

Effects of salinity and nutrient load and their interaction on *Zostera marina*

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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 wk 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‰ S, 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 d⁻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 30‰ S, and on the marine plants at 30‰ S. 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‰ S and estuarine plants at 23‰ S, 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.

KEY WORDS: Eelgrass · Eutrophication · Interaction · Nitrogen · Phosphorus · Population dynamics · Salinity stress · Seagrass · Wadden Sea

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‰ S (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‰ S, 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‰ S. Productivity of another *Zostera* species, *Z. capensis* Setchell, was negatively correlated with salinity in the range of 15 to 75‰ S (Adams & Bate 1994). Photosynthesis of *Z. japonica* Aschers. & Graebn. (sub nomine *Z. nana*) was optimal at 25‰ S (Ogata & Matsui 1965). Wasting disease, which

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destroyed many eelgrass populations on both sides of the Atlantic Ocean during the 1930s (review in den Hartog 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‰ S (Young 1943, Pokorný 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‰ S) and estuarine (15 to 25‰ S) 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 and 4 μM NO_3 , 1 and 8 μM NH_4 , 2 and 10 μM P_{tot} and in winter between 15 and 55 μM NO_3 , 7 and 11 μM NH_4 , and 3 and 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 vary between 0 and 90 μM NO_3 , 2 and 11 μM NH_4 , 7 and 25 μM P_{tot} ; in winter between 50 and 260 μM NO_3 , 15 and 55 μM NH_4 , and 8 and 20 μM P_{tot} (Ministry of Transport Water Management and Public Works unpubl. data). Furthermore, in some marine environments, seagrass distribution shifted towards areas with some freshwater influence (Burdick et al. 1993, D. J. de Jong pers. comm.). 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).

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)

MATERIAL 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‰ S (21.5 to 24.0), 26‰ S (24.0 to 27.0) and 30‰ S (27.0 to 31.5), respectively, at 3 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 $\text{kg N ha}^{-1} \text{ yr}^{-1}$, and 20, 45 and 100 $\text{kg P ha}^{-1} \text{ yr}^{-1}$, 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 pers. comm., Dutch Ministry of Transport, Public Works and Water Management unpubl. data).

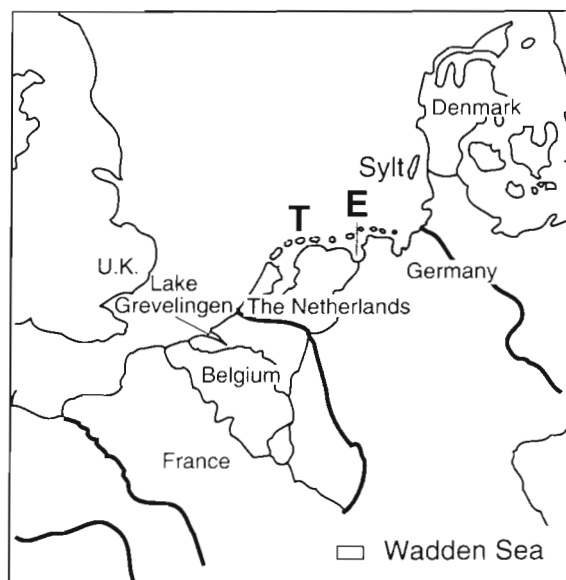


Fig. 1. Map showing locations of *Zostera marina* L. populations of interest. T: Terschelling, E: Eems

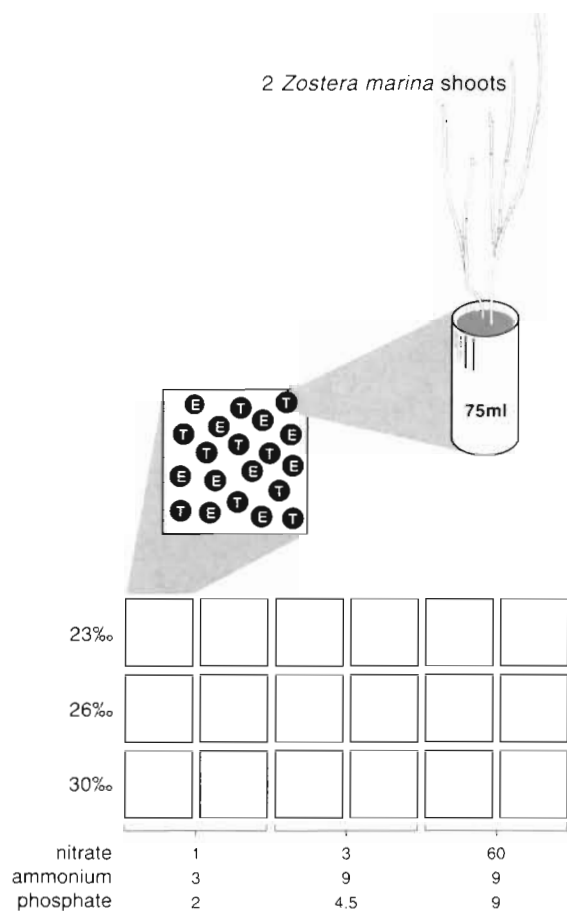


Fig. 2. Scheme of the experimental set-up. In the actual set-up, the glass containers were randomly placed. *Zostera marina* plants originating from Terschelling (T) and Eems (E). Supplied nutrient concentrations in μM

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 wk in synthetic seawater (23, 26 and 30‰ S, Wimex, 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 $95 \mu\text{E m}^{-2} \text{s}^{-1}$ (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 d, 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 wk 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 wk, 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 mo.

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 (HNO₃ 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, $r_{\max} = 19690 \times g$) and the supernatant was stored at -20°C for max. 4 mo. NH₄ content of the supernatant was measured colourimetrically with a Technicon AAI system according to Kempers & Zweers (1986). Total P was measured with an ICP spectrophotometer, type IL Plasma 200.

Water analysis. NH₄, NO₃, PO₄ and Cl content of the water samples were measured colourimetrically with a Technicon AAI 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 d⁻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 × nutrient treatment × replicate + salinity × replicate + nutrient treatment × 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 NH₄ and P_{tot} were not influenced by the nutrient treatment (ANOVA, $p > 0.05$). Over the time course of the experiment, NH₄ and P_{tot} concentrations in the sediment decreased (Table 1). NO₃ and PO₄ 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 NH₄ 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 above-ground tissue nutrient contents of the *Zostera marina*

Table 1. Sediment water-extractable nutrient concentrations ($\mu\text{mol kg}^{-1}$ dry wt) at the onset of the experiment, after 2 wk and after 5 wk. Geometric means (SE) of all treatments are presented

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

Table 2. Water nutrient concentrations and loads. Geometric means (SE) of the nutrient concentrations ($\mu\text{mol l}^{-1}$) of 8 sampling dates are presented per nutrient treatment. Nutrient loads ($\text{kg ha}^{-1} \text{ yr}^{-1}$) are presented in italics; for calculation see 'Materials and methods'

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

plants after 5 wk (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 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 wk. 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.

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

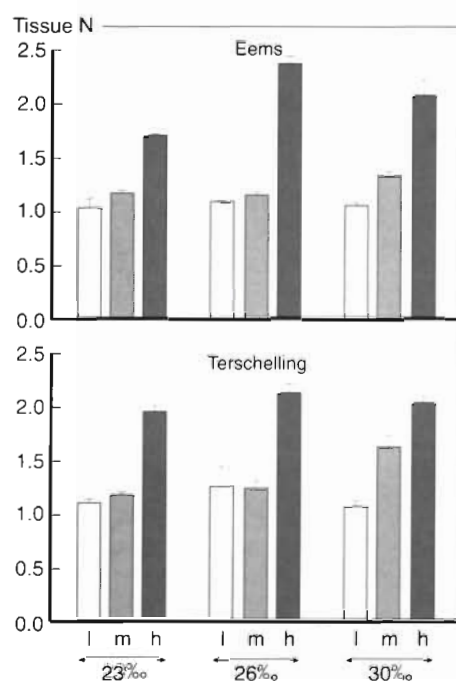


Fig. 3. % N in aboveground tissue of *Zostera marina* after 5 wk at different combinations of salinity and nutrient loads. l: low, m: medium, h: high nutrient load. Means and SEM of 2 replicates are presented

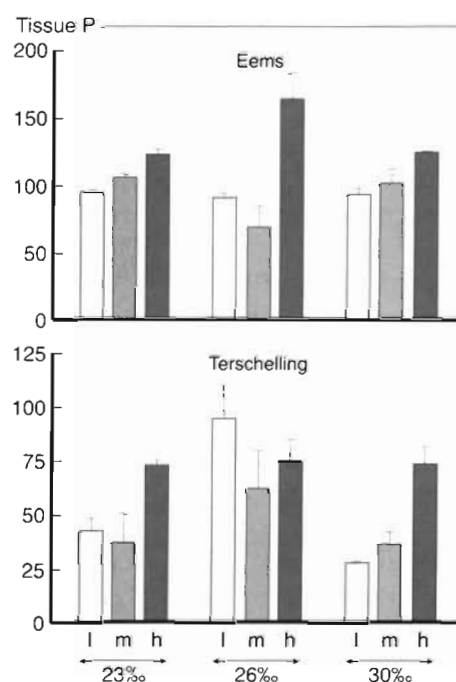


Fig. 4. P_{tot} ($\mu\text{mol g}^{-1}$ dry wt) in aboveground tissue of *Zostera marina* after 5 wk at different combinations of salinity and nutrient loads. l: low, m: medium, h: high nutrient load. Geometric means and SEM of 2 replicates are presented

Table 3. Split-plot ANOVA for effects of nutrient and salinity treatments, and population of origin of *Zostera marina* plants. Ntr: nutrient, Sal: salinity, Pop: population. * $0.01 \leq p \leq 0.05$, ** $0.001 \leq p \leq 0.01$, *** $p < 0.001$, 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 × Sal	0.090	13.4	4	0.001	**
Ntr × Pop	0.017	1.46	2	0.28	ns
Sal × Pop	0.009	0.79	2	0.49	ns
Ntr × Sal × 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 × Sal	0.083	3.13	4	0.79	ns
Ntr × Pop	0.009	0.19	2	0.83	ns
Sal × Pop	0.344	7.58	2	0.02	*
Ntr × Sal × 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 × Sal	28.50	6.42	4	0.01	*
Ntr × Pop	45.86	2.90	2	0.11	ns
Sal × Pop	20.11	1.27	2	0.32	ns
Ntr × Sal × 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 × Sal	3.80	0.77	4	0.57	ns
Ntr × Pop	1.12	0.35	2	0.71	ns
Sal × Pop	7.82	2.45	2	0.14	ns
Ntr × Sal × 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	1603	1.54	1	0.25	ns
Ntr × Sal	1558	2.08	4	0.18	ns
Ntr × Pop	319	0.31	2	0.74	ns
Sal × Pop	166	0.16	2	0.85	ns
Ntr × Sal × Pop	798	0.77	4	0.57	ns

$p < 0.01$, $n = 4$) 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 2 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

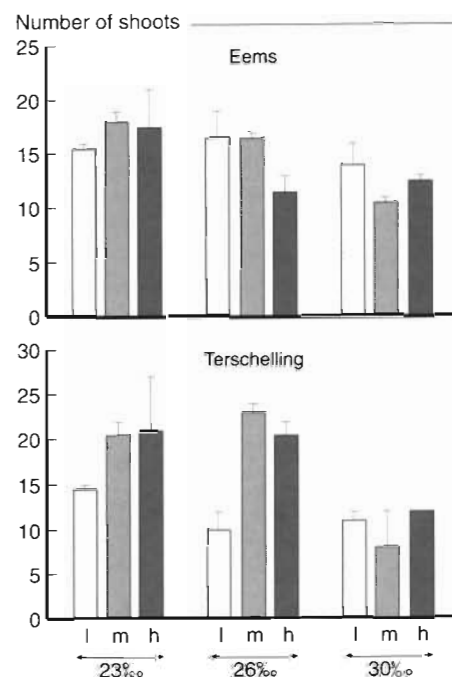


Fig. 5. Number of *Zostera marina* shoots after 5 wk at different combinations of salinity and nutrient loads. l: low, m: medium, h: high nutrient load. Means and SEM of 2 replicates are presented

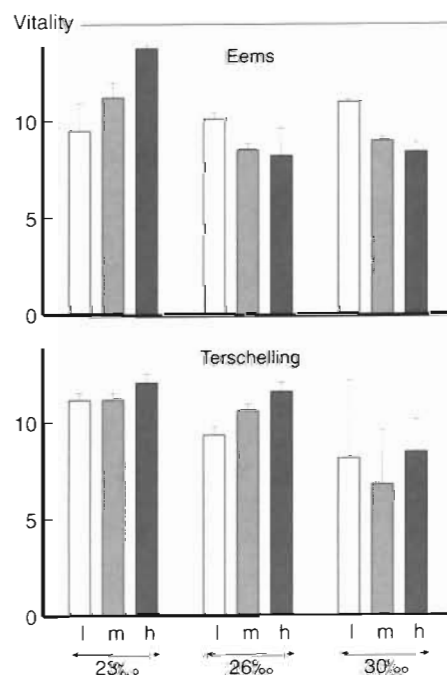


Fig. 6. Vitality of *Zostera marina*, a combined factor ('Materials and methods'), after 5 wk at different combinations of salinity and nutrient loads. l: low, m: medium, h: high nutrient load. Means and SEM of 2 replicates are presented

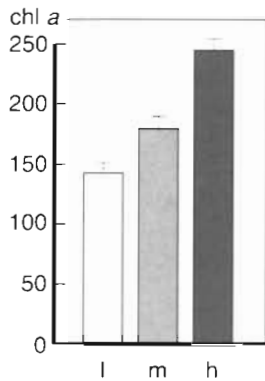


Fig. 7. Chlorophyll a concentrations ($\mu\text{g g}^{-1}$ fresh wt) in *Zostera marina* leaves in 3 nutrient treatments. l: low, m: medium, h: high nutrient load. Means and SEM of replicates, salinity treatments and population of origin are presented

when testing the overall effects in the split-plot ANOVA (Tab. 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 wk, 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 synthesised of number of shoots, plant size, necrosis and number of missing leaves) decreased at 26 and 30‰ salinity, whereas the marine population responded with a reduction of the number of shoots, at the 30‰ 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 (Pokorný 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) attribute 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 (Wijgengangs 1994, M. M. van Katwijk & D. J. de Jong unpubl. 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‰; Eems plants 23‰), 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, 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 direc-

tion 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 \times 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‰ acted adversely on *Zostera marina* plants originating from the marine Terschelling habitat, while *Z. marina* plants from the estuarine Eems habitat were negatively affected by 26 and 30‰ 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 pers. comm.). 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 unpubl. 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.

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