Potential and realized connectivity of the seagrass *Posidonia oceanica* and their implication for conservation

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Abstract

Aim: Connectivity assessments are crucial to large-scale conservation planning, in particular for establishing and monitoring connected networks of marine protected areas (MPAs). Using biophysical modelling and genetic analyses, we assessed potential and realised connectivity among MPA populations of a benthic foundation species, the Mediterranean endemic seagrass *Posidonia oceanica*.

Location: Adriatic and Ionian seas (central Mediterranean).

Methods: We assessed potential and realized connectivity among eight *P. oceanica* populations, mostly located in MPAs. Potential connectivity was assessed over a time horizon of 10 years via an individual-based biophysical model whose physical component relies on fine scale spatiotemporal ocean circulation fields. Genetic assessments of realized connectivity were carried out by means of a set of 14 neutral microsatellite loci, as well as a larger dataset of 19 loci including outlier loci that did not conform to expectations under neutrality.

Results: Our findings point out a relatively high potential connectivity through long-range dispersal of floating fruits. Genetic connectivity analyses show a complex scenario with an apparent lower realized connectivity. The *P. oceanica* meadow within Torre Guaceto MPA (TOG), a well enforced MPA within our study area, showed one of the highest levels of genotypic richness, indicative of high levels of sexual reproduction and/or recruitment of foreign genotypes. Both biophysical modelling and population genetics indicate that TOG is important to ensure the viability of the species at the local scale, and does likely play a key role as a source of propagules for the whole Adriatic area.

Main conclusions: Our results show that realised dispersal does not necessarily match with the potential for dispersal. Still, both genetic and physical connectivity analyses show good agreement in identifying hotspots of connectivity. Such information can guide management of networks of MPAs and advance conservation of marine biodiversity.

Introduction

Spatial structuring is common in the marine environment, and may often favour local adaptation (Palumbi 2004; Conover *et al.* 2006; Sanford & Kelly 2011). This is an important issue in conservation, as it supports the design of marine protected areas (MPAs) in a way that ensures seascape connectivity, so that connected networks of MPAs can effectively sustain the persistence, recovery, and productivity of marine ecosystems (McCook *et al.* 2009). For instance, to enable recovery of protected coral populations after a disturbance within an MPA, the potential sources of replenishing larvae also need to be protected (Underwood *et al.* 2007). The lack of obvious physical barriers makes the marine environment an especially good case for studying adaptation in the face of gene flow. It provides an opportunity to investigate the interaction between the diversifying effects of selection and the counteracting, homogenizing effects of gene flow (Räsänen & Hendry 2008; Nosil 2009; Cristescu *et al.* 2012). Realized connectivity, or effective gene flow, depends on the interaction between oceanographic features, species-specific life-history traits affecting dispersal, habitat availability and population demography. It can be measured by genetic approaches (Galindo *et al.* 2010; White *et al.* 2010) and complemented by assessment of potential for connectivity via individual-based biophysical models (Gallego *et al.* 2007; Cowen & Sponaugle 2009).

The increasingly recognized importance of connectivity is also reflected in the Aichi target 11 of the Convention for Biological Diversity (CBD), aimed at implementing a 'well connected system of protected areas' by 2020. Despite the increasing awareness of the importance of connectivity for MPA design, few studies have assessed connectivity among MPAs (but see for instance Christie *et al.* 2010; Hogan *et al.* 2012; Planes *et al.* 2009). Moreover, only very few MPA design processes have incorporated connectivity into planning (among them Beger *et al.* 2015; Palumbi 2003; Weeks *et al.* 2014). It is in fact difficult to include information about connectivity in MPA and marine spatial planning algorithms (Beger *et al.* 2010). A major issue is the inherent problem that connectivity assessments are usually carried out for single species (yet not exclusively, see López-Duarte *et al.* 2012; Magris *et al.* 2015; Melià *et al.* 2016 for some multi-species studies). Considering the crucial role that species-specific or population-specific demographic processes play in shaping connectivity and environment-dependent dispersal processes, focusing on a single species appears to be restrictive. However, selecting umbrella species (defined here as species with an especially important role in the investigated ecosystem, e.g. ecosystem engineers) can represent a good compromise between limiting assessment efforts and emphasising the importance of the ecosystem (Hughes & Stachowicz 2009).

Seagrass meadows are considered one of the most highly impacted coastal ecosystems on Earth (Duarte *et al.* 2008). Habitat loss is a major threat to seagrasses, causing increase in fragmentation of populations (Marbà *et al.* 2014), whose dispersal is mainly dependent on floating shoots or seeds that, at least for some species, have a low dispersal capacity (McMahon *et al.* 2014). Seagrasses are also important ecosystem engineers that provide crucial ecosystem services, such as reducing wave impact, stabilising the sediment, adding oxygen to the water, providing nursery grounds and shelter for many species (including commercially important species), exporting important amounts of carbon, nitrogen, and phosphorus to coastal food webs, stocking significant amounts of organic carbon and reducing exposure to bacterial pathogens (Beck *et al.* 2001; Heck *et al.* 2003; Duffy & Stachowicz 2006; Costanza *et al.* 2014; Lamb *et al.* 2017). Ensuring connectivity of such ecologically important habitat formers is thus crucial, given the major decline of seagrasses worldwide (Short *et al.* 2011) with important cascading effects on the associated ecosystems (Healey & Hovel 2004; Warry *et al.* 2009). Establishing networks of suitably-spaced and connected MPAs is possibly the best way to maintain effective connectivity and sustain levels of gene-flow that can avoid inbreeding and allow the spread of advantageous alleles.

In this study, we focus on the Mediterranean endemic seagrass *Posidonia oceanica*, which has experienced severe habitat loss and population fragmentation over the last decades to centuries (Short *et al.* 2011; Marbà *et al.* 2014). Our research integrates connectivity assessments based on numerical simulations of the movement of sexual propagules based on oceanographic fields forced with atmospheric data and genetic analyses: the combination of these two independent approaches provides complementary information about potential and realized connectivity of *P. oceanica* at regional levels. We sampled eight populations of *P. oceanica* mostly located in MPAs encompassing five countries in the Adriatic and Ionian seas. Previous studies in the area focused mainly on mobile species (for instance Schiavina *et al.* 2014 on the Mediterranean shore crab, Boissin *et al.* 2016 on the Black scorpionfish and Carreras *et al.* 2017 on the peacock wrasse), showing either a N–S (crab) or a W–E discontinuity (scorpionfish), or a mixture of both (wrasse). Here we assess a foundation species and aim to determine the extent to which the selected Adriatic and Ionian populations of *P. oceanica* may be connected (based on neutral genetic markers and Lagrangian simulations) – given current environmental conditions and demographic processes affecting the different populations. Specifically, we address the following questions: (1) What is the level of potential connectivity, based on biophysical modelling? (2) What is the level of realized connectivity, based on genetic differentiation and assignment test? (3) How do the potential for connectivity and realized connectivity compare? Finally, we discuss our findings in the context of regional conservation management, giving important insights in the definition of management plans and MPA network design that extend beyond our case study.

Methods

Sampling

We collected individuals of *P. oceanica* at eight sites in the Adriatic and Ionian seas in five different countries during spring 2013 (Fig. 1). Most populations were sampled within MPAs (see Table 1), in sites at distances from each other varying between 65 to 605 km. At each location we sampled *ca.* 50 individuals (spaced 5 to 8 m apart, a standard distance for this species) and according to "random walk" (Arnaud-Haond *et al.* 2007a; Arnaud-Haond *et al.* 2007b). This sampling strategy is a good compromise between avoiding the sampling of clonal replicates and assessing local genetic structure of a meadow by covering an extent of 250–400 m of the meadow.

POTENTIAL (OCEANOGRAPHIC) CONNECTIVITY

Potential connectivity by means of biophysical simulations

We investigated potential connectivity between sites where genetic sampling was carried out using Lagrangian oceanographic simulations. The individual-based biophysical model used here has been developed by Melià *et al.* (2016) and it is fully described there. The physical component of the model relies on fine-scale ocean reanalysis (in both the spatial and temporal sense) produced by the Adriatic Forecasting System, which assimilates satellite-based Earth observations and accounts for atmospheric forcing by the European Centre for Medium Range Weather Forecast (ECMWF) at 1/45° (*ca.* 13 km) and tidal signal (details at http://oceanlab.cmcc.it/afs). The ocean circulation fields are generated with the Adriatic Regional Model AREG (Oddo *et al.* 2006) at a daily temporal resolution, over a regular grid with a horizontal resolution of 1/45° (*ca*. 2.2 km) and 31 vertical sigma layers. The geographical domain encompasses the whole Adriatic Sea and extends southwards into the Ionian Sea down to the 39°N parallel. The bathymetry is based on the U.S. Navy 1/60° bathymetric database DBDB1. Being performed at large scales, such reanalyses cannot account for very local and/or extreme factors (such as tidal currents and erratic, but strong winds). However, we expect that this limitation does not strongly affect our results on connectivity. In fact, though strong winds (Ruiz-Montoya *et al.* 2012) can affect movement of floating fruits (Grech *et al.* 2016), this effect is minor (McMahon *et al.* 2014), and expected to be modest in the Adriatic considering local wind speed (Katalinić *et al.* 2014) and limited tidal currents (Poulain 2013). The biological component of the model (see Melià *et al.* 2016 for a more detailed description) accounts for the key traits affecting *P. oceanica* dispersal by sexual propagules: *P. oceanica* produces positively buoyant fruits, which are released between January and April (Buia & Mazzella 1991; Balestri & Cinelli 2003) and float in the upper layers of the water column for about 28 days before dehiscence and consequent release of the sinking seed (Serra *et al.* 2010). Lagrangian particles – passively guided within their motion according to the oceanographic fields – were released at a density of 2,000 particles per km^2 from areas of suitable habitat around the eight sampling locations, within a radius of 12.5 km. The suitable habitat was derived from the suitability model for *P. oceanica* produced by the MediSeH project (Giannoulaki *et al.* 2013) on the basis of the most up-to-date information on the distribution of seagrass meadows in the Mediterranean basin. The Lagrangian simulations covered the period from 2003 to 2013 and a total of 5×10^6 particles were released. Each particle was assigned a fixed depth between 0 and 1 m below the surface and its trajectory was stepped forward for 28 days using a 4^{th}-order Runge-Kutta integration scheme characterized by a 6-minute time step, a linear convex combination in space and a linear interpolation in time of the current velocity field.

Potential connectivity between sites was measured in terms of intensity and persistence (*sensu* Melià *et al.* 2016). Connectivity intensity was calculated as the average (over the simulation period) number of particles released from a source site and reaching the suitable area of a destination site. Connectivity persistence, expressing the continuity of a connection throughout the years, was calculated as the stabilization coefficient (i.e. the reciprocal of the coefficient of variation) of connectivity intensity. Each site can then be characterized by its retaining strength (defining as *retainer* of Lagrangian particles a place where released propagules successfully remain *in situ*), source strength (defining as *source* a place from where released propagules successfully reach other sites) or sink strength (defining as *sink* a place to where propagules released from other sites tend to successfully settle). Other details on modelling explorations of potential connectivity are described in Melià *et al.* (2016).

REALIZED (GENETIC) CONNECTIVITY

DNA extraction and microsatellite amplification

We extracted DNA from *ca.* 20 mg of silica-gel dried tissue in 96-well plates using the NucleoSpin® 96 Plant II kit (Macherey-Nagel) following a modified protocol optimized for a Biomek FX robotic station (Tomasello *et al.* 2009). We amplified twenty-two microsatellites (Procaccini & Waycott 1998; Alberto *et al.* 2003; Arranz *et al.* 2013) and ran PCRs as in Jahnke *et al.* (2015). See Table S1 and S2 in Supporting Information for details on primer sequences and PCR concentrations. Three loci were subsequently removed for most analyses, resulting in a dataset of 19 loci. We only used samples that were successfully genotyped at all loci for further analyses.

Scoring and data quality checks

We scored the fragments by hand or using GeneMapper® (Life technologies) and re-checked scoring by eye for each individual. We used Microchecker (van Oosterhout *et al.* 2004) to detect potential scoring errors and we re-visited, and adjusted if necessary, loci with possible stuttering problems. We identified clones using GenClone (Arnaud-Haond & Belkhir 2007) and removed duplicate multilocus genotypes (*MLG*s) before further analyses. Specifically, only one *MLG* for each clone was retained if the probability that the repeated genotypes do not originate from distinct sexual reproductive events, considering possible departures from Hardy–Weinberg equilibrium (HWE), was smaller than 0.05. After removal of (significant) clones, we used MicroDrop (Wang & Rosenberg 2012) to detect null alleles. We tested for Linkage Disequilibrium (LD) and HWE at each locus and across all loci in each population with Genepop 4.2 (Raymond & Rousset 1995), using 100 batches and 1,000 iterations per batch and applying Bonferroni corrections. Finally, we calculated the probability of identity (PI) in GenAlEx 6.5 (Peakall & Smouse 2012) to get an indication of the power of the marker set at each location, and we used POWSIM 4.1 (Ryman & Palm 2006) to evaluate whether the sets of microsatellites have enough power to detect population structure among locations. We used the actual allele frequencies based on unique *MLG*s to simulate drift to F_{ST} levels of 0, 0.001, 0.01 and 0.1 using an effective population size (N_e) of 500 and a varying number of generations *t* (0–100) with 200 replicates and 100,000 batches.

Outlier tests

We used Lositan (Antao *et al.* 2008) and BayeScan (Foll & Gaggiotti 2008) to test whether any of the used microsatellite markers do not behave according to expectations under neutrality. In Lositan, we ran the simulations for 50,000 iterations, with a 95% confidence interval, using the options for neutral mean F_{ST}, force mean F_{ST}, a subsample size of 40, the infinite allele model and 8 populations based on the sampling sites. In BayeScan, we used default settings, which results in the same probability threshold as used for Lositan. We used the R script provided by Foll & Gaggiotti (2008) in R 3.2.2 (R Development Core Team 2014) to analyse if any loci deviate significantly from expectation under neutrality and for plotting the posterior distribution. The two methods differ in the approach to identify outliers. While Lositan identifies outliers with higher than neutral heterozygosity conditioned on F_{ST} (Antao *et al.* 2008), BayesScan uses posterior distributions generated by MCMC to identify whether a model including selection is more likely than a model without selection for each locus (Foll & Gaggiotti 2008). We only considered as outliers those detected by both methods.

Genotypic and genetic diversity and structure

We performed *MLG* identification for each population separately and repeated the analysis combining all populations to investigate clone sharing among populations in GenClone (Arnaud-Haond & Belkhir 2007). Based on *MLG* identification, we calculated genotypic richness for each population according to Dorken & Eckert (2001). After removal of clone mates, we used GenAlEx 6.5 (Peakall & Smouse 2012) to calculate the number of alleles per locus, polymorphism and heterozygosity. We calculated allelic richness standardized to the minimum number of genotypes present in the dataset (27 *MLG*s at OTR) using the STANDARICH package in R 3.2.2 (http://www.ccmar.ualg.pt/maree/software.php?soft=sarich). We used STRUCTURE

(Pritchard *et al.* 2000) for *K* 2 to 8, to identify potential population structure based on neutral loci, all loci and loci putatively under selection. We assumed population admixture and correlated allele frequencies, but also performed runs with no admixture and independent allele frequencies. We used a burn-in of 100,000 and subsequent 1,000,000 steps, checking for run convergence. We identified the most likely number of populations based on *delta K* with STRUCTURE HARVESTER (Earl & von Holdt 2012) and used Clumpak (Kopelman *et al.* 2015) to generate graphs. We also used Adegenet (Jombart 2008) in R 3.3.2 to perform a Discriminant Analysis of Principal Components (DAPC) (Jombart *et al.* 2010) with the number of principal components set to 15, following alpha-score indication. In order to validate these two approaches, we also performed an AMOVA with 999 permutations in GenAlEx 6.5 (Peakall & Smouse 2012).

Genetic connectivity

We performed assignment tests in GeneClass2 (Piry *et al.* 2004) using the exclusion method, because this method does not require an exhaustive sampling with every possible population of origin included in the data set (Berry *et al.* 2004; Underwood *et al.* 2007). This analysis was based on the dataset of neutral loci. We calculated the probability that an individual belongs to the population from which it was sampled with a partially Bayesian criterion (Rannala & Mountain 1997) and compared the likelihood of exclusion of an individual to a distribution of likelihoods of 1,000,000 simulated genotypes in order to define a statistical threshold (Paetkau *et al.* 2004; Underwood *et al.* 2007) with a type I error of 0.05. We excluded an individual from its sampling site when the probability for exclusion was higher than 95% and we assigned the individual to another sampled population when the probability for inclusion in it was higher than 10% (Underwood *et al.* 2007). Otherwise, we assumed that the individual under study did not originate from the population where it was sampled, but originated most likely from an un-sampled source population.

Realized connectivity: Isolation By (geographical) Distance

We measured geographical distances between sampling locations using the shortest path over the sea without crossing land using Google Earth and used Arlequin 3.5 (Excoffier & Lischer 2010) to calculate pairwise Weir & Cockerham F_{ST} among populations and significance levels. We also calculated the unbiased estimator of Jost's D, D_{EST} (Jost 2008) using the diveRsity package (Keenan *et al.* 2013) in R 3.2.2 with 1,000 bootstrap replicates to test for the significance of pairwise comparisons. The two methods are to a certain degree complementary for assessing population differentiation: F_{ST} measures deviation from panmixia and is calculated based on allele frequencies; *D* measures deviation from complete differentiation and is based on the effective number of alleles (Whitlock, 2011; Meirmans & Hedrick, 2011). We tested for Isolation by Distance (IBD) for the two genetic distances separately using three datasets that contained all 19 diploid loci, only neutral loci and only outliers. We also calculated Slatkin's R_{ST} in SPAGeDI 1.4 (Hardy & Vekemans 2002) and used 10,000 permutations to test whether R_{ST} is significantly higher than the permuted value pR_{ST}, which would indicate that the mutation rate exceeds the migration rate (Hardy *et al.* 2003).

Results

POTENTIAL (OCEANOGRAPHIC) CONNECTIVITY

The Apulian region was identified as the area with the highest potential connectivity (as obtained via Lagrangian simulations), in terms of both intensity (Fig. 2, top panels) and persistence (Fig. 2, bottom panels). The three Apulian sites OTR, TOG and POC (population acronyms as in caption of Fig. 1) are the strongest retainers and sinks. However, while OTR and TOG are also the strongest sources, particles originating from POC do not reach any of the study sites. These three locations are connected by the current flowing southwards along the Adriatic coasts of Apulia and then turning around Salento towards the Gulf of Taranto. Particles released from TOG and OTR can potentially (yet through less intense and persistent connections) cross the Adriatic Sea and reach BOK and (only for TOG) KAP. There are also directional connections, driven by the southern Adriatic gyre, between the eastern and the western side of the Adriatic, with particles flowing from BOK to OTR and, through a less intense and persistent connection, from KOR to TRE. OTH acts in our modelling experiments only as a source of particles, and no particles reach this location from any other. TRE is a quite strong and constant source of particles for TOG and, to a lesser extent, OTR and KOR. KOR is a strong retainer and supplies particles to TRE. KAP is a good source, subsidizing Apulian sites (TOG, OTR, POC) via the southern Adriatic gyre. Particles released from OTH, instead, are not able to enter into the Adriatic Sea, but reach the two southernmost Italian sites (OTR and POC).

REALIZED (GENETIC) CONNECTIVITY

MLG identification, null alleles and outliers

We identified a high number of *MLG*s at each location, ranging from 27 to 42 MLG per population (Table 1), resulting in 278 genets (out of 374 ramets) that were used for all further analyses. Three loci showed frequencies of null alleles above 10% in MicroDrop (Wang & Rosenberg 2012). One of them, Poc-trn (NaF = 30.8%), is chloroplastic, i.e. haploid, and therefore expected to be always homozygous. The other two loci (Poc-5, NaF =19.6% and Pooc-330, NaF =11.1%) were removed before further analyses, while Poc-trn was retained for few descriptive statistics only (Table 1), resulting in a marker set of 19 loci. Both Lositan and BayesScan identified the same five loci to be under balancing selection (Figs. S1 and S2). As the non-conformity to neutrality of these loci can affect patterns of connectivity and migration, we used three different data sets in the following analyses: a) all diploid loci (19 markers), b) only neutral loci (14 markers) and c) only outlier loci (5 markers under balancing selection).

Linkage Disequilibrium (LD), Hardy Weinberg Equilibrium (HWE) and power of the marker set

We found significant LD in 11 out of 120 tests across all populations (9%) after applying Bonferroni corrections. In particular, we detected three markers to be in gametic linkage more than two times: Pooc-PCo45G11 (five times), Pooc-229 (four times) and Pooc-361 (three times). PCo45G11 is the locus with the highest number of alleles in the data set, while the number of alleles per locus is low for the other loci (ranging from one to seven).

Seven HWE tests per population and locus were significant after Bonferroni corrections (12%). No locus deviated from HWE at more than two locations. As the HWE deviations were found to be specific to locations rather than loci and as we did not find indications of quality control problems, we retained all loci.

The probability of identity (PI) was low, ranging from 4.6×10^{-5} in OTR to 6.7×10^{-9} in TOG. The PI for sibs was higher, ranging from 5.6×10^{-3} in OTR to 1.5×10^{-4} in TOG, which are still PI values sufficient for discerning siblings, considering the number of *MLG*s. Power simulations of the full marker set and the neutral marker set suggest that both sets of loci can provide a reasonably accurate picture of genetic structure, with population homogeneity rejected in 100% of the simulations when F_{ST} was as small as 0.01 (Table S3).

Genotypic and genetic diversity and structure

Genotypic richness varied among populations, while heterozygosity was similar (Table 1). Allelic richness was generally low, ranging from 1.8 at OTR to 2.68 at OTH (Table 1). All populations had a significant excess of heterozygosity as evident by high negative F_{IS} values (Table 1). This phenomenon has been observed previously in *P. oceanica* (Arnaud-Haond *et al.* 2007b; Serra *et al.* 2010) and is most likely linked to its life history of partial clonality (Reichel *et al.* 2016). No *MLG*s were shared among locations. Pairwise F_{ST} ranged from 0.05-0.23 for the dataset of neutral loci and all pairwise comparisons were significant (see Table S4-S10 for values for different *F*-statistics and loci sets).

The STRUCTURE analysis performed on the dataset of only neutral loci showed the presence of two populations clusters (K = 2, Fig. S3) as the most likely possibility. The two population clusters consist of the Northern populations of KOR, TRE, BOK and the more Southern OTR (Fig. 3a, mostly blue populations) and the Southern populations of KAP, POC, TOG and OTH (Fig. 3a, mostly red populations). However, several locations (OTH and KAP in particular) show a high degree of admixture between the two clusters and when assuming higher *K*s further sub-structuring becomes evident (Fig. S4) The STRUCTURE analysis for higher *K*s (particularly K = 3 to 6) also shows an interesting pattern of migrants in each populations, but few admixed individuals. DAPC confirms the separation of the two clusters for all populations but KOR (Fig. 3b, c); however, KOR is located close to KAP and OTH, which show a nearly 50-50 percentage of belonging to the Northern and Southern clusters. The DAPC also shows clearly that OTR is the most differentiated population and that all populations are differentiated from each other, which is also confirmed by the AMOVA analysis, where the “among populations” level explains most variance (Table S11). The STRUCTURE and DAPC analyses based on the 5 loci presumably under balancing selection show no detectable population structure, while the picture based on all 19 diploid loci is very similar to the analyses based on the 14 neutral loci (not shown). The identification of outlier loci is associated with high type I errors, i.e. a high rate of false positive results, especially for loci that are under balancing selection (Narum & Hess 2011). The observation that results based only on neutral or on all loci are very similar suggests that loci supposedly under balancing selection may have been falsely identified.

Realized (genetic) connectivity and IBD

For realized connectivity assessments, we only considered the neutral loci dataset, as dispersal should make the biggest contribution to the observed allele frequencies of neutral loci in the different populations (as opposed to selection in the other two datasets). The assignment tests (GeneClass) show a strong population structure with only 4% of samples assigned to populations different from those of the sampling location (Table 2). TOG is identified as the most important source population, providing one individual each to TRE, KAP and BOK (Table 2). This population has the highest possible level of genotypic richness, i.e. high levels of sexual recruitment. Conversely, OTR has the highest level of clonality and all sampled individuals get assigned to their own population (Table 1; Table 2). The IBD analysis did not reveal a positive correlation between neither F_{ST} nor D_{EST} (Tables S4-S9) and geographical distance for any of the three data sets (not shown). R_{ST} values were similar to F_{ST} values (see Supplementary Tables S5–S11) and the permuted R_{ST} did not differ significantly from the observed value (two-sided p-value = 0.69, R_{ST} = 0.17 > pR_{ST} = 0.16), i.e. there was no indication that mutations made a high contribution to population differentiation and/or mutations do not follow a step-wise pattern.

Discussion

The biophysical connectivity assessments show a high potential for dispersal of *P. oceanica* fruits across the whole study area. The presented results on potential connectivity are robust and would neither qualitatively nor quantitatively be altered by incorporating into our biophysical model minor effects, such as movements of floating fruits caused by erratic strong winds. Realized connectivity, which can serve as an important indication for conservation policies and management, shows more complex patterns, but is apparently lower. There is high genetic structuring of the eight assessed *P. oceanica* populations in the Adriatic and Ionian seas, with significant pairwise population differentiation among all locations (see Tables S5–S11), and assignment tests show only a low level of recent migrants. First-generation migrants were also evident in the STRUCTURE analysis of higher *K*s and the low number of admixed individuals in this analysis points to low sexual reproduction and/or non-random mating of immigrants in the assessed populations. Geographical distance was not a good predictor for genetic differentiation, but we identified two main population clusters that are in reasonable agreement with a latitudinal gradient, i.e. a Northern and a Southern cluster (with the exception of the southern population of Otranto that groups with the Northern cluster and is generally the most differentiated site). In the assignment tests, the meadow at the Torre Guaceto MPA (TOG) was identified as the most important source population. Interestingly, this result is corroborated by the biophysical analysis, where TOG also turns out to be the most important source population. The population of TOG is within an MPA with an enforced no-take area and an enforced no-anchoring ban above the assessed *P. oceanica* meadow, and is presumably one of the most efficiently protected meadows of the evaluated sites (Guidetti *et al.* 2008). Our results confirm the key role played by TOG as a source of propagules, a role that was already established with physical modelling for different organisms in the whole Adriatic basin (Pujolar *et al.* 2013; Melià *et al.* 2016). The location of this protected area was not only well chosen for achieving positive population dynamics at the local scale (Fraschetti *et al.* 2013), but TOG is also very well connected to other *P. oceanica* populations in the Adriatic. We thus suggest that conservation measures for this MPA should be confirmed and possibly re-enforced.

The existence of two genetic clusters was suggested by both the set of neutral microsatellite loci and the complete set of loci, including also the five outliers, but was not necessarily confirmed by the oceanographic modelling, as most populations are predicted to supply and receive propagules to and from both Northern and Southern populations. For instance, in the physical modelling the Southern population of Otranto (OTR), which groups with the Northern genetic cluster, has the highest probability of dispersal to two populations of the Southern cluster and one (BOK) of its own cluster and is expected to receive propagules only from populations of the Southern cluster. However, this population has the highest levels of clonality, the lowest levels of standardized allelic richness, and fixed allele frequencies with no private alleles. OTR is clearly the most differentiated of all populations in the DAPC, suggesting that post-dispersal (i.e. pre- or post-settlement) processes played a role in the observed differentiation.

The levels of realized connectivity, as assessed by genetics show a complex pattern with detectable levels of migration, but “mosaic” populations with few admixed individuals. This picture confirms the stochasticity of dispersal at small/medium spatial scales observed in other seagrasses (Kendrick *et al.* 2012). Possible reasons could be un-sampled populations that confound the picture, pre- and post-settlement process and non-random mating, including low levels of sexual reproduction in general. This is expected to be very pronounced for *P. oceanica*, as the partial clonality and longevity of clones translates into generation times that may be as long as thousands of years (Ruggiero *et al.* 2002; Arnaud-

Haond *et al.* 2012). Regional and basin scale population structuring, supported despite detectable recent migration and no IBD on most assessed spatial scales, was already described for *P. oceanica* over its entire distribution (Arnaud-Haond *et al.* 2007b; Rozenfeld *et al.* 2008; Serra *et al.* 2010). As an alternative to stochastic events of long-distance dispersal, this pattern of *P. oceanica* population differentiation has also been proposed to stem from a stronger influence of mutation over migration at the scale of the distribution range (Arnaud-Haond *et al.* 2014). Under this assumption, population differentiation may be explained by historic step-by-step colonization followed by local recruitment and clonal growth, rather than contemporary gene flow. Here we used a permutation test of Slatkin's R_{ST} (as suggested by Hardy *et al.* 2003) and did not find any indication that mutations played a major role for genetic differentiation; and we also show that oceanographic (potential) connectivity is high among the assessed populations. Potential connectivity may well be higher than realized connectivity, because of low sexual reproduction (estimates of oceanographic connectivity are based on dispersal of fruits), low settlement success after dispersal, or small scale hydrodynamics that could not be included into the oceanographic connectivity analysis. Indeed, the modelling analysis showed that two central populations, OTR and TOG, had the highest potential for acting as sources. TOG, which has high genotypic richness (indicating a high level of sexual reproduction), seems to realise this potential and supply sexual propagules to other populations. In addition, the biophysical modelling suggests that TOG can supply propagules to BOK, as also confirmed in the genetic assignment test. In contrast, OTR has a slightly lower potential for dispersal, but is genetically distinct and has a much lower genotypic richness, suggesting that this population supplies fewer sexual propagules to other meadows. This points out that the occurrence of sexual reproduction is an important parameter that may significantly influence the link between potential and realized connectivity in *P. oceanica*.

The biophysical modelling also indicates TOG and OTR as strong retainers, a result that corroborates the outcomes of previous analyses suggesting a strong retention potential for this area (e.g. Di Franco *et al.*, 2012, Schiavina *et al.*, 2014): indeed, both populations have one of the highest percentages of individuals assigned to their own population in the genetic assignment test.

Conclusions

Connectivity assessments are increasing rapidly in the field of conservation science (Jones *et al.* 2009) and information on connectivity is important to MPA network design. They can deliver information on actual dispersal rates, and identify populations that export propagules to other areas (source populations) or populations that rely on immigration for their sustenance (sink populations), as well as populations that retain their propagules locally. Moreover, linking connectivity assessments with information on the levels of genetic diversity could also be used to identify areas of high evolutionary potential (Vandergast *et al.* 2008). Connectivity assessments are however only one component of the MPA design process and for instance size, number, representation, replication, diversity and above all capacity are other important factors (Fernandes *et al.* 2009; Gill *et al.* 2017). In this study on connectivity we found that the potential for dispersal was considerably higher than realised migration, but both approaches coherently identified the same optimal site, which is at the same time a strong retainer, a good source and a good sink. For species which disperse mainly by sexual propagules, yet can alternate between sexual and asexual reproduction, the amount of sexual reproduction may be a very important component to take into account when assessing connectivity. So far, the majority of connectivity assessments involving MPAs have been performed on mobile species, exclusively sexual in their reproduction (in the Adriatic, see for instance Boissin *et al.* 2016; Pujolar *et al.*

2013; Paterno *et al.* 2017). Our results on potential and realised connectivity indicate that dispersal occurs at large spatial scales (100s of km) for a sessile benthic partially clonal species and suggest that potential connectivity can be insufficient *per se* to describe population structure. Rather, post-dispersal, pre-settlement and post-settlement processes have to be taken into consideration to understand discrepancies between potential and realized connectivity. Together our findings on potential and realized connectivity, genetic structure and sexual reproduction have direct conservation application and can be used for the establishment and management of MPAs and other large scale conservation strategies.

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Biosketch

The study was performed as part of the FP7 project CoCoNet (http://www.coconet-fp7.eu/), aimed to produce guidelines for designing, managing and monitoring networks of MPAs, and an enriched wind atlas for both the Mediterranean and the Black Seas.

Author contributions: M.J. and G.P. conceived and designed the research. M.J. performed the genetic analysis and led the writing. R.C., P.M. and M.S. performed the biophysical analysis. All authors contributed to manuscript writing. All authors revised the article critically and approved the final version to be published.

Supporting information

Table S1 List of microsatellites used in this analysis.

Table S2 Master-mix used for amplification of microsatellites.

Table S3 POWSIM power simulation for all 20 and the 14 neutral microsatellite loci of the 8 *Posidonia oceanica* populations assessed.

Table S4 Weir & Cockerham pairwise F_{ST} of *Posidonia oceanica* calculated based on all 19 diploid loci.

Table S5 Weir & Cockerham pairwise F_{ST} of *Posidonia oceanica* calculated based on the 14 neutral loci.

Table S6 Weir & Cockerham pairwise F_{ST} of *Posidonia oceanica* calculated based on the 5 loci under balancing selection.

Table S7 D_{EST} of *Posidonia oceanica* calculated based on all 19 diploid loci.

Table S8 D_{EST} of *Posidonia oceanica* calculated based on the 14 neutral loci.

Table S9 D_{EST} of *Posidonia oceanica* calculated based on the 5 loci under balancing selection.

Table S10 Slatkin's R_{ST} values of *Posidonia oceanica* calculated based on the 14 neutral loci.

Table S11 Analysis of molecular variance (AMOVA) of the neutral loci set, showing the distribution of molecular variance among clusters (as defined by Structure), among populations, among individuals and within individuals.

Figure S1 Lositan analysis of the *Posidonia oceanica* in the Adriatic and Ionian Sea.

Figure S2 BayeScan analysis for the assessed *Posidonia oceanica* populations in the Adriatic and Ionian Sea.

Figure S3 Delta K analysis of the Structure clustering analysis for the assessed *Posidonia oceanica* populations in the Adriatic and Ionian Sea.

Figure S4 STRUCTURE plots for *K* between 3 and 8, showing further population structuring.

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Tables and Figures

Table 1. Genetic diversity of *Posidonia oceanica* at the eight locations in the Adriatic and Ionian seas. The 374 individuals from eight populations and five countries were assessed with 20 microsatellites. After the country and the population names (including acronyms), are the geographical coordinates (latitude and longitude)), the number of samples extracted (*N*) and the number of samples successfully amplified at all loci (N_r); the number of multilocus genotypes (*MLG*); genotypic richness (*R*); the mean number of alleles per locus (N_a); allelic richness standardized to 27 genotypes (A_{27}), observed heterozygosity (H_O); expected heterozygosity (H_E); the fixation index (*F*) and the percentage of polymorphic loci in the population (%*P*). Figures in bold indicate significant *F* values. The parameters marked with * were calculated after the removal of the chloroplastic locus Poc-trn.

Country	Population	Latitude	Longitude	Location info	*N*	N_r	*MLG*	*R*	N_a	A_{27}	H_O*	H_E*	*F**	%*P*
Italy	**Otranto (OTR)**	40.109233	18.519217	Potential area for future MPA	48	45	27	0.59	1.80 (0.16)	1.8 (0)	0.45 (0.10)	0.26 (0.05)	**–0.58** (0.11)	65%
	Porto Cesareo (POC)	40.195250	17.917950	MPA established in 1997	48	43	41	0.95	2.60 (0.32)	2.39 (0.11)	0.53 (0.10)	0.34 (0.06)	**–0.44** (0.09)	80%
	Torre Guaceto (TOG)	40.716650	17.800050	MPA established in 1991	48	42	42	1	2.70 (0.40)	2.61 (0.07)	0.58 (0.08)	0.42 (0.06)	**–0.41** (0.09)	80%
	Tremiti (TRE)	42.138583	15.523950	MPA established in 1989	48	46	31	0.67	2.25 (0.25)	2.22 (0.05)	0.49 (0.10)	0.30 (0.05)	**–0.49** (0.09)	75%
Albania	**Karaburun Peninsula (KAP)**	40.392800	19.324967	MPA established in 2010	38	37	37	1	2.55 (0.35)	2.51 (0.03)	0.56 (0.10)	0.38 (0.06)	**–0.44** (0.11)	80%
Croatia	**Kornati (KOR)**	43.792250	15.281483	MPA established in 1980	48	44	33	0.74	2.30 (0.19)	2.26 (0.04)	0.42 (0.09)	0.29 (0.05)	**–0.32** (0.09)	95%
Greece	**Othonoi (OTH)**	39.836017	19.397767	No MPA	48	44	34	0.78	2.70 (0.40)	2.68 (0.03)	0.55 (0.09)	0.37 (0.05)	**–0.43** (0.09)	85%

Montenegro	**Boka Kotorska Bay (BOK)**	42.387533	18.569633	No MPA	48	45	33	0.73	2.40 (0.25)	2.29 (0.08)	0.52 (0.10)	0.31 (0.05)	**–0.50** (0.09)	80%

Table 2. Assignment test of *Posidonia oceanica* in the eight Adriatic and Ionian populations based on the neutral microsatellite set (14 loci). For each site (acronyms as in Table 1), individuals are presented in rows according to their sampling site and classified into individuals that get assigned to their own population (Self) and other sites that they get assigned to, namely Torre Guaceto (TOG), Othonoi (OTH), or unknown sources that could not be ascribed to any of the sampled populations (Unknown). The last column lists the total number and percentage of individuals that were not assigned to the population from which they were sampled.

Population	**Origin**				
	Self	**TOG**	**OTH**	**Unknown**	**Total**
OTR	27	–	–	–	0 (0%)
POC	39	–	–	2	2 (5%)
TOG	41	–	–	1	1 (2%)
TRE	30	1	–	–	1 (3%)
KAP	35	1	–	1	2 (5%)
KOR	31	–	1	1	2 (6%)
OTH	34	–	–	–	0 (0%)
BOK	31	1	–	1	2 (6%)
Total	241 (96%)	3	1	6	10 (4%)

Figure 1. Sampling locations of *Posidonia oceanica* in the Adriatic and Ionian seas. The shading shows the suitable habitat produced by the MediSeH project (Giannoulaki*et al.* 2013), including a buffer to increase visibility. Location acronyms are TRE for Tremiti (MPA was established in 1989), TOG for Torre Guaceto (MPA was established in 1991), OTR for Otranto (this site is in a potential area for a future MPA), POC for Porto Cesareo (MPA was established in 1997) (all located in Italy), OTH for Othonoi in Greece (no MPA), KAP for Karaburun Peninsula in Albania (MPA was established in 2010), BOK for Boka Kotorska Bay in Montenegro (no MPA) and KOR for Kornati in Croatia (MPA was established in 1980).

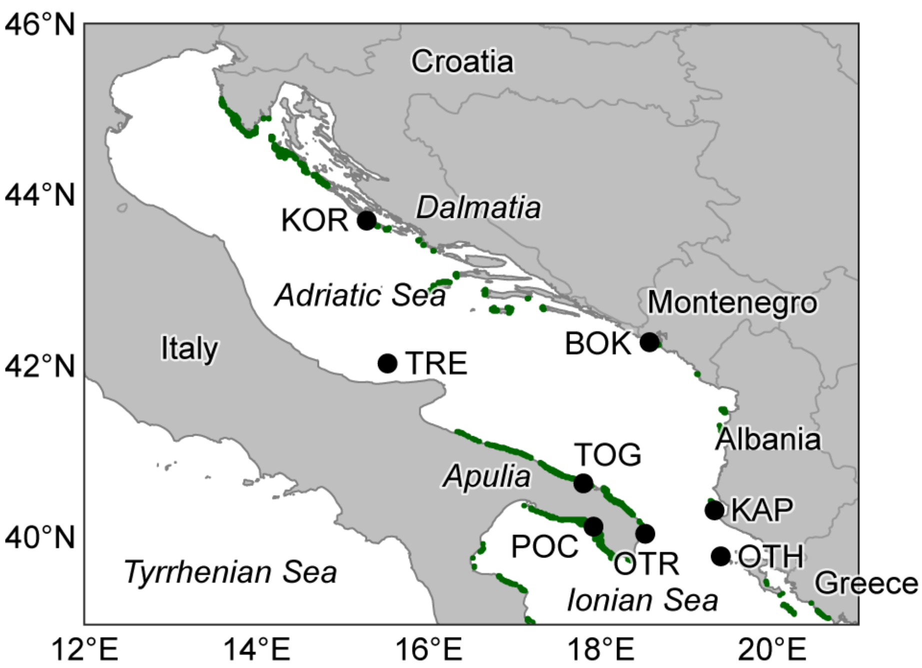

Figure 2. Oceanographic connectivity of eight *Posidonia oceanica* populations in the Adriatic and Ionian seas. Connectivity matrices (leftmost panels) show potential connectivity among sites, estimated via Lagrangian simulations, in terms of (a) intensity and (e) persistence (see text for details). Histograms show retention (b and f), source (c and g) and sink (d and h) strength of each site, as resulting by summing up the values of the corresponding matrices along the diagonal, the remaining row cells and the remaining column cells, respectively. Supplying populations are shown in the rows, receiving populations in the columns. Site acronyms as in Fig.1 and Table 1.

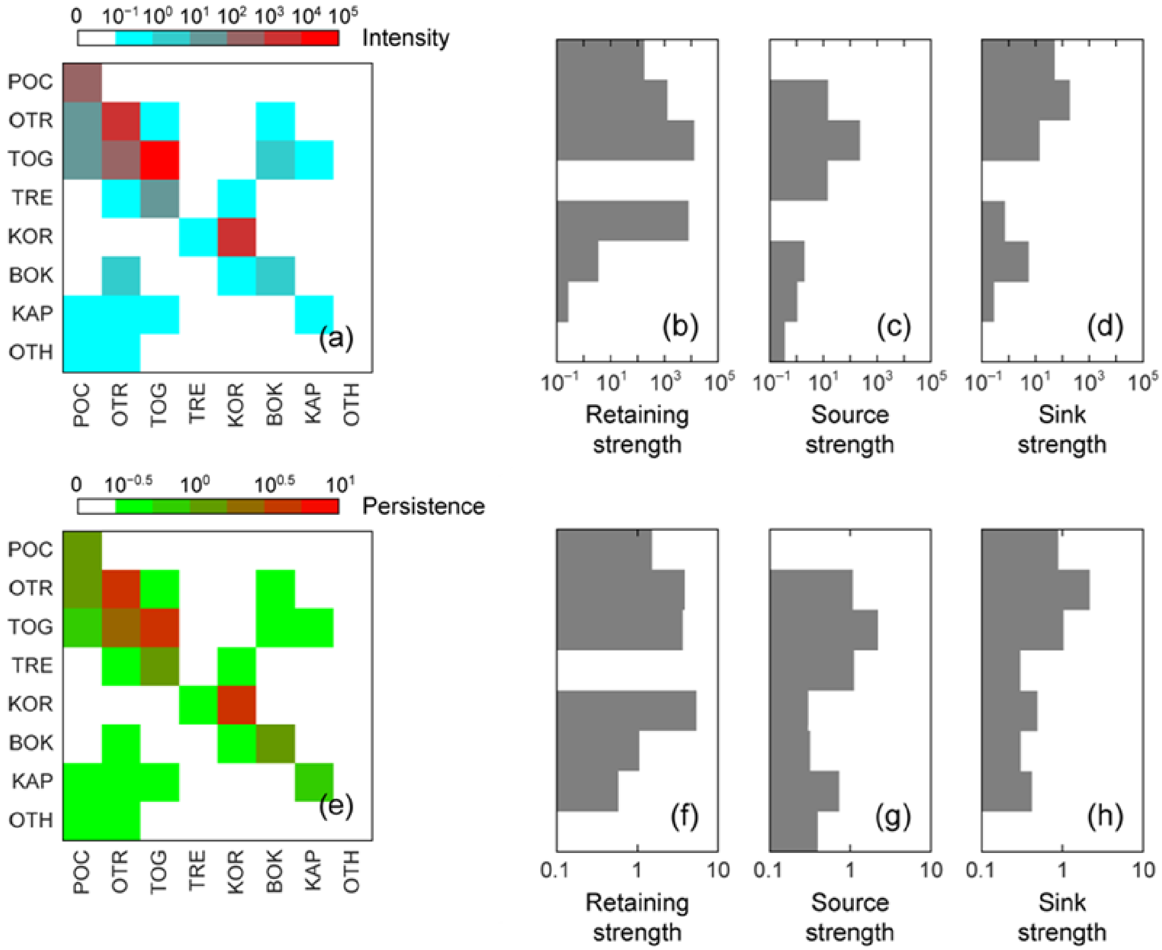

Figure 3. Clustering analyses for the eight *Posidonia oceanica* populations in the Adriatic and Ionian seas. a) population structure analysis performed using the STRUCTURE software (Pritchard *et al.* 2000) for neutral loci and based on correlated allele frequencies and admixture (K = 2); b) Discriminant Analysis of Principal Components DAPC (Jombart *et al.* 2010) for neutral loci retaining 15 principal components (PCs) as suggested in alpha score analysis (c). The STRUCTURE plot is shown for the most likely number of clusters (delta-K analysis) and plots for higher *K*s can be found in Fig. S4. Within each plot, each vertical bar represents an individual belonging to the sampling location indicated under the x-axis, clusters are colour coded, and the y-axis of each plot shows the proportion of the genotype belonging to each cluster. The DAPC analysis was performed based on the location of sampling (as opposed to defined by the cluster analysis of DAPC) and the colour of each population represents the colour of the majority of individuals of this population in the corresponding analysis performed by the STRUCTURE software. Each dot represents an individual contained into populations by a circle. Site acronyms as in Table 1 and Fig. 1.