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The use of storm fragments and biodegradable replanting methods allows for a low-impact habitat restoration method of seagrass meadows, in the eastern Aegean Sea

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SUMMARY

Seagrasses are important marine ecosystems but are vulnerable to physical damage from anthropogenic activities such as anchoring and trawling. Replanting damaged areas can represent a viable restoration strategy, yet current methods rely on the removal of plants from existing meadows and in some cases the use of non-sustainable planting materials. In this paper, we present evidence of a sustainable replanting strategy. Storm fragments of the endemic Mediterranean seagrass, neptune grass *Posidonia oceanica* were collected from the shore and shallow water, both the plagiotropic and orthotropic (horizontal and vertical) growth forms were then replanted using one of two biodegradable materials, coconut fibre pots or bamboo stakes, to secure them to the seafloor. Establishment of plagiotropic fragments were increased by bamboo anchorage ($\bar{x}$ = 89% SE ± 0%) compared to orthotropic storm fragments ($\bar{x}$ = 66.5% SE ± 6.5%). By contrast a coconut fibre method resulted in greater establishment of orthotropic fragments ($\bar{x}$ = 79% SE ± 7%) compared to plagiotropic ($\bar{x}$ = 51% SE ± 11%). Fragments showed some blade growth, but little shoot growth after 15 months. The fragment shoot and blade growth did not differ between the plagiotropic or orthotropic fragments replanted by bamboo stakes or coconut fibre pot. Our results suggest that the use of storm fragments and biodegradable anchoring materials constitutes a viable, non-destructive replanting technique in seagrass restoration. Furthermore success can be increased by selecting a growth-form appropriate planting method.

BACKGROUND

Despite seagrass meadows' ability to provide high value ecosystem services, including supporting commercial fisheries (Heck *et al.* 2003), nutrient cycling (Orth *et al.* 2006) and sediment stabilisation (Waycott *et al.* 2009), there has been a global decline in seagrass habitat since the 1970s (Orth *et al.* 2006). The positive feedback provided by seagrass ecosystem services, such as reduced turbidity, may promote the resilience of alternative stable states once seagrass is lost and explain why restoration techniques have historically had varied success (van der Heide *et al.* 2007). However, successful seagrass meadow restoration has been shown to not only restore seagrass cover, but also the ecosystem services they provide, such as carbon sequestration (Greiner *et al.* 2013). Given that seagrass regression may be caused by numerous factors, many of which are anthropogenic in origin (Boudouresque *et al.* 2009), restoration strategies may need to respond to distinct stressors.

The endemic Mediterranean seagrass, neptune grass *Posidonia oceanica* provides ecosystem services that are estimated at up to €514 ha^{-1} year^{-1} (Campagne *et al.* 2015). *P. oceanica* meadows are protected under the EU Habitats Directive 1992, where they are acknowledged as being a priority habitat requiring designated areas of conservation (Campagne *et al.* 2015).

Protection is also afforded through the EU Common Fisheries Policy (EC 1626/94, 1994), which prohibits trawling (Lachopoulou *et al.* 2013) and the use of towed fishing gear over areas of *P. oceanica* (1967/2006). Direct physical disturbance is particularly detrimental to the survival of this slow-growing species. Due to such a slow growth rate (rhizome extension rates are just 1-6 cm yr^{-1}) *P. oceanica* is particularly vulnerable to physical damage, such as that caused by anchoring or illegal trawling in the meadows; in the long term even small boats using low-impact anchors can have detrimental consequences (Milazzo *et al.* 2004) as recovery can take hundreds of years (Marbà *et al.* 1996).

Research has suggested there may be potential for using storm fragments for replanting, rather than donor meadows. During the winter storm fragments of *P. oceanica* wash ashore and form onshore banquettes. Collecting such fragments before they desiccate allows the material to be utilised for restoration. There are several advantages of using this technique over traditional methods, including greater availability with lower collection efforts, with significantly less impact on existing populations (Balestri *et al.* 2010). Of the three techniques used for seagrass restoration (seeds, shoots and bare roots with sediment intact and bare roots with shoots) (Davis & Short 1997), bare root with shoots are the most appropriate for replanting from storm fragments. Storm fragments are either

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planted with materials to secure them directly to the seafloor, or with materials that assist in stabilising the surrounding sediment. Previous transplanting research used a mixed approach in the selection of materials used to secure fragments directly onto the seafloor: whilst some have favoured biodegradable materials, such as bamboo pegs (Davis & Short 1997), others have utilised non-degradable, and potentially polluting, materials such as plastic-coated steel wire hooks (Bastyan & Cambridge 2008). In light of increasing problems of marine pollution, including plastics, replanting techniques should avoid methods that use such materials (Bastyan & Cambridge 2008) in favour of using biodegradable materials to support storm fragments. The aim of our study was to test whether storm fragments planted with biodegradable materials can provide an effective and sustainable method for restoring areas showing signs of physical damage (e.g. anchor scars).

ACTION

Seagrass fragment collection

Posidonia oceanica storm fragments were collected from February to April 2017 at three southerly sites in Samos, Greece (37°45′N 26°50′E). Loose fragments were collected from the shoreline or by snorkelers up to a depth of 5 m. Collection only took place once at the largest site, but more continuously at the two small sites near the research base, as collection at these two sites was simply to replenish the stock of viable storm fragments. Fragments were deemed viable if the blades exhibited no zones of necrosis and the rhizome length was a minimum of 5 cm. Both *P. oceanica* growth forms (plagiotropic and orthotropic) were collected (Figure 1) with a larger proportion of orthotropic fragments available. After collection, fragments were immediately deposited into containers of seawater then transferred to large transparent containers (4 boxes of 50 x 40 x 30 cm). Collection of further storm fragments was limited by the available storage, as approximately 60 fragments were stored in each container, to prevent overcrowding and shading. The seawater was changed every 1-2 days until transportation to the replanting site in April, at which point any fragments no longer deemed viable were discarded.

Replanting site

The fragments were all transported on 10th April 2017 by ferry to Lipsi Island approximately 88 km south of Samos, for replanting at Vroulia Bay, NW Lipsi (37°18′N 26°45′E). Vroulia is a sheltered bay, with limited boat traffic and occasional anchor pressure. Between arrival and replanting (24 – 43 days), the fragments were stored in the same transparent containers. These were covered with mesh and submerged in Vroulia Bay to allow for a period of acclimatisation. Two replanting sites at 4.5 m and 8 m depths were identified: an L-shaped scar within the seagrass bed and a concave indent into the seagrass bed.

Figure 1. Orthotropic fragment on site in Vroulia for pre-replanting measurements (left, photo © K. R. de Moraes) and plagiotropic fragment during health check (right, photo © E. A. Ward).

Seagrass replanting

Replanting was carried out 6th, 21st and 23rd May 2017 over three one-hour scuba dives. Prior to replanting, growth form, number of shoots, number of blades and maximum blade length for each fragment were noted. The fragments were planted 5 cm apart (Molenaar & Meinesz 1995) in four rows of six fragments on six 25 x 15 cm grids. Plagiotropic growth forms were placed preferentially on the outside of the grids, to replicate rhizome positioning in natural seagrass meadows. Plagiotropic rhizomes were planted with the horizontal rhizome orientated out from the centre of the grids (after Molenaar & Meinesz 1995).

Two biodegradable anchoring methods were used, coconut fibre plant pots and bamboo shoots (Figure 2). For method one, each fragment was pressed into the top 2 cm of sediment and secured with two pieces of bamboo (approximately 15 cm segments) inserted on either side of the fragment to form an inverted “V”. For the second method the top 5 cm layer of sediment was removed to allow for the placement of coconut fibre trays that formed a perimeter (35 x 25 cm) around the 25 x 15 grid of storm fragments. The plant pots were inverted and an incision made between each row of pots to allow the rhizomes to penetrate into the sediment below.

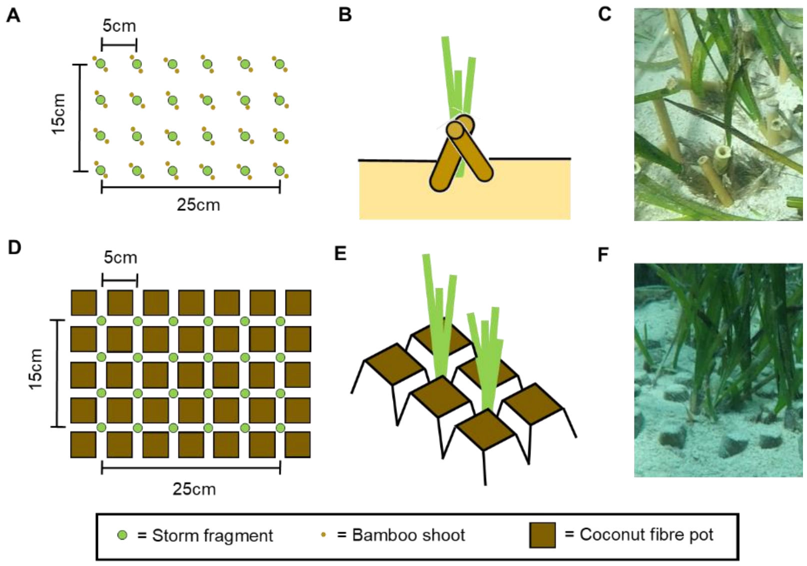

Figure 2. **A** and **D** Storm fragments are planted in four rows of six to form 15 x 25 cm grids for both replanting methods. **B** and **C,** Bamboo shoots are inserted either side of the fragment to form an inverted "V". **E** and **F,** Coconut fibre pots are inverted and covered in sediment to act as a sediment stabiliser.

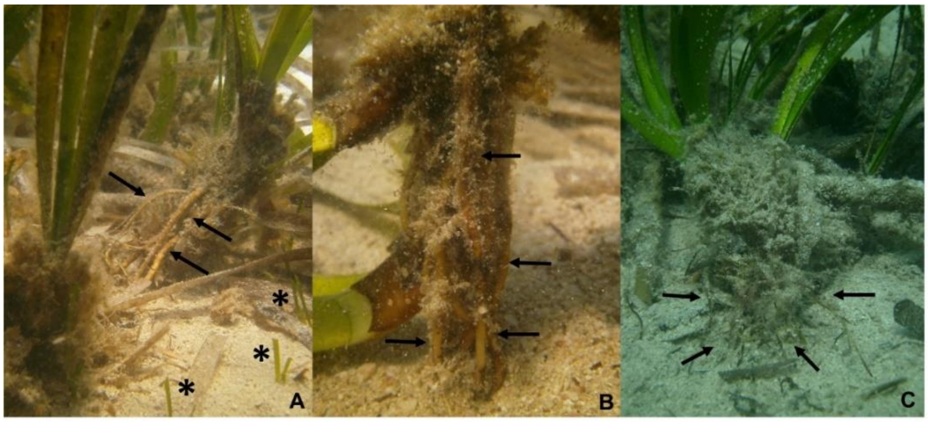

Figure 3. Replanted fragments after 15 months, arrows indicate new root growth, ***** indicates colonisation of the seagrass species, *Cymodocea nodosa*. **A** and **B** fragments at 4.5m depth. **C** fragment at 8m depth. All photos © E. A. Ward.

The trays were covered with the sediment to leave the rhizome partially buried with the shoots emerging above. *In situ* photo documentation was carried out with two GoPro Hero5 cameras (Figure 2C and 2F).

Fragments were monitored after fifteen months on the 23rd and 24th August 2018 during two one-hour scuba dives where the same measurements were noted for each fragment. *In situ* photo documentation was carried out with a Sealife DC1400 (Figure 3).

Data analysis

Generalised Linear Mixed Models (GLMM), with logit link function, accounting for binomial distribution and nesting accounted for as a random effects term were used to determine the statistical significance of factors that impacted fragment establishment (Bolker *et al.* 2008). Replant method (bamboo stake and coconut fibre pots), storm fragment growth form (plagiotropic and orthotropic) and depth (site 1 at 4.5 m and site 2 at 8 m depth) were initially included as fixed factors, including any interaction between them. However, as the deep bamboo planted fragments were likely impacted by recreational boating damage, the likelihood of storm fragment establishment between planting methods could not be determined for depth, this made it inappropriate to include depth as a fixed factor within our model for predicting fragment establishment across both replant methods. Therefore, the data for the bamboo stake planted storm fragments at the deep site were removed from the establishment data analysis. The full model was therefore:

Establishment ~ growth form + method + growth form * method + (1|Block.ID)

To determine the statistical significance of each main term and the interactions they were removed from the model and compared to the more complex model using maximum likelihood (Laplace approximations) to test our *a priori* hypotheses (Crawley 2007).

While not ideal, due to the damage to our experimental site, to predict the expected fragment establishment due to differences in the depth of replant site, a GLMM model was refit to the coconut fibre method data across both depths. Therefore, replant method was not included as a fixed factor in this model. The same stepwise model simplification methods were undertaken as above to determine the retention of factors, depth and growth form, within the maximal model for the likelihood of establishment.

The fragments, that were used for the bamboo and coconut replant methods, prior to planting into grids, were not statistically different from each other in terms of maximum blade length ($t_{(142)} = 0.75065$, p = 0.4541), number of blades ($t_{(142)} = 0.0967$, p = 0.9231) and number of shoots ($t_{(142)} = 0.35396$, p = 0.7239) – therefore we analyse the data, for the change in growth from the start to the end of the experiment.. We used ANOVA to determine the statistical significance of replant method (bamboo stake and coconut fibre pots) and storm fragment growth form (plagiotropic and orthotropic) on the change in maximum blade length, number of blades and number of shoots. We examined the residuals of each model for excessive patterning or deviations from normality and all were sound. All statistical analysis was completed using R version 3.5.1.

CONSEQUENCES

A total of 144 *P. oceanica* storm fragments were replanted, 96 at 4.5 m and 48 at 8 m depth, in six grid formations. Fifteen months later, when the sites were resurveyed evidence of a large physical disturbance (presumed anchor drag) to the grid replanted by bamboo method at 8 m was observed. The five remaining grids planted across both sites showed no signs of external physical disturbances.

There was a significant interaction between the planting method and seagrass fragment growth form ($Z_{1,5} = -2.751$, $p < 0.01$) (Figure 4). The plagiotropic storm fragments planted with bamboo stakes had a higher percentage of fragment establishment (raw data $\bar{x}$ = 89% SE ± 0%), than the orthotropic fragments planted by the same method ($\bar{x}$ = 66.5% SE ± 6.5%) (Table 1). By contrast, the plagiotropic storm fragments planted by the coconut fibre method had a lower percentage of establishment ($\bar{x}$ = 51% SE ± 11%) compared to the orthotropic fragments planted by the same method ($\bar{x}$ = 79% SE ± 7%). The success of establishment was not influenced by the depth (4.5 vs. 8 m) at which fragments were planted ($Z_{1,3} = -0.333$, p = 0.739), nor was establishment influenced by an interaction between the growth form and the depth which fragments were planted ($Z_{1,3} = -1.376$, p = 0.1688), but this is only using the storm fragments that were replanted by the coconut fibre method.

The number of blades decreased amongst the surviving plagiotropic and orthotropic fragments of both the coconut fibre (plagiotropic $\bar{x}$ = -3 SE ± 4 blades, orthotropic $\bar{x}$ = -2 SE ± 2 blades) and bamboo stake method 15 months after planting (plagiotropic $\bar{x}$ = -4 SE ± 4 blades, orthotropic $\bar{x}$ = -2 SE ± 1 blades) and there was no significant difference in the decrease in blade numbers between the fragment growth forms planted by either method ($F_{(3,8)} = 0.07114$, p = 0.9738). The maximum blade length decreased amongst the surviving plagiotropic coconut fibre ($\bar{x}$ = -4.6 SE ± 1.2 cm), orthotropic coconut fibre ($\bar{x}$ = -5.8 SE ± 1.6 cm) and plagiotropic bamboo ($\bar{x}$ = -0.7 SE ± 3.8 cm) planted fragments, whilst the orthotropic bamboo planted fragments marginally increased in maximum blade length ($\bar{x}$ = 2.8 SE ± 4.7 cm). However, there was no significant difference in the change in maximum blade length between the replant methods and fragment growth form 15 months after planting ($F_{(3,8)} = 1.527$, p = 0.2806). The surviving fragments showed marginal to no change in the number of shoots for the orthotropic and plagiotropic fragments planted by both the coconut fibre (plagiotropic $\bar{x}$ = 0 SE ± 0.1 shoots, orthotropic $\bar{x}$ = 0 SE ± 0.2 shoots) and bamboo stake method (plagiotropic $\bar{x}$ = -1 SE ± 0.5 shoots, orthotropic $\bar{x}$ = 0 SE ± 0.3 shoots) and there was no significant difference between the shoot growth for the orthotropic and plagiotropic growth forms planted by both replant methods ($F_{(3,8)} = 2.39$, p = 0.1443). Overall the fragments showed little blade growth and shoot growth after 15 months. The overall change in blade and shoot growth from the start to the end of the experiment did not vary between the orthotropic and plagiotropic fragments planted by either the bamboo or coconut fibre method.

No quantitative data concerning root growth were recorded as this would have disturbed the fragment colonisation process, but visual evidence suggested new root growth had occurred (Figure 3). It was also noted that at the time of replanting the fragments were planted within an L-shaped scar on a patch of bare sand and 15 months later alongside the replanted fragments the seagrass little neptune grass, *Cymodocea nodosa,* had begun to colonise (Figure 3).

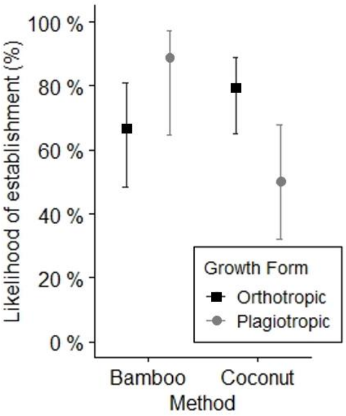

Figure 4. The modelled likelihood of establishment by orthotropic and plagiotropic *P. oceanica* storm fragments under different replanting methods. Error bars represent 95% confidence intervals of the modelled mean fragment establishment.

Table 1. Number of fragments planted by method and growth form with fragment establishment 15 months after planting. Grid 3 establishment excluded, due to physical disturbance.

Replant Method	Fragment Growth Form	Grid	Fragments Planted	Fragments Established	Establishment (%)
Bamboo Stakes	Plagiotropic	1	9	8	89
		2	9	8	89
		3	9	-	-
	Orthotropic	1	15	9	60
		2	15	11	73
		3	15	-	-
Coconut Fibre Pots	Plagiotropic	4	10	3	30
		5	9	5	56
		6	9	6	67
	Orthotropic	4	14	10	71
		5	15	14	93
		6	15	11	73

DISCUSSION

This study provides strong evidence to support the use of storm fragments as a suitable material for seagrass replanting in the Mediterranean (Balestri *et al.* 2010), negating the need to use donor meadows for provision of fragments which causes further damage to healthy meadows (Pereda-Briones *et al.* 2018). Our findings demonstrate that small areas of bare sand surrounded by seagrass, such as areas of physical damage caused by anchors, could be restored effectively using planted fragments, even in the case of the slow growing *P. oceanica.* The success of storm fragment replantation is dependent on the growth form of available fragments. In this study, a higher proportion of orthotropic storm fragments were collected, therefore using coconut fibre would have enabled better establishment of this fragment type. However, using plagiotropic fragments, even if these only represent a smaller proportion of the storm fragments collected, is important as horizontal growth by plagiotropic fragments may better assist in the colonisation of bare substrate surrounding the replanted areas. As plagiotropic fragments have improved establishment when replanted using bamboo, a mixed replanting approach is recommended between fragment growth forms.

Whilst there was little evidence of blade and shoot growth, fragment establishment combined with visual evidence of root growth suggests the redistribution of nutrient content to new roots (Balestri *et al*. 2010), which assists the stabilisation of the sediment (Christianen *et al*. 2013). Sediment stabilisation created by replanting – although not measured - may have created conditions that enabled *Cymodocea nodosa* to colonise alongside the storm fragments. These fragments therefore have the potential to assist in sediment re-stabilisation of scar areas and persist once any bamboo or coconut fibre materials have fully biodegraded, contributing to the establishment of multispecies seagrass meadows. Whilst this study highlights the positive potential in replanting strategies, the optimum conservation management strategy would be to prevent physical disturbances, such as anchoring or anchor drags. This could be achieved through the creation of anchor-free zones or provision of semi-permanent buoy-based anchors. Storm fragments are highly susceptible to damage and loss, similar to the existing seagrass beds and evidenced even in a small area during this study.

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