader to the Marine Monitoring Handbook (Davies *et al.,* 2001) which is a key guidance document covering a diverse range of survey methods and survey/monitoring requirements in general (not just for Habitats Directive monitoring). Further guidance on general intertidal survey techniques was developed by Wyn *et al.* (2006) as part of the Countryside Council for Wales' (CCW) Phase I intertidal mapping project conducted between 1996 and 2005. Whilst providing a greater level of detail than the CSM guidance, this was aimed at general biotope mapping and therefore lacks specific guidance on methods for undertaking seagrass bed assessments.

3.2. WFD monitoring

Under the WFD, NRW classify coastal and transitional waterbodies into five ecological status classes (high, good, moderate, poor and bad) that are defined by the changes in the biological community in response to disturbance. Owing to their high sensitivity to changes in environmental conditions, intertidal[10] seagrasses are monitored at a number of locations around Wales as a sub-element under the 'angiosperm' biological quality element (BQE). These beds are assessed using a multimetric tool (Foden & Brazier, 2007; UKTAG, 2014) composed of the following three key metrics:

- **Shoot density:** This is monitored by sampling at randomly selected quadrat locations (at least 30) within the ≥5 % coverage area of beds. This requires a record of percentage coverage for each seagrass taxon at each sampling location to reflect the change (% loss or gain) of seagrass cover compared with reference conditions. It should be noted that the terminology used in the guidance to describe this metric

[10] WFD seagrass monitoring in Wales does not include subtidal beds.

is misleading (UKTAG, 2014). True quantification of shoot density is conducted by recording the number of shoots per area (see section 5.1.2) rather than recording percentage coverage.

- **Taxonomic composition (of seagrass taxa):** This is assessed simultaneously with shoot density as percentage cover of each seagrass taxon at each of the quadrat sampling locations. This metric reflects the loss of seagrass taxa compared to the number of reference taxa for the specified water body (or gain where a bed is recovering).

  Despite being considered an ecotype of *Z. marina* (Becheler *et al.*, 2010), this metric requires *Z. angustifolia* to be treated as a separate species. It also requires *Ruppia* spp. to be recorded despite not being strictly considered as part of the traditional seagrass assemblage (Kuo & Den Hartoc, 2001). However, while *Zostera* plants are identified to species level, *Ruppia* plants are identified to genus only due to the difficulty of species identification, as recommended by Foden & Brazier (2007). This means that four taxa are the maximum found in any waterbody.

- **Total bed extent:** This is monitored by either aerial/remotely sensed images with ground-truthing or perimeter walking using the tracking function of a hand-held GPS. Some intertidal seagrass beds have extensive areas of very low cover (shoot density <5 %) around the periphery of the denser, continuous bed (>5 % cover). Where possible, NRW map the boundary of this peripheral low shoot density area as well as the continuous bed, although the above-mentioned metrics are not measured in this low cover area.

3.3. MESH guidance

The Mapping European Seabed Habitats (MESH)[11] project produced ‘Recommended operating guidelines’ (ROGs) for marine habitat mapping survey methods and these are hosted in the MESH archive on the EMODnet[12] website. A number of these ROGs are relevant to survey and monitoring of seagrass beds.

The MESH Atlantic Project updated the ROGs for LiDAR and side scan sonar and produced a new ROG for grab sampling. These documents will become available through the MESH archive but in the interim they need to be requested from one of the project partners who are listed on the project page of the keep.eu website.

Survey and monitoring work in relation to proposed developments and activities should have regard to the guidance provided in the ROGs. Specific ROGs are referenced where relevant in other sections of this guidance.

3.4. NMBAQC guidance

Operational guidelines for remote monitoring of epibiota using digital imagery and analysis of that data are presented within the following North-East Atlantic Marine Biological Analytical Quality Control (NMBAQC) guidance documents:

[11] The MESH project, conducted between 2004 and 2008, was a consortium of twelve partners from five European countries led by the UK’s JNCC.

[12] EMODnet is an EU network of organisations that collate and make available data relevant to Europe’s marine environment.

- Operational guidelines for remote monitoring of epibiota using digital imagery are presented in Hitchin *et al.* (2015). The guidance covers the approaches, available equipment and methods for a variety of camera systems, including towed camera sledges, drop down cameras and towed camera platforms, as well as remote-operated vehicles (ROVs) and the use of freshwater lens camera systems. It also provides information on quality control of imagery and analysis and a recommended approach for data review.
- Guidance on the analysis of remote underwater video footage and still images is provided in the epibiota remote monitoring interpretation guidelines (Turner *et al.*, 2016)

3.5. Other sources of guidance

General guidance has been developed for seagrass monitoring by SeagrassWatch in 2003 (McKenzie, 2003) and continues to act as a benchmark for designing and undertaking seagrass bed assessments worldwide.

More specific guidance for monitoring and managing European seagrasses was subsequently developed by Borum *et al.* (2004) and covers methods for assessing both intertidal and subtidal *Z. marina* and *Z. noltei* beds. This guidance provides detailed methods for monitoring key seagrass bed indicators including biochemical metrics that, to date, have been largely ignored in the UK as indicators of seagrass bed health. Further UK-specific guidance has also been developed based on a comprehensive literature review and an assessment of various approaches used to assess the vulnerability of seagrass beds in Studland Bay, UK (Jackson *et al.*, 2013).

More recently, a UK-wide study of 11 separate *Z. marina* seagrass beds recommended that measurements of a number of key biochemical and morphological indicators should be incorporated into any future monitoring of seagrass beds to understand their true environmental and ecological health (Jones & Unsworth, 2016). This includes measurement of shoot length, width and biomass combined with leaf tissue analysis of carbon (C), nitrogen (N) and phosphorus (P) content. This allows for a comparison of C:N, C:P and N:P ratios as indicators of light reduction, environmental P limitation and environmental balance of N and P respectively (also see Atkinson & Smith, 1983; Duarte, 1990; McMahon *et al.*, 2012; McKenzie & Unsworth, 2009). Other biochemical metrics such e.g. $\delta^{15}N$ have also been highlighted as useful indicators of impacts on seagrass beds resulting from effluent discharge (McKenzie, 2003; Lepoint, *et al.*, 2004).

Other examples in seagrass monitoring include the 'balisage' method of Neptune grass (*Posidonia oceanica*) monitoring within the Posidonia Monitoring Network (PMN) in France (Boudouresque, *et al.*, 2000; 2012), and the SeagrassNet global monitoring network. The Convention on Migratory Species (in conjunction with the Total Foundation) has also recently developed a freely available E-Research kit aimed at helping to select appropriate methods for assessing seagrass beds. In addition, the phone application SeagrassSpotter provides an advanced mode aimed at scientists to facilitate rapid field data collection aligned to public communication of the findings.

3.6. Data sources

Distribution data for intertidal and subtidal habitats in Wales and the UK are available from a number of sources. Our Guidance Note GN006 Marine ecology datasets for marine developments and activities (Natural Resources Wales, 2019) identifies data sources for intertidal and subtidal habitat maps. It also explains how you can access information about

Marine Protected Areas in Wales including maps and supporting documentation on protected features, as well as data and maps on protected marine habitats and species in Welsh waters.

4. Survey and monitoring design

The Guidance Note GN030 and Introductory Chapter GN030-intro explain when and why habitat characterisation and monitoring may be required in relation to development proposals and activities and over-arching principles for both of these[13]. It is important to understand the differences between characterisation surveys and monitoring when designing project-specific survey programmes.

The information provided in the following sections presumes an existing knowledge of the presence of seagrass beds in the area to be surveyed based on available ecological data and/or habitat surveys. For subtidal areas, if you have little or no seabed habitat data, a general benthic survey will be needed to record the habitats present and determine their extent and distribution; refer to chapter GN030g of the guidance which addresses subtidal habitat characterisation surveys. For intertidal areas where you have no recent habitat data refer to chapters GN030a or GN030b for guidance on undertaking intertidal Phase I survey to determine the habitats present.

4.1. Existing data

Where possible, and where timeframes allow, a comprehensive desk-based review of all available data of relevance to seagrass beds should be conducted prior to designing any habitat characterisation surveys or monitoring programmes. Ideally, historic data will be available for the area of interest to help inform identification of knowledge gaps and provide specific information to inform survey design.

Our Guidance Note GN006 (Natural Resources Wales, 2019) provides information on the marine ecology data sets we hold and routinely use and how you can access them. Further information relating to sourcing and using data is also provided in the Introductory Chapter GN030-intro (section 3.2.3.) and Noble-James *et al*. (2017).

4.2. Selecting ecological parameters

The Introductory Chapter GN030-intro (sections 3.2.7 and 4.2.1) addresses the importance of selecting suitable ecological parameters for survey (known as ‘indicators’ for monitoring programmes) and the process to determine the effectiveness, appropriateness and validity of parameters.

Seagrass bed indicators

The main ecological parameters that can be measured and evaluated for seagrass beds are set out below. In the absence of an entirely relevant Conceptual Ecological Model (CEM) (see those developed for Mediterranean and the Black Sea seagrass beds by Langmead, *et al.* (2007)), these were short-listed based on a critical review of relevant literature and the findings of a number of recent studies discussing the most robust bioindicators of impacts to seagrass beds (OSPAR, 2009; Jackson, *et al.*, 2013; Marbà, *et al.*, 2013; McMahon, *et al.*, 2013; Jones & Unsworth, 2015).

In most cases it will not be necessary to assess all or any of these indicators for habitat characterisation purposes, particularly if data already exist for particular beds. It can,

[13] Note that the Guidance Note and Introductory Chapter apply to all of the specific habitat chapters of this guidance; consequently, some parts may not be directly relevant to a specific marine habitat, and information should be evaluated as appropriate.

however, be prudent to collect and store shoot samples to ensure that indicators based on leaf metrics can be determined at a later date if not deemed a requirement initially. Such information is easily collected and can be invaluable if the actual impacts of a development or activity are different, or a greater magnitude and/or severity than the predicted impacts.

For monitoring, it may be necessary or advantageous to include a greater selection of indicators depending on the particular predicted impacts. The indicators to be measured during monitoring programmes should be clearly set out in a monitoring plan and include a full rationale for their inclusion.

- ‘Bed-scale’ indicators (indicators of long-term change)
    - Bed extent (WFD metric) and fragmentation
    - Percentage seagrass cover (WFD metric)
    - Seagrass species composition (WFD metric)
    - Maximum depth/height of bed
    - Shoot density (no/m^2)
    - Wasting disease prevalence (% infection)
    - Epiphytic cover (% cover, composition and biomass)
    - % Macroalgae cover / non-native species presence

- ‘Plant-scale’ morphological indicators (indicators of short-term change)
    - Leaf length
    - Leaf width
    - Shoot biomass (above ground biomass)

- ‘Plant-scale’ physiological indicators (indicators of short-term change)
    - Shoot C:N ratio
    - Shoot C:P ratio
    - Shoot N:P ratio

- Environmental indicators
  Light: Light is the most critical driver of seagrass productivity and it is, in most cases, likely to be identified as a suitable indicator[14] for seagrass bed assessments. The monitoring of turbidity is often used as a surrogate for light, but this has been found to have a poor correlation with actual light availability measured as Photosynthetic Active Radiation (PAR) (Sofonia & Unsworth, 2010) and is therefore not recommended. PAR is more effectively and readily monitored using well-calibrated PAR loggers (Long, *et al.*, 2012) fitted with wiper units (to prevent sediment build up and fouling) (Collier, *et al.*, 2012; Kilminster & Forbes, 2014; Chartrand, *et al.*, 2016).

  Temperature: The shallow water distribution of seagrass means they are commonly subjected to higher temperature ranges than surrounding water, particularly in the summer months and when low water coincides with periods of high atmospheric temperatures and irradiance causing superheating. Elevated temperature can be problematic for seagrasses, especially with respect to power station developments where cooling water outfalls can create plumes of elevated water temperature. The respiratory demand of seagrass is high due to the metabolic burden of the rhizome. This respiration rate rapidly increases with increasing temperature, creating a

[14] Environmental or other predictive indicators are commonly termed as ‘covariates’. These are parameters thought to influence variation in addition to the indicator(s). For consistency, environmental covariates are termed as environmental indicators in this guidance document.

greater overall carbon requirement (Hemminga, 1998). Such elevated carbon requirements can only be sustained if the plant has a means of increasing its photosynthetic rate, which, in poor water quality, is not often possible. As such, temperature will, in most cases, represent a suitable and key indicator for seagrass bed assessments.

- Other indicators

  A number of other indicators could potentially provide additional critical information for understanding specific impacts on seagrass beds and potential recovery/colonisation rates. This type of understanding will be particularly important where seagrass beds might be lost or undergo decline (for example, during dredging), as further information may indicate the potential for recovery. It may be useful to collect additional data when considering compensatory measures (for example, seeding or transplantation) to offset seagrass bed loss as a direct result of a project. The relevance of these to an assessment program will need to be determined on a case by case basis and it may be beneficial to discuss them further with an expert in the field of seagrass ecology.

  - Seagrass tissue nutrients ($\delta^{15}N$)
  - Seed bank
  - Seed viability
  - Flowering intensity
  - Sediment composition and dynamics
  - Associated species composition and diversity (benthos, epibenthos, motile fauna)

4.3. Habitat characterisation

4.3.1. Aims of habitat characterisation surveys for seagrass beds

The aim of habitat characterisation survey is to collate data to describe any seagrass bed within the survey area, identify any habitats and/or species of conservation importance and provide an up-to-date ecological appraisal to inform EcIA.

4.3.2. Design of habitat characterisation surveys for seagrass beds

Development- and activity-specific information should inform the design of habitat characterisation surveys which will also be influenced by the scale of the proposed development or activity (see Introductory Chapter GN030-intro).

The range of available survey methods for habitat characterisation of seagrass beds is indicated in Section 5.1. The methods to be used should be determined on a project-by-project basis prior to survey.

Guidance for habitat characterisation survey design is provided in a range of sources including the Marine Monitoring Handbook (Davies, 2001) and Noble-James *et al.* (2017).

Sampling design

In most cases, habitat characterisation surveys of seagrass beds will involve a single sampling ‘event’ to characterise the beds in the general study area and any potential control sites identified. As an assessment of the influence of background spatial and temporal variance is not required, these sampling designs can be relatively simple, unlike the more complex Beyond-Before-After-Control-Impact (BACI) designs (Underwood, 1992) advocated for the design of seagrass bed monitoring programmes (see section 4.4.5).

A key requirement is that all beds located within the predicted project ZoI should be characterised. The scale of the project and the potential resource constraints should be considered when determining whether a full complement of indicators need to be assessed for all the beds to be characterised. This may include indicators used to fulfil the requirements of policies or directives already in place (for example, WFD) if suitable existing data are not available.

Bed extent

Extent mapping of all known seagrass beds within the project ZoI (i.e. the impact beds) should be undertaken prior to the design of sampling arrays (unless suitable existing data are available). This is an essential first step as it provides the framework for appropriately locating sampling positions across the beds.

Bed extent can be determined using a variety of methods, for example, perimeter tracking on foot, UAV mapping and acoustic mapping (see section 5.1). The most appropriate method will depend on a number of factors such as biological zone (i.e. intertidal or subtidal), size of the bed, water depth and water clarity. Regardless of the method selected, all surveys should aim to achieve 100 % coverage and, if possible, include a buffer zone (for example, 50 m) around known bed extents (based on existing data) to ensure that any expansion in bed extent since previous mapping is included. New beds or patches found extending beyond these areas should also be mapped. Once the bed extent has been finalised it will be possible to establish a sampling array for assessing other indicators of bed condition.

Locating sampling stations

If sampling is deemed necessary, a systematic grid of points should be overlain across each bed delineated by prior extent mapping (see above). This should be used to identify the sampling stations for measuring each of the indicators to be used to assess bed condition. Where possible, triangular grid patterns should be used, as this reduces the chance of bias towards a regularly spaced feature or condition (Barry & Nicholson, 1993; Byrnes, 2000).

Habitat characterisation surveys are not aimed at detecting change, so it is not necessary to undertake *a priori* power analysis to determine the appropriate grid size for each bed (see section 4.4.3). Instead, the sampling interval (i.e. grid size) should be back-calculated by considering what is thought to be the maximum number of sampling stations that could be employed given the size, depth (or position on shore), variability and accessibility of the bed, as well as the resources available. Where SCUBA divers are needed to access subtidal beds, the final sampling grid can be treated as a series of transects.

If environmental indicators such as temperature and light (measured by loggers deployed on the seabed – see section 4.2) are necessary, they will not need to be measured at every sampling station but either at a bed scale or at points within a gradient (e.g. depth). A ‘judgement sampling’ approach (see Noble-James, *et al.*, 2017) may therefore be adopted whereby a targeted single sampling station may be positioned in the centre of the bed(s), or elsewhere within the bed when there is a desire to target a particular feature or existing pressures. Alternatively, a stratified random approach may be adopted whereby the bed(s) are delineated into distinct areas (e.g. upper, mid and lower depth limits of the bed) within which sampling stations may be randomly positioned.

4.4. Monitoring

4.4.1. Aims of monitoring programmes for seagrass beds

The aims of the monitoring need to be clearly defined and will depend on the potential impacts of a proposed development or activity as identified through the EcIA process. The monitoring methodology, including experimental design, needs to provide sufficient information to satisfy the relevant environment assessment processes and any conditions set by the regulator.

Monitoring requires repeat sampling to detect change over time in one or more indicators (i.e. selected ecological parameters). In relation to regulatory development control, monitoring usually consists of pre-construction monitoring (the 'baseline'), monitoring during construction and operational monitoring (see Introductory Chapter GN030-intro section 4.1).

As noted in section 4.2 of the Introductory Chapter, it may be beneficial to make any development-related monitoring compatible with data from existing, ongoing monitoring programmes, such as those undertaken by NRW.

For seagrass beds an 'investigative' monitoring approach will, in most cases, be the most appropriate monitoring type to adopt (see Kröger & Johnstone, 2016). The guidance provided here is therefore based around 'investigative' monitoring principals; however, 'sentinel' and/or 'operational' approaches may need to be considered when the requirement for complex sampling designs makes 'investigative' monitoring unfeasible (see Noble-James, *et al.*, 2017).

4.4.2. Defining hypotheses and trigger levels

Hypotheses to inform ecological monitoring are generally framed to detect change in a selected indicator over time, and to determine if any change observed is outside normal expectations. In the context of regulatory development control and EcIA, key thresholds known as 'trigger levels' are generally set to help assess whether impacts are evident on a given indicator over the course of a monitoring programme, along with management action(s) to be implemented if trigger levels are exceeded. The Introductory Chapter GN030-intro (sections 4.2.2 and 4.2.3) provides further detail relating to hypotheses testing and considerations associated with the potential use of trigger levels.

In relation to seagrass beds, trigger levels may be set:

- that require real time monitoring of key environmental indicators (such as light) during construction activities in order to allow for a timely implementation of mitigation measures (for example, cessation of dredging) when the indicator measurements reach levels known to exceed the tolerance of the impact seagrass bed(s) (see Chartrand *et al.*, 2016)
- for indicators that provide an insight into potential impacts over longer time periods (for example, shoot density)

In both cases it is important that the thresholds for initiating the measures are set in consideration of established thresholds (see thresholds developed by Sofonia & Unsworth (2010) and Chartrand *et al.* (2016)).

4.4.3. Determining appropriate sampling effort for seagrass beds

The onus is on the developer/seabed user to demonstrate that any proposed monitoring programme is statistically robust and has the power to detect change with an acceptable level of confidence. *A priori* power analysis can be carried out on data previously collected at the survey site and used to determine an appropriate but achievable number of sampling stations (N). In the case of seagrass beds, power analysis will not always be necessary for all indicators. For example, it would not be needed to determine the required sampling effort for monitoring bed extent and fragmentation (there may only be one impact bed), whereas it would be needed to establish how many sampling stations would be required to monitor shoot density or leaf length. The process of deducing a feasible sampling effort for appropriately measuring these indicators is a complex one and should be conducted in line with the guidance set out by Noble-James *et al.* (2017) and discussed in the Introductory Chapter.

4.4.4. Determining appropriate sampling units for seagrass beds

After generating a statistically robust sample size through power analysis, it is important to ensure that the sampling units (for example, quadrat size for shoot density counts or number of cm per pixel for UAV or acoustic mapping) provide accurate observations of the indicator(s) in question. A number of factors which can determine the effectiveness of sampling units must be considered as part of the design process. The most influential of these are the size and type of the sampling unit (Eleftheriou, 2013), and the amount of replication required within each.

Ideally, the precision and accuracy of different sampling unit sizes should be investigated in a pilot study to assess the retention of seagrass and consider spatial autocorrelation (Noble-James *et al.*, 2017). If resources do not allow this, the life history and ecology of the seagrass species (or combination of species), or a similar proxy species, should be researched to inform decisions on the sampling unit to be used (Sutherland, 1996; Underwood & Chapman, 2013).

4.4.5. Design of monitoring programmes for seagrass beds

Sampling design

Noble-James *et al.* (2017) provides a background to the variety of survey designs that can be employed to monitor impacts of developments and activities on marine habitats. Of these, the ‘Beyond-BACI’ design (Underwood, 1992) is considered as best practice for designing seagrass bed monitoring programmes and should be implemented when possible. To fulfil a true beyond-BACI design, a minimum of two control beds are needed. When multiple control beds are not available, a Before-After-Control-Impact Paired Series (BACIPS) (Stewart-Oaten *et al.* 1986) design should be considered. The control beds must be selected carefully in order to minimise the likelihood that monitoring is confounded by natural variation or changes arising from the impact itself. The suitability of potential control beds should be assessed considering the key selection principles set out in Table 7 of (Noble-James *et al.* (2017). In practice, it may be difficult to fully adhere to these recommendations if suitable control beds are scarce, in which case mitigative measures should be considered and applied if necessary or possible.

Locating sampling stations

Sampling stations should be located on a systematic grid across each impact and control bed using the process outlined for habitat characterisation survey design in section 4.3.2.

The sampling interval (grid size) should be determined on a project-by-project basis by back-calculating the number of sampling stations required to confidently detect the desired magnitude of effect derived by *a priori* power analysis (see 4.4.3).

Final positioning of sampling stations should aim to minimize the effects of pseudo-replication resulting from correlations within the indicators to be monitored (i.e. the response variables) in space (spatial autocorrelation) and/or time (serial correlation) (see Noble-James *et al.* (2017) for further explanation). There are conflicting opinions on whether sampling stations should remain fixed or re-randomised during each monitoring event (Jon Barry, Cefas, pers. Comm. 2015 cited in Noble-James *et al.*, 2017; Schultz *et al.,* 2015) in order to reduce the influence of dependency issues. This should be decided on a project-by-project basis and with careful consideration of the aims of the monitoring.

If environmental indicators such as temperature and light (measured by loggers deployed on the seabed) are deemed necessary at the monitoring stage, they will not need to be measured at every sampling station. A sampling approach similar to that described for habitat characterisation surveys (see section 4.3.2) can therefore be implemented.

4.4.6. Determining appropriate timing, frequency and duration for monitoring seagrass beds

Timing

Typical seagrass beds in Wales transition through seasonal cycles with maximum biomass (and maximum shoot density) occurring in late summer/early autumn and lowest biomass (and shoot density) in late winter. Like many terrestrial plants, seagrasses senesce in the autumn and winter with storms causing dying shoots and leaves to break off and become redistributed (Pedersen & Borum, 1993). This process is important for recolonization of disturbed beds (Borum *et al.*, 2004). At some Welsh sites (for example, Porthdinllaen), seasonal studies have revealed reductions in % cover of up to 75 % during winter months (R. Unsworth pers comm). The prevalence of epiphytes is also greater in the summer months. In healthy systems, these patterns depend upon local weather patterns and longer term climatic influences, although changes can be exacerbated in a system that is under stress.

The most relevant guidance available relating to the most appropriate timing for seagrass bed surveys in Wales is represented by the CSM guidance for inshore sublittoral sediment habitats (JNCC, 2004a), OSPAR guidance (OSPAR, 2009), and guidance for undertaking intertidal WFD seagrass monitoring (UKTAG, 2014). These suggest that surveys should be undertaken during periods of peak growth between June and September. Large seasonal variations in bed extent are common within seagrass beds globally (Vermaat & Verhagen, 1996; Rasheed & Unsworth, 2011) and as a result many monitoring programmes instead focus their efforts around the period of peak biomass (i.e. August to September) rather than peak growth. It is therefore suggested that monitoring of Welsh seagrass beds should be conducted during periods of peak biomass.

Frequency

A single annual assessment of bed-scale and plant-scale indicators will generally be sufficient for habitat characterisation and monitoring purposes. However, seagrass bed extent can vary highly between years (Bertelli *et al.*, 2017) and therefore it should be considered that a one point in time estimate may be a gross underestimate of the true extent. Monitoring surveys should always be undertaken within the same month of the year (preferably the same two weeks) as the baseline surveys.

Additional measurement of bed-scale and plant-scale indicators may be considered during the period of lowest biomass (March/April) for particularly sensitive seagrass beds (for example, those closely located to construction activities or within a MPA), or for those predicted to be subject to major impacts, in order to gain a complete understanding of the potential impacts throughout the year. More regular (for example, monthly or seasonal) measurements of environmental metrics such as light and temperature should also be considered in order to capture the marked spatio-temporal variations exhibited by these indicators (Jackson *et al.*, 2013). In practice, the sampling regime chosen for each indicator will be project- and impact-specific.

'Real-time' monitoring

During periods of suspected heavy stress on seagrass beds (for example, during a dredging campaign, construction event or high outfall discharge), it may be necessary to assess particular environmental indicators (such as light, temperature, sedimentation) in real time in order to detect exceedance of potential lethal thresholds and instigate mitigation procedures (see section 4.4.2). In such cases, monthly assessments of bed-scale indicators (for example, epiphyte coverage) and plant-scale indicators (for example, shoot biomass) may also be considered in order to closely monitor the health of the bed. This will allow for a timely implementation of mitigation measures (such as cessation or a reduction in intensity of dredging) if unexpected or unacceptable impacts are observed. 'Real-time' monitoring is commonplace in other parts of the world where anthropogenic activities in the coastal zone frequently interact with seagrass beds (for example, in Australia), and is implemented as a means of rapidly responding to any observed changes in seagrass bed status (Cardno, 2013; Chartrand *et al.*, 2016).

Monitoring duration

Although seagrasses may respond very rapidly to anthropogenic pressures, determining real changes to seagrass beds brought about by most disturbances is thought to take 5–10 years (Duarte & Kirkman, 2001). Seagrass bed monitoring should therefore be considered for a minimum of five years (post impact). The required length of any monitoring programme for a development or activity needs to be assessed in relation to the specific project and predicted impacts and should be set out in the monitoring plan. The frequency of sampling events and the requirement for ongoing monitoring should be reviewed on at least an annual basis. Any proposed changes to the monitoring programme should be documented in the project annual monitoring report if required and submitted for regulatory review each year.

4.4.7. Supporting environment

Any monitoring programme for seagrass beds needs to consider other parameters of the wider environment that may influence the presence and condition of the beds and the nature and quality of their associated species communities. Depending on the nature, scale and location of a proposed development or activity and its associated environmental pressures, these other environmental parameters may also require monitoring.

These requirements should be determined through assessment of the likely impact pathways from a proposed development or activity. Relevant environmental parameters could include elements such as patterns of sediment transport or the hydrodynamic regime within the survey area. These requirements are outside the scope of this guidance document but are identified here as they may need to be incorporated into a monitoring programme. If you need to undertake any survey or monitoring work in relation to physical processes, you may find it useful to refer to Brooks *et al.* (2018) which provides guidance on survey and monitoring requirements in relation to Environmental Impact Assessment for major development projects.

Any requirements for monitoring of the supporting environment should be described in the monitoring plan.

5. Survey and monitoring methods and analysis

5.1. Field methods

A range of survey methods can be appropriate for survey and monitoring of seagrass bed parameters/indicators. The considerable amount of literature on the methods has been a subject of a number of comprehensive reviews (Borum *et al.*, 2004; Jackson *et al.*, 2013; McMahon *et al.*, 2013; Hossain *et al.*, 2014).

The main options include:

- Phase I walkover survey and habitat mapping (intertidal beds)
- Aerial surveys / Unmanned Aerial Vehicle (UAV) (intertidal and subtidal beds)
- manta-tow / systematic sampling / diver swims (subtidal beds)
- acoustic survey (such as side scan sonar, sediment imaging sonar, single beam and multibeam echosounders) for habitat mapping (subtidal beds)
- quantitative sampling (for example, quadrats) (intertidal and subtidal beds)
- underwater image survey (such as drop-down video and still images) (subtidal beds
- dive survey for quantitative and semi-quantitative sampling (subtidal beds).
- other sampling for fine scale or environmental parameters/indicators (intertidal and subtidal beds)

These methods are discussed in further detail below, with respect to the parameters/indicators that can be surveyed using these approaches. In practice, the suitability of each of the methods will depend on the specific seagrass bed(s) to be assessed, the project scale and the predicted impacts. As such, you might want to consult established experts in the field of seagrass ecology to help you with the selection of methods employed. When impact and/or control beds are located within marine protected areas and WFD waterbodies, using the same methods as those used in the statutory feature condition monitoring will enable comparison with existing data sets.

The JNCC Marine Monitoring Method Finder, a web-based information hub, has been developed to provide a single point of access to the numerous guidance documents and tools generated both within and outside the UK and can be used in conjunction with this document to assure a consistent approach to data collection and analysis.

5.1.1. Seagrass bed parameters

Non-invasive sampling methods

5.1.1.1. Bed extent and fragmentation

A variety of methods are available for mapping the extent and fragmentation of seagrass beds (Jackson *et al.*, 2013; Hossain *et al.*, 2014; Duffy *et al.*, 2016).

Intertidal beds

Phase I mapping

Most intertidal beds can be mapped using established Phase I habitat mapping methods using a hand-held GPS to track the perimeter of beds on foot (Wyn *et al.*, 2006). Due to issues relating to surveyor bias and overestimation of extent when employing this method (Brazier, 2013; Moore, 2010), efforts have been made to employ alternative remote sensing techniques for mapping intertidal habitats around Wales (Davies & Newstead, 2013; Brazier, 2013; Ocean Ecology Limited 2016c).

Unmanned Aerial Vehicle (UAV mapping)

It is recommended that UAV mapping techniques, combined with ground-truthing observations (i.e. quadrat sampling – see below), are employed to measure extent and fragmentation of seagrass beds whenever possible. General guidance on UAV mapping techniques is provided in Kakaes *et al.* (2015) whereas specific UAV methodologies for, and the challenges faced when mapping coastal habitats are detailed in Jaud *et al.* (2016) and Duffy *et al.* (2017; 2018).

Remote sensing and manual delineation of coastal habitats through photointerpretation is becoming widely used by researchers, as it enables rapid mapping of sensitive species and habitats without disturbing them (McEvoy *et al.*, 2016). Historically this has been achieved using imagery derived from satellites (Komatsu, 2015) and/or light aircrafts. Developments in Unmanned Aerial Vehicle (UAV) technology now means that remotely sensed imagery can be easily obtained at high temporal frequencies and substantially lower cost than aircraft or satellite derived imagery. As they are not subject to the same regulations as aircraft, UAVs can be flown at low altitude, which is crucial to improve the resolution and accuracy of the data (Jaud *et al.*, 2016). This also means that mapping surveys can be more flexible and undertaken at short notice.

In the UK, UAV mapping methods have been demonstrated as a very effective and low cost option for rapidly mapping the extent of intertidal seagrass beds (Pratt, 2016) and have been employed to successfully map the extent of the largest seagrass bed in Wales (Ocean Ecology Limited, in prep). Examples of outputs from UAV mapping are shown in Figure 3.

As the majority of UAV platforms are capable of collecting elevation data alongside high-resolution photographs, UAV extent mapping also offers the potential for low cost topographic monitoring of intertidal areas (Jaud *et al.*, 2016; Ocean Ecology Limited, 2016b) including seagrass beds potentially subject to sedimentation and/or erosion. Furthermore, as seagrass beds require shallow, clear and sheltered waters, UAVs can also be used to map the extent and fragmentation of subtidal beds when conditions allow (i.e. low turbidity and flat calm sea state). For beds occurring on light, sandy bottoms, the outer perimeters can easily be distinguished.

As with satellite and aircraft derived imagery, UAV mapping outputs require ground-truthing by direct field observations but the ground-truthing effort can be much reduced due to the greater resolution of the outputs. These ground-truthing observations are essential to make sure that other underwater features such as macroalgae, bedrock reefs or mussel beds are not mistakenly identified as seagrass coverage.

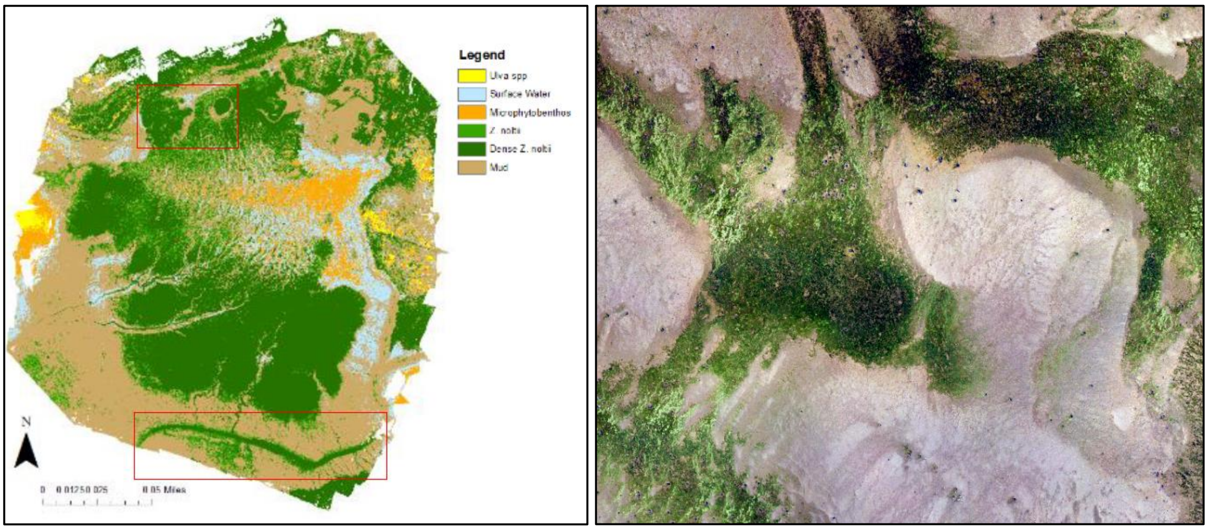

Figure 3. Orthomosaic output of seagrass bed mapped using a UAV in Milford Haven, West Wales (left) (taken from Pratt, 2016). High-resolution UAV imagery of part of the Welsh Grounds seagrass bed in the Severn Estuary, south Wales (right) (captured by Ocean Ecology Limited and © Tidal Lagoon Power).

Subtidal beds

In some cases, mapping of subtidal beds may be achieved using UAV methods however in most cases this will not be possible due to water depth, high turbidity and/or the presence of macroalgae. Mapping subtidal bed extent therefore requires the use of alternative methodologies (see McKenzie *et al.*, 2001).

Physical surveys

These alternative methods can include:

- manta-tow methods (English *et al.* 1997)
- using data derived from systematic grid or transect sampling to determine presence/absence (Burton *et al.*, 2015)
- diver swims of the bed boundary involving tracking divers via surface marker buoys or Ultra-Short Base Line (USBL) positioning systems

Acoustic surveys

Where logistical or resource constraints rule out these physical surveys (such as with large beds), boat-based acoustic surveys can represent a viable and cost-effective alternative. These methods can separate the distinctive acoustic signature (backscatter) returned by the air-filled lacunae of seagrass leaves from ambient background noise to map bed extent. A variety of acoustic methodologies have been used to map seagrass beds previously including digital echosounders (Populus & Perrot, 2007; Burton *et al.*, 2015), side scan sonars (SSS) (Montefalcone *et al.*, 2013 references therein) and sediment imaging sonars (SIS) (Lefebvre *et al.*, 2009) each having been shown to exhibit varying degrees of success (Hossain *et al.*, 2014) and provide varying levels of resolution (Kenny *et al.*, 2003).

Echosounder systems specifically designed for submerged aquatic vegetation mapping (e.g. BioSonics DT-X) have been successfully employed to rapidly map seagrass beds over several years within the Skomer MCZ resulting in outputs that matched very closely to the bed extent derived from diver swims (Burton *et al.*, 2015) (see Figure 4).

These single beam echosounder (SBES) systems do not however produce continuous data as required for software commonly used for assessing the fragmentation of seagrass beds (e.g. FRAGSTATS) (McGarigal & Marks, 1991). Advances in the multibeam echosounder (MBES) approaches mean that continuous seagrass bed data can be collected using small low cost vessels (Ocean Ecology Limited, 2014). Advances in post-processing methods also mean that bed extent and fragmentation can be mapped with high confidence (Hamana & Komatsu, 2016) particularity when refined using ground-truthing data (see Figure 4).

This can also be achieved using side scan sonar (SSS) systems that can differentially analyse the returning waves reflected by underwater features and produce two-dimensional images that can be 'stitched' together to create mosaics (i.e. continuous data) of seagrass beds and surrounding areas. These mosaics can then be 'draped' over Digital Terrain Models (DTMs) computed from MBES data for detailed interpretation. This method has been used widely to map seagrass beds (Siljeström *et al.*, 1996; Montefalcone *et al.*, 2013) and should, alongside MBES systems, be given due consideration when other lower cost methods cannot be utilised.

Detailed guidance on the specific methodologies for using acoustic mapping systems is provided in the MESH Recommended Operating Guidelines (ROG) (Hopkins, 2007; Populus & Perrot, 2007; Henriques *et al.* 2012), and in other generic guidance for conducting marine geophysical surveys (Plets *et al.*, 2013).

Although acoustic methods can be very effective for rapidly mapping seagrass bed extent it is also imperative that appropriate ground-truthing is undertaken to increase the confidence in the classification of seagrass and non-seagrass areas particularly as dense macroalgae can in some situations produce similar acoustic returns to seagrass leaves. Ground-truthing is also particularly important for accurately delineating the <5 % coverage areas commonly associated with the periphery of seagrass beds.

For all remote methods of mapping seagrass beds the error associated in the classification of the bed and the minimum detectable difference (MDD) must be carefully considered, especially when utilising modelling methods. Ground-truthing is key in minimising this error and maintaining an acceptable MDD, however the number of ground-truthing locations required to confidently detect an appropriate level of change (some recommend 10 %) may, in some cases, be prohibitively high and mean that more time-consuming methods (for example, diving) may be a more desirable option in terms of accuracy and cost (Schultz *et al.*, 2015).

5.1.1.2. Percentage seagrass cover

Seagrass cover should be derived through *in situ* estimation of % cover of quadrats by surveyors on foot (intertidal) or divers, snorkellers or snorkeller drop-down camera imagery (subtidal) positioned on a systematic grid (see section 4.4.5). Where possible it is advised that guides to % cover standards are used to accurately assign estimations and reduce observer bias (see McKenzie, 2003). Alternatively, % cover of pan-view quadrat photos/seabed images can be estimated using image analysis software packages, however caution is advised with the use of such software as these methods can in some cases lead to highly variable results at low % cover values (see 5.2.4.).

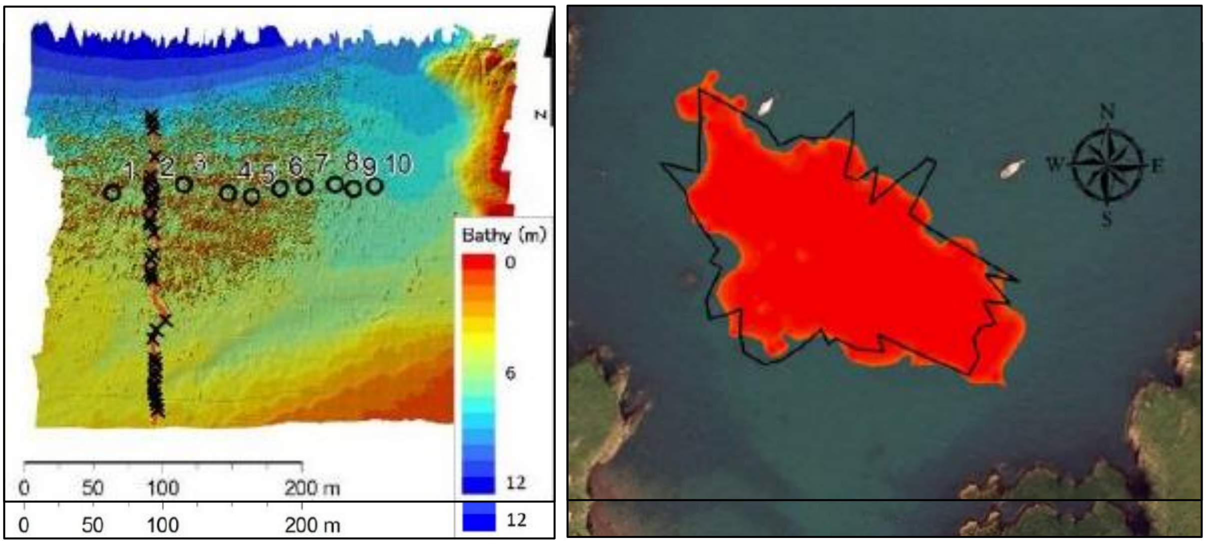

Figure 4. High-resolution bathymetry and relative abundance of subtidal seagrass classified with 95 % confidence interval using a MBES system (left) (taken from Hamana & Komatsu, 2016). Extent of subtidal seagrass mapped in the Skomer MCZ using a BioSonics DT-X echo sounder system (red) and compared to the boundary derived from a diver swim of the bed extent (black line) (right) (Burton *et al.*, 2015).

A quadrat size of 0.25 m^2 is commonly used for percentage cover estimations within MPAs around Wales (Boyes *et al.*, 2009; Duggan-Edwards & Brazier, 2015; Burton *et al.*, 2015) and other seagrass assessments (Jones & Unsworth, 2016). However, larger quadrat sizes (e.g. 1 m^2) are also used for some ongoing WFD monitoring programmes (for example, the Welsh Grounds bed in the Severn Estuary). Some UK subtidal seagrass surveys (Milford Haven and Isles of Scilly) have also used much smaller quadrats (0.125 m^2) (Bull *et al.*, 2012).

To ensure compliance with WFD and MPA methodologies, a multi-quadrat approach was used during a habitat characterisation survey related to the Tidal Lagoon Cardiff (TLC) development, employing both 0.25 m^2 and 1 m^2 quadrats (Figure 5). Measurements of WFD metrics were recorded in a 1 m^2 quadrat, then measurements for all key indicators were collected in a 0.25 m^2 quadrat that was positioned in the bottom left corner of the larger quadrat (Figure 5). Such approaches should be considered where consistency with WFD data is required. This type of approach may not necessarily be required for subtidal seagrass beds as no subtidal WFD seagrass bed monitoring is conducted in Wales. Whilst it is advisable to consider quadrat sampling sizes used previously, the final choice of quadrat size should consider the precision and accuracy of different quadrat sizes (see section 4.4.4). This will ensure that the size(s) selected will provide accurate measurements of % seagrass cover and any other indicators to be assessed at the same location (see below).

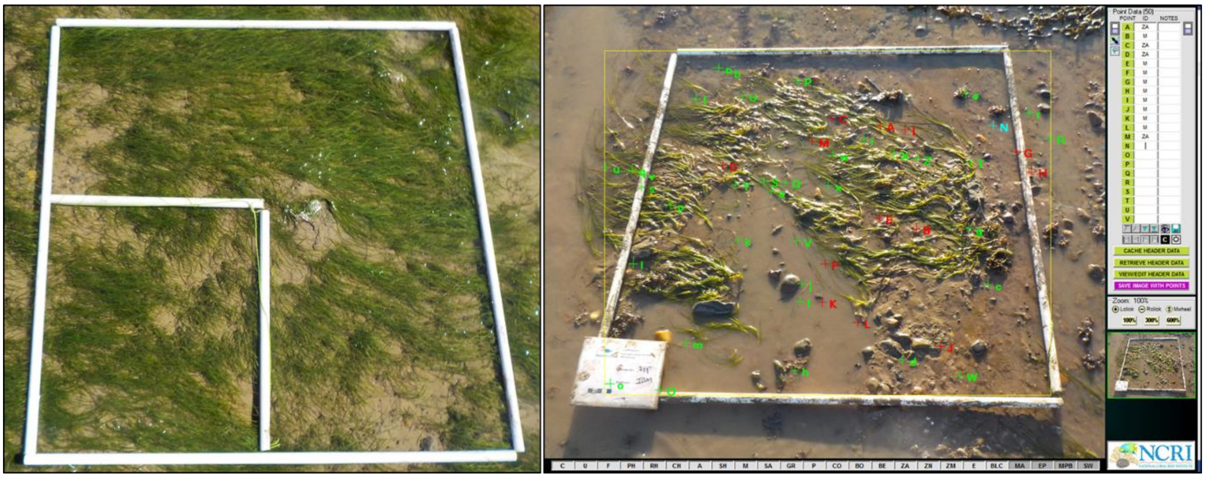

Figure 5. ‘Multi-quadrat’ approach used for measuring intertidal seagrass bed indicators consistent with WFD and MPA approaches in the Severn Estuary (left). Screen shot of % cover estimation analysis using Coral Point Count with Excel extensions (CPCe) software (right). Images captured by Ocean Ecology Limited and © Tidal Lagoon Power.

5.1.1.3. Seagrass species composition

Seagrass species composition should be derived in line with the methods described above for % seagrass cover. A record of % coverage for the following taxa should be recorded at each sampling station:

- *Z. marina*
- *Z. noltei*
- *Ruppia* sp.[15]

If consistency with WFD data is required, *Z. angustifolia* should be treated separately to *Z. marina* (UKTAG, 2014). Percentage coverage for any macroalgae taxa should also be recorded if present.

5.1.1.4. Maximum depth of bed

Methods for measuring maximum depth of bed (or lower boundary on the shore for intertidal beds) will depend on the methods used for measuring bed extent and fragmentation (see above).

- **Intertidal beds mapped on foot**: take a series of fixes along the lowest part of the bed along the shore using a Real Time Kinematic (RTK) GPS device to measure elevation with high vertical accuracy[16]
- **Intertidal beds mapped using UAV methods**: the lowest part of the bed on the shore can be derived from Digital Elevation Models (DEMs) computed from the elevation data collected alongside the imagery. This will require RTK GPS-derived Ground Control Points (GCPs) to be collected and captured in the imagery to georeferenced the orthomosaic outputs (see Kakaes *et al.*, 2015)
- **Subtidal beds mapped by diver swims:** simple depth records of the bed surface along the lower boundary of the bed can be made by divers using depth gauges or dive computers with their positions determined via surface marker buoys or USBL

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[15] Identified to genus only due to the difficulty of species identification, as recommended by Foden & Brazier (2007).

[16] Several cm accuracy rather than the 2-3 m accuracy typical of standard handheld GPS devices.

positioning systems (adjusted to chart datum). When surface mapping methods are used instead of SCUBA (for example, manta-tows), snorkelling to the seabed may be required to collect depth measurements in the same manner.

- **Beds thought to be particularly susceptible to changes to their maximum depth**: the possibility of monitoring concrete markers or iron screw anchors positioned along the lower margin of the bed should be considered (see methods described for the PMN (Boudouresque *et al.*, 2000; 2012) and SeagrassNet global monitoring network).
- **Beds mapped using acoustic techniques**: accurate depth measurements should be derived from the resulting outputs (Luzzu *et al.*, 2014)

5.1.1.5. Shoot density (no./m^2)

Shoot[17] density should be derived by counting the number of shoots within pre-defined areas (for example, quadrats[18]) (Duarte & Kirkman, 2001) positioned on a systematic grid[19].

- **Intertidal beds**: can be achieved by field surveyors on foot.
- **Subtidal beds**: can be measured by divers/snorkellers or by examining high-definition drop-down camera imagery or, when all other methods are not possible, by examining shoots collected in grab samples[20]

5.1.1.6. Wasting disease prevalence (% infection) and epiphytic cover

An average score for epiphytic cover and wasting disease prevalence (Figure 6) should be derived for each sampling station by assigning a score to a random selection of leaves (preferably 5 or more) from each quadrat, based on % epiphytic cover (Table 4) and % leaf coverage of disease lesions (Table 5) (Burdick *et al.*, 1993).

- **Intertidal beds**: can be achieved by field surveyors on foot.
- **Subtidal beds**: can be measured in the field by divers or snorkellers or by examining drop-down camera imagery if it is of suitably high resolution. When all other methods are not possible, shoots collected in grab samples could be examined[20]

Table 4. Scoring system for determining epiphytic % coverage of seagrass leaves (adapted from Irving & Worley, 2000)

Parameter	Score				
	0	1	2	3	4
Epiphytic growth of *Ulva* spp.	0 %	<10 %	10-50 %	50-80 %	>80 %
Other epiphytic cover	0 %	<10 %	10-50 %	50-80 %	>80 %
Degree of leaf deterioration	0 %	<10 %	10-50 %	50-80 %	>80 %

[17] Section of the plant that protrudes from the sediment, which splits into separate leaves.
[18] Size to be determined on a project and pressure specific basis (see Section 0).
[19] Sampling interval (i.e. grid size) to be determined on a project- and pressure-specific basis (see 0).
[20] In general, NRW would advise against the use of grab sampling due to the potential impact of this on the bed. Any such sampling would need to be assessed and fully justified before any survey or monitoring work was undertaken

Table 5. Five-point scale for determining seagrass leaf disease prevalence (adapted from Burdick *et al.*, 1993)

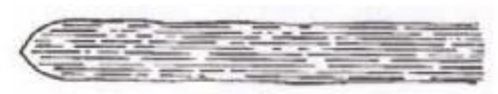
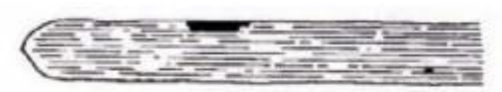
Score	Degree of deterioration	Percentage	Diagrammatic representation of score
0	No deterioration	0%	
1	Some deterioration apparent	<10%	
2	Up to half of leaf deteriorated	10 – 50%	
3	Over half of leaf deteriorated	50 – 80%	
4	Almost all of leaf deteriorated	>80%	

Figure 6. *Zostera marina* shoot showing both clean leaves, wasting disease and epiphyte cover (taken from Cook, 2011)

Invasive sampling methods

The following indicators should be measured using shoot samples collected at the level of the substrate within quadrats positioned on a systematic grid. When collecting shoots, take particular care not to disturb or damage the rhizomes or roots.

- **Intertidal beds**: shoot samples can be collected by field surveyors on foot
- **Subtidal beds**: samples can be collected by divers/snorkellers or, when all other methods are not possible, by the use of a grab sampler deployed from a boat[21]

Numerous research and monitoring programmes globally (including the highly successful long-term programme in the Isles of Scilly (Bull & Kenyon, 2015)) collect data by destructively sampling seagrass above ground tissue, with no evidence available to suggest that it causes a negative impact upon the bed as a whole. Some studies such as the SeagrassNET programme also collect complete cores that include the rhizome. This sampling method, if conducted in moderation in healthy beds, has also been found to result in no lasting impact (Short *et al.*, 2014).

5.1.1.7. Epiphytic composition and biomass
Trained taxonomists should derive the epiphytic composition by microscopic examination of all shoots collected at each sampling station (see Section 0). The cumulative dried biomass should then be measured for each of the epiphytic taxa identified (see methods described in Section 0).

'Plant-scale' morphological indicators (indicators of short-term change)

5.1.1.8. Leaf length & width
Leaf length and width measurements should be derived by measuring the longest intact leaf of all shoots collected at each sampling station (see methods described in Section 0).

5.1.1.9. Shoot biomass (above ground biomass)
Shoot biomass should be derived by measuring dried leaf biomass of all shoots collected at each sampling station (see methods described in Section 0).

'Plant-scale' morphological indicators (indicators of short-term change)

5.1.1.10. Shoot C:N:P ratios and tissue nutrients (δ15N)
Shoot C:N, C:P and N:P ratios, as well δ15N, should be determined from ground seagrass leaf tissue derived from all shoots collected at each sampling station (see methods described in section 5.2.5).

Environmental indicator sampling

[21] In general, NRW would advise against the use of grab sampling due to the potential impact of this on the bed. Any such sampling would need to be assessed and fully justified before any survey or monitoring work was undertaken

5.1.1.11. Light and temperature

Ambient light (measured as Photosynthetic Active Radiation (PAR)) and temperature should be measured using calibrated loggers that can be fitted with wiper units to prevent sediment build up and fouling (Collier *et al.*, 2012; Kilminster & Forbes, 2014; Chartrand *et al.*, 2016). These loggers should be buried into the sediment such that the sensors are positioned at approximately seagrass canopy height.

- **Intertidal beds**: these can be deployed by field surveyors on foot.
- **Subtidal beds**: the loggers can be deployed by divers/snorkellers. Additional PAR loggers should be located out of the water within the general vicinity of each bed to record the daily irradiance from the sun without influence from the water column.

Other indicators

5.1.1.12. Seed bank and seed viability

The seed bank of each bed should be measured by collecting suitably sized sediment core samples (see section 4.4.4) (typically 50 mm diameter and 10 mm depth, see McKenzie (2003), Jarvis *et al.* (2014; 2015)) at each sampling station, for subsequent enumeration and viability testing (see methods described in section 5.2.6).

- **Intertidal beds**: can be collected by field surveyors on foot.
- **Subtidal beds**: samples can be collected by divers/snorkellers by either emptying sediment cores into a mesh bag or by plugging the corer with a cap once the sample is collected (McKenzie, 2003). When all other methods are not possible, samples can be collected by the use of a grab sampler deployed from a boat[21]

5.1.1.13. Flowering intensity

To assess flowering intensity, individual shoots should be inspected for the presence of a reproductive shoot. These are clearly visible during the summer months; however, their density can be patchy and low within a bed. Sampling therefore needs to occur at large scales in order to determine the density of flowering shoots.

- **Intertidal beds**: shoots can be assessed by field surveyors on foot.
- **Subtidal beds**: shoots can be accessed by divers/snorkellers or, when all other methods are not possible samples can be collected by the use of a grab sampler deployed from a boat[21]

The state of the flowering and seed production can be assigned to each reproductive shoot according to De Cock (1980)

5.1.1.14. Sediment composition

Sediment composition should be measured by collecting suitably sized sediment samples (see section 4.4.4) at each sampling station (separate to those collected for seed bank and seed vitality assessment) for subsequent Particle Size Distribution (PSD) analysis.

- **Intertidal beds**: can be collected on foot by field surveyors using a corer
- **Subtidal beds**: samples can be collected by divers/snorkellers by plugging the corer with a cap once the sample is collected (McKenzie, 2003) or, when all other methods are not possible samples can be collected by the use of a grab sampler deployed from a boat[21]

Sedimentation can be assessed in the field by surveyors or divers/snorkellers, or by collecting and interpreting drop-down camera imagery at each sampling station and noting any evidence of sediment deposition on the seagrass shoots within suitably sized quadrats, that can then be compared over time (see section 4.4.4). Alternatively, Sediment Profile Imaging (SPI) systems can be employed to collect detailed images of the sediment profile of the upper surface sediments (see Germano *et al.*, 2011). This can provide a useful insight into the redox potential discontinuity depth layer via differences in optical reflectance (Carey *et al.*, 2015), as well as estimations of sediment grain size, seagrass root depth and root biomass.

5.1.1.15. Associated species composition and diversity (benthos)

The composition and diversity of benthic taxa should be measured by collecting sediment samples at each sampling station for subsequent macrobenthic analysis (see methods described in section 5.2.6).

- **Intertidal beds**: suitably sized core samples (see section 4.4.4) can be collected by field surveyors on foot.
- **Subtidal beds**: cores can be collected by divers/snorkellers by plugging the corer with a cap once the sample is collected (McKenzie, 2003) or, when all other methods are not possible samples can be collected by the use of a grab sampler deployed from a boat[21]

5.1.1.16. Associated species composition and diversity (epibenthos)

The composition and diversity of epibenthic taxa should be measured at each sampling station using standard methodologies for *in situ* recording or by collecting high resolution seabed imagery (subtidal) for subsequent interpretation (see methods described in section 5.2.4). All visible epibenthos within suitably sized quadrats can be recorded by field surveyors on foot (intertidal) or divers/snorkellers (subtidal).

5.1.1.17. Associated species composition and diversity (motile fauna)

Motile faunal assemblages within seagrass can be assessed using a range of methods. In shallow subtidal beds adjacent to soft shores, large beach seines (30 m) are the most suitable method. They allow data to be collected on both cryptic and large species and an accurate assessment of fish size (enabling nursery function to be established). Fyke nets and Underwater Visual Census can also be effective techniques for targeted surveys, but these are not effective in assessing all species.

The use of beam trawls is not advised due to the impacts it can have on the seagrass. Lightweight trawls have been used in the UK at sites where other methods have not been deemed suitable (Jackson *et al.*, 2006) but for development-related survey and monitoring NRW advise against the use of such approaches. Similarly, gill nets can be very effective at assessing larger predatory fish on beds where beach seines cannot be deployed, but these are destructive and are not advised. It is advised that where possible (visibility allowing) motile fauna on these beds are assessed using Baited Video techniques (see Unsworth *et al.*, 2014; Peters *et al.*, 2015; Hinder *et al.,* 2013; Griffin *et al.*, 2016).

5.1.2. Fieldwork quality control

All fieldwork should be carried out by experienced field scientists, with necessary health and safety provisions, and should observe the following points:

- there should be full sample tracking documentation and field notes for the sampling procedures

- sample collection and handling during surveys must conform to the requirements of subsequent analytical analyses
- where samples are being collected for laboratory analysis, care is needed to limit degradation in transit. A portable freezer may be necessary for preserving shoot samples in the field.
- all processes should be witnessed and documented, with documentation retained after the surveys are completed

Across all methods it is important to obtain accurate, detailed records and to retain records/data for quality control/assurance procedures. All data need to be collected using standard field data procedures (for example, time, date, observer, GPS location, site code, sample code of sample). Where samples are collected for laboratory analysis these require consistent labelling that is suitably robust (see McKenzie, 2003).

5.1.2.1. UAV mapping

UAV surveys should be undertaken by qualified UAV Pilots operating under the current CAA rules (for example see Cunliffe *et al.*, 2017). If mapping a particular seagrass bed requires the UAV to fly beyond Visual Line of Sight (VLOS) (for example, when mapping extensive beds), the UAV operator will require CAA permission to conduct Extended Visual Line of Sight (EVLOS) operations. Alternatively, extensive beds may need to be mapped during two or more flights.

Images captured by the UAV should have sufficient forward and lateral overlap so that post-processing software can identify common points between each image. Flight transect plans should allow for this. A forward overlap of 70–80 % and lateral overlap of 60–80 % is generally recommended (Kakaes *et al.*. 2015), but a higher or lower overlap may be appropriate for different seagrass beds. In practice the chosen overlap will be site- and bed-specific. For example, heterogeneous beds will require less overlap, whereas relatively featureless beds will need greater overlap.

The highest possible resolution[22] and accuracy[23] should be aimed for in order to delineate beds as precisely as possible. As with overlap, this will be constrained by bed size and may need to be traded against ensuring entire beds can be surveyed within the project resource and tidal constraints. The survey objectives will also govern the accuracy requirements. For example, habitat characterisation surveys are not likely to require high vertical accuracy, whilst monitoring surveys aiming to assess deposition or erosion will require the use of RTK-derived GCPs to detect fine scale changes in substrate elevation (i.e. several centimetres).

Flights should preferably be carried out in early morning or late evening during cloudy weather, if compatible with tide times (Jaud *et al.*, 2016; Duffy *et al.*, 2017). This will avoid sun glints and the effects of brightly illuminated water-saturated sediments. Surveys should be undertaken when the maximum extent of the intertidal bed has drained which can be achieved by starting close to the time of low water and working up the shore ahead of the flooding tide. Survey dates should be selected for when the low tide level is sufficient to exposure as much as possible of the seagrass bed present on the shore. When repeat survey events are undertaken, differences in low tide height can lead to

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[22] Measured as Ground-Sampling Distance (GSD).
[23] Measured as Root Mean Square Errors (RMSE).

differences in extent measurements. Subsequent analysis of repeat surveys needs to standardise the seaward extent to the least low tide.

For subtidal beds, surveys are best undertaken over low tide periods to minimize the depth of the water overlying the bed(s).

Table 6. Summary of recommended quality standards for UAV mapping of seagrass beds.

Requirements	Forward overlap	Lateral overlap	Resolution (GSD)	Accuracy (RMSE)
CAA PfCO and UAV pilot qualification (RPQ). Further CAA permission for EVLOS operations.	70-80 %	60-80 %	<5 cm / pixel	5-10 m or <5 cm*

*High accuracy will be required when monitoring bed elevation requiring use of RTK-derived GCPs to georeference orthomosaic outputs.

5.1.2.2. Acoustic mapping

Acoustic data collection requires advanced survey instruments together with a sound technical knowledge of their operation and the calibrations required to obtain high quality data. These surveys should therefore be undertaken by appropriately qualified and experienced personnel, preferably recognised by a professional institute (International Hydrographic Organization (IHO)) in line with relevant guidance (Populus & Perrot, 2007; Henriques *et al.*, 2012; Plets *et al.*, 2013; IMCA, 2015). For echo sounder surveys it is critical to measure movements of the vessel accurately and to understand the velocity structure of the water column in order to georeference the sounding footprint on the seafloor. This should be achieved by dimensional surveys, patch tests, and sound velocity corrections (see IMCA, 2015). Global Navigation Satellite System (GNSS) receiver verifications and Vertical Offshore Reference Frame (VORF) checks should also be undertaken to verify the positioning against local Bench Marks (BM) and verify VORF models and real-time height reductions.

5.1.2.3. Seabed imagery collection

Quality standards for seabed imagery collected on subtidal seagrass beds using drop-down and/or towed camera systems should align with those set out in the NE Atlantic Marine Biological Quality Control (NMBAQC) scheme Operational Guidelines for Epibiota Remote Monitoring (Hitchin *et al.*, 2015).

5.1.2.4. Environmental sampling

Photosynthetically Active Radiation (PAR) sensors are low-cost and useful for applications where relative differences in light are more important than absolute readings. Sensors should be programmed to accumulate pulse data over set intervals (for example, 10 minutes), and to record the accumulated value at the end of the scan time. This data integration compensates for the high degree of fluctuation in solar irradiance. Loggers that are not factory-calibrated (e.g. HOBO and Odyssey loggers) should be calibrated in line with the methods discussed by Kilminster & Forbes (2014) and Long *et al.* (2012). Keeping sensors in place can be difficult, particularly in highly tidal areas and therefore it can be prudent to attach the sensors to cement blocks either on the surface or buried in the sediment.

5.1.2.5. Diving and snorkelling
Seagrass bed surveys should adhere to the guidelines for dive surveys provided in Procedural Guideline 3-3 of the Marine Monitoring Handbook (Davies *et al.*, 2001).

5.2. Analytical methods
5.2.1. GPS tracks and fixes
Positional fixes collected during bed perimeter tracking on foot or via manta-tows and/or diver swims should be downloaded and plotted in standard GPS mapping software immediately after each survey. The resulting files should be imported into a Geographical Information System (GIS) and converted to relevant mapping formats (such as .shp, .tab) for sense checking against available base maps (for example, aerial imagery and admiralty charts).

5.2.2. UAV data
Imagery from UAV extent mapping surveys should be ‘stitched’ together to generate orthomosaic outputs for each bed, using widely available processing software packages.

For small continuous beds these orthomosaics may be sufficient for rapidly establishing the <5 % and >5 % shoot coverage boundaries (as mapped for WFD purposes) when combined with ground-truthing.

For large patchy beds, manual delineation in GIS is impractical and the orthomosaic output can instead be autonomously ‘zoned’ using a variety of image classification methods (Dugdalle, 2007; Meyer, 2008; Pratt, 2016). These include methods that use red, green and blue (RGB) values collected in standard three-band imagery (for example, the Vegetation Adjusted Reflectance Index (VARI) – see Gitelson *et al.* (2002)) and methods that require the use of specialised cameras for the calculation of the Normalised Difference Vegetation Index (NDVI) (Barillé *et al.*, 2010; Valle *et al.*, 2015). This combines the reflectance of the red and infrared red colour bands (Tucker, 1979) to assess the quantity of green biomass.

Regardless of the method used, the classifications should be fine-tuned by an appropriate interpretation of the ground-truthing data collected from each bed, as it is often difficult to distinguish between seagrass and other vegetation found on the shore or in shallow coastal waters (for example, microphytobenthos films and/or macroalgae). In most cases, the assessment of indicators using quadrat and/or seabed imagery will provide the necessary information for appropriate ground-truthing. Additional targeted sampling may be required if an assessment of such indicators is not included in the survey programme.

With the rapid advance of UAV mapping technology and processing software, it is likely that methods for delineating seagrass beds from UAV-derived imagery will be continually improved and updated. Where possible, the equipment, methods and ‘rules’ used for mapping and classifying beds should be kept consistent throughout entire monitoring programmes.

5.2.3. Acoustic data
Processing of acoustic data can be complex and will vary markedly depending on the method of collection. A variety of guidance is available (Populus & Perrot, 2007; Henriques *et al.*, 2012; Plets *et al.*, 2013; IMCA, 2015) and should be followed where possible. Other chapters of this guidance (GN030e (horse mussel *Modiolus modiolus*), GN030f (*Sabellaria*

reefs) and GN030g (subtidal habitats)) provide guidance on the interpretation of acoustic data including for specifically mapping biogenic habitats. All processing should be undertaken to International Hydrographic Organisation 1A standard (IHO, 2008).

Single beam echo sounder data

The data collected from single beam echo sounder systems can be analysed using specialist tools to output metrics that can be useful for seagrass bed mapping. For example, Burton *et al.* (2015) used the 'macrophyte' tool in Sonar 5-pro (Balk & Lindem, 2014) to output metrics on Percentage Area Inhabited (PAI) and Bioheight (m) within georeferenced 5 m Ecological Sampling Units (ESU). This produced bed extent outputs that matched very closely to the bed extent derived from diver swims. This mapping technique does rely heavily on interpolation between the ESUs in GIS (for example, Inverse Distance Weighting (IDW)), meaning the outputs should be treated with some caution, especially when assuming continuity in the data for assessing fragmentation of beds using tools such as FRAGSTATS (see below).

Multibeam Echo Sounder (MBES) data

The data collected from MBES systems are much more complex, given that it can provide full bottom coverage. The data require a great deal of post-processing to apply positional, tidal and sound velocity corrections before meaningful interpretations can be made (see IMCA, 2015). Once applied, continuous Digital Terrain Models (DTMs) can be interrogated in GIS alongside ground-truthing information to classify areas of seagrass beds. Because seagrass shoots and leaves reflect beams emitted from MBES transducers, the depth range of the reflected beams can be used as a proxy for the difference between seagrass height and the seabed (Di Maida *et al.*, 2011) and therefore used to classify areas of seagrass and/or bare substrate (see methods described in Hamana & Komatsu (2016). Unlike data derived from single beam echo sounders, the DTM outputs are normally continuous (providing 100 % coverage is achieved) and interpolation is not necessary. This means the outputs are suitable for assessing fragmentation of beds using tools such as FRAGSTATS (see below).

Side Scan Sonar (SSS) data

SSS data can be processed in real-time to provide field surveyors with composite mosaics. This is suitable for initial quality control and preliminary on-board interpretation. However, like MBES-derived data, side scan sonars are susceptible to interferences from a number of sources (such as vessel noise) meaning the recorded raw data requires post-processing before seagrass bed extent classification is attempted (Henriques *et al.*, 2012; Plets *et al.*, 2013).

Using side scan imagery interpretation in the context of seabed habitat mapping is a very complex task. In general, image interpretation is an open subject of research and there is no clearly defined 'best practice' (Blondel, 2009). Seagrass bed delineation from SSS usually involves manual tracing of areas thought to represent seagrass in GIS (Montefalcone *et al.*, 2013). To aid the interpretation, ground-truthing information should be classified into categories (e.g. <5 % coverage, >5 % coverage, substrate, macroalgae etc.) and overlain on the SSS mosaic. If available, the SSS mosaic should be 'draped' over MBES DTMs to further aid classification (Henriques *et al.*, 2012).

FRAGSTATS

The FRAGSTATS software (McGarigal & Marks, 1994) can be integrated with GIS and measures numerous metrics at the landscape (i.e. the whole seagrass bed location, across habitat types), class (habitat specific, for example, for seagrass) and patch scales (an individual separated patch of seagrass). This analysis has commonly been applied to evaluating the fragmentation of seagrass beds (Abadie *et al.*, 2015; Farina *et al.*, 2016) and should be considered if bed fragmentation is selected as an indicator of bed condition as part of an EcIA survey programme.

5.2.4. Quadrat and seabed imagery

Image analysis tools

Estimations of percentage cover taken in the field can vary substantially between surveyors (Wells, 2013). This observer bias can either be controlled using training exercises and reference cards or using image analysis tools. Percentage seagrass cover within each quadrat can be derived through post-survey analysis of plan-view quadrat photographs collected either by field surveyors and/or drop-down cameras. This can be undertaken rapidly using open source image analysis software packages such as Coral Point Count with Excel extensions (CPCe) software (Kohler & Gill, 2006) or ImageJ (Schneider *et al.*, 2012). CPCe in particular is widely used for monitoring seabed habitats throughout the world (Tabuga *et al.*, 2016), including seagrass beds (Cardno, 2013; Kloedsin *et al.*, 2016). It provides an accurate and repeatable methodology for determining percentage cover from plan view photography. As it is based on a standardised set of categories defined by the user, it substantially reduces the inherent subjectivity of analyst-derived estimates or estimates made by field surveyors. A set of categories can be defined for specific seagrass beds to provide a repeatable method for detecting change in percentage cover over time. Percentage cover of seagrass can then be estimated by assigning categories to a set of points randomly overlain across each quadrat image.

Caution is advised with the use of such software as these methods can in some cases lead to highly variable results at low % cover values. The minimum number of points necessary to ensure accurate percentage cover estimation per image (the Optimal Point Count (OPC)) should therefore be determined by a preliminary precision analysis on a subset of representative images from each bed surveyed (see Pante & Dustan, 2012). The CPCe software can then be used to produce a data matrix suitable for statistical analysis. Photo interpretation of percentage cover should be carried out by trained CPCe operators and overseen by experienced scientists. All CPCe analysts should be trained using photographic reference images. Photo interpretation and counts should be verified by a second experienced scientist on 10% of images. Where an error rate exceeds 10%, all images within that batch should be re-analysed.

All seabed imagery analysis should be undertaken by experienced ecologists and should, where relevant, follow the NMBAQC / JNCC epibiota remote monitoring interpretation guidelines (Turner *et al.*, 2016).

5.2.5. Shoot samples

The methods outlined below are based on those detailed in Jones & Unsworth (2016) and only apply when plant-scale indicators are to be assessed:

remove collected shoots (from either hand or grab sampler) from sample containers and rinse thoroughly in fresh water to remove salt water, sediment and detritus

measure leaf length and width from the longest intact leaf of each shoot:

- Length: Use a rule to measure to the nearest 1.0 mm from the meristem to the top of the leaf
- Width: Measure with callipers to the nearest 0.05 mm

record the number of leaves per shoot (if not already derived *in situ*) for all shoots collected. Trained taxonomists should examine each leaf under a stereomicroscope to identify all epiphytic taxa present.

carefully scrape all epiphytes from both sides of the leaf using a scalpel or microscope slide and pool

dry the cleaned leaf sections at 60°C for 24 hours and then ground using a pestle and mortar until homogeneous. The dry leaf mass should then be recorded using a balance accurate to four decimal places

the pooled epiphytes should also be dried at 60°C (separately) for 3-4 hour periods until they are of constant weight, then record the dry mass as described above

When seagrass shoot C:N:P ratios are to be assessed, samples should be analysed for % N and % C by weight by using a continuous flow isotope ratio mass spectrometer. Total P can be determined by inductively coupled plasma atomic emission spectrometry. Detailed method descriptions are provided in Fourqurean *et al.* (1997) and Jones & Unsworth (2016).

5.2.6. Cores and grab samples

Seed bank

Samples collected to assess the seed bank of seagrass beds should, where possible, be separated into three sections based on sediment depth (e.g. 0-20 mm, 20–-50 mm and 50-100 mm) (note that this may not be possible for grab samples). Each section should be washed over a series of sieves with fresh water to separate out large fractions (shells, detritus etc.) and smaller fractions containing the seeds (0.5 mm-1mm) (Jarvis *et al.*, 2014; 2015). The 0.5 mm -1 mm fractions should be inspected for seeds using a stereomicroscope. All seeds should be identified and measured using microscope camera software (to the nearest 0.01 mm). A catalogue of seed micrographs should also be compiled. Density data should be reported as mean ± standard error for the total number of seeds per m^2 per station, and as a percentage of seeds in each depth category per station.

Seed viability

Seed viability should be tested within one week of collection and storage, using tetrazolium chloride (Lakon, 1949; Conacher *et al.*, 1994); Sawma & Mohler, 2002). Seed embryos should be removed from their seed coats and soaked in a 0.5 % tetrazolium chloride solution for 48 hours before examination using a dissecting microscope. Seeds with a pink to brown stained cotyledon and axial hypocotyl should be counted as viable (Taylor, 1957; Harrison, 1993; Conacher *et al.*, 1994) (Figure 7). Viability data should be reported as mean ± standard error for the number of viable seeds per m^2, the percentage of viable seeds per sampling site, and as the percentage of viable seeds per depth category per station.

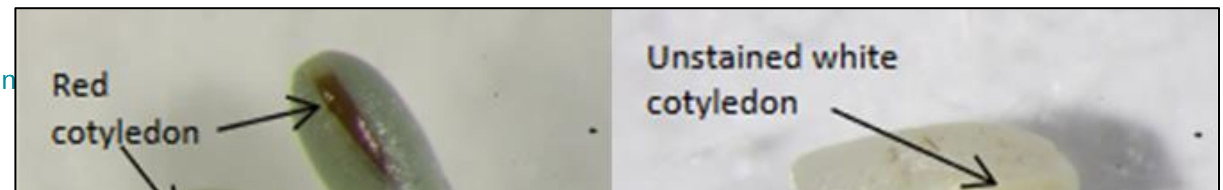

Figure 7. Examples of stained viable (left) and non-viable (right) *Zostera* sp. seeds using tetrazolium chloride (from Jarvis *et al.*, 2015).

Sediment composition
Core and/or grab samples collected to assess the composition of seagrass bed sediments should undergo PSD analysis. This should be undertaken by a laboratory participating in the Particle Size Analysis (PSA) component of the NMBAQC scheme and methods should follow those described in the NMBAQC Best Practice Guidance (Mason, 2016).

Associated communities (benthos)
Core samples collected to assess the composition of the benthic communities associated with seagrass beds should be sieved over a 0.5 mm sieve and undergo full macrofaunal analysis at a laboratory participating in the macrobenthic component of the NMBAQC scheme. Methods should follow those described in the NMBAQC Processing Requirement Protocol (PRP) (Worsfold *et al.*, 2010).

5.3. Data analysis and interpretation

5.3.1. Analysis requirements

Before conducting statistical analyses, it is important to understand why they are being conducted and what information is required, for example the data could be to inform an EcIA or could be used to test a specific hypothesis as part of a monitoring programme (see section 4.4.2). This understanding allows the most appropriate statistical test/methods for interpretation and analysis to be selected. For more details see the Introductory Chapter.

A detailed description of the main analytical methods and procedures that can be employed for analysing data are set out in Noble-James *et al.* (2017). In practice, the routines employed will be bed-specific and should be developed in consultation with an experienced statistician.

5.3.2. Habitat Characterisation and mapping

The key aim of the habitat characterisation data analysis is to provide the data outputs necessary to enable the subsequent interpretation required for EcIA and any associated assessments that are required such as Habitats Regulations Assessment and Water Framework assessment (see Guidance Note GN030, section 2.2).

Key outputs of habitat characterisation surveys for seagrass beds will include production of spatial maps of beds within the ZoI with details any other sampling outputs and photographs. Spatial data is often most usefully presented as detailed survey maps, typically using GIS software packages.

It will generally not be not necessary to undertake in-depth analysis of seagrass bed indicator data collected for habitat characterisation purposes. In most cases, simple interpretation using univariate statistics will be sufficient. Most importantly, any analysis should aim to present the data in the most suitable manner for assessing the likely impacts of the project/activity on seagrass beds within the ZoI, and for assessing potential impacts on any protected areas and/or WFD water bodies which overlap and/or are close by.

5.3.3. Monitoring

For monitoring, the statistical framework should be established at the survey design stage as this will inform decisions on appropriate effect sizes, sampling effort etc. (see section 4.4.3).

Monitoring data should be subject to in-depth statistical analysis and interpretation to test the hypotheses set out at the design stage. A plethora of suitable univariate and multivariate analysis and mapping techniques are available to achieve this and as a result those chosen are likely to vary markedly between projects. A full account of the proposed statistical tests to be used to monitor change should be set out in the project monitoring plan. Considerations for conducting statistical analysis for monitoring purposes are discussed further in the Introductory Chapter GN030-intro.

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