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Restoring charophytes is still a challenge: A call for developing successful methods

C.L. Faithfull[a,*], E. Tamarit[b], P. Nordling[c], E. Kraft[c]

[a] Institution for Aquatic Resources, Swedish University of Agricultural Sciences, Skolgatan 6, Öregrund 74242, Sweden
[b] Department of Earth Sciences, Gothenburg University, Box 460, Gothenburg 40530, Sweden
[c] Department of Nature, County board of Gävleborg, Borgmästarplan 2, Gävle 80170, Sweden

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ABSTRACT

Submerged aquatic vegetation, and especially charophytes, which are an important habitat for many species, have declined in the Baltic Sea due to changes in light climate, eutrophication and physical disturbance. Physical disturbance in the form of small-scale dredging activities is commonplace in Sweden due to land uplift, but causes fragmentation of coastal habitats. Here we test three planting methods for restoration of the charophyte *Chara aspera* on an area of deposited sediment, and a single method for restoration of *C. tomentosa* in a dredged area. We found that none of the planting methods tested was more successful than natural recolonization of *C. aspera* on the deposited sediment. *C. tomentosa* planting was unsuccessful in the dredged area and was likely outcompeted for light by taller species. The *C. aspera* meadow was resilient to smaller disturbances, as experimental removal of up to 2.5% of *C. aspera* and sediment from the donor area did not reduce *C. aspera* coverage a month after removal. Even after an uncontrolled event that removed up to 50% of *C. aspera* in the experimental plots, *C. aspera* coverage had returned to pre-removal levels a year after the disturbance. We suggest future restoration experiments test transplanting sediment rich in oocytes and bulbils into areas with suitable light climates and low competition with other species. Restoration efforts are costly and highly uncertain of success, therefore we recommend discontinuing dredging activities in charophyte meadows to protect this important habitat.

1. Introduction

Submerged aquatic vegetation has declined in many areas of the world, including the world's largest brackish inland sea, the Baltic Sea. Dramatic declines have been recorded for eelgrass (*Zostera marina*) (Moksnes et al., 2018) and charophytes, which are sensitive to physical disturbance, low light availability and nutrient concentrations (Blindow, 2000). Charophytes can live in a range of salinities from freshwater to brackish, which reflects the range of salinities found in the Baltic Sea, making it a global hotspot for charophytes, with twelve species documented (Schubert and Blindow, 2003). Charophytes are important species in this environment, and contribute to a range of ecosystem services; including carbon removal from water, storage of carbon and nutrients in biomass and sediments (Kufel and Kufel, 2002; Kufel et al., 2016), phytoremediation of organic chemicals and trace metal elements (Schneider and Nizzetto, 2012), indicators of past climate conditions (Martin-Closas et al., 2006), and provide essential habitat, food and recruitment areas for a range of organisms (Snickars et al., 2010). In the Baltic, charophyte meadows have been associated with high fish recruitment (Snickars et al., 2010) and good water quality (Blindow, 2000; Hidding et al., 2010). However, charophytes are threatened by eutrophication, as they are sensitive to changes in light availability. Also, they are impacted by physical disturbances such as boating and dredging activities, which are common in the Baltic Sea (Blindow, 2000).

The species chosen for this study are two of the most common meadow forming charophyte species in the Baltic Sea, *Chara aspera* and *Chara tomentosa*. Both species are abundant in protected shallow bays around the Baltic coast. *C. aspera* is found in a wide range of salinities in the Baltic Sea up to approximately 20 PSU, whereas *C. tomentosa* is found up to moderate salinities (Blindow, 2000). In brackish water asexual reproduction appears to be the most common method of establishment for both species (Blindow et al., 2009). *C. aspera* builds large dense mat-like meadows and can reach a height of 0.3 m (Kufel and

* Corresponding author.
E-mail address: carolyn.faithfull@slu.se (C.L. Faithfull).

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Kufel, 2002), whereas *C. tomentosa* is the largest charophyte in the Baltic Sea, with a height of up to 1.5 m (Torn et al., 2006). The distribution of both *C. tomentosa* and *C. aspera* has declined over the past decades, with disappearances from areas where turbidity has increased due to eutrophication or sediment disturbance (Blindow, 2000; Henricson et al., 2006)

The area of coastline affected by anthropogenic disturbances leading to fragmentation of habitat and loss of submerged underwater vegetation have increased by 17% in Gävleborg county, where this study was conducted, and 28% in Stockholm county between 1994 and 2016 (Törnqvist et al., 2020). This has led to the disappearance of charophytes in many areas and a subsequent interest in restoring charophyte meadows (Pitkänen et al., 2013; Baastrup-Spohr et al., 2015). With more than 80% of land and sea habitats in Europe in poor condition, the European Union has proposed the *Nature restoration law*, which aims to restore habitats, species and ecosystems (European Commission, 2022). Restoration methods for seagrasses are being developed worldwide, such as for eelgrass in Sweden (Moksnes et al., 2016). However, restoration methods for charophytes are still experimental and have mostly been attempted in freshwater lake ecosystems (Blindow et al., 2021). Examples of restoration efforts include nutrient remediation (Rodrigo et al., 2015), improving light conditions for oospore germination, and fish removal (Dugdale et al., 2006). To our knowledge there are no currently documented restoration efforts for charophytes in the Baltic Sea (Blindow et al., 2021).

Consequently, it is important that different restoration methods are tested and developed to ensure that we can restore degraded areas successfully. Here we contribute to the development of restoration methods for the charophytes *C. aspera* and *C. tomentosa* by conducting three experiments where we tested three planting methods for the restoration of *C. aspera* and one method for planting of *C. tomentosa.* It is also important to consider the effects of removing individuals from a site for planting elsewhere and the response of the *C. aspera* meadow to disturbance. Thus, we also examined the effect of a controlled removal of *C. aspera,* and the consequences of an uncontrolled disturbance on the integrity of a natural *C. aspera* meadow.

2. Methods

2.1. Study site

Our study site was Siviksfjärden, a sheltered bay in Gävleborg county in Sweden (WGS84: 61°34'34.5"N 17°2′46.0"E). The bay is 43 ha with a maximum depth of 3 m and has a catchment area dominated by mixed coniferous and deciduous forest. Relative to the size of Siviksfjärden the anthropogenic pressure on the bay is relatively small, with 12 jetties on the north side and two dredged channels 120–132 m long and approximately 10 m wide on the southern side (Fig. 1). Dredging channels to allow boat access to jetties is common practice along the Swedish coast, as land uplift occurs at approximately 7 mm per year in Gävleborg county (Vestøl et al., 2019). This has reduced the water level of the Northern Baltic Sea by approximately 2 m in the last 282 years (Weisse et al., 2021), making boat access to coastal properties difficult. Dumping of dredged sediment in the sea is illegal in Sweden, but seldom controlled, and using dredged sediment to "fill out" shallow areas and extend land area is approved by the County Board on a case-by-case basis. Here dredging and dumping alongside the dredged area took place in 2018 directly across a large meadow of *C. aspera* (Fig. 1). The bay itself has good water quality and conditions for charophyte growth, but *C. aspera* has disappeared from the dredged and dumped areas. The dumped sediment parallel to the dredged channel forms a shallow raised area approx. 75 m long and 10 m wide and 0.3–0.5 m depth.

2.2. Study species

We tested different planting techniques for two common charophytes in the Baltic Sea, *C. aspera* and *C. tomentosa*. *C. aspera* and *C. tomentosa* were chosen as they grow close to the study site and perform important ecosystem functions such as; maintaining fish nursery populations and habitats (Snickars et al., 2010; Sundblad and Bergström, 2014), stabilise sediments and regulate nutrient cycles and burial (Pełechaty et al., 2006; Chao et al., 2021).

2.3. Experimental design

We used three different experiments in three different areas to test methods for (A) replanting *C. aspera*, (B) the effects of harvesting *C. aspera* on the donor *C. aspera* meadow and (C) replanting

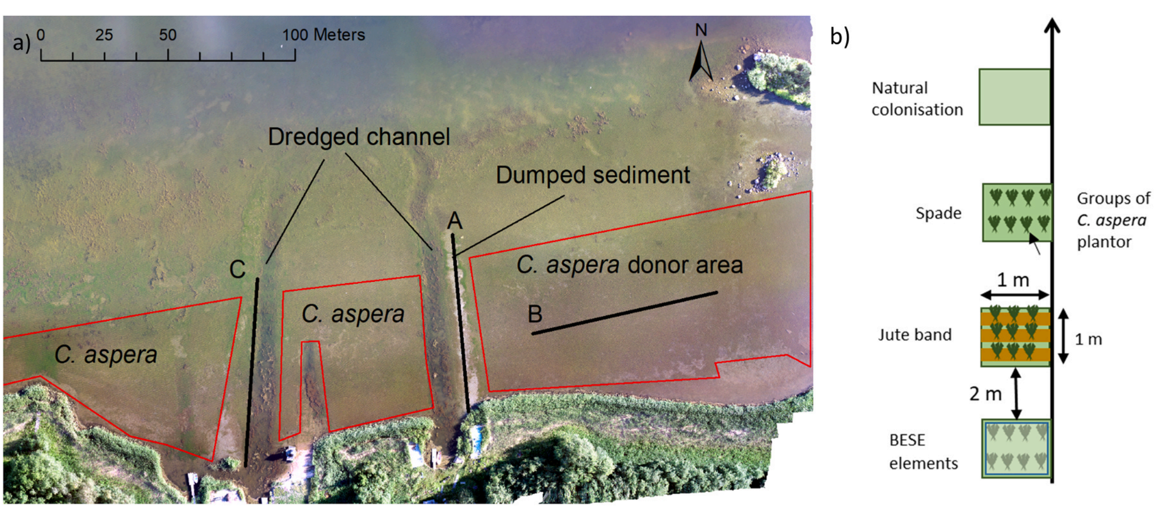

Fig. 1. The right hand figure (a) is a detail aerial photo of Siviksfjärden taken on 24–08–2021. The dredged channels (darker areas) and associated dumped sediment parallel to the channels (lighter areas) are visible. The *C. aspera* meadows surrounding the dredged and dumped areas are marked with red polygons. The layout of the experimental transects, *C. aspera* transplanting experiment (transect A), the *C. aspera* donation experiment (transect A) and the *C. tomentosa* transplanting experiment (transect C) are indicated. The left hand figure (b) shows an example of how the plots with different treatments (in this case, experiment A) were arranged along each transect.

C. tomentosa. As the dredged and dumped sediment areas were long and narrow we were obligated to place the treatments along transects to minimise changes in depth and sediment characteristics and simulate an actual restoration. The first experiment tested three planting methods of *C. aspera* and natural recolonization, which was located at transect (A) perpendicular to the shore in the dumped sediment area (Fig. 1). Depth varied along this transect from 0.3 to 0.5 m and fluctuated over time depending on atmospheric conditions. Four replicates of each *C. aspera* planting method and four plots for natural recolonization were placed randomly along transect A in 16 1 m^2 plots placed at 2 m intervals (Fig. 1). The second experiment tested the effects of harvesting *C. aspera,* whereby we placed a transect (B) parallel to shore in the *C. aspera* meadow. Depth varied along this transect from 0.4 to 0.5 m and fluctuated over time depending on atmospheric conditions. Four replicates of four harvesting levels were placed randomly along transect B in 16 1 m^2 plots placed at 2 m intervals (Fig. 1). The third experiment tested planting of *C. tomentosa* along transect (C) along the dredged area perpendicular to shore (Fig. 1). Depth varied along this transect from 0.5 to 0.7 m and fluctuated over time depending on atmospheric conditions. A single method of planting *C. tomentosa* was tested in six replicates and natural colonisation in three replicates ordered randomly across nine 1 m^2 plots placed at 2 m intervals along transect C (Fig. 1). Individuals of *C. tomentosa* were sourced from nearby in the bay where they grew patchily and *C. aspera* was sourced from the donor transect or the surrounding meadow. All planting and harvesting took place on 30 May-1 June 2021 and measuring of plots in July, August, September 2021 and September 2022. All aerial photos were taken with a DJI Phantom 4 RTK and orthophotos were built with Agisoft© software. We measured treatment plots three times in 2021 for shoot height, shoot density and percent coverage and once in 2022, details are below.

2.4. Planting and harvesting techniques

We tested three planting methods for *C. aspera*: spade planting, jute band, BESE elements© and natural recolonization (Transect A, Fig. 1). Each method was replicated in four 1 m^2 plots. 1) *Spade planting*: An area of nearby *C. aspera* meadow with both plant material and sediment 64 cm^2 x 12 cm deep was removed and transported carefully underwater to be pushed down directly into the sediment without any additional anchoring. We planted eight groups of *C. aspera* and sediment per plot. 2) *Jute band:* We placed 10 shoots with rhizoids in each of three approx. 8 cm slits in each jute band of 1 m length and 5 cm width. The bands were laid parallel approximately 25 cm apart in each plot and weighted with stones found nearby. 3) *BESE elements©* are a biodegradable three dimensional structure made of potato starch, which have been tested for replanting and restoration of for example eel grass, mangroves and mussel banks (Gagnon et al., 2021). Eight groups of 64 cm^2 *C. aspera* were laid on top of two layers of BESE elements©. A third layer was fixed on top and the BESE elements© and plants were placed in the treatment plot and pushed down into the sediment 2–3 cm (Fig. 2). The BESE elements (0.92 $\times$0.92 m^2) covered 0.78 m^2 of the 1 m^2 plots. 4) *Natural colonization*, no planting, the plots were left undisturbed.

We tested four levels of harvesting in the *C. aspera* meadow, removal of 0, 0.8%, 1.8% or 2.6% of the *C. aspera* coverage (Transect B, Fig. 1). Harvesting levels were based on removing half the amount of *C. aspera* required for the three different planting methods in the restoration plots. These levels were justified due to the uncertainty of how resilient the meadow would be to removal. In all donor treatments sediment and plant matter were removed to 12 cm deep, but varied in area: 1) removal of four squares with a spade equivalent to 256 cm^2 (2.6% of C. aspera in plot removed), 2) removal of four circles with a plastic pipe for a total removal of 78.4 cm^2 (0.8%), 3) removal of eight circles 157 cm^2 (1.6%), 4) no removal of *C. aspera* 0% removal. All removals were spaced evenly within each 1 m^2 plot and each removal treatment was replicated four times.

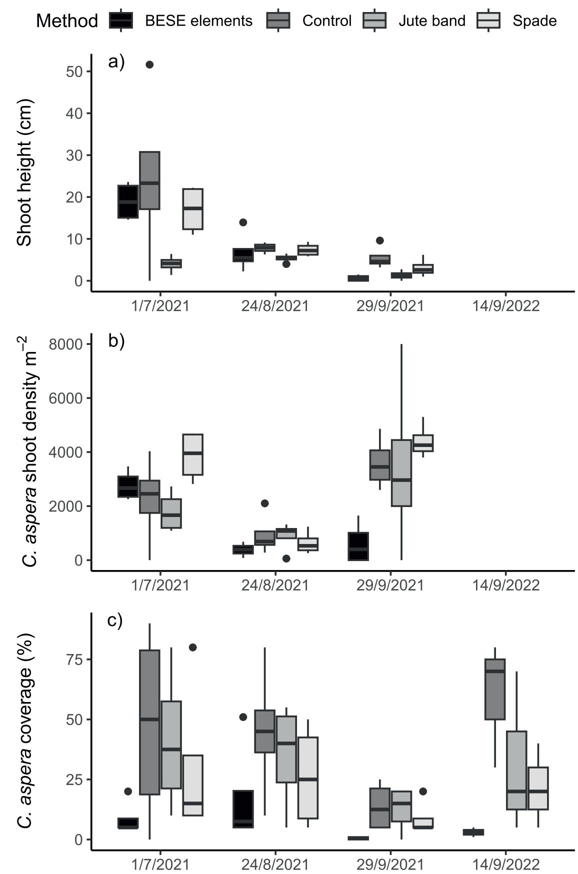

Fig. 2. Boxplot of changes in shoot height a), shoot density b) and coverage c) over time in the *C. aspera* planting experiment (transect A, Fig. 1). Planting of *C. aspera* took place on 30 May-1 June 2021. N = 4 for each treatment on each sampling date, except on the 14 Sept 2022, where N = 2 for BESE elements©, N = 3 jute, N = 3 spade and N = 3 control. *C. aspera* coverage (c) was significantly higher in the control treatment compared to the BESE elements treatment and varied over time, being lowest on 29 Sept 2021 compared to 1 July 2021 (see results section for statistics).

We tested replanting *C. tomentosa* using BESE elements© (Transect C, Fig. 1). Eight groups of *C. tomentosa* (plants and sediment) were placed on top of two layers of BESE elements© and fastened with a third layer on top. The BESE elements© were placed in six treatment plots of 1 m^2 along the dredged transect and pushed 2–3 cm down into the sediment. Three control plots were located along the transect and treatments were assigned randomly to the plots.

2.5. Monitoring

Temperature (°C) and light (Lux) were measured every 30 min from 2 June- 29 Sept with two loggers per experimental transect placed 0.2 m above the sediment (HOBO Pendant MX2202). Water depth in the Baltic Sea varies with atmospheric conditions, therefore changes in water depth were corrected using data taken from the nearest station (Ljusne) monitored by the Swedish Meteorological Institute. Measurements of turbidity (FNU), salinity (PSU), light attenuation (Kd), and pH (HANNA HI 9829) and cleaning of light loggers took place on 2 June, 1 July, 24 August and 29 September 2021. We monitored shoot height, shoot density and coverage of aquatic vegetation species in the plots on 1 June (before treatment), 1 July, 24 August and 29 September 2021 by snorkelling. We made follow up measurements of vegetation coverage 15 months after initial planting on 14 September 2022 for all the plots and in the dredged channel D (Fig. 1). However, due to missing plot markings on 14 Sept 2022 only 11 marked plots were found along the *C. aspera* planting transect (A), which were two BESE elements©, three jute, three spade and three control replicate plots. On 1 June 2021 before planting, only plots along the *C. aspera* donor transect (C) and *C. tomentosa* planting transect (B) were measured as there was no vegetation visible along the *C. aspera* planting transect (A) at that time. For all plots we also recorded the number of snails, coverage of filamentous algae and amount of bare sediment on each sampling occasion. Sediment samples of 118 cm^3 (15 cm depth) were collected with plastic tubes on 2 June and 29 Sept close to the dredged channels (transect C), the dumped sediment (transect A) and the natural *C. aspera* meadow (transect B) (Fig. 1). Water content of sediment was measured as loss of mass after drying at 60 °C for 48 h, and organic content as loss-on-ignition of dried sediment ignited at 550 °C for 6 h in a muffle furnace.

2.6. Statistical analysis

Two-way repeated measures analysis of variance (ANOVA) was used to explore differences in shoot density, shoot height and coverage of the target species between treatments over time. Data was tested for normal distribution and homogeneity of variances to meet the assumptions of ANOVA using a Bartlett test and by examining plots of residuals versus fitted values and normal versus theoretical quantile plots, and transformation was not required to meet assumptions of normality. In the cases where there was no significant difference between treatments, we combined this data to calculate the average coverage of all the underwater species over time. We also tested for differences in the number of snails and filamentous algae between treatments with a two-way repeated measures ANOVA. We used the Tukey honestly significant difference (TukeyHSD) posthoc test to determine where differences lay when the ANOVA results where significant. An error of 5% ($P = 0.05$) was used to test for significant differences. All statistical analyses were carried out in the R statistical programme (R Core Team, 2021).

3. Results

3.1. C. aspera planting experiment

None of the planting methods increased *C. aspera* coverage in any of the treatments more than natural colonization (Fig. 2, $F_{3, 43} = 4.74$, $p = 0.006$). The coverage of *C. aspera* when planted with BESE elements© was lower than coverage in the naturally colonised plots, (TukeyHSD, $p = 0.04$, Fig. 2). Shoot density and shoot height did not differ between treatments and there was no change over time depending on treatment (Treatment effect: Shoot height: $F_{1,32} = 0.035$, $p = 0.991$; Shoot density: $F_{1,32} = 0.639$, $p = 0.595$; Treatment*Time effect: Shoot height: $F_{1,32} = 0.302$, $p = 0.824$; Shoot density: $F_{1,32} = 0.392$, $p = 0.759$). Differences in *C. aspera* coverage between treatments were not explained by differences in the number of snails or the coverage of filamentous algae, which did not differ between treatments (Snails: $F_{3,51} = 1.04$, $p = 0.383$, Filamentous algae: $F_{3,40} = 0.289$, $p = 0.833$).

There was a trend towards decreasing coverage of *C. aspera* over the 2021 growing season for all treatments ($F_{3,43} = 3.58$, $p = 0.021$) (Fig. 2). The community composition of the species present in the plots changed over time (Fig. 3). *C. aspera* was the most abundant species over all sampling dates, however, *Zannichellia palustris* and *Ruppia* sp. had a high coverage in June but were nearly absent in August and September. *Najas marina* and *Callitriche hermaphroditica* appeared later in the season with highest abundances in September (Fig. 3).

The *C. aspera* planting experiment was located on deposited sediment in the middle of a natural *C. aspera* meadow. This sediment was deposited in 2018 and three years after this disturbance (28 August 2021) the coverage of *C. aspera* on the deposited sediment in the natural colonization plots had reached about half (mean ± se = 45 ± 14%) of the undisturbed meadow (*C. aspera* donor area (mean ± se = 91 ± 2.4%). By September 14 2022 this coverage had increased to 60 ± 15% (Fig. 2c).

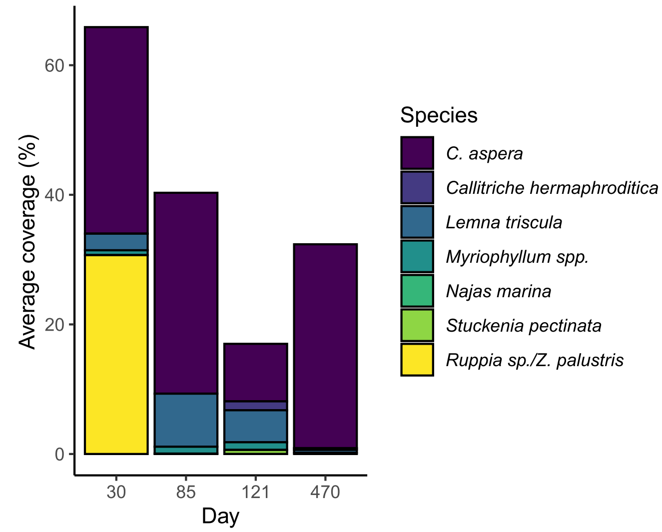

Fig. 3. Average coverage of species across all plots across the *C. aspera* planting transect (Fig. 1 A) from the 1 Jul 2021 (day 30 after planting) until 14 Sept 2022 (day 475).

3.2. C. aspera removal experiment

There were no differences between any of the *C. aspera* removal treatments and the control plots (Fig. 4a). Removing up to 2.5% of a 1 m^2 area had no effect on *C. aspera* coverage, shoot height or shoot density (Treatment effect: Coverage: $F_{3,64} = 0.051$, $p = 0.985$; Shoot height: $F_{1,48} = 0.410$, $p = 0.746$; Shoot density: $F_{1,32} = 0.306$, $p = 0.821$). Shoot height was highest on 24 Aug 2021. By the 29 Sept 2021 shoot height had declined across treatments (Time effect: Shoot height: $F_{1,48} = 9.90$, $p < 0.001$). *C. aspera* shoot height, coverage and density did not change over time depending on removal level (Treatment*Time effect: Coverage: $F_{3,64} = 0.008$, $p = 0.999$; Shoot height: $F_{1,48} = 0.134$, $p = 0.939$; Shoot density: $F_{3,32} = 0.155$, $p = 0.926$). The coverage of *C. aspera* was not affected by the experimental removal, but on 29 Sept 2021 all donor plots except one showed considerable disturbance, potentially from a boat propeller (Fig. 4b). In the disturbed plots *C. aspera* was coming loose from the sediment and there was a higher degree of bare sediment with *C. aspera* having ≤50% coverage, with mean coverage being 20% ± 18% (mean ± standard deviation). The *C. aspera* meadow at a distance from the donor transect was not as disturbed as the area along the transect. In Sept 2022 when we returned to the site the transect had fully recovered from the disturbance the previous year with a mean *C. aspera* coverage of 95% ± 3% (mean ± standard deviation) across all plots (Fig. 4a).

3.3. Abiotic conditions in the C. aspera experiments

When the light attenuation coefficient (Kd) was measured on sampling days, light attenuation did not differ significantly between the experimental areas. However, over the growing season the *C. aspera* transplant area (deposited sediment) had 22% less light (Lux) than the donor area, even though the light meter was located 10 cm closer to the surface in the planting area (Fig. 5). Temperature did not differ between the experimental areas. Sediment characteristics differed between donor and planting areas (Fig. 6). Water content of sediment in the *C. aspera* planting area, which consisted of deposited sediment, was 9.3% and 5.2% lower than the donor and dredged area respectively (Fig. 6a, Area: $F_{2,13} = 8.97$, $p = 0.004$). Water content of all sediment types increased over the growing season, being higher in September than at the end of May (Time effect: $F_{1,13} = 44.2$, $p < 0.001$). Organic content of the

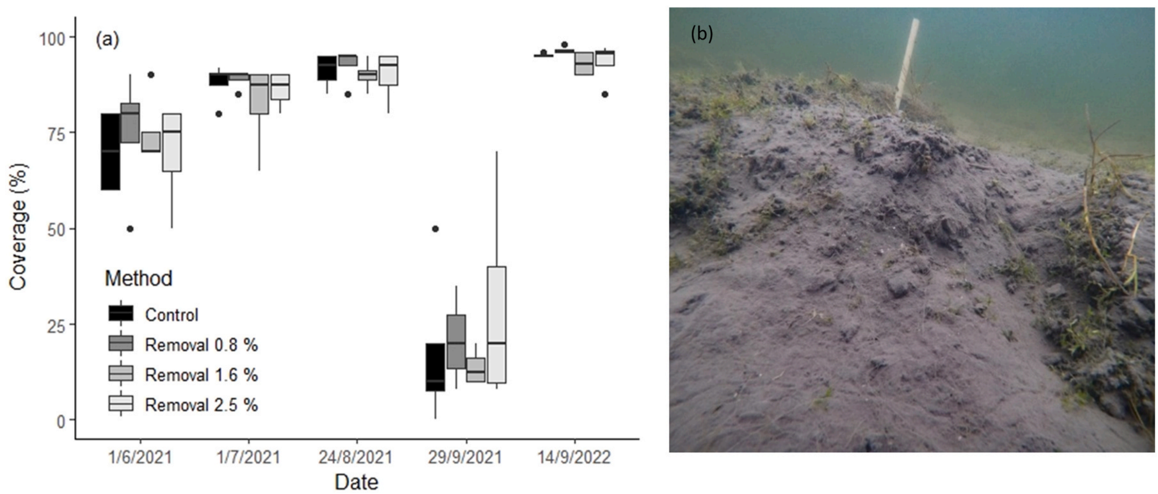

Fig. 4. a) Boxplot of *C. aspera* coverage in the donor area with three different levels of *C. aspera* removal up to 2.5% of a 1 m^2 plot. b) Photo of a plot on the 29 Sept 2021 where a disturbance had occurred, 11 of 12 plots showed signs of considerable disturbance.

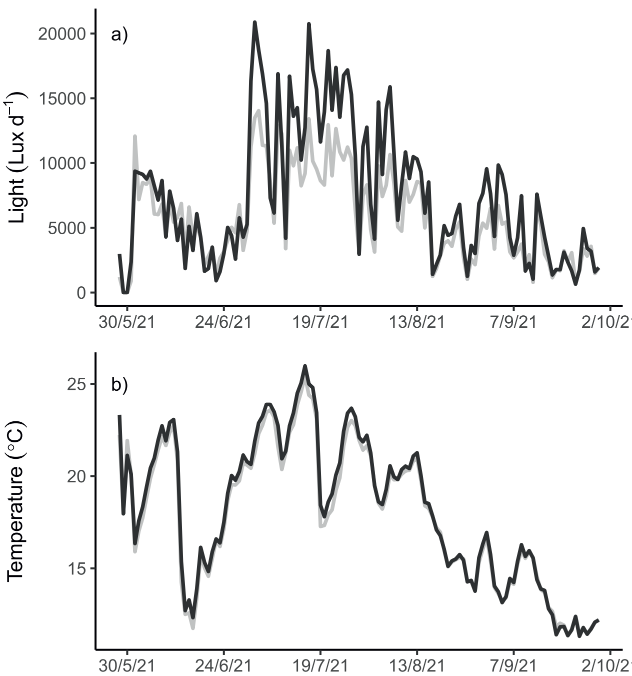

Fig. 5. Light (a) and temperature (b) from May to September in the *C. aspera* donor area (black line 0.4–0.5 m deep) and the *C. aspera* planting experiment area (grey line, 0.3–0.5 m deep). Cleaning of light loggers took place on 2 June, 1 July, 24 August and 29 September 2021.

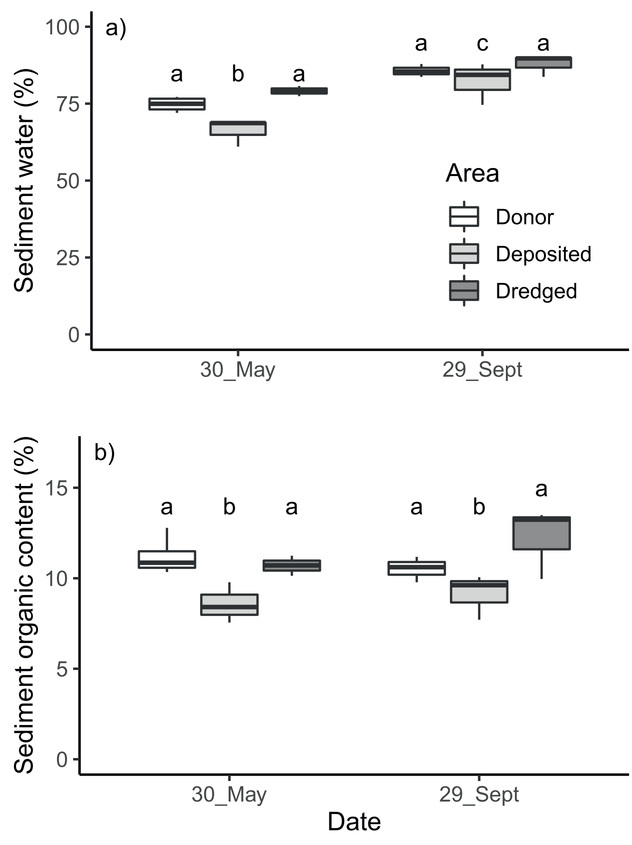

Fig. 6. Sediment water content (a) and organic content (b) in the donor area (undisturbed sediment), the dredged area and the transplant area (deposited sediment), N = 4 for each area on each date. Significant differences ($p<0.05$) between areas and over time are indicated with lowercase letters.

planting area (deposited sediment) was 2.4% lower than the donor and dredged areas and there was no change in organic content over time (Fig. 6b, Area: $F_{2,13} = 8.11$, $p = 0.005$).

3.4. *C. tomentosa* experiment

A few shoots of *C. tomentosa* initially survived and were observed on 28 Aug, however, by the 29 Sept these shoots had disappeared. When we returned in 2022 there was no sign of *C. tomentosa* along the transect and the transplantation of *C. tomentosa* was deemed unsuccessful. The total vegetation coverage was low in June, which was why this area was chosen for replanting. Coverage of submerged aquatic vegetation changed over the season with *Ruppia* sp. and *Z. palustris* having high coverage in early summer, and *Lemna triscula* achieving a high coverage in the plots from Aug to Sept. *Callitriche hemaphroditica* becoming more common at the end of Sept.

3.5. *Estimating the costs of replanting*

The most effective and cheapest method of transplanting was using a spade, simply digging up an area of *C. aspera* and transporting it to the transplant site with as much sediment as possible remaining around the rhizoids and pushing down the rhizoids into the sediment with the spade (Table 1). This method worked well when the donor and replanting area were close to each other and the plants did not need to be transferred above water, as the muddy sediment rapidly dissipated when *C. aspera* was transferred to a holding container with water. The most expensive methods of transplanting were using jute band due to the longer time required and BESE elements© due to the high cost of the consumable material. None of the transplanting methods was more successful than natural colonization, which had no associated costs.

3.6. The dredged area

We would have liked to test *C. aspera* transplantation in the dredged channel, however after our initial observations it was clear that three years after sediment removal, the dredged channel was already densely colonized with other submerged aquatic vegetation (92% coverage). This vegetation coverage is visible as a darker area in Fig. 1. Additionally, the dredged channel was 0.5–1 m deeper than the surrounding *C. aspera* meadow (0.4–0.8 m), which limited the amount of light reaching the sediment by 25–40%. The most common species found in the dredged channel were *L. triscula*, *Myriophyllum* spp., *Stukenia pectinata* and *C. hermaphroditica.*

4. Discussion

Charophyte restoration methods are currently under-studied, with only a few examples of restoration techniques tested in lakes (Dugdale et al., 2006; Blindow et al., 2021). This study highlights the high cost of restoration and the need for further method development for charophyte restoration. Restoration of *C. aspera* and *C. tomentosa* had relatively high costs and low success rates using the methods we tested. None of the transplantation methods achieved higher *C. aspera* coverage than natural colonization during a single growing season and after a second growing season the coverage in the natural colonization plots had increased to 60% of the undisturbed meadow. The rate of *C. aspera* recolonization we observed was only half the rate of eelgrass recovery when removed from 4 m^2 patches (Boese et al., 2009). However, *C. aspera* recolonization was much more rapid than the rate of seagrass recovery after large scale dredging, which often fails to recolonize areas where it has been removed due to increased turbidity and sedimentation (Erftemeijer and Lewis, 2006).

Here we chose transplantation methods we believed had a strong chance of success. However, further development of these methods is needed. We excluded methods that used weights or plaster as anchors (Rohal et al., 2021) as the rhizoids of *C. aspera* are delicate and form tight mats in the sediment, making it impossible to separate individual shoots while maintaining the integrity of the stoneworts. We also excluded methods which required rhizoids to be contained in a jute bag or peat pots (Rohal et al., 2021), as we believed this would make spreading of *C. aspera* difficult due to the fine rhizoids lacking direct contact with the sediment. Pots or bags which would biodegrade over a single growing season, which are made of a substance that will not change the sediment composition and can biodegrade even under anoxic conditions may be suitable. However, methods using pots and bags are more time consuming than those we tested and this extra cost needs to be factored into the restoration (Rohal et al., 2021).

In this study transplanting with jute band and BESE elements© were the most expensive methods and resulted in the lowest coverage and shoot numbers. The low transplant success rate with BESE elements© was not due to snail density or filamentous algal growth, as these factors did not differ with treatment. We suspect that BESE elements© reduced light availability close to the sediments, thus inhibiting *C. aspera* and *C. tomentosa* establishment, as much of charophyte biomass is close to the sediment making them sensitive to light availability (Blindow, 2000). We would not recommend three layers of BESE elements© for future transplant attempts with charophytes, however one or two layers could be tested as this should result in less shading, although the shoots will not be held in place as effectively. Here the transplant area was very sheltered and anchoring the charophytes was not a problem, but if planting in more exposed areas methods to anchor the charophytes without reducing light availability near the sediment need to be considered. In this experiment spade planting was the most cost and time effective method and we would recommend testing this method in other sheltered areas with lower natural recolonization rates.

The *C. aspera* planting experimental site had high light availability and was located beside a *C. aspera* meadow, which may have contributed to high rates of natural recolonization; therefore, it is difficult to predict how our transplantation methods would have worked in an area without these characteristics. The low shoot density relative to percent coverage of *C. aspera* on Aug 28 2021 suggests that *C. aspera* shoots were appearing from the sediment rather than spreading outwards from the groups of planted stonewarts. The relatively high natural colonization of the dumped sediment after four years could be a result of omnipotent node cells, bulbils and oospores being present in the sediment, which was previously a dense *C. aspera* meadow. These are the main modes of dispersal (Blindow et al., 2021) and the importance of oospores, bubils, node cells, or a seed bank in the sediment for establishment has been observed for both macrophyte and charophyte species (Blindow et al., 2009; Muller et al., 2013). Oospores are hardier than bubils and thus facilitate long-distance dispersal and buffer the risk of reproductive failure over time (Bonis and Grillas 2002). If oospores are taken from sediments they will be in secondary dormancy and can germinate immediately (Blindow et al., 2021). However, oospore germination success is lower than bulbils and is dependent on species-specific environmental conditions of temperature, salinity, redox potential and light (Blindow et al., 2009; Skurzyński and Bociąg, 2009; Holzhausen et al., 2018). However, sediment from charophyte meadows is also rich in

Table 1
The estimated time, material and personnel costs for the different planting methods. All material costs are one off, except the jute band and the BESE elements©, which are per m^2. There will be additional costs if charophytes need to be transported to the planting area. The estimated time is the time for all personnel required per m^2 of replanted C. aspera, with experience less time will be needed. *The estimated costs for personnel and consumables per replanted m^2 of C. aspera. This is based on a personnel cost of 50 € per hour per person.

Method	People	Materials	Estimated material costs (€)	Estimated time (min m^{-2})	*Estimated costs consumables + personnel (€ m^{-2})
Spade	2	Spade(s)	20	30 min	25
		Snorkelling gear	10 00		
Juteband	2	Spade(s)	20	90 min	155
		Snorkelling gear	10 00		
		Jute band (m^{-2})	5		
		Plastic container	10		
		Stand up paddle board	400		
BESE elements©	2	Spade	20	60 min	151
		Snorkelling gear	10 00		
		BESE elements© (m^{-2})	51		
		Stand up paddle board	400		

bubils and node cells (Van den Berg et al., 2001), and bubils can have germination rates of over 70%, compared to 40% for oocytes (Blindow et al., 2009), making bubils an effective reproduction method. We recommend testing transplanting sediment with high concentrations of node cells, bulbils and oospores as a method of restoration for *C. aspera*. Important considerations when testing this restoration method are: 1) Use the uppermost layers of the sediment as most oospores and bulbils are found in the top 5–30 cm (Van den Berg et al., 2001; Bonis and Grillas 2002; Rodrigo and Alonso Guillén, 2013). 2) Transplanted sediment should have high concentrations of node cells, bulbils and oospores, as this increases the likelihood of a successful germination (Van den Berg et al., 2001; Bonis and Grillas 2002). 3) The restoration area should be suitable habitat for *C. aspera*, especially with regards to light availability, redox potential and temperature (Van den Berg et al., 1998; Blindow, 2000; Blindow et al., 2021), lack of competition by other species (Van den Berg et al., 1998) and low potential grazing by water birds (Van den Berg et al., 2001; Noordhuis et al., 2002). 4) It may be possible use dredged sediment for restoration if it contains charophyte oospores and bulbils and has suitable sediment characteristics.

In our study the three different experimental areas had different sediment water and organic matter characteristics, slightly different depths and differed in light availability. However, within each experimental area, depth, light and sediment characteristics were very similar, allowing us to compare planting techniques and *C. aspera* removal within each experimental area. The *C. aspera* planting area, which consisted of deposited sediment, had lower organic and water content than the dredged or natural sediment areas, which can be coupled to larger grain sizes and less silt content (Frenzel et al., 2009). The deposited sediment comes from a deeper sediment layer, which could explain the lower percentage organic material. However, as *C. aspera* increases burial of organic matter, the lower coverage of vegetation on the deposited sediment may also contribute to lower sediment organic content (Chao et al., 2021). In our study, the deposited sediment area had slightly lower light availability than the surrounding *C. aspera* meadows, which is likely due to increased sediment resuspension. Depending on sediment type, water depth and currents, decreased light availability and increased turbidity resulting from dredging can extend over 3.5 km from the dredged area (Törnqvist et al., 2020). Even though the deposited sediment had different characteristics from the surrounding natural sediment, *C. aspera* was able to naturally recolonize this area to 60% of the coverage of the surrounding undisturbed *C. aspera* meadows four years after dredging and deposition of sediment.

Although other macrophyte species appeared in the *C. aspera* planting plots they were low growing species and were seasonally abundant, whereas *C. aspera* is a persistent perennial. The depth of the deposited sediment (0.3–0.5 m) also prevented establishment of taller species. The dredged channel was unsuitable habitat for *C. aspera,* being deeper (0.5–1.2 m) with low light levels and high competition with the taller growing species *L. triscula*, *Myriophyllum* spp. and *S. pectinatus* (Van den Berg et al., 1998; Blindow and Schütte, 2007). *Myriophyllum* spp. and *S. pectinatus* are considered nuisance species by boat owners as the long surface growing stalks tend to tangle in boat propellers, but were only present in high densities in the areas dredged to enable boat traffic.

C. aspera can be heavily grazed upon by swans and other water birds (Van den Berg et al., 2001; Noordhuis et al., 2002), but we did not observe obvious signs of this in the *C. aspera* meadow. The disturbance observed in September 2021, where half of the *C. aspera* coverage was removed along the donor transect, appeared to be damage by a boat propeller, as the sediment was disturbed locally along the marked transect and not in other areas of the meadow. *C. aspera* showed resilience to this disturbance, with *C. aspera* coverage reaching pre-disturbance levels just a year after the disturbance. This rapid recovery was much faster than found by Torn et al. (2010), where *C. aspera* was completely removed from 1.5 m^2 plots and had not recovered to original biomass levels a year after removal. This observation, coupled with the lack of effects of *C. aspera* removal in our donor treatments, illustrates that dense *C. aspera* meadows can be resilient to removal of isolated areas up to 0.5 m^2, which in total do not exceed 2% of the total *C. aspera* meadow extent. This suggests an otherwise undisturbed *C. aspera* meadow can be a suitable donor area for sediment for restoration activities, as long as the underlying physical attributes of the site do not change, i.e. depth, sediment characteristics, light climate.

Globally, restoration of charophyte meadows are still in a method development stage, thus even though the experimental planting methods tested here did not exceed rates of natural colonization on dumped sediment, this study contributes valuable information to future work. One of the limitations of our study is that our results are specific to the local conditions where they were tested. In the case of the *C. aspera* planting techniques, these were tested on dumped sediment obtained from dredging the surrounding meadow, which would have contained bubils, oospores and node cells. Although the dumped sediment had larger sediment grain sizes, and lower water and organic content, it still had high levels of natural colonization, which may reflect *C. asperas'* ability to cope with different types of substrate and water level fluctuations (Kovtun et al., 2011). However, natural colonization was not possible in the dredged area due to changes in depth, light availability and competition with tall growing species. Currently small-scale dredging activities in Sweden, which occur on the publicly owned seabed and often only benefit a single household, require only that the local authorities are notified, and the amount of fragmented coastal habitat is increasing at an alarming rate (Törnqvist et al., 2020). We recommend discontinuing dredging activities in charophyte meadows. Restoration is difficult and expensive and may not be successful if we do not have prior knowledge of methods that will work in the type of area or with the type of vegetation we need to restore. However, we found that a dense *C. aspera* meadow was able to recover from a smaller scale disturbance, which opens up opportunities to test transplanting sediment rich in oospores and bulbils as a future restoration method for *C. aspera*.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data Availability

The data is available from the Swedish National Data repository, https://doi.org/10.5878/gcf6-qr58.

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