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Temporal and spatial variability on rocky intertidal macrofaunal assemblages affected by an oil spill (Basque coast, northern Spain)

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A large (100 km) rocky coast intertidal was sampled several times (from 2004 to 2006) to assess the affection degree of invertebrate assemblages impacted by a continuous oil spill. Twelve locations and two intertidal heights were selected along the coast representing two spatial scales (kilometres and tens of metres). Univariate and multivariate analyses of variance were used to test whether faunal assemblages exposed to different intensities of oil disturbance differ in terms of diversity, total cover, key species cover and trophic guilds. Whereas no significant differences in midshore assemblages were noted, the low intertidal zone exhibited comparatively lower abundance values of the limpet Patella ulyssiponensis *at worst affected sites. Besides, a generalized increasing diversity trend was found in the low intertidal from 2004 to 2006. Natural variability of communities is also discussed as the cause of the differences we observed. With respect to spatial and temporal scales of variation, mid-intertidal communities showed a more consistent structure, while lowshore assemblages were markedly heterogeneous in practically all the variables measured.*

Keywords: rocky intertidal, *Patella*, oil spill, spatio-temporal variation, 5-way ANOVA, PERMANOVA.

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INTRODUCTION

Intertidal ecosystems are especially vulnerable to oil pollution since oil commonly accumulates in littoral areas leading to physical smothering and chemical poisoning (Raffaelli & Hawkins, 1996). Disturbance and recovery of communities after accidental spills can vary considerably depending on the spill magnitude and nature of the oil spilled (Antrim *et al.*, 1995), the use of dispersants or mechanical cleaning (Crothers, 1983), the timing and intensity of the spill relative to the timing and intensity of reproduction and subsequent recruitment (Forde, 2002), species sensitivity (Crowe *et al.*, 2000) and habitat sensitivity (Jackson *et al.*, 1989). Despite these differences, the noxious effects of oil spills on intertidal animal populations are similar and well chronicled in environmental disasters such as the 'Torrey Canyon' (Southward & Southward, 1978), 'Nella Dan' (Simpson *et al.*, 1995), 'Exxon Valdez' (Peterson *et al.*, 2003), 'Braer' (Newey & Seed, 1995), 'Sea Empress' (Crump *et al.*, 1998), and 'Erika' (Le Hir & Hily, 2002) oil spills. Research coincides with initial biological effects: removal of key intertidal grazing species (limpets, littorinid snails and/or sea urchins) followed by a generalized growth of opportunistic algae. In contrast, absence or minimal impact is also documented in littoral ecosystems, such as after the 'Antonio Gramsci' (Bonsdorff, 1981) and the 'Tebar V' (Lopes *et al.*, 1997) oil spills.

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In November 2002, the 'Prestige' oil tanker carrying more than 77,000 tons of heavy fuel oil sank off the Galician coast, north-west of Spain. Following the wreckage, the tanker initially released 10,000 tons of oil, but the initial catastrophe worsened after the sinking as the wrecked ship gradually released the remaining fuel resulting in a series of oil waves for 10 months (González *et al.*, 2006). Due to the prevailing winds and ocean currents, the oil reached extensive areas of the Bay of Biscay as well as Portuguese coasts affecting mainly the shoreline, where much of the spilled fuel was deposited (González *et al.*, 2006; Acuña *et al.*, 2008). The arrival of oil to the Biscay coast was continuous with two main oil waves reaching the coast in January and September 2003, that generated an extensive, but not intense, fuel deposition in the intertidal zone (ORBANKOSTA, 2004).

Some previous investigations have focused on rocky intertidal assemblages after the 'Prestige' oil spill. In the most impacted area on the Galician coast, Vazquez *et al.* (2005) established a negative relation between per cent of substratum covered by the oil and the abundance of limpets *Patella* spp. Linnaeus, the barnacle *Chthamalus montagui* Southward and the mussel *Mytilus galloprovincialis* Lamarck. Recently, a genetic study has found a reduction in genetic diversity of the snail *Littorina saxatilis* (Olivi) (Piñeira *et al.*, 2008). Rocky intertidal vegetation has also been under investigation. By contrast, there is a lack of evidence of impact on macroalgal assemblages of the entire affected area (Lobón *et al.*, 2008; Díez *et al.*, 2009a).

Monitoring for changes in the marine assemblages is beset with several difficulties (McIntyre & Pearce, 1980) and often the sampling design decisions have influenced the outcomes of those investigations (Peterson *et al.*, 2001). The absence of pre-impact data together with the deficient knowledge of natural ecological variation at spatial and temporal scales, are serious weaknesses in the assessment of any environmental impact (Terlizzi *et al.*, 2005). Furthermore, recovery from acute incidents is inevitably a long-term process, whereas monitoring studies are often too short-lived, circumstances which make fair evaluation of ecological response rather unachievable (Hawkins *et al.*, 2002).

The peculiarity of the 'Prestige' oil spill gave us a unique opportunity to investigate a continuous impact over time of oil deposition on rocky intertidals. Although our study is unfortunately lacking pre-spill data, the nested sampling design allowed us to test whether faunal assemblages exposed to different intensities of oil disturbance differ in terms of diversity, total cover, key species cover and trophic guilds. In addition, our research provides quantitative data on intertidal faunal communities that were so far unavailable for the region and fills the gap of knowledge on spatio-temporal variability of rocky assemblages of the central sector of the Bay of Biscay, locally known as the Basque coast.

MATERIALS AND METHODS

The sampling methods described here were designed for an ecological study focused on the assessment of the effects of the oil on the rocky intertidal benthic system (flora and fauna) on the Basque coast. The results of the phytobenthic component have recently been published (Díez *et al.*, 2009a).

Study area

The Basque coast is located in the North Atlantic, Western Europe. It stretches for approximately 150 km along the south-eastern corner of the Bay of Biscay (41°53′N to 43°40′N and 01°40′W to 09°20′W) (Figure 1). Wave action is predominantly from the north-west. The coast is delimited by moderate to high cliffs (20–150 m) (Pascual *et al.*, 2004). Tides are semidiurnal and the maximum range during spring tides is 4.5 m. Mean water surface temperature ranges between 12°C in February and 22°C in August (Valencia *et al.*, 2004).

Target habitat and community

The habitat and community for investigation were selected on the basis of providing a homogeneous biotope along the coast to compare and assess the putative impact of oil. The rocky intertidal zone was selected for study as the Basque coast is 70% rocky (Pascual *et al.*, 2004) and because oil affected mainly this habitat (González *et al.*, 2006). Oil spills have indirect effects on the structure and function of marine communities (Johnston & Keough, 2002), so scientific interest was also focused on species that play a dominant role in structuring the community.

Two shore levels were studied in order to gather comprehensive information of possible altered biological processes occurring at the intertidal zone. We selected the midshore zone dominated by barnacles (tidal height: 3–4 m) as it extends homogeneously along the coast. Furthermore, grazing species such as limpets typically shelter on this habitat and they have been proven to experience great reductions after oil spills (e.g. Southward & Southward, 1978) and they also play a key role in intertidal community dynamics (Branch, 1985). In addition, the *Corallina elongata* J. Ellis and Sol. community dominating the low intertidal zone (tidal height: 0.7–1.2 m) was also studied. This algae species occurs throughout the Basque coast and provides habitat for a large number of invertebrates, including limpets. The validity of coralline turf in studies analysing spatial changes in diversity and structure of associated macrofaunal assemblages has been previously assessed (Bussell *et al.*, 2007; Liuzzi & López-Gappa, 2008).

At both tidal levels, only communities inhabiting gently to moderate sloping (0°–45°) stable substrates (continuous bedrock and great blocks) were considered for sampling. In this way, we can discard the undesirable effects of rocky surface inclination in the investigated assemblages.

Sampling design

Given the absence of data from an affected population prior to the pollution event, the experimental design needs to compare polluted and control populations (Underwood, 1981). However, no suitable controls were found on the investigated area, as the prolonged continuous arrival of oil affected the

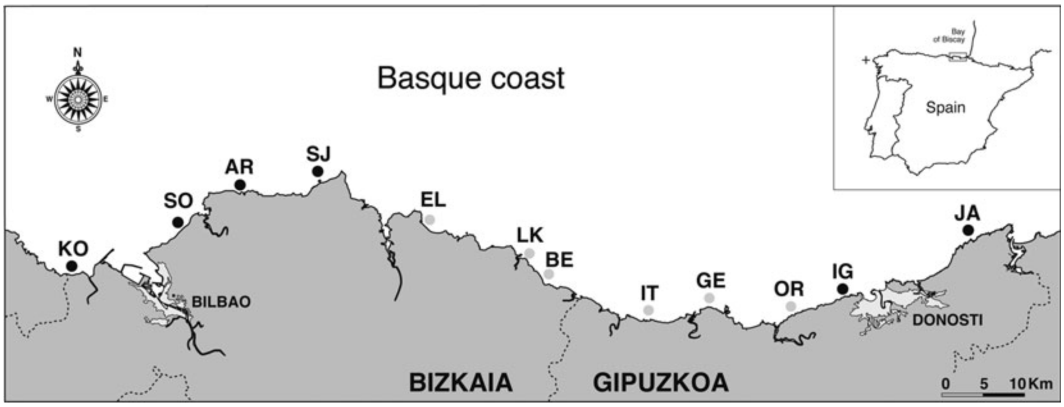

Fig. 1. Study area showing the emplacement of the sampling locations. Kobaron (KO), Sopelana (SO), Arminza (AR), San Juan de Gaztelugatxe (SJ), Elantxobe (EL), Lekeitio (LK), Berriatua (BE), Itziar (IT), Getaria (GE), Orio (OR), Igueldo (IG) and Jaizkibel (JA). Grey and black dots: slightly and moderately affected locations, respectively. The inset shows the initial leaking area of the 'Prestige' spill (indicated by a plus symbol) and the study area (delimited by a rectangle) on the northern coast of Spain.

entire coast to some degree (ORBANKOSTA, 2003). Thus, we decided to divide the coast into different sectors with two different levels of pollution: (1) slightly affected locations with small oil patches 5–50 cm in diameter and 1–3 mm thick sparsely distributed along the supralittoral and higher intertidal zones; and (2) moderately affected locations with patches measuring several tens of square metres irregularly distributed along the supralittoral and higher intertidal zones. Several locations (separated by tens of kilometres) were classified in either group on the basis of the oil arrival inventory report (ORBANKOSTA, 2003) as well as by direct inspection during repeated visits to the locations before sampling. Locations that were extremely exposed to wave action were not selected and only places accessible on foot and only very exposed or exposed shores were considered. A subset of 6 locations was randomly chosen from among the low affected possible sectors: Elantxobe, Lekeitio, Berriatua, Itziar, Getaria and Orio. Similarly, another 6 locations were randomly selected from among the possible moderately affected ones: Kobaron, Sopelana, Arminza, San Juan de Gaztelugatxe, Igueldo and Jaizkibel (Figure 1).

At each location (1 km in length, or less), several areas providing potential communities under investigation were identified. Two areas (separated by tens of metres) were selected by choosing at random from among the set of previously identified ones. At each area (15 m in length and 2–4 m wide), 3 quadrats used as replicates were randomly sampled. Quadrats were selected by moving at random distances along a measuring tape spread out on the bedrock. The no coincident points with the target surfaces (i.e. gently to moderate continuous bedrock and great blocks) were excluded. The same locations were revisited during the study, while areas were randomly selected in each sampling. All random choices were accomplished using tables of random digits. Temporal variability was studied at fixed intervals. During the whole study period, samplings were carried out twice a year at 2 opposite season conditions: spring (mainly in March and April) and the autumn (mainly in October and November).

There are some specific circumstances that partly explain the delay to the start of the study, which began almost one year after the first slicks reached the Basque coast. After the ‘Prestige’ sank, several oil waves over a ten-month period constantly worsened the reported scenario. Thus, as a result of the changing situation, we faced considerable difficulties when trying to establish a proper sampling design with sectors along 150 km of coast at a determinate level of oil affection. Furthermore, data obtained in a first sampling survey carried out in the autumn of 2003, were invalidated as some undesirable design deficiencies were soon detected.

Field sampling

A non-destructive sampling strategy was used which consisted of visually assessed estimates of animal cover in % at specific level in 40 × 40 cm quadrats using the abundance-covering scale proposed by Pérès & Picard (1964) as reference: + (<1%), 1 (2.5–5%), 2 (5–25%), 3 (25–50%), 4 (50–75%) and 5 (75–100%). The mean species cover was calculated for each replicate using the categorical mean of each of the ranges. As a result of the field sampling, a total of 432 observations were obtained: 3 years, 2 seasons, 2 oiling levels, 6 locations, 2 sites and 3 quadrats.

Species not identified in the field were preserved in formalin or alcohol according to recommended procedures. Taxonomic identification was mostly carried out at species level, but in some cases such as difficult groups, we provided identification at family level or even higher taxonomic categories. The intertidal barnacles *Chthamalus montagui* and *Chthamalus stellatus* (Poli) co-occur along the Basque coast. As these species were not easy to distinguish in some locations, particularly at juvenile stages, we grouped both as *Chthamalus* spp. Ranzani for the statistical analyses. Similarly, the midshore limpets *Patella intermedia* Murray, *Patella vulgata* Linnaeus and *Patella rustica* Linnaeus were all grouped as *Patella* spp. complex.

Response variables studied

We used 3 common metrics of diversity: species richness (i.e. the number of species within a community) (S), Shannon's diversity (H') and Pielou's evenness (J'). These univariate measures of diversity provide useful information about the loss or addition of species, the abundance of the species that are present and the skew in the distribution of abundance among the different species. As the number of species in the mid-intertidal community was limited and spatio-temporal differences detected corresponded to an extremely short range of variation, diversity measures were not taken into account for the statistical analyses in our research.

Other variables explored were the total faunal cover, the cover of key species, cover of the most abundant species and the structure of the community as a whole. In addition, species were aggregated into functional groups in order to examine for changes in the community trophic guilds. The trophic approach provides indirect information on the physical variables of the environment, as variations in the relative abundance of each strategy might respond to changes in the environmental conditions (natural or anthropogenic) (Roth & Wilson, 1998).

Statistical analyses

Data of the two tidal levels studied were treated separately. Spatio-temporal patterns of species richness (S), Shannon's diversity (H'), Pielou's evenness (J'), total cover, cover of key species, and cover of particularly abundant taxa were examined by a 5-way nested analysis of variance (ANOVA) using GMAV5 software (Institute of Marine Ecology, University of Sydney, Australia). The factors considered were: year (3 levels, fixed), season (2 levels: fixed and orthogonal), oiling level (2 levels, fixed and orthogonal to season), location (6 levels: random and nested in oiling level) and area (2 levels, random and nested in the interaction location × oiling level). Prior to analysis, Cochran's C-test was employed to assess homogeneity of variances. When appropriate, Student–Newman–Keuls (SNK) tests were used for *a posteriori* multiple comparisons of the means.

Permutational multivariate analysis of variance (PERMANOVA) was used to identify significant temporal and spatial variation in assemblage structure and trophic traits. The analyses were carried out using the PERMANOVA+ for PRIMER software package developed by Anderson *et al.* (2008). The model for the analyses (experimental details concerning factors and levels) were similar to those described for univariate analysis. In order to lessen the influence of the more abundant taxa, data were transformed beforehand in some cases. Mid-intertidal data were square root transformed for assemblage-structure analysis, while data were not transformed for trophic guilds analysis. At the low intertidal zone, data were 4th root transformed for the assemblage-structure analysis, while a square root transformation was considered more adequate for trophic guilds analysis. The procedure was based on Bray–Curtis dissimilarities which were used to calculate a distance matrix between pair of samples. Probability values were given using 999 random permutations of the residuals under a reduced model. In addition, the significant results of the permutational analyses were further investigated using the SIMPER routine. This procedure allowed us to establish which species or trophic strategy was mainly responsible for the significant differences in the factors selected by the permutational analyses.

Any evidence of significant temporal trend (from 2004 to 2006 or vice versa) was considered of importance as it could be related to the arrival of oil to the coast. If no temporal pattern was found, we only considered as significant those spatial differences constant throughout the three years, since spatial patterns can vary from time to time (Underwood & Petraits, 1993).

Temporal and spatial variation of faunal assemblages were represented graphically also using the PRIMER +add on program (Anderson *et al.*, 2008). In order to facilitate the visualization of possible patterns and trends, non-parametric multidimensional scaling ordinations (nMDS) were plotted using 72 mean measures obtained by averaging the following factors: year, season and location. Medium intertidal data were square root transformed, while low shore data were 4th root transformed.

RESULTS

Low intertidal zone

A total of 77 macrofaunal taxa were recorded, comprising 4 species of Porifera, 7 Cnidaria, 13 Annelida, 25 Arthropoda, 22 Mollusca, 3 Echinodermata and 3 Bryozoa. Few species reached mean values above 1% cover (Table 1). These were: the limpet *Patella ulyssiponensis* Gmelin, the cirripeds *Chthamalus* spp., the sea urchin *Paracentrotus lividus* (Lamarck) and the bivalve *Mytilus galloprovincialis* Lamarck. The most common species (found over a 30% of the total of replicates) were *P. ulyssiponensis*, *Chthamalus* spp., the gastropod *Bittium reticulatum* (Da Costa), *M. galloprovincialis* and the polychaete *Pomatoceros lamarckii* (Quatrefages).

DIVERSITY MEASURES

Species richness S, Shannon diversity H' and Pielou's evenness J' showed significant differences during the three years studied (Table 2). According to the SNK test comparisons, an increasing trend was detected from 2004 to 2006 (Figure 2). By contrast, significant differences between seasons, oiling level and locations were not detected. ANOVA showed strong spatial variability at the scale of tens of metres (areas) which was consistent with time as it was indicated by the absence of interaction year × area and season × area.

TOTAL FAUNAL COVER

ANOVA did not detect significant differences in the faunal cover between years, seasons or oiling level (Table 3; Figure 3A). On the contrary, the abundance of the fauna was characterized by a strong spatial variability both at scale of kilometres (locations) and tens of metres (areas), although differences between areas were not consistent over years.

SPECIES ABUNDANCE

Neither annual nor seasonal differences were found in the abundance of the limpet *Patella ulyssiponensis* (Table 3; Figure 3B). Nevertheless, significant differences between the two oiling levels were detected which were consistent over the three years studied. As indicated by the SNK test, this gastropod reached lower cover values in the moderately affected sites, although no temporal trend was detected (Figure 4). The distribution of *P. ulyssiponensis* was characterized by both a strong variability at a scale of kilometres (locations) and tens of metres (areas). However, spatial differences between locations were not consistent over years.

The distribution of the population of *Chthamalus* spp. in the low intertidal level was defined by considerable stability over time (Table 3; Figure 3C). Furthermore, their cover did not show significant variations between the two oiling levels. The cirripeds showed a homogeneous distribution at a scale of tens of metres (areas) that contrasts with the significant differences maintained over time at scale of kilometres (locations).

FAUNAL ASSEMBLAGES

The results of the permutational analyses of variance performed on the complete species abundances data set (77 variables) showed a significant effect of all factors considered in the analysis with the exception of the factor season (Table 4). Differences between the two oiling levels were constant throughout the whole period studied, whereas differences between locations and areas were not consistent over time. During the three years of the study, slightly affected locations were distinguished from moderately affected locations for their higher abundance of the limpet *Patella ulyssiponensis* (Table 5). The permutational analyses also proved a significant effect of the factor year. However, the SIMPER routine does not show evident trends in the abundance of any species among the three years of the study.

The MDS plot (Figure 5) does not show clear effects of time. However, a separation of assemblages is noted with less impacted locations being mainly placed in the right side of the diagram. The graph also illustrates a more scattered distribution for moderately than slightly affected locations.

TROPHIC GUILDS

The relative abundance of the trophic guilds of the community showed significant temporal differences between years and seasons although no differences were found between the two oiling levels (Table 4). Trophic guilds in the community were characterized by a strong variability at a scale of kilometres (locations) and tens of metres (areas). However, spatial differences between areas were not consistent over time. The contribution of trophic strategy to the significant factors obtained by the permutational analyses can be consulted in Table 5. Suspensivores and omnivores showed a slightly increasing trend over time. Seasonal trends are

Table 1. Lowshore faunal species cover (C, in %) and frequency (F, in %) in the study area according to different seasons and years (N = 72). The symbol + indicates cover values less than 1%.

Lowshore		**2004**						**2005**						**2006**					
		Spring			**Autumn**			**Spring**			**Autumn**			**Spring**			**Autumn**		
		C	**SE**	**F**	**C**	**SE**	**F**	**C**	**SE**	**F**	**C**	**SE**	**F**	**C**	**SE**	**F**	**C**	**SE**	**F**
	Porifera																		
Su	*Cliona celata* Grant, 1826	+	0.0	1.4	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·
Su	*Haliclona cinerea* (Grant, 1827)	·	·	·	·	·	·	·	·	·	·	·	·	+	0.0	1.4	+	0.0	2.8
Su	*Hymeniacidon sanguinea* (Grant, 1826)	+	0.0	6.9	+	0.1	19.4	+	0.1	23.6	+	0.0	9.7	+	0.1	16.7	+	0.0	11.1
Su	*Polymastia mammillaris* (Müller, 1806)	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	+	0.0	1.4
	Cnidaria																		
Su	*Actinia equina* (Linnaeus, 1758)	+	0.0	2.8	·	·	·	+	0.0	1.4	+	0.0	1.4	+	0.0	2.8	+	0.0	2.8
Su	*Actinothoe sphyrodeta* (Gosse, 1858)	·	·	·	·	·	·	·	·	·	·	·	·	+	0.0	2.8	·	·	·
Su	*Anemonia viridis* (Forskål, 1775)	·	·	·	+	0.2	1.4	·	·	·	+	0.0	1.4	+	0.0	1.4	·	·	·
Su	Anthozoa indet.	·	·	·	·	·	2.8	·	·	·	+	0.0	1.4	+	0.1	11.1	+	0.0	12.5
Su	*Cariophyllia smithii* Stokes & Broderip, 1828	+	0.0	1.4	+	0.0	2.8	·	·	·	·	·	·	·	·	·	+	0.0	2.8
Su	*Cereus pedunculatus* (Pennant, 1777)	+	0.0	8.3	+	0.0	6.9	+	0.0	11.1	+	0.1	6.9	+	0.1	22.2	+	0.0	6.9
Su	Epizoanthus indet.	·	·	·	·	·	·	+	0.0	1.4	·	·	·	·	·	·	·	·	·
	Annelida																		
Su	*Cirriformia tentaculata* (Montagu, 1803)	·	·	·	·	·	·	·	·	·	·	·	·	+	0.0	2.8	·	·	·
Om	*Eulalia viridis* (Linnaeus, 1767)	+	0.0	8.3	+	0.0	5.6	+	0.0	9.7	+	0.0	9.7	+	0.0	13.9	·	·	·
Ca	Eunicidae indet.	·	·	·	·	·	·	·	·	·	+	0.0	1.4	·	·	·	·	·	·
Om	*Neanthes succinea* (Frey & Leuckart, 1847)	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	+	0.0	1.4
Om	Nereidae indet.	·	·	·	+	0.0	1.4	·	·	·	+	0.0	1.4	·	·	·	+	0.0	1.4
Om	*Perinereis cultrifera* (Grube, 1840)	·	·	·	+	0.0	2.8	·	·	·	+	0.0	1.4	·	·	·	·	·	·
Om	*Platynereis dumerilli* (Audoin & Milne-Edwards, 1833)	·	·	·	·	·	·	·	·	·	·	·	·	+	0.0	1.4	·	·	·
Om	*Polyophthalmus pictus* (Dujardin, 1839)	+	0.0	15.3	+	0.0	9.7	+	0.0	4.2	+	0.0	25.0	+	0.0	23.6	+	0.0	47.2
Su	*Pomatoceros lamarckii* (Quatrefages, 1865)	+	0.0	25.0	+	0.0	19.4	+	0.0	44.4	+	0.0	31.9	+	0.1	52.8	+	0.0	33.3
Su	Sabellaridae indet.	+	0.0	9.7	+	0,1	18.1	+	0.0	18.1	+	0.1	26.4	+	0.1	23.6	+	0.0	20.8
Su	*Serpula concharum* Langerhans, 1880	+	0.0	15.3	+	0.1	16.7	+	0.0	29.2	+	0.0	26.4	+	0.0	26.4	+	0,0	20.8
Su	Spionidae indet.	·	·	·	+	0.0	2.8	·	·	·	+	0.0	1.4	+	0.6	4.2	·	·	·
Su	*Spirorbis pagenstecheri* Quatrefages, 1865	+	0.1	26.4	+	0.0	19.4	+	0.0	19.4	+	0.1	18.1	+	0.0	15.3	+	0.1	27.8
	Arthropoda																		
De	Amphipoda indet.	·	·	·	+	0.0	5.6	+	0.0	4.2	+	0.0	8.3	·	·	·	+	0.0	6.9
De	*Ampithoe ramondi* Audoin, 1826	·	·	·	+	0.0	4.2	·	·	·	·	·	·	+	0.0	1.4	+	0.0	6.9
De	*Apherusa jurinei* (Milne-Edwards, 1830)	·	·	·	·	·	·	+	0.0	2.8	·	·	·	+	0.0	2.8	·	·	·
Su	*Balanus perforatus* Bruguière, 1789	+	0.0	4.2	+	0.0	4.2	+	0.0	5.6	+	0.0	5.6	+	0.0	2.8	+	0.0	4.2
De	*Caprella penantis* (Leach, 1814)	+	0.0	6.9	+	0.0	6.9	+	0.0	9.7	+	0.0	2.8	+	0.0	1.4	+	0.0	1.4
Om	*Carcinus maenas* (Linnaeus, 1758)	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	+	0.0	2.8
Su	*Chthamalus* spp.	4.0	1.2	61.1	2.4	0.7	61.1	2.2	0.7	58.3	2.9	0.7	62.5	2.6	0.7	63.9	1.7	0,5	59.7
Om	*Clibanarius erythropus* (Latreille, 1818)	+	0.1	6.9	+	0.0	12.5	+	0.0	4.2	+	0.0	11.1	+	0.0	4.2	+	0.0	16.7
He	*Cymodoce truncata* Leach, 1814	·	·	·	·	·	·	·	·	·	+	0.0	5.6	·	·	·	+	0.0	4.2
He	*Dynamene bidentata* (Adams, 1800)	·	·	·	+	0.0	2.8	·	·	·	·	·	·	+	0.0	1.4	·	·	·
Om	*Eriphia verrucosa* (Forskål, 1775)	+	0.0	1.4	+	0.0	2.8	·	·	·	+	0.2	2.8	·	·	·	+	0.0	4.2
De	*Hyale* indet.	+	0.0	2.8	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·
De	*Hyale perieri* (Lucas, 1846)	+	0.1	16.7	+	0.0	19.4	+	0.1	30.6	+	0.2	31.9	+	0.1	29.2	+	0.1	29.2
De	*Hyale pontica* Rathke, 1837	+	0.0	4.2	·	·	·	·	·	·	·	·	·	+	0.0	4.2	·	·	·
De	*Hyale stebbingi* (Chevreux, 1888)	+	0.0	8.3	+	0.0	8.3	·	·	·	·	·	·	+	0.0	12.5	+	0.0	8.3
De	*Idotea baltica* (Pallas, 1772)	·	·	·	·	·	·	+	0.0	1.4	·	·	·	·	·	·	·	·	·
De	*Jassa falcata* (Montagu, 1808)	·	·	·	·	·	·	+	0.0	4.2	+	0.0	2.8	·	·	·	+	0.0	8.3
Om	*Pachygrapsus marmoratus* (Fabricius, 1787)	+	0.0	8.3	+	0.0	8.3	+	0.0	1.4	+	0.0	26.4	+	0.0	15.3	+	0.0	40.3
Om	*Pirimela denticulata* (Montagu, 1808)	·	·	·	·	·	·	·	·	·	+	0.0	1.4	·	·	·	·	·	·

Continued

Table 1. Continued

Lowshore		**2004**						**2005**						**2006**					
		Spring			**Autumn**			**Spring**			**Autumn**			**Spring**			**Autumn**		
		C	**SE**	**F**	**C**	**SE**	**F**	**C**	**SE**	**F**	**C**	**SE**	**F**	**C**	**SE**	**F**	**C**	**SE**	**F**
Om	*Pisa armata* (Latreille, 1803)	·	·	·	·	·	·	+	0.0	1.4	·	·	·	·	·	·	·	·	·
Om	*Pisa tetraodon* (Pennant, 1777)	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	+	0.0	1.4
De	*Sphaeroma* indet.	·	·	·	·	·	·	·	·	·	·	·	·	+	0.0	5.6	·	·	·
De	*Sphaeroma serratum* (Fabricius, 1787)	·	·	·	·	·	·	+	0.0	1.4	+	0.0	1.4	·	·	·	·	·	·
De	*Tanais dulongii* (Audoin, 1826)	·	·	·	+	0.0	1.4	·	·	·	·	·	·	·	·	·	·	·	·
Om	*Xantho* indet.	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	+	0.0	2.8
	Mollusca																		
He	*Acanthochitona crinita* (Pennant, 1777)	+	0.0	5.6	+	0.0	1.4	+	0.0	4.2	·	·	·	+	0.0	1.4	+	0.0	2.8
He	*Aplysia* indet.	·	·	·	·	·	·	·	·	·	·	·	·	+	0.0	2.8	·	·	·
De	*Bittium reticulatum* (da Costa, 1778)	+	0.2	37.5	+	0.0	41.7	+	0.0	38.9	+	0.1	48.6	+	0.1	43.1	+	0.1	55.6
He	*Gibbula cineraria* Linnaeus, 1758	·	·	·	·	·	·	+	0.0	4.2	·	·	·	·	·	·	+	0.0	1.4
He	*Gibbula pennanti* (Philippi, 1851)	·	·	·	·	·	·	+	0.0	1.4	·	·	·	·	·	·	·	·	·
He	*Gibbula umbilicalis* (da Costa, 1778)	+	0.1	29.2	+	0.0	25.0	+	0.1	23.6	+	0.1	25.0	+	0.2	38.9	+	0.1	31.9
De	*Hinia incrassata* (Ström, 1768)	+	0.0	11.1	+	0.0	16.7	+	0.0	22.2	+	0.0	18.1	+	0.1	33.3	+	0.0	30.6
He	*Lepidochitona cinerea* (Linnaeus, 1767)	+	0.0	1.4	·	·	·	+	0.0	4.2	+	0.0	1.4	+	0.0	1.4	+	0.0	4.2
Su	*Lithophaga caudigera* (Lamarck, 1797)	+	0.0	6.9	+	0.0	11.1	+	0.0	15.3	+	0.0	22.2	+	0.0	11.1	+	0.0	9.7
He	*Littorina* indet.	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	+	0.0	1.4
He	*Littorina littorea* Linnaeus, 1758	+	0.0	1.4	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·
He	*Littorina neritoides* Linnaeus, 1758	+	0.1	2.8	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·
Su	*Mytilus galloprovincialis* Lamarck, 1819	+	0.1	33.3	+	0.1	29.2	+	0.1	34.7	+	0.1	38.9	1.2	0.4	51.4	+	0.1	36.1
Ca	*Ocenebra erinacea* (Linnaeus, 1758)	+	0.0	1.4	+	0.0	9.7	+	0.0	6.9	+	0.0	4.2	+	0.0	12.5	+	0.0	6.9
Ca	*Ocinebrina aciculata* (Lamarck, 1822)	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	+	0.0	1.4
Su	*Ostrea edulis* Linnaeus, 1758	·	·	·	+	0.0	2.8	·	·	·	·	·	·	+	0.0	4.2	·	·	·
He	*Patella ulyssiponensis* Gmelin, 1791	9.2	1.7	68.1	11,3	1.9	79.2	8.0	1.4	76.4	11.3	1.7	70.8	9.8	1.7	73.6	6.4	1.2	65.3
He	*Rissoa parva* (da Costa, 1778)	+	0.0	8.3	·	·	·	+	0.0	8.3	+	0.0	2.8	+	0.0	9.7	+	0.0	16.7
He	*Rissoa parva* var. *interrupta* (J. Adams, 1798)	·	·	·	·	·	·	+	0.0	5.6	·	·	·	+	0.1	19.4	+	0.1	26.4
Su	*Rocellaria dubia* (Pennant, 1777)	+	0.0	1.4	+	0.0	4.2	+	0.0	4.2	+	0.0	8.3	+	0.0	8.3	+	0.0	2.8
He	*Tricolia pullus* (Linnaeus, 1758)	+	0.0	1.2	+	0.0	26.4	+	0.0	12.5	+	0.0	19.4	·	·	·	+	0.0	2.8
Ca	*Trivia monacha* (da Costa, 1778)	·	·	·	·	·	·	·	·	·	+	0.0	1.4	·	·	·	·	·	·
	Echinodermata																		
Ca	*Marthasterias glacialis* (Linnaeus, 1758)	·	·	·	·	·	·	·	·	·	+	0.0	2.8	·	·	·	·	·	·
De	*Ophiotrix fragilis* (Abildgaard, 1789)	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	+	0.0	1.4
He	*Paracentrotus lividus* Desor, 1856	+	0.4	9.7	+	0.4	12.5	2.0	1.0	18.1	+	0.6	6.9	+	0.4	12.5	+	0.3	12.5
	Bryozoa																		
Su	*Chorizopora brongniartii* (Audoin, 1826)	+	0.0	6.9	+	0.0	8.3	+	0.0	6.9	·	·	·	+	0.0	4.2	+	0.0	8.3
Su	*Conopeum seurati* (Canu, 1928)	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	+	0.0	1.4
Su	*Cryptosula pallasiana* (Moll, 1803)	·	·	·	·	·	·	+	0.0	1.4	·	·	·	·	·	·	·	·	·

SE, standard error; Ca, carnivores; De, detritivores; He, herbivores; Om, omnivores; Su, suspensivores.

vaguely detected for practically all the trophic strategies. Suspensivores and detritivores are more abundant in spring, while herbivores and omnivores are more abundant in autumn. It should be noted that temporal and seasonal significant trophic differences corresponded to an extremely short range of variation in the relative abundance of each strategy, so, finally, we decided not to consider them as relevant.

Mid-intertidal zone

A total of 8 macrofaunal taxa were recorded, comprising 1 Cnidaria, 1 Arthropoda, 6 Mollusca (Table 6). The assemblage was dominated by the cirripeds *Chthamalus* spp. and the limpets *Patella* spp. (mainly *Patella intermedia* and *Patella vulgata*). The aforementioned species together with the gastropod *Littorina neritoides* Linnaeus were found in nearly all the samples.

TOTAL FAUNAL COVER

The abundance of the fauna did not show any significant changes through time and there were no differences between the two oiling levels (Table 7; Figure 6A). Strong spatial variability was detected at a scale of tens of metres (areas), but was not consistent over the years.

Table 2. ANOVA results testing the effect of year (Y), season (S), oiling level (H), location (L) and area (A) on species richness S, Shannon's diversity *H′* and Pielou's evenness *J′* of lowshore faunal assemblages.

Lowshore	**df**	***S***		***H′***		***J′***	
		MS	**F**	**MS**	**F**	**MS**	**F**
Y	2	146.07	21.58***	5.07	20.98***	0.24	4.21*
S	1	11.34	0.83	0.45	1.25	0.10	1.13
H	1	37.93	0.36	3.50	0.84	3.90	5.37
L(H)	10	105.53	1.41	4.18	1.61	0.73	2.64
A(HXL)	12	74.69	26.8***	2.59	16.4***	0.28	5.78***
YXS	2	2.25	0.69	0.32	1.86	0.04	0.69
YXH	2	16.22	2.4	0.71	2.94	0.09	1.63
YXL(H)	20	6.77	1.58	0.24	1.55	0.06	0.66
YXA(HXL)	24	4.30	1.54	0.16	0.99	24	0.08
SXH	1	17.93	1.31	0.02	0.06	0.30	3.21
SXL(H)	10	13.66	6	0.36	1.98	0.09	1.73
SXA(HXL)	12	2.28	0.82	0.18	1.15	12	0.05
YXSXH	2	7.10	2.16	0,06	0.34	0.00	0.02
YXSXL(H)	20	3.29	0.45	0,18	0.65	20	0.06
YXSXA(HXL)	24	7.36	2.64***	0.27	1.72**	24	0.06
RES	288	2.79		0.16		0.05	
Cochran's test		ns		ns		ns	
Transform		None		None		None	

*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; ns, not significant.

SPECIES ABUNDANCE

The species group of *Patella* remained stable over time; likewise its abundance presented no variations between the two oiling levels (Table 7; Figure 6B). The distribution of *Patella* spp. was characterized by a marked variability between locations and areas. However, differences at a scale of kilometres (locations) varied over time as indicated by the significant season × location interaction.

Similarly, the cover of the species group of *Chthamalus* spp. did not show temporal changes nor were any differences detected between slightly and moderately affected locations (Table 7; Figure 6C). The cirripeds maintained homogeneous abundances over time at a scale of kilometres (locations). Spatial differences were found at a scale of tens of metres (areas), which were not consistent over the years.

FAUNAL ASSEMBLAGES

Permutational analyses of variance performed on the whole species data set (8 variables) did not show a significant temporal effect and differences between the two intensities of oil disturbance were not found either (Table 8). However, significant spatial variability was found between locations and areas. Differences between locations were not consistent over the seasons while differences between areas were not consistent over the years. The n-MDS ordination plot (Figure 7) shows locations without any temporal or spatial gradient between years, seasons and oiling levels.

TROPHIC GUILDS

The relative abundance of the trophic guilds of the community inhabiting the mid-intertidal zone was characterized by a strong stability between years, seasons, oiling levels and locations (Table 8). Significant differences were only found between areas, which were not consistent over the years.

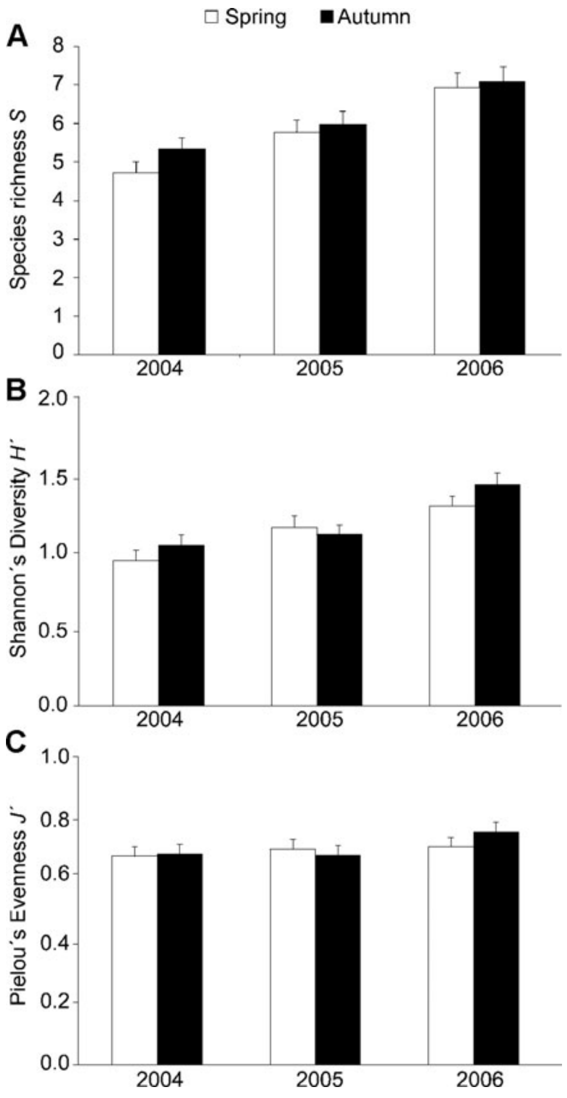

Fig. 2. Species richness *S* (A), Shannon's diversity *H′* (B) and Pielou's evenness *J′*(C) of lowshore faunal assemblages on the Basque coast at time of each sampling (from 2004 to 2006).

DISCUSSION

Previous experience has shown that impacts of oil in rocky intertidal communities lead to reductions in midshore *Patella* populations, which strongly influences the structure of the community (Hawkins & Southward, 1992; Moore, 2006). By contrast, the results presented here show that oil deposition on rocky surfaces deriving from the 'Prestige' accident did not have an appreciable quantitative effect on the abundance of *Patella* along the mid-intertidal zone. Furthermore, the composition, abundance and trophic guilds of the community at this tidal level remained notoriously similar along the Basque coast.

On the contrary, the null hypothesis that community characteristics in the low intertidal zone were invariant to oil affection was rejected in two of the variables tested: (1) the abundance of the limpet *Patella ulyssiponensis*, with lower values at moderately affected locations; and (2) the structure of assemblages. Both results are concurrent since *P. ulyssiponensis* was the sole species of the assemblage that contributed to the significant oiling effect detected by permutational

Table 3. ANOVA results testing the effect of year (Y), season (S), oiling level (H), location (L) and area (A) on faunal cover, *Patella ulyssiponensis* cover and *Chthamalus* spp. cover in lowshore habitat.

Lowshore	**df**	**Faunal cover**		***Patella ulyssiponensis***		***Chthamalus spp.***	
		MS	**F**	**MS**	**F**	**MS**	**F**
Y	2	57.05	0.16	179.56	0.64	0.565	1.04
S	1	151.12	1.18	51.05	0.65	0.04	0.05
H	1	15046.18	3.76	132093.06	5.4^{*}	7.33	1.23
L(H)	10	3998.54	6.07^{**}	2452.51	5.55^{**}	5.97	5.76^{**}
A(HXL)	12	659.25	3.69^{***}	442.16	5.82^{***}	1.04	1.72
YXS	2	1116.61	7.45	451.35	5.45	1.39	1.79
YXH	2	909.63	2.48	362.24	1.28	1.27	2.36
YXL(H)	20	366.93	1.13	282.42	2.43^{*}	0.54	0.66
YXA(HXL)	24	325.81	1.82^{*}	116.15	1.53	0.81	1.34
SXH	1	5.67	0.04	114,60	1.46	0.49	0.6
SXL(H)	10	127.98	1.14	114.60	1.18	0.82	1.78
SXA(HXL)	12	112.13	0.63	66.46	0.87	0.46	0.76
YXSXH	2	38.44	0.26	29.46	0.36	1.07	1.38
YXSXL(H)	20	149.84	0.41	82.75	0.7	0.77	0.65
YXSXA(HXL)	24	366.09	2.05^{**}	118.92	1.56^{*}	1.19	1.96
RES	288	178.53		76.01		0.60	
Cochran's test		ns		ns		$p < 0.01$	
Transform		None		None		Ln(x + 1)	

$^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$; ns, not significant.

analyses. The differences in *P. ulyssiponensis* cover could be better understood if background information existed. In spite of these, the fact that differences in *P. ulyssiponensis* are maintained for 3 years after the spill leads us to consider that the reported differences probably existed prior to the oil arrival. Another significant result that could be related to the arrival of oil to the coast was the increasing diversity trend detected from 2004 to 2006 in low intertidal fauna. Similarly, this result was found after the 'Prestige' spill for macroalgal assemblages along the Basque coast (Diez *et al.*, 2009a). Unfortunately, there were no pre-impact data on fauna for the Basque coast, but helpful information is available from macroalgal communities (Díez *et al.*, 2009b). Their results indicated particularly low diversity values at the *Corallina elongata* assemblage in 2002, prior to the 'Prestige' accident. This information together with the fact that no differences have been found between slightly and moderately oiled locations in terms of faunal diversity, total faunal cover or trophic guilds, led us to deliberate that the increasing trend in diversity is most likely associated with natural variability on the shore.

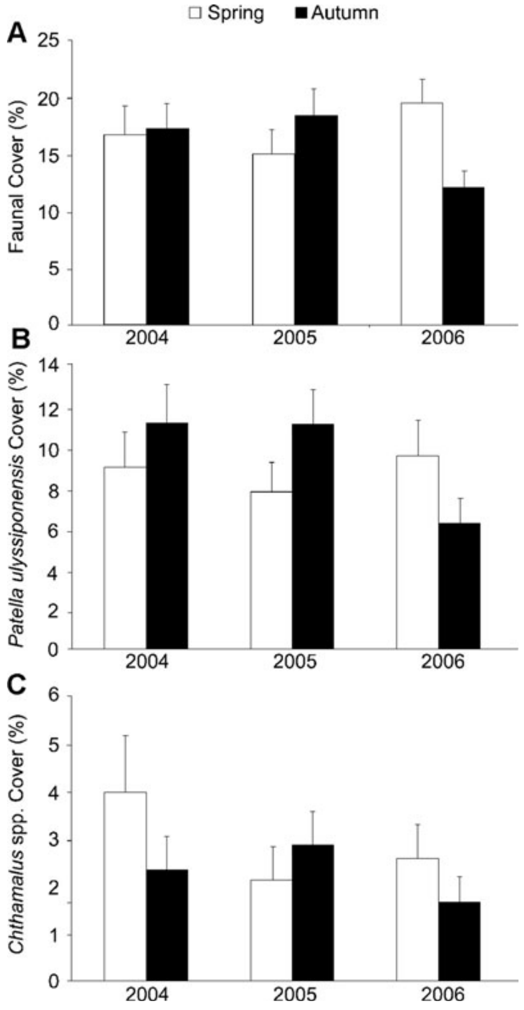

Fig. 3. Faunal cover (A), *Patella ulyssiponensis* cover (B) and *Chthamalus* spp. cover (C) in % of lowshore faunal assemblages on the Basque coast at time of each sampling (from 2004 to 2006).

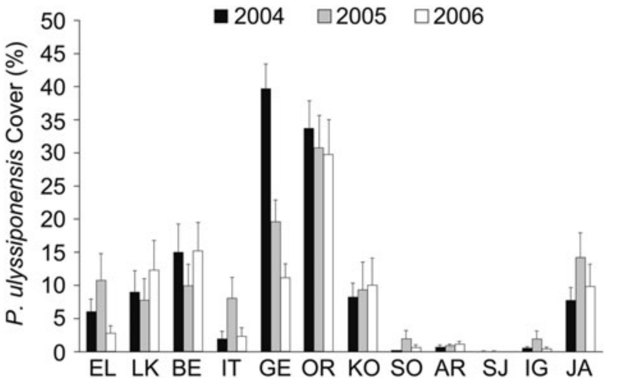

Fig. 4. Lowshore spatial changes in *Patella ulyssiponensis* cover (in %) at slightly and moderately affected locations on the Basque coast over time (from 2004 to 2006).

Table 4. PERMANOVA results showing the effect of year (Y), season (S), oiling level (H), location (L) and area (A) on lowshore faunal assemblages considering the whole species data set and the community trophic guilds.

Lowshore source	df	Species abundance			Trophic guilds		
		SS	MS	F	SS	MS	F
Y	2	22587	11293	3.42^{**}	5442.9	2721.5	2.21^{*}
S	1	7985	7985	1.94	5995.4	5995.4	3.82^{*}
H	1	97390	97390	3.49^{**}	53560	53560	3.47
L(H)	10	2.78E + 05	27846	3.62^{**}	1.54E + 05	15449	4.02^{**}
YxS	2	8851.1	4425.5	1.35	4304.7	2152.4	1.30
YxH	2	6190.1	3095	0.94	603.46	301.73	0.25
SxH	1	5093.1	5093.1	1.24	1927.3	1927.3	1.23
A(L(H))	12	92105	7675.5	6.78^{**}	46093	3841.1	5.61^{**}
YxL(H)	20	65987	3299.3	1.38^{**}	24592	1229.6	1.04
SxL(H)	10	41209	4120.9	2.04^{**}	15690	1569	1.42
YxSxH	2	10056	5027.7	1.53	2210.7	1105.4	0.67
YxA(L(H))	24	57232	2384.7	2.10^{**}	28347	1181.1	1.72^{**}
SxA(L(H))	12	24163	2013.6	1.78^{**}	13252	1104.3	1.61^{*}
YxSxL(H)	20	65611	3280.5	1.05	33080	1654	0.84
YxSxA(L(H))	24	75297	3137.4	2.77^{**}	47149	1964.6	2.87^{**}
Res	288	3.26E + 05	1131		1.97E + 05	683.87	
Total	431	1.18E + 06			6.34E + 05		

$^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$.

Table 5. Average dissimilarity between the significant discriminating factors detected by the permutational analyses in the lowshore habitat.

Lowshore					
Determining discriminating species	**Compared factors**	**Average dissimilarity**	**Standard deviation**	**Ratio**	**Contribution (%)**
Patella ulyssiponensis	H (slightly versus moderately)	11.09	9.72	1.14	14.33
Suspensivores	Y (2004 versus 2005)	11.55	10.04	1.15	22.61
Omnivores	Y (2004 versus 2005)	5.29	6.70	0.79	12.63
Suspensivores	Y (2005 versus 2006)	10.73	9.67	1.11	22.61
Omnivores	Y (2005 versus 2006)	6.69	6.89	0.97	12.52
Herbivores	S (spring versus autumn)	23.33	17.67	1.32	46.77
Suspensivores	S (spring versus autumn)	11.31	10.28	1.11	22.67
Detritivores	S (spring versus autumn)	7.26	7.12	1.02	14.55
Omnivores	S (spring versus autumn)	6.00	6.59	0.91	12.02

Y, year; S, season; H, oiling level.

Effects of pollution on biotic integrity are difficult to identify when there are overlaps between environmental gradients and pollutant effects (Rakocinski *et al.*, 1997). Clearly, the lack of previous data on affected sites and the absence of undisturbed sites (as the whole shore was affected by oil to some extent) were quite restrictive circumstances to our research. Nevertheless, we inferred conclusions about possible alterations of assemblages by contrasting our results with other spill events published in the scientific literature. Investigations of the noxious effects of oil spills on intertidal populations coincide with two major biological effects: the removal of key mid-intertidal grazing species, essentially limpets, and the consequent growth of opportunistic algae (e.g. Southward & Southward, 1978; Crump *et al.*, 1998; Le Hir & Hily, 2002). On the one hand, our results did not show quantitative impact from the 'Prestige' spill on the population of limpets at the mid-intertidal. On the other hand, macroalgal assemblages of the entire affected area did not evidence any indirect impact caused by alterations in the population of grazing species such as *Patella ulyssiponensis* (Díez *et al.*, 2008a; Lobón *et al.*, 2008). These facts led us to conclude that the significant changes detected in the low intertidal zone along the Basque coast are not deleterious and better related with natural fluctuations of these communities.

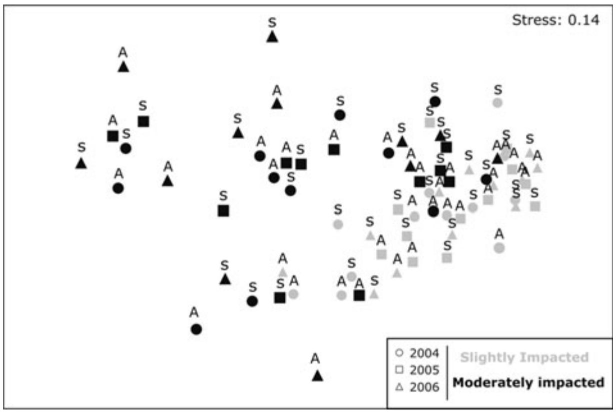

Fig. 5. Non-metric multidimensional scaling similarity plot computed for slightly and moderately affected lowshore assemblages on the Basque coast at time of each sampling (from 2004 to 2006). Spring (*S*); autumn (*A*).

The absence of major impact of the 'Prestige' oil spill on the Basque rocky coast was raised previously by other authors

Table 6. Midshore faunal species cover (C, in %) and frequency (F, in %) in the study area according to different seasons and years (N = 72). The symbol + indicates cover values less than 1%.

Midshore		2004						2005						2006					
		Spring			Autumn			Spring			Autumn			Spring			Autumn		
		C	SE	F	C	SE	F	C	SE	F	C	SE	F	C	SE	F	C	SE	F
	Cnidaria																		
Su	*Actinia equina* (Linnaeus, 1758)	+	0.0	5.6	·	·	·	+	0.0	4.2	+	0.1	2.8	+	0,0	1.4	+	0.0	4.2
	Arthropoda																		
Su	*Chthamalus* spp.	56.1	3.4	100	60.3	3.0	100	55.3	2.9	100	55.8	3.6	98.6	61.6	3.1	100	53.5	2.8	100
	Mollusca																		
He	*Gibbula umbilicalis* (da Costa, 1778)	+	0.1	12.5	+	0.1	20.8	+	0.1	19.4	+	0.0	9.7	+	0.0	18.1	+	0.0	5.6
He	*Littorina littorea* Linnaeus, 1758	·	·	·	+	0.0	5.6	·	·	·	·	·	·	·	·	·	·	0.1	·
He	*Littorina neritoides* Linnaeus, 1758	1.2	0.1	100	2.0	0.4	95.8	1.5	0.3	100	1.3	0.2	97.2	1.4	0.2	100	1.1	0.1	100

SE, standard error; He, herbivores; Su, suspensivores.

Table 7. ANOVA results testing the effect of year (Y), season (S), oiling level (H), location (L) and area (A) on faunal cover, *Patella* spp. cover and *Chthamalus* spp. cover in midshore habitat.

Midshore	df	Faunal cover		*Patella* spp.		*Chthamalus* spp.	
		MS	F	MS	F	MS	F
Y	2	179.78	0.20	0.22	0.28	264.10	0.32
S	1	1022.13	1.46	2.57	1.94	145.26	0.43
H	1	15944.45	2.33	3.78	0.23	12550.72	1.5
L(H)	10	6855.42	1.12	16.52	3.97^{*}	8374.52	1.99
A(HXL)	12	6119.16	18.19^{***}	4.16	10.18^{***}	4204.81	14.82^{***}
YXS	2	4553.60	7.22	6.56	5.36	1434.44	1.92
YXH	2	614.17	0.69	0.98	1.24	129.61	0.16
YXL(H)	20	889.57	1.29	0.78	1.24	827.26	1.35
YXA(HXL)	24	687.24	2.04^{**}	0.63	1.55	612.11	2.16^{**}
SXH	1	3807.42	5.42	1.29	0.97	1766.21	5.23
SXL(H)	10	701.91	1.29	1.33	3.29^{*}	337.79	0.95
SXA(HXL)	12	545.29	1.62	0.40	0.99	354.72	1.25
YXSXH	2	2685.92	4.26	0.97	0.79	1904.01	2.55
YXSXL(H)	20	630.62	0.51	1.23	0.81	746.50	1.37
YXSXA(HXL)	24	1245.27	3.7^{***}	1.52	3.72^{***}	543.13	1.91^{**}
RES	288	336.46		0.41		283.71	
Cochran's test		ns		$p < 0.01$		ns	
Transform		None		$\mathrm{Ln}(x + 1)$		None	

$^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$; ns, not significant.

(Lobón *et al.*, 2008; Díez *et al.*, 2009a). The use of the fishing fleet to collect the oil at sea prevented to some extent large fuel deposition on the rocky coast (González *et al.*, 2006). Indeed, communities at both tidal levels studied were not smothered by oil (ORBANKOSTA, 2004). Furthermore, fuel was partially weathered by the time it arrived at the Basque coast (Gallego *et al.*, 2006), probably producing less damaging effects in organisms (Antrim *et al.*, 1995). The limited use of aggressive cleanups was also decisive, as they sometimes cause more damage than the fuel itself (Southward & Southward, 1978; Houghton *et al.*, 1996).

However, in the cases of apparently unaltered populations (or fully recovered ones), the existence of genetic damage that could affect their long-term capability of adaptation (Piñeira *et al.*, 2008) should be noted. Bartolomé and co-workers (submitted for publication) found considerable levels of polycyclic aromatic hydrocarbons (PAH) accumulated in *Patella vulgata* tissues inhabiting the supralittoral fringe (above 4 m) of the Basque coast in several of the most affected locations. Unfortunately, no surveillance has been accomplished in the study area on the genetic impact of the oil accumulated in *P. vulgata* populations after the 'Prestige' disaster. No doubt, an integral assessment of the consequences of environmental pollution requires a complete understanding of all variables responsible for changes in the population dynamics in the affected area (Clark, 1982). Concerning limpets, they play a key role in structuring the entire intertidal ecosystem (Branch, 1985; Hawkins *et al.*, 1992). Consequently, further studies are necessary to evaluate the state of the marine environment on the Basque coast after the 'Prestige' oil spill with certainty.

With respect to spatio-temporal variation, a trend that emerges from this study is that heights on the shore involve important differences. Mid-intertidal communities exhibited a more consistent structure in space and time with a single variable showing spatially significant difference. On the

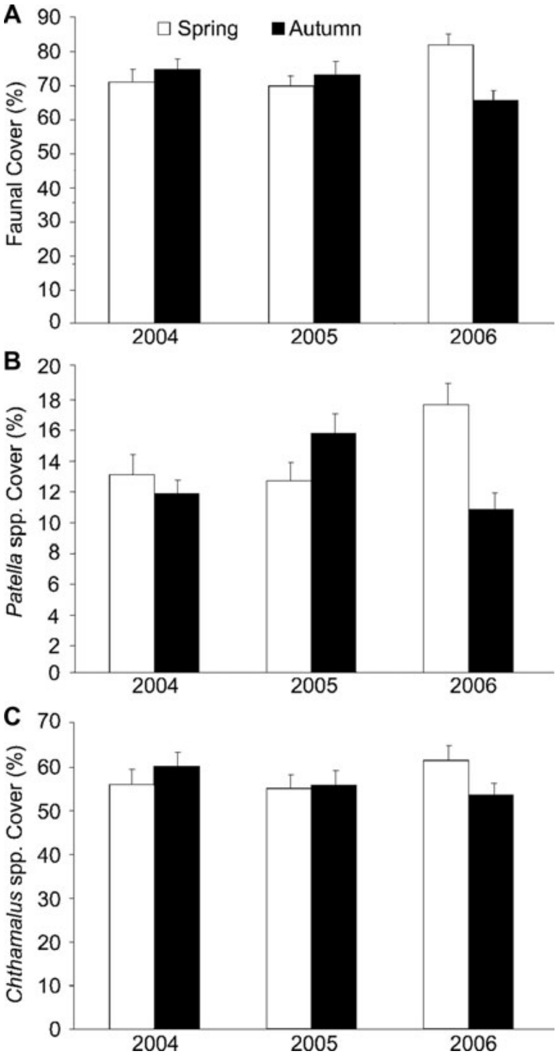

Fig. 6. Faunal cover (A), *Patella* spp. cover (B) and *Chthamalus* spp. cover (C) in % of midshore faunal assemblages on the Basque coast at time of each sampling (from 2004 to 2006).

Fig. 7. Non-metric multidimensional scaling similarity plot computed for slightly and moderately affected midshore assemblages on the Basque coast at the time of each sampling (from 2004 to 2006). Spring (*S*); autumn (*A*).

contrary, lowshore habitat was heterogeneous in practically all the variables examined. Physical stress on the harsh mid-intertidal environment has been addressed in other studies as limiting variability of midshore communities (Archambault & Bourget, 1996), while additional variation in the low intertidal zone may be attributed to the existence of a greater patchiness. Another issue resulting from the present study is that different spatial scale (kilometres and tens of metres) explains the variability of different community characteristics. Other authors (Fraschetti *et al.*, 2005) have addressed the issue of spatial variability in the rocky intertidal zone, although tests of space × time interactions are less common. Contrary to our results, many studies found significant scales of variation simultaneously at both spatial scales considered in the present study. We believe that to some extent, temporal replication in our design makes spatial patterns quite complicated to define over time.

Finally, we must summarize the lessons learned. The 'Prestige' incident highlighted many serious weaknesses in

Table 8. PERMANOVA results showing the effect of year (Y), season (S), oiling level (H), location (L) and area (A) on midshore faunal assemblages considering the whole species data set and the community trophic guilds.

Midshore source	df	Species abundance			Trophic guilds		
		SS	MS	F	SS	MS	F
Y	2	991.01	495.51	1.36	1190.4	595.2	0.82
S	1	372.49	372,.49	1.03	443.52	443.52	0.66
H	1	2632.9	2632.9	0.69	6935.9	6935.9	1.00
L(H)	10	38097	3809.7	2.09*	69476	6947.6	1.91
YxS	2	2439.3	1219.6	4.06	4254	2127	3.76
YxH	2	517.87	258.93	0.71	1284.5	642.23	0.89
SxH	1	593.65	593.65	1.64	1704.1	1704.1	2.53
A(L(H))	12	21840	1820	11.10**	43555	3629.6	10.18**
YxL(H)	20	7296.9	364.85	1.42	14477	723.84	1.14
SxL(H)	10	3611.2	361.12	2.18*	6740.7	674.07	1.64
YxSxH	2	1665.8	832.91	2.77	3276.3	1638.2	2.90
YxA(L(H))	24	6147.1	256.13	1.56*	15245	635.2	1.78**
SxA(L(H))	12	1984.7	165.4	1.01	4936.1	411.34	1.15
YxSxL(H)	20	6004.9	300.24	0.73	11.03E + 05	565.13	0.82
YxSxA(L(H))	24	9837.5	409.9	2.50**	16502	687.6	1.92**
Res	288	47214	163.94		1.03E + 05	356.38	
Total	431	1.51E + 05			3.04E + 05		

$^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$.

the marine pollution management system of the Basque coast. Those relating to scientists include serious limitations in assessing environmental damage, as a response of the lack of well-designed surveillance programmes prior to the incident. Although oil spills and pollution have nowadays raised public concern, marine environmental protection is a long-term responsibility, one that requires further research and budget effort (Chiau, 2005). In this sense, future challenges for managers and scientists should include the development of reliable long-term monitoring research to measure future impacts on the rocky shore in a more accurate way.

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