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Assessing methods for restoration of eelgrass (Zostera marina L.) in a cold temperate region

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ABSTRACT

More than 50% of eelgrass habitats have disappeared from the Swedish NW coast in the last 30 years. Restoration is being proposed to assist recovery but little is known regarding methods suitable under Scandinavian conditions; e.g. short growing seasons and scouring by ice. In the present study we evaluated different restoration methods using shoots and seeds in a Swedish fjord and assessed if eelgrass could be successfully transplanted between sites with different depth and exposure. The study demonstrates that both shoot- and seed methods can be successfully used to restore eelgrass at this latitude. Survival and growth of unanchored single shoots, transplanted without sediment in shallow habitats (1.0–1.5 m) was very high (>500% increase in shoot density after 14 months). This restoration method showed 2–3.5 times higher growth rate and was 2–2.5 times faster compared with shoots anchored in the sediment and shoots transplanted in sediment cores, respectively, and is recommended for shallow habitats in Sweden. Growth within deeper habitats (3.0–4.5 m) was substantially lower (40% loss to 50% increase) due to light limitations and high winter mortality. Restoration using seeds distributed from mesh-bags showed very low seedling establishment rates (approximately 1%) making this method less cost-effective than transplanting single shoots in shallow habitats. However, growth of seedlings was high and this method is recommended for deep habitats with soft sediment where shoot transplantation is difficult. Despite dramatic differences in eelgrass morphology between habitats with different depth and exposure, all shoots within a planting site had the same morphology at the end of the study, independent of origin. A baseline genetic survey supported that the observed changes in morphology of transplants were due to a plastic response, suggesting that donor populations do not have to exactly match the morphology of the plants targeted for restoration.

1. Introduction

Seagrasses play an important role as key ecosystems within many coastal areas and provide functions important on a local as well as global scale (Costanza et al., 1997; Orth et al., 2006a; Rönnbäck et al., 2007; Fourqurean et al., 2012). As a result of anthropogenic impact seagrass ecosystems are experiencing a rapid worldwide decline (Short and Wyllie-Echeverria, 1996; Waycott et al., 2009; Short et al., 2011). To sustain the functions that seagrass provide there is a need to improve management and conservation within marine spatial planning (Orth et al., 2006b; Douvere, 2008; Kenworthy et al., 2006; Boström et al., 2014) and many countries have realized the need to include seagrasses in their marine management plans for their importance in providing ecosystem services (Borum et al., 2004; Orth et al., 2006a; Waycott et al., 2009; Cole and Moksnes, 2016) and as functional indicators for water quality conditions (e.g. Bricker et al., 2003; Yamamuro et al., 2003; Baaner and Stoltenborg, 2011).

Eelgrass (Zostera marina L.) is one of the most widely distributed species of seagrass in the northern hemisphere, and the dominating species of the temperate North Atlantic (Short et al., 2007). Transplantation of eelgrass has been used in the USA since the 1940s as a method for restoring damaged or lost eelgrass habitats (Addy, 1947) and has since been successfully used to mitigate losses in several areas (e.g. Boston Harbour, MA, USA: Leschen et al., 2010; Chesapeake Bay, VA, USA: Orth et al., 2012; Southern California, USA: Olsen et al., 2014). Over the past 20 years, restoration experiments with eelgrass have been performed in many countries (e.g. Netherlands: van Katwijk et al., 1998; Bos et al., 2005; Korea: Li et al., 2010; Japan: Tanaka et al., 2011) and is becoming acceptable as a functional practice and as a management tool to improve coastal ecosystems (Paling et al., 2009; De Groot et al., 2013). However, the global success rate of seagrass restoration projects is still low (<50%; Fonseca, 2011; van Katwijk et al., 2015), which is also the case for Northern Europe (Cunha et al., 2012). In Scandinavian countries, no successful restoration of eelgrass has been

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performed and losses continue (Baden et al., 2003; Frederiksen et al., 2004; Jorgensen and Bekkby, 2013; Boström et al., 2014). According to a global meta-analysis on seagrass restoration, the success depends largely on the scale of the project (larger ones being more successful), the conditions at the restoration site and the technique used for planting (van Katwijk et al., 2015). The most commonly used techniques for restoring eelgrass involves transplantation of shoots within intact sediment cores (plugs) or the transplantation of shoots with bare roots and rhizomes, with and without anchoring (Fonseca et al., 1998; van Katwijk et al., 2015). Also, restoration with seeds is emerging as an efficient method for large-scale restoration (Orth et al., 2012). Guidelines regarding suitable methods and appropriate site characteristics for eelgrass restoration are generally based on local studies (e.g. Fonseca et al., 1998; Short et al., 2002; van Katwijk et al., 2009). Presently there is a lack of studies comparing methods for eelgrass restoration at high latitude areas. We are only aware of one report testing eelgrass restoration in Denmark (Christensen et al., 1995).

Along the NW Swedish coast around 60% of eelgrass meadows have been lost since the 1980s and in some areas over 80% has disappeared (Baden et al., 2003; Nyqvist et al., 2009). Eutrophication is considered a major factor behind the decline, together with cascading effects resulting from overfishing of large predatory fish (Moksnes et al., 2008; Baden et al., 2012). A successful effort to reduce nutrient loading over the past 20 years has improved water quality in many coastal areas (Anonymous, 2014, Moksnes et al., 2015) but no recovery of eelgrass coverage have been observed (Nyqvist et al., 2009). Swedish national agencies are presently discussing restoration of eelgrass, which could help meet the demands of good environmental status set by the new EU Marine Strategy Framework Directive (2008/56/EG).

So far, there have been very few studies assessing the efficiency of different restoration methods for seagrass habitats in high latitude areas (see Phillips and Lewis, 1983 for transplantation of eelgrass in Alaska; Christensen et al., 1995 for transplantation of eelgrass in Denmark) and it is unclear whether methods developed for lower latitudes are suitable. The NW coast of Sweden is characterized by low light conditions from October through to March, making the growing season relatively short, and ice cover and scouring during the winter pose potential problems for restoration in shallow areas. In this region, eelgrass grows subtidally in monospecific meadows (Boström et al., 2003), usually in sheltered areas with soft muddy sediments high in organic content (10–25%; Jephson et al., 2008). This can result in high sulfide invasion (Holmer et al., 2009), which may pose challenges for restoration. The morphology of the plants varies with exposure and depth, and it is unclear whether this is a result of a phenotypically plastic response to local conditions or a result of specifically adapted genotypes (i.e., ecotypic differentiation). The distinction is important in restoration, where selection of donor material, possibly from distant meadows or patches, should match the targeted restoration area (Fonseca et al., 1998; van Katwijk et al., 1998; Olsen et al., 2013). Fulfilling the match criteria might be difficult to accomplish in areas where large quantities of eelgrass have disappeared and available donor meadows are scarce, occur in different habitats or remain geographically distant. Therefore, it is important to investigate if morphology and origin of transplants affects the establishment success in the new environment or if eelgrass has the ability to acclimatize to new environmental conditions.

The aim of this study was to compare and evaluate restoration methods for typical conditions along the NW coast of Sweden and determine if eelgrass could be successfully transplanted between sites with different depth and exposure. Plant material was reciprocally transplanted between 4 habitat sites (shallow and deep sites within a sheltered and exposed location) to determine factors affecting growth and establishment success of transplants. Three methods of transplanting shoots (plug; transplants within intact sediment, single shoot; single transplants without sediment, anchored shoots; single transplants anchored in the sediment) and one method of planting seeds (seeds; distributed from mesh-bags) were assessed in this study. A baseline genetic survey was also conducted to assess diversity, clonality and connectivity among donor and recipient sites and locations.

2. Materials and methods

2.1. Study area and transplant sites

To evaluate eelgrass restoration methods in different environments, each method was assessed at two depths in two separate areas representing condition under which eelgrass typically grow along the Swedish west coast. The experiment was carried out between June of 2011 to September 2013 within 2 bays, Torgestad (58°19′51″N, 11°32′23″E) and Snäckebackebukten (58°21′41″N, 11°33′58″E) in the Gullmarsfjord on the NW coast of Sweden (Fig. 1). The Gullmarsfjord has only experienced minor losses of eelgrass since the 1980s, and the conditions for eelgrass growth were considered good. The two bays were chosen to contrast in exposure regimes. The level of hydrodynamic exposure was based on sediment characteristics, where coarse sediment with low organic content indicates high exposure and fine sediment with high organic content indicates low exposure (Fonseca et al., 1983). From grain size and organic content analysis (Table 1) Torgestad was considered semi-exposed and Snäckebackebukten was more sheltered from the dominating winds, and they are hereafter referred to as the “exposed” and “sheltered” bay, respectively (Fig. 1). At the sheltered location, eelgrass plants grew from 1.5 to 4.5 m deep (with a few smaller patches at 5 m depth); in the exposed location, plants grew from 0.7 to 4 m deep. The causes for the deeper upper depth limit in the sheltered bay is not clear but was likely caused by ice that could form more easily in the calm conditions of the bay. Shallow and deep unvegetated areas within each bay were chosen as planting sites as shown in Fig. 1. Donor plant material was harvested from, what is in this study defined as shallow (1.0–1.5 m) and deep (3.0–4.5 m) habitats at both the sheltered and exposed location (Table 1).

2.2. Genetic survey of diversity, clonality and connectivity within and between sites

Shoots ($n = 20$) were collected at 1–1.5 m intervals perpendicular to shore from each of the 4 donor habitats. Leaf samples were placed in tubes with silica crystals for preservation and dehydration until further DNA extraction. The shoots were genotyped with 8 neutral microsatellite loci following Olsen et al. (2013).

Genotypic/genet/clonal diversity (R; number of genets (G-1) over the number of sampled ramets (N-1) and corrected allelic richness (A_c; number of alleles$^{-\text{locus}}$) for the minimum number of genets identified among all locations (here, $n = 14$) were calculated with GenClone 2.0. (Arnaud-Haond and Belkir, 2007). Expected heterozygosity (H_{exp}) and Wright's fixation indices (F_{IS} and F_{ST} as f and θ) were calculated using Genetix 4.05 (Belkhir et al., 2001). All subsequent analyses of population structure used unique genets only, i.e., duplicate multilocus genotypes (MLG) were removed. Clone size was estimated based on the spatial resolution of the linear sampling method (i.e., 1–1.5 m), which provided a coarse minimum value only; shoots were not sampled in a grid or mapped. For example, if three consecutive samples had the same MLG, the clone was estimated as minimally 3–4.5 m in size.

2.3. Cross-transplantation experiment

An orthogonal cross-transplantation experiment was carried out between the 4 habitat sites (exposed-shallow, exposed-deep, sheltered-shallow and sheltered-deep) to assess the effect of 2 transplantation methods using vegetative shoots, and the effect of origin at several

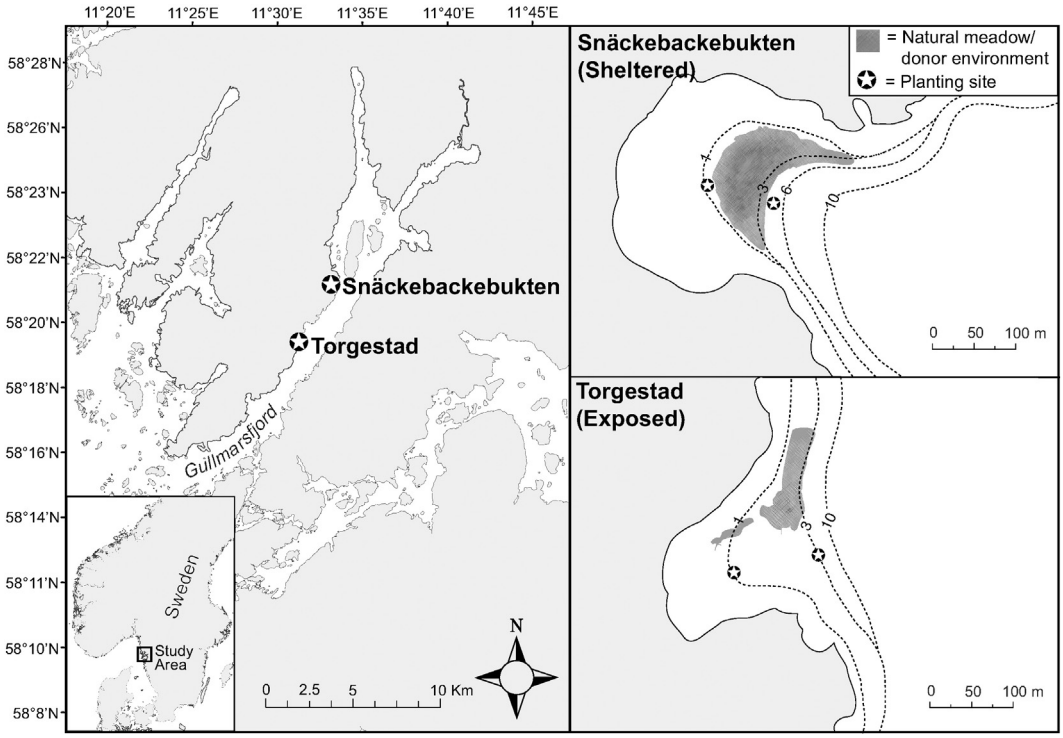

Fig. 1. Location of the two bays used as planting and donor sites in the study (Snäckebackebukten and Torgestad) inside the Gullmarsfjord on the NW coast of Sweden. Snäckebackebukten — the sheltered bay has a bottom profile with an even slope down to 10 m, with natural eelgrass meadows growing from 1.5 to 4.5 m depth. Torgestad — the exposed bay has a bottom profile with an even slope down to 2 m, after which the bottom has a steep slope down to 10 m. Natural eelgrass grow from 0.7 to 4 m in this bay. Planting of shoots and seeds took place at shallow and deep unvegetated areas of both bays, as indicated on the map (stars).

depths and hydrodynamic exposures (Table 2). The two planting methods tested were (1) the plug method in which a group of shoots was transplanted within intact sediment using corers (Fonseca et al., 1998), and (2) the single shoot method in which single shoots were planted without sediment (Orth et al., 1999). Transplanting cores has been the most commonly used method for eelgrass restoration (Fonseca, 2011) and is often considered less stressful for the plant, resulting in higher survival and growth rates compared to planting shoots without sediment. It is, however, more labour intensive and more costly (Fonseca et al., 1998).

All harvesting and planting was carried out using scuba. For the plug method, shoots were collected with a 15 cm Ø PVC corer, retrieving intact shoots and 10 cm of sediment. The cores, filled with seawater, were kept inside coolers until transplantation (maximum 6 h). Sediment plugs with shoots were planted at the new site inside pre-dug holes in the sediment surface. For the single shoot method, shoots were harvested by hand by carefully breaking off the rhizome 2–3 cm from the meristem of apex shoots. Shoots were stored inside coolers with seawater until transplantation (maximum 6 hours) and were planted as described by Orth et al. (1999) by pushing the single rhizome with two fingers into the sediment at an angle, increasing the anchoring capacity of the sediment, as undisturbed sediments falls on top of the rhizome.

Both plugs and single shoots were planted inside 0.5 × 0.5 m square plots, which were arrange linearly, parallel to the shoreline, with 1–2 rows of plots depending on available space at each planting site. A distance of 1 m separated each plot, and the transplantation methods and origin of shoots were randomly assigned amongst plots within

Table 1
Environmental conditions (mean ± SD) at the 4 planting sites, shoot morphology within the corresponding donor meadows in June of 2011, and the light attenuation coefficient (K_d), % surface light reaching the bottom, the theoretical maximum depth distribution (D_{max}; assuming 20% incident light required), temperature and lugworm abundance measured throughout the growth season (from the date of transplantation in June through October). See text for details regarding calculations of light variables.

	Exposed bay		Sheltered bay	
	Shallow	Deep	Shallow	Deep
Depth range (m)	1.0–1.3	3.0–4.0	1.2–1.5	4.0–4.5
Silt + clay (%)	1.3 ± 0.5	10.4 ± 3.6	6.6 ± 6.2	24.7 ± 3.4
Organic content LOI (%)	0.4 ± 0.1	1.4 ± 0.3	1.2 ± 0.8	11.3 ± 1.9
Water content (%)	4.0 ± 5.7	30.8 ± 4.7	24.7 ± 13.0	73.9 ± 3.4
Maximum leaf length (cm)	22.8 ± 6.3	59.1 ± 15.4	78.4 ± 12.2	86.9 ± 14.1
Maximum leaf width (mm)	4.7 ± 0.7	5.8 ± 0.4	6.6 ± 0.6	6.7 ± 0.7
Maximum root length (cm)	7.6 ± 1.3	6.0 ± 1.5	11.1 ± 2.6	13.5 ± 3.7
Shoot density (shoots m^{-2})	774 ± 117	574 ± 125	278 ± 167	184 ± 67
K_d	0.37 ± 0.12		0.47 ± 0.21	
% surface light	66.2 ± 8.2	29.7 ± 9.4	54.7 ± 13.1	17.6 ± 10.3
PPFD (mol m^{-2} day^{-1})	16.6 ± 9.0	7.4 ± 4.2	13.9 ± 8.8	4.6 ± 3.6
D_{max} (m)	4.7 ± 1.0		4.0 ± 1.5	
Temperature (°C)	18.0 ± 2.1	17.4 ± 1.8	18.2 ± 2.2	17.4 ± 1.8
Lugworm abundance (m^{-2})	18 ± 6	4 ± 4	4 ± 3	0

Table 2
Experimental design showing the cross-transplantations (with planting site and transplant origins in the exposed and sheltered bay) performed according to the 4 methods assessed in the study.

	Exposed bay			Sheltered bay		
	Planting site	Transplant origin	Replicate plots	Planting site	Transplant origin	Replicate plots
Single shoot method	Shallow	Exposed-shallow	3	Shallow	Sheltered-shallow	3
		Exposed-deep	3		Sheltered-deep	3
		Sheltered-shallow	3		Exposed-shallow	3
		Sheltered-deep	3		Exposed-deep	3
	Deep	Exposed-shallow	3	Deep	Sheltered-shallow	3
		Exposed-deep	3		Sheltered-deep	3
		Sheltered-shallow	3		Exposed-shallow	3
		Sheltered-deep	3		Exposed-deep	3
Plug method	Shallow	Exposed-shallow	3	Shallow	Sheltered-shallow	3
		Exposed-deep	3		Sheltered-deep	3
		Sheltered-shallow	3		Exposed-shallow	3
		Sheltered-deep	3		Exposed-deep	3
	Deep	Exposed-shallow	3	Deep	Sheltered-shallow	3
		Exposed-deep	3		Sheltered-deep	3
		Sheltered-shallow	3		Exposed-shallow	3
		Sheltered-deep	3		Exposed-deep	3
Anchoring method	Shallow	Exposed-shallow	3			
		Sheltered-shallow	3			
Seeds	Shallow	Exposed	3	Shallow	Sheltered	3
		Sheltered	3		Exposed	3
	Deep	Exposed	3	Deep	Sheltered	3
		Sheltered	3		Exposed	3

each planting site. For the single shoot method, 9 shoots were planted 0.25 m apart in each plot, including the corners (equivalent to 16 shoots m^{-2}). For the plug method, 3 plugs containing a total average of 13 shoots (±8 SD; equivalent to 52 shoots m^{-2}) were planted in a triangular arrangement within each plot. Shoots were cross-transplanted with the two methods between the 4 habitat sites with 3 replicate plots, resulting in a total of 96 plots (Table 2).

To assess whether anchoring of single shoots would increase shoot survival in the more exposed bay, an additional treatment was included at the shallow site of the exposed bay. Single shoots were harvested as described above and planted in pairs, adjacent to each other with rhizomes facing in opposite directions and secured in the sediment with a v-shaped bamboo skewer that was pushed down over the rhizome, as described by Davis and Short (1997). Nine units (18 shoots in total) of eelgrass shoots were planted 0.25 m apart in each 0.5 × 0.5 m plot (equivalent to 32 shoots m^{-2}). A total of 6 plots were planted, with eelgrass originating from the exposed-shallow and sheltered-shallow habitat (n = 3) (Table 2), which were randomly allocated with the other treatments in the shallow exposed planting site.

2.4. Seed method

A seed method was also tested in a smaller companion study at the 4 planting sites with seeds originating from the two bays (Table 2). In August of 2011 reproductive shoots were randomly collected throughout the natural meadows (shallow and deep) of the two bays. Shoots were brought back to the lab and mean number of seeds per reproductive shoot was counted for the two bays. Using a method modified from Pickerell et al. (2005), 15 reproductive shoots were placed inside mesh-bags, with an estimate of 400 seeds per bag. To minimize the spread of seeds after they naturally release from the reproductive shoot, the mesh-bags were anchored 0.5 m above the sediment surface at the 4 planting sites by use of ropes and metal spikes. Seed plots were placed along transects parallel to the shore at 1.3–1.6 and 3.1–4.0 m in the exposed bay and 1.1–1.2 and 4.5–4.6 m in the sheltered bay. The transects were located close (10–20 m) to the transects transplanted with vegetative shoots. Three replicate plots were planted at each site including 3 control plots without bags to control for possible natural dispersal of seeds from the adjacent meadow, resulting in a total of 24 plots with seeds (Table 2) and 12 control plots. In the study region, eelgrass seeds typically drop from the spathes in late August through to October (Infantes et al. unpubl. data) and bags were retrieved in November of 2011 after examining that the remains of the reproductive shoots were empty on seeds.

2.5. Monitoring and measurements

Survival and shoot numbers within plots were measured at 0, 1, 2, 4, 12 and 14 months, beginning at the start of the experiment in June 2011. As the number of shoots planted differed between plots, the proportional shoot increase within plots was used in the statistical analyses. At the final sampling in August of 2012, 5 shoots were carefully collected by hand from each plot and morphological measurements (leaf length, leaf width, rhizome length, number of branches and root length) were taken, to assess possible phenotypical change in response to environmental change. In September of 2013 (27 months after transplantation) all plots were revisited and the total coverage of transplanted eelgrass was estimated for each site. For the seed method, most seeds naturally germinate in March to April (Infantes et al., in press). Accordingly, the number of seedlings was recorded in late May of 2012 and the number of shoots in each seed plot was sampled again in August and September 2012, and in September 2013.

HOBO loggers (UA-002-64, Onset) were used to record light intensity (Lumen m^{-2}) and water temperature (at 15-min intervals) at two depths inside the two bays throughout the growth season. Loggers were cleaned regularly and data were reviewed before analysis to remove unreliable measurements due to fouling. Light intensity (Lumen m^{-2}) was converted to PAR measured in photosynthetic photon flux density (PPFD in μmol m^{-2} s^{-1}) by calibrating against simultaneous measurements using a quantum sensor (MQ-200, Apogee instruments). A power regression analysis was performed to convert the intensity to PPFD: $\ln(PAR) = \ln(0.089) + 0.835 * \ln(Lumen)$, ($r^2 = 0.94$, $P < 0.001$). The difference in PAR values between shallow and deep light loggers was used to calculate the attenuation coefficient (K_d) of light (Dennison et al., 1993) for each bay at each measurement point and as a mean value throughout the growth season for the planted eelgrass (June to October). The theoretical maximum depth distribution of eelgrass (D_{max}) was calculated based on the assumption that eelgrass on average requires 20% of the surface light in order to survive (Borum, 1983; Dennison et al., 1993) and a mean value throughout the growth

season was calculated for each bay. The percent surface light reaching the mean depth of each planting site was calculated as a daily and seasonal average throughout the growth season based on the K_d values. In order to calculate the mean PPFD per day, the daily average percent light reaching the mean depth at each planting site was used to transform light measurements of PPFD in air recorded by the Swedish meteorological and Hydrological institute (SMHI) at the study location.

The lugworm, Arenicola marina, can potentially have negative effects on seeds and seedlings of eelgrass (Valdemarsen et al., 2011) and therefore its abundance was measured at all planting sites in October of 2011 by counting the number of faecal deposits 3–5 h after smoothing the sediment in 0.25 m^2 quadrats (n = 3). Sediment characteristics were analysed in 3 replicate samples collected with corers to a depth of 9 cm from each planting site. Grain size was determined through wet sieving, drying and weighing the different fractions, and organic content was determined as the weight loss of dried material after combustion (loss on ignition, LOI). Water content of sediments was determined from organic content, based the exponential relationship between the two factors, as described by Lillebø et al. (2011). Abiotic conditions at the 4 planting sites and the morphology of the corresponding shoot transplants in the donor habitats are presented in Table 1.

2.6. Statistical analyses

Statistical analyses were performed using SPSS 20.0 (SPSS Inc., Chicago, Illinois, USA). Homogeneity of variance was tested using Cochran's c-test (Sokal and Rohlf, 2011) and data was square root transformed (if found to be heteroscedastic) in order to meet the assumption of homogeneity. For the two shoot methods (single and plug), the proportional shoot increase within plots and morphological measurements at the last sampling date (in August 2012) were analysed as dependent variables in a three-way analysis of variance (ANOVA) with planting site, transplant origin and planting method as fixed independent variables. To test for differences in proportional shoot increase between the unanchored single shoots and anchored single shoots, a two-way ANOVA was used, with method and transplant origin as fixed independent variables.

For the seed method, the number of seedlings (in May 2012) and number of shoots (in September 2013) were analysed as dependent variables in a two-way ANOVA with planting site and seed origin as fixed independent variables. To test for differences between the shoot and seed methods, the proportional change in shoot density (after germination for seeds) was used as the dependent variable in a two-way ANOVA with method and planting site as fixed independent variables. Multiple comparison post-hoc tests were performed using the Student–Newman–Keuls (SNK) procedure.

3. Results

3.1. Environmental conditions

The 4 sites assessed in this study showed large differences in physical properties, faunal composition and eelgrass morphology (Table 1). A gradient could be observed for grain size and organic content moving from the exposed and sheltered shallow to the exposed and sheltered deep. The morphology of shoots differed both between bays and depths, with leaf length and width increasing, and shoot density decreasing with decreasing exposure or increasing depth (Table 1). The tallest shoots were found in the sheltered-deep and the shortest in the exposed-shallow site (mean maximum length 87 and 23 cm, respectively).

As indicated by a larger attenuation of light (K_d) in the sheltered bay, the estimated D_{max} of eelgrass was lower in sheltered compared to the exposed bay (Table 1). Percentages of light at the planting depths were always above 50% for the two shallow sites (Table 1), but showed mean values below 30 and 18% respectively in the exposed-deep and sheltered-deep site. Mean photosynthetic photon flux density (PPFD) per day at the mean depth of each planting site showed large fluctuations between days and along the sampling period (Fig. 2), with mean values throughout the season of 17 and 7 mol photons m^{-2} day^{-1} at the exposed shallow and deep respectively and 14 and 5 mol photons m^{-2} day^{-1} at the sheltered shallow and deep respectively (Table 1). According to a study by Thom et al. (2008), eelgrass needs 7 mol photons m^{-2} day^{-1} to prevent light limitation. Assuming that this is valid also for eelgrass in the study region, the growth season is ~2 months shorter at 4 m compared to 1 m depth (Fig. 2).

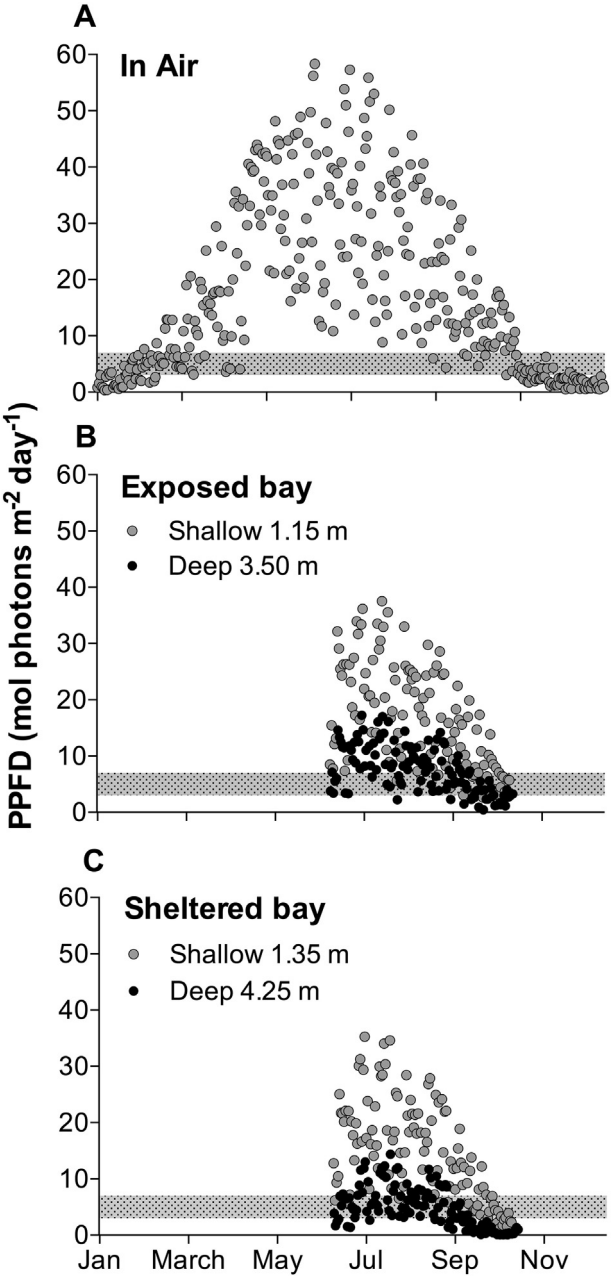

Fig. 2. Integrated estimates of daily photosynthetic photon flux density (PPFD) in A) the air for 2011 in the study area and at the 2 depths of the B) exposed and C) sheltered bay from June 2011 to October 2011. Ticks on the x-axis represent the 1st of each month. The grey bar shows PPFD between 3 and 7 mol m^{-2} day^{-1}, where 3 mol represents the minimum requirement for long-term survival of eelgrass and 7 mol represent the level above which growth is light-saturated according to Thom et al. (2008).

3.2. Genetic diversity, clonality and connectivity

Allelic richness (Table 7) was uniform among sites and considered average for the region (3.58 alleles/locus) based on previous broad scale surveys of the E Atlantic (Olsen et al., 2004; Olsen et al., 2013). Genotypic/clonal diversity, a measure of vegetative spread, was moderate with estimated linear clone sizes of 2–10 m. The highest number of ramets/genet was found at the sheltered-deep site (Table 7). Single ramets accounted for roughly 50% of the shoots sampled, indicating regular recruitment. There were no departures from Hardy-Weinberg Equilibrium. There was no significant population differentiation found between shallow and deep sites but weakly significant differentiation between locations (F_{ST} 0.0908, P = 0.05, 6 km). A Bayesian analysis of population structure, in which no a priori groupings are assumed, revealed no differentiation regardless of assumption sets (not shown).

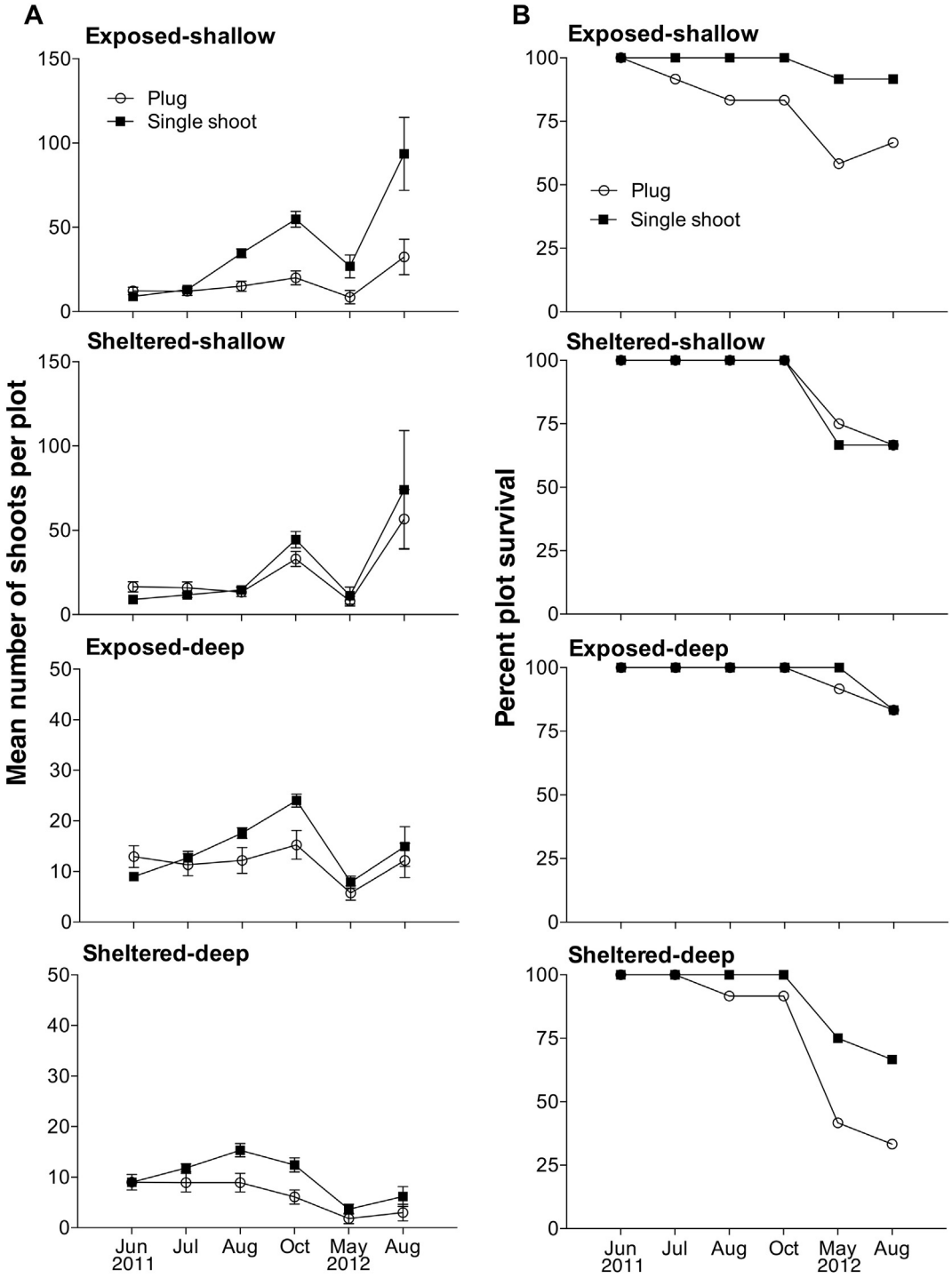

Fig. 3. Cross-transplantation study. A) Mean number of shoots per plot (+SE) at the start of the experiment (June-2011) and 1, 2, 4, 12 and 14 months after transplantation in the 4 planting sites (Exposed; shallow and deep, Sheltered; shallow and deep) with the two transplanting methods (independent of origin). Note the different scale on the y-axis (n = 12). B) The percentage of surviving plots (plots still containing shoots) throughout the experimental period at the 4 planting sites transplanted with the two methods (independent of origin) (n = 12).

3.3. Growth and survival of shoot transplants

The results from the cross-transplantation study show that transplanted eelgrass has the potential to grow and spread rapidly on the Swedish NW coast when environmental conditions are favourable. Plots transplanted with the single shoot method showed an overall positive mean growth 1 month after transplantation, whereas plots transplanted with the plug method remained unchanged for the first 2 months (Fig. 3a). Seasonal variation in shoot numbers was seen at all sites, with a decrease in shoot numbers occurring over the winter. Between October 2011 and May 2012, average shoot numbers in plots decreased with 59 and 64% in the exposed shallow and deep respectively and by 76% in both sheltered sites (Fig. 3a). This trend was also visible when examining the complete loss of all shoots within plots, which was largest over the winter (Fig. 3b). The largest loss of plots occurred in the sheltered location, and in August of 2012, 50% of all plots transplanted within the sheltered-deep site were empty. However, the shoots that remained in plots after the winter losses grew rapidly and increased in numbers throughout 2012. The overall mean percentage of shoot increase (independent of method and origin) from transplantation to the final sampling in August of 2012 was 528% and 550% in the exposed-shallow and sheltered-shallow site, respectively. The exposed-deep site showed a smaller increase of 51%, while the sheltered-deep showed a 40% loss compared to the amount of shoots planted.

The proportional shoot increase in plots at the final sampling in august 2012 showed significant differences as a function of transplantation method and an interaction effect between planting site and transplant origin (Table 3). Growth was significantly higher in plots transplanted with the single shoot method compared to the plug method (average increase of 424% and 120%, respectively) in all planting sites and irrespectively of origin (Fig. 3a).

Although there was a strong general trend of higher growth in the shallow sites (on average 539% increase) compared to the deep sites (on average 5% increase), as indicated by the significant main effect of planting site (Table 3), this trend was only significant for eelgrass originating from the exposed-shallow site which showed significantly higher growth when planted in the two shallow sites compared with the sheltered-deep site, and for shoots originating from the exposed-deep site which grew more in the exposed-shallow compared to the sheltered-deep (SNK < 0.05, Fig. 4).

Transplanting twice the number of single shoots with anchored rhizomes in the exposed bay did not result in higher proportional survival or growth rate compared to single unanchored shoots 1 month after transplantation and at the end of the experiment in August 2012. Effects from anchoring of shoots was expected during the first year, but 1 month after transplantation the proportional shoot growth was similar between anchored and unanchored plots (46 and 51%, respectively) and in October 2011 plots transplanted with singles shoots showed a larger proportional shoot increase compared to anchored shoots as indicated by the significant main effects of method (Table 4), however this trend was only significant for shoots originating from the exposed-shallow site, although a similar relationship was seen also for shoots from the sheltered origin (Table 4, Fig. 5). Similar results were observed at the final sampling in August 2012, where the average shoot number in plots transplanted with anchored shoots were comparable to those transplanted with single shoots, 105 and 106 shoots plot^{-1} on average, corresponding to an increase of 487% and 1065% for anchored and unanchored shoots, respectively. Since the anchoring technique was not tested between all habitat sites, plots transplanted with anchored shoots were excluded from further analysis.

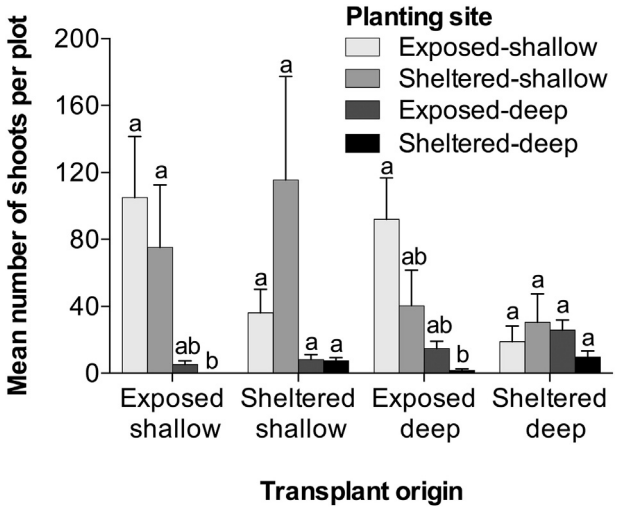

Fig. 4. Cross-transplantation study. Mean number of shoots per plot (+SE) at the 4 planting sites (exposed; shallow and deep, sheltered; shallow and deep), separated by planting origin, at the final sampling in August 2012. Different letters above bars indicate significant difference of the proportional shoot increase within transplant origin depending on planting environment (SNK-test at $P < 0.05$; $n = 6$).

3.4. Morphological changes and plasticity

Shoots collected at the final sampling, 14 months after transplantation, showed significant differences in morphology and growth patterns depending on planting site. In general, plants at the sheltered-deep site had longer and wider leaves, shorter rhizomes with fewer branches and longer roots, whereas plants at the exposed-shallow site had shorter and narrower leaves, and rhizomes with a high number of branches (Table 5, see Fig. 6 for an example of maximum shoot length). However despite dramatic differences in morphology at the time of planting (Table 1), all shoots within a planting site had the same morphology at the end of the study, independent of origin, as shown by the lack of significant main or interaction effect of origin in all but one variable (Table 6). Only leaf width showed a significant interaction between origin and planting site, where at the sheltered sites shoots originating from the exposed sites had narrower leaves compared with shoots from the sheltered origins (Table 6; SNK-test at $P < 0.05$).

Table 3
Cross-transplantation study. Three-way ANOVA table of average proportional shoot increase in plots (from June 2011 to August 2012), testing for differences between method (single shoots and plug), planting site and transplant origin and the interaction between them.

Source	df	F	P
Method	1	5.07	0.028
Planting site	3	5.22	0.003
Origin	3	1.41	0.248
Method × planting site	3	1.32	0.276
Method × origin	3	2.20	0.097
Planting site × origin	9	2.48	0.017
Method × planting site × origin	9	0.99	0.454
Error	64		

Table 4
Anchoring method. Two-way ANOVA table of average proportional shoot increase in plots (June 2011 to October 2011), testing for differences between methods (anchored and single shoots), transplant origin and the interaction between them.

Source	df	F	P
Method	1	34.1	0.000
Origin	1	8.29	0.020
Method × origin	1	19.3	0.002
Error	8		

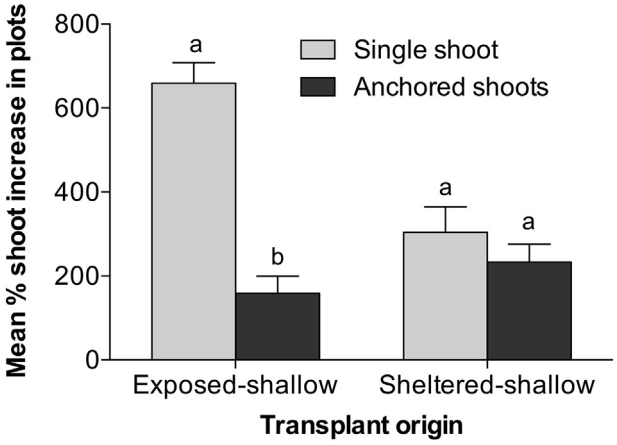

Fig. 5. Anchoring method. Mean proportional shoot increase (+SE) in October 2011 for plots within the exposed shallow sites transplanted with the single shoot method and anchored shoots originating from the exposed-shallow and sheltered-shallow site. Different letters above bars indicate significant difference between treatments. (SNK-test at $P < 0.05$; $n = 3$).

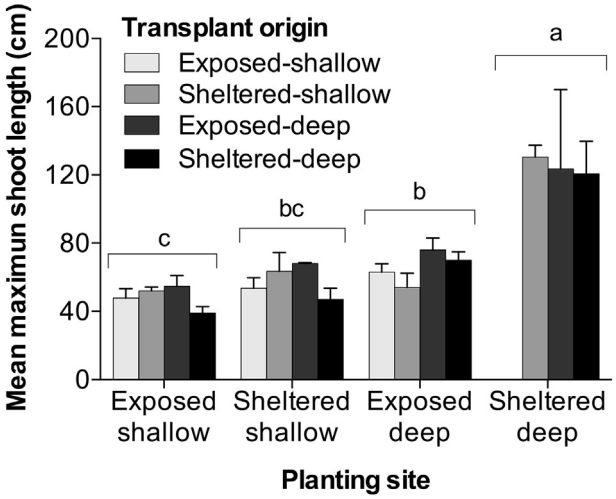

Fig. 6. Cross-transplantation study. Mean maximum shoot length (+SE) of shoots collected from the 4 planting sites (exposed; shallow and deep, sheltered; shallow and deep), separated by planting origin, at the final sampling in August 2012. Different letters above grids indicate significant different means depending on planting environment (SNK-test at $P < 0.05$; $n = 6$).

3.5. Seed method

The sampling of seedlings in May 2012 showed very low densities at all 4 planting sites indicating high losses of seeds and/or low rates of germination. Assuming that 400 seeds dropped from the mesh-bags, only around 1% of the seeds remained and germinated on average. The number of seedlings was significantly higher within the sheltered-deep compared to the exposed-shallow site (on average 7 and 4 seedlings, respectively), and compared to the exposed-deep site where no seedlings were found (Table 8, Fig. 7), possible due to the steep slope that increased advection of seeds to deeper areas at this site (Fig. 1). No seedlings were ever encountered in control plots. The growth of seedlings differed between the shallow and deep sites. In the exposed and sheltered shallow sites, shoot numbers had increased by on average 263% by September (2012), with a mean leaf length of 17 and 27 cm, respectively. In the sheltered-deep site, shoot numbers had only increased with 2.5% (Fig. 7), but with an average length of 51 cm.

At the final sampling in September 2013, shoot morphology of seedlings had changed further, obtaining morphological characteristics resembling those of natural eelgrass and shoot transplants in the 4 sites (Tables 1 and 5). In the shallow sites, particularly in the exposed bay, the shoot numbers continued to increase rapidly during the second summer reaching, on average, 65 shoots per plot. This was higher than the sheltered-deep site, although not significantly so, where shoot densities had only increased to an average of 8 shoots per plot. The overall increase in shoot numbers was 791% and 35% in the shallow and deep sites on average based on the seeds that had germinated some 16 months earlier (Table 8, Fig. 7).

Interestingly, both seedling density in May 2012 and shoot density at the final sampling differed significantly depending on the origin of the seeds (Table 8). Seeds from the exposed bay produced significantly higher numbers of seedlings and showed significantly higher shoot growth in all planting sites; compared to seeds originating from the sheltered location (on average 6 and 3 seedlings and 651% and 157% shoot increase following germination, respectively).

When comparing the proportional shoot increase among the three methods (single shoots, plugs and seeds) at the final sampling (Aug 2012 for shoots and Sep 2013 for seeds, 14 and 16 months after planting and germination, respectively), the two-way ANOVA revealed significant main effects for both method and planting site; however, a suspiciously low P-value for the interaction effect ($P = 0.066$) indicated that the difference among the methods may change with planting site

Table 5
Description of morphological characteristics (mean ± SD) of 1 whole shoot transplant retrieved per plot at the 4 planting sites at the final sampling in August 2012 ($n = 3$) and of seed transplants in September 2013 ($n = 3$). The total above ground biomass is calculated based on the average weight of one shoot times the total shoot count of the plot.

		Exposed bay		Sheltered bay	
		Shallow	Deep	Shallow	Deep
Shoot transplants AUG 2012	Planting depth (m)	1.0–1.3	3.0–4.0	1.2–1.5	4.0–4.5
	Maximum leaf length (cm)	49.3 ± 11.5	65.3 ± 16.7	58.1 ± 17.0	125.2 ± 34.1
	Mean leaf length (cm)	40.2 ± 10.2	52.4 ± 13.6	46.9 ± 14.2	98.2 ± 20.6
	Maximum leaf width (mm)	4.7 ± 0.7	4.8 ± 0.9	4.7 ± 1.1	5.4 ± 0.7
	Rhizome length main shoot (cm)[a]	46.6 ± 23.0	25.4 ± 11.6	59.7 ± 65.5	18.7 ± 7.3
	Rhizome length branches (cm)[a]	32.8 ± 21.7	19.2 ± 18.9	121.0 ± 125.4	8.8 ± 10.7
	Number of branches[a]	8 ± 5	5 ± 4	23 ± 22	3 ± 3
	Maximum root length (cm)	15.6 ± 4.3	11.8 ± 2.8	11.4 ± 3.7	12.0 ± 3.7
Seed transplants Sep 2013	Planting depth (m)	1.3–1.6	3.1–4.0	1.1–1.2	4.5–4.6
	Maximum leaf length (cm)	29.6 ± 1.2	NA	60.5 ± 3.4	98.8 ± 16.6
	Mean leaf length (cm)	21.2 ± 1.6	NA	36.0 ± 1.4	65.0 ± 2.9
	Maximum leaf width (mm)	4.4 ± 0.1	NA	4.6 ± 0.1	4.3 ± 0.3
	Rhizome length main shoot (cm)	28.0 ± 4.8	NA	36.0 ± 7.0	10.9 ± 1.9
	Rhizome length braches (cm)	30.3 ± 3.0	NA	85.0 ± 10.0	3.7 ± 1.0
	Number of braches	8 ± 2	NA	20 ± 4	2 ± 1
	Maximum root length (cm)	13.2 ± 3.3	NA	16.5 ± 0.7	13.8 ± 2.9

[a] The rhizome length might be underestimated for the exposed bay since it was difficult to retrieve the whole shoot due to compact sediment.

Table 6
Cross-transplantation study. Summary of three-way ANOVAs for shoot morphology measured in plots in August 2012, testing for differences between method, planting site and transplant origin and the interaction between them.

Source	df	Maximum shoot length		Maximum leaf width		Total rhizome length		Number of branches	
		F	P	F	P	F	P	F	P
Method	1	1.54	0.222	0.042	0.839	1.98	0.168	0.024	0.877
Planting site	3	20.2	0.000	0.534	0.661	8.56	0.000	10.9	0.000
Origin	3	1.06	0.377	2.13	0.112	0.035	0.991	0.192	0.901
Method × planting site	3	0.046	0.987	0.385	0.764	0.583	0.630	0.399	0.755
Method × origin	3	1.17	0.335	0.730	0.540	0.675	0.573	0.468	0.706
Planting site × origin	8	1.25	0.300	2.43	0.031	0.612	0.762	0.532	0.825
Method × planting site × origin	6	0.592	0.735	1.15	0.353	0.342	0.910	1.339	0.265
Error	39								

(Table 9, Fig. 8). Generally, all methods showed higher growth in the shallow compared to deep planting sites, where the single shoot method and seeds showed the highest growth rates in the exposed-shallow site and seeds were the only method that showed a mean positive growth within the sheltered-deep site, albeit small (Fig. 8).

4. Discussion

Although transplantation of eelgrass has been studied and used in restoration and mitigation projects in the USA for over 6 decades (Fonseca et al., 1998), relatively little information is available regarding restoration methods for high latitude environments, particularly for Scandinavian countries where strong seasonal variations in temperature and light, ice formation, and organic rich sediments present special challenges for restoration.

This study, which to our knowledge is the most northerly eelgrass transplantation study to date, demonstrates that both shoot- and seed methods can be used to restore eelgrass at this latitude, and that planted eelgrass exhibits growth rates that are comparable to lower latitudes despite the short growing season. The results also reveal large differences in growth and survival of transplants between methods and planting sites that will have implications for successful management and restoration of eelgrass in Scandinavia.

4.1. Challenges for eelgrass restoration at high latitudinal environments

4.1.1. Light limitation

At high latitudes, such as the study area located at 58°2′N, low light conditions during the long winter poses a special challenge for both natural growth and restoration of aquatic plants. Seasonal measurements of light at the surface suggest that available PPFD per day, under the light attenuation conditions present at these bays, may allow eelgrass growth that are not limited by light (>7 mol photons m^{-2} day^{-1}; Thom et al., 2008) between March and October in the shallow sites, but only between mid May and to the end of August at depths close to the maximal depth distribution of eelgrass (Fig. 2). Thus, eelgrass growth in deeper habitats is challenged both by low light conditions and a short growing season at high latitudes. That light availability affect the growing season was supported in the cross-transplantation study where the shoot numbers decreased between August and mid-October at the planting site with the lowest light levels, sheltered-deep, but increased in the other sites during the same period (Fig. 3a).

Eelgrass relies on carbohydrate storage in the rhizomes and roots for growth and respiration during periods of low light (Kraemer and Alberte, 1995; Burke et al., 1996; Vichkovitten et al., 2007). Storage of carbohydrates can therefore be critical for the survival of transplanted shoots during the winter, as demonstrated for Z. noltii (Govers et al., 2014). In the present study, the high losses of planted shoots during the winter (particularly in the deepest site) were most likely caused by depleted carbohydrate reserves. Thus, at high latitudes, winter survival constitutes a challenge for eelgrass restoration and transplant success should not be evaluated until after the first winter. Earlier restoration studies in Denmark (Christensen et al., 1995) and for other areas (Calumpong and Fonseca, 2001; Vichkovitten et al., 2007) have found better success when transplantation takes place in the spring or early in the summer. This may be particularity important in high latitudes to ensure that there will be a sufficient store of carbohydrates to survive through the winter.

4.1.2. Organic rich sediments

Eelgrass meadows along the Swedish and Norwegian North Sea coasts are mainly found in sheltered areas in fjords and archipelagos where the sediments typically have a high content of clay, organic material, sulfide and water at depths over 2 m (Holmer et al., 2009). In the present study, the sediment in the deep planting site in the sheltered bay had an organic content of 11.3% and estimated water content of 74%. As far as we know, eelgrass restoration has never been assessed in this type of sediment, which poses several challenges for restoration, including decreased anchoring capacity of seedlings (Lillebø et al., 2011) and increased risk of sulfide invasion and mortality of the plants (Goodman et al., 1995; Holmer and Bondgaard, 2001; Holmer et al., 2005). The high concentration of fine sediment, water and organic material make the sediment very sensitive to resuspension, which can increase turbidity for a long time following disturbance. Low visibility makes large-scale restoration using hand-planted shoots and plugs difficult. Surprisingly, the present study demonstrated that both eelgrass shoots and seeds can survive for several years in this type of organic rich sediment although they were planted at light levels of 18% surface light, which is close to the average minimum light requirement for eelgrass (20%; Dennison et al., 1993) and within the lower range of minimum light requirement measured from different study

Table 7
Genetic diversity and clonality based on 8 microsatellite loci. N = number of shoots/ramets analysed, G = number of clones/genets, R = genotypic/clonal diversity, Â = allelic richness (standardized to 14 genets), G > 1 = number of genets with >1 ramet, nR = mean number of ramets per genet (distribution of duplicate ramets per genet given in parentheses), F_{IS} = Wright fixation index estimated as f.

Site	N	G	G-1/N-1	Â (n = 8)	G > 1	nR	H_{exp}	F_{IS}
Exposed-shallow	20	10	0.474	3.973	3	4.3 (3,4,6)	0.4362	0.0571
Exposed-deep	20	8	0.368	2.643	3	5.0 (2,3,10)	0.4167	0.0267
Sheltered-shallow	20	9	0.421	3.716	5	3.2 (2-5)	0.4112	−0.0678
Sheltered-deep	20	16	0.789	3.976	3	2.3 (2,2,3)	0.4284	−0.0976

Table 8
Seed study. Results of two-way ANOVA testing the effects of planting site and seed origin on the number of shoots per plot in May 2012 and September 2013.

Source	df	May 2012		September 2013	
		F	P	F	P
Planting site	3	12.4	0.000	6.89	0.003
Origin	1	11.8	0.003	6.04	0.026
Planting site × origin	3	1.37	0.289	1.26	0.323
Error	16				

regions (10-37%; Zimmerman, 2006). In fact, seedling densities were higher in the shelter-deep site than in the other habitats suggesting that seed loss was lower and/or germination higher in the deep, organic rich sediment. However, growth was very low under these conditions (the plants merely persisted), and the eelgrass would be sensitive to temporary declines in water quality. Thus, although restoration appears to be possible of these deep habitats, recovery would be very slow, and long-term survival quite uncertain. Restoration of eelgrass close to the maximum depth distribution in this type of sediment should therefore be avoided.

4.1.3. Ice-scouring

Formation of ice in the winter has the ability to reduce above-ground biomass and shoot densities within shallow eelgrass meadows through ice-scouring (Robertson and Mann, 1984; Davis and Short, 1997; Wong et al., 2013). In this study, ice coverage was present in both bays during the first winter and some of the transplant losses appeared to have been caused by ice-scouring because marking-poles had moved and several plots of eelgrass were completely lost in shallow areas, particularly within the sheltered bay. Thus, the formation of ice during cold winters could potentially destroy eelgrass planted in shallow waters, and for the study area restoration of eelgrass is not recommended at depth < 1 m, and the long-term success of restoration in shallow habitats (<1.5 m depth) can only be assessed after the first ice-winter.

4.2. Importance of origin

As demonstrated in this and earlier studies, eelgrass can display large differences in morphology and growth characteristics depending on e.g. light conditions and wave exposure. Although previous studies have shown that eelgrass can change morphology if transplanted to a new environment (Schanz and Asmus, 2003; Li et al., 2010), the extent of the plastic response varies and guidelines for eelgrass restoration generally suggest that donor plants should be as similar as possible to the plants being restored (Fonseca et al., 1998; van Katwijk et al., 2009; Olsen et al., 2014).

Results of the genetic baseline survey indicated diverse, homogenous and connected locations, as well as coherence between shallow and deep sites at each location. Thus, suitable donor material was interchangeable among all four sites in the present study. Observed changes in morphology were consistent with a plastic response; however it should be noted that the survey was based on neutral markers and it is therefore still possible that ecotypic differences (as opposed to phenotypically plastic differences found in the leaf morphology)

Table 9
Comparison between methods. Results of two-way ANOVA testing the effects of transplantation method (single shoots, plugs and seeds) and planting environment on the proportional shoot increase in plots at the final measurements in august 2012 (shoots) and September 2013 (seeds).

Source	df	F	P
Method	2	3.15	0.047
Planting site	3	7.66	0.000
Method × planting site	6	2.05	0.066
Error	108		

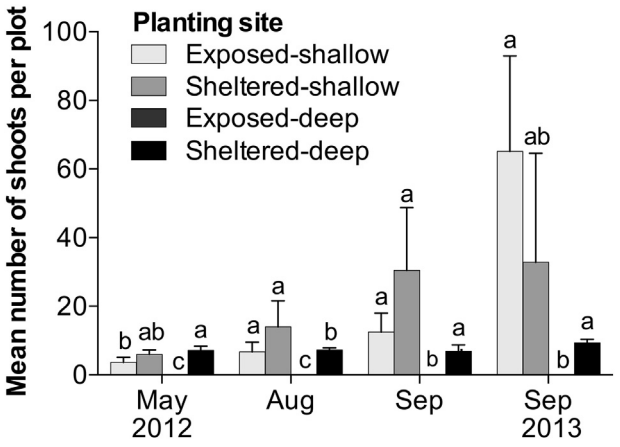

Fig. 7. Seed study. Overall mean number of seedlings (May 2012) and adult shoots (3 to 16 months after the expected germination) in plots planted with seeds (+SE) in the 4 planting sites (exposed; shallow and deep, sheltered; shallow and deep). Different letters above bars indicate significant different means depending on planting site at each sampling date (SNK-test at $P < 0.05$; $n = 6$).

affect other physiological traits (e.g., light) and were therefore not detected.

The change in both morphology and growth structure observed displays that eelgrass from the donor habitats used in this study has an strong capacity to adjust their morphology in order to acclimatize to new physical environments. For example, shoots that originated from the sheltered-deep site had a mean maximum leaf length of 120 cm and on average 3 lateral branches on the rhizome when planted within its original site, but displayed a mean maximum leaf length of 40 cm and on average 19 lateral branches when planted in the exposed-shallow site 14 months after transplantation. The leaf width was the only morphological parameter that still showed an effect of origin, which indicates that leaf widths are less plastic compared to the length of the leaves, which have previously been seen for Z. marina (Backman, 1990) and the seagrass Thalassia testudinum (van Tussenbroek, 1996). The allocation of energy for vertical leaf growth is typical for a low light, low energy environments, whereas high lateral branched growth resulting in high shoot density and smaller shoots is typical for eelgrass growing under saturated light conditions, where more energy goes into below ground biomass (Bintz and Nixon, 2001; Koch, 2001; Ochieng et al., 2010).

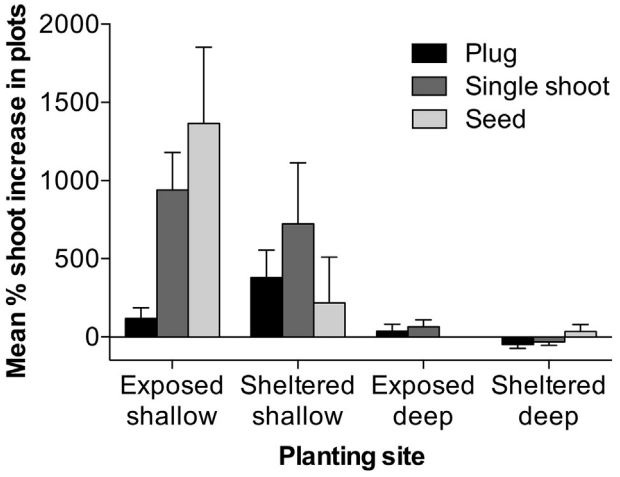

Fig. 8. Comparison between methods. Mean proportional shoot increase (+SE) in plots within the 4 planting sites (exposed; shallow and deep, sheltered; shallow and deep), comparing the 3 different methods of eelgrass transplantation ($n = 6$ for seeds, $n = 12$ for shoots).

Despite their ability to change morphology, the results from the present study also suggest that it may be prudent to avoid transplanting shoots between the most extreme environments since the shoot may perish before it has had a chance to change its morphology to the new environment. This was most clearly observed for the smallest shoots (from the exposed-shallow site), which suffered 100% mortality after the first winter when planted in the sheltered-deep site. A similar problem was observed for the opposite cross-transplantation, where the tall shoots originating from the sheltered sites on average suffered higher mortality in the exposed-shallow planting site. This was likely a result of the taller shoots generating more drag, which increased the risk of being up-rooted during strong wind conditions. Similar losses have been described after transplanting both Zostera noltii and Z. marina from sheltered to exposed locations in the Wadden Sea (van Katwijk and Hermus, 2000; Schanz and Asmus, 2003). Furthermore, cross-transplantation experiments with the seagrass Posidonia oceanica between different depths have shown similar low survival when transplants originating from shallow locations were planted deep (Molenaar and Meinesz, 1992).

For the seed method, seedling density and growth showed no interaction between origin and planting site suggesting that seeds were able to acclimatize to all environments within the first growing season. However, the origin of the seeds did affect the recruitment success and their subsequent growth. Seeds originating from the exposed bay showed higher initial densities of seedlings and 4 times higher proportional shoot growth in all planting sites. These results suggest that there were differences in seed quality between the two bays that affected loss and/or germination of seeds, as well as their subsequent lateral growth. However, since this effect was similar in all sites where germination occurred and not higher in the bay where the seeds originated from, it appears not to represent a local adaptation. That seeds from different donor populations can result in different seedling densities is consistent with studies from the Wadden Sea (van Katwijk and Wijgergangs, 2004). This suggests that if the quality of seeds cannot be estimated beforehand, the use of seeds from several different donor populations could increase the chances of success.

Taken together, the capacity of eelgrass to adjust its morphology and growth strategy according to the new environmental conditions means that it may not be necessary to find a donor population that exactly matches the morphology of the plants targeted for restoration, as long as the extreme differences are avoided. Thus, the shorter shoots (30 to 50 cm tall leaves) from shallow habitats that are easily collected and transported could be used for most environments. However, for restoration at a site close the maximum depth distribution, or in a more exposed site, a more closely matching donor population should be used to increase chances of survival.

4.3. Restoration methods for eelgrass in Scandinavian waters

Surprisingly, the simplest shoot method using unanchored single shoots without sediment showed lower losses and higher growth rates compared to transplanting shoots inside intact sediment cores, or using shoots with anchors, in all 4 planting sites. On average, the increase in shoot density at the end of the second growth season was 3.5 times higher using the single shoot method compared to the plug method. In contrast to the single shoot method, which showed high shoot growth already after 1 month, shoots planted with the plug method did not increase in density during the first 2 months. This lag-period in growth may have affected the ability of the transplanted shoots to store enough carbohydrates over the summer to survive the winter, and explain the higher winter mortality found when using the plug method compared to the single shoot method. The reason for this lag in growth is not clear, but may have been a result of initial competition for space and resources in the sediment plug, before shoot rhizomes had expanded outside of the plug. The plug method also resulted in high initial losses of shoots when transplanting plugs from deep habitats with sediment consisting of fine mud and high water content to the shallow-exposed habitat, due to wave erosion. These results were contrary to our expectations since transplanting shoots in sediment cores generally is considered the most successful and least stressful method (Phillips, 1990), as plants are left within an undisturbed rhizosphere (Fonseca et al., 1998). In addition to showing lower growth and survival, the plug method was also substantially more labour intensive than the other methods, and resulted in larger impacts on the donor beds where holes were left in the sediment. Field measures of the time required to harvest and plant shoots with the two methods showed that the plug method would require approximate 2.5× more time. Moreover, the transport and storage of heavy tubes would constitute a huge logistical challenge for large-scale restoration projects. Thus, the plug method is not recommended for restoration of subtidal eelgrass beds in Scandinavian waters.

Comparison between the single shoot method and the method where two shoots were anchored with bamboo sticks in the shallow exposed habitats suggest that erosion of shoots did not constitute a problem in the assessed area. Although the sediment composition at the exposed-shallow habitat suggests that the bay was subjected to moderate wave exposure, we found no positive effect of anchoring on shoot survival. Instead, the single shoot method showed on average 2 times higher individual growth rate. The reason for the lower growth rates of the anchored shoots is not clear, but was likely caused by competition between shoots. Although the density of the anchored shoots was not very high (equivalent of 32 shoots m^{-2}) the fact that the shoots were planted pairwise, adjacent to each other in the used method (Davis and Short, 1997) may enhance competitive interactions. Other studies suggest that eelgrass growth is limited by competitive effects at planting densities above 60 shoots m^{-2}, particularly in sheltered areas (van Katwijk et al., 1998; Granger et al., 2000). Based on these results single shoots without anchoring planted at densities of 16 shoots m^{-2} or lower is recommended for restoration of shallow habitats (1–3 m depth) for the range of physical exposure included in this study.

The lateral growth of shoots planted with the single shoot method in shallow habitats was surprisingly large considering the short growing season of the study area. At the end of the second growth season, many shoots in the exposed-shallow habitat had grown from a single shoot with a 2–3 cm long rhizome to a meter-long mat of rhizomes with over 40 shoots. This growth rate appears to be substantially higher than rates of lateral growth in natural eelgrass beds in other areas (16–31 cm yr^{-1}; Olesen and Sand-Jensen, 1994; Borum et al., 2004), likely related to the lack of conspecific competition for transplanted single shoots, and the long summer days with relatively high temperature in shallow areas along the Swedish west coast (16–20 °C).

Monitoring of the experimental plots in 2013 (after two years) showed that the rapid expansion of shoots continued in the shallow habitats, whereas the shoots in the deeper habitat merely persisted. During planting in June 2011, a total of 7.5 and 6.0 m^2 of eelgrass were transplanted within shallow and deep sites, respectively, with shoot density varying between 16 and 52 shoots m^{-2} for the different methods. In September of 2013, 27 months after shoot transplantation, many plots in the shallow areas had merged, covering approximately 46 and 26 m^2 in the exposed and sheltered shallow site respectively, with a shoot density similar to the natural beds. However, the total area in both deep sites had decreased to approximately 3 m^2. The high survival and fast expansion of eelgrass planted in shallow habitats indicate that shoot methods can be successfully used to restore these habitats at high latitude areas if the environmental conditions are favourable.

Seeds that survived the winter and germinated in the spring showed very high lateral growth in the shallow habitats that were similar to the growth found in shoots planted with the single shoot method. In some cases, a single seedling had branched into 20 shoots (including also reproductive shoots), and consisted of a meter-long mat of rhizomes by the end of the second summer. However, very few of the seeds

planted in the shallow habitats resulted in a seedling. Assuming that 400 seeds dropped from each mesh-bag, only about 1% of the seeds remained and germinated in spring in the shallow habitats. Thus, the very high loss rates of seeds in shallow sites make seed methods less cost-effective than shoot methods when it comes to the amount of donor material needed, if shoots are collected with the same technique.

The reason for the high loss of seeds, and/or low germination rate is not clear. Eelgrass seeds in Scandinavian countries germinate in Spring (March–April), 7–8 month after they are released from the reproductive shoots (Infantes et al., in press). This long dormancy period may increase the loss rate of seeds due to e.g. hydrodynamic transport of seeds or burial by sediment dynamics and bioturbators such as lugworms (Valdemarsen et al., 2011; Delefosse and Kristensen, 2012). Recent studies also suggest that seed predation by shore crabs, Carcinus maenas, can cause losses of seeds in the study area (Infantes et al., in press).

In the sheltered-deep habitat, seed losses were significantly lower than in the other habitats, resulting in almost twice as high density of seedlings in the spring, despite the potentially stressful conditions in the deep sediments. Considering the sensitivity of the lose sediment in the deep habitat to resuspension it is not well suited for planting of shoots which depend on good visibility. Seed-methods are therefore likely the best option for eelgrass restoration of deeper habitat (3 to 5 m depth) in areas with soft sediment. Before seed methods can be recommended for large-scale restoration of eelgrass also in shallow habitats, techniques have to be developed that decrease the high loss rates.

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