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Patterns and mechanisms of dispersal in a keystone seagrass species



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ABSTRACT

Mechanisms and vectors of long-distance dispersal remain unknown for many coastal benthic species, including plants. Indications for the possibility for long-distance dispersal come from dispersal modelling and from genetic assessments, but have rarely been assessed with both methods. To this end, we assessed dispersal of the seagrass *Zostera noltei*, an important foundation species of the coastal zone. We investigate whether small scale seed dispersal and long-distance propagule dispersal do play a role for meta-population dynamics, using both genetic assessments based on eight microsatellite markers and physical modelling of ocean currents. Such assessments enhance our understanding of the biology and population dynamics of an important coastal foundation species. They are relevant for large scale conservation strategies as they give insights in the maintenance of genetic diversity and connectivity that may enhance resilience and resistance to stresses associated with seagrass loss.

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1. Introduction

Quantifying connectivity in marine populations is a crucial component of the management and conservation of coastal ecosystems (Ruiz-Montoya et al., 2015). Connectivity may confer populations resilience to anthropogenic impact and climate change (Andrello et al., 2015), and changes in patterns of connectivity may affect population persistence, leading to decline and extinction. Habitat loss and fragmentation are among the main causes of population isolation, and further changes in connectivity may represent major threats to all levels of biodiversity (Fahrig, 2003).

Connectivity among populations can be assessed using genetic approaches or modelling movements of dispersal units. In the marine environment, passive dispersal vehicles such as planktonic larvae of benthic organisms or vegetative and sexual propagules of

marine plants disperse with the movement of marine currents in which they occur (Thiel and Gutow, 2005). Genetic assessments are the most straight-forward way to assess realized connectivity, which is the result of dispersal, recruitment success and selection pressures (Sanford and Kelly, 2011). Modelling approaches in contrast can give information on dispersal potential and patterns. Comparing genetic and physical connectivity assessments can be especially useful for discerning the main modes of dispersal as well as relevant time scales (White et al., 2010). With the increasing availability and coverage of operational hydrodynamic models, semi-realistic Lagrangian modelling of physical-biological interactions has become feasible for a wide range of species, the accuracy being limited by uncertainty in the knowledge of the species biology. Recent examples include eco-regionalization in the Mediterranean basin (Serra et al., 2010; Berline et al., 2014), understanding of elusive eel recruitment aspects (Bonhommeau et al., 2009) and physical-biological interaction in spatial fish population models (Christensen et al., 2013). While most oceanographic current assessments are in the range of days to months, genetic

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structure is the consequence of the level of gene flow over many generations (Benzie, 1999). Genetic diversity can therefore give information on long-lasting barriers to dispersal. On the other hand, recent gene flow (first and second generation migrants) can also be detected with genetic assignment tests (Wilson and Rannala, 2003; Paetkau et al., 2004).

Here we assess genetic and physical connectivity of the seagrass *Zostera noltei* (Hornemann, 1832), a temperate seagrass inhabiting mainly intertidal zones of the Northern hemisphere. *Zostera noltei* is monoecious (Ackerman, 2006) and, as all seagrasses, reproduce both sexually and asexually. Sexual reproduction is thought to be common, forming dense seed banks in some meadows (Diekmann et al., 2005; Zipperle et al., 2011), but other meadows are highly clonal with little sexual reproduction (Ruggiero et al., 2005). Dispersal of the filamentous hydrophilous pollen is most likely limited to a spatial scale of metres (McMahon et al., 2014). Flowering begins with the development of a long erect generative shoot from a vegetative shoot (Loques et al., 1988). Each generative shoot may have 2–8 spathes (Loques et al., 1988) and each single spathe groups an equal proportion of male and female flowers (Loques et al., 1988; Zipperle et al., 2009). The overall process of flowering and fruiting lasts somewhere around 47 days (Alexandre et al., 2006 for *Z. noltei* in Ria Formosa Portugal), but may differ depending on the local climate. More than half of this time is required for formation and maturation of the fruits (Alexandre et al., 2006). The elongated seeds are 2–4 mm long (Loques et al., 1988; Orth et al., 2006) and are negatively buoyant, leading to very local dispersal (cm to m) as currents and waves transport the seeds in the bottom boundary layer (Berković et al., 2014; Ruiz-Montoya et al., 2015). Seeds can however remain attached to floating spathes, possibly enabling long distance dispersal and connectivity (Berković et al., 2014). The floating spathes are influenced by oceanic currents as well as local wind forcing and have the potential to travel for thousands of kilometres (Berković et al., 2014; Ruiz-Montoya et al., 2015). Long distance dispersal by floating shoots (both sexual and asexual propagules) has been investigated only recently in *Zostera* spp., claiming that sexual and vegetative floating shoots may have a major impact on meta-population connectivity, being viable even after up to 55 days of detachment (Harwell and Orth, 2002; Källström et al., 2008; Berković et al., 2014; Stafford-Bell et al., 2015). Moreover, dispersal by herbivorous animals has also been shown (Loques et al., 1988; Sumoski and Orth, 2012) and, although occasional, it may represent a significant source of connectivity between distant localities. It has been argued that in terrestrial plants such occasional nonstandard dispersal may be the main factor for long-distance connectivity among populations (Higgins et al., 2003).

Seagrasses are important foundation species providing habitat for many associated species and fulfilling important ecosystem services in the coastal zone. Inhabiting highly impacted coastal areas, the functional extinction of some species (e.g. Jorda et al., 2012 for *Posidonia oceanica*) has been forecasted for the near future. Within the Black Sea, *Z. noltei* has experienced drastic declines over the last 40 years, mainly due to pollution and eutrophication (Milchakova, 1999; Surugiu, 2008). Although cumulative human impact decreased in the Black Sea over the last few years (Halpern et al., 2015), it remains nevertheless a highly impacted sea (Halpern et al., 2008) and fragmentation of population may further increase. Given the observed decline of *Z. noltei* in many parts of the Black Sea and in other regions within its distributional range, it is crucial to understand the level of connectivity among populations. Here we assess populations of *Z. noltei* in the Black Sea at a reciprocal distance ranging from dozens to hundreds of kilometres and combine genetic structure and connectivity analyses with physical connectivity assessments to discern whether local dispersal by

seeds or long-distance dispersal by floating shoots (both sexual and asexual fragments) explains observed population structure. In the former case, the expectation is to find high genetic structure and lack of connectivity between meadows. In the latter case, genetic differentiation should be lower among populations that are predicted to be physically connected and it should be possible to detect recent migrants. If asexual propagules play an important role, we would moreover expect to find identical clones at different sampling locations.

2. Materials and methods

2.1. Study region and sampling

The study was carried out within the coastal area of the North-Western Black Sea spanning 3.5 degrees of latitude, 8 degrees of longitude and four countries. Samples were collected in eleven populations at eight sites (Fig. 1) at distances ranging from ca. 2–680 km. At each location, ca. 50 individual shoots were sampled at a reciprocal distance of 1–1.5 m (see Appendix S1 in Supporting Information for further details).

2.2. DNA extraction, microsatellite amplification and data analysis

DNA extraction and microsatellite amplification was performed as in Jahnke et al. (2015b), using eight polymorphic microsatellites (Coyer et al., 2004a). See Appendix S1 for information regarding data quality control. Multilocus genotype (MLG) identification was done for each population separately as well as combining all populations to investigate clone sharing using the software GenClone (Arnaud-Haond and Belkhir, 2007). Genotypic richness was calculated for each site according to Dorken and Eckert (2001). GenAEx ver. 6.5 (Peakall and Smouse, 2012) was used to calculate the number of alleles per locus, polymorphism and expected and observed heterozygosity. The STANDRICH package (<http://www.ccmr.ualg.pt/maree/software.php?soft=sarich>) was used to calculate standardized allelic richness (A). Arlequin (Excoffier and Lischer, 2010) was used to calculate pairwise F_{ST} among populations, while SMOGD (Crawford, 2010) was used to calculate Jost's D_{EST} (Jost, 2008). Geographic distance between sampling locations was measured using the shortest path over the sea without crossing land using Google Earth (<https://earth.google.co.uk/>). Isolation by distance (IBD) was tested using Pearson's product-moment correlation between geographic and genetic distance in R (R Development Core Team, 2014). STRUCTURE (Pritchard et al., 2000) was used to identify population clusters. Given high and significant F_{ST} values, runs were performed under assumptions of no admixture and independent allele frequencies (Falush et al., 2003). Adegenet (Jombart, 2008) for R (R Development Core Team, 2014) was used to perform discriminant analysis of principal components (DAPC) (Jombart et al., 2010), with the number of principal components set to 7, following alpha-score indication. The program BayesAss (Wilson and Rannala, 2003), a Bayesian clustering algorithm that uses Markov chain Monte Carlo (MCMC) sampling to make inferences about levels of migration and population inbreeding, was used to estimate rates of first and second generation migrants. Migration rates were also calculated based on rare alleles (Slatkin, 1985) in Genepop version 4.2 (Raymond and Rousset, 1995), which only takes into account the frequencies of uncommon alleles to calculate migration rates. For confirmation, we calculated N_m also according to Alcalá et al., 2014 using the package diveRsity (Keenan et al., 2013) in R 3.2.2 (R Development Core Team, 2014). See Appendix S1 for a detailed description of the STRUCTURE and BayesAss analyses.

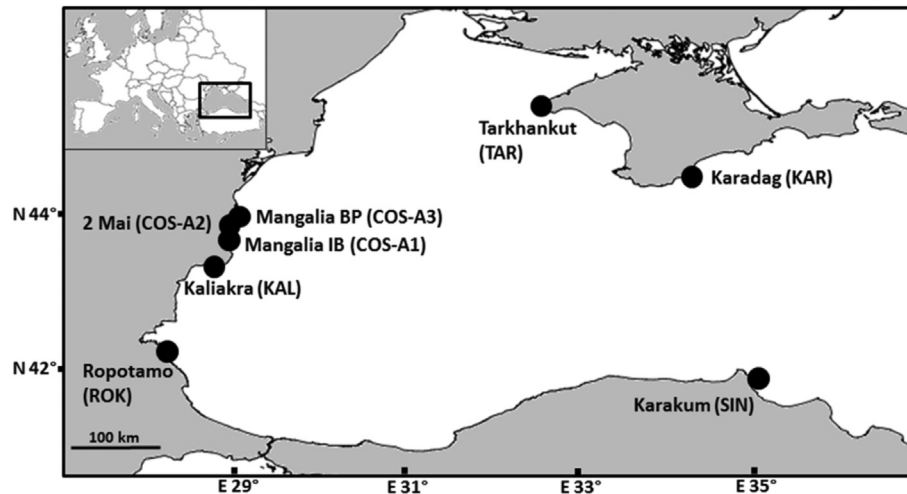


Fig. 1. Map of sampling locations of *Zostera noltei* in the Black Sea. Samples were collected at Bulgarian, Turkish, Crimean and Romanian coastlines.

2.3. Oceanographic modelling of physical connectivity

Potential dispersal of *Z. noltei* via physical mechanisms was explored by Lagrangian modelling of seeds and shoots. The basic input for these simulations was 3D ocean currents from the BIMS model for the contrasting years 1993 (cold), 2001 (warm), 2005 (unremarkable). The BIMS model is a sbPOM setup (Mellor, 2002), calibrated for the Black Sea with 6 km horizontal spatial resolution and 35 vertical sigma levels. Lagrangian particles were passive, with a buoyancy speed chosen in a relevant range for seeds and shoots. To our knowledge, buoyancy of *Z. noltei* seeds/shoots has only been characterized quantitatively (Berković et al., 2014). Lagrangian particles were advected by 3D currents from the BIMS model, and the effect of sub scale eddies was modelled by an overlaid random walk process (Visser, 1997). We conducted two sets of simulations: one for shoots with positive buoyancy focusing on mesoscale transport and one for seeds with negative buoyancy focusing on intra-meadow transport scales. More technical details of the Lagrangian simulations are provided in Appendix S1.

2.3.1. Mesoscale physical modelling

For the mesoscale simulations (shoots), we studied the potential connectivity along the full coast line of the Black Sea. The full coast line was represented by the 489 6×6 km cells of the BIMS model grid facing land. We computed the probability of transport between each of these coastal cells by releasing 5000 particles in each cell for each simulation and determining how many particles settled in each of the 489 cells, thus giving a 489 \times 489 matrix of transport probabilities, which we refer to as the coastal connectivity matrix. Further, to resolve rare long-distance dispersal events connecting the actual sampling locations in this study, we repeated runs as above, but this time releasing 50,000 particles per sampling site. For both assessments, a characteristic positive buoyant speed of 2 mm/s for shoots was assumed. In conjunction with the typical vertical diffusivity ranges in the Black Sea (assessed from BIMS model output), this implies that shoots were typically distributed within the upper 1–5 m of the water. To assess seasonal and interannual variability in coastal connectivity, we released particles on the first day of each month from April to August in 1993, 2001, and 2005, because spring and summer are the time of sexual reproduction (flowering to seed-ripening) in this species. We chose the reproductive period for physical modelling, because although we assess shoot dispersal (including reproductive shoots), we

hypothesise that seeds attached to floating shoots are an important dispersal mechanism. Shoots were allowed to drift for up to 55 days (Berković et al., 2014). The connectivity matrix represents the upper bound on transport probability, since other loss processes have not been included.

2.3.2. Local scale physical modelling

For local scale simulations (seeds), we down-scaled the BIMS hydrographic data set to near-coast meadows, since the BIMS data set does not resolve the near-coast topography. We computed for the hydrographic properties at the cell centre at each sampling site for spring/summer of 1993, 2001, and 2005. The horizontal current profiles were fitted to a linear shear model (zero current at sea bed) and parabolic model for vertical diffusivity (corresponding to a well-mixed layer). These profiles were extrapolated toward the coast linearly, applying the actual recorded sampling depth at the 11 sampling locations. Thus the hydrodynamic environment controlling seed dispersal is boiled down into four parameters: coastal current speed shear, vertical/horizontal diffusivity amplitude and water depth. These crude assumptions add some uncertainty to the simulation, but are the best that can be done in the absence of extensive high-resolution data for each sampling site.

3. Results

3.1. Quality and power of the marker set

MicroDrop (Wang et al., 2012) revealed very low rates of null alleles for all loci, ranging between 0 and 0.009, hence all loci were considered in further analyses. There was no evidence for LD after applying Bonferroni corrections. Eight percent (5 out of 84) of the HW proportion tests per locus and population were significant after Bonferroni corrections. Three of these occurred in the population TAR (Crimea), which had also the highest genetic diversity values (allelic richness and observed heterozygosity). The probability of identity (PI) of the marker set ranged from 0.141 in the uniclinal Romanian meadow (COS-A2) to 8.27 E-08 in TAR (Crimea). Power simulations of the marker set indicate that the 8 microsatellites have a 100% probability of detecting an F_{ST} of 0.01 and the α error (false significance) is below the value of 0.05, when assuming no population structure (see Appendix S2).

3.2. Genotypic and genetic diversity

Genotypic richness varied considerably among populations, ranging from zero (i.e. all sampled individuals belong to the same genotype) to one (i.e. every sampled individual has a unique genotype) (Table 1). Observed heterozygosity also varied considerably from 0.167 in COS-A3 (Romania) to 0.625 in KAL-1 (Bulgaria) (Table 1). Allelic richness was low in all locations in Romania and highest in TAR (Crimea) (Table 1). A significant excess of heterozygosity was detected in two populations with very few individuals (ROK-2 and KAL-1) while heterozygosity deficit, a sign for inbreeding, was detected in five populations (COS-A1, COS-A3, KAL-3, TAR, KAR; Table 1). No MLGs were shared among populations, indicating their distinctness.

3.3. Population genetic structure and patterns of gene flow

AMOVA and STRUCTURE analyses suggested that the combining of close-by Bulgarian sampling sites (KAL-1-3 and ROK-1,2) and the use of seven populations for connectivity assessments was appropriate (see Appendix S3). Genetic differentiation (F_{ST}) among the seven locations was very high, ranging from 0.119 to 0.534 and all pair-wise comparisons were highly significant (see Appendix S4). D_{EST} estimates were similarly high (see Appendix S4). There was no significant IBD when considering the relationship between F_{ST} (p-value = 0.968; $r = 0.009$) or D_{EST} (p-value = 0.076; $r = 0.395$) and geographic distance.

The population genetic clustering analysis of STRUCTURE showed the highest likelihood for six population groups ($K = 6$). Each sampling location represents its own population, with the exception of a Romanian and a Bulgarian site (COS-A1 and KAL), which were grouped together (Fig. 2a). The DAPC analysis confirmed the strong population structure (Fig. 2b–d). The estimated numbers of recent migrants (N_m) were similarly low among all populations analysed (Table 2). The estimation of N_m based on rare alleles gives a more differentiated picture, with some populations having higher connectivity levels (Table 3; Fig. 3a). In particular, the Bulgarian populations ROK and KAL share N_m above

one, as well as TAR with KAR and KAL, and KAR with KAL (Table 3; Fig. 3). N_m estimates according to Alcalá et al., 2014 confirm the same patterns (see Appendix S5).

3.4. Physical connectivity

The physical modelling showed limited connectivity with dispersal ranges mostly below 50 km. A fraction of the large scale 489×489 coastline connectivity matrix is shown in Fig. 4, for shoots released in June 2005 and shared main features with matrices for other release months and years (see Appendix S6). Transport scales along the coast line were very variable (rows, one pixel ~ 6 km). Most noticeable was the off-diagonal cluster connecting the Crimean Peninsula (TAR and KAR) to Bulgaria and Romania (ROK, KAL, COS-A). Long range transport probability was fairly low ($\ll 1\%$), with typical transport distances below 50 km. In our analyses over several months and in several years we found an expected inter- and intra-year variability in calculated physical connectivity (see Appendix S6). As we presumed that it is mainly rare long-distance transport that influenced genetic connectivity, we determined peak connectivity (largest transport probability) between two given sites, when release date and year was varied over the included ranges. Under this scenario, the tip of the Crimean Peninsula is connected with the Bulgarian coast, with genetic sampling sites TAR and KAR being at the boundary of the connected region (Fig. 3b, Table 4). Hydrographic and genetic connectivity were overall consistent (Fig. 3), although in the physical calculations Bulgarian and Romanian sites are connected reflecting their small geographic distance.

Seed transport at intra-meadow scales as a function of seed buoyancy was low (Fig. 5). There is some inter-site variability (Fig. 5a) and dispersal grows exponentially, as seed buoyancy approaches neutral buoyancy ($v = 0$), so that buoyancy is the most sensitive parameter determining local dispersal scale (ranging from 3 to 200 m) (Fig. 5a). Inter-site variability in scales was at factor 3, and ordering between sites changes with seed buoyancy, reflecting the fact that local dispersal scale is determined both by coastal current and eddy properties, which respond differently to buoyant

Table 1

Genetic diversity of *Zostera noltei* in the Black Sea. N, number of extracted samples; Nr, number of samples successfully amplified at all loci; MLG, number of multilocus genotypes; R, genotypic richness (MLG-1/Nr-1); Na, mean number of alleles per locus; A8, allelic richness standardized to 8 genotypes; A28, allelic richness standardized to 28 genotypes (na, not applicable); Ho, observed heterozygosity with standard error (SE); He, expected heterozygosity with standard error (SE); F, fixation index with standard error (SE); %P, percent of polymorphic loci in the population. F values in bold indicate significant deviations from expectations under HWE. All indices have been calculated after the removal of significant duplicate clones.

Coast	Pop	GPS	N	Nr	MLG	R	Na	A8	A28	Ho	He	F	%P
Romanian	2 Mai (COS-A2)	43.779367, 28.58244	48	46	1	0	1.25 -0.164	na	na	0.25 -0.164	0.125 -0.082	na	25%
	Mangalia Inner Bay (COS-A1)	43.81684, 28.59032	48	45	24	0.5	2.5 -0.378	1.8	na	0.25 -0.091	0.264 -0.088	0.082 -0.072	75%
	Mangalia Broken Pier (COS-A3)	43.804298, 28.59175	48	45	9	0.2	1.875 -0.227	1.88	na	0.167 -0.059	0.235 -0.061	0.305 -0.167	75%
Turkish	Karakum (SIN)	42.015556, 35.196944	48	45	26	0.6	3.375 -0.42	2.975	na	0.519 -0.095	0.475 -0.062	-0.074 -0.106	100%
Bulgarian	Ropotamo-Kiten (ROK-1)	42.330817, 27.755883	37	37	25	0.7	4.625 -0.8	3.712	4.94	0.49 -0.109	0.49 -0.109	-0.01 -0.044	88%
	Ropotamo-Gradina (ROK-2)	42.428100, 27.649933	10	10	8	0.8	2.375 -0.375			0.531 -0.127	0.38 -0.086	-0.386 -0.046	75%
	Kaliakra-Balchik (KAL-1)	43.399310, 28.217235	4	3	3	1	2.75 -0.164	3.45	4.96	0.625 -0.098	0.507 -0.04	-0.216 -0.138	100%
	Kaliakra-Byala (KAL-2)	43.404367, 28.235353	30	25	24	0.95	4 -0.423			0.49 -0.104	0.442 -0.071	-0.028 -0.104	100%
	Kaliakra-Kavarna (KAL-3)	43.411717, 28.356953	19	17	17	1	4.75 -0.675			0.419 -0.056	0.547 -0.067	0.197 -0.08	100%
Crimean	Tarkhankut (TAR)	45.520537, 32.709393	47	42	36	0.9	6.125 -0.639	4.638	5.88	0.604 -0.053	0.691 -0.036	0.132 -0.059	100%
	Karadag (KAR)	44.951641, 35.249375	48	44	42	0.95	4.75 -0.861	3.35	4.46	0.429 -0.072	0.485 -0.082	0.085 -0.075	100%

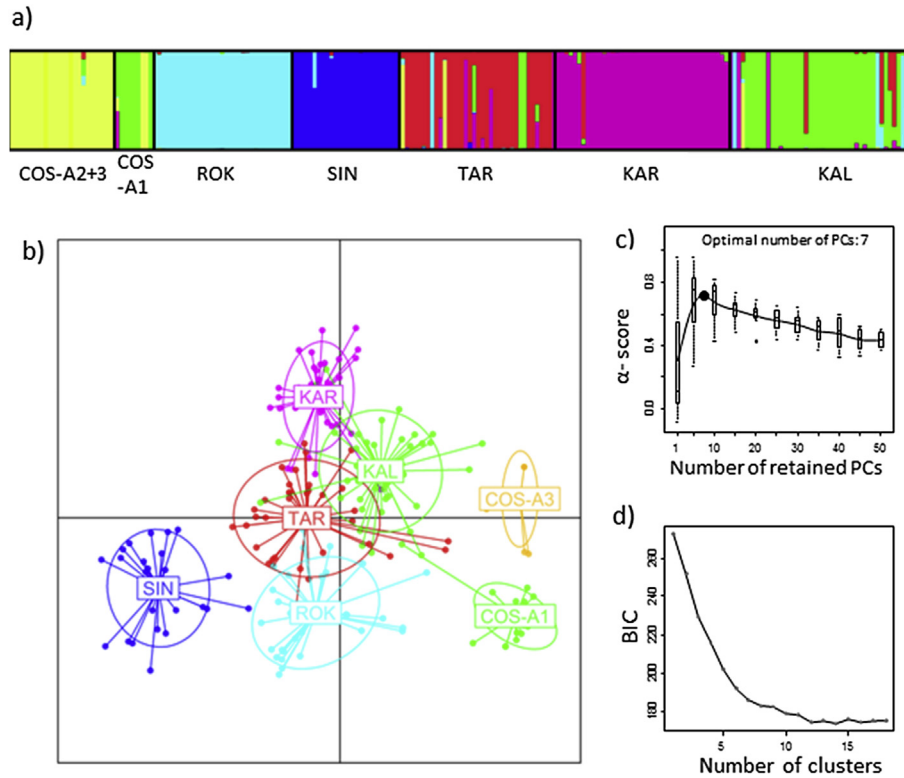


Fig. 2. Results of the population clustering analyses of *Zostera noltei* in the Black Sea. The STRUCTURE analysis (a) was performed assuming no admixture and with independent allele frequencies and shows a strong population differentiation. The DAPC (b) was performed with the original populations with 7 PCs as suggested by the alpha score analysis (c). Colours indicate the different clusters suggested by Structure. The DAPC cluster analysis also suggests a likely presence of 6–8 populations (d). Abbreviations as in Table 1.

Table 2

Number of migrants (N_m) of *Zostera noltei* in the Black Sea calculated from migration rates obtained from BayesAss and multiplied by the number of individuals per population. Three independent runs of BayesAss were performed and the run with the lowest Bayesian deviation is reported. Donor populations are shown in the columns, receiving populations in the rows. Population codes are given in Table 1.

	COS-A1	ROK	SIN	TAR	KAR	KAL
COS-A1	22.668	0.266	0.266	0.266	0.266	0.266
ROK	0.281	31.588	0.281	0.281	0.284	0.284
SIN	0.270	0.270	24.645	0.270	0.273	0.270
TAR	0.284	0.288	0.284	34.571	0.284	0.288
KAR	0.294	0.290	0.290	0.290	40.547	0.290
KAL	0.295	0.290	0.290	0.290	0.295	42.539

Table 3

Numbers of migrants (N_m) of *Zostera noltei* in the Black Sea calculated based on the rare alleles method (Barton and Slatkin, 1986). Population abbreviations are given in Table 1.

	COS-A1	ROK	SIN	TAR	KAR
ROK	0.339				
SIN	0.105	0.299			
TAR	0.340	0.751	0.423		
KAR	0.172	0.284	0.321	1.482	
KAL	0.244	1.119	0.232	1.245	1.051

velocity. Somewhat surprisingly, there was only a weak response of dispersal scale to resuspension, only affecting dispersal near neutral buoyancy (Fig. 5b). Finally, there was only a low inter-annual variability in local dispersal scale (Fig. 5c), when hydrodynamic parameters (current and diffusivity) were derived for each year, rather than averaging over years, as in Fig. 5a,b. In conclusion,

seed dispersal is limited to at most a few hundred meters in the Black Sea hydrographic regime, the exact range depending on undetermined specific physical and biological parameters.

4. Discussion

We used genetic and physical connectivity approaches to assess distance, direction, effectiveness and mechanisms of dispersal in the temperate seagrass *Z. noltei*. Overall the results show low levels of genetic connectivity at a distance ranging from dozens to hundreds of kilometres in the Black Sea and no indication of isolation by distance at the assessed spatial scale. Low levels of connectivity were also indicated by physical modelling, which showed that shoots generally drift 50–100 km, whereas seeds settle locally. Recent migration rates (first and second generation) were not noteworthy among populations. However, migration rates based on the rare allele method detected higher levels of connectivity between couples of closer sites (i.e. two Crimean sites and two Bulgarian sites), an observation also supported by physical modelling. Remarkably, complete agreement between the two approaches was also found in detecting rare migration between sites in Bulgaria and Crimea, which are several hundred km apart.

4.1. Population structure and genetic connectivity

Pairwise F_{ST} values are very high, reaching values of 0.54 for an average reciprocal distance between assessed sites of 360 km. When F_{ST} was standardized for the maximum achievable heterozygosity (Hedrick, 2005; Meirmans and Hedrick, 2011), values were as high as 0.86 (not shown). Similar high F_{ST} values at comparable scales were already reported in *Z. noltei* (Chust et al., 2013) and genetic breaks were found in previous studies at ca. 65–150 km.

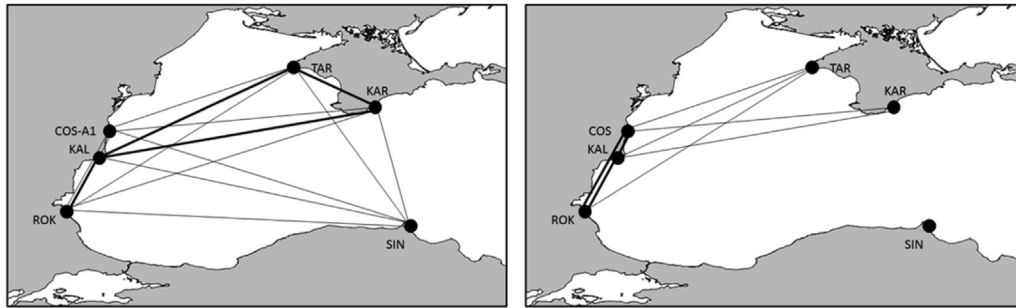


Fig. 3. Genetic and physical connectivity of *Zostera noltei* in the Black Sea. Left: visualisation of the number of migrants calculated based on the rare allele method (Barton and Slatkin, 1986). Bold lines represent Nm estimates of about one. Thin lines represent estimates of 0.1–0.7 Nm. Right: Peak direct physical connectivity strength from Table 4, for the years 1993, 2001 and 2005 and release time April–August calculated from hydrographical data with 500,000 particles per source. Bold lines represent 2% exchange probability, thinner lines down to 0.05% exchange probability. Population abbreviations are given in Table 1.

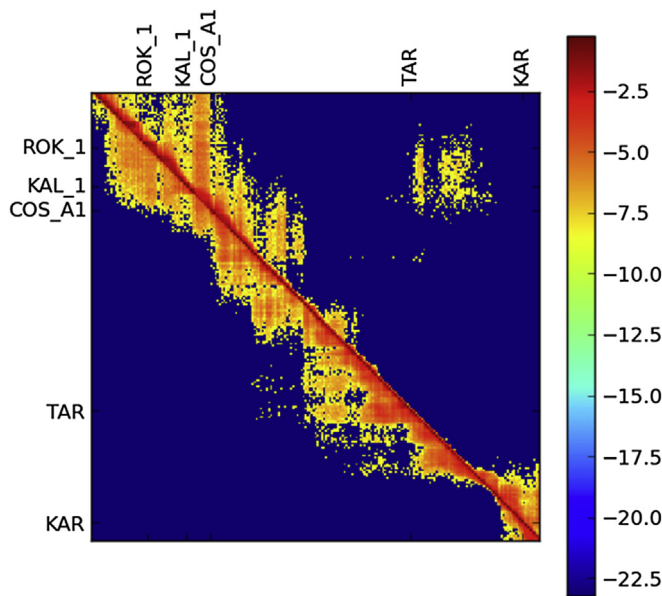


Fig. 4. Western part of the coastline connectivity matrix of *Zostera noltei* in the Black Sea, scanning the coast line from the Bosphorus to the Crimean Peninsula for shoots released June 2005. Starting sites along columns, settling sites along rows, with sampling locations indicated in the figure. The figure is displayed on log scale to emphasize long range transport. The colour is indicative of the strength of the natural log of transport probability.

Table 4

Maximal physical connectivity of *Zostera noltei* in the Black Sea for Lagrangian releases April–August for the years 1992, 2001 and 2005. Shown is the percentage of exchange probability down to 0.5%. “–” indicates no direct physical exchange between sites.

	ROK	KAL	COS	TAR	KAR	SIN
ROK		0.02217	0.01124	0.00030	–	–
KAL			0.01024	0.00047	–	–
COS				0.00103	–	–
TAR					–	–
KAR						–
SIN						

(Coyer et al., 2004b; Chust et al., 2013). Consequently, we did not find evidence for IBD and the different approaches utilized for assessing population structure (i.e. STRUCTURE and DAPC) basically grouped each sampling location into its own cluster. Genetic differentiation data are still scarce in the Black Sea. A study on the

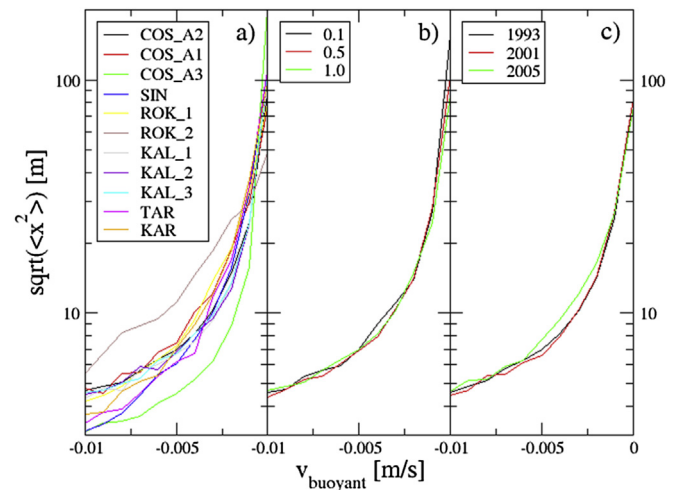


Fig. 5. Local dispersal scale as function of seed buoyancy of *Zostera noltei* in the Black Sea. Neutral buoyant seeds corresponds to $v = 0$ (right axis of figures). (a) shows intra-site variability. (b) shows sensitivity to resuspension for site COS-A2, with the parameter varied in the figure being sticking probability = 1 – resuspension probability, so that 1.0 means seeds stick for sure when they hit the sea bed. (c) shows inter-annual variability in local dispersal scale for site COS-A2. (a + c) corresponds to resuspension probability = 0.0.

scorpionfish *Scorpaena porcus* conducted in the same sites considered in this study, also found no clear spatial patterns but, in contrast to *Z. noltei*, revealed lack of population differentiation (Boissin et al., 2016).

In our analysis, we utilized different methods for estimating genetic connectivity and results were not always in complete agreement. The contrasting results likely reflect the different time-frames on which gene-flow is assessed. The assessment of recent connectivity with BayesAss indicated the almost complete absence of gene flow among the selected populations. In contrast, more historically deep estimates of Nm based on the frequency of rare alleles showed some level of migration among the Bulgarian and Crimean sites, as also nicely visualised in the DAPC (Fig. 2). The historically deepest assessment based on F_{ST} (Structure) doesn't show historical long-distance gene flow, but in contrast to all other methods groups one Bulgarian with one Romanian site (Fig. 2).

Seagrass meadows can be large (hundreds of square kilometres) and very long lived (thousands to millennia of years), and are often predominantly persisting through clonal growth (Kendrick et al., 2012). In the North Sea, an intertidal *Z. noltei* meadow persisting since before 1936 showed stable levels of high genetic and

genotypic diversity, despite a dramatic decrease in shoot density (Zipperle et al., 2009). The authors hypothesized that this stability is mainly due to the existence of a local seed bank i.e. high genet turnover and frequent seedling recruitment from the seed bank. Not much is known about the genetic stability and turn-over of *Z. noltei* meadows in the Black Sea, but the genetic connectivity assessments on different historical scales allow some inference. Generally, local populations seem to be maintained by high genet turnover and frequent seedling recruitment. The lack of recent (first and second generation) gene-flow – combined with generally very strong population isolation – indicates that long-distance dispersal is rare and has not occurred recently. However, long-distance dispersal does occur during the life span of meadows and is detectable with molecular markers, indicating that sporadic events also give an important contribution to meadows connectivity.

4.2. Comparison between genetic and physical connectivity

Results of genetic and physical connectivity assessments are in good agreement in this study, both confirming strong isolation of the assessed populations, but also indicating that rare long-distance dispersal is possible and realized. Findings from rafting kelp in New Zealand similarly showed that dispersal was realized according to oceanographic features, as shown by phylogenetic structure (Bussolini and Waters, 2015). Other studies comparing genetic and physical connectivity did not necessarily find such good agreements. A study on the giant kelp *Macrocystis porifera* in the northeast Pacific found that oceanographic transport alone could not explain genetic patterns, but that isolation by environment also played an important role (Johansson et al., 2015). Equally, a study on the seagrass *P. oceanica* in the Mediterranean Sea showed that breaks in genetic connectivity cannot necessarily be explained by contemporary currents, but are confounded by historically deep vicariance (Serra et al., 2010). In our study, we show in contrast that oceanographic features play an important role in shaping realized connectivity of the passively dispersing rafting *Z. noltei* shoots. Although it is not surprising that passively distributed propagules mainly depend on oceanographic currents, such assessments have rarely been made (but see for instance Bussolini and Waters, 2015 and Thiel and Haye, 2006) and have the potential to contribute substantially to our understanding of marine biogeography.

4.3. Sexual reproduction and dispersal

We observe high genotypic richness at many locations suggesting that sexual reproduction is an important mechanism of reproduction in *Z. noltei* in the Black Sea, but also observe one uniclinal meadow. Clonality seems to be a competitive expansion strategy of many seagrasses in stable environments, but when disturbance is put in the equation, large scale mortality renders this strategy less viable (Steneck et al., 2009), and there is some concern about the future evolutionary potential of uniclinal seagrass meadows (Procaccini et al., 2007; Jahnke et al., 2015a). Genotypic and genetic diversity are very low in all Romanian sites, where homozygosity excess (a sign for inbreeding) was also observed. As Romania has seen considerable recent declines of *Z. noltei* and these populations represent the only remaining meadows of this species along its coastline (Surugiu, 2008), these sites are preferential targets for protection to avoid further fragmentation. Our results of strong population structure indicate that very local dispersal by seeds plays a major role for population structure, as already shown in the Wadden Sea, where Zipperle et al. (2009) showed that recruitment originated mainly within the meadow. Indeed, also our modelling showed a dispersal capacity for seeds of cm to m, confirming previous findings for *Zostera* seed dispersal (Orth et al.,

2006; Berković et al., 2014). Despite generally high genotypic richness, we detected signs of inbreeding at several sites, further indicating high rates of very local dispersal. Ruggiero et al. (2005) also suggested that *Z. noltei* grows clonally through a phalanx strategy that can be associated to higher probability of selfing through geitonogamy, which could explain the high occurrence of inbreeding (Eckert, 2000).

The potential long-distance dispersal and viability of shoots (both vegetative and reproductive fragments) of *Zostera* spp. has been recently assessed (Erftemeijer et al., 2008; Källström et al., 2008; Berković et al., 2014; Hosokawa et al., 2015; Stafford-Bell et al., 2015). Here we show that dispersal and re-establishment of asexual propagules is unlikely in the study area of the Black Sea as we did not find any identical clones at more than one site. However, we do show that dispersal and germination of seeds contained in spathes of floating shoots may occasionally occur and impact gene flow among populations at a distance of up to 600 km. These events probably explain the historical and sporadic long-distance gene-flow between Crimea and Bulgaria.

Our study clearly points to the need of investigations of most basic physical properties of *Z. noltei* seeds or shoots as well as life-history characteristics that influence dispersal patterns such as frequency of sexual reproduction or re-establishment success of shoots. For instance, knowledge on the buoyancy distribution in relation to ambient conditions would clearly increase the potential of using operational hydrographic data-sets for connectivity assessments of *Z. noltei* habitat networks. First models are now being developed that include environmental parameters such as wind, temperature, rainfall and wave energy, to forecast colonization trajectories (i.e. sexual recruitment) of a given meadow (Furman and Peterson, 2015). Such models, together with assessments of dispersal such as performed here, could in the future provide information to managers regarding spatial and temporal scales of seagrass coverage and connectivity.

4.4. Wider implications

In the marine environment, rafting represents an important mechanism of long-distance dispersal and may transport a wide variety of organisms (Thiel and Gutow, 2005). The importance for population genetic dynamics is mainly dependent on temporal and spatial scales over which rafting acts (Thiel and Haye, 2006). Here, dispersal among most populations is absent or below our detection sensitivity. However, physical and genetic methods detected limited connectivity among populations at a distance of up to 600 km, theoretically at the limit of an “ecologically significant scale” (Thiel and Haye, 2006) and above genetic breaks detected in other studies for this species (Coyer et al., 2004b; Chust et al., 2013). To our knowledge this is the first time that comparisons of genetic and physical connectivity have been used to investigate the impact of vegetative propagules, sexual propagules and seed dispersal for seagrass population genetics, although dispersal of sexual propagules have been assessed with both methods for *Posidonia oceanica* (Serra et al., 2010) and *Thalassia testudinum* (van Dijk et al., 2009). The potential dispersal inferred from physical connectivity and the realized dispersal inferred from genetic connectivity estimates on three different temporal scales, are in good agreement. This indicates that the main mechanism of long-distance dispersal is indeed by rafting shoots. Other vehicles of transport, for instance by animals, cannot be excluded, but – given our results – seem less likely. The comparison between physical connectivity over the time-frame of nearly a decade and the assessment of different temporal scales of genetic connectivity also shows that infrequent long-distance dispersal affects the dynamics of populations. The results presented here have wider implications for instance for

understanding recovery after disturbance (Macreadie et al., 2014) and colonization processes (Furman et al., 2015) and for designing transplantation plans (Jahnke et al., 2015b; Valle et al., 2015). Moreover, understanding dispersal potential and realized connectivity in habitat-providing species such as seagrasses, is fundamental for establishing networks of marine protected areas (MPAs) (Andrello et al., 2014, 2015; Lagabriele et al., 2014). The major challenge, in fact, is understanding the appropriate spacing of single MPAs in a given network, in order to allow for sufficient connectivity between local populations.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at <http://dx.doi.org/10.1016/j.marenvres.2016.04.004>.

References

- Ackerman, J., 2006. Sexual Reproduction of Seagrasses: Pollination in the Marine Context. Seagrasses: Biology, ecology and conservation. Springer Netherlands, pp. 89–109.
- Alcala, N., Goudet, J., Vuilleumier, S., 2014. On the transition of genetic differentiation from isolation to panmixia: what we can learn from *Gst* and *D*. *Theor. Popul. Biol.* 93 (0), 75–84.
- Alexandre, A., Cabaço, S., Santos, R., Serrão, E.A., 2006. Timing and success of reproductive stages in the seagrass *Zostera noltii*. *Aquat. Bot.* 85, 219–223.
- Andrello, M., Jacobi, M.N., Manel, S., Thuiller, W., Mouillot, D., 2014. Extending networks of protected areas to optimize connectivity and population growth rate. *Ecography* 38.3, 273–282.
- Andrello, M., Mouillot, D., Somot, S., Thuiller, W., Manel, S., Bode, M., 2015. Additive effects of climate change on connectivity between marine protected areas and larval supply to fished areas. *Divers. Distributions* 21, 139–150.
- Arnaud-Haond, S., Belkhir, K., 2007. genclone: a computer program to analyse genotypic data, test for clonality and describe spatial clonal organization. *Mol. Ecol. Notes* 7, 15–17.
- Barton, N.H., Slatkin, M., 1986. A quasi-equilibrium theory of the distribution of rare alleles in a subdivided population. *Heredity* 56 (3), 409–415.
- Benzie, J.A., 1999. Genetic structure of coral reef organisms: ghosts of dispersal past. *Am. Zoologist* 39, 131–145.
- Berković, B., Cabaco, S., Barrio, J.M., Santos, R., Serrão, E.A., Alberto, F., 2014. Extending the life history of a clonal aquatic plant: dispersal potential of sexual and asexual propagules of *Zostera noltii*. *Aquat. Bot.* 113, 123–129.
- Berline, L., Rammou, A.-M., Doglioli, A., Molcard, A., Petrenko, A., 2014. A connectivity-based eco-regionalization method of the Mediterranean Sea. *PLoS one* 9, e111978.
- Boissin, E., Micu, D., Janczyszyn-Le Goff, M., Neglia, V., Bat, L., Todorova, V., Panayotova, M., Kruschel, C., Macic, V., Milchakova, N., Keskin, C., Anastasopoulou, A., Nasto, I., Zane, L., Planes, S., 2016. Contemporary genetic structure and postglacial demographic history of the black scorpionfish, *Scorpaena porcus*, in the Mediterranean and the Black Seas. *Mol. Ecol.* <http://dx.doi.org/10.1111/mec.13616>.
- Bonhommeau, S., Blanke, B., Tréguier, A.-M., Grima, N., Rivot, E., Vermard, Y., Greiner, E., Le Pape, O., 2009. How fast can the European eel (*Anguilla anguilla*) larvae reach the Atlantic Ocean? *Fish. Oceanogr.* 18, 371–385.
- Bussolini, L.T., Waters, J.M., 2015. Genetic analyses of rafted macroalgae reveal regional oceanographic connectivity patterns. *J. Biogeogr.* <http://dx.doi.org/10.1111/jbi.12491>.
- Christensen, A., Butenschön, M., Gürkan, Z., Allen, I., 2013. Towards an integrated forecasting system for fisheries on habitat-bound stocks. *Ocean Sci.* 9, 261.
- Chust, G., Albaina, A., Aranburu, A., Borja, A., Diekmann, O.E., Estonba, A., Franco, J., Garmendia, J.M., Iriondo, M., Muxika, I., Rendo, F., Rodríguez, J.G., Ruiz-Larriaga, O., Serrão, E.A., Valle, M., 2013. Connectivity, neutral theories and the assessment of species vulnerability to global change in temperate estuaries. *Estuar. Coast. Shelf Sci.* 131, 52–63.
- Coyer, J.A., Reusch, T.B.H., Stam, W.T., Serrão, E.A., Pearson, G., Procaccini, G., Olsen, J.L., 2004a. Characterization of microsatellite loci in the dwarf eelgrass *Zostera noltii* (Zosteraceae) and cross-reactivity with *Z. japonica*. *Mol. Ecol. Notes* 4, 497–499.
- Coyer, J.A., Diekmann, O.E., Serrão, E., Procaccini, G., Milchakova, N., Pearson, G.A., Stam, W.T., Olsen, J.L., 2004b. Population genetics of dwarf eelgrass *Zostera noltii* throughout its biogeographic range. *Mar. Ecol. Prog. Ser.* 281, 51–62.
- Crawford, N.G., 2010. SMOGD: software for the measurement of genetic diversity. *Mol. Ecol. Resour.* 10, 556–557.
- Diekmann, O.E., Coyer, J.A., Ferreira, J., Olsen, J.L., Stam, W.T., Pearson, G.A., Serrão, E.A., 2005. Population genetics of *Zostera noltii* along the west Iberian coast: consequences of small population size, habitat discontinuity and near-shore currents. *Mar. Ecol. Prog. Ser.* 290, 89–96.
- Dorken, M.E., Eckert, C.G., 2001. Severely reduced sexual reproduction in northern populations of a clonal plant, *Decodon verticillatus* (Lythraceae). *J. Ecol.* 89, 339–350.
- Eckert, C.G., 2000. Contributions of autogamy and geitonogamy to self-fertilization in a mass-flowering, clonal plant. *Ecology* 81, 532–542.
- Erfteemeijer, P.L.A., van Beek, J.K.L., Ochieng, C.A., Jager, Z., Los, H.J., 2008. Eelgrass seed dispersal via floating generative shoots in the Dutch Wadden Sea: a model approach. *Mar. Ecol. Prog. Ser.* 358, 115–124.
- Excoffier, L., Lischer, H.E.L., 2010. Arlequin suite ver 3.5: a new series of programs to perform population genetics analyses under Linux and Windows. *Mol. Ecol. Resour.* 10, 564–567.
- Fahrig, L., 2003. Effects of habitat fragmentation on biodiversity. *Annu. Rev. Ecol. Syst.* 34, 487–515.
- Falush, D., Stephens, M., Pritchard, J.K., 2003. Inference of population structure using multilocus genotype data: linked loci and correlated allele frequencies. *Genetics* 164, 1567–1587.
- Furman, B.T., Jackson, L.J., Bricker, E., Peterson, B.J., 2015. Sexual recruitment in *Zostera marina*: a patch to landscape-scale investigation. *Limnol. Oceanogr.* 60, 584–599.
- Furman, B.T., Peterson, B.J., 2015. Sexual recruitment in *Zostera marina*: progress toward a predictive model. *PLoS one* 10, e0138206.
- Halpern, B.S., Frazier, M., Potapenko, J., Casey, K.S., Koenig, K., Longo, C., Lowndes, J.S., Rockwood, R.C., Selig, E.R., Selkoe, K.A., Walbridge, S., 2015. Spatial and temporal changes in cumulative human impacts on the world's ocean. *Nat. Commun.* 6, 7615.
- Halpern, B.S., Walbridge, S., Selkoe, K.A., Kappel, C.V., Micheli, F., D'Agrosa, C., Bruno, J.F., Casey, K.S., Ebert, C., Fox, H.E., Fujita, R., Heinemann, D., Lenihan, H.S., Madin, E.M.P., Perry, M.T., Selig, E.R., Spalding, M., Steneck, R., Watson, R., 2008. A global map of human impact on marine ecosystems. *Science* 319, 948–952.
- Harwell, M.C., Orth, R.J., 2002. Long-distance dispersal potential in a marine macrophyte. *Ecology* 83, 3319–3330.
- Hedrick, P.W., 2005. A standardized genetic differentiation measure. *Evolution* 59, 1633–1638.
- Higgins, S., Nathan, R., Cain, M., 2003. Are long-distance dispersal events in plants usually caused by nonstandard means of dispersal? *Ecology* 84, 1945–1956.
- Hosokawa, S., Nakaoka, M., Miyoshi, E., Kuwae, T., 2015. Seed dispersal in the seagrass *Zostera marina* is mostly within the parent bed in a protected bay. *Mar. Ecol. Prog. Ser.* 523, 41–56.
- Jahnke, M., Olsen, J.L., Procaccini, G., 2015a. A meta-analysis reveals a positive correlation between genetic diversity metrics and environmental status in the long-lived seagrass *Posidonia oceanica*. *Mol. Ecol.* 24, 2336–2348.
- Jahnke, M., Serra, I.A., Bernard, G., Procaccini, G., 2015b. The importance of genetic make-up in seagrass restoration: a case study of the seagrass *Zostera noltei*. *Mar. Ecol. Prog. Ser.* 532, 111–122.
- Johansson, M.L., Alberto, F., Reed, D.C., Raimondi, P.T., Coelho, N.C., Young, M.A., Drake, P.T., Edwards, C.A., Cavanaugh, K., Assis, J., 2015. Seascape drivers of *Macrocystis pyrifera* population genetic structure in the northeast Pacific. *Mol. Ecol.* 24, 4866–4885.
- Jombart, T., 2008. adegenet: a R package for the multivariate analysis of genetic markers. *Bioinformatics* 24, 1403–1405.
- Jombart, T., Devillard, S., Balloux, F., 2010. Discriminant analysis of principal components: a new method for the analysis of genetically structured populations. *BMC Genet.* 11, 94.
- Jorda, G., Marba, N., Duarte, C.M., 2012. Mediterranean seagrass vulnerable to regional climate warming. *Nat. Clim. Change* 2, 821–824.
- Jost, L.O.U., 2008. *GST* and its relatives do not measure differentiation. *Mol. Ecol.* 17, 4015–4026.
- Källström, B., Nyqvist, A., Åberg, P., Bodin, M., André, C., 2008. Seed rafting as a dispersal strategy for eelgrass (*Zostera marina*). *Aquat. Bot.* 88, 148–153.
- Keenan, K., McGinnity, P., Cross, T.F., Crozier, W.W., Prodöhl, P.A., 2013. diveR: an R package for the estimation and exploration of population genetics parameters and their associated errors. *Methods Ecol. Evol.* 4 (8), 782–788.
- Kendrick, G.A., Waycott, M., Carruthers, T.J., Cambridge, M.L., Hovey, R., Krauss, S.L., Lavery, P.S., Les, D.H., Lowe, R.J., i Vidal, O.M., 2012. The central role of dispersal in the maintenance and persistence of seagrass populations. *BioScience* 62, 56–65.
- Lagabriele, E., Crochelet, E., Andrello, M., Schill, S.R., Arnaud-Haond, S., Alloncle, N., Ponge, B., 2014. Connecting MPAs – eight challenges for science and management. *Aquat. Conserv. Mar. Freshw. Ecosyst.* 24, 94–110.
- Loques, F., Caye, G., Meinesz, A., 1988. Flowering and fruiting of *Zostera noltii* in Golfe Juan (French Mediterranean). *Aquat. Bot.* 32, 341–352.
- Macreadie, P.I., York, P.H., Sherman, C.D.H., 2014. Resilience of *Zostera muelleri* seagrass to small-scale disturbances: the relative importance of asexual versus sexual recovery. *Ecol. Evol.* 4, 450–461.

- McMahon, K., van Dijk, K.-j., Ruiz-Montoya, L., Kendrick, G.A., Krauss, S.L., Waycott, M., Verduin, J., Lowe, R., Statton, J., Brown, E., Duarte, C., 2014. The movement ecology of seagrasses. *Proc. R. Soc. B Biol. Sci.* 281.
- Meirmans, P.G., Hedrick, P.W., 2011. Assessing population structure: FST and related measures. *Mol. Ecol. Resour.* 11, 5–18.
- Mellor, G.L., 2002. Users Guide for a Three Dimensional, Primitive Equation. Numerical Ocean Model.
- Milchakova, N.A., 1999. On the status of seagrass communities in the Black Sea. *Aquat. Bot.* 65, 21–31.
- Orth, R., Harwell, M., Inglis, G., 2006. Ecology of Seagrass Seeds and Seagrass Dispersal Processes. *Seagrasses: Biology, Ecology and Conservation*. Springer Netherlands, pp. 111–133.
- Paetkau, D., Slade, R., Burden, M., Estoup, A., 2004. Genetic assignment methods for the direct, real-time estimation of migration rate: a simulation-based exploration of accuracy and power. *Mol. Ecol.* 13, 55–65.
- Peakall, R., Smouse, P.E., 2012. GenAlEx 6.5: genetic analysis in Excel. Population genetic software for teaching and research—an update. *Bioinformatics* 28, 2537–2539.
- Pritchard, J.K., Stephens, M., Donnelly, P., 2000. Inference of population structure using multilocus genotype data. *Genetics* 155, 945–959.
- Procaccini, G., Olsen, J.L., Reusch, T.B.H., 2007. Contribution of genetics and genomics to seagrass biology and conservation. *J. Exp. Mar. Biol. Ecol.* 350, 234–259.
- R Development Core Team, 2014. R: a Language and Environment for Statistical Computing. R Foundation for Statistical Computing. Austria, Vienna. ISBN 3-900051-07-0, URL: <http://www.R-project.org/>.
- Raymond, M., Rousset, F., 1995. GENEPOP (version 1.2): population genetics software for exact tests and ecumenicism. *J. Hered.* 86, 248–249.
- Ruggiero, M., Capone, S., Pirozzi, P., Reusch, T.H., Procaccini, G., 2005. Mating system and clonal architecture: a comparative study in two marine angiosperms. *Evol. Ecol.* 19, 487–499.
- Ruiz-Montoya, L., Lowe, R.J., Kendrick, G.A., 2015. Contemporary connectivity is sustained by wind- and current-driven seed dispersal among seagrass meadows. *Mov. Ecol.* 3 (1), 1–14.
- Sanford, E., Kelly, M.W., 2011. Local adaptation in marine invertebrates. *Annu. Rev. Mar. Sci.* 3, 509–535.
- Serra, I.A., Innocenti, A.M., Di Maida, G., Calvo, S., Migliaccio, M., Zambianchi, E., Pizzigalli, C., Arnaud-Haond, S., Duarte, C.M., Serrao, E.A., Procaccini, G., 2010. Genetic structure in the Mediterranean seagrass *Posidonia oceanica*: disentangling past vicariance events from contemporary patterns of gene flow. *Mol. Ecol.* 19, 557–568.
- Slatkin, M., 1985. Rare alleles as indicators of gene flow. *Evolution* 39, 53–65.
- Stafford-Bell, R.E., Chariton, A.A., Robinson, R.W., 2015. Prolonged buoyancy and viability of *Zostera muelleri* Irmsch ex Asch. vegetative fragments indicate a strong dispersal potential. *J. Exp. Mar. Biol. Ecol.* 464, 52–57.
- Steneck, R.S., Paris, C.B., Arnold, S.N., Ablan-Lagman, M.C., Alcalá, A.C., Butler, M.J., McCook, L.J., Russ, G.R., Sale, P.F., 2009. Thinking and managing outside the box: coalescing connectivity networks to build region-wide resilience in coral reef ecosystems. *Coral Reefs* 28, 367–378.
- Sumoski, S., Orth, R., 2012. Biotic dispersal in eelgrass *Zostera marina*. *Mar. Ecol. Prog. Ser.* 471, 1–10.
- Surugiu, V., 2008. On the occurrence of *Zostera noltei* Hornemann at the Romanian coast of the Black Sea. *Analele științifice ale Universității “Al. I. Cuza” Iași Tomul LIV, fasc. 2, s.II a Biol. vegetal&abreve.*
- Thiel, M., Gutow, L., 2005. The ecology of rafting in the marine environment. I. The floating substrata. *Oceanogr. Mar. Biol. Annu. Rev.* 42, 181–264.
- Thiel, M., Haye, P.A., 2006. The ecology of rafting in the marine environment. Iii. Biogeographical and Evolutionary Consequences. *Oceanogr. Mar. Biol. Annu. Rev.* 44, 323–429.
- Valle, M., Garmendia, J.M., Chust, G., Franco, J., Borja, Á., 2015. Increasing the chance of a successful restoration of *Zostera noltii* meadows. *Aquat. Bot.* 127, 12–19.
- van Dijk, J.K., van Tussenbroek, B.I., Jiménez-Durán, K., Márquez-Guzmán, G.J., Ouborg, J., 2009. High levels of gene flow and low population genetic structure related to high dispersal potential of a tropical marine angiosperm. *Mar. Ecol. Prog. Ser.* 390, 67–77.
- Visser, A.W., 1997. Using random walk models to simulate the vertical distribution of particles in a turbulent water column. *Mar. Ecol. Prog. Ser.* 158, 275–281.
- Wang, C., Schroeder, K.B., Rosenberg, N.A., 2012. A maximum-likelihood method to correct for allelic dropout in microsatellite data with no replicate genotypes. *Genetics* 192, 651–669.
- White, C., Selkoe, K.A., Watson, J., Siegel, D.A., Zacherl, D.C., Toonen, R.J., 2010. Ocean currents help explain population genetic structure. *Proc. R. Soc. B Biol. Sci.* 277, 1685–1694.
- Wilson, G.A., Rannala, B., 2003. Bayesian inference of recent migration rates using multilocus genotypes. *Genetics* 163, 1177–1191.
- Zipperle, A.M., Coyer, J.A., Reise, K., Gitz, E., Stam, W.T., Olsen, J.L., 2009. Clonal architecture in an intertidal bed of the dwarf eelgrass *Zostera noltii* in the Northern Wadden Sea: persistence through extreme physical perturbation and the importance of a seed bank. *Mar. Biol.* 156, 2139–2148.
- Zipperle, A.M., Coyer, J.A., Reise, K., Stam, W.T., Olsen, J.L., 2011. An evaluation of small-scale genetic diversity and the mating system in *Zostera noltii* on an intertidal sandflat in the Wadden Sea. *Ann. Bot.* 107, 127–134.