

ACA Pen Llŷn a'r Sarnau SAC

Prosiect Morwellt Porthdinllaen Seagrass Project

Opsiynau ar gyfer monitro morwellt tymor hir ym Mhorthdinllaen, Cymru

Options for long-term seagrass monitoring at Porthdinllaen, Wales

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Crynodeb Gweithredol

Y morwellt y cyfeirir ato'n gyffredin fel 'Gwellt y gamlas' (*Zostera marina* L.) yw un o'r unig ddau 'wir' rywogaeth o forwellt a geir yn y DU a chaiff ei gyfyngu'n bennaf i uchafswm o oddeutu 7m o ddyfnder dŵr (dan siart datwm). Y rheswm dros hyn yw ei ofynion uchel am olau fel organeb ffotosynthetig. Mae amcangyfrifon ar draws yr ystod o *Zostera marina* yn awgrymu ei fod angen rhwng 12 a 37% o dywyniad wyneb i oroesi yn yr hirdymor. Mae'r gofynion hyn am lawer o oleuni a natur fregus y planhigion hyn yn gwneud cynefin morwellt yn agored i effeithiau. Ceir dros 20 hectar o forwellt ym Mhorthdinllaen a ddiffiniwyd gan Gyfoeth Naturiol Cymru fel un sydd mewn cyflwr anffafriol. Y prif bryder am effaith anthropogenig ar forwellt ym Mhorthdinllaen yw'r difrod a achoswyd gan angorfeydd parhaol a blynyddol a osodwyd yn y morwellt. Ceir hefyd materion arwyddocaol sy'n ymwneud â difrod gan angorau a'r defnydd o gerbydau a chredir bod hynny'n difrodi'r morwellt. Oherwydd hyn, mae'r grŵp o awdurdodau perthnasol o ACA Pen Llŷn a'r Sarnau yn ymgymryd â phroses o gyd-reoli gyda budd-ddeiliaid er mwyn ceisio adfer y cyflwr gwarthus hwn.

Elfen allweddol o hyn yw creu rhaglen fonitro tymor hir o'r morwellt fydd yn helpu i ddeall effaith unrhyw gamau gweithredu rheoli yn y dyfodol, yn gweithredu fel rhybudd cynnar am unrhyw bryderon yn y dyfodol ac yn cyfrannu at adrodd am gyflwr yr elfen ACA. Mae'r adroddiad hwn yn adolygu'r opsiynau sydd ar gael ar gyfer datblygu rhaglen asesu a monitro morwellt o'r fath ym Mhorthdinllaen. Mae'n ystyried gwaith blaenorol a gynhaliwyd ym Mhorthdinllaen ac yn archwilio opsiynau ar gyfer monitro morwellt drwy ystyried rhaglenni presennol mewn rhannau eraill o'r DU a'r arferion gorau o fonitro morwellt yn fyd-eang. Caiff opsiynau eu hadolygu drwy archwilio dulliau samplo a metrics morwellt a rhinweddau perthnasol gwahanol rai.

Rydym yn cynnig yma bod rhaglen fonitro ym Mhorthdinllaen yn cael ei rhannu'n chwe elfen ar wahân, rydym yn cynnig bod y pump cyntaf yn hanfodol a'r chweched yn awgrymedig. Cynigir y rhain oherwydd eu bod yn casglu data cadarn gwyddonol, wedi'u profi'n dda ac yn gallu cael eu casglu heb ormod o offer arbenigol. Mae'r gallu ganddynt hefyd i gynnwys gwirfoddolwyr. Drwy greu rhaglen rhynglanwol ac islanwol, gall gwirfoddolwyr gyda - a heb - arbenigedd SCUBA gymryd rhan yn y rhaglen. Mae angen i raglenni o'r fath barhau i adeiladu ar y cydweithio da gyda Seasearch a datblygu cysylltiadau pellach gyda grwpiau myfyrwyr Prifysgol (e.e. Cymdeithas Bioleg Forol ym Mhrifysgol Abertawe).

- 1) Asesiad o <u>faint</u> y morwellt <u>islanwol</u> sy'n pennu presenoldeb neu absenoldeb a dyfnder morwellt dro ar ôl tro drwy'r bae yn defnyddio GPS. Gall yr astudiaeth ddefnyddio plymwyr rhydd (freedivers), fideo 'dropdown' ysgafn neu 'hand grab' ysgafn.
- 2) Asesiad gofodol o <u>faint</u> y morwellt <u>rhynglanwol</u> a phresenoldeb effeithiau. Byddai'r astudiaeth yn defnyddio arsylwyr yn cerdded gyda GPS sy'n cael ei ddal â llaw. Byddai amcangyfrifon dwysedd morwellt (shoot density) a data cysylltiedig yn cael ei gasglu.
- 3) Arolygon <u>manwl</u> yn seiliedig ar SCUBA <u>islanwol</u> sy'n asesu statws y morwellt mewn cwadratau wedi'u rhannu ar hap sy'n deillio o bwyntiau samplo morwellt a bennwyd ymlaen llaw wedi'u gwasgaru mewn dull haenedig drwy'r dolydd morwellt cyfan.
- 4) Arolygon <u>manwl</u> yn seiliedig ar SCUBA <u>rhynglanwol</u> sy'n asesu statws y morwellt mewn cwadratau wedi'u rhannu ar hap sy'n deillio o bwyntiau samplo morwellt a bennwyd ymlaen llaw wedi'u gwasgaru mewn dull haenedig drwy'r dolydd morwellt cyfan.

- 5) Asesiad o <u>ayflyrau amgylcheddol</u> yn defnyddio cofnodwyr (loggers) (tymheredd a golau) a ddefnyddiwyd yn yr hirdymor.
- 6) Asesiad o <u>strwythur troffig pysqod</u> fel dangosydd o gyflwr yr ecosystem.

Byddai angen i'r holl ddata a gesglir gael ei reoli gan wyddonydd hyfforddedig a phrofiadol a chynnwys cofnod ysgrifenedig manwl. Mae hyn yn cynnwys ailwerthusiad blynyddol o effeithlonrwydd samplo. Drwy greu cyfres ddata hirdymor sy'n defnyddio ystadegau tebyg i raglenni monitro presennol yn y DU, dros amser dylai data gael ei archwilio'n gymharol i ddata DU arall er mwyn ystyried newid hirdymor sy'n cael ei yrru gan hinsawdd. Rydym hefyd yn awgrymu bod data allweddol a chanfyddiadau arwyddocaol ar gael ar unwaith i fudd-ddeiliaid a'r cyhoedd er mwyn cynyddu effaith y rhaglen.

Executive Summary

The seagrass commonly referred to as 'Eelgrass' (*Zostera marina* L.) is one of only two 'true' seagrass species found in the UK and is mostly restricted to a maximum of about 7 m of water depth (below chart datum). This is the result of its high light requirements as a photosynthetic organism. Estimates from across the range of *Zostera marina* suggest it requires between 12 and 37% of surface irradiance to survive in the long-term. These high light requirements and the delicate nature of these plants make seagrass habitat susceptible to impacts. In Porthdinllaen there exists over 20 hectares of seagrass that has been defined as being in an unfavourable condition by Natural Resources Wales. The primary anthropogenic impact of concern upon seagrass at Porthdinllaen is the damage caused by both permanent and annual moorings placed in the seagrass. There are also significant issues related to anchor damage and vehicle use that is thought to be damaging the seagrass. For these reasons the relevant authorities group of the Pen Llŷn a'r Sarnau SAC are undertaking a process of co-management with stakeholders to bring about a reversal of this degradation.

A significant component of this is the creation of a long-term monitoring programme of the seagrass that will help understand the effect of any future management actions, act as an early warning of any future concerns and contribute to reporting on condition of the SAC feature. This report reviews the options available for developing such a seagrass monitoring and assessment programme at Porthdinllaen. It considers previous work conducted at Porthdinllaen and examines options for monitoring seagrass by considering existing programmes in other parts of the UK and best practice seagrass monitoring globally. Options are reviewed by examining both seagrass sampling methods and metrics and the relative merits of different ones.

Here we propose that a monitoring programme at Porthdinllaen is split into six separate components, the first five we propose as being essential whilst the sixth is suggested. These are proposed as they collect scientifically robust data, are well proven and can be collected without too much specialist equipment. They also have the capacity to involve volunteers. By creating both an intertidal and a sub-tidal programme volunteers with and without SCUBA expertise can take part in the programme. Such programmes need to continue to build on the good collaborative work with Seasearch and develop further links with University student groups (e.g. Marine Biology Society at Swansea University).

- 1) An assessment of <u>sub-tidal</u> seagrass <u>extent</u> that determines seagrass presence or absence and depth repeatedly throughout the bay using GPS. Study can utilise freedivers, light weight dropdown video or a light weight hand grab.
- 2) Spatial assessment of the <u>intertidal</u> seagrass <u>extent</u> and the presence of impacts. Study would utilise observers walking with a handheld GPS. Seagrass shoot density estimates and associated data would be collected.
- 3) <u>Detailed subtidal</u> SCUBA based surveys that assess seagrass status within randomly assigned quadrats radiating out from pre-determined seagrass sampling points spread in a stratified fashion throughout the whole seagrass meadow.
- 4) <u>Detailed intertidal</u> walking surveys that assess seagrass status within randomly assigned quadrats radiating out from pre-determined seagrass sampling points spread in a stratified fashion throughout the whole seagrass meadow.
- 5) Assessment of <u>environmental conditions</u> using loggers (temperature and light) deployed over the long-term.
- *6)* Assessment of <u>fish trophic structure</u> as an indicator of the ecosystem state.

All data collection would need to be managed by a trained and experienced scientist and involve detailed write up. This includes annual re-evaluation of sampling efficiency. By creating a long-term data set that uses metrics comparable to existing monitoring programmes in the UK data should over time be examined relative to other UK data to consider longer-term climate driven change. We also suggest that key data and significant findings are made rapidly available to stakeholders and the general public in order to increase the impact of the programme.

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Introduction

Seagrasses are the only marine representatives of the Angiospermae and belong to the order Helobiae, in two families: Potamogetonaceae and Hydrocharitaceae. Seagrass plants are rhizomatous (they have stems extending horizontally below the sediment surface) and are modular plants composed of repeating units (ramets) that show clonal growth. In contrast to other submerged marine vegetation (e.g., seaweeds or algae), seagrasses flower, develop fruit and produce seeds. They also have true roots and internal gaseous and nutrient transport systems. The plants expand through clonal growth and sexual reproduction to form expansive seagrass meadows of high ecosystem service provision [1].

Seagrass meadows are declining at an unprecedented rate [2, 3]. This loss has been estimated to be as high as 7% of their total global area per year [2], therefore the ecosystem services they provide are also at risk including their role in fisheries production, biodiversity provision and nutrient cycling. In Europe, land reclamation, coastal development, overfishing and pollution over the past centuries have nearly eliminated seagrass meadows, with most countries estimating losses of between 50-80% of the original area. Seagrass was once abundant and widespread around the British coasts, but serious declines have occurred, in particular as a consequence of a severe outbreak of 'wasting disease' in the early 1930s [4]. Such an outbreak was probably exacerbated by poor coastal water quality [5]. Recovery of seagrass beds in the UK since the 1930s has been slow and patchy, and seagrass is now considered nationally scarce in the UK.



Plate 1. Zostera marina growing in Porthdinllaen, North Wales

UK Seagrass

The seagrass commonly referred to as 'Eelgrass' (*Zostera marina* L.) is one of only two 'true' seagrass species found in the UK (the other is *Zostera noltii*) and is widely distributed across the coastal seas of the northern hemisphere (20–70°N), where it occurs across a diversity of environments (from sheltered sandy bays to anoxic muddy estuaries and turbid high current pebbles) and shows a high diversity of life-history, morphology and growth dynamics. *Z. marina* is the only truly subtidal flowering plant species in the UK.

In the UK *Zostera marina* is most commonly restricted to a maximum of about 7 m of water depth (below chart datum). This is the result of its high light requirements as a photosynthetic organism. Estimates from across the range of *Zostera marina* suggest it requires between 12 and 37% of surface irradiance to survive in the long-term [6]. Due to its delicate physical condition, seagrass is limited to a distribution in sheltered environments where it can hold sand and fine sediment. This delicate nature also makes it susceptible to physical impacts from factors such as moorings, anchors, and vehicles. Seagrasses, like any angiosperm, require a sufficient supply of nutrients, however this is a fine balance and elevated nutrients can result in reduced water quality and smothering by macro

and microalgae [7]. *Zostera marina* generally requires sand or muddy substrate and in order to live in anoxic muddy environments generally uses a symbiotic relationship with Lucinid bivalves [8].

Seagrass meadows are in the conservation spotlight: identified as Features of Conservation Importance (FOCI) for the proposed English Marine Conservation Zones under the Marine and Coastal Access Act; as a UK Biodiversity Action Plan Habitat; as a habitat of principal importance in Wales under the Natural Environment and Rural Communities (NERC) Act 2006; as a threatened and declining habitat under OSPAR and as a sub-feature of intertidal mudflats and sandflats and subtidal sandbanks for the designation of Special Areas of Conservation under the European Habitats Directive. In spite of such protection many UK seagrass meadows remain under threat from a range of factors such as mooring and anchor damage, poor water quality and coastal development.

Porthdinllaen seagrass

In Wales, subtidal seagrass meadows are restricted to only a handful of locations (Skomer, Pen-Y-Chain, Criccieth, Milford Haven and Porthdinllaen. Additional subtidal *Z.marina* is also present in the inland sea between Anglesey and Holy island. The largest of these is in Porthdinllaen which is located within the Pen Llyn a'r Sarnau Special Area of Conservation (SAC) on the Llŷn Peninsula in North Wales, UK (52°56'35.30"N 4°33'58.74"W). It is a small natural harbour protected by a headland to the north, sheltering the bay from all except northerly through to north-easterly winds. As a result of this shelter the seagrass at Porthdinllaen is extensive, covering over 28 hectares [9, 10]. This seagrass meadow is identified as being an important feature of the SAC under Regulation 35 management advice produced by Natural Resources Wales [11]. The meadow at Porthdinllaen spans the full range from the intertidal to the sub-tidal.

Potential impacts on seagrass at Porthdinllaen

The primary anthropogenic impact of concern upon seagrass at Porthdinllaen is the damage caused by both permanent and annual moorings placed in the seagrass. These are within intertidal and subtidal environments and numerous studies have documented how these are resulting in extensive damage to the seagrass [10, 12-14]. The tourist value of Porthdinllaen has increased rapidly over the last few decades and as a result there are now a large number of summer visitors to the bay arriving in yachts and other craft, the majority of these use anchors to moor up and these potentially also damage the seagrass [15-17]. Other potential impacts on the seagrass at Porthdinllaen are elevated nutrients. These come from a small number of local properties disposing of sewage directly into the sea and an adjacent Golf Course using fertilisers, and could exacerbate the presence of an invasive macroalgal species such as *Sargassum muticum* that has the potential to out compete the seagrass [18]. Of additional concern to the seagrass at Porthdinllaen are the problems caused by intertidal use of tractors and four wheel drive vehicles [9]. These potential impacts all occur as we experience increasing levels of environmental changes associated to climate change and ocean acidification [19], potentially reducing the overall resilience of the meadow to severe weather related impacts [20].

Developing a long-term monitoring programme for seagrass at Porthdinllaen

The seagrass meadow at Porthdinllaen is unquestionably in a degraded state [9, 10, 12, 13, 21-23]. This has led the Special Area of Conservation relevant authorities group to commence a stakeholder co-management initiative in order to reverse this degradation. A key component of this co-management is to develop a monitoring program for the following reasons:

- 1. To help understand the effect of any future management actions (e.g. recovery from mooring damage).
- 2. To act as an early warning of any future concerns.
- 3. Contribute to reporting on condition of the SAC feature

This monitoring programme will need to assess both <u>sub-tidal and intertidal</u> seagrass. Additionally, investigations are required that seek to investigate seagrass degradation within the intertidal areas, referred to as the inner harbour.

Creating a co-management strategy that results in actual conservation outcomes may require costs to be incurred to both facilitate and implement that strategy, including potential costs for new or modified equipment (e.g. moorings). Behaviour change may also be required by stakeholders. If such effort is going to take place it is imperative that studies understand whether these changes are resulting in improvements to the seagrass, and these changes communicated to stakeholders.

Understanding the influence of management actions (positive, neutral or negative) requires separating the influence of natural variation from any such management actions. This is important given it is well documented that *Zostera marina* undergoes a high level of inter-annual change as a result of natural environmental variability. Studies of pristine seagrasses in the Isles of Scilly show inter-annual variation in shoot density of up to a five-fold change [24]. In Porthdinllaen, separating such management actions from natural variability is further complicated by a backdrop of multiple diverse interacting and cumulative anthropogenic impacts.

Developing a seagrass monitoring programme at Porthdinllaen needs to be sufficiently scientifically robust to isolate the multiple impacts on the seagrass and be statistically robust enough to be able to remove the influence of environmental variability. Due to the potential for additional diverse future impacts to occur, the program needs to be sensitive and broad enough to provide early warning of such potential issues. This requires using a range of indicators (metrics) that can provide different information about the status and environmental conditions of the meadow.

The present report provides a review of seagrass monitoring programmes and assessment methods used previously in Porthdinllaen, the UK and globally in order to develop an appropriate monitoring programme for Porthdinllaen and provide options for its commencement. This report considers not only different assessment methodologies but the range of potential seagrass metrics that can be utilised to understand the influence of a broad range of potential natural and anthropogenic induced changes. This study also considers how degradation of the intertidal seagrass might also be assessed and monitored.

Creating a seagrass monitoring programme at Porthdinllaen will be restricted by financial limitations and availability of trained personnel. It is important that any methods developed are clearly repeatable with basic equipment and that the intensity of sampling is achievable within a scope of only one or two weekends of survey work using a minimum small team. It is hoped that any future monitoring programme will include volunteers to collect data and/or samples and therefore key components of this need to be achievable using non-scientists. Here we present monitoring and assessment options for both sub-tidal and intertidal seagrass.

Previous seagrass studies in Porthdinllaen

Sub-tidal assessments

Seven studies have been conducted on the sub-tidal seagrass at Porthdinllaen. Two of these were faunal studies and five were assessing the actual seagrass status and spatial extent. Additional studies have been conducted on the intertidal seagrass and its associated fish fauna. The first study was a Pan-Wales survey of seagrass epiphyte communities. Although targeted at seagrass epiphytes this study additionally provided broad data on seagrass shoot density and shoot lengths at seagrass in Porthdinllaen [25]. This was a diver based survey that was limited to 25 small (25 x 25 cm) quadrats collected across 5 random locations in the meadow. The status of the epiphytes, the shoot

length and the shoot density faired favourably relative to other seagrass meadows in Wales (Skomer, Criccieth and Milford Haven).

Three seagrass studies at Porthdinllaen have been volunteer based using the logistics and networks of the Seasearch programme and the funding of CCW. These have all been coordinated by staff at Menai Bridge based consulting company Marine EcoSol and all three studies utilised SCUBA diving:

- 1. In 2008 surveys were conducted to assess the seagrass surrounding moorings and to determine the extent of the whole meadow. Assessments surrounding 5 moorings were conducted at different points of the compass using transects pointed away from the mooring. Quadrats were placed every 5m and shoot density counts created. Additional transects were conducted across the meadow to more fully understand the extent of the meadow. Aerial photography of the seagrass meadow was also available from CCW, surveys in 2008 were also used to validate the extent of seagrass within Porthdinllaen based on these maps [12]. The surveys in 2008 conclude that the seagrass meadow contains the densest seagrass in the UK, this claim is unsubstantiated and unlikely to be true.
- 2. In 2009 further seagrass surveys were conducted at Porthdinllaen, these were conducted to provide further validation of the extent of the seagrass meadow relative to aerial imagery. Seagrass transects were also established across the meadow to develop the idea of using transects to create a 'Monitoring Index' for seagrass condition. Transects were conducted with the help of underwater scooters and involved the use of shoot counts in quadrats. Two methods were used. The first method was to use divers who swam towards a fixed underwater acoustic marker (transponder) with a homing device, noting the distance and bearing to the transponder noting the key features of the bed. The second method was for the divers to follow a fixed bearing across the bed and to record where the bed starts and stops in conjunction with a surface tracking GPS buoy. This second method was found to be more effective [13].
- 3. In 2012 another series of assessments on the seagrass were conducted, these were used to identify in detail the impact of the moorings on the seagrass. 31 moorings were assessed relative to 4 control sites. At each mooring and control site a similar methodology to that used in 2008 was conducted, except that in each quadrat, canopy height and numbers of shoots infected by wasting disease were also counted [14]. The data in 2012 although collected slightly differently to 2008 and 2009 enabled a broad comparison of shoot density between years. Additional information on the sediment type, associated fauna and presence of *Sargassum muticum* was also collected.

During 2013 a study of 14 different seagrass meadows in the UK found that seagrass in Porthdinllaen ranked as potentially the least healthy. This was principally driven by a low light environment suggested by short shoot length and low carbon to nitrogen ratio [21]. This study used SCUBA to collect samples and compared seagrass meadows UK wide. It included sites in the Thames, the Isles of Scilly, the Isle of Man, Ireland and South Wales. Although the sites sampled were spatially expansive, it lacked detail at a within site level, so these findings only provide a broad assessment of the conditions at Porthdinllaen. This study was unique in the UK as no previous studies have attempted to assess a whole suite of such seagrass bioindicators at a UK wide scale.

Seagrass meadows at Porthdinllaen have also been assessed for their fish and invertebrate fauna. All three studies were conducted at different spatial scales but illustrate how the seagrass at Porthdinllaen supports greater abundance and diversity of fauna than adjacent sand habitat [22, 26,

27]. Studies utilising seine nets and Stereo Baited Underwater Video Systems reveal how the seagrass supports up to 28 species of fish, of which 10 species that are commercially important use the seagrass as valuable nursery habitat [26].



Plate 2. Transects used to assess seagrass at Porthdinllaen in 2008 (left image) and 2009 (right image) [12, 13].

Intertidal assessments

The intertidal seagrass at Porthdinllaen has been far less studied than the subtidal seagrass in spite of the ease of access at spring-tide low water. Two key assessment studies were carried out in 2004 and 2005 [9]. These characterised the seagrass as having areas of high and low impact and collected data on area and blade density. Quadrats (50 x 50 cm) were placed randomly. Additional data on epiphytes, algae and wasting disease were also collected. In addition to the surveys in 2004 and 2005, the CCW phase 1 habitat survey also surveyed the intertidal seagrass at Porthdinllaen in 1997.

During 2012 and 2013, an MRes (Masters by research degree) study of the fish utilisation of the intertidal seagrass was undertaken with respect to seagrass degradation. This found clear evidence of decreasing fish diversity and abundance in lower cover seagrass [22] (see Figure 1). Large geo-referenced plots of seagrass were recorded in detail with shoot counts, % cover and canopy height data collected. Significant areas of degraded and low coverage seagrass were recorded within areas of the intertidal boat moorings and evidence collated on the impact of vehicle use on the seagrass (see Plate 3).

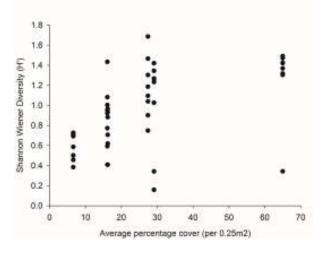


Figure 1. Relationship between fish diversity (Shannon Wiener) and intertidal seagrass % cover in Porthdinllaen. Data collected using a small seine net. Figure is taken from the MRes thesis by Rosemary McCloskey [22].



Plate 3. Photographic evidence of the impact of physical degradation of vehicles, boat moorings and keels on the seagrass at Porthdinllaen.

UK Seagrass Monitoring and Assessment

Sampling methods

Subtidal

An intensive search of the academic and grey literature using the Web of Knowledge and Google Scholar revealed the presence of at least 17 UK based sub-tidal seagrass monitoring and assessment programmes and projects (Table 1). Although we know that this is not a complete list of programmes, additional reports remain difficult to obtain from government agencies and private contractors (e.g. EIA site assessments). Additional intertidal seagrass monitoring reports are available but these are beyond the scope of the present study. 13 of these 17 programmes have used SCUBA diving teams to undertake at least part of the assessment and 6 of these programmes have used a mixture of at least 2 different methods, enabling greater detail to be collected (e.g. towed video and SCUBA diving) or in one case Sediment Imager Sonar and Towed Video. Although SCUBA requires extensive HSE considerations and can often be costly it enables the collection of a range of detailed data. Methods such as Towed video, ROV and various optical and remote assessment methods are limited by the limitations of the data that can be collected. For example, towed video footage in some UK studies hasn't always been good enough to be used for shoot density counts.

A useful guide for sub-tidal seagrass assessment in Wales and the UK was created by CCW and written by Liz Morris at Marine EcoSol [28]

Intertidal

Seagrass assessment methods for intertidal habitats are generally much more simplistic due to the capacity to walk to the site at low tide. Care is required with respect to changing tides and the nature of the substrate, but otherwise this is a reasonably simple method of assessing seagrass that enables volunteers to become involved. But in some locations other techniques are required to assist with safety, access difficult to reach sites or conduct intertidal surveys over very large areas. For example, in Australia helicopters are commonly used to rapidly survey intertidal seagrass meadows over large areas. Remote methods are more useful in intertidal areas but remain limited by their capacity to collect detailed information about the health of the seagrass.

A useful guide for inter-tidal seagrass assessment in Wales and the UK was created by CCW and written by Liz Morris at Marine EcoSol [29].

Remote sampling

Although remote sampling methods for seagrass are general expensive and the detail of the data that can be collected by such methods is largely limited (e.g. spatial extent and an index of density) with often high levels of associated error they do have a unique potential use in terms of understanding fragmentation. Studies in the Isles of Scilly are currently using very high resolution aerial imagery in order to understand the spatial meadow dynamics in terms of their fragmentation isolation and potential extinction risk [30]. Spatial mapping studies using drop down video, divers, snorkelers or grab samples will not map the levels of fragmentation present in the meadow. Such landscape issues are of paramount importance particularly in terms of issues of boat mooring and anchor damage and the flow on effects to biodiversity [22, 31].

Seagrass metrics

In these UK subtidal seagrass studies typically data on shoot density and some estimate of spatial extent are collected. Some studies have also collected information on the extent of wasting disease and shoot length, together with data on invasive macroalgae [14, 32]. Two studies have collected information on epiphyte coverage [24, 25]. Only studies in the Isles of Scilly have collected fully quantitative information on flowering and seed production [33]. A unique UK seagrass study was conducted in 2013 that used a series of biochemical and morphometric parameters to assess the seagrass in 14 meadows around the UK. This enabled information about light availability, nutrient status and general plant health to be determined [21]. The only other study we're aware of that has collected environmental information from a seagrass meadow in the UK is one in the Fleet conducted by Kew gardens [34]. The study monitored temperature and salinity over a 12 month period.

Studies in the UK have mostly not taken advantage of the range of potential seagrass bioindicators available for assessing the seagrass (See Table 2), many of which are suitable for UK use. All metrics can be assessed within intertidal and subtidal seagrass systems.

Sampling design

Subtidal

The design of the majority of these 17 UK studies was based on the assessment of quadrats along transects (for towed video and/or SCUBA diving). This enabled detailed quantitative data to be collected within these quadrats (either as photos, shoot harvest or underwater observations). Most studies have used transects perpendicular to the shore, leading to the eventual depth maxima of the seagrass meadow (e.g. Monitoring at Gelliswick Bay [35]). Such an approach is not always applicable when the meadow is very large and expands in different directions. The seagrass assessment transects at Porthdinllaen in 2012 used a series of cross transects running parallel to the shore, these were in addition to the perpendicular transects used. The use of long transects such as those used in

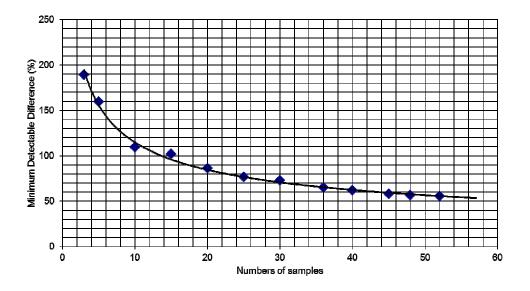
Porthdinllaen [13], Gelliswick Bay [35] and Torbay [36] although very useful in obtaining data across the full extent of the meadow and assisting with finding meadow boundaries do not provide detailed data that is individually geo-referenced. Surveys in Plymouth Harbour and the Scilly Isles used quadrat points randomly placed from a fixed line or central point using bearings and distances (m) [24, 32, 33]. The random locations of these quadrats were predetermined. The benefit of using such quadrat placement is that they can be geo-referenced.

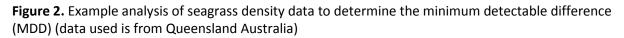
Intertidal

Seagrasses assessed intertidally are easily and very accurately mapped at their upper edge using a GPS on foot (see McKenzie et al [37], but when the meadow continues into the subtidal this can create difficulties and levels of unknown error associated to tidal changes. Mapping of the intertidal seagrass at Porthdinllaen up to the seawater edge resulted in large ranges of seagrass coverage between studies [9].

Most survey methods for the intertidal place quadrats along transects or haphazardly place them within the meadow. The methods proposed by Morris et al [29] suggest placing 50m transects perpendicular to the shore straight through the main portion of the meadow, this is similar to the established global methods used in SeagrassWatch and SeagrassNET.

There exists a wide range of intertidal seagrass assessments conducted in the UK based around the need to examine seagrass for the Water Framework Directive. Many of these are conducted by the Environment Agency, SEPA and NRW or by private contractors on their behalf. A series of frameworks have been developed in order to assess seagrass health, but these remain focussed on shoot density and spatial extent as the primary means of assessment [38, 39].





Statistical considerations

The creation of a scientifically sound and robust monitoring programme that can determine actual differences over spatial and temporal scales requires sampling effort that is statistically powerful. No seagrass monitoring programme in the UK that we are aware of has previously conducted any form of power analysis on the design and the data. This includes the long-term studies conducted in the

Isles of Scilly. It might be the case that intertidal Water Framework Directive (WFD) seagrass sampling has undergone some form of power analysis at an EU level but we are not aware of this. A common approach to understanding sampling effort and statistical power in seagrass monitoring is to determine the Minimal Detectable Difference (MDD) [40]. This enables the authors to examine and re-examine sampling effort each time data is collected and refine their sampling to suit the known variability within the system. Minimum detectable difference is calculated for a defined number of samples within your dataset. By calculating this across a range of numbers of samples a curve can be fitted and the expected MDD estimated for the collection of more samples (See Figure 2). This form of analysis is commonly utilised by groups conducting routine seagrass monitoring in Australia.

International seagrass monitoring methods

Many seagrass monitoring programs globally are using novel techniques and methods to assess ecosystem health which have not yet been considered in the UK. Seagrass monitoring programmes in the UK have rarely looked within the global scientific literature to develop best practice for seagrass assessment. Two major programs stand out as being truly novel; the Florida Keys seagrass monitoring program and the Great Barrier Reef (GBR) seagrass monitoring programme.

The website of the Florida Keys National Marine Sanctuary (FKNMS) water quality and protection program states that *"The scope and depth of this monitoring effort are without precedent or peer for seagrass ecosystems throughout the world"*. Within global seagrass research networks this statement is a widely held view [41]. Key to the Florida programme is the breadth of assessment metrics used that assess not only seagrass presence and status but also consider productivity and population demographic techniques. A key component of the Florida program is their assessment of seagrass nutrient availability using tissue concentration assays in a similar manner to that used in the UK (including Porthdinllaen) during 2013 [21]. A novel component of the Florida programme is the seagrass the seagrass cover.

The GBR seagrass programme also aims to collect an extensive range of data about the seagrass and its environment, including actual light and temperature monitoring (PAR) and again seagrass tissue nutrient concentrations. The programme in the GBR also collects quantitative data on flowering, reproduction and seed banks [42, 43]. The design of the GBR programme is fully intertidal but the methods are applicable for sub-tidal assessment too. It is based on the use of the SeagrassWatch methodology [44] but has been adapted and extended to include the environmental and reproductive metrics [45]. The collection of background PAR data has also been very useful in validating the use of various bio indicators. Temperature loggers have helped assist with understanding how climate change is increasingly impacting intertidal and shallow water seagrass meadows.

A significant difference between monitoring programs from other areas of the world is that they mostly contain some seasonal component and target repeat sampling in specific periods of the year (e.g. biomass maxima and minima). Although some studies have examined seagrass change at monthly or seasonal frequency such information largely does not provide any further information except in the case of the risk of a high intensity impact (e.g. dredging project). Assessments twice a year are usually sufficient to understand the dynamics of the system. The authors of the present study are not aware of any such seasonal sampling of UK seagrass undertaken.

Seagrass monitoring programmes using volunteers are widespread. Two major global networks exist (SeagrassWatch and SeagrassNET) that help facilitate this process and provide materials that enable volunteer education and method training, resulting in consistent data collection between sites. Both

of these methods have and are being used and developed in the UK (Cornwall and Devon). A useful aspect of both of these programmes is the central global coordination of data and the public presentation of this data online. Such independent data presentation is a useful communications method for working with stakeholders. These established monitoring protocols are especially suited to intertidal assessment.

James Cook University in Queensland also conducts extensive monitoring of nearshore seagrass meadows, these are intertidal, subtidal and deepwater (>20m) and principally relate to the assessment of commercial ports. Their methods rely mostly on two key metrics of the seagrass as an indicator of condition (seagrass biomass and macroalgal % cover). This technique uses an adaptation [46] of the seagrass visual biomass estimation method [47] and enables multiple techniques (e.g. Freediving, towed video, light weight dropdown video, helicopter, intertidal walking) to assess the same metric across varying environments and conditions. By avoiding SCUBA all the other methods can collect detailed geo-referenced data and be replicated across the meadow at high frequency enabling statistically robust data to be collected (see Figure 3) [46].By conducting the sampling at arbitrary locations spread along lines perpendicular to the coastline it is possible to determine the point at which the meadow ends, such information can be used to determine the spatial extent of the meadow [37]. Each individual sample is the mean of three random quadrats. This method using drop down video or freedivers provides a means of rapidly assessing the extent of the seagrass and mapping it to GIS to determine seagrass area (ha).

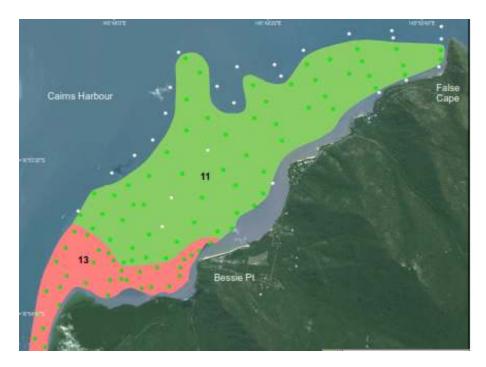


Figure 3. Seagrass sampling in Cairns Harbour, Queensland. Figure showing sampling sites stratified across and in and out of the meadow (Green dots indicate seagrass presence, White dots indicate seagrass absence).

Data collection sampling techniques suitable for Porthdinllaen

Subtidal data collection

Sixteen seagrass sampling methods were recorded in the literature (see Table 4). Of these methods 11 were deemed inappropriate for seagrass monitoring at Porthdinllaen (Table 4), principally due to the cost implications of these methods, their limitations in collecting broad data about the seagrass status, and the technical capacity required to undertake these studies. The use of SCUBA diving,

Dropdown video (very light weight), Freediving and Grabs were the four techniques deemed suitable for sample collection in the subtidal seagrass (See Table 4 and Plate 3).

SCUBA diving is the most suited to the collection of detailed seagrass metrics but is not considered an efficient method to enable the extent of the meadow to be determined. At Skomer, where the meadow is <1 ha, SCUBA diving is used to determine seagrass extent [48], but this method is very time intensive and unlikely to be viable for use at Porthdinllaen (>26ha). The use of Freediving, Grabs and Light weight drop down video all provide a rapid and simple system to determine seagrass presence and absence in geo-referenced locations and can therefore be used from a small boat to accurately assess seagrass extent.

Previous surveys at Porthdinllaen have used underwater scooters to enable SCUBA divers to rapidly cover the extent of the meadow and additionally used underwater transponders to determine the approximate location of divers. Whilst these methods are valid and very effective they limit the future repeatability of the programme to teams who don't have access to such equipment. For density and other associated metrics to be assessed the simplest way to conduct this is to observe the shoots or collect the shoots within a small quadrat (25 x 25cm). Numerous research and monitoring programmes globally (including the programme in the Isles of Scilly) collect data by destructively sampling the seagrass above ground tissue, with no evidence available to suggest that it causes a negative impact upon the seagrass. Some studies such as the SeagrassNET programme also harvest complete cores that include the rhizome, this sampling method is done in moderation in healthy meadows has also been found to result in no impact on the seagrass.

Intertidal data collection

Seagrass can readily be assessed throughout the intertidal at Porthdinllaen using observers on foot. Both spatial extent and detailed health assessments should be conducted preferentially on low water spring tides. One observer collecting data with a handheld GPS and a datasheet can cover the whole intertidal meadow rapidly.

Assessing intertidal impacts

Understanding how the intertidal seagrass at Porthdinllaen is being impacted upon by Vehicle usage, anchors and moorings requires assessment right across the meadow. This is because these impacts are not discreet, moorings are changed and moved, vehicles use a variety of routes and boats haphazardly drop anchor within the meadow. Understanding these impacts requires collecting detailed data at a spatial level and analysing this using GIS. This form of data can however be collected easily and rapidly by trained volunteers.

Table 1. Subtidal seagrass monitoring programs undertaken in the UK and the methods used.

Survey Location	Sampling Methods	Sampling Design	Metrics Recorded	Shoot Collection	Monitoring Frequency	Organisation and nature of the work	Reference
Skomer	SCUBA Diving	Quadrats (every 5m) along parallel transects	Shoot density and meadow extent	No	Every 4 years (first mapping in 1979)	CCW (now NRW) using volunteer divers	[48]
Porth dinllaen	SCUBA Diving	Quadrats along Transects	Shoot density, canopy height, wasting disease, Seagrass Depth, Presence of Sargassum muticum	No	2008. 2009. 2012	SeaSearch, Marine EcoSol, SAC and CCW (now NRW)	[10, 14]
Gelliswick Bay, Milford Haven	SCUBA Diving	Quadrats along Transects	Shoot density, meadow extent, Epiphyte cover, % of Leaves infected, Conspicuous epiflora, epifauna and other macrobenthos	Yes	One off survey (sporadically since 1986)	RPS Report (by Aquatic Survey and Monitoring Ltd)	[35]
Yarmouth	Towed video, Walking and SCUBA Diving	Quadrats every 10m	% cover, Shoot density and extent	No	One off survey	Hampshire and Isle of Wight Wildlife Trust	[49]
Solent	Towed Video	Quadrat every 10 second of footage	% Cover and extent	No	Annual	Hampshire and Isle of Wight Wildlife Trust	[50]
Solent	Sediment Imager Sonar, Walking and towed video	Quadrats along transects perpendicular to shore	Shoot density, leaf length and extent	No	One off survey	Southampton University	[51]
Plymouth Sound	Towed video and SCUBA Diving	Quadrats (every 5m) along 50m transects	Meadow extent, % Cover, Epiphyte Score, Shoot Density, Maximum plant length, % of Leaves infected, Infection Score	Yes	One off survey	ECOSPAN for Natural England	[32]
Torbay	Towed video	Quadrats every 15 seconds of footage	% Cover and extent	No	One off survey	Torbay Coast and Countryside Trust	[36]
Isles of Scilly	SCUBA diving	Random quadrat placement	% Cover, Shoot density, Meadow Extent, No Sargassum muticum, Seabed type, Epiphyte cover, % of Leaves infected, Infection Score, No of flowering plants	Yes	Annual (since 1984)	Natural England and now Swansea University	[24, 33]
Kilkieran Bay and Islands	Underwater viewer and SCUBA Diving using DPV's	Quadrats along transects	AFOR scale	No	One off survey	MERC Consultants – Private Contract	[52]
14 sites UK Wide	Walking and SCUBA diving	Random Quadrats	% cover, Shoot density, C:N, N:P, C:P, N15, Shoot length, Shoot width, Epiphyte cover	Yes	One off survey	SERG at Swansea University and Partners	[21]
North West Coast Scotland	Underwater viewer, Snorkellers, SCUBA Diving	Stratified and targeted observations	Presence-absence	No	One off survey	SNH	[53]
Helford River	SCUBA Diving	Stratified Quadrats across meadow	Presence-absence, Shoot density, % damaged leaves	No	Annually (1995- 1998)	Helford Voluntary Marine Conservation Area Group	[54]
Ventry Bay, South West Ireland	SCUBA Diving	Stratified Quadrats across meadow	Shoot density, Macroalgae	No	One off survey	University College Cork	[55]
Weymouth	Towed video	Quadrats extracted from towed video	Shoot density, relative abundance, extent	No	1999-2001	Southampton Oceanography Centre	[56]
Studland Bay	SCUBA Diving	Quadrats along transects perpendicular to shore	Shoot density, Epiphyte cover, Shoot length, % macroalgae	No	One off survey (sporadically since 1994)	SeaStar Survey Ltd and the Southampton Oceanography Centre	[57]
Long Oar Bay	SCUBA Diving	Quadrats along transects perpendicular to shore	Shoot density, presence-absence, broad extent	No	One off survey	SeaSearch and Marine EcoSol	[58]

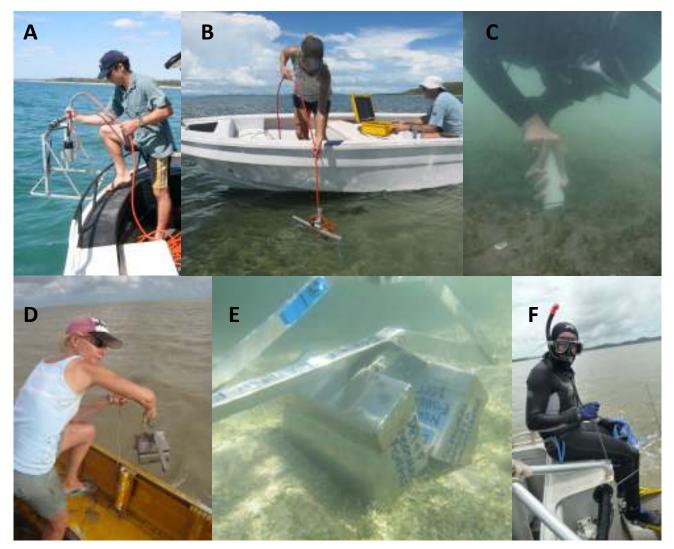


Plate 3. Techniques proposed as being suitable for seagrass sampling in Porthdinllaen: a and b) Light weight dropdown video, c) SCUBA diving, d and e) light weight grab, f) freediving. Methods a, b, d, e and f most suited to determining seagrass extent. Pictures - James Cook University Queensland.

Whilst techniques such as Remote Sensing, Side Scan Sonar and various types of dropdown video (Table 4) can provide data on the seagrass to varying degrees (e.g. biomass, shoot density), these methods cannot provide information on detailed metrics that can be used to understand the status and environmental conditions of the seagrass ecosystem.

Seagrass metrics suitable for assessing seagrass health at Porthdinllaen

Seagrass can be assessed at various different spatial and temporal scales that reflect the different response of the system to environmental change. For example the electron transport system of the photosynthetic apparatus will respond to change very rapidly, whereas the meadow may decrease in spatial extent at a much slower rate (See Figure 4).

Given the need to assess the status of the Porthdinllaen seagrass meadow for a range of potential stressors, indictors will be required to provide a broad assessment of the condition. This includes not only examining re-colonisation in degraded areas but the programme also needs to be able to examine impacts from physical stress, light reduction, nutrients, and invasive macroalgae, as well as

equally important but largely unappreciated top-down ecosystem effects from the depletion of associated fauna [59].

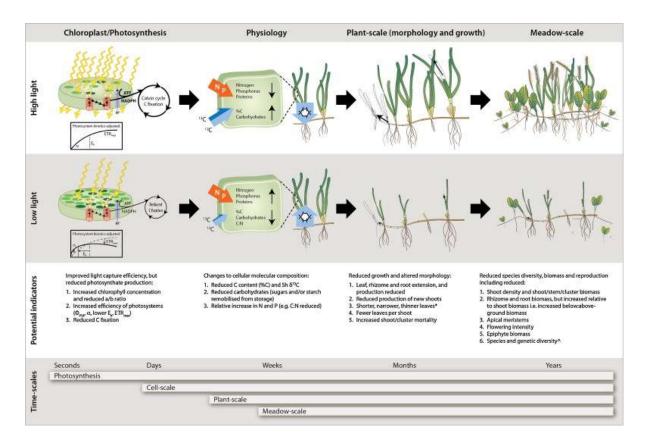


Figure. 4 Conceptual diagram of the current understanding of the of seagrass response pathway under low light conditions separated by photosynthetic, other physiological, plant-scale (growth and morphology) and meadow-scale variables. The timescales at which the responses to light reduction generally occur are indicated at the base of the diagram. Potential bioindicators are highlighted (Figure taken from McMahon et al 2013 [60]).

Meadow scale metrics

Traditional seagrass monitoring metrics (% cover, shoot density, meadow area) are useful in that they show a slow measured response to stressors well before a system reaches a point of collapse. They also provide comparative data to other sites, but such metrics don't always provide much information on what causes the issue. We propose to pursue the use of the shoot density and meadow area metrics only, but not the more subjective measure (% cover) or the difficult to measure metric (biomass). Shoot density is useful as it can be determined underwater or after sample collection.

Light metrics

Metrics are required that at least provide some bioindication of the seagrass light environment. Metrics associated to shoot length (inc canopy height) are thought to be good indicators of a changing light environment, as are the number of leaves per shoot [60]. One of the most robust indicators of the light environment is the C:N ratio of the leaf tissue. Using a PAR logger with a wiper unit can also help with such monitoring as can determining the maximum depth of the meadow (below chart datum) [61].

Nutrients

Metrics that assess different aspects of the concerns associated to excess nutrient additions are limited to assessing seagrass tissue nutrient ratios (N:P and C:P) and the consideration of relative amounts of epiphyte cover and the cover of macroalgae. Although tissue nutrient analysis is costly (\approx £30/sample) not many samples would be required and samples can be frozen for long durations prior to funds being available to analyse samples. Epiphyte cover can be assessed readily but this must follow prescribed clear methods (e.g. SeagrassWatch method).



Plate 4. Metrics commonly used to assess seagrass. Left foreground show the long-term deployment of a light (PAR) logger together with a wiper unit, left rear shows photosynthetic measurements (using Waltz Diving PAM) of seagrass being collected as an indicator of seagrass light stress. Picture right shows the typical structure of a seagrass plant and the structures such as the sheath and the root nodes that can be assessed to examine the productivity of the plant.

Resilience

Quantifying reproduction in a seagrass meadow is an important part of developing an understanding of the resilience of the meadow to future impacts. Although seed banks can be measured, these are typically of very high levels of variability and are difficult to reliably quantify. Sampling is also destructive (use of a corer) and is time intensive. Measurement of the density of either flowering or fruiting shoots provides an alternative means of collecting such information. Such metrics can be determined underwater or after sample collection.

Wasting disease

In addition to metrics of seagrass status, metrics that provide information on key issues of concern are important to consider. The seagrass wasting disease caused by the slime mould *Labyrinthula zosterae* is a particular issue of concern within all UK seagrass meadows and needs to be monitored. A clear methodology for assessing wasting disease on seagrass leaves already exists [62] and has been used extensively in the UK [63]. This should be applied at Porthdinllaen.

Climate change

Although future Sea Surface Temperatures (SST) are not expected to cause issues for UK seagrass, the super heating of shallow water over intertidal and shallow subtidal seagrass can present a potential issue resulting in seagrass 'burning' [64]. Monitoring of the seagrass water temperature is a useful means of examining these issues. Temperature can be cheaply and easily logged using long-term loggers such as the iButton.

Assessing the invasive Alien species Sargassum muticum

There exists growing evidence from throughout Europe that *Sargassum muticum* is a serious threat to seagrass [18, 65]. To date the presence of *S.muticum* in Porthdinllaen appears largely constrained to attachment to areas of course sediments [14], although no detailed full survey has examined its presence in throughout the meadow. As a result of the loss of seagrass around moorings, such course sediments have become more abundant and the extent of this invasive seaweed is thought to be growing. As its density is currently small relative to seagrass, assessing its abundance cannot be considered at the same scale as any assessment for seagrass. It may therefore be useful to include its assessment in both spatial seagrass surveys and detailed seagrass surveys. This can be conducted concurrently by observers estimating *Sargassum* density (no of plants) in an imaginary 2m x 2m quadrat surrounding the seagrass assessment quadrat.



Plate 5. *Sargassum muticum* is now abundant at Porthdinllaen and requires monitoring as part of a seagrass assessment methodology.

Assessing intertidal physical impacts (vehicles, moorings and anchors)

The assessment of the impacts of physical degradation on intertidal seagrass at Porthdinllaen by moorings, vehicles and anchors requires an initial whole spatial assessment of the issue. Some data was collected in 2004 and 2005 but this was not a full spatial and detailed dataset. As this particular assessment is proposed only within the inner harbour area (an area of approximately 250m x 200m) very detailed information can be collected on this seagrass.

Determining cause and effect for vehicle and anchor damage is arguably not possible as there is no control site to compare data against, however detailed data can be collected on the seagrass meadow to examine how it varies relative to sites of observed impacts (e.g. recent tyre track), and those locations then observed over time.

Assessing and monitoring the impacts of degradation may require the use of an additional series of metrics. Degradation of seagrass from a physical impact may result in changes to the sediment (e.g. greater compaction), and their will exist an increasing chance that a greater proportion of the plant will be exposed. Studies on trampling impacts on wetlands in Taiwan have used a penetrometer to examine sediment compactness [66]. Some studies examining the impacts of mooring damage on seagrass have quantified the density or length of exposed rhizome present in a quadrat (see

Appendix 1). Given the visual ease at spotting tyre marks from vehicles it may also be possible to quantify their presence spatially.

Assessing ecosystem effects

Although there is a growing understanding of how 'top-down' processes drive seagrass ecosystem health no seagrass monitoring programmes we are aware of actually considers such factors. For example studies in California, Norway, Indonesia and Virginia have documented how large fauna and/or mega-fauna (e.g. predatory fish, sea otters, turtles) help control the food web that helps determine the productivity of the seagrass system. Specifically within Norway, studies *on Zostera marina* have found that overfishing of Cod and Pollack reduced the capacity of the seagrass to resist the impacts of eutrophication [59]. Seagrasses in Porthdinllaen are clearly at similar risk given the poor status of the Irish Sea Cod stocks.

Given the extensive successful seine net surveys conducted within the seagrass at Porthdinllaen, such monitoring is entirely feasible.

Table 2. Metrics used to assess the health and status of seagrass and their methods of collection, advantages and disadvantages. Each metric is considered as to its suitability to be collected by volunteers at Porthdinllaen.

VARIABLE	METRIC	MEASURE/METHOD	REASONS/RESULTS	ADVANTAGES	DISADVANTAGES	APPLICABLE TO PORTHDINLLAEN MONITORING REQUIREMENTS
Meadow scale	Cover (abundance) [60, 67]	 % cover SCUBA; free-diving or walking (for intertidal meadows) transect (stratified) or random; quadrats estimating % cover or ranking categories with or without camera/video 	 Shows denseness which can describe structural role within the ecosystem (refuge etc.) Reflects patchiness (related to functioning of meadow as habitat) Indicates change over environmental gradient Robust bioindicator of light stress 	 Common practise in current monitoring programmes Understandable parameter for managers, stakeholders and public 	Expensive or labour intensive	Use of % cover standards makes metric easy to determine for volunteers
	Shoot density [68]	 Number of shoots per m² Using SCUBA; transects (stratified) or random; quadrats 	 Affects the structural role of the meadow Low densities are characteristic of eutrophic waters Robust bioindicator of light stress 	 Key parameter in assessment of meadow health Understandable parameter for managers, stakeholders and public 	Labour intensive	Data easily collected by volunteers using quadrats
	Flowering intensity	Number of flowers per m ²	Robust bioindicator of light stress [60]	Relatively easy to measure?	Seasonal limitation	Data easily collected by volunteers using quadrats
	Meadow extent and fragmentation [67]	 SCUBA; Manta –tow (snorkelling); Remote-sensing (aerial/satellite/sonar/towed- video/drop-down camera) Fragstats (McGarigal <i>et al.</i> 2002 in [67] 	 Monitoring shows changes in meadow extent indicating loss from disturbance (natural or man-made) Indicative of change in max depth which can be a sign of deteriorating water quality (turbidity) Extent allows management by way of calculating overall area and value in terms of ecosystem services 	 Understandable parameter for managers, stakeholders and public Relatively easy to measure with various methods available 	 Expensive, SCUBA labour intensive but other methods more affordable. 	Data easily collected by volunteers using dropdown video or freediving
	Max Depth [61]	SCUBA; Drop-down camera from boat with sonar depth finder	 Indicates mean annual light availability Lower maximum depth extent is characteristic of waters with minimal anthropogenic impacts 	Relatively easy to measure		Data easily collected by volunteers using dropdown video or freediving
	Canopy height [60]	Using SCUBA; transects (stratified) or random	 Describes structural role in ecosystem (refuge etc.) Signify health?? 	Easy to measure	Not considered a robust bioindicator of light stress	Data easily collected by volunteers using quadrats

VARIABLE	METRIC	MEASURE/METHOD	REASONS/RESULTS	ADVANTAGES	DISADVANTAGES	APPLICABLE TO PORTHDINLLAEN MONITORING REQUIREMENTS
	Biomass [60]	 Walking for intertidal meadows or SCUBA for subtidal; cores to take whole sections of seagrass meadow, or just removal of above-ground biomass 	 Above-ground biomass and root biomass found to be Robust bioindicators of light stress 	Could also be used to estimate living carbon storage	 Destructive sampling (not recommended for WFD [38] Requires further lab analysis 	Requires unnecessary lab analysis
	Wasting disease [62, 67]	 Wasting Index (WI) Visual census 	 Health in relation to obvious pathogen presence Quantity of lesions indicates stress from increased turbidity, low levels of insolation, and raised temps during growing periods 	Indicate any potential threat from wasting disease	Labour intensive	Data easily collected by volunteers using quadrats and wasting disease guides
	Epiphytes [67]	Cover and composition	 Indicate deterioration of water quality; increased dissolved nutrients Epiphyte biomass robust bioindicator of light stress [60] 	Rapidly indicate deteriorating water quality	 Lab analysis to ID and quantify epiphytes Destructive sampling ID training needed for composition 	Accurate assessment in field difficult, needs sample collection and lab analysis
	Invasive algae [67]	Using SCUBA; transects (stratified) or random; quadrats	 Displacement of <i>Z.marina</i> or growth in areas that prevent seagrass recovery Intrusion of invasive <i>Sargassum</i> can indicate fragmentation of meadows 	Easily combined within monitoring protocol, area assessed can be extrapolated from transect quadrat to assess presence of invasives	Labour intensive	Data easily collected by volunteers using quadrats
	Macroalgae [69]	 Using SCUBA; transects (stratified) or random; quadrats Drop-down camera 	 Monitor shift in eelgrass- to macroalgal- dominated communities Shift can indicate nutrient enrichment Causes shading of eelgrass 	Easily combined within monitoring protocol, area assessed can be extrapolated from transect quadrat to assess presence of macroalgae	 May need destructive sampling for ID purposes ID training needed 	Data easily collected by volunteers using quadrats
	Seed bank	 Walking for intertidal meadows or using SCUBA; transects (stratified) or random Sediment cores 	 Indicate the reproductive capability of the meadow 	Relatively easy to quantify	 Extremely variable Affected by environmental conditions, site etc. Analysis of sediments needed 	Data highly variable for Zostera marina
Morphological	Leaf length [60]	 Walking for intertidal meadows or using SCUBA; transects (stratified) or random, seagrass removed for measurements <i>ex situ</i> 	Low light can alter morphology; shorter leaves	Easy to measure	 May need destructive sampling Labour intensive 	Data easily collected by volunteers using quadrats
	Leaves/shoot [60]	 Walking for intertidal meadows or using SCUBA; transects (stratified) or random, seagrass removed for measurements <i>ex situ</i> Number of leaves per shoot or per cluster 	 Fewer leaves per shoot indicates low light - robust bioindicator of light stress 	Easy to measure	 May need destructive sampling Labour intensive 	Data easily collected by volunteers using quadrats
	Leaf area [60]	Walking for intertidal meadows or using SCUBA; transects	 Low light can alter morphology; shorter, narrower leaves will affect leaf area 	Easy to measure	Destructive samplingNot considered a robust	×

VARIABLE	METRIC	MEASURE/METHOD	REASONS/RESULTS	ADVANTAGES	DISADVANTAGES	APPLICABLE TO PORTHDINLLAEN MONITORING REQUIREMENTS
		(stratified) or random, seagrass removed for measurements <i>ex situ</i>			bioindicator of light stressLabour intensive	Limited value as an indicator
	Leaf width [60]	Walking for intertidal meadows or using SCUBA; transects (stratified) or random, seagrass removed for measurements <i>ex situ</i>	Low light can cause leaves to become narrower	Easy to measure	 Destructive sampling Not considered a robust bioindicator of light stress Labour intensive 	Limited value as an indicator
	Leaf thickness [60]	Walking for intertidal meadows or using SCUBA; transects (stratified) or random, seagrass removed for measurements <i>ex situ</i>	 Low light can cause leaves to become thinner Robust bioindicator of light stress 	Easy to measure	Destructive sampling Labour intensive	Limited value as an indicator
	Sheath length [70]	Walking for intertidal meadows or using SCUBA; transects (stratified) or random, seagrass removed for measurements <i>ex situ</i>	 Indicative of growth and production which can indicate effects of environmental and anthropogenic influences 	Help to determine duration of monitoring or a restoration project	Destructive samplingLabour intensive	Very useful indicator and simple to measure
	Rhizome biomass [60]	 Walking for intertidal meadows or using SCUBA if subtidal; cores to take whole sections of seagrass meadow, or just removal of above-ground biomass 	Shift in storage of carbohydrates can occur if photosynthesis is limited from light stress	Could also be used to estimate carbon storage?	 Destructive sampling not recommended for WFD [38] Not considered a robust bioindicator of light stress Labour intensive 	Limited value as an indicator
Physiological	Pigments [60]	Chlorophyll concentration; chlorophyll a/b ratio using spectrophotometry	Higher content and reduced a/b ratio indicates low light	•	 Requires lab analysis Not considered a robust bioindicator of light stress 	Limited value as an indicator
	Shoot C:N ratio [60]	Continuous flow isotope ratio mass spectrometer or C:N analyser	C:N reduction robust indicator of light stress	Widely used indicator of low light. Data comparable across sites.	Requires lab analysis	Very useful indicator but needs sample collection and lab analysis
	δ15Ν [71]	Continuous flow isotope ratio mass spectrometer	 Indicator of the origin (sewage or Fertiliser) of the elevated Nitrogen 	Effective indicator of sewage derived nitrogen	 Requires lab analysis Expensive per sample 	Very useful indicator but needs sample collection and lab analysis expensive
	Shoot N:P ratio [72]	 N - Continuous flow isotope ratio mass spectrometer P - Inductively Coupled Plasma Atomic Emission Spectrometry 	Indicator of the meadow nutrient balance	Shows nutrient enrichment from N or P.	 Can be highly variable Requires lab analysis Expensive per sample 	Very useful indicator but needs sample collection and lab analysis
	Carbohydrate stores	Extracted from below-ground biomass or above-ground biomass; analysed colorimetrically (Collier et al., 2012)	 Shifts in carbohydrate stores can indicate inbalance of respiration rate and carbon supply due to low light Leaf starch content has been found to be a moderate bioindicator of light stress 	 Indicator of the capacity of a seagrass to resist impact (e.g. available energy stores) 	 Extremely variable not considered a robust bioindicators of light stress Lab analysis needed 	Limited value as an indicator

VARIABLE	METRIC	MEASURE/METHOD	REASONS/RESULTS	ADVANTAGES	DISADVANTAGES	APPLICABLE TO PORTHDINLLAEN MONITORING REQUIREMENTS
Environmental data	Light	 Using light meter Light loggers (e.g. Hobo, Odyssey PAR logger); placed within centre of meadow in canopy, at max depth, and near surface 	 Measuring ambient light in seagrass meadow will help determine if light is limited 	 Easy to install Give good seasonal data with little time cost Could help determine impacts 	 Light varies thoughout day, tidal cycle and season so loggers more appropriate Can be expensive depending on type of instruments and number needed Loggers need wiper unit to stop biofouling 	Very useful indicator but requires seasonal or monthly logger care.
	Temperature	Temperature logger (can be combined with conductivity/salinity logger)	Log temperature shocks that can cause decrease in seagrass abundance [38]	Could help determine impacts	Can be expensive depending on type of instruments and number needed	Very useful indicator long-term logger deployment very easy
	Salinity	Salinity/conductivity data logger (e.g. Hobo U24-002-C, Odyssey temp and conductivity logger)	 Salinity shocks can cause reduction in seagrass abundance [38] 	Could determine impacts	Can be expensive depending on type of instruments and number needed	Not a significant issue at Porthdinllaen
	Sediment characteristics [67]	Walking for intertidal meadows or using SCUBA; transects (stratified) or random, sediment cores removed for analysis <i>ex situ</i>	 Particle size can be linked to turbidity and likelihood of re-suspension from impacts Nutrient content can lead to anoxia oxygen profiles can show anoxic layers Sediment depth can inhibit eelgrass recovery 	Can determine the stresses affecting the below-ground	 Further lab analysis needed Difficult to measure anoxic layers in subtidal 	Useful indicator but requires lab analysis
	Fish fauna [73, 74]	 Visual fish transects (SCUBA) Roving diver fish count Seine-netting, in shallow meadows Video/BRUV 	 Indicate change fish biodiversity Biodiversity has an impact on ecosystem function 	Useful management tool for recording commercial fish species and contribution to ecosystem services	 Difficult to quantify mobile species Labour intensive Specialist ID training needed 	Very useful indicator requires skilled personnel
	Invertebrate fauna [73, 74]	 SCUBA surveys; 1m² belt transects for larger epifauna Benthic cores for infauna 	 Indicates change in invertebrate biodiversity Biodiversity has an impact on ecosystem function 	Useful management tool for recording commercial invertebrate species and contribution to ecosystem services	 Labour intensive ID training needed ID training or sample analysis costs for benthic samples 	No value as an indicator and labour intensive
Genetic	Genetic Diversity [75-77]	 Sample collection. PCA analysis 	 High genotypic diversity allows more resilience to climate change Examples have shown diversity between meadows from different regions display different gene expression and recovery rates when exposed to extreme heat wave events 	•	Costs in UK too high to be used for monitoring purposes	No value as an indicator

Discussion

Seagrass at Porthdinllaen remains in a poor state, and developing a co-management strategy to improve the seagrass meadow requires a scientifically robust and statistically sound monitoring programme. This needs to be able to provide an early warning of issues of concern and provide evidence of the improving state of this important feature of the Pen Llŷn a'r Sarnau SAC. Here we provide evidence for the most appropriate options for this monitoring programme given the available finances, equipment and skills. The recommendations build on knowledge collected in previous assessments at Porthdinllaen and around the UK; they also build on the success of the long-term monitoring programme conducted in the Isles of Scilly in collaboration with Natural England; and use information gleaned from the international seagrass literature.

We propose that a monitoring programme at Porthdinllaen is split into six separate components, the first five we propose as being essential whilst the sixth is suggested:

1) An assessment of <u>sub-tidal</u> seagrass <u>extent</u> that determines seagrass presence or absence and depth repeatedly throughout the bay using GPS. Study can utilise freedivers, light weight dropdown video or a light weight hand grab.

2) Spatial assessment of the <u>intertidal</u> seagrass <u>extent</u> and the presence of impacts. Study will utilise observers walking with a handheld GPS. Seagrass shoot density estimates and associated data will be collected.

<u>3) Detailed subtidal</u> SCUBA based surveys that assess seagrass status within randomly assigned quadrats radiating out from pre-determined seagrass sampling points spread in a stratified fashion throughout the whole seagrass meadow.

<u>4) Detailed intertidal</u> walking surveys that assess seagrass status within randomly assigned quadrats radiating out from pre-determined seagrass sampling points spread in a stratified fashion throughout the whole seagrass meadow.

5)Assessment of <u>environmental conditions</u> using loggers (temperature and light) deployed over the long-term.

6) Assessment of <u>fish trophic structure</u> as an indicator of the ecosystem state.

The first four and the final components involve collecting data using metrics of sufficient simplicity to enable non-scientists to assist with data collection. Component five would require the assistance of on-site personnel (e.g. conservation ranger, harbour master). All components would collect data (and/or) samples that would require suitably qualified and skilled scientists to analyse and write up into an annual monitoring report. Some of this would require specialist skills such as GIS and potentially laboratory analysis of samples.

We propose that this monitoring of spatial extent and seagrass health is conducted twice each year, once in early spring (March) and once at the end of summer in order to coincide with maximum biomass and fruiting (September). If volunteer assistance and funding is available to increase this monitoring to include each season, then data would be beneficial to help develop a greater understanding of the natural variability of the meadow.

Assessing the subtidal seagrass extent and depth maxima

Using a shallow draft boat parallel transects (approx. 1km long) would be conducted in an approximatly due east direction from the beach (see Figure 5). These would be spaced 75-100m apart. The Bay will therefore contain around 10 or 11 of these transects. The frequency of these can be increased or decreased subject to available resources (e.g. boat time, staff or volunteer availability), the higher the frequency of these transects in the bay the greater the resolution of determining the meadow extent (see McKenzie et al 2001 [37]). The placement of these transects is arbitrary and their exact location not intended to be repeated each year. Their easterly direction is only intended as a guide. The aim of the placement of these transects is to cover the entire <u>potential</u> distribution of the seagrass.

Moving along each transect measurements of the presence or absence of seagrass should be determined at least every 50m (approximately) in triplicate (meaning 3 random samples at each approx. 50m sampling point) and marked with a GPS point. The use of 50m is an arbitrary length, as the aim is to spread these samples approximately equally around the meadow. This frequency could be increased or decreased dependent upon available resources. The use of triplicate sampling is to enable the sample to deduce that at that point the seagrass is either continuous or patchy.

All data should be collected on waterproof paper relative to the GPS marker number using preprepared data sheets. Again this level of sampling intensity will depend upon available resources. Each sampling point could be sampled using a free-diver, light weight dropdown video, or a light weight hand grab. Sampling along the transect should continue until at least 2 consecutive samples beyond the previous known extent of seagrass have recorded an absence of seagrass. Each sample should also report the presence or absence of *Sargassum muticum*.

This sampling of seagrass when conducted using an experienced and physically fit free-diver can be conducted rapidly within a small RHIB using a three man team (cox, data recorder, freediver) (subject to an appropriate Risk Assessment). When resources area available and volunteers or staff sufficiently trained, it is possible to determine % cover at each location as a quantitative measure of the seagrass, enabling the creation of a seagrass density map. In this instance a free-diver can also be used to assess the density of *Sargassum muticum* within a large quadrat (2m x 2m).



Figure 5. Proposed transect configuration used determine seagrass extent

Assessing the status and extent of the intertidal seagrass

A method similar to that used for assessing subtidal seagrass extent is proposed. This would utilise parallel transects in a due east direction from the beach (see Figure 5). In contrast to those in the subtidal environment these would be spaced at higher frequency and be at least every 20m apart.

The inner harbour will therefore contain around 11 or 12 of these transects. The frequency of these can be increased subject to available resources (e.g. staff or volunteer availability), the higher the frequency of these transects in the bay the greater the resolution of determining the meadow extent (see McKenzie et al 2001 [37]). The placement of these transects is arbitrary and their exact location not intended to be repeated each year. Their easterly direction is only intended as a guide. The aim of the placement of these transects is to cover the entire <u>potential</u> distribution of the seagrass within the inner harbour at low tide.

Moving along each transect quadrats (triplicate) will be assessed hap-hazardly along the length of the transect. Conducting them every 20 paces could be a means of quadrat placement. An assessment of shoot density should be conducted within each. Within an imaginary 2m quadrat surrounding each small quadrat the presence of any tyre marks, moorings, or other evidence of physical damage to the seagrass should be documented. With a team of volunteers extensive detailed data could be collected rapidly.

All data should be collected on waterproof paper relative to the GPS marker number using preprepared data sheets. Again this level of sampling intensity will depend upon available resources. Each quadrat should also report the presence or absence of *Sargassum muticum*.

In addition to the spatial assessment of the seagrass, the exact position of every mooring should be assessed so that data can be over layered onto the resultant spatial analysis. In a similar way to previous studies conducted on subtidal seagrass moorings at Porthdinllaen, we propose that the seagrass is assessed around each mooring within the intertidal area. Such assessments would be along North, South, East and West directions away from the moorings (see Figure 6). Due to the smaller length of the moorings within the intertidal we propose these quadrats be placed at higher frequency (e.g. every 2m) and last a maximum of 10m.

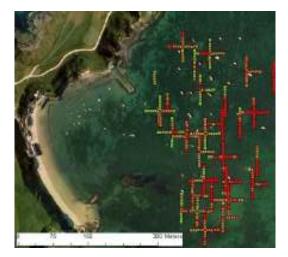


Figure 6. Placement of quadrats by SCUBA diving volunteers at Porthdinllaen during 2012 every 5m in North, South, East and Westerly directions away from each mooring.

Seagrass health assessment

In order to collect detailed quantitative data on seagrass density and other bioindicators of health in sub-tidal seagrass, SCUBA diving is the only effective means of collecting this. We propose that such an approach is used at Porthdinllaen. In shallow subtidal environments their does exist some capacity for freedivers or snorkelers to collect such data, but this is reliant on good visibility from the sea surface.

Sampling methodology

The sampling methodology utilised by Natural England in the Isles of Scilly since 1984 to collect detailed data on seagrass status provides a model to work with at Porthdinllaen. The capacity of the method to be repeated by different groups undertaking that programme of monitoring since 1984 is testament to its effectiveness.

We therefore propose that a series of locations throughout the Porthdinllaen meadow should be established. The locations will be permanent locations marked with GPS and returned to each year, although 'permanent' they will involve no physical marker. Additional accuracy of these locations can be created by triangulating the spot using landmarks and bearings. At each location, random points radiating out will be assessed for a range of seagrass metrics within 25 x 25 cm quadrats. The quadrats will be within 30m of the central point. At each location a minimum of 12 quadrats will be assessed. This is the number that can be achieved within one dive by one buddy pair of SCUBA divers. Dependent upon available resources a series of permanent locations would need to be established within the main central areas of the meadow. These would be targeted away from moorings. Targeting the locations away from moorings enables the meadow to be sampled rather than locations degraded by the presence of moorings. This also enables data to be compared against other sites in the UK.

Seagrass metrics

We propose that ten metrics are used as bioindicators to understand the status and health of the seagrass meadow at Porthdinllaen (Table 3). The majority (8) of these metrics can be determined either through underwater observation and measurement, or through sample collection and analysis in the laboratory. These first 8 metrics would need to assessed in every single quadrat throughout the meadow. The last 2 metrics (C:N and N:P ratios) require sample collection, preparation and laboratory analysis (and /or freezer storage). The variability in these ratios is likely to be low throughout the meadow, therefore only one such sample would be required at each permanent location. Given the capacity to store these samples easily in the freezer and the value of such bioindicators it is proposed that samples are collected irrespective of available funding so that later analysis can be conducted as and when finance is available.

Collection of material will require permission from the SAC and NRW. Such sample collection is common place globally and there is no evidence to suggest that collection of the seagrass shoots (without the rhizome) has any significant impact upon the meadow. This technique has been used in the Isle of Scilly since 1984, and 4 of the 17 other UK monitoring programmes conducted examined in Table 1 also use this method.

Metric	What it means	Data Collection	Method
Shoot density	Structural value of meadow	In Situ or Sample collection	Density within quadrat
Flowering and fruiting intensity	Resilience of meadow	In Situ or Sample collection	Density within quadrat
Canopy height	Light stress indicator	In Situ	SeagrassWatch Method
Sheath length	Productivity	In Situ or Sample collection	Gaeckle et al. 2006
Invasive Sargassum muticum	Invasive species	In Situ	Density in 2m x 2m quadrats
Wasting disease	Disease indicator	In Situ or Sample collection	Burdick et al. 1993
Macroalgae	Ecosystem imbalance	In Situ	% Cover
Epiphytes	Excess nutrients	In Situ	SeagrassWatch Method
Shoot C:N Ratio	Light stress indicator	Sample collection & lab analysis	Fourqurean et al 1997
Shoot N:P Ratio	Nutrient status	Sample collection & lab analysis	Fourqurean et al 1997

Table 3. Proposed metrics to be used undertake assessments of the health of the seagrass at

 Porthdinllaen.

Statistical considerations

The final design of any future monitoring programme at Porthdinllaen will require data to be collected that is statistically powerful. Predetermining how many samples are required is not possible without an understanding of the natural levels of variability. It is therefore important that numbers of samples collected at least use previous studies as a guide to inform sampling requirements.

After the first year or season of monitoring, it is important that the statistical power of the sampling strategy is considered by conducting an analysis such as the Minimal Detectable Difference (MDD) on key metrics [40]. This may result in the need to change the sampling design for the second sampling period.

The collection of data in future studies if collected as proposed here will enable data to be compared qualitatively against previous data collected at Porthdinllaen. However, data previously collected has never been used to assess the whole meadow and therefore caution will be required in its use. Specifically using previous data to make statistical comparisons to future data would likely be unwise except in the event that the seagrass at specific moorings are studied in detail and previous sampling designs repeated. It is important to recognise that all previous seagrass data from Porthdinllaen, although collected to a high standard was collected with different planned outcomes, and these were not to collect data for long-term understanding of the seagrass ecosystem.

All data collection at Porthdinllaen would need to be managed by a trained and experienced scientist and involve detailed write up using spatial GIS analysis and univariate and possibly multivariate analysis. This includes an annual re-evaluation of sampling efficiency. By creating a long-term data set that uses metrics comparable to existing monitoring programmes in the UK data should over time be examined relative to other UK data to consider longer-term climate driven change.

Intertidal seagrass health assessments

We propose that the methodology used to assess the subtidal seagrass should be extended to assess the inner harbour and the other intertidal seagrass areas. Permanent locations should be established within these intertidal seagrasses (marked by GPS) and quadrats randomly assigned at sites around the location. This would be done on foot and conducted in exactly the same way as is outlined for the subtidal assessments.

The initial placement of these locations should target both the main intertidal meadow and additionally areas determined from the initial inner harbour seagrass assessment to be potentially impacted by vehicles, moorings or anchors.

Within these intertidal assessments we propose that additional data be collected within quadrats on sediment compactness and rhizome exposure [78]. Sediment compactness can be assessed using a penetrometer. It is also possible to determine an index of fragmentation as proposed by Francour 1999 [78], this would also be a useful measure to be determined to examine the health of the intertidal meadow (see Table 5).

Assessment of environmental conditions

Light and temperature are two key drivers of seagrass productivity [79]. We propose that environmental data sets for these parameters are collected within the meadow. Recording temperature of the seagrass is a simple and low cost process using various loggers (e.g. iButton, OnSET, Tinytag) that can be deployed for up to 12 months at a time. We propose that one is deployed within the intertidal meadow and one within the subtidal meadow and records every 30 minutes.

Submersible light loggers collecting either Photosynthetic Active Radiation (PAR) or light intensity (LUX) (e.g. Odyssey, ONSET) are readily available and can be easily deployed within a seagrass meadow, however these first need calibration and can collect data every 30 minutes for 12 months. Due to the capacity of the sensors to become fouled they require the use of an attached wiper unit (See Plate 4). At Swansea University, Odyssey loggers have been integrated into an associated wiper unit to enable logging for long durations. The placement of such loggers should be at the deepest edge of the meadow and within the main meadow itself. Inspection of the logger and download of data would be advisable every three months if possible.

Deployment of all loggers can either be on a weighted base plate sited (see Plate 4) within an area of sand (within the meadow) or attached to a plastic steak permanently placed into the sediment.

Assessing the ecosystem state

We propose that an annual survey of the seagrass fish community is conducted at Porthdinllaen in much the same way as programmes have been conducted in Northern Europe (e.g. Since the 1800's in Norway). The use of a standard seine net can provide a non-destructive means of conducting this monitoring and can build on previous studies conducted at Porthdinllaen [26]. We propose that this should be conducted in September when seagrass biomass and fish community diversity are at their

highest. The aim of this is to examine the trophic structure of the faunal communities (e.g. relative presence of predators).

Communication

We propose that all data collected is made truly available and accessible to the general public and local stakeholders (e.g. key figures on the internet in a visible form, - see

<u>http://www.seagrasswatch.org/cairns.html</u>). Experience from global seagrass monitoring programmes such as SeagrassNET and SeagrassWatch suggests that such communication can be an important way of creating impact for the findings of the programme. Such communication of key data needs to be rapid so that when stakeholders witness monitoring taking place then within a short space of time they can see the results on the internet.

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Table 4. Overview of techniques available for the surveying and monitoring of *Zostera* beds in the UK (Table adapted and modified from Dale and Chesworth 2013).

SURVEY METHOD		ADVANTAGES	DISADVANTAGES	APPLICABLE TO PORTHDINLLAEN MONITORING REQUIREMENTS
1. AERIAL REMOTE SENSING	Aerial photography - A vertically mounted camera on a light aircraft takes digital natural colour transparencies, in transects across the site. Using infra-red, the methodology is the same, but this format allows better differentiation between intertidal algae and Zostera	 Complete coverage of <i>Zostera</i> bed so can indicate extent. High spatial resolution Can be cost effective due to large coverage Infra-red options allow for better discrimination between <i>Zostera</i> and algae Suited for coarse resolution mapping of bed location and extent s format 	 Expensive Requires ground-truthing as image interpretation difficult when distinguishing between <i>Zostera</i> and algae eg. <i>Ulva</i> spp. Sparse <i>Zostera</i> not easily detected Data requires geo-rectification to correct for aircraft roll, camera aspect, refraction Poor penetration below sea level, especially in areas of high turbidity, only really suitable for shallow areas with good water clarity Limited by weather – not suitable in low cloud. Not suited for detailed mapping of densities, associated flora and fauna, sexual status etc. 	Too expensive and not suitable for volunteers Provides too little seagrass information
	Satellite imagery	 Large areas may be mapped at any one time Older imagery e.g. Landsat Thematic Mapper (TM) and SPOT less expensive than aerial photography and CASI. More modern high resolution e.g. IKONOS produces 1 m panchromatic and 4 m multi-spectral data. Direct observations produced with continuous detailed coverage Suited to coarse scale mapping in tropical climes 	 Older satellites less accurate than aerial photography More modern methods as expensive as aerial photography Different habitats may not be distinguished Limited by weather conditions, light levels and operating constraints - restricted to shallow or clear water Unpredictable weather and poor water clarity mean less reliable for UK use 	Too expensive and not suitable for volunteers Provides too little seagrass information
	Compact Airborne Spectrographic Imager (CASI) - is an airborne sensor on a light aircraft providing high spectral and spatial resolution	 Most accurate, particularly for small scale studies with high spatial and spectral resolution for comparing absorbance characteristics of macrophytes Large areas may be mapped at any one time Provides an estimate of standing crop biomass Data is easily geo-referenced. Multispectral image is more appropriate than aerial photo for atmospheric and water column correction modelling. 	 Very expensive. Requires ground-truthing as sometimes difficulty in distinguishing between <i>Zostera</i> and algal species Limited by weather conditions Suitable only for intertidal or very shallow <i>Zostera</i> beds 	Too expensive and not suitable for volunteers Provides too little seagrass information
2. ACOUSTIC GROUND DISCRIMINA TION SYSTEMS	Sidescan sonar	 Useful in areas of poor water clarity Large areas covered relatively quickly Easily geo-referenced Can indicate attributes including leaf height and bed Density 	 Insensitive to sparse patches May not be able to differentiate between <i>Zostera</i> and macroalgae Insensitive to features which define biotopes Requires extensive ground-truthing Requires 8-10 m boat which can restrict movement in shallow areas 	Too expensive and not suitable for volunteers Provides too little seagrass information

SURVEY METHOD		ADVANTAGES	DISADVANTAGES	APPLICABLE TO PORTHDINLLAEN MONITORING REQUIREMENTS
	RoxAnn	 Useful in areas of poor water clarity Relatively cost efficient Large areas mapped relatively quickly Broad scale maps will display habitat lifeforms and some biotopes Easily geo-referenced 	 Rough seas may affect accuracy of data In shallow water, swath width is restricted and therefore larger numbers of transects needed to maintain full spatial coverage May not be able to differentiate between <i>Zostera</i> and macroalgae Insensitive to features which define biotopes Requires ground-truthing Requires 8-10 m boat which can restrict movement in shallow areas Rough seas may affect accuracy of data In shallow water, swath width is restricted and therefore larger numbers of transects needed to maintain full spatial coverage 	Too expensive and not suitable for volunteers Provides too little seagrass information
	Biosonics DT4000	 Accurate and quantitative measures of seagrass attributes such as canopy height (to an accuracy of 10 mm), cover, depth range and extent obtained all georeferenced in realtime Easily analysed. Differentiates between macroalgae and seagrass Easy to deploy and manoeuvre 	 Narrow beam width 6 degrees compared with other systems gives poor spatial coverage and requires large number of transects Limited to slack tide Initial equipment cost expensive Requires extensive ground-truthing 	Too expensive and not suitable for volunteers Provides too little seagrass information
	Echosounders	 Transects general quick and easy to carry out Requires minimal post processing Relatively inexpensive Easy to geo-reference 	 Not all echosounders are able to pick up seagrass May not be able to differentiate between <i>Zostera</i> and macroalgae Insensitive to features which define biotopes Requires extensive ground-truthing. 	Too expensive and not suitable for volunteers Provides too little seagrass information
3. OPTICAL REMOTE SENSING SYSTEMS	Remote Operated Vehicles (ROVs)	 No time limits, assuming good power source Highly manoeuvrable in 3D and can remain stationary for detailed inspection Can survey large areas of seabed Can provide both overview and high resolution data including density and associated species Can provide continuous data transects. Easy deployment Can record permanent digital films for visual comparisons over time easy dissemination. 	 High cost and requires specialist operators May require hard boat to operate which can restrict access to shallow areas Difficult to fly in straight transects Data analysis of video is time consuming. Field of view varies and can lead to inaccuracies of density estimates 	Too expensive and not suitable for volunteers
	Towed Video	 No depth time limits depending on power source Towed video at a known speed may provide information on the extent of bed faster than ROV Easy deployment. 	 May require hard boat to operate which can restrict access to shallow areas. Requires maintenance of slow towing speed so dependent on tidal conditions and means straight lines not easy 	Difficult to tow a sled through moorings at

SURVEY METHOD		ADVANTAGES	DISADVANTAGES	APPLICABLE TO PORTHDINLLAEN MONITORING REQUIREMENTS
		 Can provide both overview and high resolution data including density and associated species Can ground-truth acoustic and aerial surveys Can record permanent digital films for visual comparisons over time easy dissemination Cheaper than ROV 	 Speed and direction difficult to control so can not maintain position for close inspection Data analysis of video is time consuming Field of view varies and can lead to inaccuracies of density estimates Towed videos often utilise a metal sledge and stabilising chains which can damage <i>Zostera</i> beds. 	Porthdinllaen Not suitable for volunteers
	Dropdown Video	 Low cost - cheaper than ROV Can be very portable, lightweight, easily deployed and simple to use so minimum expertise required Many drops can be completed in a day Can ground-truth remote sensing surveys Can be used from small dinghies allowing shallow water data collection Some versions can be used while underway so capable of giving continuous transect data and making them manoeuvrable in 3D Can provide overview and/or detailed data Easy to geo-reference Can record permanent digital films for visual comparisons over time and easy dissemination 	 Image quality can be less than ROV and towed systems depending on equipment used Estimating field of view can be difficult 	Light weight system deployed from small boat Volunteers can assist with meadow mapping whilst on small RHIB boat
	Underwater Viewer	 Cheap and easy way of determining seagrass extent Easy to geo-reference data points Can be used from small dinghies allowing shallow water data collection Can be very portable, lightweight, easily deployed and simple to use so minimum expertise required Excellent for habitat mapping 	 Requires excellent visibility Limited to shallow waters Requires still water Not suitable for collecting quantitative data on the seagrass (e.g. shoot density, % cover) 	Suitable for volunteers habitat mapping but requires excellent visibility
4. PHYSICAL SAMPLING	Grabs and Cores	 Provides physical samples for subsequent analysis The sampling and analysis techniques are well established Can measure a number of <i>Zostera</i> attributes in each sample Can provide information on sediment 	 Potential small scale damage to the seagrass Difficult to assign quantitative data to the sample (e.g. shoot density) 	Can assist with habitat mapping if using small light weight grab Volunteers can assist with meadow mapping whilst on small RHIB boat

SURVEY METHOD		ADVANTAGES	DISADVANTAGES	APPLICABLE TO PORTHDINLLAEN MONITORING REQUIREMENTS
	Field observation (Walking)	 The most flexible survey / sampling technique for monitoring intertidal <i>Zostera</i> species Allows quantitative observation of intertidal <i>Zostera</i> species attributes such as density, shoot length, associated species and photosynthetic activity Good geo-referencing if GPS used. Several intertidal <i>Zostera</i> species attributes can be monitored on one visit Allows repeatable fixed point monitoring Can ground-truth remotely sensed data Low cost simple equipment required Simple methods can be devised to allow relatively unskilled volunteers to be used, reducing costs (volunteer training courses have been established by Hampshire and Isle of Wight Wildlife Trust) 	 Time limited due to tides Only suitable for intertidal populations Full extent can be difficult to ascertain if bed extends below low water Can only cover small areas during each site visit Time consuming if repeat visits required due to tidal window Access can be problematic on very soft sediments 	Perfect activity for volunteers Only suitable for intertidal monitoring
	SCUBA Diving Observation	 The most flexible survey / sampling technique for monitoring <i>Z. marina</i>. Allows quantitative observation of <i>Z marina</i> species attributes such as density, shoot length, associated species Several <i>Z. marina</i> attributes can be monitored in one dive Volunteer divers can be trained to carry out surveys 	 Potentially high cost Time and weather limited Can only cover small areas during each dive Can be difficult to accurately geo-reference survey stations and map bed extent. 	Perfect activity for trained diving volunteers Can collect diverse data and additional samples for further analysis
	Freediving (using snorkel gear) Observation	 Simple and flexible sampling technique for monitoring <i>Z.marina</i> Allows quantitative observation of <i>Z marina</i> species attributes such as % cover, shoot length, associated species Enables rapid assessment of meadow extent Low cost Can be done from a very small boat with two personnel Enables wide area to be assessed rapidly Several <i>Z. marina</i> attributes can be monitored in one freedive Number of dives possible per observer is high per day Volunteer divers can be trained to carry out surveys Enables sample locations to be geo-referenced easily 	 Requires physically fit, trained and able observers Time and weather limited 	Perfect activity for trained diving volunteers Can collect some data and additional samples for further analysis

SURVEY METHOD		ADVANTAGES	DISADVANTAGES	APPLICABLE TO PORTHDINLLAEN MONITORING REQUIREMENTS
	Manta-tow (pulling a snorkelling observer behind a slow steady moving vessel)	 Rapid and easy assessment of meadow area Broad quantitative assessments of % cover possible Transects easily georeferenced Possible to map meadow extent over large area rapidly Low cost 	 Limited ability to obtain detailed seagrass meadow level metrics Dependent upon excellent visibility Requires excellent helmsman Weather limited 	Suitable for volunteers but requires excellent visibility
	Very low flying helicopter survey (use of Robinson R22)	 Large areas can be sampled rapidly Each sample can be easily georeferenced Intertidal sites that are dangerous or difficult to access can be sampled easily Can map seagrass extent accurately and collect data on multiple seagrass metrics 	 Limited to intertidal seagrass Helicopter rental expensive Staff require training Weather dependent 	Too expensive and not suitable for volunteers

Table 5. Summary of the literature examining the impacts of moorings on seagrass and an explanation of the methods used in the study.

Study	Source/location	Metrics	Method	Results/observations
Impact of moorings	- Porthdinllaen Zostera marina [80]	 Extent Density Unusual features 	 Volunteer diver surveys Extent measured using rapid unmarked transects analysis using GPS surface tracking buoy Moorings/anchor chain damage measured using transects taken from centre of mooring in N,E, S, W directions 3 x quadrats (30cmx30cm) every 5m for 30m Seagrass density measured (shoots/m²) Presence of seeds, Labrynthula sp., snake pipefish Entelurus aequoreus and Sargassum muticum also noted 	Preliminary survey using volunteer divers for subtidal monitoring survey work.
	- Porthdinllaen Zostera marina [81]	 Extent Density and depth Unusual features 	 Volunteer diver surveys Rapid unmarked transect using a GPS surface tracking buoy Noting timing depth and position of features (including seagrass start/finish, mooring scars) Quadrats taken where possible along transects Presence of flowering, seeding and wasting noted along with any potentially threatening (<i>Sargassum muticum, Chorda</i> and epiphyte presence) or BAP species Methods used from (Morris & Goudge, 2008), but only recorded presence of moorings, transects not on moorings. 	Rapid unmarked transect analysis only worked within 200m of the transponder. It was not possible to record the distance if out of range, battery low or in very shallow water.
	 Porthdinllaen (outer harbour moorings) Zostera marina [82] 	Extent Density Unusual features	 Volunteer diver surveys Transects from centre of mooring in N,E, S, W directions 3 x quadrats (25cmx25cm) every 5m for 30m Seagrass density measured (shoots/m²) Band transect of 2m width to establish presence of snake pipefish <i>Entelurus aequoreus</i>, slipper limpet <i>Crepidula spp.</i>, stalked jellyfish <i>Stauromedusae spp.</i>, wire weed <i>Sargassum muticum</i>, seahorses <i>Hippocampus spp.</i>, seedlings or flowering <i>Zostera marina</i>, black slime mould <i>Labrynthula spp.</i> 	Quadrat size used different to previous, not consistent for monitoring purposes.

Study	Source/location	Metrics	Method	Results/observations
	- Australia - Posidonia australis [83]	- Density - Cover - Substrate	 Diver surveys 0.25m² (50cm x 50cm) quadrat at 3 distances along 2 transect lines taken from the centre of each mooring Number of shoots (consisting of one-several leaf blades joined at the base) Shoots with at least 50% of sheath located in the quadrat were also counted Cover estimated using point counts along the transect line 	Comparing the impacts of 'seagrass- friendly' mooring on <i>Posidonia australisl</i> . Techniques applicable to <i>Z</i> . <i>marina</i>
	- [84] - Australia	- Cover	 Aerial photography Comparison of aerial photos to estimate changes in seagrass cover from loss due to boat moorings 	Could give a general overview of effects from moorings
Impacts of anchoring	- NW Med/France - Posidonia oceanica [78]	 Direct effects of anchoring Shoot density Compactness (of rhizome matt) Extent of rhizome baring (length of rhizome above sediment) Field Comparisons Meadow cover Shoot density Extent of rhizome baring Proportion of plagiotropic rhizomes (horizontal growth) Degree of meadow fragmentation 	 Diver surveys Direct effects Seven sites used to compare the effect of tested anchoring damage on shoot density, meadow compactness and rhizome baring Experiments devised with boats of similar characteristics and anchors to the ones operating in the area Field Comparisons Five sites of different anchoring histories compared Meadow cover % of substrate covered by seagrass estimated using 40cm x 40cm transparent PVC plate (divided into 9 squares) held about 2m above the bottom Shoot density measured in 30 locations within each site using a quadrat of 0.04m² (20cm x 20cm) Extent of rhizome baring in horizontal growth (plagiotropic) is the distance from the bottom part of the rhizome and the sediment; and for vertical rhizomes (orthotropic) it is the distance from sediment to the base of the leaves, minus 2cm (indicates sedimentary loss) Plagiotropic rhizomes measured as a proportion of the total number of rhizomes in a given area, using a 0.04m² quadrat placed in the middle of the bed Degree of fragmentation measured by frequency and extent of intermats (patches of seabed without living shoots or devoid of vegetation) along a randomly placed 10m transect placed at 3 sites 	Investigating the damage caused by anchoring in <i>P.</i> <i>oceanica</i> but with some transferrable methods and interesting metrics measured.

Study	Source/location	Metrics	Method	Results/observations
	- [85] - Italy - Posidonia oceanica	 Meadow: Absolute density of shoots number of shoots per m² Rhizome baring (the distance between the rhizome and substrate, cm) Number of dead shoot per quadrat (%) Shoots: Mean length of intermediate leaves length per shoot Mean length of adult leaves per shoot Maximum length of adult leaves per shoot Coefficient A, % adult and intermediate leaves with broken apices per shoot 	 Diver surveys BACI design survey; in and out of affected areas; deep and shallow sections of meadow General cover measured in 3 levels; low (<65%), medium (65-85%) and high (>85%), by two divers swimming approx 3m above seagrass meadow over an area of 25m² to determine impacted and non-impacted sites Quadrats (40cm x 40cm) used for absolute cover in three replicates of chosen sites (impact and control) Three shoots within each quadrat removed for metric measurements in laboratory 	Measuring general damage from anchoring in and out of affected areas (in <i>P.oceanica</i>) but with some potentially transferrable methods.
	 Impacts of anchoring Studland Bay Zostera marina [86] 	 Shoot density Average blade length Seagrass cover Unusual features 	 Diver Surveys Comparison of Voluntary no anchor zones and Monitoring between 2009-2011 Four temporary, pre-marked 100m survey transects placed 20m apart in each area (in a north-south direction) Qualitative: Video footage taken along the transect line by one diver approx. 0.5m above the bottom Second diver taking notes of seagrass percentage cover, patches of bare ground, scaring, patches of dead seagrass, algal cover and any other noteworthy features. Quantitative: 	Different methodology to other UK monitoring surveys. Baseline data obtained.

Study	Source/location	Metrics	Method	Results/observations
			 On return swim, a 50cm x 50cm quadrat placed at 20m intervals along the transect, and seagrass and epiphyte density estimated Within a quarter of the quadrat shoots were counted and blade lengths measured (5 representative leaves), and flora and fauna counted and recorded Epiphyte samples on plants of interest taken for ID at the lab Video footage of each quadrat also taken Quadrat positions were used for subsequent monitoring Boat monitoring was also conducted (with the aid of students) using still photos taken from fixed positions 	
	- California - <i>Zostera marina</i> [87]	 Epifaunal density Density Community composition 	 Diver Survey assessing impact and recovery of simulated 'scarring' in a seagrass meadow Epifauna sampled using a randomly placed throw-trap (0.25m² with 1.6mm mesh) and a suction sampler (on for 1 min) 	Potential for epifauna to be integrated into monitoring programme.
Monitoring moorings	 (Monitoring of seagrass friendly moorings) Australia Zostera capricorni and Posidonia australis [88] 	- Cover - Max. leaf length	 Diver survey Underwater photograph taken of each mooring and scar 4 x 10m long measuring tapes laid out from the mooring in N, E, S and W directions Boundary of each mooring scar measured along each transect HD video camera in waterproof housing used to record seagrass coverage, diver swimming along transect lines approx. 1m above the tape measure 50cm x 50cm quadrat is placed in the scar, along the transect line at the same position as the previous year (position recorded to nearest 1cm) Quadrats also placed in the seagrass surrounding the scar, in the same positions as the previous year Digital photo taken of each quadrat (taken perpendicular to the substratum) 10 longest blades of <i>Zostera capricorni</i> and <i>Posidonia australis</i> in each quadrat measured to the nearest 1mm Stills and footage analysed in lab for presence absence of each seagrass species, algae and sand leading to 40 points for each mooring from which % cover was obtained 	Detailed monitoring programme around 'seagrass-friendly' moorings in Australia. Unique monitoring that concentrates on moorings.