



Stressors and feedbacks in temperate seagrass ecosystems

Tjisse van der Heide

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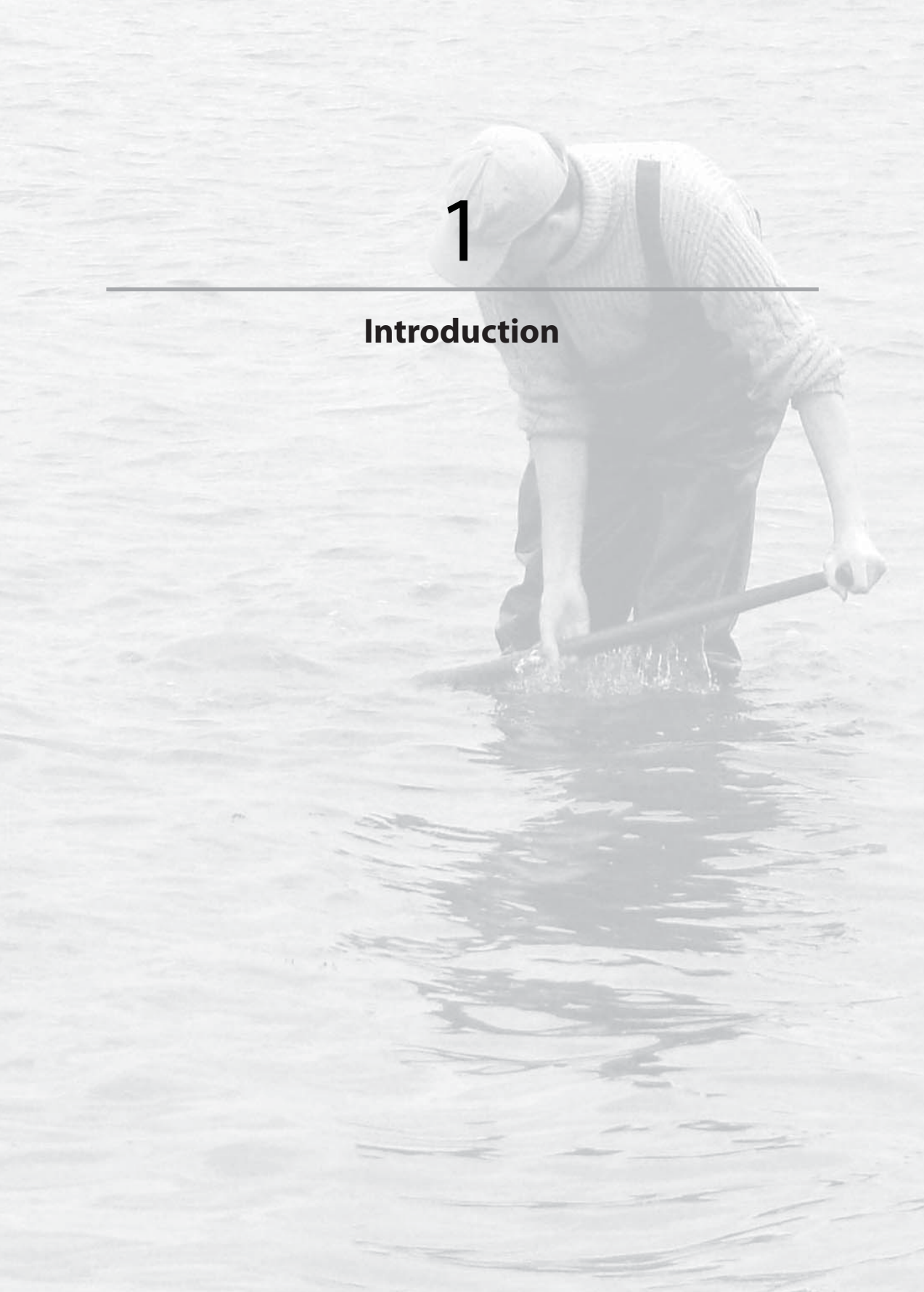
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Introduction



WHAT ARE SEAGRASSES?

Seagrasses are aquatic flowering plants, that have evolved from freshwater macrophytes to grow in brackish to marine environments (den Hartog 1970, Hemminga & Duarte 2000, Green & Short 2003). They can form extensive monospecific stands or mixed species meadows in the intertidal to subtidal littoral of temperate to tropical coastal areas (fig. 1.1). The lower depth limit of seagrasses is generally dependent on light availability and ranges from 1 m in turbid systems to over 50 m in very clear waters (Duarte 1991). The upper depth limit is mostly determined by desiccation during emersion or hydrodynamic stress (waves/currents) (Philippart & Dijkema 1995, Leuschner et al. 1998, van Katwijk et al. 2000, Greve & Binzer 2004).

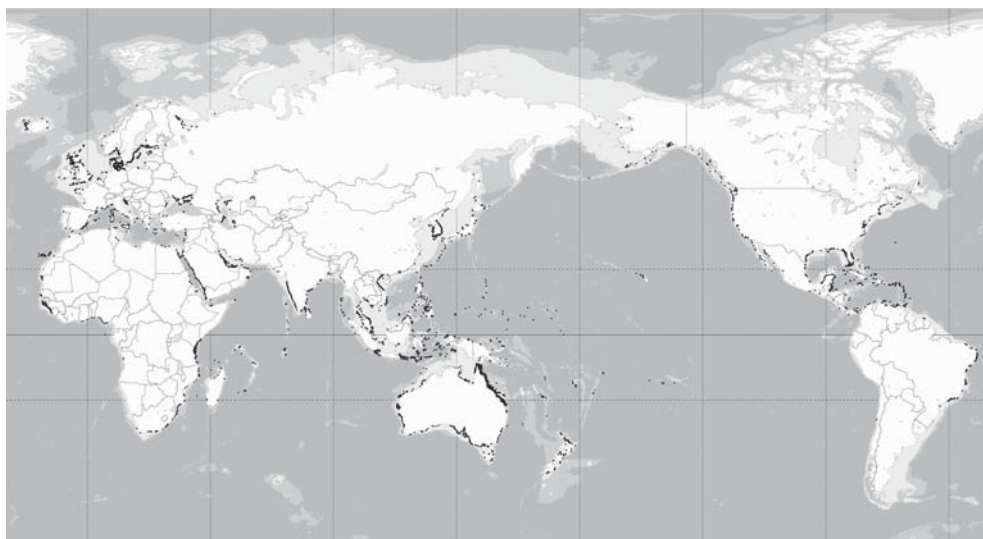


Figure 1.1. A map showing the distribution of seagrasses around the world. The map is based on observations compiled by UNEP-WCMC (Green & Short 2003).

Seagrasses comprise less than 0.02% of the total angiosperm flora, and represent a relatively small number of species compared to other angiosperm plant groups (Hemminga & Duarte 2000, Green & Short 2003, Larkum et al. 2006, Orth et al. 2006). They form an ecological group rather than a taxonomic group, implying that the included families are not necessarily closely related (den Hartog 1970, Larkum et al. 2006). Of the four families that include seagrass species, three consist exclusively of seagrasses. These are the *Zosteraceae*, *Cymodoceaceae* and *Posidoneaceae* (Green & Short 2003, Larkum et al. 2006). The fourth family, the *Hydrocharitaceae*, includes three genera of seagrasses and 14 freshwater genera (Larkum et al. 2006). In total, these four families include 12 seagrass genera (Larkum et al. 2006), consisting of 55 species (Green & Short 2003). Apart from these seagrass species, some species from two other aquatic plant families also occur in brackish or marine habitats. These families are the *Ruppiceae* and *Zannichelliaceae* (Green & Short 2003, Larkum et al. 2006). At present, it is still a subject of debate whether marine or brackish water confined species

from these families should be considered seagrasses (Green & Short 2003, Larkum et al. 2006).

Seagrasses are clonal plants that are interconnected via belowground stems called rhizomes (Hemminga & Duarte 2000). Individual seagrass units are called ramets and consist of three components (fig. 1.2). The shoot is the aboveground part and consists of a bundle of leaves attached to the rhizome. It is responsible for growth and oxygen production by photosynthesis and carbon uptake from the water layer (Hemminga & Duarte 2000, Larkum et al. 2006). The second component is the rhizome. Depending on the species, rhizomes can grow both horizontally and vertically (Hemminga & Duarte 2000). They are responsible for vegetative expansion of the plant as well as storage and translocation of resources like carbohydrates and nitrogen (Hemminga & Duarte 2000, Marba et al. 2002, Larkum et al. 2006, Marbà et al. 2006). The final component is the root system, which is responsible for anchoring of the plant and nutrient uptake from the soil (Hemminga & Duarte 2000, Peralta et al. 2005, Larkum et al. 2006). Apart from vegetative expansion through the rhizomes, seagrasses can also reproduce sexually. During sexual reproduction, the shoot of individual ramets can hold flowers or fruits (Hemminga & Duarte 2000).

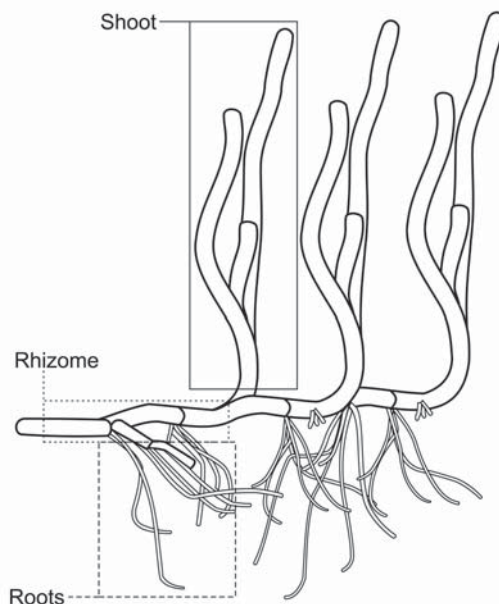


Figure 1.2. Diagram of three connected *Zostera marina* clones (or ramets). Each ramet consists of three components. 1) The shoot, responsible for photosynthesis and carbon uptake from the water layer. 2) The rhizome, responsible for clonal expansion, storage and translocation of resources. 3) The roots, responsible for anchoring of the plants and nutrient uptake from the sediment.

Most seagrass species are perennial and stay green throughout the year (Hemminga & Duarte 2000, Larkum et al. 2006). In temperate areas, however, seagrass species like *Zostera marina* and *Zostera noltii* may lose their aboveground biomass in autumn, and survive the winter with the energy (carbohydrates) stored in their rhizomes (Vermaat et al. 1987, van Lent & Verschuure 1994, Vermaat

& Verhagen 1996, Larkum et al. 2006). In some cases, *Z. marina* may even adopt a completely annual growth strategy, surviving the winter solely on the seeds produced during the growing season (van Lent & Verschuure 1994).

FUNCTIONS AND VALUES OF SEAGRASSES

Worldwide, seagrasses are of great ecological importance to the coastal zone. They are among the most productive ecosystems worldwide and harbour a high biodiversity of marine animal species (Hemminga & Duarte 2000, Nagelkerken et al. 2000, Duarte 2002, Green & Short 2003, Larkum et al. 2006, Orth et al. 2006). Seagrasses serve as nursery ground for the juvenile stages of many commercially important finfish and shellfish species (Heck et al. 2003, Orth et al. 2006), but they are also a direct food source for grazers such as sea urchins, turtles, manatees, dugongs, and many species of waterfowl and fish (Valentine & Heck 1999, Hemminga & Duarte 2000, Larkum et al. 2006). Because seagrasses are often found near other important coastal ecosystems such as salt marshes, mangroves and coral reefs, they are also important in facilitating cross-habitat utilization by many fish and invertebrate species (Nagelkerken et al. 2000, Duarte 2002, Orth et al. 2006).

Apart from their important functions as shelter and primary producers in the food chain of coastal ecosystems, seagrasses offer a range of other important services. Seagrasses provide shoreline protection by attenuating currents and waves and stabilizing the sediment (Gambi et al. 1990, Fonseca & Cahalan 1992, Granata et al. 2001, Duarte 2002). Seagrass meadows trap and store nutrients (Duarte 2002, Moore 2004, Orth et al. 2006, Bos et al. 2007) and sediment particles (Granata et al. 2001, Agawin & Duarte 2002) from the water column, thereby acting as a filter for nutrient input into the coastal zone and increasing water clarity. Additionally, seagrasses also act as a carbon sink. They are responsible for about 15 % of all carbon being stored in marine ecosystems (Duarte 2002). Apart from their obvious ecological value, all these functions and services combined also result in a high economic value. Together with macro-algal beds, seagrasses represent an economic value that is over twice as high as salt-marshes, the next most valuable coastal habitat (Costanza et al. 1997).

THREATS TO SEAGRASSES

The first seagrass species evolved nearly a 100 million years ago (Orth et al. 2006). At present, seagrasses are exposed to lower temperatures, CO₂ concentrations, and sea water levels compared to the situation in which their ancestors developed (Orth et al. 2006). Hence, recent global warming might eventually result in environments that would theoretically be more favourable for many seagrass species (Orth et al. 2006). However, environmental changes are currently much faster compared to the rate of change from the ancient circumstances to the present. Currently, it may therefore be hard for seagrasses to adapt (Orth et al. 2006). Moreover, seagrasses are at present under threat of a wide array of additional anthropogenic-based stressors.

In temperate regions, eutrophication is probably the most important cause for decline

(Nielsen et al. 2002, Kemp et al. 2005, Orth et al. 2006, Burkholder et al. 2007). Eutrophication in seagrass ecosystems is mostly caused by an increase in nitrogen and phosphorus availability due to agricultural run-off (Nielsen et al. 2002, Kemp et al. 2005, Orth et al. 2006). The enhanced nutrient levels favour growth of opportunistic, fast-growing species such as phytoplankton, epiphytes and macro-algal species. The increased abundance of these species leads to a decrease of light availability (Twilley et al. 1985, Nielsen et al. 2002, Kemp et al. 2005). Moreover, eutrophication may also cause toxicity events, caused by for instance high ammonium and nitrate levels in the water layer (Burkholder et al. 1992, van Katwijk et al. 1997, Bird et al. 1998, Brun et al. 2002), or high sulphide concentrations in the sediment pore water (Goodman et al. 1995, Holmer & Bondgaard 2001, Pedersen et al. 2004). Apart from these factors, the wasting disease epidemic of the 1930s, caused by the protist *Labyrinthula zosterae* (Muehlstein et al. 1988, Muehlstein et al. 1991, Vergeer & den Hartog 1994, Vergeer et al. 1995), also considerably reduced the most common temperate seagrass species of the North Atlantic region, *Zostera marina* (den Hartog 1987, Vergeer & den Hartog 1994).

Like in temperate seagrass ecosystems, eutrophication is also a major cause for concern in tropical areas. However, seagrasses in these areas are often also threatened by siltation events (Orth et al. 2006). These pulsed events are the result of erosion during heavy rainfall in adjacent deforested terrestrial systems. Finally, changes in salinity and hydrodynamics caused by altered water flow (e.g., construction of dams), or direct damage to seagrasses due to dredging (for instance fishery) or direct boating effects are factors that affect both temperate and tropical seagrasses (Giesen et al. 1990, Giesen et al. 1990, Orth et al. 2006).

CONSERVATION AND RESTORATION OF SEAGRASSES

Public awareness of the importance of seagrasses and their decline is currently increasing. In response to the large-scale decline, the number of marine protected areas that include seagrasses has greatly increased over the last decade (Orth et al. 2006). At the same time, the number of monitoring efforts and restoration projects has increased in a similar fashion (Orth et al. 2006, van Katwijk et al. 2009). Moreover, some countries have implemented a zero-loss policy, which means that any losses caused by direct human disturbance should be compensated (Duarte 2002).

Although efforts to protect and restore seagrasses are greatly increasing, success of such measures is at present still very uncertain (Orth et al. 2006). This is especially the case for seagrass restoration projects. Numerous projects have been carried out and are being planned, using a wide variety of techniques (Orth et al. 2006). Because costs are very high (up to \$1000,000 per ha) (Paling et al. 2009), restoration projects are typically attempted at a small scale (< 1 ha). At present, the success rate of such projects is only about 30 % (Fonseca et al. 2001).

Managers trying to protect or restore seagrass ecosystems are confronted with a number of problems. First of all, threats like eutrophication or siltation, are diffuse and have sources outside the boundaries of seagrass ecosystems themselves, often crossing jurisdictional boundaries as

well (Duarte 2002, Orth et al. 2006). This makes successful implementation of protection measures difficult in such cases. Another major problem is the large number of potential threats to seagrasses. Because seagrass decline typically seems the result of the combined effects of multiple stressors (Duarte 2002, Orth et al. 2006), separating and prioritising these stressors is often extremely difficult. Additionally, seagrass ecosystems do not seem to respond linearly to changing environmental stressors, but may often collapse without clear warning (den Hartog & Polderman 1975, Giesen et al. 1990, Jackson et al. 2001). At present, underlying mechanisms responsible for such collapses are not well understood, making prevention or even prediction of seagrass decline in many coastal ecosystems nearly impossible.

FEEDBACKS IN SEAGRASSES

Seagrasses are ecosystem engineers, which implies that they modify their abiotic environment (Jones et al. 1994). Seagrasses may improve their growing conditions with increasing vegetation density. They relieve hydrodynamic stress by attenuating currents and waves (Gambi et al. 1990, Fonseca & Cahalan 1992, Granata et al. 2001) and increase water transparency through reduction of sediment resuspension (Ward et al. 1984, Gacia & Duarte 2001, Kemp et al. 2005) and nutrient levels (Moore 2004). If such a feedback is strong enough, theory suggests that it could lead to alternative stable states (Scheffer et al. 2001, Scheffer & Carpenter 2003). This implies that a seagrass meadow with a sufficient size and biomass could be able to modify conditions so dramatically, that the bed would be able to maintain itself in an environment that would otherwise not suffice.

When an ecosystem with alternative stable states is exposed to gradually changing environmental conditions, it may at first seem highly resilient to change, because changes are buffered by a feedback (fig. 1.3). However, at some point, a critical threshold can be crossed beyond which buffering is not possible anymore, hence causing the system to collapse to an alternative stable state (Scheffer et al. 2001). A sufficiently large perturbation by for instance a storm, disease or over-exploitation may also cause a shift in the ecosystem. Notably, if the system is already close to a critical threshold, resilience is low and even a slight disturbance may trigger a shift (Scheffer et al. 2001). If such an ecosystem is to be recovered from a collapse, conditions have to improve beyond the initial point of collapse, a phenomenon called hysteresis (Scheffer et al. 2001).

If feedbacks are indeed important in seagrasses, implications for ecosystem conservation or restoration may be profound. Catastrophic shifts in ecosystems with alternative stable states are notoriously hard to predict, as the system can collapse without clear warning (Scheffer et al. 2001, Scheffer & Carpenter 2003). Furthermore, strong feedbacks will also make restoration by for instance transplantation much harder. If the conditions in a considered system are not suitable for seagrass growth without seagrass presence in the first place, it means that a certain threshold for a minimum size and density needs to be bridged.

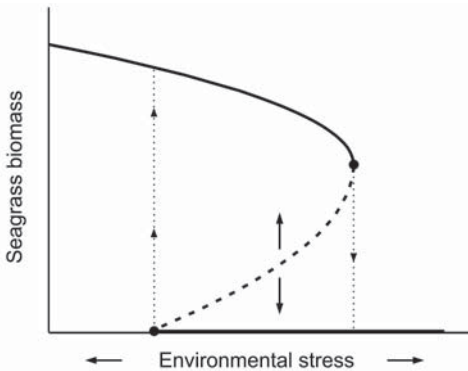


Figure 1.3. A diagram showing alternative stable states in a seagrass meadow. The solid lines represent possible equilibrium states for the ecosystem. Between two thresholds, represented by the black dots, both a vegetated and a bare state are stable. Here, the system can shift from a vegetated to a bare state when the system is pushed below the dashed line by a strong enough perturbation (for instance by disease). The system will also collapse when conditions progress beyond the right-side threshold. If a collapsed ecosystem is to be restored, conditions have to improve beyond the left-side threshold.

OBJECTIVES AND OUTLINE OF THIS THESIS

This thesis focuses on temperate seagrass ecosystems dominated by *Zostera marina* (eelgrass) and *Zostera noltii* (dwarf eelgrass). Using these ecosystems as a model, the primary objective of this thesis is to improve the understanding of the main causes and mechanisms responsible for sudden collapse in seagrasses and seagrass decline in general. Secondly, I aim to provide predictive tools for seagrass conservation and restoration.

First, the most important environmental factors determining survival and growth of *Z. marina* and *Z. noltii* will be elucidated. This is investigated by gathering and analysing vegetation and abiotic field data from a large number of locations, scattered across Western-Europe, in regions where seagrass is either present or used to be present (chapter 2). Results from these data and analyses are then used to develop theoretical and empirical tools for predicting the suitability of a site for seagrass growth (chapters 2).

Next, a case study on the collapse of the *Z. marina* meadows in the Dutch Wadden Sea in the 1930s is used to examine the potential importance of positive feedbacks and alternative stable states in explaining sudden shifts in seagrass ecosystems (chapter 3). It is hypothesised that *Z. marina* in the Dutch Wadden Sea reduced hydrodynamics and enhanced water clarity by preventing sediment resuspension. GIS analyses of historical maps are combined with analyses of recent turbidity data and a computer model to test this hypothesis.

Possible implications of positive feedbacks in seagrasses are further explored in laboratory experiments (chapter 4). These experiments focus on toxicity in eelgrass caused by elevated levels of reduced nitrogen in the water layer. This phenomenon has been suggested to cause sudden collapses by acute toxicity (van Katwijk et al. 1997, Brun et al. 2002). In the experiments, I tested the hypothesis that toxicity by reduced nitrogen is dependent on surface water pH and shoot density (or biomass) of the eelgrass meadow. Next, consequences of density-dependent toxicity for ecosystem stability are studied by implementing observed mechanisms and results from the laboratory experiments in computer models (chapter 5).

Finally, effects that feedback mechanisms may have on the spatial structure of seagrasses are tested in the field (chapter 6). In this chapter, it is investigated whether feedback mechanisms may cause regular patterning in seagrasses, a phenomenon that has been described for a range of both terrestrial and marine ecosystems (Rietkerk & van de Koppel 2008). Additionally, it is theoretically and empirically tested whether such patterns can potentially be useful in ecosystem monitoring programs to detect changes in abiotic stress (e.g., eutrophication, over-exploitation) in ecosystems.

A grayscale photograph of a long wooden pier extending from the foreground into a body of water. At the far end of the pier, there are two wooden benches. The water is slightly rippled, and the sky is overcast. The pier is made of wooden planks and has a railing on the right side.

2

Predicting habitat suitability in temperate seagrass ecosystems

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ABSTRACT

The world-wide observed dramatic decline of seagrasses has typically been attributed to multiple stressors like for instance eutrophication, disease, sedimentation, and toxicity events. Using principal components analysis and (multivariate) logistic regression, we investigated the importance of 30 commonly measured variables in explaining the presence and absence of the temperate seagrass species *Zostera marina* and *Z. noltii* at 84 locations scattered over Western-Europe, in regions that were historically suitable for seagrasses. Although many interrelated variables influence seagrass presence in our dataset, presence or absence of both species could be reliably predicted by using only two, easy to measure variables. A logistic regression model of *Z. marina* correctly predicted 77% of all observations by including water column light attenuation and pore water reduction oxidation potential (redox). The *Z. noltii* model had an 86% accuracy based on only tidal location (intertidal or subtidal zone) and pore water redox. Applying the models to 5 evaluation sites demonstrated that both models can be usefully applied as tools for seagrass ecosystem restoration and conservation.

INTRODUCTION

Seagrasses are rhizomatous marine angiosperms that form extensive meadows in temperate to tropical regions. These beds are among the most productive ecosystems on earth and harbour a high biodiversity of marine animal life. Seagrasses have become increasingly threatened by human activities in the last decades, resulting world-wide in large-scale losses of seagrass-dominated ecosystems ($>30,000 \text{ km}^2$) (Short & Wyllie-Echeverria 2000, Duarte 2002, Orth et al. 2006).

Seagrass decline has been attributed to multiple stressors. In tropical areas, seagrass losses are mainly ascribed to salinity changes and siltation events due to poor land management, eutrophication, and direct damage by boats and dredging activities (Duarte 2002, Orth et al. 2006). Degradation in temperate regions is mostly attributed to eutrophication, increased water temperature and disease (Duarte 2002, Orth et al. 2006). Whereas temperature and disease cause direct damage to seagrasses, eutrophication is, in contrast, a summarizer representing a number of indirect and direct problems. In the first place, it may lead to reduced light as growth of phytoplankton, epiphytes and macroalgae are enhanced (Twilley et al. 1985, Nielsen et al. 2002, Kemp et al. 2005, Burkholder et al. 2007). Moreover, eutrophication can also trigger toxicity events caused by for instance increased levels of ammonia (van Katwijk et al. 1997, Brun et al. 2002) or nitrate in the water column (van Katwijk et al. 1999, Touchette & Burkholder 2000) or high sulphide concentrations in the pore water (Goodman et al. 1995, Pedersen et al. 2004).

Because seagrasses are highly important for the ecology and economy in many coastal zones, numerous efforts have been carried out to restore ecosystems that were lost (Orth et al. 2006, van der Heide et al. 2007, van Katwijk et al. 2009). Even though costs for these projects are extremely high (\$ 1000,000 per ha) (Paling et al. 2009), success is limited and very uncertain (about 30% success) (Orth et al. 2006). One explanation for this low success rate may lie in the fact that seagrasses improve their own growing conditions in an environment which would otherwise not suffice (e.g., reduction of hydrodynamics, turbidity and stabilization of sediment) (Bos & van Katwijk 2007, van der Heide et al. 2007, van der Heide et al. 2008). Because these self-facilitation mechanisms only work above a certain threshold for size and density of a seagrass meadow, the scale of many transplantations may often be too small (Bos & van Katwijk 2007, van der Heide et al. 2007). A second explanation however, might be that estimations of abiotic habitat suitability of potential restoration sites are unreliable, because the complex interplay of many different stressors makes these environmental assessments too complicated.

Although a multitude of environmental variables have been found to influence seagrass growth, only a few studies have tried to disentangle the relative importance of multiple abiotic variables for explaining global seagrass decline or for predicting seagrass habitat suitability (van Katwijk et al. 2000, Kelly et al. 2001, Short et al. 2002). In this study, we investigate to what extent different abiotic factors correlate with occurrence of the seagrasses *Zostera marina* and *Zostera noltii*, species that dominate seagrass ecosystems in many temperate regions. We sampled 84 different locations scattered all over Western-Europe in regions where seagrasses have been historically

present, measuring 30 different environmental variables that are commonly used to evaluate ecosystems. The obtained dataset was analyzed using logistic regression (Ter braak & Looman 1986, Hosmer & Lemeshow 1989, Jongman et al. 1995), a method that has been successfully applied to obtain habitat suitability models for birds (Manel et al. 1999) and freshwater gammarids (Peeters & Gardeniers 1998). We constructed for both species a simple multivariate model, containing the most important environmental variables (Jongman et al. 1995, Peeters & Gardeniers 1998). To evaluate the usefulness of the models for restoration or conservation, we predicted the probability of occurrence of both species for 5 sites which had a history of seagrass loss and where recovery or applied restoration measures had different levels of success.

MATERIALS AND METHODS

Data collection. All data were collected in regions where seagrasses are present or were historically present (fig. 2.1). The sampling sites in these areas were randomly selected and each site was sampled once in the growth season (May to September) of 2005. Depth of these locations varied between 0.5 m above to 5 m below mean water level. At each site, we recorded presence or absence of both *Z. marina* and *Z. noltii* and noted whether the location was situated in the intertidal or subtidal zone. Next, we sampled and pooled 3 replicates of the surface water, pore water (top 10 cm) and sediment (top 10 cm). Samples were frozen immediately after collection for transport to the laboratory in Nijmegen (the Netherlands) where they were analyzed. As a measure for hydrodynamic exposure we calculated the maximum and modified effective fetch length and included the exposure index developed by the Physical Shore-Zone Mapping Task Force of British Columbia, Canada (Howes et al. 1999).

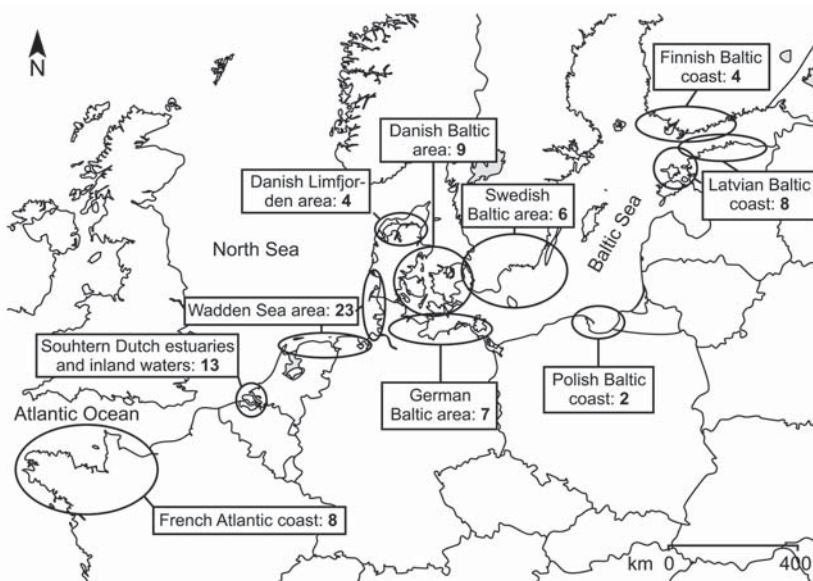


Figure 2.1. General overview of the geographical setting of the 84 sampled sites. All sites are located in regions where seagrass is present or has been present in the past.

Sample analyses. Apart from the hydrodynamic exposure variables, we included 26 additional commonly measured environmental variables. The light attenuation coefficient of the water column was measured in PAR (400 – 700 nm) with a quantum light meter (Li-192, Li-Cor). Water temperature, salinity (of both surface and pore water), reduction oxidation potential (redox) of the pore water, and surface and pore water pH were measured onsite with a multi-probe meter (556 MPS, YSI). Total sulphide (TS) levels in the pore water were determined immediately after sampling by measuring TS with an ion-selective silver-sulphide electrode in a mixed solution containing 50% Sulphide Anti-Oxidation Buffer (SAOB) and 50% sample (Lamers et al. 1998). Alkalinity of all water samples was determined by titration with 0.01 mol l⁻¹ HCl to pH 4.2 (Lamers et al. 1998). The concentrations of ortho-phosphate and ammonium in all water samples were measured colorimetrically, using ammonium-molybdate and salicylate (Lamers et al. 1998). Nitrate was determined by sulphanilamide after reduction of nitrate to nitrite in a cadmium column (Wood et al. 1967). Total nitrogen and total phosphorus in the surface water were measured as nitrate and ortho-phosphate after digestion with persulphate (Koroleff 1983). We measured total inorganic carbon (TIC) in pore and surface water as CO₂ on an infrared carbon analyzer (PIR-2000, Horiba Instruments) after conversion of all TIC to CO₂ by phosphoric acid. Organic matter content in freeze dried sediments was estimated as weight loss by ignition at 550°C. Carbon and nitrogen content in the sediment were determined on freeze dried samples by a Carbon-Nitrogen-Sulphur analyzer (type NA1500; Carlo Erba Instruments). Total phosphorus in the sediment was measured on an inductively-coupled-plasma emission spectrophotometer (ICP) (Spectroflame, Spectro Inc.), after digestion with nitric acid (Smolders et al. 2006). Grain size distribution of the sediment was measured on freeze dried samples by laser diffraction on a Beckman Coulter particle size analyzer. All devices were calibrated according to standardized procedures provided by the manufacturers. For all analyses quality assurance measures included blanks, replicate analyses and matrix spikes. Recoveries from matrix spikes ranged from 95% to 107%. Repeated analyses did not reveal differences greater than 5%.

Data analysis. We used 79 locations of the 84 location dataset for the modelling procedures, thereby excluding 5 sampling locations. These 5 sites had a recorded history of seagrass loss and recovery or restoration attempts resulting in different levels of success. After model construction, we used these locations to evaluate usefulness of the models and to exemplify how logistic regression models may be applied for restoration and conservation purposes. The excluded sites were 2 sites from the Dutch Wadden Sea (Balgzand and Mokbaai), 1 site from the Baltic Sea (Puck bay) and 2 sites from Dutch brackish closed off water bodies (Lake Grevelingen and De Bol). Balgzand and Mokbaai are both sites where restoration efforts by transplantation have been attempted (Bos & van Katwijk 2007, van Katwijk et al. 2009). *Z. marina* transplantations were unsuccessful at both sites, but *Z. noltii* was successfully transplanted at Balgzand in 1993, resulting in a population that is at present still expanding. Puck bay is a site near the city of Gdansk in Poland, where *Z. marina* is now slowly recovering after the population nearly disappeared in the last century (Boström et al. 2003).

Z. marina disappeared from De Bol at the end of the 1970s due to eutrophication and changes in the hydrology of the site (den Hartog 1994). The population at Lake Grevelingen became extinct at the end of the 1990s. Although the cause for this is still not fully understood, it has been suggested that this isolated estuarine population was (over-)adapted to low salinity and could not cope with a dramatic increase in salinity of the lake (from 22 to 32 PSU) that took place in the 1980s and 1990s (Kamermans et al. 1999).

To get an overview of the relations between all variables included in this study, we first performed a standardized Principal Components Analysis (PCA). Next, we fitted response curves by binary logistic regression to the presence/absence data for every single variable. The general equation from this analysis describes the probability P that a species can occur at a certain value for the fitted environmental variable (Ter braak & Looman 1986, Hosmer & Lemeshow 1989, Jongman et al. 1995):

$$P(x) = \frac{\exp(\beta_0 + \beta_1 x + \beta_2 x^2)}{1 + \exp(\beta_0 + \beta_1 x + \beta_2 x^2)} \quad (1)$$

The parameters β_0 , β_1 and β_2 are regression coefficients, with β_0 as intercept. The equation can either yield a symmetrical bell-shaped (Gaussian) curve if β_2 is significant or a sigmoid curve if β_2 is not significant (and thus excluded). The parameters were analyzed for significance using the likelihood ratio test ($p < 0.05$). We used logarithmically transformed data ($y = \log_{10}(x+1)$) when these gave a more significant fit. The calculated hydrodynamical exposure index and tidal location (subtidal or intertidal) were analyzed as categorical variables.

Next, we included all significant variables from the first analysis in a second logistic regression procedure to construct a multivariate model. The applied equation is similar to function 1, except that a larger number of parameters can now be included (Jongman et al. 1995):

$$P(x) = \frac{\exp(\beta_0 + \beta_{1,1}x_1 + \beta_{1,2}x_1^2 + \dots + \beta_{n,1}x_n + \beta_{n,2}x_n^2)}{1 + \exp(\beta_0 + \beta_{1,1}x_1 + \beta_{1,2}x_1^2 + \dots + \beta_{n,1}x_n + \beta_{n,2}x_n^2)} \quad (2)$$

We used a multiple stepwise regression procedure with forward selection. The likelihood ratio test was applied to determine if a variable should be included or not ($p < 0.05$). Additionally, we calculated the percentage reduction of deviance compared the null model that included only a constant term (Peeters & Gardeniers 1998), and applied leave-one-out cross-validation to assess the reliability of the resulting model. In this method, the model is built leaving out one single observation from the dataset. Next, the obtained model is used to predict presence or absence for the left out observation. This procedure is repeated for every observation in the dataset. Based on the results, we calculated prediction success, mean parameter values and standard deviation for the model parameters.

Because many variables are often related to each other in the field, we applied linear regression on continuous variables to determine correlations between variables included in the model and variables that were excluded. The F-test and R^2 were used to test whether correlations were significant. Logarithmically transformed data were used when these gave a more significant

fit. Correlations between included categorical variables and other variables were explored using single variable logistic regression. For these analyses, we followed the same procedure as for the first logistic regression analysis, with the exception that we did not include second-order terms (β_2 , x^2) in this analysis.

Finally, we applied the obtained models from the multivariate logistic regression procedure to the data from the 5 sites that were excluded from the analyses to evaluate their potential use as prediction tools for restoration success or failure.

RESULTS

Measured ranges for each variable are presented in table 2.1. Our 79-site database included 34 observations for *Z. marina* and 19 for *Z. noltii*. PCA-analysis revealed that many environmental variables correlated with each other (fig. 2.2). For instance, light attenuation showed strong positive correlations with water column variables like total nitrogen, total-phosphorus, ortho-phosphate, TIC and alkalinity. Pore water sulphide showed a strong inverse correlation with Redox. Tidal location and surface and pore water salinity were strongly related to each other, but did not correlate with variables like light attenuation, total nitrogen or Redox. Most surface water nitrogen and phosphorus variables did not relate strongly with sediment or pore water nitrogen and phosphorus variables.

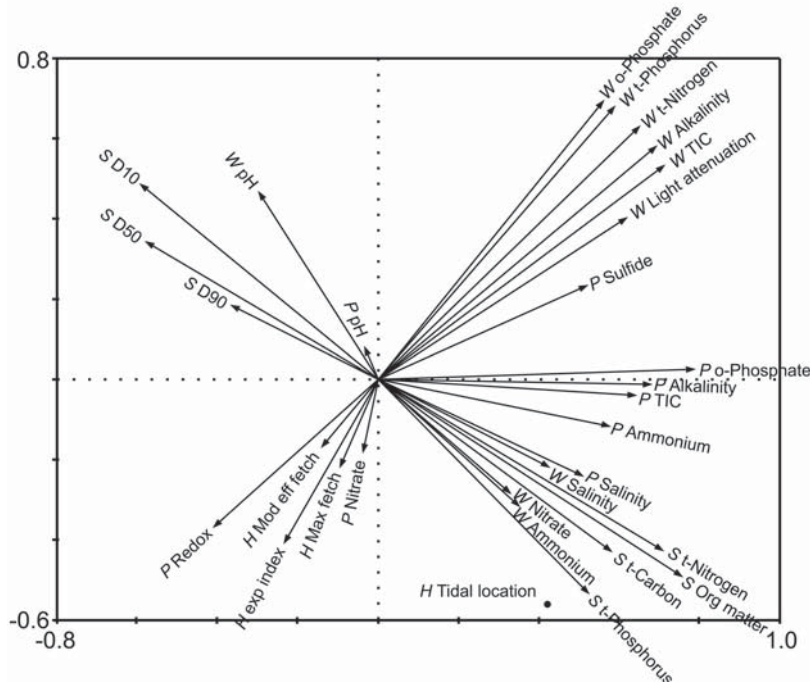


Figure 2.2. Results of the PCA-analysis showing the relations between all variables included in our analyses. W and P indicate surface and pore water variables respectively. S indicates a sediment variable and H indicates a variable related to hydrodynamics. Eigenvalues of the x and y-axis were 0.272 and 0.156 respectively, indicating that both axes together explained 42.8 % of all variance in the dataset.

Table 2.1: Measured ranges (median, 5 and 95 percentiles respectively) for the complete dataset and both seagrass species. The dataset included 79 sampling sites, with 34 observations for *Z. marina* and 19 for *Z. noltii*. Variables D10, D50 and D90 indicate the sediment grain size at which 10, 50 and 90 % respectively of the volumetric fraction is smaller.

		Complete dataset			Zostera marina			Zostera noltii		
	Unit	Med	5%	95%	Med	5%	95%	Med	5%	95%
Surface water										
Light attenuation	m ⁻¹	0.997	0.346	37.35	0.541	0.306	1.462	1.018	0.469	30.90
Salinity	PSU	22.25	3.420	34.83	16.65	4.710	34.92	29.95	10.46	35.14
pH		8.320	7.850	9.170	8.330	7.885	8.903	8.080	7.594	8.434
Alkalinity	meq l ⁻¹	2.090	1.446	4.697	1.986	1.453	2.751	2.260	1.858	2.785
TIC	mmol l ⁻¹	2.114	1.361	4.614	2.007	1.432	2.738	2.317	1.851	2.925
o-Phosphate	μmol l ⁻¹	0.444	0.048	39.61	0.296	0.056	4.830	1.604	0.112	5.321
t-Phosphorus	μmol l ⁻¹	1.475	0.275	49.30	0.969	0.171	7.543	2.900	0.207	13.98
Ammonium	μmol l ⁻¹	9.232	2.278	21.36	8.898	1.780	16.88	8.940	2.603	23.28
Nitrate	μmol l ⁻¹	2.051	1.087	28.55	2.294	1.075	5.772	1.801	1.108	30.80
t-Nitrogen	μmol l ⁻¹	33.96	12.54	191.5	29.39	9.720	53.43	33.56	11.79	105.3
Pore water										
Salinity	PSU	22.16	3.710	34.83	17.19	4.858	35.07	29.88	10.69	35.83
pH		7.460	7.040	7.940	7.480	7.140	7.733	7.490	7.120	7.900
Alkalinity	meq l ⁻¹	3.030	1.934	12.58	2.593	1.905	4.724	3.040	1.903	10.02
TIC	mmol l ⁻¹	3.283	2.018	14.90	2.791	2.025	5.121	3.288	1.987	10.56
o-Phosphate	μmol l ⁻¹	13.00	3.270	132.3	9.045	2.760	58.39	17.6	2.738	57.35
Ammonium	μmol l ⁻¹	78.73	16.71	713.5	65.37	17.14	239.8	91.05	19.13	517.8
Nitrate	μmol l ⁻¹	0.001	0.001	4.482	0.001	0.001	5.283	0.001	0.001	7.128
Redox	mV	37.00	-268.7	213.2	56.70	-179.9	235.6	96.10	-237.0	217.4
Sulphide	μmol l ⁻¹	10.63	0.000	1584	6.879	0.000	237.2	0.906	0.000	1284
Sediment										
D10	μm	82.00	9.818	183.0	114.3	14.94	199.6	54.16	8.260	158.5
D50	μm	176.7	78.50	410.1	208.6	85.69	418.6	103.8	66.58	410.4
D90	μm	366.2	152.6	684.2	411.8	149.4	733.6	239.3	143.4	663.8
Organic matter	% (g:g)	1.036	0.244	7.918	0.541	0.206	4.271	1.797	0.250	6.396
t-Carbon	% (g:g)	0.740	0.122	5.114	0.364	0.100	3.929	1.229	0.160	7.160
t-Nitrogen	% (g:g)	0.034	0.007	0.296	0.020	0.001	0.124	0.035	0.011	0.145
t-Phosphorus	% (g:g)	8.728	2.503	27.43	6.363	2.275	14.02	9.657	2.955	19.32
Hydrodynamics										
Maximum fetch	km	11.40	0.430	658.6	16.94	1.198	984.9	10.89	1.468	984.4
Effective fetch	km	3.126	0.154	47.5	3.788	0.184	25.78	3.055	0.437	61.90
Exposure index*		2	0	3	2	0	3	2	0	4
Tidal location*		0	0	1	0	0	1	1	0	1

* = categorical variable

Results from the single variable logistic regression analyses (table 2.2) show that the parameter for the second-order term (β_2) was not significant in most cases, resulting in sigmoid shaped curves for the majority of the variables. Light attenuation produced the greatest reduction in deviance for *Z. marina* (33.8 %, fig. 2.3A), followed by surface water total nitrogen (17.0 %, fig. 2.3B). Nearly all nitrogen and phosphorus content describing variables (in water layer as well as sediment) were significant for *Z. marina*, revealing a consistently negative effect of both nitrogen and phosphorus on its probability of occurrence. Nitrogen and phosphorus seem much less important for *Z. noltii*. The greatest reduction in deviance was accomplished by tidal location of the sites (24.8 %, fig. 2.3C),

followed by surface water salinity (20.5 %, fig. 2.3D), pore water salinity (20.4 %), pore water nitrate (11.6 %) and surface water pH (11.6 %). The analyses also show that *Z. noltii* mostly prefers the intertidal zone while *Z. marina* has a stronger preference for subtidal areas.

Table 2.2: Results from the logistic regression procedure on single variables for *Z. marina* and *Z. noltii*. R indicates the percentage reduction in deviance from a model with only constant β_0 . Log indicates if a variable was log-transformed (y) are not (n). See table 2.1 for units of the variables. In case of a sigmoid shaped curve (β_2 is not significant), the effect of a variable of seagrass presence is positive when $\beta_1 > 0$.

	<i>Zostera marina</i>					<i>Zostera noltii</i>				
	Log	β_0	β_1	β_2	R (%)	Log	β_0	β_1	β_2	R (%)
Surface water										
Light attenuation	y	3.327	-11.65	–	33.8	n	–	–	–	
Salinity	n	–	–	–		n	-4.458	0.139	–	20.5
pH	n	–	–	–		y	57.98	-61.20	–	11.6
Alkalinity	n	1.626	-0.867	–	5.1	n	–	–	–	
TIC	n	1.448	-0.786	–	4.5	n	–	–	–	
o-Phosphate	y	0.436	-2.383	–	7.9	n	–	–	–	
t-Phosphorus	y	0.811	-2.310	–	9.1	n	–	–	–	
Ammonium	y	2.408	-2.755	–	5.6	n	–	–	–	
Nitrate	n	0.238	-0.143	–	6.4	n	–	–	–	
t-Nitrogen	y	7.198	-4.780	–	17.0	n	–	–	–	
Pore water										
Salinity	n	–	–	–		n	-4.557	0.143	–	20.4
pH	n	–	–	–		n	–	–	–	
Alkalinity	y	3.419	-5.726	–	12.2	n	–	–	–	
TIC	y	3.414	-5.517	–	11.7	n	–	–	–	
o-Phosphate	y	0.927	–	-0.763	9.1	n	–	–	–	
Ammonium	y	1.178	–	-0.375	5.9	n	–	–	–	
Nitrate	n	–	–	–		n	-1.594	0.640	–	11.6
Redox	n	-0.309	0.004	–	4.8	n	-1.366	0.006	–	9.5
Sulphide	n	0.047	-0.003	–	7.8	y	-0.480	-0.690	–	6.1
Sediment										
D10	n	-2.623	0.041	-1.2E-4	14.1	n	-0.232	-0.012	–	5.6
D50	y	-10.56	4.566	–	10.3	y	5.795	-3.145	–	5.5
D90	n	-1.727	0.004	–	5.1	y	10.347	-4.578	–	10.8
Organic matter	y	1.113	-4.035	–	11.8	n	–	–	–	
t-Carbon	y	0.835	-3.974	–	9.9	n	–	–	–	
t-Nitrogen	y	0.427	-33.39	–	7.6	n	–	–	–	
t-Phosphorus	n	1.071	-0.157	–	8.2	n	–	–	–	
Hydrodynamics										
Maximum fetch	n	–	–	–		n	–	–	–	
Effective fetch	n	–	–	–		n	–	–	–	
Exposure index*	–	–	–	–		–	–	–	–	
Tidal setting*		0.241	-1.585		7.9		-2.752	2.959		24.8

* = categorical variable; – = not significant

Multiple logistic regression analysis revealed that most of the variance in species presence could be explained by including only 2 variables in the models of both seagrasses. For *Z. marina*, light attenuation was the most significant parameter in the model. A significant part of the remaining deviance could be explained by including redox in the model as a second parameter. The obtained

model correctly predicted 77% of all presence/absence data and reduced deviance by nearly 38% (table 2.3). Tidal location was the most important variable for predicting *Z. noltii* presence. As for the *Z. marina* model, redox was also adopted by the *Z. noltii* model to improve its explanatory potential. The model predicted over 86% of all observations correctly. Moreover, the model reduced deviance from the data with nearly 34%.

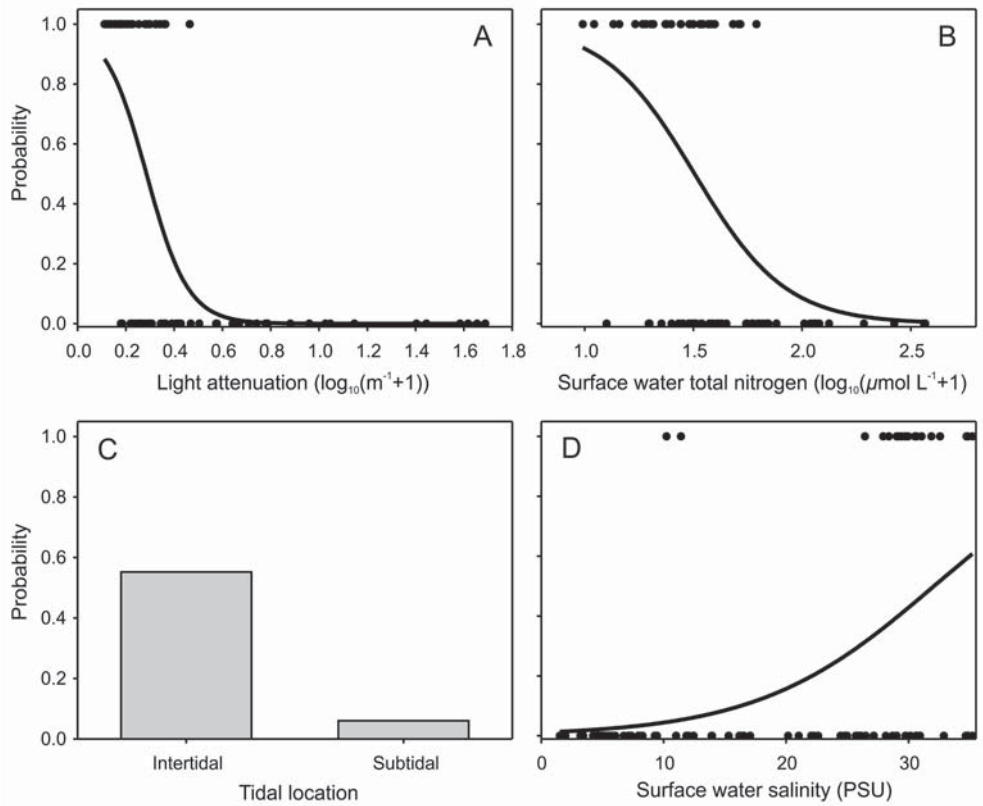


Figure 2.3. Probability of presence for *Z. marina* (A & B) and *Z. noltii* (C & D) plotted against the variables that showed the highest significance. Dots are actual observations (1 = present, 0 = absent). The x-axes for light attenuation and surface water total nitrogen are log-transformed.

Table 2.3: Results from the multivariate logistic regression analysis. Only light attenuation and pore water redox were significant in the model. R indicates the percentage reduction in deviance from a model with only a constant term (Peeters & Gardeniers 1998).

<i>Zostera marina</i>		<i>Zostera noltii</i>	
Constant	3.551	Constant	-3.090
Light attenuation	-12.95	Redox	0.007
Redox	0.005	Tidal location*	3.122
R (%)	37.7	R (%)	33.8
% Correct	77.2	% Correct	86.1

* = categorical variable

Results from the leave-one-out cross-validation (table 2.4) show that the procedure yields a prediction success similar to that of the model constructed from the full dataset (table 2.2). 76% of the observations were correctly predicted for *Z. marina*, while 86% of the predictions were correct for *Z. noltii*. The procedure yields mean parameter values that deviate only slightly from those obtained from the complete dataset. Additionally, standard deviation in the model parameters was low, being 4 and 3% of the parameters mean value at maximum for *Z. marina* and *Z. noltii* respectively.

Table 2.4: Results of the leave-one-out cross-validation procedure. Mean values deviate only slightly from results obtained from the complete dataset (table 2.3) and standard deviation is low for all model parameters.

<i>Zostera marina</i>			<i>Zostera noltii</i>		
	Mean	SD		Mean	SD
Constant	3.556	0.133	Constant	-3.095	0.094
Light attenuation	-12.97	0.545	Redox	0.007	4.12 10^{-4}
Redox	0.005	3.36 10^{-4}	Tidal location*	3.126	0.099
% Correct		75.9	% Correct		86.1

* = categorical variable

Linear regression analysis for included model parameters demonstrated that light attenuation correlated strongest with surface water total nitrogen (table 2.5; fig. 2.4A), followed by total phosphorus (fig. 2.4B). Redox related most to the sulphide concentration in the pore water (table 2.6; fig. 2.4C), but it also showed a reasonably strong correlation with alkalinity (fig. 2.4D) and pore water TIC. Logistic regression analysis demonstrated that tidal location correlated best with pore water salinity (table 2.7; fig. 2.4E) and surface water pH (fig. 2.4F).

Finally, we tested the models obtained from the multivariate logistic regression procedure on the 5 pre-selected locations. Table 2.8 shows the predicted probabilities and actual observations. The *Z. marina* model correctly predicted absence or presence in 4 out of 5 cases. The model produced a false positive reading for Lake Grevelingen, predicting a probability for presence of over 75%. The *Z. noltii* model correctly predicted all selected test sites.

Table 2.5: Results from the linear regression analysis of continuous single variables on light attenuation. Log indicates whether a variable was log-transformed (y) or not (n). a_0 the intercept, a_1 is the slope of the equation. F is the value from the F-test, p is the significance value. See table 2.1 for units of the variables.

	Log	a_0	a_1	F	p	R^2
Surface water						
Salinity	n	–	–	–	–	
pH	n	–	–	–	–	
Alkalinity	n	-13.13	7.670	81.0	<0.000	0.51
TIC	n	-13.52	7.889	85.1	<0.000	0.53
o-Phosphate	n	2.509	0.440	61.6	<0.000	0.44
t-Phosphorus	y	-4.537	17.12	98.1	<0.000	0.56
Ammonium	y	-4.996	9.588	4.4	0.039	0.05
Nitrate	y	-4.069	14.23	21.5	<0.000	0.22
t-Nitrogen	n	-2.736	0.132	207.9	<0.000	0.73
Pore water						
Salinity	y	-4.740	7.417	4.1	0.046	0.05
pH	n	–	–	–	–	
Alkalinity	y	-4.085	12.49	7.0	0.010	0.08
TIC	y	-3.397	11.10	5.6	0.020	0.07
o-Phosphate	y	-6.343	8.695	13.7	<0.000	0.15
Ammonium	y	-7.307	5.989	6.5	0.013	0.08
Nitrate	n	–	–	–	–	
Redox	n	4.448	-0.015	4.0	0.048	0.05
Sulphide	n	3.538	0.005	4.5	0.038	0.05
Sediment						
D10	n	–	–	–	–	
D50	n	9.627	-0.027	4.8	0.032	0.06
D90	n	–	–	–	–	
Organic matter	y	0.600	10.20	6.2	0.015	0.07
t-Carbon	y	0.705	12.29	7.2	0.009	0.09
t-Nitrogen	y	2.479	79.22	4.3	0.041	0.05
t-Phosphorus	n	–	–	–	–	
Hydrodynamics						
Maximum fetch	n	–	–	–	–	
Effective fetch	n	–	–	–	–	

– = not significant

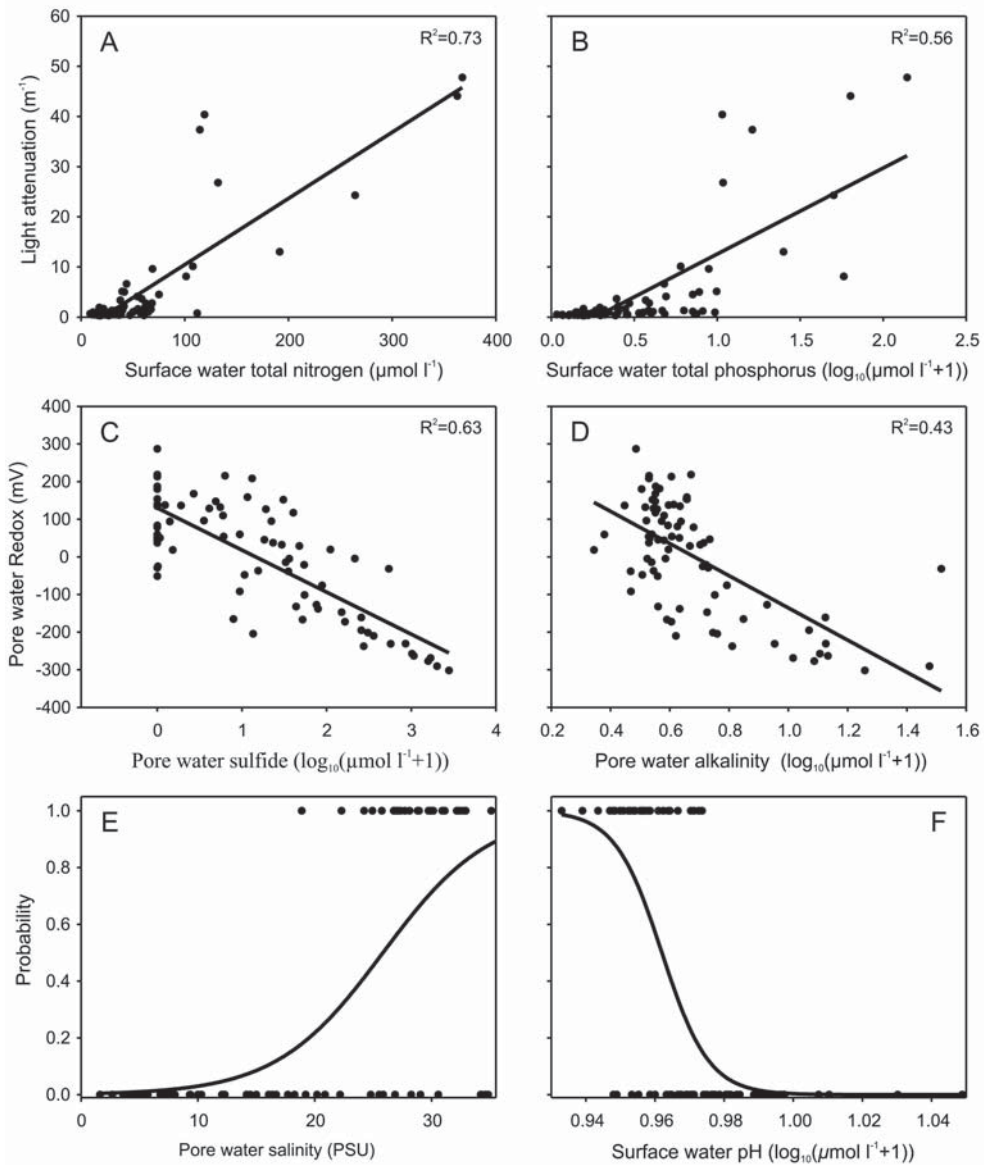


Figure 2.4. A & B: Correlations between light attenuation and its two most significant explaining variables from the single linear regression analysis (table 2.5). **C & D:** Correlations between Redox and its two most significant explaining variables (table 2.6). **E & F:** Correlations between tidal location and the variables that gave the highest reduction of deviance. The y-axis of figs. E & F indicate the probability that the location is situated in the intertidal zone. Apart from total nitrogen (A) and pore water salinity (E), all variables on the x-axes are on a log-transformed scale.

Table 2.6: Results from the linear regression analysis of continuous single variables on redox. See table 2.5 for explanation of abbreviations and table 2.1 for units of the variables.

	Log	α_0	α_1	F	p	R^2
Surface water						
Light attenuation	y	43.11	-96.18	5.4	0.023	0.07
Salinity	n	–	–	–	–	–
pH	n	791.1	-94.59	6.4	0.014	0.08
Alkalinity	n	108.5	-47.38	7.2	0.009	0.09
TIC	n	97.25	-42.75	5.6	0.021	0.07
o-Phosphate	n	14.96	-3.422	10.3	0.002	0.12
t-Phosphorus	n	17.30	-2.653	9.4	0.003	0.11
Ammonium	n	–	–	–	–	–
Nitrate	n	–	–	–	–	–
t-Nitrogen	y	316.4	-197.2	15.5	<0.000	0.17
Pore water						
Salinity	n	–	–	–	–	–
pH	y	-3020	3259	5.0	0.028	0.06
Alkalinity	y	291.9	-427.6	57.4	<0.000	0.43
TIC	y	288.6	-408.7	52.4	<0.000	0.41
o-Phosphate	y	194.5	-156.8	21.2	<0.000	0.22
Ammonium	y	319.0	-162.5	25.8	<0.000	0.25
Nitrate	y	-27.52	253.3	14.5	<0.000	0.16
Sulphide	y	129.9	-111.8	127.6	<0.000	0.62
Sediment						
D10	n	–	–	–	–	–
D50	n	–	–	–	–	–
D90	n	–	–	–	–	–
Organic matter	n	–	–	–	–	–
t-Carbon	n	–	–	–	–	–
t-Nitrogen	n	–	–	–	–	–
t-Phosphorus	n	–	–	–	–	–
Hydrodynamics						
Maximum fetch	n	–	–	–	–	–
Effective fetch	n	–	–	–	–	–

– = not significant

Table 2.7: Correlations between the dichotomous tidal setting parameter and other variables, analyzed with logistic regression. See table 2.2 for explanation of abbreviations and table 2.1 for units of the variables.

	Log	β_0	β_1	R (%)
Surface water				
Light attenuation	y	-1.269	1.571	5.6
Salinity	n	-4.618	0.179	32.2
pH	y	141.8	-147.4	36.9
Alkalinity	n	–	–	
TIC	n	–	–	
o-Phosphate	n	–	–	
t-Phosphorus	y	-1.333	1.460	5.6
Ammonium	n	-1.340	0.077	3.8
Nitrate	n	-1.120	0.130	9.2
t-Nitrogen	n	–	–	
Pore water				
Salinity	n	-5.658	0.219	37.4
pH	n	–	–	
Alkalinity	n	–	–	
TIC	n	–	–	
o-Phosphate	y	-2.836	1.803	8.5
Ammonium	y	-3.230	1.351	5.3
Nitrate	y	-0.895	3.152	7.4
ORP	n	–	–	
Sulphide	n	–	–	
Sediment				
D10	n	2.156	-0.039	32.4
D50	y	14.81	-6.944	20.2
D90	y	14.39	-5.904	15.9
Organic matter	y	-2.877	5.946	24.3
t-Carbon	y	-2.052	4.701	14.9
t-Nitrogen	y	-1.380	33.71	10.5
t-Phosphorus	n	-1.628	0.114	6.5
Hydrodynamics				
Maximum fetch	n	–	–	
Effective fetch	n	–	–	
Exposure index	n	–	–	

– = not significant

Table 2.8: Observed and predicted values from the logistic regression model for the 5 pre-selected test-sites. *P* is the probability (scaled 0 to 1) that either *Z. marina* or *Z. noltii* can grow at a site. A prediction is positive (i.e. 1) when probability *P* is over 0.5.

<i>Zostera marina</i>				<i>Zostera noltii</i>		
	<i>P</i>	Predicted	Observed	<i>P</i>	Predicted	Observed
Balgzand	0.005	0	0	0.697	1	1
De Bol	0.021	0	0	0.025	0	0
Grevelingen	0.751	1	0	0.034	0	0
Mokbaai	0.012	0	0	0.320	0	0
Puck bay	0.593	1	1	0.015	0	0
% Correct			80			100

DISCUSSION

The observed world-wide decline of seagrasses has classically been ascribed to a multitude of environmental factors (Duarte 2002, Orth et al. 2006). In this study, we show through the use of various multiple regression procedures that presence or absence of the temperate species *Z. marina* and *Z. noltii* can be predicted to a large degree of confidence by the use of only two, easy to measure variables (light attenuation/redox and tidal location/redox for *Z. marina* and *Z. noltii* respectively). This is remarkable as all sites from our dataset were sampled only once, thereby probably increasing noise due to infrequent events (e.g., algal blooms, toxicity events, enhanced turbidity with strong winds). Moreover, our analyses of 5 evaluation sites illustrate that the models can be useful tools to monitor (change in) habitat suitability for conservation purposes or for selecting suitable sites for restoration projects. For example, based upon our results, *Z. marina* transplantations at Balgzand or Mokbaai might not have been attempted. Instead, Lake Grevelingen could have been selected as our model indicates that this site is more suitable. Because Lake Grevelingen is a closed-off water body, it is also likely that natural re-establishment from more salt-tolerant *Z. marina* populations like Brittany (France) is difficult at best. This might help explain why the species has not returned to the site and illustrates why restoration may be particularly necessary at this site.

Although both multivariate logistic models include just two variables, our results do not imply that seagrass presence is only dependent on these variables. The analyses merely show that the included parameters are good indicators for general seagrass habitat suitability. Notably, single logistic regression analyses demonstrated that seagrass occurrence correlated with many variables and PCA-analysis showed that these variables were strongly interrelated. For instance, although *Z. marina* presence seems to be most reliant on light availability, it is indirectly dependent on total nitrogen content of the water layer, as this variable could explain variance in light attenuation for 73% in the linear regression analysis.

Linear regression analysis demonstrated that redox showed strong correlations with both pore water sulphide and alkalinity explaining 63 and 43% of the variance respectively in the linear regression procedure. In estuarine sediments these variables are most likely tightly linked together because organic matter breakdown is typically anaerobic. Anaerobic degradation, causes low redox potentials and stimulates release of pH-buffers like bicarbonate and organic acids (Gieskes 1975). Furthermore, the abundant sulphate, which serves as a substitute electron acceptor for oxygen, is reduced to toxic sulphide during the breakdown process (Gieskes 1975). Thus, redox may probably be interpreted as an indicator for toxins and hypoxia in the sediment and, due to diffusion also in the above-lying water (Gieskes 1975). Another reason why redox was included in both multivariate models might be due to interaction with light availability. Apart from passive diffusion of oxygen into the sediment, detoxification of sulphide, organic sulphur compounds and organic acids in seagrasses is dependent on oxygen produced by photosynthesis, that is then transported from the leaves to the root system (Pedersen et al. 2004). This detoxification mechanism is compromised when water column turbidity levels become too high, while it becomes more important as redox

values drop.

While tidal location also correlated well with several other environmental variables, it is most likely that this variable has its strongest effect on *Z. noltii* presence through light availability. During emersion, light availability is very high. Compared to for instance *Z. marina* that was found to prefer the subtidal zone, *Z. noltii* is particularly well adapted to periodical emersion. *Z. noltii* is relatively insensitive to desiccation (Leuschner et al. 1998), has a very high compensation point for photosynthesis (Philippart 1995, Vermaat & Verhagen 1996) and a large demand for CO₂ that is only available during emersion (Leuschner & Rees 1993, Silva et al. 2005). These factors make this species highly suitable for growing in the intertidal zone, but on the other hand less adapted to subtidal life with lower average light conditions and CO₂ availability. Strikingly, none of the included hydrodynamic exposure variables offered a significant fit to the presence/absence data of both seagrass species. This is puzzling at first sight, because hydrodynamics are typically described as important for seagrass development. Although we randomly selected sites in our survey ranging from very protected to exposed to hydrodynamics, we sampled locations that were situated in areas where seagrasses are or have historically been present, as our focus was on finding indicators for seagrass decline. Many of these areas offer a higher degree of protection from hydrodynamics than average for European coasts, hence causing a bias to protected areas in our dataset. Thus, based upon our analysis only, it cannot be concluded that hydrodynamics are not important for seagrasses. Our results simply imply that (altered) hydrodynamics are generally not among the most important variables in explaining the observed distribution of seagrass presence.

In summary, we showed that presence or absence of the temperate seagrass species *Z. marina* and *Z. noltii* in European coastal zones that were historically suitable, can in most cases be accurately predicted by our multivariate logistic regression models. Despite the fact that many variables seem to influence seagrass presence, the models turned out to be remarkably simple as they included only two easy to measure variables, being light attenuation and redox for *Z. marina* and tidal location and redox for *Z. noltii*. These variables are strongly correlated with other variables, and are, therefore, good indicators of general habitat suitability for seagrasses. Finally, we illustrated how both logistic regression models may be applied to evaluate seagrass ecosystem restoration and conservation plans.

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3

Positive feedbacks in seagrass ecosystems – implications for success in conservation and restoration

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ABSTRACT

Seagrasses are threatened by human activity in many locations around the world. Their decline is often characterized by sudden ecosystem collapse from a vegetated to a bare state. In the 1930s, such a dramatic event happened in the Dutch Wadden Sea. Before the shift, large seagrass beds (*Zostera marina*) were present in this area. After the construction of a large dam and an incidence of the “wasting disease” in the early 1930s, these meadows became virtually extinct and never recovered despite restoration attempts. We investigated whether this shift could be explained as a critical transition between alternative stable states, and whether the lack of recovery could be due to the high resilience of the new turbid state. We analyzed the depth distribution of the historical meadows, a long-term dataset of key factors determining turbidity and a minimal model based on these data. Results demonstrate that recovery was impossible because turbidity related to suspended sediment was too high, probably because turbidity was no longer reduced by seagrass itself. Model simulations on the positive feedback suggest indeed the robust occurrence of alternative stable states and a high resilience of the current turbid state. As positive feedbacks are common in seagrasses, our findings may explain both the worldwide observed collapses and the low success rate of restoration attempts of seagrass habitats. Therefore, appreciation of ecosystem resilience may be crucial in seagrass ecosystem management.

INTRODUCTION

Seagrasses are rhizomatous plants, forming extensive meadows in temperate to tropical coastal areas. Their ecosystems are among the most productive ecosystems on earth and serve as key-habitat in the lifecycles of many marine animal species (Duarte 2002, Orth et al. 2006). In the last decades, seagrasses are increasingly threatened by human activities in many locations around the world. These disturbances recently resulted in a widespread loss of habitat, often characterized by sudden ecosystem collapse (Jackson et al. 2001). Once seagrass beds are damaged, restoration costs are high and chances for success are remarkably low (i.e. worldwide around 30% success) (Orth et al. 2006).

Seagrasses are ecosystem engineers in the sense that they significantly modify the abiotic environment of their ecosystem. They lower nutrient levels in the water column (Moore 2004) and attenuate currents and waves (Gambi et al. 1990, Fonseca & Cahalan 1992, Granata et al. 2001). Both mechanisms decrease turbidity through reduction of phytoplankton, epiphytes and suspended sediment (Ward et al. 1984, Twilley et al. 1985, Granata et al. 2001, Kemp et al. 2005). As seagrass is often light limited, their own growth is enhanced by the increased light availability. If this positive feedback is strong enough, theory suggests that it could lead to alternative stable states (Scheffer et al. 2001, Scheffer & Carpenter 2003). This implies that through gradually changing conditions (for instance increasing turbidity), occasionally a critical threshold can be crossed, causing a collapse to an alternative stable state (Scheffer et al. 2001). Such a shift can also be triggered by a sufficiently large perturbation of the ecosystem (for instance by a rampant disease). If the system is close to a critical threshold, resilience is low and only little disturbance is needed to trigger the shift. If subsequently the conditions (e.g. turbidity) improve, they have to progress further than the point of collapse, before recovery may take place, a phenomenon called hysteresis (Scheffer et al. 2001). It is even possible that the shift is irreversible, i.e. that recovery is not possible by improving the conditions (Carpenter et al. 1999). Although these critical transitions are notoriously hard to predict, their implications for ecosystem management (i.e. conservation and restoration) can be profound (Scheffer et al. 2001, Scheffer & Carpenter 2003).

In the Dutch Wadden Sea such dramatic change took place in the 1930s (den Hartog & Polderman 1975, Giesen et al. 1990), when the large seagrass meadows of the species *Zostera marina* (commonly called eelgrass) in this area suddenly collapsed. After the construction of a large dam and a severe occurrence of the wasting disease, these meadows disappeared from the area and never returned despite positive evaluations of the general habitat suitability (van Katwijk et al. 2000) and several restoration attempts since 1950, using both plants and seeds (den Hartog & Polderman 1975, van Katwijk & Hermus 2000).

Here, we determined whether the dramatic decline of the Wadden Sea eelgrass beds and their lack of recovery afterwards can be explained by a catastrophic shift between two alternative stable states. First we show that the turbidity is currently much higher than before the shift. Secondly, we analyzed the cause of the currently high turbidity. Based on these data, we constructed a

minimal computer model describing a positive feedback between eelgrass, hydrography and light availability. We analyzed whether this model can have alternative stable states in realistic parameter ranges. We discuss the role of this feedback in the functioning and restoration of the ecosystem in the light of possible alternative explanations like increased eutrophication and changed hydrodynamics.

STUDY SITE

The Wadden Sea is a largely micro-tidal system (amplitude 1.3 to 2.5 m) sheltered by barrier islands and characterized by shallow intertidal and subtidal mudflats and deeper tidal channels. Here, eelgrass was a widely distributed species at the beginning of the 20th century. In the western part of the Dutch Wadden Sea, eelgrass beds originally covered an area of $\pm 150 \text{ km}^2$ (both subtidal and intertidal) (van Goor 1919). The dramatic collapse of the meadows at the beginning of the 1930s took place within a few years. Their massive die-off has typically been attributed to a combination of factors. In the first place, “wasting disease”, which destroyed many eelgrass stands in the North Atlantic region, reached the Netherlands in that period (den Hartog & Polderman 1975, Giesen et al. 1990). Secondly, the 5-year construction (1927 – 1932) of a large dam (“Afsluitdijk”) also took place at the time, which led to increased turbidity during its construction (den Hartog & Polderman 1975, Giesen et al. 1990). Although populations in other areas recovered from the impact of the wasting disease at the end of the 1930s, the population in this part of the Wadden Sea disappeared nearly completely, leaving only a few sparse intertidal populations that eventually disappeared in the following decades (den Hartog & Polderman 1975, Giesen et al. 1990).

ANALYSIS OF FIELD DATA

Methods. We analyzed two datasets: 1) maps of eelgrass beds in 1930 and 2) a 26-year dataset on water quality from 1980 to 2005. We used these data to estimate the difference in turbidity before and after the 1930s collapse, and to determine the current cause of the high turbidity.

To compare the turbidity in the 1930s with the current situation, we reconstructed the vertical light attenuation based on the historical eelgrass distribution, as there are no historical turbidity data available. For this, we used the eelgrass lower depth limit, defined as the 5% percentile of the eelgrass depth distribution in the early 1930s (in meters below mean water level), calculated from the maps. We assumed that eelgrasses at this lower depth limit needed at least 5.9% of the light entering the water column. This value is rather low as populations in Dutch waters were adapted to relatively poor light conditions (Pellikaan 1980, Giesen et al. 1990). Using these values, the vertical light attenuation could then be calculated using the Lambert-Beer equation.

To determine the historical depth distribution of eelgrass, we used digitized distribution (Reigersman 1939) and tidal (Klok & Schalkers 1980) maps from 1930. These data were combined with a high resolution depth map (20 x 20 m grid-size) from this period (1926 – 1934), which was kindly provided by Rijkswaterstaat (Dutch National Institute for Coastal and Marine Management,

RIKZ). We calculated the eelgrass depth distribution by spatially analyzing the acquired maps using GIS (Geographical Information System) at a resolution of 20 x 20 m.

Phytoplankton and suspended sediments are the primary factors controlling water column turbidity in estuaries (Postma 1961, Colijn 1982, Giesen et al. 1990, Kemp et al. 2005). To determine the relative influence of these factors in the Wadden Sea, we related the vertical light attenuation coefficient to suspended matter and chlorophyll *a*, using linear and nonlinear regression. As a quality measure of our fits we used the F-test and R^2 . Additionally, we determined the relative contribution of chlorophyll *a* to light attenuation, using its specific light attenuation coefficient (Gallegos 2001). For these analyses we used a 26-year dataset (1980 – 2005) from Rijkswaterstaat, which routinely sampled 13 locations scattered over the Wadden Sea area at least 4 times a year. We used year-averaged values in our calculations. Winter months were not excluded, as both summer and winter turbidity levels are important for eelgrass survival (van Goor 1919, Greve & Krause-Jensen 2005).

Internal sediment resuspension on the (shallow) mudflats and sediment transport between the channels and the mudflats are the two dominant processes with regard to turbidity caused by sediment dynamics in the Wadden Sea (Postma 1961, Janssen-Stelder 2000, Christiansen et al. 2006). Therefore, we also related turbidity factors to the depth of the sampling sites. We expected the vertical light attenuation coefficient to increase with decreasing depth as resuspension of fine material increases under the growing influence of wind-driven currents and waves on the sediment bed (Postma 1961, Christiansen et al. 2006, Condie & Sherwood 2006). We acquired the depths of the 13 locations from a high resolution depth map (20 x 20 m grid-size) of corresponding period (1997 – 2002), provided by Rijkswaterstaat.

Results. The eelgrass beds of the 1930s covered an area of 117 km² in the Wadden Sea and Zuiderzee combined. The total surface of the beds that was covered by our tidal and depth maps was 105 km² (fig. 3.1A). 90% of all beds could be found at depths between 0.5 and 2.3 m below mean water level. Over 90% of the beds were located in the subtidal zone. The mean depth of the beds was 1.1 m below mean water level. The distribution was slightly left-skewed with a median depth of 1.0 m below mean water level (fig. 3.1B). We calculated that the vertical light attenuation coefficient was at maximum 1.2 m⁻¹ inside the Wadden Sea eelgrass beds.

The vertical light attenuation coefficient showed good correlation with suspended matter and fitted best using a linear equation (table 3.1; fig. 3.2A). Further analysis revealed that both vertical light attenuation and suspended matter correlated well with depth. Both fitted best to depth by a Monod equation (table 3.1; fig. 3.2B, C). Chlorophyll *A* did not show a significant correlation with vertical light attenuation (table 3.1; fig. 3.2D). Although correlation with depth was not very strong, it was significant (table 3.1). Concentrations of chlorophyll *A* were generally slightly higher in shallow areas. Based on its specific light attenuation coefficient (Gallegos 2001), we calculated an average contribution of 9.7% by chlorophyll *A* to the total vertical light attenuation. This percentage was

highest in the channels and lowest in the shallow areas.

From the equations in table 3.1 we calculate that the present average vertical light attenuation coefficient at the lower eelgrass depth limit (2.3 m) is 6.8 m^{-1} , indicating that turbidity increased nearly 6 times since the 1930s (i.e. maximum estimated at 1.2 m^{-1}). This increase in turbidity since the 1930s cannot be explained by increased turbidity levels of the water entering the Wadden Sea. Water in channels with depths of over 30 m forms a good conservative proxy for the turbidity of water entering the Wadden Sea. The vertical light attenuation in these channels is currently as low as 0.8 m^{-1} or less. Even when we assume that the channels in the 1930s contained no suspended matter at all, calculations based on the background vertical light attenuation for seawater ($0.3 - 0.4 \text{ m}^{-1}$) ((Colijn 1982, Gallegos 2001); this study, table 3.1) clearly illustrate that turbidity in the channels increased much less compared to the shallow areas. Here, turbidity levels increased at maximum 2 to 3 times.

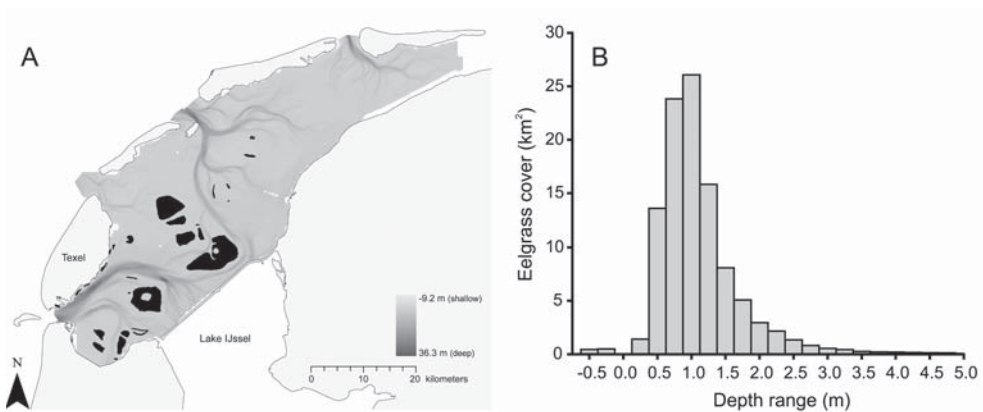


Figure 3.1. A: Distribution of the examined historical eelgrass beds (black areas) on the 1930 depth map (m below mean water level). The indicated beds completely disappeared due the 1930s collapse. The topographical map is from the present situation, showing the position of the Afsluitdijk and Lake IJssel (the former Zuiderzee). **B:** Eelgrass depth distribution below mean water level in the 1930s. The total covered area of the analyzed beds was 105 km^2 , with a mean depth of 1.1 m. The distribution was slightly left-skewed with a median of 1.0 m.

Table 3.1: Correlations of fitted equations and parameter values, describing relations between light attenuation (k) and suspended matter (SS), light attenuation and depth (D), suspended matter and depth, light attenuation and chlorophyll A (ChP) and chlorophyll A and depth. Plots are shown in figure 4.2.

	Equation type	R^2	F	p	Intercept	Slope	Maximum level	Half-saturation
$k - SS$	Linear	0.64	466.2	<0.001	0.28 m^{-1}	$36.1\text{e-}3 \text{ l mg}^{-1}$	–	–
$k - D$	Monod	0.79	1053	<0.001	–	–	18.58 m^{-1}	1.34 m
$SS - D$	Monod	0.60	464.0	<0.001	–	–	192.28 mg l^{-1}	4.81 m
$k - \text{ChP}$	Linear	0.03	2.57	0.110	2.96 m^{-1}	$-34.3\text{e-}3 \text{ l } \mu\text{g}^{-1}$	–	–
$\text{ChP} - D$	Linear	0.05	13.18	<0.001	$13.48 \text{ } \mu\text{g l}^{-1}$	$-119\text{e-}3 \text{ } \mu\text{g m}^{-1} \text{ l}^{-1}$	–	–

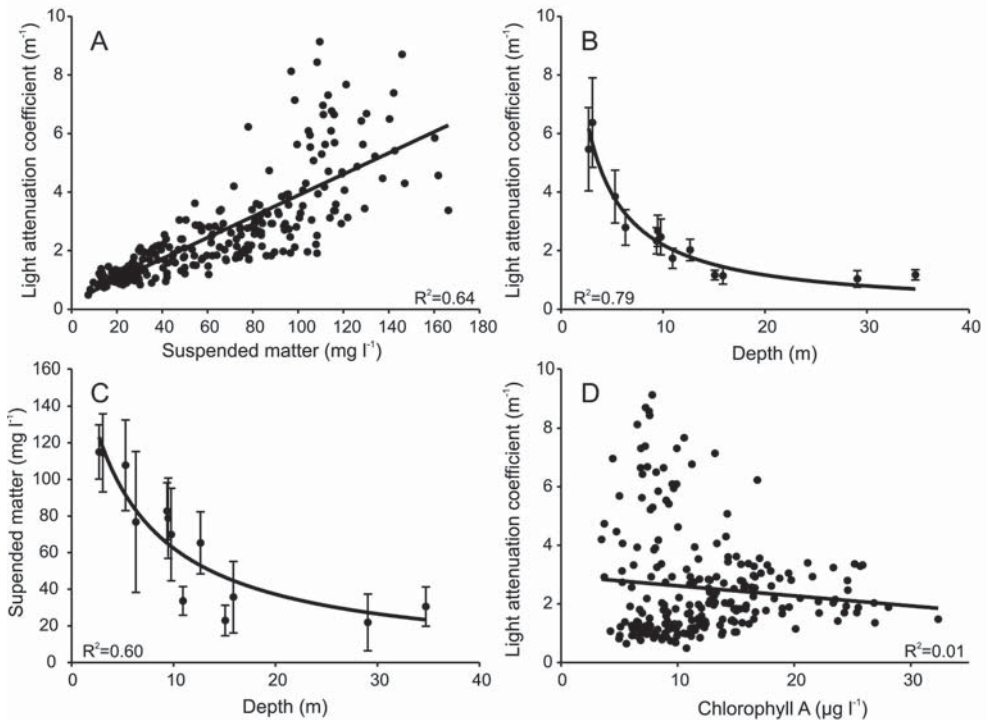


Figure 3.2: Relations between light attenuation (k), suspended matter (SS), chlorophyll A (ChP) and depth (D), based on data collected in 1980 – 2005. Light attenuation fitted well to suspended matter (A) but not to chlorophyll A (D). Both light attenuation and suspended matter correlated well with depth (B & C respectively). The error bars indicate standard deviations. Regression data are summarized in table 3.1.

A SIMPLE MODEL

Description. Analysis of our field data shows that the influence of phytoplankton on turbidity in the Wadden Sea is negligible and that suspended sediment is the dominant factor determining suspended matter concentrations and light availability. The sediment concentration increases with

decreasing depth as resuspension of sediment by a combination of waves and currents increases (Postma 1961, Christiansen et al. 2006, Condie & Sherwood 2006). The excessive increase in turbidity in shallow areas compared to turbidity levels in the channels indicates that the extensive eelgrass meadows of the 1930s significantly reduced turbidity within the beds through sediment retention. We constructed a simple model to explore the positive feedback between eelgrass shoot density (Z) and sediment-related water clarity based on logistic growth:

$$\frac{dZ}{dt} = r f(I) Z - \frac{r-m}{K} Z^2 - mZ \quad (1)$$

In this model, the maximum gross growth rate r is light limited ($f(I)$). We assumed a constant mortality rate m due to senescence. Intraspecific competition causes the population to grow to the carrying capacity K . For light limitation $f(I)$ we used the P-I (Photosynthetic Irradiation) curve for eelgrass (Zimmerman et al. 1995):

$$f(I) = 1 - e^{(-I/I_k)} \quad (2)$$

where I_k is the irradiance saturation constant. We assume that light intensity (I) at the bottom (D) determines the eelgrass growth rate (Verhagen & Nienhuis 1983). This value is calculated from the vertical light attenuation coefficient (k) and incoming irradiation (I_0) using the Lambert-Beer equation. The light attenuation coefficient is linearly dependent on the amount of suspended sediment SS in the water column (table 3.1; fig. 3.2A). In our model, the suspended sediment concentration depends on the shear stress on the sediment bed. The suspended sediment concentration is constant (SS_b) below the critical bed shear stress boundary τ_0 . Above the threshold, the amount of eroded sediment per m^2 that is suspended in the water column, relates to bed shear stress in a linear fashion (Houwing 1999). The total suspended sediment concentration can be obtained by dividing this relation with depth and adding the outcome to the background level:

$$SS = SS_b + \frac{p\tau - q}{D} \quad \text{if } \tau > \tau_0 \quad (3)$$

with p , q and τ_0 as constants depending on the sediment type. We used general formulations to describe the combined effect of currents and waves on the bed shear stress and assumed a collinear flow of waves and currents. Total bed shear stress (τ) is defined as the sum of the shear stress caused by currents (τ_c) and waves (τ_w) (Davies & Lawrence 1994, Janssen-Stelder 2000):

$$\tau = \tau_c + \tau_w \quad (4)$$

The bed shear stress caused by currents is described as (van Rijn 1990, Kleinhans & Grasmeijer 2006):

$$\tau_c = \rho g \frac{U_c^2}{C^2} \quad (5)$$

Here ρ is the fluid density, g is the gravitational acceleration, U_c is the depth averaged current velocity and C is the Chézy grain roughness (van Rijn 1990, Kleinhans & Grasmeijer 2006):

$$C = 18 \log_{10} \frac{12D}{k_{sc}} \quad (6)$$

where k_{sc} is the grain roughness constant for currents. We used the 90% cumulative grain size distribution (d_{90}), recommended for a flat bed (van Rijn 1990).

The wave related bed shear stress is calculated as (van Rijn 1990, Davies & Lawrence 1994, Kleinhans & Grasmeijer 2006):

$$\tau_w = \frac{1}{2} \rho f_w U_w^2 \quad (7)$$

where U_w is the orbital velocity amplitude. The friction coefficient f_w is described as (van Rijn 1990, Kleinhans & Grasmeijer 2006):

$$f_w = \exp \left[-6 + 5.2 \left(\frac{(U_w T) / 2\pi}{k_{sw}} \right)^{-0.19} \right] \quad (8)$$

with T as the wave period and k_{sw} is the grain roughness for waves ($2.5 d_{50}$). Finally, current and orbital velocity depend on the eelgrass shoot density. Based on multiple sources, it can be concluded that shoot density related attenuation per meter in the canopy, of both currents (Gambi et al. 1990, Worcester 1995, Peterson et al. 2004) and waves (Bouma et al. 2005), can be readily described by Monod equations (fig. 3.3):

$$U = U_{max} \frac{h_z}{h_z + Z} \quad (9)$$

where U is the actual current (U_c) or orbital (U_w) velocity, U_{max} is the maximum (free-stream) current (U_{cmax}) or orbital (U_{wmax}) velocity and h_z is the eelgrass half-constant for either currents (h_{cz}) or waves (h_{wz}). In our model we consider conditions one meter from the edge of the bed. We assume that any excess sediment particles entering our eelgrass bed will settle in the outline of the bed as observed in the historical meadows (Oudemans 1870), and that shoots in this area are supported through clonal translocation of resources (Marbà et al. 2006). Parameter settings, variables and units from our model analyses are presented in table 3.2.

Sensitivity analysis. We chose default parameter settings to mimic average Wadden Sea conditions, based on literature and our field data analysis (table 3.2). In a sensitivity analysis we analyzed the robustness of the results by varying settings of key parameters in the model. In this analysis, critical thresholds were determined by the following numerical procedure: a key parameter value was increased in small steps. After each step, the model was run to stabilize to its equilibrium point. For every step, the equilibrium biomass was plotted to find a discontinuity (critical threshold, also called a bifurcation point). Subsequently, this analysis was also performed in a backward manner (i.e. a decrease in small steps) to find the other critical (return) threshold. By combining these analyses we constructed two-dimensional bifurcation plots of various parameters.

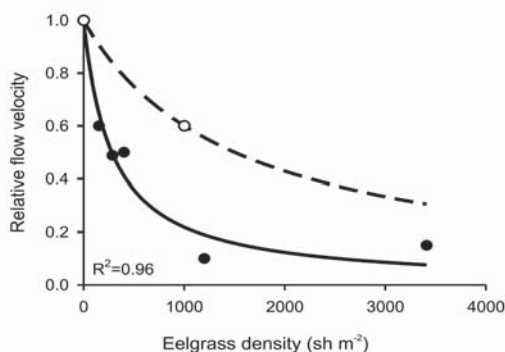


Figure 3.3: The relative remaining flow velocity (i.e. U_{wmax} & U_{cmax} = 1 in the absence of vegetation) as a function eelgrass density, one meter into the bed. The relation between U_{cmax} and eelgrass shoot density (closed dots) is based on multiple sources (Gambi et al. 1990, Worcester 1995, Peterson et al. 2004). h_{cz} has a value of 281 sh m⁻². $R^2 = 0.96$; $F = 87.80$; $p = 7e-4$. The relation between U_{wmax} and eelgrass density (open dots) was derived from data of Fonseca and Cahalan (Fonseca & Cahalan 1992). Results from Bouma et al. (Bouma et al. 2005) revealed that wave attenuation at different shoot densities can be described by a Monod equation. h_{wz} has a value of 1500 sh m⁻².

Results. The model has two stable equilibriums at the default parameter settings (fig 3.4A). The population will crash if shoot densities are below the unstable equilibrium which is between both stable equilibriums at a density of 988 sh m⁻². Above this threshold, the population will stabilize to the carrying capacity (3370 sh m⁻²).

Alternative equilibriums can be found over a wide range of current and orbital velocities. Hysteresis occurs from 0.06 to 0.39 m/s for orbital velocity (fig. 3.4B) and from 0 to 3.72 m s⁻¹ for current velocity (fig. 3.4C). In these ranges both eelgrass dominance and a bare state are stable. An eelgrass population with high shoot density reduces currents and waves (and thereby turbidity) enough to sustain itself. The population collapses when a disturbance, for instance disease, pushes the shoot density below the critical threshold (fig. 3.4B, C; dashed lines). Past the bifurcation points ($U_{wmax} = 0.39$ m s⁻¹ and $U_{cmax} = 3.72$ m s⁻¹) the population cannot reduce hydrodynamics enough to keep turbidity low. From these thresholds on, the only stable state is the bare state. When the population has collapsed, recovery can only take place when the orbital velocity is reduced below 0.06 m s⁻¹. Interestingly, the current velocity graph shows a non-reversible alternative equilibrium, i.e. it is impossible to recover the vegetated state by reducing the current velocity alone. This is because the default orbital velocity (0.2 m s⁻¹) is high enough to prevent recovery, even when current velocity is zero.

Simulations on the interactive effect of current and orbital velocity reveal that orbital velocity needs to be below average Wadden Sea conditions in order to facilitate eelgrass colonization (fig. 3.5A). Current velocity should remain below 0.37 m s⁻¹ and should even be lower if orbital velocity is above zero. Alternative equilibriums can be found over a wide range of current and orbital velocities.

We analyzed the influence of minimum levels of suspended sediment by varying the background sediment concentration constant SS_b between 0 to 120 g m⁻³ at different values for orbital velocity (fig. 3.5B). If sediment concentrations are low, an established eelgrass bed tolerates high orbital velocities. The critical orbital velocity for vegetation decreases as sediment

Table 3.2: Variables and parameters used in the model. Values for I_k and I_0 were converted into $\mu\text{mol m}^{-2}\text{s}^{-1}$ PAR (Photosynthetically Active Radiation) using standard conversion factors (Ross & Sulev 2000). Sources: *) This study, 1) Verhagen & Nienhuis 1983, 2) van Lent & Verschuure 1994, 3) Royal Dutch Meteorological Institute 2006, 4) Houwing 1999, 5) Christiansen et al. 2006, 6) Janssen-Stelder 2000, 7) Gambi et al. 1990, Worcester 1995, Peterson et al. 2004, 8) Fonseca & Cahalan 1992, Bouma et al. 2005. ^a indicates either a direct value from the source or a value obtained via straightforward calculations, ^b is a value derived from the source.

Values		Unit	Description	Sources
Default	Tested range			
Variables:				
Z		sh m^{-2}	Eelgrass density	
I		$\mu\text{mol m}^{-2} \text{s}^{-1}$	Light availability in PAR	
SS		g m^{-3}	Suspended sediment concentration	
U_c		m s^{-1}	Current velocity	
U_w		m s^{-1}	Orbital velocity	
K		m^{-1}	Light attenuation coefficient	
τ		Pa (N m^{-2})	Total bed shear stress	
τ_c		Pa (N m^{-2})	Bed shear stress by currents	
τ_w		Pa (N m^{-2})	Bed shear stress by waves	
C		$\text{m}^{1/2} \text{s}^{-1}$	Chézy grain roughness	
f_w			Friction coefficient	
Parameters:				
r	0.08	day^{-1}	Maximum growth rate	1 ^a
m	0.013	day^{-1}	Mortality rate	1 ^a
K	3500	sh m^{-2}	Carrying capacity	1 ^a , 2 ^a
I_k	31.63	$\mu\text{mol m}^{-2} \text{s}^{-1}$	Saturation irradiance in PAR	1 ^a
I_0	372.61	$\mu\text{mol m}^{-2} \text{s}^{-1}$	Year-averaged irradiation at the surface in PAR	3 ^a
D	1.0	0.5 – 3.0 m	Depth below the mean water level	*
a	0.28	m^{-1}	Background light attenuation coefficient	*
b	36.1e-3	$\text{m}^2 \text{g}^{-1}$	Constant in suspended matter – light attenuation equation	*
SS_b	26.2	0 – 120 g m^{-3}	Minimum SS. The default value is based on levels in the historical eelgrass beds	*
p	14133	g N^{-1}	Sediment erosion constant (eq. 3)	4 ^a , *
q	2120	g m^{-2}	Sediment erosion constant (eq. 3)	4 ^a , *
τ_0	0.15	0.01 – 1 Pa (N m^{-2})	Critical bed shear stress boundary	4 ^a
ρ	1000	kg m^{-3}	Fluid density	
g	9.81	m s^{-2}	Gravitational acceleration	
d_{90}	110e-6	m	90% percentile cumulative grain size distribution	4 ^a
d_{50}	80e-6	m	50% percentile cumulative grain size distribution	4 ^a
T	1.2	s	Wave period	5 ^a
U_{cmax}	0.3	0 – 5 m s^{-1}	Maximum (free-stream) flow velocity	6 ^a
U_{wmax}	0.2	0 – 1 m s^{-1}	Maximum orbital velocity amplitude	5 ^a , 6 ^a
h_{cz}	281	sh m^{-2}	Eelgrass density where U_{cmax} is reduced by 50%	7 ^b , *
h_{wz}	1500	sh m^{-2}	Eelgrass density where U_{wmax} is reduced by 50%	8 ^b , *

concentrations increase. If background levels of suspended sediment increase above 108 g m^{-3} , seagrass cannot reduce light availability enough for a sustainable population. The effect of water depth is very similar to the effect of background suspended sediment concentrations as light availability is determined by a combination of both factors. In the analysis, depth was varied between 0.5 and 3 m below mean water level. At shallow depths hysteresis occurs between 0.12 and 0.40 m s^{-1} for U_{wmax} (fig. 3.5C). For U_{cmax} these values are 0.35 and 4.47 m s^{-1} (not shown). At greater depths, the potential for alternative equilibria significantly decreases.

The effect of different degrees of cohesiveness of the sediment was simulated by varying the critical shear stress boundary (τ_0). τ_0 was tested in the range of 0.01 to 1 Pa, which can be regarded as values common for different types of sediment (Houwing 1999). Results show that at low values for τ_0 , establishment of an eelgrass bed is much more difficult than at high values (fig. 3.5D). The

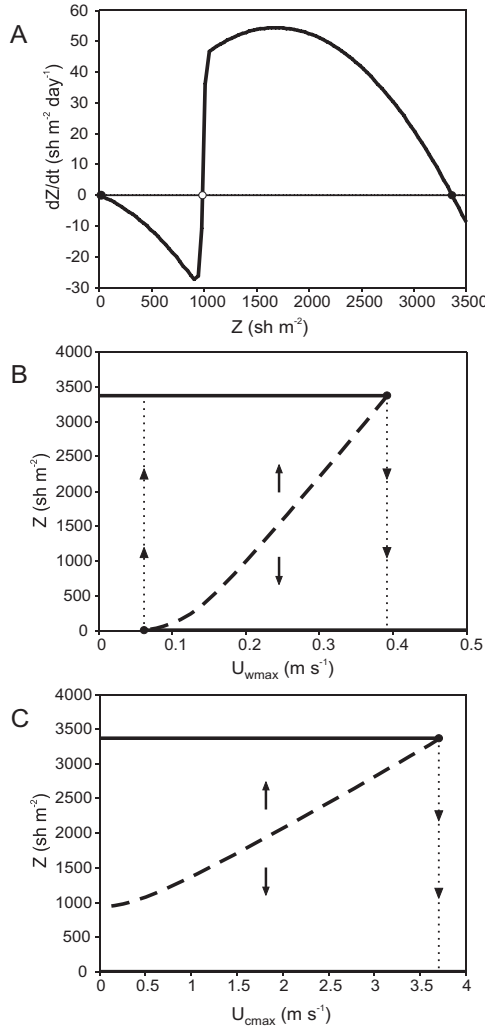


Figure 3.4. A: The change in shoot density per day (dZ/dt) for different shoot densities (Z) at default parameter settings. The dots indicate equilibrium points; the open dot represents an unstable equilibrium. Figures **3B & C** show the stable states for different maximum orbital (U_{wmax}) and current (U_{cmax}) velocities respectively. The dot indicates the bifurcation points; arrows indicate the direction of change. The dashed equilibriums are unstable. Note that the bifurcation point of 3.72 m s^{-1} for current velocity is unrealistically high for the Wadden Sea as currents reach velocities of at maximum 2 m s^{-1} in this system (Rijkswaterstaat).

hysteresis range increases when τ_0 values increase.

The most important thing to note from the bifurcation analysis is that the occurrence of alternative stable states is very robust. They occur over a wide range of maximum current and orbital velocities, even when crucial environmental parameter settings are changed.

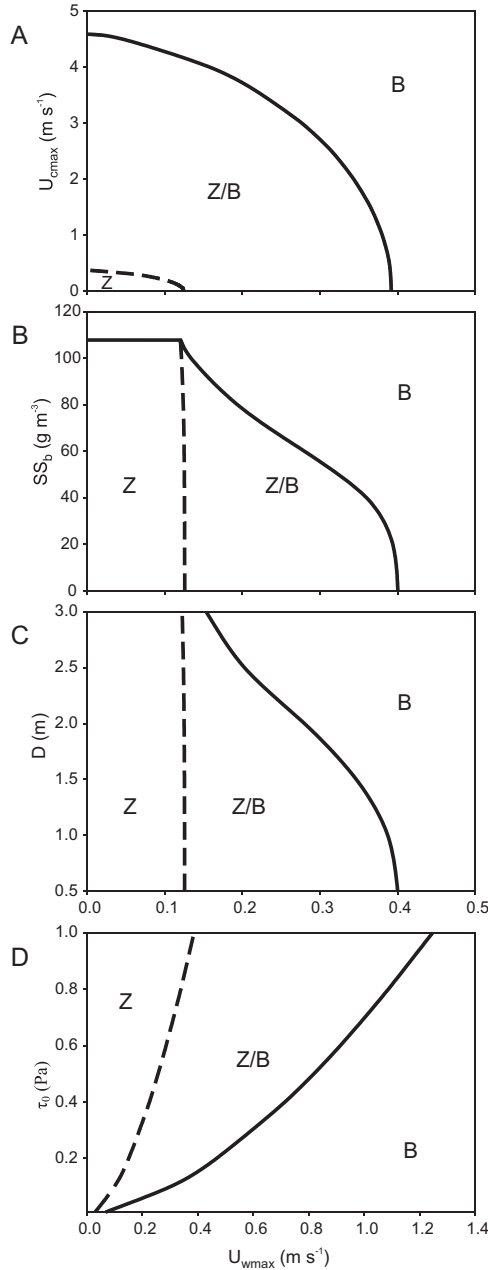


Figure 3.5. Bifurcation analysis on the maximum orbital velocity (U_{wmax}) for varying current velocity (U_{cmax}), depth (D), background levels of suspended sediment (SS_b) and the critical bed shear stress boundary (τ_c). *B* indicates a bare state; *Z/B* indicates the area where alternative stable states occur. Left of the dashed lines (indicated with *Z*), eelgrass presence is the only stable state. To get a clear view on the model behaviour, we set current velocity to zero when analyzing the effects on orbital velocity and vice versa. Note that at a set wave height, the near-bed orbital velocity decreases with depth. This effect is ignored in figure 5B, as we were solely interested in examining the sensitivity of the model to varying depths.

DISCUSSION

Marine ecosystems were among the first ecosystems to illustrate the potential importance of alternative equilibriums. Evidence has been presented from coral reefs, soft sediments and rocky substrates from both the subtidal and intertidal (Knowlton 2004). Remarkably, detailed studies on the subject in seagrass ecosystems are lacking, though there are many studies pointing at a potential positive feedback in seagrasses. From tropical to temperate species, positive effects on water quality (Moore 2004), hydrodynamics (Gambi et al. 1990, Fonseca & Cahalan 1992, Granata et al. 2001, Bos & van Katwijk 2007) and substrate (Ward et al. 1984, Gacia & Duarte 2001, Kemp et al. 2005) have been demonstrated. Our study suggests that this positive feedback is most likely strong enough to lead to alternative equilibriums in many seagrass ecosystems. This result has important implications for the management and restoration of seagrass communities in general. As shown in this study on the Wadden Sea, it might help to explain dramatic shifts such as the 1930s decline of eelgrass in the Dutch Wadden Sea and lack of recovery despite intense restoration efforts.

Our study suggests that the Wadden Sea eelgrass beds could not recover due to threshold behaviour, caused by positive feedback mechanisms in the system. In the absence of eelgrass, current and wave velocities were no longer reduced and suspended sediment concentrations and related turbidity levels became too high to sustain eelgrass growth. However, an alternative explanation could be that the conditions in general have changed in such way that eelgrass cannot grow. Indeed, the lack of recovery since the 1930s has been typically attributed to increased turbidity levels (Giesen et al. 1990). Even for the intertidal beds, light conditions proved to be too poor. The eelgrass lower depth limit for obtaining sufficient light shifted up high into the intertidal zone, beyond the upper depth limit marked by the intertidal beds. Thus, required emersion periods became too long for eelgrass to survive, as desiccation becomes a limiting factor for eelgrass at these shallow depths (Giesen et al. 1990). The poor light conditions were allegedly caused by eutrophication related high phytoplankton levels and dredging and sediment extracting activities (Giesen et al. 1990). Additionally, enhanced deposition of fine material from the river Rhine (de Jonge & de Jong 2002) and erosion caused by increased tidal currents due to the Afsluitdijk construction, may also have led to an increase in turbidity in the western part of the Wadden Sea (den Hartog & Polderman 1975, Giesen et al. 1990). Our analyses suggest that phytoplankton had no significant part in the failure of eelgrass to recover, as turbidity is only weakly related to phytoplankton. We show that the main factor determining turbidity in shallow areas is suspended sediment, a factor that is closely related to depth. This can be explained by an increasing shear stress on the sediment bed with decreasing depth, caused by the combined effects of wind-driven currents and waves (Postma 1961, Houwing 1999, Christiansen et al. 2006, Condie & Sherwood 2006). The effect of enhanced deposition from the river Rhine seems moderate at a large scale. Data used in our analyses include locations from both the western and the eastern part of the Wadden Sea, and is not restricted to the western part that is more influenced by the river. The relations we derived seem to apply to the Dutch Wadden Sea as a whole, irrespective of local

differences in sediment composition. Finally, increased tidal currents are unlikely to have caused substantial additional sediment erosion. Even at present conditions, tidal current velocities alone are not nearly strong enough to initiate sediment erosion. Instead, erosion in the Wadden Sea is primarily controlled by weather conditions (e.g. wind-driven currents and waves) (Janssen-Stelder 2000, Christiansen et al. 2006).

Our model cannot determine what triggered the disappearance of the eelgrass beds in the 1930s. We can only show that there is probably a critical density of eelgrass below which the population collapses. In the 1930s, there were two external impacts that may have pushed the eelgrass population below this critical density: the wasting disease and the raised concentrations of suspended sediment caused by the Afsluitdijk construction (Giesen et al. 1990).

As our results suggest that eelgrass recovery at default model settings is very difficult, it raises the question of how eelgrass colonized the Wadden Sea in the first place. First of all, it is obvious that the conditions in the Wadden Sea are not homogeneous, whereas our predictions were made with average conditions. This means that colonization may still be possible in optimal areas (e.g. sheltered areas with firm sediment and low concentrations of suspended solids). Secondly, it should be noted that in the past, eelgrass could develop in much calmer hydrodynamic conditions in this region. Around 7000 years ago, sheltered tidal basins developed behind an extensive barrier coast when the rate of the sea level rise in that era decreased (van der Molen & van Dijck 2000). It seems likely that initial colonization had already taken place millennia ago in these ancient lagoons (Olsen et al. 2004).

Though our analysis suggests that alternative stable states occur over a wide range of parameter settings, some processes are represented in a very simple way in our model. The most significant simplification is that we disregarded a possible turbidity gradient from the edge to the centre of the bed. Turbidity of the water entering the bed may be higher due to sediment resuspension in adjacent barren areas. Water clarity towards the centre will increase as suspended particles settle due to reduced hydrodynamics. By considering conditions near the edge of the bed we underestimated the general clearing effect of large eelgrass beds. These effects may be described by a spatially explicit model. However, at present the spatial processes involved are poorly understood. Calculations on particle settling velocities and travelling distance into the bed are extremely complex, depending on particle size and shape (Le Roux 2005), particle flocculation (Pejrup & Edelvang 1996, Edelvang & Austen 1997), water turbulence (Ward et al. 1984, Granata et al. 2001), flow velocity (Ward et al. 1984, Gambi et al. 1990, Fonseca & Cahalan 1992, Granata et al. 2001) and direct trapping of particles by seagrass canopies (Agawin & Duarte 2002).

In recent decades, restoration and protection of seagrasses have gained a strong foothold in the ecosystem management of marine ecosystems worldwide (Orth et al. 2006) as well as in the Wadden Sea (van Katwijk & Hermus 2000, van Katwijk et al. 2000). A better understanding of feedbacks and threshold behaviour in seagrasses is important as the lack of recognition may in part explain the seemingly high resilience, related sudden collapses (Jackson et al. 2001) and the high

number of failed restoration attempts that are observed worldwide (Orth et al. 2006). Currently, numerous restoration projects, using a wide variety of transplantation techniques, are being planned mostly at small scales (<1 ha) (Orth et al. 2006). Our results suggest, that it is in fact this small scale that may be responsible for the low overall success rate, as small populations cannot modify their environment sufficiently to cross critical thresholds. As our model is not spatially explicit, we can only speculate on the minimum viable population size for either transplantation or conservation efforts in seagrass ecosystems. Much will depend on the maximum expansion rate of the meadow under consideration and the spatial exchange rates of both recourses and stressors (van Nes & Scheffer 2005). With respect to the Wadden Sea situation, we estimate that future transplantations should be at least in the order of several hectares as turbidity in adjacent bare areas will be high and rhizotomous expansion rates near de edges will be relatively slow. Currently, such large-scale transplantations are most likely not feasible. Therefore, restoration may only be possible if the water layer in a considered area is temporally cleared using devices such as enclosures or artificial seagrass in order to obtain a sufficiently large eelgrass meadow that can sustain itself.

In other ecosystems, success rate of restoration is often related to the scale of management measures. In for instance shallow lake management, where the relevance of alternative stable states has been recognized for over a decade (Scheffer et al. 1993), a shift to clear water can only be achieved by large-scale manipulation, targeting the trophic structure of the ecosystem as a whole (Meijer et al. 1999). In this perspective, some important implications for the management of ecosystems emerge from these studies. 1) The possibility of positive feedback mechanisms in a target area should be studied to improve chances for conservation or restoration efforts. 2) Where there is an important feedback, the transplanted population size (and density) should be adjusted according to the ecosystem resilience to invoke a shift, whereas in conservation the focus should be on preventing the system from exceeding the critical threshold in the first place.

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4

Toxicity of reduced nitrogen in eelgrass (*Zostera marina*) is highly dependent on shoot density and pH

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ABSTRACT

In sheltered, eutrophicated estuaries, reduced nitrogen (NH_x), and pH levels in the water layer can be greatly enhanced. In laboratory experiments, we studied the interactive effects of NH_x , pH, and shoot density on the physiology and survival of eelgrass (*Zostera marina*). We tested long-term tolerance to NH_x at pH 8 in a 5-week experiment. Short-term tolerance was tested for two shoot densities at both pH 8 and 9 in a 5-day experiment. At pH 8, eelgrass accumulated nitrogen as free amino acids when exposed to high loads of NH_x , but showed no signs of necrosis. Low shoot density treatments became necrotic within days when exposed to NH_x at pH 9. Increased NH_3 intrusion and carbon limitation seemed to be the cause of this, as intracellular NH_x could no longer be assimilated. Remarkably, experiments with high shoot densities at pH 9 showed hardly any necrosis, as the plants seemed to be able to alleviate the toxic effects of high NH_x loads through joint NH_x uptake. Our results suggest that NH_x toxicity can be important in worldwide observed seagrass mass mortalities. We argue that the mitigating effect of high seagrass biomass on NH_x toxicity is a positive feedback mechanism, potentially leading to alternative stable states in field conditions.

INTRODUCTION

Seagrass meadows in tropical to temperate coastal areas are among the most productive ecosystems on earth, and harbour high biodiversity. They provide forage and refuge for a vast number marine animal species (Duarte 2002, Orth et al. 2006). At present, these ecosystems are increasingly being lost worldwide, often characterized by mass mortality of seagrasses (Jackson et al. 2001). Multiple stressors have been identified as causes for the decline (Duarte 2002, Orth et al. 2006). Seagrass losses have been ascribed to climate change (e.g., temperature and sea level rise), sedimentation, disease, turbidity, and toxicity events (Duarte 2002, Orth et al. 2006). In temperate estuaries, (anthropogenic) eutrophication is one of the most important processes fuelling turbidity and toxicity events (Goodman et al. 1995, Azzoni et al. 2001, Nielsen et al. 2002, Kemp et al. 2005, Perez et al. 2007). Seagrass mass mortality in these areas has been linked with high concentrations of sulphide in the sediment pore water near the end of the growing season often in combination with high turbidity levels in the water layer (Carlson et al. 1994, Goodman et al. 1995, Azzoni et al. 2001, Pedersen et al. 2004). However, elevated levels of inorganic nitrogen in the water layer may also be important. These compounds have been shown to cause severe toxicity in seagrasses and may therefore help explain these dramatic events (Burkholder et al. 1992, van Katwijk et al. 1997, Bird et al. 1998, Brun et al. 2002).

In this perspective, toxicity caused by reduced nitrogen compounds (NH_x) is particularly interesting, as it is a common phenomenon described for a vast number of aquatic and terrestrial plant species (Britto & Kronzucker 2002). In seagrasses, ammonium (NH_4^+) is taken up directly by both leaves and roots (Iizumi & Hattori 1982, Thursby & Harlin 1982, Rubio et al. 2007). Even though NH_4^+ is preferred over nitrate (NO_3^-) as a nutrient source (Touchette & Burkholder 2000), it has been found to cause severe toxicity within weeks when concentrations become too high (van Katwijk et al. 1997, Bird et al. 1998, Brun et al. 2002). In seagrass beds growing in sheltered eutrophicated estuaries, NH_x concentrations may be greatly enhanced ($>200 \mu\text{mol l}^{-1}$) by degradation processes inside competing macroalgal mats (Hauxwell et al. 2001), discharges of waste and river water (Brun et al. 2002) or due to natural die-off of phytoplankton, macroalgae, or seagrass itself near the end of the growing season (Landers 1982, Farnsworth-Lee & Baker 2000). Additionally, the pH in some of these stagnant lagoons may rise up to 9 or even 10 during daytime due to photosynthesis by the same species (Choo et al. 2002, Beer et al. 2006, Feike et al. 2007; our unpublished data). This in turn increases concentrations of highly toxic gaseous ammonia (NH_3), which is converted from ammonium as the pH level rises (Farnsworth-Lee & Baker 2000, Körner et al. 2001, Körner et al. 2003, Nimptsch & Pflugmacher 2007).

As NH_x is both a valuable nutrient source and a toxic substance for eelgrass, we hypothesize that its toxicity is dependent on the shoot density in meadows. At a certain load, NH_x concentrations in the canopy will most likely be significantly lower in high shoot density meadows compared to beds with low shoot densities, because concentrations will be actively lowered through the direct uptake of NH_x by the leaves (Iizumi & Hattori 1982, Thursby & Harlin 1982). Thus, it can be expected

that a certain load may cause severe toxicity in a low density bed whereas the effect will be much less severe or even absent in a high density seagrass bed.

In this study, we investigated the interactive effects of NH_x , pH and shoot density in the water layer on the physiology and survival of the seagrass *Zostera marina* (commonly called eelgrass). We conducted two laboratory experiments. First, we tested the eelgrass long-term tolerance for NH_x at natural sea water pH (pH 8.1). Secondly, we conducted an experiment to test the interactive effects of NH_x , pH and shoot density on the short-term survival of eelgrass. Finally, we discuss the relevance of our results with regard to (eelgrass) physiology and ecology.

MATERIALS AND METHODS

After collection in the field, plants were stored at 10 °C for transport to the laboratory in Nijmegen (Netherlands). Shoots (aboveground parts attached to at least 4 cm of rhizome) were acclimatized for 2 weeks in 100 L glass containers (l:w:h = 80:30:45 cm) that were placed in a climate controlled room at 20 °C. The culture medium used for acclimatization and the experiments was prepared from deionised water and Tropic Marin® synthetic sea salt. We used a salinity equal to the level measured in the field. We set light intensity at $180 \mu\text{mol m}^{-2} \text{s}^{-1}$ with a day-night cycle of 16 – 8 hours. This is comparable to average light levels in the field during the growing season around depths of 2 to 3 m (assuming a light attenuation coefficient of 0.5 m^{-1}) (van der Heide et al. 2007), which is in the normal depth ranges of eelgrass (Nielsen et al. 2002).

At the start of both experiments, we carefully selected healthy apical shoots, based on shoot length, number of leaves and the percentage mortality (black coloration) of the leaves. Only shoots with 4 or 5 leaves and a mortality of less than 5% in the 3 youngest leaves were used in the experiments. The plants were allowed to acclimatize for two additional days, after placement in the experimental units.

Long-term experiment. The goal of this experiment was to simulate the response of eelgrass to NH_x exposure in 'open ocean' conditions. Plants were obtained at the end of August from the Atlantic Ocean near the shores of Brest (France). Based on literature, we expected the plants to show a strong response to NH_x exposure due to the low ambient nitrogen concentrations ($\pm 11 \mu\text{M}$ total N) and a relatively high salinity in this area (± 33 PSU) (van Katwijk et al. 1999). The experiment was carried out in a 5-week period in 16 glass containers (l:w:h = 20:20:58 cm) placed in a 20 °C water bath. Each unit contained 20 L of medium and 8 cm of sandy sediment from the sampling site. Two shoots were placed in each container (total shoot biomass $0.68 \pm 0.04 \text{ g DW}$). The culture medium in the containers was continuously replenished from stock containers at a renewal rate of twice a week, using Masterflex peristaltic pumps. We used 4 different treatments, resulting in 4 replicates per treatment. Total NH_x concentrations in the stock solutions were 20, 60, 100 and $150 \mu\text{mol l}^{-1}$ by addition of NH_4Cl . We added $1 \mu\text{mol l}^{-1}$ cyanoguanidine to the stock solutions to prevent nitrification of ammonium (Smolders et al. 1996). The pH level was carefully kept between 8.0 and 8.2 by

aerating the units with CO₂ enriched air (5 ppt CO₂), using mass flow controllers. The total dissolved inorganic carbon concentration (DIC) in the water was 2.2 ± 0.02 mM, a close approximation of the DIC level of the sampling site (2.3 mM). Water samples for analysis of NH_x and nitrate were taken on a weekly basis. Leaf growth, leaf number and shoot biomass were determined before and after the experiment.

Short-term experiment. Alongside the 5-week experiment, we performed a 5-day experiment with eelgrass from the Atlantic Ocean (near Brest, see previous heading), focussing on the acute combined effects of pH and NH_x (setup, pH, NH_x levels and analyses were performed as described below). In this trial we found severe necrosis in eelgrass leaves due to NH_x addition at pH 9.1 but not at pH 8.1 (fig. 4.1). However, these results pose virtually no ecological relevance as raised pH levels almost only occur in sheltered eutrophicated estuaries with a much lower salinity due to nutrient-rich freshwater influence. Therefore, we repeated this experiment at a lower salinity, using plants collected from the Baltic Sea near Kiel (Germany) at the end of August 2006. We expected this population to respond somewhat more conservative to NH_x exposure compared to the Brest population, as nitrogen loads in the Baltic Sea are higher (± 27 μ M ambient total N) and salinity is much lower (± 16 PSU) compared to the waters around Brest (van Katwijk et al. 1999). The experiment was conducted in round glass columns containing 4 L of medium (\varnothing :h = 11:45 cm) placed in a water bath at 20 °C. We used four NH_x concentrations (0, 50, 100, 250 μ mol l⁻¹ added as NH₄Cl) at two pH levels (8.1 and 9.1). Additional to the initial setup of the short-term trial, we used 2 shoot densities at the highest pH to investigate a possible density dependent effect: 1 or 10 shoots per vase, corresponding with 88 and 884 shoots m⁻² (total shoot biomass: 0.44 ± 0.12 g DW and 4.25 ± 0.28 g DW respectively). At pH 8, experiments were performed using low shoot densities only, as a much lower toxicity was expected. In total, we used 48 glass columns, resulting in 4 replicates per treatment. Because of the short experimental period, plants could be maintained without sediment (Thursby & Harlin 1982, Rubio et al. 2007). This prevented NH_x loss due to nitrification or adsorption to sediment particles. Shoots with 4 cm of rhizome were positioned in the columns by attaching stainless steel nuts to the rhizomes. As NH_x affinity for leaves is much higher for leaves than for roots (Iizumi & Hattori 1982, Thursby & Harlin 1982) and because uptake via roots and rhizomes is strongly limited in eelgrass when aboveground tissues are exposed to NH_x (Thursby & Harlin 1982), we assumed that NH_x assimilation through the remaining belowground tissue was negligible in our experiment. During the experiment, the culture medium was replenished each day at the start of the light period. The pH was checked regularly during the day and kept between 8.0 and 8.2 or 9.0 and 9.2, by adding HCl or NaOH. DIC of the 16 PSU water was 1.85 ± 0.03 mM, comparable to the values measured at the sampling site (1.88 mM). Water samples for analysis of NH_x were taken daily just before replenishment.

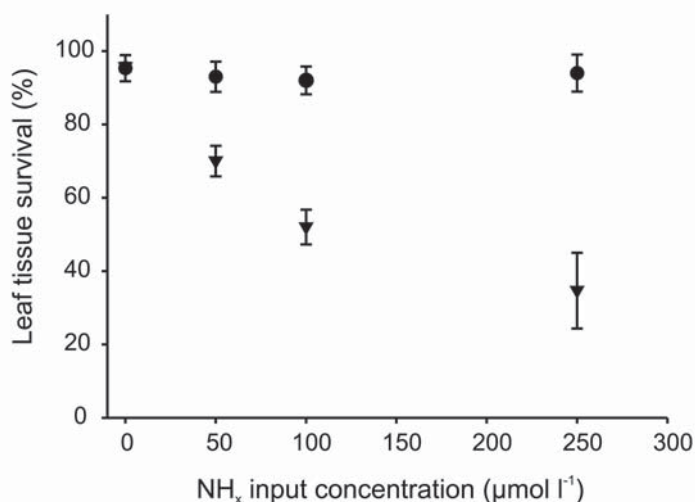


Figure 4.1. Effects of applied NH_x loads on tissue survival at a salinity of 33 PSU. Errors bars indicate \pm SEM. Treatments were carried out in 4-fold and leaf tissue was analyzed using the image analysis technique as described in Methods and Materials.

Analysis of leaf tissue fitness. After each experiment we examined the fitness of the leaves by scanning the three youngest leaves of each shoot at 600 dpi, resulting in a resolution of over half a million pixels per leaf. We subsequently analyzed the scans using Adobe Photoshop. First, we eliminated background noise in the picture using the “threshold” function. The image was then converted into a 64-color index picture (red-green-blue scheme). Next, we used the “colour range” function to distinguish between dead and living leaf tissues. The amount of green coloration per pixel was used to determine whether leaf tissue was either dead or alive. Based on tests with fit and dead leaves from the field, we found that pixels with a green value of over 70 (± 2 ; on a scale of 0 - 255) should be considered alive while lower values could be regarded as dead tissue. Based on this procedure, dead, living, and background areas in the picture were then coloured in the three primary screen colours (red, green and blue). Finally, we calculated the percentage of living and dead tissues from the histogram values of the image.

Chemical analyses. Water samples taken during both experiments were analyzed for NH_x and nitrate (Tomassen et al. 2003). NH_x concentrations were determined spectrophotometrically using hypochlorite. Nitrate was measured spectrophotometrically with sulphanilamide after reduction of nitrate to nitrite in a cadmium column.

Free amino acids in the leaves of the shoots were extracted from healthy looking tissues, according to Van Dijk and Roelofs (van Dijk & Roelofs 1988). After extraction, amino acid concentrations were measured after pre-column derivatisation with 9-fluorenylmethyl-chloroformate (FMOC-Cl),

using High Pressure Liquid Chromatography (HPLC) (Tomassen et al. 2003). C:N ratios and total N concentrations were determined in freeze-dried leaf tissues by an CNS analyzer (type NA1500; Carlo Erba Instruments, Milan, Italy).

Statistical analyses. For all relevant variables, the effects of NH_x in the long-term experiment and NH_x , pH, density and their interactions in the short-term experiment were tested by Analyses of Variance (ANOVA) and multifactor ANOVA, respectively. Prior to the analyses, outliers were omitted based on Dixon's Q-test (Dean & Dixon 1951) and data were tested for normality. For multiple comparisons of normally distributed means (posthoc tests) we used independent samples t-tests. In the long-term experiment, we compared the effect of NH_x between treatments (6 tests). For the short-term experiment, we evaluated the effect of NH_x in all pH and density treatments (18 tests), compared group means of both pH levels at low density (4 tests) and tested the effect of density in the pH 9 treatments (4 tests). Thus, 26 hypotheses were tested in total. Type I error in the testing procedures was controlled using the False Discovery Rate method ($p^* = 0.05$) (Benjamini & Hochberg 1995, Verhoeven et al. 2005).

RESULTS

Long-term experiment. Data were normally distributed and no outliers were detected. Within one week, NH_x concentrations in the different treatments stabilized at 1, 12, 38 and 75 (± 2) $\mu\text{mol l}^{-1}$. Nitrate levels were negligible in all treatments during the entire experimental period. After 5 weeks, we found no significant change in biomass, leaf growth or in number of leaves. Moreover, shoots in all treatments looked healthy and leaf survival showed no trend at all, being over 90% in all treatments. In contrast, ANOVA revealed significant differences for internal nitrogen concentrations ($F=34.9$, $p<0.001$) and C:N ratios ($F=21.9$, $p<0.001$) in the leaf tissues. Posthoc test showed that, apart from the two highest loads, all treatments differed significantly from each other for both variables. The total nitrogen content in the leaves ranged from 1.4 to 3.7 % from the lowest to the highest treatments, respectively (fig. 4.2A). Average C:N ratios varied from 25 in the lowest to 10 in the highest treatments (fig. 4.2B).

The amount of nitrogen that was stored as free amino acids in the tissues varied substantially with the applied NH_x load. Concentrations of free amino acid N increased significantly from 113 $\mu\text{mol N/g DW}$ in the lowest NH_x treatment to 1093 $\mu\text{mol/g DW}$ in the highest treatment (ANOVA: $F=10.3$, $p=0.001$). The percentage of total tissue nitrogen stored as free amino acids varied from 11 in the lowest to 41% in the highest treatment (ANOVA, $F=8.6$, $p=0.003$; fig. 4.2C). Excess nitrogen in the shoots was primarily stored as glutamine. The percentage of glutamine in the total free amino acid concentration changed from 21 in the lowest to 82 % in the highest NH_x treatment (ANOVA: $F=13.9$, $p<0.001$; fig. 4.2D). Post-hoc tests revealed significant differences between the lowest and the two highest loads for all 3 variables. Additionally, the 60 $\mu\text{mol l}^{-1}$ NH_x treatment also differed significantly with the highest load for these variables.

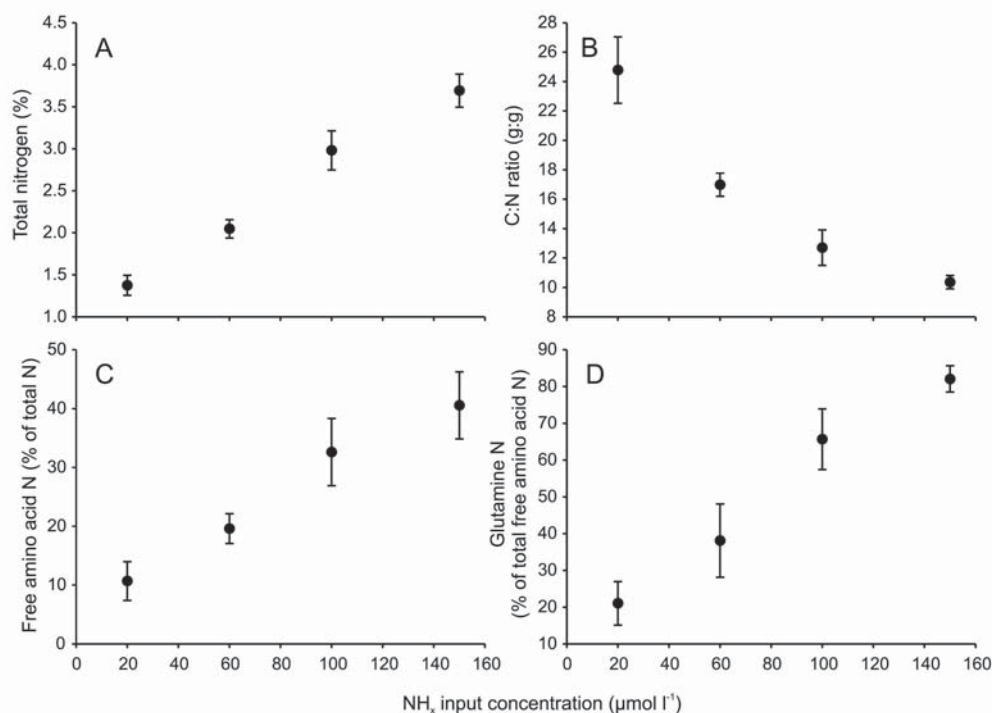


Figure 4.2. Effects of the applied NH_4^+ loads in the long-term experiment on **A**; the total nitrogen (N) content, **B**; C:N ratio, **C**; free amino acid N relative to total N content and **D**; glutamine N relative to the total free amino acid N content in the leaf tissues. Errors bars indicate $\pm\text{SEM}$. All figures demonstrate a marked response of eelgrass to the applied NH_4^+ load.

Short-term experiment. Analysis showed that all data were normally distributed, and that 2 outliers should be excluded. Survival was significantly affected by NH_4^+ , pH, density and their interactions (ANOVA, table 4.1). Leaf tissue survival ranged from 94% in the controls to 57% in the $250 \mu\text{mol l}^{-1}$ – pH 9 treatment. Necrosis in the leaf tissues was expressed as a typical brown-black discoloration (fig. 4.3). Within the pH 8 treatment, there was no significant effect of any of the NH_4^+ loads on leaf tissue survival. At pH 9, NH_4^+ significantly affected survival in both density treatments, but the intensity of the effects was clearly different. In the high density columns, only the highest NH_4^+ treatment deviated significantly from the control treatment, whereas in the low density columns both the 50 and $250 \mu\text{mol l}^{-1}$ NH_4^+ treatments differed significantly from the control (and each other) (fig. 4.4A). The effects of pH and density were significant in the 50 and $250 \mu\text{mol l}^{-1}$ NH_4^+ treatments.

Total nitrogen content ranged from 1.5% in the control treatments to 2.6% in the $250 \mu\text{mol l}^{-1}$ – pH 8 treatment (fig. 4.4B). The effect of the NH_4^+ treatments was highly significant (table 4.1). Multiple comparison tests on the NH_4^+ treatments were significant in 13 out of 16 cases. The effect of pH was only significant in the $250 \mu\text{mol l}^{-1}$ treatment, whereas density was significant in the $100 \mu\text{mol l}^{-1}$ treatment. The C:N ratio showed a trend that was inversely correlated with the total N content (fig. 4.4C). The average ratio ranged from 27 in the control treatments to 15 in the $250 \mu\text{mol l}^{-1}$ – pH

Table 4.1: Results of the multi-factor ANOVAs on all relevant variables in the short-term experiment. All data were normally distributed, except for total nitrogen. This variable obtained normality after inverse transformation. F-values and significance levels are shown for all main effects and their interactions. * $0.01 \leq p \leq 0.05$, ** $0.001 \leq p < 0.01$, *** $p < 0.001$.

	NH _x treat.	pH	Density	NH _x treat. x pH	NH _x treat. x Density
Leaf tissue survival (%)	29.1 ***	92.1 ***	82.9 ***	20.7 ***	13.6 ***
Total N (%)	92.8 ***	4.3 *	6.6 *	3.3 *	2.9 ns
C:N ratio (g:g)	64.8 ***	0.7 ns	5.9 *	0.4 ns	2.2 ns
Free amino acids N of tot. N (%)	313.8 ***	96.0 ***	7.9 **	46.1 ***	4.7 **
Total free amino acids N ($\mu\text{mol l}^{-1}$)	339.6 ***	112.0 ***	7.8 **	79.2 ***	5.8 **
Glutamine N of tot. amino acids N (%)	339.8 ***	2.1 ns	49.9 ***	1.8 ns	18.0 ***
NH _x conc. after 24 hours ($\mu\text{mol l}^{-1}$)	3514.4 ***	11.0 **	887.6 ***	0.622 ns	212.5 ***



Figure 4.3. Two scans from the low density – pH 9 series (0 $\mu\text{mol l}^{-1}$ and 250 $\mu\text{mol l}^{-1}$ NH₄⁺) at the end of the short-term experiment. The leaves from the shoot of the 250 $\mu\text{mol l}^{-1}$ treatment are necrotic, which is typified by a brown-black discoloration.

8 treatment.

The percentage of nitrogen that was stored in free amino acids increased significantly with increased NH_x loads (significant in 14 out of 16 tests). Contents ranged from 3% in the controls to a maximum of 39% in the $250 \mu\text{mol l}^{-1}$ pH 8 treatment (fig. 4.4D), equalling 36 and $702 \mu\text{mol}$ free amino acid N/g DW, respectively. The effect of pH was significant in the 50 and $250 \mu\text{mol l}^{-1}$ treatments, whereas density showed significant effects in the 50 and $100 \mu\text{mol l}^{-1}$ treatments. As in the long-term experiment, excess nitrogen was primarily stored as glutamine. The percentage of glutamine N in the total free amino acid N concentration varied from 33% in the $0 \mu\text{mol l}^{-1}$ treatments

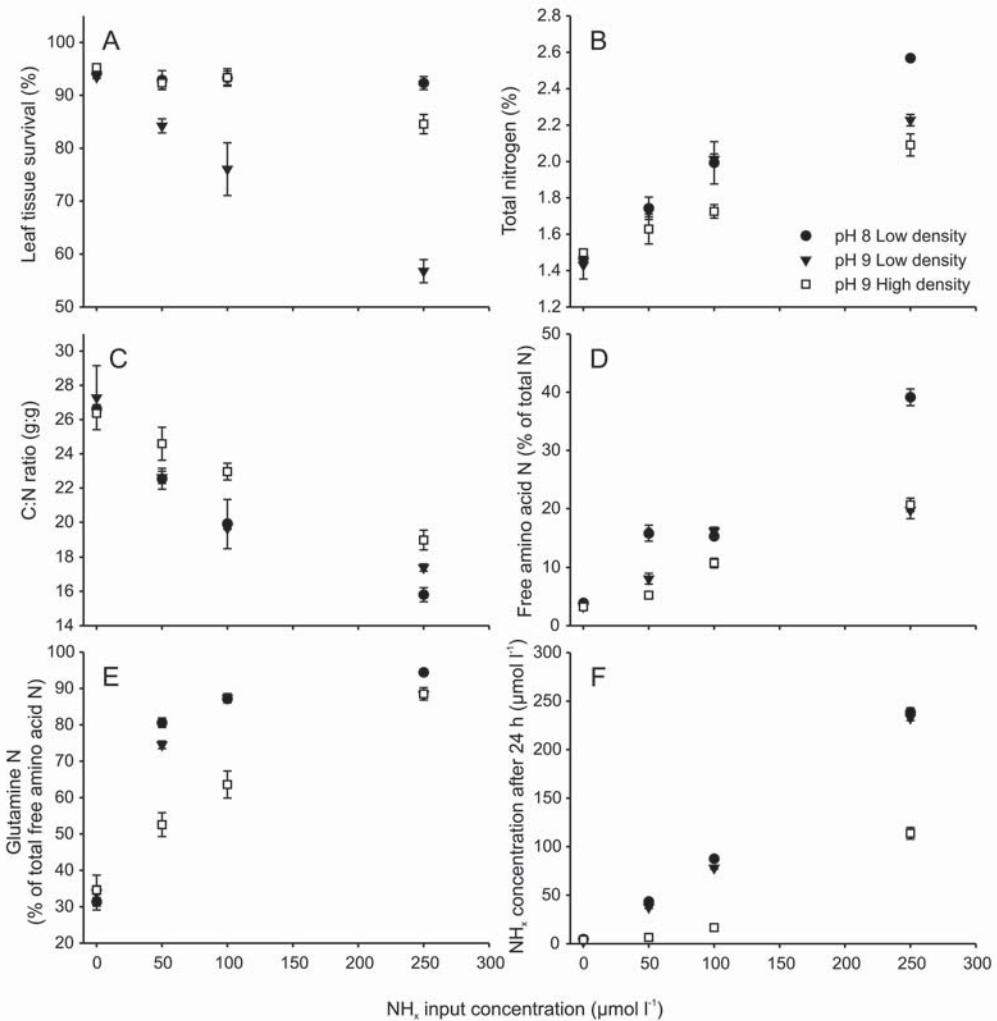


Figure 4.4. Effects of the applied NH_x loads in the short-term experiment on **A**; leaf tissue survival, **B**; total nitrogen content, **C**; C:N ratio, **D**; free amino acid N relative to total N content and **E**; glutamine N relative to total free amino acid N content. Figure **F** shows the average NH_x concentration in all treatments after 24 hours. Error bars indicate \pm SEM. The figures clearly illustrate that eelgrass response to the applied NH_x loads is strongly dependent on pH and shoot density.

to 94% in the 250 $\mu\text{mol l}^{-1}$ – pH 8 treatment (fig. 4.4E).

The NH_x concentration after 24 hours was reduced to values around 6 and 17 $\mu\text{mol l}^{-1}$ in the high density 50 and 100 $\mu\text{mol l}^{-1}$ treatment, respectively. In the 250 $\mu\text{mol l}^{-1}$ treatments, NH_x concentrations were still well above 100 $\mu\text{mol l}^{-1}$ (fig. 4.4F). The reduction of NH_x in the low density treatments was much smaller. The average total uptake ranged from around 10 $\mu\text{mol l}^{-1} \text{ day}^{-1}$ in the 50 $\mu\text{mol l}^{-1}$ treatments to 14 $\mu\text{mol l}^{-1} \text{ day}^{-1}$ in the 250 $\mu\text{mol l}^{-1}$ treatments. NH_x concentrations in the low density pH 9 treatments were slightly but significantly lower compared to their counterparts in the pH 8 treatments.

DISCUSSION

Stress caused by reduced nitrogen in the water layer in seagrasses and other macrophytes has been frequently observed (Agami et al. 1976, Smolders et al. 1996, van Katwijk et al. 1997, Bird et al. 1998, Körner et al. 2001, Brun et al. 2002, Cao et al. 2004, Cao et al. 2007, Nimptsch & Pflugmacher 2007). However, the nature of the reported symptoms differ widely. Typical responses are free amino acid accumulation, oxidative stress, inhibited growth, and structural tissue damage. Results from our experiments suggest that differences in response, may at least in part be explained by differences in pH of the medium. NH_x toxicity is the sum of the effects caused by NH_4^+ and (gaseous) NH_3 (Nimptsch & Pflugmacher 2007), and the balance between NH_3 and NH_4^+ levels in the NH_x concentration in turn depends on pH (Johansson & Wedborg 1980).

In our first, long-term experiment, conducted at a normal pH level (pH 8.1), structural tissue damage did not appear. However, internal nitrogen concentrations increased linearly with increasing NH_x loads and were much higher than commonly reported for eelgrass. In the 150 μM treatment, total N concentrations in the eelgrass leaf tissues were higher than maximum values and nearly 1.5 times higher than average values reported from the field (Duarte 1990). Moreover, free amino acid N concentrations were also much higher than the highest value reported in literature (Touchette & Burkholder 2002) and consisted mainly of glutamine (around 88%). These results indicate that, like many plants susceptible to NH_4^+ toxicity, eelgrass shoots may not be able to exclude NH_4^+ when exposed to high NH_4^+ levels (Britto et al. 2001, Kronzucker et al. 2001). To prevent accumulation, NH_x then needs to be assimilated by the plant. Glutamine is often the main compound formed in this process, because it is the first amino acid formed in the NH_x -assimilation cycle of plants (Märschner 1995).

In our second, short-term experiment, leaf survival in the low density treatments was strongly affected by the applied NH_x load at pH 9, whereas no effects were visible at pH 8. At first sight, these results may suggest that the observed necrosis should be attributed to increased diffusion of NH_3 into the tissues. After all, the concentration of this species is much higher at pH 9 (28.5% of NH_x) than at pH 8 (3.8% of NH_x) (Johansson & Wedborg 1980). However, this explanation alone does not seem satisfactory, as both the long-term experiment and the pH 8 treatments of the short-term experiment demonstrated that nitrogen also accumulated strongly at pH 8 in eelgrass at the

applied NH_x concentrations. It seems likely that disruption of the carbon assimilation (through photosynthesis) rendered the plants susceptible to enhanced external NH_x concentrations at pH 9, as the carbon consuming NH_x assimilation becomes impaired when carbon availability is limited (Märschner 1995). Even though eelgrass is able to utilize bicarbonate (HCO_3^-) next to carbon dioxide (CO_2) for carbon assimilation, carbon may become limited at pH 9 when the total dissolved inorganic carbon (DIC) concentration is at a natural sea water level (± 2.2 mM) (Sand-Jensen & Gordon 1984). This is because with rising pH, CO_2 and HCO_3^- levels drop in favour of CO_3^{2-} , a carbon species which cannot be assimilated. Next, high intracellular NH_x concentrations may cause uncoupling of photophosphorylation by NH_3 in the chloroplasts, further decreasing photosynthesis and related carbon uptake (Pearson & Stewart 1993, Märschner 1995). This hypothesis is supported by the analyses of the tissue nitrogen compounds in the short-term experiment. Free amino acid and total N concentrations were lower instead of higher in the pH 9 treatments, suggesting that, despite a possible increased intrusion of NH_3 , NH_x assimilation rates were lower at pH 9.

Another important outcome of the short-term experiment is that a high density of eelgrass was able to buffer for NH_x toxicity up to a load of $100 \mu\text{mol l}^{-1} \text{NH}_x$ per day, as necrosis only appeared in the highest NH_x treatment. Even though these high density treatments must also have experienced carbon limitation and impaired photosynthesis, survival of the plants was not affected in the three lower NH_x treatments. Additionally, total tissue nitrogen and free amino acid concentrations were significantly lower in the high density treatments compared to their low density counterparts. This indicates that the potential for NH_x toxicity was alleviated in the high density treatments through the joint uptake of NH_x by the shoots, thereby decreasing ambient NH_x concentrations below the threshold above which NH_x becomes toxic. This view is supported by the measured decrease of NH_x concentrations in the water column within 24 hours. The high density treatments were able to lower NH_x concentrations in the 50 and $100 \mu\text{mol l}^{-1}$ treatments to values well below $20 \mu\text{mol l}^{-1}$ within 24 hours.

The visual symptoms of NH_x toxicity that appeared in the pH 9 treatments, are much alike those reported by Van Katwijk *et al.* (van Katwijk *et al.* 1997). They also observed a typical brown-black discoloration of the eelgrass leaves within a period of 2 weeks. It is striking, however, is that the experimental setup of Van Katwijk *et al.* was much more similar to the design of our long-term experiment. Both experiments were performed in the same laboratories, lasted 5 weeks, and were performed in glass containers with similar light conditions. Replenishment of the medium was continuous in both experiments, resulting in similar NH_x concentrations in the containers. However, in contrast to the results of Van Katwijk *et al.*, we found no visual symptoms of NH_x toxicity in our long-term experiment. Moreover, the mean total tissue N concentration in our highest treatment was even higher than the highest reported value by Van Katwijk *et al.* In our opinion, these contradicting results can be best explained by differences in pH. In our experiment, pH was carefully controlled. Van Katwijk *et al.* mention an average pH of 8.5, which is already somewhat higher than in our experiment (8.0-8.2), but additionally pH was not controlled in their experiment. This lack of

pH control most likely allowed for pH fluctuations during the day due to photosynthetic activity. It is therefore not inconceivable that the photosynthetic activity of eelgrass plants may have caused pH spikes of around pH 9 or even higher near the end of each day, leading to carbon limitation and impaired photosynthesis.

Ecological implications: At normal pH levels (i.e., 'open ocean' conditions, pH 8.1) and sufficient light for photosynthesis and carbon fixation, acute toxic effects may at least temporarily be prevented by the assimilation of NH_4^+ into free amino acid compounds (van Katwijk et al. 1997, Brun et al. 2002). However, the observed disproportional accumulation of glutamine can be considered an indication of physiological stress due to the elevated NH_4^+ levels in the water layer (Näsholm et al. 1994, Huhn & Schulz 1996, Smolders et al. 2000). Such a chronic exposure to NH_4^+ (i.e. several months or years) will most likely lead to the classically reported visual symptoms like chlorosis of leaves and the suppression of growth (Britto & Kronzucker 2002). Moreover, phenolic content in the leaves will decrease, due to a changed allocation of carbon skeletons, making the plant more susceptible to pathogens like the "wasting disease", which destroyed many eelgrass stands in the 1930s (Buchsbaum et al. 1990, van Katwijk et al. 1997, Vergeer & Develi 1997).

Although high NH_x levels in the water layer at pH 8 most likely cause stress in eelgrass when exposed for longer periods of time, results from our experiments demonstrate that acute toxicity and sudden collapse through mass mortality will probably not occur as long as sufficient light is available. In contrast, elevated NH_x concentrations at enhanced pH levels (i.e. pH 9) can cause severe mortality in eelgrass within days. Thus, mass mortality through NH_x toxicity can be of significant importance in for instance sheltered eutrophicated estuaries, where phytoplankton, macroalgae, and eelgrass itself can cause high pH levels as well as NH_x spikes near the end of the growing season. Additionally, the mechanism may also be important at a normal pH during phytoplankton blooms or along eelgrass depth limits as light availability is poor in those cases.

Interestingly, our results show that the severity of the toxic effect is most likely strongly dependent upon the shoot density of an eelgrass bed. Especially in sheltered stagnant estuaries where mixing between the eelgrass canopy and its surroundings is poor, this could lead to a positive feedback: once there is a high density of eelgrass, its susceptibility to NH_x toxicity becomes lower. If such positive feedback is strong enough, it can lead to alternative stable states in seagrasses (Scheffer et al. 2001, van der Heide et al. 2007). However, in our case, the feedback mechanism may be more complex: a high eelgrass density can also imply a higher photosynthetic activity and thus a higher pH, which in turn may lead to a higher toxicity. Therefore, the outcome in a particular field situation will strongly depend on the NH_x loads, the photosynthetic activity, and the exchange rates between the seagrass meadow and the surrounding water layer (van Nes & Scheffer 2005). The occurrence of alternative stable states may at least in part explain the worldwide observed seemingly high resilience to change, sudden ecosystem collapses, and mass mortalities in seagrasses (van der Heide et al. 2007).

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5

Alternative stable states driven by density-dependent toxicity

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ABSTRACT

Many populations are exposed to naturally occurring or synthetic toxicants. An increasing number of studies demonstrate that the toxicity of such compounds is not only dependent on the dosage, but also on the biomass of exposed organisms. At high biomass, organisms may be able to alleviate adverse effects of the toxicant by actively lowering ambient concentrations through either a joint detoxification mechanism or growth dilution. We show in a conceptual model that this mechanism may potentially lead to alternative stable states if the toxicant is lethal at low densities of organisms, whereas a high density is able to reduce the toxicant concentrations to sub-lethal levels. We show in an example that this effect may be relevant in real ecosystems. Based on results of laboratory experiments, we constructed a model describing the complex interactions between the temperate seagrass *Zostera marina* and potentially lethal ammonia. Analyses of the model show that alternative stable states are indeed present over wide ranges of key-parameter settings, suggesting that the mechanism might be important especially in sheltered, eutrophicated estuaries where mixing of the water layer is poor. We argue that the same mechanism could cause alternative stable states in other biological systems as well.

INTRODUCTION

In their environments, organisms can be exposed to a wide range of naturally occurring or synthetic toxic substances (Moriarty 1999). Physiological or population effects of such chemicals are mostly examined in dose-response studies, where the dosage of a toxicant is varied on a certain biomass of organisms (Moriarty 1999). However, an increasing number of studies demonstrate that toxicity effects may often not only be dependent on the dose, but also on the biomass of exposed organisms. In such cases, toxicity may be alleviated by a high organism biomass because the concentration of a toxicant is reduced to sub-lethal levels due to joint uptake or active detoxification. This effect has been described for a wide range of biological systems such as heavy metal accumulation in organisms (Duxbury & McIntyre 1989, Pickhardt et al. 2002), phytotoxins in microbes and plants (Weidenhamer et al. 1989, Greig & Travisano 2008, Pollock et al. 2008), and even for drug treatments of infectious bacteria or cancer (Brook 1989, Kobayashi et al. 1992, Brandt et al. 2004). In ecotoxicological studies, this mechanism is often referred to as ‘density-dependent toxicity’ (Duxbury & McIntyre 1989, Kobayashi et al. 1992, van der Heide et al. 2008) or the ‘dilution effect’ (Karimi et al. 2007, Pollock et al. 2008), while it is described as the ‘inoculum effect’ in many pharmaceutical studies (Brook 1989, Kobayashi et al. 1992, Brandt et al. 2004).

This density-dependent toxicity implies a positive feedback of a population and the toxic substance, because a high biomass of organisms has a positive effect on their growth as they alleviate toxicity. Theory suggests that if such a positive feedback mechanism is strong enough, it could lead to alternative stable states (also called bistability) and hysteresis (Carpenter 2001, Scheffer et al. 2001, Scheffer & Carpenter 2003). This implies that environmental changes or disturbances (e.g., disease) may push the population beyond a critical threshold, causing a collapse (e.g., mass mortality) to an alternative stable state (Scheffer et al. 2001). Implications of non-linear response and threshold behavior in biological systems can be profound. Shifts in populations with positive feedbacks are typically hard to predict and recovery of a collapsed system is often difficult.

In this study, we examine whether a positive feedback caused by density-dependent toxicity may cause alternative stable states. First, we show the basic idea in a simple theoretical model, describing a generalized positive feedback system between a population of organisms and a toxic compound. We analyzed two different assumptions of how an established population can alleviate toxicity: the organisms can alleviate toxicity actively (‘joint detoxification’) or can take up and store a limited amount of the toxic substance per organism or unit of biomass (‘growth dilution’). Next, we created a more realistic model, describing ammonia toxicity in seagrass ecosystems, to test our theory for an empirical situation. We analyze whether this model can have alternative stable states using realistic parameter settings based on laboratory experiments and literature.

METHODS

Theoretical models. The basic mechanism of this feedback can be shown in a very simple model. We assume that the considered population of organisms grows logistically, but with an extra mortality due to a toxic compound. To show the basic mechanism this mortality is assumed to be simply proportional to the concentration of the toxic compound:

$$\frac{dX}{dt} = a\left(1 - \frac{X}{K_x}\right)X - bXT \quad (1)$$

X describes the biomass of the population, a is the maximum growth rate per unit of time and K_x is the carrying capacity. Parameter b is a mortality constant which, multiplied with the concentration of toxicant T represents the mortality rate in population X per unit of time. Although the linear effect of T in the model may often be an oversimplification of reality, simulations showed that the model behavior is not sensitive to the choice of this function. Moreover, in many cases the response of organisms to toxicants can well be described by this simple relation (Hendriks et al. 2005). The second equation describes the change in toxic compounds in the system per unit of time:

$$\frac{dT}{dt} = (T_{max} - T)p - Tf(X) \quad (2)$$

Where T_{max} is the maximum concentration of toxicants in the system and p is the refreshing rate (e.g., production or external input of the toxicant). We assume that the organisms reduce the concentration of toxicants as a function of their biomass. This density-dependent alleviation of toxicity is essential for the feedback.

We analyzed two different assumptions. In the first case, organisms can actively alleviate toxicity by detoxification ('joint detoxification'). Here, $f(X)$ is described by $d_1 X$, where d_1 is a constant describing the uptake rate of T per unit of X . For the second mechanism, organisms can only take up a limited amount of the toxic substance per unit of biomass and detoxification is thus dependent on growth ('growth dilution'). In this case, $f(X)$ is replaced with the term $d_2 a X (1 - X/K_x)$, where d_2 describes the uptake of T proportional to growth of X .

If we further assume that the dynamics of T are faster than those of the organisms (i.e. the quasi steady state assumption $\frac{dT}{dt} = 0$, this assumption has no consequences for the equilibrium density of organisms) this model can be simplified as:

$$\frac{dX}{dt} = a\left(1 - \frac{X}{K_x}\right)X - bX \frac{p T_{max}}{p + f(X)} \quad (3)$$

The conditions for alternative stable states of this simple model can be determined analytically for the joint detoxification assumption or numerically in case of the growth dilution model (appendix 5.1).

Specific model of NH_x toxicity in eelgrass. Recent studies have demonstrated that positive feedbacks are important mechanisms in seagrass ecosystems (van der Heide et al. 2007, van der Heide et al. 2008). This model, based on empirical data, describes a feedback mechanism between the temperate seagrass *Zostera marina* (commonly called eelgrass), reduced nitrogen (NH_x) and potentially lethal gaseous ammonia (NH_3) in the water layer. We chose this system as an empirical test for our theory since recent research demonstrated that susceptibility of eelgrass to NH_x toxicity is highly dependent on vegetation density, indicating that positive feedbacks between eelgrass and reduced nitrogen may lead to alternative stable states in sheltered estuaries with high exposure to NH_x . In these systems, high concentrations of NH_x may be caused by for instance degradation of macro-algal mats, discharges of waste or river water or natural die-off of phytoplankton, macro-algae or seagrass itself near the end of the season (van der Heide et al. 2008). Also, since ammonium uptake is well studied in eelgrass, model parameters could be reliably estimated based on these studies and results from our own experimental work.

We based the model on the joint detoxification assumption. In the first place because ammonium is used as a nutrient by the plant and it is therefore metabolized. Secondly, eelgrass may discard of excess nutrients by replacing its leaves without resorbing the nutrients stored in the leaves that are lost (Hemminga et al. 1999). In eelgrass, lifespan of leaves can be highly variable, ranging from 35 to 160 days depending on environmental conditions (Hemminga et al. 1999). In the model, survival of eelgrass is dependent on the concentration of NH_3 in the water layer. The equation describing the change in eelgrass shoot density per day (dZ/dt) is similar to equation 1:

$$\frac{dZ}{dt} = r \left(1 - \frac{Z}{K_z}\right) Z - m f(\text{NH}_3) Z \quad (4)$$

With r as the maximum growth rate (day^{-1}), K_z as the carrying capacity (shoots m^{-2}) and m as the maximum mortality rate (day^{-1}). The toxic effect of NH_3 is described by the function $f(\text{NH}_3)$. To estimate toxicity effect of NH_3 in eelgrass, we recalculated experimental data of Van der Heide et al. (van der Heide et al. 2008) to present the relation between eelgrass mortality after 5 days of exposure to various NH_3 concentrations. The data revealed that toxicity by NH_3 in eelgrasses can be readily described by a Hill-curve (fig. 5.1), an equation expressing a sigmoid response that is often used to describe toxic effects in organisms (Hill 1910):

$$M = i + M_{\max} \frac{\text{NH}_3^n}{\text{NH}_3^n + H_{\text{NH}_3}^n} \quad (5)$$

Here M describes the fraction leaf tissue mortality in eelgrass after 5 days of exposure to NH_3 , i is the background leaf mortality at zero exposure. H_{NH_3} is the half-saturation constant (mmol m^{-3}) and n is a dimensionless exponent determining the slope of the curve. To describe the effect of NH_3 in our model, we adopted the part of equation 4 describing the relative effect of NH_3 on eelgrass mortality:

$$f(\text{NH}_3) = \frac{\text{NH}_3^n}{\text{NH}_3^n + H_{\text{NH}_3}^n} \quad (6)$$

In water, the total NH_x concentrations is made of the sum of NH_3 and ammonium (NH_4^+). NH_3 and NH_4^+ are in equilibrium and the balance between these compounds is determined by the pH of the water. The concentration of NH_3 in the water can be calculated from the pH and the total concentration of reduced nitrogen in the water layer:

$$\text{NH}_3 = \frac{k_a \text{NH}_x}{k_a + 10^{-\text{pH}}} \quad (7)$$

Where k_a is the dimensionless dissociation constant of NH_x in water with a salinity of 16 PSU at 20 °C. The change of NH_x in the water layer is described by the second differential equation:

$$\frac{d\text{NH}_x}{dt} = (\text{NH}_x \text{in} - \text{NH}_x)R - U_{\max} \frac{\text{NH}_x}{\text{NH}_x + H_{\text{NH}_x}} f(Z) \quad (8)$$

With $\text{NH}_x \text{in}$ as the NH_x concentration of the water flowing into the meadow and R as the replacement rate of the water layer inside the meadow. U_{\max} is the maximum uptake rate of NH_x by eelgrass ($\text{mmol g dry weight}^{-1} \text{ day}^{-1}$) and H_{NH_x} is the half-saturation constant for NH_x uptake (mmol m^{-3}). Finally, $f(Z)$ is a function describing the conversion from eelgrass shoot density (shoot m^{-2}) to the amount of dry weight biomass per unit of volume:

$$f(Z) = \frac{Z}{C} Dw_z \quad (9)$$

Here C is the height of the canopy (m) and Dw_z is the dry weight of one eelgrass shoot (g).

Bifurcation analysis. We analyzed the robustness of model results by varying settings of key parameters. Critical thresholds were determined by a numerical procedure. The key parameter was increased in small steps, after which the model was run to stabilize to its equilibrium. Next, this analysis was also performed backwards, by decreasing the key parameter in small steps. These analyses were combined to construct bifurcation plots of various parameters.

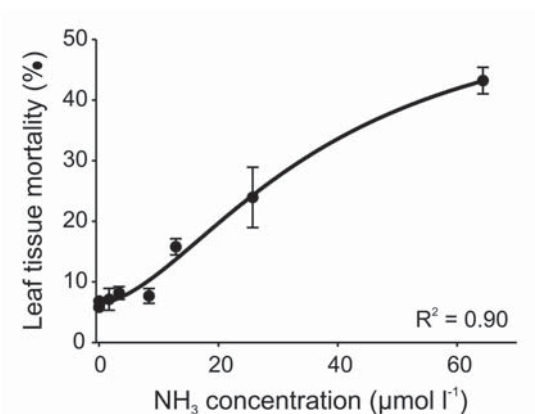


Figure 5.1. Response of eelgrass shoots to ammonia at varying concentrations after 5 days of exposure.

RESULTS

Theoretical models. Figures 5.2A and B show phase planes of the joint detoxification and growth dilution model respectively based on the default settings presented in table 5.1. Both graphs show two stable equilibria and one unstable equilibrium (saddle point). Whereas toxicant concentrations show a straightforward decrease with increasing biomass in the joint detoxification model, toxicant levels in the growth dilution model increase again when X nears its carrying capacity. This is because the population growth and therefore also the detoxification rate, is highest halfway the populations carrying capacity. Next, we analyzed the sensitivity of both models to varying values of the maximum toxicant concentration (T_{max}) in a one dimensional bifurcation plot (figs. 5.2C & D). The results demonstrate that both models can have alternative stable states, one without organisms

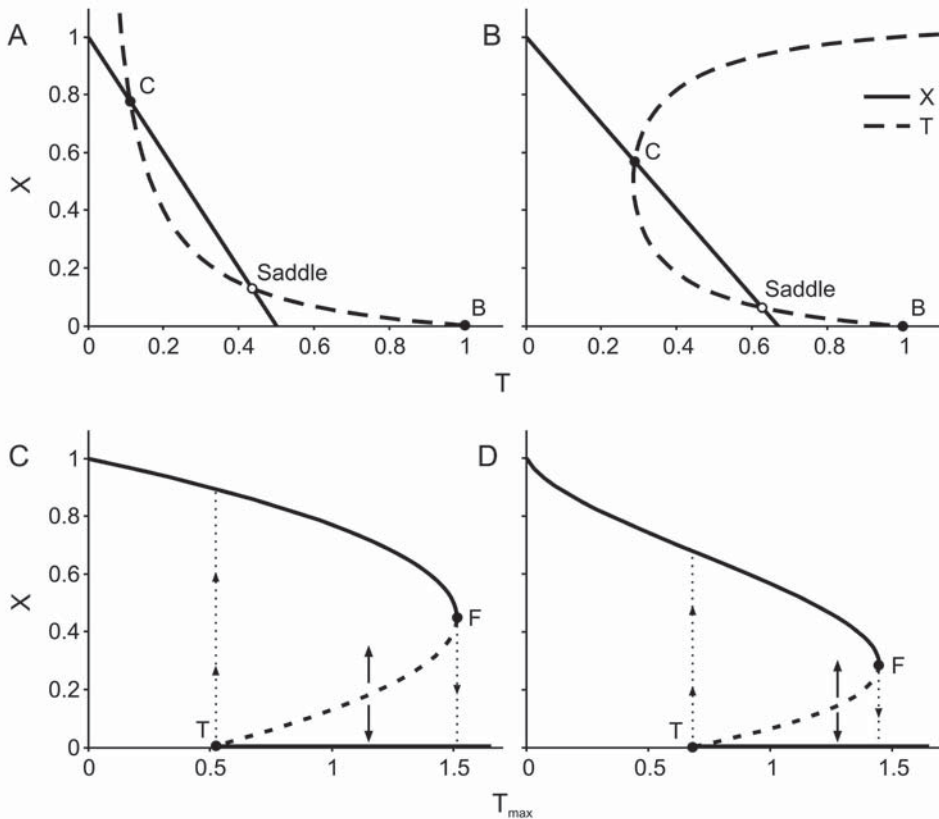


Figure 5.2. Analyses of the theoretical models. **A & B:** Nullclines at default settings of the “joint detoxification” model and “growth dilution” model respectively. The closed dots represent stable equilibria in the models, the open dots are unstable saddle points. C indicates a colonized state, B is a bare state. **C & D:** Bifurcation analyses of the “joint detoxification” model and “growth dilution” model respectively, with varying values of T_{max} . Solid lines represent stable equilibria, while the dashed line indicates unstable equilibria. Dots indicate bifurcation points (F = fold bifurcation; T = transcritical bifurcation), arrows show the direction of change. Note that all parameter settings for the “joint detoxification” model and the “growth dilution” model were identical, except for parameter b , which were set at 0.2 and 1.5 respectively.

and one with a population that can alleviate toxicity. The systems collapse to a bare state when organism density is pushed below the critical threshold (fig. 5.2C & D, dashed lines).

A more thorough bifurcation analysis of the joint detoxification model shows that the conditions for alternative stable states in this model are relatively simple (appendix 5.1). They can be summarized in a simple 2D plot, showing all parameter combinations at which alternative stable states occur (fig 5.3A). It appears that there are two prerequisites that determine whether the feedback is strong enough to cause alternative stable states. First the equilibrium concentration of toxicant without organisms (T_{max}) should be able to prevent colonization of the organism (i.e. its

Table 5.1: Variables and default parameter settings of the theoretical model. Note that in this instance, units used in the theoretical model are based upon an organism living in a water body with a constant refreshing rate, e.g., (phyto)plankton or fish.

	Default	Unit	Description
Variables			
X		$g\ l^{-1}$	Biomass of organism X per liter
T		$mol\ l^{-1}$	Concentration of toxicant T
Parameters			
a	0.1	day^{-1}	Growth rate
b	0.2 – 1.5	$l\ day^{-1}\ mol^{-1}$	Mortality constant; set at 0.2 for model 1 and at 1.5 for model 2.
K_x	1	$g\ l^{-1}$	Carrying capacity of X
T_{max}	1	$mol\ l^{-1}$	Maximum concentration of T
p	1	day^{-1}	Refreshing rate
d_1	10	$l\ day^{-1}\ g^{-1}$	Uptake constant of T
d_2	10	$l\ g^{-1}$	Uptake constant of T

effect on the organisms ($b\ T_{max}$) should be higher than the maximum growth rate of the population (a). The second prerequisite is that the effect of a full grown population (K_x) on the toxic substances should be strong enough to let the concentration of the toxicant decrease, i.e., the refresh rate of the toxic substance (p) should be less than the maximum effect of the organisms ($K_x\ d$). Note that this means that the chances for alternative equilibria increase if the turnover rate of the toxic substance (p) is low. If these two prerequisites are met, there is a range of T_{max} with alternative stable states. With increasing carrying capacity (or decreasing turnover rate), this range increases.

The growth dilution model is too complex for a similar analytical bifurcation analysis. However, we did this analysis numerically, showing very similar results (fig. 5.3B). Although the range for bistability in this model is narrower compared to the joint detoxification model, there is still a rather large parameter space with alternative stable states. Moreover, the qualitative effect of scaled toxicity and carrying capacity is remarkably similar.

Specific model of NH_x toxicity in eelgrass. The eelgrass model was parameterized to describe a sheltered estuary, where water mixing between the eelgrass meadow and its surroundings is limited (table 5.2). Water flowing into the seagrass bed has a NH_x concentration of $100 \mu\text{mol l}^{-1} \text{NH}_x$, a value comparable to various measurements in the field (Hauxwell et al. 2001, Brun et al. 2002). In these systems pH can vary strongly. At night, pH is generally around 8 while pH can rise up to 9 or even 10 during the day, due to photosynthesis of algae and seagrass itself (Choo et al. 2002, Feike et al. 2007, van der Heide et al. 2008), hence leading to higher NH_3 concentrations. For simplicity, we assumed an average pH of 8.5 for our model system.

The nullclines of this model at default settings are presented in figure 5.4A. Similar to the theoretical model, the graph shows one unstable equilibrium and two stable points. If shoot density is higher than $437 \text{ shoots m}^{-2}$, the meadow will develop towards carrying capacity, while below this

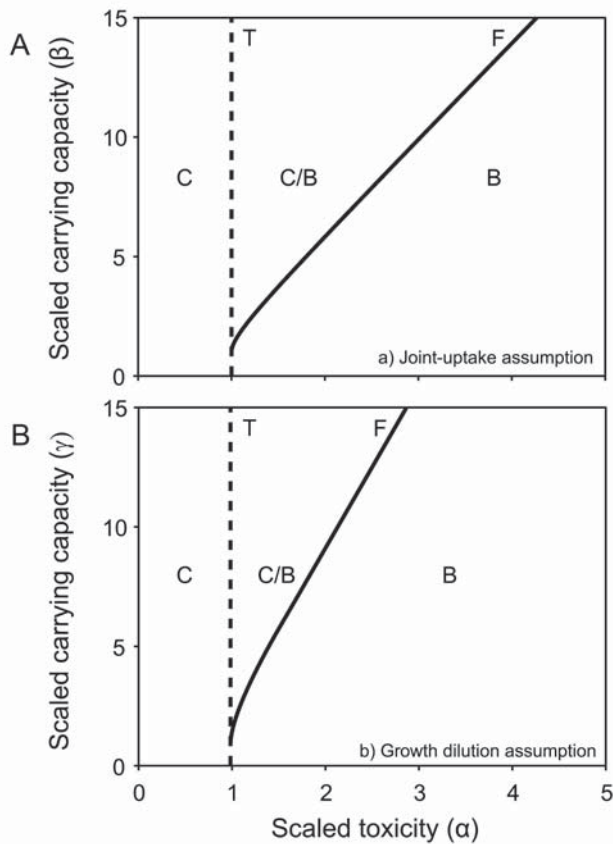


Figure 5.3: Two dimensional plots of the scaled theoretical models (see appendix 1). On the axes are the two combined parameters: the scaled toxic load $\alpha = \frac{b}{a} T_{\max}$ and the scaled carrying capacity of X, which is $\beta = \frac{d}{p} K_x$ for the "joint detoxification" model and $\gamma = \frac{d}{p} aK$ for the "growth dilution" model. The figures give all parameter combinations where we get alternative stable states. (C= colonized, C/B = alternative states; B = bare only, F (solid line) = fold bifurcation, T (dashed line) = transcritical bifurcation).

Table 5.2: Variables and default parameter settings of the empirical eelgrass model.

	Default	Unit	Description	Source
Variables				
Z		sh m ⁻²	Eelgrass shoot density	
NH _x		mmol m ⁻³ (= μmol l ⁻¹)	Reduced nitrogen concentration	
NH ₃		mmol m ⁻³ (= μmol l ⁻¹)	Ammonia concentration	
Parameters				
r	0.0105	day ⁻¹	Maximum net growth rate	1
m	0.16	day ⁻¹	Maximum mortality rate	*
K _z	2500	sh m ⁻²	Carrying capacity	2, +
H _{NH3}	37.432	mmol m ⁻³ (= μmol l ⁻¹)	Half rate constant for toxic effects of NH ₃	*
n	1.6922		Hill-curve exponent in NH ₃ toxicity curve	*
pH	8.5		pH	3, +
k _a	0.35e-9		Dissociation constant for NH ₃ /NH ₄ ⁺	4
NH _x in	100	mmol m ⁻³ (= μmol l ⁻¹)	NH _x concentration of water coming into the meadow	5
R	5	day ⁻¹	Replacement rate of the water in the meadow	±
U _{max}	0.492	mmol g ⁻¹ day ⁻¹	Maximum uptake rate per g dry weight	
H _{NHx}	9.2	mmol m ⁻³ (= μmol l ⁻¹)	Half rate constant for NH _x uptake	6
C	0.5	m	Canopy height	2, +
Dw _z	0.44	g	Dry weight per shoot	*

(1) (Olesen & Sandjensen 1994), (2) (Boström et al. 2003), (3) (Choo et al. 2002, Feike et al. 2007), (4) (Khoo et al. 1977), (5) (Hauxwell et al. 2001 , Brun et al. 2002), (6) (Thursby & Harlin 1982), (*) recalculated from original data of (van der Heide et al. 2008), (+) unpublished results, (±) Estimated

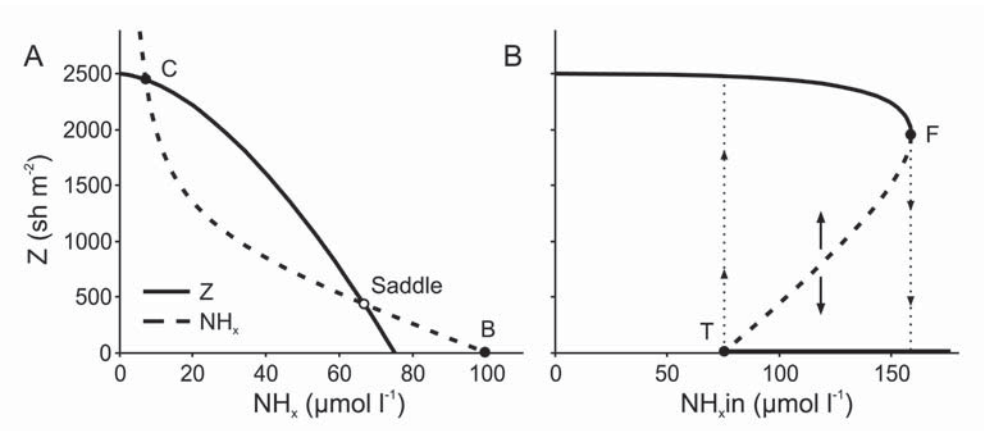


Figure 5.4: Analyses of the empirical eelgrass model. **A:** Nullclines of the model at default settings. **B:** Bifurcation analysis of the model with varying NH_x concentrations in the incoming water (NH_xin). See fig. 2 for the meaning of symbols used.

threshold vegetation will collapse to a bare state. A bifurcation analysis on the NH_x concentration of the water flowing into the eelgrass meadow ($\text{NH}_{x\text{in}}$) reveals that alternative stable states are present over a wide range of realistic concentrations, from 75 to over $158 \mu\text{mol l}^{-1}$ (fig. 5.4B). We analyzed the interactive effects of $\text{NH}_{x\text{in}}$, pH and replacement rate R , because these parameters are often variable in the field. Results demonstrate that the effect of the NH_x concentration of the incoming water is highly dependent on both pH and the replacement rate of the water inside the meadow (fig. 5.5A & B). The analysis shows that alternative stable states are present at pH values higher than 7.9 (fig. 5.5A). Below pH 7.9, the toxicity of NH_x is too low as only little NH_x is present as toxic NH_3 . Therefore, the meadow tolerates extremely high concentrations of NH_x in the incoming water. Sensitivity to NH_x exposure increases strongly with rising pH levels, as the $\text{NH}_4^+/\text{NH}_3$ equilibrium shifts towards NH_3 . At pH 10, alternative stable states exist between NH_x concentrations of 10 and $55 \mu\text{mol l}^{-1}$ in the water flowing into the meadow. Figure 5.5B demonstrates the interactive effects of $\text{NH}_{x\text{in}}$ and water replacement rate R . No alternative stable state are present when the concentration of NH_x is below $75 \mu\text{mol l}^{-1}$, since these concentrations are not lethal for the eelgrass plants at pH 8.5 (cf the first prerequisite of the theoretical model). The effect of NH_x becomes dependent on both NH_x input concentrations and the turnover rate R , when NH_x concentration of the incoming water rise above the $75 \mu\text{mol l}^{-1}$ threshold. Alternative stable states exist far beyond NH_x concentrations of $500 \mu\text{mol l}^{-1}$ for $\text{NH}_{x\text{in}}$ when R drops below 1 day^{-1} .

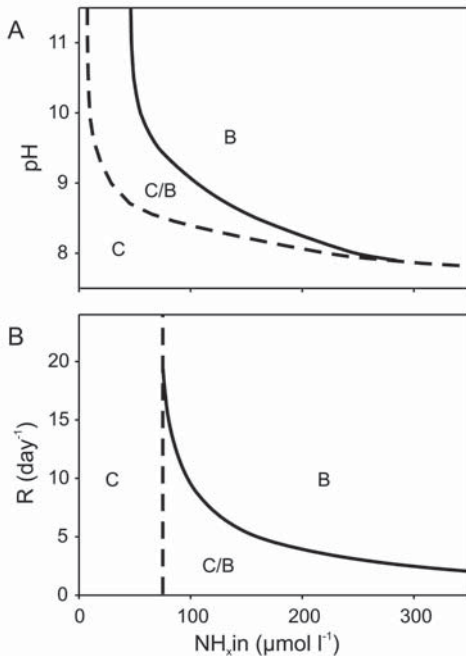


Figure 5.5: Two-dimensional bifurcation analyses of the empirical model. **A:** Bifurcation analysis with varying pH and NH_x concentrations in the incoming water ($\text{NH}_{x\text{in}}$). **B:** Bifurcation plot with varying replacement rates of the water in the meadow (R) and NH_x concentrations in the incoming water ($\text{NH}_{x\text{in}}$). Solid lines represent fold bifurcations, while the dashed lines indicate transcritical bifurcations. *B* indicates a bare state; *C/B* indicates the area where alternative stable states occur. Left of the dashed lines (indicated with *C*), eelgrass presence is the only stable state.

DISCUSSION

We show that 'density-dependent toxicity', a positive feedback mechanism between a population of organisms and a toxic compound, may lead to bistability in biological systems if the feedback is strong enough. Organisms may be able to alleviate any adverse effects of the toxicant by actively lowering ambient concentrations through either 'joint detoxification' or 'growth dilution'. Joint detoxification is a mechanism where the toxicant is actively broken down by the exposed organisms. The population can maintain itself, provided that its biomass is sufficient to reduce toxicant concentrations to a level where organism growth may equalize or exceed mortality. If the organism density drops beneath a certain threshold for biomass, toxicant concentrations will rise and any remaining individuals will die, resulting in a shift to an alternative stable state. Growth dilution is a mechanism where the toxicant is not broken down, but is stored in the organisms tissues. Because these tissues are only able to store a limited amount of toxicants, they will become saturated. In this case, reduction of the toxicant is dependent on population growth rather than the biomass present in the system. Compared to joint detoxification, growth dilution typically results in a narrower range of alternative stable states and a lower maximum biomass as now the strongest effect of the population on the toxicant is halfway the carrying capacity where growth is maximal. Note however that, despite these differences between the mechanisms, the qualitative behavior of both models turned out to be strikingly similar.

Our eelgrass model illustrates that density-dependent toxicity may indeed be important in real ecosystems. Although the model is somewhat more complicated, but its essence is identical to our theoretical joint detoxification model. Sudden die-off events caused by high reduced nitrogen (NH_x) loads, combined with a high pH may be prevented if shoot density of the meadow is high enough. Combined uptake by the shoots lowers ambient concentrations of ammonium (NH_4^+) and ammonia (NH_3) to levels that are tolerable for eelgrass. This mechanism fails if shoot densities are pushed below a certain threshold, resulting in a shift to a bare state. Our bifurcation analyses show that these model outcomes are robust. At realistic parameter settings, the model shows hysteresis over a wide range of NH_x input concentrations. Moreover, bifurcation analyses show that alternative stable states are present over a wide range of settings of key-parameters like NH_x input concentration, pH or water replacement rate.

Although we studied only one possible example, density-dependent toxicity is most likely an important mechanism in a wide range of biological systems. Reduction of toxic effects by joint detoxification has not only been reported for dynamics between reduced nitrogen and eelgrass (van der Heide et al. 2008; this paper), but for example also for ammonium in isoetid macrophytes. In these vegetations, ammonium toxicity can be prevented since ammonium concentrations in the pore water are actively lowered, not only by uptake, but also by density-dependent oxidation of ammonium to nitrate due to high radial oxygen loss of the roots (Smolders et al. 2002). Toxic effects of sulphide in salt-marshes (Webb et al. 1995, Webb & Mendelssohn 1996), seagrasses (Goodman et

al. 1995, Pedersen et al. 2004) or sulphate-rich freshwater wetlands (Lamers et al. 1998, Armstrong & Armstrong 2001, van der Welle et al. 2006) may be prevented in a similar way. In these systems, sulphide can be oxidized to harmless sulphate if oxygen loss by the root system is sufficiently high.

A second possible mechanism for density-dependent toxicity, growth dilution, may for instance reduce toxic effects of heavy metals; toxicants that cannot be broken down. The dilution effect increases tolerance of microbes to heavy metal exposure (Duxbury & McIntyre 1989), while accumulation of toxic metals in the trophic chain of aquatic food webs has been shown to be reduced with increasing concentrations of phytoplankton (Pickhardt et al. 2002) or even with increasing nutritional quality of the algae (stoichiometric dilution) (Karimi et al. 2007). Finally, there are also some biological systems with density-dependent toxicity, where the exact mechanism (joint detoxification or growth dilution) is still unclear. These systems include for instance the reduction of toxicity of allopathic compounds in terrestrial plant species (Weidenhamer et al. 1989, Pollock et al. 2008) and microbial populations (Greig & Travisano 2008). Similarly, density-dependence also affects resistance of infectious bacteria (Brook 1989) and cancer cells (Kobayashi et al. 1992, Brandt et al. 2004) to drug treatment (i.e. the 'inoculum effect').

Although our analyses suggest that the mechanism presented in this study may lead to alternative stable states in many biological systems, it should be noted that dynamics in our model are described in a simplified manner. This implies that the models may disregard or oversimplify processes that might in reality be important. These can include factors that weaken the positive feedback as well as processes that enhance it. For instance, one disregarded factor, possibly weakening the positive feedback in our eelgrass model, is that high shoot densities may imply a higher photosynthetic activity, causing a higher pH in sheltered conditions, which in turn may lead to increased ammonia toxicity (van der Heide et al. 2008). Other, factors that may weaken the feedback mechanism in biological systems may include sub-lethal physiological effects that may inhibit growth or reproduction (Moriarty 1999), limitation of resources (e.g., nutrients, water) (Weidenhamer 1996), competition with other species (Weidenhamer 1996) or disease (van der Heide et al. 2007). These factors can reduce the strength of the feedback because they negatively affect the density of the population or reduce the fitness of individuals. Additionally, spatial variation in population density or input rate of toxicants can also affect the resilience of a system (van Nes & Scheffer 2005). Processes that may strengthen the feedback include for instance symbiosis or natural selection leading to more resistant individuals (Brook 1989).

Despite the fact that the described mechanism might not lead to hysteresis in all systems, either due to interfering factors or simply because the feedback mechanism is too weak, density-dependent toxicity may still be important in such systems. Our analyses demonstrate that exposure of a system to toxicants still causes a strong non-linear response, even in case of a weak feedback (not shown). Thus, if laboratory tests on individual organisms from such systems were to be extrapolated to the field, it could lead to an overestimation of the effects of the toxicant in a natural

population. It may therefore be important to test toxicity effects for a range of relevant densities and concentrations.

In summary, we present a feedback mechanism between organisms and toxic compounds that may potentially lead to bistability in biological systems. Adverse effects of toxicants that are being produced or coming into the system may be prevented by actively lowering ambient concentrations through either rapid joint detoxification or growth dilution. The presented general mechanism may be important in a wide range of biological systems.

APPENDIX 5.1: Analytical bifurcation analysis

Joint detoxification assumption. In this case we assume that the alleviation of toxicity is only dependent on the biomass of the organisms ($f(X) = d_1 X$).

We scale the model (3) by substituting:

$$X = K_x X^*; t = \frac{t^*}{a} \text{ and defining two non-dimensional parameters: } \alpha = \frac{b}{a} T_{max}; \beta = \frac{d_1}{p} K_x$$

$$\frac{dX^*}{dt^*} = (1 - X^*)X^* - \alpha \frac{X^*}{1 + \beta X^*}$$

This simple model with two parameters can be analyzed analytically. The equilibria of this model are:

$$0 = (1 - X^*)X^* - \alpha \frac{X^*}{1 + \beta X^*}$$

$$X^* = 0 \vee X^* = \frac{(\beta - 1) \pm \sqrt{\beta^2 + 2\beta + 1 - 4\beta\alpha}}{2\beta} \quad (x)$$

The model can have three equilibria if the square root in (x) is larger than 0:

$$\beta^2 + 2\beta + 1 - 4\beta\alpha > 0 \text{ or simplified: } \alpha < \frac{1}{4} \left(\beta + 2 + \frac{1}{\beta} \right) \quad (xx)$$

Condition (xx) determines the position of the fold bifurcation, which is the upper boundary of parameter region with alternative stable states. For biological meaningful solutions, both equilibria in (x) should also be positive, which is the case if:

$$(\beta - 1) > \sqrt{\beta^2 + 2\beta + 1 - 4\beta\alpha}$$

Which is true if $\alpha > 1$ and $\beta > 1$. These conditions determine the lower boundary of parameters with alternative stable states (transcritical bifurcation). So the conditions for alternative stable states are:

$$1 < \alpha < \frac{1}{4} \left(\beta + 2 + \frac{1}{\beta} \right) \text{ and } \beta > 1$$

Substituting the original parameters:

$$1 < \frac{b}{a} T_{max} < \frac{1}{4} \left(\frac{d_1 K_x}{p} + 2 + \frac{p}{d_1 K_x} \right) \text{ and } \frac{d_1 K_x}{p} > 1 \text{ or simplified:}$$

$$\frac{a}{b} < T_{max} < \frac{a}{4b} \left(\frac{d_1 K_x}{p} + 2 + \frac{p}{d_1 K_x} \right) \text{ and } K_x > \frac{p}{d_1}$$

Growth dilution assumption. In this case we assume that the alleviation of toxicity is dependent on the growth of the population (we conservatively assumed this to be similar with the logistic growth term $f(X) = d_2 a X (1-X/K)$).

We again scale the model (3) by substituting:

$$X = K_X X^*; \quad t = \frac{t^*}{a} \quad \text{and defining two non-dimensional parameters: } \alpha = \frac{b}{a} T_{max}; \quad \gamma = \frac{d_2}{p} a K_X$$

$$\frac{dX^*}{dt^*} = (1 - X^*) X^* - \alpha \frac{X^*}{1 + \gamma X^* (1 - X^*)}$$

Again we have a model with two parameters that are similar to the parameters in the above model. However the analytical solution of the equilibria is too complex to analyze. Therefore we analyzed the bifurcations numerically using GRIND for MATLAB (fig. 5.3B).

6

Spatial self-organized patterning in seagrasses along a depth gradient of an intertidal ecosystem

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ABSTRACT

The spatial structure of seagrass landscapes is typically ascribed to the direct influence of physical factors such as hydrodynamics, light and sediment transport. We studied banded patterns, formed by elongated seagrass patches, in a small-scale intertidal ecosystem. In this system, we investigated 1) whether spatial patterns may arise from feedback interactions between seagrass and its abiotic environment and 2) whether changing abiotic stress may lead to predictable changes in these spatial patterns. Field measurements, experiments and a spatially-explicit computer model identified 'scale dependent feedback', a form of spatial self-organization, as mechanism causing the banded patterns. Increased protection from uprooting by improved anchoring with increasing seagrass density caused a local positive feedback. Sediment erosion around seagrass shoots increased with distance through the seagrass bands, hence causing a long-range negative feedback. Measurements across the depth gradient of the intertidal, together with model simulations, demonstrated that seagrass cover and mean patch size were influenced in a predictable way by additional stress caused by light limitation and desiccation. Thus, our study provides direct empirical evidence for a consistent response of spatial self-organized patterns to changing abiotic conditions, suggesting a potential use for self-organized spatial patterns as stress indicator in ecosystems.

INTRODUCTION

Seagrasses are rhizotomous marine angiosperms that cover about 0.1 to 0.2 % of the global oceans. Their meadows are among the Earth's most productive ecosystems and serve as key habitat in the life-cycles of many marine animal species (Orth et al. 2006). Seagrasses typically grow in littoral environments where they are exposed to multiple stressors. Depth zonation is mostly determined by light conditions at the lower depth limit, while tolerance to emersion often defines the upper depth limit (Philippart & Dijkema 1995, Leuschner et al. 1998). The spatial structure of the seagrass landscape within these boundaries is often mainly ascribed to the influence of physical factors such as wave action, currents, sediment transport and light (Patriquin 1975, Robbins & Bell 1994, Marba & Duarte 1995, Bell et al. 1999, Fonseca et al. 2002). However, recent studies have shown that seagrasses often improve their own growing conditions (van der Heide et al. 2007, van der Heide et al. 2008), for instance by attenuating currents and waves and reducing sediment resuspension (van der Heide et al. 2007). This suggests that the structure of seagrass landscapes may also arise from feedback interactions between seagrasses and their environment instead of straightforward physical forcing.

Studies from a wide range of terrestrial and marine ecosystems have demonstrated that spatial patterns are often self-organized (Rietkerk & van de Koppel 2008). Spatial self-organization implies that patterns in ecosystems are not generated by any underlying abiotic heterogeneity, but arise from internal organization caused by feedback interactions between organisms and their environment (Pascual & Guichard 2005, van de Koppel et al. 2008). Self-organization may involve irregular patchiness, when localized positive feedback is the dominant force shaping the ecosystem (Kefi et al. 2007, Scanlon et al. 2007), but patterns can also be highly regular. In spatially-explicit models, regular spatial patterns can be described mechanistically by an interaction between a local positive and a long-range negative feedback; a phenomenon called scale-dependent feedback (Rietkerk et al. 2004, Rietkerk & van de Koppel 2008). Because scale-dependent feedbacks seem a universal mechanism behind formation of regular patterns, several recent modelling studies suggest that such patterns may be used as stress indicator in ecosystems (Rietkerk et al. 2004, Rietkerk & van de Koppel 2008). At present, however, any empirical basis for a predictable and consistent response of self-organized regular patterns to changing abiotic conditions is lacking.

In this study, we investigated the mechanisms behind seagrass patterns and their response to changing abiotic stress on an intertidal depth gradient. In our study area, a sandy intertidal beach at Saint Efflam bay, France, elongated patches of the seagrass *Zostera noltii* form a banded pattern (fig. 6.1A) that has been present for at least a quarter of a century (unpublished data). The seagrass bands at this site are typically orientated perpendicular to the (north-south) current and wave direction. Because the seagrass patterns are situated on a depth gradient, they are subjected to small-scale gradients (<1 km) of multiple stressors caused by tide induced differences in emersion time, exposure to hydrodynamics and light availability. Therefore, the system provided a very suitable environment to empirically test 1) whether spatial self-organization may cause seagrass patterning

and 2) whether changing abiotic stress (light availability and exposure) may lead to predictable and consistent changes in self-organized spatial patterns. We first investigated the mechanisms causing seagrass patterning using a combination of field measurements and experiments. Next, we examined the effects of changing abiotic conditions (hydrodynamics, light, desiccation) on the pattern characteristics across the gradient. Based on results from this field study, we constructed a spatially explicit model to test whether the identified interactions and stressors could indeed explain the observed patterns and changes across the gradient.

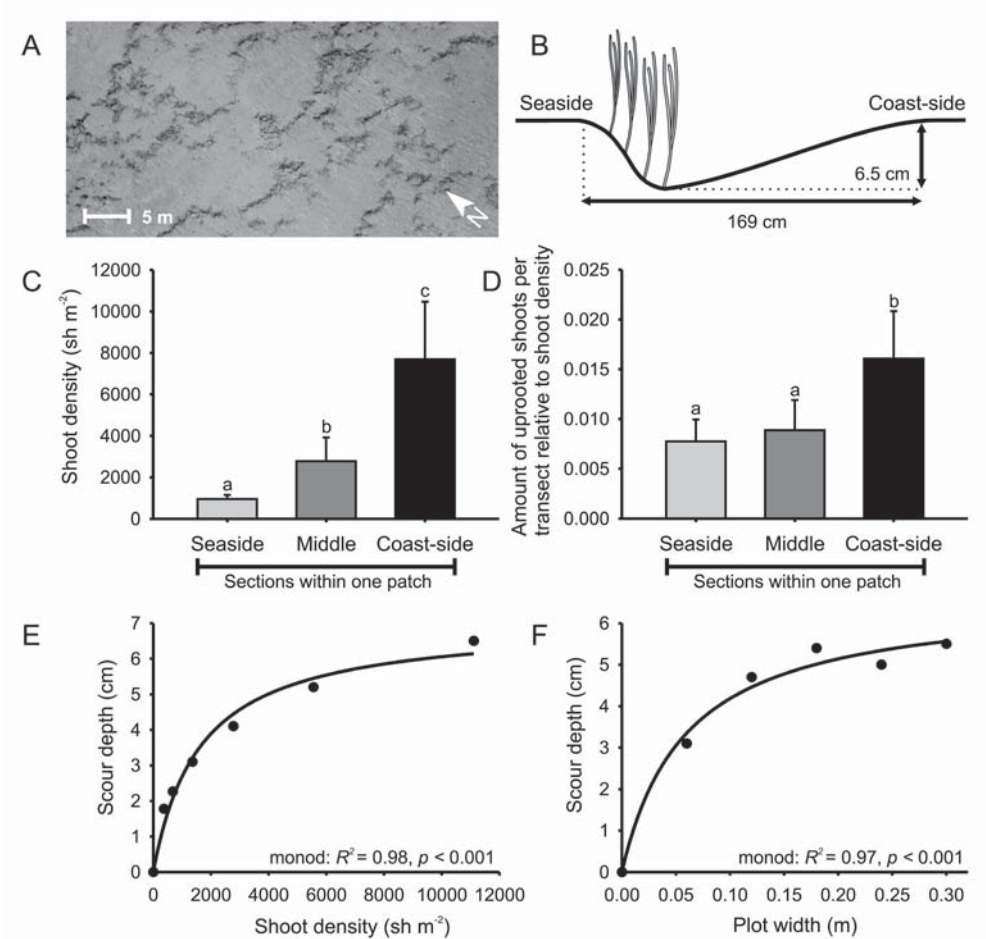


Figure 6.1. Measured characteristics of the seagrass bands. **A:** An aerial photograph of the seagrass patterns at low tide. **B:** Schematic cross-section of an asymmetric depression characterizing the seagrass bands. The mean scour depth was 6.5 (± 0.4 SD, $n=6$) cm and the total length of the disturbance was 169 (± 28 SD, $n=6$) cm. **C:** Significant change in shoot density of a seagrass band from the seaside to the coast-side of the seagrass bands (ANOVA: $F(2,10)=24.1$, $p<0.001$) and **D** in uprooting of shoots (ANOVA: $F(2,6)=11.1$, $p=0.010$). Error bars \pm SD. Different letters indicate significant differences found by post-hoc comparisons. **E:** Maximum equilibrium scour depth at varying shoot densities (plot size: 0.3 x 0.3 m) and **F:** different plot widths (shoot density: 5556 sh m⁻²). Both relations fitted best to a monod equation.

SITE DESCRIPTION

Saint Efflam bay covers an area of around 5 km² and is located in the north of Brittany (France), near the town of Lannion. The bay is enclosed by high rocky cliffs, except for the north-side of the bay, the direction from where the water enters during flood tide. Incoming waves can move unobstructed and are therefore travelling far into the bay. Due to the relatively flat incline of the beach (< 0.5 %), waves keep on travelling over a large distance of up to 500 m up to the shoreline after breaking (van Rijn 1990). The seagrass stand of *Zostera noltii* is located at the west side of the bay and covers an area of about 0.5 km² ($\pm 0.7 \times 0.7$ km). The seagrass patches at the site follow a seasonal cycle with respect to growth and development. From October until March, the seagrass bands lose their aboveground biomass and survive belowground on energy stored in the rhizomes. Aboveground biomass is regained in the growth season (from April to September).

FIELD STUDY METHODS

Analysis of patterns and mechanisms. We first tested whether patterning of the seagrass bands was regular. We mapped 15 quadrates ($\pm 15 \times 15$ m) located on a 700 m transect across the tidal gradient using RTK-GPS (Real Time Kinematic Global Positioning System). Images obtained from these mappings were subsequently analyzed for regularity by two-dimensional spectral analysis (Renshaw & Ford 1983, van de Koppel et al. 2005).

Next, we investigated the mechanism causing pattern formation. To detect any changes in the patterns over time, we tracked the spatial position and growth rate of 6 randomly selected seagrass bands over a 2-year period using belowground stainless steel markers that could be located by GPS and a metal detector. We measured the change in shoot density and sediment height across 6 individual bands by analyzing these parameters in three equally large sections for each band (seaside, middle and coast-side). The relative number of uprooted shoots per section (standardized to shoot density) was determined on a much larger number of bands, by counting shoots with exposed rhizomes along 4 transects from the coast to the sea. Onsite hydrodynamics (wave height and current velocity) measurements were carried out in twofold for 4 tidal cycles (including spring and neap tide) across 1 month using 2 simultaneously recording automated measuring frames (Bouma et al. 2005).

The effect of seagrass on sediment dynamics was first tested by removing all aboveground biomass from 6 randomly selected bands. Next, effects of density and width of the bands (north-south direction) were tested by setting up 2 experiments with 40 cm long seagrass mimics that were driven 15 cm into the sediment bed in varying configurations (Bouma et al. 2005). To examine the influence of density, we planted 0.3×0.3 m plots with 6 different shoot densities (370 to 11111 shoots m⁻²). The effect of band width was tested by planting 5 plots with varying widths (0.05×0.3 to 0.3×0.3 m; shoot density 5556 shoots m⁻²). Changes in sediment height across the plots were recorded during 6 consecutive tidal cycles.

Gradient analysis. After determining the general mechanism of pattern formation, we examined how the seagrass bands changed across the depth gradient due to changes in abiotic stress. We calculated seagrass cover and mean patch size for each of the 15 quadrates, by analyzing the RTK-GPS data in GIS (Geographical Information System). Additionally, we determined the change in wavelength of the patterns by spectral analysis (Renshaw & Ford 1983, van de Koppel et al. 2005).

Next, we performed experiments and measurements to unravel the influence of different stressors on seagrass cover and patch size across the gradient. The effects of varying exposure to hydrodynamics was tested experimentally with seagrass mimics, by planting 14 plots (0.3 x 0.3 m) across the 700 m gradient (shoot density: 5556 shoot m⁻²). The influence of light limitation was examined by determining emersion periods and measuring light availability during submersion. Average emersion periods were determined from RTK-GPS data together with calculations on the mean tidal cycle in the bay. Light availability during submersion was recorded in twofold for 4 tidal cycles (including spring and neap tide) across 1 month using 2 simultaneously recording automated measuring frames (Bouma et al. 2005).

Finally, we investigated the influence of desiccation stress on seagrass patterning across the gradient. We determined photosynthetic yield by pulse amplitude-modulated (PAM) fluorometry as an indication of the vitality of photosystem II (PSII) (Rohacek & Bartak 1999), because damage to the plants photosynthetic capacity is one of the first signs of dehydration in *Z. noltii* (Leuschner et al. 1998). Measurements were performed on dark-adapted shoots (1 hour of adaptation) from the optimum and the upper and lower depth limit. Because spatial variation was high, we used 60 randomly selected replicates for each location.

Statistical analyses. Prior to analysis, data of each tested variable was tested for normality and equality of variance, and following tests were selected accordingly. Yearly change in the spatial position of seagrass bands and change in shoot density within bands were tested with repeated measures ANOVA with Bonferroni comparisons. Counts of uprooted shoots were tested using ANOVA combined with Tukey's HSD as post-hoc test. Kruskal-Wallis and Mann-Whitney U post-hoc tests with Bonferroni significance correction were used on PAM results. Change in seagrass cover and patch size across the gradient and all experiments with mimics were analyzed by linear and non-linear regression. Tested functions were: linear, exponential and monod. We adopted the equation that fitted best to each relation. R² and the F-test were used as quality measure for each fit.

FIELD STUDY RESULTS

Analysis of patterns and mechanisms. Spectral analysis of images obtained with RTK-GPS revealed clear regularity in the patterns, suggesting a mechanism of self-organization. Individual bands were characterized by strikingly asymmetric depressions (fig. 6.1B) with a maximum depth of 6.5 (±0.4 SD, n=6) cm at the coast-side edge of the seagrass bands. The maximum depth of the depressions equalled mean seagrass rooting depth, suggesting that shoots eroded at the coast-

side. This was further supported by shoot density measurements together with counts of uprooted shoots, showing that numbers of exposed rhizomes were significantly higher on the coast-side, compared to the middle and seaside sections (fig. 6.1C, D). Measurements on the spatial position of the seagrass bands confirmed that they mainly eroded at the coast-side while expanding on the seaside, resulting in a significant net seaward migration of $17 (\pm 10 \text{ SD}, n=6) \text{ cm year}^{-1}$ (ANOVA: $F(1,5)=17.5, p=0.009$).

In all 6 bands where aboveground biomass was removed, depressions disappeared within 2 tidal cycles. Next, experiments with seagrass mimics revealed that the asymmetric depressions indeed developed as a result of seagrass presence, by erosion of sediment around seagrass shoots (fig. 6.1E, F). Equilibrium depth of the depression was always reached within 5 tidal cycles (2.5 days). The maximum erosion depth (scour depth), at the coast-side edge of the plots, was dependent on both shoot density and the width of individual bands. This indicated that erosion was caused by interactions between hydrodynamics and seagrass itself. Hydrodynamics measurements demonstrated that tidal currents were relatively slow (maximum measured water speed 0.2 m s^{-1}) and that breaking waves with an average significant wave height of 0.5 m form the main hydrodynamic force in the system.

Gradient analysis. The relative amount of overall surface covered by seagrass bands varied along the depth gradient. GIS analysis of the 15 quadrates along the 700 m transect showed that seagrass coverage increased linearly from the upper depth limit to a maximum of around 30 %, after which coverage declined again in a linear fashion in the direction of the lower depth limit (fig. 6.2A). Moreover, mean patch size showed the same trend along the gradient (fig. 6.2B) and wavelength of the patterns changed from 2 m near the maximum to about 5 m near the outer limits.

Experiments with 14 plots of seagrass mimics planted across the gradient showed that the equilibrium scour depth of the depressions was not influenced by the location of the plots (linear fit: $R^2 = 0.03$; $p = 0.591$). Hence, differences in hydrodynamic exposure were not responsible for the observed gradient in seagrass coverage. PAM measurements revealed that shoot-fitness was reduced by 43 % from the optimum to the upper depth limit, but that desiccation was no significant factor on the lower part of the depth gradient (fig. 6.2C). This indicated that desiccation is the most important stressor shaping the pattern gradient between the upper depth limit and the optimum.

Finally, turbidity measurements of the water column showed that light availability at the bottom ranged from 5 % in the first and last 15 minutes of submersion to less than 1 % halfway the submersion period. As even 5 % of light is not nearly enough for seagrass photosynthesis to even reach its light-compensation point (Philippart 1995, Vermaat & Verhagen 1996), seagrass can only grow during emersion. Average emersion periods at the upper depth limit, optimum and lower depth limit were about 7, 4.5 and 1.5 hours respectively (fig. 6.2A, B). Because the optimal emersion period of *Z. noltii* usually varies between 5 and 8 hours (Philippart & Dijkema 1995), light limitation seems to be the driving force behind the gradient in seagrass cover and mean patch size between the optimum and the lower depth limit.

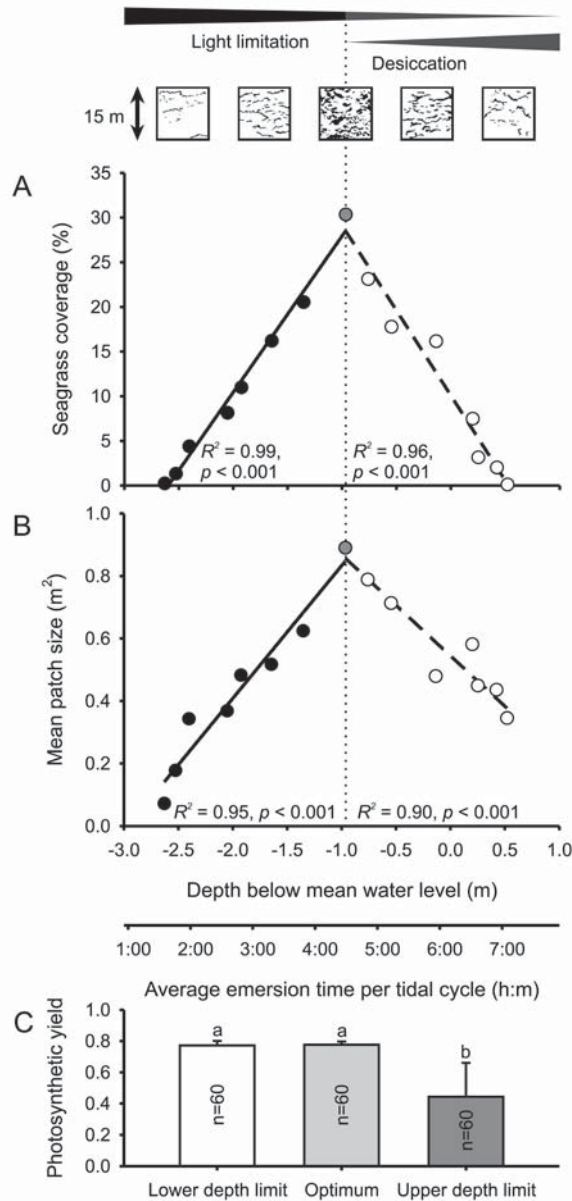


Figure 6.2. Measured characteristics of seagrass patterning across the depth gradient. Boxes above fig. 6.2A depict actual measurements on the presence of seagrass bands (dark areas) on the beach (white area) for 5 of the 15 quadrates. **A & B:** Seagrass cover and mean patch size show optima at around 1 m below the mean water level or an emersion (light) period of 4.5 hours. Both cover and mean patch size decrease linearly in the direction of the upper and lower depth limits. **C:** Measurements by pulse amplitude-modulated (PAM) fluorometry reveal that desiccation is a significant stressor in the upper (shallow) part of the gradient (Kruskal-Wallis: $\chi^2=116.7$, $df=2$, $p<0.001$). Error bars \pm SD, different letters indicate significant differences found by post-hoc comparisons. The lower part of the gradient is light limited as the optimal emersion period for *Z. noltii* is between 5 and 8 hours.

MODEL

Model description. Results from our field study suggest that the banded patterns arise due to scale-dependent feedbacks between hydrodynamics and seagrass itself. A local positive feedback, is caused by the seagrass roots and rhizomes. A high density of the root system improves sediment stability and anchoring capacity of the plants, thereby locally preventing uprooting of seagrass shoots (Peralta et al. 2005). Contrastingly, aboveground parts cause a negative feedback on a longer range. The interaction of the shoots with the hydrodynamics cause a disruption of sediment dynamics (i.e., scouring). This disruption intensifies with distance through each seagrass band (van Rijn 1990, Nepf 1999), resulting in increased erosion of both sediment and seagrass shoots towards the coast-side edge of a band. Apart from stress caused by scale-dependent feedback, field results also illustrate that additional stress caused by desiccation and light limitation causes consistent change in seagrass cover and mean patch size.

To test whether observed seagrass patterns and changes in patterns can indeed be caused by the mechanisms suggested from our field study, we constructed a minimal, spatially explicit model. The model describes changes in shoot density (dZ_{ij}/dt) and scour depth (dD_{ij}/dt) on a 200×50 grid by two differential equations:

$$\frac{dZ_{ij}}{dt} = r \left(1 - \frac{Z_{ij}}{K} \right) Z_{ij} - m f_1(D_{ij}) \frac{h_1}{h_1 + Z_{ij}} Z_{ij} + d_z \Delta Z_{ij} \quad (1)$$

$$\frac{dD_{ij}}{dt} = e \frac{f_2(Z_{ij})}{f_2(Z_{ij}) + h_2} - s D_{ij} \quad (2)$$

Here, Z_{ij} and D_{ij} are the seagrass shoot density (shoots m^{-2}) and the scour depth of grid cell ij respectively. The maximum net logistic growth rate is described by r (day^{-1}), m is the maximum mortality rate due to uprooting of shoots (day^{-1}), e is the maximum scouring rate ($m \ day^{-1}$) and s is the depth dependent sedimentation rate (day^{-1}). K is the carrying capacity (shoots m^{-2}), h_2 ($sh \ m^{-2}$) is the half rate constant for the enhancing effect of seagrass on e and d_z describes the lateral dispersal of Z_{ij} from and to neighbouring grid cells. The short-range positive effect of seagrass on their own growth is described in a Monod equation with half saturation h_1 which locally causes a lower loss rate. The function $f_1(D_{ij})$ describes the effect of scour depth on seagrass mortality m :

$$f_1(D_{ij}) = \frac{D_{ij} - D_r}{D_{max} - D_r} \text{ if } D_{ij} > D_r \quad (3)$$

With D_{max} as the maximum scour depth (m) and D_r as the minimum seagrass rooting depth (m) after which $f_1(D_{ij})$ increases in a linear fashion. Finally, $f_2(Z_{ij})$ describes the longer-range effect of seagrass on the scouring rate e . Field measurements on the depth profiles of the bands revealed that the effect of seagrass decreased exponentially with distance n . The cumulative eroding effect of all seaward seagrass can thus be described as:

$$f_z(Z_{ij}) = \int_{n=X}^{n=Y+\infty} e^{-d \cdot n} Z_n dn$$

which was approximated in our model in a discrete way:

$$f_z(Z_{ij}) = \sum_{n=0}^N (1-d/L)^n Z_{x+n} \quad (4)$$

Here, L is the grid size, which was normalized to 1 m, d (m^{-1}) describes how the eroding effect of seagrass decays towards the coast-side with increasing distance. We obtained parameter values from our field experiments and observations. The values used in the model analysis were: $K=12000$, $m=0.6$, $D_{max}=0.07$, $D_r=0.03$, $h_1=554$, $d_z=0.1$, $r, h_2=2959$, $s=2$, $e=D_{max}$, $s=0.14$ and $d=0.1$.

The effects of the light availability and desiccation gradients was analyzed in the model by using a gradual stepwise increase of r . After each small increase of the net maximum growth rate r , the model was allowed to stabilize again. Next, the same analysis was also performed in a backward manner, i.e. a stepwise decrease of r towards zero. For this bifurcation analysis, we used a range of 0 to 0.15 day^{-1} . Note that the maximum net growth rate in the field was 0.1 day^{-1} . For the calculation of seagrass cover, we defined an area as vegetated if the density exceeded $1000 \text{ shoots m}^{-2}$, which was approximately the minimum shoot density in the field (fig. 6.1B).

Model results. Model simulations demonstrated that scale-dependent feedbacks between hydrodynamics and seagrass could indeed generate the observed banded patterns (fig. 6.3A). At realistic growth rates, patterns are typically migrating towards the sea as observed in the field. Our model confirms that self-organization is a potential mechanism behind the observed spatial patterns.

The bifurcation analysis of growth rate r reveals that the model predicts alternative stable states (bistability) between a homogeneous seagrass cover and a patterned state of regularly spaced stagnant bands at high growth rates (i.e. low stress levels) (fig. 6.3B). Bistability in the model disappears when stress levels are increased and the maximum net growth rate resembles those measured in the field (max. $\pm 0.1 \text{ day}^{-1}$): only a state of migrating banded patterns remains stable. As stress levels are increased even further, the model predicts an almost linear decrease of seagrass cover as observed in the field.

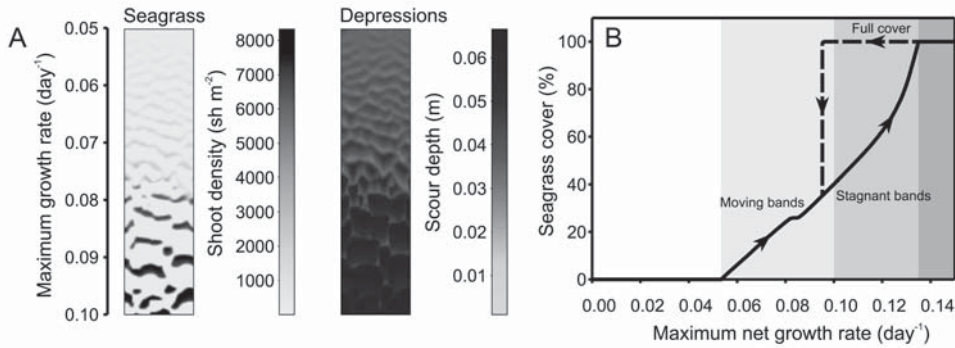


Figure 6.3. Effect of the maximum net growth rate on seagrass cover in the model. **A:** Modeling results after 2 years of simulation on a 200 x 50 grid with a gradually declining growth rate from the bottom to the top. The size of one grid cell compares to about 5x5 cm in the field. **B:** Bifurcation diagram describing the effect of the maximum net growth rate on seagrass cover. Note that the maximum net growth in the field was ± 0.1 day⁻¹. Different shades of grey indicate ranges of growth rates with seagrass absence, migrating seagrass bands, stagnant bands or full seagrass cover.

DISCUSSION

In this study, we report on self-organized, regular spatial patterns in a small-scale intertidal seagrass ecosystem. Even though our ecosystem is possibly unique in the sense of the specific interactions driving pattern formation, its general mechanism of scale-dependent feedback has been reported for a wide range of ecosystems (Rietkerk et al. 2004, Rietkerk & van de Koppel 2008), including (semi-) arid systems, peatlands and forests, but also in marine systems like mussel beds and diatom mats (Rietkerk & van de Koppel 2008). Although some degree of patterning has previously been described for seagrasses (Patriquin 1975, Marba & Duarte 1995, Fonseca et al. 2002), simple physical forces rather than spatial self-organization were proposed as an explanation for these phenomena. This implies that straightforward translation of the driving mechanism in our system to other seagrass ecosystems is difficult, because factors other than scale-dependent feedbacks may also cause patterning in seagrasses. Moreover, it illustrates that understanding the underlying driving mechanisms is critical when using regular patterns as stress-indicator for ecosystems.

Although recent modelling studies suggest that characteristics of self-organized spatial patterns might be indicative for increased stress and degradation (Rietkerk et al. 2004, Rietkerk & van de Koppel 2008), experimental evidence has so far remained scarce. In this study, we provide clear empirical evidence that regular spatial patterns can be indicative for increased stress and degradation. Our results demonstrate that properties of self-organized patterns do not only respond to the mechanisms causing pattern formation, but also respond predictably to changes in additional stressors (light limitation and desiccation in this instance). Changes in properties of regular patterns like decreasing cover and patch size may therefore be regarded as signs of increasing overall stress in ecosystems, caused by for instance eutrophication, climate-change or over-exploitation.

At present, ecological monitoring studies largely rely on abundances and range shifts of

species or functional groups (Barbier et al. 2006). Self-organized spatial patterns can make a valuable contribution to monitoring, since they can yield important information on the main stressors in ecosystems. Moreover, once self-organization has been determined, monitoring of spatial patterns may reduce costs and efforts because spatial data are relatively easy to obtain by for instance aerial photography or satellite imagery. Such a perspective is promising for many ecosystems, including seagrasses. In the last decades, seagrass ecosystems have been increasingly disturbed by human activities (e.g. eutrophication, siltation, dredging), resulting in dramatic and large-scale losses worldwide that were in many cases unexpected (Orth et al. 2006, van der Heide et al. 2007). As a result, monitoring and conservation efforts have recently greatly increased (Orth et al. 2006).

In contrast with results typically obtained from most terrestrial ecosystem models (Rietkerk et al. 2004), we found no indication in either the field data or our model suggesting a potential for catastrophic shift from a vegetated to a bare state. Instead, our model predicts bistability at high growth rates between a patterned and a fully covered state. Our results differ from analyses of terrestrial ecosystems because in contrast to these models, our model has no threshold mechanism for colonization like for instance a limiting resource (e.g. water, nutrients). Instead, feedback control is driven by adverse conditions (i.e. erosion) that are generated by interactions between hydrodynamics and the seagrass itself. Still, despite obvious differences with respect to predicted ecosystem dynamics, all existing models predict a consistent effect of abiotic stresses on spatial patterns, suggesting that self-organized spatial patterns may be used as stress indicator for ecosystems.

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7

Synthesis

Seagrasses are of great ecological importance in temperate to tropical coastal areas. Their ecosystems are among the most productive on earth and serve as a key-habitat for many marine animal species, providing forage, refuge and reproduction grounds (den Hartog 1970, Hemminga & Duarte 2000, Duarte 2002, Green & Short 2003, Orth et al. 2006). Apart from their ecological value, they offer a wide array of other services, including fisheries, shoreline protection and carbon and nutrient storage (Hemminga & Duarte 2000, Duarte 2002, Orth et al. 2006). Despite their ecological and economic relevance, seagrasses have become increasingly disturbed by human activities in the last decades, causing dramatic large-scale losses of these ecosystems worldwide (>30,000 km²) (Short & Wyllie-Echeverria 2000, Duarte 2002, Orth et al. 2006). Therefore, large efforts are made to restore seagrass beds. However, success of restoration projects has so far been limited (about 30% success, Fonseca et al. 2001) even though costs of such measures are extremely high (up to \$ 1000,000 per ha) (Paling et al. 2009).

Seagrass loss has typically been ascribed to multiple environmental stressors (e.g., eutrophication, disease, salinity changes) (Duarte 2002, Orth et al. 2006). The large number of potential stressors in seagrass beds complicates quality assessments of these ecosystems. Additionally, self-facilitating effects of seagrasses on their own growth (Fonseca & Cahalan 1992, Granata et al. 2001, Moore 2004, Bos et al. 2007) may lead to non-linearity and threshold behaviour in these ecosystems, making reliable habitat suitability assessments even more difficult (Scheffer et al. 2001, Scheffer & Carpenter 2003). In turn, unreliable assessments may frustrate restoration or conservation projects, possibly explaining their low success rate.

As stated in the introduction (chapter 1), the main objectives of the present thesis are to improve our understanding of the main causes and mechanisms responsible for the observed worldwide degradation of seagrass beds and to provide tools for seagrass conservation and restoration. These objectives are addressed using the temperate seagrasses *Zostera marina* and *Zostera noltii* as model species. This final chapter integrates the most important findings described in this thesis with existing knowledge on seagrass ecosystems, their stability and reasons for decline. First, I discuss the environmental stress factors determining seagrass growth, the (cor)relations between these factors and the potential of these variables as predictors for seagrass habitat suitability. Next, the function of seagrasses as ecosystem engineers and the impact of feedback mechanisms in these ecosystems is addressed. Finally, implications of the presented findings for seagrass ecosystem management are put forward.

MULTIPLE STRESSORS IN SEAGRASSES

In the last decades, studies on seagrass decline have identified a multitude of possible stressors in these ecosystems (den Hartog 1970, Hemminga & Duarte 2000, Duarte 2002, Green & Short 2003, Orth et al. 2006). Tropical seagrasses are mainly threatened by salinity changes, eutrophication and siltation caused by agriculture and deforestation in adjacent terrestrial areas (Duarte 2002, Orth et al. 2006). Additionally, meadows in tropical areas are also frequently damaged by boats and

dredging activities. In temperate regions, losses are mainly ascribed to eutrophication, increased water temperature and disease (den Hartog 1987, Giesen et al. 1990, Giesen et al. 1990, Duarte 2002, Orth et al. 2006). Because of the large number of potential stressors, some of them may coincide or even interact within a single ecosystem, thereby increasing total stress on seagrasses (Orth et al. 2006). In for instance the Dutch Wadden Sea, 150 km² of *Zostera marina* meadows collapsed within a few years in the 1930s when the 'wasting disease', that hit the North-Atlantic region, coincided with altered hydrodynamics and increased suspended sediment-turbidity caused by the construction of the 'Afsluitdijk' (chapter 3; den Hartog & Polderman 1975, Giesen et al. 1990).

In general, eutrophication is probably the most common stressor causing seagrass decline, especially in temperate seagrasses (Duarte 2002, Nielsen et al. 2002, Kemp et al. 2005, Orth et al. 2006). Unlike disease, hydrodynamics or salinity, it is a summarizer of a number of direct and indirect problems (fig. 7.1). First of all, increased input of nutrients into the ecosystem promote growth of phytoplankton, epiphytes and macroalgae, thereby reducing light availability (Twilley et al. 1985, Nielsen et al. 2002, Kemp et al. 2005). Secondly, eutrophication may also trigger toxicity events, especially in sheltered, stagnant lagoons. In these systems, levels of nitrate or reduced nitrogen (ammonium/ammonia) in the water can become greatly enhanced by for instance waste or river water discharges (Brun et al. 2002) or degradation of phytoplankton or macroalgae (Landers 1982, Farnsworth-Lee & Baker 2000, Hauxwell et al. 2001). Several weeks of exposure to elevated levels of nitrate or ammonium may lead to impaired growth or increased mortality in *Z. marina* and *noltii* (Burkholder et al. 1992, van Katwijk et al. 1997, van Katwijk et al. 1999, Brun et al. 2002). However, reduced nitrogen may also interact with elevated pH levels to cause severe mortality of *Z. marina* within days (chapter 4). In stagnant estuaries, pH may rise to 9 or even 10 during daytime due to increased photosynthesis of for instance phytoplankton, macroalgae or seagrass itself (Choo et al. 2002, Beer et al. 2006, Feike et al. 2007). This in turn increases levels of highly toxic gaseous ammonia, which is converted from ammonium as pH levels rise (chapters 5 & 6; Farnsworth-Lee & Baker 2000, Körner et al. 2001, Körner et al. 2003, Nimptsch & Pflugmacher 2007). Furthermore, increasing pH also causes conversion of the carbon sources CO₂ and bicarbonate, which can both be assimilated by seagrasses, to useless carbonate. As a result carbon availability, needed to detoxify ammonium, is limited (chapter 4; Sand-Jensen & Gordon 1984, Pearson & Stewart 1993, Märschner 1995).

Apart from causing stress in the water layer, eutrophication also influences pore water chemistry (Gieskes 1975, Smolders & Roelofs 1995, Lamers et al. 1998, Smolders et al. 2006). Increased production by algal species in the water layer may lead to accumulation of easily degradable organic matter in the sediment (fig. 7.1). Because oxygen penetration in aquatic sediments is often restricted to a top few millimetres, microbial breakdown of organic matter is largely anaerobic (Gieskes 1975, Drever 1997, Wetzel 2001). Anaerobic degradation results in release of dissolved phosphorus, ammonium and inorganic carbon (CO₂ and HCO₃⁻), leading to strong correlations between these compounds in the sediment pore water (chapter 2). The rate of degradation is strongly dependent on the availability of alternative electron acceptors (Smolders et al. 2006). In marine sediments, the

abundant sulphate is the main substitute for oxygen as electron acceptor (Gieskes 1975). During anaerobic breakdown, sulphate is reduced to sulphide (Gieskes 1975), which therefore also shows strong correlations with mobile nutrient pools in marine sediments (chapter 2). When sulphide levels in the sediment pore water increase, it can become highly toxic to seagrasses (Carlson et al. 1994, Goodman et al. 1995, Pedersen et al. 2004). Although seagrass plants are able to oxidize sulphide back to harmless sulphate by transporting oxygen produced by photosynthesis to the roots (Pedersen et al. 2004), this mechanism fails when light availability is low and sulphide levels are high, hence leading to mass mortality events in seagrass meadows (Carlson et al. 1994, Goodman et al. 1995, Azzoni et al. 2001, Holmer & Bondgaard 2001, Pedersen et al. 2004, Morris et al. 2008).

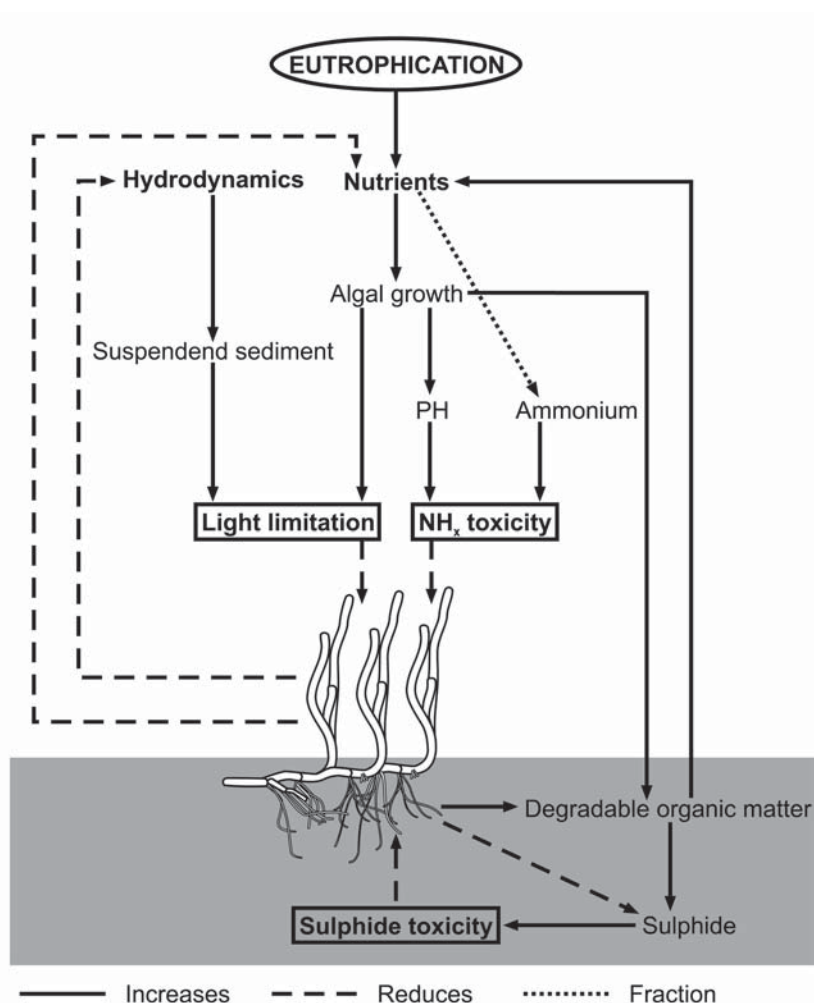


Figure 7.1. A schematic figure showing the most important environmental stressors and interactions in temperate seagrass ecosystems. Many of the environmental variables are interacting or are related to each other and therefore often show strong correlations. Additionally, the scheme shows many feedbacks, illustrating the potential for non-linear behaviour in these ecosystems.

Even though a multitude of environmental variables have been suggested to drive global seagrass decline, their relative importance has thus far never been thoroughly studied. Results presented in chapter 2 demonstrate that presence of *Z. marina* and *noltii* indeed correlates with many environmental factors. However, the analyses also reveal that many of these factors show strong inter-correlations as many processes in these ecosystems are tightly linked (chapters 2; fig. 7.1). It turned out that presence or absence of *Z. marina* or *noltii* can be predicted to a large degree of certainty by including only two variables in a logistic regression model, representing both aboveground and belowground processes (chapter 2). For *Z. marina*, these variables were light attenuation (a water clarity coefficient) and pore water Reduction Oxidation potential (Redox), a relative measure of pore water oxygen conditions. *Z. noltii* presence or absence can be predicted by pore water Redox combined with information on whether a site is situated in the subtidal or intertidal zone (chapter 2).

POSITIVE FEEDBACK IN SEAGRASSES

Seagrasses can significantly modify their environment and can therefore be regarded as ecosystem engineers (fig. 7.1) (Jones et al. 1994). In sheltered conditions, uptake by seagrasses can lower nutrient levels in the water layer (Moore 2004). This reduces growth of epiphytes, macroalgae and phytoplankton (Twilley et al. 1985, Nielsen et al. 2002, Kemp et al. 2005, Burkholder et al. 2007), thereby improving light conditions for seagrass growth. Next, reduction of for instance ammonium or nitrate concentrations may also prevent toxicity events that cause mass mortality in seagrass meadows (chapter 4; Burkholder et al. 1992, van Katwijk et al. 1997, Brun et al. 2002). In more hydrodynamically exposed environments, seagrasses predominantly alter their environment by increasing sediment stability, trapping sediment particles and attenuating waves and currents (chapter 3; Gambi et al. 1990, Fonseca & Cahalan 1992, Granata et al. 2001, Bos et al. 2007). Apart from relieving mechanical stress, the hydrodynamics attenuating and sediment stabilizing properties of seagrasses also lead to reduced sediment resuspension and thus to an improved light climate (chapter 3).

These positive effects of seagrasses on their own growth imply that their growing conditions may improve with increasing size and density of seagrass beds. Theory suggests that such positive feedbacks may lead to alternative stable states (bistability) in ecosystems if the feedback is strong enough (Carpenter 2001, Scheffer et al. 2001, Scheffer & Carpenter 2003). This means that environmental changes or disturbances may drive the ecosystem beyond a critical threshold, causing a sudden collapse to an alternative state (Carpenter 2001, Scheffer et al. 2001, Scheffer & Carpenter 2003). A case study on the 1930s collapse of the eelgrass meadows of the western Dutch Wadden sea, presented in chapter 3 of this thesis, illustrates that alternative stable states may indeed occur in seagrass ecosystems. The combined analyses of historic maps, current turbidity data and a computer model demonstrate that these seagrass meadows most likely considerably reduced turbidity by preventing sediment resuspension. After the 1930s collapse of the eelgrass beds,

caused by the 'wasting disease' and the construction of the 'Afsluitdijk', recovery was impossible because turbidity was no longer reduced by seagrass itself.

Laboratory experiments, described in chapter 4, demonstrate that a positive feedback may also arise from the interactive effects between eelgrass density and reduced nitrogen in the water layer. Apart from showing adverse effects from the interaction of elevated pH with high reduced nitrogen loads (see also previous section), outcomes reveal a clear positive effect of increased shoot density on eelgrass survival. Toxic effects were alleviated by eelgrass itself due to 'joint uptake' of reduced nitrogen, thereby actively lowering ambient concentrations of ammonium and ammonia to sublethal levels. Model analyses based on the experimental results demonstrate that this mechanism may lead to alternative stable states in sheltered estuaries (chapter 5). Moreover, a generalized model suggests that density-dependent toxicity may also cause bistability in seagrasses when interacting with other toxicants (chapter 5). For instance, toxic effects of pore water sulphide in seagrasses might also be density-dependent, since detoxification of the toxin results from oxygen supplied by the root system of the vegetation (Goodman et al. 1995, Pedersen et al. 2004).

SCALE-DEPENDENT FEEDBACKS IN SEAGRASSES

The spatial structure of seagrass landscapes is typically assumed to be shaped by direct physical forces caused by for instance wave action, currents or sediment transport (Patriquin 1975, Robbins & Bell 1994, Marba & Duarte 1995, Bell et al. 1999, Fonseca et al. 2002). Studies from both terrestrial and marine ecosystems have, however, demonstrated that spatial patterns in vegetation or sessile animals are often self-organized, implying that patterns are not generated by any underlying abiotic heterogeneity, but arise from internal organization caused by feedback interactions between organisms and their environment (Pascual & Guichard 2005, van de Koppel et al. 2008). Spatial self-organization may involve irregular patches, when localized positive feedback is the dominant force shaping the ecosystem (Kefi et al. 2007, Kefi et al. 2007), but patterns can also be highly regular. Modelling studies have indicated that regular patterning can be mechanistically described by an interaction between a local positive feedback and long-range negative feedback; a phenomenon called scale-dependent feedback (Rietkerk et al. 2004, Rietkerk & van de Koppel 2008). Because of the apparent cross-ecosystem universality of the mechanism, it has been suggested that these patterns may be used as stress-indicator in ecosystems (Rietkerk et al. 2004, Rietkerk & van de Koppel 2008), even though experimental evidence has thus far remained scarce.

In chapter 6 it is investigated whether spatial self-organized patterning may also be an important mechanism in seagrass ecosystems. At the study site in Saint Eflam bay (France), 'patchy' regular banded patterns of the seagrass *Z. noltii* are growing on an intertidal beach. Field measurements revealed that the seagrass bands eroded on the coast-side, while growing on the seaside, causing the bands to slowly migrate towards the seaside. Additional experiments combined with a spatially explicit computer model, demonstrated that interaction between hydrodynamics and seagrass growth resulted in self-organization by scale-dependent feedback. A local positive

feedback is caused by the seagrass roots and rhizomes, as a high density improves anchoring of the plants in the sediment, thereby preventing uprooting of vegetation. On a longer range, interaction of hydrodynamics and vegetation leads to a negative feedback. The interaction causes a disruption in the sediment dynamics (i.e. scouring), which intensifies with distance through the vegetation, resulting in increased erosion of both plants and sediment towards the coast-side of the vegetation. Finally, measurements and experiments along the depth gradient revealed that additional stress caused by light limitation and desiccation resulted in predictable changes in coverage and patch size of the patterns. Although this ecosystem is possibly unique in the sense of the specific interactions driving pattern formation, these results illustrate that the spatial structure of seagrass systems may not only be controlled by straightforward physical forcing, but that feedbacks interactions between seagrass and its environment can be important in shaping these ecosystems. Moreover, outcomes also provide direct empirical evidence that regular spatial patterns, formed by scale-dependent feedbacks, respond predictably and consistently to changing abiotic conditions, indicating their potential use stress-indicator.

IMPLICATIONS FOR SEAGRASS CONSERVATION AND RESTORATION

In conclusion, results presented in this thesis indeed indicate that seagrass decline is often driven by many different variables, as suggested by many studies. However, it was also demonstrated that many of these variables show strong correlations with each other (chapter 2). Therefore, seagrass habitat suitability may often be estimated using only few variables in for instance a logistic regression model (chapter 2).

Whereas strong correlations among environmental variables may simplify habitat suitability assessments, feedback mechanisms in seagrass ecosystems may complicate them. In ecosystems prone to threshold behaviour, habitat suitability might be dependent on size and density of the seagrass meadow (chapters 3, 4 & 5). An ecosystem may be unsuitable in the absence of seagrass, while self-facilitation effects in a large, high density seagrass meadow may create an environment suitable for seagrass growth. In such cases, habitat evaluation by regression tools, like logistic regression may lead to wrong conclusions as environmental differences may be caused by seagrass itself. A bare ecosystem may seem unfit to sustain seagrasses, while in reality it might be suitable for seagrass growth if a certain threshold for size and density can be bridged (chapter 3). On the other hand, an ecosystem dominated by large seagrass meadows may seem healthy, whereas it might in fact be very close to collapse. Thus, although tools like logistic regression can be very useful to provide a 'pilot evaluation' of a seagrass ecosystem, such methods should always be used in concert with other methods to scan for indications of non-linearity and thresholds.

In general, ecosystems controlled by feedback mechanisms often behave unpredictably. Apart from computer models, several methods have been proposed to detect bistability and to monitor ecosystem stability. Suggested indicators for alternative stable states are for example sudden jumps in time series or bimodality (e.g., two peaks in frequencies of vegetation densities)

(chapter 3; Scheffer & Carpenter 2003). Secondly, experiments can also provide important clues. Typical examples are hysteresis in response to forward and backward manipulation of a control variable, sudden ecosystem shifts caused by perturbation and contrasting outcomes when starting with different organism biomass or density (chapter 4; Scheffer & Carpenter 2003).

Although abovementioned methods can offer important information on whether an ecosystem is bistable or not, they do not provide tools to monitor stability in feedback controlled ecosystems. Recently, a number of monitoring indicators have been put forward. Several modelling studies (Rietkerk et al. 2004, Kefi et al. 2007, Rietkerk & van de Koppel 2008) and the empirical results presented in chapter 6 suggest that self-organized spatial patterns may indicate stress in such ecosystems, both for gradual degradation and for catastrophic shifts. Moreover, indicators have been presented from both regular and irregular patterns. In regular patterns, changes in simple and easy to measure properties like coverage and mean patch size may reveal whether an ecosystem is degrading or improving (chapter 6), whereas a deviation from a power-law distribution in patch sizes may indicate degradation in systems with irregular patterning (Kefi et al. 2007). Finally, in ecosystems with alternative stable states, (change in) resilience of an ecosystem to a shift may also be detected by (repeatedly) measuring the recovery speed of the system after slight perturbation (van Nes & Scheffer 2007, Dakos et al. 2008). Theoretically, recovery from a perturbation will become slower when a system is nearing a critical threshold and resilience is decreasing.

In summary, two important management implications for seagrass management emerge from this thesis. 1) In many cases, a large number of environmental factors threatening seagrass ecosystems are strongly inter-correlated. Therefore, habitat suitability may often be monitored by identifying and measuring only one or two variables. 2) Feedback mechanisms are often important in seagrass ecosystems. It should be emphasized here that when feedbacks are important, recovery of a collapsed ecosystem may in many cases be very difficult, and that protection of intact seagrass beds is therefore critical. In such cases, conservation should try to identify reliable indicators to monitor ecosystem resilience (monitoring spatial patterns, tests on recovery rates after perturbation) and focus on preventing the system from exceeding any critical thresholds. In case of restoration, the most important challenge will be to find possible thresholds for a minimum size and density of a seagrass meadow so that replanted patches will be able to modify their surroundings sufficiently to sustain their own growth.

8

Literature cited

The page features several decorative elements: a horizontal line below the chapter number, a series of diagonal lines of varying lengths and shades of gray extending from the bottom left towards the center, and a faint, stylized illustration of a plant or grass in the bottom left corner.

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Summary

Seagrasses are rhizomatous flowering plants that cover extensive areas in temperate to tropical coastal areas. Their meadows form the basis for some of the most productive ecosystems on earth and harbour a high biodiversity of marine animal life. In the last century, seagrass ecosystems have suffered large-scale losses worldwide, often characterized by sudden mass mortality of the beds. Despite conservation and restoration efforts and increasing public awareness of the values of seagrasses, this decline is at present still continuing. Using *Zostera marina* and *Z. noltii* dominated meadows as model systems, this thesis focuses on determining causes for sudden collapses of seagrass systems, seagrass decline in general, and on identifying predictive tools for seagrass conservation and restoration.

Seagrass loss has typically been ascribed to multiple environmental stressors like for instance eutrophication, disease, salinity changes and toxicity events. Analyses of a field survey conducted at 84 Western-European locations in regions where seagrasses were historically present, demonstrate that presence or absence of *Z. marina* and *Z. noltii* indeed correlates with many of the 30 measured variables (chapter 2). However, the analyses also reveal that many of the variables strongly correlate with each other. Therefore, seagrass presence or absence can be reliably predicted using logistic regression models that include only light attenuation and pore water reduction oxidation potential (redox) for *Z. marina* and tidal location (intertidal or subtidal zone) and pore water redox for *Z. noltii*. Moreover, application of these models to 5 evaluation sites demonstrates that both models can be effectively applied as predictive tools for seagrass ecosystem restoration and conservation success.

Although (combined) effects of multiple stressors in seagrasses may explain global seagrass decline, it does not explain why these ecosystems often suddenly collapse without clear warning. In chapters 3 to 5, I investigated whether positive feedbacks, caused by modification of the abiotic environment by seagrass itself (ecosystem engineering), may be the driving mechanism behind such dramatic shifts. In the early 1930s, such an event happened in the Dutch Wadden Sea (chapter 3). Large eelgrass beds (*Zostera marina*) collapsed within a few years after the construction of a large dam ('Afsluitdijk') and an incidence of the 'wasting disease' and never recovered despite restoration attempts. Analyses of historical maps, a long-term dataset of key factors determining turbidity and a minimal model based on these data, demonstrate that the historical eelgrass beds most likely facilitated their own growth by reducing sediment resuspension, hence increasing light availability. This self-facilitation mechanism caused alternative stable states (bistability) in the system: eelgrass could only grow if sufficient eelgrass biomass was present in the first place to reduce turbidity. Therefore it seems likely that recovery of the eelgrass beds was impossible because turbidity related to sediment resuspension had become too high after the disappearance of the beds.

In chapters 4 and 5, possible implications of ecosystem engineering and positive feedbacks were further explored by laboratory experiments and minimal models based on results from

the experiments. These experiments focussed on toxicity in eelgrass caused by elevated levels of reduced nitrogen in the water layer. Results show detrimental effects from the interaction of elevated pH with high reduced nitrogen loads, but also reveal a significant positive effect of increased shoot density on eelgrass survival (chapter 4). Toxicity is alleviated by eelgrass itself due to 'joint uptake' of reduced nitrogen, which lowered ambient concentrations of reduced nitrogen to sublethal levels. Next, model analyses demonstrate that this mechanism may lead to alternative stable states in poorly mixed, sheltered embayments (chapter 5) and that density-dependent toxicity may also cause bistability in seagrass systems when interacting with other toxicants, like for instance sulphide (chapter 5).

Finally, spatial effects of feedback mechanisms on the spatial structure of seagrasses were studied in the field (chapter 6). At the study site, an intertidal beach in Saint Eflam bay (France), regularly interspaced elongated seagrass patches (*Z. noltii*), growing perpendicular to the current and wave direction, form a banded pattern. Results from field measurements combined with a spatially-explicit computer model show that 'scale dependent feedback', a form of spatial self-organization, is the driving mechanism behind the patterns. Increased protection from uprooting by improved anchoring with increasing seagrass density causes a local positive feedback, while sediment erosion around seagrass shoots increases with distance through the seagrass bands, hence causing a long-range negative feedback. Additionally, results demonstrate that seagrass cover and mean patch size are influenced in a predictable way by additional stress caused by light limitation and desiccation, suggesting that self-organized spatial patterns can be used as stress indicator in ecosystems.

Overall, it can be concluded that although seagrasses are threatened by multiple environmental stressors, many of these factors are in general strongly inter-correlated and that habitat suitability can therefore often be estimated by measuring only one or two variables. Secondly, I found that feedback mechanisms are often important in seagrass ecosystems. In these ecosystems, recovery of a collapsed system may be very difficult. In such cases, conservation should focus on identifying indicators to monitor ecosystem resilience (for instance monitoring spatial patterns) and aim at preventing the system from collapsing in the first place.

Samenvatting

Zeegrassen zijn hogere, bloeiende planten die zich in de loop van de evolutie hebben aangepast aan het leven in brakke en zoute wateren. Ze vormen velden ('bedden') die grote oppervlaktes kunnen beslaan in gematigde tot tropische kustzones. Zeegrasbedden behoren tot de meest productieve systemen op aarde en vormen veelal de basis van het ecosysteem, aangezien ze als schuilplaats, foerageergebied en/of kraamkamer dienen voor tal van diersoorten.

In de afgelopen eeuw zijn wereldwijd echter veel zeegrasecosystemen verloren gegaan, vaak door menselijk ingrijpen. Ondanks het feit dat het publiek besef van de waarde van zeegrassen sterk is toegenomen en er in de laatste decennia veel aandacht is voor bescherming en herstel van zeegrasbedden, zijn maatregelen vaak weinig succesvol en blijft het zeegrasareaal wereldwijd nog steeds sterk afnemen. Bovendien verloopt de verdwijning van zeegraspopulaties vaak snel en onvoorspelbaar: ze storten zonder waarschuwing in zeer korte tijd (veelal enkele jaren) in elkaar. De studies in dit proefschrift onderzoeken de voornaamste oorzaken voor de wereldwijde afname van zeegrassen en de mechanismen achter het plotseling ineenstorten van zeegrasbedden. Hierbij is gekozen voor het gebruik van twee modelsoorten: groot en klein zeegras (*Zostera marina* en *Zostera noltii*). Op basis van de uitkomsten worden een aantal nieuwe inzichten gepresenteerd die het succes van beschermings- en herstelmaatregelen in de toekomst kunnen bevorderen.

De wereldwijde achteruitgang van zeegrassen wordt veelal toegeschreven aan een combinatie van verstoringen zoals eutrofiëring (overmatige nutriëntenverrijking), ziektes, veranderingen in het zoutgehalte van het water, en vergiftiging door bijvoorbeeld sulfide of ammonium. In hoofdstuk 2 wordt een veldonderzoek beschreven waarbij aan- of afwezigheid van zeegras op 84 West-Europese locaties is gerelateerd aan 30 verschillende omgevingsfactoren waaronder bijvoorbeeld troebelheid van het water, eutrofiëring en zoutgehalte. Alle locaties liggen in gebieden waar zeegras vroeger voorkwam of nog steeds voorkomt, maar zijn binnen deze regio's willekeurig geselecteerd. Uit de resultaten blijkt inderdaad dat aan- of afwezigheid van zeegras sterk correleert met veel van de 30 gemeten factoren. Dezelfde uitkomsten illustreren echter ook dat veel van de factoren sterk met elkaar samenhangen. Hierdoor bleek het mogelijk om voor zowel groot als klein zeegras relatief simpele modellen te maken die in staat zijn om aan- of afwezigheid met vrij grote zekerheid te voorspellen (77% correct voor groot zeegras en 86% correct voor klein zeegras). Beide modellen gebruiken hiervoor slechts twee variabelen. Voor groot zeegras zijn dit de troebelheid van het water (extinctiecoëfficiënt) en de zuurstoftoestand van de bodem (redoxpotentiaal), terwijl het voorkomen van klein zeegras kan worden voorspeld op basis van de getijdensituatie (droogvallend bij laag tij of permanent onder water) en de redoxpotentiaal. De uitkomsten van het onderzoek tonen verder aan dat beide modellen kunnen worden gebruikt bij onder meer herstelmaatregelen voor het selecteren van geschikte locaties.

Alhoewel de combinatie van meerdere versturende factoren de afname van zeegras uit kan leggen, verklaart het niet waarom zeegrasbedden vaak plotseling in kunnen storten. In hoofdstukken

3 tot en met 5 wordt onderzocht in hoeverre terugkoppelingsmechanismen veroorzaakt door “biobouwereigenschappen” van zeegrassen zelf hierin een rol kunnen spelen. Biobouwers zijn plant- of diersoorten die niet alleen reageren op hun omgeving, maar deze zelf ook sterk veranderen. Zeegrassen doen dit ondermeer door stroming en golfslag te remmen en sediment in te vangen. Hierdoor kan het zeegras vervolgens weer beter groeien, waardoor een positieve terugkoppeling tussen zeegrasgroei en dichtheid ontstaat.

In hoofdstuk 3 wordt het ineenstorten van de zeegrasvelden (groot zeegras) in de Nederlandse Waddenzee onderzocht. Rond 1900 kwam er in de westelijke Waddenzee 150 km² zeegras voor. Deze populatie stierf in de jaren 30 echter binnen enkele jaren uit door een combinatie van een ziekte (“wasting disease”) en de aanleg van de afsluitdijk. Ondanks de talloze herstelmaatregelen die sinds de jaren 50 zijn ondernomen, zijn de velden van weleer nooit teruggekeerd. Een analyse van historische kaarten, een grote hoeveelheid gegevens over de troebelheid van het water en een computermodel laten zien dat de vroegere zeegrasbedden waarschijnlijk hun eigen groei stimuleerden door opwerveling van sediment te remmen en daarmee de helderheid van het water te vergroten. Ook blijkt dat het water in de Waddenzee nu te troebel is voor zeegrasgroei, waarschijnlijk omdat het niet meer helder wordt gemaakt door de filterende werking van het zeegras zelf. Hierdoor is een natuurlijke terugkeer van groot zeegras momenteel nagenoeg onmogelijk.

In hoofdstukken 4 en 5 worden biobouwereigenschappen van groot zeegras verder onderzocht door middel van laboratoriumexperimenten en computermodellen. In de experimenten is getest of toxiciteit van ammonium afhankelijk is van de zuurgraad van het water (pH) en scheutdichtheid van het zeegras (aantal planten per vierkante meter). Uit de resultaten blijkt dat bij een hoge belasting met ammonium een hoge pH veel nadeliger is voor zeegras dan een lage pH. Dit komt doordat een groot deel van het ammonium bij een hoge pH chemisch wordt omgezet in het veel giftiger ammoniak. Verder blijkt dat de effecten van ammoniakvergiftiging veel minder ernstig zijn bij een hoge scheutdichtheid. Bij een hoge scheutdichtheid nemen de planten samen veel ammonium/ammoniak op, waardoor de concentratie in het water lager wordt: de belasting per plant wordt dus aanzienlijk verlaagd. Simulaties met computermodellen tonen aan dat dit mechanisme in het veld tot twee alternatieve stabiele evenwichtstoestanden kan leiden: 1) er zijn voldoende planten aanwezig; het zeegras kan zich handhaven of 2) het zeegras sterft uit omdat er onvoldoende planten zijn om het ammonium/ammoniak te ontgiften. Daarnaast laten de modellen zien dat dit mechanisme niet alleen van belang is bij ammonium/ammoniak toxiciteit, maar ook een rol kan spelen bij ontgiftiging van andere giftige stoffen, zoals bijvoorbeeld sulfide.

Als laatste is onderzocht of de biobouwereigenschappen van zeegras invloed kunnen hebben op de ruimtelijke structuur van zeegrasbedden (hoofdstuk 6). Dit onderzoek is uitgevoerd in de baai van Saint Eflam, Frankrijk. Hier bevindt zich een zeegrasveld (klein zeegras) in de vorm van een ruimtelijk strepenpatroon, waarbij de strepen dwars op de stromings- en golfrichting groeien. Uit

veldmetingen en een computermodel blijkt dat het typerende patroon ontstaat door ruimtelijke terugkoppelingsmechanismen tussen het zeegras, de stroming en het sediment. Een zeegrassstreep kan slechts een bepaalde maximale breedte bereiken (± 30 cm), omdat de zeegrasscheuten de waterstroming verstoren waardoor het sediment vervolgens erodeert. Door de erosie ontstaat aan de kustzijde van de streep een gat wat dieper is dan de wortelingsdiepte van het zeegras zelf, met als gevolg dat de zeegrasscheuten wegspoelen. Het resultaat van het mechanisme is dat de strepen "wandelen": ze groeien aan de zeezijde, en spoelen weg aan de kustzijde. Uit aanvullende veldmetingen en experimenten wordt duidelijk dat de totale oppervlaktebedekking van het patroon en de gemiddelde grootte van de strepen zeer voorspelbaar afnemen naarmate aanvullende stress door bijvoorbeeld lichtgebrek of uitdroging (door droogval) toenemen. Deze uitkomsten suggereren dat de eigenschappen van het strepenpatroon kunnen worden gebruikt om de "gezondheid" van het zeegrasbed te meten. Dit resultaat is niet alleen belangrijk voor de zeegrassen in de baai van Saint Eflam, maar ook voor zeegrassen in het algemeen en zelfs voor andere ecosystemen zoals woestijnvegetaties of venen, waar dergelijke "zelfgeorganiseerde" patronen veel voorkomen.

Samenvattend kan worden geconcludeerd dat alhoewel zeegrasbedden veelal worden bedreigd door meerdere versturende factoren, veel van deze factoren vaak onderling sterk samenhangen. Hierdoor is het mogelijk om de geschiktheid van gebieden voor zeegrasgroei te voorspellen met behulp van slechts één of twee factoren. Daarnaast is aangetoond dat terugkoppelingsmechanismen, veroorzaakt door biobouwereigenschappen van het zeegras zelf, een belangrijke rol kunnen spelen bij het plotseling ineensinken van zeegrassystemen. In ecosystemen waar deze mechanismen van belang zijn, zijn herstelmogelijkheden vaak zeer beperkt. In dit soort gevallen is het daarom uiterst belangrijk om plotseling afsterven te voorkomen en om methodes te vinden die de gezondheid van het ecosysteem te kunnen meten om zo toekomstige ontwikkelingen te kunnen volgen en voorspellen.

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List of publications

Peer reviewed publications:

Van der Heide T, Roijackers RMM, Peeters ETHM, Van Nes EH (2006) *Experiments with duckweed-moth systems suggest that global warming may reduce rather than promote herbivory*. *Freshwater Biology* 51(1): 110-116

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Curriculum Vitae

Tjisse van der Heide was born in Leeuwarden, The Netherlands, on July 29, 1979. He graduated from high school (VWO) at the Comenius College in Leeuwarden in 1997. That same year, he started his study environmental hygiene at Wageningen University. Over the course of the study, he specialized in aquatic ecology. He carried out his two master projects at the Aquatic Ecology and Water Quality Management group, during which he wrote his first two scientific publications on grazing pressure and temperature dependent growth of free-floating aquatic macrophytes.

Between January 2005 and March 2009, he carried out his PhD-research at the departments of Environmental Sciences and Environmental Biology of the Radboud University of Nijmegen. During his research, that focused on environmental stressors and feedback interactions in seagrasses, he worked in close cooperation with the Aquatic Ecology and Water Quality Management group from Wageningen University, the department of Spatial Ecology at NIOO-CEME and the section of Hydraulic Engineering from the TU-Delft. In that same period, he supervised 4 MSc students, gave several lectures and supervised 4 practical courses on marine biology at the universities of Nijmegen and Wageningen.

At present, he is employed as a post-doctoral researcher at the Community and Conservation Ecology group of the University of Groningen, where he focuses on ecosystem engineering and foodweb dynamics in the Wadden Sea.