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## On the role of turf species as refuge in disturbed environments: A case study with polychaetes (Annelida: Polychaeta) in the SW Mediterranean Sea

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### Abstract

During the summer of 2014, a field survey was conducted in several littoral rocky sites on the southeastern coast of Spain. In this area, dense canopies of *Cystoseira mediterranea* characterize undisturbed locations and cover most of the available infralittoral fringe. Turfs composed mainly of the coralline algae *Ellisolandia elongata* replace the canopy in locations where some kind of anthropogenic disturbance has occurred. The main goal of this study was to compare the polychaete fauna dwelling within the two types of phytal substratum, and several univariate and multivariate analyses were performed to detect significant differences between the two assemblages. The analyses showed that the assemblage inhabiting *E. elongata* was characterized by a higher population density of polychaetes and was dominated by herbivores and filter-feeders, especially *Fabricia stellaris* and *Amphiglena mediterranea*. Polychaetes were less abundant in pristine sites covered by *Cystoseira mediterranea*, although  $\alpha$ -diversity was slightly higher. This assemblage was characterized by a predominance of omnivores followed by herbivores, and by high relative abundances of *Syllis prolifera* and *Salvatoria clavata*. However, none of the detected differences was statistically significant and site to site comparison showed that differences between locations with the same phytal cover were similarly deep. Our data point out that the assemblage inhabiting turf algae in the region studied is not substantially different to that found in pristine areas and that this type of phytal covering can act as effective refuge for polychaetes under moderately disturbed condition.

**Keywords:** Turf algae, canopy, Polychaeta, benthos.

### Introduction

Coastal urbanization is increasing worldwide and has resulted in an extensive modification of natural shores, with changes in habitat heterogeneity, slope, and available area for littoral communities (Cacabelos *et al.*, 2016). Due to this process of physical transformation, dramatic changes in the structure of marine communities occur, and these modifications can alter the ecological functions of the species assemblages (Bustamante *et al.*, 2014). Additionally, urban and industrial effluents affect littoral communities, and those thriving in the upper littoral rocky environment of the Mediterranean shores are especially sensitive to industrial effluents (Pinedo *et al.*, 2007). In undisturbed conditions, dense canopies of larger macroalgae form the typical communities (Strain *et al.*, 2014), and their shape and structural complexity are important factors in determining patterns of abundance and size structure of associated epifaunal organisms (Buschbaum *et al.*, 2006; Pinedo *et al.*, 2007; Gestoso *et al.*, 2010). From a physical point of view, macroalgal canopies are often important ecosystem engineers. They exert positive effects providing refuges and shelter from wave action, solar radiation, extreme temperatures, or desiccation

(Asnaghi *et al.*, 2015), but also important negative impacts, such as preventing the establishment of other sessile organisms (Benedetti-Cecchi *et al.*, 2003). Evidence suggests that algae are more abundant, and more diverse assemblages of invertebrates occur in the most structurally complex canopies. This has been related to a larger availability of surface for the colonization by fauna and epiphytic algae (Chemello & Milazzo, 2002; Cacabelos *et al.*, 2010). These characteristics make macroalgal canopies a key functional element of coastal habitats, in terms of production, nutrient cycling, and food webs (Bertocci *et al.*, 2010). These assemblages have, however, suffered a steady decline around the world over the last 30 years (Benedetti-Cecchi *et al.*, 2003).

In temperate waters, Fucales (Ochrophyta, Phaeophyceae) represent the dominant canopy-forming species in pristine environments (Blanfuné *et al.*, 2016), and in the Mediterranean Sea, a number of species belonging to the genus *Cystoseira* C. Agardh, 1820 play this role (Pinedo *et al.*, 2007; Blanfuné *et al.*, 2016). Most Mediterranean *Cystoseira* species are particularly sensitive to human-induced changes in their environment (Mangialajo *et al.*, 2007; Asnaghi *et al.*, 2015), and some studies have demonstrated a negative relationship between the pres-

ence of rich and well developed *Cystoseira* assemblages and urbanization and high levels of nutrient concentration (Benedetti-Cecchi *et al.*, 2001; Sales & Ballesteros, 2009). Based on their low tolerance to environmental stress, these species are included in the ESG IA category of bioindicator phytal species proposed by Orfanidis *et al.* (2011), which encompasses slow-growing, non-plastic species characteristic of pristine locations. In the western Mediterranean Sea, the short algae that constitute the understory of the phytal community (Raffaelli & Hawkins, 1999) become dominant and form characteristic turfs when *Cystoseira* canopies disappear (Bulleri *et al.*, 2002; Mangialajo *et al.*, 2007). In some cases, the littoral invertebrate communities present in the turfs showed lower diversity than those living in the replaced canopies (Benedetti-Cecchi *et al.*, 2001). This substitution also happens in many other temperate locations and is expected to increase in the future (Asnaghi *et al.*, 2015). Due to their persistence when no severe disturbance happens, these slow-growing, shade-adapted coralline algae are classified as ESG IC (moderately tolerant) in the aforementioned study (Orfanidis *et al.*, 2011).

Previous studies have demonstrated that algal turfs can have strong effects on the distribution of macrofaunal organisms that live in the phytal matrix and that they can provide protection from wave exposure and restrict fluctuations in humidity and temperature on the shore (Thrush *et al.*, 2011) in a way similar to those of canopy-forming species. They can also adversely influence the community since their presence can inhibit the recruitment of canopy species following destruction and may therefore drive long-lasting changes in community structure (Bulleri *et*

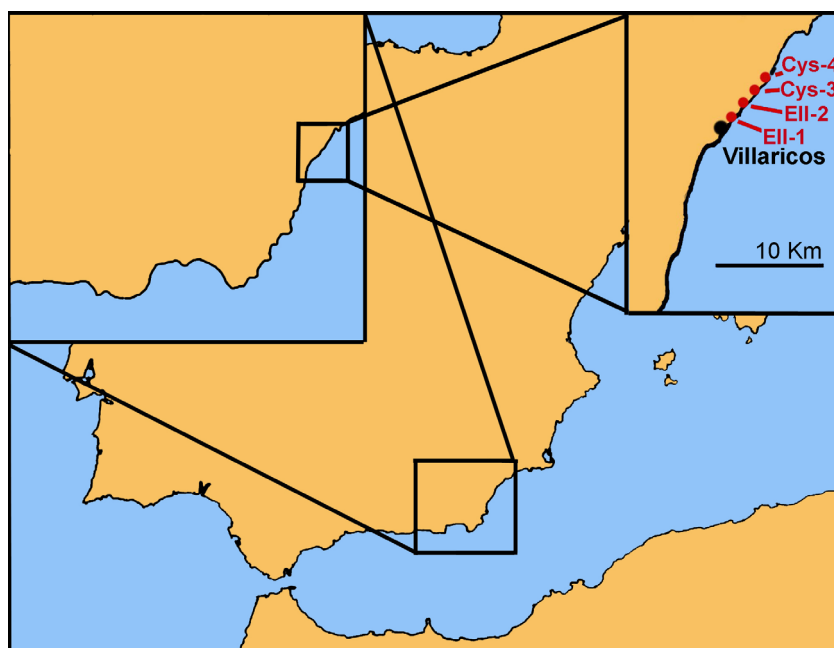
*al.*, 2002; Asnaghi *et al.*, 2015), which usually takes a long time to recover when water quality improves (Blanfuné *et al.*, 2016).

There is thus a clear need to understand the drivers underlying the loss of macroalgal canopies and their replacement by coralline alga turfs, as well as the effect of such processes on the associated animal communities (Cacabelos *et al.*, 2016). In this respect, polychaetes, which are dominant in marine benthic communities of both hard and soft bottoms (Viéitez *et al.*, 2004), can be effective surrogates for the estimation of the dynamics of benthic communities, as they play key roles in ecosystem functioning (Olsgard *et al.*, 2003; Giangrande *et al.*, 2005). The goal of this investigation was to compare the polychaete assemblages in the canopy and in turf-covered facies of the upper infralittoral rocky bottom at the southeastern Spanish coast, aiming to check for potential differences among them and to assess to what extent they are significant.

## Materials and Methods

### Study site

The study area is situated near the small town of Villaricos (Almería) on the southeastern coast of Spain (Fig. 1) and extends from the urban area of the village to about 5 km northwards. In spite of the proximity of important touristic and recreational areas, it has experienced little human perturbation due to its abrupt coastline, which has prevented intensive occupation of the landscape, and benthic communities remain relatively pristine in most of the



**Fig. 1:** Map of southeast Spain showing the sampling sites of this study. Site 1 (EII-1, 37.25454°N, 1.76346°W); site 2 (EII-2, 37.26359°N, 1.75785°W); site 3 (Cys-3, 37.26908°N, 1.74783°W); site 4 (Cys-4, 37.29087°N, 1.72297°W).

area (Luque *et al.*, 2004). Where the shoreline consists of rocky bottoms, a dense canopy of *Cystoseira mediterranea* Sauvageau, 1912 (Ochrophyta, Sargassaceae) dominates the algal assemblages of the infralittoral fringe. By contrast, in the urban area of the village and the nearby chemical factory, where significant impact has been reported (Sánchez Lizaso, 2004), the canopy-forming macroalgae are replaced by a turf composed predominantly of *Ellisolandia elongata* (J. Ellis & Solander) K.R. Hind & G.W. Saunders, 2013 (Rhodophyta, Corallinaceae).

In this stretch of coast, four sites (Fig. 1), representative of each of these facies, were chosen, covering a gradient from urban shoreline to pristine condition. Site 1 (Ell-1, 37.25454°N, 1.76346°W) was located in a semi-urban environment and site 2 (Ell-2, 37.26359°N, 1.75785°W) as situated 1.5 km north; in both places, *E. elongata* was the dominant macroalgae species. Site 3 (Cys-3, 37.26908°N, 1.74783°W) was located 2.3 km away from site 1 and was dominated by a well-developed *C. mediterranea* canopy, as was site 4 (Cys-4, 37.29087°N, 1.72297°W).

### Sampling methods and data analyses

Sampling was conducted in August 2014, with the aim to obtain a snapshot of the community during a time of high algal development and faunal abundance following the spring-early summer recruitment (Thrush *et al.*, 2011). In each site, three randomly selected samples were collected by scraping off quadrats consisting of a 10 x 10 cm area of rocky bottom from depths ranging from 0.05 to 0.15 cm. Such a small sample size has been accepted as sufficient for the study of polychaetes in the Mediterranean Sea (Fraschetti *et al.*, 2002; Musco, 2012; Casoli *et al.*, 2016). The scraped material was preserved in 70% ethanol and stored in a ZIP plastic bag. In the laboratory, the material was examined under a dissecting microscope and all the animals were sorted into higher taxonomic groups (usually class level). Polychaeta were identified to species using the updated regional keys provided in the Fauna Ibérica series (San Martín, 2003; Viéitez *et al.*, 2004; Parapar *et al.*, 2012, 2015) and enumerated, whereas the rest of the fauna were stored for further studies.

A species abundance matrix was created, summarizing actual numbers of individuals of each species. The population density, as number of individuals/sample, the species richness, and the Shannon, equitability, and Margalef indices for  $\alpha$ -diversity were computed for each sample and mean and standard deviation values were obtained for each algal facies. For analyses associated with feeding traits, the species abundance matrix was coded into six feeding guilds (carnivores, herbivores, omnivores, burrowers, surface-deposit feeders, and filter-feeders). Species assignment to guilds was performed after López & Viéitez (1999), Giangrande *et al.* (2000), and Antoniadou & Chintiriglou (2006). Relative abundance (the ratio between species abundance and total number of polychaete individuals) was calculated in every sample for all the

species and the mean value was then computed for the entire data set. Species showing a mean relative abundance over 5% were considered dominant in the community; these values were also treated as ecological descriptors of the samples.

Differences in the polychaete assemblages inhabiting the two algal facies were tested for with univariate and multivariate statistical analyses. Cochran's C test was used prior to analyses to detect heterogeneity of variances for each ecological descriptor (including relative abundance of dominant species), and data were transformed where appropriate (Underwood, 1997; Cacabelos *et al.*, 2016). If homogeneity of data was not achieved after transformation, analyses were run on the untransformed data using a more conservative significance level ( $p < 0.01$ ) (Underwood, 1997; Cacabelos *et al.*, 2016). Variables were compared by a two-way ANOVA test in order to check the weight of between-sites differences within each assemblage, considering phytal facies as Factor 1 (with two levels, orthogonal and fixed) and site as Factor 2 (with two levels, nested in one, random). Post hoc Student-Newman-Keuls (SNK) tests were used to compare means within significant terms (Cacabelos *et al.*, 2016).

A second matrix was derived from the abundance matrix, showing the similarity between samples by mean of the Bray-Curtiss coefficient after data were  $\log(x + 1)$  transformed to limit the influence of most dominant species (Clarke & Warwick, 1994). Non-metric multidimensional scaling (nMDS) was used to visually represent the results. Differences in the composition of polychaete assemblages were tested using two-way PERMANOVA (Anderson, 2001), run in Bray-Curtis similarity matrix, again using facies as Factor 1 (with two levels, orthogonal and fixed) and site as Factor 2 (with two levels, nested in one, random). Statistical analyses were conducted using the software packages PAST 3 (Hammer *et al.*, 2001) and GMAV-5 (Underwood *et al.*, 2002), except for PERMANOVA, which was computed with Primer 6 + Permanova add-on (Clarke & Gorley, 2006).

### Results

A total of 3,537 specimens, belonging to 35 species and 11 families, were collected (Table 1). The dominant polychaete species for the infralittoral fringe in the area were *Platynereis dumerilii* (mean relative abundance  $23.60 \pm 17.34\%$ ), *Salvatoria clavata* ( $22.27 \pm 14.10\%$ ), *Syllis prolifera* ( $14.98 \pm 9.07\%$ ), *Fabricia stellaris* ( $11.17 \pm 15.71\%$ ), *Sphaerosyllis hystrix* ( $7.23 \pm 6.51\%$ ), and *Amphiglena mediterranea* ( $5.97 \pm 13.14\%$ ). However, the relative abundance of these species showed a wide dispersion within each assemblage, as well as conspicuous differences between *Ellisolandia* and *Cystoseira* facies (Fig. 2). The remaining ecological descriptors calculated from the abundance matrix are presented in Figures 3 and 4.

**Table 1.** List of polychaete species collected in all the samples. A. Samples collected from *Ellisolandia elongata*. B. Samples collected from *Cystoseira mediterranea*. Abbreviations for trophic guilds.- CNV: carnivores; HBV: herbivores; OMN: omnivores; BRW: burrowers; SDF: surface deposit-feeders; FTF: filter-feeders.

|   | EII-1.1 | EII-1.2 | EII-1.3 | EII-2.1 | EII-2.2 | EII-2.3 | T. guild |
|---|---------|---------|---------|---------|---------|---------|----------|
| <i>Amphicorina armandi</i> (Claparède, 1864)              |         |         |         |         | 1       |         | FTF      |
| <i>Amphicorina eimeri</i> (Langerhans, 1881)              | 3       | 2       |         |         | 2       |         | FTF      |
| <i>Amphiglena mediterranea</i> (Leydig, 1851)             | 120     | 214     | 1       | 1       |         |         | FTF      |
| <i>Brania pusilla</i> (Dujardin, 1851)                    |         |         |         |         |         |         | OMN      |
| <i>Caulleriella bioculata</i> (Keferstein, 1862)          |         | 2       |         |         |         |         | SDF      |
| <i>Cirriiformia filigera</i> (Delle Chiaje, 1828)         |         |         |         | 1       |         |         | SDF      |
| <i>Dipolydora quadrilobata</i> (Jacobi, 1883)             |         | 2       |         |         |         |         | SDF      |
| <i>Eurysyllis tuberculata</i> Ehlers, 1864                |         | 1       |         |         |         |         | CNV      |
| <i>Exogone naidina</i> Örsted, 1845                       |         |         |         | 11      | 6       | 3       | HBV      |
| <i>Fabricia sabella</i> (Ehrenberg, 1836)                 | 167     | 77      |         | 244     | 125     | 26      | FTF      |
| <i>Hydroides elegans</i> (Haswell, 1883)                  |         |         | 1       |         |         |         | FTF      |
| <i>Lepidonotus clava</i> (Montagu, 1808)                  | 1       | 1       |         |         |         |         | CNV      |
| <i>Lumbrineris latreilli</i> Audouin & M. Edwards, 1834   |         |         | 1       |         |         |         | OMN      |
| <i>Microspio mecznikowianus</i> (Claparède, 1869)         |         |         |         |         |         |         | SDF      |
| <i>Myrianida convoluta</i> (Cognetti, 1953)               | 1       |         |         |         |         |         | CNV      |
| <i>Nereis rava</i> Ehlers, 1864                           |         |         |         |         |         |         | CNV      |
| <i>Nereis zonata</i> Malmgren, 1867                       |         |         |         |         |         |         | CNV      |
| <i>Perinereis cultrifera</i> (Grube, 1840)                | 1       | 4       | 2       | 1       |         | 20      | HBV      |
| <i>Pholoe inornata</i> Johnston, 1839                     | 1       |         |         |         | 1       |         | CNV      |
| <i>Platynereis dumerilii</i> (Audouin & M. Edwards, 1834) | 38      | 119     | 84      | 75      | 112     | 47      | HBV      |
| <i>Polyopthalmus pictus</i> (Dujardin, 1839)              | 3       | 9       |         | 9       | 4       | 7       | BRW      |
| <i>Protoaricia oerstedii</i> (Claparède, 1864)            | 51      | 68      | 3       | 9       | 8       | 1       | SDF      |
| <i>Salvatoria clavata</i> (Claparède, 1863)               | 9       | 11      | 6       | 105     | 90      | 45      | HBV      |
| <i>Salvatoria limbata</i> (Claparède, 1868)               |         | 1       |         | 10      |         |         | HBV      |
| <i>Sphaerosyllis austriaca</i> Banse, 1959                |         |         |         | 1       |         |         | HBV      |
| <i>Sphaerosyllis hystrix</i> Claparède, 1863              | 2       | 14      |         | 82      | 46      | 43      | HBV      |
| <i>Syllis armillaris</i> (O.F. Müller, 1776)              | 10      | 5       |         | 15      | 13      | 8       | HBV      |
| <i>Syllis corallicola</i> Verrill, 1900                   |         |         |         |         | 3       | 4       | HBV      |
| <i>Syllis gerlachi</i> (Hartmann-Schröder, 1960)          |         | 4       |         | 7       | 5       | 1       | CNV      |
| <i>Syllis gracilis</i> Grube, 1840                        | 3       |         |         |         |         |         | OMN      |
| <i>Syllis krohnii</i> Ehlers, 1864                        |         |         |         |         |         |         | OMN      |
| <i>Syllis prolifera</i> Krohn, 1852                       | 12      | 16      | 14      | 55      | 23      | 27      | OMN      |
| <i>Syllis variegata</i> Grube, 1860                       |         |         |         |         | 1       | 2       | SDF      |
| <i>Synmerosyllis lamelligera</i> (Saint-Joseph, 1886)     |         |         |         |         |         |         | SDF      |
| <i>Trypanosyllis coeliaca</i> Claparède, 1868             |         |         |         |         | 1       |         | CNV      |

(continued)

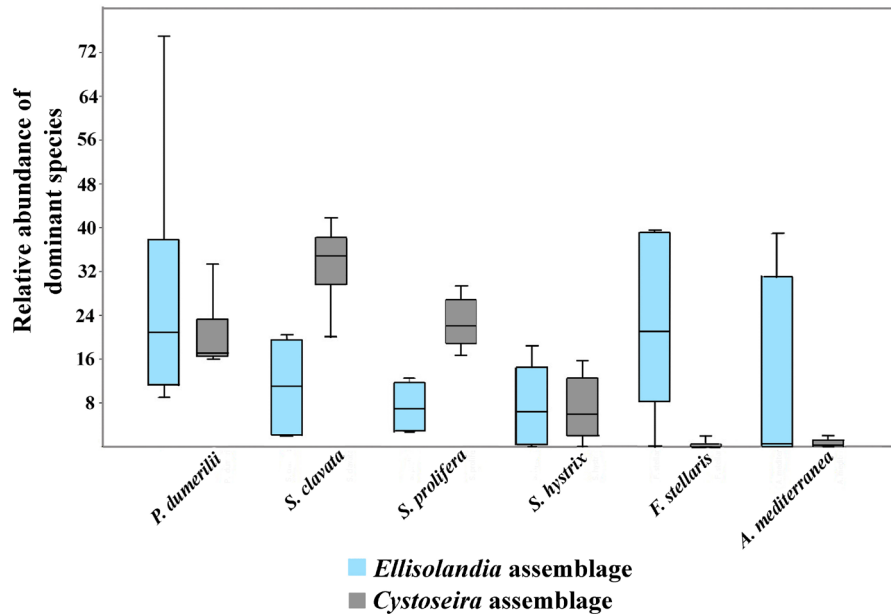
**Table 1.** (continued)

|   | Cys-3.1 | Cys-3.2 | Cys-3.3 | Cys-4.1 | Cys-4.2 | Cys-4.3 | T. guild |
|---|---------|---------|---------|---------|---------|---------|----------|
| <i>Amphicorina armandi</i> (Claparède, 1864)              |         |         |         | 5       | 6       |         | FTF      |
| <i>Amphicorina eimeri</i> (Langerhans, 1881)              | 1       | 1       | 1       |         |         | 1       | FTF      |
| <i>Amphiglena mediterranea</i> (Leydig, 1851)             |         |         | 1       | 2       |         | 1       | FTF      |
| <i>Brania pusilla</i> (Dujardin, 1851)                    |         |         |         |         | 1       |         | OMN      |
| <i>Caulleriella bioculata</i> (Keferstein, 1862)          |         |         |         |         |         |         | SDF      |
| <i>Cirriformia filigera</i> (Delle Chiaje, 1828)          |         |         |         |         |         |         | SDF      |
| <i>Dipolydora quadrilobata</i> (Jacobi, 1883)             |         |         |         |         |         |         | SDF      |
| <i>Eurysyllis tuberculata</i> Ehlers, 1864                |         |         |         |         |         | 1       | CNV      |
| <i>Exogone naidina</i> Örsted, 1845                       | 1       | 2       | 2       |         |         |         | HBV      |
| <i>Fabricia sabella</i> (Ehrenberg, 1836)                 |         |         |         |         |         | 1       | FTF      |
| <i>Hydroides elegans</i> (Haswell, 1883)                  |         |         |         |         |         |         | FTF      |
| <i>Lepidonotus clava</i> (Montagu, 1808)                  |         |         |         |         |         |         | CNV      |
| <i>Lumbrineris latreilli</i> Audouin & M. Edwards, 1834   |         |         |         |         |         |         | OMN      |
| <i>Microspio mecznikowianus</i> (Claparède, 1869)         |         | 1       | 1       |         |         |         | SDF      |
| <i>Myrianida convoluta</i> (Cognetti, 1953)               |         |         |         |         |         | 1       | CNV      |
| <i>Nereis rava</i> Ehlers, 1864                           |         |         |         |         | 2       |         | CNV      |
| <i>Nereis zonata</i> Malmgren, 1867                       |         |         |         |         | 1       |         | CNV      |
| <i>Perinereis cultrifera</i> (Grube, 1840)                | 1       |         | 1       |         |         |         | HBV      |
| <i>Pholoe inornata</i> Johnston, 1839                     |         |         |         |         |         |         | CNV      |
| <i>Platynereis dumerilii</i> (Audouin & M. Edwards, 1834) | 46      | 30      | 86      | 45      | 31      | 8       | HBV      |
| <i>Polyopthalmus pictus</i> (Dujardin, 1839)              | 31      | 4       | 11      | 1       | 2       | 4       | BRW      |
| <i>Protoarcia oerstedii</i> (Claparède, 1864)             | 5       | 4       | 3       | 2       | 10      | 1       | SDF      |
| <i>Salvatoria clavata</i> (Claparède, 1863)               | 88      | 66      | 85      | 94      | 68      | 10      | HBV      |
| <i>Salvatoria limbata</i> (Claparède, 1868)               | 1       |         |         |         | 4       | 1       | HBV      |
| <i>Sphaerosyllis austriaca</i> Banse, 1959                |         | 1       |         | 1       | 6       | 3       | HBV      |
| <i>Sphaerosyllis hystrix</i> Claparède, 1863              | 31      | 28      | 21      | 6       | 7       |         | HBV      |
| <i>Syllis armillaris</i> (O.F. Müller, 1776)              | 5       | 4       | 2       | 2       | 1       |         | HBV      |
| <i>Syllis corallicola</i> Verrill, 1900                   |         | 1       |         | 1       |         |         | HBV      |
| <i>Syllis gerlachi</i> (Hartmann-Schröder, 1960)          |         |         |         |         |         | 2       | CNV      |
| <i>Syllis gracilis</i> Grube, 1840                        |         |         |         |         |         |         | OMN      |
| <i>Syllis krohnii</i> Ehlers, 1864                        |         |         |         |         | 1       |         | OMN      |
| <i>Syllis prolifera</i> Krohn, 1852                       | 55      | 35      | 43      | 66      | 44      | 13      | OMN      |
| <i>Syllis variegata</i> Grube, 1860                       | 2       | 1       | 1       |         |         |         | SDF      |
| <i>Synmersosyllis lamelligera</i> (Saint-Joseph, 1886)    | 1       |         |         |         | 1       | 2       | SDF      |
| <i>Trypanosyllis coeliaca</i> Claparède, 1868             |         |         |         |         |         | 1       | CNV      |

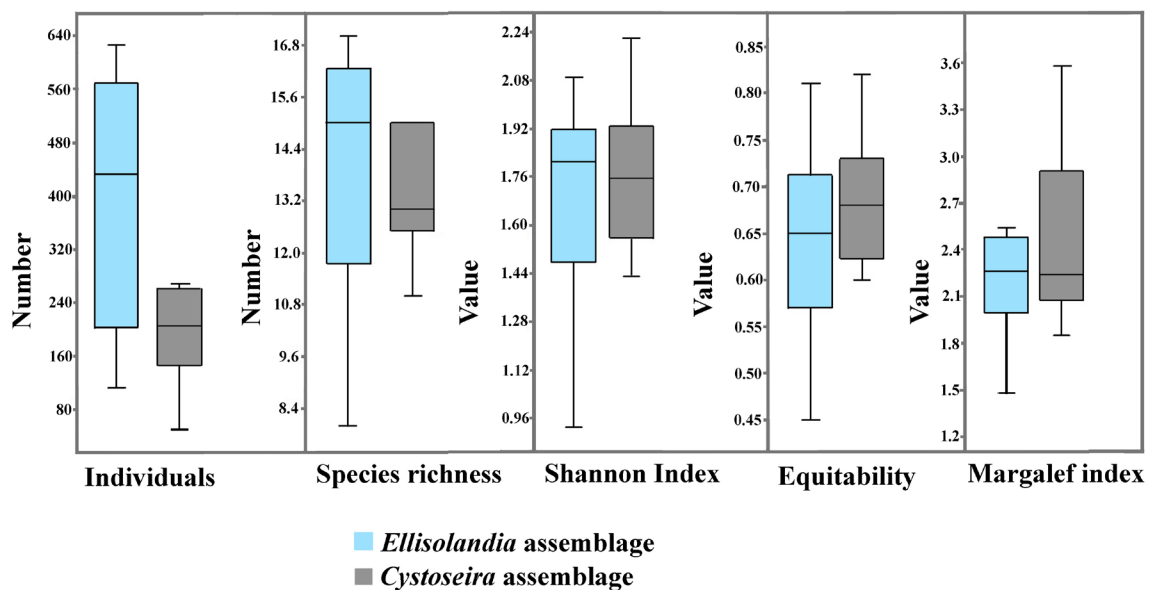
It can be concluded from these plots that the greatest difference regarding ecological indices can be observed in the number of individuals and, to a lesser extent, in the Margalef index (Fig. 3). Number of individuals ranged from 626 in Ell-2.1 to a mere 50 in Cys-4.3, with significant differences in the mean values for each facies (397.50 ± 192.98 individuals per sample in the facies of *E. elongata* vs. 194 ± 79.51 in *C. mediterranea*). Some trophic guilds also presented wide ranges in occurrence (Fig. 4). Filter-feeders accounted for 68.72% of the specimens collected in Ell-1.1 and only for 0.37% in Cys-1.1, and omnivores accounted for 29.33% of the specimens

in Cys-4.1 and for 2.91% in Ell-1.2. These differences resulted in clear differences between facies, but the extreme values for herbivores were obtained in the same station, representing 82.14% of the specimens in Ell-1.3 and 14.22% in Ell-1.1.

The results of the two-way ANOVA analyses showed that differences between facies in all the ecological descriptors were not significant (Tables 2-4). However, some significant differences between sites were detected in the relative abundances of *S. clavata*, *F. stellaris*, and *S. hystrix* (Table 2). These differences were confirmed by the SNK test, with  $p < 0.01$  for *S. clavata* between sites



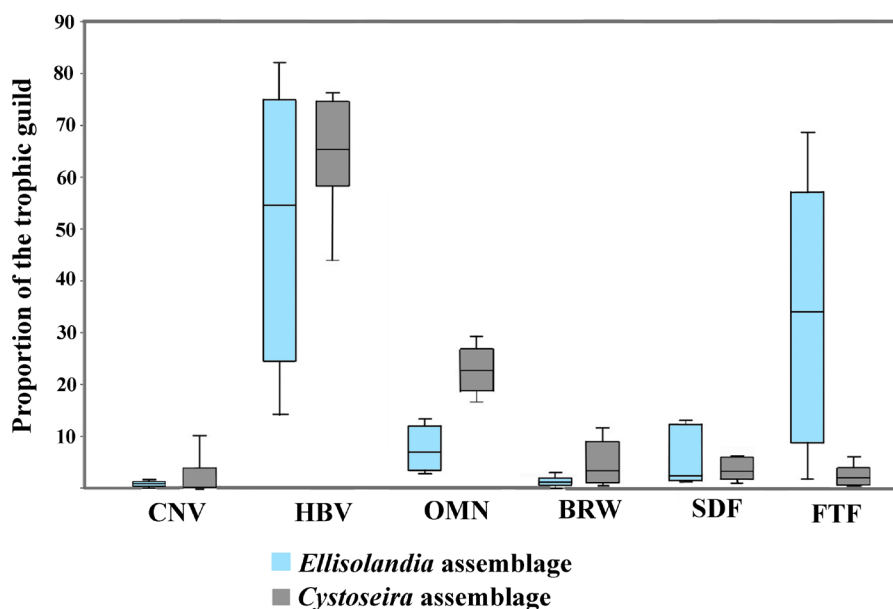
**Fig. 2:** Relative abundance of dominant species in the samples; box-and-whisker plots for values from *Ellisolandia* and *Cystoseira* facies. The whiskers represent the minimal and maximal values, the outer edges of the boxes represent the 25<sup>th</sup> and 75<sup>th</sup> percentiles, and the horizontal line within the boxes represents the median.



**Fig. 3:** Ecological descriptors; box-and-whisker plots for values from *Ellisolandia* and *Cystoseira* facies. The whiskers represent the minimal and maximal values, the outer edges of the boxes represent the 25<sup>th</sup> and 75<sup>th</sup> percentiles, and the horizontal line within the boxes represents the median.

1 and 2 and for *S. hystrix* between sites 1 and 2 and between sites 3 and 4, and with  $p < 0.05$  for *F. stellaris* between sites 1 and 2. The samples collected from each algal facies are differently placed in the nMDS plot (Fig. 5). Most of those pertaining to *C. mediterranea* sites appear in the middle of the plot, while those from *E. elongata* are

located in the left. An in-group structuration can also be mentioned, since samples from each site tend to be similar. However, while in-site distances are short in sites 2 and 3, sites 1 and 4 group more loosely in such a way that Ell-1.3 and Cys-4.3 are located far away from the rest of the samples.



**Fig. 4:** Proportions of each trophic guild; box-and-whisker plots for values from *Ellisolandia* and *Cystoseira* facies. The whiskers represent the minimal and maximal values, the outer edges of the boxes represent the 25<sup>th</sup> and 75<sup>th</sup> percentiles, and the horizontal line within the boxes represents the median. Abbreviations.- CNV: carnivores; HBV: herbivores; OMN: omnivores; BRW: burrowers; SDF: surface deposit-feeders; FTF: filter-feeders.

**Table 2.** Results of the two-way ANOVA test for relative abundance of dominant species in the samples. \*\* p<0.01, \*p<0.05, <sup>ns</sup> not significant. Abbreviations.- MS: mean square; df: degrees of freedom.

| Source         | df | <i>A. mediterranea</i> |                    | <i>S. clavata</i>    |                    | <i>S. hystrix</i>                            |                    |
|----------------|----|------------------------|--------------------|----------------------|--------------------|--|--------------------|
|                |    | MS                     | F                  | MS                   | F                  | MS   | F                  |
| Facies         | 1  | 114.08                 | 0.85 <sup>ns</sup> | 23.11                | 4.19 <sup>ns</sup> | 0.08   | 0.00 <sup>ns</sup> |
| Site (Facies)  | 2  | 134.08                 | 1.08 <sup>ns</sup> | 5.51                 | 13.08**            | 190.42                                       | 19.20**            |
| Residual       | 8  | 123.08                 |                    | 0.42                 |                    | 9.992  |                    |
| Total          | 11 |                        |                    |                      |                    |  |                    |
| SNK            |    |                        |                    | Site 1 diff Site 2** |                    | Site 1 diff Site 2**<br>Site 3 diff Site 4** |                    |
| Cochran's test |    | 0.99**                 |                    | 0.65 <sup>ns</sup>   |                    | 0.41 <sup>ns</sup>                           |                    |
| Transformation |    | None                   |                    | Sqrt (x+1)           |                    | None   |                    |

| Source         | df | <i>P. dumerilii</i> |                    | <i>S. prolifera</i> |                     | <i>F. stellaris</i> |                    |
|----------------|----|---------------------|--------------------|---------------------|---------------------|---------------------|--------------------|
|                |    | MS                  | F                  | MS                  | F                   | MS                  | F                  |
| Facies         | 1  | 96.33               | 0.64 <sup>ns</sup> | 752.08              | 16.68 <sup>ns</sup> | 675.00              | 1.98 <sup>ns</sup> |
| Site (Facies)  | 2  | 149.67              | 0.37 <sup>ns</sup> | 45.08               | 2.60 <sup>ns</sup>  | 341.67              | 5.14*              |
| Residual       | 8  | 404.75              |                    | 17.33               |                     | 66.42               |                    |
| Total          | 11 |                     |                    |                     |                     |                     |                    |
| SNK            |    |                     |                    |                     |                     | Site 1 diff Site 2* |                    |
| Cochran's test |    | 0.92 <sup>ns</sup>  |                    | 0.67 <sup>ns</sup>  |                     | 0.75 <sup>ns</sup>  |                    |
| Transformation |    | None                |                    | None                |                     | None                |                    |



**Table 3.** Results of the two-way ANOVA test for ecological descriptors. \*\* p<0.01, \*p<0.05, <sup>ns</sup> not significant. Abbreviations.- MS: mean square; df: degrees of freedom.

| Source         | df | Individuals        |                    | Species richness   |                    | Shannon index      |                    |
|----------------|----|--------------------|--------------------|--------------------|--------------------|--------------------|--------------------|
|                |    | MS                 | F                  | MS                 | F                  | MS                 | F                  |
| Facies         | 1  | 53200.08           | 1.36 <sup>ns</sup> | 10.08              | 0.33 <sup>ns</sup> | 0.37               | 0.57 <sup>ns</sup> |
| Site (Facies)  | 2  | 38988.08           | 1.17 <sup>ns</sup> | 30.42              | 1.52 <sup>ns</sup> | 0.80               | 3.27 <sup>ns</sup> |
| Residual       | 8  | 33444.83           |                    | 20.00              |                    | 0.24               |                    |
| Total          | 11 |                    |                    |                    |                    |                    |                    |
| Cochran's test |    | 0.63 <sup>ns</sup> |                    | 0.90 <sup>ns</sup> |                    | 0.81 <sup>ns</sup> |                    |
| Transformation |    | None               |                    | None               |                    | None               |                    |

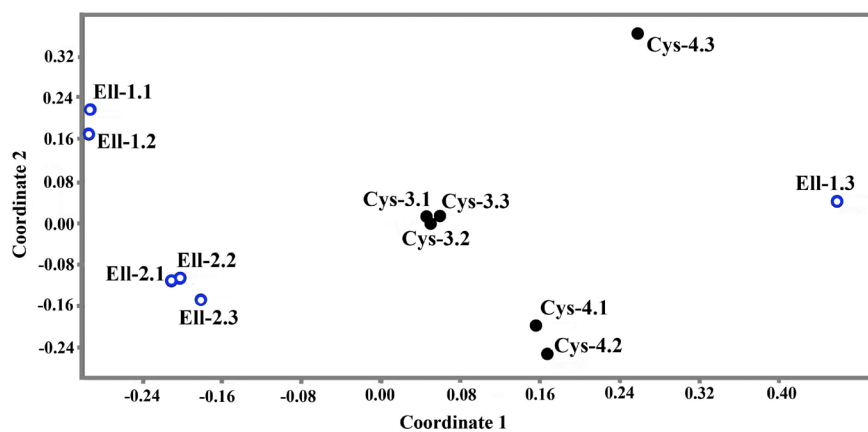
| Source         | df | Equitability       |                    | Margalef index     |                    |
|----------------|----|--------------------|--------------------|--------------------|--------------------|
|                |    | MS                 | F                  | MS                 | F                  |
| Facies         | 1  | 0.06               | 0.60 <sup>ns</sup> | 1.25               | 0.35 <sup>ns</sup> |
| Site (Facies)  | 2  | 0.10               | 3.17 <sup>ns</sup> | 0.84               | 0.30 <sup>ns</sup> |
| Residual       | 8  | 0.03               |                    | 0.60               |                    |
| Total          | 11 |                    |                    |                    |                    |
| Cochran's test |    | 0.83 <sup>ns</sup> |                    | 0.67 <sup>ns</sup> |                    |
| Transformation |    | None               |                    | None               |                    |

**Table 4.** Results of the two-way ANOVA test for proportions of each trophic guild. \*\* p<0.01, \*p<0.05, <sup>ns</sup> not significant. Abbreviations.- MS: mean square; df: degrees of freedom; CNV: carnivores; HBV: herbivores; OMN: omnivores; BRW: burrowers; SDF: surface deposit-feeders; FTF: filter-feeders.

| Source         | df | CNV    |                    | HBV    |                    | BRW                |                    |
|----------------|----|--------|--------------------|--------|--------------------|--------------------|--------------------|
|                |    | MS     | F                  | MS     | F                  | MS                 | F                  |
| Facies         | 1  | 4.55   | 0.39 <sup>ns</sup> | 777.63 | 1.36 <sup>ns</sup> | 35.43              | 4.89 <sup>ns</sup> |
| Site (Facies)  | 2  | 11.60  | 1.57 <sup>ns</sup> | 572.09 | 1.10 <sup>ns</sup> | 7.25               | 0.66 <sup>ns</sup> |
| Residual       | 8  | 7.39   |                    | 520.44 |                    | 10.92              |                    |
| Total          | 11 |        |                    |        |                    |                    |                    |
| Cochran's test |    | 0.97** |                    | 0.83*  |                    | 0.55 <sup>ns</sup> |                    |
| Transformation |    | None   |                    | None   |                    | None               |                    |

| Source         | df | SDF                |                    | OMN                |                     | FTF     |                     |
|----------------|----|--------------------|--------------------|--------------------|---------------------|---------|---------------------|
|                |    | MS                 | F                  | MS                 | F                   | MS      | F                   |
| Facies         | 1  | 0.22               | 1.80 <sup>ns</sup> | 755.09             | 14.38 <sup>ns</sup> | 1205.41 | 20.86 <sup>ns</sup> |
| Site (Facies)  | 2  | 0.12               | 0.20 <sup>ns</sup> | 52.53              | 3.04 <sup>ns</sup>  | 58.28   | 0.82 <sup>ns</sup>  |
| Residual       | 8  | 0.60               |                    | 17.28              |                     | 279.84  |                     |
| Total          | 11 |                    |                    |                    |                     |         |                     |
| Cochran's test |    | 0.73 <sup>ns</sup> |                    | 0.72 <sup>ns</sup> |                     | 0.82*   |                     |
| Transformation |    | Ln (x+1)           |                    | None               |                     | None    |                     |



**Fig. 5:** Non-metric multidimensional scaling (nMDS) ordination plot comparing assemblages from the sampling sites. Empty circles: samples of *Ellisolandia elongata*. Solid circles: samples of *Cystoseira mediterranea*.

**Table 5.** Results of the two-way PERMANOVA test for species composition run in Bray-Curtis similarity matrix. \* $p < 0.05$ , <sup>ns</sup> not significant. Abbreviations.- MS: mean square; df: degrees of freedom.

| Source        | df | MS     | Pseudo- F          |
|---------------|----|--------|--------------------|
| Facies        | 1  | 2043.7 | 1.47 <sup>ns</sup> |
| Site (Facies) | 2  | 1389.5 | 2.44*              |
| Residual      | 8  | 568.27 |                    |
| Total         | 11 |        |                    |

According to the previously described univariate analyses, the differences between the polychaete assemblages were not significant after computing the two-way PERMANOVA analysis (Table 5). Again, differences regarding sites were significant, indicating that the algal species could not be identified as the main driver of the assemblage.

## Discussion

No clear difference between the two investigated polychaete assemblages can be inferred from the nMDS graph. The faraway position of Cys-4.3 in the plot can easily be explained by the low abundance of the collected polychaetes, with only 50 specimens (Figs. 2-4). However, the reason why Ell-1.3 is separated from the rest of the samples belonging to the facies and closer to those from *C. mediterranea* remains unknown.

According to Figures 2 to 4 and to the results of the analyses of variances, the two assemblages differed in some ecological descriptors, although this difference was not significant. The trophic guild composition varied between *E. elongata* and *C. mediterranea* facies, and while herbivores were the dominant guild in both ( $51.03 \pm 26.21\%$  and  $64.67 \pm 11.47\%$ , respectively) facies, the second most dominant guild was represented by omnivores ( $22.84 \pm 4.69$ ) for *C. mediterranea* and filter-feeders for *E. elongata* ( $33.84 \pm 25.26$ ). The large proportion of herbivores in the latter is remarkable, since in other studies, the unpalatable nature of Rhodophyta rendered them unsuitable for the maintenance of large populations of mesograzers (Taylor & Steinberg, 2005), such as herbivorous polychaetes. The abundance of filter-feeders in this facies is also noteworthy, contrasting with the results of Bustamante *et al.* (2014), who detected a significant correlation between basal seaweeds and a functional group formed by detritivores, while the canopy layer of the vegetation (corresponding, among other algae, with *Cystoseira* species) was strongly correlated with the abundance of suspension feeders.

The dominant species of the assemblages were also different, although this difference was not significant

probably due to the great dispersion of the abundance values. Some species achieved clearly larger relative abundances in the turf algae, as was the case for *F. stellaris* (relative abundance in *Ellisolandia* facies  $22.0 \pm 16.15$  vs  $0.33 \pm 0.82$  in *Cystoseira*) and *A. mediterranea* ( $11.40 \pm 17.57$  vs  $0.55 \pm 0.79$ ), and this fact clearly caused the higher proportion of filter-feeders in this assemblage, as the first two species belong to this guild. *Platynereis dumerilii* was also more abundant in this facies, although the difference was not significant ( $27.18 \pm 24.22$  vs  $20.02 \pm 6.67$ ). In turn, *S. prolifera* (relative abundance in *Ellisolandia* facies  $7.30 \pm 4.26$  vs  $22.66 \pm 4.61$  in *Cystoseira*) and *S. clavata* ( $10.98 \pm 8.73$  vs  $33.57 \pm 7.41$ ) were more abundant in *C. mediterranea*. Regarding the ecological tolerance of these species according to AMBI classification (Borja *et al.*, 2000; 2008), the set of species characterizing the assemblage in *E. elongata* seems only slightly better adapted to mildly disturbed areas, since *F. stellaris* belongs to group II and *P. dumerilii* to group III (although *A. mediterranea* belongs to the least tolerant group I). The two characteristic species of *C. mediterranea* belong to group II, while no species belonging to group III was important in this assemblage.

Some studies have found evidence that different macrophyte species supported different assemblages of mobile epifauna (Chemello & Milazzo, 2002; Pinedo *et al.*, 2007), and they were more diverse when more structurally complex algal species were involved (Vazquez-Luis *et al.*, 2008; Araújo *et al.*, 2016). However, the question to what extent the species identity of dominant alga is a determinant driver has been subject of debate for the last 20 years, and some findings have pointed out that depth and hydrodynamic condition might be more important than the exact nature of the phytal species (López & Viéitez, 1999; Giangrande *et al.*, 2003). Our results point out that in the studied area, it is not possible to consider the polychaete assemblages inhabiting *E. elongata* dominated areas as a different community to those found in *C. mediterranea*. Species composition of polychaete faunas showed some differences related to the effect of site, as both two-way PERMANOVA and nMDS plot indicated, in accordance with the results of Berthelsen *et al.* (2015), who demonstrated that in some intertidal environments, faunal abundance and richness were strongly structured by site-related physical factors.

Canopy macroalgae are important components of assemblages of rocky shores, providing habitat for several species of animals and plants and preventing the establishment of other sessile organisms (Benedetti-Cecchi *et al.*, 2003). Bustamante *et al.* (2014) found that the habitat provided by the canopy-forming macroalgae enhanced the abundance of invertebrates. Bertocci *et al.* (2010) indicated that the loss of macroalgal canopies caused drastic changes in the species richness of associated assemblages, and several studies (Benedetti-Cecchi *et al.*, 2001; Bulleri *et al.*, 2002) showed that the removal of

*Cystoseira* resulted in the monopolization of low-shore habitats by turf-forming species and in a drastic loss of diversity in the northwestern Mediterranean (Mangialajo *et al.*, 2007). Some authors inferred this loss to be caused by the structure of turf algae, which are less three-dimensionally complex (Bulleri *et al.*, 2002; Asnaghi *et al.*, 2015). However, our study did not find polychaete assemblages inhabiting turf-forming algae to be an impoverished version of those found in the canopy of pristine locations. The comparison of the ecological descriptors showed at the most a slight reduction (statistically non-significant) in  $\alpha$ -diversity-related indices, but abundance was higher in *E. elongata*. This result is in accordance with that of Frascchetti *et al.* (2002), who found that *Cystoseira amenatacea* in Italy was not particularly favorable for the establishment of polychaetes. On the other hand, turf-forming algae make up a complex matrix that effectively secures space and contributes to ecological stability (Asnaghi *et al.*, 2015). Additionally, algal turfs can provide physical protection from dislocations due to waves and from desiccation in littoral environments and create an environment in which sediment (Airoldi & Virgilio, 1998) and organic matter (Bustamante *et al.*, 2014) tend to accumulate, favoring the establishment of polychaetes, which are especially tolerant to organic enrichment (Dafforn *et al.*, 2013). Moreover, the accumulation of sediment might be the cause of the high population density of *F. stellaris*, a species that has been related to high sedimentation rates (Cardell, 1990; Dubois *et al.*, 2002).

Thus, it can be concluded that turf-forming algae can act as effective refuges for the original polychaete faunas in the Mediterranean Sea; this supports previous research suggesting that calcareous algae are particularly suitable as polychaete habitats (Tena *et al.*, 2000; Dorgham *et al.*, 2014). Taking into account that polychaetes are especially pre-adapted to substrate or algal cover disturbance and that many of them can withstand even high levels of perturbation (Dauvin & Ruellet, 2007), the consideration of the slow-growing, shade-adapted coralline algae as moderately tolerant community (Orfanidis *et al.*, 2011) seems justified. A comparison of the polychaete assemblages inhabiting *E. elongata* turfs with those found in filamentous, sun-adapted algae, which the same authors consider indicators of highly disturbed environments, as well as the role of coralline algae as refuge for less tolerant animal groups (i.e. Amphipoda) would be interesting fields for future research.

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