



## Short communication

# Vulnerability to eutrophication of a semi-annual life history: A lesson learnt from an extinct eelgrass (*Zostera marina*) population

Marieke M. van Katwijk<sup>a,\*</sup>, Arthur R. Bos<sup>a,b</sup>, Peer Kennis<sup>a</sup>, Rob de Vries<sup>a</sup>

<sup>a</sup> Department of Environmental Science, Radboud University Nijmegen, PO Box 9010, 6500 GL Nijmegen, The Netherlands

<sup>b</sup> Davao del Norte State College, New Visayas, 8105 Panabo City, Philippines

## ARTICLE INFO

## Article history:

Received 26 November 2008

Received in revised form 30 July 2009

Accepted 15 August 2009

Available online 9 September 2009

## Keywords:

Annual

Demography

Eelgrass

Eutrophication

Extinction

Germination

Life strategy

Population dynamics

Restoration

Seed density

*Zostera marina*

## ABSTRACT

A semi-annual eelgrass (*Zostera marina* L.) population became extinct in 2004. It had flourished for many decades at Terschelling in the western Wadden Sea, one of the most eutrophied locations where seagrass growth has been recorded. Semi-annual populations survive the winter season by seed (annual), and by incidental plant survival (semi-annual). We compared seed bank dynamics and fate of plants between this impacted site and a reference site in the winter of 1990–1991. Seed bank density at Terschelling was extremely low (5–35 seeds m<sup>-2</sup>) in comparison to the reference site (>60 seeds m<sup>-2</sup>) and also in comparison to seed bank densities of (semi-)annual eelgrass populations in other parts of the world. Plant survival during winter was nil. Nevertheless, the population more than doubled its area in 1991, implying maximum germination and seedling survival rates. However, from 1992 onwards the decline set in and continued – while the nutrient levels decreased. To establish the cause of the low seed bank density, we conducted a transplantation experiment in 2004 to study the relationship between seed production and macro-algal cover. The transplantation experiment showed a negative relationship between the survival of seed producing shoots and suffocation by macro-algae, which is associated with light limitation and unfavourable biogeochemical conditions. The plants died before they had started to produce seeds. Thus, it is likely that macro-algal cover was responsible for the low seed bank density found in Terschelling in 1990–1991. Both the recorded low seed bank density and absence of incidental plant survival during winter were related to eutrophication. These parameters must have been a severe bottleneck in the life history of the extinct population at the impacted site, particularly as *Z. marina* seed banks are transient. Therefore we deduce that this population had survived at the edge of collapse, and became extinct after a small, haphazard environmental change. We argue that its resilience during these years must have been due to (i) maximum germination and seedling survival rates and (ii) spatial spreading of risks: parts of the population may have survived at locally macro-algae-free spots from where the area could be recolonised. As a consequence, the timing of the collapse was unpredictable and did not synchronise with the eutrophication process. The lesson learnt for conservation is to recognise that eutrophication may be a cause for seagrass population collapse and its eventual extinction, even years after nutrient levels stabilised, or even decreased.

© 2009 Elsevier Ltd. All rights reserved.

## 1. Introduction

Seagrass beds inhabit coastal zones throughout the world and many populations have declined due to eutrophication (e.g. Duarte, 1995; Hauxwell et al., 2001; Valiela and Cole, 2002; Orth et al., 2006a; Burkholder et al., 2007; Fox et al., 2008). In the western Wadden Sea, nutrient loads had increased approximately five to six times by the mid-1980s compared to the relatively pristine situation prior to the 1930s (van Beusekom, 2005). Valiela and Cole

\* Corresponding author. Tel.: +31 243652478; fax: +31 243653030.

E-mail addresses: [m.vankatwijk@science.ru.nl](mailto:m.vankatwijk@science.ru.nl) (M.M. van Katwijk), [arthurbos@yahoo.com](mailto:arthurbos@yahoo.com) (A.R. Bos), [p.kennis@groenewoud.nl](mailto:p.kennis@groenewoud.nl) (P. Kennis), [Robaj.devries@inter.nl.net](mailto:Robaj.devries@inter.nl.net) (R. de Vries).

(2002) showed that the western Wadden Sea had one of the highest nutrient loads ever recorded for natural seagrass populations. In the late 1980s, nutrient loads stabilised and slightly decreased in the 1990s and 2000s (van Beusekom, 2005; van Beusekom et al., 2008). Notwithstanding the high nutrient loads in the 1970s and 1980s, a semi-annual eelgrass (*Zostera marina* L.) population flourished for decades in Terschelling in the western Wadden Sea (Polderman and den Hartog, 1975; van Katwijk et al., 1998, 2000, Fig. 1), but became extinct in 2004 (Fig. 2). During the 1990s, this seagrass population was frequently observed to have seed-bearing shoots that died prematurely due to algal overgrowth and shading in late summer, mainly by epiphytic diatom blooms and macro-algae (van Katwijk et al., 1997, 1998). At a transplantation site at a similar type of location in the Wadden

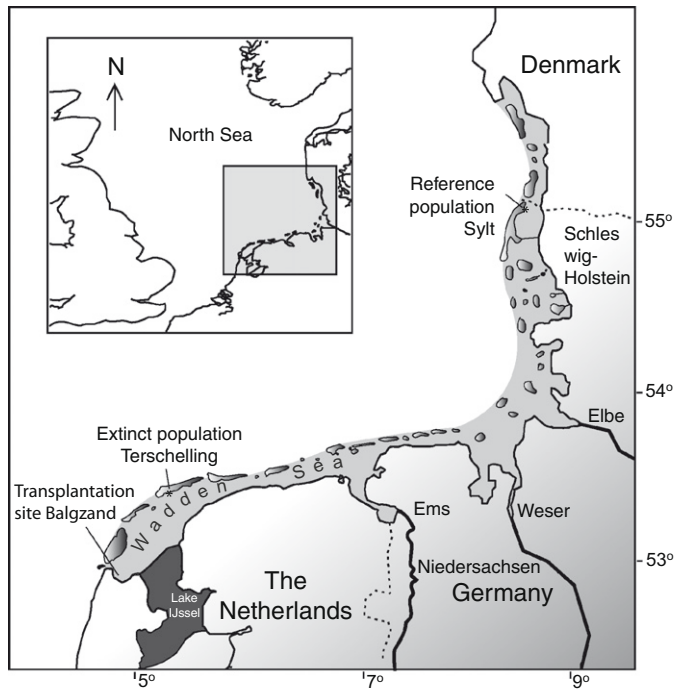


Fig. 1. Map of the Wadden Sea, showing the research locations (\*).

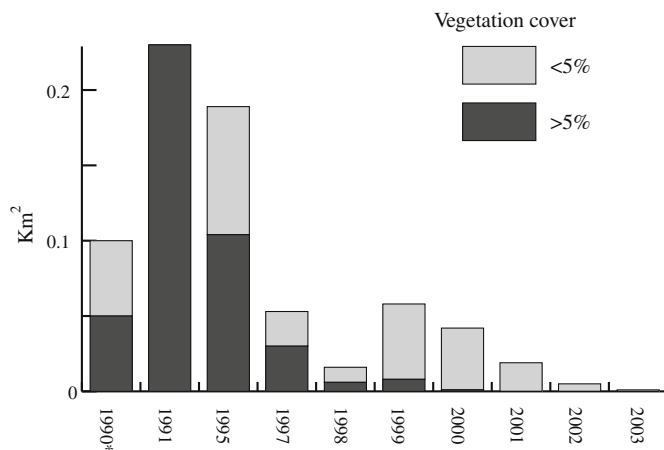


Fig. 2. Population dynamics (area and plant cover) of the semi-annual *Zostera marina* population at Terschelling, western Wadden Sea 1990\*: Surveyed by the authors, 1991–2003. Monitoring data of the Ministry of transport, public works and water management, [www.zeegras.nl](http://www.zeegras.nl).

Sea, a negative relationship between algal overgrowth and recruitment in the subsequent year had been observed (van Katwijk et al., 2009).

The seagrass *Z. marina* usually employs a perennial life history (Olesen, 1999). However, numerous examples of (semi-) annual populations are known from many parts of North-west Europe, the northern part of the Atlantic coasts of the United States and Canada and from Pacific coasts of the United States and Japan (e.g. Keddy and Patriquin, 1978; van Lent and Verschuure, 1994; van Katwijk et al., 1998; Morita et al., 2007). In these *Z. marina* populations, seed production is generally higher. Reviewing literature on a large number of eelgrass beds worldwide, Olesen (1999) found an average of 24,000 seeds  $m^{-2}$  produced in (semi-)annual populations, whereas significantly less (6200 seeds  $m^{-2}$ ) in perennial populations.

Seagrass seed losses may occur due to burial, seed predation, rotting and autonomous death (Churchill, 1983; Harrison, 1993; van Lent and Verschuure, 1994; Greve et al., 2005; Orth et al., 2006b). Seed banks of *Z. marina* are transient and seeds seldom survive longer than one year (Orth et al., 2000). Germination percentages and seedling survival rates are highly variable at a spatio-temporal scale (e.g. Keddy and Patriquin, 1978; Churchill 1983; van Katwijk and Wijgertang, 2004; Orth et al., 2006b; van Katwijk et al., 2009). For a population that relies on recruitment from seed, its traits are vital for survival. In strictly annual populations, all seedlings evolve into seed-bearing shoots (e.g. Keddy and Patriquin, 1978; Keddy, 1987). However, in semi-annual populations, a small percentage of vegetative shoots (plants) survive winter and produce new shoots in spring (van Lent and Verschuure, 1994; van Katwijk et al., 1998). Incidental rhizome survival (including the meristems) during winters also provides an additional chance for survival of semi-annual populations. Incidental rhizome survival however is restricted to coarse sediments (van Katwijk et al., 1998), whereas in hypoxic sediments, eelgrass rhizomes do not sprout (Greve et al., 2005).

We hypothesised that (i) the transient seed bank density of the Terschelling population was lowered by eutrophication as a consequence of reduced seed production due to algal cover in summer, and (ii) vegetative plant or rhizome survival was reduced in comparison to less eutrophied locations with low algal cover in late summer. If verified, this would imply that eutrophication reduces the recruitment potential of *Zostera marina* populations, thus indirectly contributing to a reduced resilience. As a result, smaller external (environmental) or internal (population dynamics) fluctuations may lead a population toward collapse. The timing of the collapse may remain unpredictable and may not be attributed to a direct and instantaneous cause.

## 2. Materials and methods

### 2.1. Study area

The Wadden Sea is one of the world's largest international marine wetland reserves (approx. 6000  $km^2$ ), bordering the coasts of The Netherlands, Germany and Denmark (Fig. 1). In the Wadden Sea, two species of seagrass occur, *Zostera marina* and *Z. noltii*, both in the mid-intertidal zone. Tides are semi-diurnal, with a tidal amplitude of ca. 1.80 m. Eutrophication levels at the western parts of the Wadden Sea are 1.5–3 times higher in comparison to the north-eastern parts (van Beusekom, 2005; van Beusekom et al., 2005). Additionally, nutrient loads vary at a local scale. At sheltered locations macro-algae accumulate during summer, by in-drifting algae as well as by local settlement and proliferation of these algae. Epiphytes develop abundantly on the seagrass leaves. Also, fine particles such as clay or organic matter, often rich in nutrients, settle out. Remineralisation, following algal mortality at the end of summer, provides an additional nutrient source in comparison to less-sheltered sites, where algae do not to accumulate and settlement of fine particles is reduced (e.g. van Beusekom and de Jonge, 2002). This process has been referred to as 'internal nutrient loading' (Duarte, 1995; Burkholder et al., 2007).

### 2.2. Overwintering dynamics and seed bank density (1990–1991)

Differences in nutrient loads within the Wadden Sea, as well as observations of premature end-of-season die-off in a seagrass bed with high loads (Terschelling, western Wadden Sea, Fig. 1; van Katwijk et al., 1998), lead us to study the overwintering features of this particular seagrass population and comparing it to a less nutrient-loaded location in the northern Wadden Sea (Sylt). The

impacted site (Terschelling) was located at a sheltered location and monthly field observations in the summers of 1989, 1990 and 1991 showed consistently high algal abundance at this site: thick epiphyte mats covering 80–100% of the leaf surface at the end of summer, and macro-algal cover locally varying between 5% and 100%, decaying at the sediment surface already at the beginning of August. The reference location (Sylt, Fig. 1) was a less-sheltered location and field observations between 1989 and 1991 showed low algal cover: maximally 20% epiphyte cover but usually none, macro-algal cover 0–20%.

Eight 16 m<sup>2</sup> permanent plots were established at both locations in September 1990. Overwintering dynamics of the seagrass population were quantified by the seed density in the sediment and the biomass of surviving plants or rhizomes in the sediment. We differentiated between sparsely and densely vegetated parts of the bed to additionally study the local dispersal of seeds. We expected that densely vegetated parts would have a higher seed bank density, but if dispersal was scattered, e.g. when a large part of the seed-containing shoots disperse over the area (e.g. Harwell and Orth, 2002a), the seeds may become evenly distributed. At the eutrophied site (Terschelling), five out of eight plots were selected in a sparsely vegetated area (>0% to ≤5% cover, cover estimated during emergence) and three of them in a densely vegetated area (20–40% cover). At the reference site (Sylt), four sparsely and four densely vegetated plots were selected (similar cover as in Terschelling). Eight sediment cores of 0.04 m<sup>2</sup> with a depth of 0.1 m were collected in each plot and immediately sieved (1 mm) at each monitoring date (Terschelling: September, October, November, December, January, March; Sylt in September and February) to measure number of seeds and plant biomass. Viable seeds in the sediments (full, rigid and undamaged, Harwell and Orth, 2002b) were counted. In the laboratory, plant material was separated in above- and belowground parts (Short and Coles, 2001) and dried for 72 h at 80 °C. Sediment samples were collected in 1998 and 2003 at three sparsely vegetated sites within the Terschelling seagrass beds in three 10-cm sediment cores with a diameter of 2.8 cm that were pooled and mixed.

### 2.3. Transplantation experiment

We conducted an experiment in 2004 at Balgzand in the western Wadden Sea (Fig. 1) to test whether abundant macro-algal cover could cause a reduced seed production. We transplanted eelgrass to (i) a sheltered area that is typically covered by macro-algae in the summer (MA), and which has ambient conditions similar to those at Terschelling (Table 1), and (ii) a control area usually devoid of macro-algae due to stronger water dynamics, both located at Balgzand. The transplantation experiment consisted of six Planting Units (PU's) of 37 bare-root plants per site. The plants originated from the Ems Estuary (Fig. 1) and were transplanted one day after collection, on 14 and 15 June 2004. They were planted 30 cm apart in a hexagon. In mid-August, we recorded % plant sur-

vival, % plant cover, % reproductive shoots and length of the reproductive shoots. Seeds were counted in five randomly chosen plants per PU, every second week between August and October 2004. We only counted ripe seeds (full and rigid). Macro-algal cover within each PU was estimated and dominant taxa were recorded. Seed bank density could not be investigated because the experiment was part of a restoration project in which sediment disturbance was not allowed. Sediment samples were collected adjacent to each of the transplantation units in three 10-cm sediment cores with a diameter of 2.8 cm that were pooled and mixed.

### 2.4. Organic matter and sediment grain size analysis

Sediment samples were freeze-dried and sieved (1 mm) to remove small pieces of shell and were analysed with a Malvern Laser Particle Sizer (Bos et al., 2007). In 1998, organic matter content was assessed by loss on ignition: after drying 48 h 60°, the samples were weighed and then ignited (4 h, 500°). In 2003 and 2004, % C was analysed by a NA-1500 NCS-analyser after decalcification by addition of 30% HCl.

### 2.5. Statistical analysis

As an estimation of the normality of biomass and seed density, we tested the normality of the residuals of a three-way ANOVA-model of location, vegetation cover and monitoring date. They were normally distributed after log-transformation. The effect of vegetation cover (densely or sparsely) on seed density at Terschelling was tested for every monitoring date in a one-way ANOVA. Effects of location (Terschelling or Sylt) and vegetation cover (densely or sparsely) on plant biomass and seed density were tested for two dates (September or February/March) in a two-way ANOVA (SAS, 1989). In the transplantation experiment, variables were not normally distributed and Mann-Whitney-U tests were performed to test the effect of site on plant survival, % plant cover, % reproductive shoots and the length of the reproductive shoots.

## 3. Results

### 3.1. Overwintering dynamics and seed bank density (1990–1991)

Aboveground seagrass biomass decreased sharply in the population in Terschelling after the first monitoring event at 4 September 1990, whereas belowground biomass decreased slowly in the course of the winter. In the reference population in Sylt, belowground biomass diminished to almost zero in February/March 1991, as did the aboveground biomass (Fig. 3). However, the aboveground biomass was still significantly higher in Sylt than in Terschelling (Table 2). Seed densities in the sediment were significantly higher in Sylt than in Terschelling, in both September and February/March (Table 2, Fig. 3). In Terschelling, the seed densities in the sediment were extremely low (Fig. 4). Seed density was still below 40 seeds per m<sup>2</sup> at the densely vegetated parts during the highest count in November 1990. Seed densities in the sediment were significantly higher in densely vegetated parts as compared to the sparsely vegetated sites (ANOVA  $p < 0.01$ ).

### 3.2. Transplantation experiment

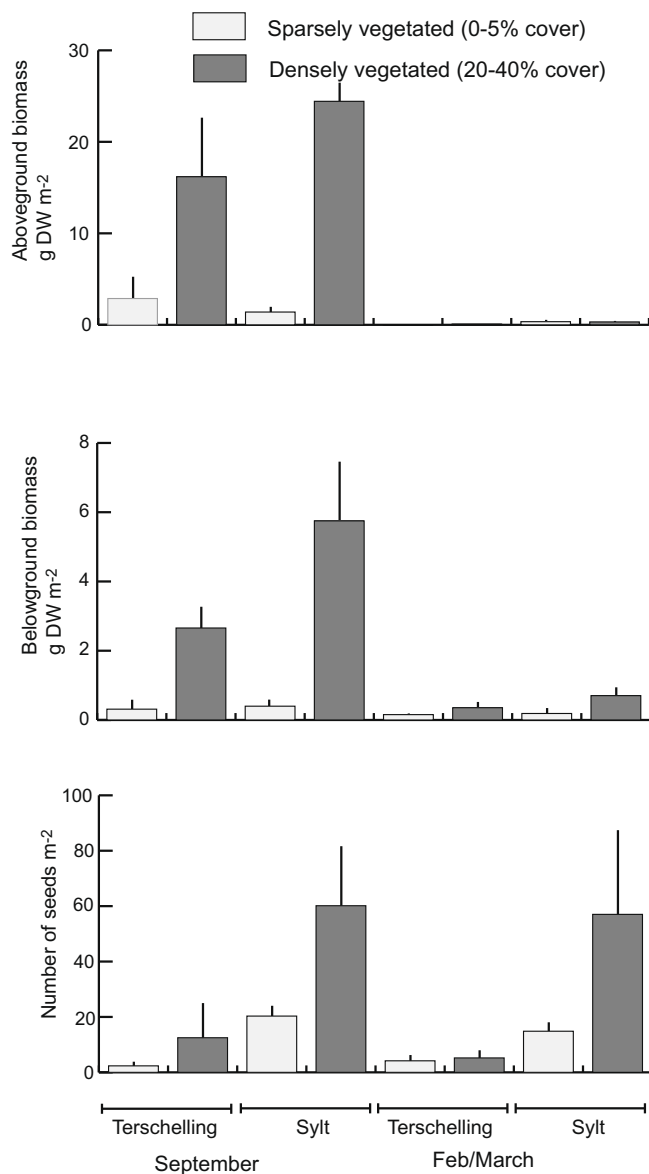
Nine weeks after transplanting, plant survival, % plant cover, % reproductive shoots and length of the reproductive shoots, were not significantly different between the PUs at site MA and the control site ( $p > 0.05$ , Table 3). Those PUs that were usually covered with macro-algae (site MA) had an actual macro-algal cover of

**Table 1**

Sediment fractions <16 µm (%), 16–63 µm (%) and organic matter (% C) in unvegetated areas within the seagrass bed (Terschelling) or adjacent to the PUs at Balgzand. Mean ± SD are presented.

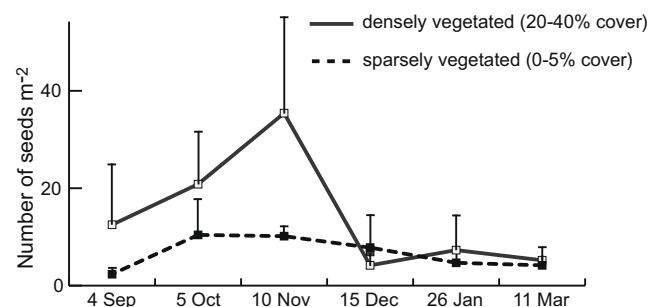
Location		Fraction <16 µm	Fraction 16–63 µm	Organic matter % C
Terschelling	1998	23.8 ± 1.6	32.6 ± 1.7	4.9 ± 0.8 <sup>a</sup>
	2003	15.0 ± 2.5	28.6 ± 5.5	1.0 ± 0.3
Balgzand (transplantation experiment)				
Control site	2003	7.3 ± 0.6	12.7 ± 1.2	0.5 ± 0.1
Site MA	2003	15.7 ± 1.4	33.9 ± 2.6	1.2 ± 0.3

<sup>a</sup> Organic matter was measured as loss on ignition in 1998.



**Fig. 3.** *Zostera marina* biomass and seed density in the sediments (mean + SE) comparing the Sylt and Terschelling populations in September 1990 and February/March 1991. Plots with 20–40% vegetation cover are compared to sparsely vegetated plots (0–5% plot cover).

40% (standard deviation 50%) in August, as compared to 5% (standard deviation 2%) at the control site (Table 3). Macro-algae con-



**Fig. 4.** *Zostera marina* seed density in the sediments (mean + SE) of the impacted site (Terschelling) during winter 1990–1991. Plots with 20–40% vegetation cover are compared to sparsely covered plots (0–5% plot cover).

sisted of: *Ulva*, *Gracilaria*, *Chaetomorpha* and *Enteromorpha* spp. (in order of decreasing dominance). In August, seed numbers were low because they were not ripe yet (i.e. full and rigid). From the beginning of September onwards, the seagrass plants started to decay at site MA. As a consequence, seed abundance was significantly reduced in comparison to the PUs at the control site which continued seed production during September and the beginning of October (Fig. 5). Note that the plant development had been similar at both sites during the preceding growing season (Table 3, Bos and van Katwijk, 2007).

#### 4. Discussion and conclusions

For the recruitment from seed, and thus for the survival of a semi-annual population, it is of importance how many seeds are deposited in the (transient) seed bank and what number is available for germination in the next year. The present study found extremely low seed bank densities in a semi-annual, intertidal *Zostera marina* population (Terschelling). The seed density in the top 10 cm of the sediment dropped from a mean of 35 seeds  $m^{-2}$  in November to an average 5–10 seeds  $m^{-2}$  in December–March. At the reference site (Sylt), seed density was 60 seeds  $m^{-2}$  at the end of the winter season. Mean seed bank densities measured in other annual *Z. marina* populations throughout the world ranged from 240 to 1157 (median 510) seeds  $m^{-2}$  and in perennial populations from 5 to 11,000 (median 250) seeds  $m^{-2}$  (Table 4, note that core depths vary and comparisons can only be indicative). Our study also showed that seed densities were about threefold higher in densely vegetated than sparsely vegetated sites, reflecting the low dispersal capacity of seeds once they dropped from the seed-containing shoots (Orth et al., 1994). On the other hand, longer distance dispersal was realised, as evidenced by the areal increase of the population (Fig. 2), probably due to the potential of *Z. marina* to

**Table 2**

The effect of vegetation cover (densely or sparsely) and of location (impacted site Terschelling versus reference site Sylt) on *Zostera marina* characteristics at the end of the growing season and at the end of winter (two-way analysis of variance).

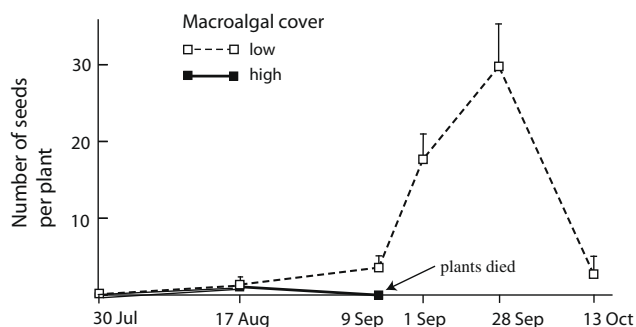
		Factor	DF	Mean square	F-value	P
September	Aboveground biomass	Location	1	1.97099	0.95	0.3489
		Vegetation	1	38.8774	18.75	0.0010
	Belowground biomass	Location	1	1.74892	1.18	0.2988
		Vegetation	1	34.3469	23.16	0.0004
	Seed density	Location	1	11.1407	18.37	0.0011
		Vegetation	1	1.60626	2.65	0.1296
February/March	Aboveground biomass	Location	1	11.6279	25.32	0.0003
		Vegetation	1	0.34673	0.76	0.4019
	Belowground biomass	Location	1	0.00019	0	0.9882
		Vegetation	1	5.33934	6.49	0.0256
	Seed density	Location	1	6.54635	13.6	0.0036
		Vegetation	1	1.13657	2.36	0.1527



**Table 3**

*Zostera marina* transplantation results, plant development and macro-algal cover at Balgzand at 16th August, nine weeks after transplantation. MA = site usually covered by macro-algae at the end of summer. Mean  $\pm$  SD are presented.

	Control site	Site MA
Survival plants (%)	60 $\pm$ 12	45 $\pm$ 41
Plant cover (%)	33 $\pm$ 16	42 $\pm$ 36
Reproductive shoots (%)	26 $\pm$ 6	26 $\pm$ 5
Length of reproductive shoots (cm)	54 $\pm$ 5	50 $\pm$ 5
Macro-algal cover (%)	5 $\pm$ 2	40 $\pm$ 50



**Fig. 5.** Relationship between seed production and macro-algal cover in late summer in Balgzand, 2004. Depicted is the seed production per seagrass plant (mean  $\pm$  SE) at a sheltered site with high macro-algal cover (mean 40%) where the plants disappeared between the first and second monitoring date, in comparison to a nearby less-sheltered site with low macro-algal cover (mean 5%).

additionally disperse through drifting seed-containing shoots (Harwell and Orth, 2002a).

The areal size of the population in Terschelling substantially increased from approximately 10 ha at the onset of our research in August/September 1990 to 23 ha in the summer of 1991 (Fig. 2). We attribute this to recruitment from seed, as inspections of the belowground parts of hundreds of plants in spring of 1989, 1990 and 1991, in both Terschelling and Sylt, consistently showed that new areas were colonised by seedlings (cotyledon still present), and not by vegetative fragments (unpublished results). Apparently the extremely low seed densities in the sediment can still sustain a population, and may even allow for a significant expansion of the seagrass bed area. More specifically, this means that the other vital

rates such as germination and seedling survival must have been at a relatively high level. A literature review in Orth et al. (2006b) about worldwide *Zostera* populations reports a maximum germination of 90%, and maximum seedling survival of 40%. Seed experiments in the Wadden Sea showed a maximum germination of 45% and maximum seedling survival of 55% (van Katwijk and Wijgergangs, 2004). Obviously, at our study site, the germination and survival percentages must have been close to these maxima.

The transplantation experiment at Balgzand in 2004 showed that the low seed density may be explained by the early end-of-season die-off related to excessive macro-algal cover in late summer. At site MA (high macro-algal cover), the eelgrass plants died when the seed production has had only started, and >95% of the seeds still had to be produced (Fig. 5). Macro-algal cover, in turn, is related to eutrophication in combination with a sheltered location that allows macro-algae to drift in and locally proliferate, eventually suffocating the *Zostera* plants. Suffocation by macro-algae is associated with light limitation and unfavourable biogeochemical conditions, like anoxia, sulphide and ammonium toxicity (den Hartog, 1994; van Katwijk et al., 1997; Hauxwell et al., 2001; Bos and van Katwijk, 2007; Reise et al., 2008). Site MA is more sheltered than the control site, and additional to in-drifting macro-algae, also fine particles such as clay or organic matter, often rich in nutrients, settle out, as such providing an additional nutrient source in comparison to less-sheltered sites like the control site (see also the section about 'internal nutrient loading' in Materials and Methods). This was reflected by the higher organic matter and fine sediment fractions at these sites (Table 1).

The semi-annual *Zostera marina* populations in the Wadden Sea largely lose their above- and belowground parts during winter, though field observations indicate that, incidentally, a small part of the new shoots in spring re-establish from apical meristems surviving on the rhizomes (van Katwijk et al., 1998), hence the term 'semi-annual' (van Lent and Verschuure, 1994). During our study, no plants survived in the plots of the Terschelling site, and the population behaved true annual. At the less eutrophied reference location (Sylt), significantly higher aboveground biomass of surviving plants was recorded in the plots, though still very low. The loss of plants during winter and the inability of sprouting from rhizome meristems in Terschelling corresponded to findings of Greve et al. (2005). In contrast, van Katwijk et al. (1998) found that plants survived winter and sprouted again in spring in a mesocosm study, as

**Table 4**

*Zostera marina* seed bank densities in the sediment worldwide, shown as mean  $\pm$  SD.

Depth of core (cm)	Seeds in sediment number (m <sup>-2</sup> )	Month	Annual/Perennial	Location	Source <sup>b</sup>
15	530 $\pm$ 430	June	P	Chesapeake Bay	1
5.5	1000	May?	P	Odense Fjord, Denmark	2
14	11,000	May?	P	Odense Fjord, Denmark	2
10	80 $\pm$ 34	Autumn/winter <sup>a</sup>	P	Lake Grevelingen 0.60 m, The Netherlands	3
10	252 $\pm$ 96	Autumn/winter <sup>a</sup>	P	Lake Grevelingen 1.25 m, The Netherlands	3
18	21 $\pm$ 19	May	P	Hamajima, Ago Bay, Japan	4
18	5 $\pm$ 9	October	P	Hamajima, Ago Bay, Japan	4
10	764 $\pm$ 141	Autumn/winter <sup>a</sup>	A	Lake Veere, The Netherlands	3
10	252 $\pm$ 175	Autumn/winter <sup>a</sup>	A	Zandkreek, The Netherlands	3
10	1120	November	A	Zandkreek, The Netherlands	5
10	240	February	A	Zandkreek, The Netherlands	5
18	219 $\pm$ 103	May	A	Tategami, Ago Bay, Japan	4
18	1157 $\pm$ 360	October	A	Tategami, Ago Bay, Japan	4
10	40 $\pm$ 34	November <sup>a</sup>	A	Terschelling, Wadden Sea	6
10	13 $\pm$ 13	September	A	Terschelling, Wadden Sea	6
10	5 $\pm$ 3	Feb/March	A	Terschelling, Wadden Sea	6
10	60 $\pm$ 21	September	A	Sylt, Wadden Sea	6
10	57 $\pm$ 30	Feb/March	A	Sylt, Wadden Sea	6

<sup>a</sup> Sampled during period of maximum seed production.

<sup>b</sup> 1 = Harwell and Orth, 2002a; 2 = Greve et al., 2005; 3 = van Lent and Verschuure, 1994; 4 = Morita et al., 2007; 5 = Harrison, 1993; 6 = this study.

did a very small part of the reference population in Sylt. The Terschelling results and the study of Greve et al. (2005) have in common that the rhizomes were collected from plants that had died back before the normal end-of-season, related to suffocation by macro-algae (see previous paragraph) or low oxygen levels respectively, whereas the mesocosm study of van Katwijk et al. (1998) was performed in coarse sediments where macro-algae had regularly been removed. The reference site in Sylt also had coarser sediments, macro-algae were present at low densities and the plants did generally not experience premature death. Thus, it is likely that the lack of plant survival is related to suffocation by macro-algae in combination with the sheltered location (this study, see previous paragraph) or by anoxia (Greve et al., 2005), both of which are related to eutrophication. Ice-scouring is not a probable cause of the difference in plant survival during winter between Terschelling and Sylt, as ice-scouring is usually more severe in Sylt than in Terschelling due to its more northern location. In conclusion, lack of incident plant survival in winter seems to be correlated to eutrophication level, as is the reduced seed bank density (previous paragraph).

The low *Zostera marina* seed density combined with the almost complete loss of plants during winter as found in our study, makes a population vulnerable to perturbations. Indeed, the population in Terschelling collapsed after several years, but the decline could not directly be related to any environmental change. Essink et al. (2003) suggested that seagrass loss might be related to increased sedimentation after maintenance dredging was reduced in a nearby channel. However, burial of plants was not observed during the growing seasons. Moreover, the sediments became more sandy between 1998 and 2003, (Table 1), which can only be marked as favourable, as the sediments were on the edge of being too muddy to support eelgrass growth (e.g. Butcher, 1934; Reise and Kohlus, 2008; Wicks et al., 2009). The coarsening of the sediment is expected to also contribute to sediment stability and reduced resuspension, i.e. increased water clarity. Thus, in this regard, the environment has rather improved than deteriorated. Eutrophication level has stabilised since the 1980s and even reduced during the 1990s and 2000s (van Beusekom, 2005; van Beusekom et al., 2008, 2009, [www.waterbase.nl](http://www.waterbase.nl)). Increasing water temperatures during the last decades (Martens and van Beusekom, 2008; van Aken, 2008) could have aggravated the consequences of eutrophication by a higher organic matter turnover. However, water temperature may not have played such a dominant role in this particular case. The average water temperature in the western Wadden was 17.2 °C in August and September 1990, the year that was followed by high seagrass recruitment in spring. Water temperatures were similar or lower in 1992, 1993, 1994, 1996 and 1998, whereas 1991, 1995 and 1997 had higher late summer water temperatures (data from Hendrik van Aken, see also van Aken, 2008). The tidal depth may have increased in this area, but is still below average tidal depths of seagrass beds in the Wadden Sea (van Katwijk et al., 2000).

Only minor environmental changes occurred over the course of the study, potentially affecting the plants negatively in some respects and positively in others. Given the spatial heterogeneity of the seagrass habitat in Terschelling, with local bathymetric variability and local variation in exposure and mud content of the sediments, one would expect only spatial shifts in seagrass distribution, if any. Therefore we hindcast that this population survived at the edge of collapse, and just any small environmental or demographical fluctuation or change could have pushed this population into extinction. The low seed bank density and the absence of plant survival during winter (both related to eutrophication) must have been a severe bottleneck in the life history of the population. The seed bank is transient (Orth et al., 2000). This life history trait makes plant populations vulnerable to fluctuations in

general as there is no spreading of risks in time (e.g. Ozinga et al., 2007). However, these vital rates are notably variable in most plant populations (e.g. Schemske et al., 1994; Jongejans et al., 2006; Ozinga et al., 2007), which is also the case in *Z. marina* (van Lent and Verschuure, 1994; Orth et al., 2006b). As a consequence of this variability, the timing of the collapse is unpredictable and might not coincide with the onset of the eutrophication. The deterioration process may have been accelerated by decreasing plant densities: early seed set of *Z. marina* is known to be reduced by pollen limitation when plant density drops below approximately 5 flowering shoots m<sup>-2</sup> (Reusch, 2003).

Why didn't eutrophication result in instantaneous collapse of this population? First, a delay in population decline is not unusual; species can exist 'below' an extinction threshold for many years (Eriksson and Kiviniemi, 1999). Second, germination and seedling survival rates must have been high, providing elasticity, as our results imply. Third, having a transient seed bank, the population could not very well spread risks in time, but the spatial heterogeneity of the habitat may have allowed for spreading of risk in space. Heterogeneity of the habitat may result from winds and bathymetry that locally influence current patterns and may create local reductions of macro-algae and epiphytes, and/or locally varying sediment composition. Some of these local circumstances may favour a large local proliferation of the plants, which may then recolonise the adjacent area. Several examples of rapid re-colonisation of *Zostera marina* exist (e.g. van Katwijk et al., 2006), and one of them actually shows a negative relationship between yearly proliferation and macro-algal cover (van Katwijk et al., 2009). In our study area, the heterogeneity of the habitat may have helped the population to survive during a number of years. Proximity of other populations as a potential source for re-colonisation (i.e. being part of a metapopulation) would also have helped to spread risks in space, but these were not available in our case.

It is well conceivable that the mechanisms described in this paper are applicable to (semi-) annual populations in various aquatic environments, because accumulating, decaying algae in late summer are a general phenomenon in eutrophic shallow waters (e.g. Sand-Jensen et al., 2008 and references therein, Viaroli et al., 2008). Freshwater macrophytes are reported to be vulnerable to their effects just like seagrasses (e.g. Lamers et al., 2002; Holmer and others, 2005; Smolders et al., 2006; Burkholder et al., 2007). Unless aquatic plants have the ability to adapt to such detrimental conditions in late summer by an early seed set (e.g. *Potamogeton pectinatus* van Wijk, 1988), seed production may be severely reduced as a result. This, and the absence of incidental survival of adult plants in winter, increases the extinction risk.

The lesson learnt for conservation is to recognise that eutrophication might be an underlying cause for seagrass population collapse, years after the eutrophication levels stabilised and decreased. In that respect, seagrass is therefore not a useful early warning bioindicator, but rather a 'too late' bioindicator. Eutrophication reduced the recruitment potential of the studied population, thus indirectly contributed to a reduced resilience. As a result, smaller external (environmental) or internal (population dynamics) fluctuations may lead a population toward collapse. The timing of the collapse may remain unpredictable and may not be attributed to a direct and instantaneous cause.

## Acknowledgements

We thank Martin Versteeg, Pim Vugteveen and Karin Hermus for assistance in the field, and Germa Verheggen-Kleinheerenbrink for assistance in the laboratory. We used seagrass monitoring data of the Ministry of Transport, Public Works and Water Management of The Netherlands, available at [www.zeegras.nl](http://www.zeegras.nl). We furthermore used temperature data made available at the website <http://www.nioz.nl/>

[public/fys/realtime\\_observations/jetty\\_t-s/tsmarsdiep.xls](#) by Hendrik van Aken of the Royal Netherlands Institute of Sea Research, website visited 16 March 2009. Part of the project was financed by this Ministry of Transport Public Works and Water Management. We thank Dr. Fons Smolders, Prof Dr. Hans de Kroon and Dr. Eelke Jongejans for stimulating discussions and critically reading previous versions of the manuscript. We thank three anonymous reviewers for their valuable comments on the manuscript.

## References

- Bos, A.R., Bouma, T.J., de Kort, G.L.J., van Katwijk, M.M., 2007. Ecosystem engineering by annual intertidal seagrass beds: sediment accretion and modification. *Estuarine Coastal and Shelf Science* 74, 344–348.
- Bos, A.R., van Katwijk, M.M., 2007. Planting density, hydrodynamic exposure and mussel beds affect survival of transplanted intertidal eelgrass. *Marine Ecology Progress Series* 336, 121–129.
- Burkholder, J.M., Tomasko, D.A., Touchette, B.W., 2007. Seagrasses and eutrophication. *Journal of Experimental Marine Biology and Ecology* 350, 46–72.
- Butcher, R.W., 1934. *Zostera* Report on the present condition of eel grass on the coasts of England, based on a survey during August to October, 1933. *Journal du Conseil/Conseil Permanent International pour l'Exploration de la Mer* 9, 49–65.
- Churchill, A.C., 1983. Field studies on seed germination and seedling development in *Zostera marina* L. *Aquatic Botany* 16, 21–29.
- den Hartog, C., 1994. Suffocation of a littoral *Zostera* bed by *Enteromorpha radiata*. *Aquatic Botany* 47, 21–28.
- Duarte, C.M., 1995. Submerged aquatic vegetation in relation to different nutrient regimes. *Ophelia* 41, 87–112.
- Eriksson, O., Kiviniemi, K., 1999. Site occupancy, recruitment and extinction thresholds in grassland plants: an experimental study. *Biological Conservation* 87, 319–325.
- Essink, K., de Vlas, J., Nijssen, R., Poot, G.J.M., 2003. Heeft mechanische kokkelvisserij invloed gehad op de ontwikkeling van zeegras in de Nederlandse Waddenzee? Rapport RIKZ/2002.026. Haren, The Netherlands.
- Fox, S.E., Stieve, E., Valiela, I., Hauxwell, J., McClelland, J., 2008. Macrophyte abundance in waquoit bay: effects of land-derived nitrogen loads on seasonal and multi-year biomass patterns. *Estuaries and Coasts* 31, 532–541.
- Greve, T.M., Krause-Jensen, D., Rasmussen, M.B., Christensen, P.B., 2005. Means of rapid eelgrass (*Zostera marina* L.) recolonisation in former dieback areas. *Aquatic Botany* 82, 143–156.
- Harrison, P.G., 1993. Variations in demography of *Zostera marina* and *Zostera noltii* on an intertidal gradient. *Aquatic Botany* 45, 63–77.
- Harwell, M.C., Orth, R.J., 2002a. Long-distance dispersal potential in a marine macrophyte. *Ecology* 83, 3319–3330.
- Harwell, M.C., Orth, R.J., 2002b. Seed bank patterns in Chesapeake Bay eelgrass (*Zostera marina* L.): a bay-wide perspective. *Estuaries* 25, 1196–1204.
- Hauxwell, J., Cebrian, J., Furlong, C., Valiela, I., 2001. Macroalgal canopies contribute to eelgrass (*Zostera marina*) decline in temperate estuarine ecosystems. *Ecology* 82, 1007–1022 (Washington, DC).
- Holmer, M., Frederiksen, M.S., Mollegaard, H., 2005. Sulfur accumulation in eelgrass (*Zostera marina*) and effect of sulfur on eelgrass growth. *Aquatic Botany* 81, 367–379.
- Jongejans, E., Soons, M.B., De Kroon, H., 2006. Bottlenecks and spatiotemporal variation in the sexual reproduction pathway of perennial meadow plants. *Basic and Applied Ecology* 7, 71–81.
- Keddy, C.J., Patriquin, D.G., 1978. An annual form of eelgrass in Nova Scotia. *Aquatic Botany* 5, 163–170.
- Keddy, C.J., 1987. Reproduction of annual eelgrass: variation among habitats and comparison with perennial eelgrass (*Zostera marina* L.). *Aquatic Botany* 27, 243–256.
- Lamers, L.P.M., Falla, S.J., Samborska, E.M., van Dulken, L.A.R., van Hengstum, G., Roelofs, J.G.M., 2002. Factors controlling the extent of eutrophication and toxicity in sulfate-polluted freshwater wetlands. *Limnology and Oceanography* 47, 585–593.
- Martens, P., van Beusekom, J.E.E., 2008. Zooplankton response to a warmer northern Wadden Sea. *Helgoland Marine Research* 62, 67–75.
- Morita, T., Okumura, H., Abe, M., Kurashima, A., Maegawa, M., 2007. Density and distribution of seeds in bottom sediments in *Zostera marina* beds in Ago Bay, Central Japan. *Aquatic Botany* 87, 38–42.
- Olesen, B., 1999. Reproduction in Danish eelgrass (*Zostera marina* L.) stands: size-dependence and biomass partitioning. *Aquatic Botany* 65, 209–219.
- Orth, R.J., Luckenbach, M., Moore, K.A., 1994. Seed dispersal in a marine macrophyte: implications for colonization and restoration. *Ecology* 75, 1927–1939.
- Orth, R.J., Harwell, M.C., Bailey, E.M., Bartholomew, A., Jawad, J.T., Lombana, A.V., Moore, K.A., Rhode, J.M., Woods, H.E., 2000. A review of issues in seagrass seed dormancy and germination: implications for conservation and restoration. *Marine Ecology Progress Series* 200, 277–288.
- Orth, R.J., Carruthers, T.J.B., Dennison, W.C., Duarte, C.M., Fourqurean, J.W., Heck, K.L., Hughes, A.R., Kendrick, G.A., Kenworthy, W.J., Olyarnik, S., Short, F.T., Waycott, M., Williams, S.L., 2006a. A global crisis for seagrass ecosystems. *BioScience* 56, 987–996.
- Orth, R.J., Harwell, M.C., Inglis, G.J., 2006b. Ecology of seagrass seeds and seagrass dispersal processes. In: Larkum, A.W.D., Orth, R.J., Duarte, C.M. (Eds.), *Seagrasses: Biology, Ecology and Conservation*. Springer, Dordrecht, The Netherlands, pp. 111–133.
- Ozinga, W.A., Hennekens, S.M., Schaminee, J.H.J., Smits, N.A.C., Bekker, R.M., Romermann, C., Klimes, L., Bakker, J.P., Van Groenendaal, J.M., 2007. Local above-ground persistence of vascular plants: life-history trade-offs and environmental constraints. *Journal of Vegetation Science* 18, 489–497.
- Polderman, P.J.G., den Hartog, C., 1975. De zeegrassen in de Waddenzee. Koninklijke Nederlandse Natuurhistorische Vereniging Wetenschappelijke Mededelingen 107, 1–32.
- Reise, K., Herre, E., Sturm, M., 2008. Mudflat biota since the 1930s: change beyond return? *Helgoland Marine Research* 62, 13–22.
- Reise, K., Kohl, J., 2008. Seagrass recovery in the Northern wadden Sea? *Helgoland Marine Research* 62, 77–84.
- Reusch, T.B.H., 2003. Floral neighbourhoods in the sea: how floral density, opportunity for outcrossing and population fragmentation affect seed set in *Zostera marina*. *Journal of Ecology* 91, 610–615.
- Sand-Jensen, K., Pedersen, N.L., Thorsgaard, I., Moeslund, B., Borum, J., Brodersen, K.P., 2008. 100 years of vegetation decline and recovery in Lake Fure, Denmark. *Journal of Ecology* 96, 260–271.
- SAS, 1989. SAS/STAT user's guide, version 6. SAS Institute Inc., Cary, NC.
- Schemske, D.W., Husband, B.C., Ruckelshaus, M.H., Goodwillie, C., Parker, I.M., Bishop, J.G., 1994. Evaluating approaches to the conservation of rare and endangered plants. *Ecology* 75, 584–606.
- Short, F.T., Coles, R., 2001. *Global seagrass research methods*. Elsevier, Amsterdam.
- Smolders, A.J.P., Lamers, L.P.M., Lucassen, E.C.H.E., van der Velde, G., Roelofs, J.G.M., 2006. Internal eutrophication: how it works and what to do about it – a review. *Chemistry and Ecology* 22, 93–111.
- Valiela, I., Cole, M.L., 2002. Comparative evidence that salt marshes and mangroves may protect seagrass meadows from land-derived nitrogen loads. *Ecosystems* 5, 92–102.
- van Aken, H.M., 2008. Variability of the water temperature in the western Wadden Sea on tidal to centennial time scales. *Journal of Sea Research* 60, 227–234.
- van Beusekom, J.E.E., de Jonge, V.N., 2002. Long-term changes in Wadden Sea nutrient cycles: importance of organic matter import from the North Sea. *Hydrobiologia* 475, 185–194.
- van Beusekom, J.E.E., 2005. A historic perspective on Wadden Sea eutrophication. *Helgoland Marine Research* 59, 45–54.
- van Beusekom, J.E.E., Bot, P., Göbel, J., Hanslik, M., Lenhart, H.J., Pätsch, J., Peperzak, L., Petenati, T., Reise, K., 2005. Eutrophication. In: Marencic, H. et al., (Ed.), *Quality Status Report Wadden Sea*. pp. 141–154.
- van Beusekom, J.E.E., Weigelt-Krenz, S., Martens, P., 2008. Long-term variability of winter nitrate concentrations in the Northern Wadden Sea driven by freshwater discharge, decreasing riverine loads and denitrification. *Helgoland Marine Research* 62, 49–57.
- van Beusekom, J.E.E., Loebl, M., Martens, P., 2009. Distant riverine nutrient supply and local temperature drive the long-term phytoplankton development in a temperate coastal basin. *Journal of Sea Research* 61, 26–33.
- van Katwijk, M.M., Vergeer, L.H.T., Schmitz, G.H.W., Roelofs, J.G.M., 1997. Ammonium toxicity in eelgrass *Zostera marina*. *Marine Ecology Progress Series* 157, 159–173.
- van Katwijk, M.M., Schmitz, G.H.W., Hanssen, L.S.A.M., den Hartog, C., 1998. Suitability of *Zostera marina* populations for transplantation to the Wadden Sea as determined by a mesocosm shading experiment. *Aquatic Botany* 60, 283–305.
- van Katwijk, M.M., Hermus, D.C.R., de Jong, D.J., Asmus, R.M., de Jonge, V.N., 2000. Habitat suitability of the Wadden Sea for restoration of *Zostera marina* beds. *Helgoland Marine Research* 54, 117–128.
- van Katwijk, M.M., Wijgergangs, L.J.M., 2004. Effects of locally varying exposure, sediment type and low-tide water cover on *Zostera marina* recruitment from seed. *Aquatic Botany* 80, 1–12.
- van Katwijk, M.M., Geerling, G.W., Rašín, R., van't Veer, R., Bos, A.R., Hermus, D.C.R., van Wieringen, M., Jager, Z., Groeneweg, A.H., Erftemeijer, P.L.A., van der Heide, T., de Jong, D.J., 2006. Macrophytes in the western Wadden Sea: monitoring, invasion, transplantations, dynamics and European policy. In: Laursen, K., Marencic, H., et al., (Eds.), *Proceedings of the 11th International Scientific Wadden Sea Symposium*. Esbjerg, Denmark, pp. 89–98.
- van Katwijk, M.M., Bos, A.R., de Jonge, V.N., Hanssen, L.S.A.M., Hermus, D.C.R., de Jong, D.J., 2009. Guidelines for seagrass restoration: importance of habitat selection and donor population, spreading of risks, and ecosystem engineering effects. *Marine Pollution Bulletin* 58, 179–188.
- van Lent, F., Verschuure, J.M., 1994. Intraspecific variability of *Zostera marina* L. (eelgrass) in the estuaries and lagoons of the southwestern Netherlands: I. Population dynamics. *Aquatic Botany* 48, 31–58.
- van Wijk, R.J., 1988. Ecological studies on *Potamogeton pectinatus* L. 1. General characteristics, biomass production and life-cycles under field conditions. *Aquatic Botany* 31, 211–258.
- Viarioli, P., Bartoli, M., Giordani, G., Naldi, M., Orfanidis, S., Zaldivar, J.M., 2008. Community shifts, alternative stable states, biogeochemical controls and feedbacks in eutrophic coastal lagoons: a brief overview. *Aquatic Conservation-Marine and Freshwater Ecosystems* 18, S105–S117.
- Wicks, E.C., Koch, E.W., O'Neil, J.M., Elliston, K., 2009. Effects of sediment organic content and hydrodynamic conditions on the growth and distribution of *Zostera marina*. *Marine Ecology Progress Series* 378, 71–80.