

Germination and seed bank analysis of *Zostera marina*

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Zusammenfassung

In den letzten Jahrzehnten hat der Bestand der einzigen echt marinen Blütenpflanzen, der Seegräser, dramatisch abgenommen. Sie nehmen zahlreiche wichtige Funktionen wahr, die zum Erhalt eines gesunden Ökosystems, besonders in den Küstenzonen, beitragen. Elementare Eigenschaften der Seegraswiesen, wie die Festigung und Stabilisierung des Sediments, gehören ebenso dazu, wie die Bereitstellung eines reichhaltigen Angebotes an Nahrung. Des Weiteren bieten sie wichtige Lebensräume für viele Meeresorganismen und spielen eine herausragende Bedeutung bei der Bindung von Kohlenstoff aus der Atmosphäre.

Der Besorgnis erregende Rückgang der Seegraswiesen, bewirkte in den letzten Jahren ein steigendes Interesse der internationalen Forschung, den Bestand dieser wichtigen Pflanzen wieder zu vergrößern. Für einen erfolgreichen Anstieg der Bestände sind Kenntnisse der sexuellen Reproduktion unabdingbar. Trotz der gut erforschten Reproduktionsmerkmale, der in den gemäßigten Zonen weit verbreiteten Art der *Zostera marina*, gibt es keine eindeutige Erklärung, weshalb die Keimrate, entgegen der grundsätzlich hohen Samenproduktion, derart niedrig ausfällt (1-20%).

Gegenstand dieser Studie ist die Untersuchung der Keimrate von *Z. marina* von fünf Ostseestandorten in Mesokosmen. Für einen überregionalen Vergleich wurden außerdem Samen aus Süditalien untersucht. Um die Menge der Samen zu bestimmen, die in der Samenbank vor der Keimung im Frühjahr vorhanden sind, wurden zusätzlich zwei eu- und ein sublitoraler Standort im nordfriesischen Wattenmeer untersucht.

Die in anderen Experimenten bereits gemessene geringe Keimfähigkeit, konnte auch hier nachgewiesen werden (5,7 %). Außerdem konnten signifikante Unterschiede in der Keimfähigkeit zwischen Standorten beobachtet werden, die ihre Ursache vermutlich in unterschiedlichen ökologischen Bedingungen der verschiedenen Habitate haben. In diesem Zusammenhang können sowohl genetische als auch phänotypische Aspekte eine wichtige Rolle spielen.

Die Keimung der Samen erhöhte sich deutlich, nachdem die Wassertemperatur gesenkt und statt künstlichem natürliches Meerwasser benutzt wurde (von 0,9 auf 2,4 Keimlinge/Tag). Eine genaue Analyse der Bedeutung von Wassertemperatur und Wasserzusammensetzung ist also nötig, um die Bedeutung dieser Faktoren zu beurteilen. Die Samenbankanalyse von *Z. marina* im Wattenmeer zeigte, dass bei

den dortigen Populationen neben der geringen Keimrate, auch ein hoher Samenverlust zu einer Reduzierung der sexuellen Reproduktion führt.

Summary

In the last decades the loss of the only true marine flowering plants- the seagrasses have dramatically increased worldwide. Important ecosystem functions like stabilizing seabeds, providing food and habitat for marine organisms and carbon sequestration make seagrasses a foundation species in the littoral zone. The loss of seagrasses has lead to strong scientific interest and efforts to restore seagrass meadows increase.

For successful restoration projects, large numbers of propagules from donor beds are needed. Even though the sexual reproduction of the most common seagrass in the temperate zone, *Zostera marina*, is well investigated, it is not really clear which factors lead to the low seed germination rates (between 1-20%) that can be observed in the field and in laboratory, although seed production is high.

In this study I have investigated the seed germination rate of *Z. marina* from five Baltic sites in a mesocosm-facility to investigate seed germination in Baltic eelgrass meadows. For comparison, seeds commonly found in distribution in the Mediterranean were also analysed. Additionally, I have also analysed the seed banks of two intertidal and one subtidal sites in the German Wadden Sea, to assess the number of seeds available in the seed bank before germinating in spring. In concordance with other studies, we found low seed germination rates (5.7 %). Moreover, germination rates of *Z. marina* seeds from different sites differed significantly. This indicates habitat differences in germination ecology that can either be genetic or phenotypic. Seed germination significantly (from 0.9 to 2.4 seedlings/ day) increased after water temperatures dropped and ambient instead of artificial seawater was used. This means that a careful analysis of the impact of water temperature and composition is needed to disentangle the impact of these factors. The seed bank analysis of *Z. marina* populations in the Northern Wadden Sea show that next to low germination rates, a strong seed loss during winter hampers sexual reproduction.

1 Introduction

Seagrasses are the only true marine angiosperms that occur in coastal waters of temperate and tropical waters (Den Hartog 1970, McRoy and Helfferich 1977) and are amongst the most productive ecosystems on earth (Duarte and Chiscano 1999). Even though seagrasses cover only 0.15% of the photic zone of the global oceans (Charpy-Roubaud and Sournia 1990), they are responsible for 1% of the ocean's primary production (Duarte and Cebrián 1996). Compared to the tropical rain forest ecosystem, seagrass meadows have a standing biomass 95 times lower than that of a rain forest (rain forest: 45000 g DW m⁻², seagrass meadows: 461 DW m⁻²) but their productivity is half of that of the rain forest ecosystem (rain forest: 5.2 g DW m⁻² day⁻¹, seagrass meadows: 2.7 g DW m⁻² day⁻¹; Duarte and Chiscano 1999).

This high productivity together with other crucial ecosystem functions such as stabilizing sea beds, providing food and habitat for many species, playing an important role as nursery grounds for juvenile fish, and providing high carbon sequestration (15% of the world's ocean; Duarte and Cebrián 1996, Duarte and Chiscano 1999), make seagrasses a foundation species (*sensu* Dayton 1972) and one of the most valuable ecosystems in the biosphere (Costanza et al. 1997). Furthermore, seagrasses provide considerable ecosystem services to us humans, which are evident in the monetary that sums up to \$ 3.500 ha⁻¹ yr⁻¹ for fisheries industry (Watson et al. 1993).

In the last few decades, the importance and awareness of seagrass meadows has increased parallel to the accelerated loss of these meadows worldwide. A global assessment of 215 studies by Waycott et al. (2009) has shown that 29% of established areal distribution has vanished since 1879. Whereas seagrass meadows have been disappearing at a median rate of 0.9% yr⁻¹ of the known area before 1940, they declined by 7 % yr⁻¹ since 1990 (Waycott et al. 2009). The biggest loss of the widespread species *Zostera marina* (eelgrass) in the northern hemisphere was caused by a pandemic disease ("Wasting disease") in the 1930s. The eelgrass along the North Atlantic coast was infected by a marine slime mould, the protist *Labyrinthula zosterae*, which nearly led to the entire eelgrass populations in North America and Europe dying out (Muehlstein et al. 1989). Symptoms are brown spots (lesions) spreading across the leaf that gradually became darker over time and which finally lead to leaf detachment and rhizome death (Den Hartog 1987). Accompanied by new breakouts, recovery was slow until 1955-65 and increased thereafter (Short et al. 1988). *L. zosterae* has also been found in apparently healthy

eelgrass (Pokorny 1967, Rasmussen 1977, Bockelmann et al. 2011 a), where between 10-67% of a population are infected with 0.5 to 15.5 *Labyrinthula*-cells per mg plant DW (Bockelmann et al. 2011 b), in review). It remains unclear which attributes and environmental factors lead to an outbreak of the disease.

Additionally, other natural and anthropogenic causes dramatically accelerated the deterioration of seagrass meadows (Orth et al. 2006). Whereas natural causes like diseases, physical damage, strong hurricanes, coastal erosion (Short and Wyllie- Eschevierra 1996) and intensely cold winters (Frederiksen et al. 2004) already have had and have a strong impact, further human- induced disturbances have been reported as a major contributory factor to declining seagrass meadows over the last decades (Duarte 2002). With an ever- growing human population concentration around coastal waters, eutrophication, which results in light limitation for seagrass beds, has been identified especially in combination with mechanical damage such as anchoring and dredging, which leads to sediment erosion, as two major human induced disturbances leading to a decline in seagrass meadows. Additionally, rising CO₂ concentration and seawater temperature, as a result of climate change, are important aspects that further exacerbate the loss of seagrasses (Duarte 2002). Due to the described processes that lead to their rapid decline, seagrass beds rank amongst the most endangered ecosystem in the world (Waycott et al. 2009)

In the Northern hemisphere, the decline of the widespread species *Zostera marina* has led efforts to restore eelgrass meadows by means of transplantation (Orth et al. 2007). It is known that *Z. marina* propagates vegetatively via rhizomes and generatively by seeds (Den Hartog 1970, Phillips and Meñez 1988). Due to advantages such as reducing damage to donor beds (Orth et al. 2008), maintaining high genetic diversity (Fonseca et al. 1998) and alleviating harvesting processes, planting eelgrass from seeds in restoration projects is a popular new practise (Tanner and Parham 2010). Therefore interest in seed development and seed germination ecology of *Z. marina* has increased (Orth et al. 2007). Since *Z. marina* has been a study object for a very long time, there are many publications on sexual reproduction of primarily North American populations. In contrast, not a great deal is known about germination ecology in European *Z. marina* populations (Hootsman et al. 1987). The majority of investigations studied the impact of salinity, temperature, light, scarification of the seed coat, and oxygen on seed germination of *Z. marina*, both in the laboratory and in the field (Moore et al. 1993, Brenchley and Probert 1998). Nevertheless it is controversial, which primary factors promote seed germination of *Z. marina*. Several laboratory studies have shown that low salinity (5-10psu)

is a main factor in inducing seed germination (Taylor 1957, Phillips et al. 1983, Hootsmans et al. 1987, Churchill 1992). In contrast, Orth et al. (1981, 1983) state that low water temperature ($< 15^{\circ}\text{C}$) is the major factor when stimulating seed germination. Additionally, stratification, anoxia, and scarification of the seed coat have also been reported as having positive effects on seed germination (Hootsmann et al. 1987, Harrison 1991, Moore et al. 1993), whereas it is shown that light does not seem to have any effect (Tutin 1938, Hootsmans et al. 1987, Harrison 1991, Moore et al. 1993).

Reproductive strategies can vary within a species according to environmental conditions. While subtidal *Zostera marina* is perennial, intertidal populations are annual and thus completely rely on sexual reproduction (Keddy and Patriquin 1978, Kuo and den Hartog 2006). In the German Baltic, where major parts of the investigations were conducted, the life-cycle of the subtidal *Z. marina* starts with the formation of reproductive shoots in May and flowering begins in June. Depending on actual weather conditions, seeds are mature by the end of July or August. After seeds fall, seeds develop dormancy, remain in a transient seed bank during winter season, (Orth and Moore 1983, Moore et al. 1993, Harwell and Orth 2002) and germinate in spring. Generally, seed germination rates are low (between 1-20%; Tutin 1938, Phillips 1971, Lamounette 1977, Orth et al. 2006, Tanner and Parham 2010) compared to the high seed production. Observations show a seed production of up to $\sim 13,000$ seeds m^{-2} year $^{-1}$ in a Danish eelgrass population (Olesen 1999), and approx. 9000 seeds m^{-2} remain in the seed bank until the following spring (Hootsmans et al. 1987, Olesen 1999). Only up to 10% of these seeds germinate (Hemminga and Duarte 2000, Orth et al. 2000) and seedling establishment is even lower ($< 10\%$; Churchill 1983, Harrison 1993, Olesen and Sand-Jensen 1994, Olesen 1999). According to Churchill (1983) the low seedling survival rate in eelgrass shows that sexual reproduction is a limiting factor for *Z. marina* by limiting genetic diversity (Waycott 1995, Reusch 2002). In light of the present decline of seagrass meadows, seed dispersal, germination, and establishment are particularly important for maintenance of existing meadows and (re)colonization of new areas (Erftemeijer et al. 2008, Harwell and Orth 2002).

In the present study, I investigated seed germination of *Zostera marina* from 5 Baltic Sea and one Mediterranean site in a mesocosm facility. My main aim was to test the applicability of germination conditions reported in the literature (e.g. Tanner and Parham 2010) to *Z. marina* seeds from my study area. Thorough knowledge of seed germination is crucial for future restorative projects in the Baltic and a prerequisite for all experiments focussing on early life stages of *Z. marina*. Additionally, I

analyzed and compared the seed bank of a subtidal and intertidal eelgrass meadow in the Wadden Sea to investigate differences in seed production and seed loss between the annual and perennial life forms.

2 Materials and Methods

2.1 Collection and seed maturation

To obtain enough ripe seeds, flowering *Zostera marina* – shoots from different sites in the Baltic Sea and one Mediterranean site were harvested after seeds had been formed by the plants (Tab. 2-1). All plants were collected via snorkelling in 1-3m depth.

Table 2-1. Overview of sampling locations and *Zostera marina* – seed harvest for this study.

Sampling site	Location	Geographic coordinates	Natural salinity (psu)	Date field harvest	Date seed harvest	Seed number	Planting date
Chioggia	Venice Lagoon, Italy	N 54°75,57' E 09°87,66'	>35	May 2010	June/July 2010	361	August 2010
Strande	Kiel Fjord, Germany	N 54°43,83 E 10°17,64'	15-20	July 2010	August 2010	360	August 2010
Kiekut	Eckernförde Bay, Germany	N 54°45,04' E 09°87,24'	15-20	July 2010	August 2010	760	September 2010
Karlsmünde	Eckernförde Bay, Germany	N 54°49,62' E 09°94,05'	15-20	June 2010	August 2010	267	August/September 2010
Gelting Bight	Flensburg Fjord, Germany	N 54°75,66' E 09°87,08'	15-20	July 2010	August 2010	800	September 2010
Maasholm	Schlei Fjord, Germany	N 54°68,82' E 10°00,80'	10-15	July 2010	August 2010	480	September 2010

For seed maturation, collected plants were cultivated in mesocosm- facility with filtrated seawater (Fig. 2-1). Salinity was adjusted to the sampling site salinity, whereas the temperature was kept at 19-23°C to accelerate the ripening process of seeds. Pumps and additional aerating via an oxygen facility ensured sufficient oxygen in the water.

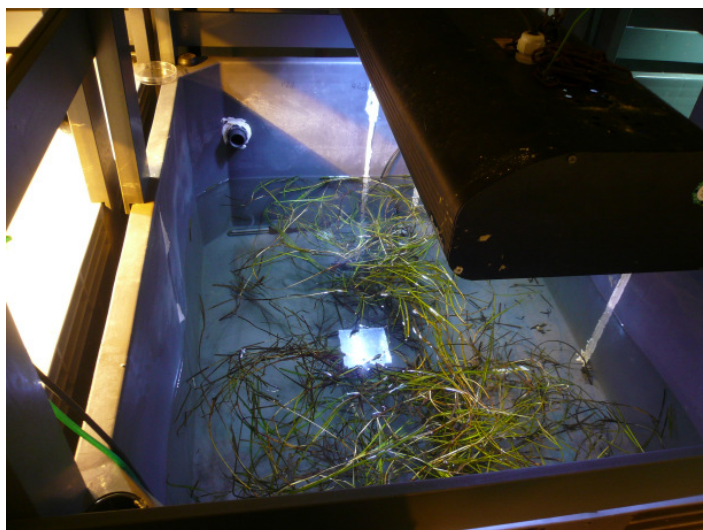


Figure 2-1. Harvested *Zostera marina* plants with maturing seeds in the mesocosm-facility at IFM- Geomar, Kiel (photo by Elma, 2010).

Between mid June and early August, mature seeds were harvested. A seed was considered mature, when the seed coat was brown, dark green or black and seeds could easily be removed from the floating florescence or fell off to the bottom. Avoiding dehydration, harvested seeds were held in Petri dishes with filtrated seawater (15psu) that was changed every week. Seeds were kept in a 5°C cold storage room to simulate a winter period in order to induce germination (stratification, Tab. 2-2).

Table 2-2. Days of stratification for each site to simulate a winter period in order to induce germination.

Site	Vernalization (days)
Chioggia	50-75
Strande	28-33
Kiekut	30-40
Karlsminde	31-40
Maasholm	31-42
Gelting bight	42

2.2 Seed germination

To keep track of the seeds and for easy operation, I worked with aquariums, in which seeds were sown and then put in the bigger mesocosms (Fig. 2-2).

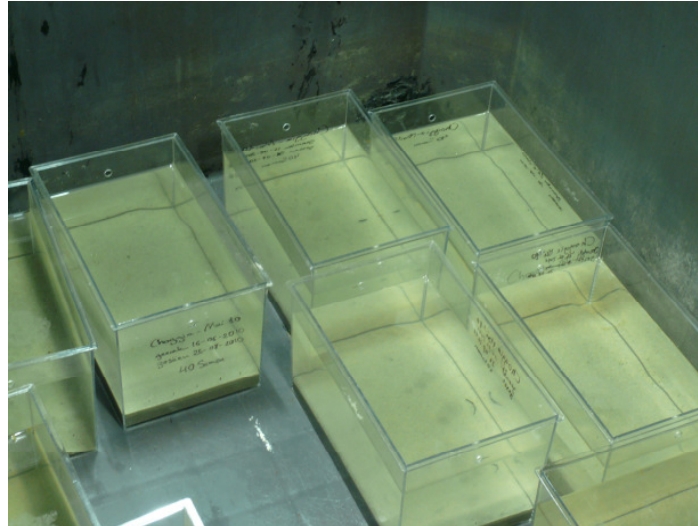


Figure 2-2. Aquaria sown with *Zostera marina* seeds (photo by Elma,

Before planting seeds, natural sediment from the Strande eelgrass meadow was sterilized for 2-4 hours at 80°C, to eliminate potential *Labyrinthula zosterae* contamination. Afterwards each aquarium (15cm x 25cm x 15 cm) was filled with 2 cm of sterilized sand and forty seeds from the same original site were planted 1 cm deep in each aquarium. To be able to identify and follow each single seed individually, I planted all seeds in a grid (coordinates given by letters and numbers) marked on every aquarium. Because of unequal seed numbers in each reproductive shoot, the number of seeds from each site was different, which leads to different number of aquaria for each site (Tab. 2-3). Before placing aquaria, mesocosms and equipment were cleaned with 0.5% sodium hypochlorite to eliminate *Labyrinthula spp.* and all other microorganisms and algae.

To keep the aquaria submerged, mesocosms were filled with ~600 litres of artificial saltwater (Fig. 2-3). I used artificial seawater prepared with Instant Ocean Salt (Aquarium systems, France) at 15psu during the first two thirds of the experiment. In the last part, all mesocosms were filled with 2xfiltered ambient seawater (13-16psu), because I observed increased death rate.

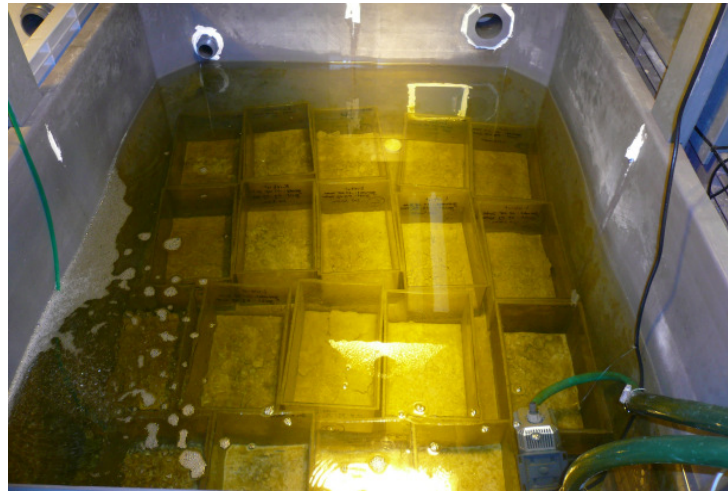


Figure 2-3. Submerged aquaria within a mesocosm (photo by Elma, 2010)

According to Tanner and Parham's (2010) large-scale study on *Zostera marina* germination, the salinity of the mesocosms were maintained at 15psu (Fig. 2-4). Initially, water temperature was 15°C. Temperature was increased by 2°C every week until a peak of 22°C was reached and then kept constant (Fig. 2-5). The reason for increasing temperature was to enhance the growth of seedlings. Temperature and salinity were measured daily or every other day to ensure constant conditions. Half of the water in each mesocosm was exchanged every week. To avoid a cold shock and a completely new water environment, the water was held in a mesocosm a couple of days beforehand and stored.

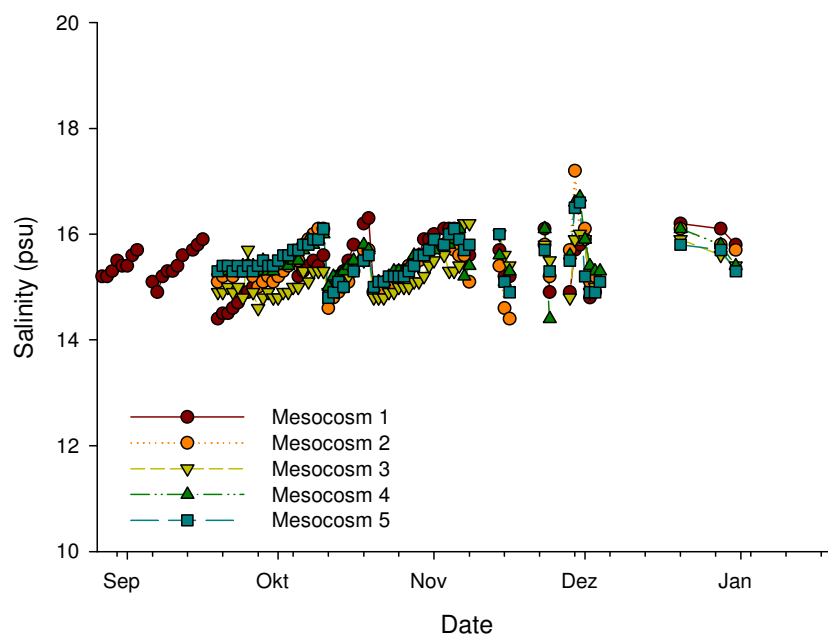


Figure 2-4. Measured salinity (psu) over time in each of 5 mesocosms.

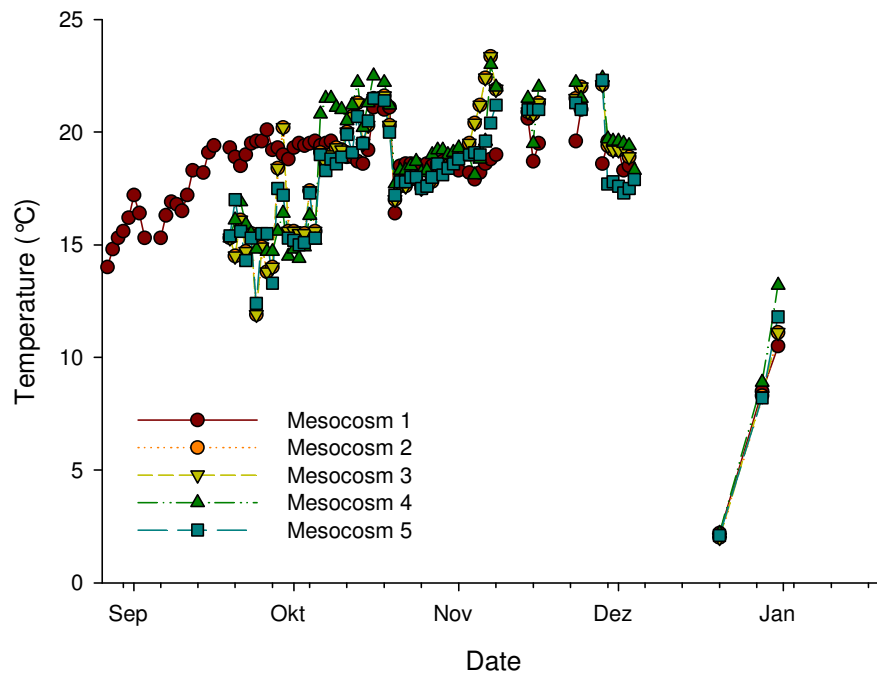


Figure 2-5. Measured temperature (°C) over time in each of 5 mesocosms. The sharp drop in the temperature on December 20th was caused by the change from artificial to ambient seawater.

I checked every few day for germinating seeds. Germination of seeds was defined as Churchill (1983) has described, i.e. the emergence and straightening of the cotyledon from the sediment. In this study, white roots very rarely appeared before the leaf. Supplying nutrients to seeds at the beginning of the experimental approach wasn't required since seedlings receive nutrients from the seed in the beginning. Additionally, the sterilized sediment contained enough organic material to supply nutrients. Forming filamentous algae were removed with a dip-net (Fig. 2-6).

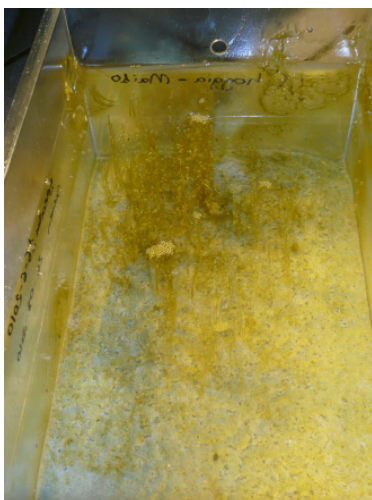


Figure 2-6. Algae formation few days after putting aquaria in mesocosms (photo by Elma, 2010).

During the experiment, nutrient concentration was measured twice (October 2010 and January 2011; Fig. 2-7). According to typical nutrient content in water column needed for seagrass growth, (Worm and Reusch 2000) nutrient concentration was very low for all sites except for ammonium in October for three of five mesocosms (Fig. 2-7)

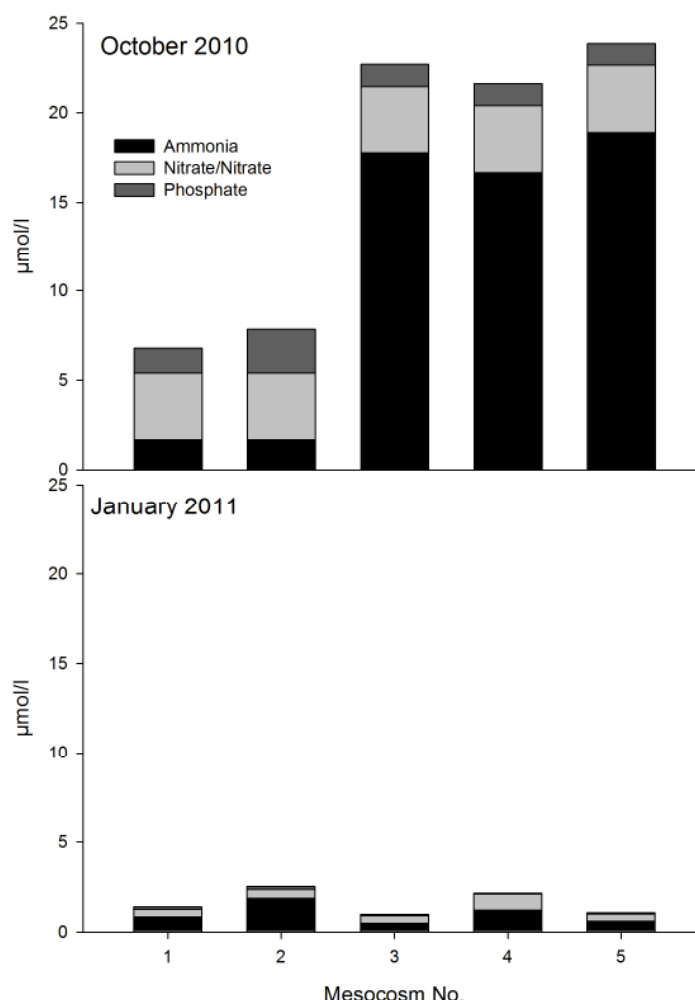


Figure 2-7. Measured nutrient concentration for all mesocosms in October 2010 and January 2011.

Subsequently in October 2010, seedlings with two green leaves were fertilized with slow-release fertilizer. Three Plantacote © Mix 4M pellets (Manna, Ammerbruch-Pfäffingen, Germany) were placed 1cm deep into the sediment in 2 cm distance from the seedling (approximately 0.02g N/seedling + approx. 0.009g P/seedling). At a size of 3-5cm, seedlings were transferred to new aquaria with 10cm sterilized ambient sediment and fertilized again.

Each mesocosm was equipped with a halogen metal vapor lamp (2x400W) with a spectrum appropriate for plant growth. Simulating springtime in this study, light was on from 6am- 8pm. After the first seedling started bleaching in October 2010, light intensity was measured 5 cm under the surface of the water. Consequently, light intensity was reduced to 400 W (Fig. 2-8; Tab. 2-3).



Figure 2-8. Bleached seedlings (photo by Elma, 2010).

Table 2-3. Measured data of irradiance for each mesocosm.

Mesocosm no. and seed origin	No. of aquaria	Light intensity with 800 W ($\mu\text{E}/\text{m}^2/\text{s}$)	Light intensity with 400W ($\mu\text{E}/\text{m}^2/\text{s}$)
1 Chioggia	10	960	870
2 Strande	9	1076	860
Kiekut	1		
Karlsminde	1		
3 Kiekut	18	1065	830
4 Maasholm	12	790	1012
Karlsminde	6		
5 Gelting Bight	20	690	1007

To control water temperature against heating above the designated temperature by the halogen-metal vapor lamp, the water in each mesocosm was cooled and kept in circulation.

2.3 Seed bank analysis

Analysing the seed bank of *Zostera marina*, sediment cores were taken in November 2010 and March 2011. Samples were taken from in three existing *Z. marina*-beds on the North Frisian island Sylt (Fig. 2-9).

The Ellenbogen eelgrass meadow is subtidal with perennial plants, whereas at Tonnenleger Bight and Puan Klent are intertidal meadows, with annual *Z.marina*.



Figure 2-9. Map of Sylt, showing the three sites where samples were taken for analysing the seed bank of *Zostera marina* beds (Source: <http://maps.google.de/maps?hl=de&tab=wl> 10.05.2011)

In Puan Klent and Tonnenleger Bight, sediment cores were taken along a horizontal and a vertical 30m transect in regular intervals (Fig. 2-10; Tab. 2-4). At Ellenbogen, samples were taken every 10m covering the whole eelgrass meadow along the shore of a creek (Fig. 2-11).

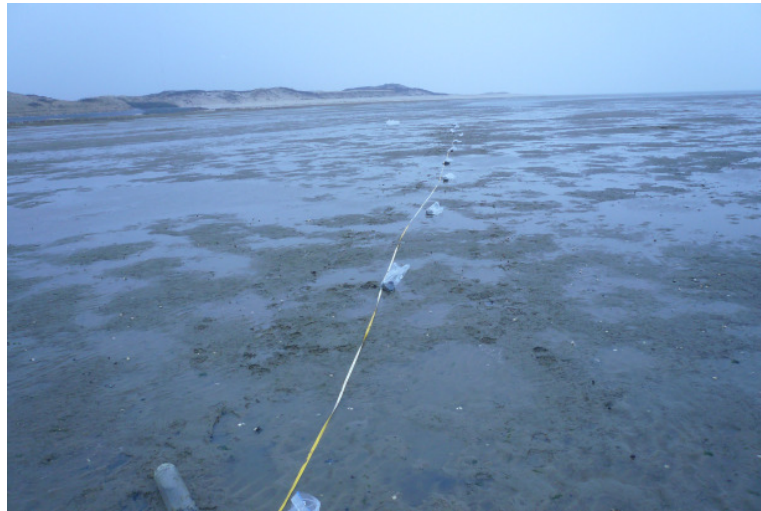


Figure 2-10. Transect with sampling sites for sediment cores at Puan Klent (photo by Elma, 2010).

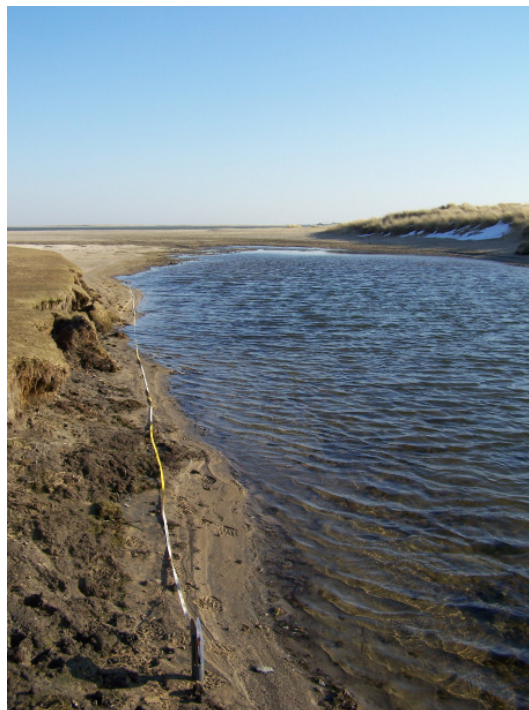


Figure 2-11. Transect with sampling sites for sediment cores at the subtidal site Ellenbogen (photo by Lopez, 2011).

I took all samples with a sediment core 5 cm deep in November 2010 (ø10cm) and March 2011 (ø 8cm).

Table 2-4. Distances and numbers of samples taken for all sites in November 2010 and March 2011.

Date	Sampling site	Transects	Distance between taken samples (m)	No.of samples	Sediment total (m ²)
Nov. 2010	Tonnenleger Bight	1 Vertical 1 Horizontal	1.5	42	32.99
March 2011	Tonnenleger Bight	1 Vertical 1 Horizontal	3	22	11.06
Nov. 2010	Puan Klent	1 Vertical 1 Horizontal	3	22	17.28
March 2011	Puan Klent	1 Vertical 1 Horizontal	3	22	11.06
March 2011	Ellenbogen	1 Horizontal	10	18	9.05

To extract seeds from the sediment, the latter was washed with a 1000µm sieve and both, *Zostera marina* and *Zostera noltii* seeds were taken out with forceps and put in Petri dishes with ambient seawater for later experiments. The two species clearly differ in morphology and colour and could easily be separated (Fig. 2-12).



Figure 2-12. *Zostera marina* and *Zostera noltii* seeds found in the sediment, differ in morphology and colour (photo by Elma, 2011).

2.4 Statistical analysis

2.4.1 Germination experiment

The binominal germination data (1= germinated, 0= no germination) were analyzed for site differences in (a) the time interval until 20.12. 2010 and (b) until the end of the experiment (18.01.2011) by logistic regression (McCullagh & Nelder 1989) using JMP 9 (SAS Institute Inc., Cary, USA). The aquarium in which 40 seeds of a site were sown was nested into site. The mesocosm as the spatial subunit was introduced as a block factor into the model. Post-hoc the six different source sites were compared by planned comparison of means and significance levels were Bonferroni corrected.

2.4.2 Seed bank analysis

Count data of seeds found in the sediment samples were log-transformed prior to statistical analysis to ensure normal distribution of the data. Differences between sample site, sampling date (spring or fall) and the different seed parts found were analyzed by ANOVA. Tukey-HSD post-hoc tests were used to investigate which of these sites were statistically different.

3 Results

3.1 Seed germination

Following stratification, the first seeds from most sites had germinated one week after being sown. An exception were the seeds from Chioggia (Italy) that had already started germinating before even having been sown, at 5°C, in darkness in Petri dishes. Because experimental conditions were changed from artificial to ambient sea water and to lower temperature (from 22 to 2-15°C) after December 20th, the analysis of the results were divided to the time before and after this date.

Before December 20th a total number of 101 seeds germinated (3.3% of 3028 seeds sown). The germination rate pooled over all sites was 0.9 seedlings/ day. After this date, germination rate increased to 2.4 seedlings/ day, so that 173 (5.7%, 1.2 seedlings/ day) had germinated by the end of January 2011. When comparing the seed germination rate (%) until January 2011 between the original sites, Strande had a significantly higher germination rate (20.6%; Fig. 3-1). The germination rates of the other sites were between 2 and 7%, with Gelting Bight showing the lowest germination rate (2.1%; Fig. 3-1; For statistical analysis see Tab. 3-1.)

Table 3-1. The germination of *Zostera marina* depending on the site of parental origin. Nominal logistic regression for the survival until 17th January 2011 (Deviance = L-R χ^2 , *** = $P < 0.01$ ** = $P < 0.02$).

SURVIVAL	df	Deviance	<i>P</i>
Site (Aquarium)	48	3587.43	<0.0001***
Mesocosm	4	863.47	<0.0001***
Aquarium	18	31.74	0.011**
Lack of Fit	68	253,18	

Whereas germination of the Strande-seeds ascends exponentially over the time, germination of seeds from the other sites increases only slowly until end of October and no more seeds germinated until end of December. After the water of mesocosms had been exchanged with ambient sea water, germination rate increased (Fig. 3-1).

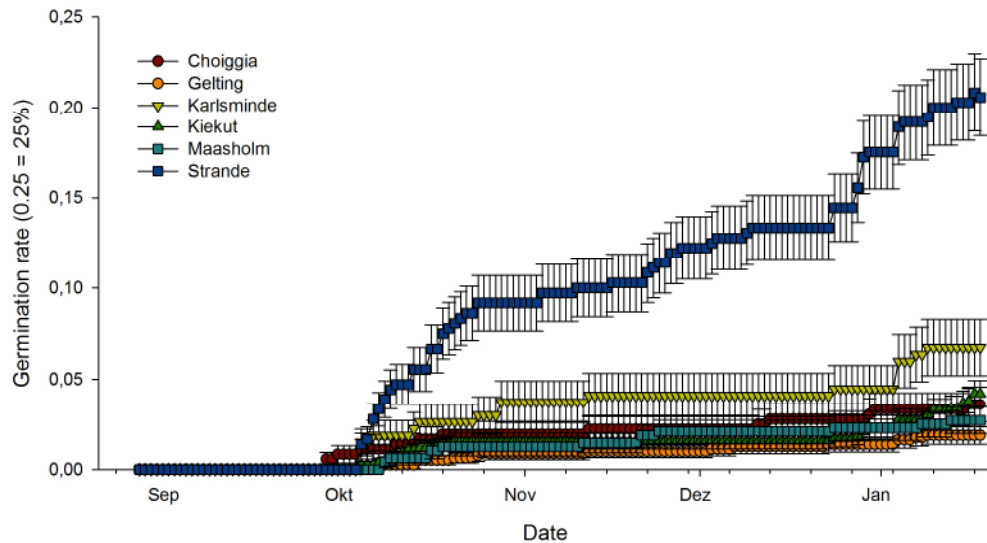


Figure 3-1. Seed germination rate over time, with the slope of the germination after 20th December for all sites.

Comparing the percentage germination (%) of the longer period before water has been changed (114days) with the shorter period afterwards (30 days), the slope of the germination of all sites strongly increased after 20th December. This is particularly true for the site Kiekut, that shows a germination rate twice as high as before the 20th December (Fig. 3-2).

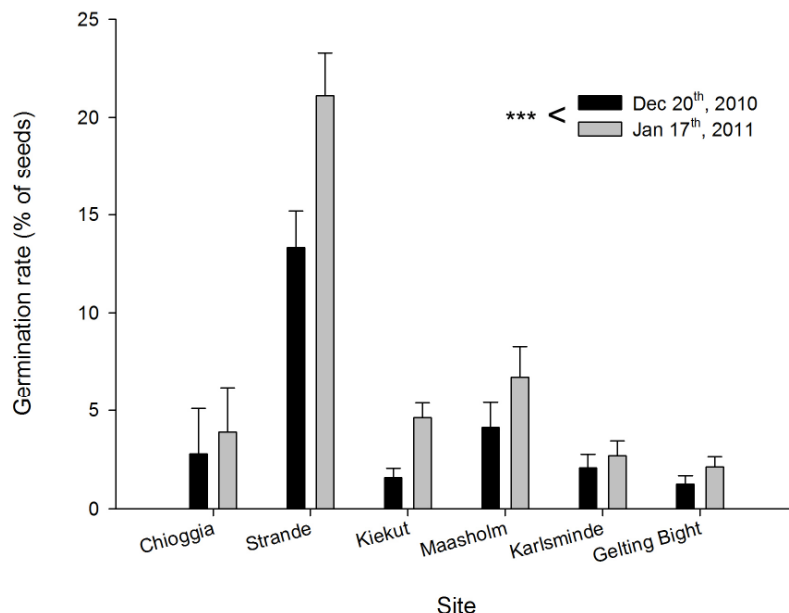


Figure 3-2. Differences in the germination rate (% of the total numbers of seeds sown) between different sites of origin. Planned comparison of sites revealed significant differences ($p < 0.0001$) between Strande and all other sites. Significant differences at $p < 0.0001$ are marked by ***)

3.2 Seed bank analysis

Analysing the seed bank of *Zostera marina* in fall 2010 and spring 2011, I found significant differences in viable seed numbers between sites. Both in November and March, the intertidal *Z. marina* in Tonnenleger Bight showed the highest number of seeds per square meter (seed density, 181.36/ m²; 39.78/ m²; Fig. 3-3). The subtidal eelgrass in Ellenbogen had the lowest amount of seeds per square meter in March 2011 (17.68/ m²; Fig. 3-3). For fall, no data was available for this site. Puan Klent eelgrass seed numbers were intermediate.

Comparing the number of viable seeds in the different seasons, the number of seeds decreased by 86% over winter, which is mostly due to decreased seed number in Tonnenleger Bight (Figure 3-3).

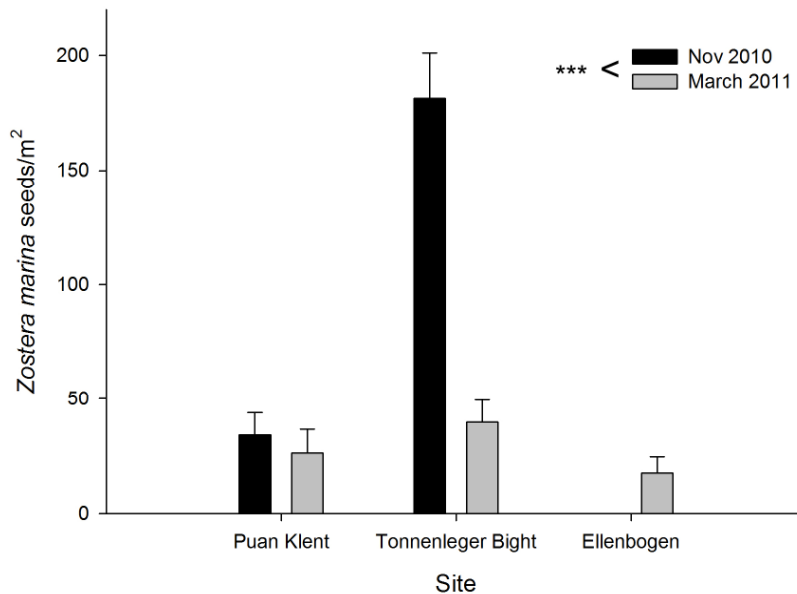


Figure 3-3. Differences in seed density of *Zostera marina* seed bank of three different Sites in the German Wadden Sea in November 2010 and March 2011. No data was available for Ellenbogen for Nov. 2011. (ANOVA_{Nov}: df=1, Sum of Squares=310284.61, F =27.42, p>0.0001, Residual SS=1000627.6; ANOVA_{Mar}: df=2, Sum of Squares 5020.88, F=1.36, p=0.26, Residual SS=113636.39, significant differences at p<0.0001 are marked by ***).

Whereas almost all seeds seemed to be viable in fall, a lot of germinated seeds and empty seed coats could be found in the sediment in March 2011 (Fig. 3-4). The amount of empty seed coats was higher than the number of viable seeds from two of three sites. Tonnenleger Bight samples showed the highest number of empty seed coats (66.90 seeds/m²) and Ellenbogen samples the lowest (11.05 seeds/ m², Fig. 3-4). Germinated seeds could only be found in the sediment of two of three sites: Tonnenleger Bight (5 seedlings) and Ellenbogen (3 seedlings; Table 3-4).

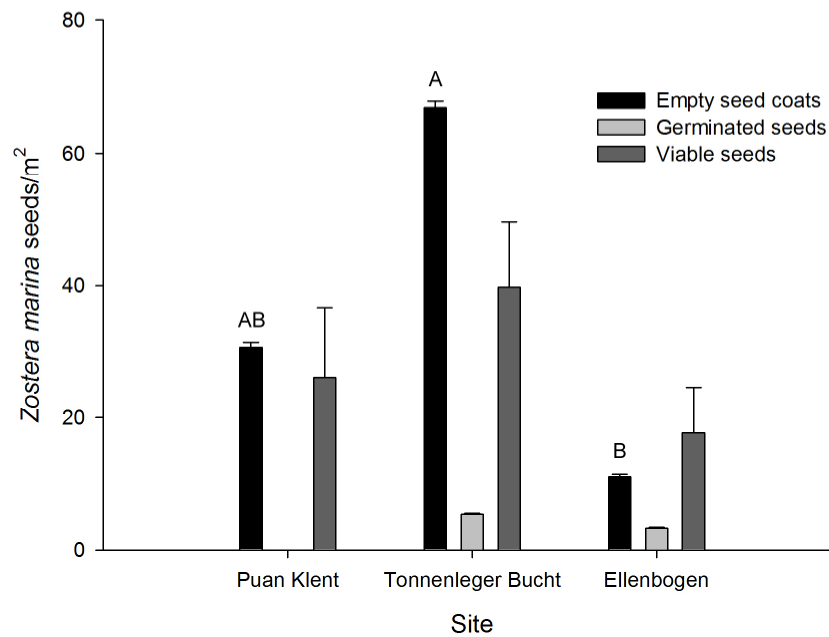


Figure 3-4. Seed bank analysis in March 2011, showing high amount of empty testa and already germinated seed. (ANOVA_{Empty seed coat}: df=2, Sum of Squares=5020.88, F =1.36, p=0.26 Residual SS=11636.39; ANOVA_{Germinated seeds}: df=2, Sum of Squares 32610.09, F=3.55, p=0.04, Residual SS=303600.38, ANOVA_{Germinated seeds}:df=2, Sum of Squares=12634, F=3.02, p=0.056, significant differences per factor are marked by different capital letters, p<0.0001)

4 Discussion

4.1. Interpretation

In agreement with seed germination rates from Chesapeake Bay (10% of 29.000 seeds sown) by Tanner and Parham (2010), the germination rates found in this study were also low (5.7%) compared to high amount of seeds (3029) sown. Moreover, other studies have also documented a germination rate between 1-20 % (Tutin 1938, Phillips 1971, Lamounette 1977). Since low seed germination rates have also been found in field test plantings (Phillips 1972, Orth et al. 1981), recruitment by seeds is low and thus successful sexual reproduction seems to be very restricted in perennial *Zostera marina* meadows.

This study has been done under the conditions Tanner and Parham (2010) described to be the optimal conditions for *Zostera marina* seed germination, factors such as stratification, salinity, temperature, and anoxia have been described that may be important. It is known, that seeds that show a dormancy to survive the winter need a cold period to break seed dormancy and enhance seed germination in natural conditions. Also Baskin and Baskin (1998) have shown in many terrestrial species that stratification is important for enhancing seed germination. Therefore, to simulate natural winter conditions in order to enhance seed germination in the laboratory, seeds have undergone stratification in this study. Observations have shown, that stratification has a positive effect by increasing seed germination in *Z. marina* seeds (Keddy and Patriquin 1978, Hootsmanns et al. 1987, Probert and Brenchley 1999), whereas Hootsmanns et al. (1987) have investigated stratification to have positive effect on seed germination only at > 20 psu. As many seeds from Chioggia have already germinated while stratification, this could indicate that *Z. marina* seeds from Chioggia don't seem to show a dormancy.

Additionally, low salinity has been shown to have a positive effect on seed germination (Arasaki 1950, Churchill 1983, Phillips et al. 1983, Hootsmans 1987, Harrison 1991, Conacher et al. 1994). Whereas seeds from Baltic sites salinity were raised in their natural salinity, seeds from Chioggia (Italy) were exposed to 15 psu whereas the origin site shows a salinity of > 35 psu. With a germination rate of 3.9%, Chioggia seeds germinated very similarly to seed from most of the Baltic sites, so that a negative effect of low salinity can be excluded.

The increase in germination rate after changing from artificial to natural seawater and a temperature drop of 2-3 °C for seed from all Baltic sites could be an in-

dication for an adaption of the seeds from the south-western Baltic to low temperatures for germination and underlines the importance of temperature as a trigger for germination. As Orth et al. (1981) have documented, low temperature can be a promoting factor for seed germination, as sharp drops in water temperature naturally occur at the study sites. Measured water temperatures in the Kiel Bight for spring indicate that the water temperature in the Baltic Sea is below 15°C when seeds start to germinate in spring (Tab.4-1).

Table 4-1. Mean water temperatures for spring, in the last decade in the Kiel Bight at the IFM– Geomar Institute (Source: http://www.ifm-geomar.de/index.php?id=3279&no_cache=1, 31.05. 2011).

Year	Mean Temp. March	Mean Temp. April	Mean Temp. May
1998	4.3	7.5	14.0
1999	4.3	7.7	11.7
2000	5.1	8.6	11.0
2001	3.6	6.0	9.9
2002	5.5	8.4	13.2
2003	4.4	8.4	18.3
2004	4.1	9.1	13.0
2005	3.3	7.4	10.8
2006	1.0	4.8	10.5
2007	6.0	9.9	12.8
2008	4.9	8.0	15.3
2009	4.1	7.9	15.9
2010	1.3	6.4	10.4
2011	2.5	6.6	No data available

The effect of low temperature is however confounded by the change from artificial to natural seawater in this study, so that a strict differentiation between these factors cannot be made.

In this study there were significant differences in germination rates between sites. Since all seeds were cultured under identical conditions, these differences could either be genetic or derived through maternal effects. Low genetic variation prevents eelgrass populations from adapting to changing environments (Fiedler and Jain 1992, Fenster and Dudash 1994). Williams (2001) has documented higher seed germination for populations with higher genetic variation. In another study Williams and Orth (1998) have shown that genetic diversity also plays an important role in the success of eelgrass restoration. One important factor that leads to the reduction of genetic diversity is that eelgrass donor plants for restoration projects are of-

ten only harvested from small areas, which restricts diversity (Ouborg et al. 1991, Ellstrand and Elam 1993, van Treuren et al. 1993, Mills and Smouse 1994, Lande 1994, Oostermeijer et al. 1994, Tanaka 1997, Young et al. 1998). Hence, low seed germination rates at some sites in this study could be related to low genetic diversity of the donor population.

Alternatively maternal effects that occur when phenotypic influences from the mother plant are translated to offspring could play a role and explain site differences (Futuyma 1986). Environmental conditions that the mother plant was exposed to and experienced can influence the phenotype of the offspring's fitness and development (Mousseau and Fox 1998). Maternal effects have also been reported to have an effect on the adaptive phenotypic plasticity over multiple generations (Mousseau and Fox, 1998). Several environmental studies have shown seeds of different populations to be different in terms of germination requirements (Bevington 1986, Dorne 1981, McWilliams et al. 1968, Petgel 1985, Van der Wegte 1987, reviewed by Roach and Wulff 1987) and therefore microclimate and site might be an important aspect in influencing seed germination (Dorne 1981) that results in maternal effects influencing the sensitivity of seeds to environmental conditions, for example for the timing of germination (Cal and Oppendorf 1972). Another aspect leading to maternal environmental effects could be nutrient content of seeds, which depends on the mother plant-growing site (Parrish and Bazzaz 1985). Increased nutrient supply can either have a positive or a negative effect on germination. It is known that nutrient enrichment can inhibit germination (Inoue and Yamamoto 1977). Therefore, eutrophication which causes nutrient enrichment indirectly can also be a reason for low germination rates of *Z. marina* seeds.

A further important factor that leads to low seed germination under natural conditions even at high seed production, is the loss of viable seeds from the seed bank before germination in spring (Harper et al. 1965, Chamber and MacMahon 1994, Fisherman and Orth 1996, Harwell and Orth 1999, Luckenbach and Orth 1999). This study found that the number of viable seeds at the study sites decreased by 86% between November 2010 and March 2011. The differences between the two intertidal sites sampled in this study can largely be explained by exposition. Whereas *Zostera marina* beds in Tonnenleger Bight are seldomly exposed to strong wave actions (Fig. 2-9), the Puan Klent meadows are more exposed (Fig. 2-9) and therefore have a higher loss of seeds. Additionally, it was observed this winter that the upper 2-3cm of the sediment containing the seed bank froze to ice sheets at low tide and drifted away at high tide, which could also be an explication for the low seed number for both intertidal sites.

Another reason that could explain the high variation of seed number in the seed bank between the subtidal and intertidal *Zostera marina* populations in this study is probably reproductive strategy. Whereas annual populations (in this study: Tonnenleger Bight and Puan Klent) depend completely on sexual propagation and therefore have higher seed productions (Kuo and den Hartog 2006), the subtidal perennial populations in Ellenbogen show generative and vegetative growth, in which propagation via rhizomes is the main strategy (Keddy and Patriquin 1987).

Several studies have shown different relevant factors for seed losses, both, within and outside the seed bank. Biotic and abiotic factors such as seed burial through hydrodynamics and bioturbation, transport of seeds away from the potential bed, predation, pathogens and drifting of the seeds to unsuitable germination sites have been described to cause the loss of seeds (Harwell and Orth 1999, Harper et al. 1965, Chambers and MacMahon 1994). Fisherman and Orth (1996) have documented the predation on *Zostera marina* seeds with the result of up to 65% loss and concluded predation to be an important cause for the low success of sexual propagation in *Z. marina* meadows. In March 53.6 % of the seeds in the seed bank were empty. It was observed that the seed coat was cut on the top, possibly as a result of predation by benthos organisms such as crustaceans (Fisherman and Orth 1996). Furthermore, deterioration, which was observed by pressing the seed, and consequently sediment squeezed out of the seed, could also be a reason for an empty seed coat.

4.2. Conclusion

Even though it is well known that sexual reproduction is carried out in *Zostera marina* populations, both in perennial and annual populations in the European waters, and that seed production has been shown to be high, recruitment is limited. The reason for low seed germination rates in the field is still not fully understood. Even though many studies have analyzed different factors, it seems unclear which of the factors are most important. Therefore, to examine more precisely the interplay between important factors such as seed production, dispersal of seeds, seed viability, seed germination, and seedling establishment, the influences of genetic diversity and seed predation need to be considered. This becomes particularly important in light of the ongoing decline of *Z. marina* beds, due to limited regeneration via seeds. However, as shown in this study, it is possible to raise *Z. marina* plants from seeds in the laboratory. The observations in this study can therefore provide a foundation for restoration projects in the Baltic sea and adjacent regions.

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Erklärung (Statement)

Hiermit Erkläre ich, dass ich die vorliegende Arbeit selbstständig und ohne fremde Hilfe angefertigt und keine anderen als die angegebenen Quellen und Hilfsmittel verwendet habe.

Die eingereichte schriftliche Fassung der Arbeit entspricht der auf dem elektronischem Speichermedium.

Weiterhin versichere ich, dass diese Arbeit noch nicht als Abschlussarbeit an anderer Stelle vorgelegen hat.

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