

Combined genetic and habitat characterization as a management tool for *Zostera noltii* seagrass populations along the Atlantic Moroccan Coast



Zarranz Elso M^a, Luque A^a, Manent P^b, Ramdani M^c, Robaina RR^a rrobaina@dbio.ulpgc.es

^a University of Palmas de Gran Canaria. Canary Islands, Spain
^b Instituto Canario de Ciencias Marinas. Canary Islands, Spain
^c Institut Scientifique Rabat. Rabat Agdal. Maroc



INTRODUCTION

Seagrass meadows provide an essential ecological service in coastal ecosystems worldwide, although they are sensitive to many human factors having been documented a serious global regression. Along the **Atlantic Moroccan Coast** we found 5 coastal lagoons with maximum environmental interest characterized by the presence of intertidal seagrass *Zostera noltii* meadows (Loukos estuary, Moulay Bousselham, Sidi Moussa, Oualidia and Nayla). In order to suggest future management strategies that promotes the conservation of these highly sensitive and valuable areas, the present work, which is conducted on the framework of TechMARAT's project (0111_TechMARAT_2_A, POCTEFEX-FEDER), has studied the genetic characterization and the connectivity between population to identify Management Units, while providing remote sensing tools to mapping the actual distribution of *Z. noltii* meadows.

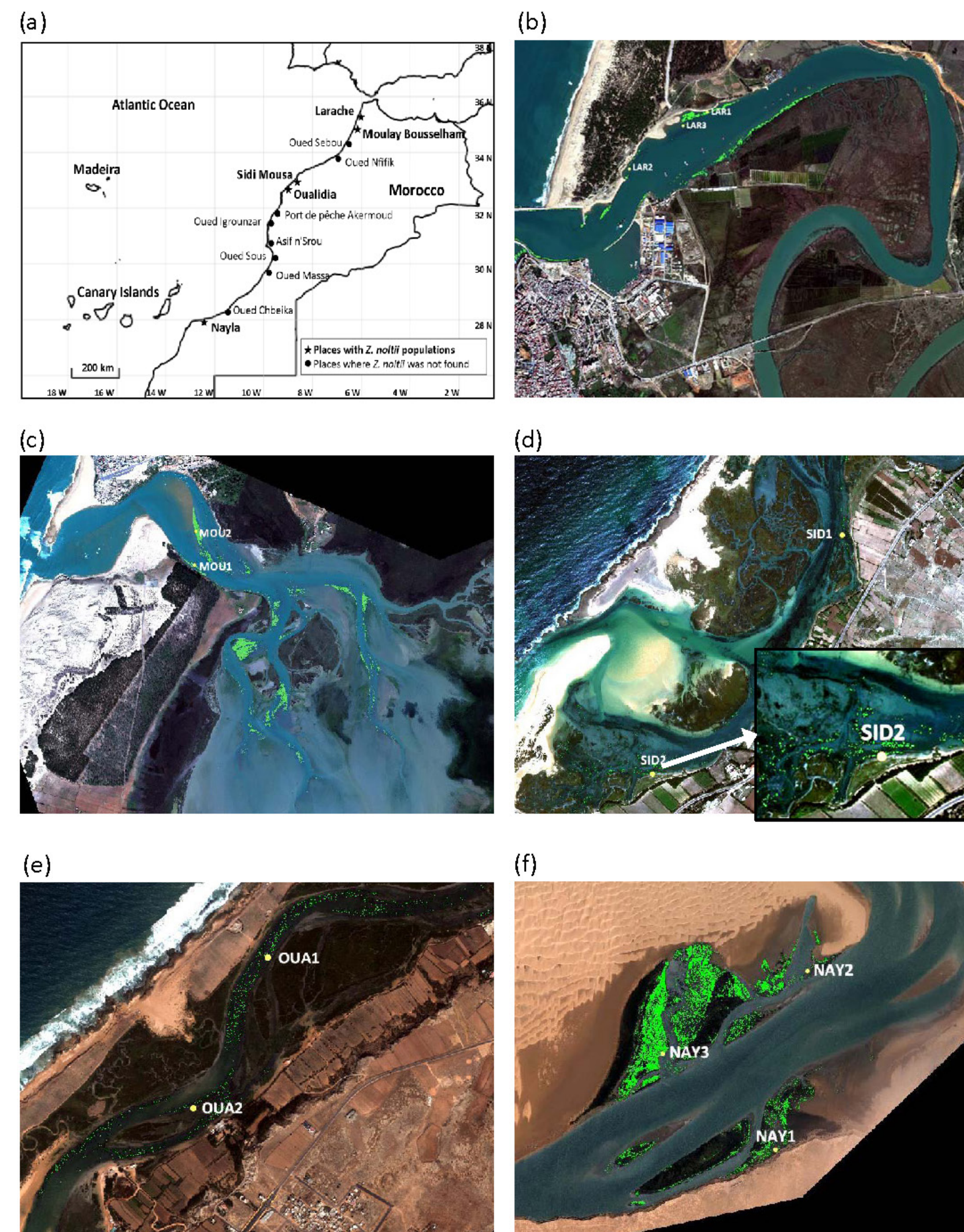
MATERIALS AND METHODS

For quantifying the area of *Zostera noltii* meadows in each bay or lagoon (Fig.1), submerged and emerged seagrasses were mapped starting firstly with in situ positioning of some samples, and then using Worldview2 satellite images pre-processing (Vermote et al. 1997; Martin et al. 2012), and applying secondly a two step mapping methodology: 1) Spectral characterization (Kruse et al. 1993) and 2) Maximum Likelihood.

For the genetic characterisation of *Zostera noltii* populations 2 to 3 subpopulations were sampled at each location by randomly collected 40 to 50 ramets (Fig.1). Silica-dried samples were used as starting plant material and genomic DNA extractions were carried out using the commercial system OMEGA (EZNA® Plant DNA Kit). Samples were PCR amplified and genotyped for nine microsatellite loci described by Coyer et al. (2004a). Raw allele sizes were scored using STRAND software. After excluding multiplies sampled ramets from the same genet, descriptive statistics of the within-population genetic diversity were calculated using the software Genetix 4.05 (Belkhir et al. 2004) and HP-RARE 1.1 (Kalinowski 2005). Among-population genetic diversity was quantified using the Fst estimator (Weir & Cockerham 1984). We tested for significant differences of allele frequencies using the Fisher exact test (Genepop 4.2, Rousset 2008). An assignment test was performed to potentially estimate direct, real-time migration rates (Genclass 2.0, Piry et al. 2004). The Markov Chain Monte Carlo Bayesian algorithm implemented in STRUCTURE 2.3.4 package (Pritchard et al. 2000) was used to make cluster assignments to estimate the genetic structure of *Z. noltii* at the Atlantic Moroccan Coast.

Figure 1. (a) Geographical location of *Zostera noltii* populations along the Atlantic Moroccan Coast. Stars: places where *Z. noltii* was found. Points: potential sites where *Z. noltii* was not found.

Figure 1. (b-f). Resulting maps of *Zostera noltii* distribution (green) in five Moroccan lagoons using Worldview2 high-resolution images after spectral characterization and Maximum Likelihood process (b). Larache. (c). Moulay Bousselham. (d). Sidi Moussa. (e). Oualidia. (f). Nayla. In each lagoon, points as LAR1, LAR2, LAR3, MOU1, MOU2, SID1, SID2, OUA1, OUA2, NAY1, NAY2, NAY3 represent sampling areas for the *Z. noltii* genetic characterization and in situ surveys points used to estimate *Z. noltii* distribution using high-resolution satellite images.



RESULTS

a) *Zostera noltii* population area estimation and mapping results from high-resolution satellite images (Table 1, Figure 1b-1f).

Table 1. *Z. noltii* area estimations (m²) and coverage rate (%) from high resolution satellite images analysis in five marine lagoons along the Atlantic Moroccan coast.

Lagoon	Image acquisition day and UTC time	Lagoon water coverage (m ²)	<i>Zostera noltii</i> coverage (m ²)	<i>Zostera noltii</i> coverage rate (%)
Larache	Nov 3, 2010 11:31	2587556	38.704	1.50
M. Bousselham	Nov 3, 2010 11:31	8020732	71.508	0.89
Sidi Moussa	Oct 10, 2010 11:39	3090212	23.918	0.77
Oualidia	Oct 3, 2012 12:03	1152792	13.392	1.16
Nayla	Sep 5, 2011 12:05	5200624	269.868	5.19

b) Genetic characterisation of *Zostera noltii* populations. Within (Table 2) and among population genetic diversity (Table 3, Figure 3).

Table 2. *Zostera noltii*. Genetic data set estimates for 9 microsatellite loci in *Z. noltii* populations (n = 5) and subpopulation (n = 12) ordered from North to South Morocco. L: Latitude, I: Longitude, N: number of collected ramets, G: number of genets, R: genotypic richness, T: total number of alleles per population, a: average number of alleles per locus and population, Ho, He: observed and expected heterozygosity, Fis: inbreeding coefficient. ** Means significant departures from Hardy-Weinberg equilibrium (p < 0.01). A, Ap: allelic and private allelic richness standardized to a minimum common sample size of 67 and 18 genotypes (the lowest G recorded for populations and subpopulation respectively).

MEADOW	L	I	N	G	R	T	a	Ho	He	Fis	A	Ap
Larache (LAR)	35° 12.558'N	06° 08.270'O	137	136	0.992	45	5.00	0.4935	0.5480	0.1031**	4.03	0.45
LAR1	35° 12.612'N	06° 08.127'O	43	42	0.976	36	4.00	0.5079	0.5295	0.0528**	3.21	0.10
LAR2	35° 12.377'N	06° 08.572'O	45	45	1	36	4.00	0.5111	0.5541	0.0887**	3.29	0.08
LAR3	35° 12.558'N	06° 08.270'O	49	49	1	37	4.11	0.4649	0.5457	0.1582**	3.22	0.05
M. Bousselham (MOU)	34° 52.159'N	06° 17.330'O	96	94	0.978	77	8.56	0.6123	0.6481	0.0606**	6.99	1.34
MOU1	34° 52.159'N	06° 17.330'O	47	45	0.956	64	7.11	0.5951	0.6307	0.0677**	4.48	0.37
MOU2	34° 52.298'N	06° 17.321'O	49	49	1	71	7.89	0.6281	0.6532	0.0487**	4.71	0.46
Sidi Moussa (SID)	32° 59.072'N	08° 44.523'O	94	88	0.935	49	5.44	0.4609	0.4796	0.0447**	4.48	0.19
SID1	32° 59.072'N	08° 44.523'O	51	47	0.92	41	4.56	0.4397	0.5044	0.1387**	3.53	0.12
SID2	32° 58.452'N	08° 45.119'O	43	41	0.952	40	4.44	0.4851	0.4404	-0.0893**	3.05	0.03
Oualidia (OUA)	32° 45.140'N	09° 01.292'O	91	76	0.833	47	5.22	0.5322	0.5375	0.0166**	4.73	0.22
OUA1	32° 45.140'N	09° 01.292'O	45	38	0.840	40	4.44	0.5906	0.5469	-0.0666**	3.70	0.13
OUA2	32° 44.810'N	09° 01.478'O	46	38	0.822	40	4.44	0.4737	0.5091	0.0827**	3.26	0.07
Nayla (NAY)	28° 01.841'N	12° 14.176'O	137	67	0.485	38	4.22	0.4196	0.4326	0.0375**	3.80	0.70
NAY1	28° 01.841'N	12° 14.176'O	47	29	0.608	33	3.67	0.4138	0.3973	-0.0238**	2.84	0.11
NAY2	28° 02.490'N	12° 14.032'O	45	32	0.704	34	3.78	0.4132	0.4162	0.0231**	2.82	0.06
NAY3	28° 02.200'N	12° 14.630'O	45	18	0.386	29	3.22	0.4383	0.4511	0.0570**	2.81	0.04

Table 3. Matrix showing pairwise Fis-estimator values θ (below diagonal) and pairwise distances in kilometres (above diagonal) among 12 *Zostera noltii* Moroccan subpopulations. All Fis values are significantly different from zero (p < 0.01) except those shown in boldface.

	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	0.0011		0.5	41	41	370	371	406	408	1117	1117	1117
LAR3	-0.0019	0.0010		42	42	371	372	408	409	1118	1118	1118
MOU1	0.1568	0.1412	0.1369		0.2	329	330	366	367	1076	1076	1076
MOU2	0.1369	0.1239	0.1251	0.0063		329	330	366	367	1076	1076	1076
SID1	0.2929	0.2904	0.2793	0.2832	0.2792		1.5	37	38	747	747	747
SID2	0.3400	0.3357	0.3245	0.3278	0.3224	0.0092		36	37	745	745	745
OUA1	0.2407	0.2420	0.2305	0.2704	0.2662	0.0617	0.0844		0.75	710	710	710
OUA2	0.2524	0.2538	0.2406	0.2792	0.2719	0.0451	0.0628	0.0219		709	709	709
NAY1	0.4135	0.4057	0.4089	0.3329	0.3046	0.3960	0.4640	0.4075	0.4209		1.2	1
NAY2	0.4178	0.4114	0.4137	0.3386	0.3088	0.3965	0.4613	0.4064	0.4193	0.0044		1.1
NAY3	0.3817	0.3741	0.3775	0.2967	0.2696	0.3809	0.4491	0.3834	0.3975	0.0050	0.014	

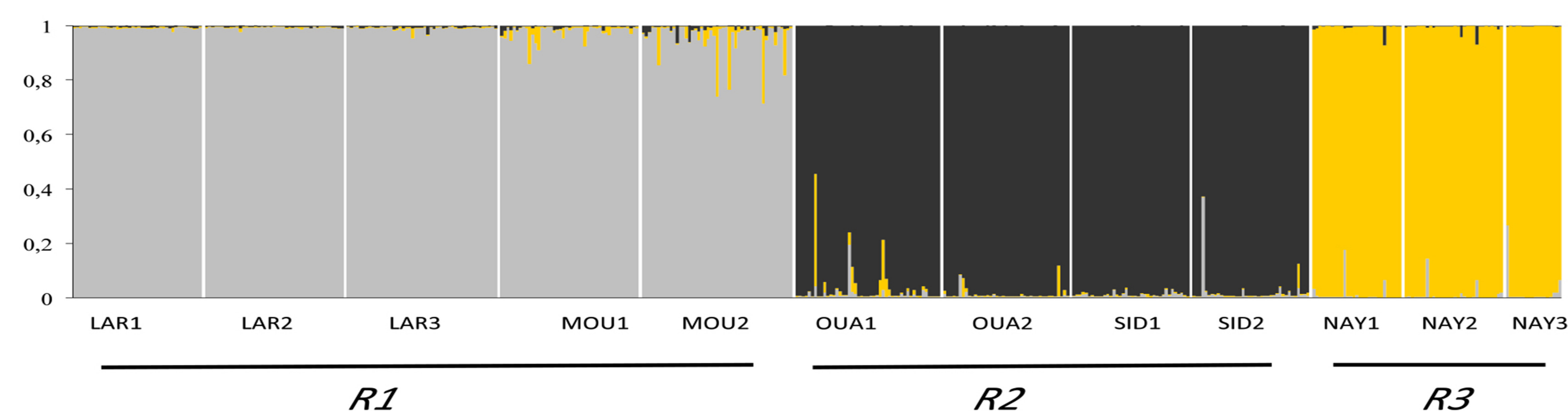


Figure 3. Bayesian assignment of individuals into K=3 genetic clusters using the program STRUCTURE v2.3.4. Each column (or vertical line) corresponds to one individual and each colour represents a single cluster. The vertical height of each column denotes the probability of each individual belonging to each of the inferred clusters. Group R1 comprised populations from the Northern Moroccan Region (LAR1, LAR2, LAR3, MOU1 and MOU2); Group R2 included population from the Centre Moroccan Region (OUA1, OUA2, SID1, and SID2), while Group R3 was formed by populations from South Moroccan Region (NAY1, NAY2, NAY3).

DISCUSSION

Area coverage of *Zostera noltii* population along the Atlantic Moroccan Coast. Seagrass ecosystems are declining worldwide due to habitat destruction and climate global change (Orth et al. 2006). Unfortunately, the Atlantic Moroccan Coast are not an exception to these threats, as we observed the completely disappearance of *Z. noltii* meadows in many estuaries (see Fig. 1a). Appropriate management and conservation strategies become necessary for the remaining seagrass habitats due to their fragility and ecological importance. The analysis of high resolution satellite images from WV-2 sensor carried out in this work has allowed the estimation of *Z. noltii* areas and its distribution in five Atlantic Moroccan lagoons (Table 1 and Fig. 1b-1f) that could be used in future management strategies as a powerful tool to evaluate their conservation status throughout the years. As expected, Nayla shows the highest conservation status, according to the greater area covered by *Z. noltii* (269868 m²) and the greater coverage rate (5.19%), probably due to high ecosystem protection degree and low human interaction. Unfortunately this is not that common to the other four lagoons: cities-ports (Larache) and many agricultural fields (M. Bouselham, Sidi Moussa and Oualidia) are probably affecting *Z. noltii* habitats (Fig. 1b-1f).

Genetic description of *Zostera noltii* population for future management strategies. Along with the traditional seagrass monitoring, it becomes necessary the inclusion of the genetic/genotypic description and the information about populations connectivity for a proper management, which should be extended in time with periodic resampling (Procaccini et al. 2007, Schwartz et al. 2007). Our results showed high genetic diversity in all Moroccan populations, being higher in the Northern Moroccan populations rather than in the South (Table 2). Moulay Bousselham and Larache presented the highest levels of genotypic richness (0.99 and 0.97), where practically each sample analysed correspond to a single genotype raised from a different seed (Arnaud-Haond et al. 2007), highlighting the importance of sexual versus clonal reproduction. This could be related with their less protective status that increase direct anthropogenic disturbance, since moderate levels of disturbance may enhance genotypic diversity due to an increase in sexual reproduction by promoting the recruitment of new seeds through gap formation (Coyer et al. 2004b, Zipperle et al. 2010). However, we consider that anthropogenic activities should be regulated, and, at the same time, the genetic monitoring of these meadows will allow the evaluation of the particular effect of human activities on their survival. By contrast, the least diverse population turned out to be Nayla, the most southern population (Table 2). The less number of different genotypes found in Nayla (R=0.48) indicates higher levels of clonal reproduction. In that sense, Coyer et al. (2004b) have shown that low levels of disturbance promote clonal growth and stability, generating very large and old clones adapted to local conditions. This is consistent with the current protection state of Nayla's Lagoon, which is included in the Khnifiss National Park, where the access is limited and human activities are regulated.

Population connectivity and Management Units. *Z. noltii* populations are shown to be highly isolated due to low long (F_{ST}) and short-term gene flow (Assignment Test, data not shown) and high distance between optimal habitats. Bio-geographical analysis (STRUCTURE) supported the clustering of *Z. noltii* subpopulations into three groups corresponding to three different geographical regions (Fig. 3): Northern Morocco (Larache and Moulay Bousselham), Central Morocco (Oualidia and Sidi Moussa) and Southern Morocco (Nayla). The clear definition of seagrass clusters should be considered for the future management of these spaces, defining Management Units that could be used to improve restoration techniques, avoiding translocation and mixing of very different populations (outbreeding depression), or to prioritize population units for conservation due to the limited economical resources (Allendorf et al. 2010).

In conclusion. Population genetic and habitat description with remote sensing has been combined to detect and describe 5 *Z. noltii* populations along the Atlantic Moroccan Coast. The results show that all of them behave as separate populations with no interaction among them and susceptible to be handled as different Management Units. As a species-ecosystem *Z. noltii* revealed as very sensitive to natural threats thus revealing a gain in genotypic richness as a result of surrounding anthropogenic activities, probably due to higher sexual propagation and recruitment, whilst remain more homogeneous in stable environments with the highest *Z. noltii* coverage. A genetic monitoring through time is necessary to assess the evolution of these meadows facing greater anthropogenic pressures and global changes in order to adopt adequate conservation strategies.

ACKNOWLEDGEMENT

The TechMARAT project is funded by the Ministry of Economy and Treasury of the Spanish Government and the European Regional Development Fund (ERDF), within the framework of the second call of the Operational Programme of Cross-Border Cooperation Spain-External Borders (POCTEFEX). The authors acknowledge the GPIT (Group of Image Processing and Remote Sensing Group) of Las Palmas University for the help in the satellite images pre-processing, and in particular to Javier Martin Abasolo. We also thank Licinia Gouveia (Marine Ecology and Evolution, Centro de Ciências do Mar, Portugal) for the assigned amplification and microsatellite genotyping protocols, and Najat Elkati (Faculté Des Sciences Ain Chock-Casablanca) and Miriam Chikhaoui (Institut Nationale Recherche Halieutique) for their help during field work along the Moroccan Atlantic coast.

REFERENCES

- Allendorf et al. 2010. Genomics and the future of conservation genetics. Nat Rev Genet 11, 697-709.
- Arnaud-Haond et al. 2007. Standardizing methods to address clonality in population studies. Mol Ecol 16, 5115-5139.
- Belkhir et al. 1996-2004. GENETIX 4.05, logiciel sous Windows TM pour la génétique des populations. Laboratoire Génome, Populations, Interactions. CNRS UMR 5171, Université de Montpellier II, Montpellier (France).
- Coyer et al. 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 et al. 2004b. Population genetics of dwarf eelgrass *Zostera noltii* throughout its biogeographic range. Mar Ecol Prog Ser 281, 51-62.
- Kalinowski, 2005. HP-RARE: A computer program for performing rarefaction on measures of allelic diversity. Mol Ecol Notes 5:187-189.
- Kruse et al., 1993. The Spectral Image Processing System (SIPS) - Interactive Visualization and Analysis of Imaging Spectrometer Data. Rem Sens Environ (44) 145 - 163.
- Martin et al., 2012. Atmospheric correction models for high resolution WorldView-2 multispectral imagery: a case study in Canary Islands, Spain. Proc. SPIE 8534.
- Coyer et al., 2006. A global crisis for seagrass ecosystems. Bioscience 56, 987-996.
- Piry et al., 2004. GENELASS2: A Software for Genetic Assignment and First-Generation Migrant Detection. J Hered 95(6): 536-539.
- Pritchard et al., 2000. Inference of population structure using multilocus genotype data. Genetics 155, 945.
- Procaccini et al., 2007. Contribution of genetics and genomics to seagrass biology and conservation. J Exp Mar Biol Ecol, 350, 234-259.
- Raymond and Rousset. 1995. An exact test for population differentiation. Evolution 49, 1280-1283.
- Rousset. 2008. Genepop'007: a complete reimplementation of the Genepop software for Windows and Linux. Mol. Ecol. Resources 8, 103-106.
- Schwartz et al., 2007. Genetic monitoring as a promising tool for conservation and management. Trends Ecol. Evol. 22, 25-33.
- Vermote et al., 1997. Second simulation of the satellite signal in the solar spectrum, 6S, an overview. IEEE Trans Geosci Rem Sens 35, 675-686.
- Weir and Cockerham. 1984. Estimating F-statistics for the analysis of the population structure. Evolution 38, 1358-1370.
- Zipperle et al., 2010. Waterfowl grazing in autumn enhances spring seedling recruitment of intertidal *Zostera noltii*. Aquat Bot 93: 202-205.