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

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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.

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
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 (Hutchison and Templeton, 1999). In contrast, in a metapopulation and populations at equilibrium between dispersal and genetic drift (Hubbell, 2001), the exchange of 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 fixation 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 (Hutchison and Templeton, 1995). 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 the 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 (Krogulec 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
2. Population Genetics: Sampling and Genotyping

2.2. 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 it is present only in three (Oka, Lea and Bidasoa) of them (Valle et al., 2011). 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, Santona, Santander) and one in Asturias (Ribadesella) regions, one estuary in France (Arcachon), and one population from Galicia region, Ria de Vigo estuary (42°21′57″N and 8°27′46″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, Lea, and Oka (Basque Country, Fig. 1) and at Carril (Ria de Arousa, Galicia, NW Spain, 42°21′57″N, 8°27′46″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, CeATC2-34, 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.

![Fig. 1.](image-url)
While COCKLE1 reaction contained 1 μl MgCl2 (25 mM), 0.30 μL of each primer (10 μM), except for 0.50 μl of CeATC2-46 ones, 1.25 μl GeneAmp® PCR Buffer II (Applied Biosystems), 1 μl dNTPs (2.5 mM each), 0.48 μl AmpliTaq Gold DNA Polymerase (5 U/μl; Applied Biosystems) and 2.07 μl ultrapure H2O, COCKLE2 reaction included 1.25 μl MgCl2, 0.30 μl of each primer, except for 0.50 μl of CeATC2-51 ones, 1.25 μl PCR Buffer, 1 μl dNTPs (2.5 mM each), 0.24 μl DNA Polymerase and 4.46 μl 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 (Tå = 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 3.7i 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 multic locus genotypes (ramets, i.e. shoots, belonging to the same genet, which is a group of genetically identical individuals), heterozygosity (H and Ho) and inbreeding coefficient (Fis) 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): \( R = \frac{(G - 1)}{(N - 1)}, \) 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 (Â) 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 and H0) and inbreeding coefficient (Fis).

The presence and frequency of null alleles were tested for both species using the expectation maximization algorithm of Dempster et al. (1977) implemented in FreeNA (Chapuis and Estoup, 2007). Genetic diversity 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 \( \Delta K \) (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 saltmarshes, 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, Ifurritizia, Ori, and 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 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, Ori, 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 1. 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): \( \beta_{\text{sim}} \) (Lennon et al., 2001), and \( \beta_{-3} \) (Williams, 1996):

\[
\beta_{\text{sim}} = 1 - \frac{a}{\min(b, c) + a}
\]

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

\[
\beta_{-3} = \frac{\min(b, c)}{a + b + c}
\]

(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 (\( = 1 – \) 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 \( F_S \) 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 Sanoña (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 \( F_S \) values in all estuaries except for Lea, Ribadesella and

| Table 1 |
| Allele number, observed heterozygosity (\( H_o \)), expected heterozygosity (\( H_e \)), and inbreeding coefficient (\( F_S \); \( p < 0.05 \)) 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 | \( H_o \) | \( H_e \) | \( F_S \) | Null allele frequency |
|-------|------------------------|--------|--------|--------|----------------------|
| ZnB1  | 18                     | 0.81   | 0.71   | 0.13   | 0.02                 |
| ZnB2  | 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   | 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 : 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 (\( \Delta K \)) when considering all populations resulted in \( K = 2 \) and a less supported solution of \( K = 3 \) and \( K = 5 \) (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 Sanoña 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 genes (\( G \)), 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 | \( N \) | \( G \) | \( R \) | \( AR(G - 10) \) | \( H_o \) | \( H_e \) | \( F_S \) |
|----------|-------|-------|-------|----------------|--------|--------|--------|
| Vigo     | 44    | 44    | 0.91  | 4.343 ± 0.485 | 0.557  | 0.508  | 0.0093* |
| Ribadesela | 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 |
| Oka      | 50    | 40    | 1.00  | 5.209 ± 0.404 | 0.646  | 0.615  | 0.0605 |
| Lea      | 40    | 40    | 0.74  | 4.263 ± 0.422 | 0.537  | 0.492  | 0.0934* |
| Bidasoa  | 47    | 10    | 0.20  | 3.857 ± 0.000 | 0.487  | 0.582  | 0.1379 |
| Arcachon | 54    | 28    | 0.51  | 2.651 ± 0.181 | 0.330  | 0.263  | 0.1716 |

*Significant \( F_S \) (\( p < 0.05 \)).
population genetics. While the number of alleles per marker showed values ranging from 11 to 51 (Table 4), $H_e$ values varied from a minimum of 0.418 to a maximum of 0.902 (meaning 1 total absence of homozygotes). Non-negligible null allele frequencies were reported for seven of the microsatellite markers (Table 4). When comparing within genetic diversity among the different estuaries, the sample from Ria de Arousa showed the highest values of $H_e$ and AR (Table 5). Nevertheless, only 3 out of 30 pairwise comparisons were statistically significant using Wilcoxon test: Arousa vs. Deba for both $H_e$ ($p = 0.028$) and AR ($p = 0.021$), and Arousa vs. Lea for AR ($p = 0.028$).

Regarding genetic differentiation among estuaries, the pairwise comparison of the fixation index ($F_{ST}$) values showed significant values only when comparing Butron estuary against both Deba and Arousa estuaries, and when comparing Arousa against Lea. This last comparison became non-significant when applying null allele correction (Table 6). The geographically farthest estuaries (Arousa and Bidasoa, Fig. 1) showed a $F_{ST}$ value of 0.0030 (non-significant; null allele corrected).

In the estuaries where more than one sampling point was surveyed, a pairwise $F_{ST}$ test was performed without obtaining any significant value. While regarding Oka estuary, $F_{ST}$ values among sampling locations ranged between 0.000 and 0.009 (11 sample points), a range of 0.000–0.011 corresponded to Deba estuary (3 sample points) and a sole value of 0.008, corresponding to the presence of two sampling points, was found within Butron estuary.

No correlation was found when comparing genetic and geographical distances (for $F_{ST}$: $r_M = 0.017$; $p = 0.553$; for D: $r_M = -0.14$, $p = 0.627$). STRUCTURE analysis was not performed since it has been described that the performance in STRUCTURE to detect the

| | Vigo | Ribadesella | Barquera | Santander | Santona | Oka | Lea | Bidasoa | Arcachon |
|---|---|---|---|---|---|---|---|---|---|
| $F_{ST}$ | | | | | | | | | |
| Vigo | — | 0.32 | 0.15 | 0.19 | 0.12 | 0.18 | 0.25 | 0.31 | 0.16 |
| Ribadesella | — | — | 0.16 | 0.21 | 0.18 | 0.14 | 0.12 | 0.47 | 0.17 |
| Barquera | — | — | — | 0.09 | 0.06 | 0.08 | 0.15 | 0.38 | 0.09 |
| Santander | — | — | — | — | 0.10 | 0.15 | 0.17 | 0.36 | 0.12 |
| Santona | — | — | — | — | 0.07 | 0.12 | 0.28 | — | 0.09 |
| Oka | — | — | — | — | — | 0.12 | 0.34 | — | 0.11 |
| Lea | — | — | — | — | — | — | 0.36 | — | 0.33 |
| Bidasoa | — | — | — | — | — | — | — | — | 0.33 |
| Arcachon | — | — | — | — | — | — | — | — | — |

| D | Vigo | — | 0.32 | 0.13 | 0.18 | 0.14 | 0.17 | 0.25 | 0.20 | 0.08 |
|---|---|---|---|---|---|---|---|---|---|---|
| Ribadesella | — | — | 0.07 | 0.17 | 0.16 | 0.11 | 0.11 | 0.12 | 0.30 | 0.09 |
| Barquera | — | — | — | 0.09 | 0.07 | 0.06 | 0.06 | 0.06 | 0.06 | 0.06 |
| Santander | — | — | — | 0.11 | 0.16 | 0.19 | 0.19 | 0.19 | 0.19 | 0.19 |
| Santona | — | — | — | — | 0.06 | 0.15 | 0.32 | 0.22 | 0.11 |
| Oka | — | — | — | — | 0.11 | 0.32 | 0.10 | 0.10 | 0.10 |
| Lea | — | — | — | — | 0.28 | 0.28 | — | 0.23 | 0.23 |
| Bidasoa | — | — | — | — | — | — | — | — | — |
| Arcachon | — | — | — | — | — | — | — | — | — |
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 $\beta$-diversity across estuaries

The Mantel correlations between species similarity (for the two measures used: $1 - \beta_{\text{sim}}, 1 - \beta_{3}$) 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,
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. Fries 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 implies a high discriminatory power when identifying subpopulations 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 larval 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

| 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 frequencies 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 ≤ $r$ < 0.20) or large ($r$ ≥ 0.20). |
|----------------------------------|--------------------------------------------------|----------------------------------|----------------------------------|
| Marker                           | 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** | 0.030                |
| CeATC1-22                 | 11             | 0.710| 0.797  | 0.109*  | 0.049                |
| CeATC1-54                 | 31             | 0.426| 0.794  | 0.464*  | 0.202                |

| 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 Arosa | 0.748 ± 0.142 | 7.15 ± 2.73 |

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.
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 the 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 tidal soft-bottom macroinvertebrates seems to move almost with no barriers as in most of the pelagic organisms (e.g. *Kinlan and Gaines, 2003*; *Cermeh 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 relation to sea level (e.g. *Friedrichs and Perry, 2001*; *Fries et al., 2012*; *Lorri 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 (*Carrata 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 prioritize 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.

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Appendix A. Supplementary data

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

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