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Significance of salinity and silicon levels for growth of a formerly estuarine eelgrass (*Zostera marina*) population (Lake Grevelingen, The Netherlands)

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Abstract Since the early 1980s, the eelgrass, *Zostera marina* L., population in the saline Lake Gevelingen, The Netherlands, is rapidly declining. An earlier study, in which long-term data on eelgrass coverage in this former estuary were correlated with several environmental variables, showed only one significant correlation: coverage was positively related to water column silicon levels. In addition, a negative correlation with salinity was observed, but this was not significant. In the present study, the effect of silicon and the effect of salinity on the development of *Z. marina* were investigated experimentally. Enhancement of dissolved silicon concentrations in the water did not stimulate *Z. marina* above-ground production or an increase in final above- and below-ground biomass. The highly significant correlation between eelgrass coverage and water column silicon levels, thus, remains to be explained. The results of the growth experiments did, however, demonstrate a clear effect of salinity on *Z. marina* growth. Plants cultured at 22 psu showed a higher production of shoots and leaves, resulting in more above-ground biomass, than plants grown at 32 psu. In addition, below-ground biomass was also higher at 22 psu. Measurements of chlorophyll *a* fluorescence, performed with a PAM-fluorometer, indicated a reduction of photosynthesis in the high-salinity treatments. Thus, low salinity stimulates development of *Z. marina* from Lake Grevelingen. Eelgrass from such a historically estuarine area may be more sensitive to high salinities than other, more marine populations. Recovery of the autochthonous eelgrass population is expected to be favoured when the estuarine conditions of the seagrass area are re-established, or when restoration programmes are carried out with allochthonous ecotypes that are less sensitive to high salinities.

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Introduction

Seagrasses have a world-wide distribution forming vast meadows in shallow coastal areas (Den Hartog 1970). Together with mangroves and coral reefs, seagrass beds belong to the most productive coastal habitats in the world. They support complex food webs and provide breeding grounds and nurseries for commercially important fish and shellfish species (Zieman and Wetzel 1980; Thayer et al. 1984; Heck et al. 1995). The canopy reduces wave action (Fonseca et al. 1982) and filters suspended sediments and nutrients from coastal waters (Fonseca and Fisher 1986; Hemminga et al. 1991). Furthermore, their rhizomes and roots stabilise sediments (Harlin et al. 1982). This valuable group of plants appears to be highly vulnerable to environmental change. In fact, seagrass meadows are declining around the world (Short and Wyllie-Echeverria 1996). The extensive losses have triggered many research efforts on the causes of seagrass disappearance. Results of those studies indicate that human-induced deterioration of water clarity and quality is the main factor responsible for seagrass habitat loss (Short and Wyllie-Echeverria 1996). Nutrient enrichment leads to enhanced development of fast-growing primary producers such as phytoplankton, epiphytic algae and macroalgae, causing reduced light levels for seagrasses (Duarte 1995). The possession of roots and rhizomes makes seagrasses especially vulnerable to the combination of increased nutrient levels and reduced light availability (see review by Hemminga 1998). Less light causes decreased photosynthetic activity of the plants. This leads to decreased oxygen supply to the roots and rhizomes, which can result in carbon starvation of these parts (Hemminga

1998; Zimmerman et al. 1995). In addition, the reduced oxygen supply to the sediment can cause an increase in sediment sulphide levels, which may be toxic for seagrasses (Hemminga 1998; Goodman et al. 1995).

Seagrass decline is also occurring in the saline Lake Grevelingen in the SW Netherlands. This lake formerly was one of the tributaries of the Rhine–Meuse estuary. In 1964, a dam in the inland (eastern) part the estuary was finished which stopped the inflow of river water. In 1970, the connection with the North Sea was blocked in the west. To avoid stratification, the connection between the North Sea and the newly formed lake was re-established in 1978 by means of a sluice in the western dam. This sluice is open every winter. Initially, the population of eelgrass, *Zostera marina*, showed large fluctuations in size, reaching a peak surface area in 1978, but since then the population has been rapidly declining (Nienhuis 1983; Nienhuis et al. 1996). This decline is remarkable, because the disruption of the inflow of river water by a dam resulted in a decrease in water nutrient concentrations and turbidity of the water (Nienhuis et al. 1996), conditions which are supposedly favourable for *Z. marina*.

The engineering works did not only change nutrient conditions and water transparency, but salinity of the water was affected as well. After the closure, salinity initially decreased from 30 psu to 22 psu due to freshwater input by land run-off and the absence of a connection with the North Sea. During this period, *Zostera marina* extended its area from 1200 to 4600 ha (Nienhuis et al. 1996). The establishment of a sluice connection between Lake Grevelingen and the sea in 1978 led to a rise in salinity again. Salinity increased to a value of 32 psu, which is higher than before the dam construction. The decline in eelgrass areal coverage started after the construction of the sluice connection (Nienhuis et al. 1996). These observations suggest that low salinities favour development of eelgrass in Lake Grevelingen.

It is known that seagrass growth may be affected by salinity. For example, Zieman (1975) observed depressed production in *Thalassia testudinum* from Biscayne Bay, Florida, at salinity values below 20 psu. Walker (1985) studied *Amphibolis antarctica* (Labill.) Sonder & Aschers. along a salinity gradient from 35 to 64 psu in Shark Bay, Western Australia, and found maximal production and biomass at 42 psu. In Florida Bay, the total biomass of *Halodule wrightii* Aschers., *Ruppia maritima* L. and *T. testidunum* together showed a negative correlation with the magnitude of salinity fluctuations (Montague and Ley 1993). Salinity optima for survival and growth have been established in the laboratory for several seagrass species (McMillan and Moseley 1967; Walker and McComb 1990; Bird et al. 1993; Adams et al. 1994; Hilman et al. 1995). Although *Zostera marina* can tolerate salinities from 5 to 42 psu (Den Hartog 1970; Giesen et al. 1990), data on growth performance of this species in relation to salinity are scarce. Field observations in the Limfjord in Denmark showed reduced leaf production rates of *Z. marina* at a site with a mean salinity of 13 psu, but did not show large differences in leaf production rates between sites with a mean salinity of 20 or 31 psu (Pinnerup 1980).

The present subtidal *Zostera marina* population in Lake Grevelingen is the continuation of an estuarine intertidal population. This could explain the observed coincidence of a decrease in size of the eelgrass population and an increase in salinity in Lake Grevelingen. Herman et al. (1996) analysed a possible correlation between the long-term data on *Z. marina* areal coverage and yearly median values of salinity, water column nutrients (ammonium, nitrate + nitrite, phosphate, silicon) and light attenuation. They indeed found a negative correlation with salinity, although this was not significant. Surprisingly, the only significant correlation was a positive relation with water column silicon levels (Herman et al. 1996). In coastal areas, low salinity and high water column silicon levels can occur simultaneously, as they may both be caused by the input of fresh water.

Silicon is an essential element for only some plant groups (e.g. diatoms and horsetails). Nevertheless, silicon is found in the tissues of many vascular plants (Sangster and Hodson 1986). Much of the silicon is located in the cell wall giving it its rigidity (Epstein 1994). Several beneficial effects of silicon addition have been described for a number of terrestrial species. Silicon addition can correct imbalances in supplied nutrient concentrations, alleviate heavy metal toxicity and salinity stress and improve resistance to diseases and herbivores (Epstein 1994). Herman et al. (1996) determined the silicon content of eelgrass leaves collected in Lake Grevelingen, the Oosterschelde estuary and the nearby Lake Veere and found a range from 0.02 to 0.46% dry weight, which was related to the silicon levels in the water column. They hypothesised that the changes in the *Zostera marina* population in Lake Grevelingen could be causally related to the fluctuating silicon levels in the water.

In the present paper, we investigate if salinity and water column silicon levels affect productivity of eelgrass from a formerly estuarine population, and, hence, if these factors may have contributed to the long-term decline of eelgrass in Lake Grevelingen. To be able to separate the effect of salinity and silicon, an experimental approach was taken. In a series of growth experiments, the production of *Zostera marina* from Lake Grevelingen was determined at different salinity and silicon levels. In addition, variable chlorophyll *a* fluorescence measurements with a PAM-fluorometer were used to determine whether the quantum efficiency of photosystem II (PSII) of eelgrass leaves responded upon changes in salinity.

Materials and methods

Zostera marina L. was cultured in the laboratory to investigate the effect of different salinity values (Experiment 1, two treatments,

65 d), different water column silicon levels (Experiment 2, two treatments, 69 d) and the combined effect of the two factors (Experiment 3, four treatments, 119 d). At the beginning of each experiment, perennial *Z. marina* plants and sediment were collected from Lake Grevelingen. Shoots were separated from their neighbouring shoots by cutting the rhizome. Each shoot was placed in a 300 ml polyethylene container filled with sieved (2 mm) sediment. To recover, the plants were placed in an outside basin for 2 to 4 weeks. The water in the basin was regularly renewed with water from the Oosterschelde estuary.

The experiments were carried out in a culture room. For each treatment, three Plexiglas cylinders were used (30 cm diam. and 50 cm height) that were closed at the bottom. Each cylinder had an inflow at 10 cm above the bottom of the cylinder and an outflow at 2 cm below the top of the cylinder. At the location of the inflow, the water was aerated. The cylinders were placed on a table under Philips 32-W 840 HF fluorescent light tubes. The photoperiod was 14 h light:10 h dark. Light intensity in the cylinders varied according to location on the table as well as location within the cylinder and ranged from 260 to 410 $\mu E\ m^{-2}\ s^{-1}$ (PAR, photosynthetically active radiation). These values are at, or above, the light saturation intensity for *Zostera marina*. Light saturation intensities reported for *Z. marina* range from 80 $\mu E\ m^{-2}\ s^{-1}$ (Dennison and Alberte 1986) to 280 $\mu E\ m^{-2}\ s^{-1}$ (Zimmerman et al. 1995). Nevertheless, to counteract possible light effects, the cylinder positions of the different treatments were either alternating (in the case of two treatments) or random (in the case of four treatments). Temperature of the water in the cylinders was 14 °C during the dark period and increased to 18 °C during the light period.

Artificial seawater was prepared from demineralised water and a sea salt mixture (Wiegandt hw Meeressalz) to produce two different salinity treatments. Low salinity was 22 psu and high salinity 32 psu. These values represent the lowest and highest value observed after the closure of the Grevelingen estuary (Nienhuis et al. 1996). Two silicon treatments were used. The low-silicon treatments consisted of medium without additional silicon. Silicon in this medium nonetheless resulted in a concentration of around 5 μM, due to impurities in the sea salt mixture and trace additions from components of the experimental set-up. For the high-silicon treatments, Si was added to a concentration of 70 or 100 μM. These levels are higher than what was maximally observed in Lake Grevelingen (40 μM; Nienhuis et al. 1996). During the first 30 d of Exp. 1, $SiO_2 \cdot nH_2O$ was added to the high-silicon treatment. As the solubility of this compound gave problems during the experiment, we switched to $Na_2SiO_3 \cdot 9H_2O$ which was subsequently used. In all three experiments, each treatment received NH_4Cl to attain a medium concentration of 10 μM. Furthermore, $Na_2HPO_4 \cdot 2H_2O$ was added to produce a concentration of 5 μM. At Day 50 of Exp. 2, the phosphate concentration was lowered to 2.5 μM to reduce cyanobacterial growth. In Exp. 3, the phosphate concentration was further reduced to 0.626 μM. The phosphate and nitrogen concentrations in the experiments, hence, were within the range of what is presently observed in Lake Grevelingen: 0.5 to 5.5 μM PO_4 and 1 to 10 μM NH_4 (Van Lent and Verschuure 1994). The artificial medium was pumped from stock basins to the cylinders at a rate of 5 ml min^{-1}. At regular intervals, water samples were collected from the basins and cylinders to determine the concentration of NO_x, NH_4, PO_4 and Si (using a Skalar autoanalyser) and the salinity (using a WTW Microprocessor Conductivity Meter).

At the beginning of each experiment, 10 (Exp. 1) or 14 (Exps. 2 and 3) 300-ml polyethylene containers each with one *Zostera marina* shoot were placed in the cylinders. The number of leaves was counted, and the length and width of each leaf was measured. A random sample of the remaining plants was used to determine the initial above- and below-ground biomass. Two to four plants were randomly selected in each cylinder to be monitored during the experiment. Every 7 to 10 d the number of shoots and leaves on each of these plants was counted. In addition, the length and width of each leaf was measured. These size measurements made it possible to identify individual leaves, and thus the number of leaves lost and gained could also be recorded. Furthermore, the size measurements were used to calculate the amount of leaf surface area produced during the experiment. On each measuring occasion, epiphytes were gently removed from the leaves of all plants present in the cylinder. In addition, all containers were given another position within the cylinder. A summary of the conditions in each experiment is given in Table 1. At the end of the experiments, the plants that had been monitored were harvested. Of each of these plants, the final biomass of above-ground plant tissue (weight of shoots and leaves together) and the below-ground material (rhizome and roots together) was determined. The relation between leaf surface area and dry weight (DW) was used to calculate production in milligrams dry weight per day for each plant during the experiment. The remaining plants, which had not been monitored, were returned to the outside basin.

At the end of Exp. 3, before harvesting the plants, we used a pulse amplitude modulated fluorometer (PAM) to determine a possible stress response in the chlorophyll *a* fluorescence of the leaves. Excitation energy, obtained by absorption of light by the pigment protein complexes responsible for light harvesting in PSII can be used for the photochemical reaction initiating photosynthesis, or is lost as heat or fluorescence. These processes compete and influence the energy conversion efficiency (Dau 1994). The variable/maximum fluorescence ratio (F_v/F_m), i.e. the quantum efficiency of PSII in dark adapted samples, is used as an indicator of stress (e.g. Ralph and Burchett 1995). Maximum energy conversion efficiency of PSII charge separation is calculated as:

$$F_v/F_m = (F_m - F_0)/F_m \ ,$$

where F_0 is the minimum fluorescence, F_m is the maximum fluorescence and F_v is the maximum variable fluorescence yield of a sample adapted to the dark for a minimum of 15 min. Variable fluorescence was measured with a PAM 101-103 fluorometer (Walz, Effeltrich, Germany), which controlled the Schott KL 1500/E light source used for administering the saturating irradiance pulses. The two best performing plants, i.e. the plants that produced most leaves, were selected in the low-salinity–low-silicon and high-salinity–low-silicon treatments. Of each of these four plants the third leaf of the main shoot was harvested for measurement of the maximal energy conversion efficiency or PSII-quantum efficiency (F_v/F_m). The tip of the PAM fiberoptic was positioned 2 mm above and perpendicular to the leaf surface. Three saturating light pulses of 0.5 s duration were given with 20-s intervals at each of three locations on the leaf: the base, the middle and the top.

Table 1 Set-up of the growth experiments in the culture room: salinity levels of artificial seawater, calculated concentrations of added nutrients and duration of each experiment. Starting dates: 16 May 1996 for Exp. 1; 2 August 1996 for Exp. 2; and 9 June 1997 for Exp. 3

Exp. No.	Salinity (psu)	Silicon (μM)	Nitrogen (μM)	Phosphate (μM)	Duration exp. (d)
1 Salinity (Sal)	22 or 32	70	10	5	65
2 Silicon (Sil)	30	5 or 70	10	5 and 2.5	69
3 Sal + Sil	22 or 32	5 or 100	10	0.625	119

In Exps. 1 and 2, a two-way nested analysis of variance (ANOVA) was used to test the effect of treatment (salinity or silicon) and of the individual cylinder number (Sokal and Rohlf 1995). Cylinder number was nested in treatment, therefore, an interaction between treatment and cylinder number could not be tested. In Exp. 3, a three-way nested ANOVA was used to test the effect of salinity, silicon, and of the individual cylinder number, as well as the interaction between salinity and silicon (Sokal and Rohlf 1995). Again, cylinder number was nested in treatment. The significance of differences between the initial and final biomass values was determined by a two-way ANOVA followed by a Tukey–Kramer procedure (Sokal and Rohlf 1995). Differences in the F_v/F_m ratio between treatments, leaves and position on the leaf were tested with a three-way ANOVA. All data were tested for heteroscedacity with a Bartlett's test for homogeneity of variances (Sokal and Rohlf 1995). Data that scored as significant were log-transformed, which yielded non-significant results in Bartlett's test. When data series contained zeros, the transformation was log $(x + 1)$. A significance level of 5% was used in all tests. The statistical analyses were conducted using the STATISTICA programme (StatSoft Inc., Tulsa, Oklahoma).

Results

Salinity and nutrient levels

During the three growth experiments, salinity levels of the water fluctuated around the planned values (Figs. 1a, b, 2a, b, 3a, b). Dissolved silicon concentrations were not always as expected. In Exp. 1, the concentration was initially lower than 70 μM (Fig. 1c, d). These low values can be attributed to the use of $SiO_2 \cdot nH_2O$ which is less soluble than $Na_2SiO_3 \cdot 9H_2O$. Silicon values exceeding the expected concentration were observed in all three growth experiments. However, differences between low- and high-silicon treatments were maintained. In Exp. 1, the concentration increased to values of up to 100 μM at the end of the experiment (Fig. 1c, d). In Exp. 2, the silicon concentrations were too high in both treatments during the first 15 d, but levels were as expected after this period (Fig. 2c, d). In Exp. 3, silicon concentrations were too high from Day 67 to 88 (Fig. 3c, d). These high values were due to malfunctioning of the demineralised water installation. In all cases the concentrations dropped to the expected levels after repair of the apparatus. Nitrogen ($NH_4 + NO_2/NO_3$) concentrations were generally somewhat higher than the expected 10 μM, but did not show differences between the treatments of the three experiments. The extra nitrogen was probably introduced in the medium with the salt, although the manufacturer claims that nitrogen is not present in Wiegandt hw Meeressalz. Phosphate concentrations did not show large deviations from the expected values. Nutrient concentrations in the cylinders were always lower than in the medium basins (Figs. 1, 2, 3). This indicates nutrient uptake by the plants.

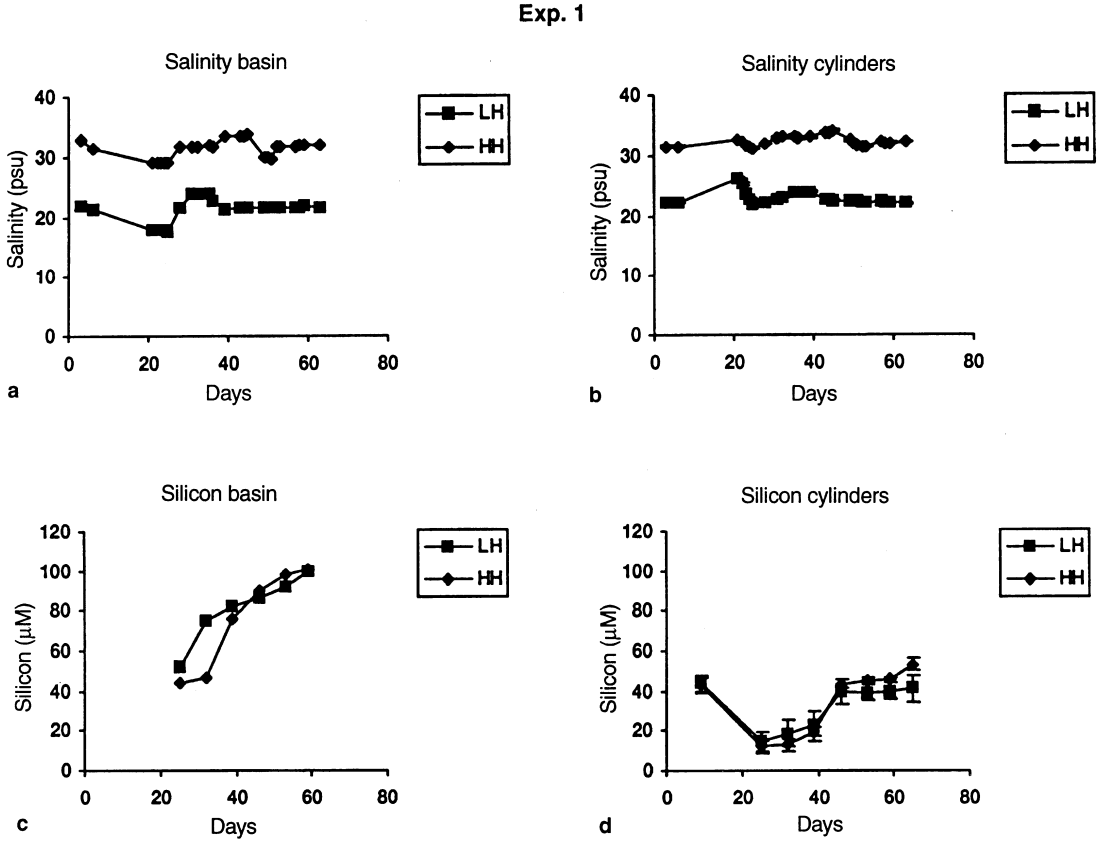

Fig. 1 Measured salinity levels (**a, b**) and dissolved silicon concentrations (**c, d**) in cylinders (mean of three cylinders with standard error) and in medium basins during Exp. 1 (*LH* low-salinity–high-silicon treatment; *HH* high-salinity–high-silicon treatment)

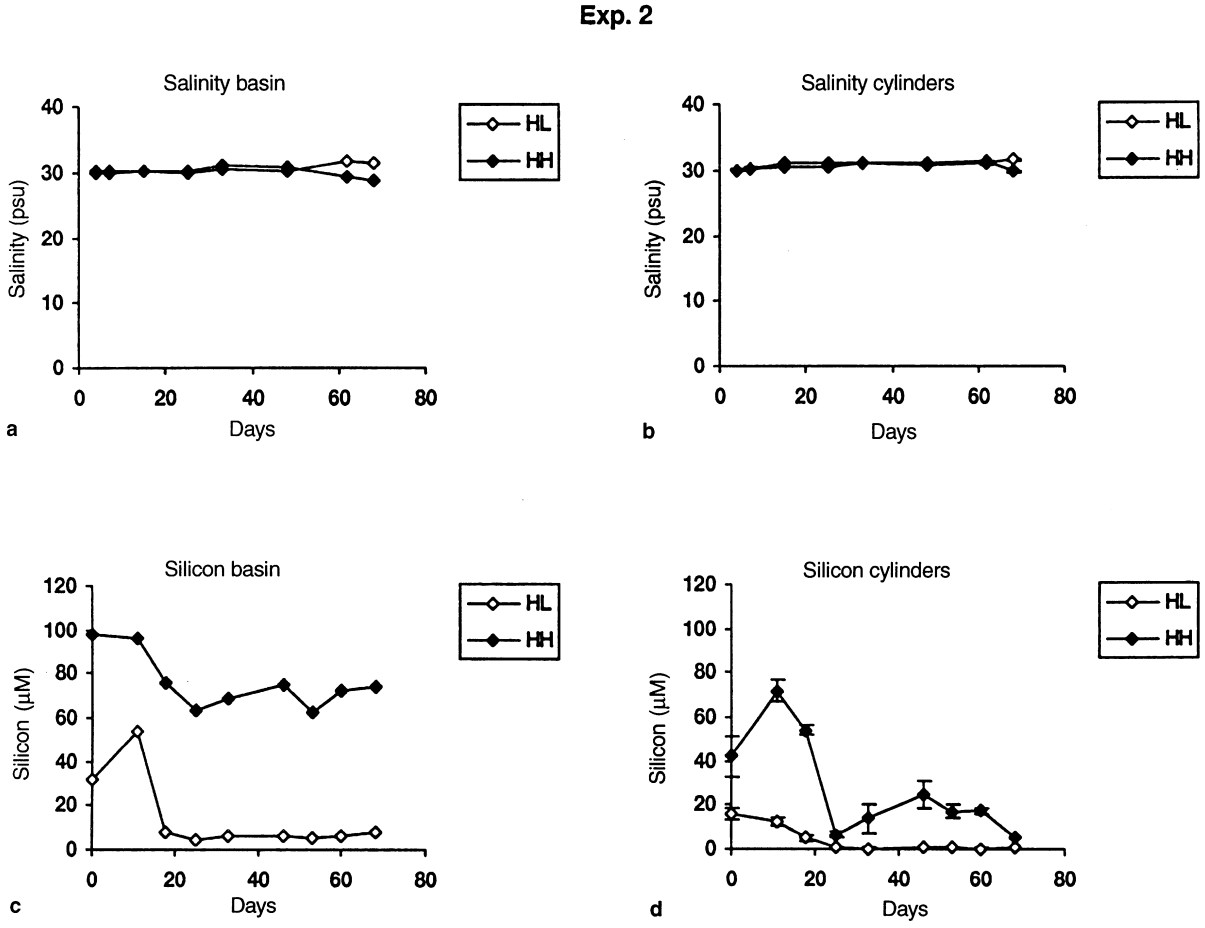

Fig. 2 Measured salinity levels (**a, b**) and dissolved silicon concentrations (**c, d**) in cylinders (mean of three cylinders with standard error) and in medium basins during Exp. 2 (*HL* high-salinity–low-silicon treatment; *HH* high-salinity–high-silicon treatment)

Above-ground production

In Exp. 1 significantly more leaves were gained and lost in the low-salinity treatment than in the high-salinity treatment (Fig. 4a, b; Table 2). The different silicon levels of Exp. 2 did not affect the leaf turnover rate of *Zostera marina* (Fig. 4c, d; Table 2). In Exp. 3, a significant effect of salinity was found for the number of leaves gained and lost (Fig. 4e, f; Table 2). Again, the low-salinity treatments gained more leaves and lost more leaves. In the high-salinity treatments of all three experiments, the mean number of leaves gained over an observation period was never more than 2.8, while in the low-salinity treatments up to five new leaves were frequently formed in the 7- to 10-d period (Fig. 4). This indicates that high salinity hampers leaf formation. Silicon did not affect leaf turnover in Exp. 3 (Fig. 4e, f; Table 2).

Despite the higher leaf production in the low-salinity treatment of Exp. 1, above-ground production, as calculated from the amount of leaf surface area produced, was not significantly higher compared to the high-salinity treatment (Fig. 5a; Table 2). This is probably a result of a significantly smaller surface area per leaf in that treatment (Fig. 5b; Table 2). Apparently, more numerous, but smaller, leaves were produced, leading to a production in milligrams of dry weight that was similar in the two treatments. In Exp. 2, silicon did not affect the production or mean leaf size of *Zostera marina* (Fig. 5c, d; Table 2). Treatment had a significant effect on production, but not on mean leaf size in Exp. 3 (Fig. 5e, f; Table 2). Plants in the two low-salinity treatments showed significantly higher production than plants in the two high-salinity treatments. Silicon levels did not affect production or leaf size in Exp. 3 (Fig. 5e, f; Table 2).

In Exp. 3, differences in leaf surface area were not present. A significant cylinder effect was found (Table 2). This means that the results obtained in one of the three cylinders within one treatment differed significantly from results of the other two cylinders. The difference in performance could not be related to differences in nutrient, light or temperature conditions. Cylinder effects were also observed for the number of standing shoots in Exps. 3 and 1 (Table 2), and for the final below-ground biomass in Exp. 3 (see Table 4). However, the cylinder causing the effect was not consistently the same, nor was the location on the table the same in each case. These effects are probably not caused

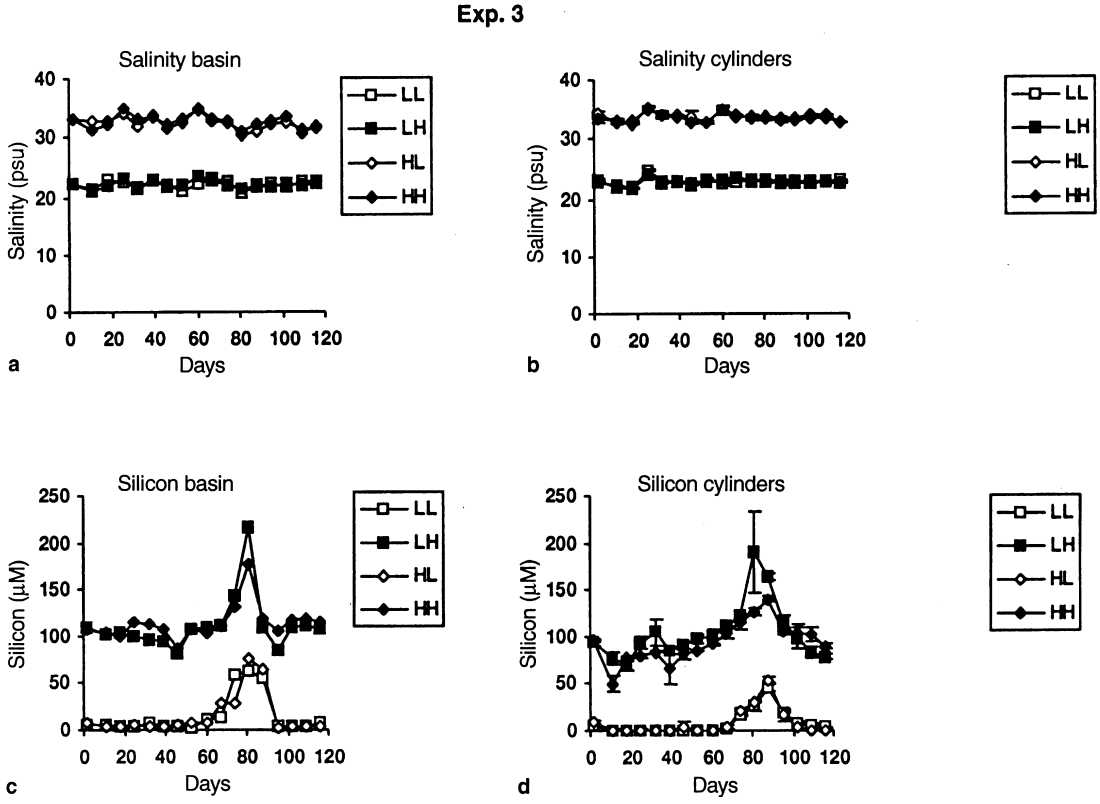

Fig. 3 Measured salinity levels (**a, b**) and dissolved silicon concentrations (**c, d**) in cylinders (mean of three cylinders with standard error) and in medium basins during Exp. 3 (*LL* low-salinity–low-silicon treatment; *LH* low-salinity–high-silicon treatment; *HL* high-salinity–low-silicon treatment; *HH* high-salinity–high-silicon treatment)

by differences between cylinders, but are a result of the natural variability between individuals. Therefore, cylinder effects will be omitted from a further discussion of the results. In Exp. 3, leaf surface area showed a significant interaction between salinity and silicon (Table 2). This is caused by differences within salinity treatments. In the low-salinity treatments, a larger leaf surface area was observed at high-silicon levels, while in the high-salinity treatments, high silicon levels resulted in smaller surface areas of the leaves (Fig. 5f).

Biomass

In all three experiments, the number of standing shoots and leaves increased during the experiments (Fig. 6). A clear treatment effect was observed in Exp. 1. Both the number of shoots and the number of leaves were significantly higher in the low-salinity treatment (Fig. 6a, b; Table 2). This is supported by the relatively high numbers of leaves gained in the low-salinity treatment of Exp. 1 (Fig. 4a). Although the plants also lost more leaves in this treatment, the difference between treatments was smaller for the lost leaves than for gained leaves. That is the reason why we find a higher number of standing leaves in the low-salinity treatments. The different silicon levels of Exp. 2 did not affect the number of standing leaves or shoots (Fig. 6c, d; Table 2). Exp. 3 showed a significant effect of treatment (Fig. 6e, f; Table 2). At the end of the experiment, the low-salinity treatments showed significantly higher numbers of shoots and leaves than the high-salinity treatments (Fig. 6e, f; Table 2). Silicon did not affect the final number of shoots and leaves (Fig. 6e, f; Table 2). Together, the results of the three experiments suggest that high salinity, and not low silicon, hampers growth of shoots and leaves in *Zostera marina*.

At the end of Exp. 1, both above-ground and below-ground biomass had increased significantly compared to the initial values (Fig. 7a, b; Table 3). Furthermore, biomass was highest in the low-salinity treatments, although not significantly so (Fig. 7a, b; Table 4). In Exp. 2, the silicon experiment, only a slight increase in biomass compared to the initial value was observed (Fig. 7c, d). This increase was not significant and not affected by treatment (Tables 3, 4). The plants in the low-salinity treatments of Exp. 3 showed an increase in above-ground biomass compared to the initial value, while the above-ground biomass of the plants in the high-salinity treatments remained the same or decreased (Fig. 7e). The observed differences were not significant (Table 3). All treatments of Exp. 3 showed an increase in below-ground biomass compared to the initial value (Fig. 7f). The increase was significant for the below-ground biomass of the low-salinity treat-

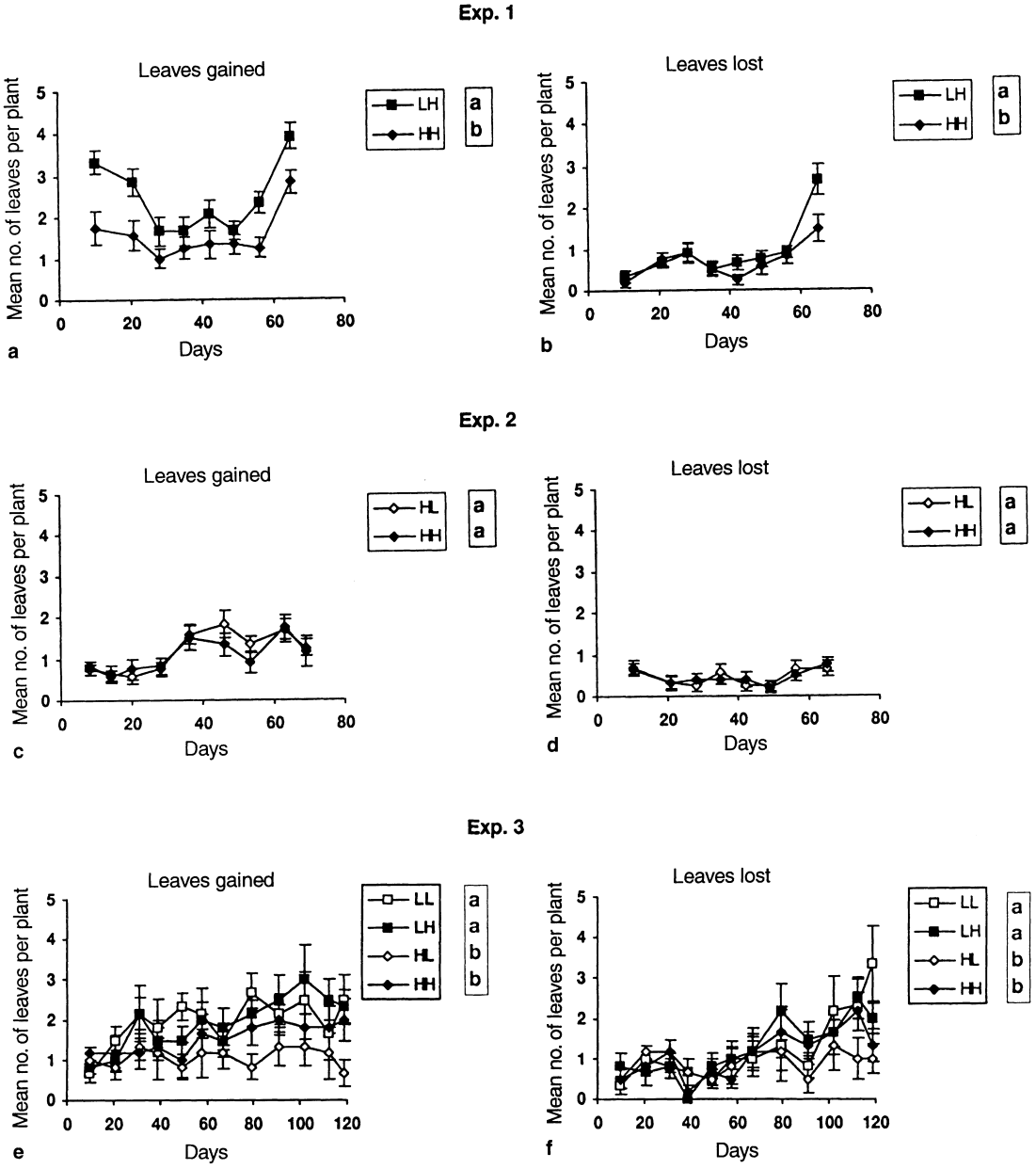

Fig. 4 *Zostera marina*. Number of leaves gained and lost every 7- to 10-d period during Exp. 1 (**a, b**; mean of 12 plants with standard error), Exp. 2 (**c, d**; mean of 12 plants with standard error) and Exp. 3 (**e, f**; mean of 6 plants with standard error) (*LL* low-salinity–low-silicon treatment; *LH* low-salinity–high-silicon treatment; *HL* high-salinity–low-silicon treatment; *HH* high-salinity–high-silicon treatment). Significance of differences between treatments are indicated by letters next to keys (see Table 2); treatments that share the same letter are not significantly different from each other ($P > 0.05$)

ments (Table 3). The above- and below-ground biomass was significantly higher in the low-salinity treatments of Exp. 3 than in the high-salinity treatments (Fig. 7e, f; Table 4). Silicon levels did not affect final biomass values (Fig. 7e, f; Table 4). A significant interaction between salinity and silicon in this experiment was caused by an increase in below-ground biomass at high silicon levels in the high-salinity treatments, while in the low-salinity treatments, a slight decrease in below-ground biomass was observed at high silicon levels. The above-ground biomass data do not support a positive effect of silicon addition at high salinity only. In addition, the interaction is opposite to the interaction observed with leaf surface area, where smaller leaves were found in the high-salinity–high-silicon treatment, than in the high-salinity–low-silicon treatment. The final biomass data support the suggestion that low salinity is beneficial for *Zostera marina* growth and that silicon does not play a role.

Energy conversion efficiency

The variable/maximum fluorescence ratio F_v/F_m was higher in the low-salinity–low-silicon treatment than in

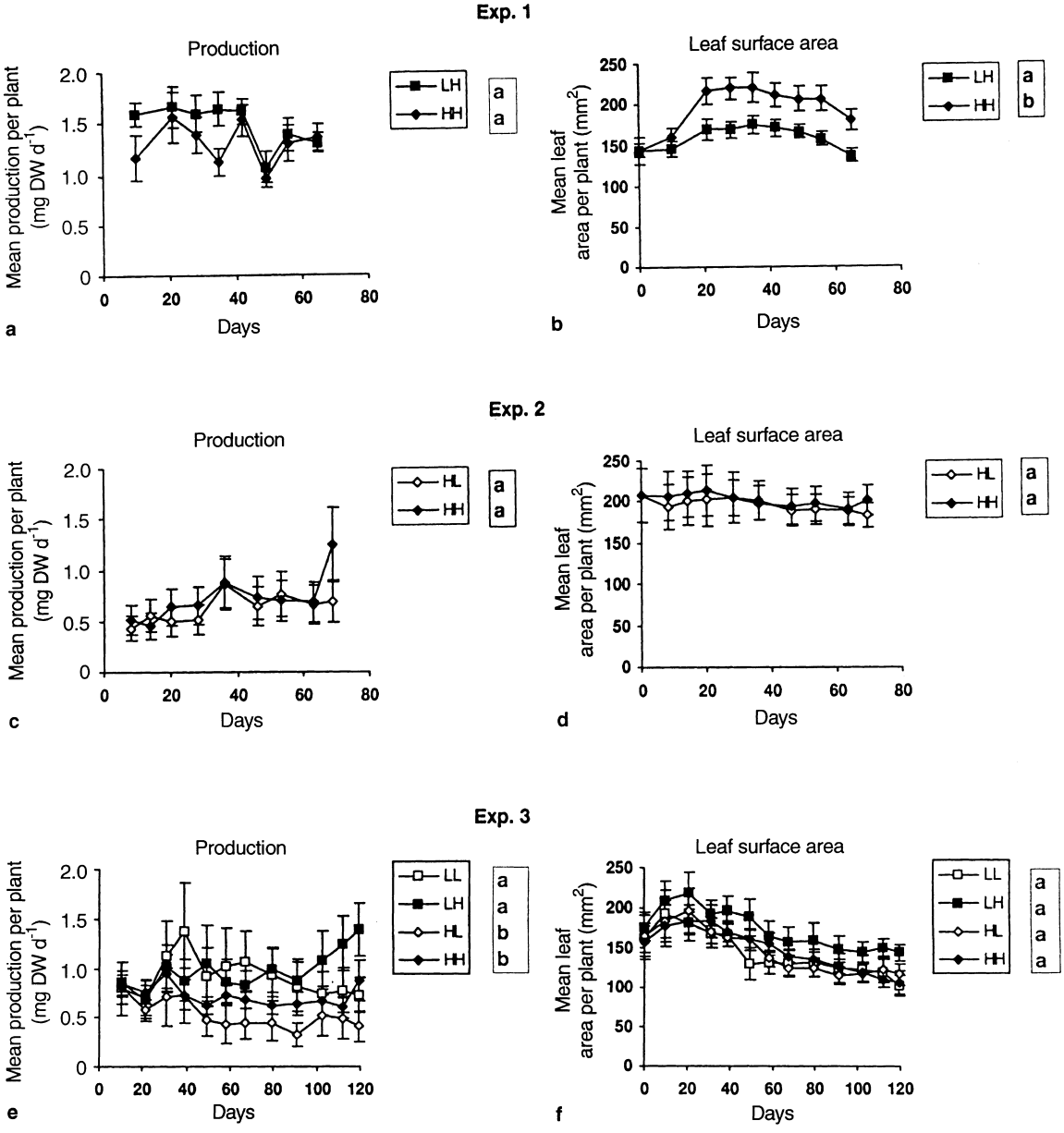

Fig. 5 *Zostera marina*. Above-ground production and surface area per leaf during Exp. 1 (**a, b**; mean of 12 plants with standard error), Exp. 2 (**c, d**; mean of 12 plants with standard error) and Exp. 3 (**e, f**; mean of 6 plants with standard error). Abbreviations and significance notation as in Fig. 4

the high-salinity–low-silicon treatment and showed a decrease from the base to the top of the leaf (Fig. 8). The differences between treatments, as well as between all locations on the leaf, were significant (three-way ANO-

Table 2 *Zostera marina*. *P*-values of ANOVA for effect of treatment and cylinder on the following dependent factors: total number of leaves gained and lost during experiment, total production during experiment, final surface area of leaf at end of experiment, final number of standing shoots and leaves at end of experiment (see Figs. 4, 5, 6) (*ns* not significant; $*P < 0.05$; $**P < 0.01$; $***P < 0.001$)

Exp. No., effect	Gained	Lost	Prod.	Surface	Shoots	Leaves
1						
Salinity	***	**	ns	*	***	***
Cylinder	ns	ns	ns	ns	***	ns
2						
Silicon	ns	ns	ns	ns	ns	ns
Cylinder	ns	ns	ns	ns	ns	ns
3						
Salinity (Sal)	*	*	*	ns	*	*
Silicon (Sil)	ns	ns	ns	ns	ns	ns
Cylinder	ns	ns	ns	*	*	ns
Sal × Sil	ns	ns	ns	*	ns	ns

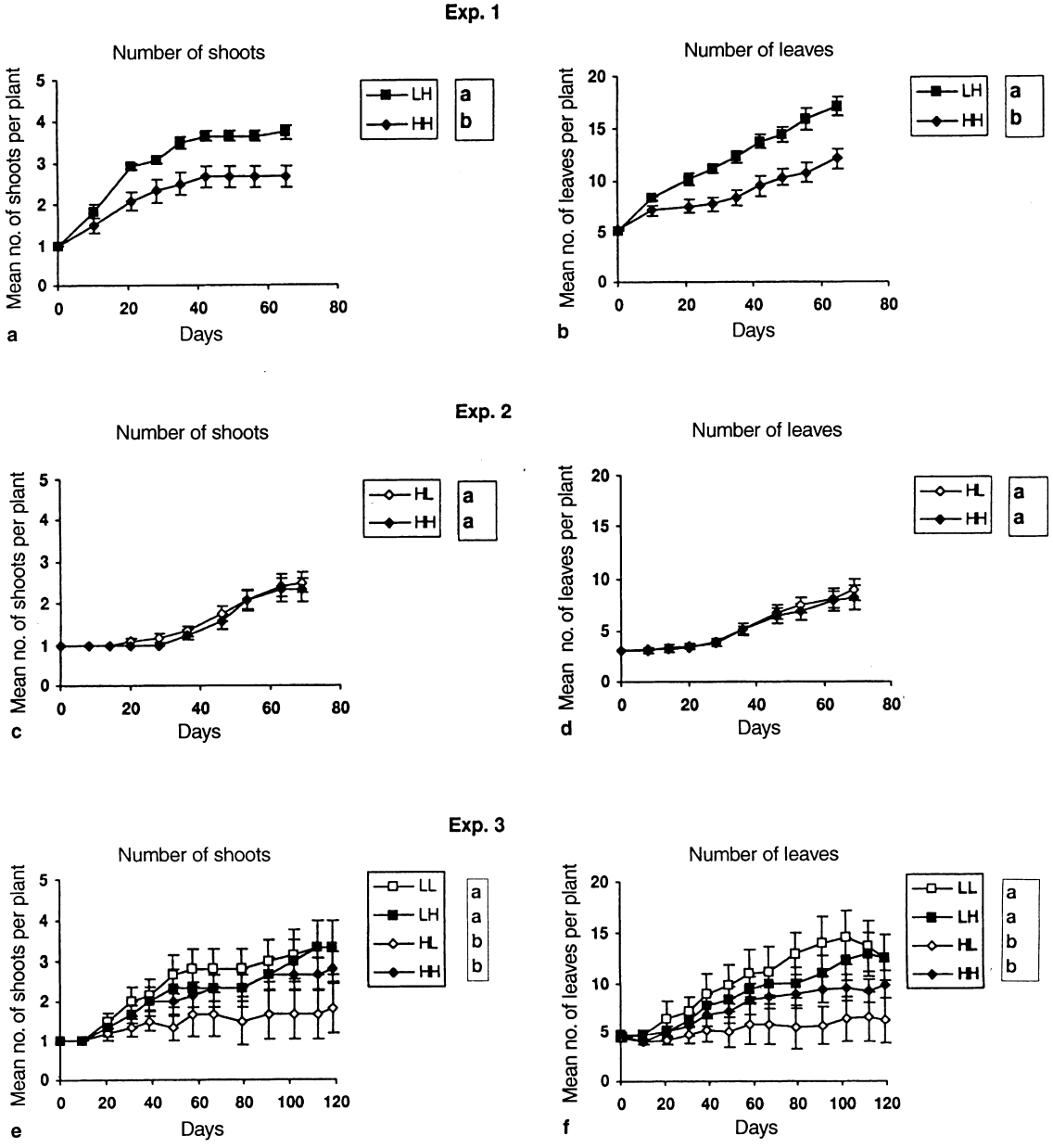

Fig. 6 *Zostera marina*. Number of standing shoots and leaves during Exp. 1 (**a, b**; mean of 12 plants with standard error), Exp. 2 (**c, d**; mean of 12 plants with standard error) and Exp. 3 (**e, f**; mean of 6 plants with standard error). Abbreviations and significance notation as in Fig. 4

VA, $P < 0.001$). Differences between leaves of the same treatment were not significant (three-way ANOVA, $P > 0.05$). These results indicate that energy is more efficiently converted to carbon at low salinity than at high salinity, and more efficiently at the base of the leaf than at the top.

Discussion

The results of the two growth experiments with different salinities (Exps. 1 and 3) demonstrate a clear effect of salinity on *Zostera marina* growth. At 22 psu, plants generally showed higher above-ground production than plants from 32 psu. This resulted in a higher number of standing shoots and leaves at low salinity values. In addition, both above- and below-ground biomass increase compared to the initial value was larger for plants cultured at 22 psu than for plants grown at 32 psu. Below-ground biomass seemed to have responded more strongly to low-salinity conditions than above-ground biomass. In eelgrass, the turnover time of below-ground material is generally slower than that of above-ground tissue (Jacobs 1979; Hillman et al. 1989). Thus, a larger proportion of below-ground production contributes to the final below-ground biomass. This is supported by higher below-ground biomass values in Exp. 3, the experiment with the longer duration, compared to the other two experiments. In Exp. 1, a remarkable, and as

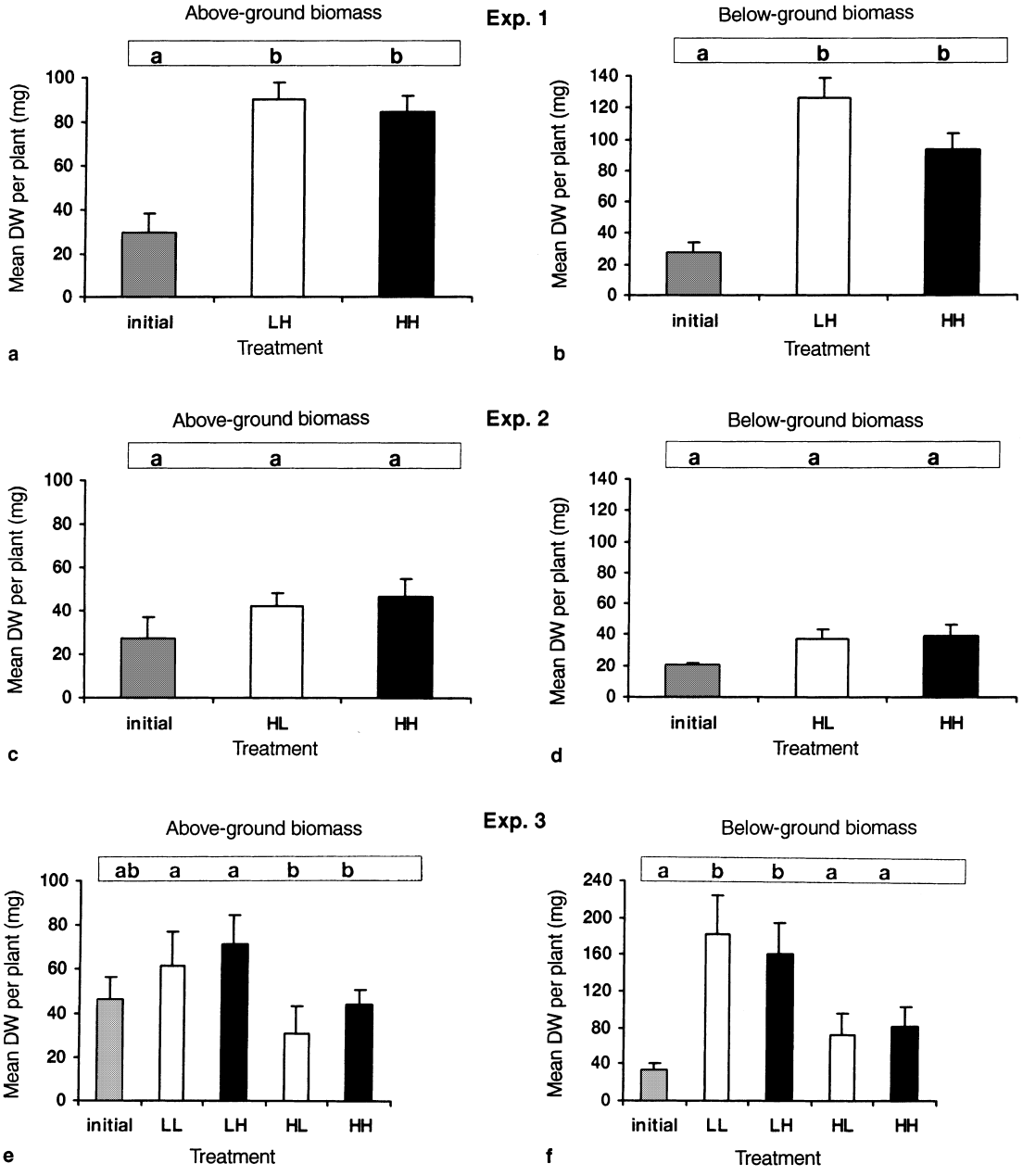

Fig. 7 *Zostera marina*. Above-ground and below-ground biomass at the end of Exp. 1 (**a, b**; mean of 12 plants with standard error) Exp. 2 (**c, d**; mean of 12 plants with standard error) and Exp. 3 (**e, f**; mean of 6 plants with standard error) (*LL* low-salinity–low-silicon treatment; *LH* low-salinity–high-silicon treatment; *HL* high-salinity–low-silicon treatment; *HH* high-salinity–high-silicon treatment). Initial values are indicated by grey bars (mean of 8 plants in Exps. 1 and 3 and mean of 4 plants in Exp. 2). Significance of differences between treatments are indicated by letters next to keys (see Tables 3, 4); treatments that share the same letter are not significantly different from each other ($P > 0.05$)

yet unexplained, smaller leaf surface area was observed at low salinity than at high salinity. This may explain the relatively small difference in above-ground biomass between treatments in that experiment. A possible salinity effect on leaf size was not confirmed in Exp. 3. Thus, the production of more leaves does not necessarily lead to the production of smaller leaves.

Zostera marina can adapt to changes in salinity by osmotic regulation of cellular solutes (Van Diggelen et al. 1987). However, salinity stress can cause reduced photosynthesis rates in *Z. marina* (Biebl and McRoy 1971). Reduction of photosynthesis may have occurred in the high-salinity treatments of the growth experiments as well. This is supported by the fluorescence measurements on eelgrass leaves carried out at the end of Exp. 3. The maximal energy conversion efficiency (F_v/F_m) of the low-salinity plants was around 0.73, which is similar to the ratio observed for unstressed *Halophila ovalis* (Ralph and Burchett 1995). Ralph and Burchett demonstrated a decrease in F_v/F_m to < 0.2 in *Halophila ovalis* (R. Br.) Hook f. subjected to high irradiance stress consisting of

Table 3 *P*-values of ANOVA for significance of differences between initial and final above-ground and below-ground biomass (see Fig. 7) (*ns* not significant; $^{**}P < 0.01$; $^{***}P < 0.001$)

Exp. No., treatment	Above-ground	Below-ground
1		
Low salinity	***	***
High salinity	***	***
2		
Low silicon	ns	ns
High silicon	ns	ns
3		
Low salinity	ns	**
High salinity	ns	ns
Low silicon	ns	ns
High silicon	ns	ns

exposure to 1000 μE m^{-2} s^{-1} for 120 min. The ratios measured at the end of Exp. 3 were significantly lower in the high-salinity treatments than in the low-salinity treatments, indicating a stress response. In comparison to the study of Ralph and Burchett (1995), however, the reduction in F_v/F_m at high salinity is small. Thus, caution is advised in the use of this ratio as an instantaneous index of salinity stress. F_v/F_m was significantly lower at the top than at the base of the leaves. This difference is expected, as in seagrasses the location of the growth meristem is found at the base of the leaf. As a result, photosynthesis decreases from the leaf base to the top (Mazzella and Alberte 1986).

The present results suggest that the observed increase in salinity in Lake Grevelingen may have contributed to the long-term decline of eelgrass in that area. Not only adult plants, but also young stages of eelgrass seem to be affected by high salinity values. A laboratory study by Hootsmans et al. (1987) indicates that salinities higher than 20 psu prevent seed germination and seedling survival of *Zostera marina*. In the entire Netherlands, eelgrass has been rapidly declining in the past decades. In the Dutch Wadden Sea and Zuiderzee, the area covered by *Z. marina* decreased from approximately 118 km^2 when it was first recorded in 1930, to less than

Table 4 *P*-values of ANOVA for effect of treatment and cylinder on final above-ground and below-ground biomass (see Fig. 7) (*ns* not significant; $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$

Exp. No., effect	Above-ground	Below-ground
1		
Salinity	ns	ns
Cylinder	ns	ns
2		
Silicon	ns	ns
Cylinder	ns	ns
3		
Salinity (Sal)	**	***
Silicon (Sil)	ns	ns
Cylinder	ns	***
Sal × Sil	ns	*

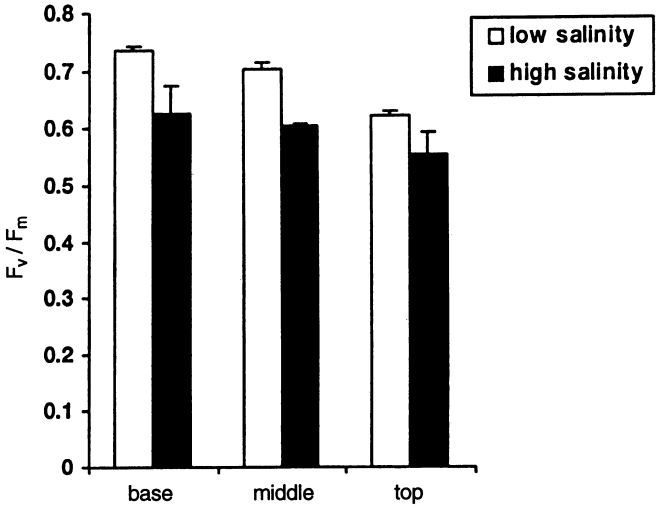

Fig. 8 The PSII-quantum efficiency F_v/F_m of eelgrass leaves from the low-salinity–low-silicon treatment and the high-salinity-low-silicon treatment at the end of Exp. 3. Mean with standard error of measurements on 2 leaves per location (base, middle and top)

1 km^2 at present (Hemminga 1998). The coastal zone of the Netherlands is the outflow area of three large European rivers, i.e. the Rhine, the Meuse and the Scheldt. As such, the distribution of *Z. marina* has always been restricted to areas with moderate salinity. There are indications that the eelgrass decline in the Netherlands can be attributed to increased salinity in areas other than Lake Grevelingen as well. For instance, dam constructions in the SW Netherlands not only changed the hydrology of the Grevelingen estuary, but also of the neighbouring Oosterschelde estuary. Since 1969, salinity in the latter estuary increased considerably. Coinciding with this increase, the distribution of *Z. marina* showed a decline (de Jong, unpublished data). Eelgrass populations in the Wadden Sea may also have responded to increased salinity. Sampling of a sediment profile in a reclaimed tidal area at Texel, the Netherlands, revealed 11 different layers, of which three contained fossil eelgrass (Kuipers and Van Noort, in preparation). The layers were dated and ambient salinity was determined from the number of radiating ribs of *Cerastoderma edule* and *Cardium glaucum* shells. This analysis indicated that *Z. marina* was present when salinity was 10 to 11 psu, and absent at salinities of 25 to 33 psu (Kuipers and Van Noort, in preparation).

Eelgrass is a euryhaline species that is able to form dense meadows in high-salinity areas. Apparently, *Zostera marina* is not always affected by high salinity values. Leaf production rate measurements in the Limfjord in Denmark indicate that *Z. marina* occurring at high salinities is not negatively affected (Pinnerup 1980). In addition, a pilot experiment in which *Z. marina* collected in high-salinity areas in France (Roscoff and Bay of Arcachon) was cultured at 22 and 32 psu did not reveal a salinity effect on growth (Kamermans, unpublished data). These results suggest ecotypic differences

between populations. An isozyme electrophoresis study showed 93 to 98% similarity among four eelgrass populations in the SW Netherlands (including the Lake Grevelingen population), while the similarity with a population from Roscoff was only 79% (De Heij and Nienhuis 1992). This supports the existence of genetic differences between estuarine and marine *Z. marina* populations.

The two growth experiments with different silicon levels (Exps. 2 and 3) did not show an effect of enhanced dissolved silicon concentrations in the water on *Zostera marina* growth. Silicon was taken up, as the concentrations in the cylinders were always lower than in the medium basins. However, it is not certain whether silicon was taken up by eelgrass or by diatoms that were present in the cylinders. Our findings do not support the hypothesis of Herman et al. (1996) that silicon levels in the water determine the size of the *Z. marina* population in Lake Grevelingen. It can be argued that, similar to the enhanced seed germination and seedling growth at low salinity demonstrated by Hootsmans et al. (1987), silicon may play a role in early stages of the life cycle. An experiment with *Z. marina* seeds collected in Lake Grevelingen, however, showed that the absence of dissolved silicon did not prevent seed germination (Kamermans and Leenders, unpublished data). In addition, seedling survival rates were not significantly higher in conditions with, than without, silicon (Kamermans and Leenders, unpublished data). These observations suggest that silicon is not important for seed germination and seedling growth of *Z. marina*.

Liang et al. (1996) demonstrated that addition of silicon to barley plants resulted in a reduction of membrane permeability in the leaves. This reduced the toxicity of salt to the plants. Results of Exp. 3, in which *Zostera marina* plants were subjected to different combinations of salinity and silicon levels, suggest a similar mechanism. The most poorly performing plants were indeed found in the high-salinity–low-silicon treatment. However, plants grown under high-salinity–high-silicon conditions did not grow as well as the plants from the two low-salinity treatments. Thus, the negative effects of exposure to high salinity were not counteracted by the presence of silicon. Hence, the remarkable observation of Herman et al. (1996) that eelgrass coverage significantly correlated with water column silicon levels, but not significantly with salinity, remains to be explained. Perhaps another factor, which was not included in the analysis and co-varies with water column silicon levels, is important.

In conclusion, enhancement of dissolved silicon concentrations in the water does not stimulate the above-ground production nor increase the final above- or below-ground biomass of *Zostera marina*. Low salinity, however, stimulates growth in *Z. marina* from historically estuarine areas such as Lake Grevelingen. Recovery of the autochthonous Dutch eelgrass populations is, therefore, expected to be favoured when the estuarine conditions of the seagrass areas are re-established, or when restoration programmes are carried out with allochthonous ecotypes that are less sensitive to high salinities.

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