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Comparative study on populations of *Zostera marina* L. (eelgrass): experimental germination and growth

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Abstract

Seeds of both perennial and (semi-)annual populations of *Zostera marina* L. originating from different habitats in the southwestern Netherlands and Roscoff (Brittany, France) were germinated and grown under identical circumstances to identify possible genotypically based differences in their population dynamical characters. Individual populations displayed a genotypic background for germination, morphology and flowering, while relative growth rate was considered to be an entirely phenotypic response. Flowering seemed to be related with a higher demand for nitrogen. Perennial versus annual populations did not show genetic variation in any of the response variables investigated. Comparisons with *in situ* data revealed that presumably the perennial populations in their habitats not fully exploited their potential for (above-ground) growth. Among individual populations, differences in morphology and especially (sexual) reproduction suggest a greater and more complex genotypic background than previously assumed through interpretation of isozyme patterns.

Keywords: Flowering; Genetic basis; Germination; Growth; Life-history strategy; *Zostera marina*

1. Introduction

The life-history strategy of *Zostera marina* L. (eelgrass) may vary between two extremes (i.e. annual and perennial) along a continuum (Jacobs, 1982; Van Lent & Verschuure, 1994a). Isozyme patterns revealed some genetic differentiation (Gagnon et al., 1980; McMillan, 1982; De Heij & Nienhuis, 1992; Laushman, 1993) among populations. Keddy & Patriquin (1978) reported that seeds of perennial populations gave rise to both annual (flowering) and perennial (non-flowering) plants and vice versa,

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concluding that some genetic basis for these characteristics should be present. Backman (1984) found genotypic differentiation in the morphology of eelgrass plants. A possible genetic basis of other specific population dynamical features (e.g. growth rate) has not been experimentally studied and is therefore unknown. The general opinion is that variation in morphological and ecological responses of *Z. marina* seems to be for the greater part due to environmentally induced (phenotypic) differences (Keddy & Patriquin, 1978; Gagnon et al., 1980; De Heij & Nienhuis, 1992).

A study on isolated populations with different life-histories in the southwestern Netherlands displayed significant differences in biomass partitioning, primary production and reproduction (Van Lent & Verschuure, 1994a). The populations occurred in habitats clearly differing in salinity, nutrient concentrations, extinction coefficient, etc. (Van Lent & Verschuure, 1994b). The present experimental study was performed to test the hypothesis that growth rate, germination, generative reproduction and morphology of eelgrass populations in the southwestern Netherlands were in principle completely phenotypically determined, annual and perennial populations alike. The results are important for further studies on intraspecific variability of *Z. marina* in the region concerning the relation between population dynamical characters and environmental factors. The question was also of interest as (perennial) eelgrass populations in one particular area of the region were declining rapidly since 1985. Concomitantly, preliminary comparisons were possible between this experimental study and the in situ studies performed (Van Lent & Verschuure, 1994a,b). A perennial population from a relatively distant region (Roscoff in Brittany, France) was used for comparison.

2. Materials and methods

2.1. Study sites

Shoots bearing spathes with seeds were collected between 17 August and 4 September 1989 from populations in the Grevelingen, Veerse Meer, Zandkreek (southwestern Netherlands, 52° N) and at Roscoff (Brittany, France, 48° N) (Fig. 1). Populations in both the Grevelingen and Veerse Meer lagoon were at a depth of 1.25 m, while in the Zandkreek the population was on a tidal flat (≈ 0.90 m above MLW). Grevelingen is a mesotrophic, non-tidal salt water lake (27.5 ± 0.2‰ S), Veerse Meer is a eutrophic non-tidal brackish water lake (16.1 ± 0.4‰ *S*) and Zandkreek is an intertidal sheltered estuary (27.3 ± 0.6‰ S), part of the Oosterschelde. Sediment silt content varies from less than 10% (Grevelingen) up to 40% (Zandkreek). For a detailed description of these sites confer to Van Lent & Verschuure (1994a,b). At Roscoff the eelgrass population grows at mean low water in a sheltered coastal area (with a tidal range of 9 m); the substrate consists of coarse sand.

The population in the Grevelingen is perennial and part of the above-ground biomass remains in winter. Reproduction takes place mainly through vegetative production of shoots from rhizomes. Flowering occurs, but extreme variation is shown between years. The Veerse Meer and Zandkreek populations have an almost exclusively generative reproduction (seeds) from one year to the next. The Veerse Meer population is regarded

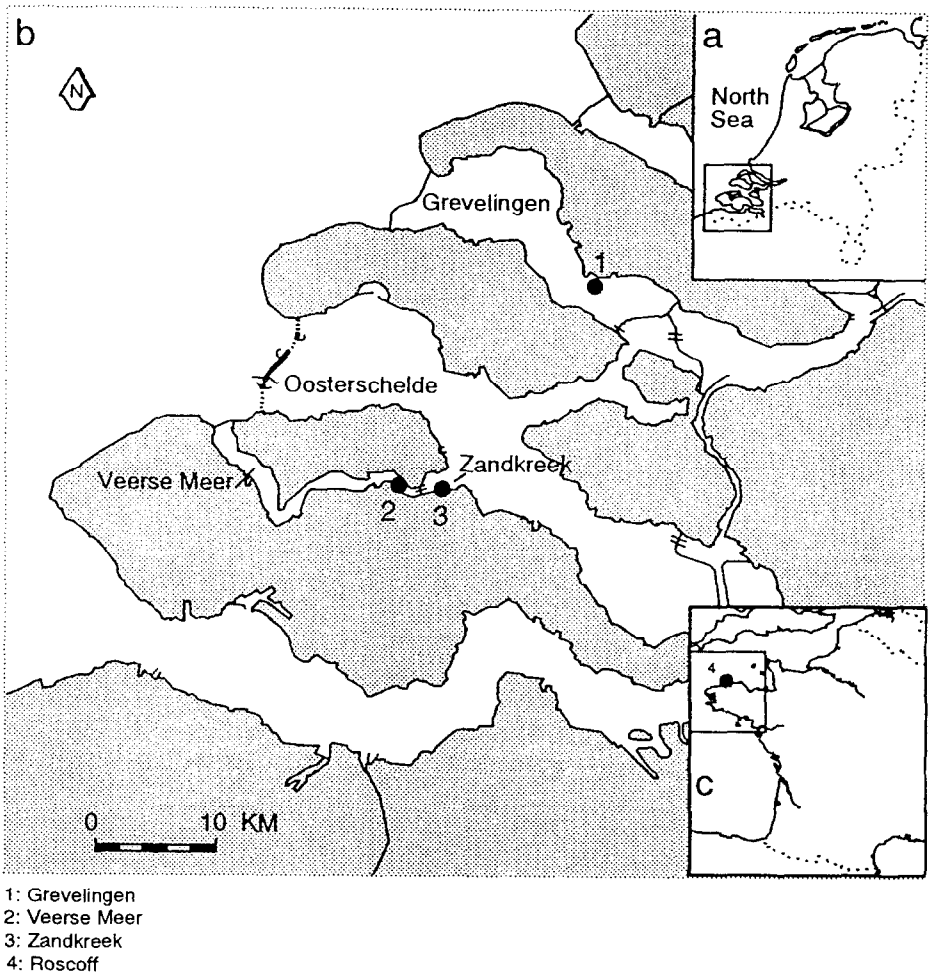

Fig. 1. The southwestern Netherlands (a) and Brittany, France (c) and the collection (study) sites (b) in the Grevelingen (1), Veerse Meer (2), Zandkreek (3) and at Roscoff (4).

as semi-annual, because occasionally vegetative shoots survive the winter. The Zandkreek population is truly annual. Both populations have a relatively low below-ground biomass (Van Lent & Verschuure, 1994a). The population at Roscoff is perennial, above-ground biomass persists in winter (Jacobs, 1979) and flowering can be abundant (Jacobs, 1981; Van Lent, pers. obs.).

During 2 wk the shoots were kept in nets submersed in an outdoor basin ($30 \times 20 \times 1.5$ m), with an inlet of seawater from the adjacent Oosterschelde estuary (Fig. 1b). The released seeds were collected and kept in filtered seawater from the Oosterschelde estuary (salinity 32.0‰ S) until January/February 1990 at 4 °C in complete darkness.

2.2. Germination experiment

On 19 January 1990, 1500 seeds from each population were divided into groups of 100 seeds. One group was put into each of 10 Petri dishes with 15.7‰ Cl^- artificial seawater (158.96 gNaCl, 100.00 g$MgSO_4 \cdot 7H_2O$, 7.00 gKCl, 0.30 gH_3BO_3, 2.00 g$NaHCO_3$) and 5 Petri dishes with 10.5‰ Cl^- artificial seawater, respectively. The chlorinity values used, were comparable with the chlorinities of the Grevelingen (15.5‰ Cl^{-1}) and Veerse Meer (9.0‰ Cl^{-1}) (Van Lent & Verschuure, 1994b). The Petri dishes were kept at 10 °C under a light regime of 14 h dark/10 h (ample) monochromatic light. Temperature and light regime were chosen according to ambient values in the region in early spring (Van Lent, unpubl. data, 1987, 1988, 1989). From 22 January until 17 April 1990, at regular intervals (22 times), the number of germinated and decayed seeds were counted. Every 10 days the water was renewed, while germinated and decayed seeds were removed.

At the beginning of the experiment, two batches of 25 seeds from each population were checked for viability with the tetrazolium test (Grabe, 1970). A pink colour of both cotyledon and axial hypocotyl was considered a positive result for vital seeds (Taylor, 1957; Harrison, 1991).

2.3. Growth experiment

In the beginning of February 1990, for each population ≈ 1000 seeds were put (5 to 9 each) into small permeable pots ($5.0 \times 5.0 \times 7.5$ cm) filled with sediment consisting of 40% fine sand and 40% silt. The pots were placed in groups of 25 to 35 into plastic containers ($32 \times 32 \times 12$ cm), which were transferred to the outdoor basin mentioned above.

The last week of April 1990, 10 groups of 42 randomly selected seedlings for each population were replanted directly into the containers, which were filled with the same sediment as the pots. Subsequently, the 10 containers per population were randomly located in the outdoor basin. The experiment lasted until 31 August 1990. The response variables were relative growth rate, reproduction as well as tissue carbon and nitrogen content.

The water depth in the outdoor basin was continuously kept at 0.90 m, while every 10 days the water was renewed at high tide from the Oosterschelde estuary (32‰ S).

2.4. Relative growth rate

The above-ground relative growth rate (day^{-1}) of the seedlings was measured using the leaf-marking method of Sand-Jensen (1975). Of each population in three randomly chosen containers 18 shoots were marked. These were collected every 9 to 13 days and new shoots were marked in other containers holding shoots from the same population. All leaves of vegetative (non-flowering) shoots were individually marked with a felt-tip pen (non-toxic) at a constant distance from a reference point (upper rim of the enclosing leaf sheath). The following data were noted: number of leaves, length (1 mm precision) of leaves and sheath as well as maximum width (0.1 mm precision). The marked shoots

harvested were examined to record the same variables, together with the number of new leaves and the number of stems and spathes. Of each sampled shoot 5 cm leaf (and 5 cm stem if present) material was taken, bundled and dried at 70 to 80 °C to constant dry weight (DW). Ash-free dry weight (AFDW) was established by combustion at 550 °C for 2 h.

Units of area (length * width) were transformed to units of biomass (g AFDW·cm^{-2}) and initial biomass (IB), leaf loss (LL), growth (LG) and final biomass (FB) were calculated (IB = FB-LG + LL).

Exponential growth of a seagrass shoot is described by the formula (Vermaat et al., 1987):

$$FB = IB \cdot e^{R \cdot t}$$

where R is the relative growth rate (day^{-1}) and t is the length of the growth period (days). In this way a net relative growth rate (RGRN, day^{-1}) was calculated (Vermaat et al., 1987):

$$RGRN = (\ln(FB) - \ln(IB)) \cdot 1/t.$$

2.5. Reproduction

All 10 containers of each population were regularly checked for any stems or spathes. On three occasions the number of flowering shoots per container and the number of spathes per 10 shoots, as well as the number of seeds (if present), were counted.

2.6. Tissue carbon and nitrogen content

At the end of the experiment for each container above-ground material was separated from below-ground material and grouped per population. Both fractions were dried (70 to 80 °C) to constant weight and ground to powder using a bullet-mixer. Organic carbon and total nitrogen were determined with a Carlo Erba NA-1500 autoanalyzer according to a method described by Nieuwenhuize et al. (1994).

2.7. Statistics

Differences in characteristics between populations were analysed by ANOVA. For individual populations, a post hoc Tukey HSD test was used. Contrasts between perennial and annual populations were tested with a nested ANOVA. The populations were nested within category, strategy (perennial or annual). Differences "between strategy" were tested against the Mean-Square of populations "within strategy". Means are given with standard error (SE), and differences were considered to be significant at a level of $p < 0.05$.

3. Results

3.1. Germination

For all populations the seeds used in the tetrazolium test were viable. The fraction of seeds germinating in seawater (Table 1) was significantly highest for seeds collected

Table 1
Mean percentage of germinated and decayed seeds and in situ percentages for germination of Grevelingen, Veerse Meer, Zandkreek and Roscoff populations. In situ data from Van Lent & Verschuure (1994a) and Van Lent (unpubl. data). N represents 10 or 5 times a group of 100 seeds each

	Maximum percentage germinated			Maximum percentage decayed	
	In situ	Exp. seawater	Exp. brackish water	Exp. seawater	Exp. brackish water
Grevelingen (perennial)					
Mean ± SE	12.5	20.5 ± 1.39	46.4 ± 3.76	35.1 ± 3.04	27.2 ± 2.24
N		10	5	10	5
Veerse Meer (semi-annual)					
Mean ± SE	74.1	14.8 ± 1.26	33.8 ± 1.30	1.3 ± 0.28	0.2 ± 0.18
N		10	5	10	5
Zandkreek (annual)					
Mean ± SE	87.2	6.5 ± 0.89	30.6 ± 5.50	0.1 ± 0.09	0.4 ± 0.22
N		10	5	10	5
Roscoff (perennial)					
Mean ± SE	no data	4.9 ± 1.33	17.0 ± 2.59	0.4 ± 0.22	0.4 ± 0.22
N		10	5	10	5

from the Grevelingen population, followed by Veerse Meer seeds. Zandkreek and Roscoff seeds showed the lowest germination. In brackish water germination of Grevelingen seeds remained significantly higher, while for Veerse Meer seeds germination was significantly higher compared with Roscoff seeds only.

For all populations alike, germination of seeds in brackish water was significantly higher than in seawater (Table 1).

In both sea- and brackish water the amount of decaying seeds (Table 1) originating from the Grevelingen population was significantly higher than from the other populations, among which no significant differences were found.

3.2. *Relative growth rate*

Between populations, no consistent differences in net relative growth rate (RGRN, day^{-1}) (Fig. 2) were apparent.

3.3. *Morphology*

Shoots grown from seeds of the Roscoff population remained significantly shorter (Fig. 3a) than shoots from other populations.

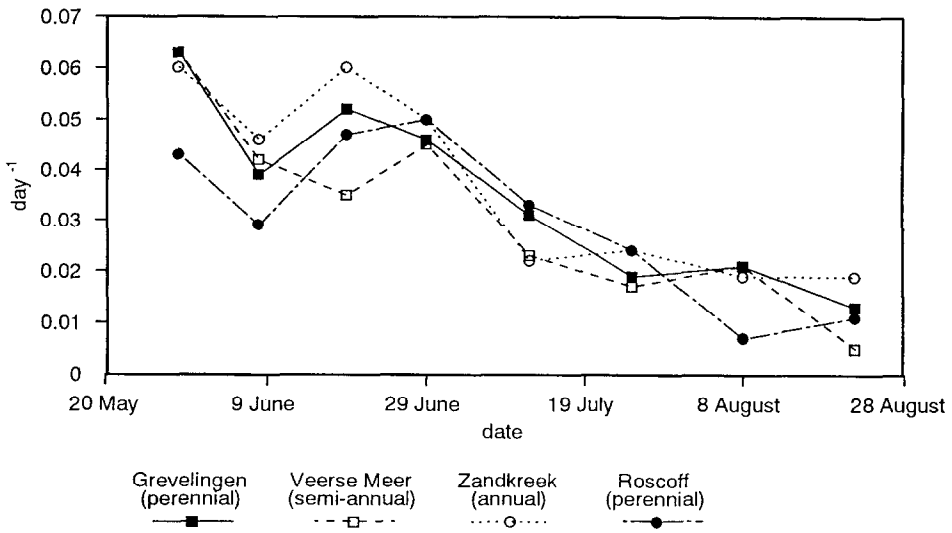

Fig. 2. Mean net relative growth rate (day^{-1}) for the Grevelingen (perennial), Veerse Meer (semi-annual), Zandkreek (annual) and Roscoff (perennial) populations.

Zandkreek leaves were significantly narrower (Fig. 3b) than Grevelingen and Veerse Meer leaves. The development in width of Roscoff leaves was conspicuous, these leaves were the narrowest in the first part of the study period and became the widest later on. In the flowering populations the first stems appeared around 18 June, while the top percentage of flowering shoots was reached around 24 July (Fig. 4a). Within these populations leaf width decreased in this period, but for the Roscoff population it increased (Fig. 3b).

3.4. Reproduction

The maximum percentage of flowering shoots (Fig. 4a) per container recorded for the Grevelingen population was significantly lower than of the Zandkreek population. The population from Roscoff showed no flowering shoots at all. Between populations with flowering shoots, no significant differences were displayed in the number of spathes per shoot (Fig. 4b). The number of seeds per spathe never exceeded five.

3.5. Tissue carbon and nitrogen content

At the end of the study period, nitrogen content (N as percent DW) (Table 3) of above-ground material was highest for the Roscoff population, followed by the Grevelingen, Veerse Meer and Zandkreek populations, respectively. Nitrogen content in below-ground material was lower compared with above-ground material, except for the Zandkreek population.

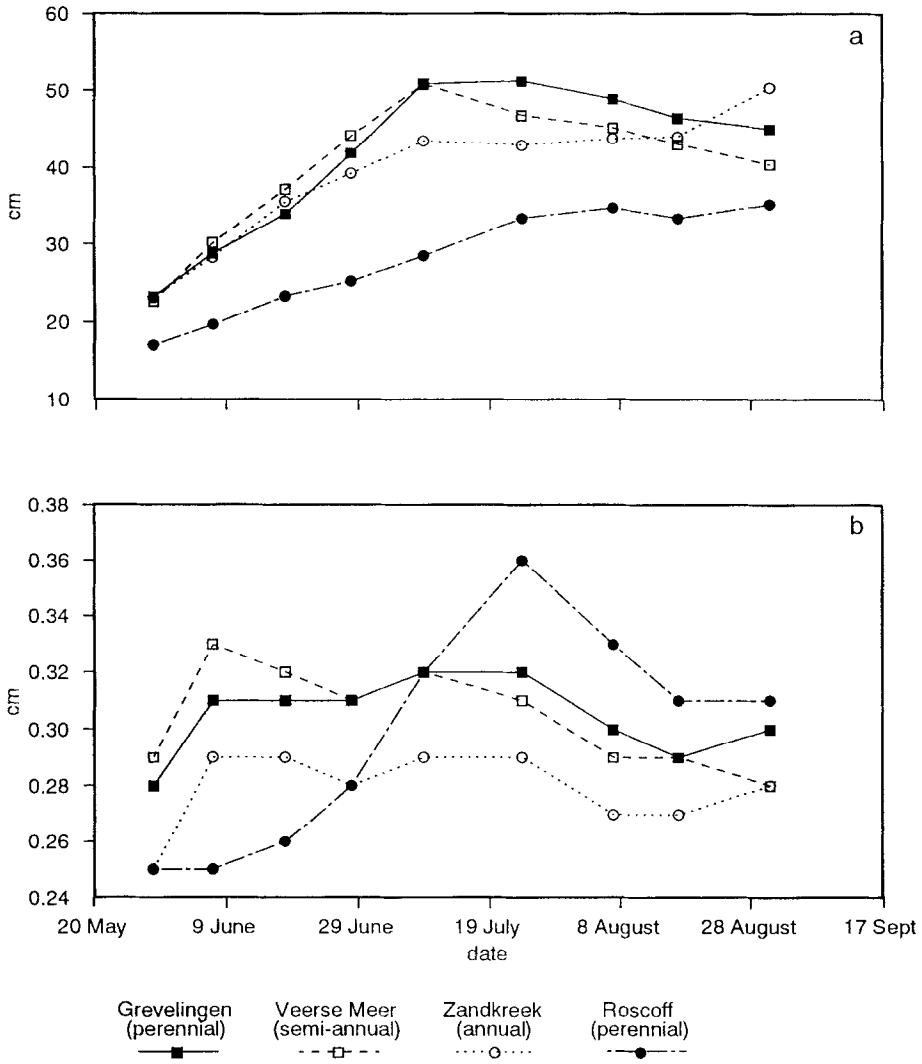

Fig. 3. Mean maximum non-flowering shoot length (a) and leaf width (b) (cm) of the Grevelingen (perennial), Veerse Meer (semi-annual), Zandkreek (annual) and Roscoff (perennial) populations.

The range for C:N (atom) ratio of above-ground material (Table 3) was wider than for below-ground material.

Between perennial (Grevelingen and Roscoff) and (semi-)annual (Veerse Meer and Zandkreek) populations no significant differences were found for any of the population dynamical response variables mentioned.

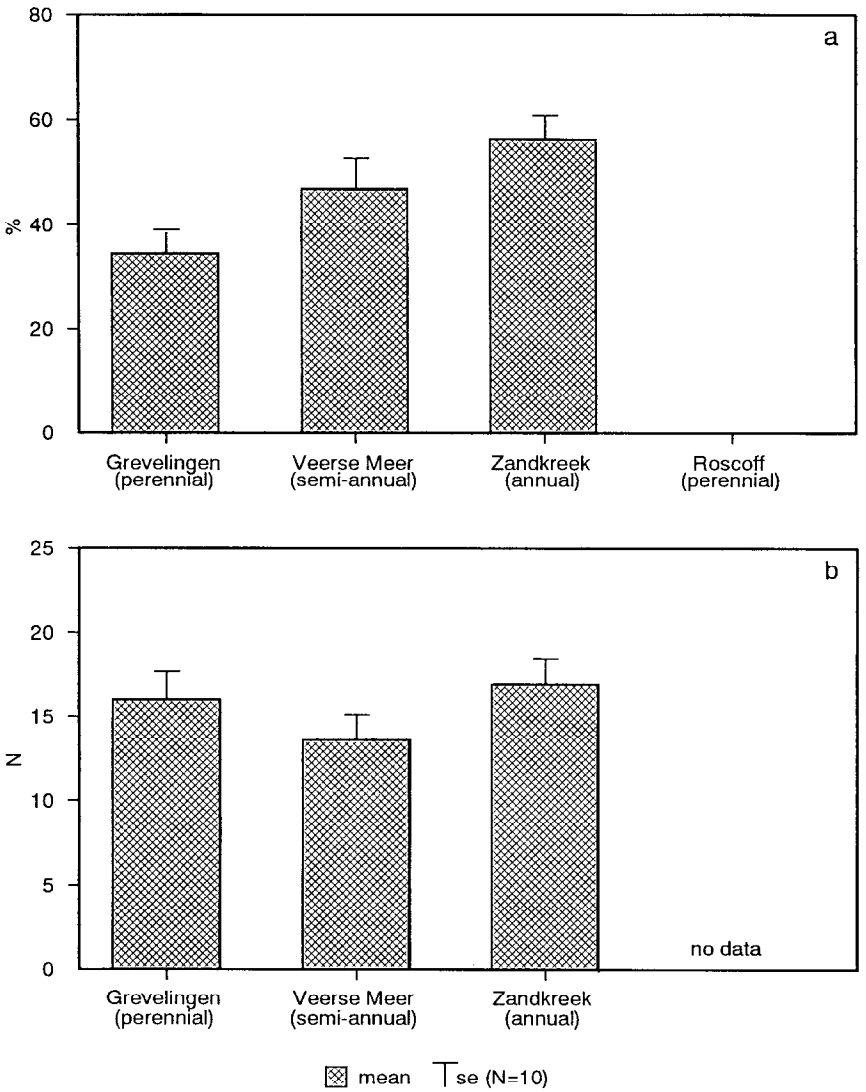

Fig. 4. Mean maximum percentage of flowering shoots (a) and number of spathes per shoot (b) for the Grevelingen (perennial), Veerse Meer (semi-annual), Zandkreek (annual) and Roscoff (perennial) populations.

4. Discussion

Shoots bearing spathes with seeds were kept under identical and controlled conditions for 2 wk in the outdoor basin, before the seeds were colllected. It is therefore assumed that any osmotic shock, which seems to promote germination, had the same influence on seeds of all populations alike. One could speculate that during seed

development, environmental factors already have their impact on the genetic information of the seeds. This would imply that the differences shown by the populations in response variables would not necessarily be the result of genotypic variability among populations only, as any future phenotypic variability could have been established before sampling and germination. If there is any truth in the speculation, even phenotypic differences could be expected among seeds originating from one population, but released relatively early or late in the season. No information adressing this subject is available. Therefore, it was assumed that the differences among populations observed were entirely genetic in nature.

Generative recruitment depends on seed production, germination and seedling survival (or net relative growth rate). Among individual populations genotypic variation in germination and flowering (as well as morphology) was present. Relative growth rates did not vary among individual populations, suggesting that relative growth rate represents a phenotypic response. Consequently, especially in the first stages of the generative recruitment proces, genotypic differences among populations appear to be of importance.

When compared with the Mean Square Error among populations, the difference between strategies (perennial versus annual) could not be shown to be significant. With only two populations in each category, the power of this test was very low. More populations from each category would be required to reveal any possible genetic differences in the characters mentioned. For germination of seeds, Keddy & Patriquin (1978) could not show a significant difference between annual and perennial populations, however.

The germination percentages found in this study are within the range reported from experimental laboratory studies (Phillips, 1971; Keddy & Patriquin, 1978; McMillan, 1983; Phillips et al., 1983; Hootsmans et al., 1987). The clearly higher percentage of decaying seeds of the Grevelingen population (Table 1) was remarkable and might indicate a lower vitality. This was not confirmed by the vitality test or germination results (Table 1), however. The large number of seeds (in particular from the Veerse Meer, Zandkreek and Roscoff populations) that remained and neither germinated nor decayed (Table 1) was conspicuous, but we can offer no explanation.

According to Duarte (1990) leaf nutrient content below 1.8 nitrogen as percent dry weight or an atomic C:N ratio above 20 predicts limitation of growth. At the end of the experiment leaf nitrogen content (Table 3) of the Veerse Meer and Zandkreek populations indicated shortage for nitrogen, while the atomic C:N ratio (Table 3) suggested nitrogen limitation for the Zandkreek population only. The Veerse Meer and Zandkreek populations showed the highest amount of flowering shoots (Fig. 4a). Since nutrient availability was similar for all populations, these results indicate that flowering and seed production involve a significant nitrogen investment (Madsen, 1991). The Roscoff population showed no flowering at all and had the highest leaf nutrient content and lowest atomic C:N ratio at the end of the experiment (Table 3). For the Grevelingen population in situ nitrogen limitation is reported for density and biomass of flowering shoots (Van Lent et al., 1995).

The results from this experiment are difficult to compare with in situ results previously recorded (Van Lent & Verschuure, 1994a,b), as in some cases different methods

were used and conditions varied considerably. Nevertheless, some results were comparable and are worth mentioning.

In situ germination was represented by established seedlings as a percentage of seeds present in spring (Table 1). Compared with the experimental germination results, in particular the relatively high in situ germination of the (semi-)annual populations is notable as seedling survival (after germination) is determined by different environmental conditions and mortality rates are usually high (Churchill, 1983). Although the Grevelingen population has very few seedlings in situ (Van Lent & Verschuure, 1994a), seeds showed the highest potential for germination under experimental conditions (Table 1).

Maximum above-ground RGRN of the Veerse Meer and Zandkreek populations approached maximum values recorded for the above-ground relative growth rate in situ, while for the Grevelingen population the maximum value reached in experimental growth was higher (Table 2). Apparently, the Grevelingen population does not fully exploit its potential (above-ground) growth rate *in situ*. The same seems to apply for the population at Roscoff. For a somewhat deeper situated *Z. marina* bed at Roscoff, Jacobs (1979) reported a maximum turnover rate of $1.8\% \cdot day^{-1}$ ($\approx 0.02\ day^{-1}$), a distinctly lower value than the maximum recorded during this experimental study (Table 2). For both the perennial populations the in situ growth rates account for vegetatively produced shoots only. In contrast with the perennial Grevelingen population, the (semi-)annual Veerse Meer and Zandkreek populations regenerate each year from seeds (Van Lent & Verschuure, 1994a). It is unknown to what extent regeneration of populations at Roscoff takes place through seeds, but seedlings do relatively seldom occur within the eelgrass beds (C. den Hartog, pers. comm.). In these comparisons it is assumed that the potential for growth is equal for vegetatively and generatively produced shoots. Furthermore, the difference between calculation methods of the in situ and experimental relative growth rates must be taken into account. The in situ relative growth rates include respiration, but the leaf loss component is unknown. No references could be found to studies comparing the ecology of seagrasses grown from seeds or vegetative plant parts such as rhizomes, while relatively few terrestrial studies touched this topic. For terrestrial plants (*Aster* and *Solidago*) Schmid & Bazzaz (1990) found no difference in population dynamical characteristics between plants grown from seeds or rhizomes of the same (maternal) parents.

The upper limit for maximum (non-flowering) shoot length in the experiment was higher than in situ values (Table 2) for the (semi-)annual Veerse Meer and Zandkreek populations. Opposed to the relatively sheltered habitat provided by the experimental set up, the (semi-)annual populations endure high physical stress in their original habitat (Van Lent & Verschuure, 1994a,b). No such an environmental explanation is available for the upper limit of maximum width of experimental shoots being lower than of in situ shoots (Table 2) for the Veerse Meer and Roscoff populations.

In the experiment the percent of flowering shoots reached by the Grevelingen population was higher than maximum percentages recorded in situ (Table 2). The opposite was true for the Veerse Meer and Zandkreek populations. For the Grevelingen in situ flowering is largely restricted to vegetatively produced shoots. Quantitative in situ data for the Roscoff population are not available.

Table 2
Comparisons between in situ and experimental data for flowering (%), net relative growth rate (day^{-1}) and morphology (cm) of Grevelingen, Veerse Meer, Zandkreek and Roscoff populations. In situ data from Van Lent & Verschuure (1994a) and Van Lent (unpubl. data)

	Maximum percentage flowering shoots		Mamimum above-ground RGRN (day^{-1})		Upper limit maximum non-flowering shoot length (cm)		Upper limit maximum width (cm)	
	In situ	Exp.	In situ	Exp.	In situ	Exp.	In situ	Exp.
Grevelingen (perennial)								
Mean ± SE	11.1	34.5 ± 3.79	0.031	0.063 ± 0.002	49.4 ± 1.10	51.2 ± 2.15	0.32 ± 0.009	0.32 ± 0.005
N		10		18	40	36	20	36
Veerse Meer (semi-annual)								
Mean ± SE	65.2	46.9 ± 5.57	0.051	0.063 ± 0.005	43.5 ± 2.35	50.8 ± 2.45	0.36 ± 0.015	0.33 ± 0.005
N		10		18	39	33	30	36
Zandkreek (annual)								
Mean ± SE	100.0	56.2 ± 4.43	0.067	0.060 ± 0.004	32.0 ± 2.85	50.4 ± 1.84	0.31 ± 0.015	0.29 ± 0.005
N		10		18	10	14	10	36
Roscoff (perennial)			*		**		**	
Mean ± SE	No data	0.0	0.018	0.050 ± 0.006	33.8 ± 1.58	35.3 ± 2.50	0.40 ± 0.008	0.36 ± 0.007
N		10		18	60	16	60	36

* Jacobs (1979); ** Data, end of growing season.

Table 3
Nitrogen content (N as percent DW) and C:N (atom) ratio of above- and below-ground material for Grevelingen, Veerse Meer, Zandkreek and Roscoff populations

	Grevelingen (perennial)	Veerse Meer (semi-annual)	Zandkreek (annual)	Roscoff (perennial)
N as percent DW				
above-ground	2.17	1.58	1.10	3.21
below-ground	2.04	1.25	1.38	1.87
C:N (at) ratio				
above-ground	17	17	23	13
below-ground	28	29	25	24

In general, isozyme studies did not reveal a high genetic differentiation among seagrass populations. McMillan (1982) did not find intraspecific variation in the enzyme systems of most of the 31 seagrass species studied. A relatively low partitioning of allozyme variation among populations of *Z. marina* compared with species from other hydrophilous taxa was found by Laushman (1993). For the populations used in this study, De Hey & Nienhuis (1992) found some differentiation at the isozyme level for the tidal Zandkreek population (similarity 93% with the Grevelingen, Veerse Meer and Roscoff populations) and the geographically isolated Roscoff population (similarity 79% with the other populations). Together with Gagnon et al. (1980) they concluded that variation found in morphology and ecology should for the greater part be attributed to phenotypic (environmentally induced) differentiation. Using different methods, Backman (1984) reported that genetic variation (14%) together with variation due to interaction between genotype and environment (distinguished from phenotypic plasticity along temporal and spatial gradients due to variable environments) explained 49% of the total variation observed in morphology. In the present study, the discrepancy between in situ and experimental data illustrates that all of the characters mentioned were more or less influenced by the environment. Nevertheless, differences in morphology and especially sexual reproduction among individual populations suggest a greater and more complex genotypic background than previously assumed through interpretation of isozyme patterns. The relative importance of genotype differentiation in displayed population dynamical characters among populations remains open to further study. It is clear, however, that in future studies concerning the impact of environmental factors on population dynamical characters (in particular sexual reproduction) of eelgrass populations, a possible significant genotypic influence can not be neglected. Laushman (1993) stated that much additional research is needed on the population genetics of aquatic plants. The results from our study imply that the conclusions may well account for other seagrass species than only *Z. marina*.

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