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Artículo de investigación

Bioremediation impact on intertidal communities affected by the Prestige oil spill at Sorrizo beach (Galicia)

El impacto del biorremedio en las comunidades del mesolitoral de la playa de Sorrizo (Galicia) afectadas por el vertido del Prestige

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Abstract

In November 2002 the sinking of the oil tanker Prestige caused one of the worst oil spills off the European coastline. The Marine Biology Station of A Graña carried out a study aimed at assessing the effect of certain bioremediating products used to remove oil from the coast as well as other cleaning techniques used (e.g. hydrocleaning) both on the flora and the fauna. This study was carried out during a year (2003-2004) at Sorrizo inlet (A Coruña, Galicia, NW Iberian Peninsula). This paper sets out the results obtained in the study of both the impact on the fauna and flora caused by bioremediating products and cleaning techniques used at the inlet and the impact caused by the Prestige fuel itself. The use of bioremediation hardly influenced the flora and fauna at Sorrizo beach. A temporary increase of fauna was observed in all sampling areas as shown in the number of species and total abundance. Besides, certain similarities between samples of September 2003 and 2004 were observed both in abundance and specific richness.

Keywords: oil spill, Prestige, bioremediation, Sorrizo beach, intertidal, NW Iberian Peninsula.

Resumen

En Noviembre de 2002, el accidente del petrolero Prestige originó una de las peores mareas negras sufridas en las costas europeas. La Estación de Bioloxía Mariña da Graña realizó un estudio cuyo objetivo era evaluar el efecto de determinados productos biorremediadores utilizados para eliminar el fuel de la costa, así como de otras técnicas de limpieza empleadas (p.e. la hidrolimpieza), sobre la fauna y flora. Este estudio fue llevado a cabo durante un año (2003-2004) en la ensenada de Sorrizo (A Coruña, Galicia, NO Península Ibérica). En este artículo se exponen los resultados obtenidos en el estudio del impacto sobre la fauna y flora provocado por los productos biorremediadores y técnicas de limpieza utilizados en la ensenada, además del impacto ocasionado por el propio fuel del Prestige. El empleo del biorremedio apenas influyó en la flora y fauna de la playa de Sorrizo. En general, existió en todas las zonas de muestreo un incremento temporal de la fauna reflejado en el número de individuos y especies. Se observaron además ciertas similitudes entre las muestras correspondientes a septiembre de 2003 y 2004, tanto en abundancia como en riqueza específica.

Palabras clave: marea negra, Prestige, biorremedio, playa de Sorrizo, mesolitoral, NO península ibérica.

INTRODUCTION

Between 1.7 and 8.8 million metric tons of oil are estimated to be spilled into the earth waters every year, of which more than 90% are directly related to anthropic activities (Zhu *et al*., 2001). However, only a small part of the oil released to waters comes from accidents of oil tankers. Since the early 1980s of last century, the number of oil spills has decreased noticeably, mainly due to the reduction of oil transport at sea and the improvements on navigation safety. However, it should be taken into account that these figures may vary greatly every year. Although most spills that took place in the last years were of lesser magnitude (inferior to 7 tons), some are noteworthy due to the great tonnage spilt (Gómez-Gesteira, 2001; Zhu *et al*., 2001).

The methods for cleaning up an oil spill may be natural, physical and chemical (Gómez-Gesteira, 2001; Zhu *et al*., 2001). Natural methods include evaporation, photooxidation and degradation by microorganisms. Within the physical methods, floating barriers, mechanical removal and hydrocleaning should be highlighted. Chemical methods comprise dispersants, solidifiers and film formers.

According to Zhu *et al*. (2001), bioremediation can be defined as the act of adding nutrients or microorganisms in polluted environments in order to speed up the natural processes of hydrocarbon biodegradation. On the one hand, the success of bioremediation depends on the premise that a great amount of hydrocarbons are biodegradable; on the other hand, it also depends on the ability to establish and keep the conditions that favour the oil biodegradation rates in a contaminated environment.

We can define two bioremediation methods (Zhu *et al*., 2001): bioaugmentation (introduction of oil degrading bacteria, both autochthonous and allochthonous, as a complement to the existing bacterial population) and biostimulation (addition of nutrients or alteration of environmental conditions to stimulate the existing oil degrading microbial populations). According to these authors, one of the main problems when it comes to applying bioremediation is the lack of guidelines or protocols on when and how to apply it, which bioremediation agents should be used, how they should be applied and how results could be considered and evaluated.

In November 2002, the sinking of the oil tanker Prestige caused one of the worst oil spills off the European coastline which affected not only the coast of Galicia, but also extended to the Cantabrian coast, Portugal and France (Urgorri, 2003; Veiga *et al*., 2009). The Prestige oil spill affected all habitats on the Galician coast, from intertidal zones to bathyal bottoms (Urgorri *et al*., 2003; Junoy *et al*., 2005; Serrano *et al*., 2006; Veiga *et al*., 2009). Several studies were carried out along the coast of Galicia to study the effects of the oil spill both on rocky areas (Urgorri & Besteiro, 2004; Besteiro *et al*., 2006; García-Regueira *et al*., 2010) and soft substrata (Mora *et al*., 2003; De la Huz *et al*., 2005; Junoy *et al*., 2005; Veiga *et al*., 2010).

In 2003 researchers of the University of Santiago de Compostela and University of Barcelona collaborated on a joint project with the objective of assessing the efficiency of bioremediation as an alternative for the environmental recovery of Sorrizo beach (Arteixo, A Coruña) after the Prestige oil spill. Thus, several tests and experiments with bioremediation products were scheduled for a year. Objectives focused not only on the efficiency of the aforementioned bioremediation techniques but also on the assessment of their impact on the environment; therefore, a monitoring plan of the local biota was drawn up. Sorrizo beach was seriously affected by the Prestige oil spill: approximately 50% of the beach was already polluted by the fuel a few days after the accident. The Prestige oil spill was characterized by affecting a wide section of coastline comprising almost all kinds of intertidal environments. Thus, Sorrizo beach was highly suitable to study the possible viability of bioremediation, as within the same area very different intertidal habitats (stones, pebbles of different sizes and sand) were present and polluted by the oil spill (Fernández-Álvarez *et al*., 2006).

The Marine Biology Station of A Graña carried out, within the aforementioned project, a study on the potential impact caused by the bioremediation products used on the macrofauna and algae of the littoral (Urgorri *et al*., 2004). This paper shows the results of the evaluation of the possible action of bioremediation products on intertidal organisms and that of the Prestige fuel itself on different points

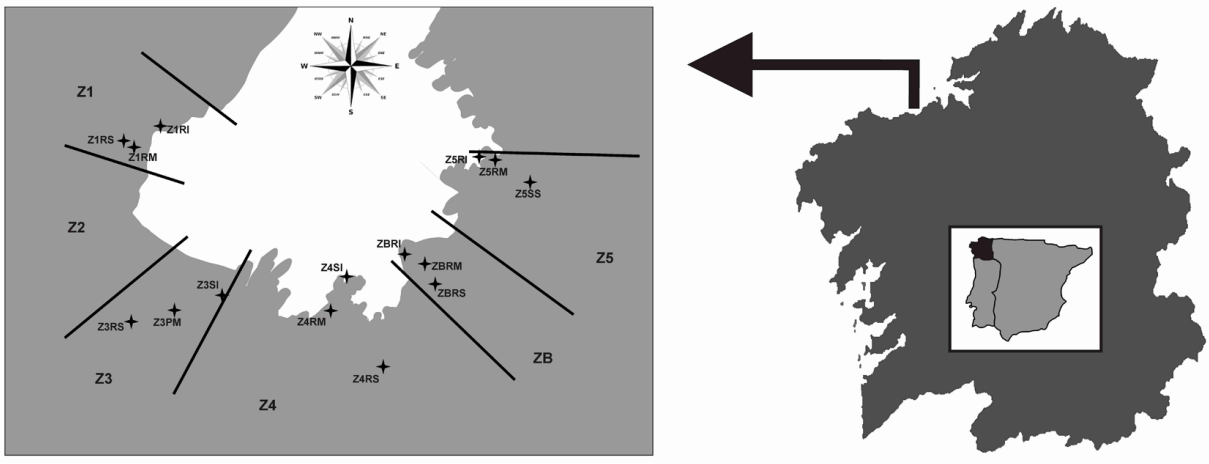

Figure 1. Map showing location of Sorrizo inlet (NW Iberian Peninsula) and study zones.
Figura 1. Mapa de situación de la playa de Sorrizo (NO Península Ibérica) y zonas de estudio.

of Sorrizo beach in the years 2003 and 2004. The full results of the research study done are gathered on electronic support in URGORRI & SEÑARÍS (2012).

MATERIAL AND METHODS

Study and sampling locality

Sorrizo beach (43º18'43" N; 008º34'07" W) is an inlet of around 200 m of coastline, facing North and located in A Coruña (NW Iberian Peninsula) (fig. 1). The area represents a natural shelter with a narrow mouth, delimited by a rocky and terrigenous shelf with agricultural crops on its upper part. At the inlet beach there are areas of thick sand, pebbles and rocks. In the western area of the inlet there is an access concrete-made ramp as well as a small river course and a drainage channel.

Two samplings were carried out (September 2003 —250903— and September 2004 —150904—, respectively) before and after applying the bioremediation treatments —between April and October 2003 (FERNÁNDEZ *et al*., 2006)—, in order to assess whether the application of the different techniques caused any impact on the biological communities of the area studied. A third sampling was also carried out in April 2004 (070404), so as to obtain information on the state of the macrobenthic communities in spring, which is considered to be more active than autumn biologically speaking.

The inlet was divided in 6 study zones: Zone 1 (Z1), Zone 2 (Z2), Zone 3 (Z3), Zone 4 (Z4), Zone 5 (Z5) and White Zone (ZB) (Fig. 1). Different bioremediation agents were applied to four of them (Z1, Z3, Z4, Z5); no product was applied to ZB which was therefore considered a control zone; Z2 was not included in the final study as a small river course and a drainage channel flew into it and this fact may cause differences between the fauna of this zone and the remaining zones, not necessarily related to the effects of the oil spill. Three levels were defined in the intertidal of each zone: upper, middle and lower, which were characterized by belts of different animal species and algae.

The codes of the sampling points refer to the level (S: upper, M: middle; I: lower) and type of substratum (R: rocky; P: pebbles; S: sandy sediment).

In the upper level of Z3 and Z4, hydrocleaning techniques with L-1800 (Bio-Systems Corporation) and freshwater at high pressure and temperature were applied in April 2003.

A 40x40 cm quadrat was sampled at each level of each zone. In the sites of sandy substratum, the sediment of the quadrat surface was collected down to a depth of 10 cm. In those of rocky substratum, sampling was carried out by scraping the covering. The most conspicuous fauna and flora were previously collected using tweezers. Samples were collected with sea water in plastic tubs previously labelled, fixed with formaldehyde at 4% (v/v) and

stained with Bengal rose. Organisms were subsequently sorted in the laboratory and identified up to species level whenever possible.

Analysis of data

A matrix samples-species was set up from the abundance data of the latter in order to compare the data obtained among the different sampling areas. The total number of specimens and the specific richness were determined for each sample (e.g. total number of species). Patterns of evolution were determined by means of multivariate analyses using the package PRIMER 5. In order to determine the biological affinities among the sampling points throughout the sampling period at each intertidal level, the Bray-Curtis measures of dissimilarity were applied after transforming the original data of the abundance matrix by square root (Clarke & Warwick, 1994). From the similarity matrix, dendrograms of classification of the sampling points were set up using the algorithm UPGMA ("Unweighted Pairgroup Method Using Arithmetic Averages"). In these dendrograms, sampling points are grouped according to their average similarity (CLUSTER program of PRIMER package). The ordination technique MDS ("Non-Metric Multidimensional Scaling") was used to contrast the results obtained in the dendrograms. Both in the dendrograms and in the graphical representations of the MDS, points have been named according to the corresponding sampling period and adding A, B or C to the quadrat code in the following way: A corresponds to the points sampled in September 2003; B corresponds to the points sampled in April 2004; C corresponds to those sampled in September 2004.

RESULTS

Abundance and specific richness

Zone 1

A total of 1024 specimens belonging to 33 animal species and 4 algae were collected in Z1.

At the upper level (Z1RS), the specific richness increased throughout the sampling period from 4 to 13 species (fig. 2). The number of specimens was larger in the second sampling (403 specimens; fig. 3), decreasing in the third sampling to a value similar to the first one (60 and 69, respectively). Gastropoda of the genus *Patella* and the barnacle *Chthamalus montagui* Southward, 1976 were the only taxa found in the three samplings. The latter showed its highest abundance in spring; the abundance of *Patella* spp. increased slightly throughout the study period; however, no defined pattern was observed for any species. For example, the highest number of specimens of *Patella depressa* Pennant, 1777 was collected in the first sampling (12 specimens); an only one specimen was collected in the second sampling whereas in the third sampling no specimens were found.

A decrease in the number of species was observed in spring at the middle level (Z1RM) (fig. 2) with a slight recovery in the third sampling. As for the number of specimens (fig. 3), it was observed no uniform pattern; the abundance of some species evolved similarly to the specific richness, whereas other species were not found at the end of the study (e.g. *P. depressa*) or were only found in the last sampling (e.g. the molluscs *Mytilus galloprovincialis* Lamarck, 1819 and *Lepidochitona cinerea* (Linneo, 1767)).

The tendency observed at the lower level (Z1RI) regarding the specific richness was similar to Z1RM (fig. 2). In the spring sampling, the number of species decreased and similar levels were observed to those found in the first sampling. The number of specimens varied depending on the species; some decreased in spring and seemed to show a slight increase in the last sampling (e.g. Nematoda and *Patella vulgata* Linneo, 1758); on the contrary, others increased their number during the spring sampling, whereas in the last sampling they showed zero or testimonial presence, as in the case of the barnacle *C. montagui*.

Zone 3

In this sampling zone, 442 specimens corresponding to 22 animal species and an only alga (*Enteromorpha* sp.) were found.

At the upper level (Z3RS) no specimens were found in any of the three samplings (figs. 2, 3).

At the middle level (Z3PM), the temporary evolution of the specific richness and the number

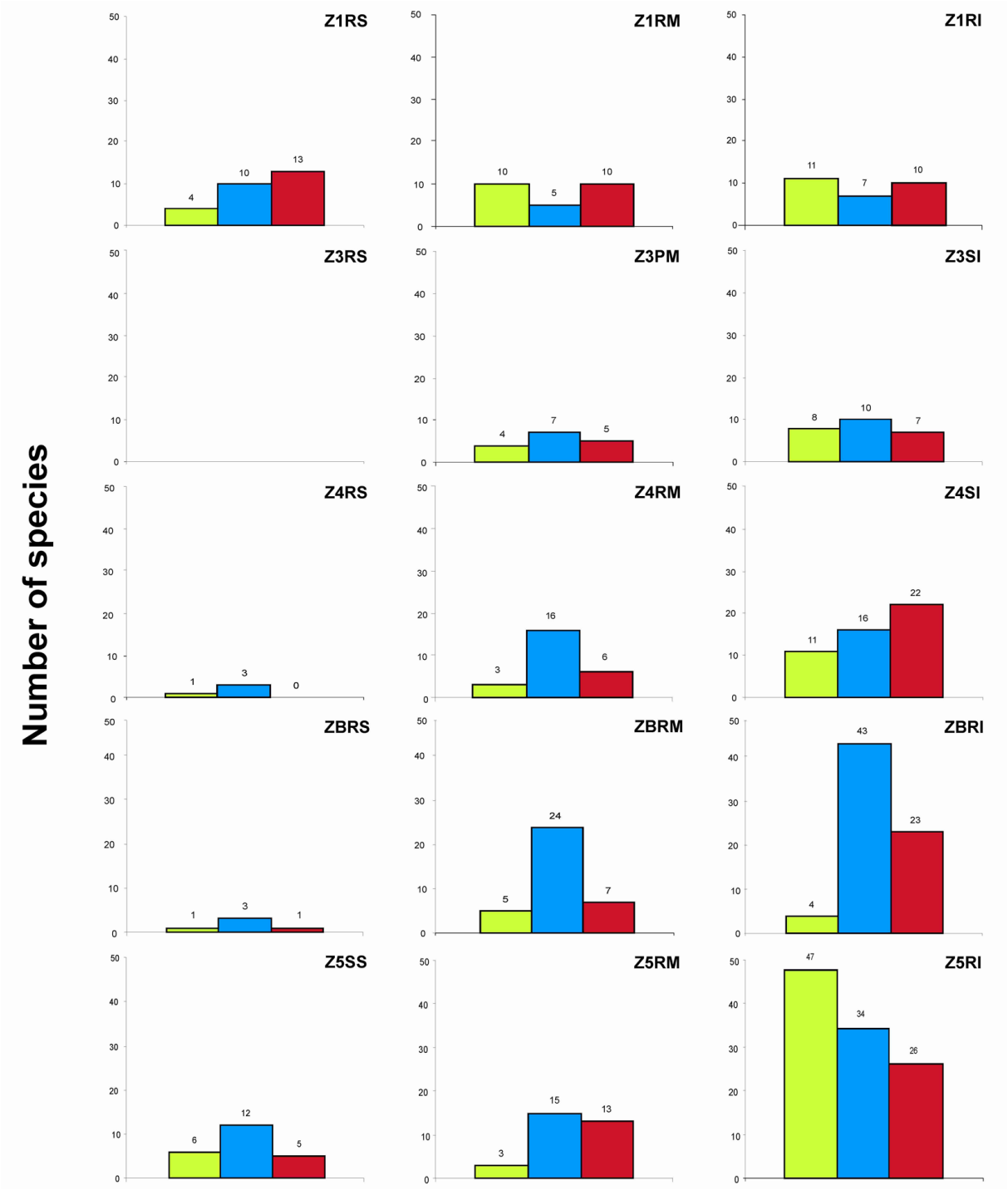

Figure 2. Temporal variation of specific richness per sample at each tidal level and zone. Sampling dates: green, 250903; blue, 070404; red, 150904. No specimen was found in Z3RS in any of the three samplings.

Figura 2. Variación temporal de la riqueza específica. Fechas de muestreo: verde, 250903; azul, 070404; rojo, 150904. En Z3RS no se encontró ningún individuo en ninguno de los tres muestreos.

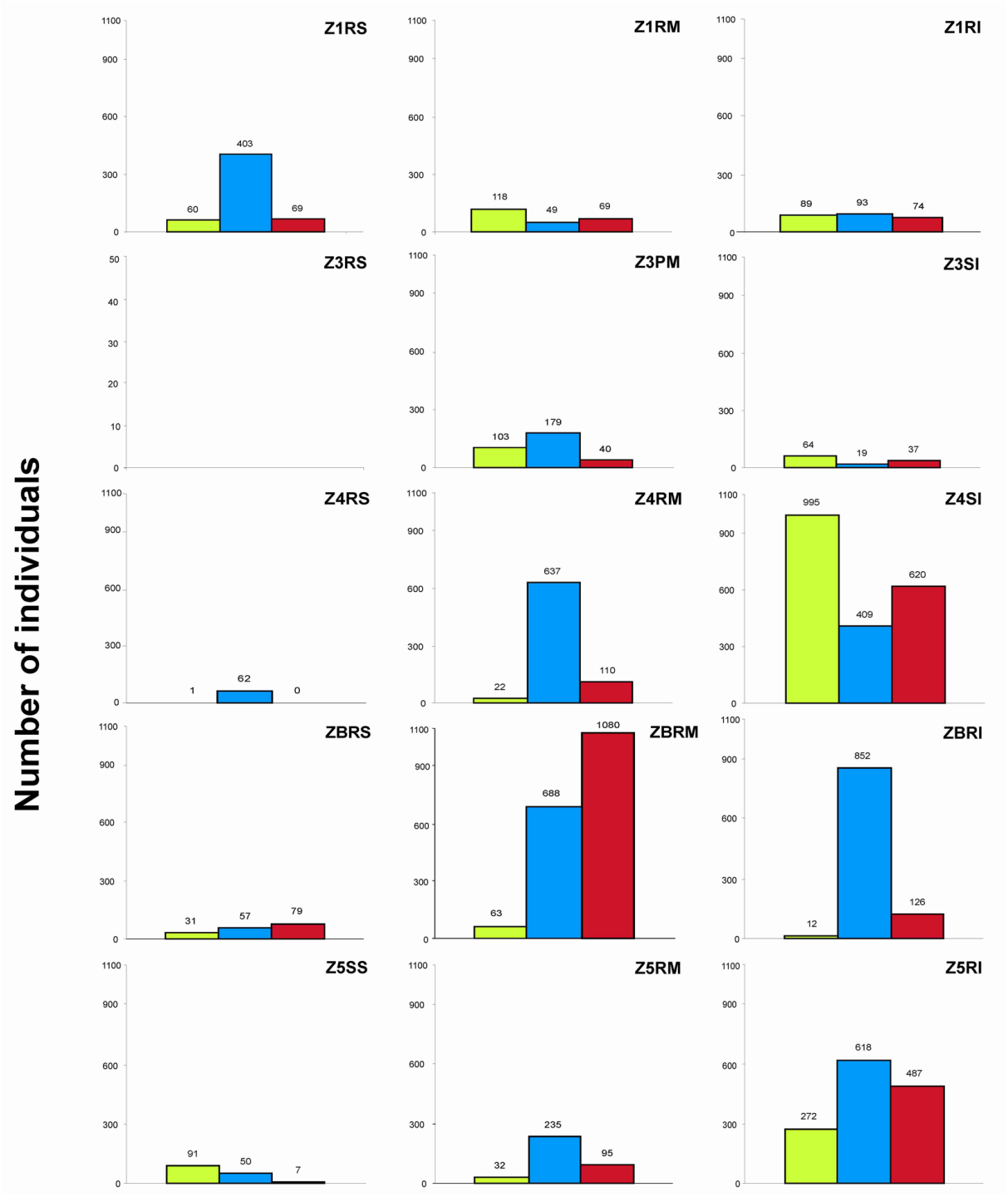

Figure 3. Temporal variation of total abundance per sample at each tidal level and zone. Sampling dates: green, 250903; blue, 070404; red, 150904. No specimen was found in Z3RS in any of the three samplings.

Figura 3. Variación temporal de la abundancia total. Fechas de muestreo: verde, 250903; azul, 070404; rojo, 150904. En Z3RS no se encontró ningún individuo en ninguno de los tres muestreos.

of specimens were characterized by showing the highest values in spring (figs. 2, 3). The most abundant groups were Nematoda and Oligochaeta, however, the numerical presence of the first decreased throughout the study period.

At the lower level (Z3SI) the specific richness values were low (fig. 2) and most of the specimens collected corresponded to Nematoda and Annelida. The total number of specimens was lower during spring, although a larger number of species were collected (fig. 3). The number of taxa represented in the three samplings was similar, however the dominant groups varied in each of them; Mollusca were more frequent in spring whereas Polychaeta appeared more frequently in the last sampling.

Zone 4

2856 specimens belonging to 44 animal species and 4 algae species were collected in Z4.

Very low values both of specific richness and abundance (figs. 2, 3) were obtained in the upper level (Z4RS). At this level, only the alga *Enteromorpha* sp., the periwinkles *Littorina saxatilis* (Olivi, 1792) and *Melarhaphe neritoides* (Linneo, 1758) and insects belonging to the Family Chironomidae were found. The most abundant taxon was *M. neritoides* (57 specimens in spring). A higher number of species and specimens were observed in spring, whereas no specimen was collected in the last sampling (figs. 2, 3).

At the middle level (Z4RM) the number of specimens and species showed their highest values during spring (figs. 2, 3). Moreover, when comparing the two samplings done in September 2003 and 2004, an increase in the last sampling both of specific richness and abundance was found. The best represented group in number of species were Mollusca, including 7 species of Gastropoda, of which the most abundant were *P. vulgata* and *Patella ulyssiponensis* Gmelin, 1791. Regarding the number of specimens, most of them were Cirripedia of the genus *Chthamalus*, as well as Nematoda and Polychaeta.

At the lower level (Z4SI), the specific richness increased progressively over time (fig. 2). The most abundant groups were Nematoda and Polychaeta; Oligochaeta reached high abundance in the last sampling; however, their presence was lower in the first sampling and non-existent in the spring sampling.

White Zone

In the control zone, 2988 specimens belonging to 66 animal species and 15 algae species were collected.

At the upper level (ZBRS) only representatives of Mollusca and Cirripedia were found; the most abundant species was *M. neritoides* with 127 specimens, and was present in the three samplings. The highest specific richness was observed in spring (fig. 2). The highest abundance (79 specimens) was recorded in the last sampling, however it corresponded entirely to *M. neritoides* (fig. 3).

The highest specific richness of the middle level (ZBRM) corresponded to Mollusca with 10 species. The most abundant mollusc was *P. vulgata* with 46 specimens, all found in spring. The greatest number of species was collected during spring (fig. 2). A similar number of species was found in the first and third samplings, although a slight increase was observed in the latter. Four species were present in all samplings: 3 species of *Patella* and the barnacle *C. montagui*. The highest number of specimens collected corresponded to *C. montagui* in the last sampling (1012 specimens).

At the lower level (ZBRI), both abundance and specific richness showed their highest values during spring (figs. 2, 3). On the other hand, there was a clear increase in both parameters in 2004 when comparing both September samplings. The number of algae species collected in the last sampling (12 species) was substantially greater than the algae species of the two previous samplings (4 species in the first sampling and 2 in the second). In the first sampling, the values of abundance and specific richness were very low: 12 specimens and 4 animal species (2 crustaceans and 2 molluscs) (figs. 2, 3). However, these values increased significantly in spring, when 852 animal specimens belonging to 43 species were collected.

Zone 5

1887 specimens belonging to 88 animal species as well as 11 algae species were collected in Z5.

At the upper level (Z5SS), the highest specific richness was recorded in spring (fig. 2). Both samplings of September showed a similar number of

species. The most abundant taxa were Nematoda, Oligochaeta and the amphipod *Talitrus saltator* (Montagu, 1808). The presence of the last two was constant in the three samplings. The greatest fauna diversity corresponded to the Hexapoda, which were represented by Collembola, Ephydridae, Psychodidae, Ceratopogonidae and Trichogrammatidae and different developmental stages of some families. However, no hexapod taxon collected in spring was found in the last sampling.

At the middle level (Z5RM), the highest values of abundance and specific richness were recorded during spring (figs. 2, 3). As in other zones, there were greater values in both parameters in September 2004 when compared to September 2003. The anthozoan *Anemonia viridis* (Forskål, 1775) and the limpet *P. vulgata* appeared in all three samplings, but showed different patterns. The abundance of *A. viridis* increased throughout time whereas *P. vulgata* was more abundant in spring; however, the latter showed higher abundance in September 2004 than in the same month of 2003.

Values of specific richness were higher than those of the remaining levels of Z5, although its values decreased (down to 50%) between the first and the last sampling. As for the abundance, the highest levels were observed during spring (figs. 2, 3). Six groups were constantly present in the three samplings: Hydrozoa, Mollusca, Polychaeta, Crustacea, Bryozoa and Echinodermata. The abundance of Hydrozoa and Polychaeta increased throughout time whereas Mollusca, Crustacea and Bryozoa presented their greatest number of specimens in spring. As regards Crustacea and Bryozoa, the number of specimens collected in September 2004 was lower than in the same month in 2003, with a more pronounced difference in the case of Crustacea.

Multivariate analysis

Upper level

In the dendrogram drawn from the presence/absence data, three clusters are made up with 20% similarity, according to the type of substratum (sandy or rocky) and the exposure degree of the zone (fig. 4A). A cluster includes the samples of Z4 and ZB of rocky substratum located on the protected area of the inlet; a second group corresponds to the samples of Z1 of rocky substratum located on the most exposed part; the last cluster comprises the samples of Z5 of sandy substratum on the protected zone of the sampling area. Considering the abundance data, the three groups are formed with a lower similarity index of 10% (fig. 4D).

The graphical representations of the MDS ordination of the presence/absence and abundance data show a similar pattern to that of the dendrograms (fig. 5A, D). Based on the presence/absence data, the samples of Z1 of 2004 show a higher degree of similarity among them than in relation to the samples of 2003. This may be due to the fact that the number of species increased throughout the sampling period and the number and identity of the species was similar in the last two samplings. However, a different pattern can be observed when considering the abundance data, with samples from September 2003 and 2004 more similar to each other. In Z4, the samples of September 2003 and April 2004 seem distant from each other; in the third sampling no organism was collected, therefore the corresponding sample has not been represented. According to the presence/absence data, samples corresponding to September 2003 and 2004 on ZB show great similarity. On the other hand, the analysis based on the abundance data indicates that the samples of April and September 2004 are more similar between them than to the corresponding abundance of the first sampling. A great variability among samples, which are distant from each other, can be observed in Z5.

Middle level

The dendrograms based on the presence/absence data show a clear arrangement of the sampling points in two clusters with 15% similarity based on the substratum type (fig. 4B). Samples of Z3 are included in one cluster (substratum of pebbles on the protected zone of the sampling area); a second cluster includes samples of Z1, Z4, ZB and Z5 of rocky substratum. As regards the analysis done based on abundance, the pattern is similar to that based on the presence/absence data; however, the same clusters are defined at a higher similarity level: 20% (fig. 4E).

In the graphical representation of the MDS analysis based on the presence/absence and abundance

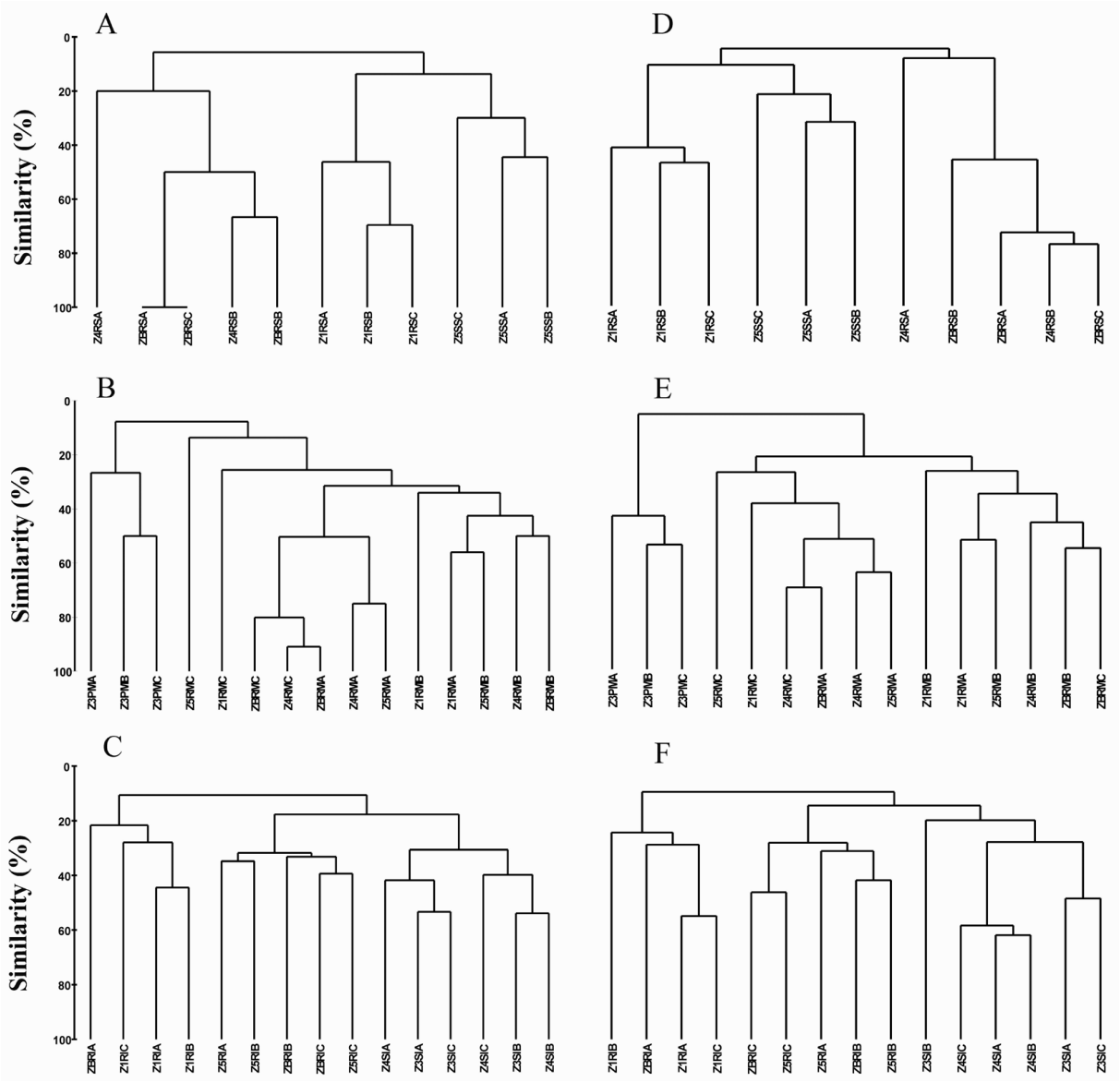

Figure 4. Classification dendrograms of the samples based on presence/absence data (A, B, C) and abundance (D, E, F) for each sampling level (upper: A, D; middle: B, E; lower: C, F). Sampling dates: A, 250903; B, 070404; C, 150904.
Figura 4. Dendrogramas de clasificación de las muestras basados en los datos de presencia-ausencia (A, B, C) y de abundancia (D, E, F) para cada nivel de muestreo (superior: A, D; medio: B, E; inferior: C, F). Fechas de muestreo: A, 250903; B, 070404; C, 150904.

data, a similar tendency to that of the dendrograms can be observed (fig. 5B, E). In Z1, samples seem distant from each other. In Z3, samples of 2004 are more similar among themselves than to those of September 2003 according to the presence/absence data; however, the MDS based on the abundance data shows differences among the three samples. In Z4, sample ordination is similar according to both types of data; thus, there is a higher similarity between samples of September 2003 and 2004 than with that of April 2004. In ZB, the MDS based on presence/absence data represents the samples of September 2003 and 2004 close to each other and distant in relation to that of spring; however, the MDS representation of the abundance data is different; the last two samplings show the highest similarity. In Z5, the MDS based on the abundance data shows higher similarity between the samples of September 2003 and 2004 than in relation to April 2004; however, the MDS representation of

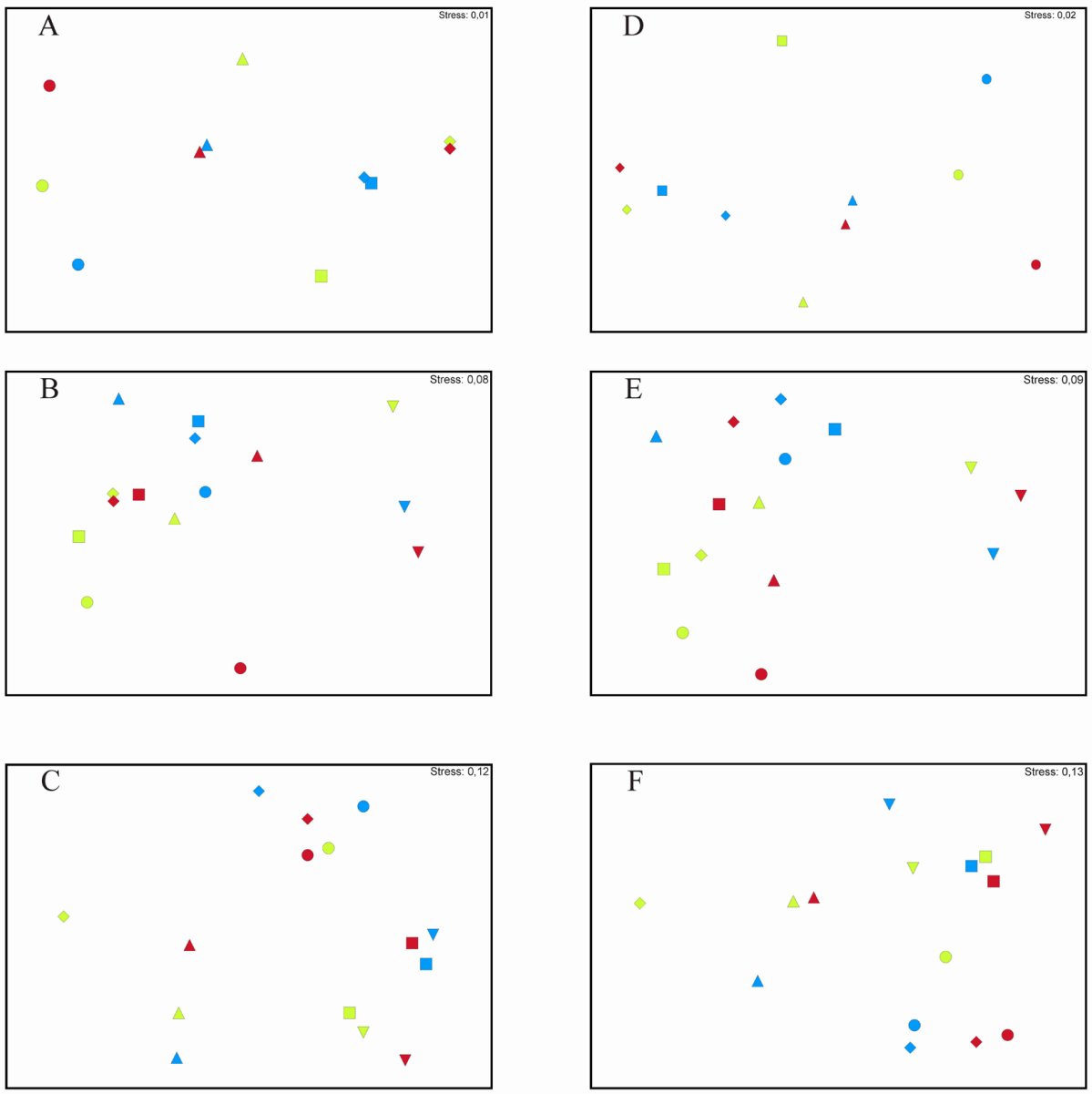

Figure 5. MDS ordination of the samples based on presence/absence data (A, B, C) and abundance (D, E, F) for each sampling level (upper: A, D; middle: B, E; lower: C, F). Sampling dates: green 250903; blue, 070404; red, 150904. ▲, Z1; ▼, Z3; ■ Z4; ♦, ZB; ●, Z5.

Figura 5. Ordenación MDS de las muestras en función de los datos de presencia-ausencia (A, B, C) y de abundancia (D, E, F) para cada nivel de muestreo (superior: A, D; medio: B, E; inferior: C, F). Fechas de muestreo: verde, 250903; azul, 070404; rojo, 150904. ▲, Z1; ▼, Z3; ■ Z4; ♦, ZB; ●, Z5.

the presence/absence data shows differences among the three samplings.

Lower level

The analyses based on the presence/absence data show the existence of three groups of samples (30% similarity) according to the type of substratum and the exposure degree of the zone (figs. 4C, 5C). Samples of Z1 are included in one group (exposed rocky substratum); a second group comprises the samples of ZB and Z5 (rocky substratum in the most protected area); a third group includes the samples of Z3 and Z4 (sandy substratum in the protected sampling zone). The analysis done based on the

abundance data (figs. 4F, 5F) shows a similar pattern to that of presence/absence, but the groups are made up at a lower similarity level (20%).

DISCUSSION

It may be concluded that the employ of different bioremediation products seems not to have influenced either the flora and fauna of the upper and middle rocky intertidal of Sorrizo beach or it has done it to a very limited extent. However, an extended study period of at least one spring sampling would have been necessary to have a greater amount of comparable data (two spring and two autumn samplings). Thus, we could have reached more reliable conclusions on the temporal evolution of the communities. Unfortunately, this was not possible due to funding limitations.

According to other studies carried out at Sorrizo beach (Fernández-Álvarez *et al.*, 2006), neither the bioaugmentation nor the biostimulation products used speeded up the degradation of the fuel present on the rocky and sandy zones compared to the natural decrease. These studies concluded that the fuel degradation on the sandy sediment was high and almost constant throughout the study. Similarly, the fuel degradation on the rocks increased with time, but it could not be concluded whether this was greater on any of the zones due to the heterogeneous nature of the beach.

The fuel remained longer on the upper rocky intertidal of Sorrizo beach. This may be due to a low cleansing action of the sea at this level. This pattern has also been observed by Fernández-Álvarez *et al*. (2006).

An increase of the fauna present at the studied levels was registered in all sampling zones throughout time; however, in some of the zones signs of recovery were unclear (e.g. *Chthamalus* spp. in ZBRM and Z5RM). In some zones, an increase in the number of specimens and/or species was observed during the spring sampling, which could be indicative of a recovery of biota. However, these results are consistent with the patterns of temporal evolution expected in intertidal communities in the Northern Hemisphere, in which spring is much more active biologically than autumn due to the recruitment of several species (e.g. Rueda *et al*., 2001). Nevertheless, in other zones, the increase of the parameters mentioned takes place in the last sampling (September 2004). In both cases, as indicated above, it would have been desirable to carry out at least one more sampling in spring in order to dispose of a more complete seasonal set of data.

In the upper level of zones Z3 and Z4 (Z3RS and Z4RS), it was observed that organisms were strongly affected by the use of hydrocleaning methods and even disappeared due to high pressure and temperature and the fresh water used during cleaning. Therefore, the use of this mechanism on natural rocky substrata would not be advisable; however, using sea water at lower pressure and temperature is recommended in port facilities and promenades mainly for aesthetic purposes (Fernández-Pulpeiro & César-Aldariz, 2003).

Considering the results of the multivariate analyses, a pattern has been observed that is repeated in most sampling zones. This pattern can be defined by the existence of certain similarities between the samples of September 2003 and 2004, either when considering the number of specimens and/or the presence/absence of species. Once again, these results reinforce the idea pointed out above about the need for a second spring sampling with the objective of proving whether the pattern observed would be applicable to spring. The results obtained would confirm the little or no impact of bioremediation products on the fauna and flora.

The possible effectiveness of the bioremediation products used on Sorrizo beach was also studied in other oil spills, e.g. Exxon Valdez (Zhu *et al.*, 2001). In that case, it was proven that the bioaugmentation did not favour the fuel biodegradation; in fact, in some zones affected by this oil spill, the limiting factor of biodegradation was not the absence of hydrocarbon-degrading microorganisms, but the concentration of certain nutrients as nitrogen (Pritchard & Costa, 1991; Venosa *et al.*, 1992). Moreover, the results obtained from the use of bioremediation on the coasts affected by the Exxon Valdez oil spill showed that these methods favoured fuel biodegradation. However, these conclusions were questioned as the applications of bioremediating methods were not properly replicated (Zhu *et al.*, 2001). In a study carried out at Fowler Beach (Delaware, United States) (Zhu

et al., 2001), different bioremediating methods were applied on a sandy beach in order to obtain statistical evidence on the effectiveness of bioremediation. These results concluded that there were significant differences between the treated and the untreated zones regarding biodegradation rates, although high biodegradation levels were found in the untreated zones. It was also determined that, as in the case of Exxon Valdez, bioaugmentation is not a determining factor in the removal of hydrocarbons (Zhu *et al.*, 2001).

In last years, laboratory research has been done to improve the effectiveness of bioremediation. Parameters such as fuel concentration, nutrients and bacterial populations are being used as independent variables in statistical analyses, thus obtaining the optimization of fuel degrading experiments (Mohajeri *et al*., 2010a). Also, the use of kinetic models allows describing the biodegradation in progress (Rončević *et al.*, 2005; Mohajeri *et al*., 2010b).

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Appendix: Systematic list of all taxa collected on Sorrizo beach (In alphabetical order from class level)

DIVISION CHLOROPHYTA
FAMILY ULVACEAE
Ulva sp.

DIVISION OCHROPHYTA
FAMILY FUCACEAE
Fucus spiralis Linneo
Fucus vesiculosus Linneo
Fucus sp.

DIVISION RHODOPHYTA
Rhodophyta indet.
FAMILY CORALLINACEAE
Corallina officinalis Linneo
Corallina sp.
Jania rubens (Linneo) Lamouroux
Jania sp.
Lithophyllum incrustans Philippi
FAMILY DASYACEAE
Heterosiphonia plumosa (Ellis) Batters
FAMILY DELESSERIACEAE
Acrosorium ciliolatum (Harvey) Kylin
Cryptopleura ramosa (Hudson) Newton
FAMILY GELIDIACEAE
Gelidium corneum (Hudson) Lamouroux
FAMILY GIGARTINACEAE
Chondrus crispus Stackhouse
Chondrus sp.
Gigartina pistillata (Gmelin) Stackhouse
Gigartina sp.
FAMILY GRACILARIACEAE
Gracilariopsis longissima (Gmelin) Steentoft, Irvine & Farnham
FAMILY HAPALIDIACEAE
Mesophyllum lichenoides (Ellis) Lemoine
FAMILY LOMENTARIACEAE
Lomentaria articulata (Hudson) Lyngbye
FAMILY PHYLLOPHORACEAE
Mastocarpus stellatus (Stackhouse) Guiry
FAMILY PLOCAMIACEAE
Plocamium cartilagineum (Linneo) Dixon

PHYLUM CNIDARIA
Class Anthozoa
FAMILY ACTINIIDAE
Actinia equina (Linneo, 1758)
Anemonia viridis (Forskål, 1775)
Class Hydrozoa
FAMILY CAMPANULARIIDAE
Clytia gracilis (Sars, 1850)
Obelia dichotoma (Linneo, 1758)
Obelia geniculata (Linneo, 1758)
Campanulariidae indet.
FAMILY CORYNIDAE
Coryne muscoides (Linneo, 1761)
Coryne sp.
FAMILY SERTULARIIDAE
Amphisbetia operculata (Linneo, 1758)
Dynamena pumila (Linneo, 1758)
Sertularella sp.
Sertulariidae indet.

PHYLUM PLATYHELMINTHES
Class Turbellaria
Turbellaria indet.

PHYLUM NEMERTEA
Nemertea indet.

PHYLUM NEMATODA
Nematoda indet.

PHYLUM MOLLUSCA
Class Bivalvia
FAMILY CARDIIDAE
Cerastoderma edule (Linneo, 1758)
FAMILY CORBULIDAE
Corbula gibba (Olivi, 1792)
FAMILY HIATELLIDAE
Hiatella arctica (Linneo, 1767)
FAMILY KELLIIDAE
Kellia suborbicularis (Montagu, 1803)
FAMILY LASAEIDAE
Lasaea adansoni (Gmelin, 1791)
FAMILY MONTACUTIDAE
Kurtiella bidentata (Montagu, 1803)
FAMILY MYTILIDAE
Musculus costulatus (Risso, 1826)
Mytilus galloprovincialis Lamarck, 1819

Class Gastropoda
FAMILY BARLEEIDAE
Barleeia unifasciata (Montagu, 1803)
FAMILY CERITHIIDAE
Bittium reticulatum (da Costa, 1778)
FAMILY CERITHIOPSIDAE
Cerithiopsis tubercularis (Montagu, 1803)
FAMILY EPITONIIDAE
Epitonium clathratulum (Kanmacher, 1798)
FAMILY LITTORINIDAE
Melarhaphe neritoides (Linneo, 1758)
Littorina obtusata (Linneo, 1758)
Littorina saxatilis (Olivi, 1792)
FAMILY PATELLIDAE
Patella depressa Pennant, 1777
Patella pellucida Linneo, 1758
Patella ulyssiponensis Gmelin, 1791
Patella vulgata Linneo, 1758
Patella sp.
FAMILY PHASIANELLIDAE
Tricolia pullus (Linneo, 1758)
FAMILY PYRAMIDELLIDAE
Odostomia scalaris MacGillivray, 1843
FAMILY RETUSIDAE
Retusa truncatula (Bruguiére, 1792)
FAMILY RISSOIDAE
Cingula trifasciata (Adams, 1800)
Onoba semicostata (Montagu, 1803)
Rissoa parva (da Costa, 1778)
Setia pulcherrima (Jeffreys, 1848)
FAMILY SKENEOPSIDAE
Skeneopsis planorbis (Fabricius, 1780)
FAMILY TROCHIDAE
Gibbula cineraria (Linneo, 1758)
Gibbula umbilicalis (da Costa, 1778)
Class Polyplacophora
Lepidochitona cinerea (Linneo, 1767)
Acanthochitona crinita (Pennant, 1777)

PHYLUM ANNELIDA

Class Clitellata
Oligochaeta indet.
Class polychaeta
FAMILY ARENICOLIDAE
Arenicolides ecaudata (Johnston, 1835)
FAMILY CAPITELLIDAE
Capitella capitata (Fabricius, 1780)
FAMILY CIRRATULIDAE
Caulleriella alata (Southern, 1914)
FAMILY EUNICIDAE
Lysidice ninetta Audouin & Milne-Edwards, 1833
FAMILY FABRICIIDAE
Fabricia sabella (Ehrenberg, 1836)
FAMILY HESIONIDAE
Microphthalmus cf. *pseudoaberrans* Campoy, 1982
FAMILY LUMBRINERIDAE
Lumbrineris funchalensis (Kinberg, 1865)
FAMILY NEREIDIDAE
Platynereis dumerilii (Audouin & Milne-Edwards, 1834)
Nereididae indet. (juvenile)
FAMILY PHYLLODOCIDAE
Phyllodoce mucosa Örsted, 1843
FAMILY SABELLIDAE
Amphiglena mediterranea (Leydig, 1851)
Branchioma lucullanum (Delle Chiaje, 1828)
Sabellidae indet.
FAMILY SERPULIDAE
Laeospira corallinae de Silva & Knight-Jones, 1962
FAMILY SPIONIDAE
Boccardia polybranchia (Haswell, 1885)
Malacoceros fuliginosus (Claparède, 1870)
Polydora sp.
Scolelepis (Parascolelepis) tridentata (Southern, 1914)
FAMILY SYLLIDAE
Eusyllinae indet.
Exogone (Exogone) naidina Örsted, 1845
Odontosyllis fulgurans (Audouin & Milne-Edwards, 1833)
Syllides convolutus Webster & Benedict, 1884
Syllis sp.

PHYLUM ARTHROPODA

Subphylum Chelicerata
Class arachnida
Order acarina
Halacarus actenos Trouessart, 1889
Lohmanella sp.
Acarina indet.
Class Pycnogonida
FAMILY AMMOTHEIDAE
Achelia echinata Hodge, 1864
Achelia simplex (Giltay, 1934)
FAMILY NYMPHONIDAE
Nymphon gracile Leach, 1814
FAMILY PYCNOGONIDAE

Pycnogonum litorale (Strom, 1762)
Subphylum Crustacea
Class Malacostraca
Subclass Eumalacostraca
Superorder Eucarida
Order Decapoda
FAMILY GRAPSIDAE
Pachygrapsus marmoratus (Fabricius, 1787)
FAMILY PAGURIDAE
Pagurus prideaux Leach, 1815
FAMILY PALAEMONIDAE
Palaemon serratus (Pennant, 1777)
FAMILY POLYBIIDAE
Liocarcinus navigator (Leach, 1814)
FAMILY PORTUNIDAE
Carcinus maenas (Linneo, 1758)
Superorder Peracarida
Order Amphipoda
FAMILY AMPITHOIDAE
Amphitholina cuniculus (Stebbing, 1874)
Ampithoe helleri Karaman, 1975
FAMILY ATYLIDAE
Atylus vedlomensis (Bate & Westwood, 1862)
FAMILY CALLIOPIIDAE
Apherusa cirrus (Bate, 1862)
FAMILY CAPRELLIDAE
Caprella linearis (Linneo, 1767)
FAMILY HYALIDAE
Hyale stebbingi Chevreux, 1888
FAMILY ISCHYROCERIDAE
Ericthonius cf. *punctatus* (Bate, 1857)
FAMILY MAERIDAE
Maera sp.
FAMILY MELITIDAE
Abludomelita gladiosa (Bate, 1862)
FAMILY OEDICEROTIDAE
Perioculodes longimanus (Bate & Westwood, 1868)
FAMILY PHOTIDAE
Gammaropsis maculata (Johnston, 1828)
Photis longicaudata (Bate & Westwood, 1862)
FAMILY STENOTHOIDAE
Stenothoe monoculoides (Montagu, 1815)
Stenothoe sp.
FAMILY TALITRIDAE
Talitrus saltator (Montagu, 1808)
Order Isopoda
FAMILY IDOTEIDAE
Idotea emarginata (Fabricius, 1793)
Idotea granulosa Rathke, 1843
Idotea pelagica Leach, 1815
FAMILY LIGIIDAE
Ligia oceanica (Linneo, 1767)
FAMILY PARANTHURIDAE
Paranthura nigropunctata (Lucas, 1846)
FAMILY SPHAEROMATIDAE
Campecopea hirsuta (Montagu, 1804)
Dynamene bidentata (Adams, 1800)
Order Tanaidacea
FAMILY APSEUDIDAE
Apseudopsis latreillii (Milne-Edwards, 1828)
FAMILY TANAIDAE
Tanais dulongii (Audouin, 1826)
Class Maxillopoda
Subclass Thecostraca
Infraclass Cirripedia
Chthamalus montagui Southward, 1976
Chthamalus stellatus (Poli, 1791)
Class Ostracoda
Heterocythereis albomaculata (Baird, 1838)
Ostracoda indet.
Subphylum Hexapoda
Class Collembola
Collembola indet.
Class Insecta
Order Diptera
Ceratopogonidae indet.
Chironomidae indet.
Dolichopodidae indet.
Ephydridae indet.
Pipunculidae indet.
Psychodidae indet.
Order Hymenoptera
Trichogrammatidae indet.

PHYLUM BRYOZOA
Class Gymnolaemata
FAMILY AETEIDAE
Aetea anguina (Linneo, 1758)
FAMILY CELLARIIDAE
Cellaria fistulosa (Linneo, 1758)
FAMILY CRYPTOSULIDAE
Cryptosula pallasiana (Moll, 1803)
FAMILY ELECTRIDAE
Electra pilosa (Linneo, 1767)
FAMILY HAPLOPOMIDAE
Haplopoma impressum (Audouin, 1826)
FAMILY HIPPOTHOIDAE

Celleporella hyalina (Linneo, 1767)
FAMILY MEMBRANIPORIDAE
Membranipora membranacea (Linneo, 1767)
FAMILY MICROPORELLIDAE
Microporella ciliata (Pallas, 1766)
FAMILY UMBONULIDAE
Oshukorvia littoralis (Hastings, 1944)
FAMILY VESICULARIIDAE
Bowerbankia gracilis Leidy, 1855
Bowerbankia sp.
Class Stenolaemata
FAMILY CRISIIDAE
Crisia denticulata (Lamarck, 1816)

PHYLUM ECHINODERMATA
Class Holothuroidea
FAMILY CUCUMARIIDAE
Stereoderma kirschbergi (Heller, 1868)
Class Ophiuroidea
FAMILY AMPHIURIDAE
Amphipholis squamata (Delle Chiaje, 1828)
FAMILY OPHIOTRICHIDAE
Ophiothrix fragilis (Abildgaard *in* O.F. Müller, 1789)

PHYLUM CHORDATA
Class Actinopterygii
Lipophrys pholis (Linneo, 1758)