Assessment of hessian seed bag planting success for *Zostera marina* within Plymouth Sound SAC, UK

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Postgraduate Certificate in Ecological Survey Techniques 2019 – 2021

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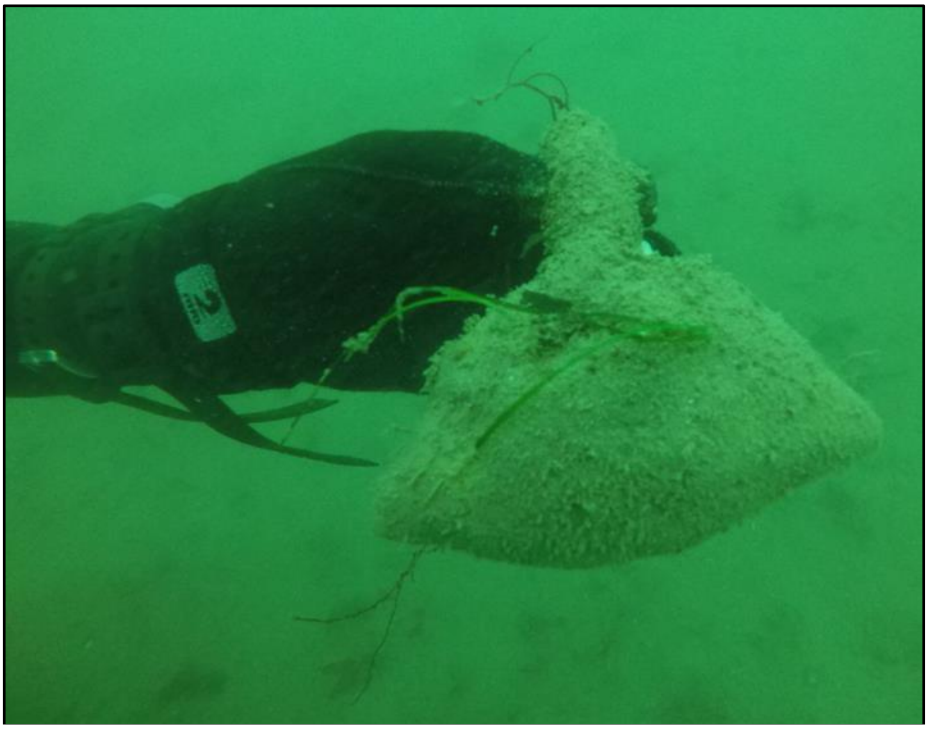
Abstract

Vegetated coastal habitats account for an estimated 50% of carbon sequestration within the marine environment, despite comprising < 0.2% of the ocean floor. In the UK, there has been an estimated 85% decline in seagrass presence within estuaries since the 1920s. The fast growth rate of seagrasses makes them ideal candidates for the restoration or planting of meadows. The Ocean Conservation Trust planted 18,200 hessian bags containing *Zostera marina* seeds in Plymouth, UK. Two months post-planting, seed germination and growth success along three permanent transects was monitored via the use of SCUBA for four months (June 2021 – September 2021). Seed germination rate was lower than anticipated, and although maximum shoot length did appear to increase over the survey period, this was not statistically significant. Delays and improvisations relating to the storage of seeds brought about by the COVID-19 pandemic, and an oversight relating to the degradation of the hessian bags, hindered the project. Presented here is a preliminary report on the first phase of a novel seagrass planting project within the UK, whereby methods and materials utilised have been assessed in order to provide feedback to the OCT for the future deployment of *Z. marina* seeds in the area. However, the restoration and creation of seagrass meadows is a developing science, and as such, the best practice methodology to assess the status and success of planting seagrass is also yet to be determined.



Emergent eelgrass from a deployed hessian seed bag

1. Introduction and Literature Review

Vegetated coastal habitats (consisting primarily of seagrass meadows, mangrove forests, and salt marshes) account for an estimated 50% of carbon burial and sequestration within the marine environment, despite comprising less than 0.2% of the ocean's total surface (Duarte *et al*., 2005; Fourqurean *et al*., 2012; Duarte *et al*., 2013). An environment consisting of ≥5% mean seagrass coverage is classified as being a seagrass bed habitat, whilst areas of <5% are considered the periphery of a bed, or patches of very low cover, if still associated with a meadow (WFD-UKTAG, 2014; Curtis, 2015; Jackson *et al*., 2016). Seagrass beds alone provide a multitude of ecosystem services, including carbon storage, enhancing substratum stability, and promoting higher biodiversity and abundance (Costanza *et al*., 2014).

Despite their importance, seagrasses are considered one of the most threatened ecosystems in the world, with large scale declines commencing in the 1930s due to wasting disease (caused by a marine slime mould, *Labyrinthula zosterae*), before being furthered from the 1970s onward principally due to anthropogenic impacts, such as nutrient pollution, dredging, anchoring, and other detrimental land management practices (Butcher, 1933; Rasmussen 1977; Davison & Hughes, 1998; Paling *et al*., 2009). In the UK alone, there has been an estimated 85% decline in seagrass presence within estuaries since the 1920s (Figure 1), with only 20 of the UK's 155 estuaries now harbouring meadows ≥ 1 ha. (Tubbs, 1995). Based upon the extent of loss of seagrass and other vegetated coastal habitats, global carbon storage has reduced by approximately 30 Tg C y^{-1} (30,000,000,000 kg per year) (Duarte *et al*., 2005).

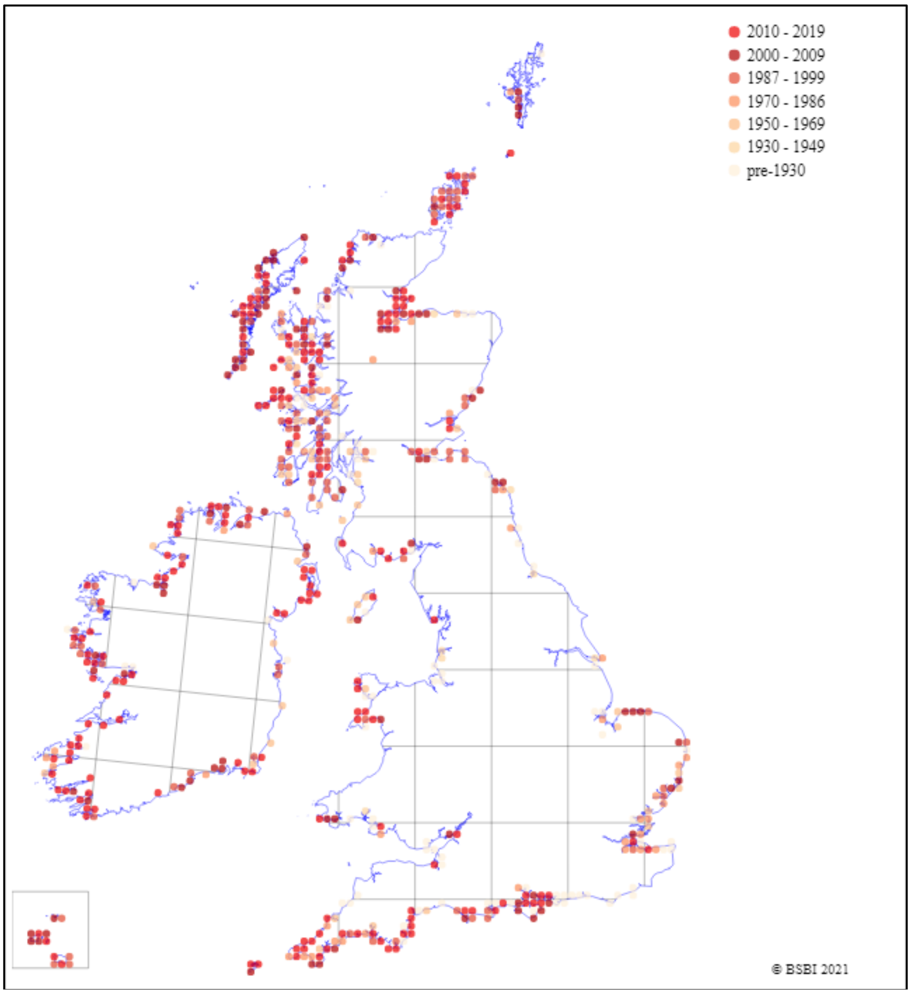


Figure 1*. Map showing the temporal shift in UK-wide distribution of eelgrass, Zostera marina, produced using point data of meadows recorded from pre-1930 up until 2019. Taken from the Botanical Society of Britain & Ireland 2021, online.*

Certain attributes of seagrasses make them ideal candidates for the restoration or even the planting of novel seagrass meadows to mitigate climate change and declines in marine biodiversity (Duarte *et al*., 2013). Primarily, the fast growth rate of seagrasses can allow for the rapid establishment of productive and functioning seagrass meadows in as little as two to three years (Orth *et al*., 2020; Tan *et al*., 2020). One of the most abundant and widespread species, *Zostera marina* – commonly referred to as eelgrass, has a shoot growth rate of 2 to 5cm per day during summer months in temperate regions, and can branch to consist of up to 12 leaves per rhizome (mean 3–6) within three months (Sand-Jensen, 1975; Borum & Wium-Andersen, 1980; Wium-Andersen & Borum, 1984; Harrison, 1993). This can potentially allow for comparatively quick results in tackling climate change whilst longer term goals are being simultaneously strived towards, such as the phasing out of high CO_2 emitting but historically reliant upon technologies and industries.

Furthermore, seagrasses are known to be resilient to sudden stressors, such as marine heatwaves – provided they are not already operating at the fringes of their max/min depth ranges (Aoki *et al*., 2020; Orth *et al*., 2020). As marine heatwave events are projected to increase in occurrence (Oliver *et al*., 2019), this resilience may prove an invaluable buffer as the efforts to slow the impact of climate change take effect. Additionally, seagrass species grow in the shallow coastal waters of every continent except Antarctica, providing opportunities for planting efforts circumglobally (Cullen-Unsworth & Unsworth, 2016).

1.1 Planting and Restoration Efforts

Since the end of the 20th century, and start of the 21st century, seagrass restoration efforts have achieved varying degrees of success, with the vast majority focusing on *Z. marina* due to its aforementioned fast growth and establishment rate (Ganassin & Gibbs, 2008). Two main methods of seagrass planting have emerged as the most promising and feasible: transplantation of mature seagrass from a donor bed, and/or seed harvesting and subsequent targeted dispersal of seeds and aquarium-reared seedlings (Park & Lee, 2007; Short *et al*., 2002a; Unsworth *et al*., 2019).

Davis & Short (1997) achieved 75 – 99% survival rates of transplanted *Z. marina* one year after transplanting into an estuary in New Hampshire, US. Short *et al*. (2002a) then developed the 'Transplanting Eelgrass Remotely with a Frame System' (TERFS) method, whereby mature donor *Z. marina* plants are attached to a rubber-coated frame via biodegradable cable ties before being deposited at the desired location, with the frame removed at a later date. The main advantage of the TERFS method is the avoidance of labour costs from manually planting the individual seagrasses, but at the cost of scale – it is limited by the size of the frame(s) that can be built and the equipment and apparatus capable of deploying such frames (Davis & Short, 1997). Short *et al*. (2002b) also recorded increased successful establishment rates compared to Davis & Short (1997) whilst using the TERFS method within the same transplanting location, whilst a project utilising this method in Deukryang Bay, South Korea, achieved 58.7–69% survival rates a little over one year after planting (Park & Lee, 2007).

With the apparent success of transplantation techniques, great care must be taken, however, as methods such as transplantation of mature plants can have an immediate and even permanent detrimental impact to donor meadow health if the health of the donor bed isn't known prior to harvesting (Nixon *et al*., 2002; Seddon, 2004). Alternatively, the systematic harvesting of seeds from donor meadows leaves rhizomes intact and is far less likely to significantly impact meadow health (Seddon, 2004).

Initial attempts using harvested seeds utilised broadcast dispersal but had low establishment rates of 3–4%, attributed to variability in local abiotic conditions (local water movement resulted in either a clumped or less focused dispersal) and biotic conditions (disturbance and/or predation, primarily by crustaceans) (Roy *et al*., 2001; Ganassin & Gibbs, 2008; Orth *et al*., 2008; Paling *et al*., 2009). To counter these drawbacks, Traber *et al*., 2003 created a mechanical sled that bores shallow planting plots in the benthos and automatically injects a gelatin/seed mixture via the use of a dosing pump as it is dragged across the seabed. This successfully increased germination and seedling establishment by 160% compared to sown broadcasting, but this was still comparatively low compared to transplanting of mature *Z. marina* and is only more efficient when the benthos topography is uniform (Short *et al*., 2002a; Orth *et al*., 2009).

The use of seeds contained within biodegradable hessian bags has been used as a labour/equipment/cost compromise, and with moderate success within the UK. Unsworth *et al*., 2019 observed a seedling germination and establishment rate of 3.5% across multiple sites off the Pembrokeshire coast, Wales, UK, comparable to that of seed broadcasting, but the authors noted the added protection from predators and local water movement as a notable advantage of the bag methodology. The fibrous nature of hessian has also been shown to increase natural recruitment of some seedlings via entrapment/entanglement (Wear, 2006). Furthermore, the hessian bag methodology has the potential to be up scaled to the attempted planting of full-sized seagrass meadows (Unsworth *et al*., 2019).

As such, the recurring primary factor seemingly influencing seagrass planting and restoration outcomes is the spatial scale of the planting effort, as this dictates the applicable methodology based upon its economic feasibility (Ganassin & Gibbs, 2008; Paling *et al*., 2009; Unsworth *et al.,* 2019). Naturally, larger scale projects incur more expense unless the methods of carrying out the restoration is altered, with the more underwater labour-intensive methods of transplantation incurring greater economic costs due to the employment of skilled workers and specialist equipment (Seddon, 2004; Unsworth *et al*., 2019). Transplanting of seeds is often the only economically feasible method for large scale planting projects, with hessian bags currently seen as the most viable method in a trade-off between cost, logistical effort, and effectiveness (Unsworth *et al*., 2019). Additionally, seeds can be stored for many months, allowing for more flexibility in their subsequent deployment, as well as for stretching out of the seed harvesting period to prevent any significant deleterious impact on the donor bed (Granger *et al*., 2000).

Additional threats to the success of restoration efforts include smothering by macroalgae and epiphytes often brought about by insufficient water movement across the planting area. High presence of macroalgae and epiphyte cover intercepts photosynthetic available light and increases competition for nutrient and CO_2 uptake (Kinney and Roman, 1998; Hauxwell *et al*., 2001), and Hauxwell *et al*. (2001) recorded a decrease in *Z. marina* growth rate of ~50% when macroalgal canopy height exceeded 9cm.

1.2. The ReMEDIES Project

The Ocean Conservation Trust (OCT) is one of five partners for the LIFE Recreation ReMEDIES Project (Reducing and Mitigating Erosion and Disturbance Impacts affecting the Seabed), an EU LIFE funded project to restore and conserve five Special Areas of Conservation (SACs) within the UK. One focus of the project is the collaborative effort to plant *Z. marina* seeds and seedlings in the Plymouth Sound and Estuaries SAC (PSE SAC). Natural England is leading the project, working with Marine Conservation Society, Plymouth City Council, Royal Yachting Association, and the Queen's Harbour Master, all of whom will provide project input relative to their jurisdiction. The seed collection, storage, bagging, planting, and monitoring/survey aspect of the project is being coordinated and conducted by The Ocean Conservation Trust.

The OCT are using best practice cultivation methods for *Z. marina* as part of a large-scale planting project, utilising harvested seeds and seedling stored and grown in aquaria, to ultimately restore 8 hectares of seagrass habitat to two Special Areas of Conservation on the south coast of England: 4 hectares each in Plymouth Sound and the Solent Maritime.

The first stage of the project saw *Z. marina* seeds collected from multiple sites along the south coast of England during the late summer of 2019 and 2020 (Table 1; Figure 2). Sites were chosen in consultation with Natural England, with permissions granted through licence application to the Marine Management Organisation (MMO).

***Table 1.** Zostera marina seed collection sites*

Date of collection	Site
31st July 2019	Little Arthur, Isles of Scilly
23rd August 2019	Lymington, Dorset
29th August 2019	Looe, Cornwall
3rd September 2019	Cawsands, Cornwall

6th – 7th August 2020	Looe, Cornwall
11th – 12th August 2020	Gyllyngvase, Cornwall
1st September 2020	Looe, Cornwall

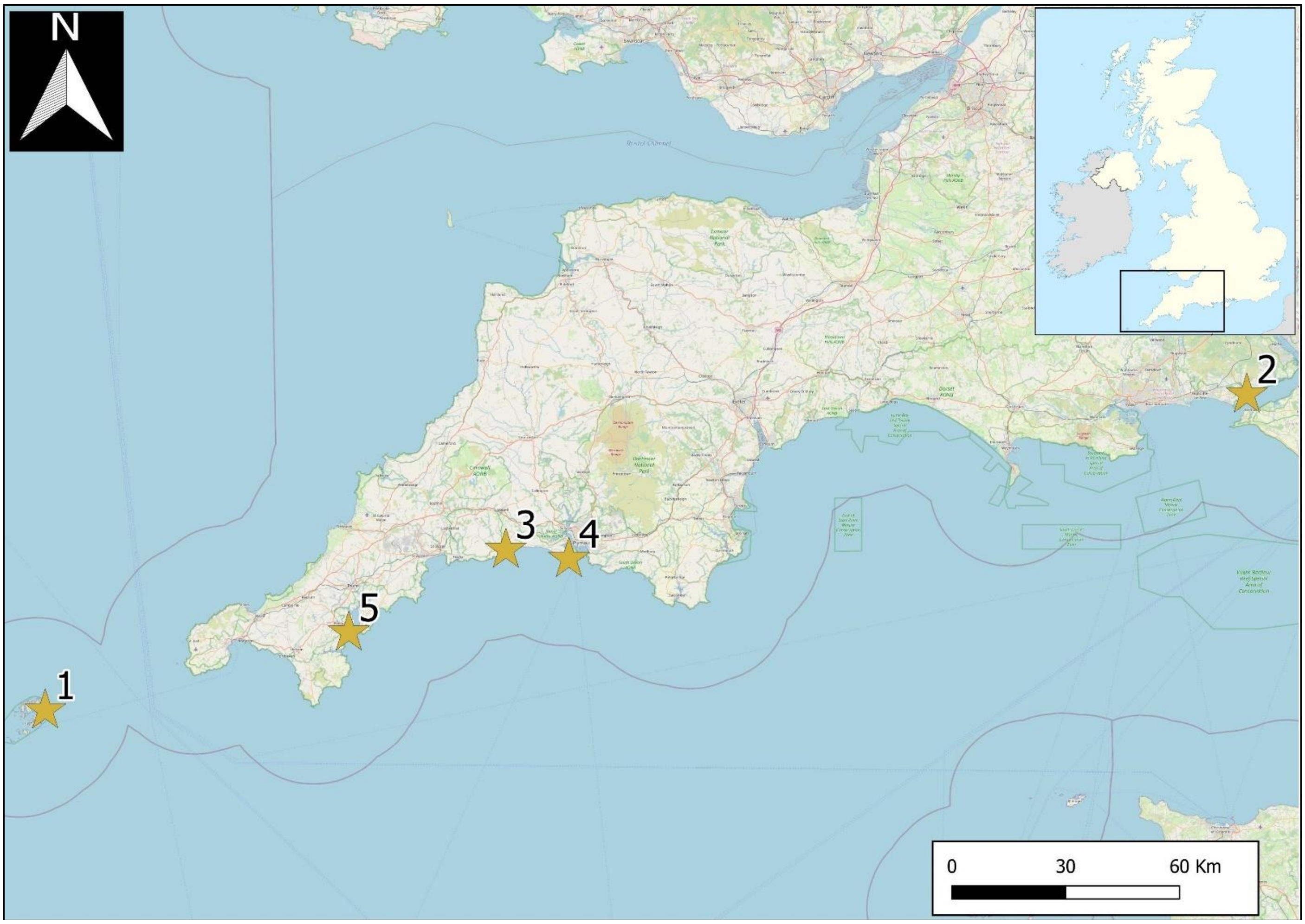


Figure 2. *Z. marina seed collection sites. Numbered in order of the date seeds were collected. 1 = Little Arthur, Isles of Scilly, 2 = Lymington, Dorset, 3 = Looe, Cornwall, 4 = Gyllyngvase, Cornwall. Produced in QGIS 3.4.10.*

The seeds were stored at the cultivation facility based at the Ocean Conservation Trust's National Marine Aquarium (NMA), Plymouth, England. The seeds were initially stored in the dark at 13^{o}C within recirculating seawater and an air stone placed on top of the seed pile to prevent the build-up of anaerobic bacteria. After about four weeks of seed storage, it was noticed that the seeds from Looe were beginning to germinate, presumably due to the temperature of water being close to local temperatures for seed germination. To halt the germination process and prevent further seed germination, all seeds were moved to refrigerated storage; seeds were stored at a temperature of 6^{o}C. After a period of two months, seed collected from Falmouth started to germinate in refrigerated conditions.

A laboratory trial was conducted to test bag degradation and seed viability. A total of 1,992 untreated hessian bags (dimensions 13cm x 14cm) were packed with a 90% sand, 10% loam mix, with the majority of bags containing 30 seagrass seeds (Figure 3). These trial bags were planted in the NMA's cultivation facilities large recirculation tanks (1m x 3m x 0.4m), containing UV sterilised seawater at constant parameters of salinity (32 ppt), temperature (14 °C), light intensity (~ 20 µmol m^{-2} s^{-1} at the water surface, 11 hr photoperiod), water circulation rate (~16,000 L/hr), and CO_2 injection rates. 207 bags were planted in the lab in July 2020, 1,335 bags in January 2021, and 450 bags in February 2021.

Figure 3*. The laboratory trial hessian bags containing 30 seagrass seeds and/or seedlings amidst a sand/loam mix. Photo by Emma Nolan*

The hessian bags lost all structural integrity within three weeks of immersion (Figure 4). July 2020 bags had a recorded germination rate of 1.59% within one month of planting, whilst January 2021 bags had a rate of 0.58%, and the February 2021 bags a rate of 1.86%.

Figure 4*. The laboratory trial hessian, once degraded, and the emergent Z. marina plants. Photo by Emma Nolan.*

The low germination rates observed are thought to be a result of the increased storage time which the seeds were subjected to due to a delay in planting permit processing time and subsequent planting; this was due to logistical and staffing issues at the MMO caused by the COVID-19 pandemic. Additionally, the decrease in germination rate for the January 2021 seeds is thought to be due to overloading of the system, where more than three times the number of seed bags were planted compared to February 2021, likely affecting water quality and the availability of nutrients within the closed aquarium system.

In April 2021, 18,200 of the hessian bags were filled with sand/loam mix, with 16,000 bags containing 30 seagrass seeds and 2,200 bags containing 30 seedlings. Within a maximum of 48 hours after being packed, the bags were taken to the designated project site.

2. Study Site

The first planting phase and subsequent survey took place within Jennycliff Bay, at the furthest coastal edge of the South Devon Area of Outstanding Natural Beauty (Figure 5).

Figure 5. *Location of Jennycliff Bay, to the south-east of Plymouth, UK.*

It was one of multiple sites identified as being suitable for seagrass planting following the completion of a habitat suitability model by Exeter University, as commissioned by Natural England, and conducted in consultation with Plymouth City Council and the Harbour commissioners (Figure 6).

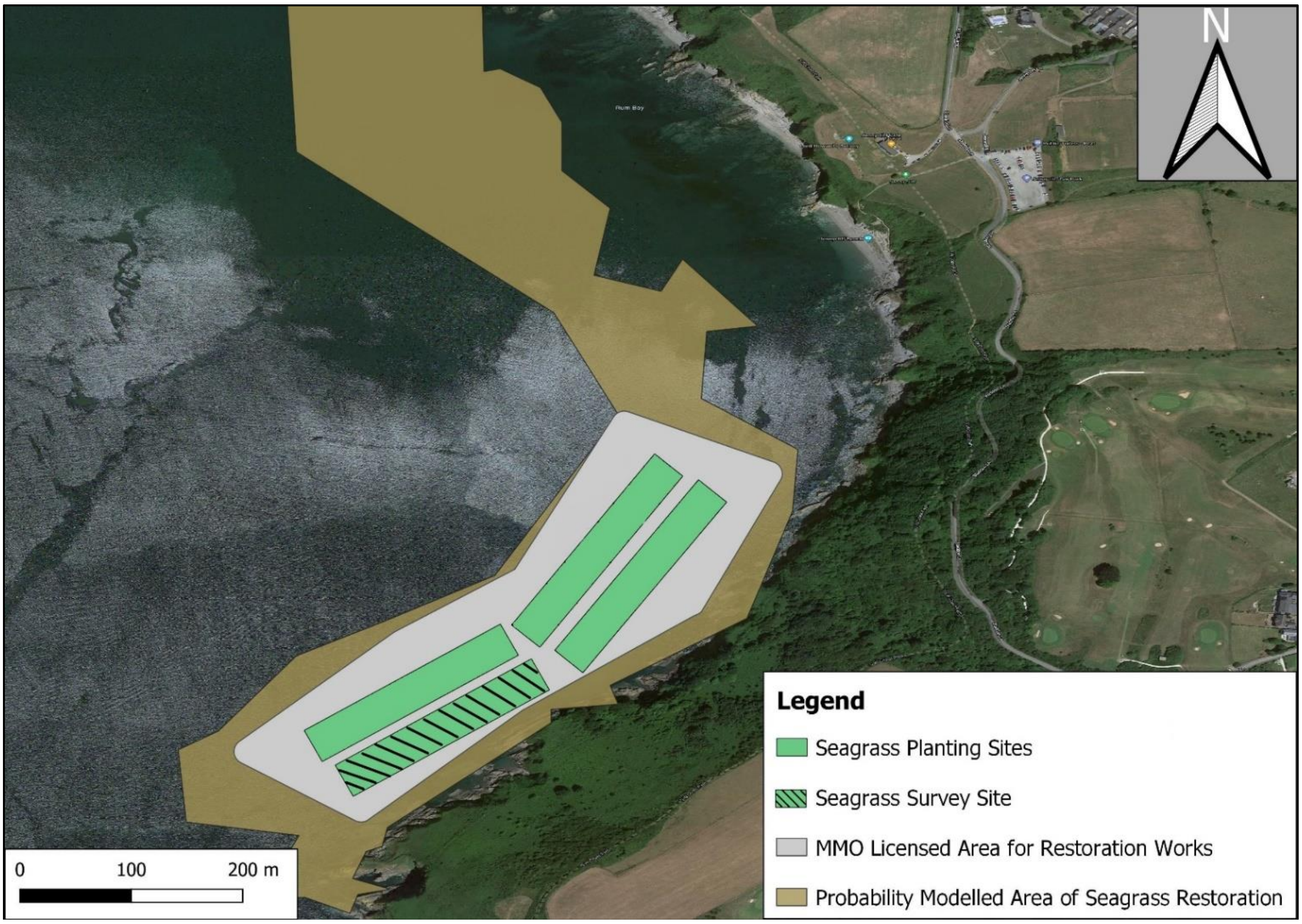


Figure 6. *Satellite map of Jennycliff Bay, Plymouth, highlighting the replanting sites and the initial 'bed' planted and subsequently surveyed in this study.*

The planting site is divided into four plots of approximately 200x50m in size, each to ultimately receive 18,200 bags per plot, with this survey covering the first plot planted – the south-westernmost plot (Figure 6).

GPS start and end points were recorded when depositing the seed bags in approximate straight lines over the course of four days (Figure 7). Initial planning had hoped to utilise divers to deposit and anchor seed bags connected in a grid format by biodegradable hessian rope to the benthos, in a variation of the TERFS method (Short *et al*., 2002a); however, COVID-19 restrictions prevented the necessary workforce for this methodology from assembling, so laying of the bags was achieved via the use of a barge navigating a series of fixed headings at a constant speed, with the bags being deployed at approximately 50cm distance intervals via a 4m chute extending down into the water column. A post-deployment dive was then conducted to confirm the successful depositing of the bags.

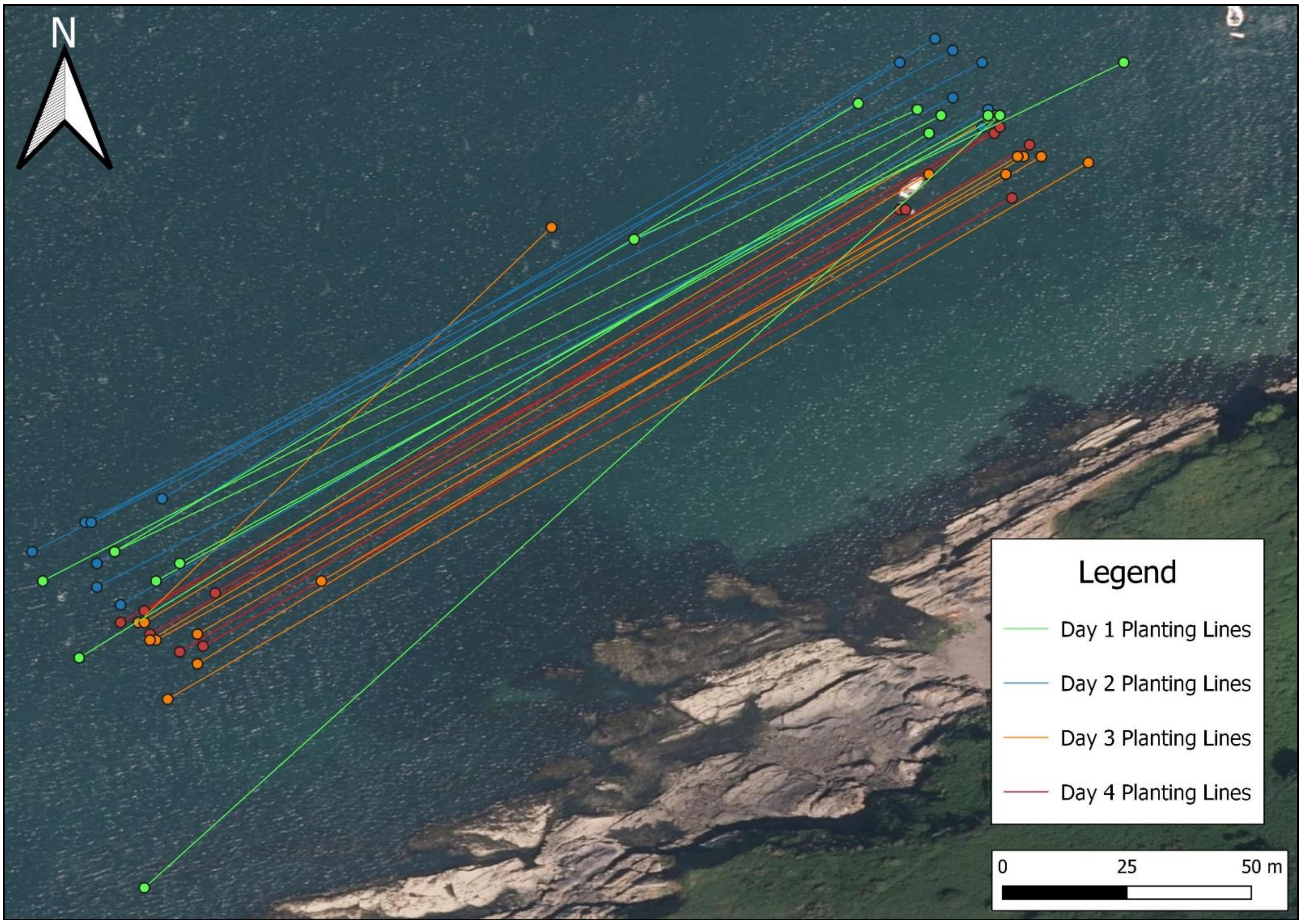


Figure 7. *Estimated lines of deployment of Z. marina seed and seedling bags over four days based upon GPS start/end points. Created with QGIS 3.4.10.*

3. Research Aims and Questions

The criteria set out to determine the condition of the planted seagrass is based upon those assessed by Natural England (2015) for the 2012 survey of PSE SAC's pre-existing seagrass beds as part of their statutory duty to periodically assess the condition status of the SAC. These included (but were not limited to):

1. Density of *Z. marina* (percentage cover and number of plants).
2. Maximum plant length.
3. Amount of infection with the slime mould, *Labyrinthula zosterae* (percentage cover on shoots/leaves).
4. Amount of epiphyte cover (percentage cover on shoots/leaves).

Therefore, this study aimed to answer the following four questions:

1. *What is the germination success of the seeds within the bags?*

- Do the hessian bags become significantly degraded and/or buried over time?

- Is there a significant change in the number of *Z. marina* plants/shoots per bag over time? I.E. Density of seedlings per bag.

2. *What is the growth rate of any emerging Z. marina plants?*

- Is there a significant change in the maximum shoot length over time?

3. *Is there evidence of the planted Z. marina establishing within the planting site?*

- Does the percentage cover of the planted seagrass 'bed' show temporal changes?

- What is the percentage cover of wasting disease and/or epiphytes on leaves from any emerging shoots and/or leaves and does this show temporal changes?

4. *Is there evidence of ecosystem engineering as a result of seagrass growth?*

- Does the abundance and/or diversity of fauna change in relation to seagrass growth?

Faunal surveys are included in the methodology as increased biodiversity is strongly correlated with the establishment of seagrass beds (Orth *et al*., 2020; Tan *et al*., 2020).

4. Survey Protocol and Methodology

Surveys were conducted by boat-based SCUBA and conformed to surveying methods used by the global seagrass monitoring network, Seagrass-Watch (McKenzie *et al*., 2003).

Three permanent submerged float lines spaced approximately 50m apart and starting 50m inward from the edges of the planting plot were installed to mark the seaward start/end points of three 50m transects, and their GPS coordinates were taken for locating on subsequent monitoring dives (Figure 8).

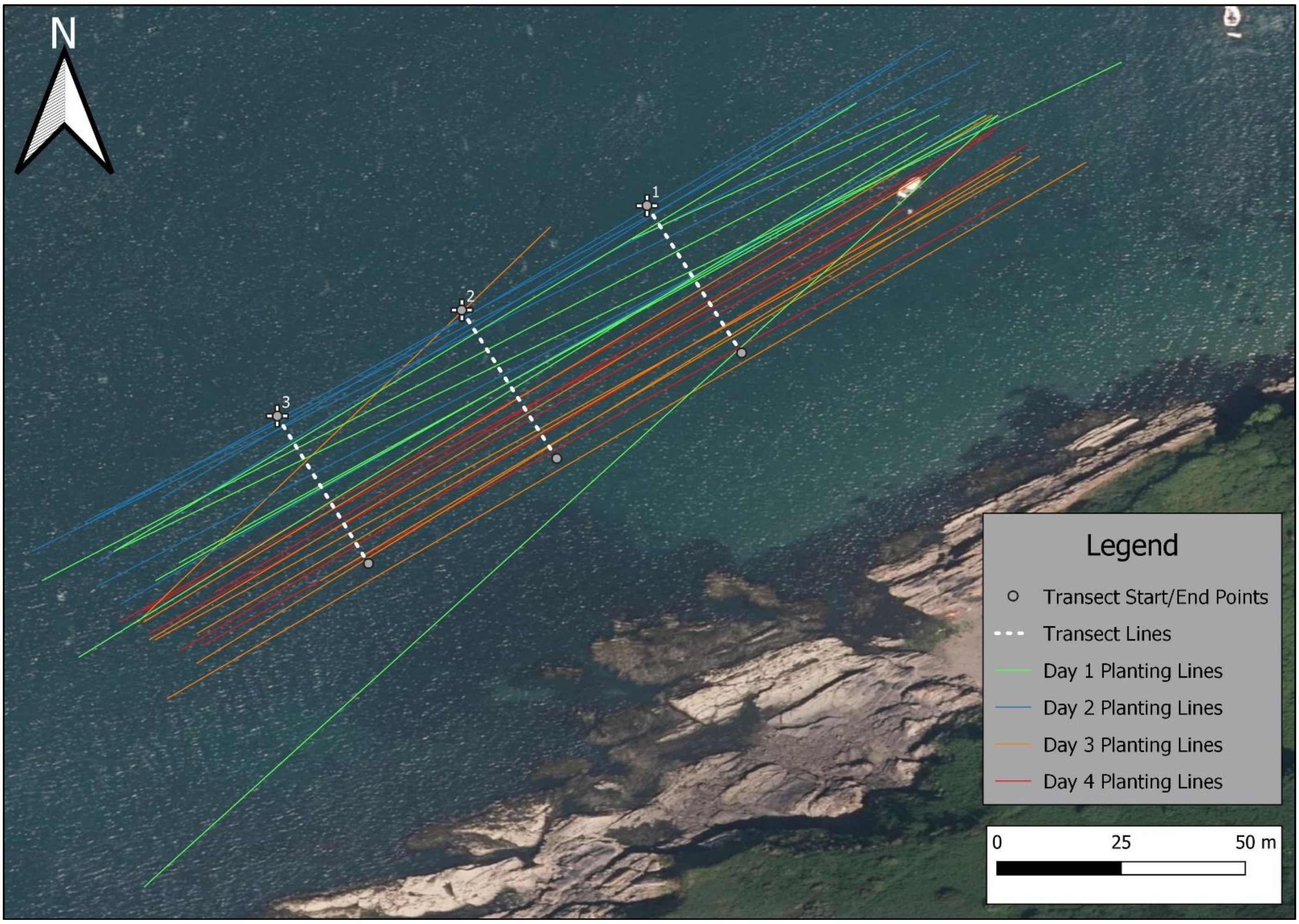


Figure 8*. The three survey transect lines created to intersect the planting area at 50m intervals based upon the estimated lines of deployment of Z. marina seed and seedling bags. Created with QGIS 3.4.10.*

Six divers, forming three buddy pairs, conducted the surveys over the course of one dive of max 60-minute duration (Figure 9). Each pair entered the water at the GPS coordinates of their respective float markers with the aid of a deployed shot line and descended to locate their corresponding marker. Once located, diver 1 swam along a 45^{o} SE onshore compass heading whilst reeling out the 50m transect. Diver 2 was positioned slightly above and behind Diver 1 and conducted a faunal count survey, videoing all visible fauna species approximately 1.5m either side of the tape (3m total belt transect width) using a

GoPro (models, Hero 5 – 8). As a secondary measure in case of camera failure or user error, faunal data was also recorded on the Fauna Survey Data Sheet by use of pencil on waterproof paper taped onto dive slates (Section 4.1).

A quadrat survey was then completed, following the same transect in reverse, with the first and last quadrat being placed at 50m and 5m, respectively. Diver 1 placed a 50x50cm quadrat at 5m intervals, with the bottom right corner of the quadrat adjacent to the tapes 5m interval, totalling 10 quadrats per transect. Each quadrat was then photographed by Diver 1, and Diver 2 proceeded to record the following parameters within each quadrat:

- Percentage cover of *Z. marina*
- Number of shoots/plants
- Shoot length(s)
- Epiphyte percentage cover
- Wasting disease percentage cover on shoots/leaves
- Macroalgae percentage cover
- Percentage of degradation and/or burial of bags falling within the quadrat

Laminated sheets of the Braun-Blanquet Percentage Cover Visual Aid were taken on dives to aid standardisation of percentage cover estimates between individual data collectors (Section 4.1). Photographs of quadrats were later analysed by the author and any obvious discrepancies in percentage estimates between transects/observers were regulated. Individual divers conducting the quadrat aspect of the survey were the same individuals between months and along the same transects to further aid standardisation of the visual estimate data.

Directly sampling individual bags was avoided by recommendation due to concerns of difficulty in locating or identifying the bags once they had broken down (Unsworth *et al*., 2019; Unsworth R., pers. comms., 18^{th} Dec 2020).

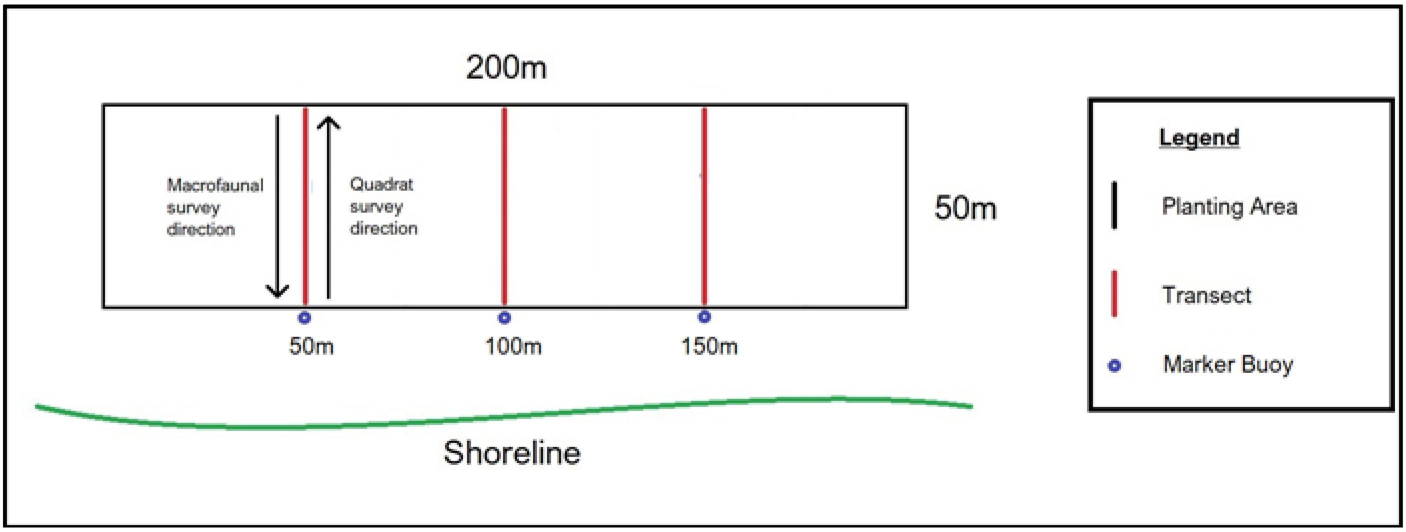


***Figure 9**. Aerial diagram of the methodology layout (not to scale).*

Environmental conditions of water temperature (Suunto Zoop dive computer), salinity (Milwaukee MA887 digital refractometer), turbidity (30cm diameter marine Secchi disk), and minimum/maximum depths (Suunto Zoop dive computer) were also be recorded during each dive.

The results in this report cover the survey period of June 2021 – September 2021 and are planned to be repeated annually for the next three years.

4.1 Field Data Recording Sheets

Seagrass Monitoring Data Recording Sheets

Environmental Variables Data Sheet

Date (dd/mm/yy):	
Time (24 hr):	
Water Temperature (°C):	
Air Temperature (°C):	
Salinity (ppt):	
Turbidity (m):	
Water Temperature (°C):	
Average Max Dive Depth (m):	
Average Min Dive Depth (m):	

	Max Dive Depth (m)
Buddy Pair 1 – Transect 1	
Buddy Pair 2 – Transect 2	
Buddy Pair 3 – Transect 3	

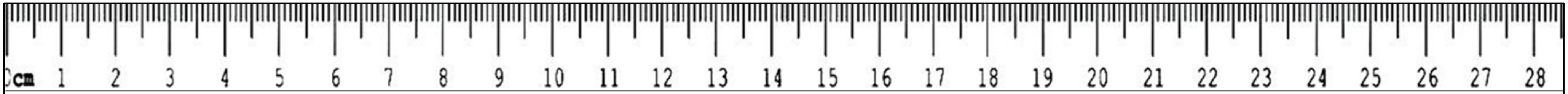
Survey/Transect Number:
Diver Buddy Pair:

Starting Survey Depth:
Starting Dive Time:

End Survey Time:
End Survey Depth:

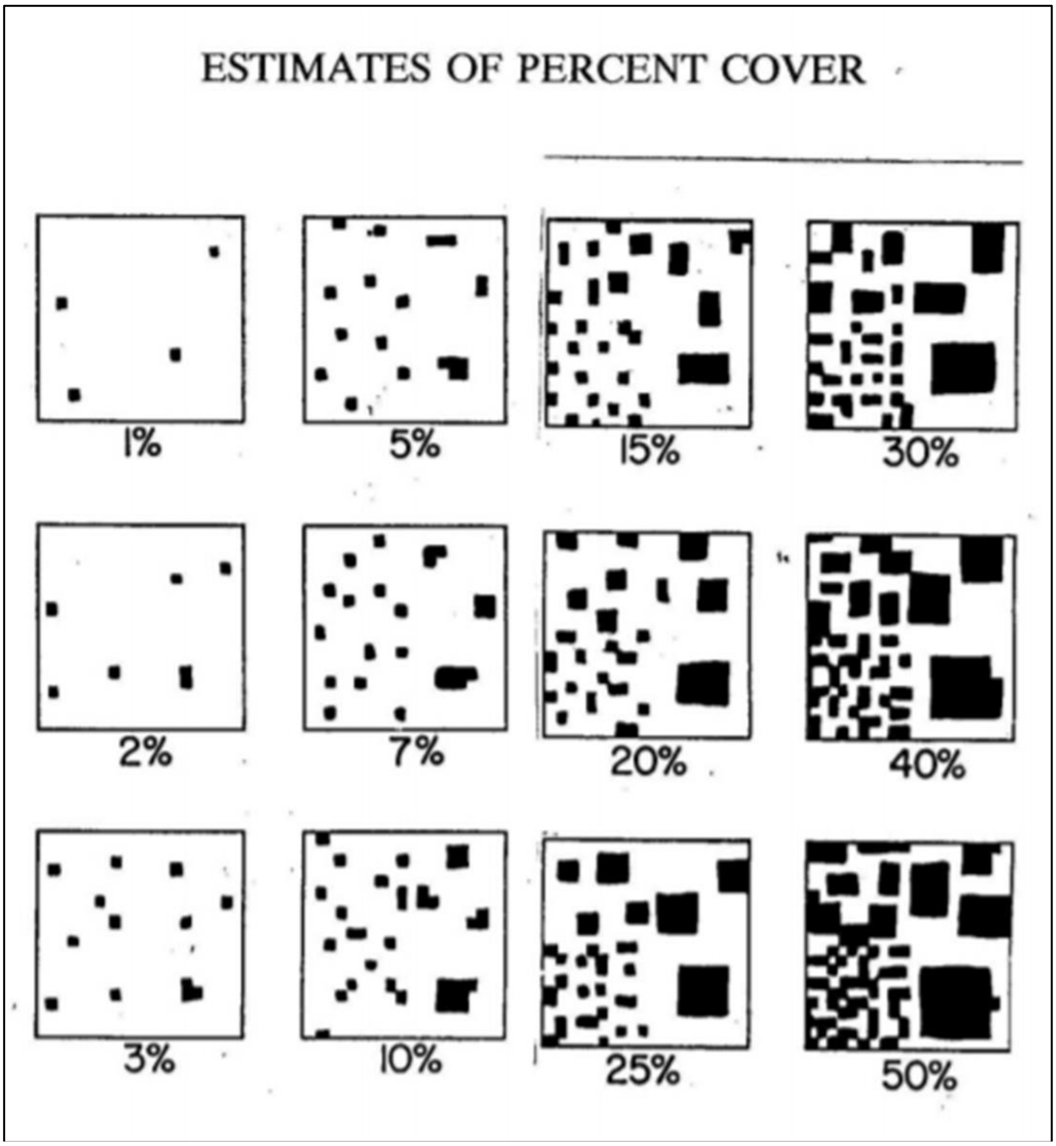
Fauna Survey Data Sheet

Species	Count (Tally)



Quadrat No.	No. of Plants	Max Plant Height (mm)	Wasting Disease % Cover	Epiphyte % Cover	% of bag degradation/ burial	Macroalgae % Cover	% Cover of Z. marina
50m							
45m							
40m							
35m							
30m							
25m							
20m							
15m							
10m							
5m							

BRAUN-BLANQUET PERCENTAGE COVER VISUAL AID



4.2 Data Analysis

Data analyses were conducted using a combination of IBM SPSS (v. 28.0.0), R (v. 3.6.1), QED Statistics (v. 1.5.4.502), and Microsoft Excel (v. 2107).

The majority of the collected data set was zero-inflated, and therefore the recorded variables of bag degradation percentage, number of shoots per bag, maximum shoot length, percentage cover of *Z. marina*, and percentage cover of macroalgae failed to adhere to a normal distribution (Shapiro-Wilk, $P < 0.05$) (Appendices 1 – 5) despite log10 transformations, and arsine square root transformations for percentage cover data (August bag degradation being the only exemption). As a non-parametric alternative, Friedman one-way repeated measure analysis of variance by ranks was selected to test for statistical significance due to the paired nature of the variables across three or more groups. Dunn's pairwise post-hoc tests with Bonferroni adjustment was used to identify where statistically significant differences lay between groups, if any.

An exact multinomial goodness of fit test was chosen as a more representative measure on any changes in shoot count numbers, as the zero-inflated count integer dataset would inevitably cause any statistical tests utilising the variability amongst medians to be insignificant, whilst a one-sample chi-square test for association was inappropriate due to all the expected values being < 5 (4.25). Chi-square test was utilised for assessing the faunal species richness count data between the four months, and an R x C contingency table was constructed in Excel and cross-referenced with results from QED in order to investigate species evenness count data across the four months using the chi-square test for goodness of fit. Chi-square values were compared with those on the table of critical values in order to identify significance (Appendix 13).

Due to a lack of seed dispersal from poor bag degradation, data analysis for quadrats regarding percentage cover of *Z. marina*, number of *Z. marina* shoots, and maximum shoot lengths, were conducted where a seed bag was present within a quadrat, so as to provide a more representative indication of immediate establishment and growth. Furthermore, the low number of emergent seagrasses posed problems in conducting statistical analysis of sufficient power and confidence, meaning any results presented here, significant, or otherwise, should be met with more uncertainty. Graphs were produced to aid in the identification of any potentially emerging trends at this early stage of the ReMEDIES PSE SAC project.

5. Results

***Table 2**. Mean environmental variable values (+ standard deviation) recorded for the June – Sept 2021 survey period.*

	Salinity (ppt)	Water Temp. (°C)	Survey Depth (m)	Turbidity (m)
Mean	32.00	15.25	10.51	4.50
SD	1.15	2.06	0.72	1.35

Percentage of bag degradation and burial across the four months of surveying showed slight statistical significance (χ^2F = 7.921, df=3, P < 0.05), but Dunn's pairwise post-hoc tests with Bonferroni adjustment (0.05/7) failed to identify any significant degradation between months (P > 0.05) (Appendix 6).

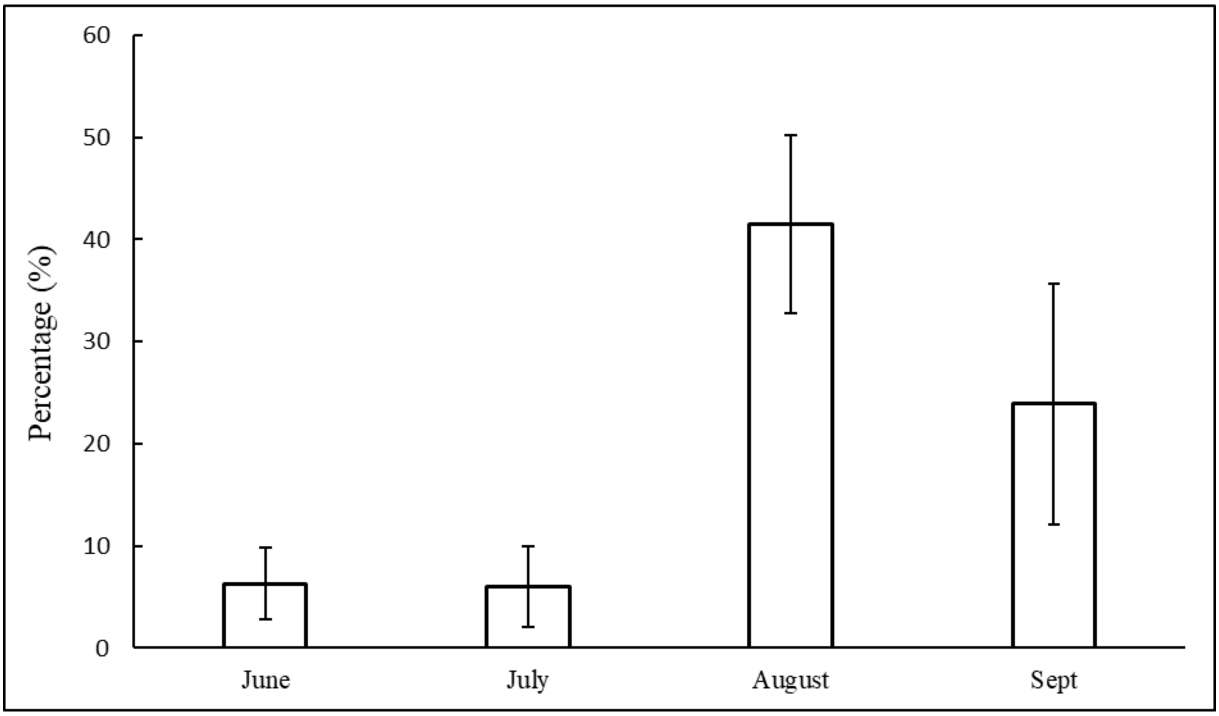


***Figure 10**. Mean bag degradation percentage ± S. E. across the four survey months. N=40 bags.*

<u>Z. marina Shoots per bag</u>

Shoots per bag between the four months did not significantly differ from the expectation of a random distribution (exact multinomial test, P > 0.05) (Appendix 7).

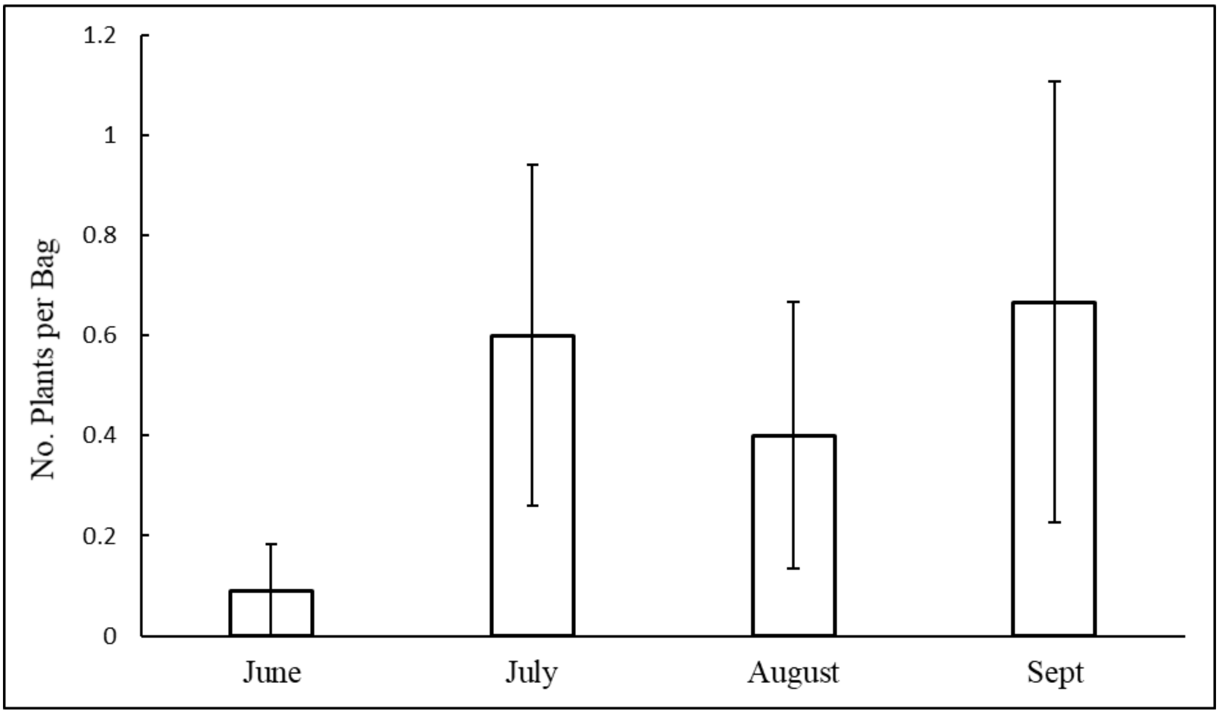


Figure 11. *Mean number of Z. marina shoots per bag ± S. E. across the four survey months. N=40 bags.*

Maximum Shoot Length

Maximum shoot length did appear to increase over the four survey months (Figure 12), but any perceived increase was not statistically significant ($\chi^2 F$ = 1.462, df=3, P > 0.05) (Appendix 8).

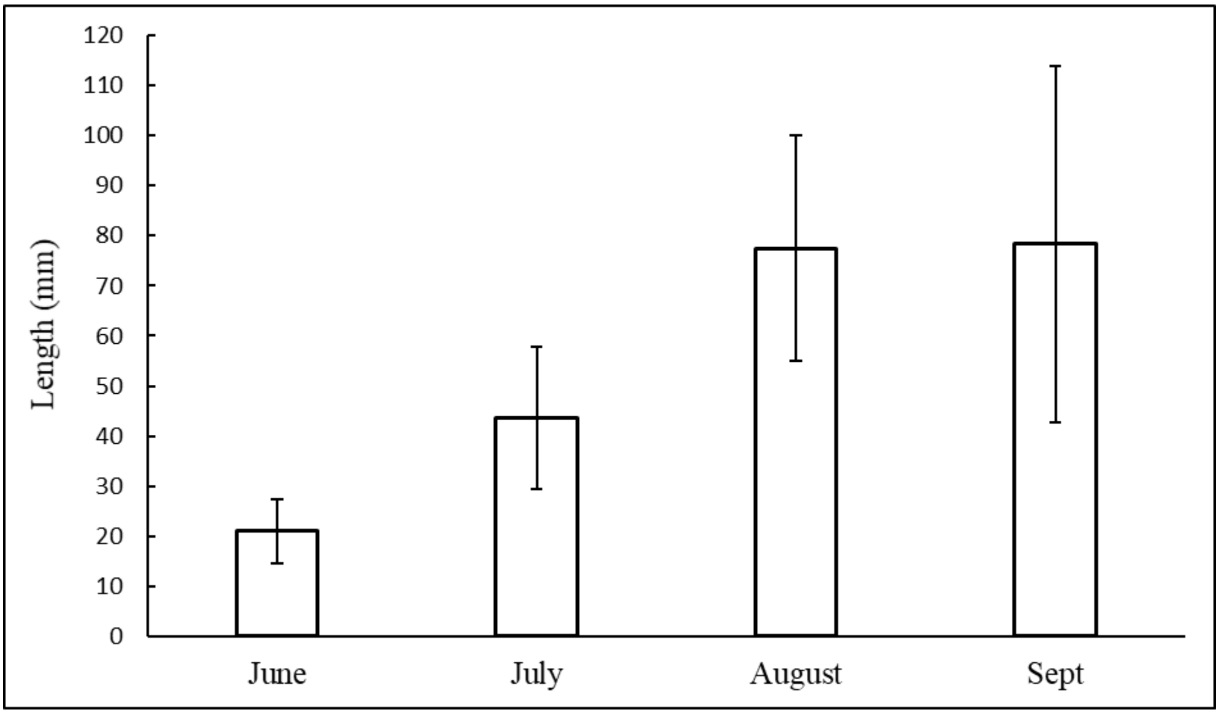


Figure 12. *Mean maximum shoot length ± S. E. of observed Z. marina across the four survey months. N=17.*

Percentage Cover of the Planted Seagrass

No statistically significant difference in the percentage cover of *Z. marina* within quadrats where a bag fell across the four survey months was observed ($\chi^2 F$ = 3.000, df=3, $P > 0.05$) (Appendix 9).

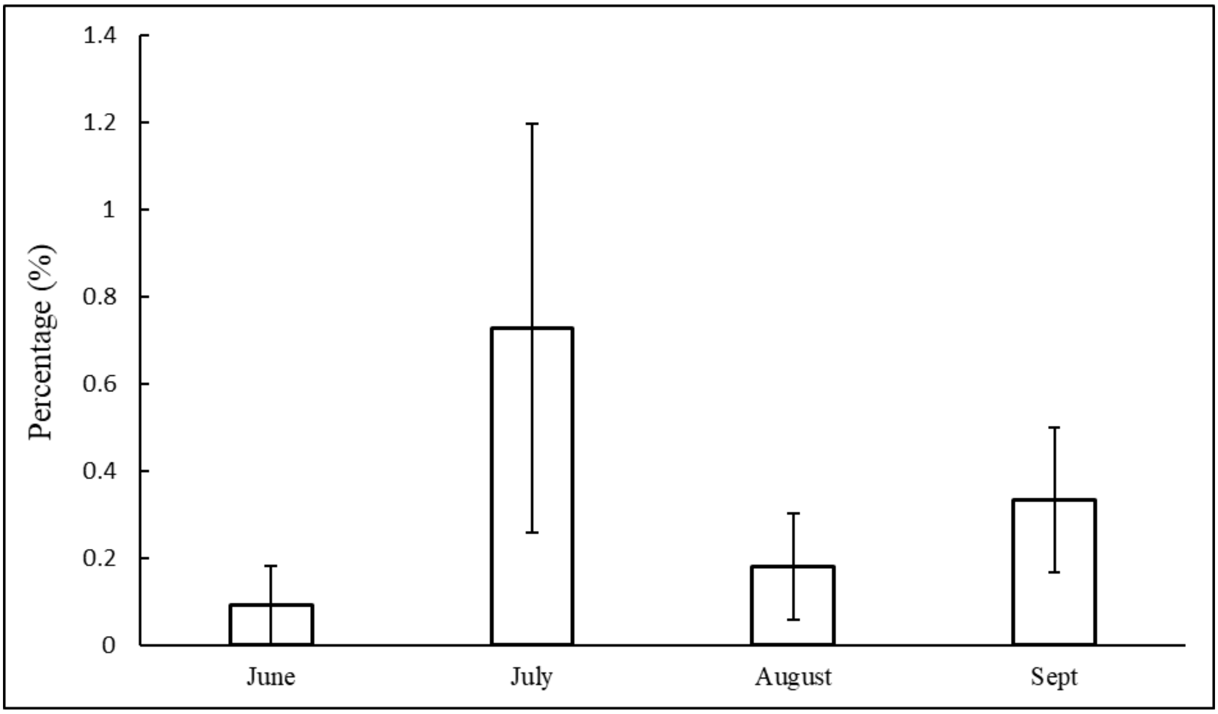


***Figure 13**. Mean percentage cover of Z. marina ± S. E. within 50x50cm quadrats in which bags fell. N=40 bags.*

Percentage Cover of Macroalgae

There was a statistically significant difference in the percentage cover of macroalgae across the four survey months ($\chi^2 F$ = 14.380, df=3, $P < 0.01$) (Appendix 10). Dunn's pairwise post-hoc tests with Bonferroni adjustment (0.05/7) identified macroalgae percentage cover as significantly higher in August compared to July (P < 0.005) (Appendix 10).

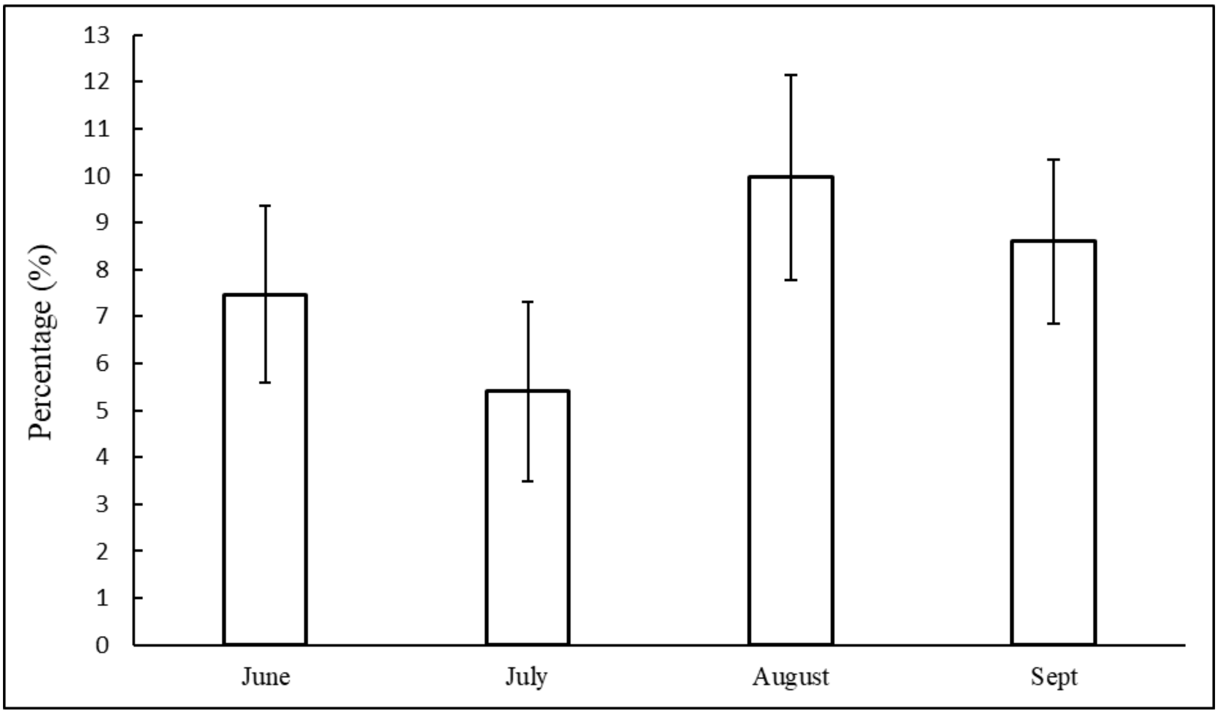


Figure 14. *Mean percentage cover of macroalgae ± S. E. within 50x50cm quadrats across the four months. N=120.*

Percentage Cover of Wasting Disease and Epiphytes

No instances of wasting disease or epiphyte cover were observed on any of the emergent *Z. marina* shoots.

Abundance and Diversity of Fauna

Table 3. *Compiled species list and abundance for the June – September 2021 survey period.*

Species	Count
Common Dragonet (*Callionymus lyra*)	46
Common Hermit Crab (*Pagurus bernhardus*)	9
Peacock Worm (*Sabella pavonina*)	232
Sand Mason Worm (*Lanice conchilega*)	4
Great Scallop (*Pecten maximus*)	3
Spiny Spider Crab (*Maja brachydactyla*)	11
Thornback Ray (*Raja clavata*)	1
Common Cuttlefish *(Sepia officinalis*)	1
Spiny Starfish (*Marthasterias glacialis*)	4

There was no significant departure from homogeneity in species richness between the survey months (*Chi-square test,* $\chi^2=0.44$*, df = 3, P > 0.05*) (Appendix 11), and no significant association between the survey month and species evenness (*Chi-square test,* $\chi^2 = 30.80$*, df = 24, P > 0.05*) (Appendix 12).

A mean Simpson's Diversity Index of 0.56 was recorded for the survey period (Appendix 14).

6. Discussion

Incidents of germination were far lower than expected, making it difficult to confidently identify any potential trends (Figure 11), and the most immediately notable observation that is likely to have influenced seed germination was the low percentage of hessian bag degradation (total survey period mean = 19.46% ± 4.34 S. E., Figure 10)., particularly when compared to the 100% degradation observed in the laboratory trials in under three weeks. Although overall bag degradation was objectively significant over the survey period, there was high variability in degradation between bags and there was still an apparent oversight in selection of hessian bag design, and it is likely that the seeds encased within the bags experienced a prolonged lack of flow, leading to anoxic and nutritiously depleted substratum; evidence of anoxic blackened sand directly beneath the bags was noted by multiple surveyors.

As the hessian bags failed to degrade as expected, with many maintaining full structural integrity, disturbance from fauna as a cause for lower survival rates of seedlings can be largely disregarded, unlike in Davis & Short (1997) who recorded comparable survival rates of as low as 1% at their less successful subtidal sites, attributing this to disturbance and predation from crabs and clam worms. Although the presence of two crab and two burrowing worm species were recorded in this survey (Table 3), the lack of bag degradation has likely maintained protection for both seeds and seedlings, so it is unclear as to whether faunal predation and disturbance would pose a threat to seagrass survival at this site in the event of rapid and complete bag degradation.

A further concern likely to have affected seed viability was the unplanned increase in storage duration. Although successful long-term storage of *Z. marina* seeds has been achieved by storing them at 0°C and 40–50ppt salinity (for up to 17 months), the unforeseeable delay in planting meant storage of the seeds was improvised at 6°C and 30ppt, and therefore likely not as successful. Storage at higher temperatures and lower salinities significantly increases seed mortality and decreases germination rates post longer term storage (Xu *et al*., 2020).

For seagrasses where germination did occur, the observed amount of growth was also considerably less than expected for the project and species (Sand-Jensen, 1975; Borum & Wium-Andersen, 1980; Wium-Andersen & Borum, 1984; Harrison, 1993). It is unclear whether unfavourable environmental conditions may be responsible, or if it is a consequence of poor seed health prior to planting. Nutrition, temperature, and irradiance are cited as the major influencing factors on *Z. marina* growth, and despite an HSI being outsourced and produced prior to site selection, there is concern that the depth of the planting site is unsuitable for the establishment of *Z. marina*. A mean maximum depth of 10.51m (± 0.72 S.D.) (Table 2) was recorded across the three transects, with all dives being conducted ± 1 hr from high tide, whilst the maximum depth tolerance of *Z. marina* is thought to be approximately 7m due to its light requirements, increasing to 12m in very clear water near its northern geographical limit (Unsworth *et al*., 2014; Scalpone *et al*., 2020). However, Lee *et al*. (2007) did find that eelgrass growing in lower temperatures had proportionately lower operating light requirements for photosynthesis compared to plants in areas of higher irradiance, so it is conceivable that the combination of operating within lower light conditions but at the lower end of its thermal tolerance (13–24°C) for optimum growth (survey period mean bottom temperature = 15.25°C (± 2.06 S.D.) (Table 2), makes significant *Z. marina* growth possible at the planting site.

Additionally, in other studies, a significant increase in seedling growth of Z. *marina* (as well as seed germination and establishment) has been observed at salinities of 35ppt, and, whilst lower salinities can be tolerated, it can significantly inhibit growth (Lee *et al*., 2007; Xu *et al*., 2016). Mean salinity across the sampling days was slightly low for the time of year (32ppt ± 1.15 S.D.) (Table 2), with rainfall for May (< 1 month after planting) having almost triple the past decades average rainfall for that month (142.8mm compared to 49.0mm) (Plymouth Live Weather Station, 2021). This may have further contributed to the poor growth and germination rates observed during the survey period. The HSI report produced is unavailable to the author for further insight. The installation of permanent data loggers at the study site would have provided more reliable insight into the potential influence of salinity and other environmental variables on the planting effort, but this was outside of the financial scope of the study.

Based on the ≥5% mean seagrass coverage definition for a meadow (WFD-UKTAG, 2014), the first planting phase of the Plymouth Sound SAC ReMEDIES project has not produced an official seagrass bed within six months of planting, with a final mean percentage cover of just 0.33% (± 0.16 S.E.) being recorded in the final survey. As percentage cover is a direct consequence of both seed germination/survival rate and growth, the failure of the planted bags to produce a seagrass bed habitat so far can be attributed to the possible influencing factors previously mentioned; chiefly lack of bag degradation, seed viability/health, and unfavourable environmental conditions.

The cover of macroalgae remained relatively low, most likely due to the less sheltered nature of the site, resulting in noticeable currents at low water and therefore preventing smothering. However, significant changes in percentage cover over the survey period were observed, but the non-linear fashion of the variation over the survey period (Figure 14) is most likely due to variability in wave action intensity occurring as a result of storm/swell events and/or spring and neep tides (Bos & van Katwijk, 2005). However, it is worth noting that Bos & van Katwijk (2005) documented poor germination and an increase to seedling mortality of *Z. marina* for unknown reasons in areas of higher flow. No epiphyte cover was recorded on any of the emergent shoots, possibly further indicating that flow at the site is sufficient to prevent this, or it may be that not enough time has elapsed to allow colonisation, and although no evidence of wasting disease, *L. zosterae*, was recorded in this study, very little is known about the mechanisms influencing the prevalence of this opportunistic pathogen (Jakobsson-Thor *et al*., 2020). Given the historical prevalence of wasting disease (it was responsible for a 99% decrease in Atlantic eelgrass populations in under three years in 1931), it is perhaps the single greatest threat to successfully established seagrass restoration projects, with the potential to decimate both existing and novel seagrass meadows (Muehlstein, 1989). Continuing to monitor its presence/absence and extent (if present) in conjunction with constant monitoring of environmental parameters recorded in this survey (sea temperature, salinity, visibility) could highlight potential causative factors worth investigating further (Jakobsson-Thor *et al*., 2020).

Lastly, it is not surprising that there was also no significant change in the abundance, nor diversity of fauna recorded at Jennycliff Bay, as such a change would be indicative of the formation of a seagrass habitat, which did not occur (Duarte *et al*., 2013). Seagrass meadows play a vital role in promoting biodiversity by providing shelter and reducing localised environmental stressors whereby facilitating fish and invertebrate settlement, but in turn attracting predators, resulting in a trophic cascade (Zhang & Silliman, 2019). Furthermore, it has been documented through isotopic niche metrics that transplanted seagrass beds provide the same degree of ecological functions as natural beds (Park *et al*., 2021). However, the degree of establishment and growth observed at the site was very likely insufficient to allow for any of these ecosystem services to develop. Nevertheless, the data collected is still potentially useful for establishing a baseline to be used against any future monitoring of fauna at the site.

7. Future Monitoring

Sediment core sampling is recommended as it would allow for the assessment of below ground conditions, providing insights into how sediment nitrogen and carbon content might influence seagrass growth rates (Lee *et al*., 2007; Fraser & Kendrick, 2017; Moreno- Marín *et al*., 2018); this was unfortunately beyond the financial scope of this preliminary monitoring project.

The next phase of the ReMEDIES Plymouth project will see the deployment of up to 1.5 million seeds, divided between the four plots, every year for the next three years. The methodology of monitoring presented here will continue each year, during the same four months post-deployment. Once, or if, a bed is established, transitioning from transect sampling to stratified random sampling would likely provide data on density with more statistical power (SAC, 2018). However, predetermining how many samples are required for the optimum trade-off between sampling effort and statistical power is difficult to quantify without an understanding of the natural levels of variability at a site, which in the case of planting of novel meadows, would be unknown.

Future monitoring should also aim to survey further additional attributes indicative of multigenerational establishment once the emergent seagrass should have matured, such as the presence of flowering seagrass and seed-bearing shoots (Davison & Hughes, 1998). Mean Simpson's Diversity indices can also be compared between the annual surveys to assess whether the planted seagrass begins to provide a significant enough habitat to encourage an increase in biodiversity.

8. Conclusion

This has been a preliminary report on the first phase of a novel seagrass planting project within the UK, whereby methods and materials utilised have been assessed in order to provide feedback to the OCT for the future deployment of *Z. marina* seeds in the area. Ultimately it seems likely that a culmination of setbacks, delays, and improvisations relating to the storage and deployment of the seagrass seeds brought about by the COVID-19 pandemic, and the oversight relating to the degradation of the hessian bags once deployed, hindered the monitoring project from providing as much meaningful data on the temporal changes of the planted seagrass as had been hoped/predicted. Had the lack of bag degradation been anticipated, direct sampling of bags would have provided a larger and subsequently more comprehensive dataset. It was hoped that further significant bag degradation would occur after the first monitoring dive, and once this was not observed to be the case on the second monitoring dive, it was decided to persevere with the current methodology for the sake of dataset consistency, as financial constraints meant monitoring was limited to a maximum of four survey dives total. As a result, it is still unclear at this stage as to whether the seagrass planting project at Jennycliff bay will successfully establish a seagrass meadow.

However, the restoration, transplanting, and creation of seagrass meadows is still a developing science, and as such, the best practice methodology to assess the status and success of planting seagrass is also still yet to be determined (Ganassin & Gibbs, 2008).

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10. Appendices

Appendix 1: Shapiro-Wilk test for normality on Arcsine transformed bag degradation percentage data, performed in R (v. 3.6.1).

```
> shapiro.test(June_Bags_Arcsine)

        Shapiro-Wilk normality test

data:  June_Bags_Arcsine
W = 0.78405, p-value = 0.005804

>
> shapiro.test(July_Bags_Arcsine)

        Shapiro-Wilk normality test

data:  July_Bags_Arcsine
W = 0.71546, p-value = 0.001357

>
> shapiro.test(August_Bags_Arcsine)

        Shapiro-Wilk normality test

data:  August_Bags_Arcsine
W = 0.93957, p-value = 0.5482

>
> shapiro.test(Sept_Bags_Arcsine)

        Shapiro-Wilk normality test

data:  Sept_Bags_Arcsine
W = 0.79575, p-value = 0.01826
```

Appendix 2: Shapiro-Wilk test for normality on Log10 transformed number of shoots per bag data, performed in R (v. 3.6.1).

```
> shapiro.test(June_Shoots_Log)

        Shapiro-Wilk normality test

data:  June_Shoots_Log
W = 0.34499, p-value = 2.243e-08

>
> shapiro.test(July_Shoots_Log)

        Shapiro-Wilk normality test

data:  July_Shoots_Log
W = 0.65553, p-value = 0.0002558

>
> shapiro.test(August_Shoots_Log)

        Shapiro-Wilk normality test

data:  August_Shoots_Log
W = 0.50927, p-value = 4.672e-06

>
> shapiro.test(Sept_Shoots_Log)

        Shapiro-Wilk normality test

data:  Sept_Shoots_Log
W = 0.67145, p-value = 0.0006581
```

Appendix 3: Shapiro-Wilk test for normality on Log10 transformed shoot length data, performed in R (v. 3.6.1).

```
> shapiro.test(June_Height_Log)

        Shapiro-Wilk normality test

data:  June_Height_Log
W = 0.34499, p-value = 2.243e-08

>
> shapiro.test(July_Height_Log)

        Shapiro-Wilk normality test

data:  July_Height_Log
W = 0.64055, p-value = 0.000169

>
> shapiro.test(August_Height_Log)

        Shapiro-Wilk normality test

data:  August_Height_Log
W = 0.52524, p-value = 7.198e-06

>
> shapiro.test(Sept_Height_Log)

        Shapiro-Wilk normality test

data:  Sept_Height_Log
W = 0.66939, p-value = 0.0006226
```

Appendix 4: Shapiro-Wilk test for normality on Arcsine transformed *Z. marina* percentage cover data, performed in R (v. 3.6.1).

```
> shapiro.test(June_Cover_Arcsine)

        Shapiro-Wilk normality test

data:  June_Cover_Arcsine
W = 0.34499, p-value = 2.243e-08

>
> shapiro.test(July_Cover_Arcsine)

        Shapiro-Wilk normality test

data:  July_Cover_Arcsine
W = 0.65952, p-value = 0.0002857

>
> shapiro.test(August_Cover_Arcsine)

        Shapiro-Wilk normality test

data:  August_Cover_Arcsine
W = 0.50927, p-value = 4.672e-06

>
> shapiro.test(Sept_Cover_Arcsine)

        Shapiro-Wilk normality test

data:  Sept_Cover_Arcsine
W = 0.61728, p-value = 0.0001526
```

Appendix 5: Shapiro-Wilk test for normality on Arcsine transformed macroalgae percentage cover data, performed in R (v. 3.6.1).

```
> shapiro.test(June_Macro_Arcsine)

        Shapiro-Wilk normality test

data:  June_Macro_Arcsine
W = 0.87849, p-value = 0.002604

>
> shapiro.test(July_Macro_Arcsine)

        Shapiro-Wilk normality test

data:  July_Macro_Arcsine
W = 0.72816, p-value = 4.118e-06

>
> shapiro.test(August_Macro_Arcsine)

        Shapiro-Wilk normality test

data:  August_Macro_Arcsine
W = 0.81048, p-value = 0.0001024

>
> shapiro.test(Sept_Macro_Arcsine)

        Shapiro-Wilk normality test

data:  Sept_Macro_Arcsine
W = 0.84572, p-value = 0.0005052
```

Appendix 6: Bag degradation percentage between months Friedman one-way repeated measure analysis of variance by ranks and Dunn's pairwise post-hoc tests with Bonferroni adjustment output, performed in IBM SPSS (28.0.0.0 (190)).

Hypothesis Test Summary

	Null Hypothesis	Test	Sig.[a,b]	Decision
1	The distributions of June, July, August and Sept are the same.	Related-Samples Friedman's Two-Way Analysis of Variance by Ranks	.048	Reject the null hypothesis.

a. The significance level is .050.

b. Asymptotic significance is displayed.

Related-Samples Friedman's Two-Way Analysis of Variance by Ranks

June, July, August, Sept

Related-Samples Friedman's Two-Way Analysis of Variance by Ranks Summary

Total N	11
Test Statistic	7.921
Degree Of Freedom	3
Asymptotic Sig.(2-sided test)	.048

Pairwise Comparisons

Sample 1-Sample 2	Test Statistic	Std. Error	Std. Test Statistic	Sig.	Adj. Sig.[a]
July-June	.273	.550	.495	.620	1.000
July-Sept	-.591	.550	-1.073	.283	1.000
July-August	-1.318	.550	-2.395	.017	.100
June-Sept	-.318	.550	-.578	.563	1.000
June-August	-1.045	.550	-1.899	.058	.345
Sept-August	.727	.550	1.321	.186	1.000

Each row tests the null hypothesis that the Sample 1 and Sample 2 distributions are the same.
Asymptotic significances (2-sided tests) are displayed. The significance level is .050.

a. Significance values have been adjusted by the Bonferroni correction for multiple tests.

Appendix 7: Number of shoots per bag between months exact multinomial goodness of fit test, performed in R (v. 3.6.1).

```
> library(EMT)
> observed =c(A = 1, B = 6, C = 4, D = 6)
> out <- multinomial.test(observed, prob, useChisq = FALSE, MonteCarlo = FALSE)

 Exact Multinomial Test, distance measure: p

    Events    pObs    p.value
      1140  0.0017     0.2223
```

Appendix 8: Maximum shoot length Friedman one-way repeated measure analysis of variance by ranks, performed in IBM SPSS (28.0.0.0 (190)).

Hypothesis Test Summary

	Null Hypothesis	Test	Sig.[a,b]	Decision
1	The distributions of June, July, August and Sept are the same.	Related-Samples Friedman's Two-Way Analysis of Variance by Ranks	.691	Retain the null hypothesis.

a. The significance level is .050.

b. Asymptotic significance is displayed.

Related-Samples Friedman's Two-Way Analysis of Variance by Ranks

June, July, August, Sept

Related-Samples Friedman's Two-Way Analysis of Variance by Ranks Summary

Total N	11
Test Statistic	1.462[a]
Degree Of Freedom	3
Asymptotic Sig.(2-sided test)	.691

a. Multiple comparisons are not performed because the overall test retained the null hypothesis of no differences.

<u>**Appendix 9**: *Z. marina* percentage cover Friedman one-way repeated measure analysis of variance by ranks, performed in IBM SPSS (28.0.0.0 (190)).</u>

Hypothesis Test Summary

	Null Hypothesis	Test	Sig.[a,b]	Decision
1	The distributions of June, July, August and Sept are the same.	Related-Samples Friedman's Two-Way Analysis of Variance by Ranks	.392	Retain the null hypothesis.

a. The significance level is .050.

b. Asymptotic significance is displayed.

Related-Samples Friedman's Two-Way Analysis of Variance by Ranks

June, July, August, Sept

Related-Samples Friedman's Two-Way Analysis of Variance by Ranks Summary

Total N	11
Test Statistic	3.000[a]
Degree Of Freedom	3
Asymptotic Sig.(2-sided test)	.392

a. Multiple comparisons are not performed because the overall test retained the null hypothesis of no differences.

Appendix 10: Macroalgae percentage cover Friedman one-way repeated measure analysis of variance by ranks, with post-hoc tests with Bonferroni adjustment, performed in IBM SPSS (28.0.0.0 (190)).

Hypothesis Test Summary

	Null Hypothesis	Test	Sig.[a,b]	Decision
1	The distributions of June, July, August and Sept are the same.	Related-Samples Friedman's Two-Way Analysis of Variance by Ranks	.002	Reject the null hypothesis.

a. The significance level is .050.

b. Asymptotic significance is displayed.

Related-Samples Friedman's Two-Way Analysis of Variance by Ranks

June, July, August, Sept

Related-Samples Friedman's Two-Way Analysis of Variance by Ranks Summary

Total N	30
Test Statistic	14.380
Degree Of Freedom	3
Asymptotic Sig.(2-sided test)	.002

Pairwise Comparisons

Sample 1-Sample 2	Test Statistic	Std. Error	Std. Test Statistic	Sig.	Adj. Sig.[a]
July-June	.517	.333	1.550	.121	.727
July-Sept	-.800	.333	-2.400	.016	.098
July-August	-1.150	.333	-3.450	<.001	.003
June-Sept	-.283	.333	-.850	.395	1.000
June-August	-.633	.333	-1.900	.057	.345
Sept-August	.350	.333	1.050	.294	1.000

Each row tests the null hypothesis that the Sample 1 and Sample 2 distributions are the same.
Asymptotic significances (2-sided tests) are displayed. The significance level is .050.

a. Significance values have been adjusted by the Bonferroni correction for multiple tests.

Appendix 11: Chi-square test for association calculation table for species richness across months, produced in Microsoft Excel.

	Species Richness					
		June	July	August	Sept	TOTAL
	Observed	7	7	5	6	25
	Expected	6.25	6.25	6.25	6.25	25
DF = 3	TOTAL	0.09	0.09	0.25	0.01	0.44

Appendix 12: Test for association RxC contingency table calculation for species evenness across months, produced in Excel and cross-referenced with QED Statistics output (1.5.4.502).

	Common Dragonet	Hermit Crab	Peacock Worm	Sand Mason Worm	Scallop	Spider Crab	Thornback Ray	Cuttlefish	Spiny Starfish	Total (row)
June Observed	11	2	47	1	2	4	1	0	0	68
June Expected	10.05787781	1.96784566	50.7266881	0.874598071	0.655948553	2.4051447	0.218649518	0.218649518	0.874598071	
x^2	0.088248657	0.0005254	0.273784959	0.017980424	2.753987769	1.0575511	2.792178929	0.218649518	0.874598071	
July Observed	8	3	36	3	0	3	0	0	1	54
July Expected	7.987138264	1.56270096	40.2829582	0.694533762	0.520900322	1.9099678	0.173633441	0.173633441	0.694533762	
x^2	2.07113E-05	1.32196022	0.455371992	7.652867095	0.520900322	0.6220891	0.173633441	0.173633441	0.134348577	
August Observed	15	1	82	0	0	4	0	0	2	104
August Expected	15.38263666	3.0096463	77.58199357	1.337620579	1.003215434	3.6784566	0.334405145	0.334405145	1.337620579	
x^2	0.009517927	1.34191126	0.25158906	1.337620579	1.003215434	0.0281069	0.334405145	0.334405145	0.328005194	
Sept Observed	12	3	67	0	1	0	0	1	1	85
Sept Expected	12.57234727	2.45980707	63.40836013	1.093247588	0.819935691	3.0064309	0.273311897	0.273311897	1.093247588	
x^2	0.026055707	0.1186306	0.203441265	1.093247588	0.039543534	3.0064309	0.273311897	1.932135427	0.007953471	
Total (Column)	46	9	232	4	3	11	1	1	4	311
x^2 =	30.47385155									
DF =	24									

Explore the Statistic: Contingency Table	
Chi-Squared	30.8019
DF	24
Prob.	0.159578
Cramer v	0.181697
Contingency coefficient C	0.300193
No. Columns	9
No. Rows	4
This means	The null hypothesis is accepted. The two variables are independent of each other (Chi-Squared = 30.8019, DF = 24, P = >0.05)

Appendix 13: Chi-square table of critical values, taken from Plant and Soil Sciences eLibrary 2021.

Degrees of Freedom	Probability of a larger value of x^2								
	0.99	0.95	0.90	0.75	0.50	0.25	0.10	0.05	0.01
1	0.000	0.004	0.016	0.102	0.455	1.32	2.71	3.84	6.63
2	0.020	0.103	0.211	0.575	1.386	2.77	4.61	5.99	9.21
3	0.115	0.352	0.584	1.212	2.366	4.11	6.25	7.81	11.34
4	0.297	0.711	1.064	1.923	3.357	5.39	7.78	9.49	13.28
5	0.554	1.145	1.610	2.675	4.351	6.63	9.24	11.07	15.09
6	0.872	1.635	2.204	3.455	5.348	7.84	10.64	12.59	16.81
7	1.239	2.167	2.833	4.255	6.346	9.04	12.02	14.07	18.48
8	1.647	2.733	3.490	5.071	7.344	10.22	13.36	15.51	20.09
9	2.088	3.325	4.168	5.899	8.343	11.39	14.68	16.92	21.67
10	2.558	3.940	4.865	6.737	9.342	12.55	15.99	18.31	23.21
11	3.053	4.575	5.578	7.584	10.341	13.70	17.28	19.68	24.72
12	3.571	5.226	6.304	8.438	11.340	14.85	18.55	21.03	26.22
13	4.107	5.892	7.042	9.299	12.340	15.98	19.81	22.36	27.69
14	4.660	6.571	7.790	10.165	13.339	17.12	21.06	23.68	29.14
15	5.229	7.261	8.547	11.037	14.339	18.25	22.31	25.00	30.58
16	5.812	7.962	9.312	11.912	15.338	19.37	23.54	26.30	32.00
17	6.408	8.672	10.085	12.792	16.338	20.49	24.77	27.59	33.41
18	7.015	9.390	10.865	13.675	17.338	21.60	25.99	28.87	34.80
19	7.633	10.117	11.651	14.562	18.338	22.72	27.20	30.14	36.19
20	8.260	10.851	12.443	15.452	19.337	23.83	28.41	31.41	37.57
22	9.542	12.338	14.041	17.240	21.337	26.04	30.81	33.92	40.29
24	10.856	13.848	15.659	19.037	23.337	28.24	33.20	36.42	42.98
26	12.198	15.379	17.292	20.843	25.336	30.43	35.56	38.89	45.64
28	13.565	16.928	18.939	22.657	27.336	32.62	37.92	41.34	48.28
30	14.953	18.493	20.599	24.478	29.336	34.80	40.26	43.77	50.89
40	22.164	26.509	29.051	33.660	39.335	45.62	51.80	55.76	63.69
50	27.707	34.764	37.689	42.942	49.335	56.33	63.17	67.50	76.15
60	37.485	43.188	46.459	52.294	59.335	66.98	74.40	79.08	88.38

Appendix 14: Simpson's Diversity Index calculation tables for each month, produced in Microsoft Excel.

Simpsons Diversity Index

June

Species	n_i	$n_i(n_i-1)$	N(N-1)	$\sum n_i(n_i-1)$	$Ds=\sum n_i(n_i-1)/N(N-1)$
Dragonet	11	110	4556	2288	0.502194908
Hermit Crab	2	2			
Peacock Worm	47	2162			
Sand Mason Worm	1	0			
Scallop	2	2			
Spider Crab	4	12			
Thornback Ray	1	0			
Cuttlefish	0	0			
Spiny Starfish	0	0			
Total	68				

July

Species	n_i	$n_i(n_i-1)$	N(N-1)	$\sum n_i(n_i-1)$	$Ds=\sum n_i(n_i-1)/N(N-1)$
Dragonet	8	56	2862	1334	0.466107617
Hermit Crab	3	6			
Peacock Worm	36	1260			
Sand Mason Worm	3	6			
Scallop	0	0			
Spider Crab	3	6			
Thornback Ray	0	0			
Cuttlefish	0	0			
Spiny Starfish	1	0			
Total	54				

August

Species	n_i	$n_i(n_i-1)$	N(N-1)	$\sum n_i(n_i-1)$	$Ds=\sum n_i(n_i-1)/N(N-1)$
Dragonet	15	210	10712	6866	0.640963406
Hermit Crab	1	0			
Peacock Worm	82	6642			
Sand Mason Worm	0	0			
Scallop	0	0			
Spider Crab	4	12			
Thornback Ray	0	0			
Cuttlefish	0	0			
Spiny Starfish	2	2			
Total	104				

Sept

Species	n_i	$n_i(n_i-1)$	N(N-1)	$\sum n_i(n_i-1)$	$Ds=\sum n_i(n_i-1)/N(N-1)$
Dragonet	12	132	7140	4560	0.638655462
Hermit Crab	3	6			
Peacock Worm	67	4422			
Sand Mason Worm	0	0			
Scallop	1	0			
Spider Crab	0	0			
Thornback Ray	0	0			
Cuttlefish	1	0			
Spiny Starfish	1	0			
Total	85				

Mean index	0.5619803