

# Seagrass amphipod assemblages in a Mediterranean marine protected area: a multiscale approach

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**ABSTRACT:** Marine protected areas (MPAs) are a key tool for conservation purposes, but few studies have assessed the responses of small macrozoobenthic assemblages to different protection levels in the Mediterranean Sea. In this study, we used a hierarchical sampling design spanning 3 orders of magnitude (1, 10 and 100 m) to investigate whether a MPA exerts an effect on amphipod assemblages associated with *Posidonia oceanica* meadows. We report spatial and temporal variability patterns of amphipod assemblages in 4 different protection levels and discuss potential confounding effects, such as habitat features. The structure of amphipod assemblages based on density data was patchy at all spatial scales investigated, but differed markedly among protection levels. Among outstanding points, multiscale analyses showed that lower densities and/or biomasses of several taxa occurred within fully protected and external areas, in comparison with partially protected areas (PPAs). Furthermore, *P. oceanica* meadow features (shoot density, leaf and epiphyte biomasses, coefficient A and litter biomass) accounted for only a low proportion of the total variability. We consequently infer that the observed patchiness is likely to occur for multiple and interconnected reasons, ranging from the ecological and behavioural traits of amphipod species to protection-dependent processes (e.g. fish predation). Long-term multiscale spatial and temporal monitoring, as well as experimental manipulations, are needed to fully understand the effects of protection on macrozoobenthic assemblages.

**KEY WORDS:** Amphipod assemblages · *Posidonia oceanica* · Seagrass · Hierarchical sampling design · Marine protected area

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## INTRODUCTION

Human activities exert strong pressures on marine coastal ecosystems (Crain et al. 2009). Over the past 30 yr, marine protected areas (MPAs) have become increasingly common worldwide, and are now among the most commonly utilised tools for the conservation of ecosystems and the management of fisheries (Agardy 1994, Lubchenco et al. 2003, Claudet 2011). MPAs have the potential to directly influence the density, biomass, size and richness of commercial species, such as fish and large mollusks (Halpern 2003, Lester

et al. 2009, Claudet et al. 2011), but may also indirectly change the whole structure of biological assemblages and thus the functioning of ecosystems (Pinnegar et al. 2000, Micheli et al. 2005, Guidetti 2006). For instance, the recovery of exploited fish species in MPAs can lead to declines in other species (Micheli et al. 2004), through trophic or competitive interactions (Claudet et al. 2011). Fish predation may be higher in MPAs, regulating the abundance of many benthic invertebrates such as gastropods (McClanahan 1990), bivalves (Langlois et al. 2005) and sea urchins (McClanahan 1995, Sala & Zabala 1996, Guidetti 2006).

The ways in which macrozoobenthic species respond to protection measures have been studied in different ecosystems around the world and remain somewhat unpredictable. In a Chilean marine reserve, the short-term cessation of fishing activities resulted in a 2- to 3-fold increase in abundance of a dominant gastropod, leading to the decline of its major prey and its replacement by other benthic communities (Moreno et al. 1984, 1986). Conversely, in Kenyan reefs protected from fishing, densities of large gastropods remained virtually identical both inside and outside the protected area (McClanahan 1989). In a large-scale study of Kenyan lagoons, McClanahan (1990) suggested that the removal of fish predators through fishing resulted in an increase in population densities of benthic invertebrates.

Few studies have specifically addressed the effects of protection on macrozoobenthic species in the Mediterranean Sea, especially on small vagile species (e.g. amphipods, small mollusks), despite their importance as food sources for fish (Bell & Harmelin-Vivien 1983). At the Scandola MPA (France), large macrozoobenthic organisms (mainly echinoderms and mollusks) showed lower abundance and species numbers within the MPA in comparison with outside reference sites (Boudouresque et al. 1992). In contrast, at the Ustica MPA (Italy), for both polychaetes and mollusks, abundances and number of species were higher within the fully protected sites compared to the outside fishing area (Badalamenti et al. 1999, Chemello et al. 1999, Milazzo et al. 2000). However, all these results should be interpreted with caution because of the low number of sites studied and the limited spatial and temporal scales investigated. The adopted sampling designs may make it impossible to distinguish between the natural variability of ecosystems and the potential variability induced by protection (Allison et al. 1998).

Natural heterogeneity of ecosystems is a major problem in the assessment of the ecological effectiveness of MPAs (Kolasa & Pickett 1991, García-Charton et al. 2000). The magnitude of this natural variability should be quantified at each scale of observation using a multiscale hierarchical approach (García-Charton & Pérez Ruzafa 1999). Characterization of heterogeneity and exploration of the relationships between species and habitat features are now considered key points to address when designing field experiments that aim to measure the potential effects of protection (García-Charton & Pérez Ruzafa 1999, García-Charton et al. 2000). Indeed, a variety of abiotic and biotic factors (e.g. habitat features) may explain an important part of the variability in some

populations, especially in heterogeneous ecosystems such as *Posidonia oceanica* seagrass meadows (Gobert et al. 2003).

*P. oceanica* forms dense meadows, which are key ecosystems in the Mediterranean sublittoral zone (Boudouresque et al. 2006, Gobert et al. 2006). These meadows play a major role in primary production and sediment stabilization, and provide a physical habitat for fish and invertebrate assemblages (Mazzella et al. 1989, Hemminga & Duarte 2000). This species is legally protected in many Mediterranean countries in accordance with European Commission Habitats Directive 92/43/EEC (Platini 2000, Boudouresque et al. 2006) and numerous MPAs include large areas of *P. oceanica* meadows (Francour et al. 2001). Despite their ecological importance, the effects of protection on small macrozoobenthic species associated with *P. oceanica* meadows have not yet been studied.

The amphipod fauna is an important constituent of *P. oceanica* meadow vagile invertebrates, comprising a large number of species with high abundance and biomass (Mazzella et al. 1989, Gambi et al. 1992). As a food source for decapod and fish populations, amphipods play a major role in the transfer of energy towards higher trophic levels (Bell & Harmelin-Vivien 1983, Pinnegar & Polunin 2000). Moreover, these organisms have already been used in the monitoring of environmental impacts in *P. oceanica* meadows (Sánchez-Jerez & Ramos-Esplá 1996, Sánchez-Jerez et al. 2000) and are generally considered, as a group, to be sensitive to a variety of pollutants (Conlan 1994, Dauvin & Ruellet 2007, De-la-Ossa-Carretero et al. 2012). Amphipods generally have low dispersal ability (Dauvin 1987). They recruit heavily and grow quickly (Bellan-Santini 1998) compared to other organisms such as corals, seagrasses and many sponges which are long-lived, slow-growing and poorly recruiting taxa. Amphipods could provide an important view of the changing communities within MPAs (Palumbi 2001).

Previous studies have investigated the potential importance of temperate seagrass features on the distribution of small macrozoobenthic organisms (Edgar & Robertson 1992, Connolly 1995, Attrill et al. 2000), including amphipod crustaceans (Sánchez-Jerez et al. 2000, Como et al. 2008, González et al. 2008). Evidence from both experimental data and field sampling strongly suggests that the density of several amphipod species correlates to the biomass of seagrasses (Attrill et al. 2000, González et al. 2008), epiphytes (Schneider & Mann 1991, Zakhama-Sraieb et al. 2011) and litter (Sánchez-Jerez et al.

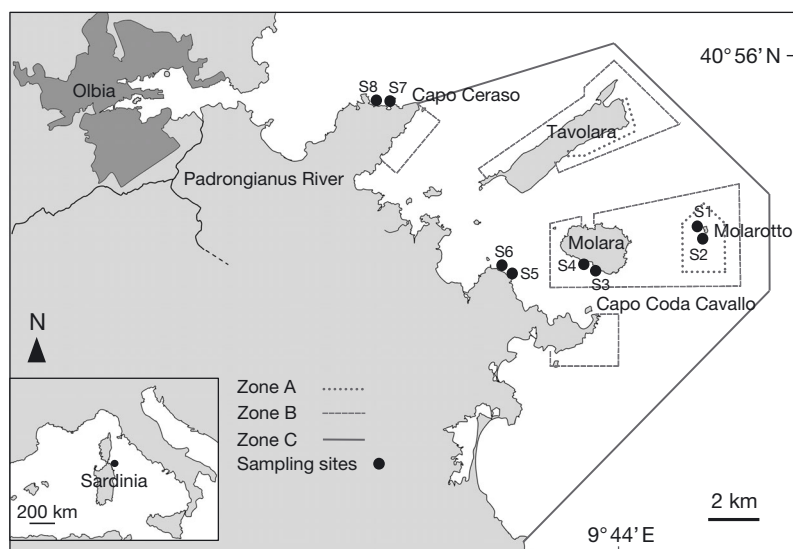


Fig 1. Location and zoning of the Tavolarara-Punta Coda Cavallo Marine Protected Area (TMPA) in Sardinia, Mediterranean Sea. Zone A: integral reserve (fully protected area); Zone B: partial reserve; Zone C: general reserve (partially protected areas). Locations of sampling sites in the 3 zones (S1 to S6) and in the External Zone (S7 and S8) outside the MPA

2000, Como et al. 2008). However, amphipod-seagrass relationships in *P. oceanica* are not yet well understood.

This study aimed to assess the effects of differing levels of protection in a Mediterranean MPA on amphipod assemblages living in *P. oceanica* meadows. To this end, we investigated the spatial variability of amphipod assemblages living in the seagrass at the different protection levels over 2 consecutive years. Specifically, we used a hierarchical sampling design at different spatial scales (1, 10 and 100 m) to describe the spatial patterns of amphipod density and biomass (total and by species), number of species, and diversity. Also, to elucidate the processes driving those patterns, the relationships between the seagrass habitat features (shoot density, leaf and epiphyte biomasses, coefficient A and litter biomass) and amphipod assemblages were examined.

## MATERIALS AND METHODS

### Study area

This study was carried out at the Tavolarara-Punta Coda Cavallo Marine Protected Area (TMPA) (40°53'N, 09°41'E) located in northeast Sardinia (Italy) (Fig. 1). The TMPA covers 15357 ha, extends along 76 km of coastline, and was established in 1997. Effective enforcement of protection started

there in 2003. The TMPA is zoned to provide 3 levels of protection: Zone A (529 ha) is a non-contiguous fully protected area (FPA) or 'integral reserve', i.e. a no-take/no-access zone. Zone B (3113 ha) is a non-contiguous 'partial reserve' and Zone C (11715 ha) is a 'general reserve'; both these zones are classified as partially protected areas (PPAs). Only scientists, reserve staff and policing authorities are permitted to access Zone A. In Zone B, only local professionals inhabiting the nearby coastal villages are permitted to fish. In Zone C, both professional and recreational fishing are permitted under restricted conditions imposed by the local Consortium Management of the TMPA. The spatial distribution of the major habitat types within the TMPA has previously been mapped by Bianchi & Morri (2006). Outside the TMPA, regulations are set according

to Italian legislation, and are less restrictive compared to the area inside the MPA. For this study, data was also collected from outside the MPA ('External Zone'), at sites located in the Gulf of Olbia, a heavily urbanized area impacted by pollutants originating from point (urban wastewater and industrial outfalls, aquaculture) and diffuse (ships, leisure tourism) sources.

### Sampling design

The sampling was conducted in the *Posidonia oceanica* meadows of Zones A, B, and C, as well as in the External Zone outside the TMPA (Fig. 1). Using a hierarchical sampling design, 2 sites (separated by ~100 m, medium scale) were chosen for each of the 4 levels of protection, and in each site 2 sectors (separated by ~10 m, small scale) were randomly selected. Each sector was delimited by a permanent frame circumscribing an area of 9 m<sup>2</sup>, where 4 replicates separated by ~1 m were sampled at constant depth interval (10 to 15 m) during the daytime (10:00 to 17:00 h local time). This sampling strategy was adopted to reduce bathymetric and nycthemeral variability. The sampling was performed during the summer months of 2 consecutive years (July to August 2007 and 2008), with the exception of the External Zone which was only sampled in 2008, yielding a total of 112 samples.

### Data collection

Amphipod samples were collected by means of SCUBA diving, using an airlift (Bussers et al. 1983, Michel et al. 2010). Each sampling area of the meadow was delimited by a PVC cylinder (height: 48 cm, diameter: 48.5 cm) to prevent the escape of mobile species. A surface of 0.185 m<sup>2</sup> was suctioned continuously for 2 min under constant airflow, collecting the amphipod and other invertebrates in a 0.5 mm mesh bag. Samples were sieved through a 0.5 mm mesh, fixed in a 4% formalin and seawater mix, and then transferred to 70% ethanol.

Amphipods were identified to species level and counted. We estimated the relative abundance and frequency of occurrence ( $f$ ) for each species. We also calculated density (ind. m<sup>-2</sup>) for each species and quantified biomass (mg dry weight [DW] m<sup>-2</sup> after drying at 60°C for 48 h) for species that contributed ≥3% of the total biomass. The amphipod assemblage was characterized according to general descriptors: number of species ( $S$ ), and the Shannon-Wiener diversity index ( $H'$ ) =  $-\sum_i p_i \log_s(p_i)$  where  $p_i$  is the proportion of the total count arising from the  $i^{\text{th}}$  species. Oviparous females were counted for all species.

In order to assess the relationships between amphipod faunal and habitat features, 3 *P. oceanica* shoots were collected in 2008 from each area previously sampled for amphipods (i.e. a total of 192 shoots). The *P. oceanica* meadow was characterized with commonly used descriptors: shoot density (shoots m<sup>-2</sup>; n = 4 per sector); leaf and epiphyte biomasses (g DW shoot<sup>-1</sup>; n = 12 per sector); and coefficient A (percentage of leaves per shoot having alteration marks; n = 12 per sector). In addition, leaf litter material and macrophyte debris were collected from airlift sampling areas and put inside plastic bags. These items were quantified as litter biomass (g DW m<sup>-2</sup>; n = 4 per sector) after drying at 60°C for 96 h.

### Data analyses

#### Univariate analyses

Nested ANOVAs were used to examine the effects of protection, site and sector on (1) amphipod variables, (2) the density of the most common species ( $f \geq 10\%$ ) and (3) the biomass of species accounting for ≥3% of the total amphipod biomass. In order to attain a comparable design between 2007 and 2008, data from the External Zone were excluded from the analysis. The data were analysed using a 3-factor

model: Protection (Pr) (fixed factor with 3 levels, corresponding to the 3 zones of the TMPA); Site (Si) (random and nested in Pr, with 2 levels); and Sector (Se) (random and nested in Si and Pr, with 2 levels). Due to the possible overlap of sampled surfaces in *P. oceanica* meadows during the 2 sampling times, 'time' was not considered as a formal factor to avoid temporal dependence of data (Underwood 1997), and the analyses were therefore conducted separately for each year of sampling. We used variance component analyses to estimate the proportion of random variation associated with each random factor (Searle et al. 1992, Underwood 1997). Seagrass habitat features were also analysed using the 3-factor model, as previously described.

Prior to these analyses, normality and homogeneity of variances were checked using the Kolmogorov-Smirnov and Cochran  $C$  tests respectively. When these assumptions were not encountered, data were transformed by  $\sqrt{(x+1)}$  or  $\log(x+1)$ . Whenever variances remained heterogeneous, untransformed data were analysed, as ANOVA is a robust statistical test and is relatively unaffected by heterogeneity of variances, particularly when experiments are balanced and contain a large number of samples (Underwood 1997). In these cases, special care was taken in the interpretation of results, and to reduce Type I error the significance level of statistics was considered at ≤0.01 (Underwood 1997). When ANOVA indicated a significant difference for the protection factor, the source of difference was identified using post-hoc Bonferroni tests to avoid the inflation of Type I errors due to multiple comparisons (Quinn & Keough 2002). The significance level was set to 0.05 when ANOVA conditions were met.

#### Multivariate analyses

Effects of different protection levels on the structure of amphipod assemblages were analysed as abundance data using a 3-way permutational multivariate analysis of variance (PERMANOVA, Anderson et al. 2008). As we did for the univariate analyses, data from the External Zone were excluded from the set in order to attain a comparable design between 2007 and 2008. In the design, Protection (Pr) was treated as a fixed factor (3 levels), Site (Si) as a random factor (2 levels) nested in Pr, and Sector (Se) as a random factor (2 levels) nested in Si. Analyses were based on Bray-Curtis dissimilarity matrices calculated from square-root transformed abundance data. The data transformation was carried out to balance

the contribution from the rarer species. Analyses were tested using 9999 random permutations of residuals under a reduced model, with appropriate units as required by the design (Anderson & ter Braak 2003). When the number of possible permutable units was not enough to get a reasonable test by permutation, a p-value was obtained using a Monte Carlo test (Anderson & Robinson 2003). A permutational test of multivariate dispersion (PERMDISP, Anderson et al. 2008) was used to test the homogeneity of multivariate dispersions.

To visualize multivariate patterns, non-metric multidimensional scaling (NMDS) ordinations were obtained from Bray-Curtis dissimilarity matrices calculated from square-root transformed abundance data. Due to the high number of total samples ( $n = 96$ ), only the 24 centroids for the combined factor Year  $\times$  Protection  $\times$  Site  $\times$  Sector were visualized. The stress value of NMDS indicated the goodness of fit of the representation of distances between samples in the 2-dimensional space of the ordination plot. A stress value  $<0.2$  is considered to provide a useful ordination. Species that contributed most to the dissimilarity among protection zones were identified using a similarity percentage analysis (SIMPER) (Clarke & Warwick 2001).

Relationships between habitat features, amphipod general descriptors and density of the most common taxa ( $f \geq 10\%$ ), were assessed using multiple linear regressions. Prior to regression analyses, data were checked for collinearity and outliers were detected (and subsequently removed) by carrying out analyses of residuals (Jongman et al. 1995, Graham 2003). The relationships between amphipod assemblages and habitat features were analysed using distance-based linear models (DISTLM) (Legendre & Anderson 1999, McArdle & Anderson 2001). Prior to analysis, the collinearity among explanatory variables was tested. The analysis was based on the Bray-Curtis distance measure after square-root transformation of the abundance data. The 'best' selection procedure, with Akaike's information criterion (AIC) as the selection criterion based on 9999 permutations was used to test habitat variables. DISTLM analysis was repeated using only significant variables ( $p \leq 0.05$ ). A distance-based redundancy analysis (dbRDA) was performed to visualize the fitted model in 2-dimensional space. Unlike NMDS, this analysis is constrained, meaning that the resulting ordination is dependent on both species composition and habitat variables. Univariate and multivariate analyses were performed using STATISTICA 10 and PRIMER 6 & PERMANOVA+ software.

## RESULTS

### Amphipod assemblages

A total of 4512 amphipod specimens belonging to 51 species and 25 families were identified, of which 10 species occurred concomitantly in all protection levels and across sampling years (Table 1). Families Caprellidae (6 species), Aoridae (5 species) and Lysianassidae (5 species) yielded the highest number of species. The 3 most frequent species ( $f \geq 80\%$ ), *Apherusa chiereghinii*, *Aora spinicornis* and *Phthisica marina*, represented 56 and 49% of the total abundance and biomass respectively. Only 4 species were frequent ( $40\% \leq f < 80\%$ ) and 8 species common ( $10\% \leq f < 40\%$ ), while 36 species were occasional ( $f < 10\%$ ). Overall, 9% of the total specimens remained unidentified.

### Multiscale variation

The general descriptors of amphipod assemblages did not differ between protection levels (Table 2, Fig. 2), except in 2007 with regards to diversity ( $p = 0.050$ ). At the suborder level, only the mean density of Gammaridea differed significantly between protection zones in 2007, with lower abundances in Zone A compared to other areas. At the species level, 5 out of 15 frequent and common species ( $f \geq 10\%$ ) showed different mean densities among zones, including 3 Gammaridea and 2 Caprellidea (Table 3). *A. chiereghinii*, *Iphimedia minuta*, *Liljeborgia della-vallei* and *Caprella* sp. (*armata* group) were more abundant within the partially protected areas (Zones B and/or C), while a higher abundance was observed for *Caprella tavolarenensis* in the FPA (Zone A) (Tables 1 & 3, Fig. 3).

At the site scale ( $\sim 100$  m), total amphipod density, total biomass and the number of species were significant in 2008 (Table 2, Fig. 2), accounting for between 0 and 54% of the total variation in Zone A (Fig. 4). The densities of 5 species, comprising 2 Gammaridea (*A. spinicornis* and *Orchomene humilis*) and 3 Caprellidea (*C. acanthifera*, *P. marina* and *Pseudoprotella phasma*), also varied at the site scale in 2008. At the sector scale ( $\sim 10$  m), 5 species showed significant differences in mean densities: 2 Gammaridea in 2007 (*Apolochus neapolitanus* and *A. chiereghinii*) and 3 Caprellidea in 2007 or 2008 (*Caprella* sp. [*armata*-group], *C. tavolarenensis* and *P. marina*). At a smaller scale ( $\sim 1$  m), the observed variability seemed to be very important for species den-



Table 1. Mean density (ind. m<sup>-2</sup>) of amphipod taxa at the 4 zones with different levels of protection (see Fig. 1) at the Tavolara-Punta Coda Cavallo Marine Protected Area (TMPA) in 2007 and 2008 (gaps indicate the species was absent)

|  | Zone A |      | Zone B |      | Zone C |      | Zone Ext |
|--|--------|------|--------|------|--------|------|----------|
|  | 2007   | 2008 | 2007   | 2008 | 2007   | 2008 | 2008     |
| <b>Gammaridea</b>  |        |      |        |      |        |      |          |
| <i>Ampelisca diadema</i> (Costa, 1853)                               |        |      |        |      | •      |      |          |
| <i>Ampelisca rubella</i> (Costa, 1864)                               |        |      |        |      |        |      | •        |
| <i>Ampithoe helleri</i> (Karaman, 1975)                              | •      | •    | •      | •    | •      | •    | •        |
| <i>Ampithoe ramondi</i> (Audouin, 1826)                              |        | •    |        |      | •      |      | •        |
| <i>Aora gracilis</i> (Bate, 1857)                                    | •      |      | •      | •    |        |      | •        |
| <i>Aora spinicornis</i> (Afonso, 1976)                               | •      | •    | •      | •    | •      | •    | •        |
| <i>Apherusa chiereghinii</i> (Giordani-Soika, 1950)                  | •      | •    | •      | •    | •      | •    | •        |
| <i>Apolochus neapolitanus</i> (Della Valle, 1893)                    | •      | •    | •      | •    | •      | •    |          |
| <i>Atylus guttatus</i> (Costa, 1851)                                 |        |      |        | •    |        | •    | •        |
| <i>Atylus vedlomensis</i> (Bate and Westwood, 1862)                  |        |      | •      | •    |        |      | •        |
| <i>Cymadusa crassicornis</i> (Costa, 1853)                           |        |      |        |      | •      |      |          |
| <i>Dexamine spiniventris</i> (Costa, 1853)                           | •      | •    | •      | •    | •      | •    | •        |
| <i>Dexamine spinosa</i> (Montagu, 1813)                              | •      | •    | •      | •    | •      | •    | •        |
| <i>Erichthonius punctatus</i> (Bate, 1857)                           | •      | •    | •      | •    | •      | •    | •        |
| <i>Eusiroides dellavallei</i> (Chevreux, 1899)                       | •      |      | •      |      |        |      |          |
| <i>Gammarella fucicola</i> (Leach, 1814)                             | •      | •    | •      |      | •      |      |          |
| <i>Gammaropsis dentata</i> (Chevreux, 1900)                          |        | •    |        |      | •      | •    | •        |
| <i>Gammaropsis palmata</i> (Stebbing & Robertson, 1891)              |        |      |        | •    |        | •    | •        |
| <i>Gammarus aequicauda</i> (Martynov, 1931)                          |        | •    |        |      |        |      |          |
| <i>Gitana sarsi</i> (Boeck, 1871)                                    |        | •    |        | •    |        | •    |          |
| <i>Guernea coalita</i> (Norman, 1868)                                | •      | •    | •      | •    |        | •    |          |
| <i>Harpinia zavodniki</i> (Karaman, 1987)                            | •      |      |        |      |        |      |          |
| <i>Hyale camptonyx</i> (Heller, 1866)                                |        | •    |        |      |        |      |          |
| <i>Iphimedia minuta</i> (Sars, 1882)                                 | •      | •    | •      | •    | •      | •    | •        |
| <i>Ischyrocerus inexpectatus</i> (Ruffo, 1959)                       | •      |      | •      |      |        | •    |          |
| <i>Lembos websteri</i> (Bate, 1857)                                  |        |      | •      | •    | •      |      | •        |
| <i>Leptocheirus guttatus</i> (Grube, 1864)                           |        |      | •      |      | •      | •    |          |
| <i>Leptocheirus pectinatus</i> (Norman, 1869)                        | •      |      |        |      |        |      |          |
| <i>Leucothoe spinicarpa</i> (Abildgaard, 1789)                       |        | •    |        | •    |        | •    |          |
| <i>Liljeborgia dellavallei</i> (Stebbing, 1906)                      | •      |      | •      | •    | •      | •    |          |
| <i>Lysianassa costae</i> (Milne-Edwards, 1830)                       |        |      |        | •    |        |      |          |
| <i>Lysianassa pilicornis</i> (Heller, 1866)                          | •      |      |        | •    | •      |      |          |
| <i>Lysianassina longicornis</i> (Lucas, 1849)                        | •      |      |        |      | •      |      |          |
| <i>Microdeutopus anomalus</i> (Rathke, 1843)                         |        |      |        |      |        | •    |          |
| <i>Microdeutopus similis</i> (Myers, 1977)                           |        |      |        |      | •      |      |          |
| <i>Nannonyx propinquus</i> (Chevreux, 1911)                          |        |      |        |      |        | •    |          |
| <i>Orchomene humilis</i> (Costa, 1853)                               | •      | •    | •      | •    | •      | •    | •        |
| <i>Peltocoxa gibbosa</i> (Schiecke, 1977)                            |        | •    | •      |      |        |      |          |
| <i>Peltocoxa marioni</i> (Catta, 1875)                               | •      |      |        |      | •      | •    | •        |
| <i>Perioculodes aequimanus</i> (Kossmann, 1880)                      | •      |      |        |      |        |      |          |
| <i>Podocerus variegatus</i> (Leach, 1814)                            |        |      |        |      |        | •    | •        |
| <i>Stenothoe eduardi</i> (Krapp-Schickel, 1976)                      | •      |      |        |      |        |      |          |
| <i>Synchelidium longidigitatum</i> (Ruffo, 1947)                     |        |      |        |      |        | •    | •        |
| <i>Tmetonyx nardonis</i> (Heller, 1866)                              | •      |      | •      |      |        | •    | •        |
| <i>Tritaeta gibbosa</i> (Bate, 1862)                                 |        | •    |        | •    | •      |      |          |
| <b>Caprellidea</b>   |        |      |        |      |        |      |          |
| <i>Caprella acanthifera</i> (Leach, 1814)                            | •      | •    | •      | •    | •      | •    | •        |
| <i>Caprella cf. acanthifera</i>                                      | •      | •    | •      | •    | •      | •    | •        |
| <i>Caprella sp. (armata-group)</i> (see Krapp-Schickel & Vader 1998) |        |      | •      |      | •      | •    |          |
| <i>Caprella tavolarensis</i> (Sturaro & Guerra-García, 2011)         | •      | •    | •      | •    | •      | •    | •        |
| <i>Phtisica marina</i> (Slabber, 1769)                               | •      | •    | •      | •    | •      | •    | •        |
| <i>Pseudolirius kroyeri</i> (Haller, 1897)                           |        |      |        |      |        | •    |          |
| <i>Pseudoprotella phasma</i> (Montagu, 1804)                         | •      | •    | •      | •    | •      | •    | •        |

• < 1    • 1–10    • 11–50    • 51–200 ind. m<sup>-2</sup>

Table 2. Nested ANOVA for amphipod general descriptors (total density, total biomass, number of species, and diversity [Shannon-Wiener  $H'$ ]) in 2007 and 2008. Pr: Protection; Si: site; Se: sector. See Fig. 2 legend for sampling design; Zone Ext (Sites S7 and S8) is excluded from the analysis. Significant ( $p \leq 0.05$ ) values are in **bold**

| Year                  | Source     | df | MS     | F     | p            |
|-----------------------|------------|----|--------|-------|--------------|
| <b>Total density</b>  |            |    |        |       |              |
| 2007                  | Pr         | 2  | 95.51  | 8.97  | 0.054        |
|                       | Si(Pr)     | 3  | 10.65  | 0.56  | 0.660        |
|                       | Se(Si(Pr)) | 6  | 18.96  | 1.79  | 0.130        |
|                       | Residual   | 36 | 10.62  |       |              |
| 2008                  | Pr         | 2  | 52.60  | 0.56  | 0.620        |
|                       | Si(Pr)     | 3  | 93.47  | 11.17 | <b>0.007</b> |
|                       | Se(Si(Pr)) | 6  | 8.37   | 0.36  | 0.897        |
|                       | Residual   | 36 | 23.01  |       |              |
| <b>Total biomass</b>  |            |    |        |       |              |
| 2007                  | Pr         | 2  | 323.07 | 5.09  | 0.109        |
|                       | Si(Pr)     | 3  | 63.51  | 1.97  | 0.221        |
|                       | Se(Si(Pr)) | 6  | 32.30  | 1.12  | 0.373        |
|                       | Residual   | 36 | 28.97  |       |              |
| 2008                  | Pr         | 2  | 18.19  | 0.20  | 0.829        |
|                       | Si(Pr)     | 3  | 91.39  | 16.59 | <b>0.003</b> |
|                       | Se(Si(Pr)) | 6  | 5.51   | 0.25  | 0.956        |
|                       | Residual   | 36 | 22.00  |       |              |
| <b>No. of species</b> |            |    |        |       |              |
| 2007                  | Pr         | 2  | 0.15   | 0.01  | 0.986        |
|                       | Si(Pr)     | 3  | 10.21  | 1.23  | 0.379        |
|                       | Se(Si(Pr)) | 6  | 8.33   | 1.78  | 0.132        |
|                       | Residual   | 36 | 4.69   |       |              |
| 2008                  | Pr         | 2  | 1.56   | 0.06  | 0.941        |
|                       | Si(Pr)     | 3  | 24.96  | 6.31  | <b>0.028</b> |
|                       | Se(Si(Pr)) | 6  | 3.96   | 0.65  | 0.690        |
|                       | Residual   | 36 | 6.10   |       |              |
| <b>Diversity</b>      |            |    |        |       |              |
| 2007                  | Pr         | 2  | 0.28   | 9.53  | <b>0.050</b> |
|                       | Si(Pr)     | 3  | 0.03   | 0.10  | 0.959        |
|                       | Se(Si(Pr)) | 6  | 0.30   | 2.79  | <b>0.025</b> |
|                       | Residual   | 36 | 0.11   |       |              |
| 2008                  | Pr         | 2  | 0.05   | 0.15  | 0.867        |
|                       | Si(Pr)     | 3  | 0.35   | 3.28  | 0.100        |
|                       | Se(Si(Pr)) | 6  | 0.11   | 0.82  | 0.564        |
|                       | Residual   | 36 | 0.13   |       |              |

sities (Fig. 3) and general descriptors accounted for 37 to 100% of the total variation (Fig. 4).

Biomass values showed some contrasting results in comparison to densities (Table 4). Biomass values for *A. chierghinii* and Gammaridea were not found to be significantly affected by protection level, whereas for both taxa differences were found between densities in 2007 and/or 2008. Biomass of *A. spinicornis* was shown to be significantly affected by the protection level in 2007, whereas no differences were found in terms of densities. In 2008,

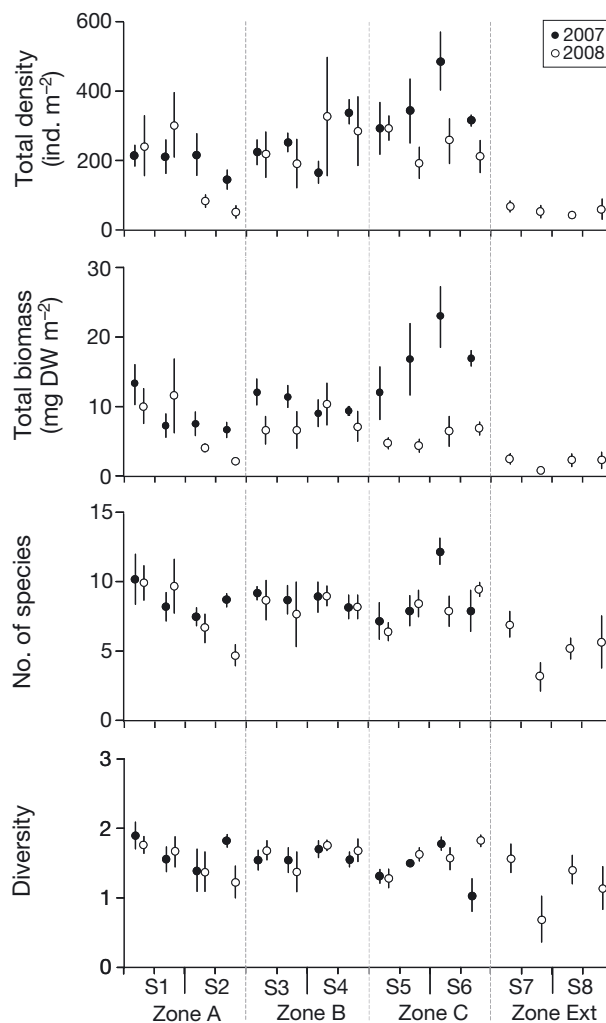


Fig. 2. Values of amphipod general descriptors at Sites S1 to S8, in 4 zones with different levels of protection inside and outside the TMPA (see Fig. 1), in (●) 2007 and (○) 2008. Within each zone, 2 sampling sites were separated by ~100 m (medium scale). Within each site, mean  $\pm$  SE values are shown for 2 sectors ( $n = 4$  replicates per sector) separated by ~10 m (small scale). Descriptors include total amphipod density (ind.  $m^{-2}$ ), total biomass (mg DW  $m^{-2}$ ), number of species per sample, and Shannon-Wiener diversity index ( $H'$ ) per sample. DW: dry weight

biomasses of *P. phasma* and Caprellidea were not found to differ between sites, while Gammaridea exhibited significant spatial heterogeneity at this scale. In 2007, at the sector scale, the abundance of Gammaridea, *A. chierghinii* and Caprellidea remained consistent between sectors, while *C. acanthifera* exhibited significant spatial heterogeneity at this scale.

In contrast to univariate statistical analyses, PERMANOVA evidenced that amphipod assemblage structures differed among protection zones in both

Table 3. Nested ANOVA for the density of the most frequent amphipod taxa (frequency [ $f$ ]  $\geq 10\%$ ) in the TMPA in 2007 and 2008. See Table 2 legend for explanation of abbreviations. Significant ( $p < 0.05$ ) values are in **bold**

| Year               | Source     | df | MS                            | F     | p                  | MS                            | F     | p                | MS                                 | F     | p            |
|--------------------|------------|----|-------------------------------|-------|--------------------|-------------------------------|-------|------------------|------------------------------------|-------|--------------|
| <b>Gammaridae</b>  |            |    |                               |       |                    |                               |       |                  |                                    |       |              |
|                    |            |    | <b>Gammaridea</b>             |       |                    | <b>Apolochus neapolitanus</b> |       |                  | <b>Ampithoe helleri</b>            |       |              |
| 2007               | Pr         | 2  | 155.30                        | 14.63 | <b>0.028</b>       | 1.19                          | 0.33  | 0.745            | 0.61                               | 0.50  | 0.650        |
|                    | Si(Pr)     | 3  | 10.62                         | 0.63  | 0.623              | 3.65                          | 0.73  | 0.572            | 1.22                               | 0.17  | 0.915        |
|                    | Se(Si(Pr)) | 6  | 16.92                         | 2.62  | <b>0.033</b>       | 5.03                          | 4.04  | <b>0.003</b>     | 7.32                               | 1.29  | 0.288        |
|                    | Residual   | 36 | 6.47                          |       |                    | 1.24                          |       |                  | 5.70                               |       |              |
| 2008               | Pr         | 2  | 28216                         | 2.06  | 0.273              | 78.74                         | 2.87  | 0.201            | 9.77                               | 2.00  | 0.281        |
|                    | Si(Pr)     | 3  | 13673                         | 9.24  | 0.011 <sup>a</sup> | 27.47                         | 0.82  | 0.529            | 4.88                               | 0.40  | 0.758        |
|                    | Se(Si(Pr)) | 6  | 1480                          | 0.16  | 0.986              | 33.57                         | 0.57  | 0.748            | 12.21                              | 0.83  | 0.552        |
|                    | Residual   | 36 | 9397                          |       |                    | 58.39                         |       |                  | 14.65                              |       |              |
|                    |            |    | <b>Aora spinicornis</b>       |       |                    | <b>Apherusa chieraghini</b>   |       |                  | <b>Dexamine spiniventris</b>       |       |              |
| 2007               | Pr         | 2  | 27.25                         | 5.83  | 0.093              | 72605                         | 22.01 | <b>0.016</b>     | 4.25                               | 7.00  | 0.074        |
|                    | Si(Pr)     | 3  | 4.68                          | 0.73  | 0.571              | 3299                          | 0.61  | 0.635            | 0.61                               | 0.53  | 0.677        |
|                    | Se(Si(Pr)) | 6  | 6.41                          | 1.86  | 0.114              | 5440                          | 2.73  | <b>0.027</b>     | 1.14                               | 1.06  | 0.403        |
|                    | Residual   | 36 | 3.44                          |       |                    | 1989                          |       |                  | 1.08                               |       |              |
| 2008               | Pr         | 2  | 0.83                          | 0.12  | 0.895              | 15900                         | 29.94 | <b>0.010</b>     | 1.03                               | 4.85  | 0.115        |
|                    | Si(Pr)     | 3  | 7.23                          | 7.34  | <b>0.020</b>       | 531                           | 0.42  | 0.743            | 0.21                               | 0.79  | 0.544        |
|                    | Se(Si(Pr)) | 6  | 0.98                          | 0.61  | 0.717              | 1255                          | 0.53  | 0.781            | 0.27                               | 0.56  | 0.756        |
|                    | Residual   | 36 | 1.60                          |       |                    | 2362                          |       |                  | 0.48                               |       |              |
|                    |            |    | <b>Erichthonius punctatus</b> |       |                    | <b>Iphimedia minuta</b>       |       |                  | <b>Liljeborgia dellavallei</b>     |       |              |
| 2007               | Pr         | 2  | 2.89                          | 1.62  | 0.333              | 90.34                         | 37.00 | <b>0.008</b>     | 115.36                             | 7.27  | 0.071        |
|                    | Si(Pr)     | 3  | 1.78                          | 1.83  | 0.242              | 2.44                          | 0.17  | 0.915            | 15.87                              | 0.45  | 0.728        |
|                    | Se(Si(Pr)) | 6  | 0.98                          | 0.84  | 0.551              | 14.65                         | 0.40  | 0.871            | 35.40                              | 1.43  | 0.232        |
|                    | Residual   | 36 | 1.17                          |       |                    | 36.22                         |       |                  | 24.82                              |       |              |
| 2008               | Pr         | 2  | 6.26                          | 6.20  | 0.086              | 4.67                          | 1.12  | 0.433            | 0.66                               | 13.36 | <b>0.032</b> |
|                    | Si(Pr)     | 3  | 1.01                          | 1.73  | 0.260              | 4.17                          | 2.76  | 0.134            | 0.05                               | 0.11  | 0.952        |
|                    | Se(Si(Pr)) | 6  | 0.58                          | 0.43  | 0.857              | 1.51                          | 1.93  | 0.103            | 0.45                               | 1.41  | 0.239        |
|                    | Residual   | 36 | 1.37                          |       |                    | 0.78                          |       |                  | 0.32                               |       |              |
|                    |            |    | <b>Orchomene humilis</b>      |       |                    | <b>Peltocoxa marioni</b>      |       |                  |                                    |       |              |
| 2007               | Pr         | 2  | 0.16                          | 0.13  | 0.885              | 12.82                         | 2.33  | 0.245            |                                    |       |              |
|                    | Si(Pr)     | 3  | 1.27                          | 4.46  | 0.057              | 5.49                          | 0.36  | 0.784            |                                    |       |              |
|                    | Se(Si(Pr)) | 6  | 0.28                          | 0.43  | 0.855              | 15.26                         | 2.14  | 0.072            |                                    |       |              |
|                    | Residual   | 36 | 0.66                          |       |                    | 7.12                          |       |                  |                                    |       |              |
| 2008               | Pr         | 2  | 4.21                          | 2.82  | 0.205              | 21.97                         | 9.00  | 0.054            |                                    |       |              |
|                    | Si(Pr)     | 3  | 1.50                          | 13.03 | <b>0.005</b>       | 2.44                          | 0.50  | 0.696            |                                    |       |              |
|                    | Se(Si(Pr)) | 6  | 0.11                          | 0.07  | 0.999              | 4.88                          | 1.33  | 0.268            |                                    |       |              |
|                    | Residual   | 36 | 1.71                          |       |                    | 3.66                          |       |                  |                                    |       |              |
| <b>Caprellidae</b> |            |    |                               |       |                    |                               |       |                  |                                    |       |              |
|                    |            |    | <b>Caprellidea</b>            |       |                    | <b>Caprella acanthifera</b>   |       |                  | <b>Caprella sp. (armata-group)</b> |       |              |
| 2007               | Pr         | 2  | 0.09                          | 0.10  | 0.906              | 35.22                         | 7.34  | 0.070            | 9.35                               | 15.14 | <b>0.027</b> |
|                    | Si(Pr)     | 3  | 0.86                          | 0.43  | 0.742              | 4.80                          | 3.34  | 0.097            | 0.62                               | 0.25  | 0.858        |
|                    | Se(Si(Pr)) | 6  | 2.03                          | 4.30  | <b>0.002</b>       | 1.43                          | 1.50  | 0.207            | 2.46                               | 3.65  | <b>0.006</b> |
|                    | Residual   | 36 | 0.47                          |       |                    | 0.96                          |       |                  | 0.67                               |       |              |
| 2008               | Pr         | 2  | 413                           | 0.02  | 0.982              | 8.93                          | 0.90  | 0.494            | 119.64                             | 12.25 | <b>0.036</b> |
|                    | Si(Pr)     | 3  | 22679                         | 7.08  | <b>0.021</b>       | 9.94                          | 25.46 | <b>0.001</b>     | 9.77                               | 0.28  | 0.841        |
|                    | Se(Si(Pr)) | 6  | 3205                          | 0.55  | 0.769              | 0.39                          | 0.25  | 0.956            | 35.40                              | 0.92  | 0.495        |
|                    | Residual   | 36 | 5865                          |       |                    | 1.57                          |       |                  | 38.66                              |       |              |
|                    |            |    | <b>Caprella tavolarensis</b>  |       |                    | <b>Phtisica marina</b>        |       |                  | <b>Pseudoprotella phasma</b>       |       |              |
| 2007               | Pr         | 2  | 49.22                         | 43.78 | <b>0.006</b>       | 1963                          | 2.08  | 0.271            | 2.41                               | 1.81  | 0.306        |
|                    | Si(Pr)     | 3  | 1.12                          | 0.73  | 0.570              | 944                           | 0.56  | 0.663            | 1.33                               | 1.13  | 0.410        |
|                    | Se(Si(Pr)) | 6  | 1.54                          | 1.07  | 0.396              | 1696                          | 7.66  | <b>&lt;0.001</b> | 1.19                               | 1.09  | 0.386        |
|                    | Residual   | 36 | 1.43                          |       |                    | 222                           |       |                  | 1.09                               |       |              |
| 2008               | Pr         | 2  | 26.88                         | 12.27 | <b>0.036</b>       | 1055                          | 0.12  | 0.895            | 2.05                               | 0.17  | 0.853        |
|                    | Si(Pr)     | 3  | 2.19                          | 0.68  | 0.597              | 9169                          | 18.99 | <b>0.002</b>     | 12.23                              | 6.97  | <b>0.022</b> |
|                    | Se(Si(Pr)) | 6  | 3.23                          | 6.29  | <b>&lt;0.001</b>   | 483                           | 0.26  | 0.951            | 1.75                               | 0.74  | 0.620        |
|                    | Residual   | 36 | 0.51                          |       |                    | 1841                          |       |                  | 2.37                               |       |              |

<sup>a</sup>Not significant at  $\alpha = 0.01$ ; this conservative level of significance was adopted because variances were heterogeneous and could not be stabilized by transformations



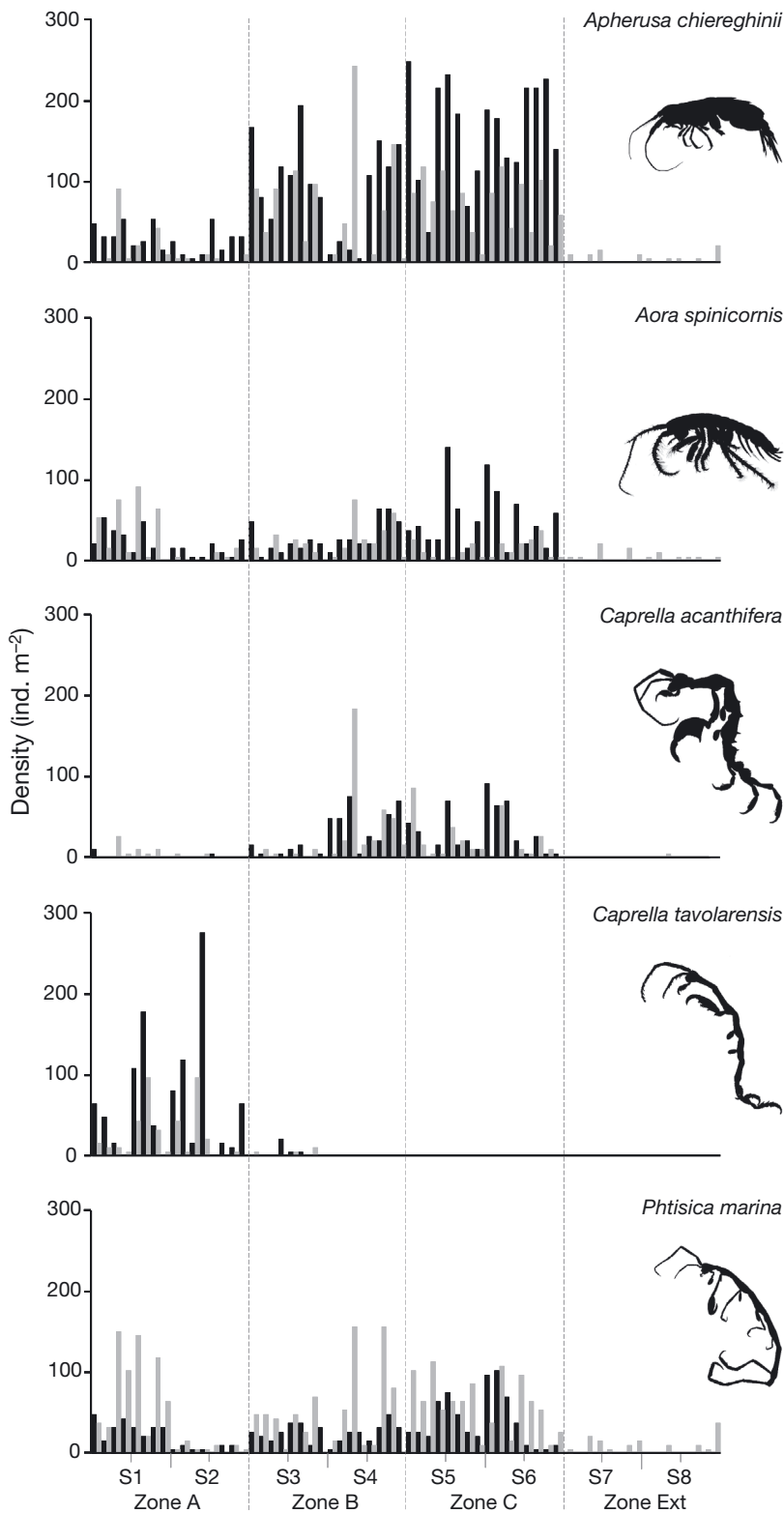


Fig. 3. Density values (ind. m<sup>-2</sup>) of the amphipod species that contributed most to the dissimilarity among the different protection levels of the 4 zones at the TMPA in 2007 (black) and 2008 (grey). Bars show the values for each replicate sample (see Fig. 2 legend for sampling design)

2007 ( $p = 0.005$ ) and 2008 ( $p = 0.020$ ) (Table 5). Pairwise comparisons showed significant differences between Zone A versus Zone B in 2007 ( $p = 0.049$ ) and Zone C in both 2007 ( $p = 0.007$ ) and 2008 ( $p = 0.031$ ). Results indicated that the sector data varied strongly in 2007 ( $p < 0.001$ ), while the site data varied in 2008 ( $p = 0.026$ ). The PERMDISP test revealed strongly significant dispersion effects across protection zones in 2007 ( $p = 0.007$ ) and 2008 ( $p = 0.011$ ), with greater variability in the structure of amphipod assemblages observed in Zone A versus Zone C for both years ( $p < 0.002$ ). General patterns of segregation showed by NMDS plots reflected the results of the PERMANOVA and PERMDISP (Fig. 5). Groups of centroids belonging to each protection level were well separated, particularly centroids of Zone A versus Zones B and C for both years. In addition, centroids from Zone C were tightly grouped, whereas those from Zone A were dispersed, suggesting that amphipod assemblages in Zone C were more homogenous than in Zone A.

The results of the SIMPER analysis performed on species abundance showed that between-zone dissimilarities were greater in 2008 (51.8 to 73.9) than in 2007 (39.2 to 59.1) (Table 6). In 2007, *A. chiereghinii* and *C. acanthifera* were the main contributors to dissimilarity among protection levels. *C. tavolarensis* and *A. spinicornis* were also important for explaining the dissimilarity between Zone A versus Zones B and C, and Zone B versus Zone C, respectively. In 2008, species that contributed the most to dissimilarity among protection zones were *A. chiereghinii* and *P. marina*. *C. tavolarensis* contributed to the differences between Zone A versus the other zones (including Zone Ext), while *C. acanthifera* played a significant role in the differences observed between Zones B, C and the External Zone outside the MPA.

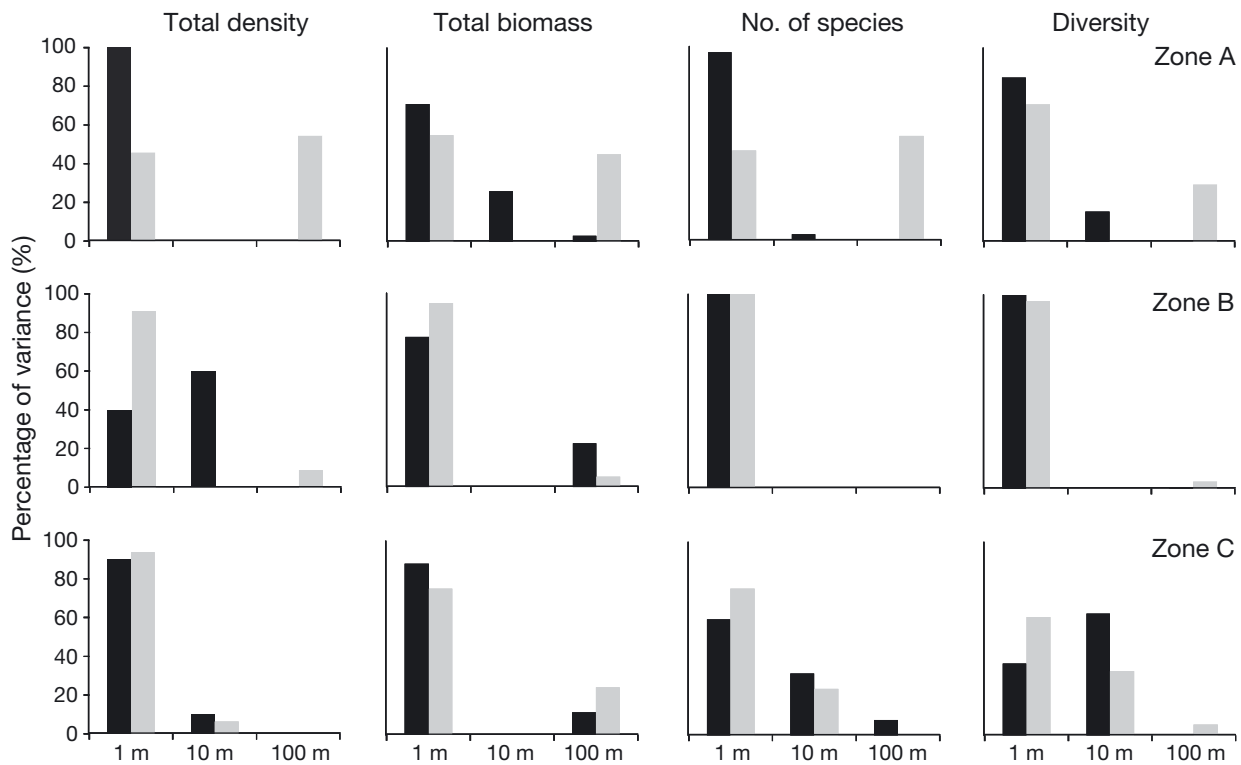


Fig. 4. Variance component analyses conducted on amphipod general descriptors in 2007 (black) and 2008 (grey), to estimate the variation among spatial scales (replicate: ~1 m; sector: ~10 m; site: ~100 m) in 3 zones in the TMPA with different levels of protection (see Fig. 1)

### Spatial variation in meadow features

No significant differences in shoot density, leaf or epiphyte biomasses were detected between protection levels. Conversely, litter biomass showed significantly higher mean values in Zone B compared to other zones, while coefficient A decreased from Zone A to Zone C and the External Zone (Fig. 6, Table 7). Shoot density and coefficient A varied significantly at sector scale (~10 m). No differences were found for any of the measured variables at site scale (~100 m).

### Influence of meadow features on amphipod assemblages

Multiple regression analyses yielded the detection of only weakly significant relationships between amphipod and habitat variables (Table 8). The number of species and diversity appeared to be unaffected by the measured habitat variables. In contrast, amphipod total density tended to be negatively correlated to leaf biomass, while amphipod total biomass correlated positively with litter biomass. The combination of habitat features accounted for between 0 and 15%

of the variation in amphipod species densities. Litter biomass appeared to have a greater influence on gammarids, while the biomass of epiphytes were more related to caprellids (Table 8).

Results from the DISTLM were consistent with those obtained from multiple regressions, indicating very weak relationships between amphipod assemblages and the habitat variables measured in this study (Fig. 7). The most parsimonious model contained 2 of the 5 measured habitat variables and explained a total of only 7.5% of the variation in the distribution and abundance of amphipods. The first dbRDA axis accounted for 4.8% of the total variation in the amphipod assemblage and allowed discriminating samples based on litter biomass. Higher values of litter biomass were associated with Zone B than with the other zones. The second dbRDA axis accounted for 2.7% of the variation in the amphipod assemblage and discriminated samples based on *Posidonia oceanica* shoot densities. This last variable was not clearly associated with a particular protection zone, but with a higher variability among the samples within Zone A. Only a few amphipod species appeared to be influenced by measured habitat variables. Some species, such as *Dexamine spiniventris*,

Table 4. Nested ANOVA for biomass of the most important taxa in terms of relative biomass ( $\geq 3\%$ ). See Table 2 legend for explanation of abbreviations. Significant ( $p < 0.05$ ) values are in **bold**

| Year                         | Source     | df | MS    | F     | p                  | MS    | F      | p                  | MS     | F    | p                  |
|------------------------------|------------|----|-------|-------|--------------------|-------|--------|--------------------|--------|------|--------------------|
| <b>Gammaridea</b>            |            |    |       |       |                    |       |        |                    |        |      |                    |
| 2007                         | Pr         | 2  | 4.62  | 3.11  | 0.186              | 21.97 | 106.72 | <b>0.002</b>       | 107.73 | 8.22 | 0.061              |
|                              | Si(Pr)     | 3  | 1.48  | 2.52  | 0.155              | 0.21  | 0.03   | 0.993              | 13.11  | 1.51 | 0.305              |
|                              | Se(Si(Pr)) | 6  | 0.59  | 1.36  | 0.258              | 7.44  | 2.78   | 0.025 <sup>a</sup> | 8.69   | 2.88 | 0.021 <sup>a</sup> |
|                              | Residual   | 36 | 0.43  |       |                    | 2.67  |        |                    | 3.01   |      |                    |
| 2008                         | Pr         | 2  | 10.65 | 0.29  | 0.765              | 3.49  | 0.42   | 0.689              | 0.68   | 6.09 | 0.088              |
|                              | Si(Pr)     | 3  | 36.34 | 41.35 | <b>&lt;0.001</b>   | 8.26  | 29.76  | <b>0.001</b>       | 0.11   | 2.67 | 0.141              |
|                              | Se(Si(Pr)) | 6  | 0.88  | 0.08  | 0.998              | 0.28  | 0.18   | 0.979              | 0.04   | 0.35 | 0.907              |
|                              | Residual   | 36 | 10.97 |       |                    | 1.51  |        |                    | 0.12   |      |                    |
| <b>Aora spinicornis</b>      |            |    |       |       |                    |       |        |                    |        |      |                    |
| <b>Apherusa chiereghinii</b> |            |    |       |       |                    |       |        |                    |        |      |                    |
| <b>Dexamine spiniventris</b> |            |    |       |       |                    |       |        |                    |        |      |                    |
| <b>Caprellidea</b>           |            |    |       |       |                    |       |        |                    |        |      |                    |
| <b>Caprella acanthifera</b>  |            |    |       |       |                    |       |        |                    |        |      |                    |
| 2007                         | Pr         | 2  | 0.73  | 4.34  | 0.130              | 0.25  | 1.38   | 0.376              | 1.08   | 4.71 | 0.119              |
|                              | Si(Pr)     | 3  | 0.17  | 0.26  | 0.854              | 0.18  | 0.43   | 0.742              | 0.23   | 1.48 | 0.312              |
|                              | Se(Si(Pr)) | 6  | 0.66  | 1.56  | 0.186              | 0.43  | 1.75   | 0.137              | 0.15   | 2.63 | <b>0.032</b>       |
|                              | Residual   | 36 | 0.42  |       |                    | 0.25  |        |                    | 0.06   |      |                    |
| 2008                         | Pr         | 2  | 0.93  | 4.60  | 0.122              | 1.58  | 0.12   | 0.889              | 0.39   | 1.15 | 0.426              |
|                              | Si(Pr)     | 3  | 0.20  | 0.53  | 0.675              | 12.97 | 3.12   | 0.109              | 0.34   | 7.24 | <b>0.020</b>       |
|                              | Se(Si(Pr)) | 6  | 0.38  | 1.60  | 0.176              | 4.15  | 1.28   | 0.291              | 0.05   | 0.37 | 0.894              |
|                              | Residual   | 36 | 0.24  |       |                    | 3.24  |        |                    | 0.13   |      |                    |
| <b>Caprella tavolarensis</b> |            |    |       |       |                    |       |        |                    |        |      |                    |
| <b>Phtisica marina</b>       |            |    |       |       |                    |       |        |                    |        |      |                    |
| <b>Pseudoprotella phasma</b> |            |    |       |       |                    |       |        |                    |        |      |                    |
| 2007                         | Pr         | 2  | 8.73  | 58.41 | <b>0.004</b>       | 0.16  | 0.24   | 0.801              | 0.64   | 0.53 | 0.636              |
|                              | Si(Pr)     | 3  | 0.15  | 0.09  | 0.963              | 0.67  | 2.53   | 0.154              | 1.21   | 0.64 | 0.617              |
|                              | Se(Si(Pr)) | 6  | 1.65  | 2.45  | 0.043 <sup>a</sup> | 0.26  | 2.44   | <b>0.044</b>       | 1.90   | 0.98 | 0.453              |
|                              | Residual   | 36 | 0.68  |       |                    | 0.11  |        |                    | 1.94   |      |                    |
| 2008                         | Pr         | 2  | 1.23  | 52.09 | <b>0.005</b>       | 0.05  | 0.09   | 0.918              | 0.51   | 0.49 | 0.653              |
|                              | Si(Pr)     | 3  | 0.02  | 0.06  | 0.977              | 0.55  | 8.29   | <b>0.015</b>       | 1.03   | 2.83 | 0.129              |
|                              | Se(Si(Pr)) | 6  | 0.37  | 3.54  | <b>0.007</b>       | 0.07  | 0.68   | 0.668              | 0.36   | 1.27 | 0.297              |
|                              | Residual   | 36 | 0.11  |       |                    | 0.10  |        |                    | 0.29   |      |                    |

<sup>a</sup>Not significant at  $\alpha = 0.01$ ; this conservative level of significance was adopted because variances were heterogeneous and could not be stabilized by transformations

*C. acanthifera* and *Ericthonius punctatus* were associated with high litter biomass. Furthermore, *A. neapolitanus* was associated with high *P. oceanica* shoot density, while *I. minuta* was related to lower shoot density (Fig. 7).

Table 5. PERMANOVA based on Bray-Curtis similarity of amphipod assemblages at the TMPA. See Table 2 legend for explanation of abbreviations. Significant ( $p < 0.05$ ) values are in **bold**; p-values given in *italics* were obtained using the Monte Carlo test

| Year | Source     | df | MS   | Pseudo-F | p                |
|------|------------|----|------|----------|------------------|
| 2007 | Pr         | 2  | 8526 | 4.13     | <b>0.005</b>     |
|      | Si(Pr)     | 3  | 2063 | 1.41     | 0.156            |
|      | Se(Si(Pr)) | 6  | 1468 | 2.05     | <b>&lt;0.001</b> |
|      | Residual   | 36 | 716  |          |                  |
| 2008 | Pr         | 2  | 7913 | 2.81     | <b>0.020</b>     |
|      | Si(Pr)     | 3  | 2815 | 1.93     | <b>0.026</b>     |
|      | Se(Si(Pr)) | 6  | 1456 | 1.02     | 0.441            |
|      | Residual   | 36 | 1426 |          |                  |

## DISCUSSION

This study demonstrated amphipod assemblage structure to be heterogeneous at all spatial scales, from metres to hundreds of metres, and to differ markedly among protection levels. In particular, the assemblage structures clearly differed between the FPA and the PPAs. Our data for the FPA also showed a reduced density and/or biomass of several taxa compared to other areas. Seagrass features only explained a small part of the variation among amphipods.

### The effect of protection

These new findings on amphipod assemblage structures are in accordance with those of previous studies conducted on mollusks at the Ustica MPA (Milazzo et al. 2000). Moreover, some studies conducted on mollusks and echinoderms (McClanahan



Fig. 5. Non-metric multidimensional scaling (NMDS) ordination of amphipod assemblages in the TMPA. Plot triangles indicate sector centroids, coded by zone (see Fig. 1) and year

1989, Boudouresque et al. 1992) showed that density and biomass can be affected by protection-dependent effects such as the rate of fish predation. An increase in abundance and/or biomass of fish predators, coupled with changes in fish assemblages within a FPA, may indeed decrease the density of prey species (Guidetti 2006, Claudet et al. 2011). In the sublittoral rocky habitats of the TMPA, previous studies have shown fish biomass (including amphipod feeders such as *Sciaena umbra* and *Diplodus sargus*) to be up to 3 times greater inside the FPA as a result of protection measures (Di Franco et al. 2009, Sala et al. 2012, Sahyoun et al. 2013). Therefore, census surveys of fish assemblages in *Posidonia oceanica* meadows, combined with an experimental

manipulation of fish predation intensity (i.e. exclusion and inclusion cages) would be needed to test the hypothesis regarding fish predation at the TMPA.

Evaluation of the effectiveness of marine protection on benthic assemblages can produce contrasting results. Our general descriptors of amphipod assemblages did not reveal any significant differences among protection levels, with the exception of diversity in 2007. At the Ustica MPA (Italy), in a study focused on mollusks and polychaetes, the abundance and number of species were found to be greater inside the FPA than in PPAs (Milazzo et al. 2000, Badalamenti et al. 1999). In contrast, at El Campello (Spain), the number of species and diversity of different crustacean taxa (including amphipods), showed similar values between control and disturbed *P. oceanica* meadows, although assemblage structures were different (Sánchez-Jerez & Ramos Esplá 1996). It is possible that functional redundancy occurs and that different species occupy the same functional role (Micheli & Halpern 2005). However, specific analyses (e.g. Claudet et al. 2011), which are beyond the scope of this study, would be required to answer this question.

**The role of meadow features**

Previous studies have demonstrated that seagrass features are among the factors likely to explain the variability of amphipod assemblages, at least for some species and general descriptors of assemblages. A substantial part of the variation in both the densities of amphipods and the assemblage structures has previously been attributed to leaf density and litter biomass, as well as to epiphyte cover and

Table 6. SIMPER routine to analyse dissimilarity among the 4 zones with different levels of protection (see Fig. 1) at the TMPA in 2007 and 2008. Species are ordered by decreasing contribution.  $\bar{\delta}$ : average dissimilarity between pair of zones;  $\bar{\delta}_i$ : contribution from the  $i^{th}$  species to the average dissimilarity, expressed as a percentage; SD $_i$ : standard deviation; S: species. Ac: *Apherusa chieraghinii*; As: *Aora spinicornis*; Ca: *Caprella acanthifera*; Ct: *Caprella tavolarenis*; Pma: *Phtisica marina*

|                                       | 2007 |                  |                       | 2008                                  |                  |                       | 2008 |  |                       |      |     |
|---------------------------------------|------|------------------|-----------------------|---------------------------------------|------------------|-----------------------|------|--|-----------------------|------|-----|
|                                       | S    | $\bar{\delta}_i$ | $\bar{\delta}_i/SD_i$ | S                                     | $\bar{\delta}_i$ | $\bar{\delta}_i/SD_i$ | S    | $\bar{\delta}_i$                         | $\bar{\delta}_i/SD_i$ |      |     |
| Zone A vs. B<br>$\bar{\delta} = 54.5$ | Ct   | 16.6             | 1.3                   | Zone A vs. B<br>$\bar{\delta} = 64.6$ | Ac               | 12.7                  | 1.5  | Zone A vs. Ext.<br>$\bar{\delta} = 73.9$ | Ct                    | 13.6 | 1.0 |
|                                       | Ac   | 12.5             | 1.7                   |                                       | Pma              | 11.2                  | 1.4  |  | Pma                   | 13.0 | 1.4 |
|                                       | Ca   | 11.0             | 1.5                   |                                       | Ct               | 9.8                   | 1.0  |  | As                    | 9.3  | 1.3 |
| Zone A vs. C<br>$\bar{\delta} = 59.1$ | Ac   | 17.1             | 2.2                   | Zone A vs. C<br>$\bar{\delta} = 62.4$ | Ac               | 17.2                  | 1.7  | Zone B vs. Ext.<br>$\bar{\delta} = 69.8$ | Ac                    | 15.1 | 1.6 |
|                                       | Ct   | 15.1             | 1.3                   |                                       | Pma              | 12.0                  | 1.3  |  | Pma                   | 13.3 | 1.5 |
|                                       | Ca   | 10.4             | 2.0                   |                                       | Ct               | 10.3                  | 1.1  |  | Ca                    | 10.1 | 1.3 |
| Zone B vs. C<br>$\bar{\delta} = 39.2$ | Ac   | 16.1             | 1.2                   | Zone B vs. C<br>$\bar{\delta} = 51.8$ | Ac               | 14.3                  | 1.2  | Zone C vs. Ext.<br>$\bar{\delta} = 69.9$ | Ac                    | 20.9 | 2.1 |
|                                       | Ca   | 10.3             | 1.3                   |                                       | Pma              | 12.1                  | 1.1  |  | Pma                   | 17.0 | 1.7 |
|                                       | As   | 8.5              | 1.4                   |                                       | Ca               | 9.2                   | 1.2  |  | Ca                    | 10.9 | 1.5 |

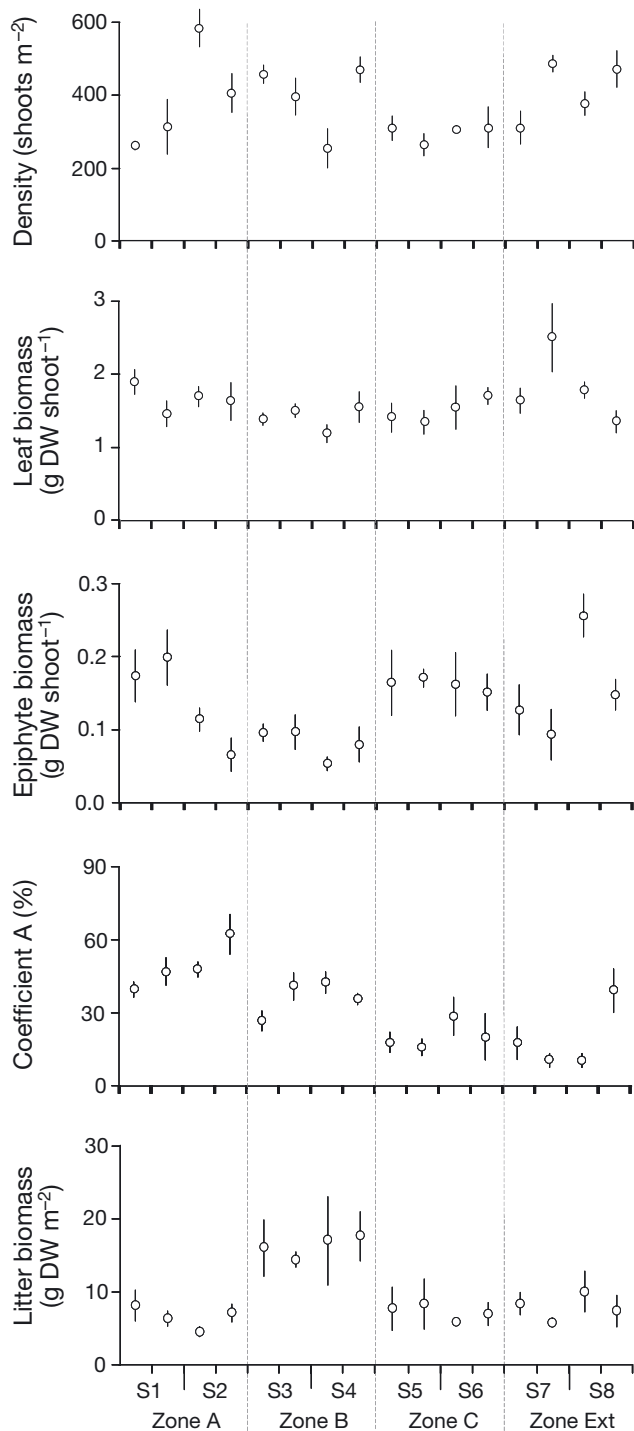


Fig. 6. Characteristics of *Posidonia oceanica* in 2008 in 4 zones with different levels of protection inside and outside the TMPA (at 2 sites in each zone and 2 sectors in each site; see Fig. 2 legend for sampling design). Values are mean  $\pm$  SE for shoot density (shoots  $m^{-2}$ ;  $n = 4$  per sector), leaf and epiphyte biomasses (g DW shoot $^{-1}$ ;  $n = 12$  per sector), coefficient A (percentage of leaves per shoot having alteration marks;  $n = 12$  per sector) and litter biomass (g DW  $m^{-2}$ ;  $n = 4$  per sector)

Table 7. Nested ANOVA for *Posidonia oceanica* variables: shoot density, leaf and epiphyte biomasses, coefficient A and litter biomass. See Table 2 legend for explanation of abbreviations. Significant ( $p < 0.05$ ) values are in **bold**

| Source                  | df | MS    | F     | p                  |
|-------------------------|----|-------|-------|--------------------|
| <b>Shoot density</b>    |    |       |       |                    |
| Pr                      | 3  | 40271 | 0.86  | 0.531              |
| Si(Pr)                  | 4  | 46893 | 1.52  | 0.283              |
| Se(Si(Pr))              | 8  | 30778 | 4.21  | <b>0.001</b>       |
| Residual                | 48 | 7309  |       |                    |
| <b>Leaf biomass</b>     |    |       |       |                    |
| Pr                      | 3  | 0.05  | 1.77  | 0.291              |
| Si(Pr)                  | 4  | 0.03  | 0.95  | 0.482              |
| Se(Si(Pr))              | 8  | 0.03  | 1.91  | 0.080              |
| Residual                | 48 | 0.01  |       |                    |
| <b>Epiphyte biomass</b> |    |       |       |                    |
| Pr                      | 3  | 0.02  | 1.16  | 0.429              |
| Si(Pr)                  | 4  | 0.02  | 4.49  | 0.034 <sup>a</sup> |
| Se(Si(Pr))              | 8  | 0.00  | 1.27  | 0.280              |
| Residual                | 48 | 0.00  |       |                    |
| <b>Coefficient A</b>    |    |       |       |                    |
| Pr                      | 3  | 3227  | 9.42  | <b>0.028</b>       |
| Si(Pr)                  | 4  | 343   | 0.94  | 0.489              |
| Se(Si(Pr))              | 8  | 366   | 2.83  | <b>0.012</b>       |
| Residual                | 48 | 129   |       |                    |
| <b>Litter biomass</b>   |    |       |       |                    |
| Pr                      | 3  | 6.46  | 35.87 | <b>0.002</b>       |
| Si(Pr)                  | 4  | 0.18  | 0.95  | 0.485              |
| Se(Si(Pr))              | 8  | 0.19  | 0.35  | 0.940              |
| Residual                | 48 | 0.54  |       |                    |

<sup>a</sup>Not significant at  $\alpha = 0.01$ ; this conservative level of significance was adopted because variances were heterogeneous and could not be stabilized by transformations

biomass (Schneider & Mann 1991, Edgar & Robertson 1992, Sánchez-Jerez et al. 2000, Scipione & Zupo 2010). Thus, distinguishing the relative contribution of habitat structures can help in elucidating the actual effects of protection (Jennings et al. 1996, García-Chariton et al. 2004). In this study, the spatial variation of meadow features (e.g. shoot density, leaf and epiphyte biomasses) remained fairly similar among protection levels, except for litter biomass and coefficient A. Litter biomass showed higher values in only one of the PPAs (Zone B), which seemed geographically more sheltered from hydrodynamism and may have represented a litter accumulation zone. Therefore, the gradual decrease in coefficient A from the FPA to the PPAs and the External Zone may be more satisfactorily explained by the grazing activity of the sparid fish *Sarpa salpa* (Prado et al. 2008). In parallel, the meadow features explained only 7.5% of the variation in the distribution and



Table 8. Multiple linear regression analyses examining relationships between measured habitat variables, and amphipod general descriptors and the density of the most frequent amphipod taxa ( $f \geq 10\%$ ) at the TMPA. Only amphipod species and general descriptors for which habitat variables presented significant partial positive (+) or negative (–) correlations and/or overall regression ( $p \leq 0.05$ ) are listed. The overall regression included shoot density, leaf and epiphyte biomasses, coefficient A and litter biomass.  $R^2_{adj}$ : adjusted  $R^2$ ; PC: partial correlation. Significant ( $p < 0.05$ ) values are in **bold**

|                               | Overall regression |              | Habitat variable |                        |
|-------------------------------|--------------------|--------------|------------------|------------------------|
|                               | $R^2_{adj}$        | p            | PC               | p                      |
| Total density                 | 0.042              | 0.195        | Leaf biomass     | -0.28 <b>0.033</b>     |
| Total biomass                 | 0.056              | 0.148        | Litter biomass   | +0.31 <b>0.020</b>     |
| <b>Gammaridea</b>             |                    |              |                  |                        |
| <i>Aora spinicornis</i>       | 0.058              | 0.147        | Litter biomass   | +0.29 <b>0.034</b>     |
| <i>Apherusa chierighinii</i>  | 0.068              | 0.113        | Leaf biomass     | -0.26 <b>0.049</b>     |
|                               |                    |              | Coefficient A    | -0.27 <b>0.042</b>     |
| <i>Dexamine spiniventris</i>  | 0.143              | <b>0.020</b> | Litter biomass   | +0.46 <b>&lt;0.001</b> |
| <i>Erichthonius punctatus</i> | 0.047              | 0.177        | Litter biomass   | +0.32 <b>0.016</b>     |
| <i>Iphimedia minuta</i>       | 0.074              | 0.101        | Litter biomass   | -0.30 <b>0.025</b>     |
| <b>Caprellidea</b>            |                    |              |                  |                        |
| <i>Caprella tavolarenis</i>   | 0.087              | 0.071        | Epiphyte biomass | +0.34 <b>0.009</b>     |
|                               | 0.148              | <b>0.016</b> | Epiphyte biomass | +0.26 <b>0.047</b>     |
|                               |                    |              | Coefficient A    | +0.41 <b>0.001</b>     |
| <i>Phtisica marina</i>        | 0.154              | <b>0.014</b> | Epiphyte biomass | +0.29 <b>0.029</b>     |

abundance of amphipods, and either did not or only weakly influenced the densities of amphipod species and general descriptors. As reported by early workers in this field, litter biomass may have greatly influenced some species (e.g. *Dexamine spiniventris* and *Erichthonius punctatus*; Sánchez-Jerez et al. 2000). Probably because of the weak amphipod-habitat relationships, there was no consistency between sta-

tistical methods (i.e. dbRDA and regression analyses) for other habitat variables.

### Spatial confounding factors

Another factor that could explain the low density of several species inside the FPA is related to both the remote geographical location of this area from the coast and to the relative isolation of its *P. oceanica* meadows (Navone et al. 1992, Bianchi & Morri 2006). Amphipods lack a pelagic larval stage and have specific habitat requirements (Thomas 1993), minimizing dispersal effects to enhance the insularity of populations (Dauvin 1987). Even with their rapid maturation and long reproduction periods in the Mediterranean Sea (e.g. from April to September for *Caprella acanthifera* and all year long for *Aora spinicornis*; Jimeno-Fernandez 1993, Bellan-Santini 1998), the insular distribution, as well as demographic characteristics of certain species, may limit the dispersion and the increase of populations within the FPA. In contrast, migratory species such as *Phtisica marina* with no insular distribution (Dauvin 1987) presented 31% of ovigerous females and did not differ among protection levels in terms of density and biomass.

Littoral amphipods are known to be greatly influenced by variations in hydrodynamism (Conradi & López-González 2001, Guerra-García & García-Gómez 2001). Despite the remote geographical location and the potentially more wave-exposed FPA, it is unlikely that differences in the amphipod assemblages observed between this protection level and the PPAs were associated with variation in hydro-

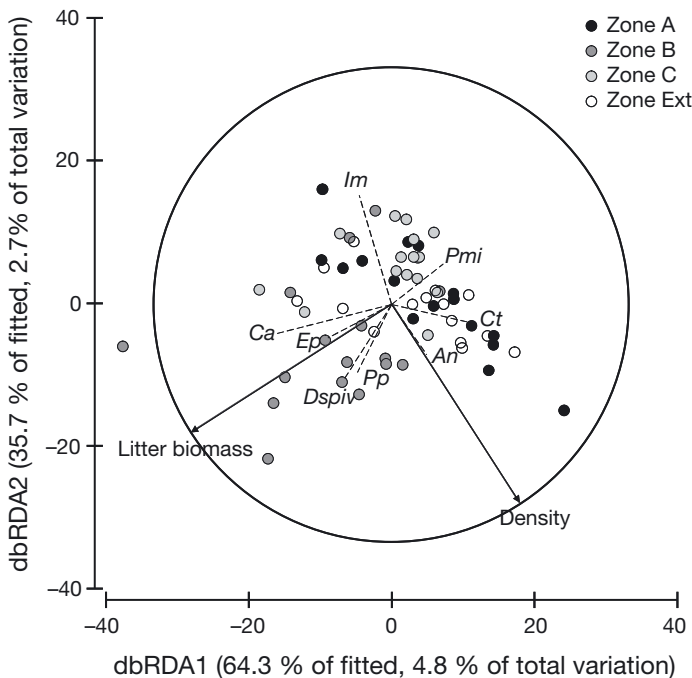


Fig 7. Distance-based redundancy ordination (dbRDA) for amphipod species and habitat features at the TMPA. Full and indented vectors indicate the direction of increasing values of the significant habitat variables (litter biomass and meadow density) and amphipod species, respectively. Only species with correlations  $\geq 0.25$  to the ordination axes are plotted. Vector length represents partial correlation strength with the dbRDA axes; the circle is a unit circle (radius = 1), whose relative size and position of origin is arbitrary with respect to the underlying plot. Plot points indicate individual samples from 4 zones with different levels of protection (see Fig. 1). An: *Apolochus neapolitanus*; Ca: *Caprella acanthifera*; Ct: *Caprella tavolarenis*; Dspiv: *Dexamine spiniventris*; Ep: *Erichthonius punctatus*; Im: *Iphimedia minuta*; Pmi: *Peltocoxa marioni*; Pp: *Pseudoprotella phasma*

dynamic conditions for the following reasons: (1) sampling was done at depths ranging from 10 to 15 m in the foliar stratum of the meadow which largely reduces hydrodynamism (Gambi et al. 1989); and (2) some species (e.g. *P. marina* and *Pseudoprotella phasma*), which are considered typical of low to intermediate hydrodynamic environments (Conradi et al. 1997, Guerra-García & García-Gómez 2001, Guerra-García et al. 2002), were abundant in the FPA.

In the External Zone, the low values of total density, biomass, number of species, and diversity of amphipods may be related to anthropogenic effluents originating from the urbanized and industrialized Gulf of Olbia. Indeed, analyses of water samples showed some higher concentrations of ammonia (with peaks  $>4 \mu\text{mol l}^{-1}$ ), phosphorus, nitrites and chlorophyll *a* in the Gulf of Olbia compared to other locations in the TMPA (Consorzio di Gestione Area Marina Protetta Tavolara-Punta Coda Cavallo 2006). Amphipods are very sensitive to various pollutants (e.g. oil spills, organic enrichment, sewage pollution and ships' wakes; see Conlan 1994 and references therein), and the disappearance of some amphipod species (or at least a decrease in abundance and diversity) can be common at impacted sites (Dauvin 1987, Conlan 1994, De-la-Ossa-Carretero et al. 2012). Some species are excluded by pollution while others increase in density (Bellan-Santini 1980). In our study, the most obvious example is the presence of the pollution-tolerant suspension feeder *Podocerus variegatus* (Bellan-Santini 1980) in the External Zone and in the PPA. Concomitantly, the densities of *A. spinicornis* and *P. marina* were greatly decreased in the External Zone compared to other sites. This corroborates previous findings on the sensitivity of these species to wastewater effluents (Borja et al. 2000, De-la-Ossa-Carretero et al. 2012). In contrast, *Hyale camptonyx* and *Caprella tavolarenis* were essentially found within the FPA. The genus *Hyale* and *Caprella liparotensis* (very closely related to *C. tavolarenis*; Sturaro & Guerra-García 2012) were found in particularly clean areas (Bellan-Santini 1980).

Most species showed the highest variation in density at the lowest spatial scale (~1 m). Many other studies have documented considerable small spatial scale variations in the distribution and abundance of macrozoobenthic populations across a wide range of habitats (Underwood & Chapman 1996, Benedetti-Cecchi 2001, Chapman et al. 2010), including seagrasses (De Biasi et al. 2003). Processes likely to be involved in such patterns include complex sets of

local physical and biological interactions such as the availability of food, behavioural aggregation, predation, competition, or different settlement features (Anderson et al. 2005, Fraschetti et al. 2005).

This study also highlighted that measuring spatial variations at both sector and site scale proved relevant for many species. The inconsistency in variability from 2007 to 2008 suggests the importance of the temporal scale. Large annual fluctuations in density have already been observed in temperate waters for *P. marina* and species of the genus *Apherusa* and *Gammarus*. This may be attributed to the variability in the available algal biomass, natural amphipod population dynamics (e.g. irregularity of recruitment) and/or fish predation pressure related to yearly variations of fish assemblages (Nelson 1979, Dauvin 1987, Costa & Costa 1999, Francour 2000, Guerra-García et al. 2000). The description of the natural variability of amphipod populations versus protection effects requires taking into account different spatial (~1, 10 and 100 m) and temporal scales, and we recommend using this approach in the context of future MPA management schemes and monitoring programs. Indeed, the choice of these relevant scales should be considered carefully to avoid a failure in the detection of protection effects.

## CONCLUSIONS

In summary, this study demonstrates the strength and usefulness of the multiscale approach, but also highlights the difficulties inherent in properly assessing protection effects versus natural variability. Analyses revealed that the structure of amphipod assemblages associated with *Posidonia oceanica* meadows was patchy at a variety of spatial scales, but differed clearly between protection levels. The low densities and/or biomasses of several taxa observed within the fully protected area compared to the partially protected areas are also noticeable results. Seagrass features only explained a small part of the variation among amphipods. Reasons for the observed patchiness in amphipod assemblages are therefore probably multiple and interconnected, encompassing a range spanning from the ecological and behavioural traits of amphipod species to protection-dependent processes (e.g. fish predation). Long term multiscale spatial and temporal monitoring of macrozoobenthic assemblages, as well as manipulative experiments, are clearly needed to fully understand macrozoobenthic responses to protection in MPAs.

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