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Genetic diversity and connectivity remain high in eelgrass *Zostera marina* populations in the Wadden Sea, despite major impacts

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ABSTRACT: Beginning in the 1930s, eelgrass meadows declined throughout the Wadden Sea, leaving populations susceptible to extinction through patchiness, low density and isolation. Additional anthropogenic impacts have altered current regimes, nutrients and turbidity — all of which affect eelgrass. Recent abiotic modeling studies suggest that poor recovery is the result of a regime shift caused by the loss of positive feedbacks between seagrass meadows and their capacity to mediate turbidity. Additionally, it is hypothesized that genetic and demographic factors — in particular, the loss of genetic diversity and patch connectivity — have contributed to lower fitness of eelgrass, thereby further diminishing recovery potential. We assessed genetic diversity and connectivity of *Zostera marina* among 19 locations, covering some 950 km of coastline between Zeeland, Netherlands and Langhölmen, Sweden. Both allelic and genotypic diversity were high. A Bayesian analysis of population structure revealed 6 significant clusters of subpopulations that are connected by varying degrees of dispersal. Although population divergence was significant at as little as 5 km, isolation by distance was very weak, indicating high connectivity at scales of 150 km. A demographic interpretation of these data suggests that realized gene flow is strong and predominantly northward, leaving the western Wadden Sea relatively isolated. The failure of eelgrass to recover in the western Wadden Sea is, therefore, due to both unsuitable physical conditions and low incoming gene flow. Nonetheless, the greater Wadden Sea can be considered a seed transfer zone providing source material for restoration efforts in any areas where abiotic conditions are more favorable.

KEY WORDS: Eelgrass · *Zostera marina* · Genetic diversity · Connectivity · Fragmentation · Dispersal rate · Wadden Sea

INTRODUCTION

Seagrasses are important habitat modifying and facilitator species (Larkum et al. 2006). Eelgrass *Zostera marina* is still the most widely distributed seagrass along European coasts, despite near mass extinction in the 1930s, which was attributed to a wasting disease caused by the slime mold *Labyrinthula zosterae* (Muehlstein et al. 1991). In the North Sea, Wadden Sea and Danish Belt Sea, losses have been further exacerbated by anthropogenic factors including habitat modification and pollution, as well as natural cycles of increased storm activity and sea surface temperatures (Frederiksen et al. 2004). The net result has been a precipitous decline in cover by ca. 99%. Today, eelgrass is restricted to only a few locations along the southern Dutch and German Wadden Sea coastline and along the northern German and Danish Wadden Sea coastline (Essink et al. 2005; www.rikz.nl). Though populations wax and wane, they have never fully recovered to pre-1930s levels.

In a conservation biology framework (Allendorf & Luikart 2007), recovery depends on both physical and biological factors. This translates to the dual importance of habitat quality (water clarity, nutrients, hydro-

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dynamics and geomorphology) and the genetic potential of the populations (genetic variation, population structure and connectivity in relation to mating systems and demography). Integration of these 2 factors is a major goal in a management context (Ouborg et al. 2006), and the power of this integration in eelgrass conservation and biodiversity is exemplified in studies that have modeled the importance of positive feedbacks between eelgrass and turbidity in the Wadden Sea (van der Heide et al. 2007) and manipulative experiments documenting the importance of genetic diversity to enhanced ecosystem function in response to disturbance (Reusch 2006) and to global warming (Reusch et al. 2005).

Over the past decade, population genetic studies of eelgrass have been greatly facilitated by the development of neutral microsatellite loci. Eelgrass reproduces both sexually and vegetatively with the consequence that the many shoots (ramets) produced in a particular area may or may not correspond to individual genotypes (genets). Thus, one of the first tasks is to establish genetic individuals in the sea of leaves. Population genetic surveys of eelgrass have documented a range of bed compositions from giant monoclones to predominantly sexual meadows (Reusch 2002). Levels of clonal diversity and their arrangement determine local bed architecture and, thus, information about fragmentation, new recruitment and turnover.

Dispersal within and between subpopulations (Procaccini et al. 2007) has been assessed directly and indirectly. Direct dispersal of pollen and seeds in *Zostera marina* is restricted to <7–15 m, even in areas with strong tidal currents of 50 cm s^{-1} (Reusch et al. 1999a). This leads to the prediction of strong population differentiation and isolation by distance at small spatial scales. However, indirect studies using genetic data and assignment tests (Reusch 2000b) show that rafting of rhizomal fragments, which may be carrying reproductive shoots with seeds, is a more important mechanism for dispersal than previously supposed. A comparison between drift-line samples with resident genotypes in the western Baltic showed that, at least for one site, 4 out of 39 drift-line genotypes were immigrants to the resident populations from a distance of at least 50 km away. Long-distance dispersal of seeds through bird droppings may also play a role, although *Z. noltii* seeds collected from droppings of Brent geese were not viable (Ehlers 2002).

Population structure and connectivity have been examined over meters to establish genet age and clonality (Reusch et al. 1999a), tens of kilometers to establish dispersal distances (Reusch 2002), and thousands of kilometers to assess the relative roles of historical processes since the last ice age (Reusch et al. 2000, Olsen et al. 2004). These studies illustrate the enormous variability (reviewed in Procaccini et al. 2007) and, therefore, the importance of place-based assessments.

Establishment of seagrasses as bioindicators/monitoring species of ecosystem health under the EU Habitats Directive, the nomination of the Wadden Sea as a World Heritage Site (Anonymous 2008), and an ongoing interest by the Dutch government in the restoration of seagrass beds in the Wadden Sea (Rijksinstituut-voor-Kust-en-Zee; www.zeegras.nl, Bos & van Katwijk 2005) has led to a reassessment of habitat quality and physical carrying capacity based on modeling (van der Heide et al. 2007). Here we complement the physical analysis with a detailed genetic assessment of eelgrass diversity, dispersal and population connectivity in the greater Wadden Sea region and show how genetic diversity contributes to restoration potential.

MATERIALS AND METHODS

Sampling sites and collection. Samples were collected from 23 locations (among 38 surveyed) in the Wadden Sea and adjacent areas (Table 1, Fig. 1). Extensive efforts were made to sample all populations of *Zostera marina* reported during the last decade. Very small (sensu a handful of plants) or very sparse patches (e.g. densities of <1 plant m^{-2}) may well have been missed, but it is unlikely that small to medium population patches were overlooked, given the extensive aerial mapping surveys and ground-truthing that are regularly conducted by the Dutch and German governments. For example, large fields of *Z. noltii* are sometimes found to contain a few *Z. marina* individuals but these cannot be considered 'patches'. Aerial resolution in these studies is 1:10 000. Small patches (on the order of a few m^2) reported up to 2004 on Terschelling and Schiermonnikoog had either disappeared entirely or were reduced to only a few shoots. Leaf pieces were collected every 1 to 1.5 m along a 50 m random walk, blotted dry and stored in silica crystals.

DNA extraction and microsatellite analysis. DNA extraction followed the silica-based method of Elphinstone et al. (2003). Microsatellite loci included *ZosmarCT3*, *GA2*, *GA6* (Reusch et al. 1999b) and *CT35*, *CT17H*, *CT12*, *CT19*, *CT20*, *GA3* (Reusch 2000a). Amplification profiles and protocols for multiplexing are given in Reusch et al. (2000). Genotyping was performed on an ABI 377 Gene Analyzer (Applied Biosystems) using GeneScan and Genotyper software (Applied Biosystems).

Data analysis. Clonal diversity (i.e. genotypic diversity) was defined as the number of genets (G) divided by the number of ramets sampled (N); 0 (all ramets belong to 1 clone), 1 (all ramets belong to separate

Table 1. *Zostera marina*. Measures of clonal and genetic diversity in populations from the Wadden Sea and adjacent regions. *N*: number of samples collected per location; *G*: genets; clonal diversity: *N*/*G*; *A*: mean number of alleles; AR: allelic richness (based on a rarefaction sample size of 25 genets); PA: number of private alleles; H_{obs}: observed heterozygosity; H_{exp}: expected heterozygosity; F_{IS}: inbreeding coefficient. Significance at ***p < 0.001, **p < 0.01 after Bonferroni corrections

Location	Sample	Tidal position	Phenotype	Cluster	*N*	*G*	Clonal diversity	*A*	AR	PA	H_{exp}	H_{obs}	F_{IS}
The Netherlands													
Zeeland	1	Subtidal	Perennial	A	70	66	0.94	4.4	3.2	0	0.44	0.41	0.073
Zeeland	2	Intertidal	Annual	A	49	46	0.94	5.7	4.5	1	0.54	0.44	0.184***
Paap	1	Intertidal	Perennial	B	50	50	1.00	7.1	5.4	1	0.49	0.41	0.161***
	2	Intertidal	Perennial	B	50	49	0.98	7.4	5.5	0	0.51	0.45	0.119***
	3	Intertidal	Perennial	B	50	49	0.98	6.8	5.0	2	0.47	0.42	0.111**
	4	Intertidal	Perennial	B	50	50	1.00	7.1	5.4	0	0.50	0.45	0.091**
Germany													
Norddeich	ND	Intertidal	Perennial	B	38	32	0.84	4.7	3.9	0	0.48	0.24	0.500***
Schleswig-Holstein	SH1	Intertidal	Annual	C	50	49	0.98	7.6	6.1	3	0.60	0.53	0.109***
	SH2	Intertidal	Perennial	C	49	49	1.00	8.3	6.3	0	0.59	0.52	0.125***
	SH3	Intertidal	Perennial	C	50	49	0.98	8.1	6.4	1	0.59	0.59	0.014
	SH4	Subtidal	Perennial	D	48	47	0.98	7.3	6.1	3	0.69	0.65	0.043
	SH5	Intertidal	Annual	C	46	46	1.00	7.8	6.4	1	0.64	0.61	0.051
	SH6	Subtidal	Perennial	D	29	29	1.00	5.2	4.7	0	0.63	0.56	0.053
	SH7	Intertidal	Annual	C	35	34	0.97	6.6	7.4	1	0.59	0.56	0.118***
	SH8	Intertidal	Annual	C	46	45	0.98	9.0	6.8	3	0.59	0.53	0.122***
Sylt	1	Intertidal	Annual	D	33	33	1.00	7.3	7.0	0	0.64	0.59	0.081
	2	Intertidal	Annual	E	48	48	1.00	8.6	7.2	3	0.67	0.62	0.089**
	3	Intertidal	Annual	E	26	26	1.00	6.7	6.4	3	0.62	0.57	0.088
	4	Subtidal	Perennial	E	27	25	0.93	5.9	6.6	0	0.63	0.63	0.011
Denmark													
Rømø	RØ	Intertidal	Perennial	D	50	50	1.00	8.4	6.4	2	0.58	0.57	0.018
Mandø	MA	Intertidal	Annual	E	51	50	0.98	8.2	6.2	5	0.62	0.58	0.050
Limfjord	LF	Subtidal	Perennial	F	51	27	0.53	6.8	6.3	0	0.55	0.53	0.047
Sweden													
Langhölmen	LH	Subtidal	Perennial	F	46	27	0.59	6.2	5.3	2	0.47	0.48	−0.027

genotypes). Genets (genetic individual) and ramets (morphological individuals that arise from vegetative reproduction) were distinguished using GENCLONE 1.0 (Arnaud-Haond & Belkhir 2007). Multiple, identical multilocus genotypes (clones) were removed from the data set.

Allelic richness was estimated after rarefaction using the CONTRIB program (Petit et al. 1998). Rarefaction was selected to equal the minimum number of genets identified for all locations (i.e. an *N* of 25 genets) in order to correct for unequal sample size (Petit et al. 1998). Calculation of allele frequencies, number of alleles, observed heterozygosity (H_{obs}), Nei's gene diversity (H_{exp}), Wright's fixation indices (F_{IS} and F_{ST} as f and θ), and linkage disequilibrium were calculated in GENETIX 4.05 (Belkhir et al. 2001). Significance was tested using permutations (N = 1000) and Bonferroni corrections were applied where necessary.

Population structure was first analyzed in a standard F_{ST} framework and using genetic distances based on Cavalli-Sforza and Edwards chord distance and Neighbor Joining (NJ) using the software package PHYLIP 3.5 (Felsenstein 1994). GENDIST was used for computing the genetic distances and NEIGHBOR for the neighbor-joining tree, CONSENCE for the consensus tree and SEQBOOT for the bootstrap analysis. In this approach, populations are pre-defined (here set at 23).

Population structure was also analyzed in a Bayesian framework using STRUCTURE v.1.0 (Pritchard et al. 2000). We used the admixture model (each individual draws some fraction of its genome from each of the K populations), as this configuration is considered the best (Falush et al. 2003) for estimating the log probability Pr (X| K) of each cluster, e.g. K = 2, 3, 4, etc., by genetically assigning the individuals to the most likely clusters. Starting with no *a priori* sample locations, i.e. all populations lumped as one, we used 2 methods to detect the true K: (1) the posterior probability of the data for a given K, Pr (X| K) (Pritchard et al. 2000); and (2) the ad hoc statistic ΔK (Evanno et al. 2005). The second method is suggested when patterns of dispersal among populations are assumed to be asymmetrical. In the second round of the anylysis, the user-defined populations (n = 23) were used as priors and assigned to the K-clusters (with the highest likelihood) determined from the first analysis. Each analysis was run 5 times

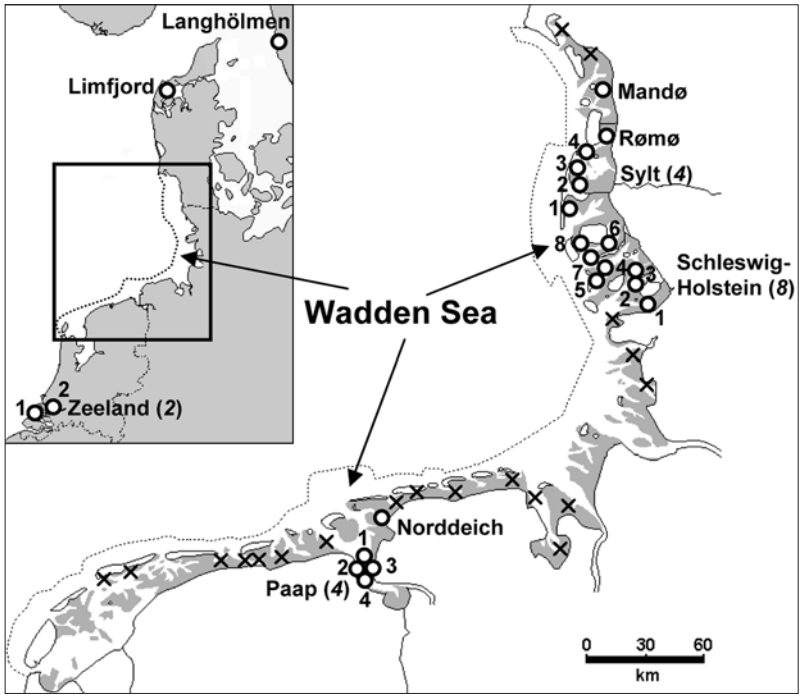

Fig. 1. *Zostera marina*. Populations sampled along the coast of the North Sea and in the Wadden Sea. Values in parentheses behind location names indicate the number of patches sampled per locality. ○: areas where *Z. marina* was sampled; ×: areas surveyed in which no populations were found. Dotted line shows the Wadden Sea area

for 1 000 000 iterations with a burn-in of 100 000 to avoid dependence on initial starting values.

Isolation by distance was tested by plotting estimates of $F_{ST}/(1-F_{ST})$, using the θ estimates obtained in GENETIX 4.05, against both the linear and log geographical distances. In our experience, 2-dimensional models generally perform better with coastal data given local, complex topography in the Wadden Sea, despite the apparently linear coastline. Matrix correlation methods based on the Mantel test were then applied using IBD 1.5 (Bohonak 2002). Reduced-major-axis (RMA) regression was used to determine the slope of significant regression.

The magnitude and direction of dispersal/gene flow (mN_e) among populations was analyzed in a coalescent framework using the program MIGRATE-2.1.0 (Beerli & Felsenstein 2001). The method provides a more accurate estimate of gene flow between populations than classical F_{ST} methods, especially when multiple loci are used. In both approaches it must be remembered that assumptions are made about discrete populations, Hardy-Weinberg equilibrium, mutation-drift equilibrium, no selective effects and a step-wise mutation model for microsatellite loci. We used 3 independent simulations to estimate the migration rates between the clusters (as identified from the STRUCTURE analysis), each consisting of 10 short-chain searches and 3 long-chain searches over 8 microsatellite loci (*GA12* was excluded due to missing data). The 3 simulations were compared to check for convergence. Because there is no formal way to check for convergence, the best that can be done is to run the simulations for a long time (a million generations) and determine at what point the standard deviation of migration rate estimates do not significantly differ from one another.

Management units (MU) sensu Palsboll et al. (2007) are defined as the level of population differentiation (F_{ST}) at which populations become demographically (estimated by dispersal) independent and not simply a rejection of panmixia. From the Wright-Fischer model $F_{ST} = 1/(4mN_e + 1)$, mN_e can be estimated using MIGRATE to define the threshold level of dispersal at which populations should be assigned to a single or multiple MUs. Actual estimates of F_{ST} that are larger than the MU value constitute separate management units; those lower are considered as one.

RESULTS

Genotypic diversity measured as number of clones

Among the 1042 ramets that were genotyped, 976 were unique and 66 represented duplicates of particular genotypes (Table 1). In the vast majority of cases, duplicates involved only 1 to 2 ramets representing a spatial scale of <3 m^2. This spatial scale was represented in both intertidal and subtidal phenotypes. Large clones (>10 m^2) were only found in the Limfjord and Langhölmen. Mean genotypic diversity (proportion of clones) was slightly higher in intertidal vs. subtidal populations, and in annual vs. perennial forms, but neither of these apparent trends was significant (Student's *t*-test; p = 0.143 and p = 0.106).

Genetic diversity measured as allelic richness

Allelic richness (AR), adjusted for sample size (Table 1) shows that genetic diversity is highest in populations between Schleswig-Holstein-7 and Sylt-2 (mean AR ± SD: 7.12 ± 0.26); and the least diverse in 2 populations from Zeeland (3.84 ± 0.90). There were no

significant differences in AR between intertidal and subtidal habitats, but there was a significant difference between annuals and perennials (*t*-test; p = 0.022).

A total of 31 unique alleles were found in 14 of the 23 populations, but with no geographic concentration. Significant heterozygote deficits (F_{IS}) were found in 11 populations. Subtidal populations show significantly less inbreeding than intertidal populations (*t*- test; p = 0.002). This pattern remained significant even when Norddeich (which had an extremely high F_{IS}-value of 0.500) was excluded from the analysis (p = 0.007). Again, if Norddeich was excluded, a significant relationship was found between positive F_{IS} values and annuals (p = 0.041).

Population structure

A neighbor-joining tree based on Cavalli-Sforza genetic distances from the allele frequencies of the 23 *a priori* identified populations recovered 3 well-supported clades around an unresolved core (Fig. 2). Averaged over replicates, the Bayesian analysis with STRUCTURE recovered 6 clusters based on a posterior probability of the data close to 1; all other probabilities were close to 0 (Fig. 2, dotted lines). Using the ad hoc statistic Δ*K*, STRUCTURE assigned the highest likelihood to 2 clusters: Cluster A (Zeeland 1 and 2), and a cluster that includes all remaining sites. The second highest likelihood recovered the same 6 clusters as in the original analysis based on the posterior probability.

Isolation by distance

Wadden Sea populations of eelgrass displayed a pattern of weak to moderately strong differentiation and high connectivity (Fig. 2). The multi-locus F_{ST} across all locations was 0.081 (0.0436 to 0.1273, 95% CI) and among the Wadden Sea locations only, F_{ST} = 0.052 (0.0286 to 0.0814, 95% CI). Although significant population differentiation among the sites was found, the low multi-locus F_{ST} estimate indicates high levels of gene flow. Pairwise F_{ST} among the 23 different locations ranged from –0.005 to 0.267. The minimum distance at which significant differentiation could be detected was 5 km. Isolation by distance was significant at the largest regional scale (p < 0.001, r = 0.729; Fig. 3), which had a spatial scale of ca. 700 km. A break was observed in the slope at 135 to 145 km. Within the Wadden Sea (Clusters B to E) isolation remained significant between the clusters, but subpopulations within Clusters C, D and E did not show significant IBD. This corresponds with the STRUCTURE analysis.

Directionality

Connectivity among clusters (Fig. 4) comes predominately from Clusters B and C. Additionally, the number of emigrants from Clusters B and C exceeds the number of immigrants into these clusters, suggesting that Clusters B and C are source populations. Within- and between-cluster distances and number of migrant (mN_e) estimates based on the analysis from MIGRATE are also given in Fig. 4. This matched with the results

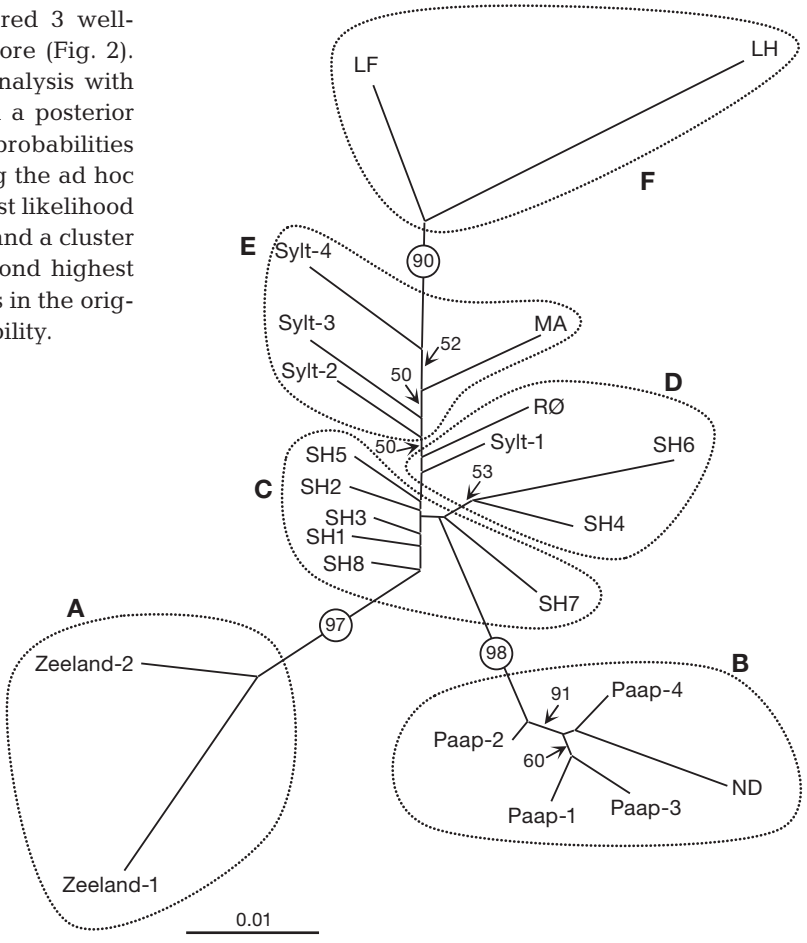

Fig. 2. *Zostera marina*. Neighbor-joining tree among the 23 populations in which branch length is proportional to genetic distance (Cavalli-Sforza & Edwards 1967) based on genets only; bootstraps based on 2000 permutations. Circled clusters are based on the probability of the data defined by STRUCTURE. See Table 1 for location names

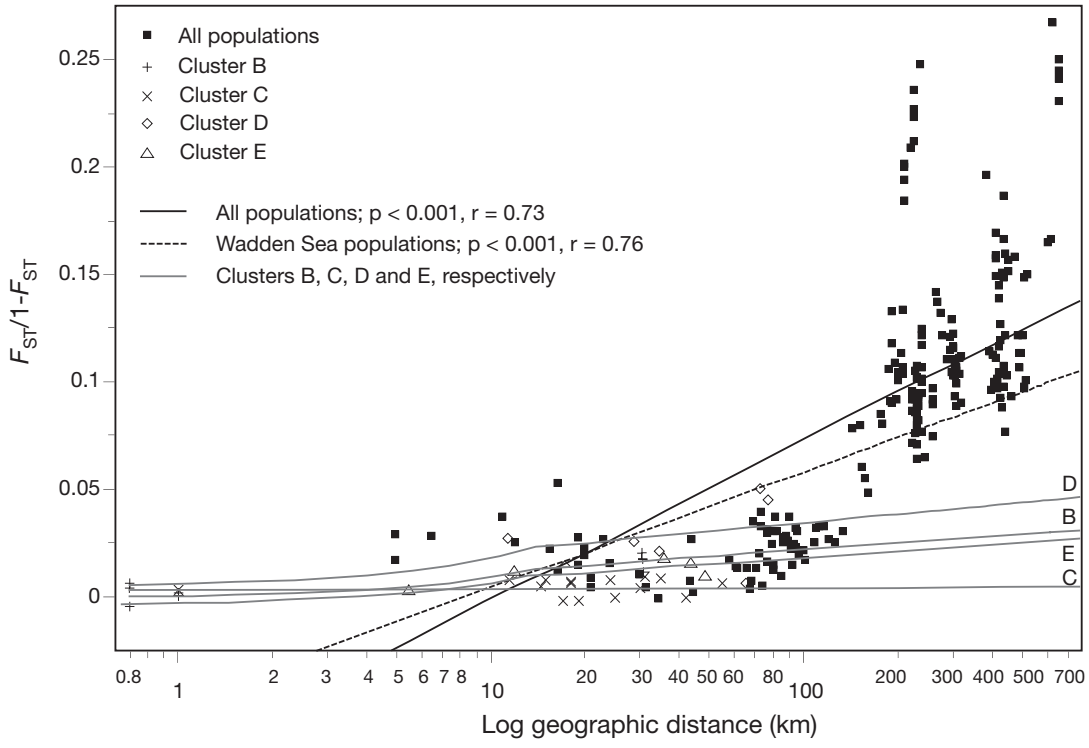

Fig. 3. *Zostera marina*. Isolation by distance. Relationships between genetic distance (θ) and log geographic distance among populations of *Z. marina*. Correlations within the Wadden Sea clusters (gray lines) were not significant

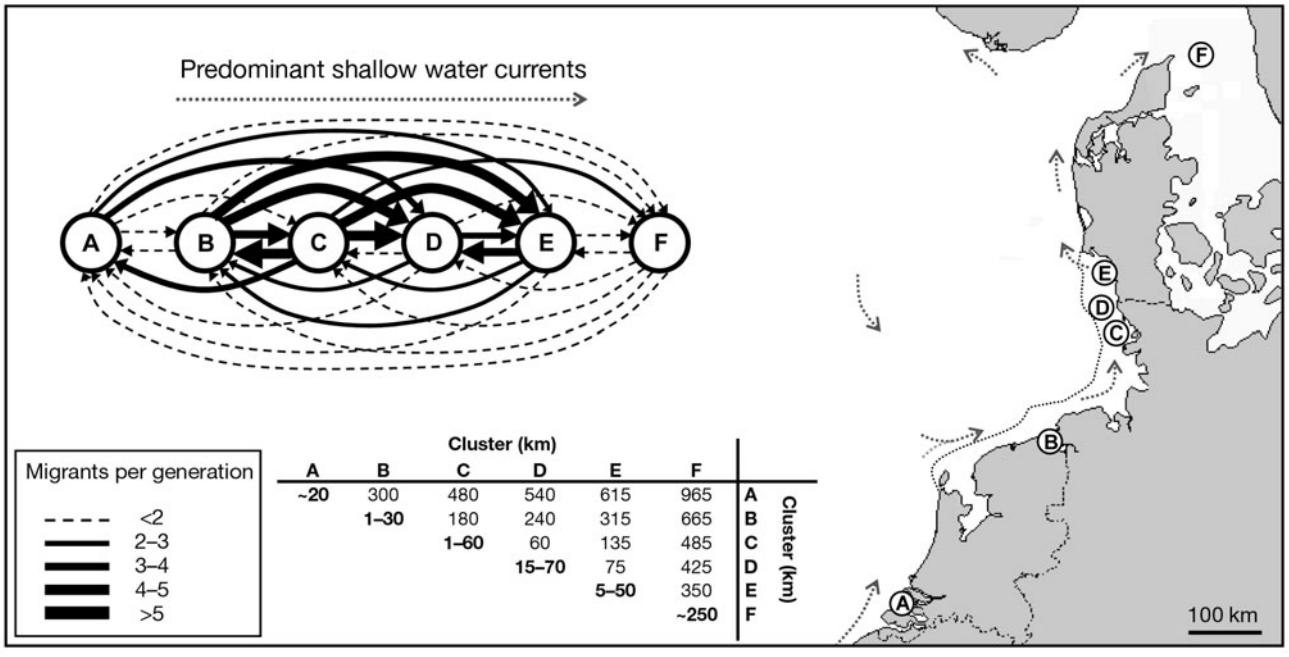

Fig. 4. *Zostera marina*. Connectivity and gene flow along the North Sea coast. Black lines indicate migrants/generation based on an analysis with MIGRATE. Gray dotted arrows show the predominant shallow water currents from south to north. Distances in kilometers within (**bold**) and between clusters are given in the matrix

from STRUCTURE, which showed that populations in Clusters B and C contain numerous individuals assigned to other clusters, whereas populations from Clusters A and F had hardly any direct migrants. The main direction of dispersal (62%) is northward, as would be predicted from the residual tidal currents in the North Sea/Wadden Sea. However, 38% of the total number of migrations is to the south. The migration to more southern clusters is generally between neighboring clusters that are affected by local current patterns.

Management units (MUs)

The total mN_e per cluster based on the MIGRATE analysis ranged from ~1 to >10 individuals per generation with an average of 7.52. Using the formula $F_{ST} = 1/(4mN_e+1)$, eelgrass populations would constitute separate MUs if $F_{ST} \geq 0.032$. The global F_{ST} across all sites was 0.081 (95% CI 0.0436 to 0.1273), larger than our threshold of 0.032, indicating that the sampled sites would be demographically isolated. However, most of the divergence comes from the populations in Zeeland and Limfjord. If these are removed, the overall F_{ST} value among the Wadden Sea locations becomes 0.052 (95% CI 0.0286 to 0.0814), while the threshold value of F_{ST} decreases to 0.030. Since the confidence interval includes the threshold value, the Wadden Sea area (Clusters B to E; Fig. 4) can be considered as a single MU, whereas Zeeland (Cluster A) and Limfjord (Cluster F) would be separate MUs.

DISCUSSION

High standing variation and clonal diversity

The mass mortality caused in part by the wasting disease *Labyrinthula zosterae* (slime mold) in the 1930s strongly reduced eelgrass numbers throughout the Wadden Sea, but the effects of this demographic bottleneck on genetic diversity were apparently negligible, having affected eelgrass meadows that were already stressed, while more resistant and resilient populations remained (Reusch et al. 2005, Ehlers et al. 2008). The Wadden Sea has the highest genetic diversity (mean AR = 6.1) of all eelgrass meadows sampled throughout the entire northern Atlantic ($3.2 < AR < 7.4$; Olsen et al. 2004, S. Ferber unpubl.), and there is, so far, no evidence of secondary admixture in relation to colonization of the North Sea area following the last glacial maximum. Similar results have been found for *Zostera noltii* ($3.8 < AR < 7.8$) based on 9 microsatellite loci (Coyer et al. 2004).

The generation and maintenance of such high diversity is most likely due to a combination of large effective (meta)population size, the effects of intermediate disturbance, and good inter-meadow connectivity (see below). In a recent experimental study in the Baltic Sea, Reusch (2006) showed, in a series of 1 m^2 plots with varying percentages of vegetation canopy removed, that genet turnover, recruitment and clonal stability were maximum at intermediate disturbance levels. Natural disturbance by waterfowl and invertebrate grazing have been noted by many (Nacken & Reise 2000, Schanz et al. 2002), and higher genetic diversity of *Zostera marina* populations in the Baltic Sea have been documented after extensive grazing by swans (Hammerli & Reusch 2003). Higher seed germination has also been found in areas where ice scouring had removed vegetation during winter months (Robertson & Mann 1984) Thus, despite lower overall abundance of eelgrass as compared with 75 yr ago, concerns about low genetic diversity, as contributory explanations for poor seagrass recovery, are unfounded.

Unlike parts of the Baltic, there are few places in the present study that were dominated by large clones. This follows with the dynamism of the Wadden Sea habitat and the presence of 2 phenotypes: an intertidal, erect, annual variant with limited lateral spread of the rhizome, and a subtidal, prostrate, perennial variant with extensive lateral spread of the rhizomes. Most seagrass ecologists consider these environmental variants to be a plastic (rather than a genetic) response (Gagnon et al. 1980) based on the fact that seeds from both phenotypes gave rise to both variants independently, and the fact that neutral genetic markers have (at least so far) been unable to distinguish the 2 phenotypes. This is true for the present microsatellite data as well.

There were, however, some weak correlations between the 2 phenotypes and allelic richness, which was slightly higher in the annual forms. This is probably due to the fact that perennial forms involve overlapping generations in which individual clones may spread and limit recruitment over time, whereas annual forms recruit from seeds each year. On the other hand, micro-evolutionary processes in the form of local selection (and thus true ecotypic differentiation) may also be at work. With the development of non-neutral markers (Oetjen & Reusch 2007), finer distinctions among the phenotypes have been detected and may become highly relevant to restoration planning, since perennial forms are generally preferred. Quantitative genetic evidence for the switch between annual and perennial forms has recently been shown in the monkey flower *Mimulus guttatus* (van Kleunen 2007), and the search for gene complexes involved in the control of this life history trait is underway in crop species (Hu et al. 2003). Likewise, subtle ecotypic differentiation

may well be found in subtidal and intertidal plants even though both annual and perennial forms are found in both areas. In conclusion, although clone size can be an indicator of longevity, it is not necessarily an indication of the annual or perennial phenotype. In continuously disturbed populations, as is the case here, clone size is often quite uniform.

Departures from random mating were found in ca. 50% of the populations sampled, which may reflect local selfing, particularly if the patch is isolated (e.g. Norddeich). In general, we interpret these departures as being related to restricted seed dispersal within patches in which mating with relatives occurs quite readily. We have found this to be the case in *Zostera noltii* populations from the same areas (A. M. Zipperle et al. unpubl.). While this will promote strong differentiation at the local scale, connectivity among patches — by incoming dispersal from rafted, fertile seeds — neutralizes this effect. Similar examples of locally high F_{IS} have been found in other European (Olsen et al. 2004) and Pacific-Mexican populations (Muniz-Salazar et al. 2005) of *Z. marina*. Alternative explanations for elevated F_{IS} values, such as null alleles, have been ruled out by extensive analyses (Olsen et al. 2004), and Wahlund effects, though never completely ruled out, seem unlikely here given, the level of population differentiation observed.

Connectivity

Independent analyses suggest that 6 population clusters best define our data, although the ad hoc statistic ΔK provided a higher likelihood for 2 partitionings. The division of the dataset into 2 groups corresponds to the strongest level of structuring, whereas subsequent analysis showed that a second level of structuring into 6 clusters was present. In a recent study, Waples & Gaggiotti (2006) have shown that the ad hoc ΔK estimate is not better than the standard estimate when gene flow is moderate ($mN_e = 5$), samples sizes are minimal ($N = 50$), and only a handful of microsatellite loci are used. Since these conditions characterize the present study, we consider the 6 cluster result to be more correct. Clusters A and F are the most isolated (sinks), whereas B to E are very well connected. Contact between Zeeland and the western Wadden Sea is minimal, given the unsuitable coastal habitat along the western Dutch coast. Likewise, the tip of Denmark and Longhölmen area of western Sweden are part of the Skagerrak–Kattegat transition.

The demographic connectivity of the central Wadden Sea cluster with the outlier A and F clusters is low, whereas connectivity within the Wadden Sea is high (Fig. 2). Based on the calculation of MUs, the Wadden Sea is a single unit, as are Zeeland and the populations occurring from the tip of Denmark into the Skagerrak area. The difference in physical size of the areas in question is striking and an important management consideration because it spans 3 national boundaries. Assignment tests (Reusch 2002) indicate that dispersal (the relevant demographic measure) of up to 50 km occurs and IBD estimates of gene flow suggest up to 150 km. The distances among clusters (Fig. 4) fall in this range with the exception of Cluster B (North Groningen), which is ca. 200 km from Cluster C populations. Results from MIGRATE show that the dispersal direction is predominantly to the north (62%) along with the tidal currents. However, southward migration was also observed. During periods of persistent northeasterly winds the residual northward currents along the North Sea/Wadden Sea coast can be slowed significantly or even reversed (de Graaf et al. 2004). Thus, occasional back-dispersal of *Zostera marina* individuals from northern to southern populations occurs, and the IBD model using log distance is, in our opinion, more explanatory than a simple linear distance.

The western Wadden Sea will remain largely upstream placing it in a 'sink' situation. Although occasional, very small patches of eelgrass arise and persist for 1 to 2 yr in some areas (Fig. 1), the abiotic conditions of the western Wadden Sea are probably no longer suitable for eelgrass (van der Heide et al. 2007). Restoration efforts (Bos & van Katwijk 2005, van der Heide et al. 2007) are likely to be successful only in the central and eastern regions. Prevention of isolation — that could occur with ongoing changes in the currents and geomorphology — is essential for maintenance of the metapopulation. The physical isolation of populations in Zeeland (The Netherlands), Limfjord (Denmark) and Langhölmen (Sweden) already show evidence for decreased diversity and increased genetic drift related to small population sizes. In all cases, the success of restoration efforts will depend on the physical state of particular areas.

In conclusion, eelgrass meadows provide the structural, biological foundation for a rich ecosystem and, when they are large and healthy, the basis for control and modification of the physical environment. The genetic potential of eelgrass throughout the Wadden Sea remains high, even though some areas are physically inadequate. Further insights into the genetic basis of adaptation to environmental conditions are underway and will be useful for conservation and management.

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