



LIFE LAESOE - "Restoration of birdlife and natural habitats at Laesoe"

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Report on control of the invasive cordgrass at Læsø







Control of the invasive Spartina anglica, at Laesoe

The invasive *Spartina anglica* control project is part of ACTION C5 tests of different methods to control of common cordgrass (*Spartina anglica*) in the EU-LIFE project "LIFE11 NAT/DK/000893 LIFE LAESOE – restoration of birdlife and natural habitats at Laesoe".

The experiments took place in the period 2014 - 2018 on locations within the Natura 2000 sites of Laesoe which consists of habitat site 10 (DK00FX118) and habitat site 9 (DK00FX010 and SPA DK00FX345) together covering about 4.400 hectares. *Spartina anglica* is combatted on about 29 ha with scattered stands of the specie.

Laesoe is a young island situated in Kattegat between the Danish mainland and the Swedish west coast. The island is very flat and consists of primarily sand on a bed of clay. There is a substantial coastline with many smaller islands and an up to 3 km wide tidal zone towards south. The invasive *S. anglica* invaded the coast in the late 1980ties and has spread to a large part of the coast around Laesoe (Figure 2).

The deliveries of ACTION C.5 have the following preconditions:

- The experiments shall enlighten which control measures are the most efficient under the preconditions at the site and similar sites.
- The experiments shall engage different measures of control and combinations hereof and include uprooting, burying and grazing as control measures.
- The experiments shall develop new and/or rarefied methods to eradicate the invasive Spartina species taking environmental as well as financial considerations into account.
- The experimental design shall ensure that the results be scientifically valid, hereunder include the necessary replica for statistics.
- The experimental design shall be replicable on other sites.
- The population of invasive *S. anglica* shall be controlled and eradicated along the seaward coastline.
- Further spreading of the invasive *S. anglica* shall be halted.

The experiments were partly made as a master student project by Nadine Rudolph supervised by the Leuphana Universität of Lüneburg and Copenhagen University. Description of the experiments and the recommendations based on the results are copied from the Master's project (Rudolph 2015) with a few editorial changes.

Background perspective

Spartina anglica – an invasive species

Spartina anglica is listed among the 100 worst invasive species worldwide (Global Invasive Species Database 2005) as it can rapidly expand into large monospecific fields, causing severe changes in the native ecosystems. An uncontrolled spread of the species is linked to the exclusion of diverse plant communities in the pioneer zone and low salt marsh. The dense and comparably high Spartina stands also create unfavourable feeding and breeding conditions for bird species like the dunlin (*Calidris alpina*) and can negatively impact fishery and tourism (Nehring and Adsersen 2006).

S. anglica, a highly productive salt marsh plant, was intentionally introduces into coastal systems outside its natural range. It was first recorded in Lymington (UK) in 1892 (Gray et al. 1991), where it has evolved from chromosome doubling of Spartina x townsendii, a sterile hybrid of native Spartina maritima and the accidentally introduced Spartina alterniflora (Ayres and Strong 2001). Due to its

attributes of stabilizing mudflats and catching large amounts of sediment, it was extensively planted for coastal protection and land reclamation purposes in many countries in the first half of the 1990's (Ranwell 1967). Beside Europe it has been spread to North America, Oceania and China (Figure 1). Climate change is likely to influence the future range of *S. anglica* (CABI 2018). The non-native *S. anglica* is highly aggressive in their new environment, and frequently becomes the dominant plant species displacing native flora and fauna (Roberts & Pullin 2006).

S. anglica is hardy and very difficult to eradicate. There have been many attempts to control the species including digging, repetitive burning, grazing and herbicide application, but there is no universally accepted management technique.



Figure 1. Distribution of Spartina anglica (CABI 2018).

S. anglica was first introduced to the Wadden Sea in Denmark in the 1930ties. The first natural spread of *S. anglica* was recorded at Vorsø in 1973 (Nehring and Adsersen 2006). It was first recorded at Laesoe in 1986 (Hansen 1993, Vestergaard 2000 in Randløv 2007).



Figure 2. Distribution of *Spartina anglica* at Laesoe in 2014.

An assessment of the salt marsh at Laesoe in 2010, revealed an invaded area of about 15 hectares. By 2014 the affected area was assessed to cover about 22 hectares with a strong eastward expansion and overgrowth of ecologically valuable habitats (Figure 2). GPS mapping of the total cover of *S. anglica* turned out to be larger than assessed. In total *Spartina anglica* is combatted on about 29 ha with scattered stands of the specie.

Ecology of Spartina anglica

S. anglica is a plant of the intertidal zone. It can tolerate a wide range of environmental conditions (Gray et al. 1991). The plant occurs on a variety of substrates, including clays, fine silts, organic muds, sands, and shingle (Gray et al. 1991), and can tolerate inundation for nine hours or more, which is long in comparison with other thematic species (NWCB 2005). As result, S. anglica can occupy the seaward edge of salt marshes where there is little or no competing vegetation (Gray et al. 1991). On bare mud seedlings may grow densely, occurring at densities up to 13,000/m².

S. anglica sprouts in the spring. In November, it produces wintering buds in the leaf axils, which is followed by rhizome development in response to short days. Seed production is quite variable both temporally and spatially. Pioneer populations often produce few seeds, but seed production increases with marsh development. Low soil temperature can delay or suppress flowering and reduce seed production. It appears that high seed production is associated with warm, late summers (Nehring and Adsersen 2006). The seeds are relatively short-lived, so it does not have a persistent seed bank. Laboratory studies have indicated that seeds stored at 4° C in a refrigerator remained viable for at least four years. Maximum germination occurred in the dark, with the germination rate increasing as temperatures increased from 7° to 25° C. Seeds buried between 1 and 3 cm have the best chance of establishing (Nehring and Adsersen 2006).



Spartina Anglica at salt marshes along the coast of Laesoe (Photo Naturstyrelsen)

S. anglica spreads via seeds, rhizomes, tillering, and rhizome fragments. Dispersal may occur by water currents, humans, shipping, or by the feet of waterfowl (Adsersen 1974, Gray et al. 1991, Eno et al. 1997). According to Reise (1998) residual currents along the Wadden Sea coast rarely exceed 0.1 m s^{-1} , but since a drifting seed of S. anglica can remain viable for weeks it could theoretically travel several thousand km before it settles down (Nehring and Adsersen 2006).

Experimental design

The experiments on control and eradication of S. anglica is divided into a demonstration project including test of different control measures as a block experiment, and a large-scale project aiming at eradication of the *S. anglica* at Laesoe.

Block experiment

The experiment was mostly set up in well-established Spartina fields in the southwestern part of the island (Figure 3). To make sure the results of our experiment are only due to the imposed treatments, the experiment was set up in a randomized block design with six experimental units (plots) nested in each block. The site conditions within each block were as homogenous as possible with respect to the topography.



Figure 3. Location of the block experiment at the southwestern coast of Laesoe.

In total 10 blocks were set up and each treatment and one control plot was represented once in each block. To guarantee random allocation of treatment and control plots in each block, a number was assigned to each treatment (Table 2) and ten random integer sets were generated containing each number once at the webpage "random.org" (Table 1).

Table 1. Randomized allocation of treatments to each block.

Block	Allocation of treatments						
1	2	4	6	1	5	3	
2	2	6	5	4	3	1	
3	4	3	1	5	2	6	
4	1	3	6	2	5	4	
5	6	4	1	3	2	5	
6	4	2	1	3	6	5	
7	1	4	5	2	3	6	
8	1	6	4	2	5	3	
9	5	6	2	1	4	3	
10	6	5	1	3	2	4	

The area of each plot measured 1 m^2 and needed to show at least 25 visible *Spartina* shoots in the beginning of the experiment.

Spartina spreads among others via clonal distribution and many above ground plant shoots are connected via the extensive below ground root and rhizome system of the species (Gray et al. 1991). Therefore all our plots were placed at least ten meters apart from each other to make sure the plants were only affected by the desired treatment of the respective plot. The distance between plots in similar experiments with *Spartina* ranged from one to five meters (Hammond 2001). The experiment was set up between June 9th and 16th, 2014 and was run for roughly one growing season until November 2014.

Selection of treatments to be tested

The treatments tested in this study are based on an analysis of different means, which have already been used in the context of *Spartina* control (Nehring and Adsersen 2006, Roberts & Pullin 2006). The effectiveness in terms of killing rates, practicability for larger scale application and stakeholder interests were the main criteria for an elimination process. Treatment with herbicides was not included in the experiments as the local community at Laesoe strongly opposed the use of herbicides due to the conservation value of the area.

Cutting *Spartina* stems down to 10 cm and smothering these with black plastic lead to a reduction in stem density of more than 95% within one year in an experiment in Ireland (Hammond and Cooper 2002). This treatment is also suitable for relatively time and cost efficient larger scale application as cutting can be done with the help of electronic mowing devices.

Uprooting and inversion of *Spartina* showed partial regrowth in an experiment conducted in France (Cottet et al. 2007), but can also be applied in a time and cost efficient manner on a larger scale with the help of a small excavator. The same is true for digging out and removing *Spartina*, which so far is primarily recognized as a very effective way to eliminate young *Spartina* (Furphy 1970 in Hammond & Cooper 2002).

As the aim of the study was to achieve full eradication of *Spartina*, we hoped to increase the documented effectiveness of uprooting and inversion of *Spartina* by also supplementing it with black plastic sheeting for the rest of the growing season. This seems especially promising for this treatment as Hammond (2001) suggests that smothering "either increases the rate of root and rhizome decomposition or kills roots and rhizomes effectively", which are a potential source of regrowth after inversion. In addition the sheeting will generate a light deficit for the plants. This is likely to induce stress as sunlight is an essential source of energy for plants to do photosynthesis (Müller-Xing et al. 2014). But bringing large amounts of plastic into a dynamic and environmentally valuable coastal system, contradicts the initial aim of nature conservation because some plastic might be washed or blown away despite fixation. Thus, we also tested whether an about 25 cm thick layer of dark sea grass, which can be found in large amounts along Laesoe's beaches, is an equally effective but environmentally friendly alternative to black plastic. This will also create a light deficit and the decay of the additional biomass might have a similar killing effect on the roots and rhizomes as plastic sheeting.

Treatments included in the block experiment

The following treatments were tested in the block experiment:

- Cutting and black plastic sheeting
- Uprooting and inversion
- Digging out

Table 2 provides a comprehensive overview of the treatments tested in this study and a brief description of its implementation.

Treatment Number	Treatment abbreviations	Implementation
1	Up + sea grass	One-time uprooting and inversion of <i>Spartina</i> with a spade; about 25 cm layer dark sea grass fixated with fine woven fish net and wooden poles for the rest of the growing season
2	Up + plastic	One- time uprooting and inversion of <i>Spartina</i> with a spade; black plastic sheeting fixated with wooden poles, sand and rocks for the rest of the growing season
3	Digging	Removal of an about 25 cm deep ground layer with a spade
4	Cut + plastic	One-time cutting down to about 2 cm above the ground with garden shears; black plastic sheeting fixated with wooden poles, sand and rocks for the rest of the growing season
. 5	Cut + sea grass	One-time cutting down to about 2 cm above the ground with garden shears; about 25 cm layer dark sea grass fixated with fine woven fish net and wooden poles for the rest of the growing season
6	.Control	No treatment

Table 2. Description of all treatments tested in this study



Nadine Rudolph demonstrating treatment no 2; Uprooting with plastic sheeting (Photo Rita Merete Buttenschøn)

Data Collection

In order to identify the most effective treatment to eradicate *Spartina*, the plant mortality rate induced by each treatment is the most meaningful source of information (response variable). This rate is based on the mean difference in the total number of living *Spartina* culms in June before treatment and in November when the experiment ended. Due to the dense growth of *Spartina* the total number of living culms in June was counted in a 20 x 20 cm square in each plot and then multiplied by 25 to receive a good estimate of the total number of *Spartina* culms in each plot.

In addition, we recorded how many plots of each treatment showed regrowth to also calculate the mortality rate on a plot level. As even a single *Spartina* plant is a potential source of reestablishment (Gray et al. 1991) this is important additional information to properly interpret the plant mortality rate and identify the most effective treatment.



Treatment no 3: Digging out. Removal of an about 25 cm deep ground layer with a spade and removing the plant (Photo Rita Merete Buttenschøn)

Besides, changes in the physical characteristics of the plant are an essential source of information, to better understand the effect of each treatment on *Spartina* in case of regrowth. Sunlight for example is "an important environmental signal to regulate growth and development" (Müller-Xing et al. 2014). Therefore, we also collected data for the following response variables on 15 randomly chosen culms in each plot. This was done in all plots in June and repeated in those with *Spartina* in November:

- Total plant height
- Number of leafs per culm
- Lengths of the longest leaf per culm

Data Analysis

For the statistical data analysis we first of all calculated for each response variable and plot the mean difference between June and November. This data was then used for further analysis in "Statistics" (R 3.1.2 GUI 1.65 Snow Leopard build (6833)). Taking into account our research design, we analyzed our data for each response variable with a linear mixed effects model (LMM). In addition we also conducted an analysis of variances (one-way ANOVA) followed by a post-hoc Tukey HSD test to identify significant differences between the treatments for each response variable. To fulfill the prerequisite of normal distribution, the "counted" response variables "number of live *Spartina* culms per plot" and "number of leaves per culm" was square root transformed (McDonald 2009)

Results

The results of the linear mixed effects models show a significant (p < 0.05) treatment effect on all four response variables. While the treatment effect on the plant mortality and the mean number of leaves per culm can be considered highly significant (p<0.0001), the treatment effect on the mean total plant height and the length of the longest leaf is slightly weaker (Table 3).

effect on all response variables (1 -value < 0.05)								
Resp. Variable	Num. DF	Den. DF	F-value	P-value				
Plant mortality	5	45	9,103	<0.0001				
Plant height	4	27	6.953315	0.0006				
Number of leaves	4	27	27.215	<0.0001				
Length of longest leaf	4	27	6.726912	0.0007				

Table 3. Results of the Linear Mixed Models (LMM) showing a highly significant treatment effect on all response variables (P-value < 0.05)

In addition to this, the results of the analysis of variances (one-way ANVOA) and post-hoc Tukey HSD test reveal partially significant differences between the treatments with respect to its effect on each of the response variables. These differences will be described for each response variable in more detail in the following.

Mortality rates on a plant and plot level

The mortality rate on a plant level provides information on how much the number of living *Spartina* shoots was reduced in average by each treatment in the course of one growing season. The results show that under natural conditions, the amount of living *Spartina* culms increased in average by 3.42 % between June and November. Opposed to that, all of the treated plots have less living *Spartina* in November than they had before treatments were applied in June. Still, the plant mortality rate varies between treatments.

"Digging" is the only treatment with a mortality rate of 100% on a plant and plot level. But the treatments "up+plastic" and "up+sea grass" also show very high plant mortality rates of nearly 100% (Figure 4). Therewith their mortality rate on a plant level does not differ significantly from "digging". Although the difference between "up+plastic" (99.62%) and "up + sea grass" (98.03%) is statistically not significant, the plant mortality rate of "up+plastic" still is slightly higher. This becomes more obvious when taking into account the mortality rate on a plot level. While "up+plastic" shows regrowth in two out of ten plots (80% plot mortality), "up+sea grass" shows regrowth in all but one of the plots (10% plot mortality).



Figure 4. Mean plant mortality rates in % achieved by each treatment between June and November 2014. Lower case letters indicate significant differences between treatments on the level of p < 0.05

In contrast to that, the plant mortality rates of both cut treatments are clearly lower and all of the treated plots show regrowth of *Spartina* in November (0% plot mortality). The total number of living *Spartina* culms in plots treated with "cut+sea grass" only decreased by 26.64% in average. But the error bar indicates very large variability in the data of this treatment. With that "cut+sea grass" is the only treatment where the amount of living *Spartina* culms has not developed significantly different than under natural conditions. "Cut+plastic" on the other hand reached a mean plant mortality rate of 60.21%. However, this result is statistically neither significantly different from "cut+sea grass" nor from the other three treatments. It only differs significantly from the control.

Difference in the mean total plant height

The difference in the mean total plant height of *Spartina* between June and November is one of the response variables providing information how each treatment affects *Spartina* that was able to regrow despite treatment. As for the previous the results show that the mean total plant height increased by 8.62 cm under natural conditions in the course of the growing season. Opposed to that, the mean total plant height of regrown *Spartina* was less in almost all of the treated plots in November than it was before treatment application in June. However, "up+sea grass" is the only treatment where the mean total plant height developed significantly different than under natural conditions (see lower case letters in Figure 5).

Even though statistically not significant, there is also a clear difference between the two "uprooting" treatments. *Spartina* shoots, which have recovered despite "up+plastic", are only 7.36 cm shorter than before the treatment application. The ones recovered despite "up+sea grass" in the contrary are 12.46 cm shorter than in June. A similar pattern also exists between the two cut treatments, although less clear. Regrown *Spartina* shoots treated with "cut+plastic" are in average only 0.80 cm shorter than they were in June. With that,

Plant mortality (%)

This treatment shows the least difference in the mean total plant height. In addition, the error bar

indicates large variability in the data and a tendency towards an increase in the mean total plant height in the course of the growing season. *Spartina* treated with "cut+sea grass" on the other hand is in average 2.08 cm shorter than it was in June.

Comparing the differences in the mean total plant height of the two cut treatments with both uprooting treatments, plants regrown despite uprooting have overall regained less height than those that were cut.



Treatments

Figure 5. Difference in the mean total plant height achieved by each treatment between June and November 2014. Lower case letters indicate significant differences between treatments on the level of p < 0.05

Difference in the mean number of leaves per culm

Another indicator for the effect of each treatment on regrown *Spartina* culms is the difference in the mean number of leaves per culm between June and November. Consistent with the previous results, the mean number of leaves per culm increased in average by 0.67 leaves under natural conditions and each of the treatments counteracted this natural development. But the extent varies in parts significantly between treatments.

Regrown *Spartina* despite "cut+sea grass" treatment shows the least difference in the mean number of leaves per culm. With an average of 0.13 less leaves per culm than before treatment application, this is the only treatment where the mean number of leaves per culm has not developed significantly different than under natural conditions (Figure 6).

In contrast to that *Spartina* regrown on plots treated with "cut+plastic" have in average 2.13 fewer leaves per culm. With that the development in the mean number of leaves per culm is significantly different between the two cut treatments.



Figure 6. Difference in the mean number of leaves per culm achieved by each treatment between June and November. Lower case letters indicate significant differences between treatments on the level of p < 0.05

Plant height (cm)

The same pattern exists between the two uprooting treatments. While regrown *Spartina* treated with "up+sea grass" has in average 0.89 fewer leaves per culm than before treatment, plants treated with "up+plastic" show in average 1.84 fewer leaves. However, this difference is statistically not significant. But the results further show that the mean number of leaves per culm has not developed significantly different in plots treated with "cut+sea grass" and in those treated with "up+sea grass". The same outcome holds true for the treatments "cut+plastic" and "up+plastic".

Difference in the mean length of the longest leaf per culm

Lastly, the difference in the mean length of the longest leaf per culm also provides information about the effect of each treatment on *Spartina*. As for all other response variables the mean length of the longest leaf increased by 1.47 cm under natural conditions.

However, nearly the same is true for both cut treatments. While the longest leaf on *Spartina* culms treated with "cut+sea grass" is in average 1.01 cm longer than it was in June, it is even 1.49 cm longer in plots treated with "cut+plastic". With that, the mean length of the longest leaf in plots treated with "cut+plastic" has increased even slightly more than under natural conditions. Nevertheless, this is statistically not significant.

In contrast to that, the mean length of the longest leaf per culm of both uprooting treatments is clearly shorter in November than it was before treatment in June. While the mean length of the longest leaf of regrown *Spartina* treated with "up+plastic" is in average 6.24cm shorter than before treatment, it is in average 5.01 cm shorter on culms regrown despite "up+sea grass" treatment. In addition, the error bar of the latter treatment indicates relatively large variability in the data. Also this is the only treatment, where the mean length of the longest leaf developed significantly different than under natural conditions.



Figure 7. Difference in the mean length of longest leaf achieved by each treatment between June and November 2014. Lower case letters indicate significant differences between treatments on the level of p < 0.05

Summary of results

In summary, the results show that under natural conditions the amount of living *Spartina* culms, the mean total plant height, the mean number of leaves per culm and the mean length of the longest leaf per culm increase in the course of one growing season. In contrast to that, all of the treatments counteract the natural development of *Spartina* to some extent. But there are some significant differences between the treatments and its effect on the plants.

"Digging" is the only treatment with no regrowth of *Spartina* at the end of the growing season. Both uprooting treatments also show plant mortality rates close to 100% but differ significantly from each other with respect to the mortality on a plot level. Also viewing the other response variables there are clear differences between "up+plastic" and "up+sea grass", even though these are statistically not significant.

The general pattern of these differences is also evident between the two cut treatments. *Spartina* treated with "cut+sea grass" is the only case where the plants did not develop significantly different than under natural conditions with respect to any of the response variables. In the following, we will discuss for each response variable what these results reveal about the effectiveness of each treatment and draw on implications for the management of *Spartina*.

Discussion

With respect to our research aim of identifying the most effective treatment to eradicate *Spartina*, the mortality rate of each treatment on a plant and plot level is the most valuable source of information in this study. "Digging" is the only treatment with a mortality rate of 100% on a plant and plot level and can therefore be considered the most effective treatment. Even though the treatments "up + plastic" and "up + sea grass" do not differ significantly from "digging" with respect to the plant mortality rate, they still need to be considered less effective than "digging" because on a plot level the mortality rate is 80% for "up+plastic" and only 10% for "up+sea grass". As a single alive *Spartina* plant is enough for large- scale reestablishment and further spread of the species (Gray et al. 1991), full recovery of *Spartina* is likely to occur in 20% of the plots treated with "up+plastic" and in

90% of the plots treated with "up+sea grass" in the near future.

Taking the mortality rates of "cut+plastic" and "cut+sea grass" into account it becomes even more obvious that "digging" is the most effective treatment to eradicate *Spartina*. With a mean mortality rate of 26.64% on a plant level and a mortality rate of 0% on a plot level, *Spartina* treated with "cut+sea grass" did not develop significantly different than under natural conditions. Consequently, this treatment can even be considered completely ineffective to eradicate *Spartina*.

Also "cut+plastic" can only be considered slightly more effective with a mortality rate of 60.21% on a plant level. With that, the treatment is statistically not significantly less effective than "digging", "up+plastic" and "up+sea grass" but the plant mortality rate is still much lower and does not differ significantly from "cut+sea grass" (26.64%) either. In addition, "cut+plastic" also shows a mortality rate of 0% on a plot level. Therewith we can expect full recovery of *Spartina* in all the plots in the near future.

These findings somewhat contradict with present literature and current management recommendations for *Spartina*. Digging out and removing all plant material is claimed only to be effective for smaller *Spartina* clumps, < 50 cm in diameter. This is due to the fact that all root and rhizome material needs to be removed from the ground to avoid regrowth (Hedge et al. 2003). On larger clones this is simply not practical as roots and rhizomes can be found as deep as one meter below the surface (Furphy 1970 in Hammond & Cooper 2002). In line with that, mechanical excavation on a large scale is reported unsuccessful due to rhizome fragments left in the ground (Shaw and Falls 1999). A management report from British Columbia further supports this by stating that manual removal by digging out *Spartina* clumps exceeding three meters in diameter is not doable. Even on smaller clumps it is extremely labor intense and time consuming to remove all roots and rhizomes (Williams et al. 2004).

However, the experimental plots in this study measured 1 m^2 and the vast majority was placed in large well-established *Spartina* fields. As a result, it was not practical to dig out all roots and rhizomes from the ground. Instead we excavated about 25 cm of the top ground layer, leaving many root and rhizome fragments, as a viable source for regrowth, left in the ground below.



Pictures of plots treated with "digging" where about 25 cm of the top ground layer was removed. Picture (a) shows how the plot starts to fill up with water right after the treatment was applied in June. Picture (b) shows how it is still completely flooded in August, even though the surrounding Spartina field is ,, dry". Picture (c) shows the plot in November where the rest of the Spartina field also is flooded (Photos Nadine Rudolph).

But as the pictures above show, the plots filled up with water right after the removal and remained mostly inundated throughout the entire growing season. Inundation exceeding nine hours in a row is

known as a limiting factor for *Spartina* and waterlogged anaerobic conditions for the roots are related to natural die-backs of the species (Gray et al. 1991; Li et al. 2011). Therefore, it is likely that the long- term inundation plays a key role for the great effectiveness of "digging" in this study. In reverse this also implies that excavating about 25 cm of the ground and leaving root and rhizome fragments left in the ground, might only be as effective as in this study when followed by longer-term submersion of the area.

Uprooting and inversion of *Spartina* on the other hand is reported to show partial regrowth (Cottet et al. 2007). In the process of inversion, roots and rhizomes of treated *Spartina* are cut off about 25 cm deep in the ground and the above ground biomass becomes buried by the inverted layer of sand and below ground biomass. This starts a decay process of the above ground plant material in the ground and creates "anoxic conditions in the sediment with a strong sulphide smell" (Cottet et al. 2007). These conditions are also related to natural die-backs of *Spartina* (Gray et al. 1991) and are likely to kill remaining roots and rhizomes in the ground.

Nevertheless, the inverted root and rhizome fragments are also a source for potential regrowth. To avoid this matter we supplemented the treatment with plastic sheeting and a layer of dark sea grass respectively for the rest of the growing season. Even though, both supplemented uprooting treatments still show partial regrowth, the results indicate that plastic sheeting enhances the effectiveness more than a layer of sea grass. As already mentioned above the difference between the two with respect to the mortality rate on a plant level is not significant but on a plot level the difference is crucial. We can expect full recovery of *Spartina* in 9 out of 10 plots treated with "up+sea grass" but only in 2 out of 10 plots treated with "up+plastic". This makes the latter treatment clearly more effective than "up+sea grass" to eradicate *Spartina*.

A very similar pattern, a little less distinct, exists between the two cut treatments. *Spartina* is likely to fully recover under both cut treatments (0% mortality rate on plot level). Also the difference in the mortality rate on a plant level between the two is statistically not significant due to large variability in the data (see error bar of "cut+sea grass" in Figure 4. Yet, "cut+plastic" (60.21%) shows clearly greater mean mortality on a plant level than "cut+sea grass" (26.64%).

In addition, the ineffectiveness of "cut+sea grass" in eradicating *Spartina*, suggests that neither cutting nor a layer of sea grass have a significant negative effect on the species. This is supported by findings from an experiment in Ireland which reveals that one time clipping of *Spartina* can even increase the stem density (Hammond 2001). Further findings from Tasmania state that cutting of *Spartina* has little effect from a management point of view except for the prevention of seed production (Bishop 1996; Lane 1996 in Shaw & Falls 1999). Baker et al (1990) even propose cutting as a good technique to help *Spartina* recovering after oil spillages. All this indicates that the black plastic sheeting is most likely the main driver for the slightly greater effectiveness "cut+plastic".

Nonetheless, the observed mortality rate on a plant level for "cut+plastic" (60.21%) is distinctly lower than what has been achieved by this treatment in previous experiments elsewhere. Hammond 2001, observed killing rates of more than 95% in plots treated with "cut+plastic" and Roberts & Pullin (2006) even report a mean density decline of 97.9 % for this treatment. Considering that black plastic sheeting is thought to "either increase the rate of root and rhizome decomposition or kill roots and rhizomes more effectively" (Hammond 2001), there is reason to believe that the setup of our experiment decreased the effectiveness of "cut+plastic" in this study. We only cut and covered an area of 1m² situated in a larger *Spartina* field with black plastic. Due to the clonal expansion of *Spartina* (Gray et al. 1991) it is very likely that many of the treated plants were below ground still connected to vital plant shoots outside the treated area. This connection might have been essential for the treated plant shoots and rhizomes to stay alive. Therefore "cut+plastic" might turn out a

lot more effective when applied on single Spartina clumps or on an entire field.

Difference in the mean total plant height

Comparing the mean total plant height of *Spartina* before treatment application in June and the mean total plant height of regrown *Spartina* in November, is a valuable source of information to draw more precise conclusions on the effect of each treatment on *Spartina*. The result of the linear mixed model already indicates that the treatment effect on the mean total plant height is slightly less significant (p<0.006) than on the plant mortality rate. In line with that "up+sea grass" is the only treatment where the mean total plant height of *Spartina* developed significantly different than under natural conditions.

In addition, the results reveal two interesting findings. First of all, regrown *Spartina* despite cut treatments generally regrew closer to its initial mean total plant height in June than *Spartina* treated by either one of the uprooting treatments (see Figure 5). This is an indicator that it simply takes longer for a new shoot to sprout from a rhizomes fragment than it takes for cut culms to regrow. Secondly, the mean total plant height of *Spartina* culms is closer to its initial mean total plant height for both treatments covered with plastic than it is for those covered with a layer of sea grass. The error bar of "cut+plastic" even indicates a tendency towards an increase in the mean total plant height compared to June (see Figure 5). This is another indicator that black plastic is more effective than a layer of sea grass by maintaining a light deficit stress for the plants. As a reaction to the lack of light, plants typically stretch out and increase its total plant height (Pfadenhauer 1997).



Pictures of experimental plots in November after Spartina was cut and covered with black plastic (a) vs. cut and covered with a layer of sea grass (b) (Photos Nadine Rudolph).

This becomes even more obvious when considering the pictures from November. These show regrown *Spartina* despite "cut+plastic" treatment (Picture a) and regrown *Spartina* despite "cut+sea grass" treatment. The bright yellow color of the plants, which were covered with black plastic, makes it evident that these suffered a light deficit. In the absence of light plants reduce its chlorophyll production, which is responsible for its green color (Carter and Knapp 2001). In addition, *Spartina* in picture (a) lies mostly flat on the ground, while regrown *Spartina* in picture (b) appears in bright green and upright position. This is a clear indicator that the material of black plastic is more effective in suppressing the plant and maintaining a light deficit than a layer of sea grass. The latter is more permeable and Spartina does not seem to have any difficulties to simply grow its way through the layer of sea grass. This puts emphasize on the fact that *Spartina* is a very stout and tolerant plant (Thompson 1991).

However, sea grass is very rich in nutrients and therefore might even have a fertilizing effect on *Spartina* fostering its recovery (Chapman and Roberts 2006). All in all; these findings further support our previously stated assumption that a layer of dark sea grass is not as effective as black plastic to eradicate *Spartina*.

Difference in the mean number of leaves per culm

Different environmental stresses also influence the development of leaves on a plant. The result of the LMM reveals a highly significant treatment effect (p<0.0001) on the mean number of leaves per *Spartina* culm (see Tab 3). This also reflects in the fact that "cut+sea grass" once again is the only treatment where the mean number of leaves per culm did not develop significantly different than under natural conditions (see Figure 6). With that it becomes inevitable "cut+sea grass" is completely ineffective to eradicate *Spartina* and that a layer of sea grass does not pose a serious stress factor for *Spartina*. Moreover, the results clearly indicate a distinction in the development of the mean number of leaves per culm beween "cut+sea grass" and "cut+plastic" as well as between "up+sea grass" and "up+plastic". Both treatments covered with black plastic show in November in average fewer leaves per culm compared to June than its respective counterparts covered with a layer of sea grass. Even though, only the results of "cut+sea grass" and "cut+plastic" differ significantly from each other, the general pattern is very obvious. Statistical evidence is added by the fact that neither the results of "cut+plastic" nor the results of "cut+sea grass" and "up+sea grass" and "up+sea grass" differ significantly from each other.

The results provide additional evidence for our previously stated assumption that black plastic is more effective than sea grass to eradicate *Spartina*. Plants develop leaves to absorb sunlight and it is a common reaction that plants' suffering a light deficit shows fewer leaves per culm than under optimal conditions. This is in line with our previous findings and supports our previously stated assumption that in the contrary to a layer of sea grass, black plastic sheeting creates stress in form of a light deficit on *Spartina*.

Difference in the mean length of the longest leaf

Lastly, the results show that the treatments have a comparably small effect on the development of the longest leaf per culm. "Up+sea grass" is the only treatment showing a significant difference opposed to the natural development. On plants treated with "cut+sea grass" and "cut+plastic" the length of the longest leaf increased almost exactly like under natural conditions. This is another indicator for the ineffectiveness of "cut+sea grass" as *Spartina* shoots develop like under natural conditions once they grew through the layer of sea grass. The significant difference between "cut+sea grass" and "up+sea grass" on the other hand supports our previously stated assumptions that a layer of sea grass does not have a significant negative effect on *Spartina* and that it simply takes longer for new shoots to sprout and develop from root and rhizome fragments than it does for cut *Spartina* to regrow. The observed mean increase in the length of the longest leaf on regrown *Spartina* despite "cut+plastic" might be due to an inaccuracy in the data collection because these plant shoots mostly consisted of only one or two leaves (see Figure 7) and it was difficult to distinguish between the stem and the longest leaf.

Management implications

From an environmental management perspective it is reasonable to base decisions not only on the effectiveness of a treatment, but to also consider further implications related to larger scale application of the treatment. With respect to the effectiveness "digging" is most successful in eradicating *Spartina*, but "up+plastic" also turned out highly effective. Therefore, both of these treatments are worth considering for larger scale application.

The intertidal zone at Laesoe invaded by *Spartina* is an extremely dynamic system, characterized by an ongoing land rise. This promotes a constant expansion of salt marsh area and the emergence of new small islands (Danish Nature Agency 2014). In this context it is important to note that *Spartina* is also considered an ecosystem engineer because it stabilizes mudflats and enhances sediment accretion (Gray et al. 1991; Balke et al. 2012). It is even associated to "progressively transform medio-littoral marine areas into supra-littoral salt marshes" (Cottet et al. 2007) and was initially planted to prevent coastal erosion (Doody 1990). Therefore, one need to be aware that *Spartina* might play a considerable role in the emergence of new salt marsh and that the removal is likely to have a reverse effect. Particularly the removal of *Spartina* along the shoreline will make the area more vulnerable for wave erosion and might contribute to a loss of saltmarsh area in the southern part of Laesoe (Paramor and Hughes 2007).

Especially when applying "digging" on a larger-scale, the removal of about 25 cm of the top ground layer is a substantial intervention into the existing system, as it intentionally lowers the topographical level and inundates the affected area. Inundation seems inevitable for the effectiveness of the treatment at this point, but it's not clear how long it will take for these areas to fill up with sediment again and become a stable mudflat.

But the probably greatest challenge related to "digging" is the proper disposal of huge amounts of "waste" generated by the removal. One potential solution to this is burying it in 2-3m deep holes as it was done in British Colombia (Williams et. al. 2004). "Up+plastic" on the other hand does not produce any waste material except for plastic, which can easily be disposed in a recycling center. Also this treatment does not impact the topographical level of the treated area and the inversed ground might be recolonized by native vegetation in the course of following growing seasons (Andreu and Vilà 2011).

However, the treatment certainly requires greater management effort than "digging", as single regrowth of *Spartina* is likely to occur. As a result, the area will require careful monitoring and targeted re- treatment e.g. by manual removal for at least one more growing season. But regular monitoring of the area should take place anyway in the following years after large-scale eradication because early detection is the best way to control *Spartina* (Nehring and Hesse 2008). Nevertheless, "up+plastic" will also require careful monitoring during the treatment process to make sure the plastic remains in its desired location and is not released into nature. As we did not have any problems with that during our experiment it seems desirable to split the sheeting for larger *Spartina* fields into several smaller pieces. Besides, the large-scale application of "up+plastic" will also impact users of the area. The plastic certainly is a distraction in the scenic landscape and the decay generated by this treatment might create an unpleasant smell (Cottet et al. 2007). Also, the uprooting and inversion creates an uneven surface, which can become a potential source of injury for users of the area. At this point we cannot reliably estimate how long it will take for the ground to become even and stable again.

Other than this, there are no hints that a large-scale application of either one of these treatments will have negative impacts on the native ecosystem. *Spartina* "has poor influence on endobenthic assemblages" and a removal of the species hardly impacts macrozoobenthos because of its minor presence and ability to quickly recolonize (Cottet et al. 2007). In the contrary the negative impact of *Spartina* on infauna abundance might have severe implications for water birds and the overall biodiversity and functioning of the system (Tang and Kristensen 2010).

Conclusion and Recommendations

Summarizing the findings of this study with respect to our research aim of identifying the most effective mechanical management technique (treatment) to eradicate *Spartina*, "digging" is certainly

the most effective technique, closely followed by "up+plastic". "Cut+plastic" might be worth another try when applied on single clumps or an entire field but all other tested techniques can be neglected at least for the purpose of full eradication.

Large-scale experiment

Digging was chosen as the main method to be used in the large-scale eradication experiment based on results and recommendations from the block experiments. Digging was started in spring 2015 as soon as weather allowed the work to begin. Digging started at the island north-eastern corner and followed the coastline and inlets towards south-west (Figure 8). The Spartina plants were dug up by an excavator and they were buried in deep ditches excavated nearby (described as Dutch ditching).



Figure 8. Distribution of S. anglica registered in 2014 and S. anglica eradicated in 2018.

The digging seemed very successful and monitoring in the spring of 2016 showed no regrowth of S, anglica. Unfortunately the project experienced – as mentioned in progress report 2016 - public resistance which quite rapidly also turned into a political issue, bringing the work to a standstill. This also led to a demand for further discussion with authorities and their subsequence assessment. The project was granted permission from the Danish Coastal Authority by 15th June 2016, but accept was also needed from private landowners. There was some public concern about the use of "Dutch ditching" in fear the seabed could turn soft causing problems for horse riders. To counteract this some tests were made and the treated areas monitored. There have been no signs of the seabed turning soft.

The treatment with digging up Spartina and bury the biomass was continued in 2017, and the clearing on 29 hectares with scatterd stands of S. anglica has been conducted.

Grazing has been used in combination with the digging up in part of the project area. The majority of the areas infested with S. anglica are outside the enclosures established for husbandry grazing. A grazing project with shepherded sheep grazing outside the fences areas (Action C6) demonstrated some effect at the S. anglica reducing seed production and foliage in general. This assists the mechanical removal of the plant as the sheer volume of foliage and upper root systems is reduced before digging the plants up and afterwards bury the plant material.

Grazing alone cannot eradicate established populations of *S. anglica*, but the grazing with cattle, sheep and horses, which has been established at the main part of the open nature areas at Laesoe (Action A1 and C7) will reduce the possibility of new invasion of Spartina to take place.

Practical experience in combating common cordgrass (Spartina anglica)

Preparation of the control Mapping

All the stands of common cordgrass were map by GPS in the field to facilitate control management and follow-up treatment. The mapping showed that the total cover of the invasive plant was larger than expected. A total of 29 hectares of common cordgrass has been eradicated during the LIFE project.



The stands of cordgrass were marked with sticks before the control management was started. (Photo Naturstyrelsen).

Marking of the stands of common cordgrass

Prior to the treatments, all stands of common cordgrass were marked in the field with bamboo sticks with markers bound to the top. The marking makes it easier for the engineer of the machine to find the cordgrass-stand and see the extension of the stand while working.

Treatment

Time for carrying out the control

The opportune time for treatment is very dependent on the weather conditions, as low tide is needed when the part of the beach which is flooded at high tide is dry. The work is primarily performed in late summer to avoid disturbances of the birds in their breeding season from 1st of April to 15th July. The dependency of the right weather situation means that the task requires great flexibility from the contractor responsible for the control.

Types of machines used in the control

Depending on the terrain and soil conditions different size and type of machines were used: a 6-tons caterpillar, a 9-tons back-hoe or 14-tons excavator. Shovel without teeth were used to reduce the detachment of roots, which may establish new stocks of cordgrass.

Control of scattered stands of cordgrass

In areas with scattered stands the following methods have been used:

- 1. Close by the occurrence of the common cordgrass stand of cordgrass a deposition hole for dugout plant material was dug on the dry beach, approximately 1 m deep and maximum 10 m² in area.
- 2. The dugout soil from the hole is placed close by. The common cordgrass plants along with their root-system are scraped off till a depth of at least 10 cm below soil surface. The scraped off plants are placed evenly in the deposition hole leaving the upper 50 cm of the hole open. The process of scraping off the plants must be done with as few scrapes as possible to avoid the break off of roots, which may establish new cordgrass stands.
- 3. The hole is refilled with the sand as quick as possible. The excess sand is leveled on top of the grave and its nearest perimeter. The surface of the grave is even out and compacted.

The average time consumption in areas with scattered stands has been 14 hours per hectare.



The hole with the common cordgrass is being refilled (Photo Naturstyrelsen).

Control of large stands of cordgrass

In areas where there are large stands of cordgrass there is often not enough space for deposition holes to contain all the plant material. Therefore a different control method was applied: turning the soil 180 degrees so that the cordgrass was turn up-side down with the clean layer of sand at the top.

- 1. The cordgrass with roots is dug up and placed in a pile
- 2. A hole to be used for the deposition of the plant is dug where the cordgrass was dug up. The soil from the hole is placed close by.
- 3. The scraped off plants are placed evenly in the hole leaving about 50 cm of the upper part of the hole open. Scraping off the plants must be done with as few scrapes as possible to avoid the roots to break off and establish new cordgrass populations.
- 4. The hole is refilled with the sand as quick as possible. The excess sand is leveled on top of the grave and its nearest perimeter. The surface of the grave is even out and compacted.
- 5. The top of the treated area is examined for loose fragments of roots.

The average time consumption in areas with large stands has been 26 hours per hectare.



Turning the soil 180 degrees. (Photo Naturstyrelsen).

Marking of the buried cordgrass

For the security of the general public's traffic along the area where the cordgrass is buried are the graves marked with landmarks. The landmarks are moved away when the sediments are stable.



The graves are marked with landmarks till the sediment is stable (Photo Rita M: Buttenschøn)

Control follow-up

It is important quickly to follow-up on the control by removing new and small populations of cordgrass, as they may develop new and larger populations of cordgrass in short time.

Information

Many of the treated areas are popular recreational areas for the local population and for tourists. Therefore the project has learned that information about the control is very important. The project has informed the public on the project homepage, by signs, leaflets, public meetings, excursions and through a local stakeholder group.

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