Possibilities for restoration of *Zostera marina* beds in the Dutch Wadden Sea

Possibilities for restoration of *Zostera marina* beds in the Dutch Wadden Sea

Een wetenschappelijke proeve op het gebied van de
Natuurwetenschappen, Wiskunde en Informatica

Proefschrift

Ter verkrijging van de graad van doctor
aan de Katholieke Universiteit Nijmegen,
volgens besluit van het College van Decanen in het
openbaar te verdedigen op woensdag 11 oktober 2000,
des namiddags om 1.30 uur precies

door

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geboren op 12 september 1958 te Zaandam

Ecoscience Nijmegen

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van Katwijk, M.M. (2000) Possibilities for restoration of *Zostera marina* beds in the Dutch Wadden Sea. PhD thesis, University of Nijmegen, The Netherlands. 160 pages. ISBN 90-9013913-3

Cover design: H. Munnik
Cover: quotation Martinet (1782), map of the distribution of *Zostera marina* beds in the Wadden Sea in 1869 with notes by an unknown person in 1931.

Realisatie: Mediagroep KUN/UMC

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Onderwaterwoud van zeegras. Waddenbulletin 2000-2: 29-31.

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VOORWOORD

Vanaf 1989 heb ik onderzoek verricht in het kader van het project ‘Herintroductie zeegras in de Nederlandse Waddenzee’, gefinancierd door Rijkswaterstaat. De belangrijkste resultaten van dit onderzoek zijn in dit proefschrift vervat. Het project is ontsproten uit een lange traditie van zeegrasonderzoek onder leiding van Prof. dr. C. den Hartog. Hij is degene die, samen met Prof. dr. V.N. de Jonge, het herintroductieonderzoek geïnitieerd en voorbereid heeft.

Behalve mijzelf hebben zes mensen een aanstelling gehad binnen het project. Mijn voorganger, Wim Giesen, betrok mij bij het modelmatige deel van zijn onderzoek. Hieraan heb ik zowel het coauteurschap van twee artikelen als mijn aanstelling in het zeegrasonderzoek te danken. In het eerste jaar werkte Lucien Hanssen mee in het project, daarna gedurende circa 5 jaar Fred Schmitz, aanvankelijk als assistent, later als collega in uitvoering en management van de projecten. Karin Hermus werkte gedurende circa 3 jaar aan transplantatie-experimenten. Janneke Wijgergangs heeft een drietal bureaustudies verricht gedurende circa 2 jaar. Anne-Marie Gasseling en Pim van Avesaath hebben bijgedragen aan de tweede laboratoriumproef. Allen wil ik bijzonder danken voor hun grote inzet en betrokkenheid.

In de projecten hebben de volgende studenten vol enthousiasme meegedraaid: Janneke Wijgergangs, Peer Kennis, Rob de Vries, Saskia Westerveld, Dick Verschuren, Antoine Janssen, Menno Hornman en Erik Crins. De hulp van vele vrijwilligers was onontbeerlijk. Het waren tientallen studenten, collega’s, Rijkswaterstaatmedewerkers, vrienden, familieleden, vrienden van collega’s, vrienden van vrienden, familieleden van collega’s, etc. etc., en zelfs diverse mensen die we niet eerder kenden. Veel dank allemaal!

Begrotingen, contracten, personeelsaanstellingen, declaraties en de ongeveer honderd reisaanvragen en -declaraties werden snel afgehandeld door het Contract Management Bureau, Personele Zaken en de Financiële Administratie, waarbij ik met name wil noemen: Rein Lehmann, Theo Goldsmid, Henny Claassen, Henk Radstaake, Frans Houben, Martin Wiecherink, Gerard Geerlings, Hans Michels en Marion Verriet. Als ik iemand achter de schermen vergeet te noemen, spijt me dat. De medewerkers van de bibliotheek wil ik danken voor hun vriendelijke ondersteuning. Voor statistisch advies kon ik terecht bij Theo de Boo (afdeling MIES van de KUN) en Cajo ter Braak (Centrum voor Biometrie, Wageningen). De afdelingen Grafische Vormgeving en Fotografie van de KUN hebben een groot deel van de illustraties in dit proefschrift verzorgd. De omslag van het proefschrift is gemaakt door Hetty Munnik en dochters.

Met veel mensen van andere instituten heb ik prettig samengewerkt. Ik noem met name Dr. Norbert Dankers, Koos Zegers, Piet-Jan van Leeuwen, André Meijboom van Alterra Texel, Wim Visser en Tiemen Top van het Rijksinstituut voor Kust en Zee (RIKZ) Haren, Annemieke van der Pluym van RIKZ Middelburg, Prof. dr. Marten Hemminga, Dr. Pauline Kamermans, Jos van Soelen en Joop Nieuwenhuize van het Centrum voor Estuariene en Mariene Oecologie, Yerseke, en Dr. Ragnhild en Dr. Harald Asmus van het Alfred Wegener Institut te Sylt, Duitsland.

Prof. dr. Victor de Jonge van het RIKZ in Haren begeleidde de projecten vanuit Rijkswaterstaat. Met Dick de Jong (RIKZ Middelburg) vormden wij gedrieën de harde kern van de zeegrasprojectgroep. Alle projectvoorstellen, en de veelvuldige bijsturingen die tijdens de projecten nodig bleken te zijn (*Zostera marina* is een wispelturige plant) zijn uit onderling overleg voortgekomen. Het is aan Victor's bureaucratische stuurmanskunsten te danken, en aan de genoemde snelheid en flexibiliteit bij de administratieafdelingen van de KUN, dat de projecten nog net in het beoogde seizoen konden worden uitgevoerd. Ook de wetenschappelijke instelling van Victor en Dick zijn de projecten ten goede gekomen. Ik heb veel aan hen te danken.

Alle medewerkers van de afdeling Aquatische Oecologie en Milieubiologie wil ik bedanken voor de zeer prettige samenwerking. Met name wil ik Prof. dr. Gerard van der Velde noemen, die mij op allerlei terreinen geholpen heeft. Het is inspirerend om deel uit te maken van zijn breed georiënteerde werkgroep. Barry Kelleher wil ik bedanken voor zijn correcties van de engelse taal en het kritisch doorlezen van alle hoofdstukken die deel uitmaken van dit proefschrift. Martin Versteeg, Germa Verheggen-Kleinheerenbrink en Jelle Eygensteyn (Gemeenschappelijk Instrumentarium) wil ik bedanken voor hun royale ondersteuning bij het veldwerk en de analyses. Met Luc Vergeer heb ik prettig samengewerkt in het eerste laboratoriumexperiment. Dr. Fred van den Brink wil ik bedanken voor zijn collegialiteit in de periode dat we kamergenoten waren. Leon Lamers en Esther Lucassen, mijn paranimfen, hebben mij vaak geholpen en waren altijd belangstellend. Prof. dr. Jan van Groenendael heeft mij faciliteiten ter beschikking gesteld om mijn proefschrift af te maken, waarvoor veel dank.

Promotor Prof. dr. C. den Hartog wil ik bedanken voor het vertrouwen en de vrijheid die hij mij gaf. Hij heeft me de kans gegeven me op mijn eigen manier in de wetenschap te ontwikkelen. Voorts voel ik me bevoorrecht om te promoveren bij een zeegrasonderzoeker die, van duizenden zeegrasonderzoekers ter wereld, tot de bekendste twee of drie behoort.

Co-promotor dr. Jan Roelofs heeft mij 14 jaar geleden aangenomen op de afdeling Aquatische Ecologie en Milieubiologie, zodat ik een carrière in het bankwezen ben misgelopen, waar ik hem nog altijd dankbaar voor ben. In diverse fasen van het zeegrasonderzoek kon ik bij hem terecht voor waardevolle adviezen. Ik heb grote bewondering voor zijn ecologisch inzicht en zijn vermogen helderheid aan te brengen.

Veel vrouwen dragen aan de maatschappij bij door een ondersteunende rol te vervullen. Veel mannen hebben daar profijt van, maar soms ook andere vrouwen. Ik heb het geluk gehad om niet alleen door mijn man, maar ook door vrouwen ondersteund te zijn geweest: Tilly, die niet alleen mijn kinderen, maar soms ook mijzelf liefdevol opving, en Marian, die mij vurig kon aanmoedigen.

Tot slot, Lucien, ik dank je voor al je steun. Ik ben je ook dankbaar dat jij, net als ik, je eigen carrière op een lagere snelheid hebt gezet toen we kinderen kregen, zodat wij onze zorgtaken gelijk konden verdelen. Hoewel ik dat vanzelfsprekend vond, heb ik om me heen gezien dat het dat nog niet is. Steef en Ivo, jullie hebben me het meest geleerd en zullen me nog meer leren, dat weet ik.

CHAPTER 1

GENERAL INTRODUCTION

GENERAL INTRODUCTION

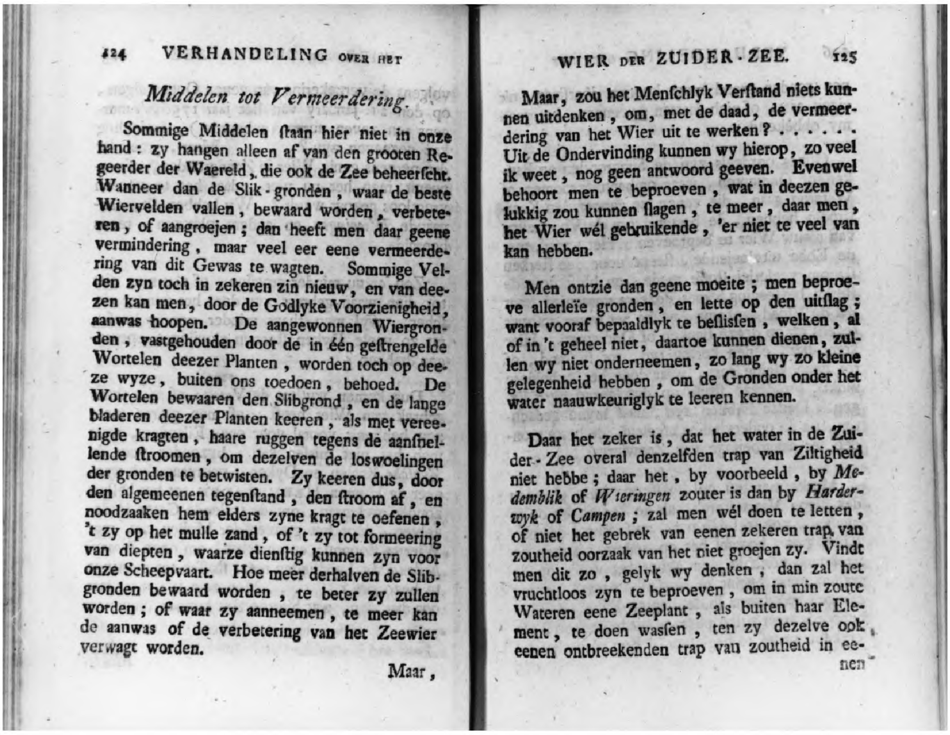
Seagrass restoration

Worldwide, many seagrass beds have disappeared (e.g. Short & Wyllie-Echeverria 1996, http://www.seagrass.unh.edu/). This is a large loss, not only from an ecological, but also from an economical point of view. The plants are known to stabilise sediments (e.g. Rasmussen 1977, Fonseca 1996), reducing particle loads (e.g. Gacia et al. 1999) and act as a sink for nutrients (e.g. Asmus & Asmus 1998), in this way improving water quality. Seagrass beds are a source of food for young fish and for crustaceans: particularly its luxuriant epiphytic flora and fauna are the main source of food for many small fish and invertebrates, which in their turn are eaten by commercially important fish species, whereas their remains, the detritus, form the basis for a complex food web (e.g. van Goor 1919, den Hartog 1970, Thayer et al. 1984, Fonseca et al. 1990, Heck et al. 1995, Horinouchi & Sano 1999, Mattila et al. 1999). This nutrient cycling ability of seagrass and algal beds led Costanza et al. (1997) to estimate their value as 19000 US$ per ha per year (in comparison: coral reefs 6100; forests 969, cropland 92 US$ ha^{-1} yr^{-1}). Priceless, however, is their contribution to biodiversity and habitat diversity of coastal waters.

Fortunately, not only scientists and lovers of nature, but also policy makers recognise the gravity of seagrass loss in many parts of the world. Many seagrass meadows are now protected by strict governmental regulations. Furthermore, restoration efforts of diminished seagrass beds have been performed in the North and Central America (review in Fonseca et al. 1998, Sheridan et al. 1998, Orth et al. 1999), Australia (e.g. Larkum & West 1983, Paling et al. 1998), Japan (e.g. Watanabe & Terawaki 1986, Kawasaki et al. 1986) and Europe (F. Duinker in Reigersman et al. 1939, Ranwell et al. 1974, Christensen et al. 1995, Balestri et al. 1998, Piazzi et al. 1998, Meinesz personal communication, this dissertation).

Seagrass in the Wadden Sea

The Wadden Sea is one of the world's largest international marine wetland reserves (approx. 6000 km^2), bordering the coasts of The Netherlands, Germany and Denmark. In the Wadden Sea, two species of seagrass occur, *Zostera marina* L. and *Z. noltii* Hornemann. Before the 1930s, The Dutch Wadden Sea contained large beds of subtidal and low-intertidal seagrass (*Z. marina*) covering an area between 65 and 150 km^2 (Oudemans et al. 1870, den Hartog & Polderman 1975). These seagrass beds were of great economic importance. The seagrass was used as roofing and isolation material, and to fill mattresses and cushions. Before 1857 it was used to build dikes (Martinet 1782, Sloet tot Oldhuis 1855, Oudemans et al. 1870). Considering the importance of dikes to The Netherlands, it is no wonder that in the past a proverb was used to describe the harvest ('good hay grass, good sea grass'), a special prayer day was held to invoke a bumper crop, and lyrical descriptions and poems about seagrass were written during the 18th and 19th century (Martinet 1782, Sloet tot Oldhuis 1855, respectively). Already in the 18th century it was urged to develop a method to multiply eelgrass, as 'one cannot have too much of it' (Fig. 1, Martinet 1782). Less is known about the past German and Danish beds. They had small or no economic value (van den Hoek et al. 1979).



124 VERHANDELING OVER HET

Middelen tot Vermeerdering.

Sommige Middelen ſtaan hier niet in onze hand: zy hangen alleen af van den grooten Regeerder der Waereld, die ook de Zee beheerſcht. Wanneer dan de Slik-gronden, waar de beste Wiervelden vallen, bewaard worden, verbeteren, of aangroejen; dan heeft men daar geene vermindering, maar veel eer eene vermeerdering van dit Gewas te wagten. Sommige Velden zyn toch in zekeren zin nieuw, en van deezen kan men, door de Godlyke Voorzienigheid, aanwas hoopen. De aangewonnen Wiergronden, vastgehouden door de in één geſtrengelde Wortelen deezer Planten, worden toch op deeze wyze, buiten ons toedoen, behoed. De Wortelen bewaaren den Slibgrond, en de lange bladeren deezer Planten keeren, als met vereenigde kragten, haare ruggen tegens de aanſnellende ſtroomen, om dezelven de loswoelingen der gronden te betwisten. Zy keeren dus, door den algemeenen tegenſtand, den ſtroom af, en noodzaaken hem elders zyne kragt te oefenen, 't zy op het mulle zand, of 't zy tot formeering van diepten, waarze dienſtig kunnen zyn voor onze Scheepvaart. Hoe meer derhalven de Slibgronden bewaard worden, te beter zy zullen worden; of waar zy aanneemen, te meer kan de aanwas of de verbetering van het Zeewier verwagt worden.

Maar,

WIER DER ZUIDER-ZEE. 125

Maar, zou het Menſchlyk Verſtand niets kunnen uitdenken, om, met de daad, de vermeerdering van het Wier uit te werken? . . . . . . Uit de Ondervinding kunnen wy hierop, zo veel ik weet, nog geen antwoord geeven. Evenwel behoort men te beproeven, wat in deezen gelukkig zou kunnen ſlagen, te meer, daar men, het Wier wél gebruikende, 'er niet te veel van kan hebben.

Men ontzie dan geene moeite; men beproeve allerleïe gronden, en lette op den uitſlag; want vooraf bepaaldlyk te beſlisſen, welken, al of in 't geheel niet, daartoe kunnen dienen, zullen wy niet onderneemen, zo lang wy zo kleine gelegenheid hebben, om de Gronden onder het water naauwkeuriglyk te leeren kennen.

Daar het zeker is, dat het water in de Zuider-Zee overal denzelfden trap van Ziltigheid niet hebbe; daar het, by voorbeeld, by *Medemblik* of *Wieringen* zouter is dan by *Harderwyk* of *Campen*; zal men wél doen te letten, of niet het gebrek van eenen zekeren trap van zoutheid oorzaak van het niet groejen zy. Vindt men dit zo, gelyk wy denken, dan zal het vruchtloos zyn te beproeven, om in min zoute Wateren eene Zeeplant, als buiten haar Element, te doen wasſen, ten zy dezelve ook eenen ontbreekenden trap van zoutheid in ee-

nen

Fig. 1. Photograph of 2 pages of Martinet's treatise on eelgrass in the Zuiderzee (1782) (courtesy Library of University of Groningen). In these and following pages, Martinet discusses the possibilities to multiply eelgrass

Restoration of seagrass beds in the Wadden Sea

During the 1930s, the seagrass cover in the Wadden Sea was largely lost and the beds never recovered (e.g. den Hartog 1987, Reise et al. 1989). Presently, *Z. marina* occurs only in the mid-littoral; approximately 0.87 km^2 of *Z. marina* and 0.26 km^2 *Z. noltii* in the Dutch Wadden Sea (de Jong 2000); in the German Wadden Sea, *Z. noltii* and *Z. marina* together cover approximately 170 km^2, and in the Danish part ca 30 km^2 (Reise & Buhs 1991). The large-scale decline of *Z. marina* coincided with (1) the outbreak of wasting disease caused by the slime-mold *Labyrinthula zosterae* (2) increased diking and damming activities and (3) two subsequent years with a considerable deficit of sunlight. There is no consensus about which of these events (or combination of events) caused this decline (reviews in den Hartog 1996, de Jonge et al. 1996). The Dutch government is currently attempting to return seagrass to the Wadden Sea, in order to 'restore natural values' (Anonymous 1989).

Conditions for successful restoration

The presence of potential seagrass habitats is the first condition for successful restoration. Therefore, an important question preceding transplantation efforts is:

Is the environment (again) suitable for the seagrass?

Main causes for the lack of recovery of eelgrass stands in the Dutch Wadden Sea were thought to be high turbidity, and later shellfish fishery (van den Hoek et al. 1979, Giesen et al. 1990a,b, de Jonge & de Jong 1992). In the late 1980s, the eelgrass habitat in the Wadden Sea had partly been restored with respect to these crucial factors: turbidity had decreased and shellfish fishery was prohibited in some areas (de Jonge & de Jong 1992, Philippart 1993). 25 % of the intertidal area was closed for shellfish fishery in 1993 (Dankers 1998). For this reason, restoration was thought to be feasible.

A second major question preceding transplantation, in case only remnants or no seagrass beds at all have been left at the target location:

Are there suitable donor populations?

Zostera marina populations differ with regard to reproductive strategy and morphology, which may be due to phenotypic adaptations to the habitat, but may also be partly attributed to genetic differences (Biebl & McRoy 1971, McMillan & Phillips 1979, Gagnon et al. 1980, McMillan 1982, Phillips & Lewis 1983, Backman 1991, Fain et al. 1992, de Heij & Nienhuis 1992, Olesen 1993, Alberte et al. 1994, van Lent & Verschuure 1994a,b, 1995). Therefore, it is probable that populations differ with regard to their donor suitability for recolonisation elsewhere.

To address both questions, a mesocosm (=outdoor tank) experiment was conducted, testing water quality and light climate in the Dutch Wadden Sea, and 5 potential donor populations originating from Roscoff (France), Lake Grevelingen and Terschelling (The Netherlands), Sylt (Germany) and Yderfjorden (Denmark). The results of this study are presented in chapter 2.

The mesocosm experiment gave positive results, leading to the first transplantation experiments (van Katwijk & Schmitz 1993, Hermus 1995, also described in chapter 5). Transplantation success appeared to be inversely related to the depth of the transplantation zone. We had learned from the mesocosm experiment (chapter 2) that light was not limiting, which was confirmed by transplantation experiments (Hermus 1995) from which we hypothesised that either some water quality factor was unsuitable to sustain eelgrass, or physical disturbance was too high at larger depths. Probably, the most influencing water quality factor is nutrient load. Nutrient loads have severely increased in the Wadden Sea (review in chapter 6), and particularly the following factors, that are part of or interacting with nutrient load, were of interest:

- Nitrate: at that time a speech-making article appeared in the United States, reporting a negative, probably toxic effect of nitrate on *Z. marina* (Burkholder et al. 1992).
- Ammonium: it is well known that high ammonium levels can be toxic to plants. However, ammonium toxicity to submerged aquatic plants has been given little attention. It had been observed in a few freshwater aquatic plants (Glänzer 1974, Grube 1974, Agami et al. 1976, Glänzer et al. 1977, Roelofs 1991, Smolders et al. 1996). It had not been reported for *Z. marina* or any other seagrass.
- Salinity: patterns of distribution and decline of *Z. marina* in the Netherlands indicated a negative effect of a high salinity, particularly above 30.5 PSU

(Wijgergangs & van Katwijk 1993, Wijgergangs 1994, Wijgergangs & de Jong 1999, D.J. de Jong personal communication). Furthermore, an interactive effect of nutrients and salinity on *Z. marina* was suspected from distribution patterns observed in The Netherlands (outlined in chapter 4).

This resulted in two laboratory experiments, (1) to test the effects of nitrate and ammonium on *Z. marina* (chapter 3), and (2) to test the effects of salinity, nutrients and their interaction on *Z. marina* (chapter 4).

The effects of water dynamics and the ensuing sediment instability as well as bioturbation were tested in 3 transplantation experiments, described in chapter 5.

The results presented in chapters 2 to 5 had implications for reintroduction efforts, potential eelgrass habitats and eelgrass ecology in general, which were synthesised in a conceptual model and led to recommendations for future research in chapter 6. A distinction has been made between a higher and a lower zone of potential habitats, each suitable for different morphotypes of *Zostera marina*. The conclusions are summarised in the 'Summary, recommendations and implications' with an international approach, and in the 'Samenvatting, aanbevelingen voor het beleid en toekomstvisie' in Dutch, with a regional approach.

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CHAPTER 2

SUITABILITY OF *ZOSTERA MARINA* POPULATIONS FOR TRANSPLANTATION TO THE WADDEN SEA AS DETERMINED BY A MESOCOSM SHADING EXPERIMENT

van Katwijk MM, Schmitz GHW, Hanssen LSAM, den Hartog C (1998)
Aquat Bot 60:283-305

SUITABILITY OF *ZOSTERA MARINA* POPULATIONS FOR TRANSPLANTATION TO THE WADDEN SEA AS DETERMINED BY A MESOCOSM SHADING EXPERIMENT

ABSTRACT: As part of eelgrass reestablishment efforts in the Wadden Sea, 5 northwest European eelgrass (*Zostera marina* L.) populations were studied in a mesocosm filled with Wadden Sea water over a 2 year period. The original habitats of these populations varied with regard to salinity, tidal regime, latitude, substrate type and water nutrient content. The reproductive strategies ranged from semi-annual to perennial. Experimental parameters studied were: planting density (64 and 121 plants m^{-2}) and irradiance corresponding to 75 %, 89 %, 95 % and 98 % light reduction at the bottom. Three out of 5 *Z. marina* populations were successfully transplanted. Planting density, although initially positively correlated, had no effect on final transplantation success. The populations maintained their original reproductive strategy. The reproductive strategy of the donor population did not show a clear correlation with transplantation success. The maximum light reduction tolerated at the bottom was 75 % to 89 %. The populations did not differ in shading tolerance. Assuming a light attenuation coefficient in the Wadden Sea of $k=2.0\ m^{-1}$, this corresponds to a maximum transplantation depth of between 0.45 m and 0.85 m below mean sea level. The differences in transplantation success, and the conservative reproductive strategy of different *Z. marina* populations, indicate genetic differences and/or low phenotypic plasticity. Moreover, this implies that the characteristics of a donor population must be carefully considered prior to transplantation.

INTRODUCTION

Eelgrass (*Zostera marina* L.) occurs along the coasts of the entire northern hemisphere (den Hartog 1970). Over the last decades, substantial eelgrass losses have been recorded throughout its distribution area (see review in Short & Wyllie-Echeverria 1996). This has led to revegetation programmes in several areas (e.g. Zimmerman et al. 1991, 1995, Batiuk et al. 1992, Kenworthy & Fonseca 1992, van Katwijk 1993, Fonseca et al. 1994, Moore et al. 1996). Until now, little attention has been paid to possible differences in the suitability of eelgrass populations to act as a donor for reestablishment. *Z. marina* populations differ with regard to reproductive strategy and morphology, which may be due to phenotypic adaptations to the habitat, but may also be partly attributed to genetic differences (McMillan & Phillips 1979, Gagnon et al. 1980, McMillan 1982, Phillips & Lewis 1983, Backman 1991, Fain et al. 1992, de Heij & Nienhuis 1992, Olesen 1993, Alberte et al. 1994, van Lent & Verschuure 1994a,b, 1995). Therefore, it is hypothesised that populations also differ with regard to their donor suitability for revegetation elsewhere.

What features determine the suitability of a population to be a donor in a reestablishment programme? Firstly, the population should be large enough so that it will not be markedly reduced by the donation. Secondly it should survive transplantation to the new environment. Thirdly, the transplants should be able to expand (sexually or vegetatively). Intertidal *Z. marina* populations usually display an annual life cycle, while subtidal populations usually are perennial (Harmsen 1936, Keddy & Patriquin 1978, Jacobs 1982, Phillips et al.

1983). This implies that transplantation to an intertidal area requires a population with a high rate of seed production and germination, whereas transplantation to a subtidal area requires a perennial population. In some cases, both strategies may be desirable, i.e. when transplantation in intertidal zones is the initial objective, but subsequent expansion to subtidal areas is hoped for. Also, if seed production fails, at least a part of the population should survive through vegetative propagation (this dual strategy is also found in natural habitats, i.e. in the Wadden Sea). This may require more than one donor population, which is also preferential from the point of view of increasing genetic diversity of the transplants, the importance of which was already pointed out by Williams & Davis (1996).

The present study was part of an eelgrass reestablishment programme for the Wadden Sea. The Wadden Sea is one of the world's largest international marine wetland reserves (approximately 6000 km^2). In the Dutch part, approximately 150 km^2 of eelgrass cover was lost during the twentieth century, especially during the outbreak of wasting disease in the 1930s, presently leaving, at most, 1 km^2 (van Goor 1921, den Hartog & Polderman 1975, personal observation). Main causes for the lack of recovery were thought to be high turbidity and shellfish fishery (van den Hoek et al 1979, Giesen et al. 1990a,b, de Jonge & de Jong 1992). In the late 1980s, the eelgrass habitat in the Wadden Sea had partly been restored with respect to these crucial factors: turbidity had decreased and shellfish fishery was prohibited in some areas (de Jonge & de Jong 1992, Philippart 1993).

A Wadden Sea mesocosm experiment was carried out using 5 northwest European *Z. marina* populations that were selected from a variety of habitats (different tidal regimes, high and moderate salinity, type of substrate and water nitrogen concentrations) and possessed different reproductive strategies (annual, perennial) and morphologies. Four questions were tested: (A) what, if any, are the differences between, and changes in, the survival and reproductive potential of the 5 populations after transplantation to the Wadden Sea mesocosm? (B) What habitat transitions can be tolerated by *Z. marina*? (C) What is the effect of light on the survival and reproductive potential of these 5 populations, and what would be the maximum depth of eelgrass occurrence? (D) What is the effect of planting density on the survival and development of *Z. marina* in the mesocosm?

MATERIALS AND METHODS

Experimental design. Five northwest European eelgrass populations were selected from a variety of habitats (Table 1). The plants were placed in a mesocosm basin in spring 1990 and monitored during 2 growing seasons (until September 1991). Two planting densities were used to study its effect on survival and maintenance of the plants and to gain some insight in the minimal planting density required for survival. Four shading categories were employed to investigate the maximum depth of potential eelgrass survival. Survival of the plants was investigated by monitoring the percentage cover, frequency, and biomass of transplanted populations. The generative potential of the populations was assessed by estimating the percentage of generative shoots, seed production and germination. During 1989 and 1991, partly similar transplantation experiments were carried out, from which we gained an impression of year-to-year differences and the effect of planting date (van Katwijk 1992).

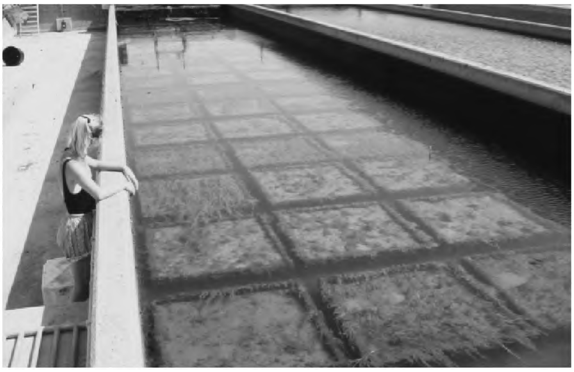

Fig. 1. Mesocosm basin with trays with *Zostera marina*. Nets were removed and the water was lowered on this photograph

Mesocosm. The mesocosm experiment was carried out in a basin at the Institute for Forestry and Nature Research (IBN/DLO) on the island of Texel, in the Dutch Wadden Sea. The mesocosm was controlled with regard to water movement, shading and water level. It was uncontrolled with regard to sunshine, precipitation, water quality and temperature. The basin measured 50 x 5 m (Fig. 1). The height of the water column was 70 cm. Unfiltered Wadden Sea water was allowed in at a rate of 50 m^3 per hour (i.e. residence time circa 4 h). Netting provided 4 shading categories, viz. 0 %, 54 %, 79 % and 90 %.

Environmental data. Water temperature in the mesocosm was measured weekly or biweekly from May to November in 1990 and 1991. Temperature data for December to April, recorded daily from the vicinity of the mesocosm inlet at 0800 h, was supplied by the Netherlands Institute for Sea Research (NIOZ).

Daily solar radiation (J cm^{-2} d^{-1}, 30 to 3000 nm) was recorded by the Royal Dutch Meteorological Institute (KNMI) at station De Kooy, 10 km away from the mesocosm. In order to calculate PAR (Photosynthetically Active Radiation, wavelength 400 to 700 nm), a conversion factor of 0.45 was used (Lüning 1981, Colijn 1982).

To compare the results of shading with existing information on growth saturating irradiance, we derived from the data of Pellikaan (1980), that saturating irradiance for growth is achieved in summer at approximately 100 J cm^{-2} d^{-1} of incident irradiance. The solar irradiance required to reach this value at the bottom was calculated for the 4 shading categories by determining the light attenuation due to reflection by the water column and the shading nets. After Pellikaan (1980), loss of light due to reflection at the water surface was assumed to be 10 %. The light attenuation coefficient of the water in the mesocosm was measured by means of a Sea-Tech transmissometer (wave length 660 nm, 5 cm water path length). Light reduction by the water column was calculated using the equation (Lambert-Beer):

$$I_Z = I_0 \exp^{-cZ} \qquad \text{(Equation 1)}$$

where: I_Z = irradiance at depth Z, I_0 = irradiance just below the water surface, c = beam attenuation coefficient, which was assumed to equal the in situ light attenuation coefficient (k).

Table 1. Habitat and reproductive characteristics of the donor populations and abiotic characteristics of the mesocosm

Donor population	Roscoff	Yderfjorden) (Vemmingbund)	L. Grevelingen (Battenoord)	Terschelling (West-Terschelling)	Sylt (Königshafen)	Mesocosm
Coordinates	3°59' W 48°44' N	9°43' E 55°54' N	4°07' E 51°42' N	5°13' E 53°22' N	8°24' E 55°03' N	4°48' E 53°00' N
Average tidal range (m)	5.5	0	0	1.8	1.7[a]	0
Emergence time	0 %[b]	0 %	0 %	Ca 50 %	Ca 50 %	0 %
Collection depth below mean sea level (m)	2 – 4	0.9 – 1.3	0.9 – 1.2	0	0 – 0.2	–
Salinity (PSU)	32	11	30	30	30	30
Visibility (m) or light att. coeff. (m^{-1})	6-10[c] m	4-8[c] m	0.2-0.5[d] m^{-1}	1.0-2.3[e] m^{-1}	0.5-1.9[f] m^{-1}	see Fig. 2
Water body	Atlantic Ocean	Baltic Sea	North Sea[g]	Wadden Sea	Wadden Sea	Wadden Sea[h]
Sea water nitrogen[i]	low	low	low	moderate	low	moderate
Type of substrate	variable[j]	sandy[j]	fine sand 8 % silt 8 % organic[k]	fine sand 8 % silt 2.5 % organic[k]	coarse sand 8 % silt 1 % organic[k]	coarse sand 0 % silt 1 % organic[k]
Reproductive strategy	perennial evergreen	perennial evergreen	perennial not-evergreen[l]	semi-annual[m]	semi-annual[m]	–
Morphology	robust	robust	flexible	flexible	flexible	–

Notes to Table 1:

[a] Reise (1985)

[b] Most of the eelgrass plants were collected in a low intertidal zone that was never exposed, because bedform features prevent the full recedence of water at low tide

[c] Visibility (m) was estimated when we sampled the location

[d] Nienhuis (1989)

[e] Measured in a nearby channel in the period 1985-1990 (de Jonge & de Jong 1992)

[f] Personal communication R.M. Asmus

[g] In connection with the North Sea by means of a small sluice

[h] Wadden Sea water is let in

[i] Low: winter: median DIN < 50 µM; summer: median DIN < 8;
Moderate: winter: 70 < median DIN < 50 µM; summer: 18 < median DIN < 8

[j] Not analysed

[k] Grain size: Sieving through 210 µm and 50 µm mesh after destruction; organic contents: loss on ignition (see Jacobs & Huisman 1982)

[l] The Grevelingen estuary was closed in 1971 and became a brackish lake. In the estuarine phase, the small *Z. marina* population was semi-annual (personal observations, Beeftink 1965). In the 1970s, the population was perennial but the rapid expansion of the beds originated largely from the germination of seeds (Nienhuis & de Bree 1977, Pellikaan 1980, Nienhuis 1983). In the past decade the population has been perennial (personal observations, van Lent & Verschuure 1994a). The population overwinters largely underground

[m] Semi-annual: most of the plants are annual; 2 to 10 % of the plants have sprouted from rhizomes

Transplantation to the mesocosm. At the donor sites (Table 1), *Zostera marina* plants were dug out and rinsed with sea water to remove sediment. The time interval between collection and planting in the mesocosm was 2 days, during which the plants were maintained at approximately 10°C in a shallow layer of sea water. Plants from perennial populations were collected at the beginning of the growing season, planting dates were: Roscoff: 31 March, Lake Grevelingen: 6 April, Yderfjorden: 14 April. Plants from semi-annual populations were collected at the time that most of seedlings were 4 to 6 weeks old, planting dates were: Terschelling: 23 May, Sylt 22 June.

Zostera marina plants were planted in trays of 1 x 1 x 0.2 m. The trays were filled to the edge with fine sand. Planting densities were 64 and 121 plants m^{-2} (= per tray). A plant usually consisted of 1 rooted shoot. Occasionally, two-shoot plants were not separated, as this would have severely damaged them. Two replicates were made of every combination of population and planting density. The populations and planting densities were randomly distributed over the trays within each shading category. The 4 shading categories were not replicated and were placed at random in the mesocosm.

Monitoring. In the mesocosm, samples were taken 5 times during the first growing season, and 6 times during the second growing season. Number of shoots, phenology and above and belowground biomass were determined in samples of 0.04 m^2. The resulting gap in the sediment was filled with clean fine sand. During the first growing season, eelgrass cover was estimated every 2 to 3 weeks in the mesocosm. During the second growing season, the total number of shoots per tray was counted and corrected for the removal of shoots during previous sampling. This was done every 5 to 6 weeks.

At the donor sites (Table 1), the eelgrass populations were monitored every 5 to 7 weeks during the growing season. Fifty plants were collected randomly in an area of ca 1000 m^2. Above and belowground biomass and phenology were determined. In order to obtain biomass values, eelgrass material was dried (80°C; 48 h) and weighed. Values for above and belowground biomass in the mesocosm at the date of planting were extrapolated from the first set of values taken at the donor sites.

In the mesocosm, macroalgae were removed every 2 to 3 weeks during the growing season. Sediment traps were laid down and their contents collected every 2 to 4 weeks. Sedimentation appeared to be equally distributed throughout the mesocosm basin. Seeds on the plants were counted in July of the first growing season; more seeds are likely to be found at this time than in August or September (personal observation 1991, van Katwijk 1992). Due to the large number of seeds, counts were made during a limited period of time (an arbitrary $2^1/_2$ minutes per tray by 2 persons). In some cases, no more than 10 % of the eelgrass plants could be investigated during this period of time. This means that the count values have only a relative value. At the beginning of the second growing season (March to May 1991), seedlings were removed as they may have been the product of unknown cross-fertilizations. Their abundance was recorded qualitatively for each population.

Data analysis. Data processing and statistical calculations were performed using the Statistical Analysis System (SAS 1989). Analyses of Variance (ANOVA) were carried out using a factorial model with the factors being: population, shading category, planting density and the interaction between population and shading. Shading categories were not replicated, therefore their effects could not be statistically tested. However, interaction effects of shading with other factors were validly tested with ANOVA. The Yderfjorden population and the 90 % shading category were not included in the analysis because their many zero values interfered with the required normal or lognormal distribution of the dependent variables. Dependent variables were: number of shoots, cover, biomass and percentage of generative shoots. In order to test differences between the means per category, Tukey's studentised range test was carried out (Sokal & Rohlf 1981). Plant cover and percentage of generative shoots approached a normal distribution. The number of shoots and biomass showed a lognormal distribution, and were, therefore, log-transformed before carrying out statistical tests (Jongman et al. 1995, Slob 1987). As a central measure for normally distributed variables, the mean is used; for variables with a lognormal distribution we used the geometric mean. Standard errors of logarithmically transformed variables were calculated following Mood et al. (1974).

RESULTS

Light

The mean light attenuation coefficient between May and October 1990 in the mesocosm basin was 2.00 m^{-1} (Fig. 2). This implied that the average light reduction by the water column of 70 cm (without shading) was 75 % (equation 1). Shading of 54 %,

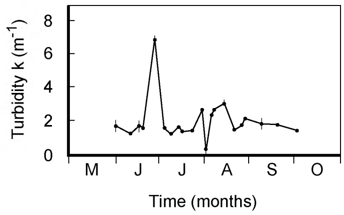

Fig. 2. Light attenuation coefficient in the mesocosm experiment during 1990

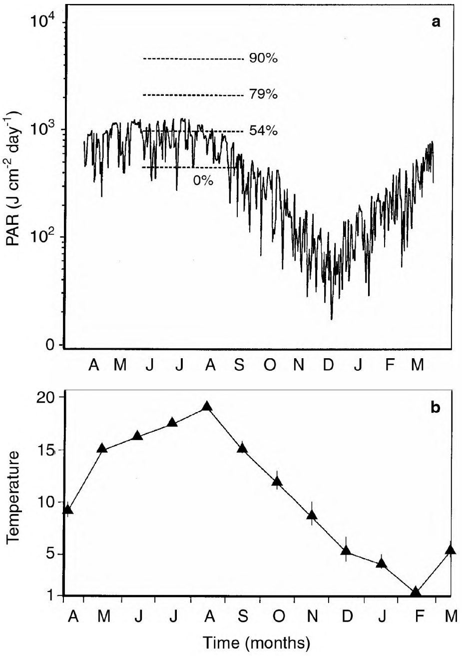

Fig. 3. a. Daily insolation above the water surface (400 to 700 nm; rugged curve) and minimal insolation that was required for growth saturating irradiance to reach to the bottom, for the 4 shading categories during summer (broken lines). This growth saturating irradiance to the bottom during summer was assumed to be 100 J cm^{-2} d^{-1}, derived from the data of Pellikaan (1980) who performed a summer experiment with Lake Grevelingen plants. Light attenuation by the water column and shading nets resulted in 4 levels of insolation above the water surface that were required to reach growth saturation at the bottom, corresponding with the 4 shading categories. **b.** Water temperature in 1990 (at mesocosm or near inlet mesocosm complex)

79 % and 90 % resulted, therefore, in a light reduction at the bottom of 89 %, 95 % and 98 %, respectively. Plants growing without shading should have encountered light saturation during summer, whereas those growing under shading experienced limiting light conditions (Fig. 3).

Zostera marina **shoot and biomass development**

During the first growing season, the highest cover and highest number of shoots in the mesocosm were attributable to Lake Grevelingen and Sylt plants (Fig. 4, Tukey's comparison of means: $p < 0.05$). Plants from Terschelling reached a higher number of shoots than those from Roscoff, while the latter achieved higher cover percentages, due to their larger dimensions. The majority of plants from Yderfjorden died during the first growing season.

The differences between the 0 % and 54 % shading categories were small. Plants in the higher shading categories, however, developed poorly. At 90 % shading, plants died

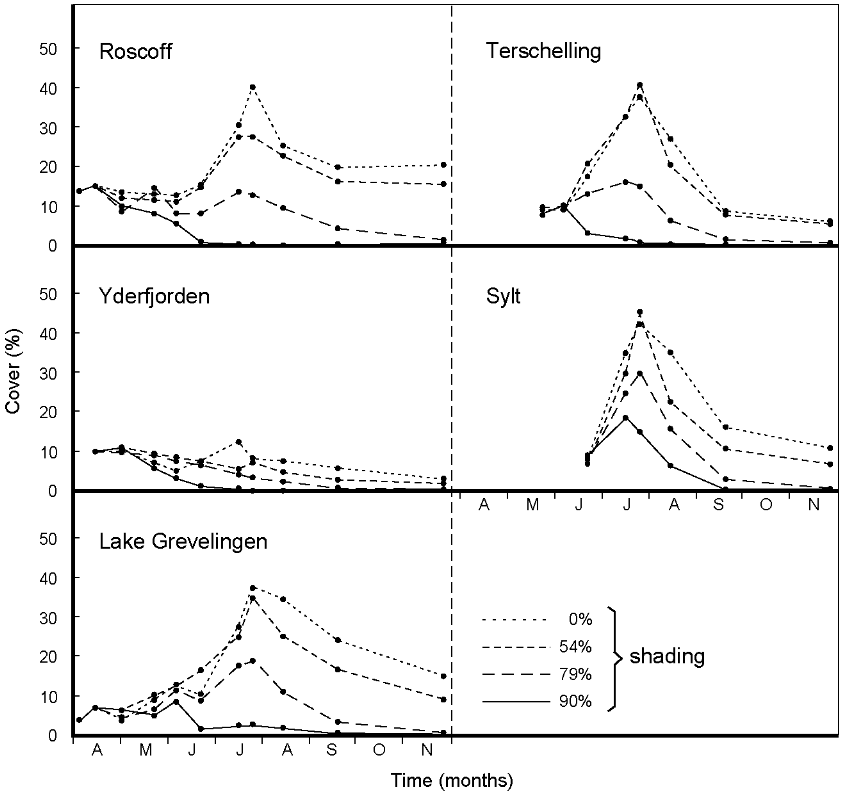

Fig. 4. Eelgrass cover (average of 2 replicates) in the mesocosm during the first growing season. Thin lines: low planting density (64 plants m^{-2}), bold lines: high planting density (121 plants m^{-2})

immediately after planting. In July and August, Sylt plants still had rather high cover percentages in the high shading categories.

A high planting density initially resulted in a higher cover and larger number of shoots in July and August. In September, however, the difference in density and cover between the planting densities had disappeared (Table 2).

The above- and belowground biomass (Figs. 5 & 6) were highest at higher light intensities. The origin of the population had no effect on the aboveground biomass (Table 2). This was due to the fact that larger plants (Roscoff plants in particular) had a relatively low transplantation success, while the smaller plants (Sylt plants in particular) had the largest increase in number of shoots. The origin of the population did, however, have an effect on the belowground biomass: Roscoff plants had a significantly higher belowground biomass than Lake Grevelingen, Sylt and Terschelling plants ($p < 0.05$ ANOVA). In August, belowground biomass of Lake Grevelingen plants was significantly

Table 2. ANOVA tests of cover, number of shoots, above- and belowground biomass and percentage generative shoots with factors; shading category (0 %, 54 %, 79 %), population (Roscoff, Lake Grevelingen, Terschelling, Sylt) and planting density (64 and 121 plants m^{-2}), in the mesocosm over the first growing season. Number of shoots, above- and belowground biomass were log-transformed prior to analysis. * $0.01 \leq p \leq 0.05$, ** $0.001 \leq p < 0.01$, *** $p < 0.001$, empty space: not significant

Dependent variables	Date	Factors				
		Shade[a]	Population	Density	Shade x Population	Shade x Density
Cover	July 16	**		***		
	Aug. 14	***		***		
	Sept. 18	***	**			
Number of shoots	July 16		**			
	Aug. 14	**	**	***		
	Sept. 18	***	*			
Above-ground biomass	July 16					
	Aug. 14	***		**		*
	Sept. 18	**				
Below-ground biomass	July 16		***			
	Aug. 14	**	***	***		
	Sept. 18	***	***			
% Generative shoots	July 16	**	***		*	
	Aug. 14	*	***			
	Sept. 18	*	***			

higher than that of Sylt and Terschelling plants ($p < 0.05$ ANOVA). The differences in belowground biomass reflected the differences between the populations at the time of planting. The belowground biomass was sustained for a longer period than the aboveground biomass because it disappeared only after decomposition, while the aboveground material drifted away soon after death or damage. This fact was particularly well borne out by the Yderfjorden population. The aboveground plant matter disappeared largely during the first growing season. However, dead rhizomes were still to be found in the second season.

Lake Grevelingen, Sylt and Terschelling plants survived the winter at 0 % shading, whereas only a marginal number of Yderfjorden and Roscoff plants emerged (Table 3). In a number of trays, the plants had disappeared during the second growing season. In the remaining trays, the number of shoots increased by a factor of 3 (Table 3). Initially, plants from Lake Grevelingen and Sylt grew in larger numbers than those from

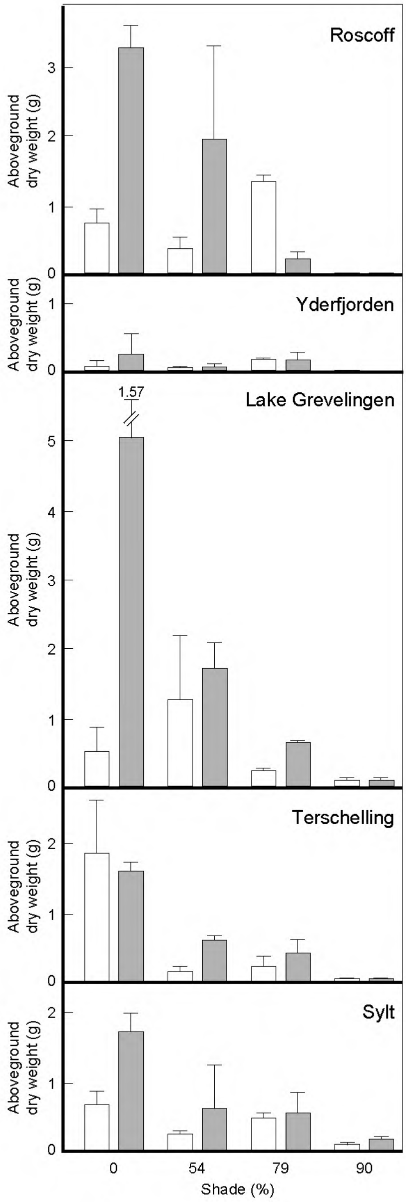

Fig. 5. Aboveground biomass in August of the first growing season in the mesocosm, where plants from different populations grew under different shadings. (0.04 m^2 samples, geometric mean of 2 replicates, SEM is presented)

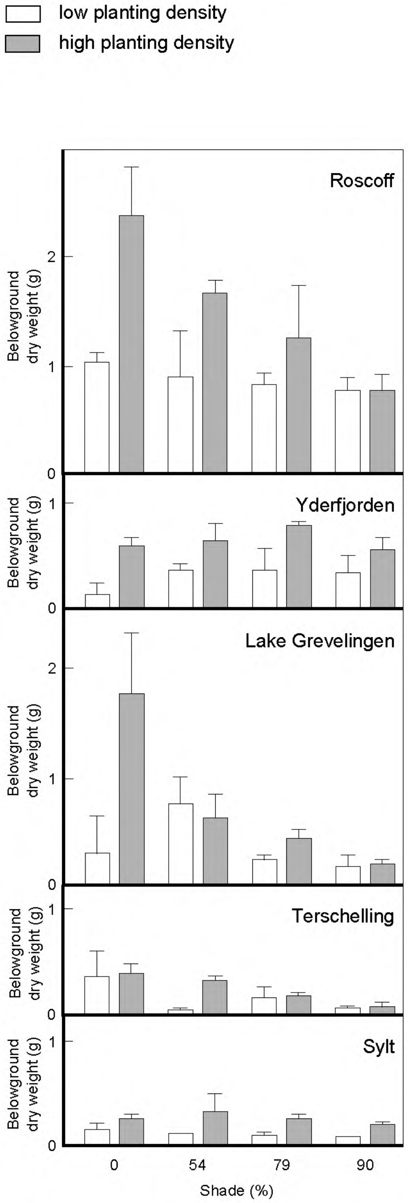

Fig. 6. Belowground biomass in August of the first growing season in the mesocosm, where plants from different populations grew under different shadings. (0.04 m^2 samples, geometric mean of 2 replicates, SEM is presented)

Table 3. Number of shoots m^{-2} in the second growing season in the mesocosm (average of 2 replicates and 2 planting densities, ±SEM). Counts also were made in June, July and August, but the maximum number of shoots was reached in October. Numbers were corrected for the loss due to sampling

Donor Site	Month Measured	Shoots (no. in m^{-2})	
		0 % Shade	54 % Shade
L. Grevelingen	May	26±3	20±3
	Oct.	102±25[a]	23±5[a]
Sylt	May	26±3	15±3[a]
	Oct.	99±22[a]	10±4[b]
Terschelling	May	14±3[a]	3±0
	Oct.	50±7[a]	–
Roscoff	May	3±1[a]	1[c]
	Oct.	23[c]	–
Yderfjorden	May	3±0	1±0[b]
	Oct.	23[c]	–

[a] The shoots were found in 3 trays, [b] the shoots were found in 2 trays, [c] the shoots were found in only 1 tray, no note: the shoots were found in 4 trays

Terschelling ($p < 0.05$ ANOVA). This difference had disappeared in October. Also, in the 54 % shading category, plants from Lake Grevelingen, Sylt and Terschelling developed foliage in spring. However, the numbers were lower than in the 0 % shading category, and decreased further during the second growing season. Planting density had no effect on the number of shoots in the second growing season. There was no correlation between planting density and number of shoots in the second growing season ($p > 0.05$ ANOVA). Furthermore, the populations did not respond differently to shading (0 % and 54 %; $p > 0.05$ ANOVA). In comparison with the first growing season, the number of shoots was about a threefold lower in the second growing season.

Zostera marina flowering, seed production and germination

Donor sites

Seagrasses in the Wadden Sea had high percentages of generative shoots when compared to the perennial populations in Lake Grevelingen, Roscoff and Yderfjorden (Fig. 7, Tukey's comparison of means: $p < 0.05$). Flowering peaks were reached in July/August. Secondary vegetative growth began in late summer. In populations with a semi-annual reproductive strategy (the Wadden Sea populations at Terschelling and Sylt, Fig. 7), secondary vegetative growth was also observed; the leaves, however, were poorly developed. In spring, a small part of semi-annual populations typically sprouted from rhizomes: at Terschelling 5 to 10 % (locally 20 %), and 2 to 3 % (locally 5 %) at Sylt. At both locations, perennial plants dominated high in the eulittoral in areas with coarse substratum. In this zone, plants were brown and small in summer, but appeared

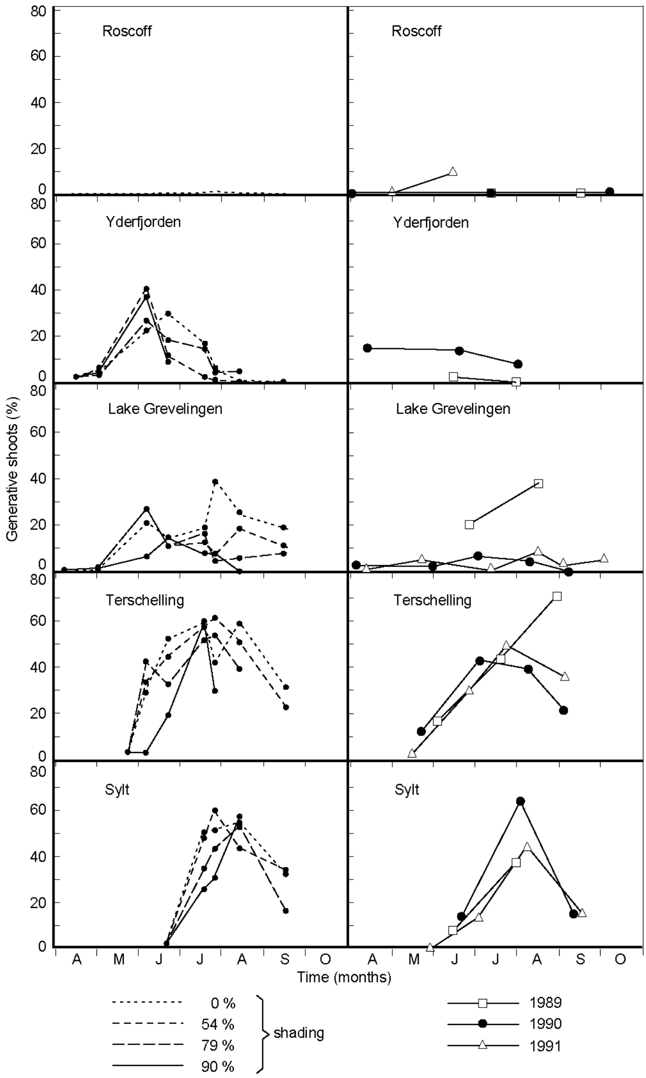

Fig. 7. Percentage of generative shoots in the mesocosm (left) and at the donor site (right). The percentage at the donor site was calculated from a sample of 50 randomly collected plants. At the mesocosm, first growing season, the percentage of generative shoots was estimated on the basis of counts in each tray. The average of 2 replicates and 2 planting densities is given. Trays with less than 2 % cover (see Fig. 4) were excluded

healthy green in spring, autumn and winter (observations in Sylt were made only in spring and summer). In Lake Grevelingen, the percentages of generative shoots varied among years: amounting to 40 % in 1989; below 10 % in 1990 and 1991, and in 1988 up to 70 % of the shoots were generative (W.B.J.T. Giesen, unpublished data).

Mesocosm

During the first growing season, the percentage of generative shoots of each of the 5 populations was generally similar to the percentage of its donor population (Fig. 7, $p > 0.05$ ANOVA for all populations). In the second growing season, the plants flowered less frequently than in the first growing season (Table 4, $p < 0.05$ ANOVA). Shading had a negative effect on the percentage of generative shoots (Fig. 7, Table 2).

Table 4. The maximum percentage of inflorescences in the mesocosm in the first and second growing season. Average ± SEM of 2 replicates and 2 planting densities in the 0 % shading category

Donor site	Inflorescences (%)	
	First growing season	Second growing season
Sylt	56±6[a]	21±9[a]
Terschelling	60±7[a]	36±11[a]
L. Grevelingen	29±7[b]	18±5[c]

[a] maximum in August, [b] maximum in September, [c] maximum in July

Eelgrass plants from Sylt had the highest production of seeds: at average 50 seeds could be counted per tray in 2½ minutes, whereas in trays with Terschelling and Lake Grevelingen plants, an average of 35 and 25 seeds were counted during this time interval, respectively. In trays with Roscoff plants, no seeds were detected. In the second growing season, seedlings had germinated in the mesocosm. They probably originated from plants in the same tray (at least maternally) due to the low advection in the mesocosm and because seeds, having a high specific gravity, will sink to the bottom immediately. This is supported by our findings that the number of seedlings was strongly related to the seed production in a tray. Most of the seedlings were found in the trays with Sylt plants (only qualitative data are available). Many Terschelling seedlings, few Lake Grevelingen seedlings and no Roscoff and Yderfjorden seedlings were found. Most seedlings were removed at the end of March. At the end of April almost no seedlings were found. No differences were observed between the number of seedlings in the 54 % shading category and in the 0 % shading category. Few seedlings were found in either of the higher shading categories.

DISCUSSION

Survival and development of 5 *Zostera marina* populations in the mesocosm

The 5 donor populations of *Zostera marina* population showed differences in survival and reproductive strategy after transplantation to the mesocosm, these are summarised in Table 5. The plants from the perennial Baltic population (Yderfjorden) died off rapidly. This occurred also in a similar experiment in 1989. Therefore the die-off is not likely to be the result of an unfavourable planting date (which differed considerably: 15 May 1989 and 12 April 1990) nor year-to-year fluctuations or chance events. Temperatures between the mesocosm and donor site were similar and so temperature differences can also be discounted. Differences in latitude or in nitrogen availability are unlikely to have caused the die-off, as the population from Sylt experienced the same transition to the lower latitude and higher nitrogen availability of the mesocosm. Possibly, the plants were unable to adapt to the high salinity in the mesocosm in such a short time (salinity Yderfjorden: ca 12 PSU; mesocosm ca 30 PSU). The adaptation difficulties of the Yderfjorden population may also be attributed to genetic characteristics

Table 5. Comparison of the development of the 5 northwest European eelgrass populations in the mesocosm. +++ Very good/high, ++ good/high, + moderate, – negligible

	Flowering	Seed production	Development first growing season[a]	Overwintering + second growing season[b]	Germination
Sylt	++	+++	+++	+++	+++
L. Grevelingen	+	+	+++	+++	+
Terschelling	+++	++	++	++	++
Roscoff	–	–	++	–	–
Yderfjorden	+[c]	–	–	–	–

[a] On the basis of percentage cover and number of shoots
[b] on the basis of number of shoots
[c] remnants of inflorescences that were formed prior to transplantation

Plants from the perennial evergreen Roscoff population did not successfully overwinter in the mesocosm; most of the aboveground parts died off and no new shoots emerged in spring. In the mesocosm, a combination of low light intensities in winter and hypoxic conditions near the bottom (as a result of the low water movements) may have caused the loss of aboveground parts. It was observed that Roscoff plants in the mesocosm suffered from sulphur precipitation on healthy leaves and sheaths which subsequently decayed. This occurred at the locations where macroalgae were abundant: (1) in early summer in the top and middle of the canopy; (2) in late summer at the base of the plants, where macroalgae were decaying. Healthy green plants were observed floating with a disrupted base. This was observed to a lesser extent with plants from the other populations, both in 1990 and in an experiment in 1989 (van Katwijk 1992). At Roscoff, water dynamics were high, thereby preventing hypoxia, and light intensity was, most probably, higher than in the mesocosm. Winter temperatures in Roscoff were higher than in the mesocosm (minimum of 9°C and 1°C, respectively; the former being measured in 1976 and 1978 by Jacobs (1979) and Jacobs & Pierson (1981)). The adaptability to anoxia or to temperature may both be genetically determined. Isoenzyme analysis has identified slight genetic differences between the Roscoff population and populations in SW-Netherlands (de Heij & Nienhuis 1992).

The plants from the semi-annual populations (Terschelling and Sylt), and the perennial Lake Grevelingen population, survived winter and sprouted in the second growing season. The Terschelling plants performed slightly less than the Sylt and Lake Grevelingen plants, but this may be attributable to year-to-year differences (van Katwijk 1992). Both semi-annual populations had a higher reproductive potential (flowering, seed production, germination) than the perennial population. The success of the 3 populations infers that neither the type of substrate at the donor site (which ranged from muddy sand to coarse sand, and from 1 to 8 % organic content), nor water nitrogen availability (which ranged from low to moderate), precluded successful transplantation to the low organic, sandy substrate, and moderately high water nitrogen availability in the mesocosm.

Planting density was initially positively correlated with the transplantation success. However, in autumn and in the next growing season, planting density was seen to have no effect.

During the first growing season, the percentage of generative shoots in the mesocosm was generally similar to that in the corresponding donor site populations. Plants from the perennial Lake Grevelingen populations flowered somewhat more in the mesocosm (both during the first and second growing season) than at the donor site in 1990 and 1991. There were, however, large year-to-year differences in flowering percentages of eelgrass in Lake Grevelingen in the period 1987 to 1991 (personal observations, W.B.J.T. Giesen unpublished data, van Lent & Verschuure 1994a). The semi-annual Wadden Sea populations from Sylt and Terschelling both flowered less in the second growing season when compared to the first growing season and to the percentage at the donor site. This may be due to the fact that all plants of the second growing season developed from rhizomes (seedlings were removed in spring). This implies that perennial plants employ a largely vegetative strategy, while seed develops more often in annual plants, as was also found in Nova Scotia by Keddy & Patriquin (1978).

Maximum light reduction tolerated by *Zostera marina*

Plants growing without shading encountered light saturation during summer, whereas those growing under shading experienced limiting light conditions. Maximum photosynthesis, and the rate of light-saturated photosynthesis, decrease in the cold season (Marsh et al. 1986, Zimmerman et al. 1995) and photosynthesis shows light acclimation to low light conditions (Dennison & Alberte 1982, 1985, 1986, Goodman et al. 1995). The winter temperatures in the mesocosm may have given rise to an eightfold decrease in saturation irradiance as compared to summer values (Marsh et al. 1986). This probably also holds for the growth-insolation relationship; therefore plants growing without shading may have experienced light saturation during most of the study. Plants growing under 54 % shading may have encountered saturating light conditions in spring, when temperatures were low but insolation was already high.

In the 0 % shading category (= 75 % light reduction at the bottom), the populations developed well in the first and second growing season. An 89 % reduction in irradiance at the bottom (category 54 % shading) was survived by the 3 successful donor seagrass populations. Long-term transplantation success was, however, doubtful, because vegetative growth was almost absent in the second growing season. The number of seedlings that emerged in the second growing season was comparable in the 0 % and 54 % shading category. At lower light intensities, the plants died immediately after planting, with the exception of the Sylt plants. The latter can be ascribed to the late transplantation date (June). This date was chosen because the Sylt seagrasses begin their growth phase later than the other populations. However, the plants were already relatively large at that time. This probably enabled them to allocate reserve material from their rhizomes so as to continue growth. Secondly, in June, the number of hours of sunshine and solar radiation are higher than in April/May, therefore the plants may have received enough light to survive for a short period.

Table 6. Values from literature concerning turbidity, maximum depth and the maximum light reduction at the bottom that is tolerated by *Zostera marina*. Light attenuation coefficients presented in this table are in some cases calculated from the Secchi depth (D_{sd}): k=1.7 / D_{sd} (Poole & Atkins 1929, Giesen et al. 1990a). At Secchi depth, light transmission equals 18 %. In brackets: geometric mean, MSL: mean sea level, M(L)LT: mean (lower) low tide

Location	Tidal amplitude[a] (m)	Number of populations	Minimum light transmission %	Turbidity k (m^{-1})	Maximum depth D_{max} (m)	D_{max} in relation to:	
The Netherlands (L. Grevelingen)	0	1	30	0.40[b]	3.0[c]	MSL	Systems with approx. constant water level
The Netherlands (L.Grevelingen)	0	1	7*	0.50[d]	5.2[e]	MSL	
The Netherlands (L. Veere)	0	1	29**	0.54[d]	2.3[e]	MSL	
Denmark	0	4	15-22 (18)	0.33-0.94[f]	2.0-5.0[f]	MSL	
Denmark	0?	20	5-44 (17)	0.22-1.216[g]	1.5-9.0[g]	MSL	
Denmark	0	several	18[h]			MSL	
Denmark	0	1	11[i]			MSL	
This study	0	5	11-25	see text	see text	MSL	
Massachusetts	0.25	1	21	0.28[j]	5.5[k]	MLT	Tidal systems
California	0.85	1	21	0.57[l]	2.75[l]	MLLT	
San Francisco	0.90	5	4-21 (8)	1.5-3.1[m]	0.5-2.0[m]	MLLT	
Japan	?	2	25[n]		2.0-5.0[n]	?	
Québec	1.50	3	18	0.43[o]	4.0[o]	MLT	
The Netherlands (Oosterschelde)	1.60	1	49***	0.9[p]	0.8[p]	MSL	

Notes to Table 6:

* More than 5 % cover, ** depth limit probably determined by steep decline towards channels, *** depth is related to MSL: the population does not occur sublittorally.

[a] Tidal amplitude: MSL-ML(L)T

[b] Calculated from data Rijkswaterstaat 1980-1990

[c] Apon 1990

[d] Calculated from data Rijkswaterstaat 1972-1975 in Pellikaan 1980

[e] Pellikaan 1980

[f] Borum 1983

[g] Nielsen et al. 1989 in Duarte 1991

[h] Ostenfeld 1905

[I] Olesen & Sand-Jensen 1993

[j] Dennison 1987

[k] Dennison & Alberte 1986

[l] Backman & Barilotti 1976

[m] Zimmerman et al. 1991

[n] Mukai et al. 1980

[o] Lalumière et al. 1994

[p] de Jonge & de Jong 1992

The 75 to 89 % light reduction tolerated at the bottom corresponds well with the values found in the literature (Table 6). It should be noted that large variations in turbidity reduce the depth limits of *Zostera marina* (Zimmerman et al. 1991). This was not taken into account in Table 6. Duarte (1991) found the following relationship between maximum depth (D_m) and light attenuation coefficient (k), based on literature values from 30 seagrass species: $D_m=1.86\ k^{-1}$, which indicates that seagrasses can colonize depths where light is reduced by 84.4 % compared to subsurface values (equation 1).

The populations did not differ in shading tolerance. The percentage of generative shoots was not notably influenced by shading, except when the plants were heavily shaded: in that case the plants ceased to grow and neither inflorescences nor new vegetative parts were formed. Van Lent et al. (1995) found that the percentage of generative shoots in the *Zostera marina* population of the Veerse Meer was lower when shaded. In our experiment, shading did not induce an increase in wasting disease in the plants during the first growing season (van Katwijk 1992), which corresponds to the finding of Vergeer et al. (1995). However, in artificially infected plants, Vergeer and coworkers did find a positive correlation between shading and wasting disease infection rate. Therefore, *Z. marina* may be more susceptible to wasting disease due to shading, as hypothesized by Giesen et al. (1990a,b), but this effect would take more than one growing season to manifest.

The maximum depth at which transplantation may succeed could be calculated from the maximum light reduction at the bottom that was tolerated by eelgrass (equation 1, Lambert-Beer). A shortcoming of this calculation would be that tidal fluctuations of the water level would not be considered. Fluctuation of the water level influences the insolation at the bottom and so negatively effects the amount of light which may be used by plants. The effect is most intense when the irradiance at the bottom is near the saturation point of the plant. To deal with this problem, a calculation model was made for the Wadden Sea situation (van Katwijk 1992). Fluctuations in turbidity following the spring-neap tidal cycle (Dring & Lüning 1994) were not included in the model. In the period 1985 - 1992, k varied between 1.0 and 1.6 m^{-1} (Anonymous 1991, de Jonge & de Jong 1992). The model calculated that when the turbidity above the tidal flats was $k=1.5\ m^{-1}$, the maximum survival depth would lie between 0.80 and 1.30 m below mean sea level (corresponding to 75 % and 89 % light reduction at the bottom). When the turbidity was $k=2.0\ m^{-1}$, these values shift to 0.45 and 0.85 m below mean sea level, respectively.

Conclusions

The donor suitability of 5 northwest European *Zostera marina* populations differed considerably. The plants from the evergreen perennial populations died during the first year of study. Plants from the remaining 3 populations developed well in the second growing season, especially in the 0 % shading category. The 3 successful donor populations shared two features: (1) the perennial part of the population overwintered belowground and sprouted from rhizomes in spring, (2) the plants had narrow leaves, the perennial Lake Grevelingen population being developed from a typically estuarine, semi-annual, form which was encountered before 1971 (personal observations, Beeftink 1965, Nienhuis 1983). Possibly, a perennial evergreen broad-leaved type of eelgrass - usually associated with subtidal or submersed habitats (Harmsen 1936, Keddy & Patriquin 1978, Jacobs 1982, Phillips et al. 1983) - was (1) not able to grow in the submersed mesocosm, which would, interestingly, indicate that some parameter other than permanent submersion is crucial to this distinction of the two forms, (2) had a narrower amplitude, or (3) was more sensitive to changes in its environment. On the other hand, the differences in donor suitability may also have been caused by an intolerance of *Z. marina* to particular habitat transfers, for example, in case of the Yderfjorden population, transplantation from a low to a high salinity environment.

The percentage of generative shoots in the mesocosm was generally similar to that in the corresponding donor site populations. The perennial populations flowered less than the semi-annual ones, both at the donor sites and in the mesocosm. In the mesocosm, seed production of the plants from semi-annual populations was high, the perennial Lake Grevelingen plants occupied an intermediate position, and the plants of the 2 evergreen perennial populations produced no seeds, which was, in the case of the Yderfjorden populations, due to the general die-off. Apparently, *Z. marina* does not rapidly change its reproductive strategy in response to a changed environment. This means that the reproductive strategy that is desired at the transplantation site, should be employed by the donor population. In case of the Wadden Sea, where a semi-annual reproductive strategy was desirable, the perennial Roscoff plants were not suitable (even if they had survived winter), because seed production remained nil.

Acknowledgements. This study has been financed by the National Institute for Coastal and Marine Management of the Ministry of Transport, Public Works and Water Management, The Netherlands. We thank Prof. Dr. V.N. de Jonge, D.J. de Jong, Dr. F.W.B. van den Brink, Dr. J.G.M. Roelofs and B. Kelleher for fruitful discussions and critically reading the manuscript, the latter also for correcting the English. We thank workers of the Ministry of Transport, Public Works and Water Management, students and workers of the Catholic University especially M.G. Versteeg, and several volunteers for their efforts in the fieldwork. D.P.J. Verschuren, S. Westerveld, and L.J.M. Wijgergangs are thanked for their contribution to the project in all its aspects. We acknowledge workers of the Institute for Forestry and Nature Research (IBN/DLO) in Texel, for cooperation. We are much obliged to A. Meijboom (IBN/DLO) and H. van Aken (Netherlands Institute for Sea Reasearch) for providing turbidity and temperature data. We thank Dr. R.M. Asmus and Dr H. Asmus for their support during our fieldtrips to Sylt. The Illustration Service of the Faculty of Science, Catholic University Nijmegen, is thanked for preparing the figures and Th. de Boo (Department of Statistical Consultation, Catholic University Nijmegen) for help with the application of statistical methods.

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CHAPTER 3

AMMONIUM TOXICITY IN EELGRASS *ZOSTERA MARINA*

van Katwijk MM, Vergeer LHT, Schmitz GHW, Roelofs JGM (1997)
Mar Ecol Prog Ser 157:159-173

AMMONIUM TOXICITY IN EELGRASS *ZOSTERA MARINA*

ABSTRACT: Seagrasses are declining all over the world, resulting in a substantial loss of biodiversity, coastal sediment stabilisation and nursery areas of economically important fish. The seagrass decline has often been associated with increasing eutrophication of coastal areas. We tested possible toxic effects of high nitrogen concentrations in the water layer on the seagrass *Zostera marina*, which is often the sole higher plant inhabiting coastal zones in the northern hemisphere. Plants grown in either mud or sand were subjected to various water ammonium and nitrate concentrations, whereby ammonium and nitrate supply were balanced (both 25 µM or 75 µM), or unbalanced (ammonium 125 µM and nitrate 25 µM, and vice versa). We used 2 temperatures, 15 and 20°C. Analyses were made after 2 and 5 weeks of exposure. In an additional experiment, 9 µM ammonium and 3 µM nitrate were supplied. An ammonium concentration of 125 µM in the water layer was toxic for *Z. marina*: the plants became necrotic within 2 weeks. After 5 weeks, plants in all treatments except for the 9 µM treatment were either necrotic or had died. This suggests that toxicity occurs at ammonium concentrations as low as 25 µM. Nitrate treatment had no effect. Ammonium toxicity effects were more pronounced in plants grown on sand and at the higher temperature. It is argued that the ammonium toxicity effects on *Z. marina* are expected to be strongest in autumn when irradiance decreases, temperature is still high, and ambient ammonium concentrations rise.

INTRODUCTION

Seagrasses are the only submerged vascular plants inhabiting shallow coastal seas. They contribute substantially to biodiversity and coastal sediment stabilisation and provide nursery areas for economically important species (e.g. Heck et al. 1995). Like most seagrasses throughout the world, many eelgrass *Zostera marina* L. populations in coastal areas of the northern hemisphere are under great pressure, or have already disappeared. This may be due to eutrophication, which elevates nitrogen. *Z. marina* is adapted to low nitrogen concentrations (Borum et al. 1989, Hemminga et al. 1991, Pedersen & Borum 1992). Increased nitrogen levels often induce increased growth and biomass (Short 1983a, 1987, Williams & Ruckelshaus 1993, Bohrer et al. 1995, van Lent et al. 1995) and affect plant morphology, tissue C/N ratios, density and reproductive strategy (Harlin & Thorne-Miller 1981, Short 1983a, 1987, van Lent et al. 1995). In some cases no significant growth response to nitrogen enrichment has been reported (Dennison et al. 1987, Murray et al. 1992). In other cases, however, decline in eelgrass was observed, not only as a consequence of shading due to increased algal growth (Neckles et al. 1993, Williams & Ruckelshaus 1993, Harlin 1995, Short et al. 1995, Taylor et al. 1995), but also as a direct effect of increased nitrogen in the form of nitrate (Burkholder et al. 1992, 1994). It is well known that high ammonium levels can be toxic to plants. Submerged aquatic plants may be particularly vulnerable to high water nitrogen concentrations because not only the roots, but also the leaves, are encompassed by water. Leaves, unlike roots, cannot regulate nitrogen uptake, e.g. through the reduction of root hairs (Short 1983a, Marschner 1995). Until recently, ammonium toxicity to submerged aquatic plants has been given little attention. It has been observed in a few freshwater aquatic plants (Grube 1974, Glänzer 1974, Agami et al. 1976, Glänzer et al. 1977, Roelofs 1991, Smolders et al. 1996); however, it has not, as yet, been reported for *Z. marina* or any other seagrass.

Increased external nitrogen concentrations may result in an increased tissue nitrogen content of *Zostera marina* (Harlin & Thorne-Miller 1981, Short 1987, Borum et al. 1989, Burkholder et al. 1992, 1994). This may cause a lowering of the concentration of phenolics, which increases the susceptibility for wasting disease (unpublished results L.H.T. Vergeer, Buchsbaum et al. 1990). In this way, increased nitrogen concentrations may have an indirect adverse effect on plant vitality. However, Ravn et al. (1994) found a positive correlation between phenolic acid content and tissue nitrogen content.

It is well known that, in many terrestrial and freshwater plant species, the presence of high ammonium concentrations can decrease the uptake of other cations like potassium, magnesium or calcium, or even lead to their exclusion (e.g. Pearson & Stewart 1993, Marschner 1995, Smolders et al. 1996). It is not known whether this also occurs in the marine environment with its high availability of potassium, sodium, magnesium and calcium.

Optimal growth for most plant species is usually obtained with a mixed supply of ammonium and nitrate (Marschner 1995); ammonium toxicity can be alleviated by nitrate supply (e.g. Hecht & Mohr 1990, Ikeda 1991, Feng & Barker 1992, Adriaanse & Human 1993).

Both roots and leaves of *Zostera marina* are capable of nitrogen absorption (Iizumi & Hattori 1982, Thursby & Harlin 1982, Short & McRoy 1984, Pedersen & Borum 1992, Hemminga et al. 1994). Ammonium is preferred above nitrate (Thursby & Harlin 1982, Short & McRoy 1984, Hemminga et al. 1994). *Z. marina* leaves have a greater affinity for ammonium than do the roots (Thursby & Harlin 1982, Short & McRoy 1984, Pedersen & Borum 1992, Hemminga et al. 1994). However, depending on the nutritional status of the sediment and overlying water, and presumably on the root:shoot ratio, root uptake is more important in some ecosystems (Iizumi & Hattori 1982, Short & McRoy 1984) and leaf uptake in others (Hemminga et al. 1994), or they may be equally important (Pedersen & Borum 1992).

In this study, the effect of nitrogen form on size, condition and a number of shoot tissue constituents of *Zostera marina* was examined through the supply of ammonium and nitrate in balanced and unbalanced ratios. It was hypothesised that excess ammonium and/or nitrate may be toxic to *Z. marina*, which would indicate why this seagrass is absent in eutrophicated areas. It would also indicate why perennial populations in northwest Europe are found in locations with low water nitrogen concentrations throughout the year, whereas annual populations are found in submersed habitats with higher nitrogen concentrations (in the Netherlands: autumn and winter concentrations rising above 15 µM ammonium and 50 µM nitrate, unpublished data Dutch Ministry of Transport, Public Works and Water Management 1980-1990, van Lent & Verschuure 1994a). Due to contamination by ammonium and nitrate of the various synthetic sea salt mixes tested, low levels of ammonium and nitrate could not be applied to the plants.

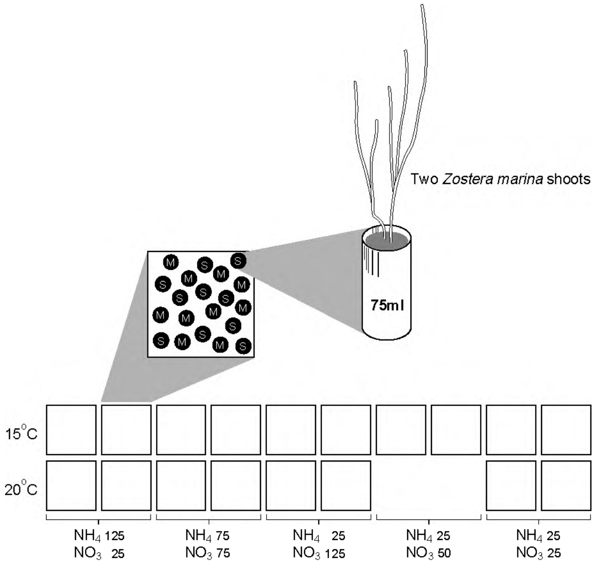

Fig. 1. Scheme of the experimental set-up. In the actual set-up, the containers were randomly placed. S: sand, M: mud. N-treatments in µM

MATERIALS AND METHODS

Zostera marina L. plants were subjected to water ammonium and nitrate concentrations of 9:3, 25:25, 25:50, 25:125, 75:75 and 125:25 µM, respectively. We used 2 sediment types, mud and sand, and 2 (growing season) temperatures, 15 and 20°C. The ammonium:nitrate treatment of 25:50 µM was applied at 15°C only. The ammonium:nitrate treatment of 9:3 µM was applied at 17°C and on mud only. Sampling was conducted after 2 and 5 weeks. The 9:3 treatment was sampled after 3 and 6 weeks.

Culture experiment. *Zostera marina* plants were collected and rinsed in the harbour-canal of Goes (SW Netherlands, 51°32' N, 3°50' E, 12 October 1992). The plants for the ammonium:nitrate 9:3 µM treatment were collected in the Wadden Sea (Eems and Terschelling, 53°22' N, 5°13' E, 11 August 1993). The plants were transported (at 8°C, corresponding to the temperature in Goese Sas) to the laboratory, and maintained overnight at 4°C. The following day, pairs of plants were placed in 75 ml jars filled with coarse sand or mud originating from dune sand or from a *Z. marina* habitat in Zandkreek, respectively (both in the Netherlands). A thin layer of sand was put over the mud to counter nutrient exchange with the overlying water. Twenty jars per container were placed in 18 containers filled with synthetic sea water (25.4 PSU, Wimex, of Wiegandt GmbH, Krefeld, Germany), and maintained under an 8 h dark:16 h light cycle corresponding to growing season conditions; light intensity just below the water's surface averaged 90 µE m^{-2} s^{-1}. The containers were placed in random sequence in a temperature controlled (15°C) water bath (Figs. 1 & 2). For technical details of the set-up, see Roelofs et al. (1984). The plants were allowed to acclimate for 3 weeks. Macroalgae were removed, with caution taken not to damage the plants, from the second

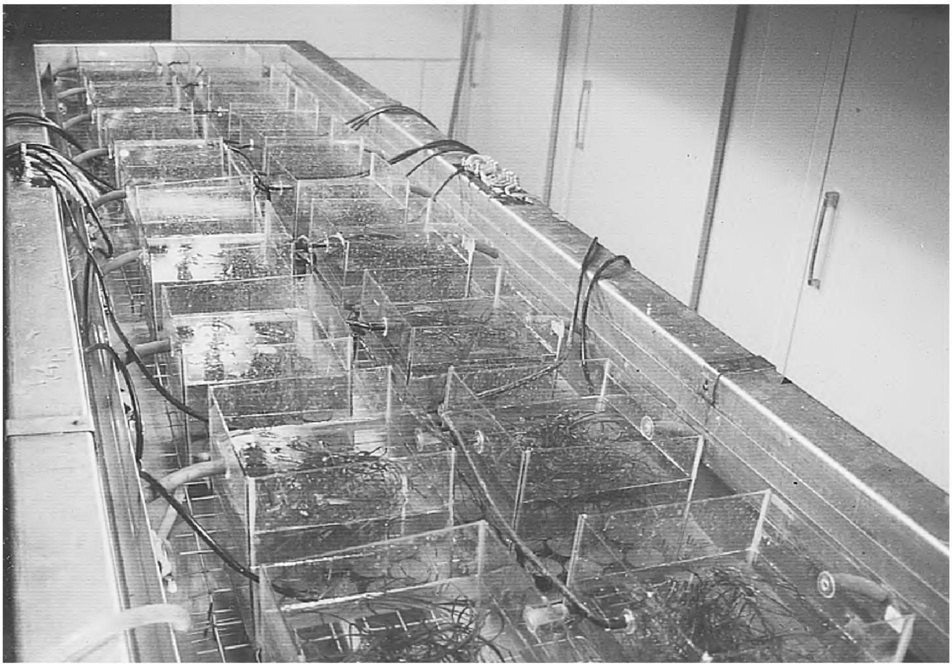

Fig. 2. Photograph of the experimental set-up

week onwards (*in situ*, water dynamics would have impeded macroalgal growth). On 5 November 1992 (9:3 treatment: 27 August 1993), the treatments started: $NaNO_3$ and NH_4Cl enriched synthetic sea water was pumped through the containers. [In making the stock solutions, it was calculated that the 25.4 PSU synthetic sea water (Wimex, 3 batches tested) already contained 10 µM ammonium and 25 µM nitrate on average. Sea salts of Reef Crystals (Aquarium Systems, Sarrbourg, France, 3 batches) were also tested, and appeared to contain even more ammonium and/or nitrate on average, which was also the case with Rila sea salts, tested by Rohrmann et al. (1992). Also, the variation was larger in Reef Crystals sea salt than in Wimex sea salt.] The ammonium:nitrate 9:3 µM treatment was applied using a self-prepared saltmix, derived from uncontaminated 'pro analysi' salts. Regrettably, it was too expensive and time-consuming to prepare this mix for all treatments. Each container was replenished once a day from its own stock container. The containers for the 20°C treatment were thermostatically heated. Water in the containers was gently aerated to ensure complete mixing.

Sampling. In Goese Sas, water samples were taken on 3 September and 12 October (date of collection). Water was sampled in the culture experiment at the onset of the treatments, and after 2 and 5 weeks. Three water samples were taken per occasion per container. Alkalinity and pH were measured instantaneously in one sample, the 2 other samples were filtered, whereafter citric acid was added to one of them in order to prevent precipitation of metals (for ICP analysis, see below). The samples were stored at –20°C until further analysis.

Table 1. *Zostera marina*. Leaf scores for necrosis or wasting disease-like lesions, based on the percentage of damaged leaf surface, from which shoot values were calculated (based on Giesen 1990)

	Damaged leaf surface					
	0 %	1 %	2-5 %	6-10 %	11-30 %	> 30 %
First leaf	0	0.2	0.4	0.6	0.8	1.0
Second leaf	0	0.1	0.2	0.4	0.6	0.8
Third leaf	0	0	0.1	0.2	0.4	0.6
Only the first 3 leaves were included in the calculation						

Eight to 10 *Zostera marina* plants (5 jars) per sediment type were sampled from each container after 2 and 5 weeks. The sediments were stored at 4°C. The sediments of the 5 jars were then mixed and water content was calculated from weight loss after drying 25 to 40 g of the wet sediment at 105°C, over 48 h. Seventy to 80 g of fresh sediment were placed in a 500 ml polyethylene bottle with 200 ml of double-distilled water, and shaken for 1 h. This mixture was centrifuged for 20 min at 11 000 rpm (r_{max} = 19 690 x *g*) and the supernatant stored at –20°C.

Plant analysis. The plants were sampled to measure length, width, wasting disease-like lesions, necrosis and dry weight. Leaf scores for wasting disease-like lesions and necrosis, based on the percentage of total leaf surface (Table 1), were estimated for each leaf separately. The leaf scores were averaged to calculate shoot values, whereby damage on young leaves was given a higher weight than damage on older leaves as the duration of their exposure to possible negative influences is less than that of older leaves (Giesen 1990). Distinction was made between discoloured leaf surface and infected leaf surface, the former being used to assess necrosis (including wasting disease-like lesions), the latter to assess only wasting disease-like lesions (examples in, amongst others, den Hartog et al. 1996). Three plants were dried at 70°C over 48 h to determine dry weight.

Chemical analysis. Two or 3 of the sampled plants were used to determine the concentration of phenolics in the shoots, using the method of Hagerman & Butler (1978), as described in Mole & Waterman (1987). The plants were freeze-dried for 2 days and ground with the use of liquid nitrogen. Ten milligrams were extracted with 5 ml 80 % ethanol for 10 min at 80°C. One millilitre of the extract was mixed with 2 ml sodium dodecyl sulphate (SDS) and 1 ml $FeCl_3$. The absorption was measured at 510 nm using a Schimadzu spectrophotometer (UV-120-01). Tannic acid (Sigma Chemical Company) was used as the standard.

Chlorophyll was extracted from leaf segments of 3 to 5 cm length which were taken from 3 cm below the top of the first fully grown leaf. The pieces of 2 shoots were taken together. The pieces were blotted dry and weighed. Chlorophyll *a* (chl *a*) was measured spectrophotometrically after extraction in 80 % ethanol. Ca. 2 ml extractant per 10 mg (fresh weight) of leaf material was used. The acidification method was used to correct for phaeophytin (Moed & Hallegraeff 1978). Calculations of chl *a* concentrations were performed according to Roijackers (1981).

Two or 3 shoots were used to analyse various chemical constituents of the shoots. The shoots were digested with sulphuric acid and hydrogen peroxide: 50 mg of oven-dried (48 h, 70°C) and ground shoot tissue were dissolved in 5 ml concentrated H_2SO_4, incubated at room temperature for 24 h, heated to 150°C and digested by slowly adding 2 ml 30 % H_2O_2. The volume of the digest was brought to 100 ml with double-distilled water.

In the water samples, sediment extracts and shoot digests, total P, Mg, Ca, Fe, Mn, total S (only in sediment extracts), and Zn (only in shoot digests) were measured with an Inductively Coupled Plasma spectrophotometer (ICP), type IL Plasma 200. Ammonium, nitrate and chloride were measured colorimetrically with a Technicon AAII system according to Kempers & Zweers (1986), Grasshoff et al. (1983), and O'Brien (1962), respectively. In the shoot destruction process, nitrogen is partly converted to ammonium, and partly to nitrate; therefore, for shoots, total N is presented, i.e. ammonium+nitrate contents. K^+ and Na^+ were determined by flame photometry. pH of the water samples was measured with a pH electrode; alkalinity was estimated by titration with 0.01 M HCl down to pH 4.2.

Statistical analysis. Water and sediment parameters were normally distributed, as were length, width, biomass, phenolic contents and chl *a* of the shoots. Necrosis, wasting disease-like lesions and water content, being (based on) percentages, were normally distributed after arcsine transformation. The remaining chemical parameters of the shoots were lognormally distributed. The back-transformed means were used as a central measure. As a measure of variance, standard error of the mean was used. For lognormal parameters, this was calculated according to Mood et al. (1974).

Analysis of Variance was used in this split-plot experiment (sediment type being a subplot factor, the containers being the experimental unit), whereby N-treatment, temperature and their interaction were tested with the following error-term: N-treatment x temperature x replicate + N-treatment x replicate + temperature x replicate, and sediment type and the interactions of sediment type with the other parameters were tested against the residual error (Steel & Torrie 1980, Freund & Littell 1985). For comparison of means, Tukey's test was used. The ANOVA and Tukey's test were carried out using the Statistical Analysis System, procedure GLM (SAS 1989).

The results were ordinated using Redundance Analysis (RDA, which is the canonical form of Principal Components Analysis, PCA) to detect and illustrate the correlations between the various shoot response parameters and the effects of the treatments on them. RDA extracts ordination axes on the basis of the shoot response variables, like in PCA, but with the constriction that they should be a linear combination of the explanatory variables, i.e. the treatments (Jongman et al. 1995). We chose scaling 2 (covariance biplot), because we were also interested in the correlations of the shoots responses, rather than on the 'distances' between the containers, which would require scaling 1 (ter Braak 1994). The ordination was carried out with help of the program CANOCO (ter Braak 1987).

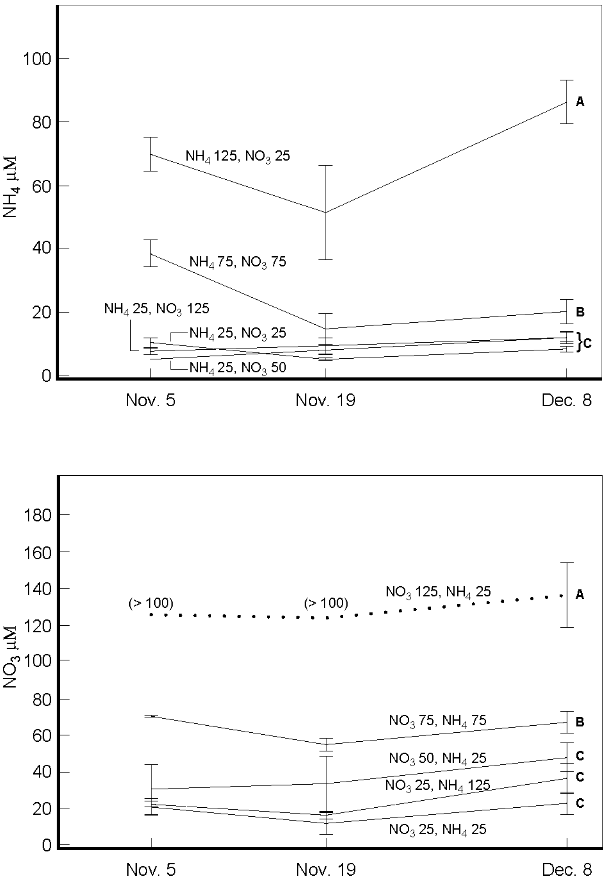

Fig 3. Ammonium and nitrate in the water at the onset of the treatment and after 2 and 5 weeks. N-treatments are presented in µM. Means and SEM are plotted. Different letters denote significant differences at the 5 % level according to Tukey's test

Results

Water

The measured chemical properties of the water were not influenced by the treatments, except pH and alkalinity, and, of course, ammonium and nitrate (Table 2). At 20°C, pH was lower than at 15°C, whereas alkalinity was higher. In the high ammonium treatment, pH tended to be lower than in the other treatments.

Ammonium concentrations in containers were lower than the concentrations applied (Fig. 3): application of 125, 75, 25 and 9 µM ammonium resulted in an actual concentration in the water of ca 70, 20, 10 and 3 µM, respectively. With pH values of the water being 8.5 on average (Table 2), associated ammonia must have been formed in appreciable quantities and its loss into the air could account for some of the missing nitrogen. Also, large quantities were probably absorbed by the plants. This is supported by our finding that on the last sampling date, when the majority of plants in the highest ammonium concentration had died, the ammonium concentration was higher than on the previous dates (Fig. 3). The nitrate concentrations in the water corresponded with the

Table 2. pH and chemical composition of the water and the effects of N-treatment and temperature and their interactive effect (ANOVA). Values are means of sampling results at the onset of the treatment and after 2 and 5 weeks; data pooled due to sampling date having no effect. nd: not detectable. * $0.01 \leq p \leq 0.05$, **$0.001 \leq p < 0.01$, all other effects were non-significant

	Mean	SEM	Significance		
			N-treat.	Temp	N-treat. x Temp
pH	8.5	0.0	**	**	
Alkalinity (meq l^{-1})	2.03	0.04		*	
Cl (mM)	402	6			
S (mM)	17.7	0.3			
P (mM)	nd	nd			
Na (mM)	334	4			
Mg (mM)	33.1	1.0			
K (mM)	7.5	0.1			
Ca (mM)	5.45	0.17			
Fe (µM)	0.35	0.04			
Mn (µM)	0.30	0.02			

concentrations applied, indicating that no large losses of nitrate occurred (Fig. 3). The nitrate concentrations measured on the first 2 sampling dates were found to be higher than 100 µM, but unfortunately no discrete analyses were made.

At the collection site, the ammonium concentration was 16 µM on the day of collection, nitrate was not measured. One month earlier, ammonium concentration was 15 µM and nitrate 0.8 µM.

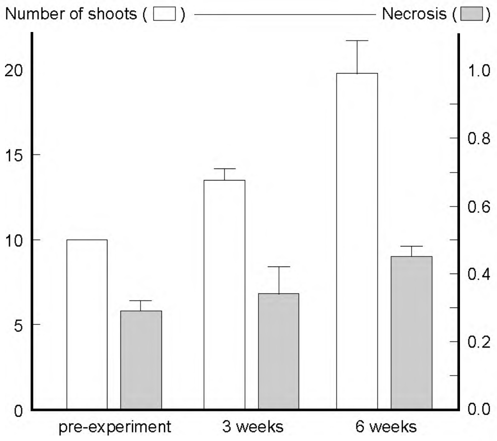

Fig. 4. Number of shoots and necrosis of *Zostera marina* in the 9 µM ammonium: 3 µM nitrate treatment

Table 3. Average water-extractable nutrient concentrations (µmol kg^{-1} DW) in the sediments measured after 2 and 5 weeks. Values are pooled exclusive of the 9:3 treatment

	2 weeks		5 weeks	
	Mud	Sand	Mud	Sand
NH_4	101	37	213	70
K	164	119	239	161
Ca	1060	940	1370	1310
Mg	3400	3500	5100	5500
Fe	2.0	1.6	2.8	3.3
Mn	0.06	20.5	0.08	13.9
P_{tot}	3.2	5.9	2.8	3.5
SO_4	2600	1900	3500	3300
Water content %	22	21	23	22

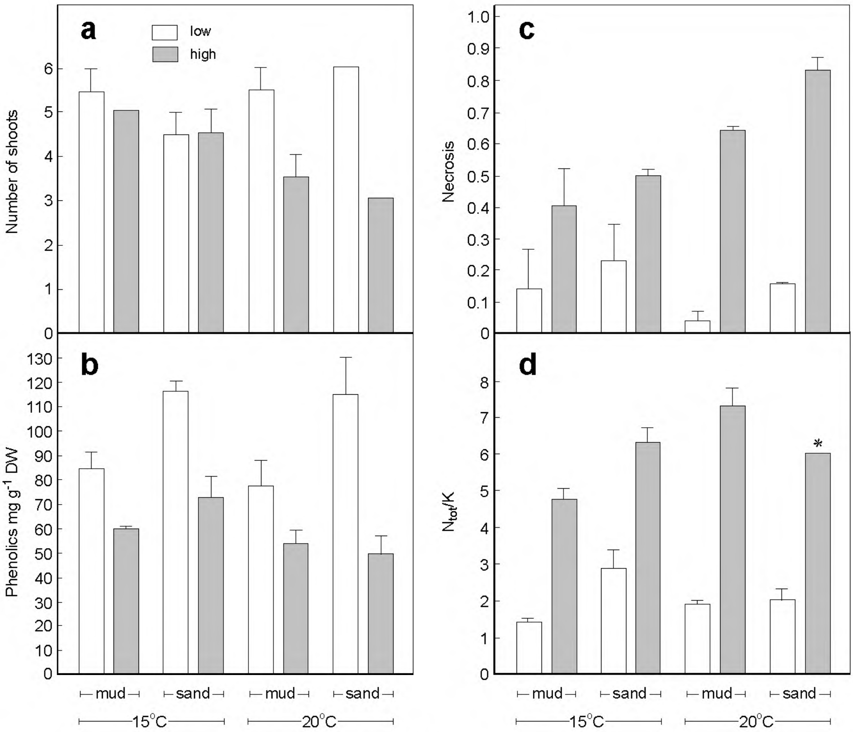

Fig. 5. Effect of 'high' (125 µM) and 'low' (25 µM) ammonium and of sediment type on (a) number of shoots, (b) phenolic content, (c) necrosis, and (d) N_{tot}:K ratio of *Zostera marina* after 2 weeks of treatment. NO_3 supply for both treatments was 25 µM. Index of necrosis: see 'Materials and Methods'. *Only 1 replicate

Table 4. *Zostera marina*. Characteristics and chemical composition of seagrass shoots under different N-treatments (NH_4:NO_3, µmol l^{-1}) after 2 weeks. Means or back-transformed means (for arcsine- and log-transformed parameters) are presented. The index for necrosis was calculated from % brown colouration of leaf surface, including infected leaf surface, and the index for wasting disease was calculated only from % infected leaf surface ('Materials and Methods'). nm: not measured (insufficient plant material)

	25:25				25:50		25:125				75:75				125:25			
	15°C		20°C		15°C		15°C		20°C		15°C		20°C		15°C		20°C	
	Mud	Sand	Mud	Sand	Mud	Sand	Mud	Sand	Mud	Sand	Mud	Sand	Mud	Sand	Mud	Sand	Mud	Sand
No.of shoots	5.5	4.5	5.5	6.0	6.5	4.5	6.5	4.0	5.0	4.0	5.0	4.0	5.5	6.0	5.0	4.5	3.5	3.0
Length (cm)	44	40	38	34	39	34	36	42	40	36	43	37	42	32	38	43	21	29
Width (mm)	2.3	2.5	2.1	2.3	2.3	1.8	2.0	2.6	2.4	2.4	2.5	2.3	2.4	2.0	2.0	2.4	1.5	1.9
Biomass (mg)	79	56	65	55	69	54	56	43	72	66	76	69	85	45	57	68	23	45
Necrosis	0.14	0.23	0.04	0.15	0.03	0.07	0.03	0.04	0.24	0.22	0.13	0.16	0.30	0.36	0.40	0.50	0.64	0.83
Wasting disease	0.01	0.03	0.02	0.04	0.03	0.07	0.03	0.04	0.16	0.05	0.07	0.06	0.08	0.04	0.01	0.01	0.02	0.00
Water content (%)	84	85	82	82	85	79	83	87	83	85	87	84	85	86	86	87	89	81
Phenolics (mg g^{-1} DW)	85	117	78	115	113	138	88	123	103	91	111	107	79	81	60	73	54	49
Chl *a* (µg g^{-1} FW)	43	74	28	24	46	27	38	27	67	25	87	50	35	39	52	64	nm	39
N_{tot} (µmol g^{-1} DW)	1080	1720	1390	1200	1280	1250	1570	1600	1310	1560	1960	2370	2240	2430	2920	2880	2830	2740
P_{tot} (µmol g^{-1} DW)	53	85	50	80	64	84	60	86	61	99	53	75	58	82	56	103	45	62
Na (µmol g^{-1}DW)	1190	960	1140	1050	1150	1160	1220	1140	1330	1320	1140	980	1210	1200	1170	1230	1640	1250
K (µmol g^{-1} DW)	690	600	740	610	780	680	810	780	720	660	880	720	670	610	610	460	450	460
Mg (µmol g^{-1} DW)	213	256	181	251	173	260	194	235	193	258	192	218	212	277	209	343	226	336
Ca (µmol g^{-1} DW)	230	242	207	180	139	186	125	140	206	261	125	126	131	227	166	187	191	222
Fe (µmol g^{-1} DW)	2.7	1.2	1.5	1.0	1.4	1.5	1.2	0.5	1.8	1.4	2.0	0.6	2.2	1.3	1.9	2.9	6.1	1.4
Mn (µmol g^{-1} DW)	2.6	4.1	4.1	4.4	2.7	5.0	3.5	2.8	2.3	2.7	3.7	4.0	2.7	5.7	3.3	4.8	1.4	3.6
Zn (µmol g^{-1} DW)	0.7	1.1	0.8	1.3	0.9	1.2	1.3	1.5	1.2	1.8	1.9	2.3	1.5	3.5	0.7	3.0	0.5	3.4
N_{tot}:K	1.6	2.9	1.9	2.0	1.6	1.8	1.9	2.0	1.8	2.4	2.2	3.3	3.4	4.0	4.8	6.3	6.3	6.0
N_{tot}:Mg	5.1	6.7	7.7	4.8	7.4	4.8	8.1	6.8	6.8	6.0	10.2	10.8	10.5	8.8	14.0	8.4	12.5	8.2
N_{tot}:P_{tot}	20	20	28	15	20	15	26	19	21	16	37	32	39	30	52	28	63	44

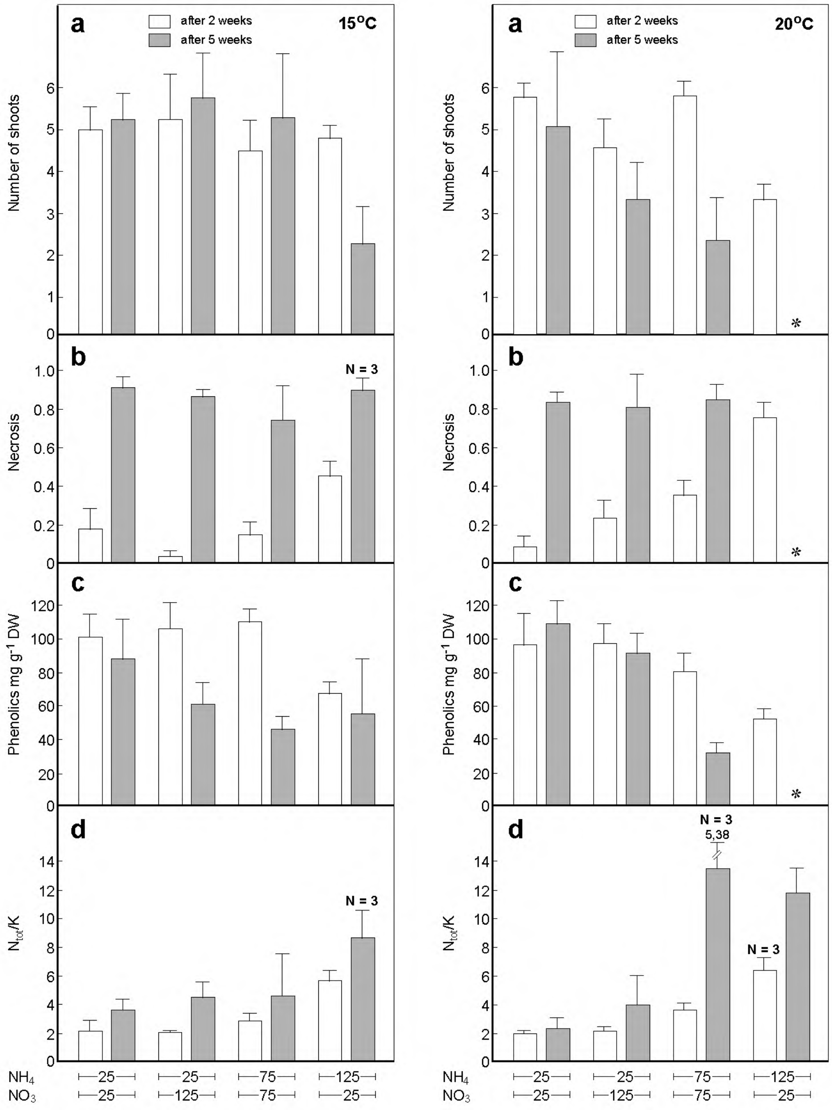

Fig. 6. Effect of N-treatments after 2 and 5 weeks on (a) number of shoots, (b) necrosis, (c) phenolic content, and (d) N_{tot}:K ratio of *Zostera marina* at 2 temperatures. NH_4 and NO_3 treatments are in µM. Averages (back-transformed) of the 2 sediment types are presented. *Not measured due to the limited number of plants that survived

Sediments

Sediment nutrient values at 2 and 5 weeks are shown in Table 3. Nitrogen and sulphate were found in lesser quantities in sandy sediment than in muddy sediment. Phosphorus and manganese were higher in the sandy sediment, probably due to its terrestrial origin.

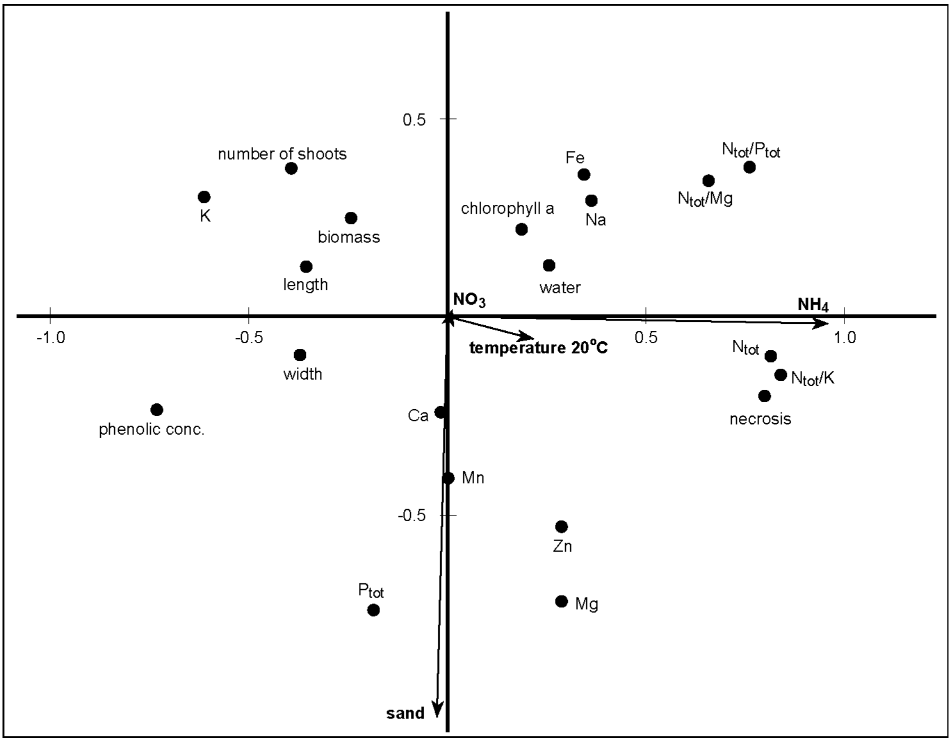

Fig. 7. Ordination diagram based on a redundancy analysis (RDA) displaying the effect of combined treatments of NO_3 (25, 50, 75 and 125 μM) and NH_4 (25, 75 and 125 μM), sediment type (sand vs. mud) and temperature (15°C vs. 20°C) on various plant parameters

Over the time course of the experiment, magnesium, calcium and sulphate concentrations rose considerably, indicating sulphide oxidation. Nitrate could not be measured due to the high sulphur concentration.

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The number of shoots increased over time in the 9 μM ammonium treatment (Fig. 4). In the other treatments, the number of shoots had decreased by ca 50 % after the acclimation period and the first 2 weeks of treatment. After 5 weeks, a further decrease was observed in the higher ammonium treatments, especially those at 20°C, in which the majority of plants died. No substantial change in shoot numbers occurred in the other treatments (Figs. 5 & 6).

High ammonium concentration in the water layer was toxic for *Zostera marina*. The plants became necrotic within 2 weeks, especially those at 20°C and on sand (Fig. 5). Necrosis was typified by brown-black discolouration. After 5 weeks, plants of all treatments were necrotic, except for those in the 9 μM ammonium treatment (Figs. 4 & 6). The plants were generally smaller (shorter, leaves narrowed, weight lost) after 5 weeks as compared to

Table 5. p-values of the ANOVA model for the effect of N-treatment, sediment type, temperature and interactions (see Table 4) on the characteristics and chemical composition of *Zostera marina* plants after 2 weeks. * $0.01 \le p \le 0.05$, ** $0.001 \le p < 0.01$, *** $p < 0.001$, all other effects were non-significant. There were no significant effects on width, biomass, wasting disease, chl *a*, Ca and Fe

	N-treat.	Sedim.	Temp.	N-treat. x Sedim.	N-treat. x Temp.	Sedim. x Temp.	N-treat. x Sedim. x Temp.
No. of shoots		**				*	
Length			*				
Necrosis	**	*	*				
Water content		*		**			*
Phenolic conc.	***	**	*				
N_{tot}	***					*	*
P_{tot}		***					
Na		*	*				
K	**	**	*				
Mg		***					
Mn		*					
Zn	*	**					
N_{tot}:K	***	*		*		**	**
N_{tot}:Mg	*	**		*			
N_{tot}:P_{tot}	**	***		*			

the first sampling date. This was more severely the case in the 125 µM ammonium 15°C treatment and the 75 µM ammonium 20°C treatment (Tables 4 to 7). In the lower ammonium treatments, on the other hand, temperature effect on plant size seemed to be the reverse, i.e. the plants were somewhat larger at 20°C (Table 6). Hence, the ammonium and temperature treatments showed an interactive effect on plant length (Table 7).

After 2 weeks, the nitrogen treatments had influenced the shoot tissue nitrogen, potassium, phenolics, zinc and the ratios of shoot tissue nitrogen to potassium, magnesium and phosphorus (Tables 4 & 5, Figs. 5 & 6). These nitrogen treatment effects can be attributed to the ammonium treatment, rather than the nitrate treatment (Fig. 7, Tukey's Comparison of Means). The nitrogen content in the leaves amounted to 3.5 % of the dry weight at the highest ammonium treatment (Table 4). After 5 weeks, most ions and nutrient concentrations measured in the shoot tissue were highly variable, which may be the result of tissue damage and/or physiological breakdown. The effects that occurred after 5 weeks were therefore less pronounced than the effects after 2 weeks (Table 7). However, clear effects of ammonium treatments after 5 weeks were still found with regard to plant number and size (smaller in high ammonium treatments), shoot tissue phenolics (lower in high ammonium treatments), and nitrogen and nitrogen:potassium ratios (higher in high ammonium treatments) (Tables 6 & 7, Fig. 6).

Table 6. *Zostera marina.* Characteristics and chemical composition of seagrass shoots under different N-treatments after 5 weeks. Means or back-transformed means (for arcsine- and log-transformed parameters) are presented

	25:25				25:50		25:125				75:75				125:25			
	15°C		20°C		15°C		15°C		20°C		15°C		20°C		15°C		20°C	
	Mud	Sand	Mud	Sand	Mud	Sand	Mud	Sand	Mud	Sand	Mud	Sand	Mud	Sand	Mud	Sand	Mud	Sand
No. of shoots	5.5	5.0	7.0	3.0	7.0	6.5	6.5	5.0	3.5	3.0	7.0	3.5	3.5	1.0	2.0	2.5	0[a]	0[a]
Length (cm)	35	28	36	35	33	26	38	28	43	29	33	33	19	12	14	14	-	-
Width (mm)	2.4	2.0	2.4	2.6	2.0	2.1	2.4	2.0	2.6	2.1	2.0	2.5	2.3	2.3	1.8	1.9	-	-
Biomass (mg)	46	31	54	58	50	42	38	32	58	29	49	51	22	25	15	27	-	-
Necrosis	0.88	0.93	0.77	0.87	0.83	0.85	0.81	0.90	0.87	0.66	0.53	0.90	0.79	0.85	0.94	0.80	-	-
Wasting disease	0.06	0.04	0.06	0.09	0.04	0.01	0.02	0.01	0.31	0.16	0.07	0.16	0.00	0.00	0.00	0.00	-	-
Water content (%)	86	86	85	82	84	84	87	87	88	88	87	87	88	90	91	88	-	-
Phenolics (mg g^{-1} DW)	89	86	117	100	88	114	58	62	79	103	47	43	36	27	19	90	-	-
N_{tot} (µmol g^{-1} DW)	1600	1520	1630	1510	1200	1900	1840	2450	690	1600	2050	1060	1970	1520	2480	2520	2000	870
Na (µmol g^{-1} DW)	1300	1500	1600	1200	1090	940	930	960	1370	1210	820	1320	1620	1760	1230	1100	1850	1040
K (µmol g^{-1} DW)	560	380	740	760	620	590	480	510	430	320	390	460	220	90	250	250	170	80
Mg (µmol g^{-1} DW)	458		223		99		117		390		417		388		292		466	205
N_{tot}:K	2.8	4.0	2.2	2.0	1.9	3.2	3.8	4.8	1.6	4.9	5.2	2.3	9.0	17.7	9.9	10.3	12.1	11.1
N_{tot}:Mg	3.5		7.3		12.1		15.7		1.8		4.9		5.1		8.5		4.3	4.2

[a] Only fragments of shoots were available

Table 7. p-values of the ANOVA model for the effect of N-treatment, sediment type, temperature and interactions (see Table 6) on characteristics and chemical composition of *Zostera marina* plants after 5 weeks. * $0.01 \leq p \leq 0.05$, ** $0.001 \leq p < 0.01$, all other effects were non-significant. There were no significant effects on width, biomass, necrosis, water content, Mg and N_{tot}:Mg (Mg measurements were mostly missing for sandy sediment: tested was the effect of N-treatment, temperature and N-treatment x temperature on muddy sediment)

	N-treat.	sediment	tempera-ture	N-treat. x sediment	N-treat. x temp	sediment. x temp.	N-treat. x sediment x temp.
No. of shoots	**	**	**				
Length	**	*			**		
Wasting disease					*		
Phenolic conc.	*	*		*			
N_{tot}			*				
Na						*	
K	**		*				
N_{tot}:K	*	**	*		*		**

Phenolic content was inversely correlated with shoot tissue nitrogen content, nitrogen ratios and necrosis (Fig. 7, $p < 0.001$). However, no correlation with wasting disease was found. Necrosis was further correlated with shoot tissue nitrogen content and the nitrogen ratios, and inversely correlated with number of shoots (Fig. 7, $p < 0.001$).

In treatments with sandy sediment, plants had fewer shoots, were more necrotic and had higher shoot tissue magnesium, zinc, phosphorus, and phenolic contents and lower shoot tissue potassium concentrations than plants in the muddy sediment (Tables 4 & 5, Figs. 5 & 7).

DISCUSSION

High ammonium concentration in the water layer was toxic for *Zostera marina*: the plants became necrotic within 2 weeks. After 5 weeks, plant size was much reduced, and a number of plants had died. At this time, plants in all treatments were necrotic, except for those in the 9 μM ammonium treatment (applied in a separate experiment), implying that the 25 μM ammonium treatment was also toxic.

Toxic effects of ammonium on *Zostera marina* have not been previously recorded. However, many indications of toxic effects can be found in the literature. For example, growth inhibition by high ammonium concentrations is suggested by the results of Dennison et al. (1987). Also, eelgrass plants used in an experiment by Borum et al. (1989) may have suffered from ammonium toxicity, as it was reported that all older leaves 'showed signs of progressing senescence' after 3 weeks of exposure to a water ammonium concentration of 50 μM (and additional pulses of 90 μM).

The brown-black discolouration of the *Zostera marina* leaves that we observed has also been reported for *Callitriche* spp., *Potamogeton* spp., *Elodea canadensis,* aquatic *Ranunculus* spp. and *Nymphaea caerulea* as a symptom of ammonium toxicity (Glänzer 1974, Grube 1974, Agami et al. 1976, Glänzer et al. 1977). Additionally, these investigators reported brown discolouration of the chloroplasts, which are the sites of ammonia assimilation in leaves (Marschner 1995), with necrosis starting at the leaf tips, and being more severe on older leaves than on younger ones. We also observed the latter symptom with *Z. marina*.

Generally, plants do not regulate nitrogen uptake as there is no feedback inhibition mechanism (Rabe 1990). *Zostera marina* roots appear to have a saturating ammonium level in the sediment, but at higher concentrations uptake begins again (Iizumi & Hattori 1982). This was also found to be the case in the roots of terrestrial plants, where a Michaelis-Menten type uptake system operates at low concentrations, and a linear system operates at higher ammonium concentrations (for review see Kronzucker et al. 1996). The leaves of terrestrial plants also take up ammonium (for reviews see Pearson & Stewart 1993, Fangmeier et al. 1994, Marschner 1995). The leaves of *Z. marina* rapidly take up ammonium, mostly in direct proportion to the ammonium concentration in the water column (Iizumi & Hattori 1982, Thursby & Harlin 1982, Short & McRoy 1984, Asmus 1986). However, low concentrations were applied in these studies: maxima of 20, 45, 12 and 10 µM, respectively. Our experiment shows increasing shoot tissue nitrogen concentration in *Z. marina* at increasing ambient ammonium levels, indicating non-saturating uptake to at least 125 µM ammonium (this was not found for increasing ambient nitrate concentrations). Ammonium uptake by the tropical seagrass *Thalassia hemprichii* showed a gradually saturating uptake curve in a study in which maximal supply was 140 µM ammonium (Stapel et al. 1996). In *Cerastophyllum demersum* (an aquatic plant without roots), no saturation occurs at concentrations as high as 2.4 mM, and biphasic uptake, as described for roots, seems to occur (Toetz 1973). This was also found to be the case for *Lemna gibba* (Ullrich et al. 1984).

The physiological mechanism of ammonium toxicity is thought to lie in the ability of ammonia to uncouple photosynthetic electron transport, which may lead to necrosis through inhibition of photosynthesis. A secondary toxic effect may result from an inability to buffer the protons released from ammonium assimilation, affecting the function of many enzymes and membrane processes (e.g. Pearson & Stewart 1993, Marschner 1995).

The ammonium continuously entering the cells must be rapidly assimilated to prevent physiological damage (e.g. Magalhäes & Huber 1989, Marschner 1995). This requires carbon skeletons, which may become limiting (e.g. Givan 1979, Magalhäes & Huber 1989, Flaig & Mohr 1991, Marschner 1995). Carbon skeletons are provided by photosynthesis, the rate of which is dependent on illumination. Low light availability has been shown to decrease carbohydrate stores in *Zostera marina* (Kraemer & Alberte 1995, Zimmerman et al. 1995a). Also, glutamine synthetase, which is a key enzyme in ammonium assimilation (e.g. Miflin & Lea 1976, Givan 1979, Magalhäes & Huber 1989), is stimulated upon illumination (Pregnall et al. 1987, Marschner 1995). As a result, low light availability will aggravate the effect of ammonium toxicity. In our experiment, illumination was presumed to be saturating as plants were collected at the end of autumn; they are able to adapt to low irradiance by decreasing their light saturation level (Zimmerman et al. 1995a). However, when light saturation is at a low level, the total amount of carbon fixed is lower than that fixed at a high saturation level. We would therefore expect that ammonium toxicity effects will be less severe in spring and summer.

Another aggravating factor is the relatively high temperature in the experiment (15 and 20°C), which would have caused enhanced metabolic activity, i.e. ammonium uptake (e.g. Lycklama 1963, Toetz 1973, Wang et al. 1993), and to a lesser extent carbon-consuming respiration (Drew 1979, Marsh et al. 1986, Zimmerman et al. 1989). It is not surprising, therefore, that we find a stronger toxic effect at 20°C than at 15°C.

Why are eelgrass leaves so susceptible to ammonium in the water layer when, in contrast, the roots are exposed to relatively high concentrations [usually between 10 and 300 µM ammonium (Harlin & Thorne-Miller 1981, Iizumi et al. 1982, Kenworthy et al. 1982, Dennison et al. 1987, Williams & Ruckelshaus 1993, Hemminga et al. 1994, van Lent & Verschuure 1994a) and occasionally even above 1000 µM ammonium (Short 1983b, Pedersen & Borum 1993)]? Firstly, leaves take up ammonium at a higher rate than roots (Thursby & Harlin 1982, Short & McRoy 1984, Pedersen & Borum 1992, Hemminga et al. 1994). Secondly, the pH of the water varies, but is ca 8.2 on average (Stumm & Morgan 1981), whereas the pH in typical marine sediments is around 7.5 (Berner 1980). A higher pH may result in an increased uptake rate of ammonium, as was found for *Ceratophyllum demersum* and *Lemna gibba* (Toetz 1973, Ullrich et al. 1984), probably owing to an increase in the proportion of the molecular species (NH_3) (Marschner 1995). It should be noted that, in most studies of terrestrial plants, no such relationship between pH and uptake rate could be established (for review see Wang et al. 1993). Thirdly, eelgrass is known to aerate its rhizosphere to a small extent, thereby, through nitrification, very locally decreasing the ammonium concentration (Iizumi et al. 1980, Smith et al. 1984).

Phenolic content was inversely correlated with shoot tissue nitrogen concentration, which is in agreement with results of Buchsbaum et al. (1990) and L.H.T. Vergeer (unpublished results). Contrary to their findings, however, no correlation with wasting disease was found. Necrosis, on the other hand, did show a significant correlation with phenolic content. However, the rapidity of plant deterioration argues against any causality: the plants were probably injured before any effect of decreased phenolic content had time to manifest in the plants.

The shoot tissue nitrogen content in *Zostera marina* leaves increased to 3.5 % of the dry weight at the highest ammonium treatment (125 µM). Possibly, at these values free ammonium begins to accumulate and protein breakdown increases. In natural habitats, nitrogen content of *Z. marina* lies between 1 and 3 % nitrogen during the growing season (Thayer et al. 1977, Harlin & Thorne-Miller 1981, Kenworthy & Thayer 1984, Short 1987, Pellikaan & Nienhuis 1988, Pedersen & Borum 1993, van Lent & Verschuure 1994a). Shoot tissue nitrogen:potassium ratio increased at high ammonium treatments and was highly correlated with necrosis, as is often observed in terrestrial plants (e.g. Marschner 1995), as well as in the aquatic *Stratiotes aloides* (Smolders et al. 1996). Interestingly, the increase of the shoot tissue nitrogen:potassium ratio at high ammonium treatments resulted not only from an increase in shoot tissue nitrogen concentration, but also from a significant ($p < 0.01$) decrease in shoot tissue potassium concentration, despite the high availability of potassium in the surrounding sea water. Shoot tissue magnesium concentrations were not influenced by the nitrogen treatments, but showed a remarkable correlation with necrosis and sediment type. Generally, the altered ion composition in the plants probably does not cause necrosis, but merely accompanies it.

Unlike the studies of Burkholder et al. (1992, 1994), no effects of nitrate on plant vitality or any other plant parameter were found in this study. Nitrate uptake by roots, and probably also by leaves, is inhibited by the presence of ammonium in the water (Iizumi & Hattori 1982, Zimmerman et al. 1987). Possibly the 25 µM ammonium present in the nitrate treatments inhibited nitrate uptake, and so suppressed any nitrate effects.

Compared to muddy sediment, plants in sandy sediment showed higher mortality, were more necrotic, and had higher shoot tissue magnesium, zinc, phosphorus, and phenolic contents and lower shoot tissue potassium concentrations. The tissue phosphorus and potassium effects corresponded to the differences in sediment properties. Zinc concentration was not measured in the sediment. The magnesium concentration was equal in both sediment types, leaving the strongly increased shoot tissue magnesium concentration on sand an interesting but unexplained phenomenon.

We did not expect that sandy sediment would have a negative influence on plant vitality under ammonium stress, because the lower ammonium concentration and higher phosphate concentration in sand were thought to give a better nutrient balance. Also, the expectedly more aerobic condition of sand was thought to be advantageous for the plants, as less energy would be needed for aeration of the root/rhizomes. A possible explanation may be that photosynthesis is carbon limited. Eelgrass is known to increase photosynthesis with an extra supply of carbon dioxide (Madsen et al. 1993, Zimmerman et al. 1995b), which demonstrates that carbon limitation is not an uncommon phenomenon in *Zostera marina*. When carbon is limiting, muddy sediment may favour the plant in 2 ways: Firstly, muddy, organic sediment contains higher inorganic carbon levels than sandy sediment (e.g. Valiela 1984). Inorganic carbon can be taken up by the roots of *Z. marina* and transported to the leaves through the lacunal gas spaces (Wetzel & Penhale 1979, Penhale & Thayer 1980, Penhale & Wetzel 1983), thereby providing an extra carbon source, analogous with the isoetid plants (e.g. Søndergaard & Sand-Jensen 1979, Roelofs et al. 1984, Madsen et al. 1993). Secondly, plants grown on the anaerobic muddy sediment generally possess larger lacunal gas spaces (Penhale & Wetzel 1983) and, therefore, a larger capacity for carbon dioxide storage and recycling of respiratory-CO_2 than do plants grown on sand. The importance of C-skeletons for the handling of excess ammonia has been outlined above.

CONCLUSIONS AND ECOLOGICAL IMPLICATIONS

Ammonium was shown to be toxic to *Zostera marina* at an applied concentration of 125 µM and probably also at 25 µM ammonium. At 9 µM ammonium, no toxic effects were found. The effect was stronger at a higher temperature (20°C as compared to 15°C) and on sand (as compared to mud). It was argued that the toxicity will increase with lower irradiance. Toxic effects of ammonium will therefore be felt primarily in autumn.

In eutrophicated areas, this coincides with rising ambient nutrient concentrations due to decreased algal growth. It is expected that this will culminate in intensification of the normal end-of-season decline of *Z. marina* populations.

Earlier ammonium increase may cause a premature die-off, as was observed year after year in the small annual *Zostera marina* population of the western Wadden Sea (D.C.R. Hermus personal communication, van Katwijk personal observations). At this location, ammonium usually increases in August/September, in contrast to other eelgrass habitats (e.g. eastern Wadden Sea and southwestern Netherlands), where ammonium increase begins in October (Helder 1974, Asmus 1986, van Lent & Verschuure 1994a, unpublished data 1980-1990 from the Dutch Ministry of Transport, Public Works and Water Management).

High ammonium concentrations may prevent an annual population from adopting a perennial reproduction strategy. For example, in the Netherlands, 2 non-tidal brackish water lakes, Lake Veere (eutrophic, average ammonium concentration in summer below 5 µM, but in autumn increasing towards 23 µM) and Lake Grevelingen (oligotrophic, average ammonium concentration during autumn increasing from 3 µM to 11 µM), are inhabited by an annual and perennial eelgrass population, respectively [van Lent & Verschuure 1994b, ammonium data (unpublished): 1980-1990 from the Dutch Ministry of Transport, Public Works and Water Management]. Formerly, both lakes were estuaries inhabited by an annual eelgrass population (Beeftink 1965, personal communication C. den Hartog). In Lake Grevingen, the population developed a perennial strategy after its habitat became submerged. In Lake Veere this may have been prevented by recurrent ammonium intoxication in autumn.

Generally, ammonium toxicity may be one of the underlying causes of the disappearance of many *Zostera marina* populations in eutrophicated coastal seas throughout the northern hemisphere. It may prevent eelgrass recovery and hamper revegetation efforts, especially at greater depth and in the case of perennial plants. The importance of sufficient carbon reserves for transplantation success has already been pointed out by Zimmerman et al. (1995a). In eutrophicated areas, this will become even more important due to (and may be partly explained by) the ammonium susceptibility of *Z. marina* as shown in this study. As seagrass species are closely related (Larkum & den Hartog 1989), it is likely that other seagrasses are also susceptible to ammonium toxicity. It would therefore be worthwhile to estimate ammonium toxicity in other seagrasses so as to gain a better understanding of global seagrass decline.

Acknowledgements. We thank G.M. Verheggen-Kleinheerenbrink and J. Eygensteyn for their assistance with the analysis. D.J. de Jong and A. van der Pluijm (Rijks Instituut voor Kust en Zee, Rijkswaterstaat, Middelburg) are acknowledged for providing data on ammonium and nitrate in Dutch coastal waters. We are much indebted to Ir. Th.M. de Boo of the Department of Statistical Consultation and Dr. C.J.F. ter Braak of the DLO Agricultural Mathematics Group Wageningen, who kindly advised us on statistics. We thank B. Kelleher for critically reading the manuscript and correcting the English. J. van Westreenen (Illustration Department of the Faculty of Science) is thanked for drawing the figures.

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CHAPTER 4

EFFECTS OF SALINITY AND NUTRIENT LOAD AND THEIR INTERACTION ON *ZOSTERA MARINA*

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Mar Ecol Prog Ser 190:155-165

EFFECTS OF SALINITY AND NUTRIENT LOAD AND THEIR INTERACTION ON *ZOSTERA MARINA*

ABSTRACT: Generally, seagrass *Zostera marina* L. distribution in the Wadden Sea and south-west Netherlands is limited to waters with low to moderate nutrient concentrations. However, it is known that *Z. marina* also occurs at high nutrient concentrations when growing in low salinity environments. In this study, we investigated the separate and interactive effects of nutrients and salinity on *Z. marina* plants in a 5 week experiment. Two populations were tested; one originating from a relatively marine habitat and the other from an estuarine habitat. Supplied salinities were 23, 26 and 30 PSU, and supplied water nutrient levels were nitrate:ammonium:phosphate, 1:3:2, 3:9:4.5 and 60:9:9 (μM at a refreshment rate of 1 day^{-1}, corresponding with a load of 20, 95 and 625 kg N ha^{-1} yr^{-1}. *Z. marina* was negatively influenced by high salinity. The estuarine plants showed a decreased 'vitality' (calculated from 6 plant response parameters), whereas the marine plants showed a lesser number of shoots at high salinity. The negative effect acted on the estuarine plants at 26 and at 30 PSU, and on the marine plants at 30 PSU. At these high salinities, a high nutrient load had no detectable effect on the marine plants, whereas the estuarine plants were negatively influenced by high nutrient loads. At low salinity levels, i.e. marine plants at 23 and 26 PSU and estuarine plants at 23 PSU, plants from both populations were positively influenced by higher nutrient loads. It is argued that these results may explain the distribution and decline of *Z. marina* in many areas of the northern hemisphere. Examples from both sides of the Atlantic Ocean are presented.

INTRODUCTION

Deterioration of the seagrass *Zostera marina* L. has frequently been attributed to increased nutrient loading (e.g. Boynton et al. 1996, Short & Burdick 1996). *Z. marina* is vulnerable to high nutrient concentrations in the water, either indirectly through algal blooms reducing light intensity (Neckles et al. 1993, Williams & Ruckelshaus 1993, Harlin 1995, Short et al. 1995, Taylor et al. 1995) or directly via the adverse effects of either nitrate (Burkholder et al. 1992, 1994) or ammonium (van Katwijk et al. 1997).

Zostera marina occurs in waters with salinities ranging between 5 and 42 PSU (Tutin 1938, Luther 1951), and seagrasses are adapted to cope with high salinity both physiologically and anatomically (e.g. Jagels 1983, Tyerman 1989, Arai et al. 1991, Pak et al. 1995, Fukuhara et al. 1996). However, few records exist of the effect of salinity on *Zostera* species. Pinnerup (1980) found a positive correlation between salinity and eelgrass productivity when investigating 3 Danish eelgrass beds situated at salinities between 13 and 31 PSU, while Wium-Andersen & Borum (1984) found no effect of seasonal salinity variations on the annual life cycle of an eelgrass bed ranging in salinity from 9 to 23 PSU. Productivity of another *Zostera* species, *Z. capensis* Setchell, was negatively correlated with salinity in the range of 15 to 75 PSU (Adams & Bate 1994). Photosynthesis of *Z. japonica* Aschers. & Graebn. (sub nomine *Z. nana*) was optimal at 25 PSU (Ogata & Matsui 1965). Wasting disease, which destroyed many eelgrass populations on both sides of the Atlantic Ocean during the 1930s (review in den Hartog

Fig. 1. Map showing locations of *Zostera marina* L. populations of interest. T: Terschelling, E: Eems

1996), did not occur in low salinity areas, which was generally attributed to the salinity optimum of the presumed disease-causing slime mold *Labyrinthula*: 22 to 40 PSU (Young 1943, Pokorny 1967, Rasmussen 1977). There is no consensus whether *Labyrinthula* was a causing agent, or merely a stress symptom in this large-scale decline (review in den Hartog 1996). *Labyrinthula* is present in most eelgrass beds (Vergeer & den Hartog 1994)

In The Netherlands, with its variety of marine (ca. 30 PSU) and estuarine (15 to 25 PSU) environments, we observed that the distribution of *Zostera marina* in marine environments was limited to waters with low to moderate nutrient concentrations, viz. in summer, monthly median values varied between 0-4 µM NO_3, 1-8 µM NH_4, 2-10 µM P_{tot} and in winter between 15-55 µM NO_3, 7-11 µM NH_4, 3-8 µM P_{tot}. Surprisingly however, *Z. marina* was observed to flourish in estuarine environments with relatively high nutrient concentrations, viz. in summer, monthly median values varied between 0-90 µM NO_3, 2-11 µM NH_4, 7-25 µM P_{tot} and in winter between 50-260 µM NO_3, 15-55 µM NH_4, 8-20 µM P_{tot} (Ministry of Transport Water Management and Public Works unpublished data). Furthermore, in some marine environments, seagrass distribution shifted towards areas with some freshwater influence (Burdick et al. 1993, D.J. de Jong personal communication). Finally, large scale *Z. marina* disappearance was recorded in Lake Grevelingen, coinciding with a salinity increase (Wijgergangs 1994, Nienhuis et al. 1996). From this, we hypothesised that (1) a relatively low salinity is favourable for *Z. marina*, (2) nutrient availability and salinity have an interactive effect on *Z. marina*, the plants being able to tolerate higher nutrient concentrations at low salinity, but not at high salinity. Also, we were interested in knowing whether marine and estuarine populations exhibited a differential response when exposed to various salinity and nutrient treatments, as *Z. marina* populations are known to differ in habitat adaptation traits (e.g. Biebl & McRoy 1971, van Katwijk et al. 1998).

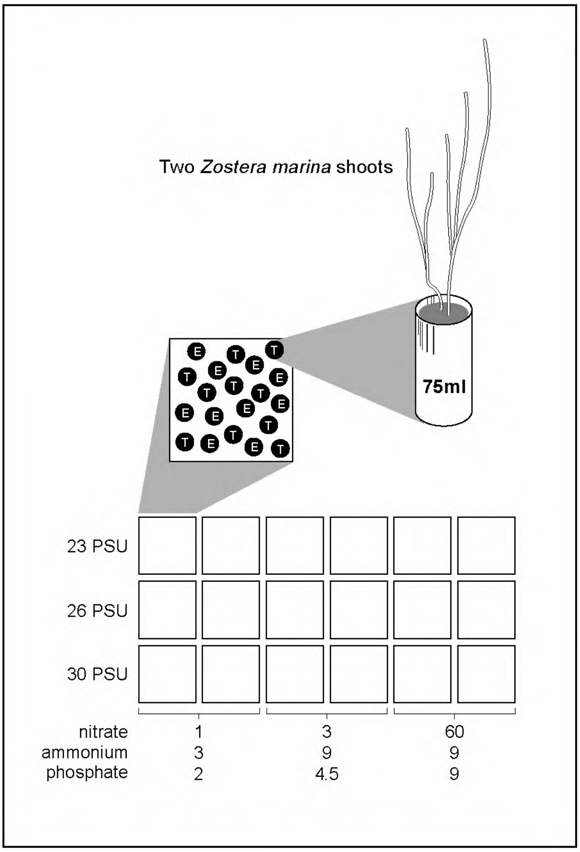

Fig. 2. Scheme of the experimental set-up. In the actual set-up, the glass containers were randomly placed. Zostera marina plants originating from Terschelling (T) and Eems (E). Supplied nutrient concentrations in μM

In this study, the combined effect of salinity and nutrient load on *Zostera marina* survival was examined in plants originating from a marine and an estuarine habitat. Supplied salinity and nutrient levels ranged between late summer levels in a marine and an estuarine situation, in a relatively undisturbed and eutrophicated system. Late summer values were chosen, because (1) it was observed that Dutch Wadden Sea plants died prematurely in late summer, so late summer conditions seemed to be important for the condition of eelgrass, and (2) adverse effects of high nutrient loads were expected to be strongest when irradiance decreased and temperature was still high, as this results in a lower carbon fixation rate. Carbon is required to assimilate ammonium, which continuously enters the cells (Marschner 1995, van Katwijk et al. 1997).

MATERIALS AND METHODS

Zostera marina plants originating from 2 intertidal populations of a relatively marine (Terschelling) habitat and an estuarine (Eems) habitat, both located in the Dutch Wadden Sea (Fig. 1), were subjected in a laboratory set-up (Fig. 2) to salinities of 23 PSU (21.5

to 24.0), 26 PSU (24.0 to 27.0) and 30 PSU (27.0 to 31.5), respectively, at three nutrient levels in the culture medium, nitrate:ammonium:phosphate, viz. 1:3:2, 3:9:4.5 and 60:9:9 µM, which was supplied to glass containers at a refreshment rate of once per day, resulting in loads of ca 20, 95 and 625 kg N ha^{-1} yr^{-1}, and 20, 45 and 100 kg P ha^{-1} yr^{-1}, respectively (see 'Results'). Salinities applied ranged between the median summer level in a marine and an estuarine environment. The nutrient levels in the culture medium corresponded to the median late summer levels in the channels of the relatively undisturbed northern German Wadden Sea, the more eutrophicated Dutch Wadden Sea and the eutrophic Eems Estuary, respectively (R.M. Asmus personal communication, Dutch Ministry of Transport, Public Works and Water Management unpublished data).

Collection and culture conditions. *Zostera marina* plants were collected in Terschelling (11 August 1993) and Eems (10 August 1993). The plants, all having a growing apex and a rhizoom of ca 5 cm length, were maintained overnight at 16°C. Wasting disease-like lesions were present in the leaves. The following day, pairs of plants were placed in 75 ml jars filled with sieved (mesh 0.5 cm), muddy sand originating from Eems. A thin layer of sand was placed over the sediment to prevent nutrient exchange with the overlying water. Twenty jars per container (10 with Terschelling and 10 with Eems plants) were placed in 18 glass containers of 15 l, and were allowed to acclimate for 3 weeks in synthetic seawater (23, 26 and 30 PSU, Wimex, of Wiegandt GmbH, Krefeld, Germany). From 3 September 1993, seawater composed of a self-prepared salt mix, derived from uncontaminated 'pro analysi' salts (Merck, Darmstadt, Germany) composition according to Pytkowicz et al. (1977) was used, to avoid nitrogen contamination found in all synthetic sea salt mixes that we tested (van Katwijk et al. 1997). Ammonium, nitrate and phosphate were added as potassium or chloride salts. The culture medium was continuously refreshed from stock containers using masterflex multichannel peristaltic pumps. Water in the containers was gently aerated to ensure complete mixing. For technical details of the set-up, see Roelofs et al. (1984). The plants were maintained at 17°C under an 8 h dark:16 h light cycle; light intensity just below the water surface averaged at 95 µE m^{-2} s^{-1} (measured with a LiCor LI-185a with quantum sensor), which is saturating for *Z. marina* at this temperature (Marsh et al. 1986). Macroalgae and epiphytes were carefully removed daily or once in 2 days, as we were interested in the effects of salinity and nutrients on *Z. marina* plants only. Simultaneously, detached leaves were removed, so hardly any leaf debris developed.

Sampling. Prior to the experiment, a sample of *Zostera marina* plants was taken from each population. Two and 5 weeks after the treatments commenced, 10 *Z. marina* plants (5 jars) of each population were sampled from each glass container, to measure the number of shoots, number of missing leaves, leaf length, width, dry weight, wasting disease-like lesions, necrosis, chlorophyll *a* (chl *a*), % N and % P in the aboveground parts. The sediments were sampled prior to the experiment, and after 2 and 5 weeks, to measure water-extractable NH_4 and total P. The sediments of the 5 jars were mixed and stored at 4°C. Water in the glass containers was sampled 8 times between 9 September and 5 October, to measure NH_4, NO_3, PO_4 and Cl concentrations. The samples were filtered and stored at –20°C for a maximum of 4 months.

Plant analysis. When there were 3 or more leaves, we supposed that there were no missing leaves; 2 leaves present indicated 1 leaf was missing and 1 leaf present indicated 2 missing leaves. Leaf scores for wasting disease-like lesions and necrosis were based on the percentage of total leaf surface, estimated for each of the first 3 leaves separately. The leaf scores were averaged to calculate shoot values, whereby damage on young leaves was given a higher weight than damage on older leaves (the method of calculation as described in van Katwijk et al. 1997). A distinction was made between discoloured leaf surface and infected leaf surface, the former being used to assess necrosis (including wasting disease-like lesions), the latter to assess only wasting disease-like lesions. Three plants were freeze-dried over 24 to 48 h to determine dry weight.

To obtain a more complete and general description of the plant response per container, we calculated 'vitality':

$$\text{vitality} = \text{number of shoots} + \text{size} - \text{necrosis} - \text{number of missing leaves}$$

in which size was the average between leaf length, width and total biomass of the plants. All parameters were standardised to mean 2 (to avoid negative values) and unit variance prior to the calculation (Jongman et al. 1995). The parameters were added rather than multiplied, because of their additive nature: they had a normal distribution (Slob 1987). A large number of missing leaves was considered to be a negative indication of plant vitality. Young shoots were not measured until they had roots of their own. Generally, they possessed 3 leaves at this stage.

Chl *a* was measured in leaf segments of 3 to 5 cm length which were taken from 3 cm below the apex of the first fully grown leaf. Epiphytes were removed by hand; however, this was seldom necessary. The segments of 2 shoots per glass container were pooled. The segments were blotted dry, weighed, ground in 80 % ethanol and centrifuged. Chl *a* content in the supernatant was measured spectrophotometrically. Ca 2 ml extractant per 10 mg (fresh weight) of leaf material was used. The acidification method was used to correct for phaeophytin (Moed & Hallegraeff 1978). Calculations of chl *a* were performed according to Roijackers (1981).

% N was measured in duplo, in 4 mg of freeze-dried and ground plant material, using a Nitrogen, Carbon, Sulphur Analyzer (Carlo Erba Instruments NA 1500). Two or 3 shoots were used to analyse total P contents of the aboveground parts. Epiphytes were scraped off. Ca 100 g ashed plant material (550°C, 4 h) was digested with 1 ml aqua regia (HNO_3 and HCl, 65 and 35 % vol., respectively, diluted 1:2 with double-distilled water). When the material was not completely digested, the sample was evaporated and another ml of aqua regia was added. This process was repeated 3 times maximum. The volume of the digest was brought to 50 ml with double-distilled water. Total P was measured with an Inductively Coupled Plasma (ICP) spectrophotometer, type IL Plasma 200.

Sediment analysis. Seventy to 100 g of fresh sediment were placed in a 500 ml polyethylene bottle with 200 ml of double-distilled water and shaken for 1 h. This mixture was centrifuged for 20 min at 11 000 rpm (maximum intrinsic rate of natural change, r_{max} = 19 690 x *g*) and the supernatant was stored at –20°C for max. 4 months. NH_4 content of the supernatant was measured colourimetrically with a Technicon AAII system according to Kempers & Zweers (1986). Total P was measured with an ICP spectrophotometer, type IL Plasma 200.

Water analysis. NH_4, NO_3, PO_4 and Cl content of the water samples were measured colourimetrically with a Technicon AAII system according to Kempers & Zweers (1986), Grasshoff et al. (1983), Henriksen (1965) and O'Brien (1962), respectively. Salinity was calculated from Cl according to Stumm & Morgan (1981). Nutrient loads were calculated by subtracting the output concentrations (as measured in the glass containers) from the input concentrations in the supply containers, and subsequently converted to ha^{-1} and yr^{-1} reckoning with a refreshment rate of 1 day^{-1} and height of the overlying water of 0.2 m.

Statistical analysis. All parameters were statistically analysed per glass container (number of missing leaves, leaf length, width, biomass, wasting disease-like lesions and necrosis of the shoots were averaged per glass container prior to statistical analysis). Plant parameters were normally distributed, except for total P, which was lognormally distributed. Sediment and water parameters were lognormally distributed. The means or geometric means were used as a central measure. As a measure of variance, standard error of the mean was used. For lognormal parameters, the standard error of the mean was calculated according to Mood et al. (1974).

Analysis of Variance (ANOVA) was used in this split-plot experiment (population of origin being a subplot factor, the glass containers being the experimental unit), whereby salinity, nutrient treatment and their interaction were tested with the following error-term: salinity x nutrient treatment x replicate + salinity x replicate + nutrient treatment x replicate, and the population and the interactions of population with the other parameters were tested against the residual error (Steel & Torrie 1980, Freund & Littell 1985). For comparison of means, Tukey's test was used. The ANOVA and Tukey's test were carried out using the Statistical Analysis System, procedure GLM (SAS 1989).

RESULTS

Sediment and water

The sediment NH_4 and P_{tot} were not influenced by the nutrient treatment (ANOVA, $p > 0.05$). Over the time course of the experiment, NH_4 and P_{tot} concentrations in the sediment decreased (Table 1). NO_3 and PO_4 concentrations in the overlying water in the glass containers were positively influenced by nutrient treatment (ANOVA, $p < 0.001$ on almost all dates of measurements), whereas water NH_4 concentrations showed no correlation. The nutrient concentrations in the glass containers were lower than in the supply medium containers (Table 2).

Zostera marina

The nutrient treatments positively influenced aboveground tissue nutrient contents of the *Zostera marina* plants after 5 weeks (Figs. 3 & 4, Table 3). Aboveground tissue nitrogen content of the highest nutrient treatment was significantly higher than in shoots of the medium treatment, which, in turn, had higher nitrogen content than in the lowest

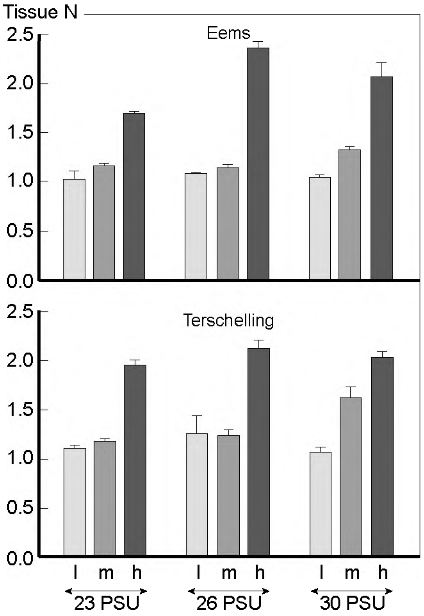

Fig. 3. % N in aboveground tissue of Zostera marina after 5 weeks at different combinations of salinity and nutrient loads. l: low, m: medium, h: high nutrient load. Means and SEM of 2 replicates are presented

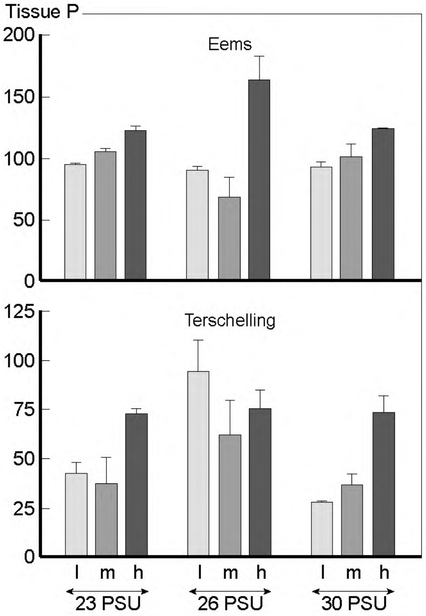

Fig. 4. P_{tot} (μmol g^{-1} dry wt) in aboveground tissue of Zostera marina after 5 weeks at different combinations of salinity and nutrient loads. l: low, m: medium, h: high nutrient load. Geometric means and SEM of 2 replicates are presented

nutrient treatment ($p < 0.05$, Tukey's test for comparison of means). Furthermore, the tissue nitrogen content at median nutrient treatment was higher at the highest salinity than at both lower salinities (Fig. 3), which is reflected in a significant effect of salinity and an interaction effect (Table 3). This effect was not present after 2 weeks. Aboveground tissue phosphorus content of the intermediate salinity treatment was higher than in the low and high salinity treatments. In the Eems plants, tissue phosphorus was higher than in the Terschelling plants (Fig. 4, Table 3), which was already the case prior to the experiment.

Table 1. Sediment water-extractable nutrient concentrations (μmol kg^{-1} dry wt) at the onset of the experiment, after 2 weeks and after 5 weeks. Geometric means (SEM) of all treatments are presented

	Pre-experiment	2 weeks	5 weeks
NH_4	400 (20)	580 (50)	210 (16)
P_{tot}	89 (0)	31 (2)	29 (4)

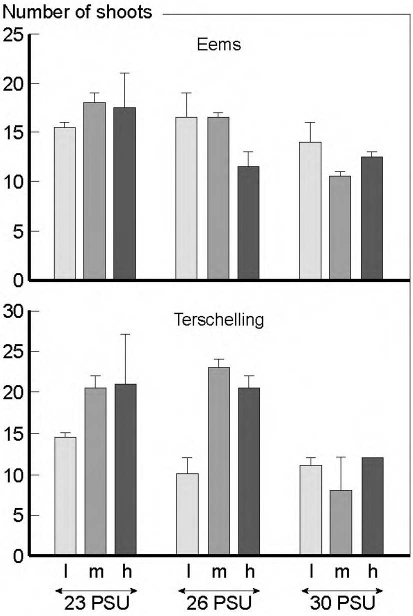

Fig. 5. Number of *Zostera marina* shoots after 5 weeks at different combinations of salinity and nutrient loads. l: low, m: medium, h: high nutrient load. Means and SEM of 2 replicates are presented

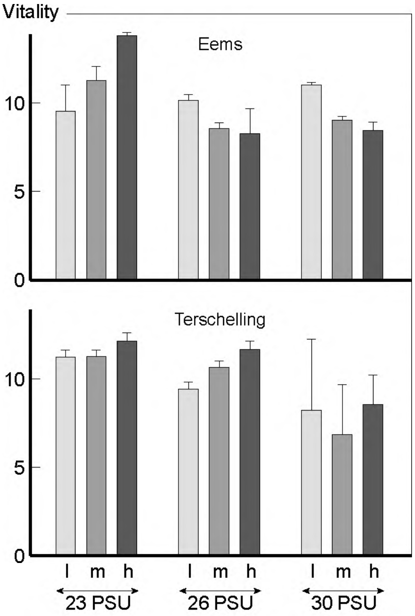

Fig. 6. Vitality of *Zostera marina,* a combined factor ('Materials and methods'), after 5 weeks at different combinations of salinity and nutrient loads. l: low, m: medium, h: high nutrient load. Means and SEM of 2 replicates are presented

Salinity had a negative effect on the number of *Zostera marina* shoots after 5 weeks of treatment (Fig. 5, Table 3). The marine and estuarine populations responded differently to the salinity and nutrient treatments. The marine Terschelling plants

Table 2. Water nutrient concentrations and loads. Geometric means (SEM) of the nutrient concentrations (µmol l^{-1}) of 8 sampling dates are presented per nutrient treatment. Nutrient loads (kg ha^{-1} yr^{-1}) are presented in italics, for calculation see 'Materials and methods'

	Low	Medium	High
NH_4	1.6 (0.2) *15*	2.0 (0.4) *70*	1.8 (0.4) *75*
NO_3	0.4 (0.1) *6*	0.4 (0.1) *25*	6.3 (3.2) *550*
PO_4	1.1(0.1) *20*	2.5 (0.1) *45*	4.5 (0.4) *100*

Table 3. Split-plot ANOVA for effects of nutrient and salinity treatments, and population of origin on *Zostera marina* plants. Ntr: nutrient, Sal: salinity, Pop: population. *$0.01 \le p \le 0.05$, **$0.001 \le p < 0.01$, *** $p < 0.001$, ns: $p > 0.05$

Treatment	MS	*F*	df	p	Sign
%N in aboveground tissue					
Ntr	3.012	448.8	2	< 0.001	***
Sal	0.125	18.7	2	0.001	**
Pop	0.032	2.81	1	0.13	ns
Ntr x Sal	0.090	13.4	4	0.001	**
Ntr x Pop	0.017	1.46	2	0.28	ns
Sal x Pop	0.009	0.79	2	0.49	ns
Ntr x Sal x Pop	0.037	3.28	4	0.06	ns
P_{tot} in aboveground tissue					
Ntr	0.768	29.17	2	< 0.001	***
Sal	0.232	8.83	2	0.009	**
Pop	3.715	82.0	1	< 0.001	***
Ntr x Sal	0.083	3.13	4	0.79	ns
Ntr x Pop	0.009	0.19	2	0.83	ns
Sal x Pop	0.344	7.58	2	0.02	*
Ntr x Sal x Pop	0.187	4.13	4	0.04	*
Number of shoots					
Ntr	22.75	5.13	2	0.04	*
Sal	139.00	31.3	2	< 0.001	***
Pop	7.11	0.45	1	0.52	ns
Ntr x Sal	28.50	6.42	4	0.01	*
Ntr x Pop	45.86	2.90	2	0.11	ns
Sal x Pop	20.11	1.27	2	0.32	ns
Ntr x Sal x Pop	15.36	0.97	4	0.46	ns
Vitality (see 'Materials and methods')					
Ntr	2.31	0.47	2	0.64	ns
Sal	24.87	5.06	2	0.04	*
Pop	0.003	0.00	1	0.98	ns
Ntr x Sal	3.80	0.77	4	0.57	ns
Ntr x Pop	1.12	0.35	2	0.71	ns
Sal x Pop	7.82	2.45	2	0.14	ns
Ntr x Sal x Pop	4.24	1.33	4	0.33	ns
Chl *a*					
Ntr	32096	42.92	2	< 0.001	***
Sal	1658	2.22	2	0.17	ns
Pop	1602	1.54	1	0.25	ns
Ntr x Sal	1558	2.08	4	0.18	ns
Ntr x Pop	319	0.31	2	0.74	ns
Sal x Pop	166	0.16	2	0.85	ns
Ntr x Sal x Pop	798	0.77	4	0.57	ns

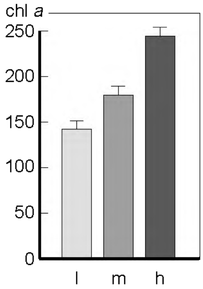

Fig. 7. Chlorophyll a concentrations (µg g^{-1} fresh wt) in *Zostera marina* leaves in 3 nutrient treatments. Means and SEM of replicates, salinity treatments and population of origin are presented

responded merely by differences in the number of shoots. The highest salinity tested had a negative effect on the number of shoots. At this high salinity, nutrients had no effects (ANOVA, $p > 0.1$, $N = 2$). In the 2 lower salinity treatments, nutrients had a positive effect (ANOVA, $p < 0.01$, $N = 4$) on the number of shoots (Fig. 5). The estuarine Eems plants responded to the treatments by differences in 'vitality' (see 'Materials and methods', Fig. 6). The two higher salinity treatments had a negative effect on vitality. In these treatments, nutrients had a negative effect (ANOVA, $p < 0.01$, $N = 4$). In the low salinity treatment, the Eems population tended to respond positively to nutrients (ANOVA, $p = 0.1$, $N = 2$). This complex response of vitality to the treatments resulted in hardly any significant treatment effects when testing the overall effects in the split-plot ANOVA (Table 3).

Chl *a* was highest at the highest nutrient treatment in both Eems and Terschelling shoots (Fig. 7, Table 3).

DISCUSSION

In the sediment, water-extractable ammonium and total phosphorus decreased during the experiment, probably due to plant uptake. Phosphate decrease in the sediment may also have been caused by precipitation of iron phosphate due to oxygenation of the rhizosphere by *Zostera marina* roots. Nutrient uptake caused the nutrient levels in the water to drop below the levels that were continuously supplied from the culture medium, although the replenishment rate of the glass containers was high. This has no effect on the nitrogen loads. In this experiment the low, medium and high nutrient treatments corresponded with 20, 95 and 625 kg N ha^{-1} yr^{-1}. In comparison, the N-load in the Dutch and German coastal zone of the North Sea, including the Wadden Sea, is estimated to be 340 kg N ha^{-1} yr^{-1} on average (Höpner 1991). In 10 lagoons and estuaries along the eastern coast of the United States N-loads were 24, 41, 64, 65, 157, 175, 310, 397, 520 and 624 kg N ha^{-1} yr^{-1}; *Z. marina* occurred only at the sites with 24, 41 and 64 kg N ha^{-1} yr^{-1} (Boynton et al. 1996, McClelland & Valiela 1998). Nutrient loads may have larger impacts on seagrass than nutrient concentrations (Tomasko et al. 1996).

After 5 weeks, tissue nitrogen contents showed an interaction effect as a consequence of the salinity treatment. At high salinity, plant biomass per container was lower, leaving more ammonium in the water layer, resulting in higher tissue nitrogen contents. This artefact had only started to develop at the end of the experiment, and showed no correlation with the interaction effect of nutrients and salinity on the number of shoots and vitality of *Zostera marina* mentioned below.

Zostera marina plants, originating from the marine Terschelling habitat and the estuarine Eems habitat, were negatively influenced by high salinity. Vitality of the estuarine population (a factor synthetised of number of shoots, plant size, necrosis and number of missing leaves) decreased at 26 and 30 PSU salinity, whereas the marine population responded with a reduction of the number of shoots, at the 30 PSU level only.

We did not find any effects of salinity on wasting disease. However, a positive relationship between salinity and the spread of wasting disease was observed in the 1930s (Pokorny 1967, Rasmussen 1977). Probably, *Zostera marina* populations occurring along the Atlantic coast in the 1930s, were susceptible to wasting disease, whereas some of the present *Z. marina* populations on which the populations tested in our study, may have descended from plants that survived the disease, and may therefore be resistant to wasting disease. During the outbreak of wasting disease in the Wadden Sea, Harmsen (1936) observed that the intertidal narrow-leaved form of *Z. marina* seemed to be unaffected. There may still be populations that are susceptible to wasting disease; for example, Burdick et al. (1993) found correlations between salinity and wasting disease in Great Bay, New Hampshire.

The recent mass decline of *Zostera marina* in Lake Grevelingen (Nienhuis et al.1996), which Herman et al. (1996) attributed to the depletion of silicate, is in our opinion more likely explained by increased salinity. Salinity has increased in Lake Grevelingen as a consequence of changes in hydrological management (Nienhuis et al. 1996). This is further demonstrated by the inverse relationship between salinity and eelgrass cover in the period 1968 to 1992 (Wijgergangs 1994, M.M. van Katwijk & D.J. de Jong unpublished results).

Chl *a* content of the *Zostera marina* shoots increased with increasing nutrient loads in the water, as was found by Pedersen (1995).

When stressed by salinity, high nutrient loads did not benefit the marine Terschelling plants. The estuarine Eems plants were even negatively influenced by the high nutrient treatment. This is supported by field observations (see ‘Conclusions and ecological implications’), but contradicts studies of salt sensitive crop species, which showed an alleviating effect of nitrate on salinity stress (e.g. Marschner 1995). At low salinity levels (i.e. Terschelling plants 23 and 26 PSU; Eems plants 23 PSU), plants from both populations were positively influenced by the higher nutrient treatments.

Positive effects on *Zostera marina* from nutrient enrichment of the sediment have been reported previously (Orth 1977, Short 1983, 1987, Roberts et al. 1984, Short 1987, Kenworthy & Fonseca 1992, Murray et al. 1992, Williams & Ruckelshaus 1993, van Lent et al. 1995). Enrichment of the water column may also lead to increased growth of *Z. marina* (Harlin & Thorne-Miller 1981, Bohrer et al. 1995), but may also negatively affect *Z. marina* (Burkholder et al. 1992, 1994, Williams & Ruckelshaus 1993, Taylor et al. 1995, Boynton et al. 1996, Nelson & Waaland 1997, van Katwijk et al. 1997, McClelland & Valiela 1998). Van Katwijk et al. (1997) found that toxic effects of ammonium were correlated with a shoot tissue nitrogen content in *Z. marina* leaves of 3.5 % of the dry weight. In natural habitats, nitrogen content of *Z. marina* lies between

1 and 3 % during the growing season, while in the present study, the shoot tissue nitrogen content in *Z. marina* leaves increased from 1.0 to 1.5 % of the dry weight in both lowest nutrient treatments, to about 2.2 % in the highest nutrient treatment. Therefore, the negative effects of water nutrient additions were not correlated to any toxic effect of NH_4. Moreover, it is more likely that the plants were nutrient limited, causing a positive effect of water nutrient additions at low salinity. It remains to be explained why there is no positive effect, and even a negative effect in the case of the estuarine plants, of nutrient enrichment at high salinity.

The measured chemical composition of plant tissue (viz. aboveground and belowground tissue N, P, C, S, Na K, Ca Mg Fe and Mn) gave no suggestion of a physiological mechanism which could be responsible for our results (see van Katwijk & Schmitz 1999). However, a physiological explanation is not unlikely. It is known that nutrient-dependent processes like ammonium assimilation and amino acid metabolism are integrally involved in seagrass responses to salinity (Joshi et al. 1962, Pulich 1986). When subjected to high nitrogen loads, plants usually synthesise amino acids with a high N:C ratio to prevent shortage of carbon which is required for ammonium assimilation (for example arginine, asparagine and glutamine, with N:C ratios of 0.66, 0.50 and 0.40, respectively, see Smolders et al. 1996). The amino acid proline accumulates in response to high salinity in *Zostera marina* and most other seagrass species, thereby acting as an osmoticum (Pulich 1986, van Diggelen et al. 1987, Adams & Bate 1994). As proline has a N:C ratio of only 0.20, carbon costs per nitrogen molecule will be high, which may cause NH_4 toxicity due to carbon shortage.

Another explanation for the observed interactive effect of nutrients and salinity may arise from the growth rate and morphology of *Zostera marina*. Immature *Z. marina* leaf tissue is sensitive to salinity and is protected from seawater by tightly enveloping sheaths (Arai et al. 1991, Fukuhara et al. 1996). This was also found in other seagrasses by Tyerman (1989), who found an osmotic gradient in the 'sheath solution', i.e. the seawater that had diffused into the sheath, surrounding the immature tissue. In the direction of the base, the osmolality decreased. Uptake of ions by the immature leaf caused this gradient, which, in turn, lessened the uptake load on the base (the expansion zone). Tyerman (1989) argues that the expansion zone of the leaf is shielded from high salinities provided that it continues to grow, since it is growth and the concomitant ion uptake which develop the gradient in the first place. Reversely, one may argue that a high growth rate may prematurely expose the leaf to seawater, thereby adversely affecting the plant. This is likely to occur at high nutrient loads and high salinity, and may therefore explain our results.

A third explanation may arise when considering that stressed plants (e.g. at high salinity) will have a lower growth rate, e.g. Grime (1979). The extra tissue nitrogen resulting from enrichment can be used for growth by plants with a high growth rate, in contrast to plants with a low growth rate, as was found by Pedersen (1995) in an enrichment experiment comparing fast-growing macroalgae with slower-growing *Zostera marina* plants. After enrichment, Pedersen found increased tissue N and chl *a* in both fast- and slow-growing plants, which is consistent with our findings. Assuming that the low salinity plants in our experiment show the same response as Pedersen's macroalgae, *viz.* using the extra N for growth, and furthermore assuming that the extra N may eventually burden the stressed high salinity plants, this would explain the nutrient x salinity effect found in our study.

The effect of salinity and the interactive effect of salinity and nutrients differed between the 2 populations, the marine (Terschelling) population responded negatively only to the highest salinity applied, whereas the estuarine (Eems) population responded negatively to both the highest and the intermediate salinity applied. In the present study, most of the differences measured between the populations at the onset of the experiment remained similar during the experiment, or became larger (see van Katwijk & Schmitz 1999). This indicates either genotypic differences or phenotypic plasticity.

CONCLUSIONS AND ECOLOGICAL IMPLICATIONS

A salinity of 30 PSU acted adversely on *Zostera marina* plants originating from the marine Terschelling habitat, while *Z. marina* plants from the estuarine Eems habitat were negatively effected by 26 and 30 PSU salinity. When stressed by salinity, the plants responded either indifferently or negatively to nutrient enrichment, whereas at relatively low salinity, the plants were stimulated by enrichment. Although in a laboratory experiment only part of reality can be simulated, our findings are supported by, and may therefore explain, distribution patterns and dynamics of *Z. marina* observed (mentioned in the 'Introduction'). The results from this study indicate that an increased nutrient input in coastal areas will restrict the distribution of *Z. marina* to areas with relatively low salinity. Without freshwater influence, a eutrophicated system will not be able to support *Z. marina* populations. Recent declines of *Z. marina* in areas at both sides of the Atlantic Ocean confirm this (Short et al. 1986, D.J. de Jong personal communication). Nutrient loads and salinities applied in this experiment cover the range present in the Wadden Sea (Höpner 1991, Ministry of Transport Water Management and Public Works unpublished data), a range that is also frequently encountered along the eastern shores of the United States (e.g. Short et al. 1993, McClelland & Valiela 1998), making our conclusions of interest to studies of *Z. marina* populations over a large geographical area.

Acknowledgements. This study has been financed by the Ministry of Transport, Public Works and Water Management. Thanks are due to Prof. Dr. VN. de Jonge of this Ministry for his skilful involvement in the project. We thank Dr. J.G.M. Roelofs and L.S.A.M. Hanssen for fruitful discussions. Prof. Dr. M.A. Hemminga, Dr. J.G.M. Roelofs, Prof. Dr. G. van der Velde, Prof. Dr. C. den Hartog and B. Kelleher are thanked for critically reading the manuscript, the latter also for correcting the English. We thank D.C.R. Hermus, F. Looyen, E. Crins, M. Hornman, A. Janssen and workers of the Ministry of Transport, Public Works and Water Management for their assistance during the experiment. We thank J. Eygensteyn and G. Verheggen-Kleinheerenbrink for their help with the analyses. Dr. R.M. Asmus (Biologische Anstalt Helgoland) and A. van der Pluym (National Institute for Coastal and Marine Management) kindly supplied us with nutrient and salinity data. The Illustration Service of the Faculty of Science, Universtity Nijmegen, is thanked for preparing the figures and T. de Boo

(Department of Statistical Consultation, University Nijmegen) for help with the application of statistical methods.

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CHAPTER 5

EFFECTS OF WATER DYNAMICS ON *ZOSTERA MARINA*: TRANSPLANTATION EXPERIMENTS IN THE INTERTIDAL DUTCH WADDEN SEA

van Katwijk MM, Hermus DCR (in press) Mar Ecol Prog Ser

EFFECTS OF WATER DYNAMICS ON *ZOSTERA MARINA*: TRANSPLANTATION EXPERIMENTS IN THE INTERTIDAL DUTCH WADDEN SEA

ABSTRACT: Pilot experiments in the tidally dominated Dutch Wadden Sea indicated a negative relationship between *Zostera marina* L. transplantation success and tidal depths. As light availability was sufficient, we hypothesised that water dynamics (particularly waves) and ensuing sediment mobility (movement or resuspension of the sediment), was the major cause for the loss of transplants at larger depths. Transplantation experiments were carried out at intertidal flats and depths under conditions of normal and reduced water dynamics and sediment mobility, using exclosures that reduced water dynamics and shells that stabilised the sediment. To test bioturbation effects, cages that excluded large biota were used. Without protection, *Z. marina* plants were successfully established in a belt within the intertidal zone (0 to –0.20 m mean sea level, MSL) at 2 out of 3 transplantation sites during the first growing season. Reduced water dynamics by exclosures, prevented the loss of plants in the zone –0.40 to –1.15 m MSL, and removal of the exclosures after 1 month caused loss of all plants within a few days. Light limitation nor bioturbation could explain these results. We conclude that prevalent water dynamics, particularly the relative period of exposure to wave dynamics, were too high for establishment and maintenance of intertidal *Z. marina* at depths below –0.20 m MSL at these sites of intermediate exposure. At the third, exposed, transplantation site, water dynamics prevented transplantation success along the entire depth gradient studied. Reduction of sediment mobility by shells had a positive effect on transplant survival, particularly at –0.20 m MSL. Shell armour can therefore be recommended in transplantations near the lower limit of potential intertidal habitats of *Z. marina*. Anchorage of the plants with pegs had no positive effect. Depth limitation of intertidal *Z. marina* populations by water dynamics can explain zonation patterns that occur in several tidal systems in northwest Europe.

INTRODUCTION

The economical and ecological importance of seagrass beds is widely acknowledged and the worldwide losses of seagrass beds during recent decennia have led to numerous restoration and mitigation projects. The Wadden Sea in northwest Europe, one of the world's largest international marine wetlands, lost at least 150 km^2 of *Zostera marina* L. (eelgrass) during the twentieth century (den Hartog & Polderman 1975; Reise et al. 1989). Lack of recovery has been attributed to increased turbidity and increased fisheries activities (Giesen et al. 1990; de Jonge & de Jong 1992). Recently, turbidity has decreased and fisheries activities have been forbidden in some areas (e.g. van Katwijk et al. 1998, in press). Restoration of the *Z. marina* biotope in the Dutch Wadden Sea is regarded as one of the major ecological targets of Dutch government policy (Anonymous 1989).

Pilot transplantation experiments in the Dutch Wadden Sea along a depth gradient revealed that transplants vanished within 3 weeks below a depth of –0.20 m below mean sea level (MSL) (van Katwijk 1993, van Katwijk & Schmitz 1993). At present, irradiance in the Dutch Wadden Sea should be sufficient for *Zostera marina* to sustain to at least –0.45 m MSL (the effect of tides was included in the calculation of this depth, see van Katwijk 1992), and the quality of Wadden Sea water does allow growth of *Z. marina* (van Katwijk et al. 1998). In the Wadden Sea, average wave action at the sediment surface (orbital velocity ms^{-1}) increases with depth down to just below mean low tide (Hermus 1995, van Katwijk et al. in press). Therefore, we hypothesise that water dynamics, particularly wave action, and the ensuing sediment mobility (movement and/or resuspension of the sediment) in the intertidal flats of the Wadden Sea are too severe for eelgrass establishment at depths greater than –0.20 m MSL. This hypothesis is supported by a frequently observed zonation pattern of *Z. marina* in northwest Europe, particularly at the beginning of the twentieth century. At sheltered locations, the intertidal eelgrass beds protruded to greater depths and joined with the subtidal eelgrass zone, whereas at less sheltered locations, the mid-intertidal eelgrass beds were separated from the subtidal beds by an unvegetated zone. Depth limitation of the mid-intertidal zone could not have been caused by light limitation, as can be inferred from the presence of the subtidal beds. Depth limitation by increasing water dynamics, on the other hand, would offer a logical explanation (van Katwijk et al. in press, see also discussion).

To our knowledge, the effects of water dynamics and sediment mobility on *Zostera marina* transplantations have never been tested, although the importance of these factors to seagrasses is indicated by the inverse correlations of seagrass cover with hydrodynamics (e.g. Fonseca & Bell 1998) and with sedimentation and erosion processes (e.g. Boley 1988), as well as by results from mesocosm and laboratory studies (Watanabe & Terawaki 1986, Koch 1999). On the other hand, stagnancy does have a negative effect on seagrass productivity and biomass (Fonseca & Kenworthy 1987, Koch 1999). However, stagnancy does not occur in the Wadden Sea. Semi-diurnal tides, with tidal amplitudes varying between 1.36 and 3.73 m, ensure continuous water movement (Postma & Dijkema 1982).

Bioturbation is an unlikely cause of the disappearance of the transplanted eelgrass in the pilot experiment. Bird predation would have resulted in high losses in the higher zones where the emergence period is longest, and would decrease with depth, which is the opposite of the pattern observed. Also, observations indicated minimal bird predation during the growing season (Hermus 1995). *Arenicola marina* is the main bioturbator in the Wadden Sea, however, it was never observed in densities that may have been damaging to *Zostera marina* transplantations (personal observation). Adult green crabs, *Carcinus maenas*, which damage eelgrass transplants when present in higher densities than 1 ind. m^{-2} (Davis et al. 1998), are only present in much lower densities in intertidal areas of the Wadden Sea (van der Veer et al. 1998, Dittmann & Villbrandt 1999). Rays, potentially damaging to eelgrass transplantations (Fonseca et al. 1994), are nowadays absent from the Wadden Sea (Lozán et al. 1994, Rijnsdorp et al. 1996). Most of the other sediment disturbing animals (e.g. flatfish) are only abundant as juveniles, which are hardly able to destroy seagrass plants (H. Asmus, R.M. Asmus, K. Reise personal communication).

In the present study the effects of water dynamics and sediment mobility on *Zostera marina* were tested by means of transplantation experiments along depth gradients in the Wadden Sea, using shell armouring, pegs and exclosures. Part of the pilot experiment was repeated to assess year-to-year differences. Exclosures with different tops were used to test the effect of water dynamics with exclusion of potentially bioturbating organisms.

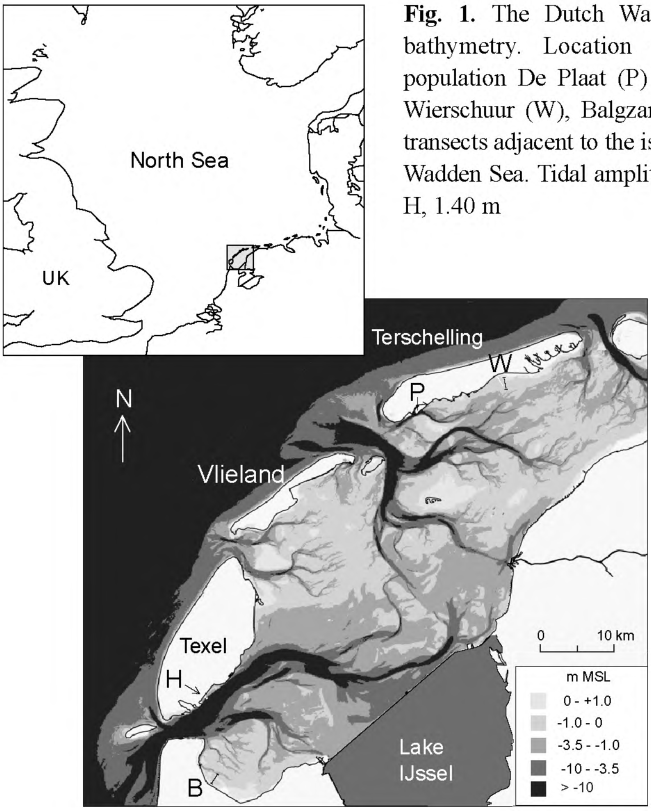

Fig. 1. The Dutch Wadden Sea with its complex bathymetry. Location of *Zostera marina* donor population De Plaat (P) and the transplantation sites Wierschuur (W), Balgzand (B) and 't Horntje (H) in transects adjacent to the islands, all located in the Dutch Wadden Sea. Tidal amplitudes P and W, 1.80 m; B and H, 1.40 m

MATERIALS AND METHODS

Three transplantation experiments with *Zostera marina* were carried out in the Dutch Wadden Sea (Figs. 1 & 2). In all experiments, the bare root transplantation method was employed (Davis & Short 1997). The donor population was mainly annual (van Katwijk et al. 1998).

In the first experiment, the effect of depth gradient and sediment stabilisation was tested. Part of the experiment was similar to a previous transplantation experiment (van Katwijk & Schmitz 1993), in order to assess year-to-year differences. The experiment began in June and was carried out at 3 sites (Fig. 1), over a depth gradient of +0.15 to –0.90 m MSL, on untreated and shell-armoured sediments (Fig. 2a). A control transplantation was carried out at the donor site (De Plaat, Terschelling) to test the effect of transplanting. At 2 out of 3 locations (B and W, Fig. 1) *Zostera marina* was present until the 1970s in the mid-intertidal zone (den Hartog & van der Velde 1970, den Hartog & Polderman 1975, Dijkema 1989). Near the third location (H) *Z. marina* was present until the 1930s (Oudemans et al. 1870, den Hartog & Polderman 1975). It was located near a deep channel (Fig. 1). As a consequence, the exposure to water dynamics (e.g. chance of breaking of waves) was high in

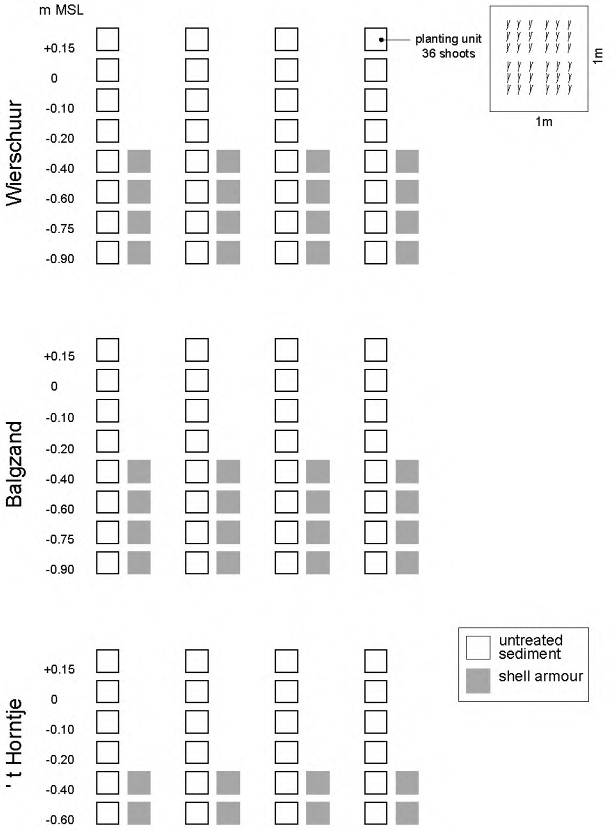

Fig. 2a. Experimental set-up of experiment 1. MSL Mean Sea Level

comparison to the 2 other sites. Table 1 and Fig. 3 summarise the main hydrodynamic features of the transplantation sites at different tidal depths.

In the second experiment, plexiglass-topped exclosures and shell armouring of the sediment were used to test the effect of water dynamics in comparison to the effect of sediment stabilisation and no treatment (Fig. 2b). The experiment began in June at Wierschuur over a depth range of 0 to –0.60 m MSL. The exclosures lessened wave activity to 80 to 100 % (H.J. Verhagen personal communication), but still allowed free exchange of water. To test the effect of water dynamics on established plants, the exclosures were removed after 1 month (Watanabe & Terawaki 1986). Water dynamics consists of wave action and tidal currents. Wave action is depth related, it increases towards just below the low tide level (between 0.10 and 0.30 m below low tide) and decreases when going deeper (van Katwijk et al. in press, Fig. 3). Orbital velocity generated by waves is expected to have a much larger (traction) effect on seagrass shoots as compared to current velocity, as wave action results in constant flapping movements of the plants, in contrast to the single bending of the plants in currents. Therefore, and because the tidal current velocities above tidal flats are low: 0 to 0.15 ms^{-1} (Postma 1961, Asmus & Asmus 1991), tidal currents are not considered in this study.

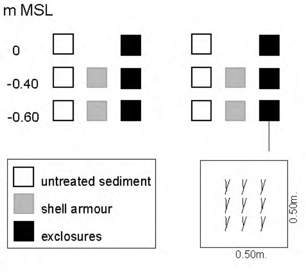

Fig. 2b. Experimental set-up of experiment 2. MSL Mean Sea Level

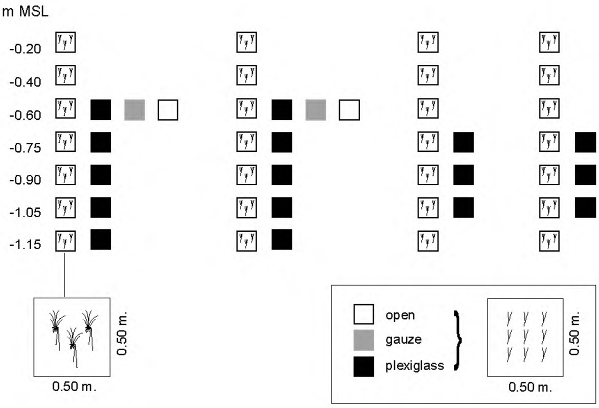

Fig. 2c. Experimental set-up of experiment 3. Peg-anchored shoots were bundled per 3

A third experiment was performed in July at Wierschuur at –0.60 m MSL to test the effect of water dynamics with exclusion of bioturbating organisms, using plexiglass-topped, gauze-topped and open exclosures, thus creating largely (80 to 100 %), intermediate (40 to 50 %) and barely (0 to 5 %) reduced water dynamics (expert opinion H.J. Verhagen, Fig. 2c). It was assumed (and later confirmed by observations during the experiment) that large animals like crabs and juvenile flatfishes did not enter the open exclosures (H. Asmus, R.M. Asmus, K. Reise personal communication). Secondly, the effect of water dynamics over a depth range of –0.60 to –1.15 m MSL was tested in this experiment, using the plexiglass-topped exclosures. The effect of peg anchoring on the transplantation success of *Zostera marina* was tested over a depth gradient of –0.20 to –1.15 m MSL.

Table 1. Hydrodynamic characteristics of the 3 transplantation sites in 1993 between June, 2 and August, 31. Only days with fetch > 0.5 km were included. Presented are the tidal amplitude in m (a), the number of days with fetch > 0.5 km (total: 91 days) (N), the mean (SD) and *maximum* (over this period) of the maximal orbital velocity at sediment surface U_{max} (ms^{-1}), the wave height H_S (m) and the wave period T (s) during a tidal cycle. U_{max} values did not vary over the depth gradient studied, except when indicated

	a	N	U_{max}	H_S	T
Wierschuur	1.90	53	0.42 (0.065) 0.54	0.15 (0.030) 0.24	1.57 (0.263) 2.03
Balgzand	1.40[a]	27	0.35 (0.044) 0.43	0.37 (0.155) 0.65	2.27 (0.531) 3.11
't Horntje	1.40[a]	47	0.63 (0.145) 0.85[b]	0.21 (0.052) 0.33	1.88 (0.389) 2.71

[a] Mean low tide at –0.80 m MSL and mean high tide at +0.60 m MSL.

[b] From –0.60 m MSL upwards U_{max} decreased towards mean (SD) 0.53 (0.074) and maximum 0.59 ms^{-1} at +0.15 m MSL

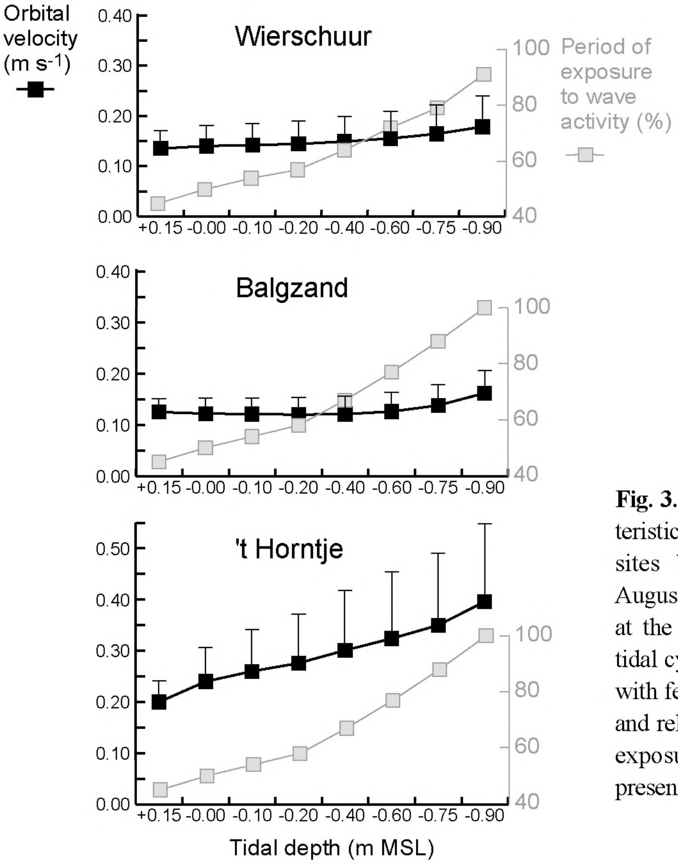

Fig. 3. Tidal depth related characteristics of the 3 transplantation sites between 2 June and 31 August. Average orbital velocity at the sediment surface during a tidal cycle (mean and SD) at days with fetch > 0.5 km (see Table 1), and relative period (% of time) of exposure to wave action are presented

Transplantation. *Zostera marina* plants for experiments 1 and 2 were collected and rinsed with seawater at the donor site De Plaat, Terschelling, on the first 2 days of June 1993, and transplanted to 3 transplantation sites and the donor site (Fig. 1). The plants were transported and kept in boxes with a shallow layer of seawater with a temperature of 10 to 15°C during 1 day. In experiment 1 and the control transplantation at the donor site, a planting unit (PU) consisted of 36 shoots (4 replicates per depth), in experiments 2 and 3, a PU consisted of 9 shoots (2 and 4 replicates per depth, respectively, Fig. 2). Peg anchored PUs consisted of 3 shoots attached in a bundle to 3 pegs (peg length 0.22 m). All PU's were planted at least 7 metres apart. Each shoot consisted of a rhizome segment with 1 shoot.

Shell armouring was achieved by mixing a 5 cm layer of shells (mainly cockles) with local sediment, which was subsequently spread and compacted over the plot. The exclosures consisted of a wooden frame of 0.4 x 0.4 x 0.3 m with the sides covered with 1 mm plastic gauze to allow free exchange of water. The exclosures were anchored by iron plates pressed into the sediment on 2 sides. The top of the exclosures was sealed with either plexiglass, or 1 mm gauze, or the tops remained open.

At –0.40 m and –0.60 m MSL, diatoms were cleaned from the plexiglass covering of the exclosures during every monitoring event (at least monthly during the growing season, see below). This was not necessary at 0.0 m MSL. The exclosures were removed after 1 month.

Monitoring. In experiments 1 and 2, the *Zostera marina* transplants at Wierschuur were monitored once every 2 days during the first 2 weeks. The monitoring was gradually reduced to once a week. From the second half of September monitoring occurred monthly. Balgzand and ’t Horntje were monitored weekly during the establishment phase. After 4 weeks, monitoring was reduced to once or twice a month. In autumn, no monitoring occurred, while we monitored monthly from December to April. Experiment 3 was monitored weekly. The peg-anchored transplants were monitored a further 5 times during the first 3 weeks of the experiment.

Vegetative and generative shoots were counted. New shoots were recorded as such when the distance to the parental plant was > 2 cm. The length of 15 randomly chosen eelgrass shoots (the longest leaf + sheath) was measured after 1 and 4 weeks, at Wierschuur (experiments 1 and 2), the donor site transplantation and the natural donor vegetation at De Plaat. No observations were made of the belowground parts after disappearance of the aboveground parts, as they may still have been viable, and replanting would diminish the chance of shooting.

Hydrodynamic characteristics. Daily values of the orbital velocity at the sediment surface were calculated for all tidal depths studied, using the effective fetch and the wind velocity. Wind direction and velocity data were obtained from the Royal Dutch Meteorological Institute at station “De Kooy”, which was situated at a distance of 4, 10, 57 and 67 km from Balgzand, ’t Horntje, De Plaat and Wierschuur, respectively. The effective fetch was calculated for each wind direction (rounded to 5°) using the Saville formula (Shore Protection Manual) of directions from –30° to +30° in steps of 10° (see also Keddy 1982). Wave height and wave period were calculated using the Bretscheider method (e.g. Verhagen 1998, Equations 1 & 2), assuming an fetch depth and breaker index of 1.0 m and 0.5, respectively, at Wierschuur, 0.8 m and 0.5, respectively, at Balgzand, and 10 m and 0.63, respectively, at ’t Horntje. These figures are based on the bathymetry of the surrounding area (Fig. 1): fetch depth is based on the average depth of the fetch area, and the breaker index is 0.5 at gently sloping areas like Wierschuur and Balgzand, but increases when slopes are steep like at ’t Horntje (Verhagen & van der Wegen 1998, H.J. Verhagen personal communication).

$$gH_S/u^2 = 0.283 \text{ x } \tanh[0.530 \text{ x } (gd/u^2)^{0.750}] \text{ x } \tanh\{0.0125 \text{ x } (gF/u^2)^{0.42}/\tanh[0.530 \text{ x } (gd/u^2)^{0.750}]\} \qquad \text{(Equation 1)}$$

$$gT/u = 2\pi \text{ x } 1.2 \text{ x } \tanh[0.833 \text{ x } (gd/u^2)^{0.375}] \text{ x } \tanh\{0.077 \text{ x } (gF/u^2)^{0.25}/\tanh[0.833 \text{ x } (gd/u^2)^{0.375}]\} \qquad \text{(Equation 2)}$$

Where: T=wave period (s), u=wind velocity (ms^{-1} at 10 m above the surface), d=depth (m), g=gravitational accelation, F=fetch (m) and H_S=wave height (m).

At each tidal depth where we transplanted eelgrass, we calculated the horizontal orbital velocity U at the sediment surface from wave height (H_S) and wave period (T) using the formula of Visser (as used in the program CRESS Verhagen & van der Wegen 1998, H.J. Verhagen personal communication):

$$U = H_S/2 \times \omega \times 1/\sinh(k \times d) \qquad \text{(Equation 3)}$$

Where: $\omega=2\pi/T$, $k=2\pi/L$, $L=\mathrm{sqrt}(gd) \times (1-d/L_0) \times T$, $L_0=1.56\ T^2$, d=depth and g=gravitational acceleration.

To calculate the average orbital velocity at the sediment surface per tidal cycle, we used a sinusoidal function to simulate the tidal curves, and calculated U (Equation 3) at all water levels from +[tidal amplitude] to –[tidal amplitude] in steps of 2 cm (Equation 4). In the case of the transplantation sites Balgzand and 't Horntje, the sinusoidal function was flattened at the high tide levels to simulate the actual tidal curve at these sites by use of the condition "if water level > 58 then water level = 58". The maximal orbital velocity was the highest orbital velocity reached during 1 tidal cycle.

$$U_{\text{average}} = \sum_{i=-(a-1),2}^{(a-1)} \{ U \times [\arcsin((i+1)/a) - \arcsin((i-1)/a)]/\pi \} \qquad \text{(Equation 4)}$$

Where: a=amplitude (cm) and i=water level (cm).

Statistical analysis. The number of *Zostera marina* shoots, day of death and percentage of inflorescences were normally distributed, and shoot length was lognormally distributed. The latter was log-transformed prior to analysis, the back-transformed means were used as a central measure, and the standard errors were calculated according to Mood et al. (1974). The effect of tidal gradient (experiment 1 and 3) was tested with repeated measurements analysis of variance (ANOVA, procedure MIXED, SAS 1989). Monitoring events involved in the statistical analysis were, in experiment 1, weeks 4, 7, 9, 11, 14 and 27, and in experiment 3, weeks 1, 2, 4, 6, and 9. When the data were similar on several tidal depths (i.e. all plants had disappeared within 2 weeks), only results from the greatest depth were included in the analysis. The effect of stabilising the sediment with shell armour on the day of death of the *Z. marina* shoots was tested with ANOVA (procedure GLM, SAS 1989), with Bonferroni correction for testing 4 times, for each tidal depth. Day of death was calculated by averaging the last day that any seagrass plant was present and the first day of its absence. Analysis of variance and Tukey's test for comparison of means were used to test to the percentage of inflorescences and shoot length in experiment 1, the effect of exclosures, shell armour or no treatment on the number of *Z. marina* shoots 4 weeks after transplantation at various tidal depths in experiment 2, and the effect of different kinds of exclosures and peg-anchoring on the number of *Z. marina* shoots 4 weeks after transplantation in experiment 3.

RESULTS

Depth gradient and sediment stabilisation

The transplanted *Zostera marina* plants established successfully in the zone between 0.0 m and –0.20 m MSL at Wierschuur and Balgzand (experiment 1, Fig. 4, repeated measures ANOVA $p < 0.001$ for depth, day and depth x day at both locations, except the effect of day at Wierschuur: $p < 0.05$). The low initial losses of transplants at those depths and at the control site indicated a low transplantation stress, although the plants were shorter at the control site relative to the natural vegetation of the donor site. At Wierschuur, transplantation was also successful at +0.15 and 0 m MSL, in contrast to Balgzand. The absence of sediment stabilisation at depths of –0.40 m MSL and deeper resulted in the complete loss of transplants within 2 weeks. Sediment stabilisation

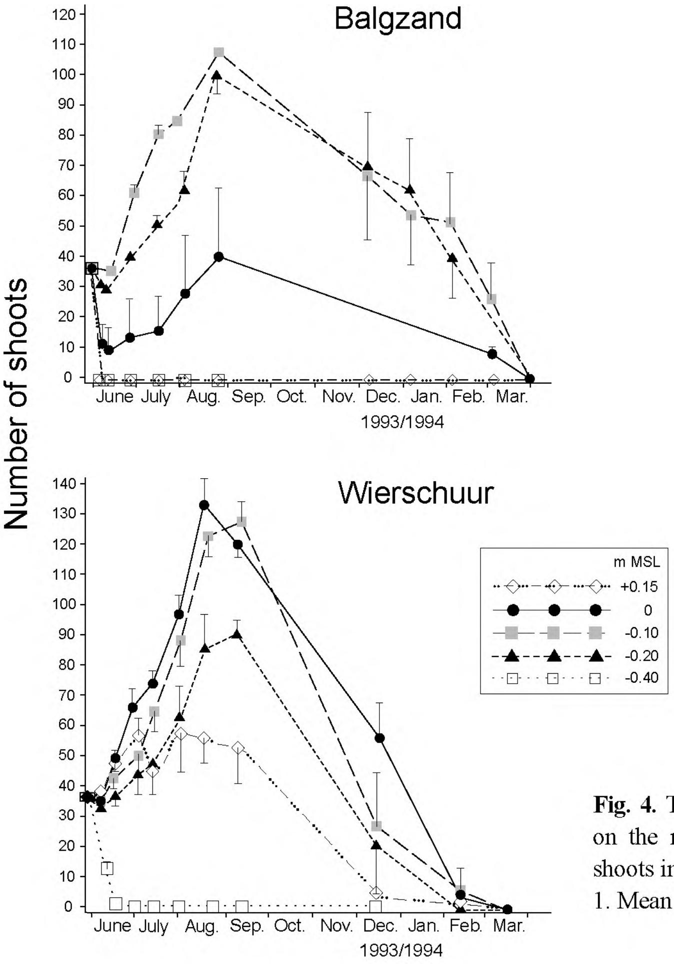

Fig. 4. The effect of the tidal depth on the number of *Zostera marina* shoots in transplantation experiment 1. Mean and SEM are presented

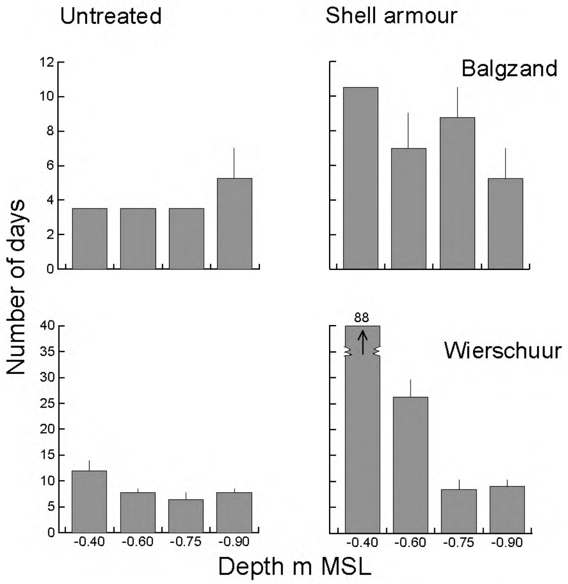

Fig. 5. Number of days after which death occurred of *Zostera marina* transplants in untreated and shell armoured sediment, along a depth gradient. Mean and SEM are presented

retarded the loss of transplants for a number of weeks, especially at –0.40 m MSL (Figs. 5 & 6, $p < 0.001$ at both locations at –0.40 m MSL, and $p < 0.01$ at –0.60 m MSL at Wierschuur). At the relatively exposed transplantation site ('t Horntje) all eelgrass plants below 0.0 m MSL disappeared within 1 week, while at +0.15 m and 0.0 m MSL some desiccated plants with dark brown leaves survived for a further 1 to 2 weeks.

At 't Horntje, the average maximal orbital velocity at the sediment surface during a tidal cycle was between 0.53 and 0.63 ms^{-1} (Table 1), while these values were 0.35 and 0.42 ms^{-1} at Balgzand and Wierschuur, respectively. At Balgzand and Wierschuur, eelgrass transplantations were successful when the relative period of exposure to wave action was lower than ca 60 % (at –0.20 m MSL, Fig. 3).

The relationship between transplantation success and tidal depth were already apparent after 1 week (Fig. 4). At Balgzand and Wierschuur, the highest wave action during the first week occurred on the very day of transplantation, with a maximal orbital velocity at the sediment of 0.41 and 0.44 ms^{-1}. These maxima occurred at all investigated tidal depths during the tidal cycle. Average orbital velocity increased with depth from 0.14 at +0.15 m MSL to 0.23 ms^{-1} at –0.90 m MSL. Loss of plants occurred at tidal depths where the average orbital velocity at day 0 was higher than 0.165 ms^{-1}. At 't Horntje, the highest wave action occurred on day 6, when maximal orbital velocity at the sediment surface varied between 0.59 (+0.15 m MSL) and 0.82 ms^{-1} (–0.60 m MSL). The average orbital velocity varied between 0.23 and 0.50 ms^{-1} on day 6.

Exclosures

No losses occurred in the exclosures that reduced water dynamics (experiment 2 and 3, Figs. 6 to 8), independent of depth (Figs 6 & 7, repeated measures ANOVA $p > 0.1$). The *Zostera marina* plants in the exclosures at –0.60 m MSL performed better than the plants in the shell armoured or untreated plots at –0.40 and –0.60 m MSL

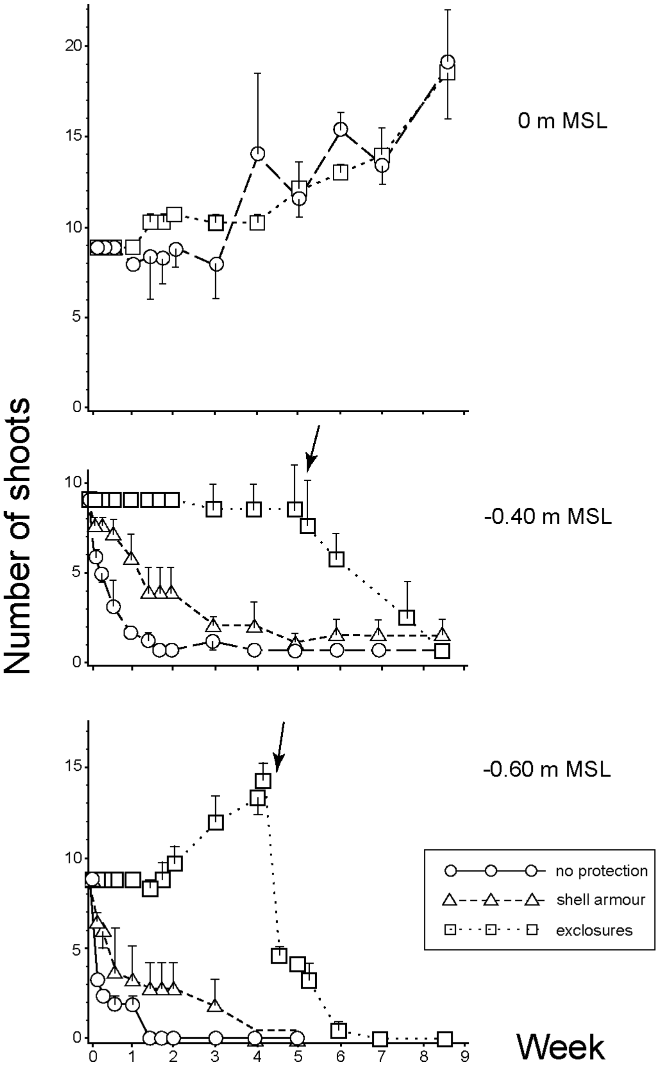

Fig. 6. Effect of sediment stabilisation (shell armour) and protection from water dynamics (by exclosures with plexiglass tops) on the number of *Zostera marina* shoots (mean and SEM), along a depth gradient. The arrow indicates the removal of the exclosures

(Tukey's test $p < 0.05$), and even tended to perform better than the exclosure plants at –0.40 m MSL (Fig. 6, ANOVA $p < 0.1$). At –0.40 m MSL, sedimentation in the exclosures was ca 8 cm month^{-1}, and circa 5 cm month^{-1} at –0.60 m MSL. At 0 m MSL no difference between unprotected and exclosures was observed (Fig. 6, Tukey's test $p < 0.05$). Anchoring of the eelgrass plants with pegs did not prevent their loss within 2 weeks at depths greater than –0.40 m (experiment 3, Fig. 7, repeated measures ANOVA for the effect of depth, day and depth x day: $p < 0.001$).

In exclosures sealed with gauze, half of the eelgrass shoots had established after 4 weeks in the duplicate exclosures (experiment 3, Fig. 8). In exclosures sealed with

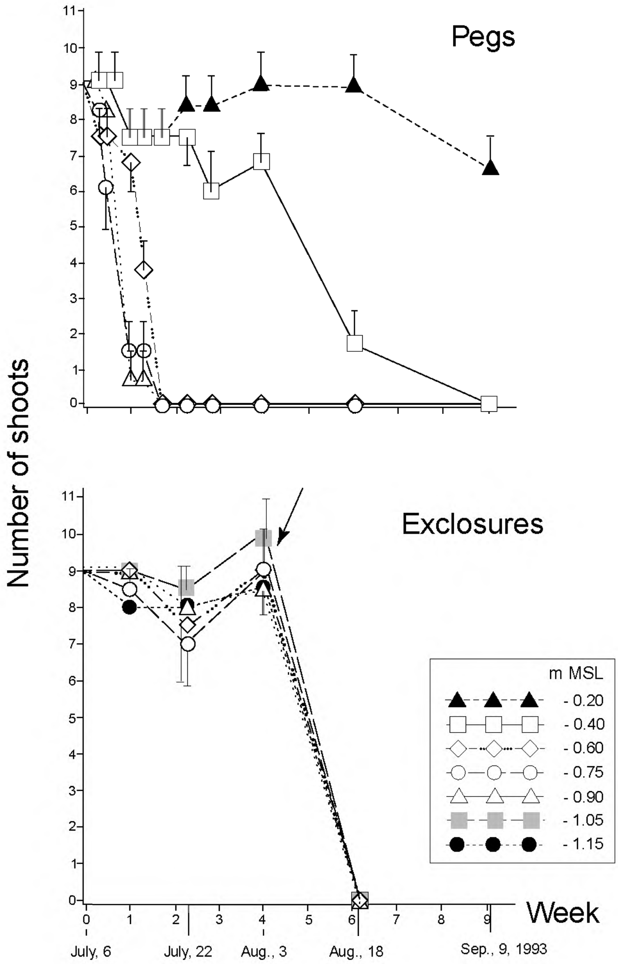

Fig. 7. The effect of reduced water dynamics (exclosures) and peg-anchoring on the number of *Zostera marina* shoots (mean and SEM) along a depth gradient. The arrow indicates the removal of the exclosures

plexiglass, all plants were still present after 4 weeks, whereas in the open exclosures, the plants disappeared within 2 weeks. All differences were statistically significant (Tukey's test $p < 0.05$).

Removal of the exclosures, after 1 month, resulted in the complete loss of *Zostera marina* plants within 2 weeks (both in experiments 2 and 3).

Length of *Zostera marina* shoots

After 4 weeks, the transplanted *Zostera marina* plants (both Wierschuur 0 m MSL and control site) were shorter than the plants in the natural vegetation (Table 2, ANOVA $p < 0.05$). The length of the unprotected *Z. marina* plants, with or without sediment stabilisation, decreased with depth, in contrast to the protected exclosure plants that showed no depth-related differences in shoot length (Table 2, ANOVA $p < 0.05$).

Fig. 8. The effect of high (open top), intermediate (gauze top) and low (plexiglass top) water dynamics on the number of *Zostera marina* shoots (mean and SEM) in exclosures that excluded large potentially bioturbating organisms after 4 weeks

Generative phase, overwintering and germination

The highest percentage of *Zostera marina* plants with inflorescences (ca 60 %) was recorded at the end of August in the 0.0 m MSL zone, both at the transplantation sites and the donor site. The percentage of inflorescences decreased significantly with depth ($p < 0.05$): from around 60 % at +0.15 and 0.0 m MSL, to around 40 % at –0.20 m MSL and 0 % at –0.40 m MSL. Eelgrass in exclosures with plexiglass coverings formed inflorescences synchronously with plants at higher levels (see also Hermus 1995).

By the end of November air temperatures dropped to –1 to –6°C, and the tidal flats

Table 2. The effect of sediment stabilisation with shells, reduction of water dynamics by exclosures, and transplantation effects on the length of *Zostera marina* shoots (cm) along a depth gradient, 5 days (7 June) and 4 weeks (29 June) after transplanting. Geometric mean (SD) is presented. n=15

Depth MSL	0.0 m	–20 m	–40 m	–60 m	–90 m
7 June					
Wierschuur					
No protection	15.8 (1.4)	15.6 (1.3)	12.8 (1.5)	9.5 (1.6)	9.5[3] (1.6)
Shells			10.1 (1.8)	9.7 (1.4)	6.2 (2.0)
Exclosures	19.2[1] (1.2)		15.0[1] (1.4)	16.4[1] (1.3)	
29 June					
Wierschuur					
No protection	22.6 (1.3)	17.6 (1.3)	Absent	Absent	Absent
Shells			14.3 (1.3)	10.2[2] (1.5)	Absent
Exclosures	20.2 (1.2)		20.5 (1.2)	21.3 (1.3)	
Donor site					
Natural vegetation			39.7 (4.3)		
Control transpl.			28.0 (1.2)		

[1] n=10 plants; [2] n=11 plants; [3] n=6 plants.

were covered with ice, still many seagrass plants survived (Fig. 4). In February, a second extremely cold period occurred with day temperatures below –15 °C (without ice-cover). The remaining plants were lost subsequently.

At the donor site, a mass appearance of white cotyledons in January 1994 indicated large-scale germination. Most of them seemed able to develop into adult plants during spring, having generative shoots in summer. The seedlings at the control transplantation developed synchronously with seedlings from the natural donor population (Hermus 1995). At the transplantation sites, all *Zostera marina* seedlings emerged within a few meters from the former eelgrass plots. At Balgzand 3 seedlings were recorded in February, which did not survive the subsequent cold period. At Wierschuur, 87 seedlings were recorded in the period February to May, with a maximum number in April (66 seedlings), predominantly between 0 and –0.10 m MSL. They did not survive. The cotyledons had a very dense epiphyte cover (50 %) during spring, which was not observed at donor site. Subsequently, relatively few seeds germinated in June and July, and developed into generative plants forming seeds. They disappeared during the following spring.

DISCUSSION

Water dynamics

No losses of *Zostera marina* transplantations occurred in exclosures that reduced water dynamics, in contrast to the complete loss of the unprotected transplants at depths greater than –0.20 m MSL. The failure of plants to establish at deeper sites, where the water dynamics were more severe, and the rapid disappearance of *Z. marina* plants after removal of the exclosures after 1 month (Watanabe & Terawaki 1986 found that 1 month was sufficient to establish), must have been due to the water dynamics being too severe at these depths. No differences were found between the number of *Z. marina* shoots in exclosures along the tidal gradient from –0.60 to –1.15 m MSL. Apparently, during the growing season, light availability is still sufficient at a depth of –1.15 m MSL and light limitation could not have caused the loss of unprotected seagrass at all depths greater than –0.20 m MSL.

Bioturbation, often causing transplantation losses along the eastern and southern shores of the United States (Fonseca et al. 1994, 1998, Davis et al. 1998, Hammerstrom et al. 1998), had no effects on the transplantations in the Dutch Wadden Sea (see 'Introduction'). This was further evidenced by our finding that the plants disappeared also in enclosures without tops (0 to 5 % reduced water dynamics, entrance of large animals unlikely and not observed), half of the plants disappearing in exclosures with gauze tops (40 to 50 % reduced water dynamics, no animals larger than 1 mm could enter), whereas all plants remained in the plexiglass-topped exclosures (80 to 100 % reduced water dynamics). The differences between these 3 treatments were significant.

In exclosures at –0.60 m MSL, *Zostera marina* tended to perform better than at –0.40 m MSL, which may be due to the sedimentation rate being higher in the exclosures at –0.40 m MSL. In the latter, 8 cm of sediment was deposited in 1 month as compared to 5 cm in the former. Increased sedimentation is obviously caused by the reduced water dynamics in the exclosures.

Depth gradient at 3 transplantation sites

The successful establishment of *Zostera marina* transplants at Balgzand and Wierschuur between 0.0 m and –0.20 m MSL during the growing season showed that these locations met the habitat requirements of the plants, which corresponded to results from a similar experiment conducted at Wierschuur one year earlier (van Katwijk & Schmitz 1993). Transplantation success was low at higher tidal levels, which was probably due to bird foraging in this zone (Hermus 1995). All unprotected transplants with or without peg-anchoring disappeared within a few days at –0.40 m MSL and deeper, which corresponds to a relative exposure period to any wave action of more than circa 60 %. Before their disappearance, the plants were shorter than at higher tidal levels, presumably because they lost their leaves. At the transplantation site 't Horntje, plants disappeared at all depths, which was probably due to its higher exposure to water dynamics in comparison to the 2 other sites. At 't Horntje, maximal orbital velocities at the sediment surface frequently exceeded 0.60 ms^{-1} during the summer months, and the chance of breaking of waves is present. At Balgzand and Wierschuur, maximal orbital velocities at the sediment surface were circa 0.40 ms^{-1} at average during the first months after transplantation.

Water dynamics and zonation in natural *Zostera marina* beds

In intertidal *Zostera marina* beds in the Dutch and German Wadden Sea, southwest Netherlands, and, for example, the Thames estuary (at present, but also in the period that subtidal eelgrass was still present, so light was not limiting), plant cover diminished with increasing depth (Harmsen 1936, C. den Hartog personal communication, personal observation). In all cases, except perhaps in the present Thames estuary, this could not be attributed to light limitation (Harmsen 1936, Wijgergangs & de Jong 1999, this study). Our results indicate that, in non-sheltered areas, this can be attributed to increasing water dynamics along the depth gradient. This was supported by the observation that intertidal *Z. marina* beds penetrate to larger depths than usual when shelter is present, i.e. behind a mussel bed at the low tide level in Sylt, Germany (K. Reise personal communication), behind the dam encompassing the eelgrass bed at The Plaat, Terschelling, located at –0.50 m MSL and directly behind an island at Roscoff, France (C. den Hartog personal communication).

In a seeming contrast with our findings is the luxurious growth of *Zostera marina* around low tide in the Wadden Sea at the beginning of the twentieth century (Harmsen 1936, de Jonge & Ruiter 1996). At that time, 2 (not necessarily genetically based) morphotypes of *Z. marina* were present, a robust, perennial morphotype, and a flexible (often annual) morphotypes of *Z. marina* (Harmsen 1936). These morphotypes were also described for other parts in the world (Harmsen 1936, Tutin 1938, Keddy & Patriquin 1978). The morphotype that occurred in the subtidal zone can withstand higher water dynamics, because of its robustness, and its relatively larger belowground biomass providing better anchoring facilities. It could not extend towards higher tidal levels because it is more susceptible to desiccation than the flexible type of *Z. marina* that grows in the mid-intertidal zone (Harmsen 1936, Tutin 1938, Keddy & Patriquin 1978, van Katwijk et al. in press). Knowing this, our results can explain why non-vegetated zones existed at non-sheltered locations in the Wadden Sea and in the Thames estuary in the 1930s (Harmsen 1936): the water dynamics was too high for the flexible type of *Z. marina*, and the period of emergence during low tide was too long for the robust type of *Z. marina*.

Sediment stabilisation

Sediment stabilisation with shells delayed, but did not prevent, the loss of the transplanted plants at –0.40 m MSL, and in Wierschuur also at –0.60 m MSL. The delay varied between 6 and 76 days. At greater depths, no effect of stabilisation by shells was recorded. These results are consistent with previous findings at Wierschuur (van Katwijk & Schmitz 1993). Furthermore, this previous experiment showed a distinct positive effect of shell stabilisation at –0.20 m MSL, whereas no effect was found at 0.0 and +0.15 m MSL, which may be due to a water draining effect of the shells (larger interstitial spaces in the sediment) causing desiccation of the plants (van Katwijk & Schmitz 1993). Therefore, the application of shell armour is particularly useful at the lower limit of potential mid-intertidal habitats of *Zostera marina*.

Generative phase, overwintering and recruitment from seeds

The transplanted eelgrass plants expanded vegetatively and reached the generative phase in synchrony with the natural vegetation at the donor site (Hermus 1995). The number of *Zostera marina* plants gradually decreased during autumn, which is the normal period of die-off and can be explained by dropping temperatures, storms, and ice-cover (den Hartog & Polderman 1975, D.J. de Jong & R.M. Asmus personal communication, personal observation) and grazing by overwintering birds (Jacobs et al. 1981).

At the donor site at De Plaat, an untimely large-scale germination of *Zostera marina* was observed in January (April is normal), as a result from unusually low salinities (Hootsmans et al. 1987, Hermus 1995), caused by exceptionally high river discharges at this time. Likewise, at Balgzand, the majority of seeds may have germinated in January, and the seedlings were possibly lost during a subsequent severe storm period in February. Unfortunately, the storm period prevented monitoring to confirm this. At the transplantation site Wierschuur (which is less influenced by freshwater discharges), we frequently noted small numbers of seedlings during February to May that became covered by epiphytes and did not survive; unlike the seedlings at the control site and donor site at De Plaat. Therefore, the Wierschuur habitat did not fulfil the requirements for *Z. marina* seedlings during the spring period. However, some seeds did germinate successfully later in the season, and developed into generative plants forming seeds. Apart from the reasons mentioned above, the failure of the transplantations to establish from seeds may due to the low number of shoots used in these small-scale experiments.

CONCLUSIONS AND RECOMMENDATIONS

Water dynamics in the Dutch Wadden Sea is too high for the establishment and maintenance of intertidal Zostera marina when maximal orbital velocity at the sediment frequently exceeded 0.60 ms^{-1}. At sites where the average maximal orbital velocity at the sediment reached ca 40 ms^{-1}, transplantation success was depth related, which was attributed to increasing periods of exposure to water dynamics at increasing depth. Transplantation failed below –0.20 m MSL, corresponding to a relative period of exposure to wave action of ca 60 %. Light limitation nor bioturbation could explain these results. To increase transplantation success of Z. marina, the availability of a sheltered location is recommended. At a local scale, a stable mussel bed could provide such a shelter (N. Dankers personal communication). Also, offering local and temporal shelter by (degradable) dam-like structures, can provide refugia from where a transplantation could expand. In the Wadden Sea, eelgrass experiments with such stabilising constructions are planned. Sediment stabilisation by shell armour is recommended at the lower depth limit of an eelgrass transplantation. Depth limitation of intertidal Z. marina by water dynamics can explain zonation patterns that occur in natural eelgrass beds in several tidal systems in northwest Europe.

*Acknowledgements.*This study has been financed by the Ministry of Transport, Public Works and Water Management. We thank Prof. Dr. V.N. de Jonge, Prof. Dr. C. den Hartog, D.J. de Jong, Dr. J.G.M. Roelofs, Prof. Dr. G. van der Velde and B. Kelleher for useful discussions and comments on the manuscript. Hydraulic information was kindly supplied by Prof. Ir. H.J. Verhagen of the International Institute for Infrastructural, Hydraulic and Environmental Engineering in Delft. We thank Dr. R.M. Asmus, Dr. H. Asmus and Prof. Dr. K. Reise of the Alfred Wegener Institute in Sylt, Germany, for advise. We thank workers of the Ministry of Transport, Public Works and Water Management, students of the University of Nijmegen and several volunteers for their efforts in the fieldwork. G.H.W. Schmitz, L.J.M. Wijgergangs and P.H. van Avesaath are thanked for useful consultation and assistance during the research. The Technical Department, the Illustration Service and the Department of Statistical Consultation of University of Nijmegen are thanked for constructing the exclosures, preparing the figures and providing statistical advise, respectively.

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CHAPTER 6

GENERAL DISCUSSION

HABITAT SUITABILITY OF THE WADDEN SEA FOR RESTORATION OF *ZOSTERA MARINA* BEDS

van Katwijk MM, Hermus DCR, de Jong DJ, Asmus RM, de Jonge VN (in press)
Helgol Mar Res 54

GENERAL DISCUSSION

HABITAT SUITABILITY OF THE WADDEN SEA FOR RESTORATION OF *ZOSTERA MARINA* BEDS

ABSTRACT. A conceptual model is proposed describing potential *Zostera marina* habitats in the Wadden Sea, based on reported data from laboratory, mesocosm and field studies. Controlling factors in the model are dynamics, degree of desiccation, turbidity, nutrients and salinity. A distinction has been made between a higher and a lower zone of potential habitats, each suitable for different morphotypes of *Z. marina*. The model relates the decline of *Z. marina* in the Wadden Sea to increased sediment and water dynamics, turbidity, drainage of sediments (resulting in increased degree of desiccation) and total nutrient loads during the twentieth century. The upper and lower delineation of both the higher and the lower zone of potential *Z. marina* habitats appear to be determined by one or a combination of several of these factors. Environmental changes in one of these factors will therefore influence the borderlines of the zones. The lower zone of *Z. marina* will be mainly affected by increased turbidity, sediment dynamics, degree of desiccation during low tide and nutrient load. The higher zone will be affected by increases in water and sediment dynamics, desiccation rates and nutrient loads. Potential *Z. marina* habitats are located above approx. –0.80 m mean sea level (when turbidity remains at the same level as in the early 1990s), at sheltered, undisturbed locations, and preferably where some freshwater influence is present. At locations with a high, near-marine, salinity, the nutrient load has to be low to allow growth of *Z. marina*. The sediment should retain enough water during low tide to keep the plants moist. Our results suggest that the return of *Z. marina* beds within a reasonable time scale will require not only suitable habitat conditions, but also revegetation measures, as the changes in the environment resulting from the disappearance of *Z. marina* may impede its recovery, and the natural import of propagules will be unlikely. Furthermore, the lower zone of *Z. marina* may require a genotype that is no longer found in the Wadden Sea.

INTRODUCTION

Seagrass beds have drastically declined during the last century. Most of the recorded declines are attributable to human activity (Short & Wyllie-Echeverria 1996). In the western part of the Wadden Sea, one of the world's largest international marine wetland reserves (appr. 6000 km^2), an area of subtidal and low-intertidal *Zostera marina* L., ranging between 65 and 150 km^2, was lost during an outbreak of wasting disease in the 1930s (van Goor 1921, den Hartog & Polderman 1975, den Hartog 1987, de Jonge & Ruiter 1996). No old records exist for the eastern and northern Wadden Sea, except for Königshafen in Sylt (Nienburg 1927, Fig. 1). Here also, a subtidal *Z.marina* bed was damaged beyond recovery by wasting disease during the 1930s (Wohlenberg 1935, den Hartog 1987, Reise et al. 1989). At present, approximately 2 km^2 of *Z. marina* remains in the Dutch Wadden Sea, at Terschelling and in the Ems estuary (D.J. de Jong unpublished results, Fig. 1). In the German Wadden Sea, *Z. noltii* and *Z. marina* together

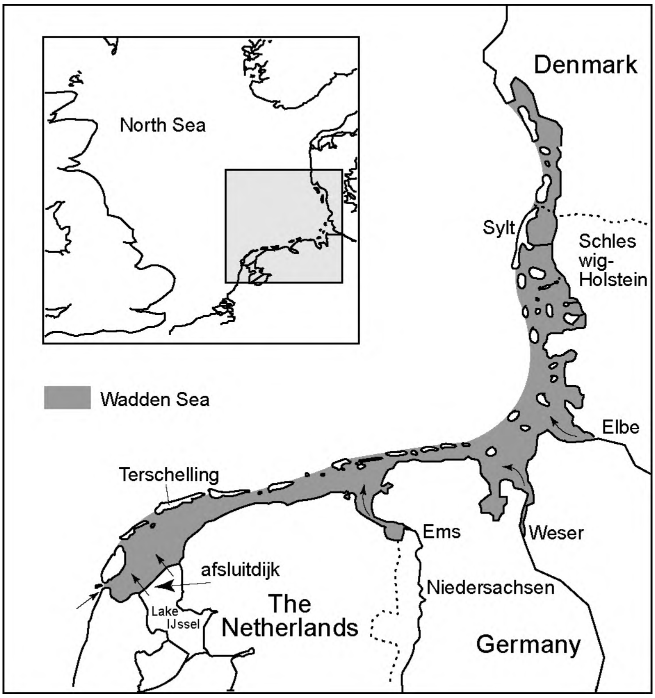

Fig. 1. The Wadden Sea with locations mentioned in the text. Indirect and direct riverine influences are indicated by arrows

cover approximately 170 km², and in the Danish part about 30 km² (Reise & Buhs 1991). In 1987, the Ministry of Transport, Public Works and Water Management in The Netherlands initiated the project "Reintroduction of seagrass in the Dutch Wadden Sea". The present literature study is a part of this project.

The presence of potential seagrass habitats is a prerequisite for successful revegetation. Important factors influencing the occurrence of *Zostera marina* are: turbidity (e.g. Giesen et al. 1990a, b, van Katwijk et al. 1998), disturbance, which in the Wadden Sea is mainly caused by shellfish exploitation (de Jonge & de Jong 1992), water dynamics (Hermus 1995, Fonseca & Bell 1998, van Katwijk & Hermus in press), sediment dynamics (Boley 1988, Fonseca 1996), degree of desiccation (Harmsen 1936), nutrient level (e.g. Short & Wyllie-Echeverria 1996, van Katwijk et al. 1997) and salinity (Kamermans et al. 1999, van Katwijk et al. 1999).

As well as the role played by wasting disease in the large-scale decline of *Zostera marina* in the western Wadden Sea, the construction of the Afsluitdijk dam closed off the Zuyderzee (now the freshwater Lake IJssel) from the Wadden Sea (Fig. 1), altered water dynamics, erosion and sedimentation patterns and increased turbidity temporarily (de Jonge & de Jong 1992). Furthermore, two subsequent years with a considerable deficit of sunlight occurred (Giesen 1990). There is no consensus over which of these events (or combination of events) caused the decline in seagrass beds (review in den Hartog 1996, de Jonge et al. 1996). Main causes for the lack of recovery of eelgrass stands in the Dutch Wadden Sea were thought to be high turbidity and disturbance caused by shellfish fishing (van den Hoek et al. 1979, Giesen et al. 1990a, b, de Jonge & de Jong 1992). In the northern part of the Wadden Sea, the same three factors coincided with *Z. marina* losses;

the outbreak of wasting disease (Wohlenberg 1935), sunlight deficit in spring (Giesen 1990), and the construction of the Hindenburg dam in 1927, although the latter had less impact than the construction of the Afsluitdijk dam, as it was built on tidal flats and no large channels were dammed.

In the present study, knowledge on the habitat requirements of *Zostera marina* was derived from published results of laboratory, mesocosm and field experiments. This was used to construct a conceptual model which assessed the influence of the relevant factors on the potential habitats of *Z. marina* along a tidal gradient in the Wadden Sea. The model considered the physical, chemical and biological properties of the Wadden Sea.

The model creates a basis for the selection of potential sites for restoration, and for measures and policies to increase the chance of successful restoration of *Zostera marina*. Moreover, the likelyhood of a natural recovery of *Z. marina* will be discussed on the basis of the model.

ZOSTERA MARINA ZONATION IN THE WADDEN SEA

In the 1930s, Harmsen (1936) investigated *Zostera marina* at several locations in the Wadden Sea. Two *Z. marina* zones, separated by a large bare strip of sediment, were noted. This was also observed by Nienburg in Königshafen, Sylt, Germany (in Wohlenberg 1935, Reise et al. 1989). The zones are inhabited by different morphotypes of *Z. marina* (Fig. 2), and can be described as follows:

1. *Zostera marina* beds in the intertidal zone (around mean sea level, the mid-intertidal zone) consist mostly of annual plants. When exposed, the plants lay flat on the moist sediment, and so are protected against desiccation (Harmsen 1936). The degree of desiccation during low tide determines the upper limit of *Z. marina* occurrence (Hermus 1995, Leuschner et al. 1998). The lower end of this zone is most probably limited by higher water dynamics (Hermus 1995, van Katwijk & Hermus in press, Fig. 2).

   The tidal depth of the upper limit varies between mean sea level and 30 cm above it (Schellekens 1975 combined with unpublished results Rijkswaterstaat, Boley 1988, Hermus 1995) and was even higher before the closure of the Zuyderzee in 1932 (Harmsen 1936). The lower limit is maximally situated a few decimetres beneath mean sea level (Harmsen 1936, Schellekens 1975, Boley 1988, Hermus 1995, de Jonge et al. 1996). Only in sheltered areas (Königshafen) it may have extended to the low tide line (Nienburg 1927).

2. *Zostera marina* in the low intertidal to subtidal zone are perennial plants (van Goor 1919, Harmsen 1936). Harmsen (1936) observed throughout the Wadden Sea that this zone never occurred more than 0.20 m above Mean Low Tide. The plants were more robust than the annual forms, and possessed stiff sheaths that could not lie flat on the sediment. The plants tolerated only short periods of emersion before the sheaths desiccated (Harmsen 1936). This morphotype of *Z. marina* protruded maximally one or two decimetres above low tide (van Goor 1920, Wohlenberg 1935, Harmsen 1936). Recent calculations with old data suggest that about half these plants were located

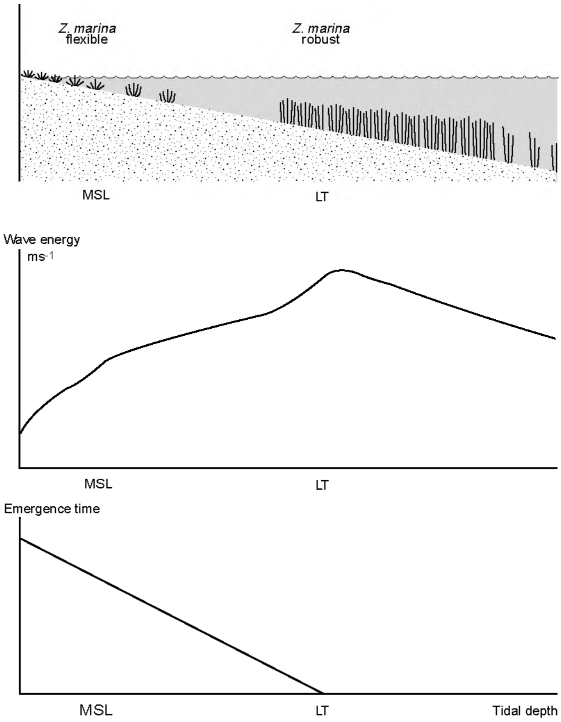

Fig. 2. Zonation of *Zostera marina* in the Wadden Sea in relation to emergence time during low tide (LT) and wave energy (orbital velocity at the sediment surface, based on model calculations by M. van Helvert and D.J. de Jong). The shape of the wave energy profile is independent of slope, fetch and tidal range, although the height of the profile varies depending on these factors. With increasing wind velocity the maximum wave energy shifts down from –0.1 m to –0.4 m beneath low tide during severe storms. MSL Mean sea level

above low tide and the other half beneath it (de Jonge & Ruiter 1996). The lower limit of this zone was probably determined by light limitation and strong currents (e.g. Oudemans 1870).

Using the model, this means that at most locations in the Wadden Sea, a bare intertidal zone exists which is not suitable for *Zostera marina* growth. This zone is located somewhere between mean sea level and low tide and its width varies, depending on water dynamics and degree of desiccation. Water dynamics in this bare zone are too high for the mid-intertidal morphotype, and the robust morphotype from the lower zone will desiccate during prolonged periods of low tide.

Areas with reduced water dynamics are an exception to this proposition. For example, the population at “de Plaat”, Terschelling (Fig. 1) is located at –0.50 m mean sea level. This tidal flat is protected by embankments. Also, in areas with barriers which retain water at all tidal levels, the robust morphotype of *Zostera marina* may extend to higher levels, as has been observed in Roscoff, France (van Katwijk 1992). In Königshafen, in the German Wadden Sea, a permanent water layer of a few centimetres covers the intertidal flats. However, the robust morphotype of *Z. marina* was not encountered here (Wohlenberg 1935). Probably, the sheaths would have still protruded above the water surface and desiccated.

The extent of genetic differentiation between these morphotypes is unknown. In the United States, some genetic differentiation between intertidal (not necessarily annual) and subtidal populations was found with regard to aspects of habitat response, morphology, and DNA sequence (McMillan & Phillips 1979, Backman 1991, Fain et al. 1992, Alberte et al. 1994), although limited gene flow may occur (Laushman 1993). Along the North Atlantic coast, seeds from perennial plants developed into annual plants and vice versa, although 90 to 95 % of the seeds developed into plants of the parent morphotype (Keddy & Patriquin 1978).

In the Wadden Sea, reciprocal transplantation experiments between high intertidal and subtidal populations were carried out by Harmsen (1936). He used intertidal seedlings, but subtidally he could only find mature plants, so he used rhizome cuttings with young shoots for his transplantations (bare root). The transplanted seedlings were lost in the subtidal zone, whereas they survived in the intertidal zone. The transplanted subtidal plants died in the intertidal environment, but survived in the subtidal. These results may indicate genetic differences, but there is also another possible explanation: the sheaths of subtidally-raised plants were probably already too stiff and desiccated in the intertidal area. When raised in the intertidal zone from seed, this might not have happened. The loss of seedlings originating from the intertidal that were transplanted to the subtidal, may have been caused by high water dynamics or shading by other plants. This is supported by the absence of seedlings in natural subtidal *Z. marina* beds in the Dutch Wadden Sea (van Goor 1919). A mesocosm experiment, using five west-European populations originating from subtidal and intertidal populations, showed partial reductions in morphological, physiological and reproductive differences after transplantation to the mesocosm, suggesting both phenotypic plasticity and genetic differentiation (van Katwijk 1992, van Katwijk et al. 1998).

DYNAMICS AND DESICCATION

The term dynamics entails both water and sediment dynamics. Fig. 3 depicts the causes and consequences of increased dynamics. Increased dynamics occur mainly as a consequence of disturbances like increased construction and fishery activities, and the disappearance of subtidal seagrass beds. Water dynamics affect a number of other parameters. Firstly, increased water dynamics may result in increased sediment dynamics, intensified sedimentation and erosion cycling, until a new equilibrium between bottom morphology and water dynamics has been reached (de Jonge 1983). Secondly, an increased suspension of fine material may occur, subsequently resulting in increased turbidity and a larger average grain size of the sediment (e.g. Ehlers 1988). The latter may increase water drainage and so lead to rapid desiccation of plants in the intertidal zone during low tide (Fig. 4).

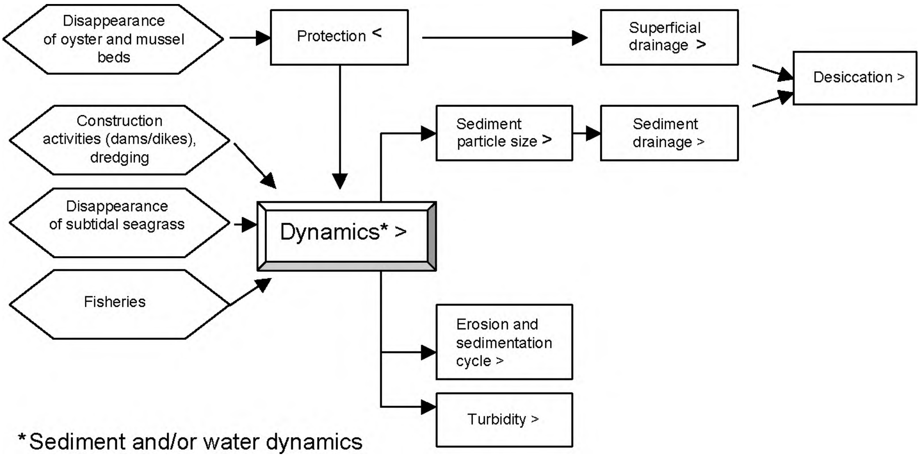

Fig. 3. Causes and consequences of increased dynamics in the Wadden Sea

Dynamics

The importance of low water dynamics, both wave energy and current velocity, to seagrass distribution in the western Atlantic is well documented (e.g. Fonseca & Bell 1998). In the Wadden Sea, it is likely that water dynamics directly determine the lower limit of the high *Zostera marina* belt (van Katwijk & Hermus in press).

Increased erosion and sedimentation negatively affect *Zostera marina* establishment. On the west coast of the United States, it is suggested that erosion rates of 0.5 mm day^{-1} and burial rates of 0.3 mm day^{-1} are the limits for *Z. marina* survival (Merkel 1992 in Fonseca et al. 1998). However, little is known about detrimental erosion and sedimentation rates in the Wadden Sea. To our knowledge, only one study, performed in the German Wadden Sea, dealt with this subject. It was found that *Z. marina* disappeared when the sedimentation rate exceeded 1 cm year^{-1} on average, over a period of 13 years (Boley 1988).

Oyster beds disappeared in the intertidal zone of the Wadden Sea at the beginning of the twentieth century. Stable mussel beds disappeared around 1990, except for a few in the German Wadden Sea (Beukema 1992, Rudfeld 1992, Dankers 1993, Nehls & Thiel 1993, Beukema & Cadée 1996, Reise 1998b). Stable, elevated oyster and mussel beds act as effective wave breakers, especially when located around low tide level (Consemulder 1984, van der Linden 1985). Their decrease may, therefore, have contributed to an increase in wave dynamics, resulting in an upward shift of the lower limit of the high belt of potential *Zostera marina* habitats (Fig. 4). Secondly, the removal of mussel beds around 1990 resulted in increased phytoplankton blooms, which increased turbidity (Beukema & Cadée 1996).

In the western Wadden Sea, water dynamics changed following the closure of the Zuyderzee. Since then, the tidal amplitude has increased by 0.3 to 0.5 m (Thijsse 1972, de Jonge & de Jong 1992). Furthermore, the closure of the Zuyderzee resulted in a change in the locations of the channels and, as a consequence, altered erosion and sedimentation patterns. Turbidity also increased temporarily (Thijsse 1972, van den

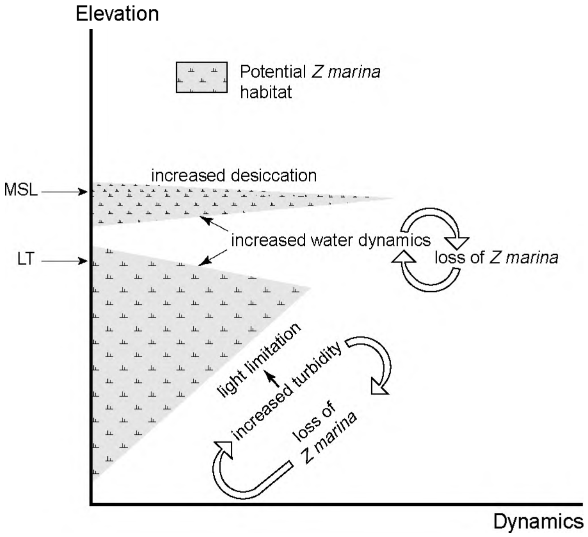

Fig. 4. Potential *Zostera marina* habitats in relation to dynamics and related factors (see Fig. 3 and text). MSL Mean sea level, LT low tide

Hoek et al. 1979, Glim et al. 1987, den Hartog 1987, Ehlers 1988, de Jonge & de Jong 1992). In other parts of the Wadden Sea, the construction of dams and dikes have had similar effects (e.g. Asmus & Asmus 1998, Reise 1998a).

Desiccation

The Wadden Sea sediments have become coarser, as a result of (1) the loss of vast areas of *Zostera marina* (Rasmussen 1977, Fonseca 1996), (2) the extensive coastal engineering works in the southwest of The Netherlands that have resulted in a reduced transport of fluvial mud to the sea (de Jonge et al. 1993), and (3) the increased intensity of land reclamation and dike construction activities during the last century (e.g. Flemming & Nyandwi 1994). Increased sediment grain size, and the rapid superficial runoff of tidal water due to the disappearance of elevated mussel beds, may have resulted in an increased degree of desiccation of the sediment surface during low tide (Fig. 3). A more rapid desiccation in the intertidal zone will result in a downward shift of the upper limit of the low *Z. marina* belt (Fig. 4).

Conclusion

The effects of the direct and indirect consequences of altered dynamics on potential *Zostera marina* habitats along the tidal gradient are depicted in Fig. 4. The horizontal axis "dynamics" includes water dynamics, sediment dynamics and the derived effects, grain size of the sediment, turbidity and the degree of desiccation. We have assumed that increased water dynamics will also affect the upper limit of the low *Zostera* belt, as the zone just below low tide experiences the highest wave energy at the sediment surface (Fig. 2, van Katwijk & Hermus in press). We speculate that increased water dynamics may reach a level that is too high, even for these robust *Z. marina* plants, to withstand. In this case, we conclude that disappearance of *Z. marina* belts will begin at the upper limit of this zone (Fig. 4).

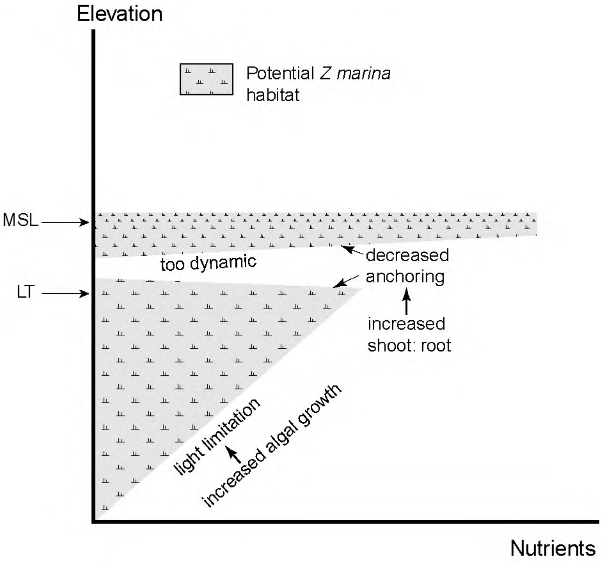

Fig. 5. Potential *Zostera marina* habitats in relation to nutrients

NUTRIENTS, SALINITY, TURBIDITY AND DIRECT DISTURBANCE

Nutrients

Zostera marina is adapted to low nitrogen concentrations (Borum et al. 1989, Hemminga et al. 1991, Pedersen & Borum 1992). Enrichment of the water column, with either nitrate, ammonium or phosphate, or a combination of them, can lead either to increased growth of *Z. marina* (Harlin & Thorne-Miller 1981, Bohrer et al. 1995), or to negative effects on its growth or productivity (Burkholder et al. 1992, 1994, Neckles et al. 1993, Williams & Ruckelshaus 1993, Taylor et al. 1995, Boynton et al. 1996, Nelson & Waaland 1997, van Katwijk et al. 1997). The effects may be direct (Burkholder et al. 1992, 1994, van Katwijk et al. 1997) or indirect, as a consequence of light limitation or oxygen deprivation due to increased algal growth (Neckles et al. 1993, Williams & Ruckelshaus 1993, den Hartog 1994, Harlin 1995, Short et al. 1995, Taylor et al.1995). Fig. 5 shows the direct and indirect effects of nutrient enrichment on *Z. marina* along the tidal gradient in the Wadden Sea.

In general, experimental nutrient enrichment of sediments has positive effects on *Zostera marina* (Orth 1977, Short 1983, Roberts et al. 1984, Short 1987, Kenworthy & Fonseca 1992, Murray et al. 1992, Williams & Ruckelshaus 1993, van Lent et al. 1995). However, in many cases, nutrient enrichment of the total system (increased loads) leads to the disappearance of *Z. marina* (e.g. Taylor et al. 1995, Boynton et al. 1996, Short & Wyllie-Echeverria 1996). It seems that prolonged exposure to increased nutrient loads has a negative effect on *Z. marina*, whereas temporary increases have a stimulating effect. N-enrichment of the water layer effects *Z. marina* negatively. Leaves, unlike roots, cannot regulate nitrogen uptake, and assimilation requires energy from the plant (see van Katwijk et al. 1997).

The processes that contribute to eutrophication are related to increased human populations and have significantly increased with industrialisation, and these were further accelerated when artificial fertilisers were introduced. In the Wadden Sea, nutrient loads increased until the mid-1980s, as a consequence of increased inputs of riverine origin (e.g. de Jonge & Postma 1974, Höpner 1991, de Jonge 1997). Nutrients reach the Wadden Sea directly from Lake IJssel, the Rivers Ems, Weser and Elbe, and indirectly from the River Rhine via the Dutch coastal zone (Fig. 1).

Secondly, internal eutrophication occurs as a consequence of mineralisation processes. These processes are driven by the input of organic matter (Postma 1966), for which the Wadden Sea acts as a sink (e.g. Postma 1954). The result of mineralisation is increased levels of N (Helder 1974) and P (de Jonge & Postma 1974). The input of living and dead organic matter into the Wadden Sea has increased, mainly due to increased phytoplankton levels in the coastal zone of the North Sea. (de Jonge & Postma 1974, de Jonge 1990, de Jonge et al. 1993). Thirdly, the nutrient load of the Wadden Sea has increased due to a 2 to 4-fold increase of the atmospheric deposition of N, assuming a pristine load of 5 kg N $ha^{-1}yr^{-1}$ (de Jonge & van Meerendonk 1990, Isermann 1990, Houdijk & Roelofs 1991, Schlünzen 1994, Mansfeldt & Blume 1997).

Since the 1980s, nutrient levels in the Wadden Sea have stabilised or decreased, although in the more unspoilt areas in the north, an increase has been measured in recent years (Martens 1989a,b, Bakker et al. 1991, Schneider & Martens 1994, de Jonge 1997, Martens & Elbrächter 1998).

Increasing nutrient loads in the Wadden Sea will have consequences for potential *Z. marina* habitats. In cases where increased algal concentrations decrease light penetration, the depth limit for the lower *Z. marina* belt may rise (e.g. Taylor et al. 1995). Secondly, the high *Zostera marina* belt may be hindered by the increased amount of opportunistic macroalgae such as *Ulva* spp. and *Enteromorpha* spp., that can suffocate the plants (e.g. den Hartog 1994). In both belts, nitrogen loads may eventually become toxic (Burkholder et al. 1992, 1994, van Katwijk et al. 1997) (Fig. 5).

Frequently, increased nutrient concentrations in nutrient-limited aquatic systems result in an increased shoot:root ratio of water plants (e.g. Roelofs et al. 1984, 1994). In *Z. marina*, this was found in a field experiment in which the sediment was enriched with ammonium (van Lent et al. 1995). In another field study, Short (1983) observed that root development and the number of root hairs were lower when sediment ammonium concentrations were high, as compared with low sediment ammonium concentrations. As a consequence of the increased shoot:root ratio, anchoring ability decreases, making the plants more vulnerable to water dynamics. Therefore, the zone, where no *Z. marina* growth is possible due to increased water dynamics, is expected to widen (Fig. 5).

In the high *Zostera marina* zone, the plants can probably tolerate the adverse effects of water quality better than when the plants are permanently submerged, because the plants are exposed to the water for a shorter period (during high tide). During low tide, they remain photosynthetically active as long as the leaves remain moist (e.g. Leuschner & Rees 1993). Therefore, we assume that the low *Z. marina* belt will disappear at lower nutrient loads than the high *Z. marina* belt (Fig. 5).

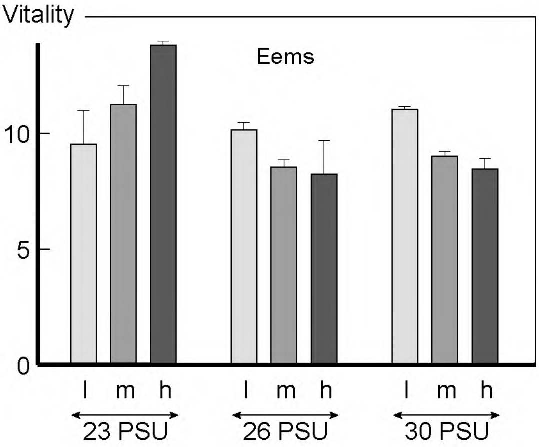

Fig. 6. Vitality of *Zostera marina* originating from an intertidal estuarine bed in the Eems estuary, after 6 weeks at different combinations of salinity and nutrient loads. L low, m medium, h high nutrient load, N:P, 20:20, 95:45, 625:100 kg $ha^{-1}yr^{-1}$, respectively. Means and SEM are indicated

Salinity

High (near-marine) salinity is unfavourable to *Zostera marina* (Kamermans et al. 1999, van Katwijk et al. 1999). Assuming that plants living in unfavourable conditions will tolerate less stress from other factors, high salinity will magnify the negative effects of high water dynamics and high nutrient loads. Van Katwijk et al. (1999) found an interaction between salinity and nutrients; at a salinity of 26 or 30 PSU, a moderate nutrient enrichment of the water layer had a negative effect, while the same nutrient application had a stimulating effect at a lower salinity (Fig. 6). 'Vitality' in Fig. 6 is a combined factor derived from the number of shoots, size, necrosis and number of missing leaves (van Katwijk et al. 1999). At high salinity, *Z. marina* performed well when nutrient loads were low. This finding is reflected in the distribution pattern of *Z. marina* in The Netherlands where it is absent at high nutrient loads, except when salinity is low (van Katwijk et al. 1999).

Although some adaptation to high salinity, genetically or phenotypically, is likely (van Katwijk et al. 1998, Kamermans et al.1999), we hypothesise that *Zostera marina* will still be more vulnerable to other stress factors when subjected to high salinity. Salinity-stressed plants, having a lower growth rate (Kamermans et al. 1999) will not be able to use the extra nutrients for growth (Pedersen 1995). These surplus nutrients will actually burden the plants (Burkholder et al. 1992, 1994, van Katwijk et al. 1997, van Katwijk et al. 1999). This hypothesis concerning potential habitats with regard to salinity and nutrients is depicted in Fig. 7. We assumed that the plants will respond to nutrients in a bell-shaped gaussian manner (log-transformed - see Slob 1987) at each salinity level.

The salinity regime of the Dutch Wadden Sea changed after the closure of the Zuyderzee in 1932. Based on yearly averages, salinity has decreased since 1932 (van der Hoeven 1982). However, the estuarine gradient was replaced by a variable salinity regime, with the input of fresh water being regulated by sluices. In the Northern Wadden Sea (Königshafen, Sylt), salinity was greater in the early 1990s than in the early 1980s (Schneider & Martens 1994).

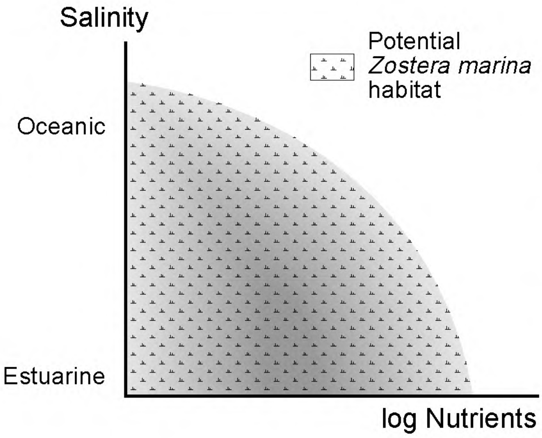

Fig. 7. Interactive effect of nutrients and salinity on potential *Zostera marina* habitats, deduced from a laboratory experiment and field observations (van Katwijk et al. 1999). Darker shades indicate greatest *Z. marina* vitality

Turbidity

Turbidity can increase due to (1) increased phytoplankton growth and (2) increased water/sediment dynamics (see previous paragraphs). Increases in turbidity during the twentieth century are thought to be the main reason for the lack of recovery of *Zostera marina* in the Dutch Wadden Sea (van den Hoek et al. 1979, Giesen et al. 1990 a,b, de Jonge & de Jong 1992). At present, turbidity has decreased (de Jonge & de Jong 1992, de Jonge et al. 1996). A mesocosm experiment with *Z. marina* plants originating from amongst others Terschelling and Sylt, showed that light was not limiting to a depth of at least –0.80 m mean sea level, assuming a tidal range of 1.80 m (van Katwijk 1992, van Katwijk et al. 1998), provided that the average turbidity factor *k* is less than 1.5 m^{-1}. This has been the case in the Dutch Wadden Sea since 1989 (de Jonge et al. 1996). This was supported by calculations by de Jonge & de Jong (1992). To our knowledge, no information is present about trends in turbidity in the German and Danish Wadden Sea.

At locations where the depth limit of the low *Zostera marina* belt is determined by light limitation rather than by the presence of channels with strong currents, increased turbidity will result in an upward shift of the depth limit of the low *Z. marina* belt. When the downward expansion of the low *Z. marina* belt is limited by the presence of a channel, increased dynamics in this channel may also shift the lower limit of *Z. marina* upwards (Figs. 4 & 5).

Direct disturbance

Fishery activity directly removes seagrass beds (de Jonge & de Jong 1992, van Katwijk 1993, de Jonge et al. 1997), and is unrelated to tidal depth. In the Wadden Sea, direct disturbance of *Zostera* is mainly caused by shellfish exploitation, e.g. mussel seed and cockles. Increased fishery activities during the twentieth century have contributed to the lack of recovery of *Z. marina* beds in the lower intertidal and subtidal zones (de Jonge & de Jong 1992). At present, shellfishing is prohibited in some areas of the Wadden Sea (Essink 1992, Rudfeld 1992, Phillipart 1993, Wonneberger 1996, Borchardt 1997).

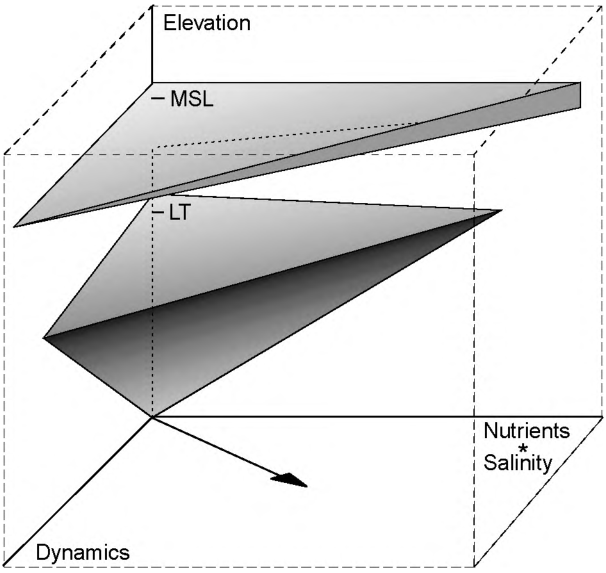

Fig. 8. Conceptual model of the potential *Zostera marina* habitats (shaded) along a tidal gradient in the Wadden Sea, in relation to dynamics (sediment and water dynamics and related factors, turbidity, grain size and degree of desiccation), and the interactive effect of nutrients (direct and indirect effects of the enrichment of the total system) and salinity. MSL Mean sea level, LT low tide

CONCEPTUAL MODEL FOR POTENTIAL *ZOSTERA MARINA* HABITATS IN THE WADDEN SEA

Model

The previously discussed effects of dynamics, desiccation, nutrients and turbidity on the distribution of *Zostera marina* over a tidal gradient are summarised in Figs. 4 & 5. The interactive effect of salinity and nutrients is shown in Fig. 7. We have integrated these 'modules' into one conceptual model, depicted in Fig. 8. The axis 'dynamics' is the same as that shown in Fig. 4, and involves water dynamics, sediment dynamics and derived effects, grain size of the sediment, turbidity and the degree of desiccation. The axis 'nutrient★salinity' represents the interactive (multiplicative) effect of nutrients and salinity: plants growing at near-marine salinity are negatively affected at moderately high nutrient loads, whereas at lower salinity, the plants tolerate much higher nutrient loads and their growth can even be stimulated. Direct disturbance caused by fishing activities was not integrated in the model as it acts on a local scale and is not related to tidal depth.

In Fig. 8, the environmental changes in the Wadden Sea during the twentieth century are indicated with an arrow in the direction of increasing dynamics, and increasing nutrients★salinity. In the western Wadden Sea, the latter is a consequence of increased nutrient loads, not salinity. Salinity in the western Wadden Sea has slightly decreased on average, but the degree of variation has increased (van der Hoeven 1982). Nutrients and salinity have both increased in the northern Wadden Sea (e.g. Schneider & Martens 1994). The various aspects of 'dynamics' (Fig. 3) have also increased in the Wadden Sea.

The conceptual model shows that the complex interaction between increased dynamics, degree of desiccation and nutrient loads in the Dutch Wadden Sea during the last century have reduced the number of potential *Zostera marina* habitats. In fact, all

Table 1. Restoration of *Zostera marina* habitats in the Wadden Sea

Factor	Required[a]	Present situation in the Wadden Sea	Recommendations[a]
Dynamics and desiccation	<	In The Netherlands, plans are discussed to restore stable mussel banks[b]	Construction of stabilisation devices or barriers (artificial, and/or stable mussel or oyster banks)
Nutrients	<	Generally reduced recently, although increased in the north[c]. Still high in comparison to the beginning of the twentieth century[d]	Further reduction
Salinity	<	Some decline, although more fluctuation in The Netherlands[e]; In N-Germany increase[f]. In The Netherlands, plans are discussed to restore the estuarine gradient[g]	Restoration of estuarine gradients; stimulation of groundwater influence
Turbidity	≤	Present turbidity allows *Z. marina* establishment to at least –0.80 m MSL[h]	Further reduction increases chances for subtidal *Z. marina*
Direct disturbance	<	Shell fishery activities are prohibited in some areas in the Wadden Sea[i]	Further limitation, with particular avoidance of potential *Z. marina* habitats

[a] This paper
[b] Faber 1999
[c] Martens 1989a, b, Schneider & Martens 1994, de Jonge 1997, Martens & Elbrächter 1998
[d] e.g. Isermann 1990, Höpner 1991, de Jonge et al. 1993, de Jonge 1997
[e] van der Hoeven 1982
[f] Schneider & Martens 1994
[g] Anonymous 1998
[h] van Katwijk et al. 1998
[i] e.g. Phillipart 1993, Wonneberger 1996, Borchardt 1997

Z. marina beds around low tide and deeper were lost in the 1930s, coinciding with a disease epidemic, and these beds have failed to recover. Also, intertidal *Z. marina* beds have suffered in the Wadden Sea, but at a much later date (den Hartog & Polderman 1975, de Jonge et al. 1996, Kastler & Michaelis 1997, Reise 1998c).

Present situation in the Wadden Sea and potential *Zostera marina* habitats

Since the end of the 1980s, the turbidity of the Wadden Sea has decreased, nutrient levels have decreased or stabilised, and shellfish fisheries have been prohibited in some areas (see previous paragraphs, Table 1).

Potential *Zostera marina* habitats in the Dutch Wadden Sea are located above approx.

–0.80 m mean sea level (when the turbidity remains at the same level as in the early 1990s). In the northern Wadden Sea, potential habitats are situated below –0.80 m, as turbidity is lower. Furthermore, potential habitats are located in undisturbed, sheltered locations, preferably where some freshwater influence is present (e.g. in estuaries, near local freshwater sources or in groundwater upwelling zones). At locations with a near-marine salinity, the present nutrient load is probably too high to allow growth of *Z. marina* in most parts of the Wadden Sea (van Katwijk et al. 1999). The sediment should retain enough water during low tide to keep the plants moist, e.g. sand containing some mud (without being unstable), or the presence of barriers. In general, areas with a diverse geomorphology are suitable for *Z. marina*, as this provides shelter and prevents rapid water runoff.

Our model suggests that the area of habitats suitable for *Zostera marina* can be increased by a (further) decrease of nutrient loads, the increase of freshwater influence (e.g. restoration of estuarine gradients), a (further) decrease in activities that rework the sediment surface (fishery on mussel seedlings, mussel culture, cockle fishing, mining of shells and sand). The enhancement of geomorphological diversity would also aid *Z. marina* recovery. This could be achieved by the application of stabilisation measures such as the recovery or restoration of stable mussel banks at the low tide level. Increased geomorphological diversity would reduce water dynamics and grain size of the sediment, and increase the water retention capacity of the area, thereby preventing desiccation of plants. Table 1 summarises these recommendations.

Natural or active restoration of *Zostera marina*

Some processes causing *Zostera marina* disappearance are accelerated by the loss of *Z. marina* itself. Among restoration ecologists, and increasingly among conservation ecologists, it is recognised that thresholds may exist in the process of degradation, where crossing a threshold precludes the return to an undegraded state without management intervention (see review in Hobbs & Norton 1996). This is probably the case for *Z. marina.* Once the bed is lost, turbidity and erosion from non-stabilised sediments and/or increased water dynamics will impair natural recovery (see Fig. 4, e.g. Fonseca et al. 1998). The return of the plants may only be possible after several consecutive years of favourable climatic circumstances at undisturbed locations; however, the time-scale required is still unknown (e.g. den Hartog 1996, Fonseca et al. 1998). Moreover, natural populations in the Wadden Sea are located in the middle, eastern and northern part. However, the prevailing winds are westerly, so propagules will have severe difficulty in reaching the western area. In general, *Z. marina* seed movement is limited to short distances (Orth et al. 1994). Finally, the genetic variation of remaining populations may be too low to cope with the unpredictable, dynamic environment of the Wadden Sea. In particular, the morphotypes with stiff sheaths, that formerly covered the lower tidal zone and became extinct in the Wadden Sea, may not evolve from the present mid-intertidal populations.

For restoration aims, it is important to gain more insight into the genetic differences among *Zostera marina* morphotypes growing at different tidal levels. If there is strong genetic differentiation in functionally important traits, it will be necessary to use donor populations that are located at approximately the same tidal level as the target location.

Also, it is unlikely that an intertidal donor stock transplanted to the intertidal will expand to the lower intertidal and subtidal regions. If genetic differentiation is low, a transplanted bed will have a greater chance of survival when an intertidal and a subtidal bed are created simultaneously: if one of them is destroyed (for instance by ice scour or severe storms) recovery will be possible from the other.

In conclusion, we estimate the chance of natural restoration of mid-intertidal *Zostera marina* in the western Wadden Sea on a human time-scale to be low. In other areas, careful site selection and possibly habitat restoration may be sufficient. The chance of natural recovery of *Z. marina* of the lower zone depends upon the ability of the present populations to adopt the morphology that is typical for the low *Zostera* belt.

Active restoration of the seagrass beds should be preceded by careful site selection. Natural restoration can only be expected at undisturbed locations, which means that sites having large potential for seagrass establishment should be protected from fishing activities. To serve both ends (active as well as natural restoration), it is essential to quantify the presented conceptual model, and map the *Zostera marina* habitat suitability in the Wadden Sea. A start in this direction was made for the Dutch Wadden Sea by de Jonge et al. (1997, in press), using the factors wave energy, currents, grain size and tidal depth in a GIS model. Combining this model with the information used in the conceptual model presented here would provide a valuable policy instrument.

Acknowledgements. We thank Prof. Dr. C. den Hartog, Prof. Dr. G. van der Velde and Dr. J.G.M. Roelofs for critically reading the manuscript, B. Kelleher for correcting the English, and L.S.A M. Hanssen for stimulating discussions. M. van Helvert is thanked for providing model results on water dynamics. Dr. H. Asmus is thanked for the information provided. The Illustration Department of the University of Nijmegen is thanked for preparing the figures.

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SAMENVATTING, AANBEVELINGEN VOOR HET BELEID EN TOEKOMSTVISIE

Een bewerkte versie van dit hoofdstuk werd gepubliceerd
in van Katwijk MM Onderwaterwoud van zeegras.
Waddenbulletin 2000-2:29-31

SUMMARY, RECOMMENDATIONS AND IMPLICATIONS

Partly published in van Katwijk MM *Zostera marina* and the Wadden Sea.
In: Phillips RC Seagrass at the millennium. In: Sheppard C (ed) Seas at
the Millenium. Warwick Univ UK/Elsevier Amsterdam (in press)

Samenvatting, aanbevelingen voor het beleid en toekomstvisie

Is herstel van Groot zeegras in de Waddenzee mogelijk?

Sinds 1987 wordt door de Katholieke Universiteit Nijmegen onderzoek gedaan naar de mogelijkheden tot herstel van Groot zeegrasvelden in de Waddenzee en de standplaatsvoorwaarden van Groot zeegras. Het onderzoek gebeurt in opdracht van het Rijksinstituut voor Kust en Zee (RIKZ) van Rijkswaterstaat. Resultaten van verschillende veld-, bassin-, laboratorium- en bureaustudies zijn verwerkt in dit proefschrift en worden hieronder kort samengevat.

Groot zeegras vroeger

Tot in de 1930er jaren kwam er in de westelijke Waddenzee 6.000 tot 15.000 hectare Groot zeegras (*Zostera marina* L.) voor (Fig. 1). Veel mensen kennen het zeegras nog

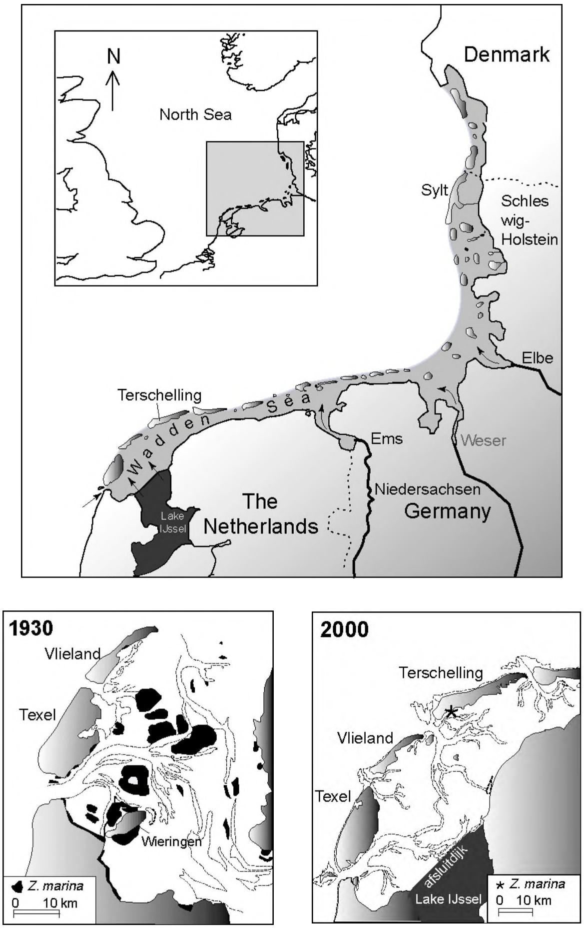


Fig. 1. The Wadden Sea and the decline of *Zostera marina* in the western part during the thirties, derived from den Hartog & Polderman (1975). Presently, one mid-intertidal *Z. marina* bed of 5.8 ha remains in this area (de Jong 2000). Black arrows indicate major freshwater influences

Groot zeegras in het westelijk deel van de Waddenzee in de dertiger jaren (afgeleid van den Hartog & Polderman 1975). Momenteel is er in dit gebied alleen nog een zeegrasveld van 5.8 ha bij Terschelling. Zwarte pijlen geven de voornaamste zoetwaterinvloeden weer

SUMMARY, RECOMMENDATIONS AND IMPLICATIONS

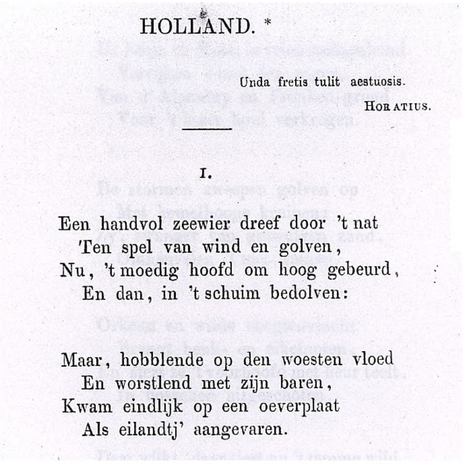
THE WADDEN SEA AND *ZOSTERA MARINA* BED RESTORATION

The Wadden Sea is one of the world's largest international marine wetland reserves (appr. 6000 km^2), bordering the coasts of The Netherlands, Germany and Denmark. Before the 1930s, The Dutch Wadden Sea contained large beds of subtidal and low-intertidal *Zostera marina* L. covering an area between 65 and 150 km^2 (Fig. 1, Oudemans et al. 1870, den Hartog & Polderman 1975). These seagrass beds were of great economic importance (Fig. 2). The seagrass was used as roofing and isolation material, and to fill mattresses and cushions. Before 1857 it was used to build dikes (Martinet 1782, Sloet tot Oldhuis 1855, Oudemans et al. 1870). Considering the importance of dikes to The Netherlands, it is no wonder that in the past a proverb was used to describe the harvest ('good hay grass, good sea grass'), a special prayer day was held to invoke a bumper crop, and lyrical descriptions and poems about seagrass were written during the 18th and 19th century (Fig. 3, see also Fig. 1 in chapter 1, Sloet tot Oldhuis 1855). Less is known about the past German and Danish beds. They had small or no economic value (van den Hoek et al. 1979).

During the 1930s, the seagrass cover was largely lost and the beds never recovered (e.g. den Hartog 1987, Reise et al. 1989). Presently, *Z. marina* occurs only in the mid-littoral; approximately 2 km^2 of *Z. marina* in The Netherlands (Wijgergangs & de Jong 1999); in the German Wadden Sea, *Z. noltii* and *Z. marina* together cover approximately 170 km^2, and in the Danish part ca. 30 km^2 (Reise & Buhs 1991). The large-scale decline of *Z. marina* coincided with (1) the outbreak of wasting disease associated with the slime-mold *Labyrinthula zosterae* (2) increased diking and damming activities and (3) two subsequent years with a considerable deficit of sunlight. There is no consensus about which of these events (or combination of events) caused this decline (reviews in den Hartog 1996, de Jonge et al. 1996). Main causes for the lack of recovery of eelgrass stands in the Dutch

Fig. 2. Landing of eelgrass crop at the beginning of the twentieth century

Lossen van de wieroogst aan het begin van de twintigste eeuw



HOLLAND. *

Unda fretis tulit aestuosis.
HORATIUS.

I.

Een handvol zeewier dreef door 't nat
Ten spel van wind en golven,
Nu, 't moedig hoofd om hoog gebeurd,
En dan, in 't schuim bedolven:

Maar, hobblende op den woesten vloed
En worstlend met zijn baren,
Kwam eindlijk op een oeverplaat
Als eilandtj' aangevaren.

Fig. 3. Part of a poem by Bilderdijk (1858)

Deel van een gedicht van Bilderdijk (1858)

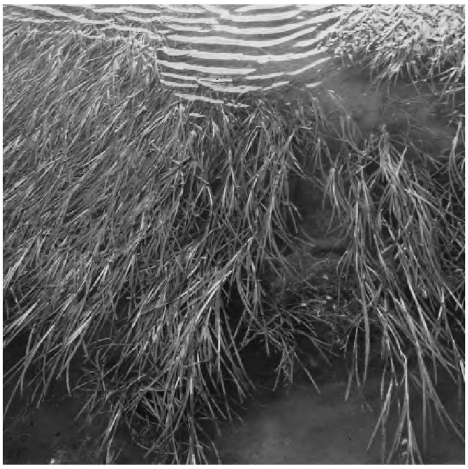


Fig. 4. Annual, flexible eelgrass (*Zostera marina*) at Terschelling

Eenjarig, slap Groot zeegras (*Zostera marina*) bij Terschelling

door de zeegrasindustrie (wiervisserij en wiermaaierij), die vroeger rond de Waddenzee floreerde (Fig. 2). Matrassen en kussens werden gevuld met zeegras, en verder werd het gebruikt als isolatiemateriaal en mest. Tot in de 19^e eeuw werden er in Noord-Holland zelfs dijken van gebouwd. Een spreekwoord (“Veel hooigewas, veel wiergewas”), een speciale gebedsdag, en lyrische beschrijvingen en gedichten in de 18^e en 19^e eeuw van Martinet en Bilderdijk getuigen van het belang dat aan zeegras (wier) werd gehecht (Fig. 3, zie ook Fig. 1 in hoofdstuk 1). De zeegrasvelden in Duitsland en Denemarken hadden weinig economische waarde. Er is daarom minder over deze velden bekend.

Groot zeegras nu

Het Groot zeegras is in de 1930er jaren grotendeels verdwenen en heeft zich nooit hersteld. In het Nederlandse deel van de Waddenzee groeit nog 87 hectare Groot zeegras en 26 hectare Klein zeegras (1999). In de Duitse Waddenzee komen nog 17.000 hectare zeegras voor, en in de Deense Waddenzee 3.000 hectare zeegras (Groot en Klein zeegras tezamen, 1991). Het afsterven van Groot zeegras in de 1930er jaren viel samen met (1) het uitbreken van de zeegrasziekte in het gehele Noord-Atlantische verspreidingsgebied, (mogelijk samenhangend met toenemende druk van de mens op het milieu), (2) het bouwen van dijken en dammen zoals de Afsluitdijk en bijvoorbeeld de Hindenburgdam en (3) een aantal jaren met weinig zonlicht in het groeiseizoen. Er is geen consensus over de bepalende factor(-en).

Het uitblijven van herstel na de catastrofe werd toegeschreven aan de toegenomen troebelheid van het water, en de later toenemende schelpdiervisserij. In het kader van ‘herstel natuurwaarden’ wil de overheid Groot zeegras graag terughebben in de

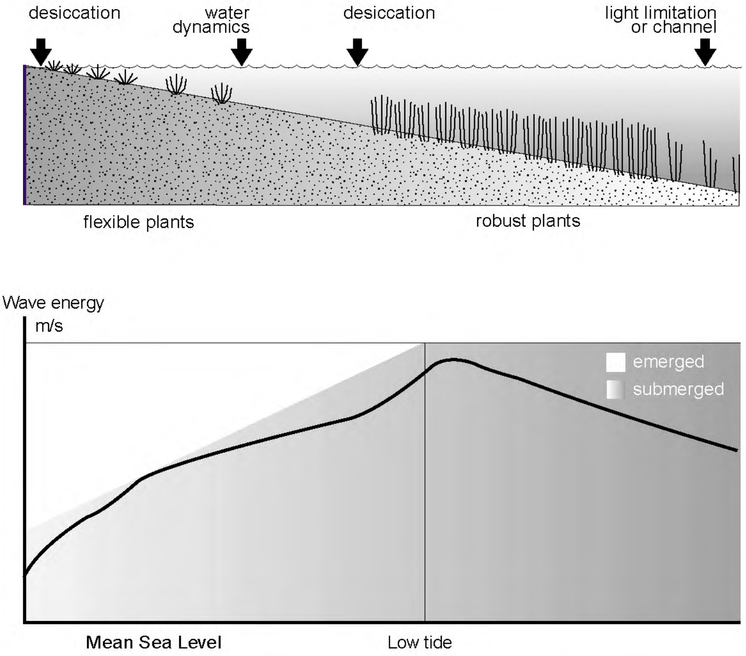


Fig. 5. Zonation of *Zostera marina* in the Wadden Sea along the tidal gradient in relation to wave energy (orbital velocity ant the sediment surface, based on model calculations) and emergence time during low tide. Delimiting factors are indicated for the upper and lower limit of both zones

Zonatie van Groot zeegras in de Waddenzee in relatie tot de golfsnelheid (gemiddelde orbitaalsnelheid aan de bodem) en droogvalduur tijdens laagwater. Beperkende factoren (van links naar rechts: uitdroging, waterdynamiek, uitdroging en lichtlimitatie/geul) voor de boven- en ondergrens van beide zones zijn aangegeven

Wadden Sea were thought to be high turbidity, and later shellfish fishery (van den Hoek et al. 1979, Giesen et al. 1990a, b, de Jonge & de Jong 1992). The Dutch government is currently aiming at restoration of seagrass beds in the Wadden Sea, in order to ‘restore natural values’ (Anonymous 1989). Seagrass beds are important as a nursery, shelter and feeding area for many fish and crustacean species (e.g. van Goor 1919, Heck et al. 1995, Horinouchi & Sano1999, Mattila et al. 1999, Valentine & Heck 1999).

The presence of potential seagrass habitats is the first condition for successful restoration. In the Wadden Sea, a distinction can be made in a higher and a lower zone of potential habitats along the tidal gradient, each suitable for differing morphotypes of *Z. marina* (Figs. 4 & 5, chapter 6). The higher zone is inhabited by mostly annual plants. When emerged, the plants lay flat on the moist sediment, in this way protected from desiccation. The lower zone (that disappeared during the thirties) was inhabited by perennial plants, with their stiff sheaths being vulnerable to desiccation during low tide, but more resistant to high water dynamics than the former morphotype (Harmsen 1936).

Waddenzee. Groot zeegras vormt een onderwaterwoud waarin vele soorten vissen een schuilplaats vinden, een kinderkamer voor krabben en schelpdieren en een foerageermogelijkheid voor alikruikjes en andere slakjes die van algen op het zeegras leven.

Twee zones met Groot zeegras

Om uit te zoeken welke plekken in de Waddenzee geschikt zijn voor Groot zeegras, maken we onderscheid in een hoge en een lage zone met potentiële habitats (leefgebieden), die elk geschikt zijn voor een andere vorm van Groot zeegras (Fig. 4 & 5). De hogere zone, rond NAP, is geschikt voor slappe (vaak eenjarige) planten. Als ze bij laagwater droogvallen liggen ze plat op de vochtige wadbodem, waardoor ze niet uitdrogen. De lagere zone, strekt zich uit vanaf iets boven laagwater tot een diepte waar licht limiterend wordt. Hier groeiden, tot in de 1930er jaren, meerjarige, enigszins stevige planten, waarvan het onderste deel, de schede, rechtop bleef staan bij droogvallen. Ze waren daardoor gevoelig voor uitdrogen. Tegelijkertijd waren ze beter bestand tegen hoge waterdynamiek.

Tussen deze twee zones is een 'kale zone' die niet geschikt is voor zeegras: het is er té dynamisch voor het slappe type zeegras dat de hoge zone kenmerkt, en de droogvalperiode is té lang voor het stevige type zeegras in de lage zone. De bovengrens van het zeegras in de hoge zone wordt bepaald door de mate van uitdrogen, terwijl de ondergrens van het zeegras in de lage zone bepaald wordt door de beschikbaarheid van licht (vóór de 1930er jaren was dat zo'n 3 meter onder NAP), of de aanwezigheid van geulen (Fig. 5). Momenteel is het water in de Waddenzee helder genoeg om Groot zeegrasgroei tot tenminste 0.80 m onder NAP mogelijk te maken.

Groeiwensen van Groot zeegras

Belangrijke factoren voor de groei van Groot zeegras zijn licht, verstoring, waterdynamiek, sedimentdynamiek, mate van uitdrogen, nutriënten (meststoffen) en zoutgehalte. Wat is de samenhang tussen deze factoren en de geschiktheid van de Waddenzee voor Groot zeegras? We onderscheiden twee hoofdinvloeden:

1. *Dynamiek.*

   Hieronder worden water- en sedimentdynamiek en afgeleide effecten verstaan, zoals de korrelgrootte van het sediment (hoe meer waterdynamiek hoe grover het sediment), troebelheid van het water (hoe meer waterdynamiek, hoe troebeler het water) en mate van uitdroging (hoe grover het sediment hoe sneller het water wegzakt in de bodem). Bij verhoogde water- en sedimentdynamiek kunnen planten wegspoelen of onderzanden, of gaat hun productiviteit omlaag waardoor ze gevoelig worden voor andere stressfactoren. Als het water troebeler is kunnen de planten minder diep groeien omdat licht limiterend wordt. Een verhoogde mate van uitdroging heeft vooral effect op de bovengrens van beide potentiële zeegraszones.

   Dynamiek in de Waddenzee is verhoogd als gevolg van bouwactiviteiten, het verdwijnen van de laaggelegen zeegrasvelden en de verhoogde visserijactiviteiten, waaronder de schelpdiervisserij. Door het verdwijnen van oester- en mosselbanken is er minder beschutting op het achtergelegen wad en vloeit het water sneller af, waardoor de mate van uitdrogen tijdens laagwater toeneemt.
2. *Het gezamenlijk effect van nutriënten en zoutgehalte.*

Between the 2 seagrass zones, a bare zone existed, where the habitat is too dynamic for the high morphotype, and the periods of emergence last too long for the low morphotype. The upper limit for *Z. marina* growth in the high zone is delineated by the degree of desiccation, whereas the low zone is limited by light availability and/or strong currents due to the presence of channels (Fig. 5, chapter 6).

Important factors influencing the occurrence of *Z. marina* are: turbidity (chapter 2), disturbance (e.g. de Jonge & de Jong 1992), water dynamics (chapter 5), sediment dynamics (e.g. Boley 1988, Fonseca 1996, chapter 5), degree of desiccation (e.g. Harmsen 1936, Hermus 1995), nutrient level (e.g. chapters 3 & 4) and salinity (chapter 4). To show how these factors determine the suitability of potential habitats, 2 main factors are distinguished:

1. Dynamics, involving water dynamics, sediment dynamics and derived effects, grain size of the sediment (positively correlated with water dynamics), turbidity (idem) and degree of desiccation (positively correlated with water dynamics via coarsening of the sediment, see below). Increased water and sediment dynamics will remove plants or will lower their productivity, making them more vulnerable to other stresses. Increased turbidity negatively influences *Z. marina* when light is near to limiting. Increased degree of desiccation has greatest effects on the high edge of both zones (Fig. 5). Dynamics may increase as a consequence of construction activities, disappearance of subtidal seagrass and increased fishery activities, including shellfish fisheries. The latter may additionally increase the degree of desiccation when the disappearance of oyster and mussel beds results in an increased superficial drainage.
2. The interactive effect of nutrients and salinity. High nutrient loads negatively effect *Z. marina*. High salinity stresses the plants, which will aggravate the negative effects of high nutrient loads. Also, nutrients stimulate algal growth, which subsequently causes increased light limitation. Finally, increased nutrient loads in the water cause an increased shoot:root ratio which makes the plants more vulnerable to high water dynamics.

Disturbance, in the Wadden Sea mainly caused by shellfish exploitation, acts locally and indiscriminately in both *Z. marina* zones.

In the Wadden Sea, dynamics, disturbance and nutrient loads have increased during the 20th century, whereas the overall salinity has remained equal. As a result, the area suitable for reestablishment of *Z. marina* has decreased. However, since the end of the 1980s, turbidity levels in the Wadden Sea have decreased, nutrients loads have decreased or stabilised, and shellfish fisheries are prohibited in some areas. Restoration of *Z. marina* beds seems now more feasible, although further decreases of turbidity, nutrient loads and shellfish fisheries are desirable so as to increase the area of potential *Z. marina* habitats. Until then, potential *Z. marina* habitats will be confined to undisturbed sheltered locations and locations with freshwater influence.

Net als andere planten groeit zeegras goed met extra nutriënten, maar bij teveel kwijnen ze weg. Onderzoek wees uit dat ze in normaal zeewater (30 PSU) minder nutriënten kunnen verdragen dan in brak water (Fig. 9). Daarnaast veroorzaakt een hoge nutriëntenbelasting sterke algengroei. Zowel de kleine algen op de zeegrasbladeren, als de vrij zwevende algen en de grote algen (zeesla, darmwier) gaan harder groeien, waardoor het zeegras niet voldoende licht krijgt. Tenslotte zorgt een verhoogde nutriëntenbelasting van het systeem ervoor dat het zeegras minder wortel en méér blad aanmaakt, waardoor de planten minder goed verankerd zijn in de bodem en sneller losslaan bij storm. De nutriëntenbelasting van de Waddenzee is enorm toegenomen in de afgelopen eeuw. Het zoutgehalte is min of meer gelijk gebleven.

Is de Waddenzee geschikt voor Groot zeegras?

Als gevolg van de toegenomen dynamiek en nutriëntenbelasting in de Waddenzee worden de twee potentiële Groot zeegraszones steeds smaller en verdwijnen uiteindelijk. Daarnaast is er de factor verstoring, die in de Waddenzee vooral veroorzaakt wordt door de schelpdiervisserij. Verstoring kan in beide zeegraszones voorkomen. Momenteel zijn potentiële groeiplaatsen beperkt tot beschutte, ongestoorde gebieden met (plaatselijke) zoetwaterinvloed. Zoetwaterinvloed is nodig omdat de nutriëntenbelasting nog steeds hoog is, en we zagen in het voorgaande dat een hoge belasting in normaal zeewater slecht verdragen wordt.

Er is ook goed nieuws. Het water in de Waddenzee is helderder geworden sinds eind jaren tachtig, de nutriëntenbelasting blijft gelijk of neemt af, en de schelpdiervisserij is op een aantal plekken verboden. Om meer geschikte Groot zeegraslocaties in de Waddenzee te verkrijgen zullen de nutriëntenbelasting, zoutgehalte, dynamiek, mate van uitdroging, troebelheid en de mate van verstoring verder omlaag gebracht moeten worden. Aanbevelingen hiervoor worden gegeven in Tabel 1.

SPONTAAN HERSTEL VAN GROOT ZEEGRAS, OF MOETEN WE EEN HANDJE HELPEN?

"Als we de negatieve menselijke invloeden terugdraaien, herstelt de natuur zich vanzelf wel" zeggen sommigen. Anderzijds is een groeiend aantal natuurbeschermers van mening dat er drempels zijn overschreden in het degradatieproces die spontaan herstel onmogelijk maken zonder menselijk ingrijpen. Dit geldt ook voor Groot zeegras. Is een veld eenmaal verloren, dan zullen de toegenomen troebelheid, de erosie van de onbeschermde bodem en de verhoogde waterdynamiek herstel belemmeren. Voorts zijn er in de Nederlandse Waddenzee nog maar twee veldjes met Groot zeegras die als zaadbron kunnen dienen, bij Terschelling en in de Eemsmonding. De kans dat zaden of zaaddragende stengels van deze veldjes de westelijke Waddenzee kunnen bereiken is uiterst klein.

Er is een derde probleem: een gezond zeegrasveld bestaat uit planten die onderling enigszins verschillend zijn. Er moet een genetische diversiteit zijn, anders ontstaat inteelt en is het veld kwetsbaar voor veranderingen in de omgeving. Een geïsoleerde spontane vestiging zal ontstaan vanuit één of enkele planten, zodat de genetische diversiteit in het

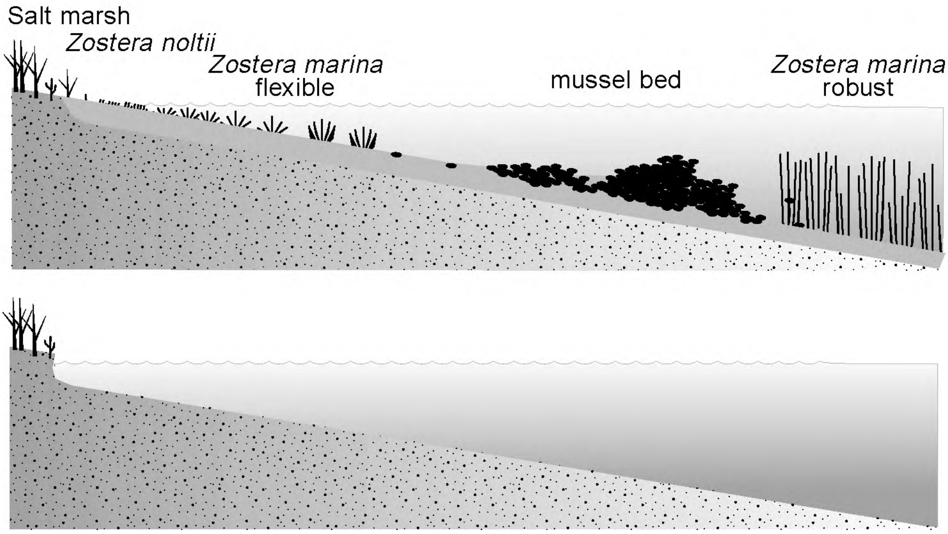


Fig. 6. Above: zones of communities protecting each other from deep to shallow against water dynamics and erosion. Below: disappearance of the communities will cause erosion of sediments and salt marshes

Boven: verschillende gemeenschappen beschermen elkaar van laag naar hoog tegen waterdynamiek en erosie. Onder: als de gemeenschappen verdwijnen, spoelt fijn slib weg en kwelders eroderen

ZOSTERA MARINA BED RESTORATION BY RESTORING A COASTAL GRADIENT

Ecosystems in a natural coastal gradient often protect each other: sublittoral *Zostera marina* beds can protect mussel beds against storms (Reusch & Chapman 1995), mussel beds can provide shelter to mid-littoral *Z. marina* and *Z. noltii* populations (chapters 5 & 6, pers. comm. N. Dankers, Figs. 6 & 7), as is evidenced by the extension of *Z. marina* beds towards mussel beds at Low Tide level (pers. comm. K. Reise). The shelter that is provided by mussel beds will additionally stimulate the accumulation of fine sediments and a lesser degree of desiccation of the sediment (chapter 6), which is favourable to *Z. marina* (van Katwijk & Wijgergangs 2000). In turn, the presence of mid-littoral *Z. marina* and *Z. noltii* beds can reduce erosion of salt marshes, as they accumulate sediments, in this way providing a natural barrier in front of the salt marsh edge (e.g. Rasmussen 1977, Beardall et al. 1988, Gacia et al. 1999, pers. comm. D.J. de Jong, see also chapter 6).

The coherence of these zones makes restoration of one of the separate zones less feasible than simultaneous restoration of the complete zonation. However, restoration of the sublittoral *Z. marina* beds is complex, as the morphotype that is suitable for this zone probably has become extinct in the Wadden Sea (chapter 6). A practical solution would be to first restore stable mussel beds, as these can maintain themselves without sublittoral seagrass. Secondly, mid-littoral *Z. marina* and *Z. noltii* can be transplanted, which will

Tabel 1. Herstel van Groot zeegras (*Zostera marina* L.) in de Waddenzee. (An English translation of this table is presented in Chapter 6, Table 1. Restoration of *Zostera marina* habitats in the Wadden Sea.)

Factor	Vereist	Huidige situatie in de Waddenzee	Aanbevelingen
Dynamiek en uitdroging	Afname	In Nederland bestaan plannen om stabiele mosselbanken te herstellen	Aanleg van stabilisatietechnieken of barrières (kunstmatig en/of natuurlijke stabiele mossel- of oesterbanken)
Nutriënten	Afname	In het algemeen verlaagd, in N-Duitsland echter verhoogd. Nog steeds hoog in vergelijking met het begin van de 20^e eeuw	Verdere afname
Zoutgehalte	Afname	Enige afname, hoewel meer fluctuatie in Nederland. In N-Duitsland toename. In Nederland bestaan plannen om estuariene gradiënten te herstellen	Herstel van estuariene gradiënten, ook op lokale schaal. Bevorderen van grondwaterinvloeden
Troebelheid	Gelijk of afname	Huidige troebelheid laat Groot zeegrasvestiging toe tot tenminste –0.80 m NAP	Een verdere afname verhoogt de kans op groei van Groot zeegras onder de laagwaterlijn
Directe verstoring	Afname	Schelpdiervisserij is verboden in een aantal gebieden in de Waddenzee	Verdere beperking, met name in potentiële zeegrasgebieden

nieuwe veldje niet groot zal zijn. In het bestaande Terschellingse zeegrasveld hebben we mogelijk te maken met zo'n inteeltgemeenschap: Terschellings zeegras vertoont lagere zaailingoverleving in het voorjaar, een lager percentage winteroverleving, en is slechter bestand tegen organisch verrijkte bodems dan bijvoorbeeld Groot zeegras afkomstig van het veld in de Eemsmonding. Dit maakt het Terschellingse zeegras ook minder geschikt om als zaadbron te dienen voor hervestiging.

Tot slot is er een vierde probleem. In het voorgaande hebben we gezien dat er twee potentiële Groot zeegraszones in de Waddenzee bestaan (Fig. 5), waar twee verschillende types planten kunnen groeien. Waarschijnlijk zijn deze planten ook genetisch verschillend en kunnen de slappe planten die kenmerkend zijn voor de hoge zone, zich niet aanpassen aan de omstandigheden (m.n. hoge waterdynamiek) in de lage zone, en omgekeerd. In de Waddenzee is het dieper groeiende, steviger en meerjarige type zeegras uitgestorven. Dat maakt spontaan herstel van de lage zone vrijwel onmogelijk.

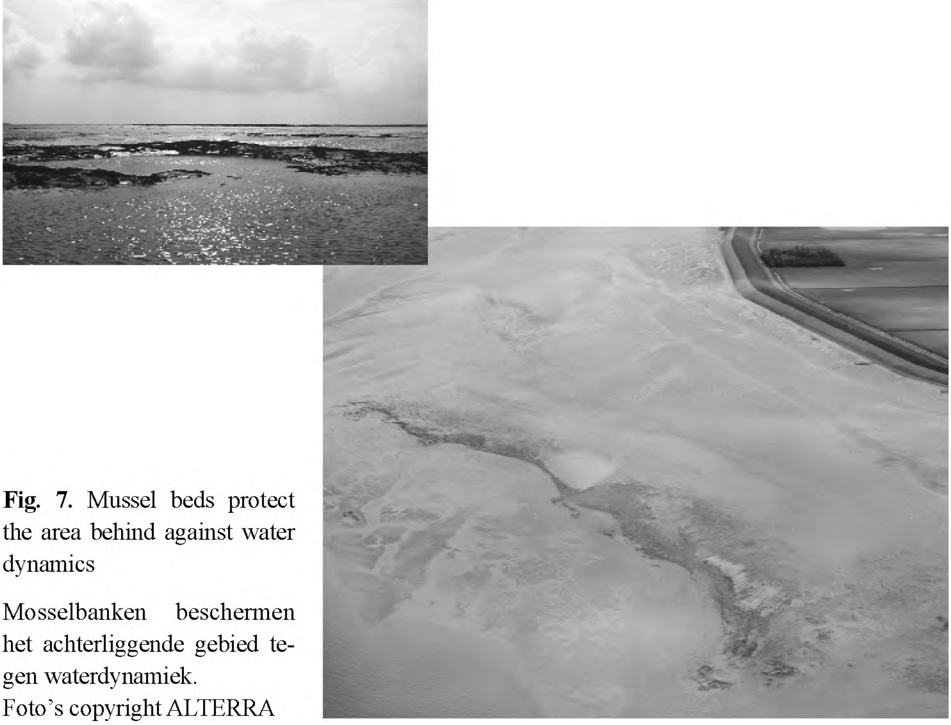


Fig. 7. Mussel beds protect the area behind against water dynamics

Mosselbanken beschermen het achterliggende gebied tegen waterdynamiek.
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probably reduce salt marsh erosion. Finally, to complete the gradient, sublittoral *Z. marina* can be transplanted, provided a suitable donor population has been found.

IMPLICATIONS FOR THE UNDERSTANDING OF *ZOSTERA MARINA* ECOLOGY IN GENERAL

The studies presented in this thesis have implications for the understanding of *Zostera marina* ecology in general, listed below.

1. It is known that *Zostera marina* populations differ with regard to reproductive strategy and morphology in several parts of the world. We have shown that this holds also for 5 northwest European populations (chapter 2). These differences may be due to phenotypic adaptations to the habitat, but may also be partly attributed to genetic differences, this remains to be investigated. Additionally, our study has shown that *Z. marina* populations appeared to differ in habitat adaptation traits, i.e. adaptation to hypoxia in the water layer near the sediment (chapter 2) and adaptation to high salinity (chapter 4).
2. Ammonium toxicity was found in *Zostera marina* (chapter 3). Until recently, ammonium toxicity to submerged aquatic plants has been given little attention, and it had never before been reported for any seagrass species. In a second laboratory experiment (chapter 4), we found a negative effect of increased nutrients (NO_3, NH_4 and PO_4), in combination with a relatively high salinity. The N-loads tested in

Zeegrasaanplant

Op dit moment kunnen kleinschalige aanplanten van Groot zeegras overleven gedurende één groeiseizoen, in een smalle strook rond NAP. Om langer te overleven moeten er (1) voldoende zaden ter plaatse terechtkomen, (2) moeten deze kiemen, en (3) moeten de zaailingen overleven. De zaden blijven voor het gebied behouden als de zaaddragende stengels niet wegdrijven. Dit hangt af van toevalligheden als windrichting en -kracht.

Kieming en zaailingoverleving zijn optimaal in modderige bodems, op beschutte plaatsen, waar liefst een permanent laagje water aanwezig is zodat de jonge planten niet uitdrogen tijdens laagwater. Toevallige klimatologische factoren spelen ook een rol. Zo was er in 1993 een extreem natte winter (denk aan de evacuaties), waardoor het zeewater verzoette. Hierdoor kiemden zaden in een kleine aanplant op Balgzand voortijdig, namelijk in januari. De zaailingen zijn daarna doodgevroren tijdens een latere vorstperiode.

Wat nu?

Spontaal herstel van Groot zeegras is alleen te verwachten op een evolutionaire tijdschaal en het succes van een aanplant hangt af van klimatologisch bepaalde toevalligheden, waardoor het een riskante onderneming wordt. Moeten we dan maar leren leven met een (westelijke) Waddenzee zonder noemenswaardige zeegrasvelden? Er is nog een derde mogelijkheid. De invloed van klimatologische toevalligheden kan worden verminderd door de aanplant te beschermen, zie intermezzo. Tegelijkertijd kan risicospreiding (kleinschalige aanplant op meerdere plaatsen en in meerdere jaren) de resterende invloed van het toeval verminderen.

Hoe creëert men beschutting? Een elegante mogelijkheid doet zich voor. Hiervoor moeten we zeegras in een bredere context zien, de context van een kustgradiënt.

Kwelders, zeegras, mosselbanken

In een natuurlijke kustgradiënt komen de volgende elementen idealiter voor (van hoog naar laag): kwelders, Klein en Groot zeegras, mosselbanken, ondergedoken Groot zeegras. Deze zones beschermen elkaar van laag naar hoog (Fig. 6): ondergedoken Groot zeegras beschermt mosselvelden tegen storminvloeden, mosselbanken beschermen Groot en Klein zeegras in de hogere zone tegen waterdynamiek (zie intermezzo en Fig. 7), Klein zeegras kan kwelders beschermen tegen erosie. Deze samenhang maakt ook duidelijk dat herstel van de afzonderlijke delen veel moeilijker is dan gelijktijdig herstel van alle delen samen.

Herstel kustgradiënt

Hoe herstel je zo'n kustgradiënt? Een logische volgorde zou zijn: herstel eerst de ondergedoken zeegrasvelden, dan mosselbanken, vervolgens hooggelegen zeegrasvelden en tot slot zal kwelderafslag vanzelf verminderen. Herstel van ondergedoken zeegrasvelden is echter complex. Omdat het daarvoor geschikte type zeegras waarschijnlijk is uitgestorven in de gehele Waddenzee, moet eerst een geschikte donorpopulatie worden gevonden. Het is om die reden praktischer om de mosselbanken

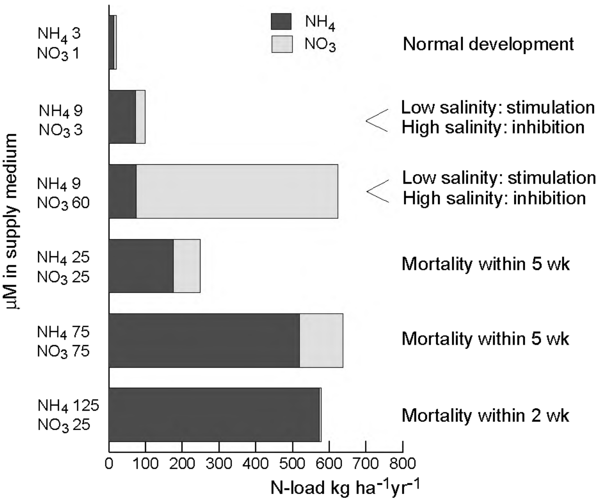

Fig. 8. The nutrient treatments applied in 2 laboratory experiments, described in chapters 3 & 4, with varying NH_4 and NO_3 concentrations in the supply medium (vertical axis), resulted in the nitrogen loads that are shown in the horizontal axis. Right: treatment effects on *Zostera marina*

chapters 3 and 4 (Fig. 8) are similar to N-loads in field situations. The N-load in the Wadden Sea was estimated to be 340 kg N $ha^{-1}yr^{-1}$ on average (Höpner 1991). In 10 lagoons and estuaries along the eastern coasts of the United States N-loads were 24, 41, 64, 65, 157, 175, 310, 397, 520 and 624 kg N $ha^{-1}yr^{-1}$; *Z. marina* occurred only at the sites with 24, 41 and 64 kg N $ha^{-1}yr^{-1}$. Therefore, the direct negative effects of nutrients reported in chapters 3 and 4 may be one of the main underlying causes of the disappearance of many *Z. marina* populations in eutrophicated coastal seas throughout the northern hemisphere.

What are critical levels of N-loads ('critical loads') to *Zostera marina*? This depends on temperature (chapter 3), the ratio between NO_3 and NH_4 (chapter 3), which is, amongst others, related to water dynamics and sediment properties, salinity (chapter 4), probably irradiance (chapter 3), and possibly the availability of phosphate (as was found for *Z. noltii*, J.L. Perez-Llorens, pers. comm., Fig. 9). Young, fast growing plants can assimilate more nitrogen without negative effects than older plants (which may explain why Wadden Sea *Z. marina* populations are mainly annual). Therefore, distinctive assessment of critical loads is required. Furthermore, it should be noted that N-loads of coastal systems are difficult to calculate and require arbitrary assumptions. The N-load is the difference between the N-input and the N-output. Generally the input comes from the land (runoff or river outflow) and nitrogen fixation by Cyanobacteria. The output goes to the open sea. However, with increasing eutrophication (also atmospheric deposition), and by tidal motion, there is an increasing input from the open sea, e.g. in the form of living or dead phytoplankton (see chapter 6). Finally, delineation of the area is often arbitrary in open systems.

3. The interactive effect of nutrients and salinity on *Zostera marina* that has been shown in chapter 4 may explain the distribution and decline of *Z. marina* in many areas of the northern hemisphere. It was found that when stressed by salinity, the plants responded either indifferently or negatively to nutrient enrichment, whereas

(en/of oesterbanken) te herstellen. Deze blijken zich tenslotte ook zonder ondergedoken zeegras te kunnen handhaven, zolang ze niet weggevist worden. (Ze zijn mogelijk wel stabieler als ze beschermd worden door ondergedoken zeegrasvelden.) Vervolgens kan het hooggelegen Groot en Klein zeegras worden aangeplant. Als dit aanslaat kan de kwelderafslag verminderen. Indien een geschikte donorpopulatie voor ondergedoken Groot zeegras is gevonden, met planten die in het Waddenzeemilieu kunnen groeien en die resistent zijn tegen de zeegrasziekte, kan de gradiënt worden gecompleteerd door aanplant hiervan.

INTERMEZZO

Zeegras en stabilisatie

De aanleg van stabilisatietechnieken ten behoeve van Groot zeegrasherstel, bijvoorbeeld door stabiele mosselbanken te herstellen, heeft een groot aantal voordelen. Achter en tussen goedontwikkelde mosselbanken ontstaan namelijk luwtes. Op die plaatsen (1) is de productiviteit van de plant hoger dan in turbulent water, (2) gaan er minder planten verloren bij storm, (3) blijft een groter deel van de losdrijvende zaadstengels in het gebied zelf, (4) ontstaan modderige plekken (fijne textuur), waardoor de kieming bevorderd wordt, en (5) blijft de wadbodem langer vochtig, zowel door fijnere textuur waardoor het water minder snel in de bodem zakt, als door de vertraging in de oppervlakkige afstroming van het water door de barrière. In een vochtige bodem komen zaailingen sneller tot ontwikkeling en breiden zich sneller uit.

Op het Noord-Duitse Waddeneiland Sylt zijn twee of drie voorbeelden bekend van goed ontwikkelde zeegrasvelden achter stabiele mosselbanken. Hier bereiken de velden een diepte tot aan de laagwaterlijn. Zonder beschutting groeit dit type zeegras niet dieper dan tot circa 20 cm onder NAP.

Onderwaterdijken?

Omdat mossel- en/of oesterbanken niet van vandaag op morgen hersteld zijn, en bovendien met name in de vestigingsfase erg gevoelig zijn voor storm en ijsgang, zou de aanleg van tijdelijke onderwaterdrempels een oplossing kunnen bieden. Zowel beginnende mosselbanken als vestigende zeegrasveldjes (zie intermezzo) hebben profijt van de luwte die achter de onderwaterbarrière ontstaat. Na verloop van 5 of 10 jaar kan de onderwaterdrempel geleidelijk worden afgebroken.

Toepassing bij kustbescherming

Een treurig verschijnsel in de Waddenzee is het domino-effect van de voortschrijdende dijkbouw. Kustafslag, meestal aan de wadkant van een Waddeneiland, maakt bedijking over een zeker traject noodzakelijk. Onmiddellijk ontstaat dan nieuwe kustafslag aan het uiteinde van de dijk, en binnen een aantal jaren dient de dijk te worden verlengd. Aan het nieuwe uiteinde ontstaat weer kustafslag enzovoort. Hier verdwijnen vaak grote stukken kwelder. Enkele voorbeelden hiervan zijn de zuidkant van Vlieland richting Posthuis, de zuidkant van Terschelling richting Wierschuur, en de oostkant van Sylt (Noord-Duitsland) tussen Kampen en List. Gelijktijdig herstel van mosselbanken en

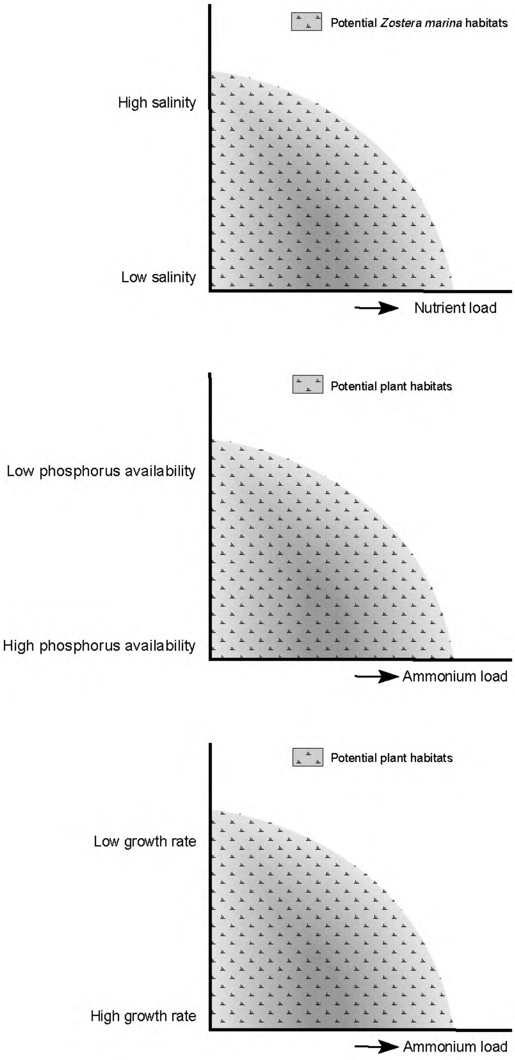

Fig. 9. The effect of nutrient load on plants depends on several factors, for example salinity as was shown for *Zostera marina* in chapter 4 (above). The effect of particularly ammonium probably depends on the availability of phosphorus, pers. comm. J.L. Perez-Llorens and A.J.P. Smolders (middle). In general, it is very likely that this pattern reflects the relationship between nitrogen load and the growth rate of plants (below), where a reduction of growth rate can be caused by a multitude of reasons (see text)

Het effect van nutriëntenbelasting op planten hangt van vele factoren af, bijvoorbeeld het zoutgehalte, zoals in hoofdstuk 4 werd aangetoond (boven), en waarschijnlijk de fosfaatbeschikbaarheid (bij ammoniumbelasting), pers. comm. J.L. Perez-Llorens en A.J.P Smolders (midden). Het is goed mogelijk dat deze figuur de relatie tussen stikstofbelasting en de groeisnelheid van planten in het algemeen beschrijft (onder). Een afname in groeisnelheid kan vele oorzaken hebben, zie tekst

at relatively low salinity, the plants were stimulated by enrichment. This probably relates to a general principle: extra tissue nitrogen resulting from nutrient enrichment can be used for growth by plants with a high growth rate, in contrast to plants with a low growth rate. This was also found when comparing slow-growing seagrasses with fast-growing macroalgae (Pedersen 1995) and in several terrestrial and freshwater species and vegetation types (e.g. Roelofs 1986, Roelofs et al. 1996, Bobbink et al. 1998). This means that in the process of eutrophication, relatively slow-growing species can maintain themselves only when conditions are optimal in all other aspects, keeping their growth rate as high as possible (Fig. 9). It also means that of course, in the end, fast-growing species will outcompete them, as history tells us repeatedly.

zeegrasvelden, eventueel met aanleg van een tijdelijke onderwaterdrempel, zou de noodzaak tot dijkverlengingen mogelijk kunnen uitstellen en zelfs voorkomen.

Huidige plannen

In 2001 zal RIKZ in samenwerking met de Katholieke Universiteit Nijmegen kleinschalig experimenteren met de aanleg van onderwaterdrempels en later zeegrasaanplant. Op deze manier wordt een begin gemaakt met de aanleg van groeikernen van waaruit het zeegras zich spontaan kan herstellen. Ook kan ervaring worden opgedaan met waterdynamische aspecten zoals boven geschetst. Mogelijk kan dit gecombineerd worden met een kleinschalig herstel van stabiele mosselbanken. In 2005 worden de resultaten geëvalueerd.

De locaties voor de groeikernen worden zorgvuldig geselecteerd. RIKZ ontwikkelt momenteel een GIS-model waarmee de optimale groeilocaties van Groot zeegras in de Waddenzee kunnen worden berekend op basis van nutriëntenbelasting, zoutgehalte, beschutting, bodemtextuur en droogvalduur.

Kortom, het drempelmodel

Samenvattend: door de voortschrijdende degradatie van het Waddenzee-ecosysteem door menselijke invloeden zijn ecologische drempels (zoals de afwezigheid van zaadbronnen) ontstaan die de weg terug voor Groot zeegras belemmeren. Door de aanleg van onderwaterdrempels (bijvoorbeeld in de vorm van mosselbanken) in combinatie met kleinschalige zeegrasaanplant kunnen groeikernen ontstaan van waaruit het zeegras zichzelf kan herstellen. Minder meststoffenaanvoer naar de Waddenzee, minder schelpdiervisserij, meer herstel van zoetwatergradiënten en meer beschutte gebieden, maken de kans groter dat het zeegras zich vanuit de groeikernen over de Waddenzee zal verspreiden.

Omdat de Waddenzee dynamisch is, en ons klimaat ook, zal de overleving van herstellend zeegras altijd deels aan toeval onderhevig zijn. Risicospreiding door de aanleg van groeikernen op verschillende plaatsen en gedurende meer dan één jaar is daarom noodzakelijk. Dit doet de natuur ook: er zijn door de eeuwen heen altijd zeegrasvelden en mosselbanken weggevaagd door ijsgang of storm. Er bleven echter altijd groeikernen over van waaruit herstel kon optreden (‘refugia’). Door de destructieve wijze waarop de mens te werk is gegaan zijn de meeste groeikernen echter verloren gegaan. Wachten op spontaan herstel is daarom niet reëel.

Onderwaterdrempels zijn niet alleen belangrijk voor het herstel van zeegrasvelden, ze bieden ook de mogelijkheid om gehele kustgradiënten bestaande uit kwelders, zeegras en mosselbanken te herstellen. Hierdoor kunnen dure en natuuronvriendelijke dijkverlengingen in kweldergebieden mogelijk worden uitgesteld of zelfs voorkomen worden.

4. Water dynamics (mainly caused by wave activity), and the ensuing sediment instability were shown to have a negative effect on *Zostera marina* transplantations (chapter 5). At exposed sites, *Z. marina* establishment was impeded, whereas at sheltered locations, *Z. marina* is known to develop well (see also chapter 6). At sites of intermediate exposure, *Z. marina* was related to the period of exposure to wave activity, i.e. to the depth gradient. This can explain present and past *Z. marina* zonation pattern in several tidal systems in northwest Europe. The construction of stabilisation measures to increase *Z. marina* transplantation success will be tested in experiments that are planned for 2001-2005.

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CURRICULUM VITAE

Marieke (Margaretha Maria) van Katwijk werd op 12 september 1958 geboren te Zaandam. In 1976 heeft zij het Atheneum B diploma behaald, waarna ze met de studie biologie in Nijmegen is begonnen. Tijdens de doctoraalfase werd achtereenvolgens geobotanisch, bodemkundig en koraalecologisch onderzoek verricht, dat laatste in het kader van de met medestudenten georganiseerde “Watamu Reef Expedition”. Na het behalen van het doctoraalexamen in 1985 was zij als gastmedewerker werkzaam op het Nederlands Instituut voor Onderzoek der Zee bij de afdeling Autoecologie. In 1986 werd ze aangesteld als toegevoegd wetenschappelijk onderzoeker bij de werkgroep Milieubiologie van de Universiteit Nijmegen, in project “Vegetaties van waterplanten in relatie tot het milieu”. In 1988 heeft zij bij het ingenieursbureau Witteveen+Bos gewerkt aan de ecologische karakterisering van diverse meren en kleine wateren. In 1988-1989 is meegewerkt aan de fysisch-chemische karakterisering en biotische samenstelling van watertypen in Noord-Holland, op de afdeling Aquatische Ecologie en Milieubiologie van de Universiteit Nijmegen. Vanaf 1989 heeft ze op deze afdeling als projectleider onderzoek verricht en gecoördineerd aan de mogelijkheden tot herintroductie van zeegras in de Waddenzee in opdracht van Rijkswaterstaat. Een deel van de onderzoeksresultaten hiervan zijn verwerkt in dit proefschrift. In deze periode is intensief samengewerkt met het Rijksinstituut voor Kust en Zee in Haren en Middelburg, Alterra op Texel, het Centrum voor Estuariene en Mariene Oecologie in Yerseke en het Alfred Wegener Institut in Sylt, Duitsland. In 2000 heeft ze het bureau Ecoscience voor ecologisch onderzoek opgericht.