SEACAMS2, Swansea University

Methodological trials for the restoration of the seagrass *Zostera marina* in SW Wales

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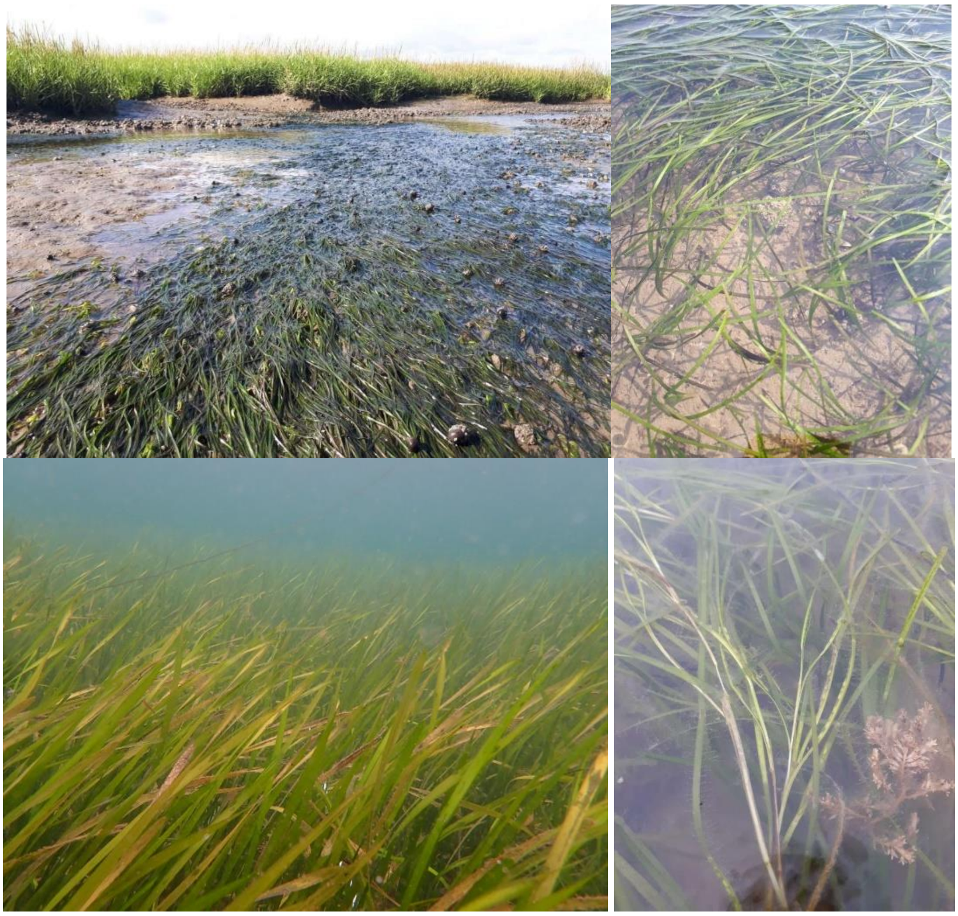

Intertidal and Subtidal Seagrass meadows (Pictures from SeagrassSpotter.org)

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Contents

Introduction

What are seagrasses?

Seagrasses are the only marine representatives of the Angiospermae and belong to the order Helobiae, in two families: Potamogetonaceae and Hydrocharitaceae [1]. 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 [2]. In contrast to other submerged marine vegetation (e.g., seaweeds or algae), seagrasses flower, develop fruit and produce seeds [3]. They also have true roots and internal gaseous and nutrient transport systems [4].
Seagrass meadows are important for their ecological functions and ecosystem services, such as their role in food web dynamics, seascape interactions and ecological resilience[5]. They are critical components of coastal and marine environments, thought to provide some of the most economically important ecosystem services of any marine habitat. Recent evidence from the UK and throughout the North Atlantic has found strong evidence of the value of these systems in supporting juvenile fish of economic importance [6-8].

Despite low floral diversity they support thousands of vertebrate and invertebrate taxa, including charismatic faunal species such as the seahorse. They also stabilise sediments whilst producing large quantities of organic carbon, and thus have an additional function in the food web. Although seagrasses cover only 0.15% of the oceans [2] they represent 1.13% of the total marine primary production, potentially acting as a sink for CO^2 [9]. A large proportion of the high primary productivity gets stored in sediments creating vast potential stores of carbon, recent evidence from the UK and the North Atlantic finds these stores to be potentially significant [10,11].

Seagrass in the UK

There are two species of seagrass found in UK waters, both belonging to the genera Zostera (Family: Zosteraceae). *Zostera marina* is the largest of the British seagrasses and typically occurs in the shallow sublittoral down to about 4 m depth, in fully marine conditions and on relatively coarse sediments. Dwarf eelgrass, *Z. Noltii* occurs higher on the shore than the other two species, typically on mixtures of sand and mud. Historically a third species of seagrass was recognised from the UK (*Z. angustifolia*, narrow-leaved eelgrass), however genetic analysis has confirmed that this is a narrow leafed variant of Z. marina that lives within the mid- to low-tide mark, usually in poorly draining muddy sediments[12].

Seagrass meadows are declining at an unprecedented rate [13,14]. This loss has been estimated to be as high as 7% of their total global area per year [13], 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[15]. In the UK a series of research papers have clearly defined seagrasses to be under threat and in a perilous state[16-18] with particularly studies citing the inaction of key stakeholders in managing these resources[19]. Seagrass was thought to be once abundant and widespread around the British coasts, but serious declines have occurred, in particular due to poor water quality (eutropication and other pollutants) , land reclamation and a severe outbreak of 'wasting disease' in the early 1930s [20,21]. Such an outbreak of disease was probably exacerbated by poor coastal water quality [22]. Recovery of eelgrass beds in the UK has been slow and patchy, with loss still continuing in many places, although case of extensive recovery have occurred such as within intertidal *Zostera noltii* beds in the Milford Haven Water way in West Wales [23].

The majority of seagrass beds around Wales are thought to be representative of two intertidal and two subtidal biotopes listed in the European Nature Information System (EUNIS) habitat classification system[1] (Table 1. EUNIS biotopes listed for sublittoral seagrass beds, these are all recognised under OSPAR (UK biotopes only). Those thought to represent the majority of Welsh seagrass beds are displayed in boldTable 1). These mostly occur on muddy sand sediments however some beds have also been recorded on mixed sediments (e.g. the 'Welsh Grounds' bed in the Severn Estuary) representing an alternative EUNIS

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[1] http://eunis.eea.europa.eu/index.jsp

Level 6 biotope (A2.611x '*[Zostera noltii]* beds in littoral mixed sediment'). Biotope mosaics also exist where two or more of the listed biotopes occur over small spatial scales (<25 m^2). The most common seagrass mosaic biotope occurs on the lower shore where the lower portions of *Z. notlii* beds merge with the upper portions of *Z. marina* beds. This is represented as either A2.6111/ A5.5331 or A5.5331/ A2.6111 depending on the predominant biotope.

Table 1. EUNIS biotopes listed for sublittoral seagrass beds, these are all recognised under OSPAR (UK biotopes only). Those thought to represent the majority of Welsh seagrass beds are displayed in bold (Table from **Ocean Ecology 2018**).

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

Seagrass management and legal status in the UK

Seagrass meadows have been defined as having biodiversity and habitat value. Specifically, they have been identified as Features of Conservation Importance (FOCI) for Marine Conservation Zones under the Marine and Coastal Access Act; as Biodiversity Action Plan (BAP) Habitats; as a threatened and declining habitat under OSPAR and as a sub-feature of subtidal sandbanks for the designation of Special Areas of Conservation under the European Habitats Directive. In 2008, the predominantly Zostera spp. associated culturally important Long snouted Seahorse (*H.guttulatus*) was given legal protection from disturbance under the Wildlife and Countryside Act (England only) due to their high cultural value, providing indirect legal protection for seagrass [24]. Seagrasses are now present within a range of marine protected Areas such as in the Mounts Bay meadow in Cornwall. In Wales seagrasses are present within the Penllyn A'r sarnau SAC, the Carmarthen Bay SAC and the Pembrokeshire SAC. They are also present with in the Severn River SAC and are located in the highly protect marine conservation zone at Skomer.

Seagrass restoration targets

Conservation designation and protection afforded to seagrass under UK and European law are of increasing importance given the 2010 release of the EU Biodiversity strategy to 2020. This sets out highly ambitious targets for where biodiversity conservation will head throughout the 21st century. Key actions within this strategy are the targets of 'No net loss of biodiversity and ecosystem services' and the need for member states to develop strategic frameworks to set priorities for ecosystem restoration at sub-national, national and EU level. Many of these targets represent local integration of the Aichi targets. The Convention on Biological Diversity identified restoration as key to delivering essential ecosystem services (Aichi Biodiversity Target 14), and has a global target of restoring at least 15% of degraded ecosystems by 2020 (Aichi Target 15; CBD 2014) [25]

Ecological restoration, defined as the process of assisting or allowing the recovery of an ecosystem that has been degraded, damaged, or destroyed creates a major opportunity for seagrass ecosystems. Given that across the UK many historical losses of seagrass have not recovered from extensive long-term loss [20] intervention is required to ensure that this happens.

A brief history of seagrass restoration

Seagrass planting guidelines were first developed during the late 1970's, but the track record for successful mitigation of impacts to seagrass beds remains variable [26]. Some spectacular failures of seagrass planting have created a lasting impression that restoration of seagrass beds is still an experimental management tool. In the UK, the only known seagrass restoration trials were a failure and have left a similar impression upon regulators and conservation organisations. Unfortunately, the causation behind such failures is unknown. Although the literature documents many failures, seagrass restoration has been successful in many plantings [27]. Planted seagrass meadows have often come to perform much as naturally-propagated meadows [28]. A consistent finding of all planting initiatives has been the expense. As a result, resource managers and developers have become more educated in the value of seagrass systems and the realities of their costly repair, more emphasis appears to now be placed on impact avoidance and minimisation. But unfortunately, loss continues and previous losses in many areas of the world (e.g. Europe and N America) have never been restored. Over the last decade increasing levels of understanding about the reproductive biology of seagrasses and their environmental requirements has led to vast improvement in the capacity of scientists to restore seagrass meadows. But despite improved techniques, many restoration efforts using either whole plants or seeds result in the number of losses exceeding gains [29].

Globally, seagrass restoration methodology is improving rapidly with an increasing chance of success, however many projects have failed [30]. A recent review of all known restoration efforts highlights the need for restoration to occur at sufficient scales in order to facilitate positive feedbacks and to spread the chances of success (see Figure 1).

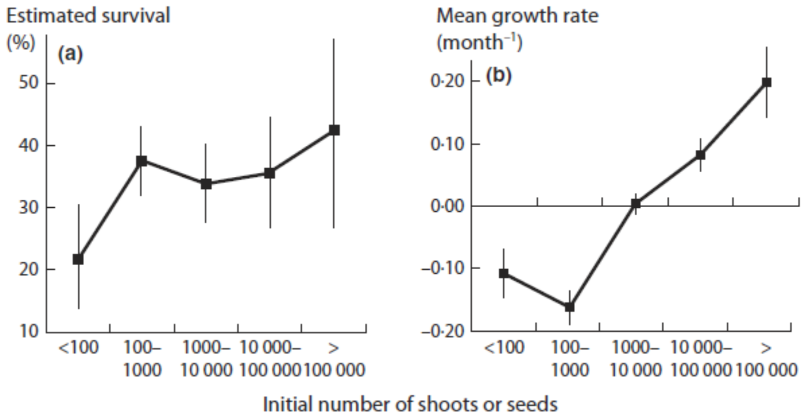

Figure 1. Influence of restoration scale on seagrass survival and growth [30].

Seagrass restoration in the UK

There have been limited attempts at seagrass restoration in the UK. One project was conducted in Breydon water in Norfolk in the early 1970's and involved transplanting plots of seagrass from the Morston. They had a high success rate after the first year (100%) and after 2 years this reduced to 35% with some plantings flowing and producing seeds [31]. The only other restoration experiment that the authors of this report are aware of comes from a project in the Helford river (Falmouth) where *Zostera marina* was planted within a ring stones after a likely water quality loss. Seagrass rapidly declined and wasn't present 12 months later. The reason for its failure is not clear however it is fair to expect that poor water quality may have been the main cause of loss.

Seagrass restoration techniques

Seagrass restoration has been conducted for over 50 years and the means of doing this can principally be split into two major techniques: 1) replanting 2) reseeding. Both techniques have their relative merits and have exhibited varying levels of success. A broad overview of the literature illustrates that although a lot is now known about seagrass restoration, much more remains to be researched and as a result the success rate of restoration projects is still often very low. The use of re-seeding generally relates to the collection and targeted redistribution (and sometimes processing) of wild seed. Adult shoot replanting normally involves harvesting plants from an existing meadow and transplanting them to the restoration site. This is because there is no readily available source of nursery grown plants.

In most cases, some means of anchoring the shoots to the bottom is necessary until the roots can take hold (root into the bottom). Replanting uses either labour intensive diving techniques or various mechanistic approaches to planting various sizes and ages of seagrass plants into new localities. In the US, reseeding and replanting techniques have sometimes been used together. Using seeds possibly in conjunction with adult plants, may in some instances prove more effective [30].

Seagrass restoration has the capacity to be both very expensive and have the capacity for project failure. Failures in many projects historically have been the result of limited consideration of the habitat requirements for seagrass and the continued presence of the stressor that caused the original seagrass loss. A recent review of the success of restoration projects globally found that success relates to the severity of the habitat degradation (eutrophication is worse than the impacts of dredging + filling or construction) [30]. Seeds, adult plants and sods are not significantly different, although seedlings show lesser planting results. A short distance to the donor site is also related to success. Whereas transplantations (replanting) frequently fail (60%) or have limited success, a substantial number of transplantations show huge expansion rates as well [30]. A recent study within the Wadden Zee [32] incorporated a series of guiding principles laid out by the Wadden Zee restoration project in order to maximise success rates:

1. Ensure long-term survival by promoting self-facilitation through implementation at a large-enough scale (hectares)
2. Focus on facilitating natural recovery through alleviating recruitment limitation (‘let nature work for you’)
3. Spread risks through space and time by restoring multiple sites on multiple occasions
4. Keep the costs of restoration (per hectare) as low as possible to achieve an as-large-as-possible scale of success
5. Minimize impacts on source meadows while avoiding introductions of invasive species at restoration sites

In addition to these guiding principles it may be prudent (dependent upon the impact type) to ensure that the restored seagrass will not be subjected to the impacts that caused their loss. Given the extensive water quality problems continuing to threaten UK seagrass it is imperative that proponents of any future restoration projects incorporate a thorough assessment of water quality and environmental conditions prior to developing any planting.

Previous restoration experiments at Swansea University

During the first SEACAMS project, field-based experiments planting seeds in different types of hessian bags were conducted in the Helford River and in Porthdinllaen (North Wales). These experiments suggested that seed bags were a potentially viable means of planting seeds, however the major lessons learnt from that work was that the smaller bags were more easily managed during dive work and bags need to be secured to prevent loss.

In addition, significant experiments were conducted in the aquaculture centre to grow seedlings. Although seeds germinated seedling development was stunted and they never developed into mature plants. Although we do not have quantitative evidence for why this occurred we expect this may to be the result of a lack of soil microbiome.

Project Aims

Given the limited understanding of seagrass restoration in the UK and the successful small restoration trials during SEACAMS1 a series of further field-based trials were proposed in collaboration with Tidal lagoon Power to demonstrate the viability of seagrass restoration methods for future use in large scale projects. The project had the following aims:

1) Test the viability of seagrass seed bag methods for planting seagrass.
2) Compare a range of different seagrass restoration methods.
3) Trial the use of protective mesh to enhance seagrass restoration.

Methods

Based on the modelling study of Greg Brown who determined potential habitat suitability of seagrass around the Welsh Coast [33] three sites were picked for potential restoration trials. These were Dale (Milford Haven Waterway, Freshwater East, and Longoar Bay). Dale was known to have an historic record of seagrass, and Freshwater East is suspected to have a small patch due to fragments commonly washing up on its beach. Longoar Bay has a small meadow of seagrass, with areas surrounding it thought likely good potential seagrass habitat. The sites were all in the range of 1-3m depth (below low water spring). During this project we recorded the location of an historic seagrass (*Zostera marina*) record in Dale which was found to still have dense seagrass (approx. 5m^2). At these locations a series of trials were conducted using seed bags, BESE plates and sods to plant the seagrass *Zostera marina*.

Seed bags

The seed laden shoots of *Zostera marina* were collected from the large seagrass meadow at Littlewick Bay by hand using SCUBA divers. Using aquaria at Swansea University seed laden shoots were left in running seawater to allow for them to reach maturity and fall out of their spathes. These were then separated from detrital material ready for placement in seed bags. Approximately 50 seeds (2cm^3) were placed into small hessian bags (see Plate 2) along with 100cm^3 of sediment (collected from nearby to existing seagrass at Littlewick bay). In addition, 50cm^3 of detritus was added to the sediment bag to assist with microbial inoculation of the seagrass microbiome. Bags were then tided and fixed along hessian rope at 1m intervals. Each line of rope contained 6 seed bags.

Two lines of seed bags were placed at Dale, whereas 1 line was placed at both Longoar Bay and Freshwater East (see Table 2). The lines were held down using steel pegs and marked using GPS.

Plate 2. Small hessian bags filled with seagrass seeds. Sediment and seagrass detritus were also added. Bags were strung into lines for deployment at potential restoration sites.

BESE plates

There is increasing evidence of the role of negative feedbacks reducing the success of seagrass restoration projects [34], namely feedbacks from bioturbating organisms (e.g. crabs and polychaete worms) and sediment redistribution. To facilitate a reduction in the associated physical pressures to these feedbacks biodegradable mesh plates have been trialled as a planting substrate for seagrass transplants as part of a Dutch led EU funded research project. The present project utilised these BESE (Biodegradable Elements for

Starting Ecosystems) plates made from potato starch to help stabilise sediments.

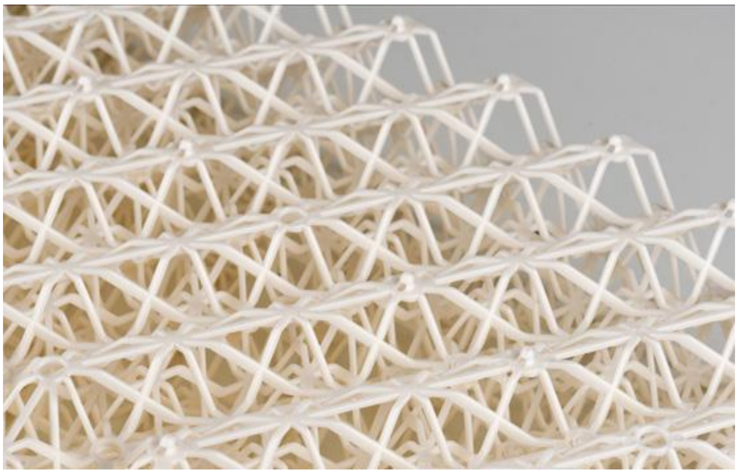

Plate 3. Example BESE (Biodegradable Elements for Starting Ecosystems) plates used to stabilise sediments for seagrass restoration.

BESE plates were placed at two sites of contrasting hydrodynamics within the Milford Haven Waterway. The first site was Dale (low hydrodynamic activity), the second was an exposed section of Longoar bay. At each site four replicates of three treatments were established, these were BESE buried under sediment, BESE on the surface of the sediment and control (no BESE).
Within each BESE plate comprising two sections of three plates (stacked) covering a 1m^2 area a 20cm diameter hole was established to enable the transplanting of seagrass fragments into the area of the BESE protection. Seagrass apical meristem sections of rhizome containing at least three shoots were carefully collected from within the Littlewick Bay meadow. These were attached using cable ties to steel u-shaped pins. These were carefully pushed into the sediment within each plot. Each BESE plate was assessed for the number of shoots, leaves and canopy height. Each plot had between 3 and 6 shoots in it.

Seagrass Sods

Many historical seagrass restoration projects have simply dug up plots of seagrass and moved them to a new site. During this present study we dug up 6 plots of seagrass at Littlewick Bay and moved them to Dale. Each plot was held to the sediment using 2 steel u-shaped pins.

Monitoring

All seagrass seed bags, BESE plates and sods were monitored over time using divers on SCUBA. Measurements of shoot density, numbers of leaves and canopy height were collected. Strings of seed bags were deployed in November 2017 and assessed during May and August 2018. BESE plates and Sods were deployed in summer 2017 and assessed during 2017 and 2018.

Table 2. Experimental design and replicates of the different restoration methods at each of the three sites (Freshwater east, dale and Longoar).

		Restoration location		
		Dale	**Longoar bay**	**Freshwater East**
Restoration Method	**Seed bags**	**2 strings of six bags**	**1 string of six bags**	**1 string of six bags**
	BESE Plates	**4 buried**	**4 buried**	
		4 on sediment surface	**4 on sediment surface**	
		4 control (no plate)	**4 control (no plate)**	
	Sods	**6 Sods**		

Plate 4. Pictures of seagrass restoration methods used in this study and their success and failures: 1) BESE plates covered in Algae at dale, 2) Seedlings developing in a hessian seed bags at Longoar Bay, 3) Shore crab taking up residence in a BESE plate, 4) remaining shoots on the seagrass Sod transplants at Dale.

Results

Seed bags

Twenty four seed bags were deployed and sixteen of these were recorded by May 2018 to have developed at least one seedling. By August 2018 this number remained the same. Density of seedlings by May 2018 was on average (2.95 ± 3.22 per bag), this declined slightly to 2.37 ± 2.41 per bag in August 2018. Failed seed bags were mostly at Freshwater east where no bags developed any seedlings, we believe this is the result of poor siting of bags leading to sand movement burying them too deep. Of those bags where shoots had developed there was an average 3.65 ± 2.09 shoots per bag by May 2018 with many of those shoots looking mature (Figure 2). This high rate of successful germination and mature plant development is similar to our previous trials in North Wales.

The hessian rope used in this experiment broke down quickly and by May 2018 was mostly breaking up making monitoring individual bags difficult but illustrating the value of using such kit for deployment. The hessian bags were highly fragmented and had mostly broken down by the end of August 2018.

BESE Plates

All BESE plates declined in shoot density throughout the trial. At Longoar all plants in the BESE were decimated during Autumn 2017 as a result of storm damage. At Dale, BESE plates declined by 72%. Of the 12 BESE plots created a Dale only 2 contained seagrass in August 2018 (12 months after deployment), all control plots were completely bare. All BESE plots were covered in attached macroalgae that had colonised the BESE surface. In addition, the Green Shore Crab (*Carcinus maenas*) had taken up residence within the central core of many of the BESE plates within a month of planting. Of the two remaining BESE plots in August 2018, one of these was growing very rapidly and had developed into a small but mature stand of seagrass.

The BESE plates had in some instances been blown away by storms but the majority had stayed in place. There was limited evidence of the potato starch breaking down however the plates had become highly brittle after 12 months in the water.

Seagrass Sods

Six sods of seagrass were planted at Dale averaging 10.33 ± 3.72 shoots per plot in Summer 2017 (See Figure 2). By August 2018 the density of these had decreased by 83% to an average of 1.66 ± 1.83 shoots per plot. Of the six plots only three contained shoots in August 2018.

Plate 4. Germinating seedlings at Dale planted in Hessian Bags.

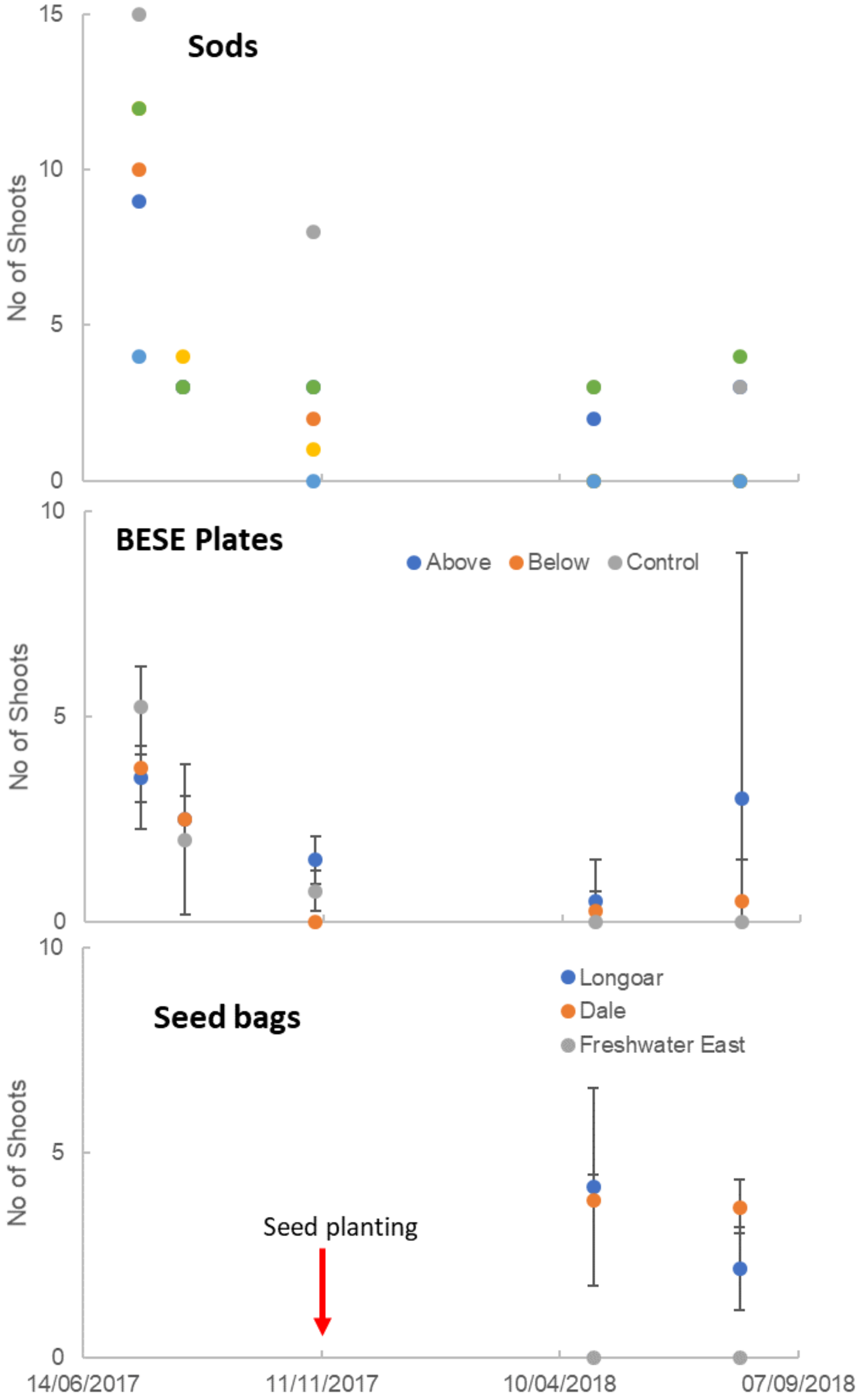

Figure 2. Shoot densities recorded for three seagrass restoration methods at three sites in and around the Milford Haven Waterway.

Discussion

The present project provides the first evidence of the potential viability of seagrass restoration in Wales as a means of creating new and restored marine habitats. Seagrass restoration projects globally suffer from poor success rates, our transplants using Sods and BESE plates have equally poor success with shoot densities declining by 72 and 83% after one year. In contrast, our seed bag deployments have shown 66% success rates after one year indicating the potential viability of this method. Whilst we have recorded success using these methods it is important to be clear that successful restoration projects require suitable environmental conditions and only when conditions can be deemed suitable should projects be conducted.

The BESE plates do potentially offer shelter to seagrass transplants, as the only remaining shoots in these plots were within the BESE plates rather than in the control plots, however algal over growth and bioturbation from Crab appeared to be major threat to any plants surviving in the long-term. Due to the lack of hard substrate in Dale, the appearance of the BESE plates created a magnet for small crabs and a site for drift algae to catch.

Experience of the team deploying the BESE plates was mostly negative as they were difficult to deploy, especially in conditions that were prone to poor visibility. Siting eight separate plates at each site took a three-person dive team at least two extended dives each. The potential minimal success of these deployments didn’t justify the amount of time and logistics taken to deploy these plates.

Like the BESE plates the Sods were also difficult to set up due to the need to slowly and carefully collect the Meristems and transplant the whole sod as one (including the sediment). In spite of not having the protection of the BESE plates 50% of the sods are still renaming after 12 months suggesting that transplanting the sediment along with the whole plants may be of benefit. Whether this relates to sediment stability and rooting or whether it relates to a less disturbed microbiome is not clear. Although 50% were surviving shoot density had declined sharply from deployment.

The seed bags were the most successful method of restoration with a very high proportion of bags showing mature plants after 12 months of deployment. The compete failure of a group of these bags reflected a poor site choice rather than a failed method, as one of the locations we picked was possibly too close to the intertidal and subject to moving sands. This highlights the need for detailed information to be collected in order to make informed site choices for seagrass restoration.

Whilst success has been observed in the use of seed bags for planting seagrass seeds a more mechanistic understanding is still required as to the processes driving the germination and development of *Zostera marina* seeds.

In conclusion, the evidence from this research project suggests that seagrass (*Zostera marina*) can be restored to sites in Wales and that the most viable method available at present in the use of seed bags. The costs in time and effort and the poor success rate suggest BESE isn’t a worthwhile method. A range of reviews and reports about global experience of seagrass restoration points towards the need to spread the risk as much as possible, for this reason we propose that future restoration might use a combination of seed bags mixed in with some seagrass sods. Again, we highlight the need for any future projects to consider the environmental conditions prior to project commencement. Sight selection is a critical part of project success.

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