Aquatic Botany 144 (2018) 9–19

Contents lists available at ScienceDirect

Aquatic Botany

journal homepage: www.elsevier.com/locate/aquabot

Eelgrass seed harvesting: Flowering shoots development and restoration on the Swedish west coast

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ARTICLE INFO

Keywords:
Seagrass
Flowering
Zostera marina
Seed
Harvesting
Seed viability
Sweden
Restoration
Seasonality
Eelgrass
Flume
Sinking velocity

ABSTRACT

Eelgrass (*Zostera marina*) flowering development and seed production were assessed along a depth gradient at three sites during 2012 and 2013 to 1) describe the flowering seasonality in the Swedish west coast, and 2) evaluate methods using seeds for large-scale restoration, including harvesting, storage and separation of viable seeds using a vertical flume. Eelgrass flowering shoots were found in the field from June to October, reaching the highest densities in July and August (average 3.8 ± 0.5 shoots m^{-2}). Flowering shoot densities decreased with depth, whereas shoot length, number of spathes, seeds/shoot and seed size increased with depth, resulting in the highest seed production at intermediate depths (2 m) in most bays and years. Because of low densities of flowering shoots, seed production in Sweden (on average 39–126 seeds m^{-2}, Jul–Sep) was an order of magnitude lower than in other studied areas. Results showed that seed production differed 2.5–3.4 times between meadows and years, mainly driven by variation in seed production per shoot. This variation was only partly explained by temperature over the growing season, suggesting that other factors such as light and the amount of filamentous algal mats might also be important in flower development. Results suggest that flowering shoots should be harvested when > 50% of the spathes have developing seeds, and that shoots should not be stored longer than 40 days in tanks to obtain an optimal release of viable seeds. A new mechanized method using a vertical flume to separate large amounts of viable seeds from the harvest is also presented.

1. Introduction

Seagrass habitats are one of the world's most threatened ecosystems and they are disappearing in many parts of the world at an alarming rate. It has been estimated that nearly 30% of the global seagrass area has been lost since the early 1900s, with an accelerating loss (Hughes et al., 2009; Waycott et al., 2009). Along the Swedish northwest coast more than 60% of the eelgrass meadows have vanished since the 1980's (Baden et al., 2003; Nyqvist et al., 2009) with little natural recovery and continuing losses in the southern part of the region (Moksnes et al., 2016). Studies suggest that the primary mechanism behind the decline in this area is caused by eutrophication in combination with overfishing, which has caused a trophic cascade that promotes the growth of algae (Moksnes et al., 2008; Baden et al., 2010, 2012). As environmental conditions are improving (SwAm, 2012), interest to restore the lost eelgrass habitats are growing (SwAm, 2015), but so far, large-scale restoration of eelgrass has not been carried out.

Seagrasses can reproduce by vegetative cloning and sexually through the production of flowers and seeds (Den Hartog 1970; Kendrick et al., 2012; Kendrick et al., 2017). Sexual reproduction is the main way to colonize new areas and it sustains existing beds (Thayer et al., 1984; Marbà and Walker 1999; Greve et al., 2005). Seeds can also be used for restoration, which can be very effective in certain locations. For example, large additions of seeds in Virginia coastal bays in the USA resulted in the development of a 125 ha of eelgrass beds, which increased to over 1700 ha during a 10 year period (Orth et al., 2012). In this successful restoration effort, seeds were produced by harvesting flowering shoots that were stored in tanks until seeds were released. Programs using seeds for large-scale restoration will require assessment of the number of seeds needed for specific planting efforts, and to identify donor sites where flowering shoots can be harvested. It is also necessary to estimate the number of seeds that are available in a donor meadow and the optimal time for harvesting. At the moment, little is known about the seasonality of flower development of eelgrass in Sweden and in Northern Europe it has only been described in Denmark (Olesen 1999; Olesen et al., 2017). Eelgrass flowering, seed development and seed viability have been largely described (Churchill and Riner, 1978; De Cock 1980, 1981) and methods for harvesting

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http://dx.doi.org/10.1016/j.aquabot.2017.10.002
Received 10 May 2017; Received in revised form 4 August 2017; Accepted 9 October 2017

flowering shoots and seeds are well described (Marion and Orth, 2010). However, a method to select the optimal time for harvesting flowering shoots to maximize the number of seeds collected is currently missing.

Flowering or reproductive shoots mature first at the base of the plant and on the main axis of the stem, then progresses upward and outward toward terminal inflorescences (De Cock, 1980). Flowering and seed development does not occur equally over the entire shoot and some judgment is required to choose a harvest time that ensures the greatest yield of seeds. Harvesting too early might reduce the yield of seeds collected since many seeds might not fully develop. On the other hand, harvesting after the seeds have matured might not be efficient since many seeds could be already released in the field. Selecting the best harvesting time is currently done by experience personal judgment of the donor site bed (Marion and Orth, 2010), but there is not a standard method to select these dates. Temperature appears to be critical for all phases of the flowering event, such as the flower appearance, seed production, seed germination and seedling development (De Cock, 1981; Orth et al., 2000; Ackerman, 2006). This relationship between flowering and water temperature could be further explored to possibly predict seed ripening time. In addition, an effective method to separate large amounts of viable seeds after harvesting is needed.

The aim of the study was to 1) describe eelgrass flowering seasonality in the Swedish West coast, 2) evaluate methods for large-scale restoration such as collection, storage and separation of viable seeds. In addition, we assess if growing degree-days, i.e. heat accumulation during the growing season, could be used to predict seed maturation and the optimal harvesting time.

2. Methods

Eelgrass *Zostera marina* (L.) is the dominant angiosperm throughout the northern hemisphere, extensively distributed throughout Scandinavian coastal waters (Boström et al., 2014). The broad-scale presence of eelgrass in this region follows the 5–30 salinity gradient from the northern Baltic Sea to the Skagerrak (Boström et al., 2014). On the Swedish NW coast, eelgrass is found mainly in muddy and sandy sediments between 0.5–4.5 m depth (Baden et al., 2003). Surface water temperature can range from below 0 °C in winter to 20 °C in summer.

Four large meadows were selected in three regions of the Swedish west coast, the Gullmars Fjord, the Stig Fjord and the Hake Fjord (Fig. 1). In the Gullmars Fjord, two meadows were selected, Lindholm and Gåsö. Lindholm represents a sheltered fjord environment where the water can be more stratified, while Gåsö represents a coastal area well flushed with water form the Skagerrak-Kattegat Sea. The meadow in the Hake Fjord, outside the port of Wallhamn is located near the Marstrand area, which has the largest documented decline of eelgrass in Sweden, where over 90% of eelgrass cover was lost since the 1980’s (Baden et al., 2003; Moksnes et al., 2016). The Wallhamn meadow could be potentially targeted as a donor meadow for restoring the area of Marstrand. In the Stig Fjord, Viks Kile was selected since it is a relatively unaffected by the large eelgrass losses. Viks Kile is located near Marstrand and is within a Nature 2000 marine protected area.

2.1. Flowering seasonality and flower development

Flowering shoot densities and the flowering development were measured monthly between Jul-Sep in 2012 at two meadows (Gåsö, Lindholm) and between May-Oct in 2013 at three meadows (Lindholm, Gåsö, Wallhamn) (Table 1). At each site, eelgrass samples were taken along 50 m transects at five different depths from the upper depth limits (1–1.5 m) to the lower depth limits (3–4 m) by snorkeling or SCUBA diving. At each depth-specific transect, the number of flowering shoots were counted in 25 quadrats of 1 m^2 separated by 1 m. Flowering stages were assessed by collecting seven shoots along the transects at each time and classifying all the spathes from each shoot as described by De Cock, (1980). Flowering stages were classified as 1) styles are erect from the spadix, 2) styles bend back after pollination, 3) pollen is released from the anthers, 4) seed maturation during 4–5 weeks and 5) seeds are released (Fig. 2). Morphological characteristics such as shoot length, number of spathes, number of developing and mature seeds per spathe were also measured on every flowering shoot.

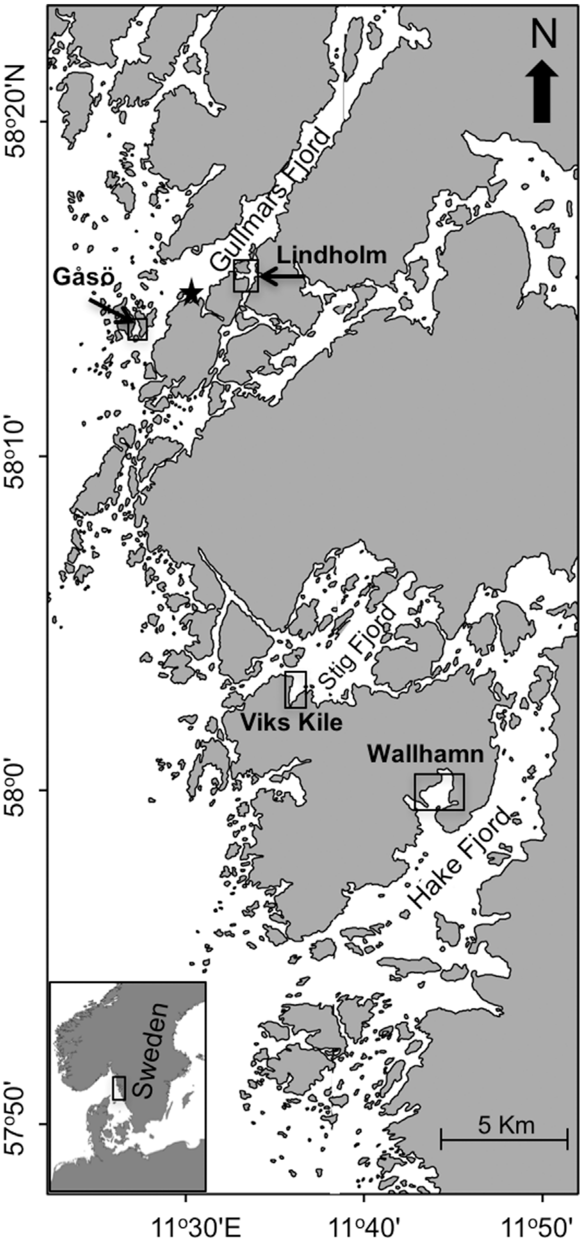

Fig. 1. Map of the study sites in the Swedish northwest coast. Gullmars Fjord and Wallhamn were the main study areas, whereas Viks Kile was used for the large-scale harvesting study. Kristineberg station is shown with a star.

Seed maturation and seed release after harvesting was assessed in 2012 and 2013. Flowering shoots were harvested between Jul-Sep and stored in outdoor tanks over 2–3 months (see below for tank storage details). Ten flowering shoots were collected at 1–1.5 m (shallow) and 3–4 m (deep) at all sites (Table 1). Flowering stages were assessed and shoots were placed in mesh bags of 500 μm in outdoor seawater flow-through tanks. Seeds released in the mesh bags after maturation were collected and counted. Water temperature and salinity in the tanks was monitored using data-loggers (HOBO, Onset®).

The relation between water temperature, light and flower development was further explored using growing degree-days (GDD) and the percentage of surface light reaching the bottom. Water temperature and