

Connectivity, neutral theories and the assessment of species vulnerability to global change in temperate estuaries

Guillem Chust^{a,*}, Aitor Albaina^b, Aizkorri Aranburu^a, Ángel Borja^a, Onno E. Diekmann^c, Andone Estonba^b, Javier Franco^a, Joxe M. Garmendia^a, Mikel Iriondo^b, Iñigo Muxika^a, Fernando Rendo^b, J. Germán Rodríguez^a, Otsanda Ruiz-Larrañaga^b, Ester A. Serrão^c, Mireia Valle^a

^a AZTI-Tecnalia, Marine Research Division, Herrera kaia portualdea z/g, 20110 Pasaia, Spain

^b Laboratory of Genetics, Dpt. Genetics, Physical Anthropology & Animal Physiology, University of the Basque Country (UPV/EHU), Leioa 48940, Spain

^c CCMAR-CIMAR, University of Algarve, Gambelas, P-8005-139 Faro, Portugal

ARTICLE INFO

Article history:

Received 23 August 2012

Accepted 6 August 2013

Available online 17 August 2013

Keywords:

Zostera
Cerastoderma
diversity
climate change
estuary
neutral theory

ABSTRACT

One of the main adaptation strategies to global change scenarios, aiming to preserve ecosystem functioning and biodiversity, is to maximize ecosystem resilience. The resilience of a species metapopulation can be improved by facilitating connectivity between local populations, which will prevent demographic stochasticity and inbreeding. This investigation estimated the degree of connectivity among estuarine species along the north-eastern Iberian coast, in order to assess community vulnerability to global change scenarios. To address this objective, two connectivity proxy types have been used based upon genetic and ecological drift processes: 1) DNA markers for the bivalve cockle (*Cerastoderma edule*) and seagrass *Zostera noltei*, and 2) the decrease in the number of species shared between two sites with geographic distance. Neutral biodiversity theory predicts that dispersal limitation modulates this decrease, and this has been explored in estuarine plants and macroinvertebrates. Results indicate dispersal limitation for both saltmarsh plants and seagrass beds community and *Z. noltei* populations; this suggests they are especially vulnerable to expected climate changes on their habitats. In contrast, unstructured spatial pattern found in macroinvertebrate communities and in *C. edule* genetic populations in the area suggests that estuarine soft-bottom macroinvertebrates with planktonic larval dispersal strategies may have a high resilience capacity to moderate changes within their habitats. Our findings allow environmental managers to prioritize the most vulnerable species and habitats to be restored.

© 2013 Elsevier Ltd. All rights reserved.

1. Introduction

The most important direct drivers of global biodiversity loss and ecosystem service changes are habitat change, climate change, invasive alien species, overexploitation and pollution (Millennium Ecosystem Assessment, 2005). The undergoing destruction and degradation of natural habitats (Halpern et al., 2008) and, as a consequence, the increasing number of threatened species (He and Hubbell, 2011), are particularly severe at the coast, where human population is concentrated (Costanza et al., 1997; McGranahan et al., 2007) and the artificialization and urbanization is increasing at a greater rate than for inland areas (European

Environment Agency, 2006). Climate change is also considered as one of the major threats to coastal and estuarine ecosystems for the 21st Century (FitzGerald et al., 2008; Defeo et al., 2009; Reid et al., 2009; Philippart et al., 2011).

In this context, the conservation of estuarine biota is a priority. One of the main adaptation strategies to global change scenarios to preserve ecosystem functioning and biodiversity is to maximize ecosystem resilience (Mawdsley et al., 2009). The resilience is the system ability to absorb rapid environmental change (Heller and Zavaleta, 2009). The resilience of a species metapopulation can be improved by facilitating connectivity among local populations, which will prevent demographic stochasticity and inbreeding (Loew, 2000). Despite the fundamental role played by dispersal and population connectivity in determining the resilience of populations (e.g. Hastings and Botsford, 2006; Cowen et al., 2007; Bradbury et al., 2008; Cowen and Sponaugle, 2009), there is still

* Corresponding author.

E-mail address: gchust@azti.es (G. Chust).

a limited knowledge about the dispersal strategies of many marine species due to the difficulty of tracking the trajectory and fate of propagules (e.g. Shanks, 2009; Weersing and Toonen, 2009; Selkoe and Toonen, 2011). In the seascape, the connectivity among populations is mainly determined by the ocean currents and the dispersal capacity of organisms (e.g. Gawarkiewicz et al., 2007; Alberto et al., 2011; Coleman et al., 2011), as well as by the demographic prospects of reproduction, mortality and migration rate (Hubbell, 2001). In the particular case of ecotones such as estuaries, the movement of organisms or propagules can be very different between species depending on both habitat specificity and dispersal strategies (Boström et al., 2010). Thus, it is difficult to anticipate, for a given spatial scale, whether estuarine species circumvent habitat discontinuities, or on the contrary, are dispersal limited. The analysis of highly polymorphic molecular markers is a powerful tool for the study of connectivity among marine populations (e.g. Bradbury et al., 2008; Galindo et al., 2010; Selkoe et al., 2010; Alberto et al., 2011). Although dispersal limitation factors has been recognized to be a common process shaping both species and genetic diversity (Vellend and Geber, 2005), the first empirical evidence has been recently proved in aquatic beetles (Baselga et al., 2013).

Our main objective here is to estimate the degree of connectivity among the structural estuarine species (plants and macro-invertebrates) along the Basque coast (south-eastern Bay of Biscay), in order to assess community vulnerability in the face of global change scenarios. Basque estuaries have been radically transformed by anthropogenic activities during the 20th century (Cearreta et al., 2004; Chust et al., 2009), and they are expected to be impacted throughout the 21st century by a sea level rise of 29–49 cm (Chust et al., 2010), storm surges (Marcos et al., 2012), warming of surface air (especially heat wave episodes) and sea of 1.5–2.0 °C (Chust et al., 2011) and the intensification of extreme daily rainfall of 10% (Chust et al., 2011). Thus, the specific objectives are: 1) to determine and compare the connectivity between species communities across two estuarine taxa groups (i.e. plants of saltmarshes and intertidal seagrass meadows, and soft-bottom macroinvertebrates in intertidal and shallow waters); 2) to determine and compare the genetic connectivity between estuarine populations along the North Atlantic Iberian coast of two species with different dispersal strategies and life traits: the seagrass *Zostera noltei* and the bivalve cockle (*Cerastoderma edule*). Recent studies determining population genetic structure of sympatric marine species (e.g. Kinlan and Gaines, 2003; Selkoe et al., 2010) have provided valuable information on gene flow through the seascape. Estimating and understanding marine connectivity can also help environmental managers to guide and prioritize local restoration plans for both species and habitats, as well as to define strategies for climate change adaptation.

To address these objectives, two proxies of connectivity have been used, based upon genetic and ecological drift processes and dispersal limitation factors (Vellend and Geber, 2005). Under a 'stepping stone model' of dispersal, in which populations tend to exchange migrants (or propagules) with nearest neighbours along the coastline, the Isolation By Distance (IBD) theory suggested that pairwise genetic variation (for instance the Wright's fixations index F_{ST}) will increase with the geographic distance between the pair of populations (Wright, 1943; Kimura, 1953; reviewed in Selkoe and Toonen, 2011). IBD assumes neutral alleles (not affecting fitness) and populations at equilibrium between dispersal and genetic drift (Hutchinson and Templeton, 1999). In contrast, in a metapopulation where all populations exchange migrants equally, F_{ST} is not correlated with geographic distance. Therefore, the slope of IBD is commonly used for estimating dispersal distance with genetic markers. The IBD theory is a central concept of the neutral theory of

molecular evolution (Kimura, 1983), which states that most of evolutionary changes at the molecular level is the result of randomly genetic drift acting on neutral alleles (not affecting fitness). In ecology, although disentangling whether the regional distribution of species arises from dispersal limitation (MacArthur and Wilson, 1967) or by niche adaptive processes (Hutchinson, 1957) have long debated, the emergence of the concept of neutrality (Hubbell, 2001) appeared more recently than in population genetics. The neutral theory of biodiversity developed by Hubbell (1997, 2001) is *neutral* because all individuals are assumed to have the same prospects for reproduction and death (Chave, 2004). Under Hubbell's model, where a metacommunity is composed by a set of communities exchanging individuals of different species with a migration rate, the variability in relative abundances across species is solely due to demographic stochasticity or 'ecological drift'. When migration rate is low in such a model (i.e. species are dispersal limited), species cross-site similarity (i.e. the opposite of β -diversity) is predicted to decline logarithmically with increasing geographical distance (Hubbell, 2001; Chave and Leigh, 2002). This pattern, named 'distance decay', has been observed for different taxonomic groups: rainforest trees (e.g. Condit et al., 2002; Chust et al., 2006a), coral reefs (Hubbell, 2001), marine bacteria (e.g. Martiny et al., 2011) and plankton (Irigoin et al., 2011; Chust et al., 2013; but see; Cermeño et al., 2010). The estimated correlation and rate of change (i.e. slope) in species similarity with increasing geographic distance, hence, can be used to compare the degree of dispersal limitation among different communities (Chust et al., 2006b). However, species distributions and environmental variables tend to be spatially autocorrelated (Legendre, 1993), hence distance decay might be a result from the relation between species composition and environmental niche factors. In such a case, appropriate statistical techniques need to be applied in order to disentangle both effects (e.g. Chust et al., 2013). Within this framework, our expectations in estuarine species is that the group of species more limited by dispersal at the community level should also have more fragmented genetic population structure, in accordance to recent findings in aquatic beetles (Baselga et al., 2013).

2. Material and methods

2.1. Study area

The study area is located within the Basque Country (Spain), in the southeastern part of the Bay of Biscay (Fig. 1). The total length of this section of the coast is ca. 150 km, with 13 main estuaries (Borja and Collins, 2004) (Fig. 1). The maritime climate along the Basque coast is related mainly to its location within the Bay of Biscay and the NE Atlantic (González et al., 2004). In relation to its location and orientation, this part of the coast is exposed to large storms from the NW, produced by evolution of the North Atlantic low pressure systems. Strong NW swell waves dominate and are the most common sea state within the study area. The tidal wave is semi-diurnal in character within the Bay of Biscay (Uriarte et al., 2004). Along the Basque coast, the mean tidal range is approximately 1.65 m on neap tides and 4.01 m on springs (Chust et al., 2010). Despite the importance of tidally-induced surface water fluctuations, the contribution of the tides to the generation of currents is somewhat modest (except within the estuaries) (Uriarte et al., 2004). The coast is dominated by rocky substrata with vertical cliffs intercalated with small estuaries and sandy beaches. The continental shelf in the Basque Country is characterized by its narrowness; it ranges from 7 km to 20 km (Galparsoro et al., 2010). As a consequence of this coastal configuration, salt-marshes are

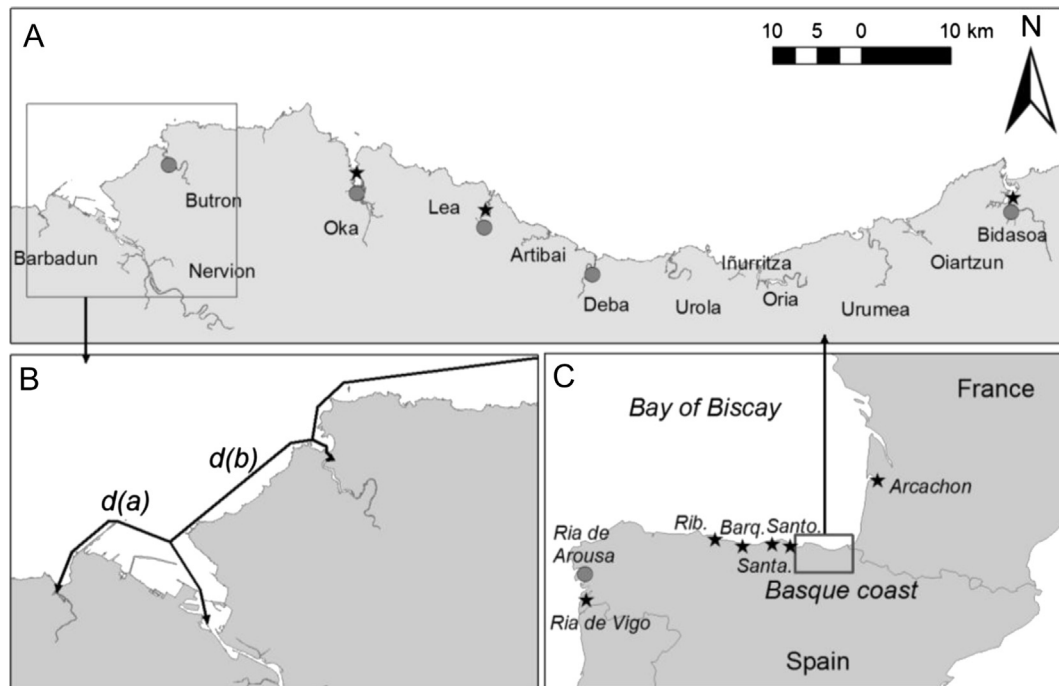


Fig. 1. A) Estuaries of the Basque coast, C) Galician (Ria de Vigo and Ria de Arousa), Asturian (Rib.: Ribadesella), and Cantabrian (Barq.: Sant Vicente de la Barquera, Santa.: Santander, Santo.: Santoña) sampling stations, in the northwestern Spain, and Arcachon (France) station. B) Detail of the minimum path distances (d) between adjacent estuaries. Stars: genetic sampling stations for *Z. noltei*. Circles: genetic sampling stations for *C. edule*.

few, fragmentary and restricted to the inner parts of the estuarine areas (Cearreta et al., 2002).

2.2. Population genetics: sampling and genotyping

2.2.1. *Zostera noltei*

The seagrass *Zostera noltei* (Hornemann, 1832) is an estuarine flowering plant which forms meadows at the muddy and sandy flats within the intertidal zone. The seagrass colonization mainly proceeds through clonal growth through rhizomes. It is distributed widely within the coastal zones from the southern coasts of Norway to the Mediterranean Sea, the Black Sea, the Canary Islands, with the southern limit on the Mauritanian coast (Valle et al., 2011). *Z. noltei* has been reported to occur along most northern Spanish estuaries, and there are also historical references about its presence along the Basque Country estuaries, but nowadays is present only in three (Oka, Lea and Bidasoa) of them (Valle et al., 2011; Fig. 1). The general degradation in quality of Basque estuaries over the last two centuries, due to human pressures (Borja et al., 2006a), might explain the decline (from 6 to 3 vegetated estuaries) of this species within the region (Valle et al., 2011).

Zostera noltei samples were collected from nine estuaries in Northern Spain and Western France: three in the Basque Country region (Oka, Lea and Bidasoa) (Fig. 1) in which the plant is still present (Valle et al., 2011), three estuaries in Cantabria (St. Vicente de la Barquera, Santoña, Santander) and one in Asturias (Ribadesella) regions, one estuary in France (Arcachon), and one population from Galicia region, Ria de Vigo estuary ($42^{\circ}21'2.95''\text{N}$ and $8^{\circ}36'57.76''\text{W}$) as a distant reference population. Shoots (between 17 and 70 per estuary) were collected randomly with at least 2 m distant between samples.

Genomic DNA was extracted from silica dried plant material by a CTAB (2% hexadecyl trimethylammonium bromide) method. Seven microsatellite loci (Genbank accession codes: ZnB1, ZnB8, ZnD6, ZnF11, ZnF8, ZnH10, ZnH8) were PCR amplified using fluorescently

labelled primers (Coyer et al., 2004). PCR products were visualized using an ABI-3130XL automated sequencer (Applied Biosystems). Raw allele sizes were scored with STRAND (<http://www.vgl.ucdavis.edu/informatics/STRand/>), binned using the R package MsatAllele (Alberto, 2009) in R software (R Development Core Team, 2011), and manually reviewed for ambiguities.

2.2.2. *Cerastoderma edule*

The common cockle, *Cerastoderma edule* (L.), is a bivalve with a north Atlantic distribution. This species inhabits in estuaries and sandy bays and its development include a planktonic larval phase (Young et al., 1996). Planktonic larvae dispersal has been reported to last up to 3–6 weeks in the water column (Marine Life Information Network; available at: <http://www.marlin.ac.uk>). A total of 479 individuals of *C. edule* were sampled at the estuaries of Butron, Bidasoa, Deba, Lea and Oka (Basque Country, Fig. 1) and at Carril (Ria de Arousa, Galicia, NW Spain, $42^{\circ}21'57''\text{N}$, $8^{\circ}27'46''\text{W}$). Cockles were sampled at intertidal sandy areas by sieving, and froze in a freezer until analyses.

Genomic DNA was extracted from 25 mg of muscle tissue for each individual using Nucleospin® 96 Tissue Kit (Macherey–Nagel). The DNA from each sample was subsequently quantified in a NanoDrop ND-8000 spectrophotometer (Thermo Fisher Scientific). The 12 polymorphic microsatellites developed by Martínez et al. (2009) were genotyped for the 479 *Cerastoderma edule* individuals to account for genetic diversity and relatedness. These markers were amplified in two independent multiplex polymerase chain reactions (PCR). The two multiplexed reactions, namely COCKLE1 and COCKLE2, included, respectively, 8 and 4 markers. While COCKLE1 included CeATC1-5, CeATC1-36, CeATC1-52, CeATC2-4, CeATC2-11, CeATC2-34, CeATC2-44 and CeATC2-46, microsatellite loci, COCKLE2 consisted of CeATC1-22, CeATC1-54, CeATC2-12 and CeATC2-51 ones. All PCR reactions were carried out in a final volume of 12.5 μl in a Veriti™ Thermal Cycler (Applied Biosystems). Each multiplex PCR reaction contained 5–10 ng DNA.

While COCKLE1 reaction contained 1 μ l MgCl₂ (25 mM), 0.30 μ l of each primer (10 μ M), except for 0.50 μ l of CeATC2-46 ones, 1.25 μ l GeneAmp® PCR Buffer II (Applied Biosystems), 1 μ l dNTPs (2.5 mM each), 0.48 μ l AmpliTaq Gold DNA Polymerase (5 U/ μ l; Applied Biosystems) and 2.07 μ l ultrapure H₂O, COCKLE2 reaction included 1.25 μ l MgCl₂, 0.30 μ l of each primer, except for 0.50 μ l of CeATC2-51 ones, 1.25 μ l PCR Buffer, 1 μ l dNTPs (2.5 mM each), 0.24 μ l DNA Polymerase and 4.46 μ l ultrapure H₂O. Both multiplex PCRs were performed using the following conditions: 5 min initial denaturation at 95 °C, followed by 34 cycles of denaturation for 30 s at 95 °C, 30 s at annealing temperature (T_a = 60 °C), and 30 s at 72 °C for extension. An extra final extension for 10 min at 72 °C was included after the last cycle. PCR products were analysed in two independent electrophoresis runs with an ABI Prism 3100 Avant Genetic Analyzer (Applied Biosystems). Raw data were processed with GENESCAN 3.7.1 and allele calling was performed with GENOTYPER 3.7 software (Applied Biosystems). Only the nine, out of twelve, microsatellite loci that were successfully genotyped, meaning that were polymorphic and reliably scored, were considered for further analysis (CeATC1-5, CeATC1-36, CeATC2-4, CeATC2-11, CeATC2-34, CeATC2-44, CeATC1-22, CeATC1-54 and CeATC2-12).

2.3. Statistical analysis: genetic diversity and divergence

In *Zostera noltei*, after removal of duplicate multilocus genotypes (ramets, i.e. shoots, belonging to the same genet, which is a group of genetically identical individuals), heterozygosity (H_e and H_o) and inbreeding coefficient (F_{IS}) were calculated using Genetix version 4.05 software (Belkhir et al., 2004). Genotypic diversity, the proportion of different genets in each sample, was estimated as R following Dorken and Eckert (2001): $R = (G - 1)/(N - 1)$, where N corresponds to the number of collected individuals and G to the number of genets (i.e. clonal colony). R ranges from 0 (all samples belong to the same genet) to 1 (all samples are unique genetic individuals). Also, the genetic variation was calculated as the allelic richness (\hat{A}) standardized for sample size (standArich package for R language, see Alberto et al., 2006). In *Cerastoderma edule*, FSTAT v.2.9.3 software (Goudet, 2001) was used to estimate allelic richness (AR), heterozygosity (H_e and H_o) and inbreeding coefficient (F_{IS}).

The presence and frequency of null alleles was tested for both species using the expectation maximization algorithm of Dempster et al. (1977) implemented in FreeNA (Chapuis and Estoup, 2007). Genetic divergence between populations was assessed by means of calculating two differentiation measures: 1) fixation index (F_{ST} ; Weir and Cockerham, 1984) using FreeNA software (Goudet, 1995, 2001) that provides null allele corrected F_{ST} values, and 2) D (Jost, 2008). Statistical significance of F_{ST} was obtained bootstrapping over loci; probability was $p < 0.05$ when the 95% confidence interval did not include 0 (F_{ST} statistically different from zero).

2.4. Genetic metrics of dispersal

Interconnectivity of populations was examined through use of cluster analysis and by calculation of isolation by distance. Isolation by distance was tested for both *Zostera noltei* and *Cerastoderma edule* species by searching for correlation between genetic and geographic distance matrices with Mantel approach (Legendre and Legendre, 1998). This test computes a statistic r_M which measures the correlation between two matrices and is tested by a non-parametric permutation test. Geographic distance was measured as the minimum path distance between two estuaries along the coastline, i.e. circumventing the terrestrial zone, using geographic information system tools (Fig. 1b). Genetic distance was estimated by $F_{ST}/1 - F_{ST}$ (Rousset, 1997).

Population structure was analysed using the Bayesian clustering techniques implemented in the software STRUCTURE 2.3.1 (Pritchard et al., 2000). STRUCTURE uses individual multilocus genotype data to cluster individuals into K groups while minimising Hardy–Weinberg disequilibrium and gametic phase disequilibrium between loci within groups. First we calculated the mode of ΔK (Evanno et al., 2005) which is a good predictor for the real number of clusters in the data. Then STRUCTURE was run for the modal value with 100,000 burnin and 1,000,000 Markov Chain Monte Carlo (MCMC) iterations.

2.5. Floristic and faunal β -diversity across estuaries

The floristic (flowering plants) inventory of mudflats and salt-marshes, and soft-bottom macroinvertebrates have been analysed in order to test whether population isolation among estuarine communities takes place at the geographic scale of the study area (Basque coast). To this end, the correlation strength and slope between species similarity among estuaries and the oceanographic distance is interpreted as a surrogate of dispersal limitation (Chust et al., 2006b). Thus, the greater the isolation the lower the capacity of the community species, on average, to adapt to new conditions. The dataset on these species, together with information on dispersal modes, is given below.

Plant (angiosperms) inventory of saltmarshes and seagrass beds was obtained from Silván and Campos (2002). The sampling considered all plant species found in saltmarshes, exclusive of halophytic wetlands and intertidal seagrass meadows, and excluded exotic species and those associated to inland habitats. The sampling was undertaken in 2001 over 9 estuaries (Barbadun, Butron, Oka, Lea, Deba, Urola, Iñurritza, Oria, Bidasoa) since Nervion, Urumea and Oiartzun did not present saltmarsh habitats. Overall dataset presented 31 plant species (Table S1). The estuarine occurrence of *Zostera noltei* seagrass was updated from Valle et al. (2011). The flowering plants have different dispersal modes (Table S1): 13 species (41%) are dispersed by water exclusively or also by other modes, 5 by wind only, 4 by animals only and 9 are unassisted (i.e. seeds drop to the ground close to or beneath the parent plant). In terms of dispersal efficiency, it is also important to take into account the pollinization modes (Table S1): 20 species (65%) by wind only, 7 by insects only, 2 by water only and 2 species use multiple modes.

The soft-bottom macroinvertebrate data were obtained from the abovementioned water monitoring network (see Borja et al., 2009b; Pascual et al., 2011). The sampling considered all macroinvertebrates taxa found in 3 replicates of 0.25 m² within the lower to mid intertidal zone (circa 1–2 m above minimum astronomical low tide), except for Nervion and Oiartzun, where the subtidal was sampled, using a van Veen grab of 0.07 m² (for details, see Borja et al., 2009b). Data considered here corresponded to the period from 2002 to 2008, with samples taken once a year, in winter, over 12 estuaries (Barbadun, Nervion, Butron, Oka, Lea, Artibai, Deba, Urola, Oria, Urumea, Oiartzun, Bidasoa). The number of sampling sites per estuary was different (from 2 to 5 sites), depending on the size of the estuary. For the analysis, species presence was counted over all sites of an estuary. Only species taxonomically identified to, at least, the genus level were considered. Overall dataset presented 321 species (Table S2). Among all species found in Basque estuaries, 49 of them had information available on the dispersion mode of their larvae (Table S2): 39 species (80%) are planktonic, 4 planktonic and benthic, 3 benthic only, 2 with no larvae phase and 1 reptant.

The correlation between species similarity and oceanographic distance across estuaries was estimated using the Mantel test (Legendre and Legendre, 1998). In our case, the number of species of each estuary greatly differs mainly because of different sampling

effort, as a consequence of different estuary size. This is due to the well-known species–area relationship (e.g. Rosenzweig, 1995; Rumohr et al., 2001; Ricotta et al., 2002; Sabetta et al., 2007). Hence, the quantification of similarity (i.e. β -diversity) is problematic (Koleff et al., 2003). One of the most used similarity measure in equal sampling areas is the Jaccard index (Koleff et al., 2003), which is the number of species shared between the two sites (a), divided by the total number of species observed ($a + b + c$), where b is the total number of species that occur in site 1 and c is the total number of species that occur in site 2. If there is a large difference in richness between sites (here, estuaries), the Jaccard index will always be small. To address this problem, we used two “narrow sense” dissimilarity indices that focus on compositional differences independent of species richness gradients (Koleff et al., 2003): β_{sim} (Lennon et al., 2001), and β_{-3} (Williams, 1996):

$$\beta_{\text{sim}} = 1 - \frac{a}{\min(b, c) + a} \quad (\text{Lennon et al., 2001})$$

which express the proportion of shared species with respect to the minimum number of species of the two sites; and

$$\beta_{-3} = \frac{\min(b, c)}{a + b + c} \quad (\text{Williams, 1996,})$$

re – expressed by Koleff et al., 2003)

which was proposed to map species turnover adjusting for sites with different sizes. Dissimilarity measures were converted to similarity indices ($=1 - \text{dissimilarity}$).

Oceanographic distance was measured as in the case of *Zostera noltei* and *Cerastoderma edule* populations. We also used Mantel tests to determine the correlation between species similarity matrices and geographic distance. The rate of change (i.e. slope) in species similarity with increasing geographic distance (i.e. distance decay) was calculated fitting a linear model.

Since distance decay may also result from the relation between species composition and environmental niche factors (Chust et al., 2013) of the estuary, partial Mantel tests were undertaken to determine the relative contribution of geographic distance and environmental distance in accounting for species composition similarity. Environmental features comprised eight geomorphological and hydrological variables of the estuary extracted from Uriarte et al. (2004): drainage area, mean river flow, estuary length, estuary volume, subtidal volume, estuary area, intertidal area and tidal prism. Pairwise environmental distances was computed using Euclidean distance.

3. Results

3.1. Genetic analysis of *Zostera noltei*

The number of alleles per marker showed values ranging from 11 to 18, H_e values varied from a minimum of 0.29 to a maximum of 0.81 (meaning 1 total absence of homozygotes). All loci show significant positive F_{IS} except for ZnF8 (Table 1). Null allele frequencies estimated for *Zostera noltei* loci were negligible ($r < 0.05$, according to Chapuis and Estoup (2007)) or nearly negligible (0.06–0.07). Genotypic diversity (R) ranged from a low (0.20) in Lea to a high value (1.00) in Arcachon and Santoña (Table 2). *Z. noltei* patches in Lea were dominated by a single clone, while in Ria de Vigo almost each collected shoot was a genetically a unique individual. Observed heterozygosity ranged from 0.26 to 0.62. There was significant deviation from Hardy–Weinberg equilibrium as shown by positive F_{IS} values in all estuaries except for Lea, Ribadesella and

Table 1

Allele number, observed heterozygosity (H_o), expected heterozygosity (H_e), and inbreeding coefficient (F_{IS} ; * $p < 0.05$, NS = Not Significant) for three sampling locations of *Zostera noltei* from the Basque estuaries and one from a distant location in the Galicia region (Ria de Vigo, NW Spain).

Locus	Number of alleles/locus	H_e	H_o	F_{IS}	Null allele frequency
ZnB1	18	0.81	0.71	0.13*	0.02
ZnB8	18	0.71	0.47	0.35*	0.06
ZnD6	15	0.68	0.44	0.35*	0.07
ZnF11	15	0.64	0.46	0.27*	0.05
ZnF8	10	0.29	0.27	0.07 ^{NS}	0.01
ZnH10	15	0.74	0.60	0.20*	0.02
ZnH8	12	0.64	0.55	0.14*	0.06

San Vicente de la Barquera, resulting in heterozygote deficiency. Allelic richness, when standardized for the minimum genet number (Lea, $G = 10$), was 2.65 in Bidasoa but much higher in Oka, Ria de Vigo and Lea.

Although no correlation was observed between geographic distance and genetic distance ($r_M = 0.12$ and $p < 0.223$ for F_{ST} , $r_M = 0.04$ and $p = 0.294$ for D , see Fig. 2), populations of *Zostera noltei* in the studied estuaries were highly differentiated as estimated by high pairwise F_{ST} and D , indicating distinct and dynamically independent populations (Table 3). Moreover, estuaries closer than 65 km had F_{ST} and D lower than those separated farther than that distance (t -test p -value = 0.0011, and 0.0002, respectively; see Fig. 2). These results indicate low connectivity. Regarding patterns within estuaries, we detected large clones in all three Basque estuaries (results not shown). Each site contained at least one dominant clone covering an area of ca. 3 ha (i.e. shoots collected across the sampling area in different patches belonged to the same multilocus genotype).

In STRUCTURE analysis, the most probable number of clusters (ΔK) when considering all populations resulted in $K = 2$ and a less supported solution of $K = 3$ and $K = 8$ (Fig. 3a). The $K = 2$ plot (Fig. 3b) shows clustering between Vigo and Bidasoa separated from the others. However, after repeating the STRUCTURE analysis with only those two populations resulted also in $K = 2$ and clearly show that Bidasoa and Vigo are different. With $K = 8$, all populations are separated from each other, except Lea that is clustered with Ribadesella (Fig. 3b). The $K = 3$ plot, which has a similar $L(K)$ than $K = 8$, shows Vigo and Bidasoa together and also Santander and Santoña are clustered.

3.2. Genetic analysis of *Cerastoderma edule*

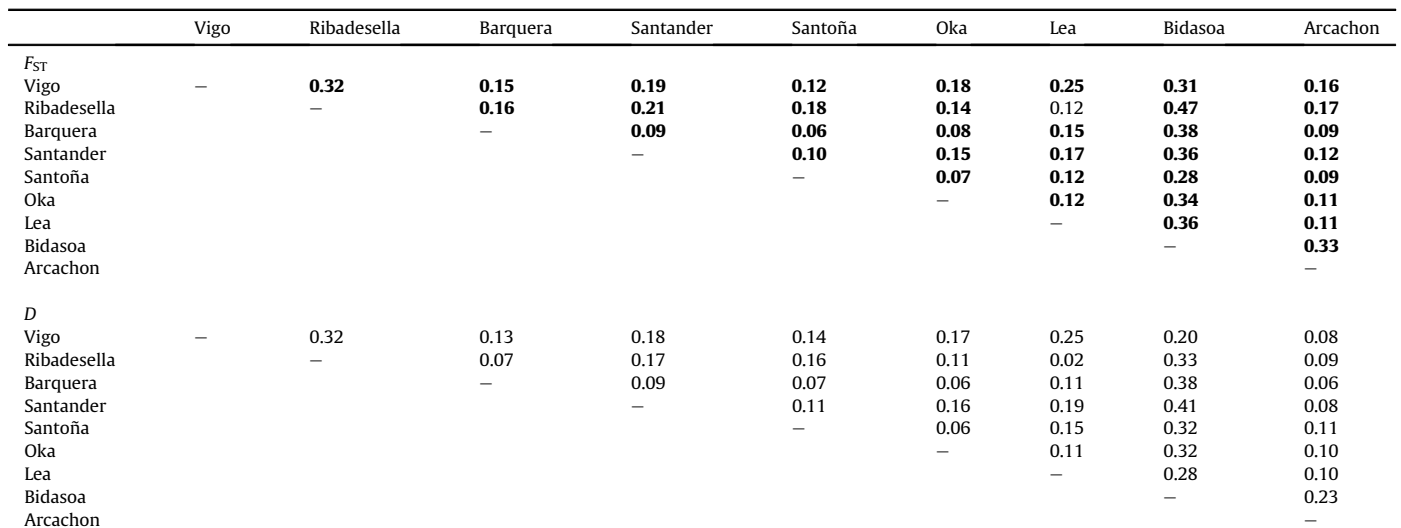
As stated previously, only nine microsatellites (out of the twelve that were genotyped) were considered for *Cerastoderma edule*

Table 2

Number of collected individuals (N), number of genets (G), genotypic diversity (R) and standardized genetic diversity or allelic richness (AR), inbreeding coefficient, and observed (H_o) and expected (H_e) heterozygosities for three sampling locations of *Zostera noltei* from the Basque estuaries and one from a distant location in the Galicia region (Ria de Vigo, NW Spain).

Location	N	G	R	$AR (G = 10)$	H_e	H_o	F_{IS}
Vigo	48	44	0.91	4.343 ± 0.485	0.557	0.508	0.0993*
Ribadesella	40	14	0.33	2.654 ± 0.112	0.350	0.415	−0.1496*
San Vicente de la Barquera	50	35	0.69	4.463 ± 0.335	0.537	0.548	−0.0076
Santander	40	38	0.95	4.623 ± 0.305	0.587	0.544	0.0867
Santoña	40	40	1.00	5.209 ± 0.404	0.646	0.615	0.0605
Oka	70	52	0.74	4.263 ± 0.422	0.537	0.492	0.0934*
Lea	47	10	0.20	3.857 ± 0.000	0.487	0.582	−0.1379
Bidasoa	54	28	0.51	2.651 ± 0.181	0.310	0.263	0.1716
Arcachon	17	17	1.00	5.08 ± 0.336	0.569	0.424	0.2864

*Significant F_{IS} ($p < 0.05$).



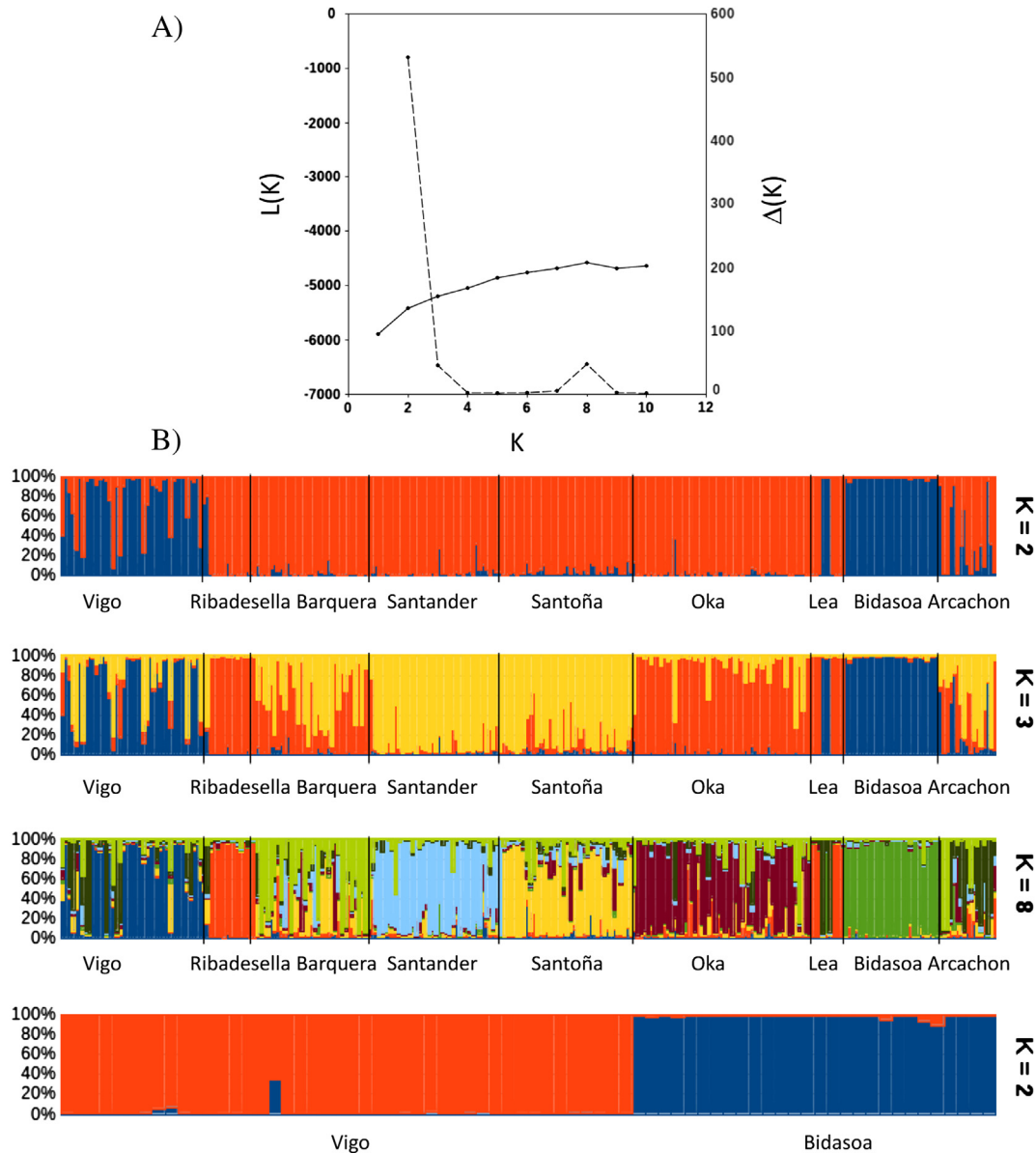


Fig. 3. A) $\Delta(K)$ as a function of K groups in STRUCTURE analysis. B) Barplot showing results from STRUCTURE with K groups (colours) for *Zostera noltei*. On the y-axis is the individual membership proportion. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

correct clusters number within a given dataset increases with F_{ST} , and that the test fails at F_{ST} values of 0.01–0.02 (Latch et al., 2006).

3.3. Floristic and faunal β -diversity across estuaries

The Mantel correlations between species similarity (for the two measures used: $1 - \beta_{sim}$, $1 - \beta_{-3}$) and the oceanographic distance was statistically significant in plants, whilst in macroinvertebrates were not statistically significant (Fig. 4, Table 7). Environmental distances were not statistically correlated with species similarity in any of the taxa group. The partial Mantel correlations between plant species similarity and the oceanographic distance, partialling out environmental distance, were statistically significant. This indicates that plants were dispersal limited. In contrast, macroinvertebrates did not present a patchy structure, suggesting that species did not have important movement barriers and populations (in average) were highly connected.

4. Discussion

Results indicate that similarity of plant assemblages of salt-marshes and seagrass beds decreases with oceanographic distance among estuaries, while it is unrelated with environmental niche factors. This is interpreted as plants are more isolated between estuaries than soft-bottom macroinvertebrates. As shown here, 59% of salt-marsh species are dispersed by modes other than water (i.e. wind, animals or unassisted) and 23% of species are pollinated by insects. Given the steep geomorphological configuration of the Basque coast, with few and fragmentary salt-marshes restricted to inner parts of the estuaries, and separated apart by hilly reliefs and seacliffs, it is possible that most of these plant species (especially those having wind, animal or unassisted modes of seed dispersal, and those pollinated by insects) are constrained to spread (Friess et al., 2011). In contrast, most of the soft-bottom macroinvertebrates (80%) spread through planktonic larvae; this could explain the unstructured spatial distribution of those communities,

Table 4

Allele number, observed heterozygosity (H_o), expected heterozygosity (H_e), inbreeding coefficient (F_{IS} ; * $p < 0.001$, NS: Not Significant) and estimated null allele frequency values for each of the markers considered for population genetics analysis in *C. edule* (see Material and Methods for further information). According to Chapuis and Estoup (2007) non-negligible null allele frequencies (in bold) are categorized as moderate ($0.05 \leq r < 0.20$) or large ($r \geq 0.20$).

Microsatellite	Allele number	H_o	H_e	F_{IS}	Null allele frequency
CeATC1-5	26	0.445	0.902	0.507*	0.240
CeATC1-36	11	0.346	0.418	0.171*	0.075
CeATC2-4	15	0.400	0.682	0.413*	0.171
CeATC2-11	51	0.658	0.887	0.259*	0.122
CeATC2-34	15	0.620	0.790	0.215*	0.095
CeATC2-44	11	0.600	0.633	0.051 ^{NS}	0.030
CeATC1-22	11	0.710	0.797	0.109*	0.049
CeATC1-54	31	0.426	0.794	0.464*	0.202

suggesting no dispersal barriers at this regional scale. Chang et al. (2007) also concluded that medium to long distance diaspore dispersal is rare in saltmarsh systems except in extreme weather events.

Zostera noltei had highly population differentiation in north Iberian estuaries as estimated by high pairwise F_{ST} and D values and clustering techniques. Although no correlation was observed between geographic distance and seagrass genetic distance, estuaries closer than 65 km had F_{ST} and D lower than those separated farther than that distance. Highly-structured populations of this seagrass could be attributed to: 1) small effective population size (the resulting genetic drift may be higher than migration rates), and 2) founder effects (*Z. noltei* is a clonal plant with potential long-lived generations). This suggests a low recolonization rate in agreement with Thayer et al. (1975) and Diekmann et al. (2005). In particular, the latter found genetic population differentiation in the West Iberian coast, as a result of habitat isolation, small populations and near-shore currents. Friess et al. (2012) also pointed out that large-scale exchange of genetic material is potentially constrained by distance between saltmarsh populations due to poor diaspore buoyancy and flotation. The combination of limited dispersal capacity of *Z. noltei*, its clonal strategy and the genetic drift, might be problematic for long-term conservation perspective in the face of habitat changes, which might exacerbate its declining situation (from 6 to 3 vegetated estuaries through the 20th Century) within the Basque Country (Valle et al., 2011). At the community level, saltmarshes and seagrass beds, and especially those species with low occurrence, and those with animal-based dispersal mode or unassisted, can be also considered as vulnerable to habitat changes because of dispersal limitation.

Pairwise F_{ST} and D results for *Cerastoderma edule* individuals collected from five different Basque estuaries and a NW Spain location showed a lack of genetic structure for this species in the studied area. Therefore, *C. edule* in the Northern coast of the Iberian Peninsula would conform a panmictic population. The high degree of polymorphism (denoted by allelic richness and observed heterozygosity values) of the molecular markers applied in this study

Table 5

Expected heterozygosity (H_e) and allelic richness (AR) when pooling individuals per estuary in *C. edule*.

Estuary	H_e	AR
Bidasoa	0.722 ± 0.160	6.21 ± 2.37
Deba	0.729 ± 0.154	6.51 ± 2.30
Lea	0.711 ± 0.152	5.91 ± 2.28
Oka	0.725 ± 0.155	6.43 ± 2.38
Butron	0.725 ± 0.152	6.66 ± 2.41
Ria de Arousa	0.748 ± 0.142	7.15 ± 2.73

Table 6

Pairwise F_{ST} values with (below diagonal) and without (above diagonal) applying null allele correction in *C. edule* (see Material and Methods; significant values in bold). Matrix below show pairwise D values in *C. edule*.

	Bidasoa	Deba	Lea	Oka	Butron	Arousa
F_{ST}						
Bidasoa	—	0.0040	0.0017	−0.0006	−0.0044	0.0024
Deba	0.0043	—	0.0065	0.004	0.0096	0.0038
Lea	0.0017	0.0038	—	0.0023	0.0039	0.0121
Oka	−0.0008	0.0042	0.0013	—	0.0053	0.0055
Butron	−0.0026	0.0098	0.0044	0.0065	—	0.0057
Arousa	0.0030	0.0031	0.0074	0.0048	0.006	—
D						
Bidasoa	—	0.0078	0.0134	0.0013	0.0000	0.0081
Deba		—	0.0154	0.0090	0.0218	0.0092
Lea			—	0.0068	0.0101	0.0301
Oka				—	0.0087	0.0082
Butron					—	0.0138
Arousa						—

implies a high discriminatory power when identifying sub-populations and thus gives further support to the reported pattern. Moreover, present results point to a high degree of connectivity among *C. edule* assemblies along the north Iberian estuaries and suggest cockle being a highly mobile species, through the planktonic larvae dispersal, that has been reported to last up to 3–6 weeks in the water column (Marine Life Information Network; available at: <http://www.marlin.ac.uk>). On the other hand, large effective population size may also explain the lack of significant

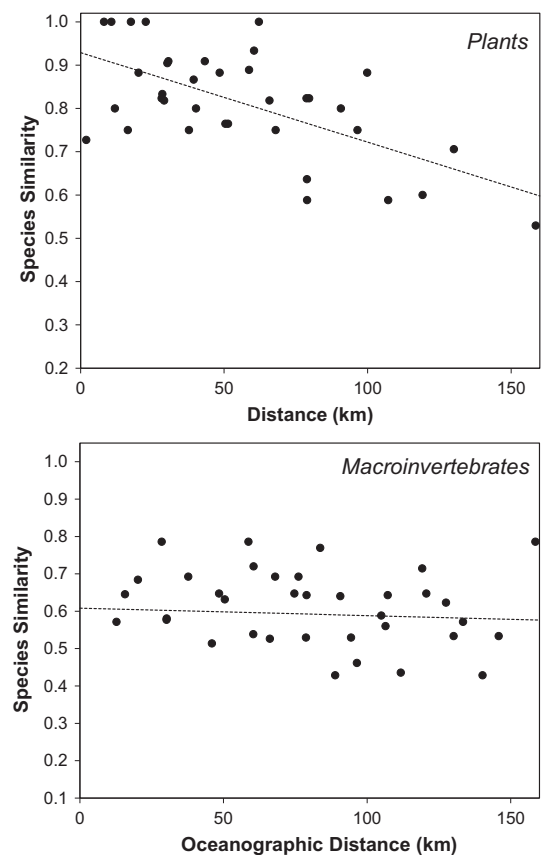


Fig. 4. Pairwise species similarity ($1 - \beta_{sim}$) of plants (saltmarshes and seagrass meadows) and benthic macroinvertebrates against the oceanographic distance. The Mantel correlation between species similarity and geographic distance are given in Table 7.

Table 7

Mantel and partial Mantel tests between species similarity indices ($1 - \beta_{sim}$, $1 - \beta_{-3}$), oceanographic distance and environmental variables. r_M : Mantel correlation; p : probability of significance. Significant values are highlighted in bold.

		$1 - \beta_{sim}$		$1 - \beta_{-3}$	
		r_M	p	r_M	p
Plants (saltmarshes and seagrass beds)	Species \times Distance	−0.63	<0.001	−0.58	0.001
	Species \times Environ.	−0.15	0.176	−0.14	0.259
	Species \times Distance (Environ. partially out)	−0.62	0.002	−0.57	0.002
Macroinvertebrates	Species \times Distance	−0.06	0.323	−0.01	0.519
	Species \times Environ.	−0.08	0.673	−0.29	0.937
	Species \times Distance (Environ. partially out)	na	na	na	na

structure. The genetic analysis of *C. edule* coincides with the community analysis in terms of high connectivity at population and community levels, respectively, suggesting no dispersal barriers at this regional scale for the soft-bottom macroinvertebrates, most of them spreading through planktonic larvae. Within this panmictic population of *C. edule* along the north Iberian coast, all estuarine populations would contribute a certain percentage of offspring to a larval pool with homogeneous redistribution to all populations (Selkoe and Toonen, 2011). Lack of population structure applying a similar number of microsatellites over long coastal areas has been also reported for other mollusc species (e.g. Bester-van der Merwe et al., 2011; Donald et al., 2011; but see; Coscia et al., 2013). High connectivity would imply a high resilience and, therefore, a low vulnerability to moderate environmental change and the putative loss of intertidal habitat. On the other hand, high connectivity can also impede local adaptation (e.g. Lenormand, 2002; but see; Clarke et al., 2010; Sanford and Kelly, 2011). On the contrary, warming of the sea of 1.5–2.0 °C within the area (Chust et al., 2011) might trigger a reduction in the dispersion period of planktonic bivalve larvae such as *C. edule*. Moreover, bivalves are not able to regulate osmotic pressure and the intensification of extreme daily rainfall of 10%, would provoke punctual changes in salinity and intense mortality of the post-larvae.

Our findings suggest that populations of species dwelling exclusively within the estuarine soft-bottom intertidal zone, such as the seagrass *Zostera noltei* and most of salt-marsh plants, can be separated apart by large functional distances along the coastline. In contrast, other estuarine sessile species but with planktonic larval dispersal and dwelling different habitats, such as *Cerastoderma edule* and other soft-bottom macroinvertebrates, could exchange migrants from the metapopulation pool without strong barriers to gene flow. In comparison with other ecosystems, the role played by dispersal limitation in saltmarsh plants is similar to the one reported for inland plants (e.g. Condit et al., 2002; Chust et al., 2006a,b), whilst the gene flow in intertidal soft-bottom macroinvertebrates seems to move almost with no barriers as in most of the pelagic organisms (e.g. Kinlan and Gaines, 2003; Cermeño and Falkowski, 2009).

The projected scenario of sea level rise induced by climate change (Chust et al., 2010), could threaten the smallest populations of some of saltmarsh species. Although the morphodynamic evolution of mudflats with sea level rise in the estuaries is site specific, and saltmarshes are capable of responding to sea level rise being near equilibrium in relation to sea level (e.g. Friedrichs and Perry, 2001; Friess et al., 2012; Leorri et al., 2013), the suitable habitat for vegetation could be reduced in some cases. This is the case, for instance, where the coastal margin is squeezed between the fixed landward boundary (artificial or natural) and the sea level

rise, i.e. the so-called ‘coastal squeeze’ effect (Schleupner, 2008). In the Basque estuaries, habitat squeeze induced by present artificial barriers is expected to be more severe in the upper tidal zone than in the mid or lower intertidal zone (Chust et al., 2011).

Basque estuaries have been greatly transformed by anthropogenic activities during the 20th Century (Cearreta et al., 2004), supporting many human pressures (Borja et al., 2006a). However, in recent times, these estuaries have experienced some recovery, due to water treatment (Borja et al., 2009a, 2010). Hence, some habitat restoration experiments have been undertaken, after recovery of the aquatic quality (Marquiegui and Aguirrezabalaga, 2009). Since genetic connectivity of some species is very low, it may affect recovery and restoration success, as shown in other areas for *Zostera* species (Campanella et al., 2010a; Tanaka et al., 2011). Moreover, the low genetic diversity in *Zostera noltei* at the estuary level weakens the population ability to overcome physical disturbances (Campanella et al., 2010b), including also habitat loss due to sea-level rise. In contrast, the connectivity of soft-bottom macroinvertebrate communities indicate high recolonization capacity, in agreement with that reported in azoic parts of some Basque estuaries after 10–15 years (Borja et al., 2006b, 2009b; 2010). Hence, the proxies of connectivity used and compared in the present study, based on genetic and ecological drift processes and dispersal limitation factors, can be applied to assess the vulnerability of species to global change; similarly, to help environmental managers to prioritise local restoration plans for the most vulnerable species and habitats. Further research should focus on applying the genetic analysis to a large number of species sufficient to represent the diversity of estuarine communities, thus, in order to test if species dispersal limitation drives similarly both genetic population structure and community patterns, as recently shown in aquatic beetles (Baselga et al., 2013).

5. Conclusions

Two proxies of connectivity at community and species levels, based on genetic and ecological neutral theories, respectively, provided similar results in estuarine biota. Although salt-marsh and seagrass plants share a sedentary life history with soft-bottom macroinvertebrates and they inhabit a similar environment, the latter disperse at larger scales and this is attributed to differences in biological dispersal modes between these two taxa. The proxy at community level indicates that plant populations of saltmarshes and seagrass beds are partially isolated among estuaries, as a result of dispersal limitation. One of these species is the seagrass *Zostera noltei*; their populations within the Basque estuaries were found genetically differentiated. This suggests that main processes operating to the seagrass populations are genetic drift and low gene flow, especially for the most distant populations, probably because seagrass colonization mainly proceeds locally through clonal growth. The chances that recolonization would occur naturally if populations get extinct would be highly unlikely. In contrast, estuarine soft-bottom macroinvertebrates did not present a spatial structure, suggesting that species do not have strong movement barriers and populations, in average, are highly connected. Accordingly, the cockle (*Cerastoderma edule*) assemblages within the Basque estuaries were found genetically undifferentiated, indicating they own to a unique panmictic population. Thus, *C. edule* seemed to be a highly mobile species, probably throughout the planktonic larvae dispersed by ocean currents, with a high recolonization capacity.

Overall, our findings suggest that *Cerastoderma edule*, and other similar estuarine soft-bottom macroinvertebrates, with planktonic larval dispersal strategies, would have a high resilience capacity in the face of moderate changes within their habitats. In contrast,

saltmarsh plants and seagrass beds are especially vulnerable to these changes because of their dispersal limitation, in particular those changes reducing species habitat extent such as those induced by anthropogenic impacts and sea level rise. As shown, the seagrass *Zostera noltei* is a particularly threatened species within the Basque estuaries because of fragmented, few and genetically low diverse populations. These findings can help environmental managers to guide species and habitat restoration plans as well as climate change adaptation strategies.

Acknowledgements

This study was supported by a contract undertaken between the Basque Water Agency—URA and AZTI-Tecnalia, likewise, financed by the Ministry of Science and Innovation of the Spanish Government (Project Ref.: CTM2011-29473). This investigation was also partially supported by the project DEVOTES (DEVELOPMENT of innovative Tools for understanding marine biodiversity and assessing good Environmental Status) funded by the EU 7th FP 'The Ocean of Tomorrow' Theme (grant agreement no. 308392), www.devotes-project.eu. OED was supported by a Fundação para a Ciência e Tecnologia (FCT, Portugal) postdoctoral grant (SFRH/BPD/64990/2009) and research project POCI/MAR/60044/2004 financed by FCT and FEDER. This is contribution number 652 of the Marine Research Division (AZTI-Tecnalia).

Appendix A. Supplementary data

Supplementary data related to this article can be found at <http://dx.doi.org/10.1016/j.ecss.2013.08.005>.

References

- Alberto, F., 2009. MsatAllele 1.0: an R package to visualize the binning of microsatellite alleles. *Journal of Heredity* 100, 394–397.
- Alberto, F., Arnaud-haond, S., Duarte, C.M., Serrão, E.A., 2006. Genetic diversity of a clonal angiosperm near its range limit: the case of the case of *Cymodocea nodosa* at the Canary Islands. *Marine Ecology Progress Series* 309, 117–129.
- Alberto, F., Raimondi, P.T., Reed, D.C., Watson, J.R., Siegel, D.A., Mitarai, S., Coelho, N., Swrroa, E.A., 2011. Isolation by oceanographic distance explains genetic structure for *Macrocystis pyrifera* in the Santa Barbara Channel. *Molecular Ecology* 20, 2543–2554.
- Baselga, A., Fujisawa, T., Crampton-Platt, A., Bergsten, J., Foster, P.G., Monaghan, M.T., Vogler, A.P., 2013. Whole-community DNA barcoding reveals a spatio-temporal continuum of biodiversity at species and genetic levels. *Nature Communications* 4, 1892.
- Belkhir, K., Borsa, P., Chikhi, L., Raufaste, N., Bonhomme, P., 2004. 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). <http://www.genetix.univ-montp2.fr/genetix/genetix.htm>.
- Bester-van der Merwe, A., Roodt-Wilding, R., Volckaert, F., D'Amato, M., 2011. Historical isolation and hydrodynamically constrained gene flow in declining populations of the South-African abalone, *Haliotis midae*. *Conservation Genetics* 12, 543–555.
- Borja, A., Collins, M., 2004. Oceanography and marine environment of the Basque Country. *Elsevier Oceanography Series* 70, 616.
- Borja, A., Galparsoro, I., Solaun, O., Muxika, I., Tello, E.M., Uriarte, A., Valencia, V., 2006a. The European Water Framework Directive and the DPSIR, a methodological approach to assess the risk of failing to achieve good ecological status. *Estuarine, Coastal and Shelf Science* 66, 84–96.
- Borja, A., Muxika, I., Franco, J., 2006b. Long-term recovery of soft-bottom benthos following urban and industrial sewage treatment in the Nervión estuary (southern Bay of Biscay). *Marine Ecology Progress Series* 313, 43–55.
- Borja, A., Bald, J., Franco, J., Larreta, J., Muxika, I., Revilla, M., Rodríguez, J.G., Solaun, O., Uriarte, A., Valencia, V., 2009a. Using multiple ecosystem components, in assessing ecological status in Spanish (Basque Country) Atlantic marine waters. *Marine Pollution Bulletin* 59, 54–64.
- Borja, A., Muxika, I., Rodríguez, J.G., 2009b. Paradigmatic responses of marine benthic communities to different anthropogenic pressures, using M-AMBI, within the European Water Framework Directive. *Marine Ecology* 30, 214–227.
- Borja, A., Dauer, D., Elliott, M., Simenstad, C., 2010. Medium- and long-term recovery of estuarine and coastal ecosystems: patterns, rates and restoration effectiveness. *Estuaries and Coasts* 33, 1249–1260.
- Boström, C., Törnroos, A., Bonsdorff, E., 2010. Invertebrate dispersal and habitat heterogeneity: expression of biological traits in a seagrass landscape. *Journal of Experimental Marine Biology and Ecology* 390, 106–117.
- Bradbury, I.R., Laurel, B., Snelgrove, P.V.R., Bentzen, P., Campana, S.E., 2008. Global patterns in marine dispersal estimates: the influence of geography, taxonomic category and life history. *Proceedings of the Royal Society of London* 275, 1803–1810.
- Campanella, J., Bologna, P., Smith, S., Rosenzweig, E., Smalley, J., 2010a. *Zostera marina* population genetics in Barnegat Bay, New Jersey, and implications for grass bed restoration. *Population Ecology* 52, 181–190.
- Campanella, J., Bologna, P., Smalley, J.V., Rosenzweig, E.B., Smith, S.M., 2010b. Population structure of *Zostera marina* (Eelgrass) on the Western Atlantic Coast is characterized by poor connectivity and inbreeding. *Journal of Heredity* 101, 61–70.
- Cearreta, A., Irabien, M.J., Ulibarri, I., Yusta, I., Croudace, I.W., Cundy, A.B., 2002. Recent salt marsh development and natural regeneration of reclaimed areas in the Plentzia estuary, N. Spain. *Estuarine, Coastal and Shelf Science* 54, 863–886.
- Cearreta, A., Irabien, M.J., Pascual, A., 2004. Human activities along the Basque coast during the last two centuries: geological perspective of recent anthropogenic impact on the coast and its environmental consequences. In: Borja, A., Collins, M. (Eds.), *Oceanography and Marine Environment of the Basque Country*, Elsevier Oceanography Series, vol. 70, pp. 27–50.
- Cermeño, P., Falkowski, P.G., 2009. Controls on Diatom biogeography in the ocean. *Science* 325, 1539–1541.
- Cermeño, P., de Vargas, C., Abrantes, F.T., Falkowski, P.G., 2010. Phytoplankton biogeography and community stability in the ocean. *PLoS ONE* 5, e10037.
- Chang, E., Veeneklaas, R., Bakker, J.P., 2007. Seed dynamics linked to variability in movement of tidal water. *Journal of Vegetation Science* 18, 253–262.
- Chapuis, M.-P., Estoup, A., 2007. Microsatellite null alleles and estimation of population differentiation. *Molecular Biology and Evolution* 24, 621–631.
- Chave, J., 2004. Neutral theory and community ecology. *Ecology Letters* 7, 241–253.
- Chave, J., Leigh, E.G., 2002. A spatially explicit neutral model of beta-diversity in tropical forests. *Theoretical Population Biology* 62, 153–168.
- Chust, G., Chave, J., Condit, R., Aguilar, S., Lao, S., Perez, R., 2006a. Determinants and spatial modeling of tree beta-diversity in a tropical forest landscape in Panama. *Journal of Vegetation Science* 17, 83–92.
- Chust, G., Perez-Haase, A., Chave, J., Pretus, J.L., 2006b. Floristic patterns and plant traits of Mediterranean communities in fragmented habitats. *Journal of Biogeography* 33, 1235–1245.
- Chust, G., Borja, A., Liria, P., Galparsoro, I., Marcos, M., Caballero, A., Castro, R., 2009. Human impacts overwhelm the effects of sea-level rise on Basque coastal habitats (N Spain) between 1954 and 2004. *Estuarine, Coastal and Shelf Science* 84, 453–462.
- Chust, G., Caballero, A., Marcos, M., Liria, P., Hernández, C., Borja, A., 2010. Regional scenarios of sea level rise and impacts on Basque (Bay of Biscay) coastal habitats, throughout the 21st century. *Estuarine, Coastal and Shelf Science* 87, 113–124.
- Chust, G., Borja, A., Caballero, A., Irigoien, X., Sáenz, J., Moncho, R., Marcos, M., Liria, P., Hidalgo, J., Valle, M., Valencia, V., 2011. Climate change impacts on coastal and pelagic environments in the southeastern Bay of Biscay. *Climate Research* 48, 307–332.
- Chust, G., Irigoien, X., Chave, J., Harris, R.P., 2013. Latitudinal phytoplankton distribution and the neutral theory of biodiversity. *Global Ecology and Biogeography* 22, 531–543.
- Clarke, L.M., Munch, S.B., Thorrold, S.R., Conover, D.O., 2010. High connectivity among locally adapted populations of a marine fish (*Menidia menidia*). *Ecology* 91, 3526–3537.
- Coleman, M.A., Roughan, M., Macdonald, H.S., Connell, S.D., Gillanders, B.M., Kelaher, B.P., Steinberg, P.D., 2011. Variation in the strength of continental boundary currents determines continent-wide connectivity in kelp. *Journal of Ecology* 99, 1026–1032.
- Condit, R., Pitman, N., Leigh Jr., E.G., Chave, J., Terborgh, J., Foster, R.B., Núñez, P., Aguilar, S., Valencia, R., Villa, G., Muller-Landau, H.C., Losos, E., Hubbell, S.P., 2002. Beta-diversity in tropical forest trees. *Science* 295, 666–669.
- Coscia, I., Robins, P., Porter, J., Malham, S., Ironside, J., 2013. Modelled larval dispersal and measured gene flow: the common cockle in the southern Irish Sea. *Conservation Genetics* 14, 451–466.
- Costanza, R., d'Arge, R., de Groot, R., Farber, S., Grasso, M., Hannon, B., Limburg, K., Naem, S., O'Neill, R.V., Paruelo, J., Raskin, R.G., Sutton, P., van den Belt, M., 1997. The value of the world's ecosystem services and natural capital. *Nature* 387, 253–260.
- Cowen, R.K., Sponaugle, S., 2009. Larval dispersal and marine population connectivity. *Annual Review of Marine Science* 1, 443–466.
- Cowen, R.K., Gawarkiewicz, G., Pineda, J., Thorrold, S.R., Werner, F.E., 2007. Population connectivity in marine systems: an overview. *Oceanography* 20, 14–21.
- Coyer, J.A., Reusch, T.B.H., Stam, W.T., Serrao, E.S., Pearson, G., Procaccini, G., Olsen, J.L., 2004. Characterization of microsatellite loci in the dwarf eelgrass *Zostera noltii* (Zosteraceae) and cross-reactivity with *Z. japonica*. *Molecular Ecology* 4, 497–499.
- Defeo, O., McLachlan, A., Schoeman, D.S., Schlacher, T.A., Dugan, J., Jones, A., Lastra, M., Scapini, F., 2009. Threats to sandy beach ecosystems: a review. *Estuarine, Coastal and Shelf Science* 81, 1–12.
- Dempster, A.P., Laird, N.M., Rubin, D.B., 1977. Maximum likelihood from incomplete data via the EM algorithm. *Journal of the Royal Statistical Society, Series B* 39, 1–38.

- Diekmann, O.E., Coyer, J.A., Ferreira, J., Olsen, J.L., Stam, W.T., Pearson, G.A., Serrao, E.A., 2005. Population genetics of *Zostera noltii* along the west Iberian coast: consequences of small population size, habitat discontinuity and near-shore currents. *Marine Ecology-Progress Series* 290, 89–96.
- Donald, K.M., Keeney, D.B., Spencer, H.G., 2011. Contrasting population makeup of two intertidal gastropod species that differ in dispersal opportunities. *Journal of Experimental Marine Biology and Ecology* 396, 224–232.
- Dorken, M.E., Eckert, C.G., 2001. Severely reduced sexual reproduction in northern populations of a clonal plant, *Decodon verticillatus* (Lythraceae). *Journal of Ecology* 89, 339–350.
- European Environment Agency, 2006. State and Pressures of the Marine and Coastal Mediterranean Environment. European Environment Agency, Copenhagen, p. 44.
- Evanno, G., Regnaut, S., Goudet, J., 2005. Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. *Molecular Ecology* 14, 2611–2620.
- FitzGerald, D.M., Fenster, M.S., Argow, B.A., Buynevich, I.V., 2008. Coastal impacts due to sea-level rise. *Annual Review of Earth and Planetary Sciences* 36, 601–647.
- Friedrichs, C.T., Perry, J.E., 2001. Tidal salt marsh morphodynamics: a synthesis. *Journal of Coastal Research* (27), 7–37.
- Friess, D.A., Krauss, K.W., Horstman, E.M., Balke, T., Bouma, T.J., Galli, D., Webb, E.L., 2012. Are all intertidal wetlands naturally created equal? Bottlenecks, thresholds and knowledge gaps to mangrove and saltmarsh ecosystems. *Biological Reviews* 87, 346–366.
- Galindo, H.M., Pfeiffer-Herbert, A.S., McManus, M.A., Chao, Y., Chai, F., Palumbi, S.R., 2010. Seascape genetics along a steep cline: using genetic patterns to test predictions of marine larval dispersal. *Molecular Ecology* 19, 3692–3707.
- Galparsoro, I., Borja, A., Legorburu, I., Hernández, C., Chust, G., Liria, P., Uriarte, A., 2010. Morphological characteristics of the Basque continental shelf (Bay of Biscay, northern Spain); their implications for Integrated Coastal Zone Management. *Geomorphology* 118, 314–329.
- Gawarkiewicz, G., Monismith, S., Largier, J., 2007. Observing larval transport processes affecting population connectivity: progress and challenges. *Oceanography* 20, 40–53.
- González, M., Uriarte, A., Fontán, A., Mader, J., Gyssels, P., 2004. Marine dynamics. In: Borja, A., Collins, M. (Eds.), *Oceanography and Marine Environment of the Basque Country*, Elsevier Oceanography Series, vol. 70, pp. 133–157.
- Goudet, J., 1995. FSTAT (version 1.2): a computer program to calculate F-statistics. *Journal of Heredity* 86, 485–486.
- Goudet, J., 2001. FSTAT, a Programme to Estimate and Test Gene Diversities and Fixation Indices, Version 2.9.3. <http://www2.unil.ch/popgen/softwares/fstat.htm>.
- Halpern, B.S., Walbridge, S., Selkoe, K.A., Kappel, C.V., Micheli, F., D'Agrosa, C., Bruno, J.F., Casey, K.S., Ebert, C., Fox, H.E., Fujita, R., Heinemann, D., Lenihan, H.S., Madin, E.M.P., Perry, M.T., Selig, E.R., Spalding, M., Steneck, R., Watson, R., 2008. A global map of human impact on marine ecosystems. *Science* 319, 948–952.
- Hastings, A., Botsford, L.W., 2006. Persistence of spatial populations depends on returning home. *Proceedings of the National Academy of Sciences of the United States of America* 103, 6067–6072.
- He, F., Hubbell, S.P., 2011. Species-area relationships always overestimate extinction rates from habitat loss. *Nature* 473, 368–371.
- Heller, N.E., Zavaleta, E.S., 2009. Biodiversity management in the face of climate change: a review of 22 years of recommendations. *Biological Conservation* 142, 14–32.
- Hubbell, S.P., 1997. A unified theory of biogeography and relative species abundance and its application to tropical rain forests and coral reefs. *Coral Reefs* 16, S9–S21.
- Hubbell, S.P., 2001. *A Unified Neutral Theory of Biodiversity and Biogeography*. Princeton University Press, Princeton, NJ.
- Hutchinson, G.E., 1957. Concluding remarks. *Cold Spring Harbor Symposia on Quantitative Biology* 22, 415–427.
- Hutchison, D.W., Templeton, A.R., 1999. Correlation of pairwise genetic and geographic distance measures: inferring the relative influences of gene flow and drift on the distribution of genetic variability. *Evolution* 53, 1898–1914.
- Irigoin, X., Chust, G., Fernandes, J.A., Albaina, A., Zarauz, L., 2011. Factors determining the distribution and biodiversity of mesozooplankton species in shelf and coastal waters of the Bay of Biscay. *Journal of Plankton Research* 33, 1182–1192.
- Jost, L., 2008. G_{ST} and its relatives do not measure differentiation. *Molecular Ecology* 17, 4015–4026.
- Kimura, M., 1953. 'Stepping-stone' model of population. *Annual Report National Institute of Genetics* 3, 62–63.
- Kimura, M., 1983. *The Neutral Theory of Molecular Evolution*. Cambridge University Press, Cambridge, ISBN 0-521-23109-4.
- Kinlan, B.P., Gaines, S.D., 2003. Propagule dispersal in marine and terrestrial environments: a community perspective. *Ecology* 84, 2007–2020.
- Koleff, P., Gaston, K.J., Lennon, J.J., 2003. Measuring beta diversity for presence-absence data. *Journal of Animal Ecology* 72, 367–382.
- Latch, E., Dharmarajan, G., Glaubitz, J.C., Rhodes, O.E.J., 2006. Relative performance of Bayesian clustering software for inferring population substructure and individual assignment at low levels of population differentiation. *Conservation Genetics* 7, 295–302.
- Legendre, P., 1993. Spatial autocorrelation: trouble or new paradigm? *Ecology* 74, 1659–1673.
- Legendre, P., Legendre, L., 1998. *Numerical Ecology*. Elsevier, Amsterdam.
- Lennon, J.J., Koleff, P., Greenwood, J.J.D., Gaston, K.J., 2001. The geographical structure of British bird distributions: diversity, spatial turnover and scale. *Journal of Animal Ecology* 70, 966–979.
- Lenormand, T., 2002. Gene flow and the limits to natural selection. *Trends in Ecology & Evolution* 17, 183–189.
- Leorri, E., Cearreta, A., García-Artola, A., Irabien, M.J., Blake, W.H., 2013. Relative sea-level rise in the Basque coast (N Spain): Different environmental consequences on the coastal area. *Ocean & Coastal Management* 77, 3–13.
- Loew, S.S., 2000. Role of genetics in conservation biology. In: Ferson, S., Burkman, M. (Eds.), *Quantitative Methods for Conservation Biology*. Springer-Verlag, New York, USA, pp. 226–258.
- MacArthur, R.H., Wilson, E.O., 1967. *The Theory of Island Biogeography*. Princeton University Press, Princeton, MA.
- Marcos, M., Chust, G., Jordà, G., Caballero, A., 2012. Effect of sea level extremes on the western Basque coast during the 21st century. *Climate Research* 51, 237–248.
- Marquiegui, M.A., Aguirrezabalaga, F., 2009. Colonization process by macrobenthic infauna after a managed coastal realignment in the Bidasoa estuary (Bay of Biscay, NE Atlantic). *Estuarine, Coastal and Shelf Science* 84, 598–604.
- Martínez, L., Arias, A., Méndez, J., Insua, A., Freire, R., 2009. Development of twelve polymorphic microsatellite markers in the edible cockle *Cerastoderma edule* (Bivalvia: Cardiidae). *Conservation Genetics Resources* 1, 107–109.
- Martiny, J.B.H., Eisen, J.A., Penn, K., Allison, S.D., Horner-Devine, M.C., 2011. Drivers of bacterial beta-diversity depend on spatial scale. *Proceedings of the National Academy of Sciences of the United States of America* 108, 7850–7854.
- Mawdsley, J.R., O'Malley, R., Ojima, D.S., 2009. A review of climate-change adaptation strategies for wildlife management and biodiversity conservation. *Conservation Biology* 23, 1080–1089.
- McGranahan, D.A., Balk, D., Anderson, B., 2007. The rising tide: assessing the risks of climate change and human settlements in low elevation coastal zones. *Environment and Urbanization* 19, 17–39.
- Millennium Ecosystem Assessment, 2005. *Ecosystems and Human Well-being: Synthesis*. Island Press, Washington, DC.
- Pascual, M., Borja, A., Eede, S.V., Deneudt, K., Vincx, M., Galparsoro, I., Legorburu, I., 2011. Marine biological valuation mapping of the Basque continental shelf (Bay of Biscay), within the context of marine spatial planning. *Estuarine, Coastal and Shelf Science* 95, 186–198.
- Philippart, C.J.M., Anadón, R., Danovaro, R., Dippner, J.W., Drinkwater, K.F., Hawkins, S.J., Oguz, T., O'Sullivan, G., Reid, P.C., 2011. Impacts of climate change on European marine ecosystems: observations, expectations and indicators. *Journal of Experimental Marine Biology and Ecology* 400, 52–69.
- Pritchard, J.K., Stephens, M., Donnelly, P., 2000. Inference of population structure using multilocus genotype data. *Genetics* 155, 945–959.
- R Development Core Team, 2011. *R: a Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna, Austria, ISBN 3-900051-07-0. <http://www.R-project.org/>.
- Reid, P.C., Fischer, A.C., Lewis-Brown, E., Meredith, M.P., Sparrow, M., Andersson, A.J., Antia, A., Bates, N.R., Bathmann, U., Beaugrand, G., Brix, H., Dye, S., Edwards, M., Furevik, T., Gangsto, R., Hatun, H., Hopcroft, R.R., Kendall, M., Kasten, S., Keeling, R., Le Quere, C., Mackenzie, F.T., Malin, G., Mauritzen, C., Olafsson, J., Paull, C., Rignot, E., Shimada, K., Vogt, M., Wallace, C., Wang, Z., Washington, J., 2009. Chapter 1. Impacts of the oceans on climate change. *Advances in Marine Biology* 56, 1–150.
- Ricotta, C., Carranza, M.L., Avena, G., 2002. Computing [beta]-diversity from species-area curves. *Basic and Applied Ecology* 3, 15–18.
- Rosenzweig, M.L., 1995. *Species Diversity in Space and Time*. Cambridge Univ. Press.
- Rousset, F., 1997. Genetic differentiation and estimation of gene flow from F-statistics under isolation by distance. *Genetics* 145, 1219–1228.
- Rumohr, H., Karakassis, I., Jensen, J.N., 2001. Estimating species richness, abundance and diversity with 70 macrobenthic replicates in the Western Baltic Sea. *Marine Ecology-Progress Series* 214, 103–110.
- Sabetta, L., Barbone, E., Giardino, A., Galuppo, N., Basset, A., 2007. Species-area patterns of benthic macro-invertebrates in Italian lagoons. *Hydrobiologia* 577, 127–139.
- Sanford, E., Kelly, M.W., 2011. Local adaptation in marine invertebrates. *Annual Review of Marine Science* 3, 509–535.
- Schleupner, C., 2008. Evaluation of coastal squeeze and its consequences for the Caribbean island Martinique. *Ocean & Coastal Management* 51, 383–390.
- Selkoe, K.A., Toonen, R.J., 2011. Marine connectivity: a new look at pelagic larval duration and genetic metrics of dispersal. *Marine Ecology-Progress Series* 436, 291–305.
- Selkoe, K.A., Watson, J.R., White, C., Ben Horin, T., Iacchi, M., Mitarai, S., Siegel, D.A., Gaines, S.D., Toonen, R.J., 2010. Taking the chaos out of genetic patchiness: seascape genetics reveals ecological and oceanographic drivers of genetic patterns in three temperate reef species. *Molecular Ecology* 19, 3708–3726.
- Shanks, A.L., 2009. Pelagic larval duration and dispersal distance revisited. *Biology Bulletin* 216, 373–385.
- Silván, F., Campos, J.A., 2002. Estudio de la flora vascular amenazada de los estuarios la Comunidad Autónoma del País Vasco. Departamento de Agricultura y Pesca, Gobierno Vasco, p. 100. <http://www9.euskadi.net/biodiversidad/datos/estuarios.pdf>.
- Tanaka, N., Demise, T., Ishii, M., Shoji, Y., Nakaoka, M., 2011. Genetic structure and gene flow of eelgrass *Zostera marina* populations in Tokyo Bay, Japan: implications for their restoration. *Marine Biology* 158, 871–882.

- Thayer, G.H., Wolfe, D.A., Williams, R.B., 1975. The impact of man on seagrass systems. *American Science* 63, 288–296.
- Uriarte, A., Collins, M., Cearreta, A., Bald, J., Evans, G., 2004. Sediment supply, transport and deposition: contemporary and Late Quaternary evolution. In: Borja, A., Collins, M. (Eds.), *Oceanography and Marine Environment of the Basque Country*, Elsevier Oceanography Series, vol. 70, pp. 97–131.
- Valle, M., Borja, Á., Chust, G., Galparsoro, I., Garmendia, J.M., 2011. Modelling suitable estuarine habitats for *Zostera noltii*, using ecological niche factor analysis and Bathymetric LiDAR. *Estuarine, Coastal and Shelf Science* 94, 144–154.
- Vellend, M., Geber, M.A., 2005. Connections between species diversity and genetic diversity. *Ecology Letters* 8, 767–781.
- Weersing, K., Toonen, R.J., 2009. Population genetics, larval dispersal, and connectivity in marine systems. *Marine Ecology Progress Series* 393, 1–12.
- Weir, B.S., Cockerham, C.C., 1984. Estimating F-statistics for the analysis of population structure. *Evolution* 38, 1358–1370.
- Williams, P.H., 1996. Mapping variations in the strength and breadth of biogeographic transition zones using species turnover. *Proceedings of the Royal Society of London. Series B: Biological Sciences* 263, 579–588.
- Wright, S., 1943. Isolation by distance. *Genetics* 28, 114–138.
- Young, E.F., Bigg, G.R., Grant, A., 1996. A statistical study of environmental influences on bivalve recruitment in the Wash, England. *Marine Ecology-Progress Series* 143, 121–129.