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# Eelgrass *Zostera marina* populations in northern Norwegian fjords are genetically isolated and diverse

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**ABSTRACT:** Populations along the northern boundary of a marine species' distributional range in the NE Atlantic are expected to harbor lower standing genetic variation as a consequence of post-glacial expansion following the last glacial maximum. Founder events and marginal habitat availability may render the edge populations more vulnerable to anthropogenic stress and less capable of rapid adaptation to global climate change, a concern for conservation and management. We analyzed meadow architecture, persistence and connectivity within and among 15 locations (600 samples genotyped with 8 microsatellite loci) in 3 fjords in Troms County, Norway (69° N). Whereas global mean allelic diversity (standardized for sample size) was in accordance with previous studies using the same markers, more extensive sampling revealed a broader range of allelic richness (mean = 2.85; range = 1.84 to 4.21) in the regional pool. Genotypic diversity was typically high, whereas large genets were rare (2 out of 15 locations). Population differentiation ( $F_{ST}$ ) was 2 to 6 times higher between fjords than within fjords. A Bayesian (STRUCTURE) analysis also strongly supported the genetic distinctness of each fjord. Although 9 locations within the 60 km long Balsfjord were connected by gene flow, demographic connectivity may actually be low, as fixed differences were observed at 6 of the 9 locations, along with significantly positive inbreeding coefficients and strong substructure. Overall, our results suggest that these northern, leading-edge meadows are healthy, but vigilance is required to avoid further losses. Fjord-level management, especially of the larger fjords, will be sufficient to capture the range of variation.

**KEY WORDS:** Seagrass · *Zostera marina* · Populations · Genetic diversity · Management · Leading-edge

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## INTRODUCTION

Seagrass meadows provide foundational habitat by supporting high biodiversity (Hughes & Stachowicz 2004, Waycott et al. 2006) and numerous ecosystem services, ranging from erosion protection and carbon

sequestration to nursery functions and recreation (Costanza et al. 1997, Heck et al. 2003, Joseph et al. 2006). Unfortunately, seagrasses are suffering a global decline due to a variety of changes related either indirectly (through climate change) or directly (as a consequence of habitat destruction, reduced water

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quality, physical disturbance from commercial fishing, aquaculture and invasive species) to anthropogenic activities (Orth et al. 2006, Waycott et al. 2009).

Eelgrass *Zostera marina* L. is the most widely distributed seagrass in temperate, northern hemisphere regions of both the Pacific and Atlantic Oceans. It is the dominant seagrass along European shores, ranging from northern Norway to southern Portugal. Along the Norwegian coast, eelgrass has been considered relatively common in the south, but progressively scattered and less abundant in the northernmost areas, as a consequence of habitat limitation (Lid & Lid 1994). However, a mapping project initiated in 2007 under the National Program for Mapping and Monitoring of Marine Biodiversity (Bekkby et al. 2008, 2011) has revealed a more extensive distribution in the higher latitudes than expected, recording >3000 beds ranging in size from 100 m<sup>2</sup> to 6 km<sup>2</sup>. Consequently, the Norwegian Directorate for Nature Management ([www.naturbase.no](http://www.naturbase.no)) and Akvaplan-NIVA ([www.akvaplan.niva.no](http://www.akvaplan.niva.no)) have initiated an action plan to establish legislation through the Nature Diversity Act of 19 June 2009 that will ensure sustainability of and promote basic research on seagrass habitat.

Although *Zostera marina* has been recorded in northern Norway since the 1880s (Norman 1900), very little research has been conducted on the biology or ecology of eelgrass itself or its role in community dynamics. Duarte et al. (2002) found no limitation on growth rate associated with nutrients, irradiance or day length, while working in Hopavagen Lagoon (63° 35' N, 9° 32' E), suggesting that long term persistence was not physiologically limited. The only other studies we are aware of were performed in southern Norway and focused on epiphytes, epifauna, infauna and grazing effects on eelgrass (Fredriksen & Christie 2003, Fredriksen et al. 2004, 2005, 2010). It should also be noted that, although our study area lies well above the Arctic Circle (68° to 70° N), it is considered sub-Arctic from a biogeographic perspective, based on the influence of the Gulf Stream and the 10°C July isotherm, which skirts just north of the country (Stonehouse 1989, [www.amap.no](http://www.amap.no)). This suggests that suitable habitat and temperatures supportive of larger and possibly higher diversity populations than predicted have been present for many thousands of years.

Recolonization of *Zostera marina* throughout the NE Atlantic commenced at the end of the last glacial maximum (LGM) (18 000 yr BP) (Bradwell et al. 2008) from more southerly refugia in parts of Ireland, the Brittany peninsula of western France and northern

Spain (Olsen et al. 2004, Becheler et al. 2010). Northerly refugia in Iceland and the Lofotan peninsula of Norway have also been proposed (see Maggs et al. 2008, Coyer et al. 2011). As the glaciers retreated, northerly expansion ensued via 'leading-edge' populations (Ibrahim et al. 1996), derived from the refugial pool(s). Leading-edge populations are expected to exhibit lower allelic diversity (reviewed in Hewitt 2004) as a consequence of founder effects and small population sizes that are strongly affected by genetic drift. Many benthic marine species present this phylogeographic gradient of 'southern richness and northern purity' (Hewitt 2004, Maggs et al. 2008).

Knowledge of population genetic structure in seagrasses provides inferences about meadow architecture and ecological processes related to meadow dynamics, as well as growth and persistence through sexual reproduction and vegetative spread of genets to produce large clones (Waycott et al. 2006, Procaccini et al. 2007). Meadows may be heterogeneous as a consequence of temporal admixture events of repeated recruitment through time (originally proposed by Petit et al. 2003 for oaks, Becheler et al. 2010); limited dispersal of pollen and seeds, leading to patchiness (Hämmerli & Reusch 2003); and local habitat selection (Oetjen & Reusch 2007, Oetjen et al. 2010, Winters et al. 2011). Alternatively, they can be homogeneous as a consequence of dominance by a few large genets/clones (Reusch et al. 1999, Coyer et al. 2008) and limited recruitment (Duarte et al. 2006).

Isolation of meadows further affects the maintenance of diversity, especially if populations are small and dispersal is limited (Waycott et al. 2006, Procaccini et al. 2007). The complexity of the Norwegian coastal fjord system creates vast amounts of dissected and patchy habitat, which conceivably could enhance or reduce diversity and gene flow within the regional pool, depending on local currents and physical barriers. Since, from a conservation perspective, the overall effect of reduced genetic variation is a reduction in adaptive potential, it is necessary to determine whether fjord populations in the high latitudes may be exceptionally vulnerable.

In the present study we focus on northern fjord populations (68° to 70° N) in Troms County, Norway. Our aims were to (1) test, via more extensive sampling, the degree to which northern fjord populations conform to the predicted low allelic diversity profile associated with the leading-edge hypothesis, (2) assess the relative contributions of sexual reproduction and the vegetative spread of large genets/clones to meadow architecture as an indicator of long-term

persistence, (3) test the level of population differentiation and isolation as an indicator of connectivity within and between fjords and (4) identify whether ecotypes (e.g. *Zostera marina* var. *angustifolia*) are specific to subtidal or intertidal habitats.

## MATERIALS AND METHODS

### Sample collection and DNA extraction

Samples ( $n = 50$  per location) of *Zostera marina* were collected from 15 locations in 3 fjords: Balsfjord ( $n = 9$ ), Sør-Lenangen (2) and Sagfjord in Salangen (4) (Fig. 1, Table 1). Samples were collected both by wading and diving (tidal difference in the area  $\sim 3$  m), at 1 to 1.5-m intervals in which direction was determined by a random walk. The only exceptions to this sampling strategy were the Laksvatn samples in Balsfjord. These were collected along 3 transects (high, medium, low intertidal), which ran parallel to the shore. Leaves from individual shoots (ramets) were cut into two or

three 2 cm pieces, blotted dry and placed into tubes with silica crystals for dehydration and preservation. Leaf tissue was pulverized in the laboratory, using an MM 301 mixer mill (Retsch). DNA was extracted using a silica-based method as described by Hoarau et al. (2007) and Coyer et al. (2009), except that the cetyl trimethylammonium bromide (CTAB)/sample slurry was heated at  $60^{\circ}\text{C}$  for 1 h.

### Microsatellite amplification and genotyping

Template DNA for polymerase chain reactions was obtained from 1  $\mu\text{l}$  of the final DNA solution (typically 2 to 10 ng). Eight species-specific microsatellite loci were multiplexed (CT2, CT23, CT35, CT12, CT16, CT17D, CT19, CT20) for PCR amplification (Reusch 2000, Reusch et al. 2000). A recent study of *Zostera marina* revealed that 2 loci commonly used (CT17H and CT35) sometimes revealed signs of genetic mosaicism (somatic mutation producing  $>2$  alleles per ramet) among populations at the northern and southern limit of its European distribution (Reusch & Boström 2011). The complexity of the microsatellite genotypes suggested that there were one or more divergent cell lineages present within a single ramet. In the present study, complex genotypes were found for CT17H and this locus was eliminated, whereas CT35 displayed biallelic patterns for the 14 alleles and was retained. Genotypes were visualized on an ABI 3730 gene analyser (Applied Biosystems) and analysed using GENOTYPER (Applied Biosystems) software.

### Data analysis

A genetic individual (genet) consists of many shoots (ramets), which can extend for several meters. Large genets are referred to as clones (see Procaccini et al. 2007 for discussion of terminology). Thus, sampled shoots in an area can have the same multilocus genotype (MLG) if part of the same large clone. The number of genets and ramets sampled in a given area were distinguished with GENCLONE 2.0 (Arnaud-Haond & Belkhir 2007). Prob-

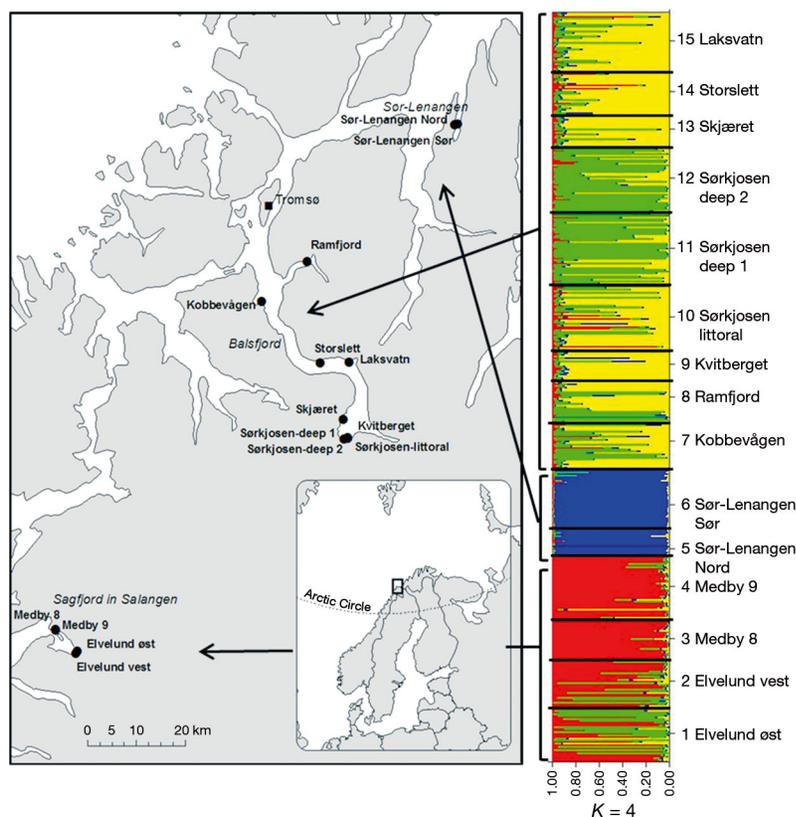


Fig. 1. Location of sampling sites for *Zostera marina* including the results of the STRUCTURE analysis (Pritchard et al. 2000). Each individual is represented by a horizontal bar partitioned into colored segments, the length of which is proportional to the individual's membership in each of 4 clusters ( $K = 4$ ). See 'Materials and methods: Data analysis' for determination of  $K$

Table 1. *Zostera marina*. Collection sites and general information. Sampling dates are given as dd/mm/yyyy

Fjord region Name of location	Location description	Date of sampling	Coordinates (N, E)	Collection depth (m)
<b>Sagfjord</b>				
Elvelund-øst	River outlet, sand, subtidal	17/06/2011	68° 51.93', 17° 49.85'	0.4
Elvelund-vest	River outlet, sand, subtidal	17/06/2011	68° 52.20', 17° 50.28'	0.4
Medby WP8	Tidal flat, mud, subtidal	18/06/2011	68° 54.58', 17° 43.95'	0.5
Medby WP9	Tidal flat, mud, subtidal	18/06/2011	68° 54.60', 17° 43.98'	0.5
<b>Sør-Lenangen</b>				
Sør-Lenangen Nord	Tidal flat, river outlet 2 km away, mud, intertidal	21/06/2011	69° 47.42', 19° 59.39'	0.3
Sør-Lenangen Sør	Tidal flat, river outlet 2 km away, mud, intertidal	21/06/2011	69° 47.35', 19° 58.39'	0.3
<b>Balsfjord</b>				
Kobbevangen	Tidal flat (RAMSAR site), mud	16/08/2011	69° 29.54', 18° 53.47'	2
Ramfjord	Tidal flat, soft, mixed sand/mud	16/08/2011	69° 33.56', 19° 08.44'	1.5
Kvitberget	Tidal flat, soft, mixed sand/mud	17/08/2011	69° 13.92', 19° 17.42'	0.3
Sørkjosen-littoral	Tidal flat (RAMSAR site), mud	17/08/2011	69° 13.83', 19° 17.47'	0.3
Sørkjosen-deep 1	Tidal flat (RAMSAR site), mud	17/08/2011	69° 13.86', 19° 17.32'	2.5
Sørkjosen-deep 2	Tidal flat (RAMSAR site), mud	17/08/2011	69° 13.76', 19° 16.13'	4–5
Skjæret	Shallow, soft, mixed sand/mud	17/08/2011	69° 15.96', 19° 16.32'	3
Storslett	Tidal flat, very muddy	18/08/2011	69° 22.36', 19° 10.34'	0.3
Laksvatn	Tidal flat, soft, mixed sand/mud	18/08/2011	69° 22.18', 19° 19.38'	0–1

abilities of identity by chance ( $P_{sex}(F_{IS})$ ) were calculated for each sample to avoid false assignment of individual ramets, sharing the same MLG by chance, to the same genet (clone).  $P_{sex}(F_{IS})$  accounts for departure from Hardy-Weinberg equilibrium (HWE) and provides the most conservative estimates of clonal identity (Arnaud-Haond & Belkhir 2007).

Genotypic diversity,  $R$  (number of genets,  $G-1$ , over number of sampled ramets,  $N-1$ ); and allelic richness,  $A_c$  (number of alleles per locus, corrected for the minimum number of genets identified among all locations (here  $n = 17$ ), were also calculated with GENCLONE 2.0. Expected heterozygosity ( $H_e$ ) and Wright's fixation indices ( $F_{IS}$  and  $F_{ST}$ ) were calculated using GENETIX 4.05 (Belkhir et al. 2001). All subsequent analyses of population structure used unique genets only, i.e. duplicate MLGs were removed.

Linkage disequilibrium (LD) was assessed in ARLEQUIN 3.5 (Excoffier et al. 2005). Pairwise comparisons of all loci ( $n = 8$ ) per population ( $n = 15$ ) were compared using a likelihood ratio test (Slatkin & Excoffier 1996) and tested for significance ( $p = 0.05$ ) with 10 000 permutations.

Clone size was estimated based on the spatial resolution of the linear sampling method (i.e. 1 to 1.5 m), which provided a coarse minimum value only; shoots were not sampled in a grid or mapped. For example, if 3 consecutive samples had the same MLG, the clone was estimated as minimally 3 to 4.5 m in size.

Population structure was first analyzed in a classic  $F_{ST}$ -based format, using genetic distances based on the Cavalli-Sforza and Edwards chord distance and neighbor-joining, using the software package PHYLIP 3.5 (Felsenstein 1994). We used GENDIST for computing genetic distances, NEIGHBOR for constructing the tree, CONSENSE for constructing the consensus tree and SEQBOOT for the bootstrap resampling.

Isolation by distance (IBD) (Wright 1943, Slatkin 1993) was evaluated by correlating estimates of  $F_{ST}/1 - F_{ST}$  (Rousset 1997), using the  $\theta$  estimator (Weir & Cockerham 1984), with geographic distances. This was done using matrix correlation methods based on the Mantel test (Manly 1994) and 10 000 randomizations with IBD Web Service v.3.23 (Jensen et al. 2005). Linear distances were determined with waypoints taken in the field and ArcGIS. Geographic distances (km) were log-transformed in accordance with a 2-dimensional stepping stone model, which we deemed more appropriate, given the dissected nature of the fjord system.

Population structure was also analyzed in a Bayesian framework implemented in the software STRUCTURE 2.3.3 (Pritchard et al. 2000). In this approach there is no *a priori* designation of 'populations'. The admixture model was used to estimate the log probability  $P(X|K)$  of encountering each user-determined set of clusters/populations ( $K = 2, 3, 4$ , etc.), by genetic assignment of individuals to the most

Table 1. (continued)

Water temp. (°C)	Sample numbers	Additional observations
5–7	0–49	Patchy distribution. Plant length max. 20–25 cm.
5–7	50–99	Patchy distribution. Plant length max. 20–25 cm.
5–7	100–149	Large, dense meadow. Plant length max. 20–25 cm.
5–7	150–199	Large, dense meadow. Plant length max. 20–25 cm.
5–7	200–249	Small patches. Plant length max. 15 cm.
5–7	250–299	Small patches. Plant length max. 15 cm.
11	300–349	Small, dense, subtidal meadow. Plant length max. 25–40 cm. Diving.
11	350–399	Small subtidal meadow. Patchy plant distribution. Plant length max. 25 cm. Diving.
9	400–449	Sparse but continuous intertidal meadow. Small <i>Z. marina</i> var. <i>angustifolia</i> type morphology.
9	450–499	Moderately dense, intertidal meadow. Small <i>Z. marina</i> var. <i>angustifolia</i> type morphology.
9	500–549	Large, dense, subtidal meadow. Plant length max. 89 cm. Flowers and seed capsules observed. Diving.
9	550–599	Large, subtidal meadow, ~700 m from littoral. Flowers and seed capsules observed. Diving.
9	600–649	Subtidal meadow. Large but sparse patches of plants. Diving.
9	650–699	Large, intertidal meadow. Sparse patches of brown and unhealthy looking plants.
9	700–747	Dense meadow. Sampling from 3 zones; shallow –0 m (700–715), intermediate –0.5 m (717–732), deep –1 m (734–749). Healthy looking plants.

likely clusters (see Fig. S1 in the Supplement at [www.int-res.com/articles/suppl/m486p121\\_supp.pdf](http://www.int-res.com/articles/suppl/m486p121_supp.pdf)). The true number of clusters was estimated under 2 assumption sets using the web-based STRUCTURE HARVESTER (Earl & vonHoldt 2012). In the first analysis, posterior probabilities for a given  $K$ ,  $P(X|K)$  (Pritchard et al. 2000), were determined directly, whereas in the second analysis the ad hoc statistic  $\Delta K$  (Evanno et al. 2005) was used. The latter is recommended when asymmetrical dispersal patterns exist among given locations. The  $\Delta K$  method is based on the rate of change of  $P(X|K)$  values between different  $K$ s, with the number of sampling locations used as priors and assigned to the most likely  $K$ . Each analysis was repeated 5 times ( $10^6$  iterations; burn-in = 100 000) to avoid dependence on starting values.

## RESULTS

Mean allelic richness ( $\hat{A}$ ), a measure of genetic variation that was adjusted for a sample size of 17, was 2.85 with a range of 1.82 to 4.21 (Table 2). Richness values varied between locations within all 3 fjords and were uncorrelated with substrate (sand or muddy) or depth (shore or subtidal position). Highest allelic richness was recorded at Elvelund-øst ( $\hat{A} = 4.00$ ) in the Sagfjord and the 2 Sørkjosen-deep sites ( $\hat{A} = 4.05$  and 4.21) situated inside a Ramsar wetland

site at the head of Balsfjord. The lowest values were found at Medby WP9 ( $\hat{A} = 1.82$ ), Kvitberget ( $\hat{A} = 1.84$ ) and Skjæret ( $\hat{A} = 1.98$ ) in Balsfjord. Nine of the 15 locations sampled had putative private alleles (mean = 3.60; range = 1 to 8) and 10 locations showed at least 1 fixed allele (Sør-Lenangen had 5.) (Table 2).

Genotypic diversity ( $R$ ), a measure of clonality via vegetative spread of genets, was also highly variable, ranging from 0.319 at Sør-Lenangen Nord, where 3 large clones dominated, to 1.0 at Sørkjosen-deep 1 and 2, and Ramfjord, where every shoot sampled belonged to a unique genotype (Table 2). Genotypic diversity was high ( $R = 0.872$  to 0.935) at Sagfjord, while at Sør-Lenangen, 1 meadow consisted of 3 large clones extending to 21 m in size. Most genets, however, ranged minimally from 2 to 5 m in size, depending on the resolution of the sampling. Genotypic/clonal diversity was mostly high at Balsfjord, with a mix of moderately sized clones and many smaller ones at most locations. The highest allelic and genotypic diversities ( $\hat{A} = 4.21$ ;  $R = 1.00$ ) were observed subtidally at the Sørkjosen Ramsar site, which hosts a large intertidal–subtidal meadow system of >400 000 m<sup>2</sup>, whereas the contiguous intertidal site was less allelically diverse with slightly more clones ( $\hat{A} = 2.58$ ;  $R = 0.957$ ). The largest clones were at Sør-Lenangen Nord, which is near a river outlet. No genets were shared among the locations sampled.

Table 2. *Zostera marina*. Genetic diversity and clonality, based on 8 microsatellite loci.  $N$  = number of shoots analyzed,  $G$  = number of genets,  $R$  = genotypic diversity ( $G-1/N-1$ ),  $\hat{A}$  = allelic richness (standardized to 17 genets),  $pp$  = putatively private alleles found only at that location,  $fixed$  = number of fixed alleles (frequency > 0.97),  $G>1$  = number of genets with >1 ramet,  $nR$  = mean number of ramets per genet (distribution of duplicate ramets per genet given in brackets),  $H_e$  = expected heterozygosity,  $F_{IS}$  = Wright's fixation index estimated as  $f$  (Weir & Cockerham 1984, \* $p < 0.05$ ), LD = linkage disequilibrium, i.e. proportion of pairwise comparisons that were significantly linked

Location	$N$	$G$	$R$	$\hat{A}$	$pp$	$fixed$	$G>1$	$nR$	$H_e$	$F_{IS}$	LD
Elvelund-øst	29	27	0.93	4.00	8	0	2	2	0.443	0.157*	0.64
Elvelund-vest	40	35	0.87	3.04	4	0	3	1.8 (2,3,4)	0.342	0.069	0.36
Medby WP8	48	42	0.87	2.12	0	3	2	4 (2,6)	0.214	-0.171	0.00
Medby WP9	47	44	0.93	1.82	2	3	1	4	0.248	-0.041	0.07
Sør-Lenangen Nord	48	16	0.31	–	0	5	3	11.7 (3,15,17)	0.183	0.101	0.00
Sør-Lenangen Sør	48	42	0.87	2.62	4	5	2	3 (2,4)	0.183	-0.025	0.00
Kobbbevågen	35	34	0.97	2.75	4	2	1	2	0.273	0.167*	0.43
Ramfjord	29	29	1.00	3.50	0	1	0	–	0.265	0.241*	0.28
Kvitberget	47	46	0.97	1.84	4	3	1	2	0.202	0.315*	0.07
Sørkjosen-littoral	48	46	0.95	2.58	0	0	2	2.5 (2,3)	0.264	0.221*	0.14
Sørkjosen-deep 1	41	41	1.00	4.21	3	0	0	–	0.430	0.215*	0.21
Sørkjosen-deep 2	41	41	1.00	4.05	2	0	0	–	0.387	0.100	0.00
Skjæret	48	46	0.95	1.98	3	4	2	2	0.162	0.067	0.00
Storslett	48	40	0.83	2.26	0	2	2	4 (2,6)	0.183	-0.007	0.03
Laksvatn	47	42	0.89	2.98	0	1	3	2.7 (2,3,4)	0.219	0.050	0.00

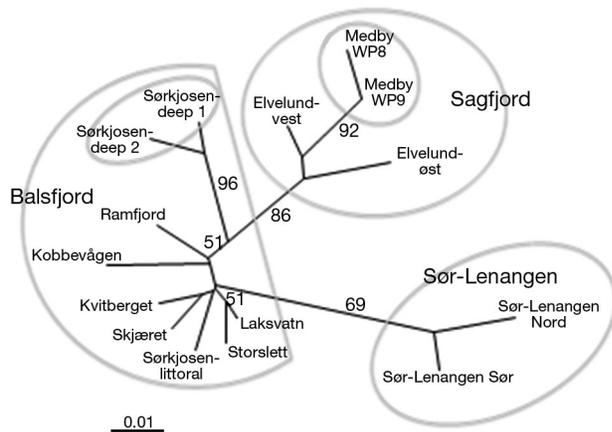


Fig. 2. *Zostera marina*. Relationships among populations in the study area of Troms County. The tree was based on pairwise Cavalli-Sforza and Edwards' chord distances (Cavalli-Sforza & Edwards 1967) between genets only. Bootstrap values were derived from 1000 resamplings

Mean  $H_e$  varied significantly, ranging from 0.162 at Skjæret to 0.430 at Sørkjosen-deep 1, sites separated by only 4 km (Table 2). Significant departures from HWE (6 locations) and linkage disequilibrium (LD) (7 locations) were observed (Table 2). Significantly positive values of  $F_{IS}$  may be due to inbreeding and kinship (null alleles have seldom been encountered with these loci) or a possible Wahlund effect, although the latter explanation is not favored, given the sampling scale and strongly correlated LD (Table 2). Pairs of loci contributing to the LD within each population were not the same loci contributing to LD among

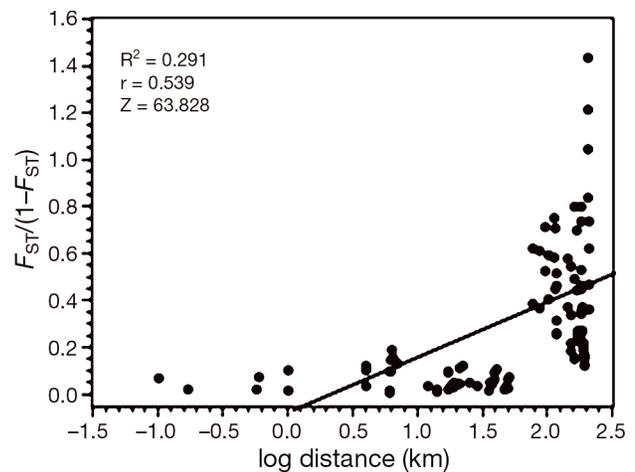


Fig. 3. *Zostera marina*. Isolation by distance (IBD). The genetic and geographic distance matrix (Table S1 in the Supplement) for *Z. marina* was compared with the Mantel Test and 10 000 randomizations; the estimate of  $R^2$  was calculated using reduced major axis (RMA) regression (Jensen et al. 2005) among all pairwise comparisons. Within-fjord distances ranged from 0.6 to 50 km; between-fjord distances ranged from 77 to 208 km

populations, and the number of loci involved was 1 or 2. In contrast, the 2 Elvelund locations showed strong LD with 18 out of 28 comparisons significant at Elvelund-øst and 10 out of 28 significant at Elvelund-vest. Significant LD, involving 2 pairs of loci (GA2 and GA12, and GA23 and GA17D), were matched between the 2 locations; all other comparisons involved different pairs of loci.

Population differentiation ( $F_{ST}$ ) was 2 to 6 times higher among fjords than within fjords (see Table S1 in the Supplement). The trend was further supported by 3 well-supported clusters in the neighbor-joining tree (Fig. 2). There was no true IBD among fjords, as the regression line in Fig. 3 reflects differences in spatial scaling of the sampling. Likewise, the Bayesian STRUCTURE analysis (Fig. 1) strongly separated each of the fjords with  $K = 3$  or  $K = 4$ . Populations were also substructured within fjords, as almost all pairwise  $F_{ST}$  comparisons were significant ( $p < 0.05$ ) following Bonferroni correction and all but 4 were significant without Bonferroni correction (Table S1 in the Supplement).

There was no IBD within fjords (Fig. 3). Pairwise  $F_{ST}$  values ranged from 0.0921 to 0.1611 within Sagfjord. The significant values found for the Elvelund-øst-Elvelund-vest, and Medby WP8-Medby WP9 pairs reflect geographic distances of  $< 1$  km, and within Sør-Lenangen, significant differentiation ( $F_{ST}$  value = 0.0717) was found between 2 locations only, which were 0.6 km apart. Populations within the entire 60 km length of Balsfjord revealed a mean  $F_{ST}$  of 0.0536 (range = 0.0079 to 0.1179), with Kobbvågen differing most. The 2 deep sites at Sørkjosen, within the Balsfjord, were comprised of many genotypes and formed a well-supported group in all analyses (Figs. 2 & 3).

Small plants, morphologically attributable to *Zostera marina* var. *angustifolia*, were collected in the mid-high intertidal at Kvitberget and Sørkjosen-littoral. Both of these locations are close to, and/or contiguous with, the Sørkjosen-deep sites (Sørkjosen-littoral is 120 m from the deep sites and 6 km from Kvitberget). Microsatellite loci did not distinguish the '*angustifolia*' morphotype from *Z. marina* (Fig. 3).

## DISCUSSION

Scientists and conservation managers alike need to understand the determinants of seagrass health, specifically what affects persistence, sustainability, and extent (area and density). Although they only form one component of conservation and management, population genetic analyses provide evolutionary insights about both historical and contemporary processes that have shaped, and are likely to shape, future sustainability. These include a snapshot of relative recruitment, turnover and genetic potential, averaged over several generations, thus providing baseline information about potential vulnerability, past resistance and stability (Procaccini et al. 2007). Genetic characterization of population structure also

provides partial (although incomplete) information about connectivity (Gaggiotti 2010) and ecological coherence that are relevant to marine spatial planning and protected area design (Nilsson-Jacobi & Jonsson 2011, 2012). And finally, genetic surveys provide a practical guide for seagrass mitigation and restoration (Reynolds et al. 2012) by identifying allelic and clonal diversity. In short, the 'evol-eco' approach (Pennisi 2012) is gaining importance in both primary research and conservation management, because it is recognized that both occur in contemporary time (Spielman et al. 2004, Allendorf & Luikart 2007).

## Past informs present

Signatures of past climate change (such as relatively lower genetic diversity) remain visible in some northern populations of *Zostera marina*, as predicted by phylogeographic theory (Hewitt 2000). For example, mean values of allelic diversity in our study (mean  $\hat{A} = 2.85$ , range = 1.84 to 4.21, normalized to  $n = 17$ , 15 locations, 200 km range) compared favorably with values from Iceland ( $64^\circ$  N, mean  $\hat{A} = 1.59$ , range = 1.51 to 1.64, normalized to  $n = 22$ , 2 locations, 50 km range) (Olsen et al. 2004) and southwestern Greenland ( $64^\circ$  N, mean  $\hat{A} = 2.20$ , range = 1.38 to 3.00, normalized to  $n = 10$ , 4 locations, 50 km range) (Diekmann & Serrão 2012). The finding of a few locations with values of  $\hat{A} > 4$  (Table 2) are more typical of southerly locations such as western Sweden, the Skagerrak and the western Atlantic coast of America (Olsen et al. 2004, Diekmann & Serrão 2012). Three non-mutually exclusive explanations may explain the diversity patterns. First, the higher diversity may have resulted in part from biased sampling in that global surveys generally assess diversity on a per population basis over the entire distributional range (Olsen et al. 2004), with most sampling occurring in the middle of the range (Diekmann & Serrão 2012). Second, the highly dissected coastline of the Norwegian fjord system and large meadow sizes in some fjords, combined with strong population differentiation and evidence for sexual recruitment, rather than vegetative expansion (particularly in large, dense meadows such as Sørkjosen; see next subsection), may foster higher levels of diversity, as also observed in Brittany (Becheler et al. 2010). Finally, the higher diversity may be related to a cryptic refugium on the coastal island of Andøya (near Lofoten, Troms County), which has been suggested for both terrestrial and marine species (reviewed in Maggs et al. 2008, Coyer et al. 2011). We conclude that, while overall mean allelic di-

versity is consistent with the leading edge hypothesis, pockets of higher diversity may ensure stability, as well as expansion further north (e.g. Jan Mayen, Eastern Spitzbergen) and east along the Siberian coast.

### Clonal diversity and population structure

Understanding the genetic structure of populations provides insights into meadow dynamics, growth and persistence through sexual reproduction and vegetative spread. In our study, within-meadow allelic diversities varied widely and were roughly proportional to meadow size, whereas genet/clonal diversity was more uniform (Table 2). Our initial prediction for the Norwegian populations was low genotypic diversity (i.e. presence of a few large genets/clones), a characteristic of isolation and reduced sexual reproduction (Duarte et al. 2006) typically present in marginal populations such as those in the northern Baltic (Reusch et al. 1999), the Black Sea (Olsen et al. 2004) and the southern-most distribution in Portugal (Billingham et al. 2003). To the contrary, only 1 of the 15 sampled locations, an intertidal river outlet at Sør-Lenangen Nord with 5 fixed differences, was dominated by large genets/clones.  $R$  values below 0.50 were mostly intertidal (4 out of 6) with the exception of the two Medby sites (Sagfjord) and Skjæret (Balsfjord). However, the remaining 9 locations in both Sagfjord and Balsfjord displayed  $R$  values  $> 0.60$  with Sørkjosen-deep (Balsfjord) displaying maximal genet/clonal diversity (every shoot a different genet) and no fixed differences. In general, the larger, denser meadows had higher allelic diversity and were subtidal, whereas genet/clonal diversity was independent of meadow size or depth. The independence of genet/clonal diversity was unexpected, as subtidal populations were assumed to be protected from annual ice scour; nevertheless, the large Sør-Lenangen Nord genets/clones were in 0 to 30 cm of water. Although we have no direct measures of growth rate, the presence of small to medium sized genets/clones (2 to 4 m) and an expansion rate of 10 cm yr<sup>-1</sup> in the Baltic (Reusch et al. 1994, 1999), suggests an age of 20 to 40 yr in the fjords and thus, a slow turnover rate. The large genets/clones at Sør-Lenangen Nord could therefore be considerably older.

Flowering was widespread from June to August, indicating the potential for new recruitment, which is less common in marginal habitats (Procaccini et al. 2007). However, the presence of fixed allelic differences at 10 of the 15 locations, and significant inbreeding coefficients at 6 locations (Table 2) suggest that individual meadows within a fjord were

more isolated than indicated by gene flow (see next subsection). Although selfing occurs in *Zostera marina*, outcrossing remains the main reproductive strategy (Reusch 2001). Mating among relatives is common in relatively closed (dense) meadows (e.g. *Z. noltii*; Zipperle et al. 2009) and biparental inbreeding may or may not reduce fitness in *Z. marina* (Hämmerli & Reusch 2003). In principle, a Wahlund effect could account for the positive  $F_{IS}$  values at some locations, although this seems less likely given the spatial scale of the sampling. Instead, the correlation between positive  $F_{IS}$  and strong LD (Table 2) at 6 locations is more consistent with non-random mating. With the exception of 3 comparisons between the 2 Elvelund populations, the loci contributing to the LD within each population were not the same loci contributing to LD among populations, which is consistent with population rather than physical linkage effects. This is also in agreement with the lack of significant results observed in Olsen et al. (2004) and the many other studies utilizing these loci.

Considering both allelic and genotypic diversity together, we conclude that present conditions for *Zostera marina* in the northern fjords are stable and that these meadows are not on the edge of local extinction as might be predicted based on distributional-edge assumptions. Strong inbreeding coefficients and LD suggest that selfing and mating with relatives may be unavoidable at some locations.

### Isolation or connectivity

Significant population differentiation existed between fjords, typically 2 to 6 times higher than differentiation found within fjords (Table S1 in the Supplement, Figs. 1 & 2). Consequently, fjords are strongly isolated from one another, whereas meadows within fjords may or may not be isolated. For example, the 2 Medby, 2 Elvelund, 2 Sør-Lenangen and 2 Sørkjosen locations are  $< 1$  km apart and distinct, whereas Laksvatn, Skjæret and Storslett are 6 to 20 km apart and well-connected by gene flow. This uncertainty stems from the fact that the genetic results (gene flow) conflict with demographic inferences drawn from the genotypic diversity part of the analysis and both form part of population structure (Lowe & Allendorf 2010). Simulation studies of dispersal distances of *Zostera marina* suggest that 50% of the floating rhiphidia stay within 500 m, with a highly skewed tail of long distance dispersal over a few km (Källström 2006). Using assignment tests, Reusch (2002) found rafting of seed-bearing shoots 30 to 54 km away from the

source and IBD estimates of 100 to 150 km have been documented along the Wadden Sea coast (Olsen et al. 2004, Ferber et al. 2008).

However, despite the critical role of dispersal (Kendrick et al. 2012), meadows within fjords (e.g. Balsfjord) are likely to be more demographically isolated than gene flow or IBD (Fig. 3) suggest. For example, Kobbevågen (Balsfjord) is highly isolated, situated in an inlet in which local current flow probably induces entrainment. Isolation also is likely at the innermost portion of a fjord such as the Sørkjosen sites (Balsfjord) and Elvelund (Sagfjord). Other meadows in Balsfjord, however, are connected by current flows and not differentiated. Laksvatn, Skjæret and Storslett are less dense meadows, situated along the fjord edges. Whereas these may be subject to more successful recruitment, dense meadows may effectively be isolated islands (see discussion below), even though gene flow is high. Because we currently know little about actual recruitment and turnover rates of eelgrass in these fjords, it remains unclear how demographically connected particular meadows are. However, the presence of significant genetic substructure and inbreeding suggests considerable meadow isolation. Clarification of actual connectivity remains fundamental to establishing ecological coherence models, an area that is in active development and for which genetic data are highly desirable.

#### Substructure within meadows — stochastic processes or habitat selection

Genetic substructure was present within all 3 fjords. At Sørkjosen (Balsfjord), intertidal and subtidal plants (700 m apart and morphologically indistinguishable) were strongly differentiated in all analyses, as were the plants from the Medby sites in Sagfjord (Figs. 1 & 2) and the 2 Sør-Lenangen sites. Sporadic recruitment and limited dispersal are 2 factors contributing to substructure. Both are affected by shoot density of the meadow, which itself can become a recruitment barrier (Duarte et al. 2006, Neiva et al. 2012). Mosaic patterns of *Zostera marina* genotypes, attributed to sporadic recruitment and limited dispersal, have been documented in Brittany (Becheler et al. 2010) and Schleswig-Holstein (Hämmerli & Reusch 2003). Both Medby and Sørkjosen are dense meadows, suggesting that recruitment may be limited. Creation of a large area of distinct substructure requires the opening of large gaps that favor new recruitment, such as destructive (as opposed to normal) waterfowl grazing (Zipperle et al. 2010) or past habitat destruction by an-

chors or fishing traps. Although destructive waterfowl grazing could be an explanation applied to the intertidal Medby sites, it is unlikely at the subtidal (2.5 to 5 m deep at low tide) Sørkjosen-deep site which is too deep. Alternatively, substructure may result from ecotypic differentiation, as fjords and specific habitats within fjords can promote local selection. Although the presence of distinct subgroups within the Sørkjosen and Medby sites suggests that ecotypic differentiation is possible, demonstration of local habitat selection (Stockwell et al. 2003) requires experimental support, which putatively neutral microsatellite loci cannot provide. However, genome scans of common garden and reciprocal transplant experiments have revealed selection between intertidal and subtidal, and depth-associated genotypes of *Z. marina* (Oetjen & Reusch 2007, Oetjen et al. 2010, Winters et al. 2011), illustrating that ecotypic differentiation is common.

The narrow-leaved *Zostera marina* var. *angustifolia* and the wide-leaved *Z. marina* at Kvitberget and Sørkjosen-littoral could not be distinguished by the microsatellite analysis, a conclusion that further supports the results of Becheler et al. (2010) in Brittany and was also reached using chloroplast and nuclear DNA sequences of the 2 morphotypes in Denmark and Orkney (J. A. Coyer unpubl. data). Clearly, ecotypes with respect to leaf width do exist and may be an indication of local selection that cannot be detected with the microsatellite loci used in our study. Furthermore, leaf width is a stable phenotypic characteristic and cannot be attributed to morphological plasticity. Since one of the goals of conservation is to preserve the integrity of gene pools, including infraspecific categories that may have no official taxonomic or legal status (Groom et al. 2006, Allendorf & Luikart 2007), it is important to protect areas sufficiently large to capture as much of the full range of diversity (genetic and/or morphological) as possible. Thus, cryptic infraspecific taxa that are morphologically indistinguishable from one another, as well as infraspecific taxa that are morphologically distinguishable (e.g. *Z. marina* var. *angustifolia*) can be protected within a management area, without assignment of any special legal status.

#### Eelgrass and the larger eco-evo conservation context

The widely held notion that populations in the high latitudes and/or at the extreme northern edge of a species' distribution are genetically depauperate and 'struggling' is unsubstantiated, as our study showed that meadows of *Zostera marina* along northern Nor-

wegian fjords have a higher than expected level of regional allelic diversity, are genotypically/clonally diverse, display some evidence of local ecotypes and were generally healthy in appearance. The importance of high genotypic diversity for community function is well-documented for *Z. marina* and can lead to enhanced growth rates and competitive ability (Hämmerli & Reusch 2003); greater biodiversity of the associated biota (Reusch et al. 2005); greater biomass production following grazing by geese (Hughes & Stachowicz 2004); greater shoot density (reflective of habitat quality) and biomass of epiphytic algae (as a measure of food resource availability) (Hughes & Stachowicz 2009a,b); enhanced 'high disturbance' response, leading to better resilience (Hughes & Stachowicz 2011); and increased restoration success (Reynolds et al. 2012). In addition, all of the aforementioned outcomes of higher genotypic diversity translate to enhanced ecosystem services (Kenworthy et al. 2006, Reynolds et al. 2012), of which habitat nursery function is one of the most important (Heck et al. 2003). High abundances of juvenile cod are associated with eelgrass meadows along the southern coast of Norway (Fjøsne & Gjørseter 1996) and eelgrass in the Balsfjord meadows provide spawning ground for herring and capelin, as well as grazing areas for numerous waterfowl (Strann et al. 2011, www.naturbase.no). Thus, loss of eelgrass is of general concern.

While the Norwegian mapping project is discovering many meadows in places not previously investigated, temporal comparisons in areas that were mapped 100 years ago (and/or after the wasting disease of the 1930s) indicate as much as a 36% reduction in the areal extent of meadows (N. M. Jørgensen & T. Bekkby unpubl. data). Some of the losses are directly attributable to landfill operations, whereas others are thought to be the result of agricultural runoff from farms along the fjords. Threats to eelgrass sustainability can be ameliorated through greater public awareness. This is especially important because more extensive fjord usage and consequent pressure on eelgrass ecosystem services can be expected in the coming decades. It is also increasingly evident that individual meadows must be protected as much as is reasonably possible, as our study suggests that demographic isolation may be present even in the presence of gene flow. This was also found in other studies of *Zostera marina* meadows in close proximity to one another (Muñiz-Salazar et al. 2006, Baja California peninsula; Coyer et al. 2008, California Channel Islands; Wyllie-Echeverria et al. 2010, San Juan Archipelago; Ort et al. 2012, San Francisco Bay). Thus, large and dense meadows such as those at Sørkjosen

and Medby, as well as smaller and sparser inter- and subtidal meadows, are of equal importance, because degraded meadows may not reestablish.

In conclusion, our ultimate ability to effectively evaluate and manage seagrass ecosystems will depend upon a better understanding of how genetic diversity and population structure affect ecological function and landscape coherence in real time. Genetic surveys add an important evolutionary dimension towards the conservation of genetic-level diversity, an explicit goal of the International Convention on Biological Diversity (Laikre et al. 2010).

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