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Genetic Description and Remote Sensing Techniques as Management Tools for *Zostera noltii* Seagrass Populations along the Atlantic Moroccan Coast

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ABSTRACT

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Seagrass meadows provide an essential ecological service in coastal ecosystems worldwide, although they are sensitive to many human factors, as a serious global regression has been documented. During sampling along the Atlantic Moroccan coast, five coastal lagoons were found characterized by the presence of intertidal monospecific seagrass *Zostera noltii* meadows (from south to north, Nayla, Oualidia, Sidi Moussa, Moulay Bousselham, and Larache). Two descriptive methods used to characterize *Z. noltii* populations could be used as management tools for future monitoring implementations: (1) the estimation of surface area covered by *Z. noltii* meadows using remote sensing techniques and *in situ* field surveys, and (2) the genetic characterisation of *Z. noltii* populations using simple sequence repeats (microsatellites) as molecular markers. Results revealed that the Nayla lagoon showed the largest area covered by *Z. noltii* (269,868 m^2) and the highest coverage rate (5.19%), while presenting the lowest genetic/genotypic diversity values ($T = 36$, $\hat{A} = 3.58$, $G = 50$; $R = 0.544$; $H_e = 0.43$). On the other hand, northern populations displayed lower rates of seagrass coverage (~1%) and higher values of genetic/genotypic diversity. Further genetic characterization also revealed that *Z. noltii* populations seem to be highly isolated in three geographically independent regions: northern Morocco (R1, Larache and Moulay Bousselham), central Morocco (R2, Oualidia and Sidi Moussa), and southern Morocco (R3, Nayla), which should be considered independent management units. Both seagrass coverage rate and the genetic description of seagrass populations along the Atlantic Moroccan coast seem to be useful management tools that could be used to evaluate changes in seagrass meadows over time to further establish appropriate conservation strategies.

ADDITIONAL INDEX WORDS: *Conservation, microsatellite, satellite images, marine plants.*

INTRODUCTION

The seagrass *Zostera noltii* (Hornemann) is a widely distributed marine plant forming intertidal mudflat prairies along the European and North African Atlantic coasts, from temperate southern Norway to Mauritania, including the Canary Islands, as well as throughout the Mediterranean, Black, Azov, Caspian, and Aral seas (Short *et al.*, 2007). Seagrass beds are known for their essential ecological and economical roles in coastal ecosystems, where they act as nursery habitats and enhance protection from coastal erosion. Moreover, seagrasses feature high rates of primary production and low decomposition rates, playing a significant role in the regulation of the global carbon cycle (Fourqurean *et al.*, 2012). However, *Z. noltii*, like other seagrasses, are very sensitive to human impacts, which is the main cause for the regression of seagrass populations worldwide (Orth *et al.*, 2006).

Along the NW Atlantic Moroccan coast, five enclaves of maximum environmental interest in the form of coastal lagoons shelter great biodiversity, including *Z. noltii* meadows: Nayla, Sidi Moussa, Oualidia, Moulay Bousselham, and Larache (Figure 1). The existence of *Z. noltii* no doubt contributes significantly to the biologic, ecological, and environmental values of these lagoons, which, except for Larache, are included in the Ramsar List of Wetlands of International Importance (Hammada, 2007). Additional protection is given to Moulay Bousselham and Nayla, as they were officially classified a permanent biological reserve in 1978 and a national park in 2006, respectively. Despite their protected status, human impacts are evident in the form of growth of human settlements, intensive agriculture, fishing, tourism, and/or oyster farming in the environs, especially in northern (Moulay Bousselham and Larache) and central (Sidi Moussa and Oualidia) populations. All these human activities should certainly have affected *Z. noltii* populations over time, but there is a total lack of knowledge about their current status, not to mention how these *Z. noltii* prairies have evolved or whether their ecological status is declining.

Several authors agreed that, in order to provide appropriate conservation and management tools for seagrass ecosystems, genetic factors should be taken into account (Frankham, 2003; Procaccini, Olsen, and Reusch, 2007), incorporating long-term genetic monitoring that includes a genetic description of the populations and knowledge of the connectivity between them (Evans *et al.*, 2014; Schwartz, Luikart, and Waples, 2007). Moreover, effective conservation and management of natural

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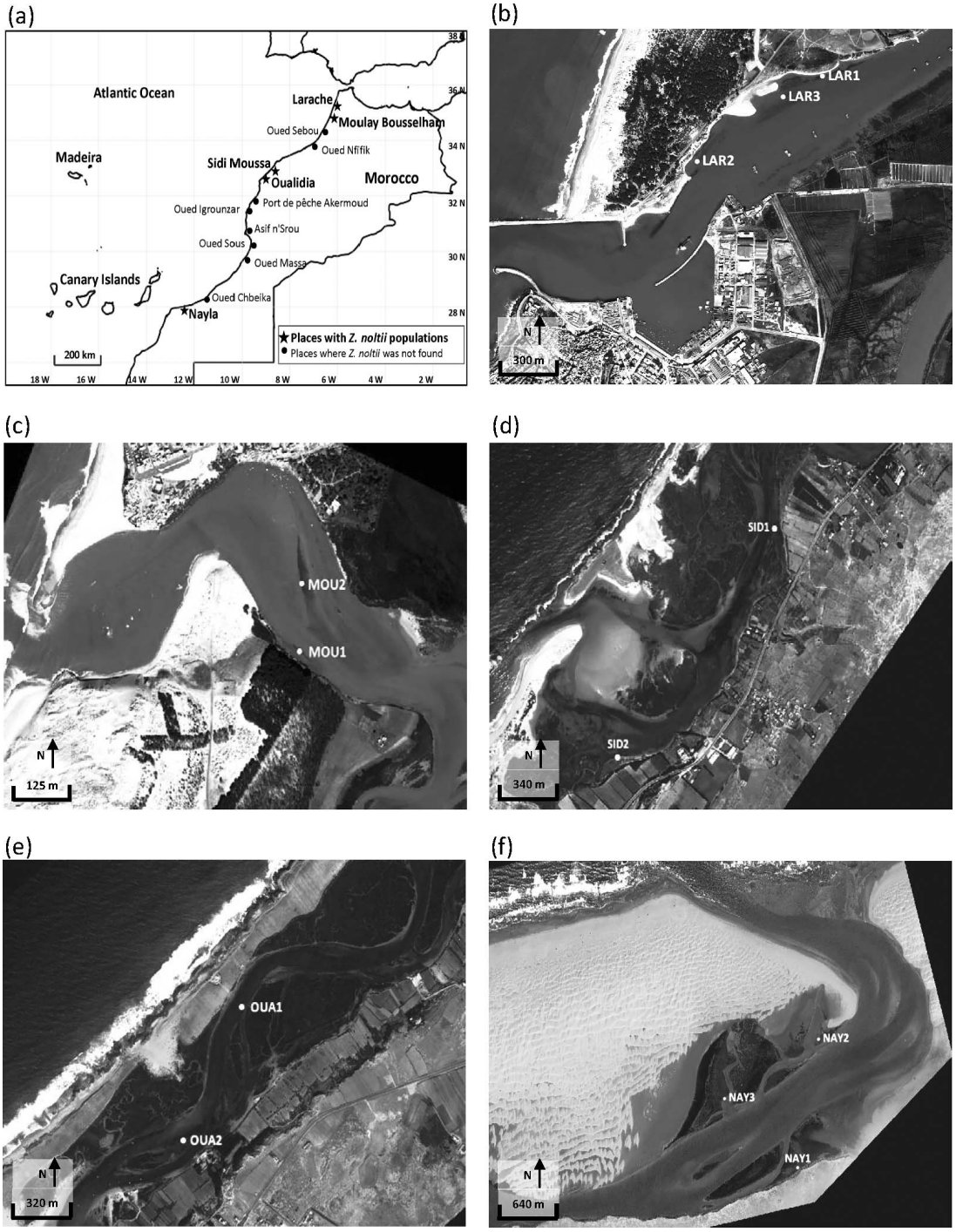

Figure 1. (a) Location of *Z. noltii* populations along the Atlantic Moroccan coast. ★ indicates places where *Z. noltii* was found; • indicates sites where *Z. noltii* was not found. (b) Larache. (c) Moulay Bousselham. (d) Sidi Moussa. (e) Oualidia. (f) Nayla. In Figures 1b–f, • represents sampling areas for the genetic characterisation of *Z. noltii* populations/subpopulations and for *in situ* surveys used to estimate *Z. noltii* distribution using high-resolution satellite images.

populations often depend on the identification of management units within species that can be outlined according to the genetic structure and the degree of genetic connectivity between populations (Allendorf, Hohenlohe, and Luikart, 2010; Palsbøll, Berube, and Allendorf, 2007). Additionally, the spatial representation of the distribution of seagrass species can serve as a powerful tool for conservation planning, providing managers and administrators with the best under-

standing of the extent of the habitat (Johnson, Seip, and Boyce, 2004). Remote sensing techniques combined with field surveys are suitable for monitoring seagrass status and changes in area distributions in large regions and over long time scales (Dingtian and Chaoyu, 2012; Meyer and Pu, 2012; Roelfsema *et al.*, 2009).

The purpose of this work is to provide a first insight about the current status of these five Moroccan Atlantic lagoons, using two different management tools: (1) measuring the extension of *Z. noltii* meadows using remote sensing techniques and *in situ* field surveys and (2) compiling a genetic description of *Z. noltii* populations and the connectivity between them, as well as the genetic structure in the region that enables us to define management units. The results will provide precise parameters for future conservation and management strategies based on the current status of this sensitive species along the Moroccan coast.

METHODS

An *in situ* ground campaign was carried out to locate *Z. noltii* populations along the Atlantic Moroccan coast from Aaiun to Larache. Among all possible locations with historical evidence for the presence of this seagrass, only Nayla, Oualidia, Sidi Moussa, Moulay Bousselham, and Larache were characterized by the presence of intertidal monospecific *Z. noltii* meadows (Figure 1).

Detection and Estimation of *Z. noltii* Surface Area Using High-Resolution Satellite Images

Remote sensing calibrated with *in situ* identification of specimens was used to estimate the area covered by the seagrass. *Zostera noltii* patches in each lagoon had to be precisely identified *in situ* during June 2012. As reported by Tulldahl, Philipson, and Tolt (2011), the Wordview2 sensor with 2.5 m spatial resolution and eight spectral channels is suitable for seagrass identification. Submerged and emerged seagrasses were mapped starting with *in situ* positioning of some samples and subsequently using Worldview2 preprocessed satellite images and a two-step mapping methodology. This methodology uses the eight spectral channels from Worldview2 and comprises (1) spectral characterization and (2) maximum likelihood. Spectral characterization delineates areas with *Z. noltii* spectra similar to those obtained from *in situ* sampling points. These areas are then used to define training areas for maximum likelihood. The maximum likelihood algorithm assigned the maximum probability level, while the minimum probability level was intentionally adjusted until all probable *Z. noltii* points shown in Figure 1 were appropriately covered. The final *Z. noltii* population in the lagoon was screened between the two probability levels, and specific areas (in m^2) were calculated after a pixel count process (further details about the *Z. noltii* mapping process can be found in the online Supplementary Material). To distinguish from plain water in the lagoon, the water spectrum was also processed from the Worldview2 data set by applying a simple bands comparison algorithm to detect water surfaces: reflectivity in band 3 was subtracted from the same in band 8; thus the more negative the result, the higher the probability of having a water pixel.

Genetic Characterization of *Z. noltii* Populations

Five *Z. noltii* meadows (populations) were sampled within five bays along the Moroccan Atlantic coast (Figure 1). The distances between bays along the coast range from 38 to about 1118 km. Within each bay, the largest two to three patches found were sampled (further considered to be subpopulations, $n = 12$; see Figure 1) by randomly collecting 45 to 50 ramets in each ($n = 555$) through walking or diving, and assuring maximum physical separation between them depending on the size of each patch. The intention was to obtain widespread genetic characterization of populations rather than pursuing a fine-scale spatial genetic structure. For each ramet, leaves were blotted dry and placed in plastic bags with silica crystals for dehydration and subsequent storage.

DNA Isolation and Microsatellite Genotyping

Genomic DNA was extracted from 5–10 mg of silica-dried leaf tissue using the EZNA® Plant DNA Kit (Omega Bio-tek, Inc., Norcross, Georgia, U.S.A.). Forward 5′ fluorochrome–labeled primers of nine *Z. noltii* microsatellite loci (Coyer, Reusch, *et al.*, 2004) were used for polymerase chain reaction (PCR) amplifications. Each reaction (15 μL total volume) contained 5 μL DNA template, 26 μM of each dNTP, 2 mM $MgCl_2$, 3 μL PCR buffer (5×), and 0.75 U GoTaq® Flexi DNA Polymerase (Promega Corporation, Madison, Wisconsin, U.S.A.). Individual primer concentrations ranged from 0.03 to 0.33 μM and were assembled as follows: mix 1, ZnB1, ZnF8, ZnE7; mix 2, ZnH8, ZnF11, ZnB3; and mix 3, ZnD6, ZnB8, ZnH10. Cycling conditions consisted of an initial denaturation step of 5 minutes at 95°C; followed by 35 cycles of 30 seconds at 95°C, 30 seconds at 57.5°C, and 40 seconds at 72°C; and a final elongation step at 72°C for 20 minutes. Fluorescent-labeled PCR fragments were run in an ABI3500 automated capillary sequencer (Thermo Fisher Scientific, Waltham, Massachusetts, U.S.A.) using the GeneScan-500 LIZ-Size standard (Thermo Fisher Scientific). Allele sizes were scored manually by several independent analyses using STRand (University of California, Davis, 2015).

Clone Identification

Zostera noltii is a clonal marine plant that reproduces both sexually through seeds and vegetatively through clonal propagation, resulting in *genets* (genetic individuals which have stemmed from a zygote) and *ramets* (morphological or clonal individuals produced by vegetative reproduction), respectively. Thus—before performing population genetic analyses and only when two or more ramets with identical multilocus genotypes (MLGs) were detected at a sample site—two additional steps were performed in order to identify those MLG replicates that were part of the same genet or conversely belonged to distinct genets with identical MLGs. Briefly, the probability of a given genotype (P_{gen}) was calculated using the round-robin method to estimate the probability that identical MLGs originated from different zygotes (P_{sex}) (Arnaud-Haond *et al.*, 2007). A P_{sex} lower than 0.05 led us to reject the null hypothesis that MLG replicates belonged to distinct zygotes, removing them for subsequent genetic analyses. All these calculations were performed using GENCLONE 2.0 (Arnaud-Haond and Belkhir, 2007).

Within-Population Genetic Diversity

After excluding clones from the data set, the Hardy-Weinberg equilibrium for each subpopulation was tested using an exact probability test (Raymond and Rousset, 1995) available in Genepop 4.2 (Rousset, 2008). Due to the clonal nature of seagrasses, their genetic variation can be organized in two components: the allele variation as a result of sexual recombination events and the genotype variation as a result of the influence of vegetative reproduction (Reusch, 2001). Thus, genotypic or clonal diversity (R) was estimated to be the proportion of sampled genets (G) in each subpopulation in relation to the number of sampling units (N), following Dorken and Eckert (2001): $R=(G-1)/(N-1)$. Allelic and private allelic richness ($\hat{A}$, $\hat{A}_p$) from subpopulations were estimated to be the average number of (private) alleles per locus, using the rarefaction method (Kalinowski, 2004; Leberg, 2002) implemented in HP-RARE v.1.1 (Kalinowski, 2005). To make the sample sizes equal, $G=18$ and $G=50$ were used in the analysis based on the minimum number of genets detected in the subpopulation and population, respectively. Furthermore, the total number of alleles (T), the observed (H_o) and expected (H_e) heterozygosities, and Wright's inbreeding coefficient F_{IS} (Weir and Cockerham, 1984) were estimated using GENETIX v.4.05 (Belkhir *et al.*, 1996). Since sharp differences in sampling size between populations could affect the comparison of the levels of genetic diversity, random resampling was used to standardize N to the minimum number of ramets detected in Oualida ($N=91$).

Genetic Diversity among the Population

Levels of genetic differentiation between populations and subpopulations were described by the F_{ST} estimator θ, and the null hypothesis of no differentiation was tested for significant differences in allele frequencies (genic differentiation) using the Fisher exact test (Raymond and Rousset, 1995), available in GENEPOP v.4.2 (Rousset, 2008), with 10,000 dememorizations, 100 batches, and 5000 iterations per batch. Additionally, Jost's D (Jost, 2008) measure of population differentiation between subpopulations was estimated and tested in 999 permutations in GenAlEx v.6.5 (Peakall and Smouse, 2012). The more familiarized θ estimator was used, although pairwise Jost's D is presented in the online supplemental material.

The isolation by distance model (IBD) (Wright, 1943) was analysed to estimate the proportion of genetic divergence within the Atlantic Moroccan coast area as a function of geographic distance between subpopulations. Thus, θ was correlated with the geographic distance using a matrix correlation method and was tested by the Mantel test (Manly, 1994), implemented in isolation by distance web service (IBDWS v.3.23; see Bohonak, 2002; Jensen, Bohonak, and Kelley, 2005) with 10,000 randomizations. The IBD slope and intercept were estimated using reduced major axis regression (Sokal and Rohlf, 1981).

To further examine the genetic divergence among subpopulations, a principal coordinate analysis (PCoA) was carried out based on the pairwise F_{ST} matrix using the covariance-standardized settings implemented in GenAlEx v6.5 (Peakall and Smouse, 2012). Furthermore, the Markov Chain Monte Carlo Bayesian algorithm implemented in STRUCTURE v.2.3.4 (Pritchard, Stephens, and Donnelly, 2000) was used to estimate the genetic structure of *Z. noltii* on the Atlantic Moroccan coast. Given the number of clusters (K) and minimizing Hardy-Weinberg and linkage equilibrium within them, STRUCTURE estimated the probability that each *genet* belonged to each cluster. In order to assist the clustering, the admixture (Pritchard, Stephens, and Donnelly, 2000) and the correlated allele frequencies (Falush, Stephens, and Pritchard, 2003) models were selected without any prior information about sampling location. After exploratory analyses, 10 independent runs with 500,000 repetitions after a burn-in period length of 100,000 Markov chain Monte Carlo were performed to achieve consistent convergence of the posterior distribution probabilities. Subsequently, the number of K populations was detected using an *ad hoc* statistic ΔK (Evanno, Regnaut, and Goudet, 2005), implemented in the STRUCTURE HARVESTER Web site (Earl and von Holdt, 2012). Additionally, the hierarchical partitioning of genetic variation was estimated by analysis of molecular variance (AMOVA) implemented in GenAlEx v.6.5 (Peakall and Smouse, 2012), using the *Codom-Genotypic* option. Firstly, Φ_{PT} was estimated to quantify the genetic differentiation among subpopulations and tested using a permutation test ($n = 999$). Secondly, the genetic variation was partitioned among regions (Φ_{RT}), among subpopulations within regions (Φ_{PR}), and within subpopulations (Φ_{PT}). Three regions (south, central, and north Morocco) were defined according to both Bayesian and PCoA clustering analysis.

Finally, for an enhanced understanding of the genetic connectivity of *Z. noltii* populations along the Atlantic Moroccan coast, an assignment test was performed using Genclass 2.0 (Piry *et al.*, 2004). The aim of genetic assignment methods is to assign or exclude reference populations as possible origins of individuals on the basis of multilocus genotypes over one or more generations. When the method's assumptions are not violated, and given strong statistical reliability in the balance between type I and type II errors, assignment tests can quantify effective dispersal events and estimate direct, real-time migration rates (Paetkau *et al.*, 2004). First-generation migrants were detected using the ratio of likelihood, $L=L_{home}/L_{max}$, computed from the population in which an individual was sampled (L_{home}) over the most likely value among all population samples, including the population in which the individual was sampled (L_{max}) (Paetkau *et al.*, 2004). The likelihood was estimated using the frequency-based method (Paetkau *et al.*, 1995). A Monte Carlo resampling method ($n = 10{,}000$) was performed to generate critical values for rejecting the null hypothesis that an individual was born in the population in which it was sampled (Paetkau *et al.*, 2004). The type I error threshold was set at 0.01 (Paetkau *et al.*, 2004).

RESULTS

The first part of the results describes the estimation of the *Z. noltii* population area and mapping results obtained from high-resolution satellite image analysis in five marine lagoons along the Atlantic Moroccan coast. The second part describes the results of the genetic description of *Z. noltii* populations and the connectivity between them, as well as the genetic structure in the region.

Table 1. Zostera noltii *area estimates and coverage rate from high-resolution satellite image analysis in five marine lagoons along the Atlantic Moroccan coast*.

Lagoon	Image Acquisition Day and UTC Time	Lagoon Water Coverage (m^2)	*Z. noltii* Area Estimates (m^2)	% *Z. noltii* Coverage
Larache	3 November 2010 11:31	2,587,556	38.704	1.50
Moulay Bousselham	3 November 2010 11:31	8,020,732	71.508	0.89
Sidi Moussa	10 October 2010 11:39	3,090,212	23.918	0.77
Oualidia	3 October 2012 12:03	1,152,792	13.392	1.16
Nayla	5 September 2011 12:05	5,200,624	269.868	5.19

Zostera noltii Population Area Estimation and Mapping Results from High-Resolution Satellite Images

The estimation of the *Z. noltii* population area obtained after the spectral and maximum likelihood process is shown in Table 1. Note that all the satellite images cover a total area of 25 km^2. *Z. noltii* area estimates (in m^2) add the results of three defined classes according to previous spectral analyses: submerged, emerged, and surface (lying on water) seagrass. The *Z. noltii* coverage rate is the quotient between *Z. noltii* coverage and lagoon water coverage, and the result is scaled by 100 in order to show percentage units. Table 1 clearly shows that the *Z. noltii* population estimate in the Nayla lagoon is the largest in area and also in lake coverage (269.868 m^2 and 5.19%, respectively) in relation to other lagoons, where a significantly lower coverage rate was observed. *Zostera noltii* mapping results and population distribution in the five Moroccan lagoons are presented in the online Supplementary Material. The methodology suffers from a limitation regarding high-resolution satellite image analysis when the water is very turbid, as was the case at Oualidia and Sidi Moussa. The spectra of these two lagoons are shown in the online Supplementary Material. Turbid water is observed in spectra by the almost flat tail (from band 5 to 8) and greater reflectivity, shown clearly by the OUA1-2 spectrum. Further evidence of the poor water quality is the slight difference in the probability level obtained in the two lagoons, as explained in section 3 of the online Supplementary Material for the maximum likelihood algorithm. Therefore, even screening the highest maximum likelihood probabilities, *Z. noltii* area estimations for these two lakes have to be interpreted with caution due to the turbid water conditions.

Genetic Characterization of *Z. noltii* Populations

A total of 555 ramets were genotyped for 9 microsatellite loci from 12 *Z. noltii* subpopulations located in 5 populations along the Moroccan Atlantic coast, revealing 90 alleles and 461 MLGs. As far as populations are concerned, the most and least genetically diverse populations were in Moulay Bousselham bay in the north ($\hat{A} = 6.43$, $H_e = 0.645$) and Nayla bay in the south ($\hat{A} = 3.58$, $H_e = 0.434$). However, Nayla is highlighted by its important endemic (private) component of its allelic variation (Table 2). The effect of clonality in the region showed a clear latitudinal trend (Table 2), diminishing the clonal diversity values gradually from north (LAR, $R = 1$) to south (NAY, $R = 0.544$). The fixation indices of all five Moroccan populations were positive and showed significant departures from the Hardy-Weinberg equilibrium; these values ranged from $F_{IS} = 0.017$ in Oualidia to $F_{IS} = 0.081$ in Sidi Moussa. In addition, descriptive statistics for both genetic and genotypic diversity within subpopulations are shown in Table 2.

Major genetic differentiation was observed between northern (Moulay Bousselham and Larache) and central (Sidi Moussa and Oualidia) Moroccan populations and the population located farther south (Nayla), as shown by high pairwise F_{ST} estimator values (LAR-NAY, $\theta = 0.397$, 1118 km; MOU-NAY, θ

Table 2. *Genetic data set estimates for 9 microsatellite loci in* Z. noltii *populations (*n = *5) and subpopulations (*n = *12) ordered from north to south Morocco.* N = *resampled ramets standardized to a minimum common sample size (original number of collected ramets),* G = *resampled genets (original number of genets),* R = *genotypic diversity,* T = *total number of alleles per population or subpopulation,* H_o, H_e = *observed and expected heterozygosity, respectively,* F_{IS} = *inbreeding coefficient,* $\hat{A}$, $\hat{A}_p$ = *allelic and private allelic richness, respectively, standardized to a minimum common sample size of 67 and 18 genotypes for populations and subpopulations, respectively*.

Meadow	Latitude	Longitude	*N*	*G*	*R*	*T*	H_o	H_e	F_{IS}	$\hat{A}$	$\hat{A}_p$
Larache (LAR)	35°12.558′ N	06°08.270′ W	91 (137)	91 (136)	1	42	0.505	0.540	0.075**	3.72	0.39
LAR1	35°12.612′ N	06°08.127′ W	43	42	0.976	36	0.507	0.529	0.052**	3.21	0.10
LAR2	35°12.377′ N	06°08.572′ W	45	45	1	36	0.511	0.554	0.088**	3.29	0.08
LAR3	35°12.558′ N	06°08.270′ W	49	49	1	37	0.464	0.545	0.158**	3.22	0.05
M. Bousselham (MOU)	34°52.159′ N	06°17.330′ W	91 (96)	89 (94)	0.977	77	0.608	0.645	0.069**	6.43	1.25
MOU1	34°52.159′ N	06°17.330′ W	47	45	0.956	64	0.595	0.630	0.067**	4.48	0.37
MOU2	34°52.298′ N	06°17.321′ W	49	49	1	71	0.628	0.653	0.048**	4.71	0.46
Sidi Moussa (SID)	32°59.072′ N	08°44.523′ W	91 (94)	87 (88)	0.955	49	0.457	0.481	0.081**	4.25	0.20
SID1	32°59.072′ N	08°44.523′ W	51	47	0.92	41	0.439	0.504	0.138**	3.53	0.12
SID2	32°58.452′ N	08°45.119′ W	43	41	0.952	40	0.485	0.440	−0.089**	3.05	0.03
Oualidia (OUA)	32°45.140′ N	09°01.292′ W	91 (91)	76 (76)	0.833	47	0.532	0.538	0.017**	4.51	0.25
OUA1	32°45.140′ N	09°01.292′ W	45	38	0.840	40	0.590	0.546	−0.066**	3.70	0.13
OUA2	32°44.810′ N	09°01.478′ W	46	38	0.822	40	0.473	0.509	0.082**	3.26	0.07
Nayla (NAY)	28°01.841′ N	12°14.176′ W	91 (137)	50 (79)	0.544	36	0.429	0.434	0.042**	3.58	0.73
NAY1	28°01.841′ N	12°14.176′ W	47	29	0.608	33	0.413	0.397	−0.023**	2.84	0.11
NAY2	28°02.490′ N	12°14.032′ W	45	32	0.704	34	0.413	0.416	0.023**	2.82	0.06
NAY3	28°02.200′ N	12°14.630′ W	45	18	0.386	29	0.438	0.451	0.057**	2.81	0.04

** Indicates significant departures from Hardy-Weinberg equilibrium ($p < 0.01$).

Table 3. *Matrix showing pairwise* F_{ST}*-estimator* θ *values (below diagonal) and pairwise distances in km (above diagonal) among* Z. noltii *Moroccan subpopulations. All* θ *values were significantly different from zero (*$p < 0.01$*) except those shown in bold.*

	LAR1	LAR2	LAR3	MOU1	MOU2	SID1	SID2	OUA1	OUA2	NAY1	NAY2	NAY3
LAR1		0.8	0.2	42	42	371	372	408	409	1118	1118	1118
LAR2	**0.001**		0.5	41	41	370	371	406	408	1117	1117	1117
LAR3	**−0.001**	**0.001**		42	42	371	372	408	409	1118	1118	1118
MOU1	0.156	0.141	0.136		0.2	329	330	366	367	1076	1076	1076
MOU2	0.136	0.123	0.125	0.006		329	330	366	367	1076	1076	1076
SID1	0.292	0.290	0.279	0.283	0.279		1.5	37	38	747	747	747
SID2	0.340	0.335	0.324	0.327	0.322	0.009		36	37	745	745	745
OUA1	0.240	0.242	0.230	0.270	0.266	0.061	0.084		0.75	710	710	710
OUA2	0.252	0.253	0.240	0.279	0.271	0.045	0.062	0.021		709	709	709
NAY1	0.413	0.405	0.408	0.332	0.304	0.396	0.464	0.407	0.420		1.2	1
NAY2	0.417	0.411	0.413	0.338	0.308	0.396	0.461	0.406	0.419	**0.004**		1.1
NAY3	0.381	0.374	0.377	0.296	0.269	0.380	0.449	0.383	0.397	**0.005**	**0.014**	

$= 0.311$, 1076 km; SID-NAY, $\theta = 0.420$, 747 km; OUA-NAY, $\theta = 0.402$, 709 km), all of which were highly significant ($p < 0.001$). Only Sidi Moussa and Oualidia showed moderate genetic differentiation ($\theta = 0.055$, 38 km, $p < 0.001$). By contrast, little genetic differentiation was found between subpopulations from the same bay or lagoon (at <2 km, $\theta < 0.05$), especially between subpopulations from Larache and Nayla, where no significant differentiation was detected ($p > 0.05$) (Table 3). This genetic differentiation pattern between populations and subpopulations was confirmed with a statistical test for IBD ($p < 0.001$, $r^2 = 0.683$), in which the regression coefficient revealed that a large proportion of the genetic divergence variance between subpopulations depended heavily on geographic distance.

The principal coordinate analysis of the F_{ST} matrix (see online Supplementary Material) allowed grouping the subpopulations into three sets corresponding to three different geographic regions: R1, north Morocco (LAR and MOU); R2,central Morocco (OUA and SID); and R3, south Morocco (NAY). Likewise, STRUCTURE analysis (Figure 2) clearly clustered the populations into three K ancestry populations corresponding to three similar geographic regions (R1, R2, and R3). The genetic structure of *Z. noltii* populations along the Atlantic Moroccan coast was also confirmed with AMOVA, which revealed considerable genetic differentiation among regions (Φ_{RT}=0.416; $p < 0.001$) (see online Supplementary Material).

Finally, the assignment tests revealed low short-term estimates of dispersal, since 98.3% of the individuals (genets) were assigned to the population from which they were sampled (see online Supplementary Material). Between populations, only one individual (genet) from LAR was designated a potential first-generation migrant (F_0) to MOU, the most likely population of origin ($p < 0.01$). The remaining individuals ($n = 8$) were assigned potential F_0 to other subpopulations of the same bay or lagoon.

DISCUSSION

Seagrass ecosystems are declining worldwide due to habitat destruction, population fragmentation, degraded water quality, and climate global change (Short *et al.*, 2011; Waycott *et al.*, 2009). Unfortunately, *Z. noltii* on the Atlantic Moroccan coast is no exception to those threats. A reduction in meadow surface area over the past 20 years has been reported (R. Flower, *personal communication*), including complete disappearance in the Oued Chbeika, Oued Sous, Asif n'Srou, Oued Massa, Oued Igrounzar, Oued Sebou, and Oued Nfifikh bays, as was observed *in situ* during sample collection in the present study (Figure 1). Appropriate management and conservation strategies are now required for the remaining seagrass habitats,

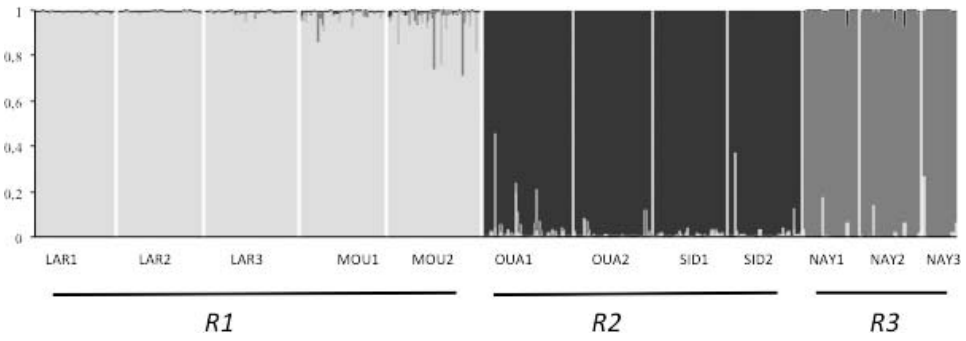

Figure 2. Bayesian assignment of individuals into $K = 3$ genetic clusters using the program STRUCTURE v.2.3.4. Each column corresponds to one individual and each color represents a single cluster. The vertical height of each column denotes the probability of each individual belonging to each of the inferred clusters. Group R1 comprised subpopulations from the northern Moroccan region (LAR1, LAR2, LAR3, MOU1, and MOU2); group R2 included subpopulations from the central Moroccan region (OUA1, OUA2, SID1, and SID2); and group R3 was formed by subpopulations from the south Moroccan region (NAY1, NAY2, and NAY3).

given their fragility and ecological importance. In the present work, two different management tools are proposed as a starting point in conservation planning that could be used in international conservation programs to evaluate changes in seagrass meadows over time: (1) the analysis of high-resolution satellite images and (2) the genetic characterization of *Z. noltii* populations.

High-Resolution Satellite Images to Estimate *Z. noltii* Population Area and Coverage Rate

The analysis of high-resolution satellite images from the Worldview2 sensor carried out in this work has enabled us to estimate the remaining *Z. noltii* areas and the distribution of this species in five Atlantic Moroccan lagoons (Table 1 and online Supplementary Material). Results have shown that the Nayla lagoon showed the largest area covered by the *Z. noltii* population (269,868 m^2) and the highest coverage rate (5.19%), probably due to a higher level of ecosystem protection and lower amount of human interaction. Unfortunately, significantly less area and lower coverage rates were observed in the other four lagoons, where city ports (Larache) and abundant agricultural fields (Moulay Bousselham, Sidi Moussa, and Oualidia) are probably affecting *Z. noltii* habitats, as observed in satellite images (Figure 1) and during field campaigns. Even though the actual estimation of the *Z. noltii* population area and its coverage rate was done with satellite images obtained from different years (Table 1), the present results could be used in further analysis using the same techniques to asses changes in *Z. noltii* meadows through time (seasonally or annually, depending on objectives and available resources). Therefore, analysis of high-resolution satellite images could be used in future management strategies as a powerful tool to evaluate and monitor spatial changes in seagrass meadows over time (covered area, patches distribution, or fragmentation) that may be related to anthropic or natural factors (Dingtian and Chaoyu, 2012; Meyer and Pu, 2012; Roelfsema *et al.*, 2009).

Genetic Description of *Z. noltii* Populations for Future Management Strategies

Along with traditional seagrass monitoring that comprises biological descriptors, it is necessary to include genetic/genotypic descriptions of the populations and information about their connectivity for proper conservation and management of threatened populations (Evans *et al.*, 2014; Procaccini, Olsen, and Reusch, 2007; Reusch, 2001). Additionally, the genetic monitoring of seagrass meadows should be extended in time with periodic resampling to evaluate changes in population genetic metrics (Evans *et al.*, 2014; Schwartz, Luikart, and Waples, 2007). Accordingly, in this work, *Z. noltii* populations along the Moroccan Atlantic coast were characterized genetically to link the genetic characterization performed in this paper to future management strategies.

Our results showed higher genetic (allelic richness and expected heterozygosity) and genotypic (clonal richness) diversity values in northern and central Moroccan populations, in contrast with those of the south (Table 2), even though the Nayla lagoon presented the highest protection status, as it belongs to Khnifiss National Park, where the access is limited and human activities are regulated. Particularly, clonal diversity values in the more disturbed populations of Larache and Moulay Bousselham (disturbances including agricultural and human settlement, besides direct physical disturbance induced by local fisherman, as observed *in situ* during field campaigns) are close to 1 ($R = 1$ and $R = 0.977$, respectively), highlighting the importance of sexual versus clonal reproduction. Several authors have reported that moderate levels of disturbance may enhance genotypic diversity in seagrass meadows due to an increase in sexual reproduction by promoting the dispersal of pollen and seeds, favoring the recruitment of new seeds and seedlings through gap formation in dense meadows, and indirectly increasing the level of outcrossing due to clone size reduction (Coyer, Diekmann, *et al.*, 2004; Reusch, 2006; Zipperle *et al.*, 2009). High expected heterozygosity values (H_e) have also been related to an increase in seedling recruitment in *Z. noltii* populations in Northern Ireland, confirming that within-population genetic diversity could be associated with differences in mating systems (Provan *et al.*, 2008). On the other side, the more protected and undisturbed population of Nayla turned out to be the least diverse population, with lower genetic diversity values ($\hat{A} = 3.58$, $H_e = 0.43$) and lower numbers of different genotypes ($R = 0.544$), meaning higher levels of clonal reproduction. In that sense, Coyer, Diekmann, *et al.* (2004) have shown that low levels of disturbance promote clonal growth and stability in *Z. noltii* meadows, generating very large and old clones adapted to local conditions that are also characterized by low levels of genetic diversity. Therefore, the balance of sexual *vs.* clonal reproduction could elucidate different population strategies with relevant ecological and evolutionary implications.

The current genetic diversity status of *Z. noltii* meadows may be related to their fitness and their ability to react and survive after future environmental and anthropogenic changes. Seagrass conservationists have demonstrated that higher values of genetic and genotypic diversity (as seen in the higher polymorphic populations of northern Morocco) promote fitness and survival likelihood against any type of environmental stress, increasing resilience and recovery of seagrasses after disturbances (Hughes and Stachowicz, 2004; Massa *et al.*, 2013; Reusch *et al.*, 2005). However, the relative importance of clonal growth and higher clonal size (as occurs in the more southern and stable population of Nayla, leading to higher vegetative growth and less genotypic diversity) should not be ignored in estimating future resilience, as it affects genet fitness through both genet persistence and seed production (Diaz-Almela *et al.*, 2007; Pan and Price, 2001). Overall, anthropogenic activities should be regulated in protected areas such as the bay of Moulay Bousselham, and, at the same time, genetic monitoring in those meadows will enable the evaluation of changes in genetic descriptors over time and assessment of how genetic/genotypic diversity will affect seagrass fitness, especially in those highly disturbed meadows.

Another major genetic factor that should be measured and particularly avoided in future management strategies is increased homozygosity resulting from inbreeding (Reed and Frankham, 2003), which may result in a loss in fitness (reproduction and survival), indicating risk of future extinction (Frankham, 2003; Spielman, Brook, and Frankham, 2004). Most Moroccan populations are characterized by low levels of inbreeding, with values ranging from a maximum in Sidi

Moussa ($F_{IS} = 0.081, p < 0.01$) to a minimum in Oualidia ($F_{IS} = 0.017, p < 0.01$). Nevertheless, these values should be tracked and evaluated in future genetic monitoring, as they may increase with increasing habitat fragmentation due to low levels of outcrossing (see below).

Population Connectivity, Genetic Structure, and Management Units

Knowledge about connectivity between populations (fragmentation and/or reduction in gene flow) and the main evolutionary process (genetic drift *vs*. natural selection) are equally important genetic issues for conservation biology (Balloux and Lugon-Moulin, 2002; Evans *et al*., 2014; Frankham, 2003). Seagrasses exist as metapopulations and are thus very sensitive to fragmentation (Procaccini, Olsen, and Reusch, 2007). Therefore, connectivity between populations seems to be highly relevant to conservation, as isolated populations with limited gene flow will lose genetic diversity and can be particularly susceptible to inbreeding depression and random genetic drift, increasing the risk of extinction (Frankham, 2003; Kramer and Havens, 2009). *Zostera noltii* meadows along the Atlantic coast of Morocco are located in suitable habitats in geographically isolated semienclosed bays, forming a singular, highly fragmented landscape in which the bays have played an important role in genetic population differentiation, probably limiting the arrival of new sexual recruits or vegetative fragments from nearby populations. These discrete spots strictly limit bay-to-bay long-range dispersal at the same time that short-range dispersal is promoted within bays. Genetic differentiation among populations was confirmed by high pairwise F_{ST} estimator values ($\theta > 0.05$; see interpopulation genetic diversity results in Balloux and Lugon-Moulin, 2002) and significant IBD, showing that, as geographic distance among populations increases, genetic differentiation also increases (Jensen, Bohonak, and Kelley, 2005). Accordingly, much lower pairwise θ values between subpopulations from the same bay or lagoon (θ values < 0.05; see Balloux and Lugon-Moulin, 2002; see Table 3) supports the results of the assignment test (see online Supplementary Material), which reflected a greater genetic cohesion within and among subpopulations of the same bay. Furthermore, both STRUCTURE (Figure 2) and PCoA results (online Supplementary Material) clearly revealed a homogenizing effect of the historical gene flow between subpopulations separated by 30 to 50 km within northern and central regions. Thus three geographic regions where admixture could not be rejected were defined: northern, central, and southern, separated by 30 to 300 km. The homogeneity of the three ancestry clusters and the significantly high proportion of variation detected between regions ($\Phi_{RT} = 0.416$; online Supplementary Material) suggested an ancestral genetic isolation of those three geographic regions.

For management purposes, all the above genetic information may support the existence of three management units along the Atlantic coast of Morocco: a northern unit (Larache and Moulay Bousselham), a central unit (Oualidia and Sidi Moussa), and a southern unit (Nayla). The data also suggests that these management units should be monitored and managed separately (Allendorf, Hohenlohe, and Luikart, 2010; Palsbøll, Berube, and Allendorf, 2007). Nevertheless, preservation of the bay as an important spot for conservation should also be highly recommended in order to preserve the current, highly fragmented *Z. noltii* landscape on the Atlantic coast of Morocco, in which functional gene flow becomes critically important to offset the deleterious effects of human impacts (Segelbacher *et al*., 2010).

CONCLUSIONS

The present work provided a first insight about the current status of five Atlantic Moroccan lagoons using two descriptive methods that could be used as management tools for future monitoring implementations. The analysis of high-resolution satellite images from Worldview2 sensors allowed the estimation of surface area covered by *Z. noltii* meadows within bays, confirming that this technique could be used to quantify changes in seagrass meadow coverage over time. The genetic considerations presented here—the genetic characterisation and the definition of genetic structure among *Z. noltii* populations—may also provide valuable guidelines for the conservation of these seagrass meadows. Further analysis of high-resolution satellite images and genetic monitoring through time (recommended on an annual basis) are necessary to assess the evolution of seagrass meadows facing greater anthropogenic pressures and global changes in order to adopt adequate management strategies.

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