The impact of nitrogen fertilisation and salinity treatments on populations of *Elymus athericus* on Schiermonnikoog.

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The impact of nitrogen fertilisation and salinity treatments on populations of *Elymus athericus* on Schiermonnikoog.

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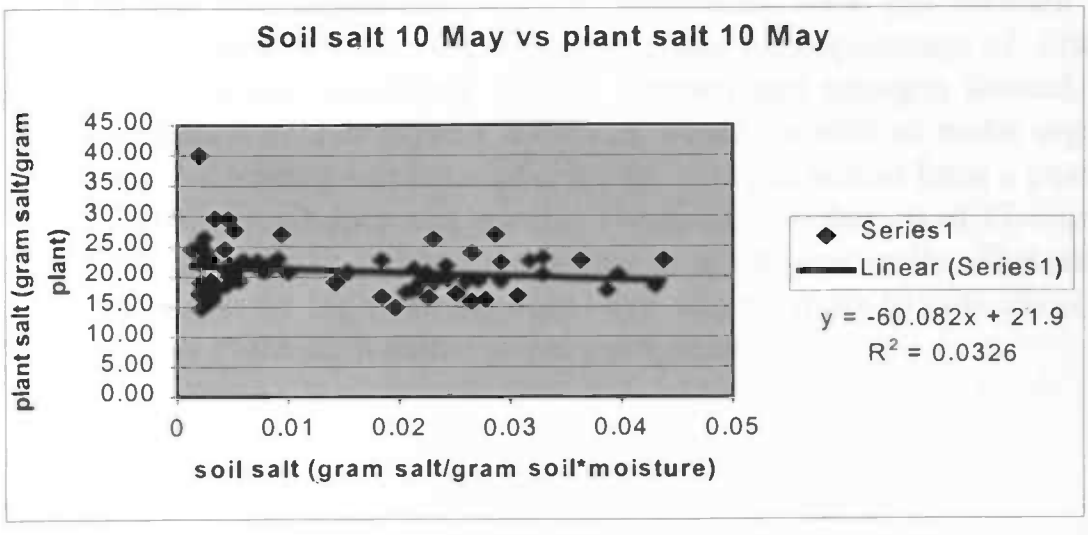
Summary

Elymus athericus has extended its distribution within the Wadden Sea salt marshes over the past 20 years. In the upper marsh, *Elymus* species almost form a monoculture and also the lower marsh with higher salinity stress is being invaded. In the salt marsh soil salinity is thought to be the major stress factor for plant growth. Plants can develop different mechanisms to cope with salt stress and one of them is the production of organic solutes, which contain nitrogen. But also nitrogen is a limiting factor for plant growth on the salt marsh. Therefor we might expect an interaction between salinity stress and nitrogen availability. Our first hypothesis was that *Elymus athericus* would be able to make such organic solutes with increasing salt stress. Secondly, nitrogen would have a positive effect on salinity resistance and growth. Third, the plants on the high marsh might have adapted more to salt stress because the populations of *Elymus* are older in the high marsh. To test our hypothesis, I did an experiment with nitrogen fertilisation and salinity manipulation at the high and low marsh on Schiermonnikoog. These plots were treated with different concentrations of salt or fresh water and different concentrations of ammonium sulphate with labelled ^{15}N. Plant growth was measured and soil and plant samples were taken to control the treatment effects. We found no significant differences in plant growth for the different nitrogen treatments. The nitrogen data to control the treatment effects are not yet available, but some results show that some labelled nitrogen did end up in the plants. Differences only occur between habitats, low marsh plants had a bigger shoot length and a higher growth rate. In most cases the salinity treatments did not influence plant performance, because we did not manage to increase or decrease soil salinity with our treatments. The temporal and spatial background variation in soil salinity was very high. However, while the soil salinity in the low marsh is ten times higher than the high marsh, the plant salinity is the same. This indicates the production of organic solutes. It also indicates the adaptation of *Elymus* to its habitat. Finally, drought also seemed to be an important stress factor during my experiment.

Introduction

Over the past 20 years clonal grasses of the genus *Elymus* have extended their distribution within Wadden Sea salt marshes, leading to a considerable loss in species and community diversity (Bakker 1989; Leendertse 1995). *Elymus* species form almost a monoculture in the upper marsh and have invaded lower salt marsh communities at several sites (Bockelmann and Neuhaus 1999). In salt marshes, one of the major factors governing zonation and succession is soil salinity, which strongly depends on inundation with seawater (Rozema et al.1985). Soil salinity is thought to be the major stress factor for plant growth. It has been recognised that in saline environments the adverse effects of a low external redox-potential can be relaxed through the uptake of electrolytes by the plants. But this uptake also creates the danger of ion excess (Greenway et al.1980). In this potentially disastrous situation different species may develop diverse mechanisms of adaptation. One way of adaptation is characteristic for halophytes. Those plants have a high ion uptake and store them in the vacuole to maintain the turgor pressure. An other way of adaptation to salt stress is avoidance of excess ion concentration, through controlled uptake and transport of ions to the shoot. Plants can finally remove ions by dying leaves or shoots. A third way of adaptation is avoidance of internal water deficit by maintaining the turgor pressure through the synthesis of organic solutes that counterbalance the osmotic pressure of the ions (Greenway et al. 1980). These organic solutes like proline, sucrose, glycine and betaine could have not only a contribution to the osmotic balance (Stewart et al. 1974), they could also have a protective effect on enzymes in the presence of high electrolyte concentration in the cytoplasm (Pollard et al. 1979). In the course Vegetatie Dynamica at Groningen University in June 2000, no elevation in salt concentration of plants with 10 times higher soil salinity was found.



This means that *Elymus athericus* could produce organic solutes with increasing salt stress. Organic solutes contain nitrogen. Nitrogen also plays a roll as a limiting nutrient. At salt marshes often nitrogen depletion is found (Kiehl et al 1997; Van Wijnen 1999). So nitrogen is also a stress factor in salt marshes. The size of the nitrogen pool of the salt marsh soil depends on the input and leaching during flooding with seawater. Furthermore, it is determined by the amount of clay, sand and organic matter in the soil because this controls the capacity to absorb the nitrogen and the nitrogen mineralisation. Due to longer periods of inundation, the thickness of the clay layer and the related nitrogen pool is larger on the low than at the high salt marshes (Olff et al. 1997). So the amount of available nitrogen can differ very much between two places in the same area on the salt marsh if the soil is heterogeneous or differing in elevation.

In the absence of salinity, plant growth in relation to the concentration of an essential nutrient in the root media is often described by a generalised dose response curve (Berry and Wallace, 1981). There is a nutrient concentration window where plant growth is optimal. Under saline conditions the range of this window may be widened, narrowed or it may shift, depending on the salinity level (Grattan and Grieve, 1994). Reviewers as Adams and Doerge, (1987) and Feigin, (1985) found that plant growth was increased by nutrient application regardless of whether the plants were salt stressed or not. Previous experiments with *Elymus athericus* showed different results. In some cases *E. athericus* built up more biomass after nitrogen addition, only if higher doses (100 – 300 kg N ha^{-1}) were used(Leendertse 1997; van Wijnen 1999). But Leendertse (1995) found an increase in biomass at 60 kg N/ha. In other cases, mostly with lower concentrations (30-40 kg N ha^{-1}), no effect on growth or biomass was found (Hennings 1995; Bockelmann and Neuhaus 1999: Bockelmann unpublished results;). However, none of these experiments controlled whether the added nitrogen really entered the plants.

Based on the aforementioned, several hypotheses were put forward and tested in this research. Under the assumption that the populations of *Elymus athericus* on Schiermonnikoog are salt stressed and nitrogen limited, our first hypothesis was that *Elymus athericus* would be able to make organic solutes with increasing salt stress. Secondly, nitrogen would have a positive effect on salinity resistance and growth. Third, the populations of *Elymus* are older in the high marsh and had more time to adapt genetically. This means that the plants on the high marsh might have adapted more to salts stress and therefor plants in the high marsh might grow better.

Methods

The site of research.

This research was carried out at the salt marsh near Kobbeduin, which is situated on the eastern part of Schiermonnikoog, The Netherlands. The salt marsh can be characterised by a gradient from low to high elevation. The lower part of the salt marsh is close to the 3rd creek (see Fig. 1). This part of the salt marsh is flooded 280-x yr^{-1} . The soil is consisting of sand and a 20-cm thick clay layer. The vegetation is dominated by an *Artemisia maritima, Atriplex portulacoides, Puccinellia maritima, Salicornia europaea* and some clusters of *Elymus athericus*. The higher parts are less flooded than the low salt marsh (90-x yr^{-1}). The soil of the high salt marsh is heterogeneous. At certain places, a thick clay layer is present. At other places only sand and organic matter is present. These big differences are for a part due to insects, especially ants which you don't find on the low salt marsh. The vegetation on the high salt marsh contains mainly *Elymus athericus* (almost 100% cover of the vegetation), with some *Artemisia maritima, Atriplex hastata* and *Festuca rubra.*

Experimental design.

At both the high and the low salt marsh 5 replicate blocks were installed randomly. The blocks on the high salt marsh contained almost 100% cover of *Elymus athericus*. On the low salt marsh 3 of the blocks (no. 2,3,5) contained almost 100% of *Elymus athericus* and the other blocks also contained *Artemisia maritima, Atriplex portulacoides* and *Puccinellia maritima.* Each block contained 7 square plots of 30x30 cm. The plots in the blocks were treated with different combinations of fresh or salt water and different amounts of labelled nitrogen (see Fig. 2). All the plots were placed at a minimum distance of 75 cm from each other to avoid that different treatments could influence each other (see Fig. 3). The treatments were applied 6 times, every second or third week.

The fertiliser (250 ml.) was slowly added to the plots with a watering can. Rainwater and water from a cattle drink water through was used as fresh water treatment whereas water from the creek was used as salt water treatment. Water from the creek was used as salt water. I tested the effect of the creek water on the plants beforehand to check if they could survive the

salinity. Three days, 1 litre of creek water was given to the plants. They all survived well.
As fertiliser, ammonium sulphate, NH_4SO_4, was applied. It contained 10% ^{15}N to enable tracking of the fertiliser in the plants and soil. For this experiment, I used higher and lower doses of nitrogen. 40 kg N ha^{-1} was used because the atmospheric nitrogen deposition is approximately 30 kg N/ha (Leendertse 1995). I wanted to know if this atmospheric nitrogen deposition can have an influence on the growth of *Elymus athericus*. I used 100 kg N ha^{-1} to be sure there would be an effect of the fertilisation.
To learn about the interaction between nitrogen and salinity stress, I manipulated the soil salinity by adding fresh or salt water. I hypothesised that with less salinity stress, plants would grow better with the same amount of nitrogen, than with high salinity stress. Measuring the soil and plant salinity also controlled the effect of the salinity treatments. In this experiment we did not use labelled NH_4NO_3 because NO_3^- is negatively charged and will not stick to soil particles but flushes away very easily.
In every plot, 5 replicate plants were marked with different coloured straws. The plants were randomly chosen. Before the experiment and each time before the fertiliser was added, all plants were measured to find a difference in growth for the different treatments. The shoot length of the whole plants, the length and width of the 2nd and 3rd leaves were measured. In order to find the same leaves back, the leaves were perforated with a needle. Additionally the number of leaves was counted.

Control of the experimental design.

In order to control whether the plants took up the applied nitrogen, we took soil samples and plant samples in each plot before the experiment and 3 days, 1 week and 2 weeks after a fertilisation. The soil samples were taken with a soil corer of 1 cm ∅. Only the first 10-cm of soil was taken because the roots are most present in the upper 10 cm of the soil. The samples were weighted and dried at 80 °C for 24 hours. Subsequently, soil samples were ground in a mill till the soil was homogenous. The plant samples were ground in a pulverisette till all the fibres were gone. All the samples were measured with a automated C/N analyser at the Biological Centre of Groningen University. The nitrogen samples taken during the experiment, were also weighted, dried and ground as described above. Seven milligrams of the plant samples and 23 milligrams of the soil were taken and folded in tin cups. The concentration of normal ^{14}N and the ^{15}N isotope was measured with a mass-spectrometer at the Centre of Isotope Analysis (CIO) of Groningen University.

To control the salinity treatment we also took soil samples, which were weighted and dried as described above. I ground 5 grams of soil and added 20 ml of demimineralsised-water afterwards. The salinity was measured with a conductivity meter (y=(x-4.6)/70.48). When they were taken, the first 140 plant samples were ground and 40 ml of demi-water was added afterwards. The salinity was measured with a conductivity meter. The other 140 plant samples were first dried before grinding and 40 ml of demi-water was added before measuring the salinity.
We also tried to get soil water samples to be able to track fertiliser in the soil water. Unfortunately they could not be taken because the soil was too dry for the zippers we used.

Statistics.
The plant growth data were processed in the statistical program SPSS. Assumed was that plants grow following a normal distribution (homogeneity of variances). For the plant data different factorial ANOVA's were made. The block factor was nested in habitat. The first measurements were set as covariant to remove differences at the beginning from the model. The salinity data were also processed in SPSS with different ANOVA's. For the correlation between soil salinity and plant salinity I used a Linear Regression (Microsoft EXCEL).

Results

Plantgrowth.

The total lengths of the plants increase over time. The growing season for adult *Elymus athericus*-shoots from 2000 apparently ends around the beginning of august (Fig. 4).

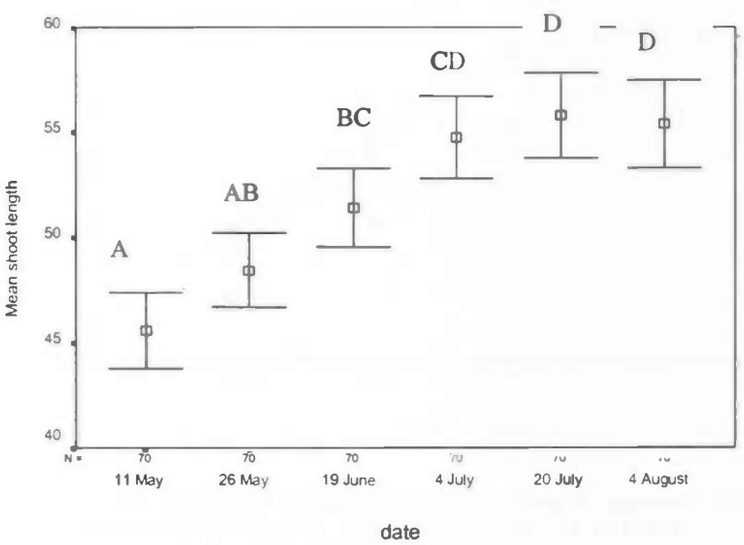


Fig. 4 : The mean total length of the plants over time, significant differences are indicated with the different letters. (ANOVA : df=5, MS=1226.091, F=18.942 ; post hoc Tukey HSD).

The addition of different concentrations of nitrogen had no significant effect on the shoot length of the plants, only a small trend of the adverse effect of 100-kg N/ha on plant growth is visible (Fig. 5).

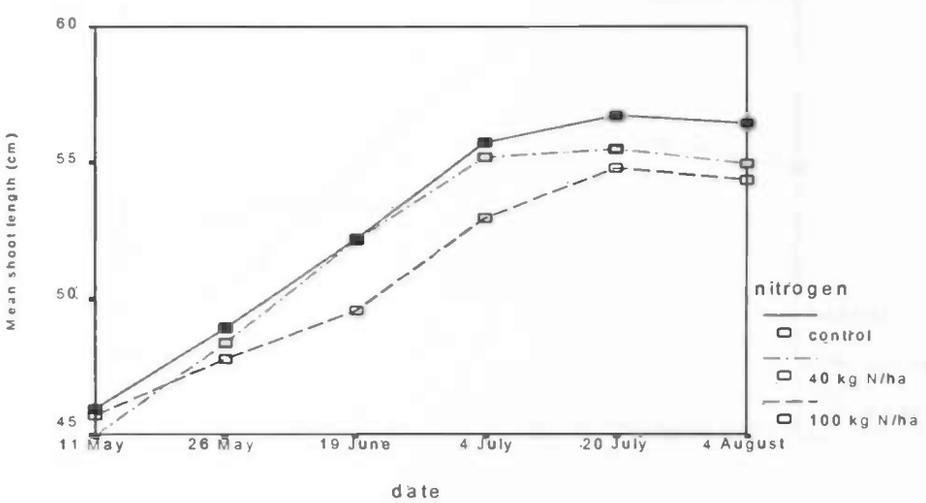


Fig. 5 : No significant differences in shoot length between different nitrogen treatments (Repeated Measurements ANOVA: df=2, MS=107.075 F=1.146, p=0.327).

The addition of fresh or salt water had no significant effect on the shoot length of the plants, only a small trend of the adverse effect of salt water on plant growth is visible (Fig. 6). However, the interaction between habitat and salinity was significant.

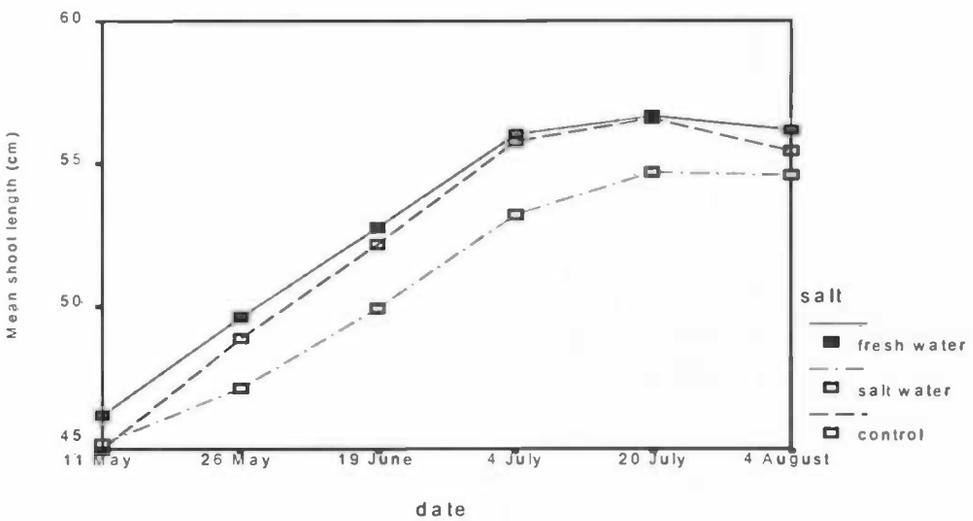


Fig. 6 : No significant differences in shoot length between different salinity treatments (Repeated Measurements ANOVA: df=2, MS=106.445, F=1.139, p=0.329).

The different habitats had a significant effect on the shoot length of the plants. In the beginning the shoot lengths on the high salt marsh are taller, like other years, but in June the shoots of the low marsh become taller. Plants on the low salt marsh have a bigger increase in shoot length than plants on the high salt marsh (Fig.7).

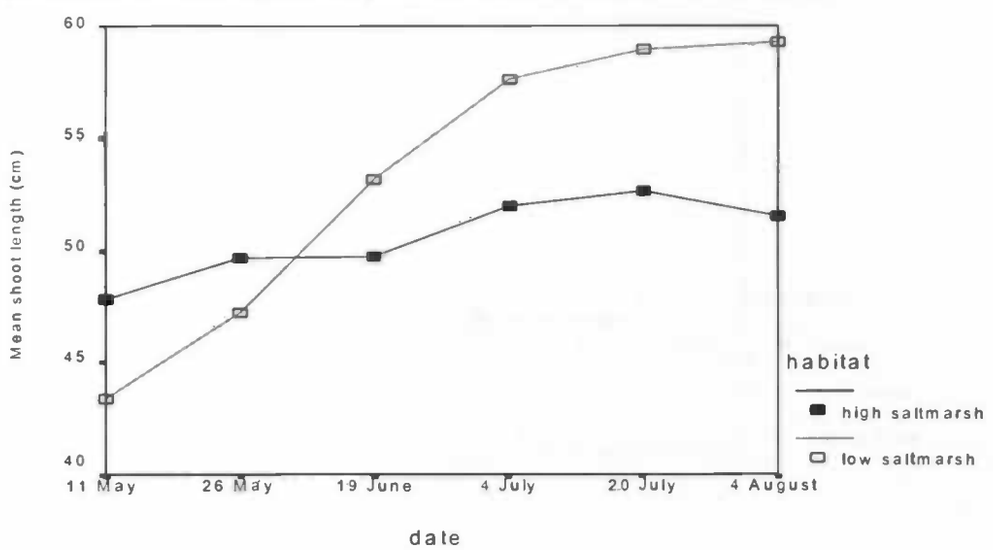


Fig. 7 : Significant differences in shoot length between different habitats (Repeated Measurements ANOVA: df=1, MS=4157.872, F=44.5, p=0.020).

The lengths of the second leaves decrease in time, in contrast to the total length of the plants (Fig. 8). When the first leaf becomes second leaf, the leaf stops growing and starts dying off, growth only occurs in the top of the plants

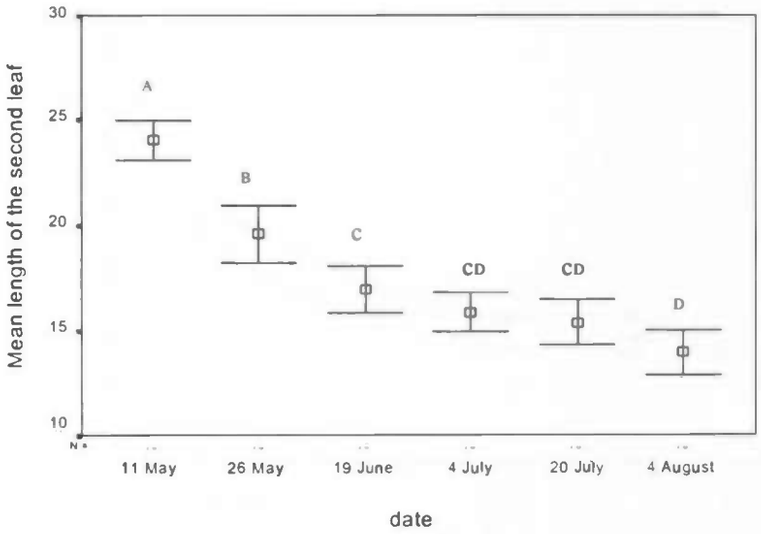


Fig. 8 : The mean length of the second leaves of the plants in time, significant differences are indicated with the different letters. (ANOVA : df=5, MS=942.327, F=44.604 ; post hoc Tukey HSD).

The addition of different concentrations of nitrogen had no significant effect on the length of the second leaves of the plants (Fig. 9).

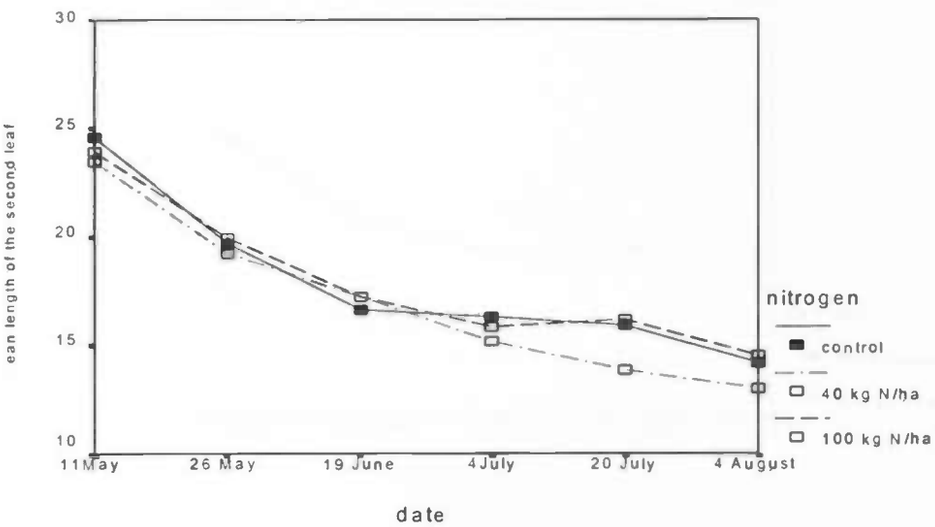


Fig. 9 : No significant differences in the mean length of the second leaf between different nitrogen treatments (Repeated Measurements ANOVA: df=2, MS=18.197, F=0.577, p=0.566).

The addition of fresh or salt water had no significant effect on the mean length of the second leaf of the plants, only a small trend of the adverse effect of salt water on plant growth is visible (Fig. 10).

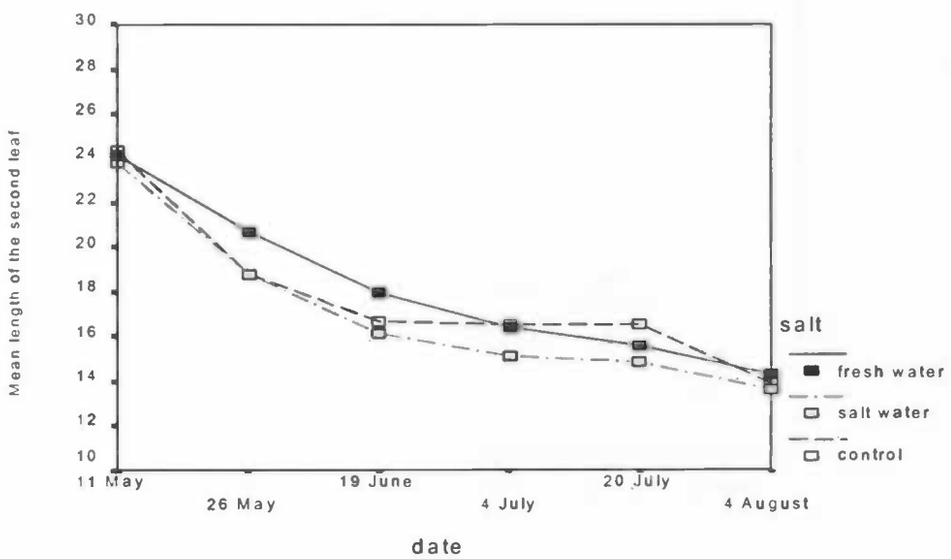


Fig. 10 : No significant differences in the mean length of the second leaf of the plants, between different salinity treatments (Repeated Measurements ANOVA: df=2, MS=50.049, F=1.586, p=0.215).

The differences between habitats were significant for the mean length of the second leaf of the plants. The second leaves of the plants on the low salt marsh are smaller than on the high salt marsh (Fig.11).

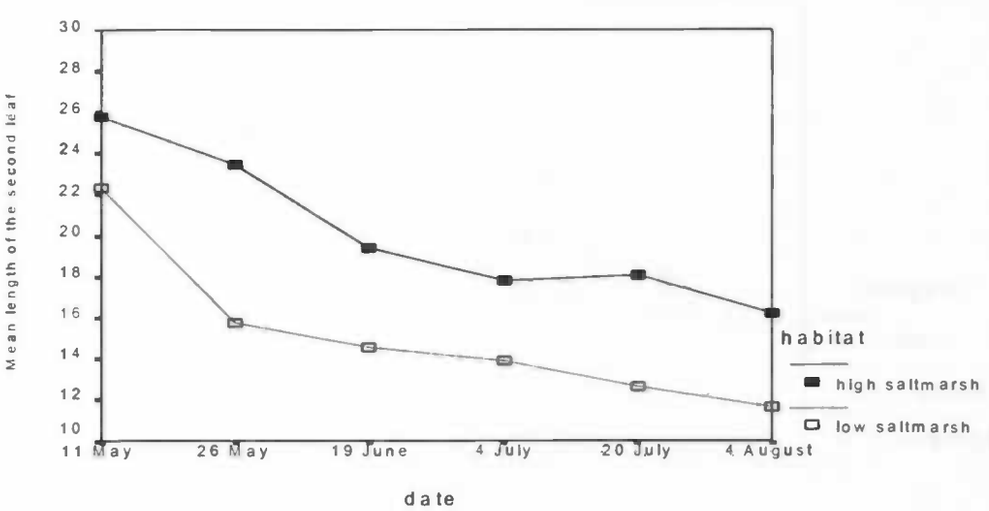


Fig. 11 : Significant differences in the mean length of the second leaf of the plants between different habitats (Repeated Measurements ANOVA: df=1, MS=629.573, F=19.956, p=0.0001).

As the lengths of the second leaves, the lengths of the third leaves also decrease in time, in contrast to the total length of the plants (Fig. 12).

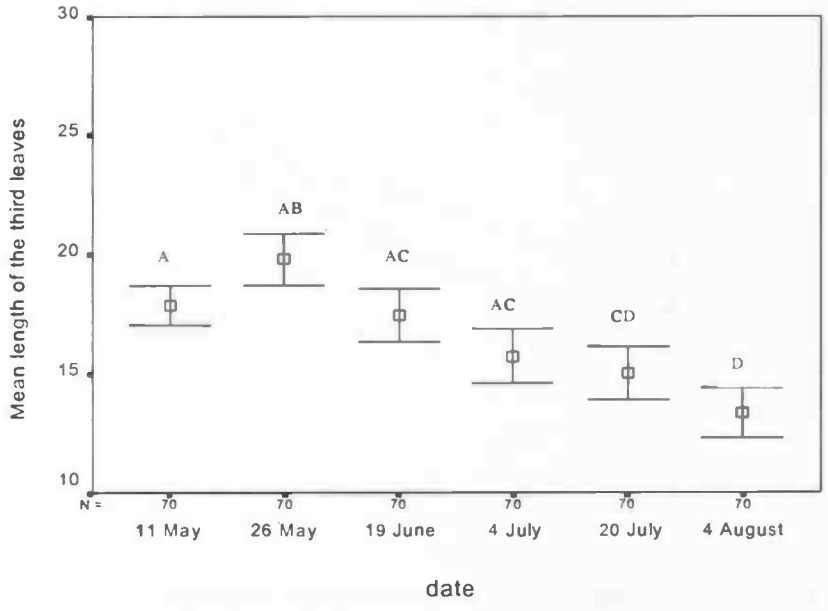


Fig. 12 : The mean length of the third leaves of the plants in time, significant differences are indicated with the different letters.(ANOVA : df=5, MS=369.527, F=18.503 ; post hoc Tukey HSD).

The addition of different concentrations of nitrogen had no significant effect on the mean length of the third leaves of the plants (Fig. 13).

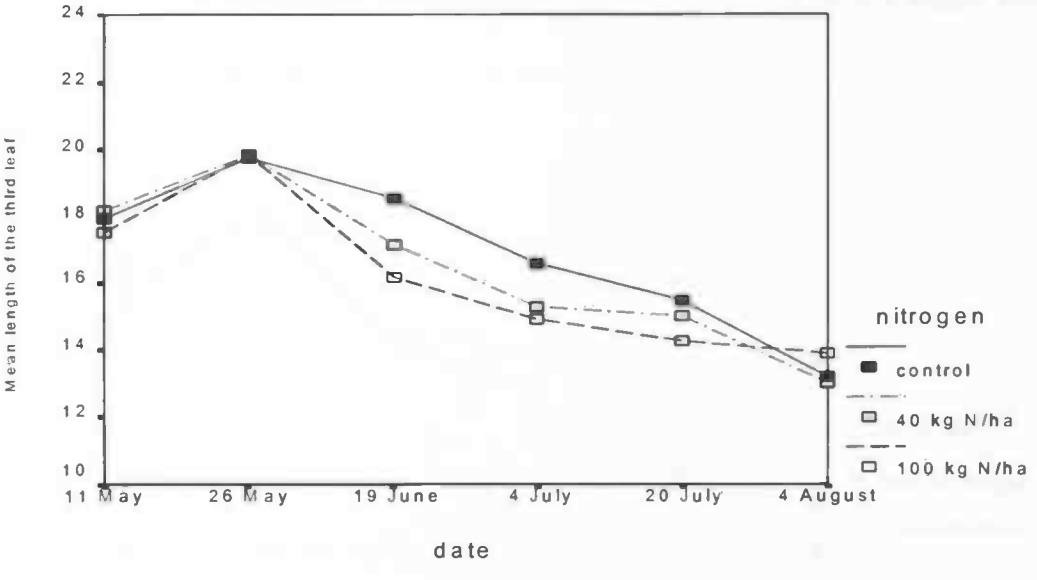


Fig. 13 : No significant differences in the mean length of the third leaf between different nitrogen treatments (Repeated Measurements ANOVA: df=2, MS=11.597, F=0.409, p=0.667).

The addition of fresh or salt water had no significant effect on the mean length of the third leaf of the plants (Fig. 14).

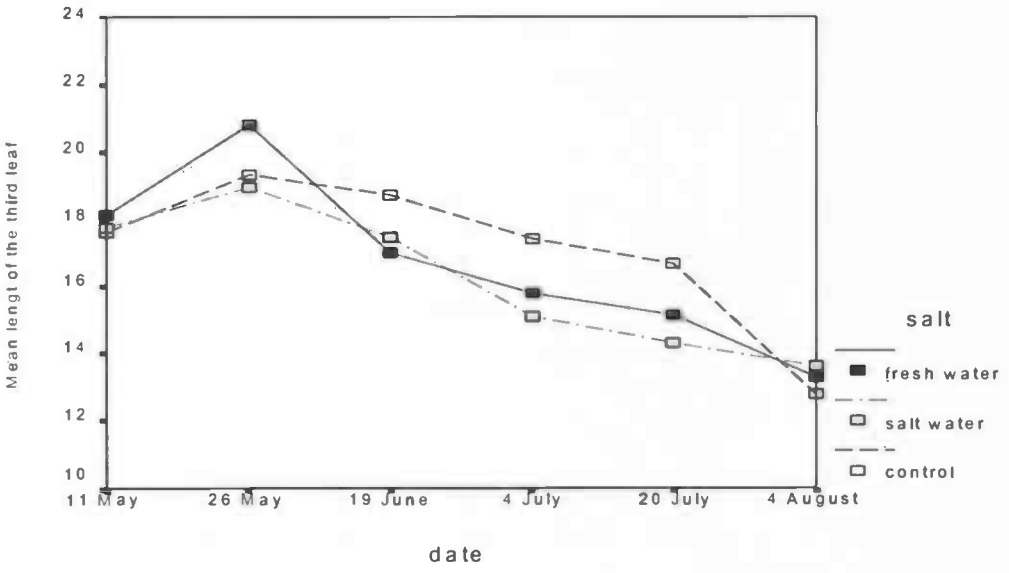


Fig. 14 : No significant differences in the mean length of the third leaf of the plants, between different salinity treatments (Repeated Measurements ANOVA: df=2, MS=10.886, F=.384, p=0.683).

The differences between habitats were significant for the mean length of the third leaf of the plants. The third leaves of the plants in the low salt marsh are smaller than on the high salt marsh from 26 May onwards. Here a same crossing of the lines is present as in the shoot lengths of the plants. The leaves on the low salt marsh have a bigger decrease over time (Fig.15).

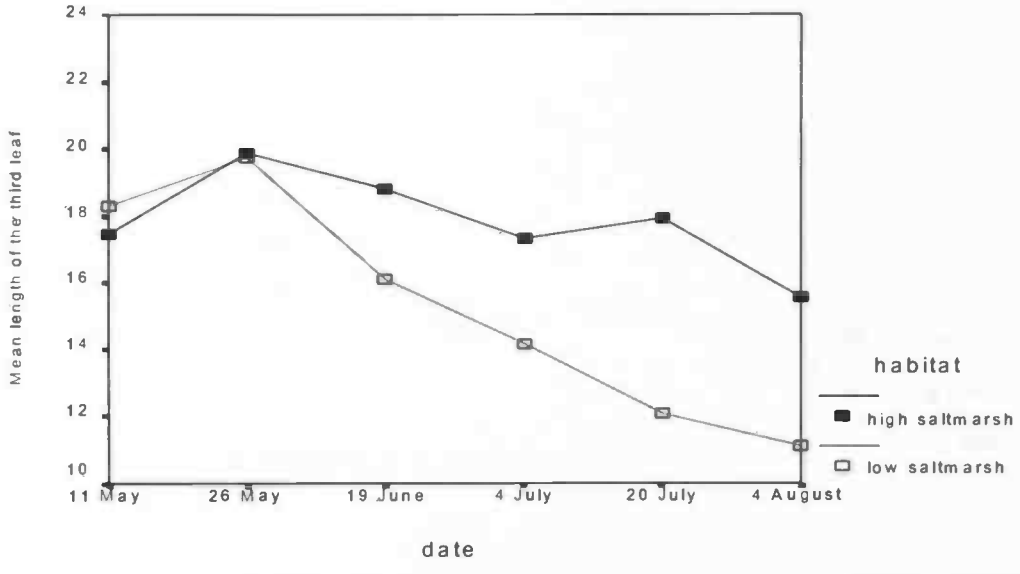


Fig. 15 : Significant differences in the mean length of the third leaf of the plants between different habitats (Repeated Measurements ANOVA: df=1, MS=783.965, F=27.630, p=0.0001).

As the lengths of the leaves, the mean number of leaves per plant decreased in time (Fig. 16).

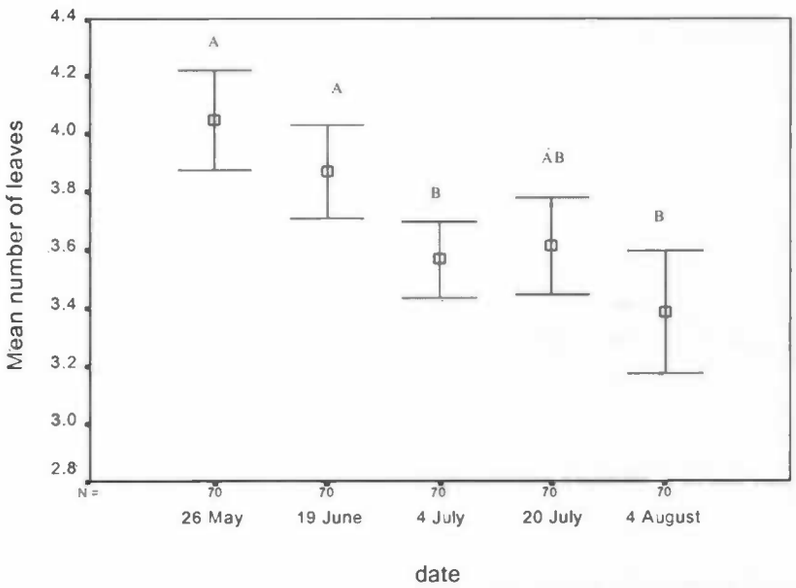


Fig. 16: The mean number of leaves per plants in time, significant differences are indicated with the different letters. (ANOVA : df=4, MS=4.811, F=9.356 ; post hoc Tukey HSD).

The addition of different concentrations of nitrogen had no significant effect on the mean number of leaves per plant (Fig. 17).

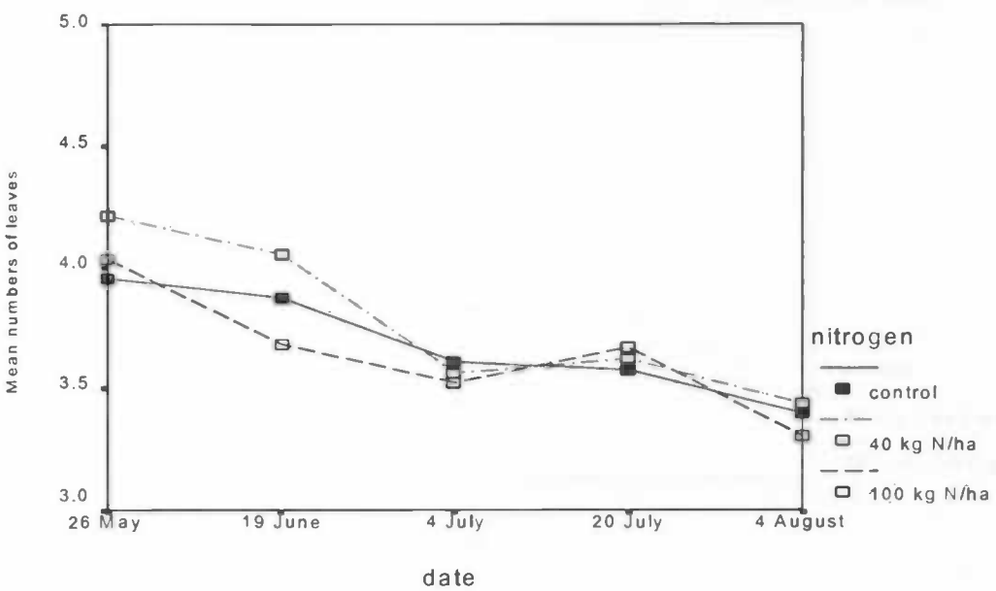


Fig. 17 : No significant differences in the mean number of leaves between different nitrogen treatments (Repeated Measurements ANOVA: df=2, MS=0.290, F=0.527, p=0.594).

The addition of fresh or salt water had no significant effect on the mean number of leaves per plant (Fig. 18).

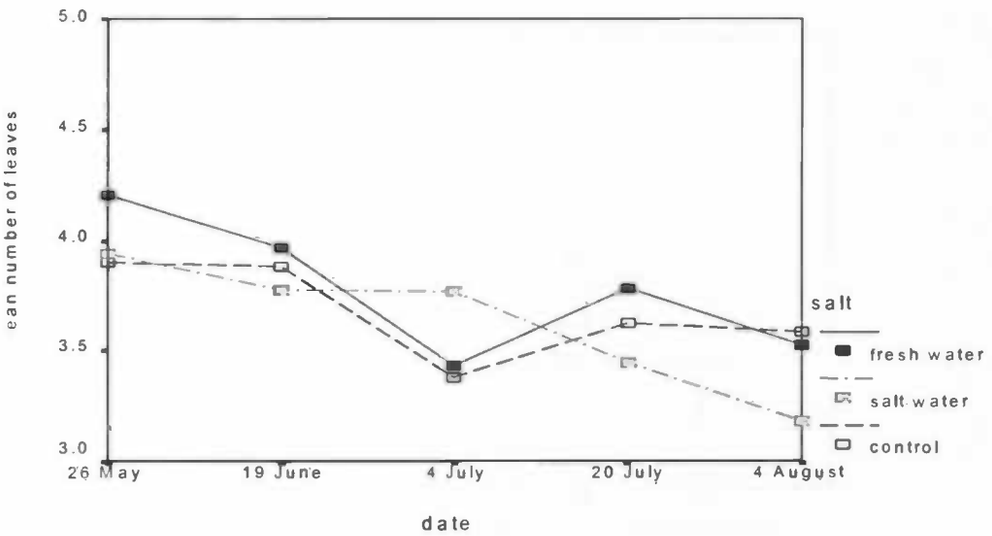


Fig. 18 : No significant differences in the mean number of leaves of the plants, between different salinity treatments (Repeated Measurements ANOVA: df=2, MS=0.470, F=0.855, p=0.432).

The differences between habitats were significant for the mean number of leaves of per plant. Plants on the low salt marsh had on average more leaves per plant than plants on the high salt marsh. This stands in contrast to the length of the leaves (Fig.19). it is also visible that the number of leaves on the high salt marsh stayed more or less constant, while the number of leaves in the low marsh had a big decrease over time.

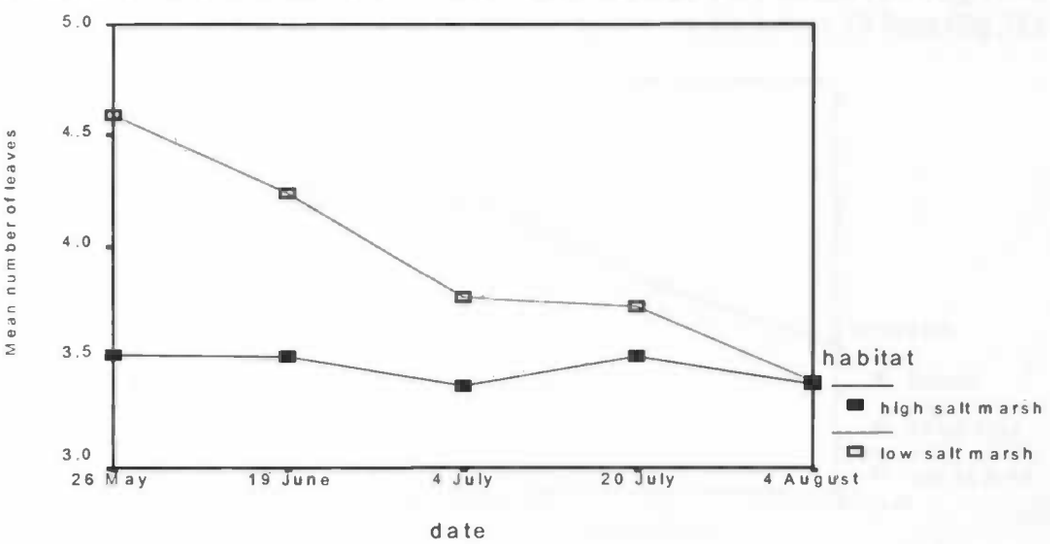


Fig. 19 : Significant differences in the mean number of leaves of the plants between different habitats (Repeated Measurements ANOVA: df=1, MS=2.211, F=4.021, p=0.0001).

The mean relative growth rate of the shoot lengths of the plant decreases over time from the beginning of July onwards. This stands in contrast to the total length of the plants (Fig. 20).

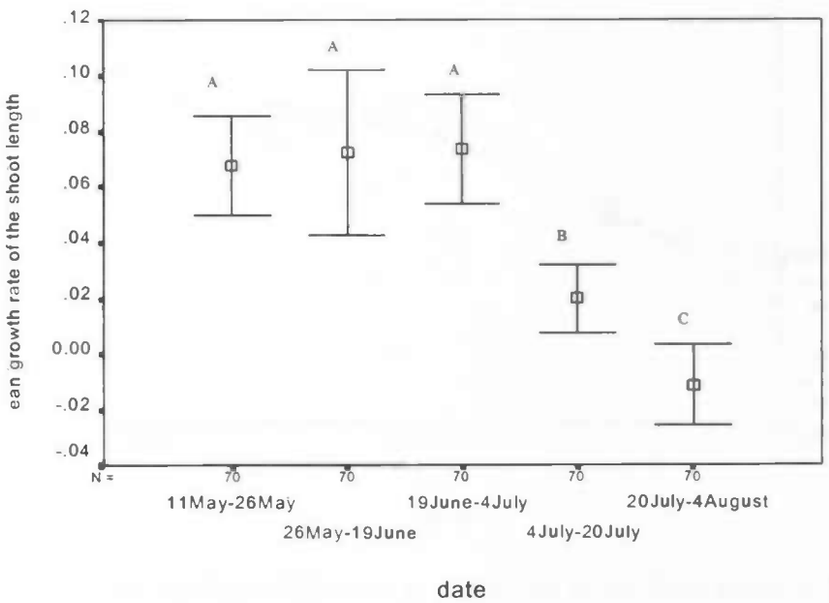


Fig. 20 : The mean growth rate of the shoot length of the plants in time, significant differences are indicated with the different letters (Univariate ANOVA : df=4, MS=0.103, F=14.746 ; post hoc Tukey HSD).

The addition of different concentrations of nitrogen had no significant effect on the growth rate of the plants. Only a small trend of a positive affect of 40-kg N/ha and an adverse affect of 100-kg N/ha on the growth rate is visible before 19 June (fig 21).

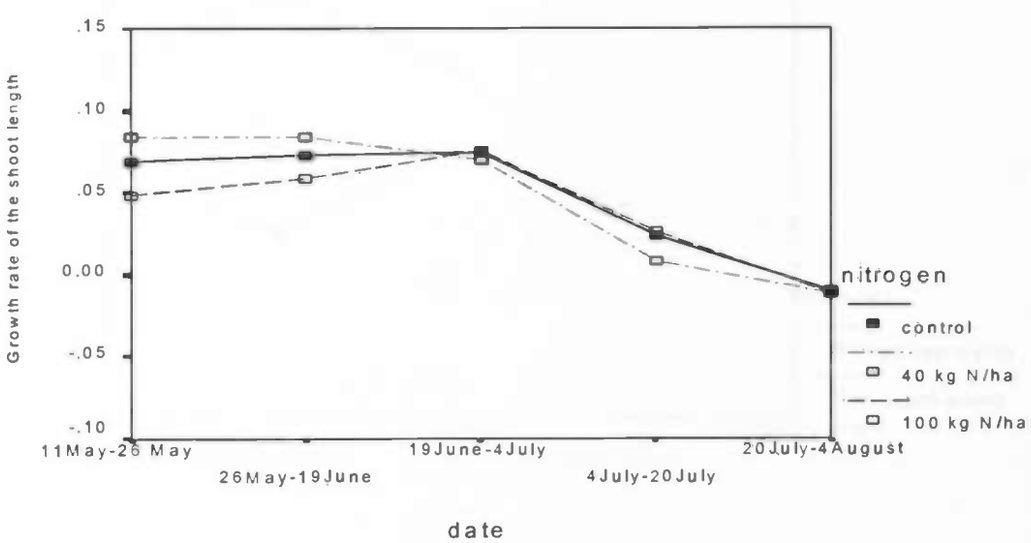


Fig. 21 : No significant differences in growth rate of the shoot length of the plants between different nitrogen treatments (Repeated measurements ANOVA : df=2, MS=1.283E-0.3, F=0.553, p=0.579).

The addition of different concentrations of fresh or salt water had no significant effect on the growth rate of the plants (fig 22).

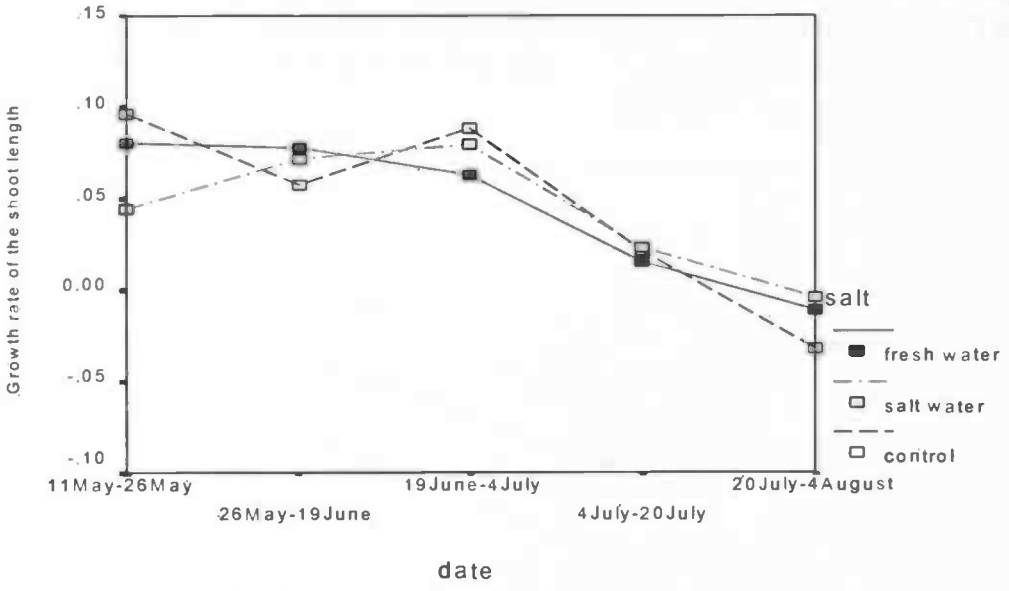


Fig. 22 : No significant differences in growth rate of the shoot length of the plants between different salinity treatments (Repeated measurements ANOVA : df=2, MS=3.734E-0.3, F=1.608, p=0.211).

Plants show different growth rates between different habitats. The plants on the low salt marsh have a higher growth rate, this is comparable with the results for shoot length (fig 23).

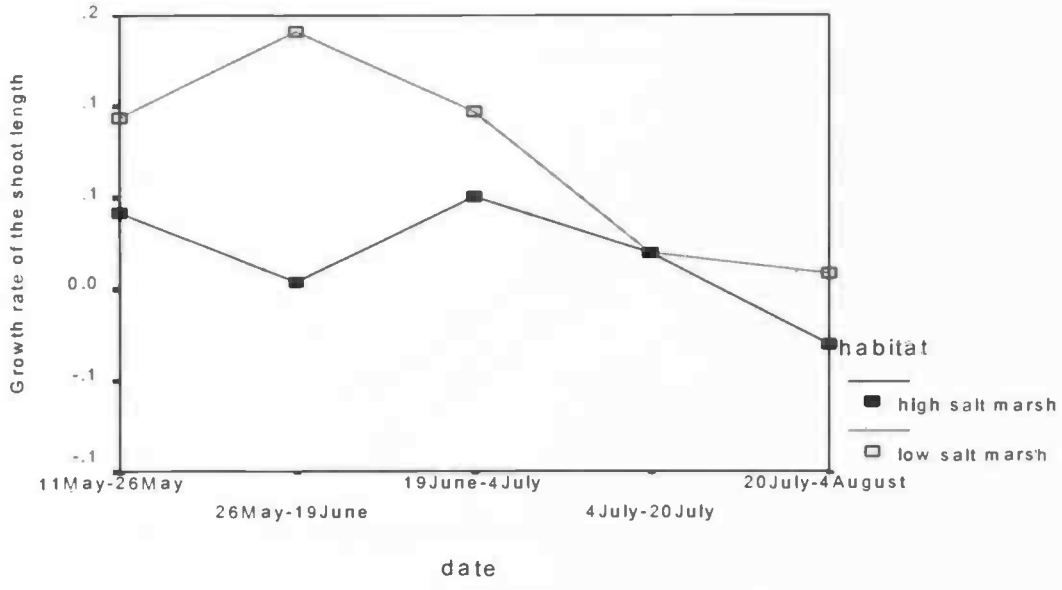


Fig. 23 : Significant differences in growth rate of the shoot length of the plants between different habitats (Repeated measurements ANOVA : df=2, MS=.126, F=54.335, p=0.0001)

Salinity control of soil and plants.

The salinity treatments had no significant effect on the soil salinity at the high salt marsh. Only a small trend is visible that salt water leads to a higher soil salinity. The soil salinity decreased very much at 22 June (Fig. 24).

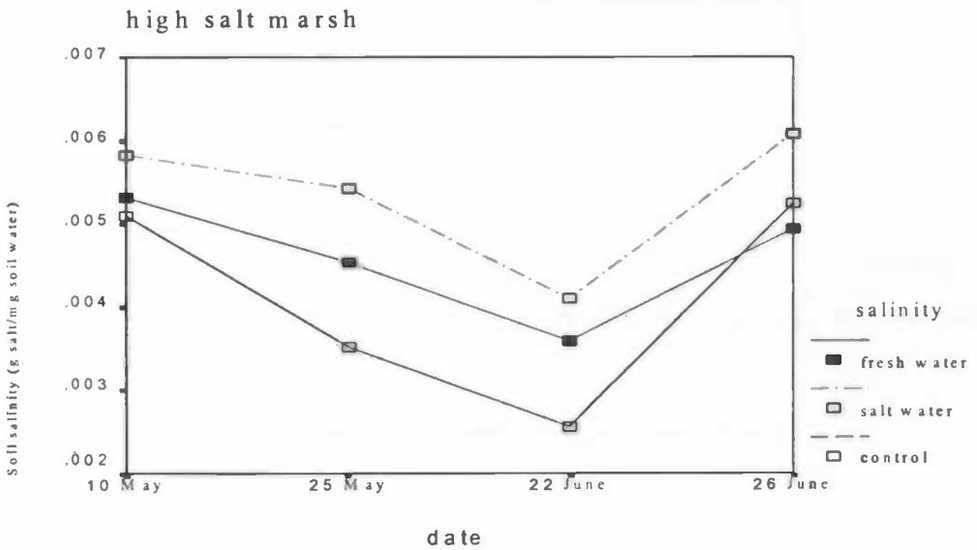


Fig. 24 : No significant differences in soil salinity between the different salinity treatments (Repeated measurements ANOVA : df=2, MS= 8.492E-0.6 F=1.253, p=0.314)

Plant salinity was also not influenced by the salinity treatments (Fig. 25).

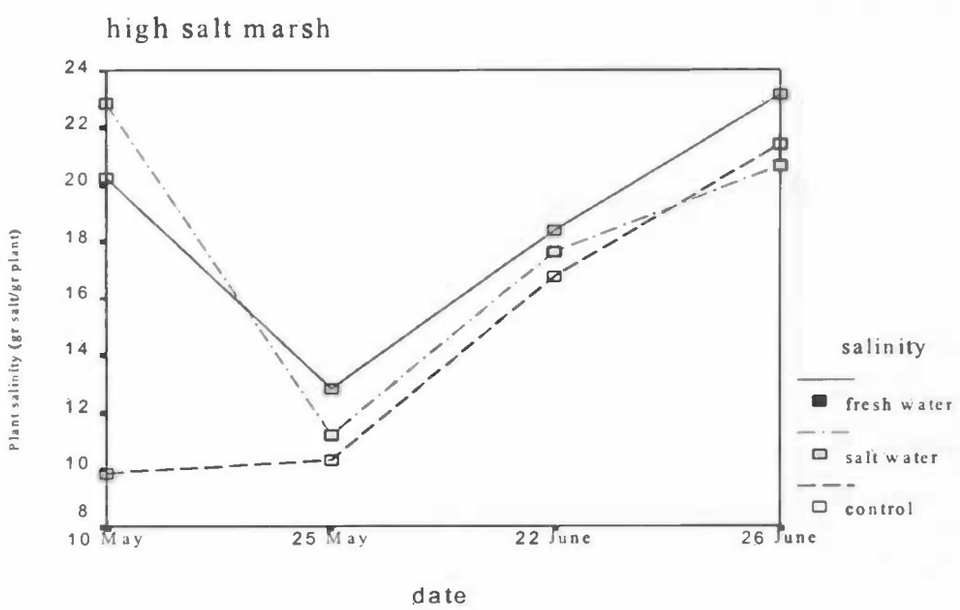


Fig. 25 : No significant differences in plant salinity between the different salinity treatments (Repeated measurements ANOVA : df=2, MS= 101.827, F=0.441, p=0.648)

The salinity treatments had also no effect on the soil salinity at the low salt marsh. However, soil salinity on the low marsh is ten times as high as on the high marsh! The soil salinity also decreases at the low marsh at 22 June (Fig.26).

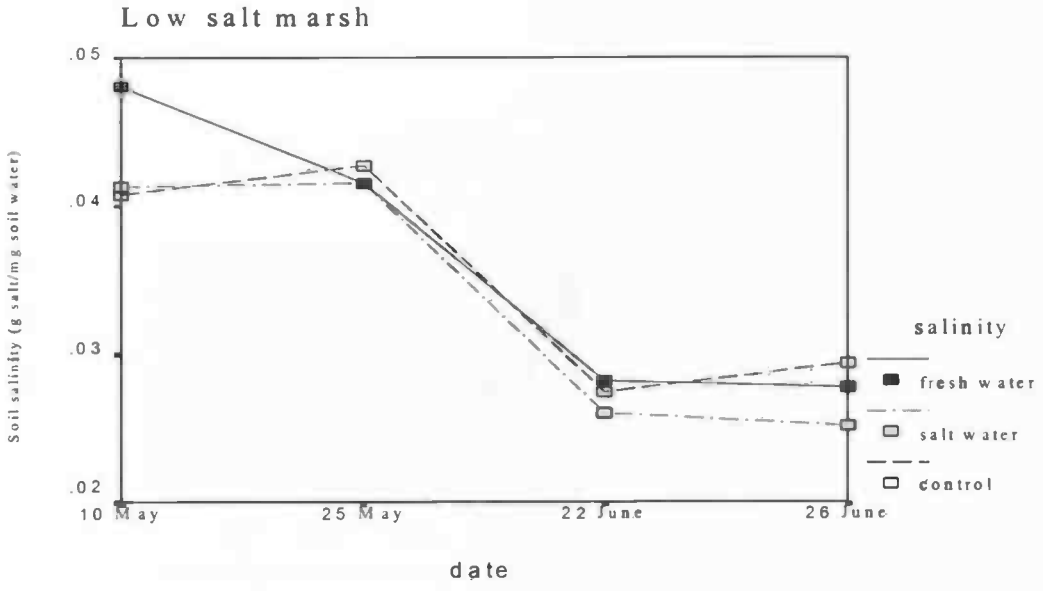


Fig. 26 : No significant differences in soil salinity between the different salinity treatments (Repeated measurements ANOVA : df=2, MS= 1.138E-0.4 F=1.563, p=0.228)

Plant salinity was also not influenced by the salinity treatments (Fig.27).

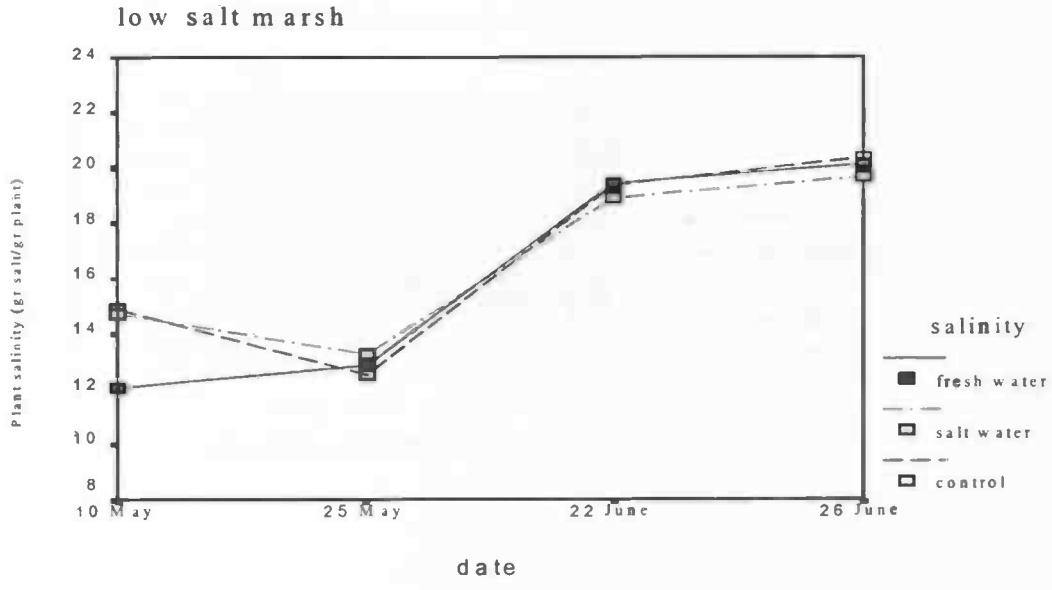


Fig. 27 : No significant differences in plant salinity between the different salinity treatments (Repeated measurements ANOVA : df=2, MS= 5.004, F=0.363, p=0.699)

I also compared the relation between plant and soil salinity at the begin and during the experiment. Only the graphs of the first and the last measurements are shown, the other graphs are similar. I found that plant salinity was not influenced by the salinity of the soil. This is consistent with the previous four graphs.

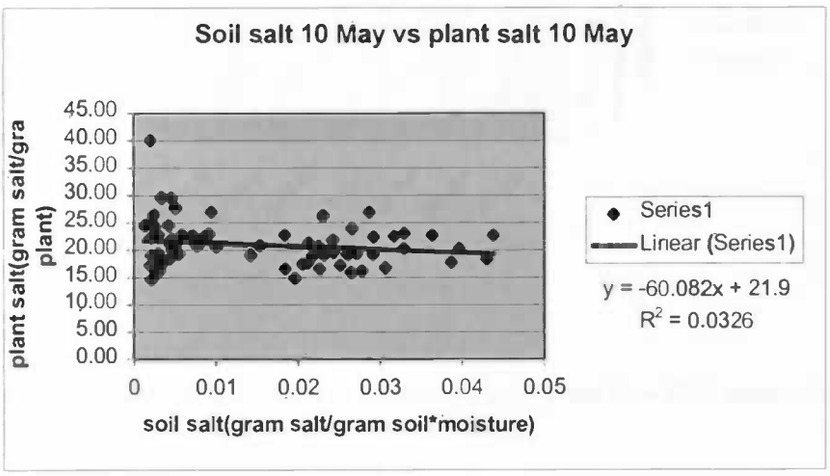


Fig. 28: Relation between salt concentrations in the plants and in the soil on May 10th 2000(R^2=0.0326).

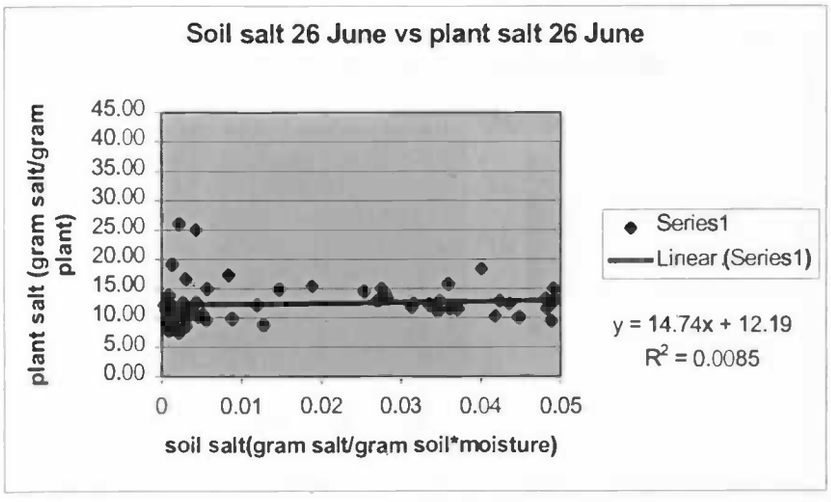


Fig. 29 : Relation between salt concentrations in the plants and soil at June 26th 2000(R^2=0.0085)

The rainfall and temperature fluctuate very much during the season (Fig.30).
I made this graph to show how the rainfall and the temperature fluctuated during my experiment.

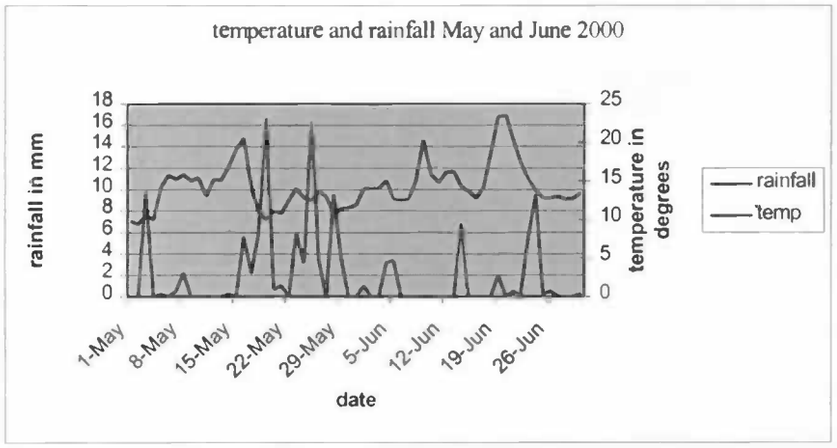


Fig 30 : Temperature and rainfall fluctuations in May and June 2000 measured by the weather station of the University of Amsterdam on Schiermonnikoog.

Rainfall and temperature in 1999 (Fig. 31).
No indication was found, that 2000 was a dryer year than 1999 (see fig. 30). However, the soil could be dryer in 2000 because the temperature is much higher in 2000. This leads to a higher evaporation of water from the soil.

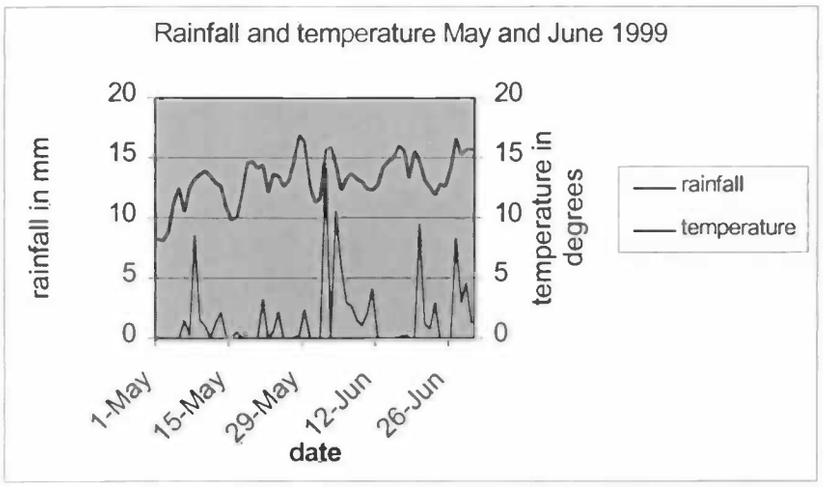


Fig 31 : Temperature and rainfall fluctuations in May and June 1999 measured by the weather station of the University of Amsterdam on Schiermonnikoog.

The percentage of water in the low salt marsh soil was higher than in the high marsh (Fig. 32).

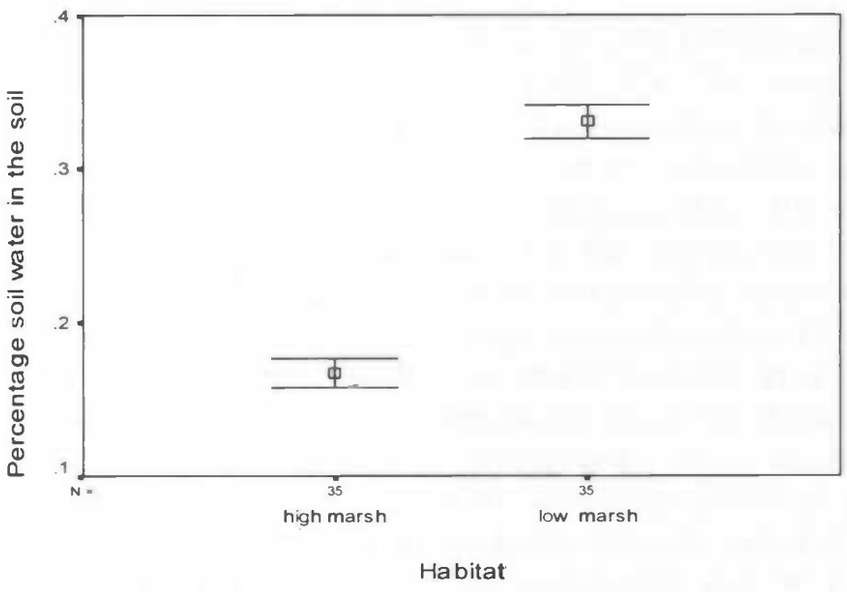


Fig. 32: The soil water percentage for the high and the low marsh plots (Univariate ANOVA : df=1, MS=0.445, F=0.445, p=0.0001)

Discussion

Plant growth and salinity.

In contrast to my hypothesis, no significant effect of the salinity treatment on plant growth was found (Fig. 6,10,14,18,22). The main reason that the treatments had no effect was caused by the failure to change soil salinity (Fig. 24, 26). Apparently, the application frequency was too low in comparison with the strong temporal changes (Fig. 30) of salinity in the environment. In other experiments on the interaction between nitrogen availability and salt stress this problem was avoided by growing the plants in greenhouses (Hodson et al. '85), experimental gardens (Van Diggelen '91) and growth chambers (Botella '94 and Cordovilla et al, '96). However, experiments under controlled conditions have the disadvantage that the environment (soil substrate, temperature etc.) is highly artificial. Furthermore, most experiments work with single plants or groups of ramets instead of intact clones. We do not know what the effect of a lack of clonal integration on the outcome of the experiment will be (see for example Wijesinghe and Handel '94).

Low marsh plants grew taller (Fig. 7,11,15,19,23) while the soil salinity in the low marsh was higher (Fig. 24, 26). The low marsh plants invested also more in reproduction by producing more spikes, but they didn't have a higher biomass (appendix). In contrast to these findings, low marsh plants showed a inferior growth when compared with high marsh populations in the same area in 1999(A.-C. Bockelmann, unpublished) found. Supporting my data, Jefferies and Perkins ('77) found that plants in the high marsh at Norfolk, England grew slower in the summer than the plants in the low marsh. They assumed that this slower growth was an adaptation to summer drought. Kiehl ('97) also mentioned that next to salinity stress, water, weather and inundation are limiting factors on plant growth. So it is not unlikely that *Elymus* athericus suffered more from water deficiency than salinity stress in the high marsh. This is supported by the fact that it was very dry in June (Fig. 30). By the end of June, there was a heavy rainfall. The soil salinity dropped very much in both, the high and low marsh (Fig. 24,26). The salinity in the plants however, went up. I assume, the water deficiency stress was that extreme that the plants took up as much water as possible during the heavy rainfall. Salt stress was probably in this situation less important. I conclude that the salt marsh ecosystem seems to be not only extreme in terms of salinity and inundation stress, but that water deficiency can be as important and should not be neglected.

I have to stress the fact that the salinity was measured only four times, the graphs 24 to 27 could be misleading. The salinity could vary much in between the measurements. It is therefore necessary to do more intense salinity measurements in the future.
But even if the salinity treatment did not fail, it is not unlikely that *E. athericus* is adapted to its habitat by not taking up more salt in the low marsh, although the salinity is higher in the low marsh (see introduction). This is supported by my findings that plant salinity stays constant at different soil salinities (Fig. 28,29).

Plant growth and nitrogen.
Also in contrast to my expectations, no effect of the nitrogen treatments on the growth of *Elymus* was found (Fig. 5,9,13,17,21). There could be several reasons for my findings.
First, the nitrogen did not reach the plants like I hypothesed, because drought and other weather conditions probably limited the plant production and nutrient uptake. I thought that the highest doses I used (100 kg/ha/yr.) would be enough to get an effect (see methods) however, Kiehl et al ('97) found the same in the first year of his fertilisation experiment (they used 250 kg/ha/yr.). They assumed that the limiting water availability explained the lack of any significant effects of nutrient addition in the first year. Bockelmann and Neuhaus ('99) also found no effect of fertilisation in a German mainland salt marsh (they used 40 kg/ha/year). Hennings ('95) fertilised with 30kg/ha on Nordstrand, Germany, and found no effect as well. So maybe the salt marsh at Schiermonnikoog was not nitrogen limited. But other fertilisation experiments by Hennings ('95) on Nordstrand, Germany and Leendertse et al. ('95) on Terschelling, with higher doses nitrogen (100-250 kg/ha/yr.) resulted in a significant increase in biomass for *Elymus athericus*. Van Wijnen ('99) found an effect of fertilisation even with 250kg/ha/year, but with lower doses (50 kg/ha/year) only in some years and not in others. I conclude, that it is unlikely that there was no nitrogen limitation. This is also supported by other findings of Van Wijnen ('99). However, the nitrogen pool of the salt marsh soil is depending on the input and leaching during flooding with seawater and is determined by the amount of clay, and organic matter (see introduction).
It is also possible that the nitrogen didn't effect the plants because it was washed away too soon. But this is in contrast to the previous argument, that drought was limiting. Moreover, the ^{15}N analysis revealed that the plants took up some of the nitrogen (preliminary results).

A third reason could be that the plants did not use the nitrogen for growth, but for salinity tolerance like I hypothesised (see introduction). Then, lower marsh plants could profit more because they have more salinity stress. Botella ('94) found that salt stressed wheatlings had 2 folds higher nitrogen took up than control plants. However, I found no differences between the different nitrogen concentrations and the control in the low or high marsh. There is even a small trend that the control plants grew better (Fig. 5,9,13,17,21). It is likely that the plants in the low marsh do not need extra nitrogen for salinity tolerance because they are already adapted to the salt stress and the nitrogen availability of their habitat. This corresponds with the hypothesis in the introduction and is also supported by previous discussion.
Also a possibility is that *E. athericus* used the nitrogen for reproduction like the higher seed production in the low marsh indicates. Finally, they could have transported the nitrogen to other parts of the clones outside the plots that we did not investigate.

Differences between the habitats.
For all measurements, significant differences were found between the plants of the high and low salt marsh (Fig. 7, 11,15,19,23).
This could be caused by the fact that the habitat induces by far the strongest selection pressure on plant performance. The differences in the environment are probably stronger than the differences created by my treatments (salinity, inundation, and soil water content). Water content could be the factor that over rules all other factors during our experiment in 2000 (Fig 32). However, I compared the weather conditions of May and June 1999 on Schiermonnikoog with the weather conditions of May and June 2000 and found that there was not a big difference in rainfall, but in temperature (see Fig.30,31). This could lead to higher evaporation in 2000 and therefor also to more water stress. However, it can be doubted that drought was the over ruling factor during the whole year. More likely is, that there is an influence of summer drought, like Jefferies and Perkins ('77) assumed.
A second reason for the difference between the plants of the low and high habitat could be that plants in the low marsh are adapted to their habitats as mentioned before in the discussion. This effect cannot be seen in very young marshes. My results indicate that *Elymus athericus* has developed a mechanism to adapt to salt stress by producing organic solutes. This means, than in combination with the summer drought, the adapted low marsh plants could have an advantage and grow better than high marsh plants in the summer and in a few years the low marsh could be invaded also by *Elymus*

athericus. However this is in contrast to other findings of A.-Chr. Bockelmann (1999, unpublished results) from previous years.

Conclusion

I conclude that the salinity and nitrogen treatments of my experiment had no effect on plant growth in my experiment. Most likely, this was caused by strong environmental fluctuations in case of the salinity treatment. The plants in the low marsh seem to be adapted to the high salinity and grew better than the plants in the high marsh this year. Nitrogen fertilisation had no effect on their growth either, but was taken up and maybe let to a higher spike production in the low marsh.

Acknowledgements

I want to thank all the people on the Herdershut for a nice time and the discussions and the input they gave on my research. Especially I want to thank Thies, Nicole, Jardy, Jeltje, Janneke, Hannah and Christophe and others, for helping me with fieldwork and Anna Bockelmann for her supervision.

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Appendix

Calculation for nitrogen fertilisation

Calculation for 15-fertilization

	diameter	area		area
plotsize	0.3	0.07065	=	3.14*(0.5*0.3)^2

	g/plot	g/20plots	kg/ha->g/plot
40kg N/ha=	0.2826	5.652	40/10000*0.07065*1000
100kg N/ha=	0.7065	14.13	

	g N	g NH4SO4	gN->gNH4SO4
nitrogen	19.782	161.082	19.782/14*114
40kg N/ha=	5.652	46.02343	
100kg N/ha=	14.13	115.0586	

N	n=14
NH4SO4	n=114

NH4SO4 per fertilization		40kg plots	100kg plots	gNH4SO4/6
(6 in total)		7.670571	19.176429	
	per plot	0.383529	0.9588214	

concentration		40kg plots	100kg plots	
per plot	M (mol/250ml)	0.000841	0.002103	M=m/n
	M (mol/l)	0.003364	0.008411	

Plant growth.

The growt rates of the shoot length are calculated with the formula:
(new shoot length-old shoot length)/ old shoot length

Univariate Analysis of Variance

Tests of Between-Subjects Effects
Dependent Variable: shoot length

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	6130.457	5	1226.091	18.942	.000
Intercept	1131804.113	1	1131804.113	17485.081	.000
DATE	6130.457	5	1226.091	18.942	.000
Error	26798.097	414	64.730		
Total	1164732.666	420			
Corrected Total	32928.554	419			

a R Squared = .186 (Adjusted R Squared = .176)

	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
(J) date of measuring				Lower Bound	Upper Bound
2.00	-2.8614	1.3599	.285	-6.7368	1.0140
3.00	-5.8600	1.3599	.000	-9.7354	-1.9846
4.00	-9.1907	1.3599	.000	-13.0661	-5.3153
5.00	-10.2143	1.3599	.000	-14.0897	-6.3389
6.00	-9.7921	1.3599	.000	-13.6676	-5.9167
1.00	2.8614	1.3599	.285	-1.0140	6.7368
3.00	-2.9986	1.3599	.235	-6.8740	.8768
4.00	-6.3293	1.3599	.000	-10.2047	-2.4539
5.00	-7.3529	1.3599	.000	-11.2283	-3.4774
6.00	-6.9307	1.3599	.000	-10.8061	-3.0553
1.00	5.8600	1.3599	.000	1.9846	9.7354
2.00	2.9986	1.3599	.235	-.8768	6.8740
4.00	-3.3307	1.3599	.140	-7.2061	.5447
5.00	-4.3543	1.3599	.017	-8.2297	-.4789
6.00	-3.9321	1.3599	.044	-7.8076	-5.6728E-02
1.00	9.1907	1.3599	.000	5.3153	13.0661
2.00	6.3293	1.3599	.000	2.4539	10.2047
3.00	3.3307	1.3599	.140	-.5447	7.2061
5.00	-1.0236	1.3599	.975	-4.8990	2.8518
6.00	-.6014	1.3599	.998	-4.4768	3.2740
1.00	10.2143	1.3599	.000	6.3389	14.0897
2.00	7.3529	1.3599	.000	3.4774	11.2283
3.00	4.3543	1.3599	.017	.4789	8.2297
4.00	1.0236	1.3599	.975	-2.8518	4.8990
6.00	.4221	1.3599	1.000	-3.4533	4.2976
1.00	9.7921	1.3599	.000	5.9167	13.6676
2.00	6.9307	1.3599	.000	3.0553	10.8061
3.00	3.9321	1.3599	.044	5.673E-02	7.8076
4.00	.6014	1.3599	.998	-3.2740	4.4768

General Linear Model

Repeated measurements ANOVA for shoot length
Tests of Between-Subjects Effects
Measure: MEASURE_1
Transformed Variable: Average

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Intercept	1363.559	1	1363.559	14.594	.000
NITROGEN	214.150	2	107.075	1.146	.327
SALINITY	212.890	2	106.445	1.139	.329
HABITAT	4157.872	1	4157.872	44.500	.000
BLOCK(HABITAT)	1765.391	8	220.674	2.362	.032
TOTLENG1	3635.949	1	3635.949	38.914	.000
NITROGEN * SALINITY	391.087	2	195.544	2.093	.135
SALINITY * HABITAT	612.649	2	306.325	3.278	.046
NITROGEN * HABITAT	129.253	2	64.626	.692	.506
NITROGEN * SALINITY * HABITAT	380.584	2	190.292	2.037	.142
Error	4391.463	47	93.435		

Univariate Analysis of Variance

Tests of Between-Subjects Effects
Dependent Variable: the second leaf

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	4711.634	5	942.327	44.604	.000
Intercept	130580.770	1	130580.770	6180.939	.000
DATE	4711.634	5	942.327	44.604	.000
Error	8746.315	414	21.126		
Total	144038.719	420			
Total	144038.719	420			
Corrected Total	13457.949	419			

a R Squared = .350 (Adjusted R Squared = .342)

Dependent Variable: Tukey HSD

		Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval
f g	(J) date of measuring				Lower Bound
0	2.00	4.4494	.7769	.000	2.2354
	3.00	7.0801	.7769	.000	4.8661
	4.00	8.1891	.7769	.000	5.9751
	5.00	8.6668	.7769	.000	6.4528
	6.00	10.1073	.7769	.000	7.8933
0	1.00	-4.4494	.7769	.000	-6.6634
	3.00	2.6307	.7769	.009	.4167
	4.00	3.7396	.7769	.000	1.5256
	5.00	4.2174	.7769	.000	2.0034
	6.00	5.6579	.7769	.000	3.4439
0	1.00	-7.0801	.7769	.000	-9.2941
	2.00	-2.6307	.7769	.009	-4.8447
	4.00	1.1089	.7769	.710	-1.1051
	5.00	1.5867	.7769	.318	-.6273
	6.00	3.0271	.7769	.001	.8131
0	1.00	-8.1891	.7769	.000	-10.4031
	2.00	-3.7396	.7769	.000	-5.9536
	3.00	-1.1089	.7769	.710	-3.3229
	5.00	.4777	.7769	.990	-1.7363
	6.00	1.9182	.7769	.133	-.2958
	1.00	-8.6668	.7769	.000	-10.8808
	2.00	-4.2174	.7769	.000	-6.4314
	3.00	-1.5867	.7769	.318	-3.8007
	4.00	-.4777	.7769	.990	-2.6917
	6.00	1.4405	.7769	.431	-.7735
	1.00	-10.1073	.7769	.000	-12.3213
	2.00	-5.6579	.7769	.000	-7.8719
	3.00	-3.0271	.7769	.001	-5.2411
	4.00	-1.9182	.7769	.133	-4.1322

General Linear Model

Repeated measurements ANOVA for the second leaf length
Tests of Between-Subjects Effects
Measure: MEASURE_1
Transformed Variable: Average

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Intercept	198.863	1	198.863	6.303	.016
NITROGEN	36.394	2	18.197	.577	.566
SALINITY	100.099	2	50.049	1.586	.215
HABITAT	629.573	1	629.573	19.956	.000
BLOCK(HABITAT)	403.917	8	50.490	1.600	.150
NITROGEN * SALINITY	.484	2	.242	.008	.992
SALINITY * HABITAT	290.200	2	145.100	4.599	.015
NITROGEN * HABITAT	14.359	2	7.180	.228	.797
NITROGEN * SALINITY * HABITAT	66.988	2	33.494	1.062	.354
BLADL2.1	393.438	1	393.438	12.471	.001
Error	1482.754	47	31.548		

Univariate Analysis of Variance

Tests of Between-Subjects Effects
Dependent Variable: the third leaf

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	1847.635	5	369.527	18.503	.000
Intercept	114839.158	1	114839.158	5750.186	.000
DATE	1847.635	5	369.527	18.503	.000
Error	8268.152	414	19.971		
Total	124954.946	420			
Corrected Total	10115.788	419			

a R Squared = .183 (Adjusted R Squared = .173)

Post Hoc Tests for the third leaf

Multiple Comparisons

Dependent Variable: BLADL3 Tukey HSD

		Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
(I) date of measuring	(J) date of measuring				Lower Bound	Upper Bound
1.00	2.00	-1.9130	.7554	.115	-4.0656	.2397
	3.00	.4212	.7554	.994	-1.7314	2.5739
	4.00	2.1429	.7554	.052	-9.7304E-03	4.2955
	5.00	2.8824	.7554	.002	.7297	5.0350
	6.00	4.5259	.7554	.000	2.3732	6.6785
2.00	1.00	1.9130	.7554	.115	-.2397	4.0656
	3.00	2.3342	.7554	.025	.1816	4.4868
	4.00	4.0559	.7554	.000	1.9032	6.2085
	5.00	4.7953	.7554	.000	2.6427	6.9480
	6.00	6.4388	.7554	.000	4.2862	8.5915
3.00	1.00	-.4212	.7554	.994	-2.5739	1.7314
	2.00	-2.3342	.7554	.025	-4.4868	-.1816
	4.00	1.7217	.7554	.203	-.4310	3.8743
	5.00	2.4611	.7554	.014	.3085	4.6138
	6.00	4.1046	.7554	.000	1.9520	6.2573
4.00	1.00	-2.1429	.7554	.052	-4.2955	9.730E-03
	2.00	-4.0559	.7554	.000	-6.2085	-1.9032
	3.00	-1.7217	.7554	.203	-3.8743	.4310
	5.00	.7395	.7554	.925	-1.4132	2.8921
	6.00	2.3830	.7554	.020	.2303	4.5356
5.00	1.00	-2.8824	.7554	.002	-5.0350	-.7297
	2.00	-4.7953	.7554	.000	-6.9480	-2.6427
	3.00	-2.4611	.7554	.014	-4.6138	-.3085
	4.00	-.7395	.7554	.925	-2.8921	1.4132
	6.00	1.6435	.7554	.249	-.5091	3.7961
6.00	1.00	-4.5259	.7554	.000	-6.6785	-2.3732
	2.00	-6.4388	.7554	.000	-8.5915	-4.2862
	3.00	-4.1046	.7554	.000	-6.2573	-1.9520
	4.00	-2.3830	.7554	.020	-4.5356	-.2303
	5.00	-1.6435	.7554	.249	-3.7961	.5091

General Linear Model

Repeated measurements ANOVA for the third leaf
Tests of Between-Subjects Effects
Measure: MEASURE_1
Transformed Variable: Average

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Intercept	875.443	1	875.443	30.854	.000
NITROGEN	23.194	2	11.597	.409	.667
SALINITY	21.771	2	10.886	.384	.683
HABITAT	783.965	1	783.965	27.630	.000
BLOCK(HABITAT)	778.115	8	97.264	3.428	.003
NITROGEN * SALINITY	1.811	2	.906	.032	.969
SALINITY * HABITAT	102.615	2	51.308	1.808	.175
NITROGEN * HABITAT	22.285	2	11.143	.393	.677
NITROGEN * SALINITY * HABITAT	9.183	2	4.592	.162	.851
BLADL3.1	128.488	1	128.488	4.528	.039
Error	1333.582	47	28.374		

Univariate Analysis of Variance

Tests of Between-Subjects Effects
Dependent Variable: total number of leaves

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	19.243	4	4.811	9.356	.000
Intercept	4784.473	1	4784.473	9304.293	.000
DATE	19.243	4	4.811	9.356	.000
Error	177.407	345	.514		
Total	4981.123	350			
Corrected Total	196.650	349			

a R Squared = .098 (Adjusted R Squared = .087)

Post Hoc Tests for total number of leaves

Multiple Comparisons

Dependent Variable: TOTNRLEA

Tukey HSD

		Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
(I) date of measuring	(J) date of measuring				Lower Bound	Upper Bound
1.00	2.00	.1779	.1212	.584	-.1528	.5085
	3.00	.4800	.1212	.001	.1494	.8106
	4.00	.4343	.1212	.003	.1036	.7649
	5.00	.6643	.1212	.000	.3336	.9949
2.00	1.00	-.1779	.1212	.584	-.5085	.1528
	3.00	.3021	.1212	.092	-2.8493E-02	.6328
	4.00	.2564	.1212	.213	-7.4208E-02	.5871
	5.00	.4864	.1212	.001	.1558	.8171
3.00	1.00	-.4800	.1212	.001	-.8106	-.1494
	2.00	-.3021	.1212	.092	-.6328	2.849E-02
	4.00	-4.5714E-02	.1212	.996	-.3764	.2849
	5.00	.1843	.1212	.549	-.1464	.5149
4.00	1.00	-.4343	.1212	.003	-.7649	-.1036
	2.00	-.2564	.1212	.213	-.5871	7.421E-02
	3.00	4.571E-02	.1212	.996	-.2849	.3764
	5.00	.2300	.1212	.319	-.1006	.5606
5.00	1.00	-.6643	.1212	.000	-.9949	-.3336
	2.00	-.4864	.1212	.001	-.8171	-.1558
	3.00	-.1843	.1212	.549	-.5149	.1464
	4.00	-.2300	.1212	.319	-.5606	.1006

Based on observed means.

* The mean difference is significant at the .05 level.

General Linear Model

Repeated measuremenst ANOVA for the number of leaves
Tests of Between-Subjects Effects
Measure: MEASURE_1
Transformed Variable: Average

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Intercept	26.679	1	26.679	48.525	.000
NITROGEN	.580	2	.290	.527	.594
SALINITY	.940	2	.470	.855	.432
HABITAT	2.211	1	2.211	4.021	.051
BLOCK(HABITAT)	28.821	8	3.603	6.553	.000
NITROGEN * SALINITY	1.250	2	.625	1.137	.329
SALINITY * HABITAT	1.008	2	.504	.916	.407
NITROGEN * HABITAT	.719	2	.360	.654	.525
NITROGEN * SALINITY * HABITAT	.177	2	8.843E-02	.161	.852
NRLEAF2	1.884E-03	1	1.884E-03	.003	.954
Error	25.840	47	.550		

Univariate Analysis of Variance

Tests of Between-Subjects Effects
Dependent Variable: growth rate

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	.410	4	.103	14.746	.000
Intercept	.691	1	.691	99.387	.000
DATE	.410	4	.103	14.746	.000
Error	2.398	345	6.951E-03		
Total	3.499	350			
Corrected Total	2.808	349			

a R Squared = .146 (Adjusted R Squared = .136)

Post Hoc Tests

Multiple Comparisons
Dependent Variable: TOTGROWR
Tukey HSD

		Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
(I) date of measuring	(J) date of measuring				Lower Bound	Upper Bound
1.00	2.00	-4.9020E-03	1.409E-02	.997	-4.3344E-02	3.354E-02
	3.00	-6.0839E-03	1.409E-02	.993	-4.4526E-02	3.236E-02
	4.00	4.765E-02	1.409E-02	.006	9.211E-03	8.610E-02
	5.00	7.864E-02	1.409E-02	.000	4.020E-02	.1171
2.00	1.00	4.902E-03	1.409E-02	.997	-3.3540E-02	4.334E-02
	3.00	-1.1818E-03	1.409E-02	1.000	-3.9624E-02	3.726E-02
	4.00	5.256E-02	1.409E-02	.002	1.411E-02	9.100E-02
	5.00	8.355E-02	1.409E-02	.000	4.510E-02	.1220
3.00	1.00	6.084E-03	1.409E-02	.993	-3.2358E-02	4.453E-02
	2.00	1.182E-03	1.409E-02	1.000	-3.7260E-02	3.962E-02
	4.00	5.374E-02	1.409E-02	.001	1.530E-02	9.218E-02
	5.00	8.473E-02	1.409E-02	.000	4.629E-02	.1232
4.00	1.00	-4.7653E-02	1.409E-02	.006	-8.6096E-02	-9.2114E-03
	2.00	-5.2555E-02	1.409E-02	.002	-9.0998E-02	-1.4113E-02
	3.00	-5.3737E-02	1.409E-02	.001	-9.2179E-02	-1.5295E-02
	5.00	3.099E-02	1.409E-02	.180	-7.4521E-03	6.943E-02
5.00	1.00	-7.8643E-02	1.409E-02	.000	-.1171	-4.0201E-02
	2.00	-8.3545E-02	1.409E-02	.000	-.1220	-4.5103E-02
	3.00	-8.4727E-02	1.409E-02	.000	-.1232	-4.6285E-02
	4.00	-3.0990E-02	1.409E-02	.180	-6.9432E-02	7.452E-03

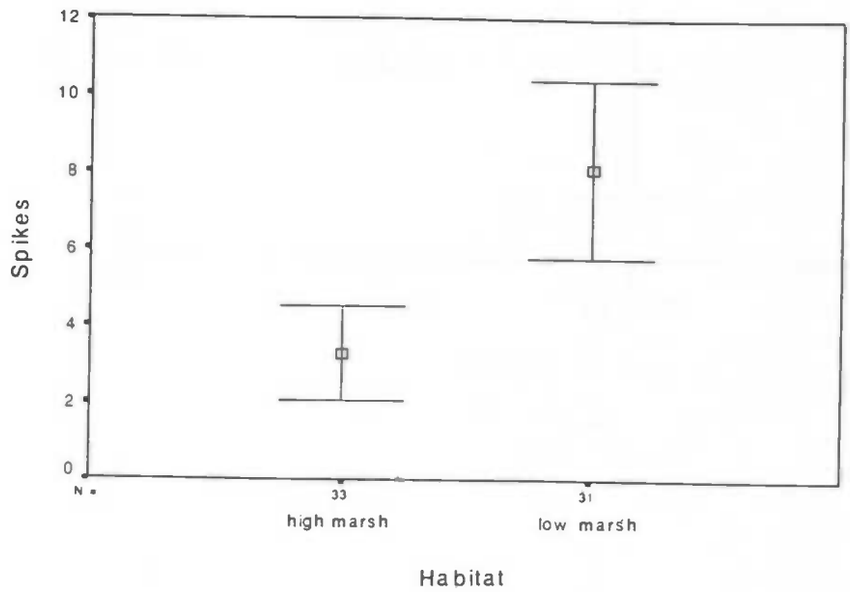
Based on observed means.
* The mean difference is significant at the .05 level.

General Linear Model

Repeated measuremenst ANOVA for the growth rate
Tests of Between-Subjects Effects
Measure: MEASURE_1
Transformed Variable: Average

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Intercept	9.707E-02	1	9.707E-02	41.816	.000
NITROGEN	2.566E-03	2	1.283E-03	.553	.579
SALINITY	7.467E-03	2	3.734E-03	1.608	.211
HABITAT	.126	1	.126	54.335	.000
BLOCK(HABITAT)	7.546E-02	8	9.433E-03	4.063	.001
NITROGEN * SALINITY	1.780E-03	2	8.899E-04	.383	.684
SALINITY * HABITAT	1.877E-03	2	9.383E-04	.404	.670
NITROGEN * HABITAT	3.151E-03	2	1.576E-03	.679	.512
NITROGEN * SALINITY * HABITAT	2.722E-03	2	1.361E-03	.586	.560
GROWR12	2.512E-03	1	2.512E-03	1.082	.304
Error	.109	47	2.321E-03		

The number of spikes and biomass for the high and low marsh. In the low marsh more spikes are present but the biomass is not higher. It seems that the plants in the low marsh invest more in reproduction by producing new spikes than the plants in the high marsh.



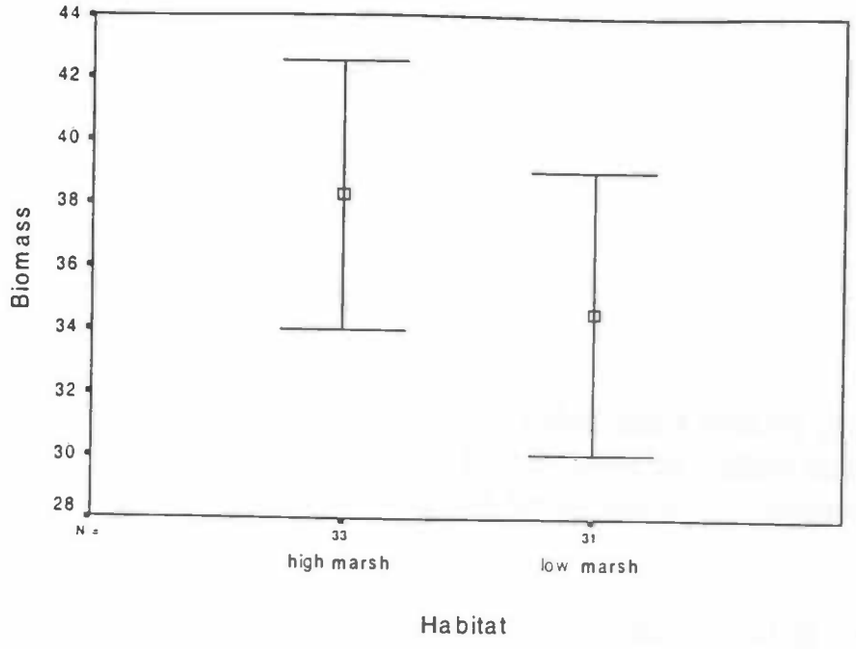
Tests of Between-Subjects Effects

Dependent Variable: SPIKES

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	1049.017[a]	21	49.953	2.255	.012
Intercept	1055.872	1	1055.872	47.673	.000
HABITAT	200.265	1	200.265	9.042	.004
NITROGEN	102.855	2	51.427	2.322	.111
SALINITY	7.967	2	3.983	.180	.836
BLOCK(HABITAT)	368.933	8	46.117	2.082	.059
HABITAT * NITROGEN	42.423	2	21.211	.958	.392
HABITAT * SALINITY	31.131	2	15.566	.703	.501
HABITAT * NITROGEN * SALINITY	150.275	4	37.569	1.696	.169
Error	930.217	42	22.148		
Total	3993.000	64			
Corrected Total	1979.234	63			

a. R Squared = .530 (Adjusted R Squared = .295)

The biomass for the high marsh and the low marsh. The biomass in the high marsh seems to be higher but there was no significant difference.



Tests of Between-Subjects Effects

Dependent Variable: SPIKES

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	1049.017[a]	21	49.953	2.255	.012
Intercept	1055.872	1	1055.872	47.673	.000
HABITAT	200.265	1	200.265	9.042	.004
NITROGEN	102.855	2	51.427	2.322	.111
SALINITY	7.967	2	3.983	.180	.836
BLOCK(HABITAT)	368.933	8	46.117	2.082	.059
HABITAT * NITROGEN	42.423	2	21.211	.958	.392
HABITAT * SALINITY	31.131	2	15.566	.703	.501
HABITAT * NITROGEN * SALINITY	150.275	4	37.569	1.696	.169
Error	930.217	42	22.148		
Total	3993.000	64			
Corrected Total	1979.234	63			

a. R Squared = .530 (Adjusted R Squared = .295)

Salinity data.

The salinity of the soil and plants was caluculated with an ykline : (y= (x-4.6) /70.48). This equation is calculated by adding different amounts of grams salt to 20 ml demi-water. X stands for micro Siemens and Y stands for gram salt.

For the soil salinity, I took 5 grams soil and added 20 ml of demi-water. From this, I calculated the soil concentration in the water, i.e. 0.25 gram/ ml. Than I multiplied this number by 4, so I got gram soil/ ml. I divided the mS by gram soil/ml, so I had now the mS per gram soil per ml. Wat I wanted to get is gram salt per gram soil, so I used the equation of the ykline here to calculate grams salt per gram soil/ml and after that I multiplied this number by the amount of moist in the soil (wet weight minus dry weight, ml) so I had now gram salt per gram soil.
The same method is used for the plant salinity.

General Linear Model

Repeated measurements ANOVA for soil salinity at the high marsh
Tests of Between-Subjects Effects
Measure: MEASURE_1
Transformed Variable: Average

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Intercept	7.242E-04	1	7.242E-04	106.848	.000
BLOCK(HABITAT)	2.137E-04	4	5.343E-05	7.882	.001
HABITAT	.000	0	.	.	.
SALINITY	1.698E-05	2	8.492E-06	1.253	.314
HABITAT * SALINITY	.000	0	.	.	.
Error	1.017E-04	15	6.778E-06		

a habitat = high saltmarsh

General Linear Model

Repeated measurements ANOVA for soil salinity at the low marsh
Tests of Between-Subjects Effects
Measure: MEASURE_1
Transformed Variable: Average

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Intercept	.127	1	.127	1741.662	.000
BLOCK(HABITAT)	8.497E-03	4	2.124E-03	29.192	.000
HABITAT	.000	0	.	.	.
SALINITY	2.275E-04	2	1.138E-04	1.563	.228
HABITAT * SALINITY	.000	0	.	.	.
Error	1.892E-03	26	7.277E-05		

a habitat = low saltmarsh

General Linear Model

Repeated measurements ANOVA for plant salinity at the high marsh
Tests of Between-Subjects Effects
Measure: MEASURE_1
Transformed Variable: Average

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Intercept	26874.935	1	26874.935	116.279	.000
BLOCK(HABITAT)	694.597	4	173.649	.751	.566
HABITAT	.000	0	.	.	.
SALINITY	203.654	2	101.827	.441	.648
HABITAT * SALINITY	.000	0	.	.	.
Error	6240.380	27	231.125		

a habitat = high saltmarsh

General Linear Model

Repeated measurements ANOVA for plant salinity at the low marsh
Tests of Between-Subjects Effects
Measure: MEASURE_1
Transformed Variable: Average

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Intercept	28966.536	1	28966.536	2099.629	.000
BLOCK(HABITAT)	107.351	4	26.838	1.945	.132
HABITAT	.000	0	.	.	.
SALINITY	10.008	2	5.004	.363	.699
HABITAT * SALINITY	.000	0	.	.	.
Error	372.493	27	13.796		

a habitat = low saltmarsh

Tests of Between-Subjects Effects

Dependent Variable: SOILWATE

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	.496[a]	21	2.360E-02	31.397	.000
Intercept	3.336	1	3.336	4437.616	.000
HABITAT	.445	1	.445	591.389	.000
NITROGEN	2.245E-05	2	1.122E-05	.015	.985
SALINITY	4.676E-04	2	2.338E-04	.311	.734
BLOCK(HABITAT)	1.869E-02	8	2.336E-03	3.108	.007
HABITAT * NITROGEN	2.101E-03	2	1.050E-03	1.398	.257
HABITAT * SALINITY	3.203E-03	2	1.601E-03	2.131	.130
HABITAT * NITROGEN * SALINITY	3.559E-03	4	8.897E-04	1.184	.330
Error	3.608E-02	48	7.516E-04		
Total	4.860	70			
Corrected Total	.532	69			

a. R Squared = .932 (Adjusted R Squared = .902)