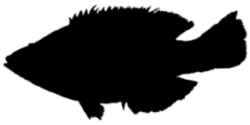
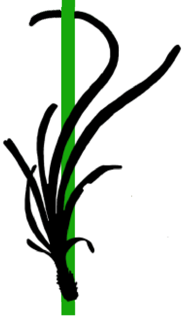
Doctoral dissertation



Multiscale variability of amphipod assemblages in *Posidonia oceanica* meadows

A comparison between different protection levels



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Multiscale variability of amphipod assemblages in *Posidonia oceanica* meadows

A comparison between different protection levels

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Dissertation

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by

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Nicolas Sturaro

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Summary

Despite the rapid expansion of marine protected areas in the Mediterranean Sea, very few studies have addressed macrozoobenthos responses to protection. The aim of this work was to assess and better understand the potential responses of amphipod assemblages in *Posidonia oceanica* meadows between different protection levels.

Two approaches were used. First, multiscale variability patterns of amphipod assemblages were investigated at the Revellata Bay (France) and the Tavolara-Punta Coda Cavallo Marine Protected Area (TMPA, Italy), over spatial scales spanning five orders of magnitude (~1 m to >100s of km) for two consecutive years. Second, the role of fish predation in affecting amphipod assemblages was evaluated using experimental manipulations of predation intensity.

Amphipod assemblages of *P. oceanica* meadows were typified by high density and number of species. Our research revealed that amphipod natural variability was great at small and large scales in *P. oceanica* meadows. At small scales (from ~1 m to ~10 m), this pattern was in relation to both total amphipod density and/or several species densities, which may be explained by behavioural traits of amphipods. At large scale (>100 km), the structure of amphipod assemblages was different between meadows and may be related to hydrodynamic forces.

During this research, a new caprellid, *Caprella tavolarensis* was discovered and described. The species is close to *Caprella liparotensis*, but can be clearly distinguished by smaller size, presence of a short rostrum, body elongate and dorsally smooth, absence of serrate carina on the basis of gnathopod 2 and pereopods, mouthparts scarcely setose, absence of fine setae on peduncle of antenna 1 and absence of swimming setae on antenna 2.

At the TMPA, the structure of amphipod assemblages differed markedly among protection levels. Moreover, it was observed lower densities and/or biomasses of several frequent taxa within the fully protected area and outside the MPA compared to partially protected areas. Meadow features account only for a low proportion of the amphipod variability, while predation by fish seemed to be an important factor in structuring *P. oceanica* amphipod populations.

Overall, this work suggests that full protection at the TMPA is likely to contribute partially (primarily via fish predation) to the observed variability patterns among zones. However, superimposed factors including behavioural traits of amphipod species and surrounding habitats are likely to be also significant. Whether these changes are representative of all fully protected areas and whether those effects are positive or negative to the meadows, are still unknown

Chapter 1

General introduction

Ecological heterogeneity

Almost a quarter of a century has passed since "space" has been considered as the last limit for ecological problems and theory (Levin 1992, Kareiva 1994, Nakaoka & Noda 2004). In nature, ecological patterns can change with spatial scale of observation (O'Neil & King 1998). To understand the "picture", the appropriate scale should be taken into account (Wiens 1989). Botanists already recognized long time ago the usefulness to integrate spatial scales in the study of species distributions (Greig-Smith 1952), but ecologists were insensitive to this issue (Wiens 1989). Nevertheless, in the last decades a large number of studies have been performed in a wide range of topics (Kotliar & Wiens 1990, Levin 1992, Underwood & Chapman 1996, Fraschetti et al. 2005, Bell et al. 2006, Boström et al. 2006, Gillanders 2006), and the scaling issue has become pervasive.

Distribution patterns of species (plant or animal) are rarely uniform and continuous in space and time, leading to species assemblages' structure and dynamics highly variable. Multiple factors can be the cause of these variations and operate at different scales, making the perception of properties of an ecological assemblage dependent on the scale of observation. Thus, there is no a single scale at which the structure and dynamics of assemblages can be described (Wiens 1989, Levin 1992).

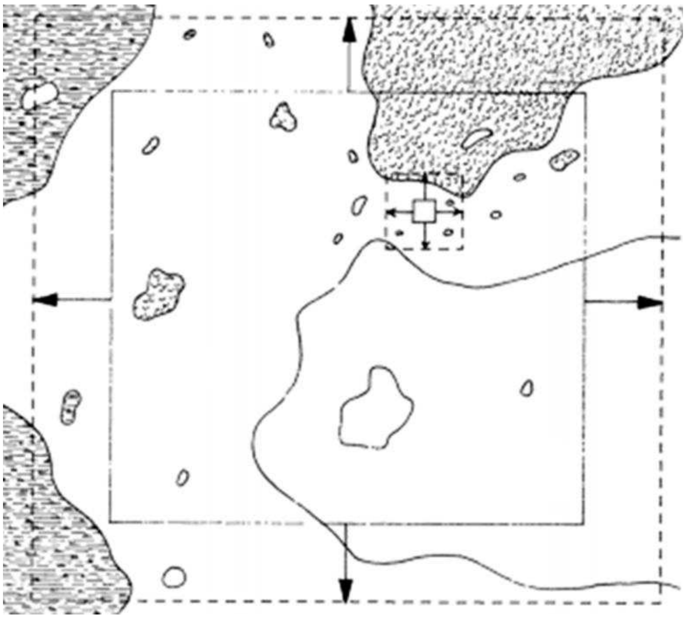
The documentation of multiscale variability patterns of populations can give clues on the processes at the origin of these patterns (Underwood & Chapman 1996). Potential processes that drive the spatial and temporal variation in assemblage structure can be both physical (e.g. habitat structure, hydrodynamism) and biological (e.g. predation, competition, behaviour, reproduction) in nature. The challenge rests on evaluating the relative importance of such processes in affecting assemblage structures (Menge & Farrel 1989).

Such documentation of patterns requires an appropriate method (Underwood & Chapman 1996). Spatial hierarchical approaches that incorporate a range of spatial scales in sampling designs is a powerful tool (Fraschetti et al. 2005) to better understand complex processes of populations (Nakaoka & Noda 2004). However, the ability to detect patterns depends of the "grain" and the "extent" of a research (O'Neill et al. 1986). According to Wiens (1989), grain is the size of the individual unit of observation. For instance, this could be the quadrat of a field ecologist. The extent corresponds to the overall area encompassed by a study or the largest scale (Fig. 1). Grain and extent define the lower and upper limits of resolution of a study. The increase of the extent allows elements of the landscape to appear, while these were not present in the original study area. On the other hand, as the grain of samples is increased, small patches are now included within samples. Beyond these two limits, any inferences are not able to make clear the real patterns leading possibly to incorrect interpretations.

Multiscale analyses of spatial patterns have been limited to a narrow range of habitats and taxa (Fraschetti et al. 2005). However, the literature is growing (Tanaka & Leite 2003, García-Charton et al. 2004, Di Franco et al. 2009, Vasapollo 2009), and the use of the spatial hierarchical approach can be an invaluable tool in the assessment of the multiscale responses of marine populations to the establishment of MPAs. In fact, in order to separate the effects of management from other sources of variation, it is essential to quantify and understand the magnitude and range of natural variability of populations at different scales of observation. Clearly, the understanding of how MPAs work as a management tool has to be gained in this framework (García-Charton & Pérez-Ruzafa 1999).

Figure 1.

The effects of modifying the grain and extent of a study in a landscape (Wiens 1989).



Marine protected areas

Coastal marine environments host key habitats for many marine populations, but are subject to strong anthropogenic pressure (Claudet & Pelletier 2004). The impact of human activities on ocean resources has become more and more evident. Declining fish stocks, loss of biodiversity, the most frequent algae blooms, degradation of fragile habitats (i.e. coral reefs or seagrass meadows), are just a few signs of marine environmental degradation (Agardy 1997). In recent years, marine protected areas (MPAs) has been increasingly seen as one of the most preferential tools for the conservation of marine biodiversity and to maintain fisheries (Agardy et al. 2003, Claudet 2011).

Definition, categories and objectives

The most widely used definition of MPA internationally is that provided by the International Union for Conservation of Nature (IUCN), "any area of intertidal or subtidal terrain, together with its overlying water and associated flora, fauna, historical and cultural features, which has been reserved by law or other effective means to protect part or all of the enclosed environment"(Kelleher & Kenchington 1992).

In some cases, this generic description has changed somewhat according to discussions and treaty negotiations (Agardy et al. 2003). For instance, documents necessary for the Convention on Biological Diversity state that "MPAs are coastal or oceanic management areas designed to conserve ecosystems together with their functions and ressources" (deFontaubert et al. 1996).

Generally, MPAs can vary widely in sizes, shapes and their objectives. The goals of the establishment of a MPA are very numerous, but some appear more frequently than others, such as maintain biodiversity (ecosystem diversity, species richness and genetic diversity) and the conservation of habitats. Promote research and education, but also sustainable development, is also one of the common targets. More specific objectives are to protect rare species, safeguard coastal areas, historical and cultural sites, promote and monitor tourism and their recreation activities, and restore habitats. MPAs can be described as tools to accomplish conservation goals and promote sustainable development (considering social and economic aspects). To attempt to ensure more consistency and order, IUCN established six management categories (IUCN 1994) (Table 1).

These categories include "no-take" and/or "no-access" zones. In no-take zones, extraction of marine life is prohibited except for purposes of approved scientific research. These restrictions are applied to commercial, recreational, traditional fishing or collection of organisms. These zones are established for scientific research, biodiversity protection, endangered species or habitats, protection of critical fisheries stocks and protection of representative ecosystems (Ballantine 1994). Generally, no-take zones are smaller and less common because they require a strong regulation to protect against the harvest on marine life. In some marine reserves, some or all non-extractive uses (e.g. swimming, boating) can also be excluded. Those areas can be called no-take/no-access zones, synonym to fully protected areas (FPA) and marine reserves (Claudet 2011). In this research we will use the term FPA.

The most recent form of MPAs is “multiple-use” (Agardy 1994). These zones are generally larger than no-take and/or no-access zones with multiples economic, social and/or conservation goals. A zoning system is often applied to these zones, in order to specify the degree of protection and accessibility of each part of the reserve. No-access or no-take zones can be considered such as zones forming part of a multiple-use area.

Table 1.

Categories of protected areas management described by their main objective (IUCN 1994).

Category	Primary objective
I. Strict nature reserve and wilderness areas	Protect the long-term ecological integrity of natural areas and furnish reference areas for scientific work and environmental monitoring
II. National park	Protect natural biodiversity along with its underlying ecological structure and supporting environmental processes, and to promote education and recreation
III. National monument or feature	Protect specific outstanding natural features and their associated biodiversity and habitats
IV. Habitat/species management area	Maintain, conserve and restore species and habitats
V. Protected landscape or seascape	Protect and sustain important landscapes (or seascapes) and the associated nature conservation, and other values created by interactions with humans through traditional management practices
VI. Protected area with sustainable use of natural resources	Protect natural ecosystems and use natural resources sustainably, when conservation and sustainable use can be mutually beneficial

MPAs in the Mediterranean Sea

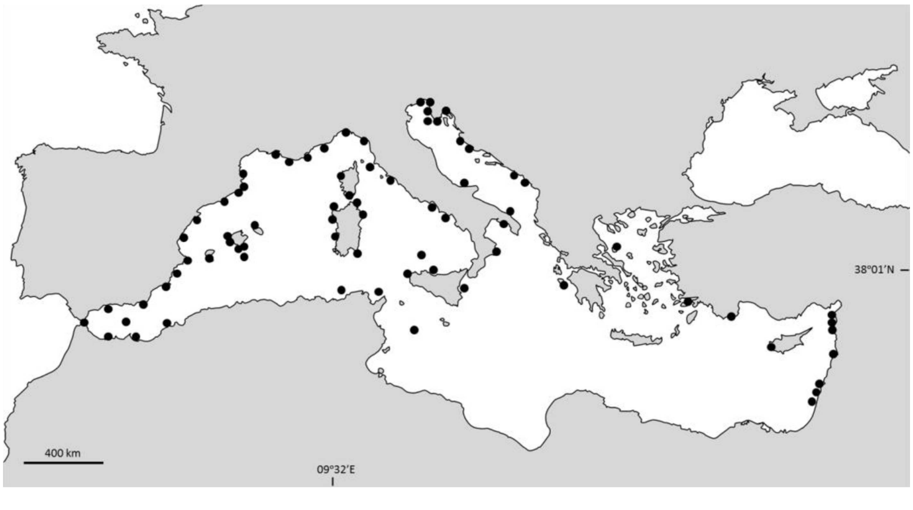
The great diversity of situations described above is also true for Mediterranean MPAs (Francour et al. 2001). In recent years, there has been an important increase in the number of MPAs established in the Mediterranean Sea (Juanes 2001). In 2005, more than 74 MPAs have been officially recognized (Mabile & Piante 2005) (Fig. 2). However, their implementation is based on many uncertainties and theoretical unverified (Allison et al. 1998). Site selection is based on insufficient scientific knowledge. There is little information available on the distribution of biodiversity in MPAs. This information is fairly abundant on fish, but there is still a lack of knowledge for small macrozoobenthic species (Fraschetti et al. 2005, see chapter 4).

In Italy, there are 25 MPAs officially established and more than 20 in the process of becoming established. The MPAs total surfaces are comprised between 120 and over 50.000 hectares (Guidetti et al. 2008). There are composed by one or more "no-take/no-access" zones defined as “Zone A”, surrounded by buffer zones called “Zone B” and “Zone C” where restrictions decrease (Villa et al. 2002). Only a few of these MPAs are effectively managed (Guidetti et al. 2008).

Assesment of the potential effects of MPAs is crucial for adaptive management and decision-making. Actually, there remains considerable uncertainty about the effects of MPAs, in spite of a large number of assessments (Osenberg et al. 2006). This essential topic, focusing mainly on macrozoobenthic species, will be largely introduced and discussed in chapter 4.

Figure 2.

Distribution of Mediterranean marine protected areas (data from Mabile & Piante 2005).



Seagrass meadows

Definition and systematics

Seagrasses are higher plants that have evolved to live in marine environments. They are angiosperms and belong to group of monocotyledons. Seagrasses spend their full lifecycle underwater and present five common features: the capacity to grow when completely submerged, the adaptation to saline environments, an anchoring system able to withstand of wave action, hydrophilus pollinination and have a full reproductive cycle in sea water.

They are distributed over 4 families: Cymodoceaceae, Hydrocharitaceae, Posidoniaceae, and Zosteraceae. The Cymodoceaceae family contains only seagrasses. It counts 5 genera (*Halodule, Cymodocea, Syringodium, Thalassodendron,* and *Amphibolis*). Hydrocharitaceae includes mainly freshwater environments (14 genera) and 3 genera of seagrass (*Halophila, Thalassia, and Enhalus*). The Posidoniaceae family is constituted by only 1 genus, *Posidonia*. It contains 9 species, including the Mediterranean *Posidonia oceanica* L. (Delile) and 8 species from Australian coasts. Finally, the Zosteraceae family regroup 3 genera entirely of seagrass (*Zostera*, *Phyllospadix*, and *Heterozostera*).

They have been observed along all coastal areas of the world except the Antarctic (Hemminga & Duarte 2000). The total area of seagrass meadows distributed in the world is estimated at 500.000 km^2 (Duarte & Chiscano 1999), but that represent less than 0.02% of the angiosperm species. If the number of seagrass species is hardly any (64 species according to Den Hartog & Kuo 2006), their ecological role is essential in coastal environments: a large number of them are ecosystem engineers, provide shelter for large number of organisms, and their extraordinarily high rate of primary production makes them a basis of food chains (Hemminga & Duarte 2000).

Heterogeneity

Marine seagrass habitats present a series of hierarchical scales components, ranging from single leaves to individual shoots to aggregation of shoots, composing a seagrass meadow. The meadow is integrated within a matrix of other habitats (e.g. sandy or rocky bottoms), which is part of a large coastal area (Robbins & Bell 1994, Gillanders 2006). Results and conclusions of studies depend and can be influenced by the scale of observation (Turner 1989). Patterns and processes that are found to be influent at one scale may not be at another scale (Wiens 1989). Therefore, observations made at different scales are the best way to allow relevant interpretation of patterns and focus attention on the range of potential processes that may be important to species (Underwood & Chapman 1996). Few studies in marine ecosystems have used a landscape scale (Table 2) considering the scale of 10s of m to km. However, the body of marine research on this theme is growing (Bell et al. 2006, Gillanders 2006 and references therein) and most studies have focused on the proximity to other habitats or the position of seagrass meadows within a bay. Little research has addressed the regional scale of variation (Fraschetti et al. 2005).

Mediterranean seagrasses

In Mediterranean Sea, we can find 5 seagrass species. The *Cymodocea nodosa* seagrass is found in shallow water, but can reach a deep of 30 to 40 m. It is usually observed in sandy substrate and sheltered sites. It tends to grow in patches and is sometimes associated with the other seagrasses, *Zostera noltii* and *Posidonia oceanica.* A small Mediterranean seagrass is *Zostera noltii* who grows in intertidal zones and subtidal areas (den Hartog 1970). These species are found in areas with high organics loads, and with euryhaline conditions. Like *Z. noltii*, *Zostera marina* is found in intertidal to sublittoral of marine waters. These seagrass are uncommon in the Western part of the Mediterranean, and even more in the Eastern Mediterranean (Lipkin et al. 2003, Procaccini et al. 2003). In the Mediterranean Sea, *Halophila stipulacea* seagrass occurs from Egypt to southern Italy, and it may have been introduced through the Suez Canal. This species grows only on soft substrates, mainly in shallow waters (Lipkin et al. 2003). Settlements of this seagrass have also been reported on dead matte of *P. oceanica.* In the end, the *Posidonia oceanica* is an endemic seagrass to the Mediterranean Sea. It's one of the largest, slowest growing, and longest-lived plants. It is a former habitat for many other marine organisms and thus plays a significant role in littoral Mediterranean ecosystems (Gobert et al 2006, Kendrick et al. 2005, Pergent et al 1994).

Table 2.

Summary of factors influencing abundance of organisms at different spatial scales from patches to region, in seagrass meadows (Gillanders 2006).

Spatial Scale	Distances	Variable
Within patches	cm to m	Shoot density, shoot length and width, above/below-ground biomass
Among patches	m to 10s of m	Patch size Patch shape Orientation of patches Percent cover of seagrass Edge *vs.* center
Landscape scale	10s of m to km	Degree of heterogeneity or patchiness Patch isolation Position of patches within landscape Adjacent habitats
Regional and bio-graphic scales	10s to 100s km and greater	Among estuaries Among biogeographic regions Tropical *vs.* temperate systems

Posidonia oceanica

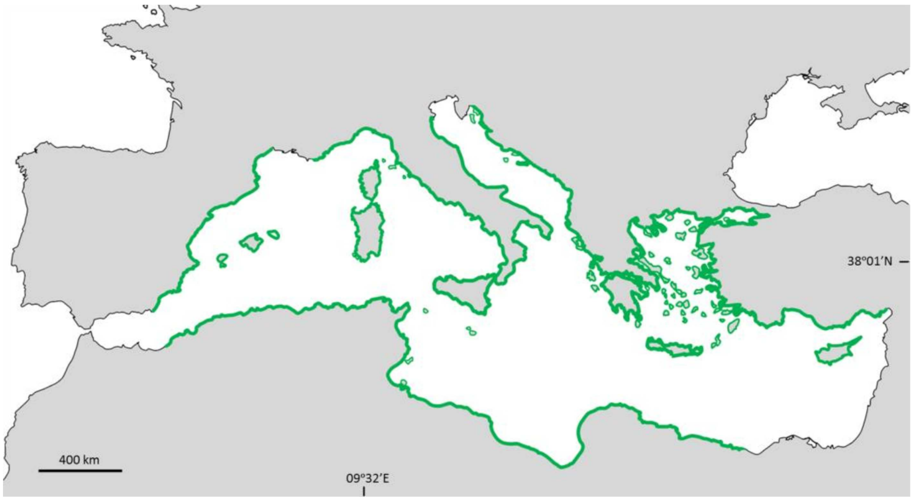
Posidonia oceanica (L.) Delile is a seagrass species belonging to the family of Posidoniaceae. The *Posidonia* genera comprises 9 species (Den Hartog 1970): *P. oceanica*, endemic to the Mediterranean Sea, and other 8 species found in Australian coastal waters. In the Mediterranean Sea, *P. oceanica* is the most common marine seagrass (Den Hartog 1970). It forms large underwater meadows covering a surface area of between 2.5 and 5.5 million hectares (Pasqualini et al. 1998) (See Fig. 3 for distribution).

Role of P. oceanica meadows

The role of *P. oceanica* meadows is comparable to forests on land. They form a "hot spot" of unique biodiversity. They constitute a refuge for many species, even a spawning ground. They are strong productive ecosystems. Part of this production (nearly 40%) is exported to other ecosystems in the form of dead leaves, allowing feed organisms associated to meadows, but also other distant and deeper organisms. *Posidonia oceanica* meadows are an important factor in the oxygenation of the water through the high photosynthetic activity. They also play a very important role in the physical balance because they act as vegetal barriers retaining the suspended sediments and contributing to the transparency of the coastal waters. Furthermore, the erosive action of waves and currents is attenuated by meadows which protect the coastline, and the benches (accumulation of dead leaves on the shore) and so the beaches (Boudouresque et al. 2006).

Figure 3.

Distribution of *Posidonia oceanica* in the Mediterranean Sea (solid green line) (Gobert et al 2006, modified).

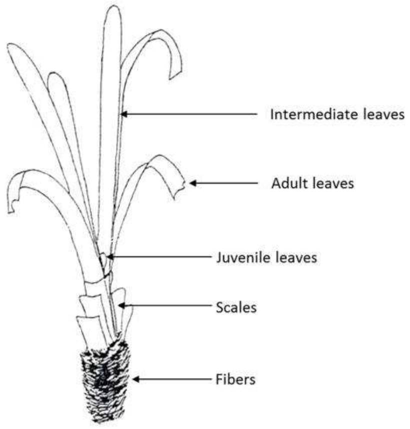


Biology and ecology

The *Posidonia* meadows are found from the coast to forty meters deep, according to water transparency. It can withstand temperatures ranging from 10 to 28 °C (Boudouresque & Meinesz 1982). However, does not support the freshening (Gobert 2002), it is thus absent in the mouths of rivers (otherwise, it can subsists in the lagoons) (Fig. 3). It colonizes sandy and rocky bottoms, and it fixed through its rhizomes. While the primary productivity of seagrass leaves is high (i.e. 68 to 147 gC/m^2 year), it is lower for rhizomes (i.e. 8.2 to 18 gC/m^2 year). Between 3 and 10% of this productivity is consumed by herbivores (in certain case up to 50%). A higher percentage is used by decomposers (Pergent et al. 1997).

The *P. oceanica* is composed by shoots of leaves located at the end of vegetal axes, called rhizomes. Each shoot (Fig. 4) has generally between 4 and 8 flat and elongated leaves (banded). Leaves can reach dimensions from 8 to 11 mm in width and from 20 to 180 cm in length. Roots also leave from the rhizomes to the sediment (up to 70 cm deep) (Boudouresque et al. 2006).

Figure 4. - Schema of a *Posidonia oceanica* shoot (Cinelli et al 1995, modified).

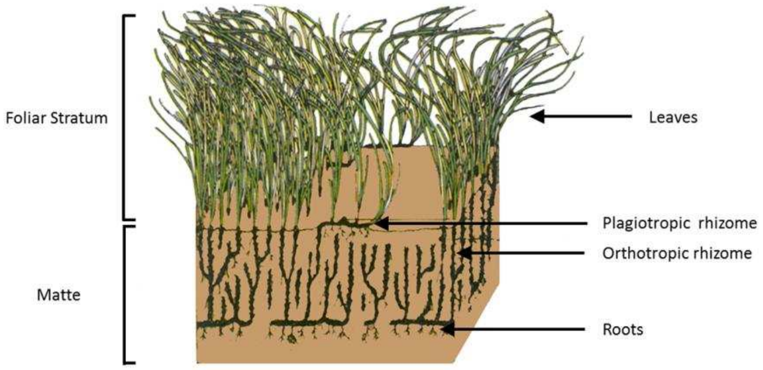


Structure of P. oceanica meadows

Posidonia oceanica meadows can be considered as a set of three interconnected compartments (Fig. 5): the foliar stratum, the root/rhizome system and matte, and the litter. Each compartment is characterized by particular conditions, determining the type of organism that can live there.

Figure 5.

Structure of a *Posidonia oceanica* meadow (Boudouresque & Meinesz 1982, modified).



The foliar stratum or canopy is characterized by values of shoot density ranging from ~100-1200 shoots/m^{-2} according to depth, but within the year values remain similar (Gobert et al. 2006). Leaf biomass production is high (160-720 g dw/year^{-1} m^{-2}), and exhibit fluctuations between seasons (Pergent-Martini et al. 1994, Buia et al. 2000). Leaf life span is between 70-350 days. Principally adult leaves can be degraded by hydrodynamics forces and grazing and present a percentage of leaves per shoot having alterations marks between 5-51% according to season (Gobert et al. 2006). The foliar stratum is inhabited by vagile fauna (see next section) and epiphytic organisms (flora and fauna). The long leaves shelter calcareous red algae, brown algae and diatoms, hydrozoans and bryozoans (Mazzella et al. 1989).

Rhizomes play an important role in the plant anchoring, but also in the vegetative growth (Hemminga & Duarte 2000). It exist two types of rhizomes: plagiotropic rhizomes, responsible for the horizontal growth (enabling colonization), and orthotropic rhizomes, responsible for the vertical growth (avoiding burial). Roots are an essential organ to assimilate nutriments present in the sediments (Gobert et al. 2006). The matte (composed of rhizomes, roots and a large amount of sediments) is formed by the progressive deposition of suspended particulate matter (Boudouresque et al. 2006). Below biomass (rhizomes and roots) can be large, and have a range between ~1600-6500 gdw.m^{-2} (Buia et al. 2000, Duarte & Chiscano 1999).

At the end of summer, *P. oceanica* leaves fall and contribute to the formation of a leaf litter that remains within the meadow, in sand patches, and/or are exported to other ecosystems (e.g. deep coastal waters) where it may represent an important trophic input (Fenchel 1977). Such leaf litter, often mixed with *P. oceanica* rhizomes and drift macroalgae within the meadow may persist for a few days only or for several months or even years, depending on its degree of exposure to hydrodynamic forces and biological phenomena (e.g. bacterial degradation and activity of the detritivorous macrofauna; Wittmann et al. 1981). Large accumulations of *P. oceanica* litter provides a structural habitat as well as potential food sources for an abundant animal community (Gallmetzer et al. 2005, Sturaro et al. 2010, Michel 2011). Crustaceans (mainly amphipods and isopods) are the dominant component of the macrozoobenthos. Therefore, the three compartments are a particularly diversified ecosystem with multiple micro-habitats (Kikuchi 1980, Garcia-Raso 1990).

Spatial variability

The *P. oceanica* spatial variability is very complex and irregular. Seagrasses often appear as a landscape similar to *leopard skin* spots (Den Hartog 1970), which can be explained by different phenomena. First, sexual and vegetative reproduction patterns (cuttings and multiplication) could influence the distribution of spots, because rhizome growth is centrifugal and the shoot density increases towards the periphery (Duarte et al. 1990). Processes as currents, grazing and human activities, also influence the distribution of *P. oceanica* meadows (Gobert et al. 2003).

The *P. oceanica* spatial variability shows very large variations of different biometric parameters (density, length, width, and number of leaves per shoot, leaf biomass and epiphytes, area and leaf index) at different scales (Zupo et al. 2006, Balata et al. 2007). Many interrelated factors modulate spatial heterogeneity and make complex the study of *P. oceanica* dynamics (Gobert et al. 2003). This spatial variability exists at scales below the metre, indicating that changes the very local habitat quality may affect the growth and morphology of *P. oceanica*, even at same depths (Panayotidis 1981, Balestri et al. 2003). Changes to large scales (order of km) may reflect differences in habitat, such as wave exposure, substrate type, sediment characteristics and grazing pressure. Factors like physical disturbance, topographic complexity and nutrient availability could have an impact on meadows at smaller scales (Zupo et al. 2006). However, variations observed at very small scales (from cm to m) are highly complex to explain and require more techniques and measures of physical and chemical variables (Balestri et al. 2003). This demonstrates the importance of applying rigorous sampling methods including all spatial scales to studies related with *P. oceanica* meadows (Balata et al. 2007).

The vagile fauna

In this study, amphipod crustaceans analysed belong to vagile fauna. They are defined as the mobile fauna maintaining strong links with the benthos (Ledoyer 1968) and it is one of the most important components of the ecosystem (Kikuchi & Pérès 1977). It is composed by many species (larvae or adults) of molluscs, crustaceans, polychaetes, echinoderms and fish (Mazzella et al. 1989, Gambi et al. 1992). Crustaceans are the most diverse group of vagile fauna, where the most important taxa are the amphipods, isopods and decapods (Gambi et al. 1992). The vagile fauna is consumed by fish and other larger organisms, so therefore play a fundamental role in the mechanism of energy transfer to higher trophic levels (Buia et al. 2000). The abundance of vagile fauna varies with predation, and it has been observed that in dense meadows, where there is a better protected from predation, the abundance of vagile fauna increases (Hemminga & Duarte 2000).

Amphipod crustaceans

Systematics

Usually treated as a subphylum, Crustacea forms a large and diverse group of arthropods. The current estimate of the number of described species is between 52.000 and 67.000. Probably that the total potential number of species may be 10 to 100 times greater (Monod & Laubier 1996, Ruppert et al. 2003). Crustaceans mainly occupy marine environments, and they are also represented on terrestrial and in freshwater habitats.

Peracarida is still treated as a superorder. Nevertheless, relationships among peracarid groups (and of peracarids to other crustaceans) are very debated. Some suggestions have been made to abandon or revise the group (Dahl 1983). According to the updated classification of crustaceans by Martin & Davis (2001), Peracarida contains 9 orders, mostly in keeping with Bowman & Abele (1982).

Among these orders, the most diversified is Amphipoda, covering more than 8.000 described species (Bellan-Santini 1999). It is traditionally divided into four suborders well characterized: the Gammaridea, the most numerous with over 6.000 species; the suborder of Hyperiidea which is represented by around 250 marine and planktonic species; Caprellidea with about 250 marine and benthic species; and Ingolfiellidea, living in the interstitial and freshwater (Bellan-Santini 1999, Barnard & Karaman 1991). However, Bowman & Abele (1982) considered 3 suborders including Ingolfiellidea within Gammaridea. Shortly after, a new classification is proposed with 3 suborders: Gammaridea (gammarids and ingolfiellids), Hyperiidea and Corophiidea (caprellids and certain gammarids) (Barnard & Karaman 1983).

Morphology

There are great morphological variations within the order Amphipoda. However, a "typical amphipod" can be described, possessing certain features common, to a certain degree, to the order. The typical amphipod has a body of around 10 mm usually arched and laterally compressed, and divided in 3 principal parts: the head, the pereon and the pleon (Bellan-Santini 1999) (Fig. 6).

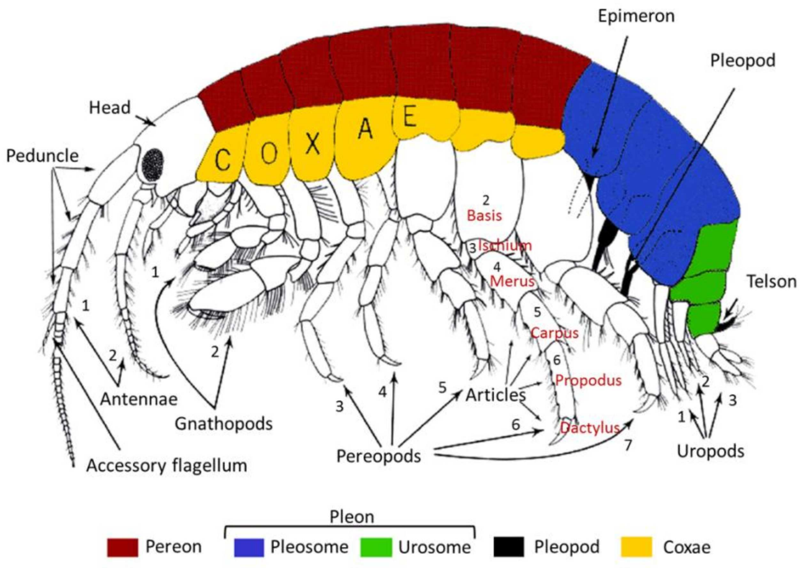
The head corresponds to a cephalothorax, and bears sessile compound eyes, two pairs of antennae terminated with pluriarticulated flagella, and mouthparts. Mouthparts include mandibles and two pairs of maxillae followed by the maxillipeds.

The pereon is divided into 7 segments and each segment bears two sets of pereopods (4 anterior and 3 posterior). Each pereopod can be divided in the 7 following parts (starting from the proximal to the distal part): the coxa, the basis, the ischium, the merus, the carpus, the propodus and the dactylus. The first 2 pairs of pereopods are converted into prehensile appendages: the gnathopods. In general, pereopodes 2 to 7 bear a gill inserted at the junction with the body, but the number can vary. In females, coxa present lamellar projections called oostegites and form a brood pouch (marsupium), a common feature proper to peracarids.

The pleon is composed of 6 segments. The 3 first segments constitute the pleosome which bears each a pair of natatory appendages (pleopods). The 3 last segments correspond to the urosome. Each of them bears a pair of appendages (uropodes). The pleon is ended with the telson, very short (Bellan-Santini 1999)

Figure 6.

Classic morphological features of an amphipod crustacean (Barnard & Karaman 1991)



General biology and ecology

Amphipods occupy almost all aquatic habitats. They have been collected in rivers, lakes and underground waters. In marine environments, they are present from littoral areas to abyssal trenches or be beneath the polar sea ice. Amphipod species may be planktonic or benthic. Planktonic species, mainly represented by Hyperiidea, live most often associated with other planktonic organisms such as jellyfish. Benthic species, most numerous and best studied colonize all types of substrates from biocoenosis of coastal detritic or muddy bottoms to corralligenous, photophilic algae and seagrass ecosystems, including *P. oceanica* meadows (Bellan-Santini 1998).

Amphipods in P. oceanica meadows

Amphipod assemblages in *P. oceanica* meadows have been extensively sampled (Scipione & Fresi 1984, Mazzella et al. 1989, Gambi et al. 1992, Sánchez-Jerez et al. 1999a, b, Sánchez-Jerez et al. 2000, Scipione 1999, Zakhama-Sraieb et al. 2006, Como et al. 2008, Vázquez-Luis et al. 2009, Michel et al. 2010, Scipione & Zupo 2010, Michel 2011, Zakhama-Sraieb et al. 2011). Amphipods are an abundant and diverse group of the vagile fauna (Fig. 7 and Fig. 8). According to the abundance, the best represented families are: Calliopiidae, Amphilochidae, Aoridae, Ischyroceridae, Dexaminidae, Amphitoidae, Talitridae and Caprellidae (Scipione & Fresi 1984).

Different studies focused on spatial and temporal distribution (e.g. bathymetric and seasonal patterns; Scipione & Fresi 1984, Mazzella et al. 1989, Gambi et al. 1992, Michel 2011, Zakhama-Sraieb et al. 2011), vertical migrations (Sánchez-Jerez et al. 1999a), comparative analyses with other habitats (Sánchez-Jerez et al. 1999b, Como et al. 2008, Vázquez-Luis et al. 2009, Scipione & Zupo 2010), feeding habits (Michel 2011), effects of bottom trawling (Sánchez-Jerez et al. 2000) and sampling methods (Michel et al. 2010, Scipione & Zupo 2010).

The temporal variation at small scale (nychthemeral variation) is well known. After the sunset, migration takes place from the lower layers of the *P. oceanica* meadow (rhizomes, matte) to the leaf stratum, which increases the abundance and diversity at the canopy during the night (Ledoyer 1969, Sánchez-Jerez et al. 1999a, Michel 2011). At larger scale, the seasonal variation is not so clear. In late summer and autumn, amphipod abundance and diversity within the meadow is generally high, while low in winter and early spring (Mazzella et al. 1989, Gambi et al. 1992, Scipione et al. 1996). Some other studies disagree with this pattern and show that the assemblage is more developed (more individuals from more species) in June (Michel 2011). Often authors relate the changes to meadow features (e.g. epiphytic biomass, litter cover).

Previous studies showed that amphipods are more abundant and/or more diverse in *P. oceanica* meadows, than in other macrophytes such as *C. nodosa* (Scipione et al. 1996, Sánchez-Jerez et al. 1999b, Como et al. 2008, Vazquez-Luis et al. 2009, Scipione & Zupo 2010), *Z. marina* (Scipione & Zupo 2010), *C. prolifera* and *C. racemosa* (Vazquez-Luis et al. 2009). Moreover, the structure amphipod assemblages are different among these ecosystems (Sánchez-Jerez et al. 1999b, Vazquez-Luis et al. 2009).

For bathymetric variations, amphipods show a non homogeneous vertical distribution. There appear to be 3 assemblages: one included between the surface and 2 m depth, which is characterized by a low number of species and individuals. Species seem to be specialized in high hydrodynamics forces such as *Hyale schmidti*. The other between 5-10 m to 20-25 m is considered as the typical assemblage of *P. oceanica* meadows. Typical species with general high values are *Apherusa chiereghinii*, *Aora spinicornis*, *Dexamine spinosa*, *Phtisica marina*, *Ischyrocerus inexpectatus*, and *Pseudoprotella phasma*. At last, the third assemblage is found at depth greater than 25 m and is characterized by species such as *Guernea coalita* and

Peltocoxa gibbosa found in bare soft bottoms (Scipione & Fresi 1984, Mazzella et al. 1989, Gambi et al. 1992).

Although some studies focus on *P. oceanica* litter accumulations at proximity of the meadow (Gallmetzer et al. 2005, Dimech et al. 2006, Como et al. 2008), some information is available for litter cover present within the meadow at the Revellata Bay (Michel 2011). In this area, *Apherusa chiereghinii* (20%), *Gammarella fucicola* (17%), and *Ampelisca rubella* (12%) were the principal species in terms of relative abundance. Gammarella fucicola is the dominant species in large accumulation in the same area (Gallmetzer et al. 2005). Other studies showed that large accumulations outside of the meadow present species such as caprellids *Pseudolirius kroyeri* and *Phisica marina* in Sardinia (Como et al. 2008) and *Atylus guttatus* in Malta (Dimech et al. 2006). In the rhizome layer, only few anecdotal evidences are present. However, *Erichtonius puntactus* and *Leptocheirus pilosus* seems to be associated with this layer (Chimenz et al. 1989). Generally, the abundance and diversity of crustaceans are important in these compartiments (Kikuchi 1980, Buia et al. 2000). In the matte, abundances and number of species of amphipods may be relatively low. *A. rubella* and *Siphonoecetes dellavalllei* are cited as the dominant species (Borg et al. 2006, Harriague et al. 2006).

Oveall, studies on spatial variations, have focused mainly on spatial patterns in relation to depth, comparison with other habitats or punctual sampling at one scale. The spatial variability at several scales of amphipod assemblages in *P. oceanica* meadows has never been described in detail (but see Sturaro 2007 for a preliminary study), while this topic is essential in community ecology (Underwood 1997) and provides valuable basis for management and conservation.

Fig. 7

Some amphipod species associated to *Posidonia oceanica* meadows that have been considered all along this study. Left to right, top to bottom. *Ampelisca rubella*, *Ampithoe helleri*, *Aora spinicornis*, *Apherusa chiereghinii*, *Apolochus neapolitanus*, *Atylus guttatus*, *Dexamine spiniventris*, *Dexamine spinosa*, *Erichtonius punctatus*, *Eusiroides dellavallei*, *Gammarella fucicola*, and *Hyale camptonyx* (Photo: Sturaro N).


2 mm

Fig. 8

Some amphipod species associated to *Posidonia oceanica* meadows that have been considered all along this study. Left to right, top to bottom. *Iphimedia minuta*, *Leucothoe spinicarpa*, *Caprella acanthifera*, *Phtisica marina*, *Leptocheirus guttatus*, *Orchomene humilis*, *Peltocoxa marioni*, *Liljeborgia dellavallei*, *Caprella sp. (armata-group)*, *Pseudolirius kroyeri* (Photo: Sturaro N).


2 mm

Objectives

The general purpose of this research is to assess and better understand the potential responses of amphipod assemblages in *P. oceanica* meadows to different protection levels, in and outside a MPA. The assessment of responses of marine populations to the establishment of MPAs depends on the ability to distinguish the natural variability from potential effects of protection. Therefore, it is essential to quantify and understand the magnitude and range of natural variability of populations at different scales of observation, notably in seagrass meadows which are heterogeneous environments.

In this context, to achieve this general purpose, specific objectives include: (1) examine variability patterns of amphipod assemblages, over spatial scales spanning five orders of magnitude (1 metre to 100s of kilometres) and the consistency of observations between two consecutive years; (2) identify one or more relevant scales that contributed most to spatial variation, providing clues on important processes for these assemblages; (3) explore the relationships between amphipod faunal variables and habitat features, as a factor likely to account for an important part of the variability; and (4) evaluate the role of fish predation in affecting amphipod assemblages associated with *P. oceanica* meadows.

This work is organized according to the following framework:

After a general introduction (**chapter 1**), and a general materials and methods (**chapter 2**), the following part (**chapter 3**) focuses on the study of natural multiscale variability patterns of amphipod assemblages at the Revellata Bay (Corsica, France). This little polluted area, part of the European Natura 2000 network, provides a comparative reference to a MPA at the regional scale, and gives a valuable basis for the monitoring of *P. oceanica* amphipod assemblages. This part includes specific objectives 1 to 3.

In order to compare different protection levels, **chapter 4** investigates the multiscale variability patterns of amphipod assemblages at the Tavolara-Punta Coda Cavallo Marine Protected Area (Sardinia, Italy) and an adjacent external zone. This part also includes specific objectives 1 to 3.

In the course of the extensive sampling at the Tavolara-Punta Coda Cavallo Marine Protected Area, we discovered a new amphipod species. The content of **chapter 5** describes this new species.

In MPAs, the potential increase of fish predator abundance may have indirect effects that can influence the structure of whole assemblages. **Chapter 6** evaluates the role of fish predation on amphipod assemblages in *P. oceanica* meadows, by means of experimental manipulations of predation intensity. This part corresponds to the specific objective 4.

Finally, **chapter 7** intends to provide an integrated overview by summarizing, discussing and confronting results detailed in previous chapters, of both mensurative and manipulative experiments.

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Chapter 2

General materials and methods

The aim of this chapter is to present the common methodology used in the different studies of this work. All studies were conducted in two different areas of the Western Mediterranean Sea: the Tavolara-Punta Coda Cavallo Marine Protected Area (TMPA, Sardinia, Italy) and the Revellata Bay (Corsica, France). A complete description of study areas can be found in the following chapters.

Sampling designs

In the view of the survey, we applied one of the basic tools needed to analyse the spatial variability of populations at different scales (Underwood 1997), a spatially hierarchical sampling design, both at the TMPA and the Revellata Bay. This approach is the most accurate way to obtain a realistic interpretation of spatial patterns when larger scales are considered (García-Charton et al. 2004). All sampling designs are described in detail in the following chapters.

The choice of comparative study sites is of major importance assessing the potential effects of protection and deserves careful consideration. Although, it is almost impossible that any control is truly comparable with protected ones (Fraschetti et al. 2002), sites were chosen in view of the most possible apparent similarity, in terms of seagrass structure, substrate, topography, depth and other temporal conditions (weather, season, and sampling). At the TMPA, study sites were chosen based on prior knowledge of *P. oceanica* seagrass distribution by the staff of the Management Consortium of the TMPA. Several exploratory dives took place in June and July 2007 to identify the most suitable sampling sites to implement the spatially hierarchical sampling design.

Within each site, permanent frames (delimiting a sector) were set up so that we could avoid any spatial potential variation in meadow features between the two consecutive years of sampling. Frames consisted of PVC tubes of 16 mm diameter. Each sector was delimited by 4 tubes of 3 m long, fixed to the bottom with metal stakes of variable length (30 to 70 cm and section 10 mm) and arranged to form a square of 9 m^2. This surface will be called a sector, along this work.

Sampling campaigns

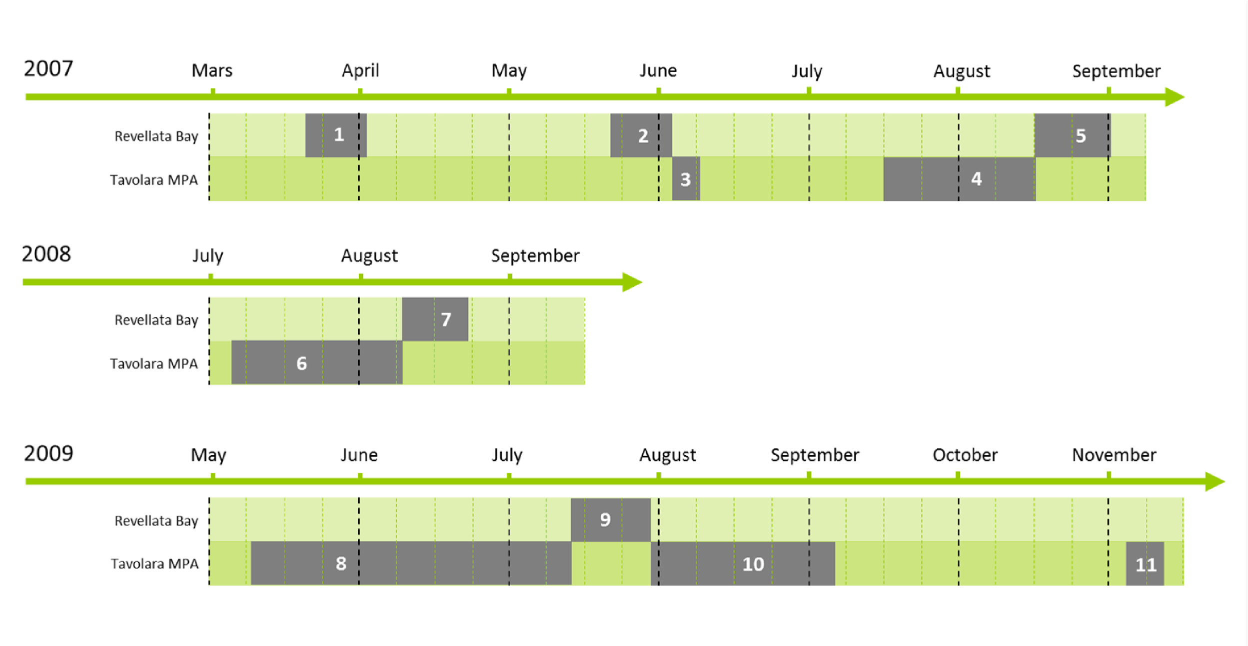
Overall, we carried out 11 sampling campaigns distributed in 251 days of fieldwork during the years 2007, 2008, and 2009 (Fig. 1). The whole team achieved a total of 532 dives (258 dives by the author) for this research. The main activities realized during each campaign were:

1. Selection of study sites, set up of permanent frames, and assessment of the sampling method
2. Finalize the set up of permanent frames and adaptation of the sampling method protocol
3. Exploration and selection of the study sites
4. Set up of permanent frames and sampling of amphipod and *P. oceanica* features
5. Sampling of amphipod and *P. oceanica* features
6. Sampling of amphipod and *P. oceanica* features
7. Sampling of amphipod and *P. oceanica* features
8. Building and set up of exclusion and inclusion cages
9. Assessment of the sampling method
10. Sampling of amphipod and *P. oceanica* features in exclusion and inclusion cages, and fish visual census
11. Remove all the equipment (permanent frames and cages) set up at the TMPA

Fig. 1

Sampling campaigns carried out during this research for 2007, 2008, and 2009 at the Revellata Bay (Corsica) and the TMPA (Sardinia).

Figure 1.


2007
Mars
April
May
June
July
August
September
Revellata Bay
Tavolara MPA
1
2
3
4
5
2008
July
August
September
Revellata Bay
Tavolara MPA
7
6
2009
May
June
July
August
September
October
November
Revellata Bay
Tavolara MPA
9
8
10
11

Sampling methods

The most widely methods used for the vagile fauna in *P. oceanica* meadows are the hand-towed net, the light trap, the corer, and the airlift (see Michel et al. 2010 for comparison). The technique of hand-towed net, developed by Peres & Picard (1964) and standardized by Russo et al. (1985), is the most used. It consists of a net hung on a rectangular metal frame (40 x 20 cm). The diver is responsible for providing a series of strokes in the canopy of the meadow to collect motile fauna. In view of the collection of amphipods, this technique gives adequate results: samples with large number of animals, low species richness and low diversity (Michel et al. 2010). Although, the hand-towed net is a “semi” quantitative method and useful on large areas (see Vinci & Russo 1991). Thus, it was not appropriate for the sampling design applied in this study that takes into account a spatial scale of ~ 1m. The light trap gives good results (diversified sampling, rich with a large number of individuals), but leaves some doubt concerning the representativeness of samples, and do not allow quantitative estimation. The corer provides a quantitative estimate and collects the aboveground component of the plant (leaf and rhizome layer) and the belowground part (rhizome/root covered by sediment). But this method is destructive and time consuming for sorting samples. Therefore, we opted for the airlift sampler, which was reported by Michel et al. (2010) as the more efficient in capturing amphipods, and interesting in biodiversity surveys.

The airlift sampler

Method

The airlift has been originally used for sandy bottoms (Massé 1970), and then was applied for seagrass meadows (Brook 1979). In *P. oceanica* meadows, it was very often used for the collection of vagile fauna (Darchambeau 1995, Como et al. 2008, Vasapollo 2009), polychaetes (Giangrande 1985, Gambi et al. 1995), molluscs (Russo & Terlizzi 1998, Micha 2009), decapod crustaceans (Sánchez-Jerez et al. 2000) and amphipods (Sánchez-Jerez et al. 2000, Sturaro 2007, Scipione & Zupo 2010, Michel et al. 2010).

The airlift used in this research is the one described by Bussers et al. (1983) (Fig. 2). It is made of a PVC sampling tube of 104 cm long and 6.6 cm internal diameter. This tube is provided at one end with a flexible tube of 76 cm long and 7 cm internal diameter, and corresponds to the sampling tube. At the other end, a device allows the fixation of a nylon collecting bag (mesh-size 0.5 mm), which is interchangeable during the dive. The operating principle of the airlift is simple. The air supplied by a scuba tank through a pipe arrives at the base of the PVC sampling tube. The air arrives with pressure in the PVC tube and expands when ascending, which causes suction at its base. The air flow is constant, but can be easily adjusted by controlling the pressure regulator.

Protocol

In each sector, four “bases” of 48.5 cm internal diameter were fixed with metal bars at least 24 hours before the amphipod sampling, in order to delimit previously the sampling area of the meadow. The bases were randomly arranged at the corners of the sector, so have an approximate distance of ~1 m between each base.

In the field, the airlift sampling required the presence of two divers. Arriving near the sampling sector, the first diver was responsible for placing a PVC cylinder (height: 48 cm, diameter: 48.5 cm) on the previously placed "base" in the *P. oceanica* meadow (Fig. 3 left). Once the cylinder enclosed the leaves, it was necessary to press down slightly in the sediment to stabilize it and prevent leakage of organisms between the sediment and the lower edge of the cylinder. At this point, the second diver came up with the airlift and maintained it vertically above the cylinder. The first diver turned on the air, while the second diver targeted the airlift into the cylinder (Fig. 3 right). The operator proceeded to periodic rhythmic upward movements of the sampler to keep the mouth of the device free from the leaves. Collecting time was standardised to two minutes for each sampling unit. The aspiration was limited to the foliar stratum and rhizome layer. Particular attention was taken to avoid the accumulation of large amounts of sediment in the collecting bag.

Figure 2.

Schema of the airlift sampler. 1, collecting nylon bag; 2, fixing device for the collecting bag; 3, PVC tube; 4, pressure regulator; 5, regulating screw for the suction force; 6, compressed air injector; 7, flexible sampling tube. All measures are expressed in centimetres (Schema: Sturaro N).

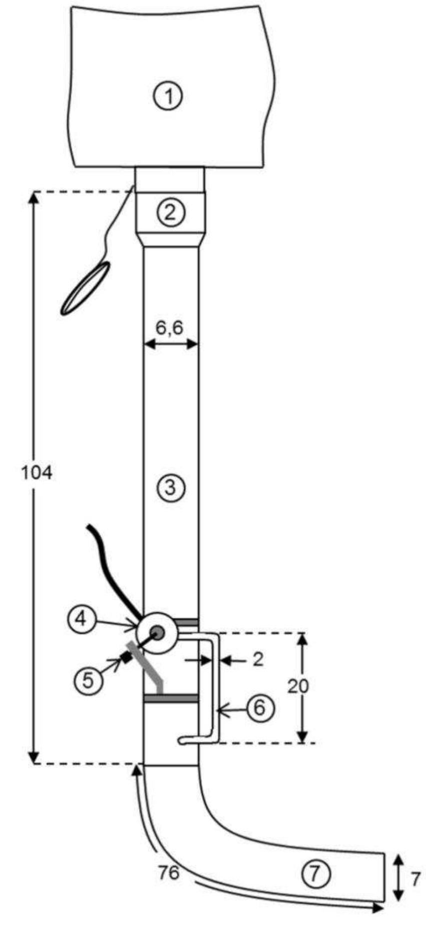


Figure 3.

Set up of the cylinder enclosing *Posidonia oceanica* leaves (left), and airlift sampling (right). (Photos: Trainito E).

Assessment

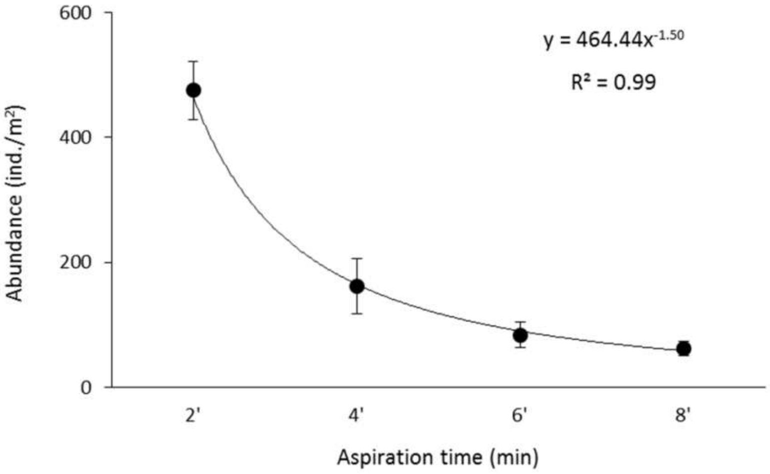
An assessment of the method was based on the analysis of 9 samples taken in March 2007 at the Bay of Revellata. A total of 429 amphipods divided into 24 species were determined. This non destructive method, allowed a near quantitative estimate of the amphipod fauna, and the ability to compare samples at small spatial scales (~ 1 m). The airlift method could collect many amphipods, ranging from 9 to 97 individuals for a sampling area of 0.129 m^2. Numerous samples could be taken on a single dive at low depth using a scuba tank of 15 liters inflated to 200 bars (i.e. 5 samples of 2 minutes each at 12 m depth). Species richness (from 3 to 13 species per sample) and diversity (Shannon-Wiener index (H') from 0.94 to 1.98) were considered low to medium. These values were explained probably by a subsampling of the community studied (too low sampling effort, community complex and dynamic). The dominant species were always the same for all samples: *Apherusa chiereghinii*, *Aora spinicornis,* and *Apolochus neapolitanus*. The risk of including species from the matte and sediment was considered low. Our results showed that samples had not been "contaminated". Indeed, no species characteristics of these environments were identified. This method seems to be not so delicate due to the high turbulence present in the collecting bag during the sampling that could severely damage organisms. However, our analysis showed that the proportion of indeterminate amphipods was relatively low (3.7%) and can only weakly affect the quality of future results and conclusions in this research. Thus, results showed that the airlift was an effective method for collecting amphipods associated with *P. oceanica* seagrass meadows.

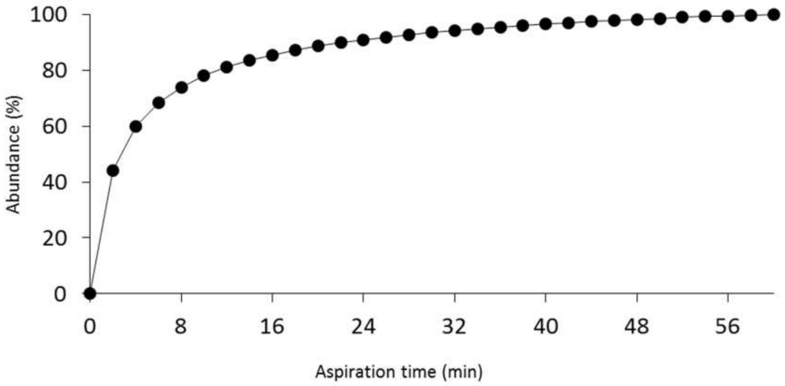
A more precise assessment was based on the analysis of 16 samples taken in July 2009 at the Revellata Bay. As generally described in the literature, this method allows a quantitative estimation of the amphipod fauna. The aim of the assessment was to identify the percentage of the assemblage that was captured during successive samples (2, 4, 6 and 8 minutes) on a previously defined surface. For each aspiration time, 4 replicates were performed. The main results showed that 44% of the amphipod fauna was collected after an aspiration time of 2 minutes and it would take almost 60 minutes to harvest the entire assemblage. However, caution should be taken on this estimated time, as it is an extrapolation (Fig. 4).

Fig. 4

Temporal evolution of the mean abundance ± SE (number of individuals. m^{-2}) of total amphipod, after 2, 4, 6, and 8 minutes of aspiration with the airlift (above). Estimated percentage of the total amphipod assemblage captured according to the aspiration time.

Figure 4.


y = 464.44x-1.50
R² = 0.99
Abundance (ind./m²)
600
400
200
0
2'
4'
6'
8'
Aspiration time (min)

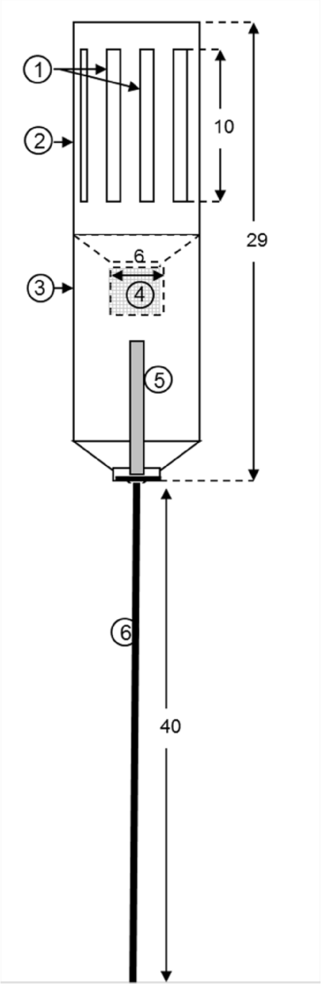

Abundance (%)
100
80
60
40
20
0
0
8
16
24
32
40
48
56
Aspiration time (min)

Light traps

In the view of the new species description (chapter 5), we harvested amphipod specimens at the TMPA using light traps. The device is well known for being less destructive than the airlift sampler (percentage of indeterminate individuals 0 %; Michel et al. 2010). The light traps used in this study are adapted from the latter author. Light traps were made of two nested 1 litre translucent plastic containers, placed in reverse position (Fig. 5). At night time, a diving emergency light stick was placed in the bottleneck. The upper container, pierced with slits, allows organisms attracted by the light to come in. The device was anchored (favor to the metal stake) in the sediment within the meadow at sunset, and gathered just before the sunrise the next morning.

Figure 5.

Schema of the light trap device. 1, slits; 2, top container; 3, bottom container; 4, nylon net; 5, diving emergency light stick; and 6, metal stake. All measures are expressed in centimetres (Schema: Sturaro N).



Sample treatment

Conditioning

All amphipod samples were sieved through a mesh of 500 µm. Then, they were fixed with 4% formaldehyde solution diluted with sea water filtered through a mesh of 0.22 µm at least for 24 hours. Samples were examinated first by eye and then under a binocular microscope. Amphipods collected were placed in a preservative solution (70% ethanol of which was added 1% glycerin).

Species identification

Once at the laboratory, amphipods were counted and identified to species level (with some exceptions) under a microscope. Amphipods were identified using primarily the keys of the Mediterranean amphipod fauna from Ruffo et al. (1982, 1989, 1993, 1998). The interactive key of Myers et al. (2001) was also very useful. In some cases, more recent diagnoses and redescriptions of species were also used, notably the works of Krapp-Schickel & Sorbe (2006) (for the genera *Apherusa*), Krapp-Schickel & Vader 1998, Guerra-García & Takeuchi (2002) and Krapp-Schickel & Takeuchi (2005) for the genera (*Caprella*).

The actual taxonomic status was checked using the WORMS (World Register of Marine Species) database (www.marinespecies.org) and the ERMS (European Register of Marine Species, www.marbef.org/data/erms.php) consulted from March to June 2012. New records for the Mediterranean Italian coast were checked using the check-list of the Amphipoda in Italian seas (Ruffo 2010) and a recent published work of Scipione & Zupo 2010. New records for the Mediterranean French coast were checked using the check-list of Dauvin & Bellan-Santini (2002).

Note that for the genera *Caprella*, some specimens were different from the typical *Caprella acanthifera.* Unfortunately, no very large males were collected to know if it could be a different species. So, we considered as *Caprella cf. acanthifera* (that could be *C. acanthifera* or maybe a new species). Other specimens were categorized as *C. acanthifera* and *Caprella sp.* (armata group) (see Krapp-Schickel & Vader 1998).

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Chapter 3

Multiscale variability of amphipod assemblages in *Posidonia oceanica* meadows (Northwestern Mediterranean Sea)

Abstract

The study of spatial patterns is of ecological importance in order to understand the causes of the distribution and abundance of organisms, and it also provides valuable basis for management and conservation. Amphipod crustaceans are vital to ecological functions in seagrass ecosystems. However, little attention has been given to spatial scales at which amphipod assemblages vary. We examined variability patterns of amphipod populations in *Posidonia oceanica* meadows, over spatial scales spanning four orders of magnitude (1 to 1000s of m) for two consecutive years. This study reports the relevant scales that contributed most to spatial variation of amphipod assemblages and explores the potential processes of the observed patterns, with particular emphasize on habitat features. The number of species, diversity and certain amphipod population densities, exhibited high variation between years. Furthermore, most of the species showed the highest spatial variation in density at the smallest scales (~1 and 10 m), but no differences were observed in the structure of amphipod assemblages at any scales. This patchiness may have been related to habitat features in a weak contribution, while fish predation and behavioural processes are likely to be significant potential factors. Thus, small scale spatial variability might be an important feature of *Posidonia oceanica* amphipod assemblages, and should be considered in designs of future studies.

Keywords

Amphipoda • Hierarchical design • Heterogeneity • Habitat features • Mediterranean Sea

Introduction

One of the main problems ecologists have to face is the inherent heterogeneity of ecosystems (Kolasa & Pickett 1991, Levin 1992, García-Charton & Pérez-Ruzafa 1999). Natural populations are patchy at several spatial and temporal scales (Dayton & Tegner 1984, Schneider 1994), with some scales fluctuating more than others (García-Charton et al. 2004, Fraschetti et al. 2005). Thus, modifications in the structure of assemblages are more evident at some scales (Underwood & Chapman 1996). This variability, particularly at small scales, should not be viewed as a bias or an impediment, but as a valuable information to ecological understanding of ecosystems (Coleman 2002, Fraschetti et al. 2005). The study of spatial patterns is vital in order to elucidate processes at the origin of the distribution and abundance of organisms (Levin 1992), and provide a basis for management and conservation.

To help to sort out spatial patterns, nested hierarchical sampling designs are a powerful tool, and ensure an appropriate replication (Underwood 1997). The spatial scale of observation is methodological based on the observer decision and not on inherent characteristics of ecological processes (Allen & Hoekstra 1991). Patterns can be different depending on the scale of observation (Hewitt et al. 1998), but at a specific scale, various factors could be responsible for the same pattern (Underwood 1997). These factors have each a certain relative importance and a preferential domain of scale at which they act (Wiens 1989, Steele 1997). The approach of investigating patterns of interest at multiple and simultaneous scales, allowed to identify relevant scales of natural variability, and a series of hypotheses concerning potential factors determining a certain spatial patchiness, can be listed and ultimately tested.

Seagrass meadows are some of the most productive ecosystems of coastal areas (Hemminga & Duarte 2000), hosting a wide variety of associated fauna (Mazzella et al. 1992, Williams & Heck 2001). The spatial and temporal variability of the structure of macrozoobenthos assemblages have been attributed to different biological and/or physical factors. At large scales, the position of meadows within a bay, adjacent habitats, as well as hydro-climatic conditions and environmental anthropogenic/natural disturbances are likely to vary (Gillanders 2006, Bell et al. 2006). At small to intermediate scales the habitat structure (with ressources such as food and shelter) is more likely to be responsible for a large part of the variability macrozoobenthos assemblages (Gillanders 2006). Some studies have investigated the potential importance of seagrass structure in the distribution of small macrozoobenthos (Stoner & Lewis 1985, Edgar 1992, Edgar & Roberstson 1992, Worthington et al. 1992, Connolly 1995, Attrill et al. 2000), including amphipod crutaceans (Russo 1989, Sánchez-Jerez et al. 2000, Como et al. 2008, González et al. 2008, Zakhama-Sraieb et al. 2011). For instance, the density of several species was correlated to seagrass (Attrill et al. 2000, González et al. 2008), epiphyte (Schneider & Mann 1991, Zakhama-Sraieb et al. 2011) and litter biomasses (Sánchez-Jerez et al. 2000, Como et al. 2008). However, small macrozoobenthos-seagrass relationships are not well understood in certain systems (e.g. *Posidonia oceanica*). Other factors such as recruitment, competition and predation would act at several spatial scales (Turner et al. 1999, Gillanders 2006). The difficulty remains on evaluating the relative importance of such processes at each scale in influencing assemblage structure (Menge & Farrel 1989).

In the Mediterranean Sea, the endemic seagrass *P. oceanica* forms large meadows, whose ecological and economic roles are widely acknowledged (Boudouresque et al. 2006). These meadows are characterized by a great heterogeneity at small and medium scales (Panayotidis et al. 1981, Balestri et al. 2003, Gobert et al. 2003, Zupo et al. 2006), which could biased the generalization of biological variables, if studied at inadequate spatial scales. *Posidonia oceanica* meadows are important habitat for a large number of species of which the abundant and diverse amphipod crustaceans (Mazzella et al. 1989, Gambi et al. 1992). Amphipods are important food sources for higher level predators such as fishes (Bell & Harmelin-Vivien 1983, Pinnegar & Polunin 2000) and possibly decapods (Lepoint et al. 2000, Vizzini et al. 2002). Furthermore, they are sensitive to anthropogenic and natural disturbances (Thomas 1993, Conlan 1994) and are consequently considered as good potential indicators of ecosystem degradation (Conradi et al. 1997, Guerra-García & García-Gómez 2001), notably in *P. oceanica* meadows (Sánchez-Jerez et al. 2000).

Amphipod assemblages associated with *P. oceanica* meadows have been extensively studied regarding spatial and temporal distribution (e.g. bathymetric and seasonal patterns respectively; Scipione & Fresi 1984, Mazzella et al. 1989, Gambi et al. 1992, Michel 2011, Zakhama-Sraieb et al. 2011), vertical migrations (Sánchez-Jerez et al. 1999a), comparative analyses with other habitats (Sánchez-Jerez et al. 1999b, Como et al. 2008, Vázquez-Luis et al. 2009, Scipione & Zupo 2010), feeding habits (Michel 2011), effects of bottom trawling (Sánchez-Jerez et al. 2000, González et al. 2008) and sampling methods (Michel et al. 2010). However, the spatial variability at several scales of amphipod assemblages in *P. oceanica* meadows has never been described in detail, while this topic is essential in community ecology (Underwood 1997). Patterns that can be observed and the processes that lead to them depend on the extent to which a system is examined (Sale 1998). Moreover, this knowledge can help to choose carefully sampling scales in the design and interpretation of monitoring programs, and save a huge amount of energy avoiding the misdirection of studies of irrelevant processes (Underwood 1997).

This study used a hierarchical sampling design for two consecutive years to: (1) examine variability patterns of the structure of amphipod assemblages, general descriptors (total density, total biomass, number of species, diversity, equitability), as well as species densities and biomasses in

P. oceanica meadows, over spatial scales spanning four orders of magnitude (1 to 1000s of m); (2) identify one or more relevant scales that contributed most to spatial variation, providing clues on potential important processes for these assemblages; and (3) explore the relationship between amphipod faunal variables and habitat features, as a factor likely to account for an important part of the variability.

Materials and methods

Study area

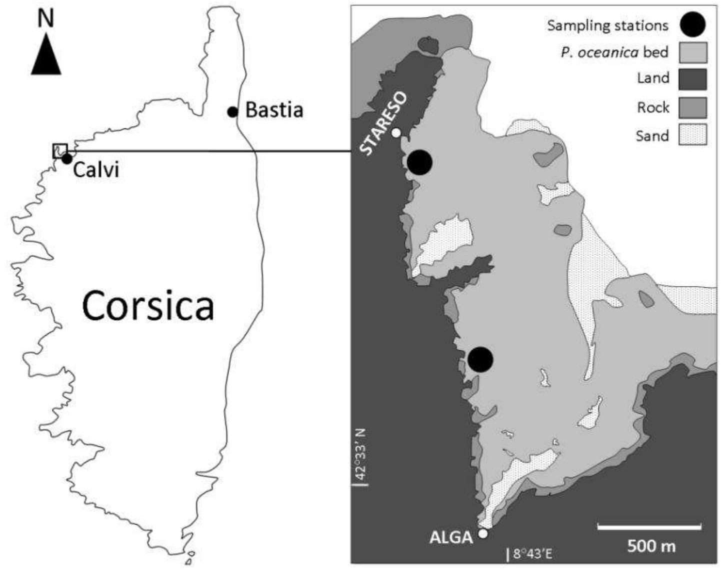
The study was carried out in the Revellata Bay (Corsica, Northwestern Mediterranean Sea; 42°34′N, 8°44′E; Fig. 1) near the oceanographic station STARESO (Station de Recherches Sous-Marines et Océanographiques). The bay has approximately 7.8 km of shoreline, fringed by 53.1 ha of rocky sublittoral habitat covered partially by photophilic macroalgae (e.g. *Halopteris scoparia*, *Dictyota dichotoma*, *Padina pavonica*, *Cystoseira balearica*), 14 ha of sandy substrate and 179 ha of *P. oceanica* seagrass meadows (Sargian 1997), reaching a depth of 40 m (Janssens 2000). Despite the oligotrophic character of coastal Corsican waters, this meadow is one of the most productive in the Northwest Mediterranean Sea (Pergent-Martini et al. 1994). The seawater surface temperatures range from ~13°C in February to ~ 26°C in August. The area is weakly urbanized with a local population of about 5500 people but tourism during summer results in a 10-fold increase. Fishing is allowed and it is estimated that around 4.47 tonnes of fish are removed from the bay each year (Pinnegar & Polunin 2004). The Revellata Bay is part of the European Natura 2000 network since 2008. Overall, the ecological status of seawater in this area is considered as good (Gobert et al. 2009)

Sampling design

The sampling followed a hierarchical sampling design and focused on variability on 4 spatial scales, ranging from m to 1000s of m among two zones (i.e. sampling stations) of the Revellata Bay (Fig. 1, see annexe). For each zone, 2 sites (separated by ~ 100 m) were chosen. In each site, 2 sectors (separated by ~ 10 m) were randomly selected. Each sector was delimited by a permanent frame circumscribing an area of 9 m^2, where 4 replicates separated by ~ 1 m were collected. The study was conducted between 11 and 13 m depth, and between 10 am and 3 pm (local time), to limit respectively bathymetric and nycthemeral variability. The sampling was performed in two consecutive years (August 2007 and 2008), for a total of 64 samples. Weather conditions (sunny and calm) were the same during the two sampling periods.

Figure 1.

Location of the two sampling stations (black points) and distribution of the different benthic ecosystems at the Revellata Bay according to Pasqualini (1997) (Gulf of Calvi, NW Corsica, Mediterranean Sea).



Data collection

Amphipod samples were collected by scuba diving using an airlift (Bussers et al. 1983, Michel et al. 2010). The sampling areas of the meadow were previously 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^2 was suctioned continuously for 2 minutes 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 seawater formalin 4%, 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, classifying them as very frequent ($f \geq 80\%$), frequent ($f\ 40\% \leq f < 80\%$), common ($f\ 10\% \leq f < 40\%$) and occasional species ($f < 10\%$). Amphipod species were quantified as density (individuals. m^{-2}) and biomass (mg dry weight. m^{-2}) after drying at 60°C for 48 h. The amphipod assemblage was characterized according to general descriptors: number of species (S), Shannon-Wiener diversity index $(H') = -\sum_i p_i \log(p_i)$ where p_i is the proportion of the total count arising from the i^{th} species, and Pielou's evenness index $(J') = \frac{H\prime}{\log S}$ where S is the total number of species.

In order to assess relationships between amphipod faunal and habitat variables, 3 *P. oceanica* shoots were collected in 2008 in the same surface area as for previously sampled amphipods, for a total of 96 shoots. The *P. oceanica* meadow was characterized with 5 common used descriptors: shoot density (shoots.m^{-2}; n = 4 per sector), leaf and epiphyte biomasses (g dw.$shoot^{-1}$; n = 12 per sector), as well as Coefficient A (percentage of leaves per shoot having alteration marks; n = 12 per sector). In addition, leaf litter material along with macrophytes debris, were collected from airlift sampling area and was put inside plastic bags, and quantified as litter biomass (g dw.m^{-2}; n = 4 per sector) after drying at 60°C for 96 h.

Data analyses

Univariate analyses

Analysis of variance (ANOVA) was used to examine the effects of the factors year, zone, site and sector on amphipod general descriptors, on the density of the most common species ($f \geq 10\%$) and on the biomass of species accounting for more than 3% of the total amphipod biomass. The model used was $X_{ijklm} = \mu + Y_i + Z_j + Y_i \times Z_j + S_k(Y_i \times Z_j) + Se_l\left(S_k(Y_i \times Z_j)\right) + T_{ijklm}$ where the site (S) factor was nested within year and zone, the sector (Se) factor was nested within site. All factors were random. T_{ijklm} were the replicates (the error term in the model). X_{ijklm} represented each replicate (m) of the dependent variable in any sector (l) and site (k) in a given zone (j) and year (i). μ was the overall mean.

Habitat descriptors available for 2008 were analyzed using a nested analysis of variance (Underwood 1997). The model used was $H_{ijkl} = \mu + Z_i + S(Z)_{ij} + Se(S(Z))_{ijk} + T_{ijkl}$ where zone (Z) was a random factor, the site (S) factor was random and nested within zone, the sector (Se) factor was random and nested within site, and T_{ijkl} were the replicates. H_{ijkl} represented each replicate (l) of the dependent habitat variable in any sector (k) and site (j) in a given zone (i). μ was the overall mean.

Prior to these analyses, normality and homogeneity of variances were checked using Kolmogorov-Smirnov's and Cochran's tests respectively. When these assumptions were not met, data were transformed by $\sqrt{(x+1)}$ or $\log(x+1)$. Whenever variances remained heterogeneous, untransformed data were analyzed, as ANOVA is a robust statistical test and is relatively unaffected by heterogeneity of variances, particularly when the experiments are balanced and contains 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 reduced to ≤ 0.01. When ANOVA indicated a significant difference for the year and zone factors, the source of differences was identified using the post-hoc Student–Newman–Keul (SNK) test (Underwood 1997). The significance level was set to 0.05 when ANOVA conditions were met.

Multivariate analyses

Effects of year and different spatial scales on the structure of amphipod assemblages were analyzed as abundance data using a 4-way permutational multivariate analysis of variance (PERMANOVA, Anderson et al. 2008). In the design, all factors (*i.e.* year, zone, site and sector) were treated as random with 2 levels, site was nested in zone and sector in site. 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 run using 9999 random permutations. 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 high number of total samples (n = 64), only the 16 centroids for the combined factor year x zone x site x 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 similarity within each group of each factor (i.e. year, zone, site and sector), and dissimilarity among these groups, were identified using a similarity percentage analysis (SIMPER) (Clarke & Warwick 2001).

Relationships between habitat variables, amphipod general descriptors and density of the most common taxa (f ≥ 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 variables were analyzed using distance-based linear models (DISTLM; Legendre & Anderson 1999, McArdle & Anderson 2001). DISTLM performs variation partitioning for sets of explanatory variables, and allows for significance testing of explanatory variables for a multivariate response variable in the form of a resemblance matrix (Anderson et al. 2008). Prior to analysis, the collinearity among explanatory variables was tested. The analysis was based on the Bray-Curtis distance measure after square-root transforming the abundance data. The "best" selection procedure, with AIC (Akaikes's information criterion) 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+ softwares.

Results

Amphipod assemblages

A total of 3337 amphipod specimens belonging to 36 taxa and 22 families were identified in this study, of which 13 species were found in all zones and across sampling years (Table 1). Gammaridea and Caprellidea represented respectively 89% and 11% of the total number of species, and 83% and 17% of the total number of individuals. Families Caprellidae, Dexaminidae and Lysianassidae showed the highest number of species (4 species in each family). The 3 most frequent species (f > 80%; *Apherusa chiereghinii*, *Aora spinicornis* and *Phtisica marina*) represented respectively 65% and 62% of the total abundance and biomass. Only 5 species were frequent and 9 species common, while 19 species were occasional (f < 10%).

Multiscale variation

Amphipod general descriptors did not show any significant effects at the zone scale (Table 2, Fig. 2). At the species level, only 1 species (*D. spiniventris*) out of 17 (f ≥ 10%), showed different mean densities between zones (zone 1 > zone 2; Table 2). Gammaridae and Caprellidae did not show any significant effects at the zone scale, but their relative abundances were unequally distributed in both zones (mean ± SE; 84 ± 3% for Gammaridae and 16 ± 3% for Caprellidae). Several occasional or common species appeared zone specific. Seven species were found only in zone 1, whereas 6 other species were found only in zone 2 (Table 1).

Table 1.

Mean density (ind. m^{-2}) of the amphipod taxa in the two zones of the Revellata Bay in 2007 and 2008 (gaps indicates absent).

	Zone 1		Zone 2	
	2007	2008	2007	2008
Gammaridea				
Ampelisca rubella (Costa, 1864)			·	·
Amphilochus neapolitanus (Della Valle, 1893)	•	●	•	●
Ampithoe helleri (Karaman, 1975)	•	●	•	•
Aora gracilis (Bate, 1857)	·			
Aora spinicornis (Afonso, 1976)	⬤	⬤	●	●
Apherusa chiereghinii (Giordani-Soika, 1950)	⬤	⬤	⬤	⬤
Atylus guttatus (Costa, 1851)	·	•	·	·
Cymadusa crassicornis (Costa, 1853)			·	
Dexamine spiniventris (Costa, 1853)	•	●	•	•
Dexamine spinosa (Montagu, 1813)	•	•	·	·
Ericthonius punctatus (Bate, 1857)		•		•
Eusiroides dellavallei (Chevreux, 1899)	•	•	•	·
Gammarella fucicola (Leach, 1814)	·	·		·
Guernea coalita (Norman, 1868)	·			
Hyale camptonyx (Heller, 1866)		•		
Iphimedia minuta (Sars, 1882)	•	•	•	•
Jassa ocia (Bate, 1862)				·
Leptocheirus guttatus (Grube, 1864)		·	·	•
Leucothoe spinicarpa (Abildgaard, 1789)		•	•	·
Liljeborgia dellavallei (Stebbing, 1906)	•	•	•	•
Lysianassa pilicornis (Heller, 1866)				•
Lysianassina longicornis (Lucas, 1849)		•		·
Maera grossimana (Montagu, 1808)			·	
Microdeutopus sp.			·	
Orchomene humilis (Costa, 1853)	•	·		
Orchomene similis (Chevreux, 1912)		·		
Peltocoxa marioni (Catta, 1875)		·	·	·
Siphonoecetes dellavallei (Stebbing, 1899)		·	•	·
Stenothoe monoculoides (Montagu, 1815)		·		·
Synchelidium longidigitatum (Ruffo, 1947)		·		
Tmetonyx nardonis (Heller, 1866)	·	•		·
Tritaeta gibbosa (Bate, 1862)	·			
Caprellidea				
Caprella acanthifera (Leach, 1814)	•	•	·	•
Caprella sp. (armata-group) (see Krapp-Schickel & Vader, 1998)		•		·
Phtisica marina (Slabber, 1769)	●	⬤	●	●
Pseudoprotella phasma (Montagu, 1804)	•	●	•	•

· < 1 • 1-10 ● 11-50 ⬤ 51-200 ind.m^{-2}

The highest spatial heterogeneity was found at smaller scales. At the sector scale (~ 10 m), results showed that the density of total amphipod and 8 species (5 Gammaridea and 3 Caprellidea) exhibited significant large heterogeneity (Table 2, Fig. 2). The observed variability in species densities seemed to be also very important at the spatial scale of 1 m (Fig. 3). No spatial variability of general descriptors and species densities were detected at the site scale (~ 100 m; Table 2).

The number of species and diversity differed significantly between year (Table 2), with higher mean values in 2008 (Fig. 2). At the species level, only 3 species (*A. neapolitanus*, *D. spiniventris* and *E. punctatus*) showed significant higher densities in 2008. Patterns of *E. punctatus* were not consistent across zones (Y x Z, $p \leq 0.001$; Table 2). No significant differences among years were recorded for *A. helleri* and *P. marina*, in spite of higher values in 2008 vs 2007 in terms of relative abundance (89 vs 11% for *A. helleri* and 74 vs 26% for *P. marina*, respectively).

Biomass values showed some contrasted results compared to densities (Table 2). *D. spiniventris* did not show significant effect at the zone and year level, whereas differences were found in terms of density. Biomasses of the taxa Gammaridea, and the species *A. chiereghinii* and *P. phasma* were not different anymore between sectors, while the taxa Caprellidea exhibited significant spatial heterogeneity at this scale.

Results of PERMANOVA evidenced that amphipod assemblage structures did not differed between years, zones, sites and sectors (Table 3). In addition, PERMDISP tests did not reveal significant dispersion effects, suggesting that amphipod assemblages were homogenous across these factors. General patterns showed by nMDS plot reflected the results of the PERMANOVA and PERMDISP (Fig. 4). Groups of centroids belonging to each zone and year were not clearly separated.

Table 2.
Summary of factorial nested ANOVA showing the effects of the factors year (Y), zone (Z) and their interaction (Y x Z), site (S) and sector (Se) on amphipod general descriptors (total density and biomass, number of species, Shannon–Wiener diversity index and Pielou's evenness), on the density of the most frequent amphipod taxa (f ≥ 10%), and on the biomass of the most important taxa in terms of relative biomass (≥ 3%). For biomass data, the level of significance is indicated after the sign "/". ns: not significant; a: not significant at α = 0.01, this conservative level of significance was adopted because variances were heterogeneous and could not be stabilized by transformations; * p ≤ 0.05; ** p ≤ 0.01; *** p ≤ 0.001.

	Y	Z	Y x Z	S(Y x Z)	Se(S(Y x Z))
Total density	ns	ns	ns	ns	***
Total biomass	ns	ns	ns	ns	ns
Number of species	*	ns	ns	ns	ns
Diversity	*	ns	ns	ns	ns
Equitability	ns	ns	ns	ns	ns
Gammaridea	ns/ns	ns/ns	ns/ns	ns/ns	**/ns
Ampithoe helleri	ns	ns	ns	ns	ns
Aora spinicornis	ns/ns	ns/ns	ns/ns	ns/ns	ns/ns
Apherusa chiereghinii	ns/ns	ns/ns	ns/ns	ns/ns	***/ns
Apolochus neapolitanus	*	ns	ns	ns	*
Dexamine spiniventris	**/ns	*/ns	ns/ns	ns/ns	ns/ns
Dexamine spinosa	ns	ns	ns	ns	ns
Ericthonius punctatus	*	ns	***	ns	*
Eusiroides dellavallei	ns	ns	ns	ns	ns
Iphimedia minuta	ns	ns	ns	ns	***
Leptocheirus guttatus	ns	ns	ns	ns	ns
Leucothoe spinicarpa	ns	ns	ns	ns	*
Liljeborgia dellavallei	ns	ns	ns	ns	ns
Orchomene humilis	ns	ns	ns	ns	ns
Caprellidea	ns/ns	ns/ns	ns/ns	ns/ns	ns/***
Caprella acanthifera	a	ns	ns	ns	ns
Caprella sp. (armata-group)	ns	ns	ns	ns	**
Phtisica marina	ns/ns	ns/ns	ns/ns	ns/ns	*/***
Pseudoprotella phasma	ns/ns	ns/ns	ns/ns	ns/ns	**/a

Fig. 2

Mean ± SE values (n = 4) of amphipod general descriptors in each sector within each site of the two zones in the Revellata Bay in 2007 (black) and 2008 (white). Descriptors include total amphipod density (number of individuals per m^2) and biomass (mg dry weight per m^2), and number of species, Shannon–Wiener diversity index (H′) and Pielou's evenness (J′) per sample.

Figure 2.

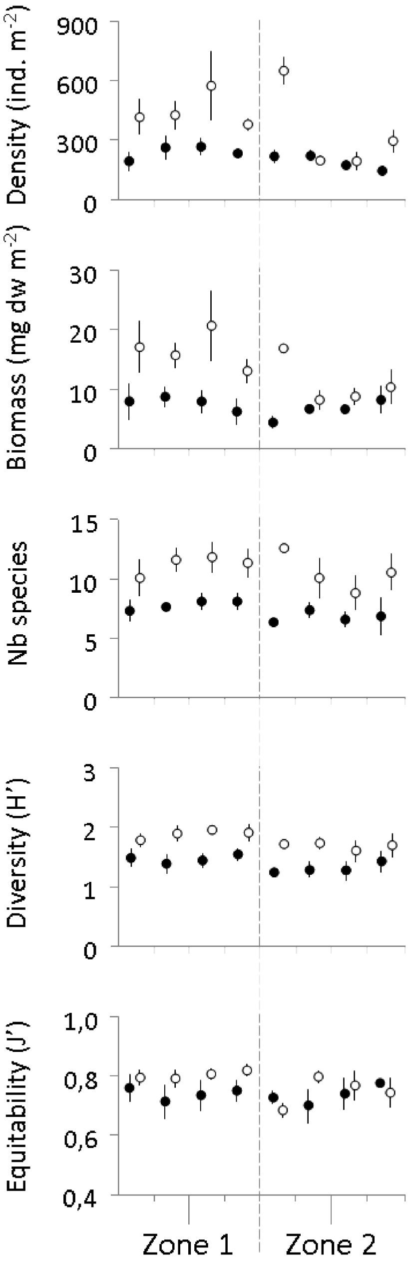

Density (ind. m^{-2})
900
600
300
0
Biomass (mg dw m^{-2})
30
20
10
0
Nb species
15
10
5
0
Diversity (H')
3
2
1
0
Equitability (J')
1,0
0,8
0,6
0,4
Zone 1
Zone 2

Fig. 3

Density values (number of individuals m^{-2}) of the four amphipod species that contributed most to the similarity/dissimilarity between zones in the Revellata Bay in 2007 (black) and 2008 (grey).

Figure 3.

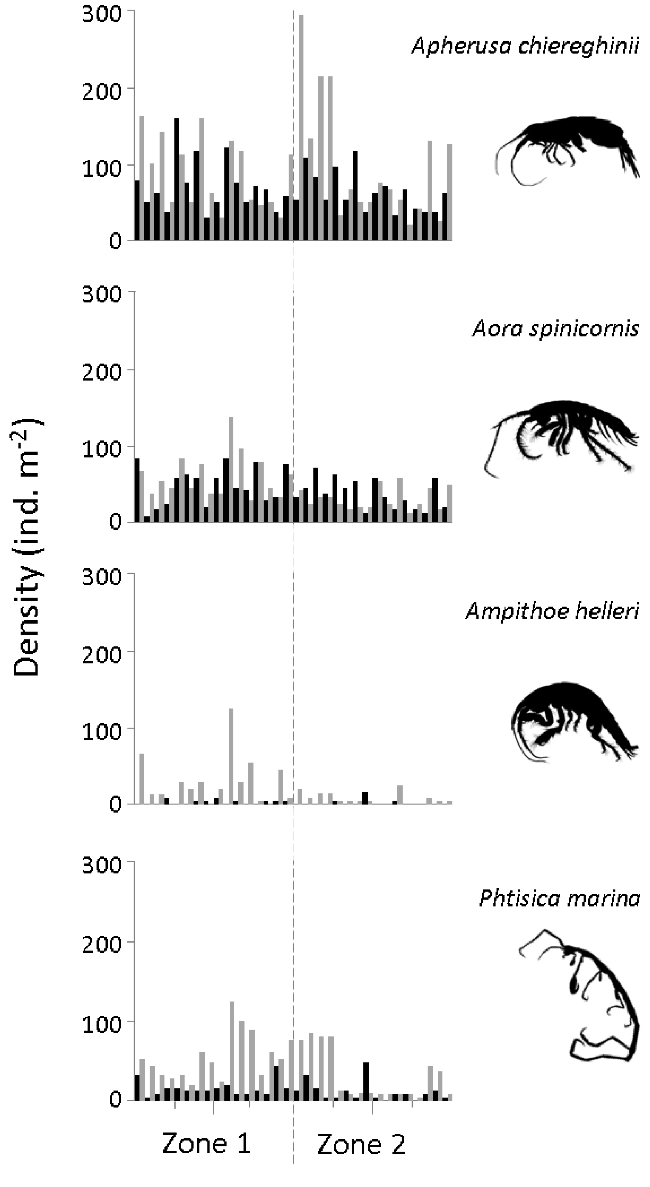

Density (ind. m-2)
300
200
100
0
Apherusa chiereghinii
Aora spinicornis
Ampithoe helleri
Phtisica marina
Zone 1
Zone 2

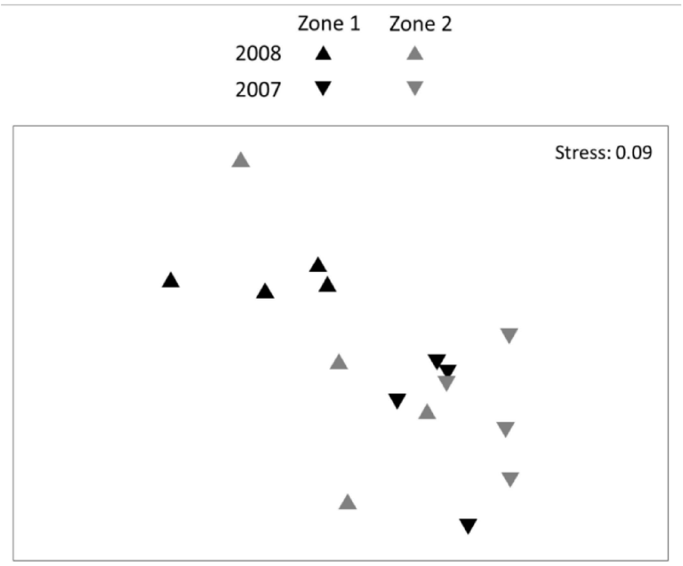
Table 3.

Results from PERMANOVA analysis of amphipod assemblages at the Revellata Bay. Mean square (MS), pseudo-F statistic and level of significance (p) are indicated. ns: not significant; Y: Year; Z: Zone; S: Site; Se: Sector.

Source	MS	Pseudo-F	p
Y	7187	5.90	ns
Z	2946	2.42	ns
Y x Z	1218	1.53	ns
S(Y x Z)	803	1.10	ns
Se(S(Y x Z))	732	1.14	ns

Figure 4.

Non-metric multidimensional scaling (nMDS) ordination of amphipod assemblages in the Revellata Bay. Plot triangles indicate sector centroids, coded by zone and year.



The SIMPER results indicated that for all groups of each factor (i.e. year, zone, site and sector), values of similarity among samples were > 55.4, and made up mainly of contributions from only 3 species: *A. chiereghinii*, *A. spinicornis* and *P. marina*, with a cumulative contribution of about 65 to 85% of the total similarity. These species were the most important in the similarities among all group samples and were considered as typical. The dissimilarities among zones and years (average dissimilarity of 40.7 and 42.3 respectively) were mainly due to *P. marina* (contributing to 10% of the total dissimilarity between zone 1 vs zone 2 and 2007 vs 2008), *A. chiereghinii* (9%) and *A. helleri* (9%).

Spatial variation in meadow structure

Nested ANOVA performed on *P. oceanica* descriptors showed that shoot density, leaf and epiphyte biomasses, coefficient A and litter biomass did not vary significantly at the spatial scale of zones and sites. Significant differences were only detected in leaf biomass at the spatial scale of sectors within site (Table 4, Fig. 5). This implies that leaf biomass potentially generate variability in the amphipod faunal variables measured at the spatial scale of sectors.

Influence of meadow structure on amphipods

Only few weak significant relationships between amphipod faunal and measured habitat variables were identified using multiple regression analyses (Table 5). The number of species, diversity and equitability appeared unaffected by the measured habitat variables. In contrast, amphipod total density and biomass tended to be positively related with respectively *P. oceanica* shoot density and epiphyte biomass. The habitat features accounted between 0 and 30% of the variation in amphipod species densities. No habitat variables were related to gammarids, while *P. oceanica* shoot density and coefficient A appeared to have greater influence on caprellids (Table 5). Indeed, densities of the caprellids *P. marina* and *P. phasma* were positively related to *P. oceanica* shoot density (PC = 0.47 and 0.55 respectively, $p \leq 0.05$). Density of *P. marina* also showed a positive association with Coefficient A.

The distance-based linear regression model indicated weak relationships between amphipod assemblages and habitat variables. The most parsimonious model contained 3 of the 5 measured habitat variables (litter, leaf and epiphyte biomasses) and explained a total of 25.8% of the variation in the distribution and abundance of amphipods, with no significant collinearity among the habitat variables. The first dbRDA axis accounted for 18.6% of the total variation in the amphipod assemblage and distinguished samples based essentially on litter biomass. The second dbRDA axis accounted for 6.1% of the variation in the amphipod assemblage and discriminated samples based on *P. oceanica* leaf biomass (Fig. 6). When looking at the amphipod species, it can be appreciated that all of them (except *Liljeborgia dellavallei*) have negative values in the first axis. Therefore they seem to have some positive relationship with litter biomass. Yet *A. spinicornis*, *A. chiereghinii* and *L. dellavallei* show a stronger relationship with the second axis, and consequently with the factor leaf biomass (positive for *A. spinicornis* and *L. dellavallei* and negative for *A. chiereghinii*; Fig. 6). On the other hand, zone 1 included samples positively associated with the 3 habitat variables (i.e. litter, leaf and epiphyte biomasses).

Table 4.

Results of the nested ANOVA for *Posidonia oceanica* descriptors: shoot density (shoots.m^{-2}), leaf and epiphyte biomasses (g dw.shoot^{-1}), Coefficient A (percentage of leaves per shoot having alteration marks) and litter biomass (g dw.m^{-2}). Mean square (MS), F-statistic and level of significance (p) are indicated. ns: not significant; a: not significant at $\alpha = 0.01$, this conservative level of significance was adopted because variances were heterogeneous and could not be stabilized by transformations; * $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$.

	Zone			Site			Sector		
	MS	F	p	MS	F	p	MS	F	p
Shoot density	111	0.00	ns	30230	2.41	ns	12530	0.81	ns
Leaf biomass	0.93	6.69	ns	0.14	0.49	ns	0.28	7.38	***
Epiphyte biomass	0.22	74.07	a	0.00	0.13	ns	0.02	3.04	a
Coefficient A	285	4.20	ns	68	1.92	ns	35	0.47	ns
Litter biomass	1077	1.52	ns	708	1.56	ns	455	1.93	ns

Table 5.

Results of multiple linear regression analyses examining relationships between measured habitat variables, and amphipod general descriptors and the density of the most frequent amphipod taxa (f ≥ 10%) in the Revellata Bay. Only general descriptors and amphipod species for which habitat variables presented significant partial correlation(s) ($p \leq 0.05$) are listed. The overall regression included shoot density, leaf and epiphyte biomasses, coefficient A and litter biomass. The values of adjusted R^2 and significances (p) are presented. + = positive relationships. ns: not significant; * $p \leq 0.05$.

	Overall regression		Habitat variable			
	R^2adj	p		+ / -	PC	p
Total density	0.235	ns	Density	+	0.49	*
Total biomass	0.116	ns	Epiphyte biomass	+	0.45	*
Caprellidea	0.263	ns	Density	+	0.53	*
			Coefficient A	+	0.47	*
Phtisica marina	0.174	ns	Density	+	0.47	*
			Coefficient A	+	0.44	*
Pseudoprotella phasma	0.209	ns	Density	+	0.55	*

Fig. 5

Mean ± SE values of *Posidonia oceanica* 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), in each sector within each site in each zone of the Revellata Bay in 2008.

Figure 5.

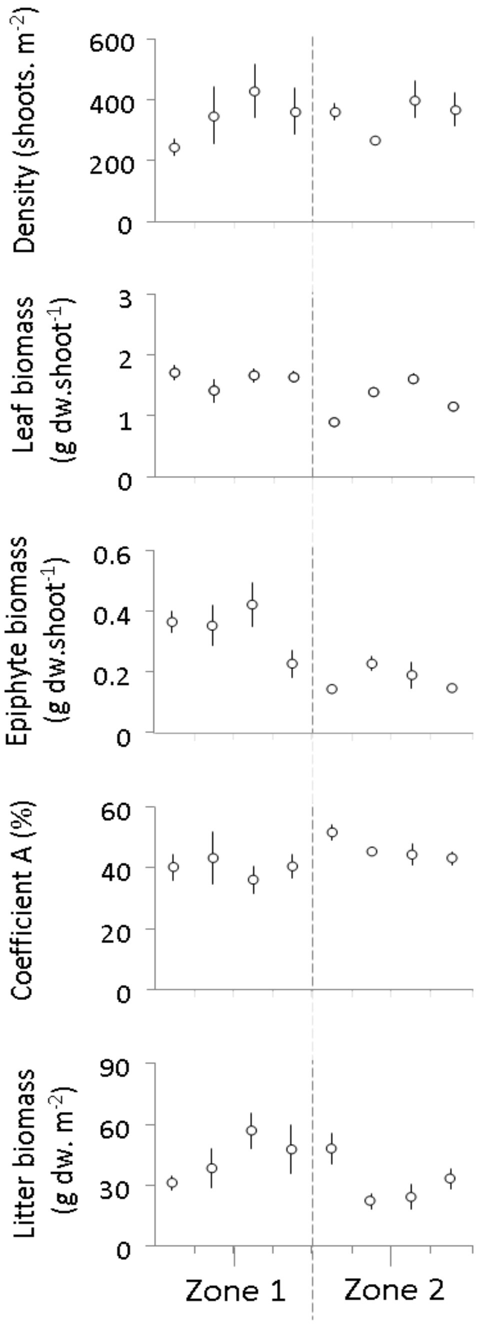
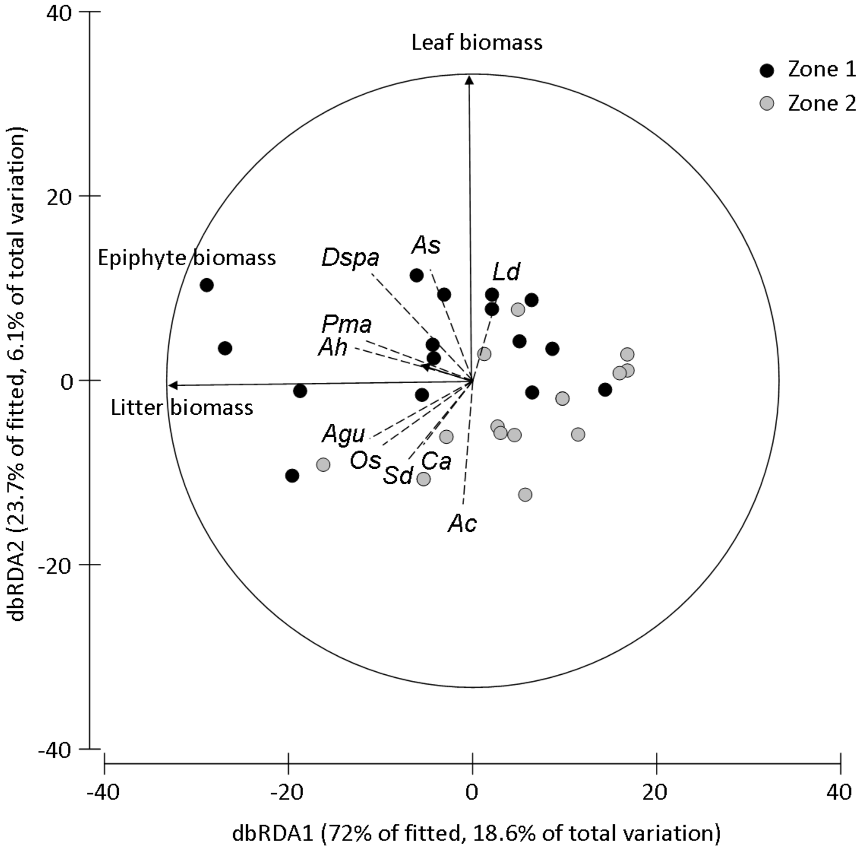

Density (shoots. m-2)
600
400
200
0
Leaf biomass (g dw.shoot-1)
3
2
1
0
Epiphyte biomass (g dw.shoot-1)
0.6
0.4
0.2
0
Coefficient A (%)
60
40
20
0
Litter biomass (g dw. m-2)
90
60
30
0
Zone 1
Zone 2

Fig. 6

Distance-based redundancy ordination (dbRDA) for amphipod species and habitat features at the Revellata Bay. Full and indented vectors indicate the direction of increasing values of the significant habitat variables ($p \leq 0.05$; litter, leaf and epiphyte biomasses) and amphipod species, respectively. Only species with correlations ≥ 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 amphipod samples, coded by zone. Species code: *Ah, Amphithoe helleri*; *As, Aora spinicornis*; *Ac, Apherusa chiereghinii*; *Agu, Atylus guttatus*; *Ca, Caprella acanthifera*; *Dspa, Dexamine spinosa*; *Ld, Liljeborgia dellavallei*; *Os, Orchomene similis*; *Pma, Phtisica marina*; *Sd, Siphonoecetes dellavallei*.

Figure 6.


Leaf biomass
● Zone 1
○ Zone 2
Epiphyte biomass
Litter biomass
Dspa
As
Ld
Pma
Ah
Agu
Os
Sd
Ca
Ac
40
20
0
-20
-40
-40
-20
0
20
40
dbRDA2 (23.7% of fitted, 6.1% of total variation)
dbRDA1 (72% of fitted, 18.6% of total variation)

Discussion

This study of amphipod assemblages in *P. oceanica* meadows of the Revellata Bay has highlighted high values of abundance and number of species, especially when compared with similar studies (in terms of sampling method, depths and periods) from elsewhere in the Mediterranean Sea (Sánchez-Jerez et al. 2000, Como et al. 2008, Scipione & Zupo 2010). Data from Otranto (Southern Italy) in the Adriatic Sea, El Campello (Southeast Spain) and the Gulf of Oristano (Sardinia, Italy) revealed lower values of total number of species (29, 32 and 15 species respectively) compared with our results. This could be explained by our high sampling effort and the idea "the harder you look, the more species you find" (Clarke & Warwick 2001). The estimation of total amphipod density was very variable, in particular at the sector scale (~10m), and ranged from ~ 100 to 600 individuals.m^{-2}, which is the range of values reported in the literature (Sánchez-Jerez et al. 2000, Scipione & Zupo 2010). The assemblage was typified by a few species *A. chiereghinii*, *A. spinicornis* and *P. marina*. Similar results have been obtained in some *P. oceanica* meadows (Scipione & Fresi 1984, Mazzella et al. 1989, Gambi et al. 1992, Michel 2011), but dominant species seems to be very variable according to different geographic areas (e.g. Sánchez-Jerez et al. 2000, Como et al. 2008, Scipione & Zupo 2010). At the regional scale, a common amphipod assemblage structure to several areas cannot be recognized in the Mediterranean Sea (Scipione 1999).

Most of the species in this study showed the highest variation in density at the smallest spatial scales, between replicates (~1m) and sectors (~10m). Analyses in marine coastal ecosystems have documented considerable small scale spatial variation in the distribution and abundance of macrozoobenthos populations across a wide range of habitats (Underwood & Chapman 1996, Menconi et al. 1999, Benedetti-Cecchi 2001, Coleman 2002, Fraschetti et al. 2005), including seagrasses (De Biasi et al. 2003, Sturaro 2007). Potential processes which have been invoked for these patterns include complex sets of local physical and biological interactions

(Fraschetti et al. 2005). Seagrass meadows are heterogeneous habitats, consisting of patches and gaps of different sizes (Nakaoka 2005), especially in *P. oceanica* ecosystems (Panayotidis et al. 1981, Balestri et al. 2003, Gobert et al. 2003, Zupo et al. 2006). Patchiness of microhabitats and/or other resources may create patchiness in the density of an organism (Underwood 1997). At small to intermediate spatial scales seagrass habitat features are more likely to be responsible for a part of the observed differences (Gillanders 2006). However, our data revealed only few weak relationships between amphipod faunal and measures habitat variables. Although shoot density and litter biomass seemed to play a non-negligible contribution, the two statistical methods (i.e. multiple regressions and distance-based linear models) gave some different results, since they are based on different assumptions. Thus, it is difficult here to validate any relationships. In previous studies, amphipod distribution and diversity were positively correlated with shoot density (Vasapollo 2009). Epiphyte (Zakhama-Sraieb et al. 2011) and litter biomasses (Sánchez-Jerez et al. 2000, Como et al. 2008) seemed to affect also amphipod assemblages. Conversely, in the Gulf of Naples, other authors (Mazzella et al. 1989, Gambi et al. 1992, Scipione et al. 1996) did not succeed to link abundance and diversity of amphipods with meadows features and proposed other biotic and abiotic factors to explain patterns observed.

Trophic ressource availability, fish predation and behavioural traits of amphipod species are alternative explanations to explain these amphipod density patterns. However, food availability is unlikely to affect the observed variation at small scales. *Posidonia oceanica* epiphytic macroalgae constitute the main fraction of the diet of all amphipod dominant species (Michel 2011). Although, small scale spatial variability is widely known for epiphytes associated with *P. oceanica* leaves (Pardi et al. 2006, Balata et al. 2007, Castejón-Silvo & Terrados 2012), our results did not show any significant spatial variation at the small scale, and accounted to 20% of the variation of total amphipod biomass and only 1.1% of the amphipod assemblage. In addition, epiflora biomass is high in summer (Lepoint et al.

1999) and this abundant amount of available epiphytes may not be a limiting factor for amphipods in the *P. oceanica* ecosystem (see Michel 2011), at least during our sampling period.

Fish predation is likely to affect the observed abundance pattern at small scales. Fish have been showed to prey upon amphipods in seagrass meadows (Nelson 1979, Caine 1991), and amphipods can constitute a high proportion of the diets (Nelson 1979, Pinnegar & Polunin 2000, Woods 2009), notably in *P. oceanica* meadows (Bell & Harmelin-Vivien 1983, Labropoulou & Plaitis 1995, Zupo & Stübing 2010, see chapter 6). However, predation of fish can be affected by structural complexity, reducing predation rates or efficiency (Heck & Orth 2006 and references therein). Due the high heterogeneity of *P. oceanica* meadows (Gobert et al. 2003, Zupo et al. 2006), fish predation may operate in patchy ways creating gaps and localized differences in the abundance of prey species (Underwood 1997). The labrid *Symphodus rostratus* is adapted to swing among the sparse leaves and feed on a great quantity of amphipods during the day (Bell & Harmelin-Vivien 1983). The gammarid *I. minuta* and the caprellid *P. marina* constituted common preys in the diet of several *P. oceanica* fishes (Labropoulou & Plaitis 1995, Zupo & Stübing 2010, see chapter 6), and presented in our study significant variability at small scales. This factor could mask any relationships with habitat features, especially in summer when fish activity is potentially higher (Garcia-Rubies 1996), and may contribute partially to the high small scale variability of the density of several species.

Given the fact that amphipod species are small scale variable, behavioural processes are likely to alter spatial variation in density (Underwood & Chapman 1996). All types of behavioural responses to other individuals and/or to the habitat may influence the distribution of a species (Underwood 1997, Nakaoka 2005). We have no observations to support this hypothesis and no data are, to our knowledge, available for amphipods living in *P. oceanica* meadows. In *Cymodocea nodosa* and *Zostera marina* meadows, preliminary observations on the vertical distribution revealed that *P. marina* is distributed on both blades and sediments, while *P. phasma* and *C. acanthifera* live mainly on leaves (González et al. 2008). The latter species can spent up to 20% of the time crawling over the substratum with an inchworm-like movement. Caprellid amphipods have poor swimming capabilities (compared to most gammarids) due to reduce pleopods (Caine 1979, González et al. 2008), and can move to other areas distances < 1 m (Keith 1971, Caine 1991). Moreover, most caprellids present substrates preferences. For instance, *P. marina* preferred algae and hydroids, but usually lived also on sponges and bryozoans (Guerra-García 2001). *Posidonia oceanica* meadows comprise these various substrates which may determine the distribution of some species. However, our results did not seem to show major differences between gammarid and caprellid species, certainly because these processes seems to operate at scales smaller than a m. Variability in spatial patterns at the scale of cm (i.e. plant or leaf scales) has not yet been quantified may, but may exist in *P. oceanica* ecosystem. This topic is neglected in the seagrass literature and would deserve further investigation.

In our study, the significant annual variation in density of some gammarid species may be regulated by reproduction and/or habitat availability. Fluctuations in population density of gammarids are frequently observed and related to periods of intense reproduction (Cardoso & Veloso 1996). Several authors have reported that temperature contribute to differences in growth and reproduction of amphipods (Bellan-Santini 1999, Cunha et al. 2000, Maranhão et al. 2001, Maranhão & Marques 2003). In the Revellata Bay, higher water temperatures were recorded in August 2008 compared to August 2007 (Champenois & Borges 2012), with a monthly mean difference of 1.51°C at 3 m depth (STARESO unpublished data). Nevertheless, only three gammarid species showed higher abundance in 2008: *A. neapolitanus*, *D. spiniventris* and *E. punctatus*. The first two, presented more ovigerous females in 2007 (59 and 3% respectively) than 2008 (32 and 1% respectively), suggesting a highest reproductive activity in 2007.

Moreover, high temporal variation in density was already observed for *A. neapolitanus* (Leite 2002). Reproduction is generally confined to strict temperature limits (Bellan-Santini 1999). Higher water temperatures may result in increased metabolic maintenance costs that only allow a decreased allocation of energy for reproduction (Cunha et al. 2000). Thus, the second hypothesis of habitat availability may provide a more plausible explanation.

In August, litter accumulates and reaches the maximum occurrence at the end of summer (or the beginning of the autumn; Romero et al. 1992). It is a highly dynamic environment and can exhibit great spatio-temporal variability within the meadow (Lepoint Pers. Comm., see also Champenois & Borges 2012 for unquantified observations), which may explain the high quantity of litter observed in August 2008. *A. neapolitanus*, *D. spiniventris* and *E. punctatus* are frequently described in studies as species associated to rhizomes of *P. oceanica* (Ruffo et al. 1982, Chimenz et al. 1989, Michel 2011). *E. punctatus* is also observed in areas of detritus accumulation of phanerogams (Ruffo et al. 1982) and showed high monthly and yearly variability (Delgado et al. 2009). Therefore, these species could follow the temporal litter availability, but other more complex interactions may also occur. For instance, *E. punctatus* showed a negative correlation with the abundance of the mysid *Mesopodopsis slabberi* (Delgado et al. 2009), a species living at the edge of *P. oceanica* meadows and sandy bottoms (Barberá-Cebrián et al. 2002). More generally, litter increases habitat complexity, which may also explained the higher number of species and diversity in 2008.

In conclusion, the general picture emerging from this study is that most spatial variability in amphipod density is at the smallest scales (~1 and 10 m). Underlying processes which create these patterns are probably multiple and include habitat features in a weak contribution, while fish predation and behavioural processes are likely to be significant potential factors in the observed variability. However, information needed to estimate the relative importance of these factors is scarce for seagrass associated amphipods, and need further manipulative experiments. This information on spatial patterns might influence the way we will design future experiments. Because most variation in density was found at small spatial scales, replicates and sectors must be the focus of our attention in order to represent adequately amphipod assemblages within sites, notably in environmental monitoring and assessment programmes (e.g. marine protected area surveys). Since spatial patterns do not necessarily remain constant over time (Underwood & Petraitis 1993) and the observed significant annual variation in density of some taxa and general descriptors (number of species and diversity), temporal replication on long term surveys (i.e. 5-10 years) is needed before drawing any relevant conclusions on the dynamics of *P. oceanica* amphipod assemblages.

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Chapter 4

Amphipod assemblages associated with *Posidonia oceanica* meadows in a marine protected area: a multiscale analysis

Abstract

Despite rapid expansion of marine protected areas (MPAs) in the Mediterranean Sea, very few studies have addressed macrozoobenthos responses to protection. Using a hierarchical sampling design spanning four orders of magnitude, we investigated whether a Mediterranean MPA affects amphipod populations associated to *P. oceanica* meadows. This study reports spatial and temporal variability patterns of the structure of amphipod assemblages in four different protection levels and discusses potential confounding effects, in particular habitat features. The composition of amphipod assemblages was patchy at all spatial scales, but differed markedly among protection levels. The multiscale analyses also revealed lower densities and/or biomasses of several frequent taxa within the fully protected area and outside the MPA compared to partially protected areas. Meadow structures account only for a low proportion of the amphipod variability. Factors likely to explain these patterns are probably multiple and may include processes dependent of protection, but also ecological traits of amphipod species (dispersion capabilities and recruitment variability). We support the hypothesis that amphipods seemed to be affected essentially by severe natural and anthropogenic disturbances. Fish predation may be a dominant regulating force of amphipod populations within the fully protected area, whereas point and diffuse water pollutions may greatly reduce several populations in the unprotected study area. Long term multiscale spatial and temporal monitoring of macrozoobenthos assemblages (notably in *P. oceanica* meadows), as well as experimental manipulations, are imperatively needed to better understand potential effects of protection on macrozoobenthos in Mediterranean MPAs.

Keywords

Marine Reserve • Amphipoda • Hierarchical designg • Seagrass habitat

Introduction

Marine coastal ecosystems are subject to strong threats from human activities (Crain et al. 2009). Over the past thirty years, marine protected areas (MPAs) have become heavily promoted worldwide as one of the most preferential tools for ecosystem conservation and fishery management (Agardy 1994, Lubchenco et al. 2003, Claudet 2011). MPAs can indeed have the potential to directly affect density, biomass, size and richness of commercial species (Halpern 2003, Lester et al. 2009, Claudet et al. 2011), but may also indirectly influence the whole structure of biological assemblages and thus the functioning of ecosystems (Pinnegar et al. 2000, Shears & Babcock 2003, Micheli et al. 2005, Guidetti 2006). For instance, the recovery of exploited fish species in MPAs can lead to declines of other species (Micheli et al. 2004), typically mediated through trophic or competitive interactions (Claudet et al. 2011). Fish predation may be higher in MPAs and controls the abundance of many benthic invertebrates (especially sea urchins; McClanahan 1995, Sala & Zabala 1996, Sala et al. 1998, Shears & Babcock 2003, Guidetti 2006). Yet the structure of prey populations may also be strongly affected by other ecological processes such as recruitment, pollution, availability of food and shelters (Sala et al. 1998).

In the Mediterranean Sea, there are only few studies that have adressed the potential effects of protection on macrozoobenthic species (excluding sea urchins). At the Scandola MPA (France) the large macrozoobenthos (essentially echinoderms and molluscs) had lower abundance and species numbers in the MPA than outside, in agreement with the trophic cascade hypothesis that greater predator abundances within the MPA may control invertebrates populations (Boudouresque et al. 1992, Francour 1994). In contrast, at the Ustica MPA (Italy), it was noted that abundance and number of species of polychaetes and molluscs were higher within fully protected sites than outside where fishing was allowed (Badalamenti et al. 1999, Chemello et al. 1999, Milazzo et al. 2000). This coincided with an increase of piscivorous fish abundances (e.g. groupers) leading to lower abundances of small fish feeding on benthic invertebrates (Vacchi et al. 1998, La Mesa & Vacchi 1999).

In these studies, conclusions should be considered with caution because of inappropriate sampling designs (i.e. low site replication and limitation on spatio-temporal scales) which may not distinguish natural variability of ecosystems and the potential variability induced by protection (Allison et al. 1998). Natural heterogeneity of ecosystems is a major problem (Kolasa & Pickett 1991) and makes the evaluation of MPA ecological effectiveness very difficult (García-Charton et al. 2000). Thus, the magnitude of this natural variability should be quantified at each scale of observation using a multiscale hierarchical approach (García-Charton & Perez Ruzafa 1999). Characterization of heterogeneity and exploration of relationships between species and their habitats are now seen as a matter of substantial importance when designing field experiments to measure the potential effects of protection (García-Charton & Perez 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 of some populations, especially in heterogeneous ecosystems such as seagrass meadows.

The seagrass *Posidonia oceanica* forms extensive meadows which constitute key ecosystems in the Mediterranean sublittoral zone (Boudouresque et al. 2006, Gobert et al. 2006). This species is legally protected in many Mediterranean countries (notably in Italy; Platini 2000) and numerous MPAs include large meadows (Francour et al. 2001, Boudouresque et al. 2006). These meadows play a major role in primary production, sediment stabilization, and provide a physical habitat for fish and invertebrate assemblages (Mazzella et al. 1989, Hemming & Duarte 2000). Despite their ecological and economic importance, assessment of the potential effects of protection on macrozoobenthic species associated to *P. oceanica* meadows has been rarely studied.

The amphipod fauna is an important fraction of *P. oceanica* vagile invertebrates, comprising a large number of species with substantial abundance and biomass (Mazzella et al. 1989, Gambi et al. 1992). As food for decapod and fish populations, it plays major role in the energy transfer to higher trophic levels (Bell & Harmelin 1983, Pinnegar & Polunin 2000). Amphipods were already used for monitoring environmental impact in *P. oceanica* meadows (Sánchez-Jerez & Ramos-Esplá 1996; Sánchez-Jerez et al. 2000) and are generally considered as a sensitive group to different kinds of pollutions (Dauvin 1987, Conlan 1994, Bellan-Santini 1999, Gómez Gesteira & Dauvin 2000, Dauvin & Ruellet 2007, de-la-Ossa-Carretero et al. 2012). Moreover, amphipods have generally a weak capacity for dispersion at large scale (Dauvin 1987), recruit heavily and grow quickly (Bellan-Santini 1998) compared to other organisms, which could provide an important view of the changing communities within MPAs (Palumbi 2001).

This study aimed at assessing the effects of different protection levels of a Mediterranean MPA on the structure of amphipod assemblages. We compared the spatial and temporal evolution of potential descriptors of ecological effects among 4 zones with different regulation status. More specifically, we examined (1) the spatial variability patterns at different scales in order to quantify natural variability versus potential protection effects; (2) the consistency of observations between two consecutive years; and (3) to what extent habitat features may account in amphipod variability.

Materials and methods

Study area

This study was carried out in *P. oceanica* meadows at the Tavolara-Punta Coda Cavallo MPA (hereafter TMPA; 40°56´N, 09°44´E) located in north-east Sardinia (Italy; Fig. 1). The MPA covers 15,357 ha, extends along 76 km of coastline and was formally established in 1997, but effective enforcement of protection started in 2003-04. It includes 3 protection zones: zone A (fully protected area = integral reserve = no-take/no-access zone: 529 ha), zone B (partially protected area = partial reserve: 3.113 ha) and zone C (partially protected area = general reserve: 11.715 ha). In zone A, access is restricted to scientists, reserve staff and police authorities. In zone B, only local professional fishermen inhabiting the nearby coastal villages within the MPA are allowed to fish. In zone C, both professional and recreational fishing are allowed under restricted conditions defined by the local Consortium Management of the MPA, with the exception of spearfishing which is totally banned. Outside the MPA, regulations are under the Italian legislation and are less restrictive compared to the inside. The external study zone is located in the Gulf of Olbia, a heavily urbanized area impacted by pollutants originating from point (wastewater and industrial outfalls) and diffuse (ships, leisure tourism) sources. In the MPA, the spatial distribution of major habitat types has been mapped by Bianchi & Morri (2006).

Sampling design

The sampling was done in zones A, B, and C of the TMPA and just outside (External zone: Ext; Fig. 1, see annexe) following a hierarchical sampling design. For each zone, 2 sites (separated by ~ 100 m, medium scale) were chosen. 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^2, where 4 replicates separated by ~ 1 m were collected at constant depth interval (10-15 m) and daytime (10 am-15 pm local time), to limit respectively bathymetric and nycthemeral variability. The sampling was performed in two consecutive years (July-August 2007 and 2008), with the exception of the external zone which was only sampled in 2008, for a total of 112 samples.

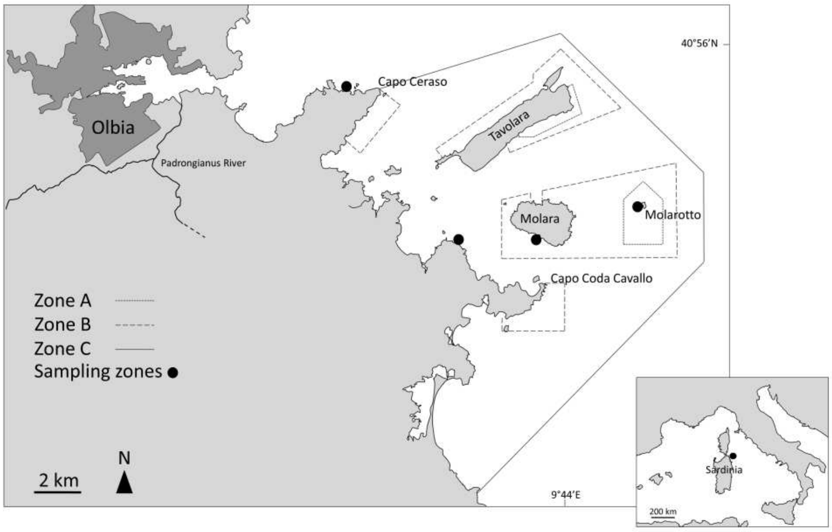
Data collection

Amphipod samples were collected by scuba diving using an airlift (Bussers et al. 1983, Michel et al. 2010). The sampling areas of the meadow were previously 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^2 was suctioned continuously for 2 minutes 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 seawater formalin 4%, 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, classifying them as very frequent ($f \geq 80\%$), frequent ($f\ 40\% \leq f < 80\%$), common ($f\ 10\% \leq f < 40\%$) and occasional species ($f < 10\%$). Amphipod species were quantified as density (individuals. m^{-2}) and biomass (mg dry weight. m^{-2}) after drying at 60°C for 48 h. The amphipod assemblage was characterized according to general descriptors: number of species (S), Shannon-Wiener diversity index $(H') = -\sum_i p_i \log(p_i)$ where p_i is the proportion of the total count arising from the i^{th} species, and Pielou's evenness index $(J') = \frac{H'}{\log S}$ where S is the total number of species. Size (i.e. distance along the dorsal side, from the distal end of the rostrum to the base of the telson) was recorded for the 4 most frequent species and measured for a maximum of 100 individuals for each species, using a DeltaPix camera connected to a stereomicroscope and DpxView Pro Image Management Software (100 microns precision).

Figure 1.

Location and zoning of the Tavolara-Punta Coda Cavallo Marine Protected Area (TMPA) in Sardinia, Mediterranean Sea. Black points represent sampling stations which are showed in zone A (fully protected area), zone B and zone C (partially protected areas), and external zone to TMPA.



In order to assess relationships between amphipod faunal and habitat variables, 3 *P. oceanica* shoots were collected in 2008 in the same surface area as for previously sampled amphipods, for a total of 192 shoots. The *P. oceanica* meadow was characterized with 5 common used descriptors: shoot density (shoots.m^{-2}; n = 4 per sector), leaf and epiphyte biomasses (g dw.shoot^{-1}; n = 12 per sector), as well as Coefficient A (percentage of leaves per shoot having alteration marks; n = 12 per sector). In addition, leaf litter material along with macrophytes debris, were collected from airlift sampling area and was put inside plastic bags, and quantified as litter biomass (g dw.m^{-2}; n = 4 per sector) after drying at 60°C for 96 h.

Data analyses

Univariate analyses

A mixed factorial and nested analysis of variance (ANOVA) was used to examine the effects of the factors year, protection zone, site and sector on amphipod general descriptors, on the density of the most common species ($f \geq 10\%$) and on the biomass of species accounting for more than 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 model used was $X_{ijklm} = \mu + Y_i + Z_j + Y_i \times Z_j + S_k(Y_i \times Z_j) + Se_l\left(S_k(Y_i \times Z_j)\right) + T_{ijklm}$ where year (Y) and zone (Z) were fixed factors, the site (S) factor was random and nested within year and zone, the sector (Se) factor was random and nested within site, and T_{ijklm} were the replicates (the error term in the model). X_{ijklm} represented each replicate (m) of the dependent variable in any sector (l) and site (k) in a given zone (j) and year (i). μ is the overall mean.

Habitat descriptors available for 2008 and comprising data from the external zone, were analyzed using a nested analysis of variance (Underwood 1997). The model used was $H_{ijkl} = \mu + Z_i + S(Z)_{ij} + Se(S(Z))_{ijk} + T_{ijkl}$ where zone (Z) was a fixed factor, the site (S) factor was random and nested within zone, the sector (Se) factor was random and nested within site, T_{ijkl} were the replicates. H_{ijkl} represented each replicate (l) of the dependent habitat variable in any sector (k) and site (j) in a given zone (i). μ is the overall mean.

Prior to these analyses, normality and homogeneity of variances were checked using Kolmogorov-Smirnov's and Cochran's tests respectively. When these assumptions were not met, data were transformed by $\sqrt{(x+1)}$ or $\log(x+1)$. Whenever variances remained heterogeneous, untransformed data were analyzed, as ANOVA is a robust statistical test and is relatively unaffected by heterogeneity of variances, particularly when the experiments are balanced and contains 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 reduced to ≤ 0.01 (Underwood 1997). When ANOVA indicated a significant difference for year and zone factors, the source of differences was identified using the post-hoc Student–Newman–Keul (SNK) test. 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 analyzed as abundance data using a 4-way permutational multivariate analysis of variance (PERMANOVA, Anderson et al. 2008). In order to attain a comparable design between 2007 and 2008, data from the external zone were excluded from the analysis. In the design, year was treated as a fixed factor (2 levels), zone (Z) as a fixed factor (3 levels), site (S) as a random factor (2 levels) nested in Z, sector as a random factor (2 levels) nested in S. 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 run using 9999 random permutations. 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 high number of total samples ($n = 96$), only the 24 centroids for the combined factor year x zone x site x 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 zones were identified using a similarity percentage analysis (SIMPER) (Clarke & Warwick 2001).

Relationships between habitat variables, 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 analyzed using distance-based linear models (DISTLM; Legendre & Anderson 1999, McArdle & Anderson 2001). DISTLM performs variation partitioning for sets of explanatory variables, and allows for significance testing of explanatory variables for a multivariate response variable in the form of a resemblance matrix (Anderson et al. 2008). Prior to analysis, the collinearity among explanatory variables was tested. The analysis was based on the Bray-Curtis distance measure after square-root transforming the abundance data. The "best" selection procedure, with AIC (Akaikes's information criterion) 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+ softwares.

Results

Amphipod assemblages

A total of 4512 amphipod specimens belonging to 51 species and 25 families were identified in this study, of which 10 species were found in all zones and across sampling years (Table 1). Gammaridea and Caprellidea represented respectively 88% and 12% of the total number of species, and 64% and 36% of the total number of individuals. Families Caprellidae (6 species), Aoridae (5 species) and Lysianassidae (5 species) showed the highest number of species. The 3 most frequent species (f > 80%; *Apherusa chiereghinii*, *Aora spinicornis* and *Phtisica marina*) represented respectively 56% and 49% of the total abundance and biomass. Only 4 species were frequent and 8 species common, while 36 species were occasional (f < 10%).

Multiscale variation

Zone scale

Amphipod general descriptors did not show any significant effects at the zone scale (Table 2, Fig. 2). At the species level, 6 species out of 15 (f ≥ 10%), showed different mean densities between zones, of which 3 Gammaridea and 3 Caprellidea. (Table 2). *A. chiereghinii*, *Liljeborgia dellavallei, Peltocoxa marioni, Caprella acanthifera* and *Caprella sp. (armata-group)* were more abundant within partially protected areas (zones B and/or C), while higher abundance was observed for *Caprella tavolarensis* in fully protected area (zone A) (Table 1, 2; Fig. 3). At the suborder level, only mean density of Gammaridae differed significantly among zones with lower abundances within zone A compared to other zones. The relative abundance of Gammaridae and Caprellidae were equally distributed in zone A (50% for Gammaridae and 50% Caprellidae), which was not the case in all other zones (mean ± SE; 67 ± 2% for Gammaridae and 33 ± 2 for Caprellidae). Several occasional species appeared zone specific. For example *Gammarus aequicauda*, *Harpinia zavodniki*, *Hyale camptonyx*, *Leptocheirus pectinatus*, *Perioculodes aequimanus*, *Stenothoe eduardi* were found only in zone A, whereas other species *Ampelisca diadema*, *Cymadusa crassicornis*, *Microdeutopus anomalus*, *Microdeutopus similis* and *Nannonyx propinquus* were found only in zone C. Other occasional species were observed only in zone B (*Lysianassa costae*) and outside the TMPA (*Ampelisca rubella*) (Table 1).

Total amphipod biomass differed significantly between year (Table 2), with higher mean values in 2007 (Fig. 2). This was mainly attributable to gammarids which exhibited significant differences in density across year. *A. chiereghinii*, *D. spiniventris*, *L. dellavallei* showed higher density in 2007 (Fig. 3), while *Ampithoe helleri* was more abundant in 2008, patterns that were consistent across zones (Y x Z, not significant; Table 2). The relative abundance of *A. chiereghinii* consistently showed the same pattern with higher values in 2007 *vs* 2008 for zone A (15 *vs* 8%), B (38 *vs* 24%) and C (46 *vs* 30%). Relative abundance of *P. marina* showed lower values in 2007 *vs* 2008 for zone A (10 *vs* 27%), B (11 *vs* 19%) and C (11 *vs* 26%). In contrast, relative abundance of *C. tavolarensis* in zone A was higher in 2007 (33%) than in 2008 (15%).

Site and sector scales

For smaller spatial scales (~ 10 or 100 m), results showed that only total amphipod biomass and a few species densities exhibited significant large heterogeneity (Table 2, Fig. 2). Densities of 3 species were spatially variable at the site-scale (~ 100 m), of which 1 Gammaridea (*A. spinicornis*) and 2 Caprellidea (*C. acanthifera* and *Pseudoprotella phasma*). At the sector-scale (~ 10 m), 3 species showed significant differences in mean densities, of which 2 Gammaridea (*Apolochus neapolitanus* and *P. marioni*) and 1 Caprellidea (*C. tavolarensis*). No spatial variability in species density was detected at both site and sector-scale (Table 2). At smaller scale (~ 1 m), the observed variability in species density seemed to be very important (Fig. 3).

Biomass values showed some contrasted results compared to densities (Table 2). *Caprella acanthifera* and *D. spiniventris* did not show significant effect at the zone and year level respectively, whereas differences were found in terms of density. A significant variability was detected across years for *A. spinicornis* and *C. tavolarensis* (in a different way between zone A and zone B for *C. tavolarensis*: Y × Z, $p \leq 0.01$; Table 2, Fig. 3). Biomasses of the species *A. spinicornis, C. acanthifera* and *P. phasma* were not different anymore between sites, while *P. marina* exhibited significant spatial heterogeneity at this scale.

Results from the external zone were consistently lower than in protected areas, except for equitability (Fig. 2). The total density, biomass, number of species and diversity of amphipods were respectively 3.9, 3.4, 1.5 and 1.3 times lower in the external zone compared to protected areas. Species densities showed also general lower values (Table 1, Fig. 3).

In contrast to univariate statistical analyses PERMANOVA evidenced that amphipod assemblage structures strongly differed between zones ($p = 0.006$), and to a lesser extent between years, sites and sectors ($p < 0.05$, Table 3). Pair-wise comparisons showed significant difference between zone A *vs* zones B ($p = 0.038$) and C ($p = 0.031$), and suggested a difference between zone B *vs* zone C, although the p-value obtained was borderline ($p = 0.056$). Results indicated that the year and zone factors did not interact together ($p = 0.473$), which allowed us performing PERMDISP test for each of the main effects separately. These revealed strong significant dispersion effects across zones ($p < 0.001$), with greater variability in the structure of amphipod assemblages observed in zone A *vs* zone C ($p < 0.001$) and zone B *vs* zone C ($p < 0.05$), but not for zone A *vs* zone B ($p = 0.118$). Significant differences in dispersion also occurred across years ($p = 0.01$), with an overall greater variability in the amphipod assemblages in 2008. General patterns of segregation showed by nMDS plots reflected the results of the PERMANOVA and PERMDISP (Fig. 4). Groups of centroids belonging to each zone were well separated, particularly centroids of zone A *vs* 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 nMDS plot also showed a clear separation between centroids from 2007 and 2008.

The results of the SIMPER analysis performed on abundances showed that between-zone dissimilarities were greater in 2008 (51.8 to 73.9) than in 2007 (39.2 to 59.1; Table 4). In 2007, *A. chiereghinii* and *C. acanthifera* were the main species contributing to dissimilarity between zones in the MPA. *Caprella tavolarensis* and *A. spinicornis* were also important for explaining respectively the dissimilarity between zone A versus B and C, and zone B versus C. In 2008, species that most contributed to dissimilarity between zones were slightly different: *A. chiereghinii* and *P. marina*. *Caprella tavolarensis* contributed to the differences between Zone A *vs* other zones, while *C. acanthifera* had an important contribution between zones B, C and external.

Size-frequency distributions of amphipod species were unimodal with the mode represented by individuals belonging to the classes 3-4 mm in size for all zones and both years, except in 2007 for zone A, where the first mode was represented by smaller individuals (2-3 mm). In 2007, larger individuals (5-11 mm) were found in zones B and C (Fig. 5).

Spatial variation in meadow structure

No significant differences in shoot density, leaf and epiphyte biomasses were detected between zones. Conversely, litter biomass presented significantly higher mean values in zone B compared to all other zones, while Coefficient A decreased from zone A to zone C and outside the MPA (Fig. 6, Table 5). Shoot density and Coefficient A varied significantly at the scale of sectors (~10 m). All measured variables did not differ at the scale of sites (~100 m).

Influence of meadow structure on amphipods

Multiple regression analyses allowed detecting only few weak significant relationships between the amphipod faunal and measured habitat variables (Table 6). The number of species, diversity and equitability appeared unaffected by the measured habitat variables. In contrast, amphipod total density tended to be negatively related with leaf biomass, while amphipod total biomass was positively related to litter biomass. The combination of habitat features accounted between 0 and 15% of the variation in amphipod species densities. More precisely, litter biomass appeared to have greater influence on gammarids, while biomass of epiphytes were more related to caprellids (Table 6). Indeed, densities of the gammarid *A. spinicornis*, *D. spiniventris* and *E. punctatus* were positively related to litter biomass (PC = 0.29-0.46, $p < 0.05$), while the opposite trend occurred for *I. minuta* (PC = -0.30, $p = 0.025$). There was also some evidence to suggest a positive relationship between the densities of *O. humilis* and *C. acanthifera* and litter biomass, although the p-value obtained was borderline ($p = 0.051$ and 0.059 respectively, Table 6). Density of *A. chiereghinii* was negatively related with leaf biomass and Coefficient A. The caprellid density was positively associated with epiphyte biomass, in particular the species *C. tavolarensis* and *P. marina*. *Caprella tavolarensis* also showed a positive association with Coefficient A.

Results from the distance-based linear regression model were consistent with those obtained for multiple regression, indicating very weak relationships between amphipod assemblages and habitat variables (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 distinguished samples based on litter biomass. Higher values of litter biomass were associated with zone B. The second dbRDA axis accounted for 2.7% of the variation in the amphipod assemblage and discriminated samples based on *P. oceanica* shoot density. This latter variable was not clearly associated with a particular zone, but with a higher variability among the samples within zone A. Only few amphipod species appeared to be influenced by measured habitat variables. Some species, such as *D. spiniventris*, *C. acanthifera* and *E. punctatus* were associated with higher litter biomass, while *I. minuta* was related to lower *P. oceanica* shoot density (Fig. 7).

Table 1.

Mean density (ind. m-2) of amphipod taxa at the 4 different zones at the TMPA in 2007 and 2008 (gaps indicate absent).

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

• < 1 ● 1-10 ⚫ 11-50 ⬤ 51-200 ind.m^{-2}

Table 2.

Summary of factorial nested ANOVA showing the effects of the factors year (Y), zone (Z) and their interaction (Y x Z), site (S) and sector (Se) on amphipod general descriptors (total density and biomass, number of species, Shannon–Wiener diversity index and Pielou's evenness), on the density of the most frequent amphipod taxa ($f \geq 10\%$), and on the biomass of the most important taxa in terms of relative biomass ($\geq 3\%$). For biomass data, the level of significance is indicated after the sign "/". Results of Student-Newman-Keuls (SNK) tests are also showed for the factors year (Y) with two levels (2007 vs 2008), and zone (Z) with three levels (zones A, B and C). ns: not significant; a: not significant at $\alpha = 0.01$, this conservative level of significance was adopted because variances were heterogeneous and could not be stabilized by transformations; * $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$.

	Y	Z	Y x Z	S(Y x Z)	Se(S(Y x Z))	SNK test Y	SNK test Z
Total density	ns	ns	ns	ns	ns		
Total biomass	*	ns	ns	**	ns	2007 > 2008	
Number of species	ns	ns	ns	ns	ns		
Diversity	ns	ns	ns	ns	ns		
Equitability	ns	ns	ns	ns	a		
Gammaridea	*/*	*/ns	ns/ns	ns/**	ns/ns	2007 > 2008	A < B = C
Amphilochus neapolitanus	ns	ns	ns	ns	*		
Ampithoe helleri	**	ns	ns	ns	ns	2007 < 2008	
Aora spinicornis	ns/*	ns/ns	ns/ns	*/ns	ns/ns	2007 > 2008	
Apherusa chiereghinii	**/**	***/**	a/ns	ns/ns	ns/a	2007 > 2008	A < B < C
Dexamine spiniventris	**/ns	a/ns	ns/ns	ns/ns	ns/ns	2007 > 2008	
Ericthonius punctatus	ns	ns	ns	ns	ns		
Iphimedia minuta	ns	ns	ns	ns	ns		
Liljeborgia dellavallei	**	**	ns	ns	ns	2007 > 2008	A < B = C
Orchomene humilis	a	ns	ns	a	ns		
Peltocoxa marioni	ns	*	ns	ns	*		A = B < C
Caprellidea	ns/ns	ns/ns	ns/ns	ns/ns	ns/ns		
Caprella acanthifera	ns/ns	*/ns	ns/ns	***/ns	ns/ns		A < B = C
Caprella sp. (armata-group)	a	***	ns	ns	a		A = B < C
Caprella tavolarensis	ns/**	***/***	ns/**	ns	**/**	2007 > 2008	A > B = C
Phtisica marina	ns/ns	ns/ns	ns/ns	a/**	ns/ns		
Pseudoprotella phasma	ns/ns	ns/ns	ns/ns	*/ns	ns/ns		

Fig. 2

Mean ± SE values (n = 4) of amphipod general descriptors in each sector within each site of the 4 different zones at the TMPA in 2007 (black) and 2008 (white). Descriptors include total amphipod density (number of individuals per m^2) and biomass (mg dry weight per m^2), and number of species, Shannon–Wiener diversity index (H′) and Pielou's evenness (J′) per sample.

Figure 2.

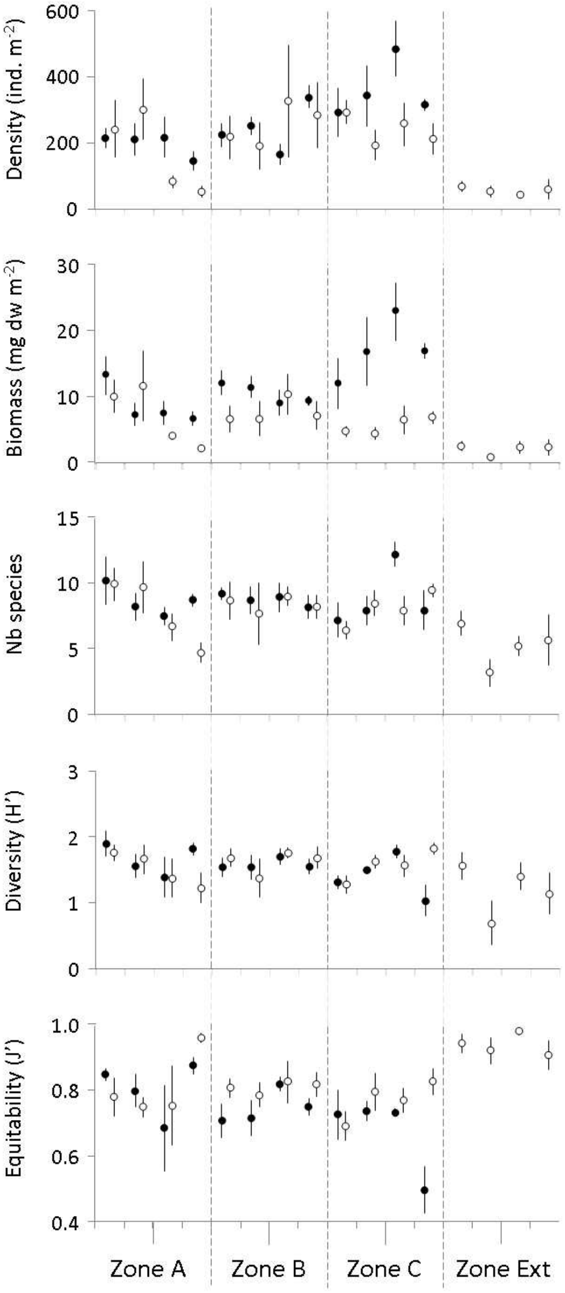

Density (ind. m^{-2})
600
400
200
0
Biomass (mg dw m^{-2})
30
20
10
0
Nb species
15
10
5
0
Diversity (H')
3
2
1
0
Equitability (J')
1.0
0.8
0.6
0.4
Zone A
Zone B
Zone C
Zone Ext

Fig. 3

Density values (number of individuals m^{-2}) of the amphipod species that contributed most to the dissimilarity between zones in each plot of the 4 different zones at the TMPA in 2007 (black) and 2008 (grey).

Figure 3.

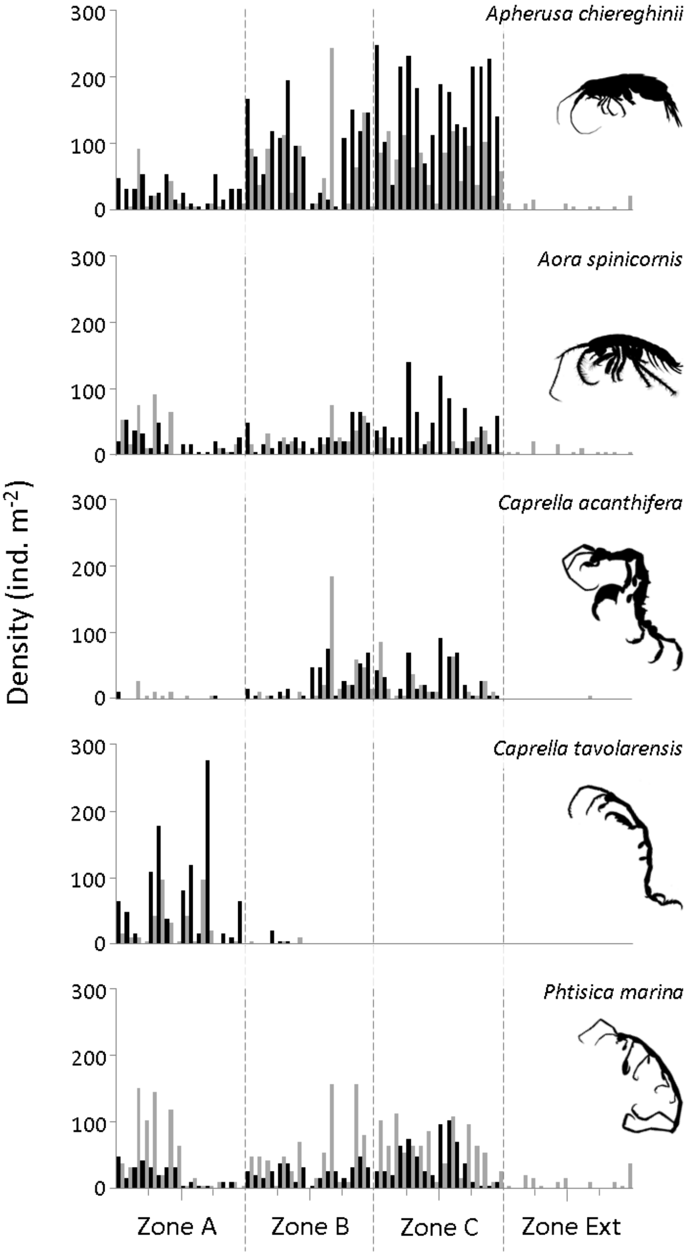

Density (ind. m-2)
300
200
100
0
Apherusa chiereghinii
Aora spinicornis
Caprella acanthifera
Caprella tavolarensis
Phtisica marina
Zone A
Zone B
Zone C
Zone Ext

Table 3.

Results from PERMANOVA analysis of amphipod assemblages at the TMPA. Mean square (MS), pseudo-F statistic and level of significance (p) are indicated. ns: not significant; * p ≤ 0.05; ** p ≤ 0.01. Y: Year; Z: Zone; S: Site; Se: Sector.

Source	MS	Pseudo-F	p
Y	6812	2.79	*
Z	13949	5.72	**
Y x Z	2490	1.02	ns
S(Y x Z)	2439	1.67	*
Se(S(Y x Z))	1462	1.36	*

Figure 4.

Non-metric multidimensional scaling (nMDS) ordination of amphipod assemblages at the TMPA. Plot triangles indicate sector centroids, coded by zone and year.

Table 4.

Results of the SIMPER routine to analyse dissimilarity between the zone types 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 ith species to the average dissimilarity, SD: standard deviation, S: species. Ac: Apherusa chiereghinii, As: Aora spinicornis, Ca: Caprella acanthifera, Ct: Caprella tavolarensis, Pma: Phtisica marina.

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

Figure 5.

Size-frequency distributions of the 4 most frequent amphipod species (*A. chiereghinii*, *A. spinicornis*, *C. acanthifera* and *P. marina*) in the different zones at the TMPA, in 2007 (zone A, n = 173; zone B, n = 296; zone C, n = 394) and 2008 (zone A, n = 185; zone B, n = 290; zone C, n = 287).

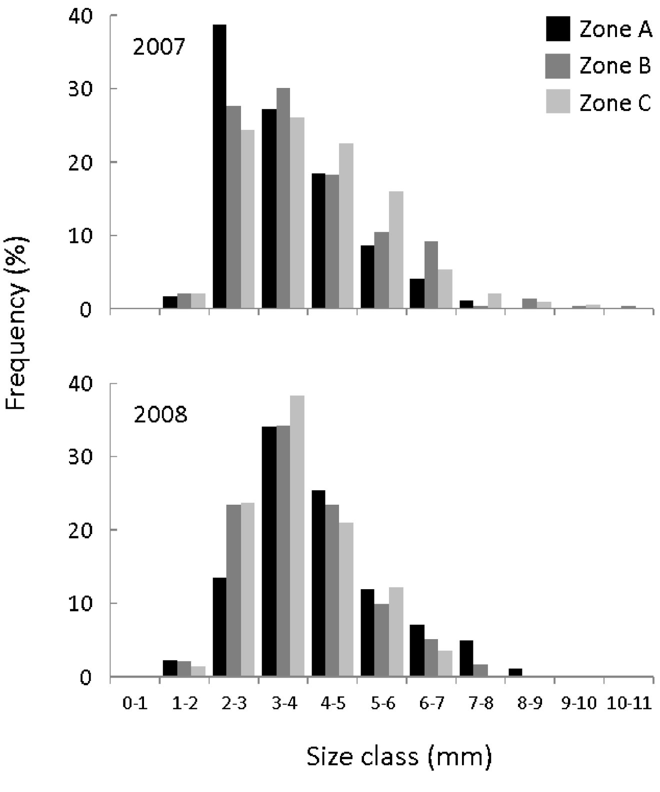


Fig. 6

Mean ± SE values of *Posidonia oceanica* 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), in each sector within each site in each zone at the TMPA in 2008.

Figure 6.

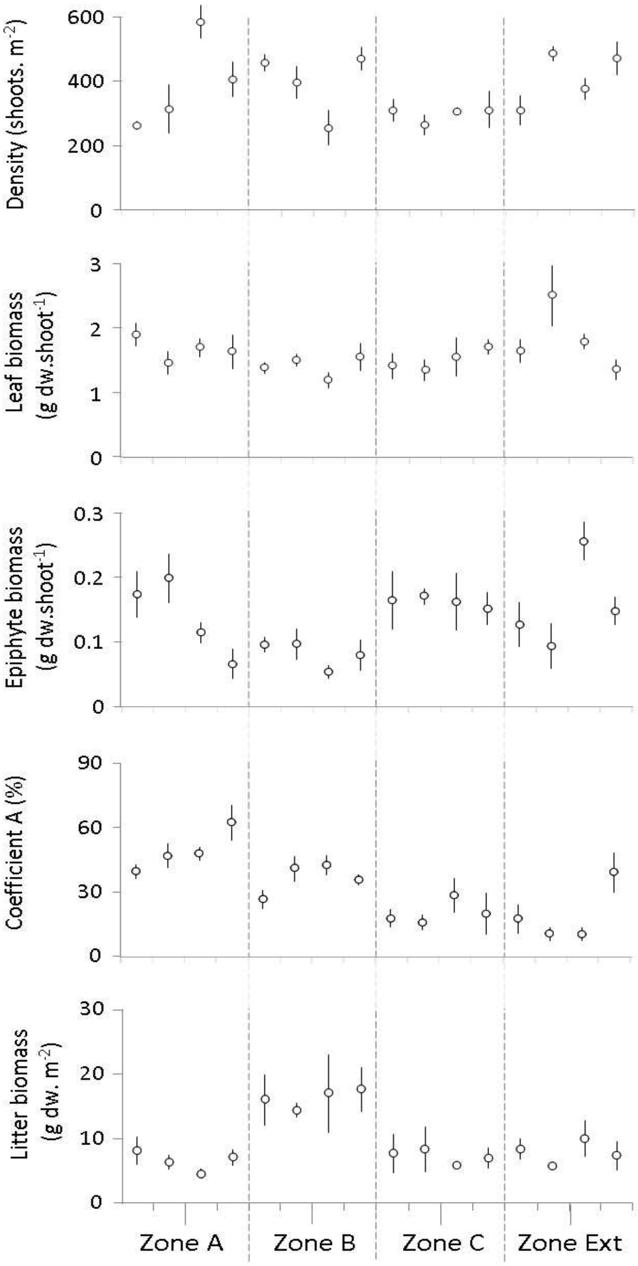

Density (shoots. m^{-2})
0
200
400
600
Leaf biomass (g dw.shoot^{-1})
0
1
2
3
Epiphyte biomass (g dw.shoot^{-1})
0
0.1
0.2
0.3
Coefficient A (%)
0
30
60
90
Litter biomass (g dw. m^{-2})
0
10
20
30
Zone A
Zone B
Zone C
Zone Ext

Table 5.

Results of the nested ANOVA for *Posidonia oceanica* descriptors: shoot density (shoots.m^{-2}), leaf and epiphyte biomasses (g dw.shoot^{-1}), coefficient A (percentage of leaves per shoot having alteration marks) and litter biomass (g dw.m^{-2}). Mean square (MS), F-statistic and level of significance (p) are indicated. ns: not significant; a: not significant at $\alpha = 0.01$, this conservative level of significance was adopted because variances were heterogeneous and could not be stabilized by transformations; * $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$.

	Zone			Site			Sector		
	MS	F	p	MS	F	p	MS	F	p
Shoot density	40271	0.86	ns	46893	1.52	ns	30778	4.21	***
Leaf biomass	0.54	1.69	ns	0.32	0.98	ns	0.33	1.93	ns
Epiphyte biomass	0.02	1.16	ns	0.02	4.49	a	0.00	1.27	ns
Coefficient A	3227	9.42	*	343	0.94	ns	366	2.83	*
Litter biomass	6.46	35.87	**	0.18	0.95	ns	0.19	0.35	ns

Table 6.

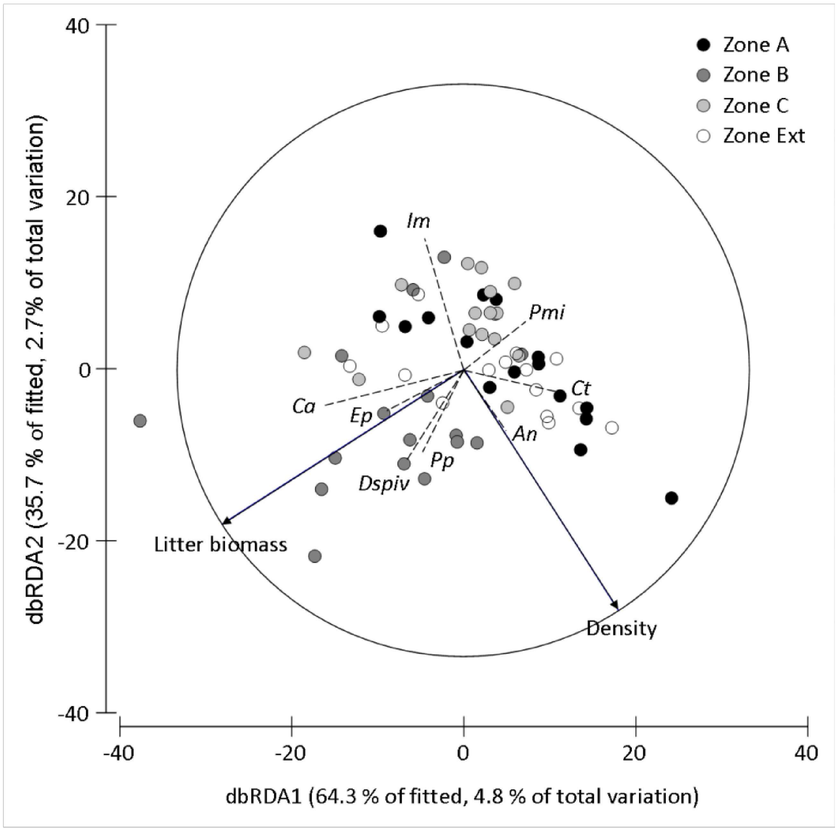
Results of multiple linear regression analyses examining relationships between measured habitat variables, and amphipod general descriptors and the density of the most frequent amphipod taxa (f ≥ 10%) at the TMPA. Only amphipod species and general descriptors for which habitat variables presented significant partial correlations and/or overall regression ($p \leq 0.05$) are listed. The overall regression included shoot density, leaf and epiphyte biomass, coefficient A and litter biomass. The values of adjusted R^2 and significances (p) are presented. + = positive and - = negative relationships. ns: not significant; * $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$.

	Overall regression		Habitat variable			
	R^2adj	p		+ / -	PC	p
Total density	0.042	ns	Leaf biomass	-	0.28	*
Total biomass	0.056	ns	Litter biomass	+	0.31	*
Gammaridea						
Aora spinicornis	0.058	ns	Litter biomass	+	0.29	*
Apherusa chiereghinii	0.068	ns	Leaf biomass	-	0.26	*
			Coefficient A	-	0.27	*
Dexamine spiniventris	0.143	*	Litter biomass	+	0.46	***
Ericthonius punctatus	0.047	ns	Litter biomass	+	0.32	*
Iphimedia minuta	0.074	ns	Litter biomass	-	0.30	*
Caprellidea	0.087	ns	Epiphyte biomass	+	0.34	**
Caprella tavolarensis	0.148	*	Epiphyte biomass	+	0.26	*
			Coefficent A	+	0.41	***
Phtisica marina	0.154	*	Epiphyte biomass	+	0.29	*

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 ≥ 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, coded by zone. Species code: *An*, *Apolochus neapolitanus*; *Ca*, *Caprella acanthifera*; *Ct*, *Caprella tavolarensis*; *Dspiv*, *Dexamine spiniventris*; *Ep*, *Erichthonius punctatus*; *Im*, *Iphimedia minuta*; *Pmi*, *Peltocoxa marioni*; *Pp*, *Pseudoprotella phasma.*

Figure 7.


40
20
0
-20
-40
dbRDA2 (35.7 % of fitted, 2.7% of total variation)
-40
-20
0
20
40
dbRDA1 (64.3 % of fitted, 4.8 % of total variation)
● Zone A
● Zone B
● Zone C
○ Zone Ext
Im
Pmi
Ct
An
Ca
Ep
Pp
Dspiv
Litter biomass
Density

Discussion

The present study showed that amphipod assemblages associated with *P. oceanica* meadows at the TMPA, is typified by high values of density and number of species, as reported for other Mediterranean areas (Scipione & Fresi 1984, Mazzella et al. 1989, Gambi et al. 1992, Sánchez-Jerez et al. 2000, Scipione & Zupo 2010, Michel 2011). The amphipod fauna was mainly represented by *A. chiereghinii*, *A. spinicornis* and *P. marina*, three species which are commonly found in *P. oceanica* meadows and considered as typical of the leaf stratum (Scipione & Fresi 1984, Mazzella et al. 1989, Gambi et al. 1992).

Multiscale spatial variation

The composition of amphipod assemblages was patchy at a variety of spatial scales, from m to 1000s of m, but differed markedly among zones, especially the fully protected area (FPA) clearly differed from partially protected areas (PPAs). Our data also revealed a reduced density and/or biomass of several frequent taxa within the FPA. This is in agreement with the outcomes of previous studies on macrozoobenthos (excluding the numerous studies on sea urchins; McClanahan 1989, Boudouresque et al. 1992), and may be explained considering that density and biomass can be affected by process dependent of protection such as fish predation rate. Increase in abundance and/or biomass of fish predators, and changes in fish assemblages within a FPA may reduce the density of prey species (Shears & Babcock 2003, Guidetti 2006, Claudet et al. 2011). In the sublittoral rocky reefs of the TMPA, Di Franco et al. (2009) showed that fish biomass was greater within the FPA as a consequence of protection. Moreover, some studies have illustrated that predator-prey relationships often cross ecosystem boundaries, connecting among habitats (Knight et al. 2005, Langlois et al. 2006). However, it is well known that fishes in *P. oceanica* meadows do not respond as rocky reef fishes (Francour 1994, Harmelin-Vivien et al. 2008, Valle & Bayle-Sempere 2009), and fish species were probably not the same (i.e. *P. oceanica* shelters predominantly juveniles and adults of small sized fishes; Francour 1997, Del Pilar Ruso & Bayle-Sempere 2006), as well as the location of study sites. In contrast to our results, at the Ustica MPA, abundance and number of species of molluscs and polychaetes were greater inside the FPA than in PPAs (Milazzo et al. 2000, Badalamenti et al. 1999). This coincided with lower abundance of small microcarnivorous fishes and an increase of piscivorous and macrocarnivorous fish abundance (e.g. groupers; Vacchi et al. 1998, La Mesa & Vacchi 1999). However, the composition of the mollusc assemblages differed between the FPA and PPAs (Milazzo et al. 2000), which agree with our observations. Therefore, censuses of fish associated to *P. oceanica* meadows at the TMPA, combined with an experimental manipulation of fish predation intensity (i.e. exclusion and inclusion cages) would be useful to better understand trophic relationships among fish and amphipod assemblages. For instance, the small sized *C. tavolarensis* is little predated in comparison with other accompanying species such as *C. acanthifera* (Sturaro & Guerra-García 2012) and is the only species to present higher values of density and biomass in the FPA compared to the adjacent PPA.

Another plausible factor that could contribute to explain low densities of several species within the FPA would be related to its remote geographic location (see Fig. 1) and isolated *P. oceanica* meadow (Navone et al. 1992). Amphipods lack a pelagic larval stage and have specific habitat requirements (Thomas 1993), which minimizes dispersal effects, and therefore generally form insular populations (Dauvin 1987). Even with their rapid maturation and long reproduction periods in the Mediterranean Sea (e.g from April to September for *C. acanthifera* and the all year for *A. spinicornis*; Jimeno-Fernandez 1993, Bellan-Santini 1998), the insular distribution, as well as some demographic characteristics of certain species (e.g. in our study *L. dellavallei* presented only 14% of ovigerous females with 1-2 eggs per individual) may limit the recolonization and increase of populations within the FPA. In contrast, migratory species such as *P. marina* with no insular distribution (Dauvin 1987) presented 31% of ovigerous females and did not differ between zones in terms of density and biomass.

General descriptors of amphipod assemblages did not show any significant differences among zones of the TMPA. The most simple measurements of assemblage complexity, such as number of species and diversity, could be good descriptors of environmental impacts, but they do not adequately address the problem of changes in patterns of assemblage complexity (Maguran 1988). Sánchez-Jerez & Ramos Esplá (1996) also observed in *P. oceanica* meadows that the number of species and diversity of different crustacean taxa (including amphipods), showed similar values between control and disturbed area. On the other hand, in our study, total amphipod density and biomass was on average respectively 1.5 and 1.3 times greater in PPAs than in FPA. Values of total amphipod density and biomass varied greatly at the scale of replicates, as well at the scale of sites for biomass. This combined with the high annual variation (particularly for biomass), probably obscured any difference between zones. In addition, despite the high level of replication in this study, univariate tests for the main effect of zone type had a low number of degrees of freedom. This influenced negatively the power of the tests, and thus probably reduced the detection of any significant response in these variables.

Comparing protected and unprotected sites suggest clear differences in all general descriptors and species densities. The most likely explanation may be that the main industries and most of the human population are located close to the external study zone in the Gulf of Olbia, so this zone receives anthropogenic and industrial effluents. Water samples analyses in the Gulf of Olbia showed very high concentration in ammonia (with peak > 4μmol/L), total phosphorous, nitrites and Chlorophyll-a compared to values obtained within the TMPA (Consorzio di Gestione Area Marina Protetta Tavolara-Punta Coda Cavallo, 2006). In addition, it is the only zone where the boat traffic (including cruise and pleasure boats) is continuous throughout the year. Amphipods are very sensitive to different kinds of pollution (e.g. oil spills, organic enrichment, sewage pollution and ships' wakes; see Conlan 1994 and references therein) and the disappearance of amphipods or at least a decrease in abundance and diversity is common (Dauvin 1987, Conlan 1994, Gómez Gesteira & Dauvin 2000, de-la-Ossa-Carretero et al. 2012). In rocky bottoms from the French Mediterranean coast, changes in amphipod assemblage composition were related to the degree of pollution. Certain species were excluded by pollution while some other species increased. (Bellan-Santini 1980). In our study, the most obvious example is the presence of the suspension feeder *P. variegatus* in the external zone (and also in the potentially more polluted PPA), a species considered to prefer more or less polluted water (Bellan-Santini 1980). Moreover, *A. spinicornis* and *P. marina* decreased drastically their densities in the external zone compared to sites within the TMPA, as reported also close to sewage outfalls (de-la-Ossa-Carretero et al. 2012). *Phtisica marina* is capable of resisting stress conditions in harbours (Conradi et al. 1997, Guerra-García & García-Gómez 2001), but according to the list of the biotic indice AMBI, this species is very sensitive (Borja et al. 2000). In contrast, *H. camptonyx* and *C. tavolarensis* were essentially found within the FPA, as reported for the genus *Hyale* and *Caprella liparotensis* (very close species to *C. tavolarensis*; Sturaro & Guerra-García 2012) which are restricted to pure and very pure water (Bellan-Santini 1980). The *P. oceanica* meadow of the external zone, presented no significant differences compared to protected meadows in terms of shoot density, leaf and epiphyte biomasses. Other authors reported that a relatively healthy *P. oceanica* meadow can coexist with a variety of human activities (Leriche et al. 2006). Given the particularity of this zone, it is difficult with our data to generalize results of the studied external zone to other potential adjacent sites outside the TMPA (e.g. southern part).

It is obviously not possible to explain all the ecological mechanisms accounting for the observed variability at small and intermediate scales for the various species. Such processes include local biological interactions (e.g. recruitment, competition), and heterogeneity in the physical features of the habitat. Explaining this variability from one to another taxa is not an easy task and it is a common pattern also reported by other authors (Fraschetti et al. 2005 and references therein).

Potential confounding effects

Seagrass meadow structure is one of the factors likely to explain the variability of amphipod assemblages, at least for some species and assemblage general descriptors. Previous studies attribute to habitat features (leaf density, epiphyte cover and biomass, and litter biomass) a substantial part of the observed variation in densities of single amphipod species and the overall assemblage structure (Russo 1989, Schneider & Mann 1991, Edgar & Robertson 1992, Connolly 1995), notably in *P. oceanica* meadows (Sánchez-Jerez et al. 2000, Scipione & Zupo 2010, Zakhama-Sraieb et al. 2011). The influence of habitat features might mask protection effects (García-Charton et al. 2004, Osenberg et al. 2011), but the differences we have detected among protection zones are not likely to be confounded by meadow structure. Spatial variation of meadows features (e.g. shoot density, leaf and epiphyte biomasses) was quantitatively assessed, and they were very similar among protection zones, except for Coefficient A and litter biomass. Litter biomass presented higher values in one of the PPA (Zone B), which seemed geographically more sheltered from hydrodynamic forces and may be associated as a litter accumulation zone. Therefore, the gradual decrease of coefficient A from the FPA to PPAs and outside zone, may be more likely explained by grazing of *Sarpa salpa* than hydrodynamism. A comparison between fish exclusion cages and controls in the FPA showed a significant difference in coefficient A (see chapter 6). On the other hand, meadow features explained only 7.5% of the variation in the distribution and abundance of amphipods, and did not influence or very weakly densities of amphipod species and general descriptors. Litter biomass appeared to have the greatest influence on some species (e.g. *D. spiniventris* and *E. punctatus*), as also observed by Sánchez-Jerez et al. (2000), but there was no consistency between statistical methods for other measured habitat variables, probably because of the weak amphipod-habitat relationships.

Littoral amphipods are known to be greatly influenced by variations in hydrodynamism (Conradi et al. 1997, Bellan-Santini 1998, Guerra-García 2001, Conradi & López-González 2001, Guerra-García & García-Gómez 2001). Despite the remote geographical location and potentially more exposed FPA, it is unlikely that differences in the amphipod assemblages observed between this zone and PPAs, were associated with variation in hydrodynamic conditions for the following reasons: (1) as we have said above, *P. oceanica* meadows features were very similar between zones (with the exception of zone B); (2) sampling was done at depth of 10-15 m in the foliar stratum of the meadow which reduces largely hydrodynamic forces (Gambi et al. 1989); (3) some species (e.g. *P. marina* and *P. phasma*), which are considered as typical of low to intermediate hydrodynamic environments (Conradi et al. 1997, Guerra-García & García-Gómez 2001, Guerra-García et al. 2002), were abundantly present at the potentially more exposed FPA.

Several amphipod populations (e.g. *A. chiereghinii*) showed high annual variation, suggesting that factors other than fish predation could underlie species densities. Large annual fluctuations of amphipod populations (including *P. marina* and species of the genus *Apherusa* and *Gammarus*) have already been observed in temperate waters (Dauvin 1987, Costa & Costa 1999, Guerra-García et al. 2000, Krapp-Schickel et al. 2011), but little had been explored in *P. oceanica meadows* at this temporal scale (variations exist for day-night and seasonal scales; Mazzella et al. 1989, Gambi et al. 1992, Scipione et al. 1996, Sánchez-Jerez et al. 1999a). At the year-to-year scale, populations fluctuations may be tightly coupled with algal biomass (Costa & Costa 1999, Guerra-García et al. 2000), natural amphipod population dynamics (e.g. irregularity of recruitment; Dauvin 1987) and/or fish predation pressure related to yearly variations of fish assemblages (Francour 2000). In addition, Mediterranean amphipod species have fast growths and relatively short life spans (4-6 months up to ~2 years; Bellan-Santini 1998, 1999, Delgado et al. 2009), which could increase this variability. Although sampling data of densities over the year is uncommon in ecological literature because it is time-consuming and costly (Simkanin et al. 2005), much longer temporal series (5 to 10 years), combined with studies on recruitment, would be important to enhance our understanding of interannual variability of species densities. Strategies for managing and monitoring potential effects of MPAs on macrozoobenthos, should be established with a long term perspective in order to support relevant conclusions.

In summary, this study demonstrated that the structure of amphipod assemblages associated with *P. oceanica* meadows was patchy at a variety of spatial scales, but differed clearly among zones characterized with different protection levels. The low densities and /or biomass of several frequent taxa within the FPA and the external zone compared to PPAs are also a notable result of this study. Factors likely to explain these patterns are probably multiple and may include processes dependent of protection, but also biological traits of amphipod species such as dispersion capabilities and recruitment variability. Knowledge on these factors is scarce and usually accessible for a few species. We suggest that fish predation may be a dominant controlling force to regulate amphipods within the FPA, whereas point and diffuse water pollutions may greatly reduce several amphipod populations in the external zone. This study emphasizes the utility of the multiscale approach and the necessity to include measurements such as quantification of potential anthropogenic (e.g nutrient enrichment) and natural disturbances (e.g. fish feeding activity). In front of the rapid expansion of MPAs in the Mediterranean Sea (Juanes 2001), long term multiscale spatial and temporal monitoring of macrozoobenthos assemblages, as well as manipulative experiments, are urgently needed to better understand macrozoobenthos responses to protection in MPAs.

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Chapter 5

A new species of *Caprella* (Crustacea: Amphipoda) from the Mediterranean Sea

Abstract

A new caprellid amphipod, *Caprella tavolarensis* n. sp., is described based on specimens collected from a *Posidonia oceanica* seagrass meadow at the Tavolara-Punta Coda Cavallo Marine Protected Area (Sardinia, Mediterranean Sea). The species is close to *Caprella liparotensis*, but can be clearly distinguished by smaller size, presence of a short rostrum, body elongate and dorsally smooth, absence of serrate carina on the basis of gnathopod 2 and pereopods, mouthparts scarcely setose, absence of fine setae on peduncle of antenna 1 and absence of swimming setae on antenna 2. The number of caprellid species reported from the Mediterranean Sea has increased from 23 (1993) to 41 (2010), consequently, further taxonomical studies should be addressed to properly estimate the total amphipod diversity in the Mediterranean Sea.

Keywords

Amphipoda • Caprellidae • New species • Marine protected area • *Posidonia oceanica* • Mediterranean Sea

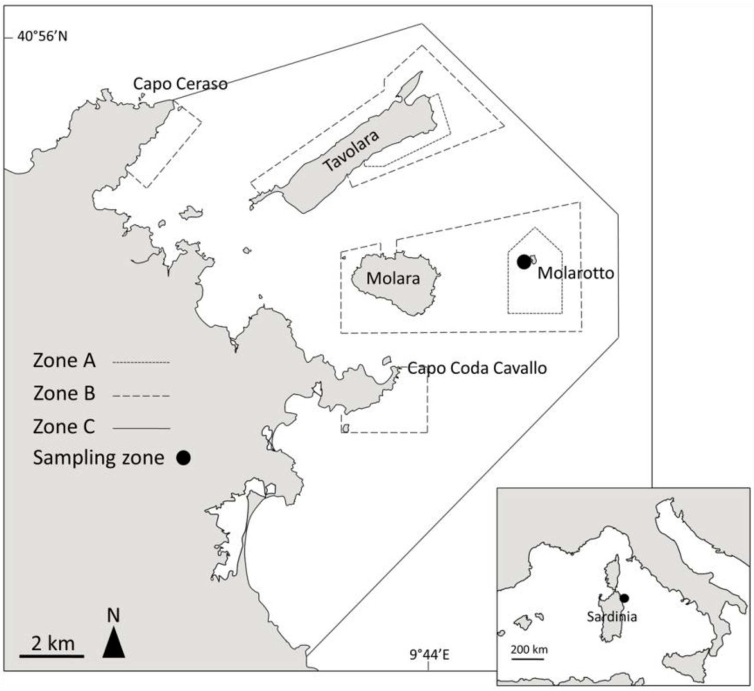
Introduction

Seagrasses are distributed worldwide (600.000 km^2 of the marine bottoms are covered by these spermatophytes) and play an important role in the general coastal dynamics and biology (Larkum et al. 1989, Templado 2004, González et al. 2008). When compared with neighbouring areas, the meadows reveal higher abundances and species richness (Edgar et al. 1994). The main factors contributing to this improvement in biodiversity are availability of microhabitat, protection from predators, trophic resources, sediment settling, hydrodynamic force reduction (see Pranovi et al. 2000). Seagrass meadows of the temperate zone support large numbers of invertebrate species and individuals, thereby providing abundant food for fishes, compared to adjacent unvegetated areas (Nakamura & Sano 2005). Along the coasts of the Mediterranean Sea, five species occur: *Posidonia oceanica* (Linnaeus) Delile, *Zostera marina* Linnaeus, *Zostera noltii* Hornemann, *Cymodocea nodosa* (Ucria) Ascherson and a lessepsian species *Halophila stipulacea* (Forsskål) Ascherson (Buia et al. 2000). In spite of the abundance of these seagrass meadows in the Mediterranean, caprellid communities associated to these plants have been scarcely studied, and the only records of caprellids from these habitats come from general faunistic or ecological studies (see González et al. 2008).

As a part of a general project assessing the effects of protection status (fully, partially and non protected areas) on amphipod communities associated to *Posidonia oceanica* meadows along the Sardinian coast (Italy), several specimens of the genus *Caprella* were collected. Detailed examination revealed that these specimens belonged to a new species close to *Caprella liparotensis*, here described as *Caprella tavolarensis* n. sp.

Figure 1.

Study area showing the sampling site in the Tavolara-Punta Coda Cavallo Marine Protected Area (Sardinia, Mediterranean Sea).
Zone A (integral reserve), *zone B* (general reserve) and *zone C* (partial reserve) are showed



Materials and methods

The sampling was conducted at Molarotto island, an integral reserve area located at the Tavolara-Punta Coda Cavallo Marine Protected Area, NE Sardinia, Italy (Fig. 1). Samples were collected from *Posidonia oceanica* bed (10–15 m) by scuba diving using an airlift sampler and light traps (about methods see Michel et al. 2010). The samples were sieved through a 0.5 mm mesh and fixed in formalin 4%, then transferred to 70% ethanol. Selected specimens were dissected under a Leica dissecting microscope. Appendages of selected specimens were mounted in polyvinyl-lactophenol and camera lucida drawings were made using a Leica compound microscope.

Although the phylogeny and higher classification of the caprellids are still under debate (see Laubitz 1993, Takeuchi 1993, Ito et al. 2008), Myers & Lowry (2003) have recently proposed a new phylogeny and classification for the suborder Corophiidea Leach, 1814. Based on the hypothesis of the evolution of different feeding strategies, the Corophiidea are divided into two infra-orders, the Corophiida and the Caprellida. In their new classification, the superfamily Caprelloidea contains five families: Caprellidae, Caprogammaridae, Cyamidae, Dulichiidae and Podoceridae. The Caprellidae are subdivided into three subfamilies: Caprellinae, Paracercopinae and Phtisicinae. In the present paper, we have adopted the classification of Myers & Lowry (2003) considering the family Caprellidae, and the subfamily Caprellinae.

The symbols used in the present work are: A1, 2 = Antenna 1, 2; UL = Upper lip; LL = Lower lip; LMd = Left mandible; RMd = Right mandible; Mx 1, 2 = Maxilla 1, 2; Mxp = Maxilliped; Gn 1, 2 = Gnathopod 1, 2; P5–7 = Pereopod 5–7; Ab = Abdomen. All the type material is deposited in the Museo di Storia Naturale di Verona, Italy (MVR).

Results

Family Caprellidae Leach, 1814
Subfamily Caprellinae Leach, 1814

Caprella tavolarensis n. sp. (Figs. 2, 3, 4, 5, 6, 7, 8, 9)

Type material Holotype male (used for drawings) (MVRCr 500.0). Paratype female (used for drawings) (MVRCr 500.1). Paratypes dissected (used for drawing the mouthparts) (3 males and 3 females, MVRCr 500.2–500.7). Other paratypes (10 males, 8 females, 2 juveniles, MVRCr 500.8). All the material was collected from the type locality (July–August 2008).

Additional material examined 17 males, 13 females, 2 juveniles, collected from the type locality (July–August 2007).

Type locality All the material has been collected from a *Posidonia oceanica* meadow between 10 and 15 m depth at the Tavolara-Punta Coda Cavallo Marine Protected Area, A zone of Molarotto island (40°52′25′′N, 9°46′35′′E), NE Sardinia, Italy (Fig. 1).

Etymology The specific name refers to the area where the species was found.

Diagnosis Head with very short rostrum. Pereonites 2, 3 and 4 with anterolateral projections. Body dorsally smooth. Peduncular articles of antenna 1 not setose. Antenna 2 without swimming setae. Basis of gnathopod 2 without serrated carina but with inner projection.

Figure 2.

Caprella tavolarensis n. sp. Lateral view of holotype male (MVRCr 500.0) and paratype female (MVRCr 500.1)

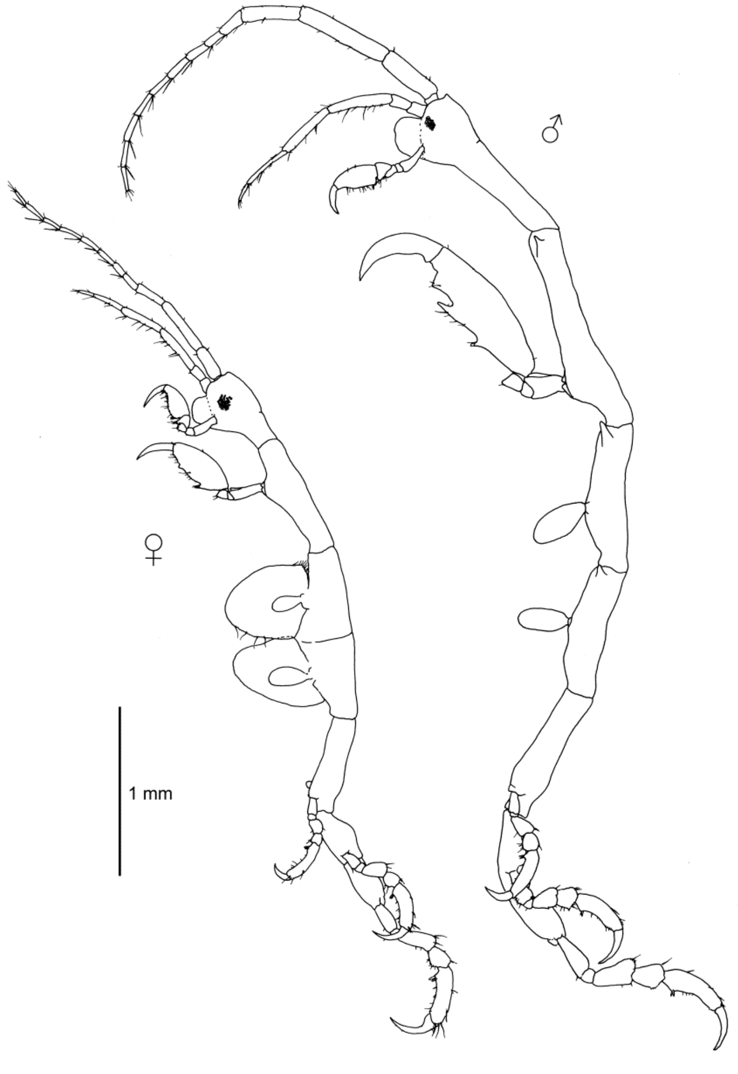
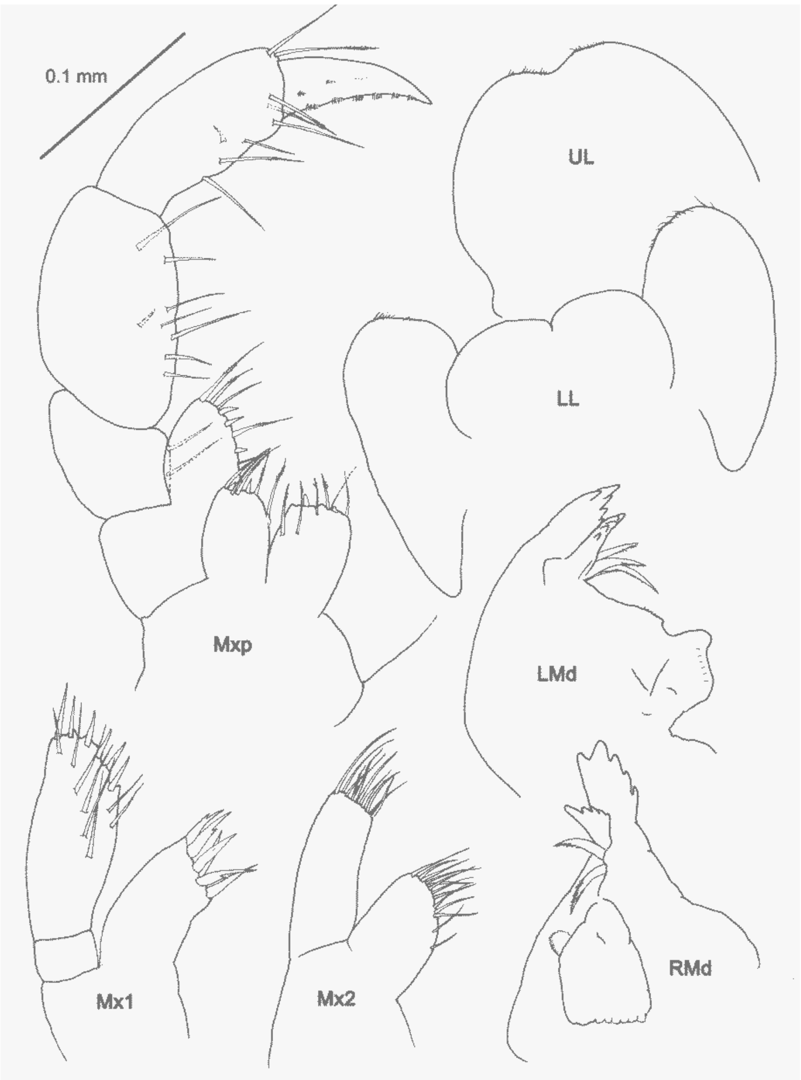


Figure 3.

Caprella tavolarensis n. sp. Mouthparts of paratype male (paratype 1, MVRCr 500.2)



Description

Holotype male (MVRCr 500.0)

Body length 5.8 mm.

Lateral view (Fig. 2). Head with a short rostrum (almost absent in subadult males), suture between head and pereonite 1 present. Body dorsally smooth, without tubercles. A pair of anterolateral projections on pereonites 2, 3 and 4.

Gills (Fig. 2). Present on pereonites 3–4, elongate, length about twice width.

Mouthparts (Fig. 3, figured from paratype 1). Upper lip symmetrically bi-lobed, small setulae apically. Mandibular molars process strong; incisor and lacinia mobilis 5-toothed; left mandible with three pectinated setae, right mandible with only two; small molar flake present on right mandible. Lower lip with inner lobes poorly demarcated and outer lobes scarcely setose. Maxilla 1 outer lobe with six robust and stout setae; distal article of palp with six setae distally and eight lateral setae. Maxilla 2 inner lobe oval and outer lobe rectangular, about 1.5 times as long as the inner lobe. Maxilliped inner plate with three robust and short setae (similar to "teeth") and five plumose setae; outer plate with long, plumose setae and three robust setae (like "teeth"); palp four-articulate, with long plumose setae, article 4 with rows of setulae on its grasping margin.

Antennae (Fig. 4). Antenna 1 about half of the body length; peduncle scarcely setose; flagellum 9-articulate. Antenna 2 flagellum two-articulate, with scarce setae; swimming setae absent.

Gnathopods (Fig. 4). Gnathopod 1 basis as long as ischium, merus and carpus combined; propodus palm with two proximal grasping spines, grasping margin smooth; dactylus elongate. Gnathopod 2 inserted near distal end of pereonite 2; basis short, about one-sixth of pereonite 2 in length, provided with a ventral projection and lacking serrate carina; propodus elongate, length about 3 times width; palm concave with one proximal projection provided with grasping spine; another projection one-quarter length from distal end, followed by "U" notch distally.

Pereopods (Fig. 5, figured from paratype 1). Pereopods 3 and 4 absent. Pereopod 5, 6 and 7 increasing in length; basis without serrate carina; carpus of pereopod 5 and 6 with an inner tooth, and pereopod 7 with two teeth; propodus provided with a pair of grasping spines proximally.

Penes (Fig. 5). Short, length about 1.2 times the width. Abdomen with a pair of two-articulate appendages, a pair of lateral lobes and a single dorsal lobe.

Paratype female (MVRCr 500.1)

Body length 3.5 mm. Similar to male, except for the following characters: flagellum of antenna 1 eight-articulate (Fig. 2); oostegites present (Fig. 2), being slightly setose on pereonite 3; gnathopod 2 (Fig. 4) inserted on the anterior half of pereonite 2, basis about one-fourth of pereonite 2 in length, length of propodus about 2 times width, U-notch lacking; abdomen without appendages, only the lateral lobes and dorsal lobe present.

Figure 4.

Caprella tavolarensis n. sp. Male holotype (MVRCr 500.0) antenna 1, antenna 2 and gnathopods 1 and 2. Female paratype (MVRCr 500.1) gnathopod 2

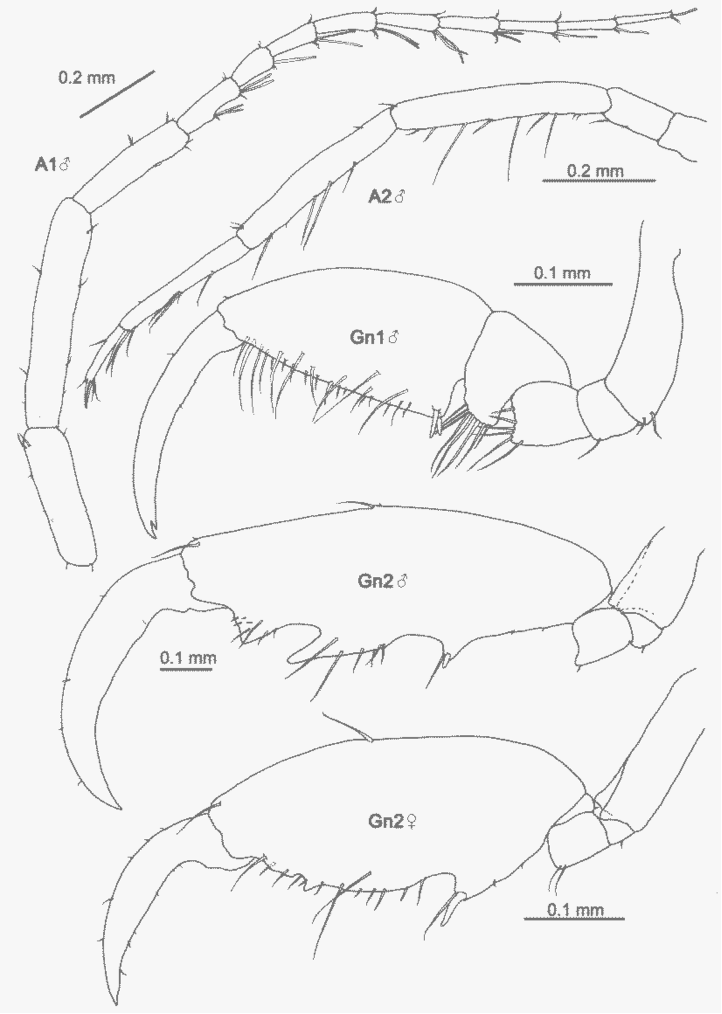


Figure 5.

Caprella tavolarensis n. sp. Pereopods 5–7 and abdomen of male paratype (paratype 1, MVRCr 500.2). Abdomen of female paratype (paratype 4, MVRCr 500.5)

Intraspecific variation

Length varied from 2.8 to 6.3 mm (males) and 2.0–4.3 mm (females). Most of the morphological characters studied in the present paper were rather constant. All the specimens examined had the body smooth dorsally, lacking tubercles. The rostrum was very short in both males and females, being extremely reduced (almost absent) in sub-adult specimens. Insertion of gnathopod 2 was near distal end of pereonite 2, although the insertion could vary from half to distal end of pereonite 2, depending on the maturation stage of the specimen. Length of pereonite 1 and 2 vary also according to the stage of development (Figs. 6b, 8d). All the specimens were lacking serrate carina on the basis of gnathopod 2 and basis of pereopods. Carpus of pereopods 5–7 was provided by tiny teeth (Figs. 7f, 9e), although the number of these internal teeth could vary between 1 and 3. Six paratype specimens (3 males and 3 females) were dissected for examination of mouthparts. The structure of mouthparts is very constant, except for the following: (a) the number of strong setae (similar to "teeth") in the outer lobe of the maxilliped could vary between 1 and 3; (b) the number of lateral setae of distal article of the palp of maxillae varied also from 4 to 8.

Ecological remarks

The specimens of *Caprella tavolarensis* n. sp. were collected from a well-preserved *Posidonia oceanica* bed between 10 and 15 m depth, located in a fully protected area. The meadow under investigation occurs on sandy bottoms and granitic rocks, and extends to a lower limit of about 27–30 m in depth. At sampling depth, mean meadow density reached 259 ± 98 shoots m^{-2} (mean ± standard deviation of 16 measurements).

The amphipod fauna of this meadow is dominated by the species *Caprella tavolarensis* n. sp. (37.2%), *Apherusa chiereghinii* Giordani-Soika (17.4%), *Aora spinicornis* Afonso (12.1%) and *Phtisica marina* Slabber (11.3%).

Caprella tavolarensis mean density was 64 ± 75 individuals m^{-2} (mean ± standard deviation of 16 samples) and reached maximum density of 271 individuals m^{-2}.

The examination of horizontal spatial variability patterns, using a hierarchical nested design with spatial scales ranging from m to 100s of m, revealed that *C. tavolarensis* density exhibited the larger variation at the small spatial scale (~1 m).

Although the existence of small scale patchiness in density within *P. oceanica* meadows has long been recognised (Panayotidis et al. 1981), no correlation has been found between *C. tavolarensis* abundance and meadow density. Previous studies revealed that there is no correlation between parameters of the vagile fauna such as number of individuals and features of the *P. oceanica* meadow such as density (Scipione et al. 1996).

In the same area, several experiments dealing with fish predation have been conducted. This species is little predated in comparison with other accompanying species such as *Caprella acanthifera*. Probably, the small size could let the specimens camouflaged among *Posidonia* leaves and escape from predators. Three caprellid species were found *living* in *P. oceanica* bed together with *Caprella tavolarensis*: *Caprella acanthifera*, *Phtisica marina* and *Pseudoprotella phasma*.

Fig. 6

Scanning electron microscopy (SEM) pictures of *Caprella tavolarensis* n. sp. male adults.

A	Head region with antennae, mouthparts and gnathopods 1, lateral view.
B	Head and pereonites 1–4 with gnathopods 1, 2 and gills, lateral view; large male length: 6.1 mm.
C	Head region with mouthparts and insertion of gnathopods 1, lateral view.
D	Mouthparts with maxilliped, ventral view.
E	Maxilliped, lateral view.
F	Maxilliped dactylus outer margin

Figure 6.

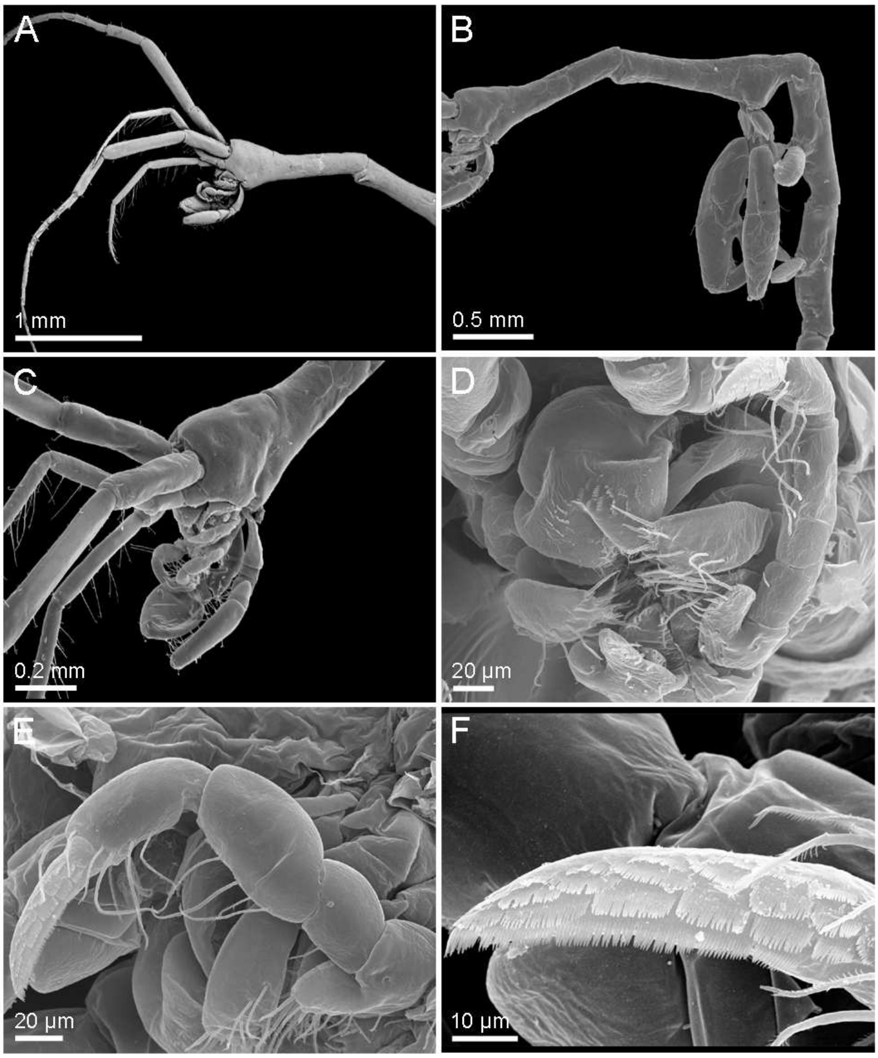
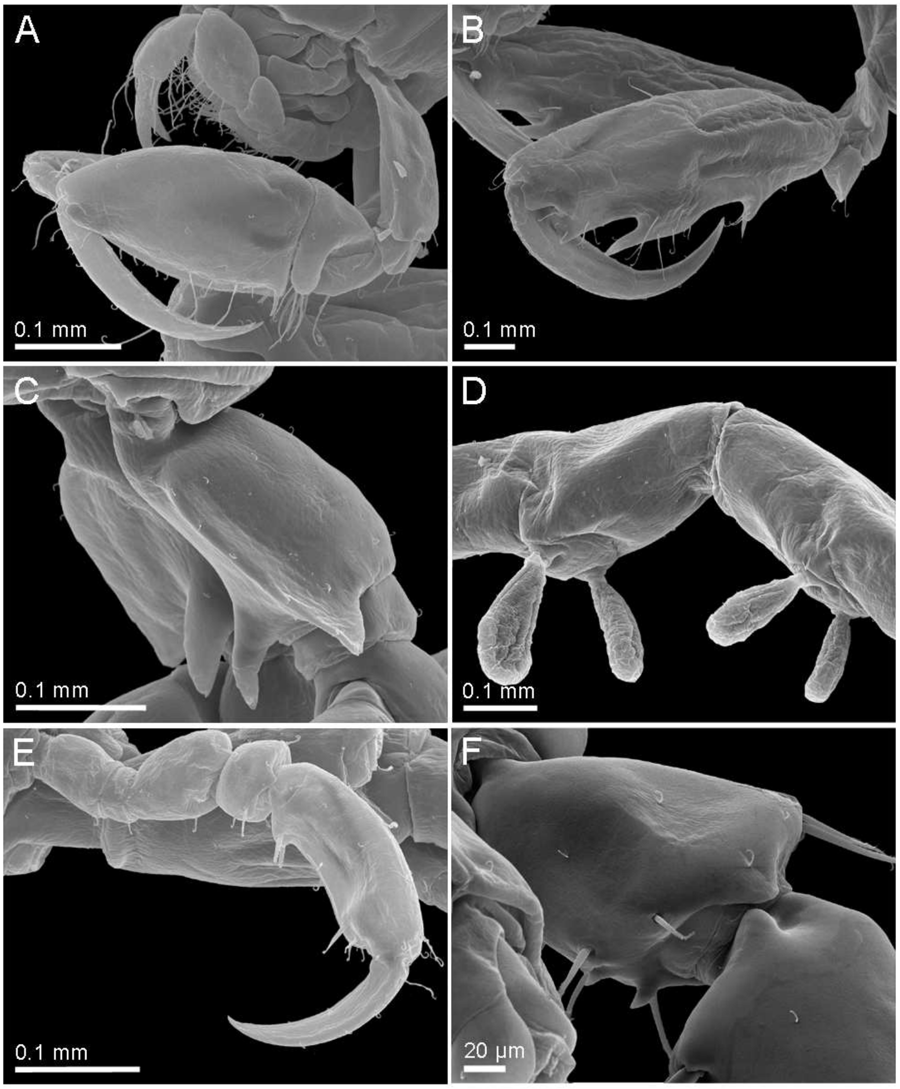


Fig. 7

SEM pictures of *Caprella tavolarensis* n. sp. Male adults, lateral views.

A	Gnathopod 1 and maxilliped.
B	Gnathopods 2.
C	Basis of gnathopods 2 with projections.
D	Pereonites 3–4 with gills.
E	Pereopod 5
F	Pereopod 6 carpus with acute teeth

Figure 7.



Discussion

The new species *Caprella tavolarensis* n. sp. is close to *Caprella liparotensis* Haller, 1879, and *Caprella wirtzi* Krapp-Schickel & Takeuchi 2005. *Caprella liparotensis* was described based on specimens collected from Lipari, Italy and has since been reported from Portugal to the whole Mediterranean, including the Adriatic and Black Seas (Krapp-Schickel & Takeuchi 2005). Additional descriptions of this species have been provided by Chevreux & Fage (1925), Krapp-Schickel (1993), and Guerra-García & Takeuchi (2002). Krapp-Schickel & Takeuchi (2005) described *Caprella wirtzi* from Cape Verde Islands (Atlantic), close to *C. liparotensis*, but showing differences on antenna 2, gnathopod 2 and pereonites.

The new species *Caprella tavolarensis* clearly differs from *C. liparotensis* and *C. wirtzi* on the following characters: (1) smaller size in *C. tavolarensis* (males 3–6 mm, females 2–4 mm) than in *C. liparotensis* (males 7–12 mm, females 4–7 mm) and *C. wirtzi* (males 8–12 mm, females 4–6 mm); (2) body more elongate and dorsally smooth in *C. tavolarensis*, while dorsal tubercles or acute projections are present on pereonites 5–7 on the other two species; (3) very short rostrum in *C. tavolarensis*, while well developed in *C. liparotensis* and *C. wirtzi*; (4) maxilliped less setose in *C. tavolarensis*; (5) peduncle articles 2 and 3 of antenna 1 lacking dense setae in *C. tavolarensis*; (6) antenna 2 lacking swimming setae in *C. tavolarensis*; (7) basis of gnathopod 2 and pereopods lacking serrate carina in *C. tavolarensis* and *C. wirtzi*, while a developed serration is present in *C. liparotensis*.

The elongate shape of the body together with the scarcely setose antennae and very short rostrum make *C. tavolarensis* to resemble the species *Caprella mitis* and *C. paramitis*. However, the gnathopod 2 is totally different in these species.

Based on the considerable degree of intraspecific morphological variation in *C. liparotensis*, Krapp-Schickel & Takeuchi (2005) already doubted about the wide distribution of *C. liparotensis* within the Mediterranean and northeast Atlantic and pointed out that careful checking of rich series taken from many different localities should shed more light on what is variable and what are small but constant differences. Probably, the new species described in the present paper may have been misidentified as *C. liparotensis* in the past. So far, *C. liparotensis* had been recorded from different species of algae and hydroids (Krapp-Schickel 1993, Guerra-García 2001, Guerra-García & Takeuchi 2002), and also from *P. oceanica* seagrass meadows (Scipione & Fresi 1984, Zakhama-Sraieb et al. 2006). The most common caprellid species recorded from seagrasses at Mediterranean sites are *Caprella acanthifera* Leach, 1814, *Caprella rapax* Mayer, 1890, *Phtisica marina* Slabber, 1769, *Pseudoprotella phasma* Montagu, 1804 and *Pariambus typicus* Krøyer, 1844 (Scipione 1998, Sánchez-Jerez et al. 1999b, Luque et al. 2004, González et al. 2008). However, these caprellid species are not exclusive of these habitats since they have been collected from many different substrates (Guerra-García 2001). During the present study, the new species *Caprella tavolarensis* was found together with *Caprella acanthifera*, *Pseudoprotella phasma* and *Phtisica marina*. Probably, further studies on *Posidonia oceanica* meadows of other Mediterranean areas could reveal the presence of *C. tavolarensis*, although we cannot exclude that the species could be an endemism of the area, and this would explain that the species has not been reported so far in the Mediterranean. Monitoring of the amphipod fauna in other comparable partially protected localities (B and C zones) of the Tavolara-Punta Coda Cavallo Marine Protected Area (Fig. 1) showed that specimens of *Caprella tavolarensis* have been found in only very rare cases, while it is the dominant species in the A zone with 37.2% of the total amphipod fauna. We cannot exclude a possible role of the protection status of this locality, highlighting the importance of fully protected areas in species conservation.

So far, 41 caprellid species have been recorded at the Mediterranean Sea (Table 1). Krapp-Schickel (1993) included 23 species in her revision. After these, several taxa were described, especially from the Strait of Gibraltar area, doubling the number of species included in the monographs edited by the late Sandro Ruffo. *Caprella scaura* Templeton, 1836 has also been recorded recently from the Mediterranean as invader species (Krapp et al. 2006). Most species (51%) have been collected only in Mediterranean waters and can be considered Mediterranean endemic so far (Table 1), with many species restricted to the Strait of Gibraltar area. Bellan-Santini and Ruffo (1998) reported 37% of amphipod species endemic to the Mediterranean Sea. Consequently, more caprellid studies are needed since, probably, further researches along the Atlantic Coast of North Africa and the Iberian Peninsula would likely reduce the number of caprellids that are considered endemic to the Mediterranean. It is the case, for example, of *Caprella rapax* and *Parvipalpus major*, which have been collected recently on the Atlantic Iberian coast (see Guerra-García & Takeuchi 2002). Although *Caprella grandimana* and *C. hirsuta* have also been reported from nearby areas of the Atlantic side of the Strait of Gibraltar (Bellan-Santini & Ruffo 1998), we have considered these species as truly Mediterranean endemics since a recent study of Guerra-García et al. (2010) showed that the distribution of these two species is clearly restricted to Mediterranean waters. Only a few species (i.e. *Caprella andreae*, *C.danilevksii*, *C. equilibra*, *C. penantis* and *Phtisca marina*) show a wide distribution area. On the other hand, species such as *Caprella erethizon*, *C. fretensis* or *Caprella tuberculata*, typically distributed along the North Atlantic, have been recently collected at the Mediterranean side of the Strait of Gibraltar.

Figure 8.

SEM pictures of *Caprella tavolarensis* n. sp. Male adults.

A Abdomen, ventral view.

B Abdomen, ventroposterior view.

C Detail of penes and abdominal appendages, ventral view.

D Head and pereonites 1–2 with gnathopods 1, 2, lateral view; young male length: 3.6 mm

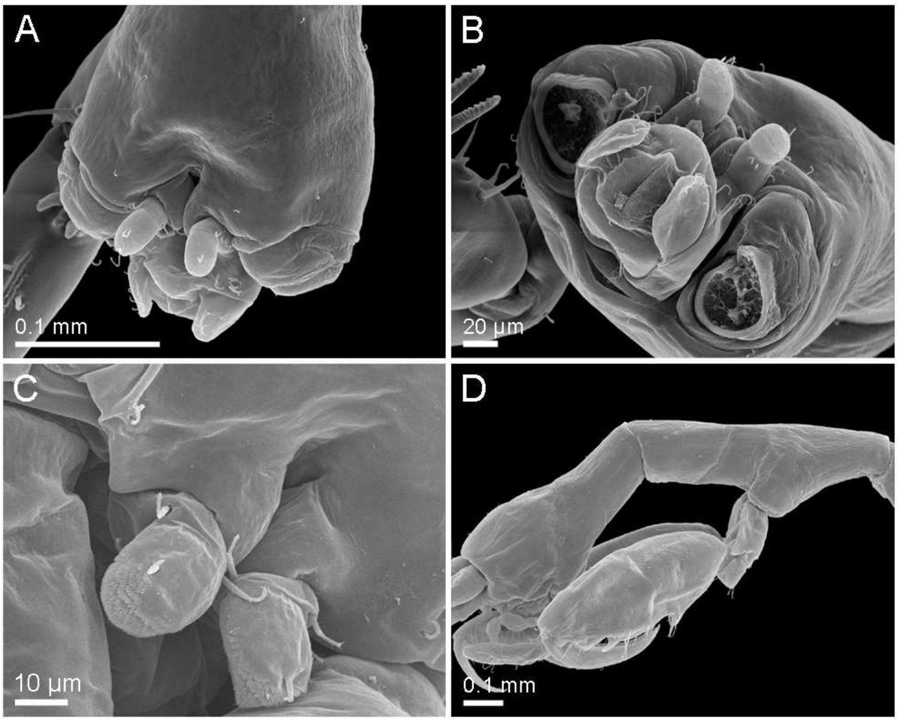


Fig. 9

SEM pictures of *Caprella tavolarensis* n. sp. Female adults.
A Entire animal, lateral view.
B Entire animal, ventral view.
C Gnathopod 2, lateral view.
D Pereonites 5–6 showing genital papillae on pereonite 5, ventral view.
E Pereopod 5, lateral view.
F Oostegites and gills, lateral view.

Figure 9.

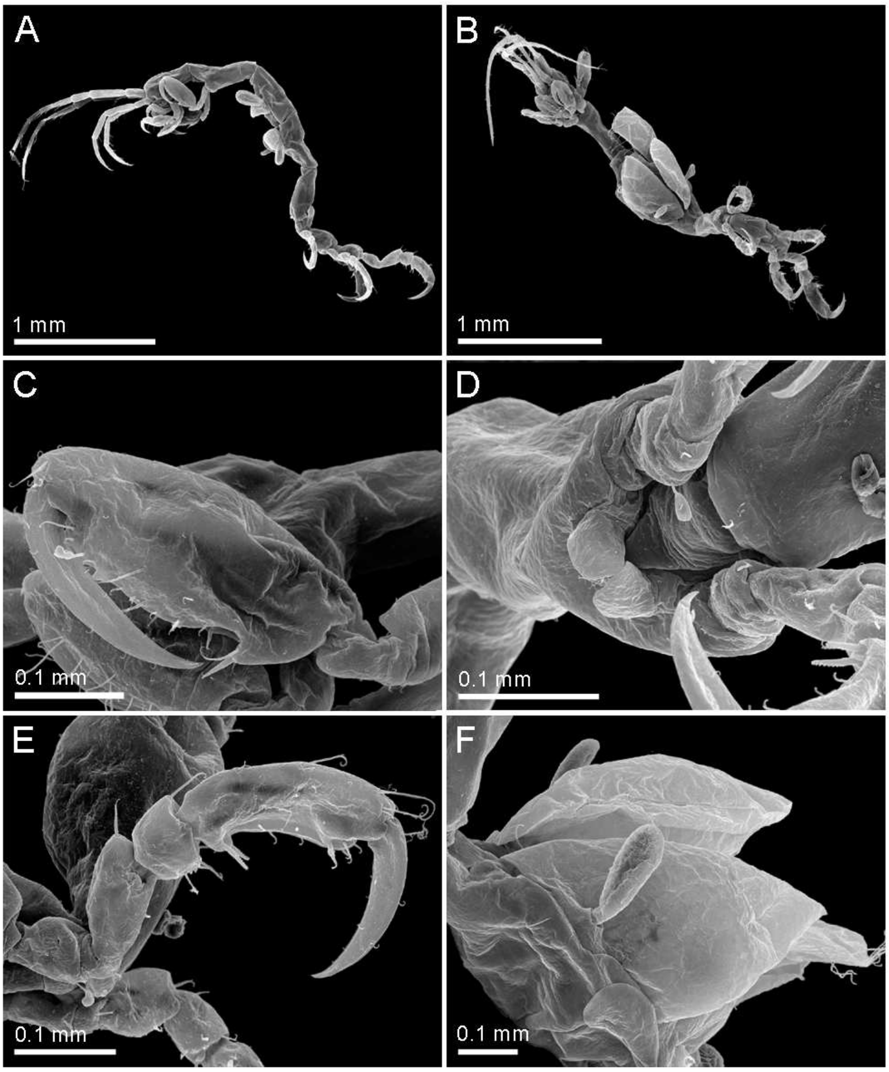


Table 1. Caprellids cited in Mediterranean waters

Name of species	Distribution
Caprella acanthifera Leach, 1814	M, A
Caprella andreae Mayer, 1890	M, A, P
Caprella caulerpensis Guerra-García, Sánchez-Moyano and García-Gomez, 2002	E*
Caprella cavediniae Krapp-Shickel and Vader, 1998	E
Caprella ceutae Guerra-García and Takeuchi, 2002	E*
Caprella danilevskii Czerniavski, 1868	M, A, P, I
Caprella dilatata Krøyer, 1843	M, A
Caprella equilibra Say, 1818	M, A, P, I
Caprella erethizon Mayer, 1901	M, A
Caprella fretensis Stebbing, 1878	M, A
Caprella grandimana Mayer, 1882	E
Caprella hirsuta Mayer, 1890	E
Caprella lilliput Krapp-Shickel and Ruffo, 1987	E
Caprella linearis Linnaeus, 1767	M, A
Caprella liparotensis Haller, 1879	M, A
Caprella mitis Mayer, 1890	E
Caprella monai Guerra-García, Sánchez-Moyano and García-Gómez, 2001	E*
Caprella paramitis Guerra-García, Sánchez-Moyano and García-Gómez, 2001	E*
Caprella penantis Leach, 1840	M, A, P, I
Caprella pseudorapax Guerra-García, Sánchez-Moyano and García-Gómez, 2001	E*
Caprella rapax Mayer, 1890	M, A
Caprella sabulensis Guerra-García, Sánchez-Moyano and García-Gómez, 2001	E*
Caprella santosrosai Sánchez-Moyano, Jiménez-Martín and García-Gómez, 1995	E*
Caprella scaura Templeton, 1836	M, A, P, I
Caprella takeuchii Guerra-García, Sánchez-Moyano and García-Gómez, 2001	E*
Caprella tavolarensis n.sp.	E
Caprella telarpax Mayer, 1890	E
Caprella tuberculata Bate and Westwood, 1868	M, A
Caprella sp (armata-group) (see Krapp-Shickel and Vader, 1998)	M, A
Deutella schieckei Cavedini, 1982	E
Liropus elongatus Mayer, 1890	E
Liropus minimus Mayer, 1890	E
Pariambus typicus Krøyer, 1844	M, A
Parvipalpus linea Mayer, 1890	M, A
Parvipalpus major A. Carausu, 1941	M, A
Pedoculina bacescui A. Carausu, 1940	E
Pedoculina garciagomezi Sánchez-Moyano, Carballo and Estacio, 1995	E*
Phtisica marina Slabber, 1769	M, A, P
Pseudolirius kroyerii Haller, 1879	E
Pseudoprotella inermis Chevreux, 1927	M, A
Pseudoprotella phasma Montagu, 1804	M, A

Modified from Guerra-García and Takeuchi (2002)

E	Mediterranean endemic;	M	Mediterranean Sea;	A	Atlantic Ocean;
P	Pacific Ocean	I	Indian Ocean		

* *Indicates that the species has a restricted distribution area to the Strait of Gibraltar*

Summarising, in spite of the amphipod fauna from the Mediterranean can be considered as very well studied, still new species are being described. Further sampling, especially from sediments, maerl bottoms and corraligène (below 40–50 m) should be conducted to complete our knowledge about Mediterranean caprellids.

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Chapter 6

The role of fish predation on *Posidonia oceanica* amphipod assemblages

Abstract

Despite the major ecological importance of *Posidonia oceanica* seagrass meadows in the Mediterranean Sea, little attention has been given to the role of fish predation in structuring amphipod assemblages. Experimental manipulations of predation intensity (inclusion and exclusion cages) were set up in a marine protected area. In the absence of predatory fishes (exclusion cages), total amphipod density increased and a trend existed for the number of species. *Caprella acanthifera* and *Iphimedia minuta* densities responded to predation. One enclosed labrid fish predator (inclusion cages) reduced density of *Aora spinicornis* and *Phtisica marina*, although total amphipod density was unaltered. The decrease of larger individuals over small ones, suggests that larger body length amphipods were preferentially consumed. In both experiments total biomass, diversity, and amphipod assemblage structures were unaffected. The present study provides evidence that predation by fish can be an important factor in structuring *P. oceanica* amphipod populations. Patterns observed at the species level, suggest complex interactions probably mainly related to behaviour of amphipod and fish species. Overall, these results give new piece of support to the hypothesis that overexploitation of top predators contribute to cascading effects.

Keywords

Predation, Seagrass, Marine reserves, Caging experiments, Trophic cascades, Coastal zone, Mediterranean Sea.

Introduction

The role of predation in regulating community structure has been for a long time a central point in ecology (Hairston et al. 1960, Duffy & Hay 2001). Such a centrality is attributable to the paramount implications this subject has not only in terms of basic ecological knowledge, but also for management and conservation of natural systems (Valentine & Duffy 2006). Predation can be one of the main factors affecting invertebrate assemblages in aquatic habitats (Shurin et al. 2002, Morin 2011), often in a variety of ways. These include the direct removal of prey (i.e. density-mediated interaction), leading to reductions in the numerical abundance of prey populations (Murdoch et al. 2003). However, predation can also induce morphological, physiological, and behavioural effects on prey populations (i.e. trait-mediated interaction; see review of Sih et al. 1985, Preisser et al. 2005).

Predator-prey relationships are well documented in a range of marine ecosystems such as soft bottoms areas, kelp forests, temperate rocky reefs, coral reefs and seagrass meadows (Pinnegar et al. 2000, Duffy & Hay 2001, Heck & Orth 2006). In these habitats, the abundance and diversity of vagile fauna are influenced particularly by fish predation. The efficiency of a predator is a function of detection, pursuit, and capture of the prey (Mattila 1995), all of which may be hindered by increasing habitat complexity (Orth et al. 1984, Heck & Orth 2006). Structural complexity usually provides refuges for prey populations and thereby lowers the number of encounters with predators, reducing the risk of predation. Hence, habitat complexity determines the intensity and nature of predator-prey interactions, and affects the structuring capacity of predation (Nelson 1979, Mattila 1995).

In seagrass systems, although the role of fish predation has received considerable attention (Orth et al. 1984, Heck & Orth 2006), the frequency, importance and consequences on benthic small invertebrate assemblages are still debated. It was recently demonstrated that communities associated to *Zostera marina* seagrass meadows can be dominated by strong predation pressure controlling biomass of mesograzers and, down to the food web, macroalgae (Moksnes et al. 2008). The available evidence is far from allowing to draw any general conclusion, but the potential functional role of consumers in seagrass systems may have relevant management / conservation implications. In meadows protected from heavy fishing impacts (especially in meadows included in fully protected sites within marine protected areas, MPAs), a greater abundance of some fish species may be accompanied by a greater predation intensity on small fish and invertebrates (Boudouresque et al. 1992, Francour 1994, 2000). Lower abundance and species number of macroinvertebrates associated to rocky and seagrass ecosystems were observed within Scandola MPA in France (Mediterranean Sea; Boudouresque et al. 1992) where the abundance, biomass and diversity of predatory fish were greater than in adjacent fished areas (Francour 1994, 2000).

In the Mediterranean coastal zone, *Posidonia oceanica* seagrass meadows represent one of the most common and productive ecosystem (Pergent et al. 1997, Boudouresque et al. 2006). *Posidonia oceanica* meadows have received in the recent decades an increasing attention in terms of conservation, which justified their recent inclusion into MPAs and/or within Sites of Community Importance (SCIs) as defined in the European Commission Habitats Directive (92/43/EEC) (Boudouresque et al. 2006, Gobert et al. 2006).

Posidonia oceanica meadows form a unique three-dimensional spatially complex habitat that provides a wide variety of microhabitats to benthic communities. Amphipod crustaceans count more than 80 species in *P. oceanica* meadows (Bellan-Santini 1998) and are an important group within the vagile fauna (Mazzella et al. 1989, Gambi et al. 1992) from different perspectives. They are one of the most useful groups of crustaceans used for monitoring environmental impact in *P. oceanica* meadows (Sánchez-Jerez & Ramos-Esplá 1996, Sánchez-Jerez et al. 2000). From an ecological point of view, they are an important trophic resource for fish (Bell & Harmelin-Vivien 1983, Pinnegar & Polunin 2000), which involves an essential role within the communities associated to *P. oceanica* (Scipione et al. 1996) in terms of energy transfer from lower to higher trophic levels within the food web. Such a relevant trophic role poses, in addition, another relevant point: the structure of amphipod assemblages could be influenced by the local level of fish predation. This aspect, quite poorly investigated until now, could be particularly interesting in the context of MPAs studies. A number of studies have investigated the cascading effects, on rocky reefs, of an increased fish predation on sea urchins within MPAs (where fish predators are more abundant and bigger in size) compared to outside fished areas (Guidetti & Sala 2007 and references therein). Conversely, only few works specifically investigated the response of vagile invertebrates to protection (Boudouresque et al. 1992, Badalamenti et al. 1999, Milazzo et al. 2000), and none of them at our knowledge focused on *P. oceanica* systems.

Previous investigations conducted at the Tavolara-Punta Coda Cavallo Marine Protected Area (hereinafter TMPA, Italy; NW Mediterranean Sea) revealed that: 1) fish assemblages mostly associated to rocky reefs clearly respond to protection, with greater abundance and size of fish in fully protected areas (where fishing is totally banned, FPA) compared to partially protected areas (PPAs) inside the TMPA (where fishing is allowed but regulated) and fished areas outside the TMPA (where fishing occurs simply according to national laws) (Di Franco et al. 2009); 2) *P. oceanica* amphipod fauna was on average 1.5 times more abundant within to the PPAs than in FPA. Further more, the multiscale analyses revealed lower densities and/or biomasses of several frequent taxas within the FPA compared to the PPAs (see chapter 4).

The aim of this study, therefore, was to evaluate the role of fish predation in affecting amphipod assemblages associated with *P. oceanica* meadows. By means of experimental manipulations of predation intensity, we tested in this study the hypothesis that structure of amphipod assemblages may change in relation to predatory fish abundance.

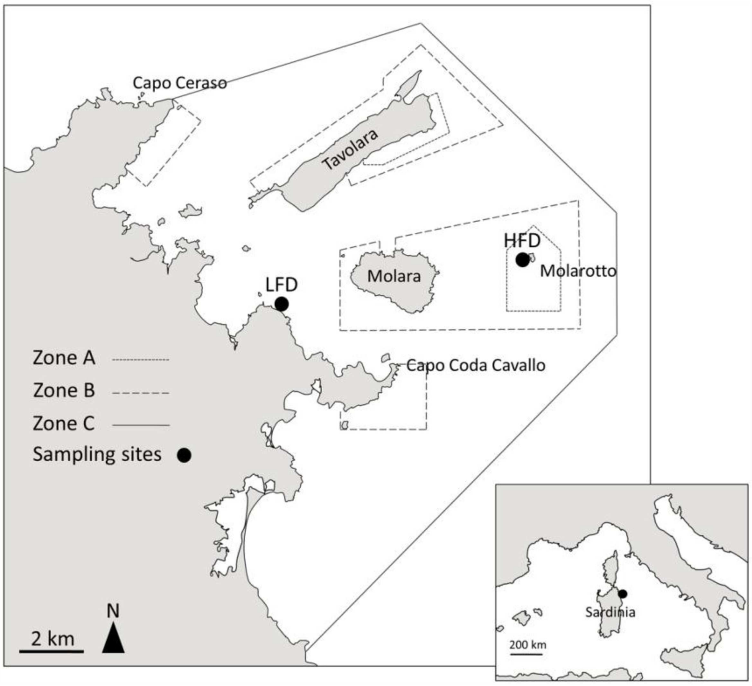
Materials and methods

Study area

This study was carried out in *P. oceanica* meadows at the Tavolara-Punta Coda Cavallo MPA (40°56´N, 09°44´E) located in north-east Sardinia (Italy, Fig. 1). The MPA covers 15,357 ha, extends along 76 km of coastline and was formally established in 1997, but effective enforcement of protection started in 2003-04. It includes 3 protection zones: zone A (integral reserves or fully protected area = no-take/no-access zone: 529 ha), zone B (partial reserves or partially protected area: 3.113 ha) and zone C (general reserves or also partially protected area: 11,715 ha). Two sites were chosen in this study: (1) Molarotto Island in the zone A, where access is restricted to scientists, reserve personnel and police authorities; and (2) Monte Pedrosu in the zone C, where professional and recreational fishing are allowed under restricted conditions defined by the local Consortium Management of the MPA, with the exception of spearfishing which is totally banned. In the study area, the rocky-reef fish assemblages were studied in detail by Di Franco et al. (2009). Taking this study as a reference point, Molarotto Island presented the highest fish density and is hereinafter referred to as the HFD (High Fish Density) site, while Monte Pedrosu had the lowest fish density and is hereafter referred to as the LFD (Low Fish Density) site. Both study sites had similar depth (8-13m) and *P. oceanica* meadows' structure in terms of shoot density, leaf surface area, leaf and epiphyte biomass, and litter biomass (authors' unpublished data).

Figure 1.

Study area and location of sampling sites (HFD and LFD) at the Tavolara-Punta Coda Cavallo Marine Protected Area (Italy, Mediterranean Sea). Zone A (fully protected area), zone B and zone C (partially protected areas) are showed. HFD: high fish density site, LFD: low fish density site.



Characterization of HFD and LFD

Fish assemblages

Visual censuses were performed by scuba diving to assess the densities of fishes along 8 transects of 20 m long x 2 m wide (Harmelin-Vivien et al. 1985) each of 7 to 8 minutes duration, randomly located in the meadow of each study site. All censuses were recorded on the 2nd and 3rd September 2009 between 10:00 and 13:00 (local time) to avoid confounding effects of temporal variation in fish assemblages and under optimum meteorological and hydrological conditions. Density of fishes was estimated by counting single specimens to a maximum of 30 individuals of each species encountered, while abundance classes (31 to 50, 51-100, 101-200 individuals) were used for more abundant species. Densities were expressed as mean (± SE) per 100 m^2. Fish species were considered to have a predation impact on amphipods when their amphipod Index of Relative Importance (IRI) > 100 (IRI from Bell & Harmelin-Vivien 1983, Kabasakal 2001). The amphipod $IRI = F\ (N + W)$ evaluates the importance of amphipods as food type in the diet of each fish species, incorporating the percentage frequency of occurrence (F), percentage number (N) and percentage weight (W) of amphipods to the total of all food types ingested (Bell & Harmelin-Vivien 1983). Finally, a Fish predation index (FPI) = $\sum(N_i * IRI_i)/1000$ was calculated for each site, where N is the mean number of individuals per 100 m^2 and IRI the amphipod index of relative importance for the i^{th} fish species. The bigger the index value, the higher the fish predation is on amphipods.

Amphipod assemblages

Amphipod samples were collected by scuba diving using an airlift (Bussers et al. 1983, see Michel et al. 2010 for further details on this sampling technique applied to amphipod assemblages). This was done in HFD and LFD (n = 5 per site) from the 28th August to the 3rd September 2009 during the day between 10:00 and 18:00 (local time) to avoid day-night variability. Sampling areas of the meadow was previously 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^2 was suctioned continuously for 2 minutes under constant airflow, collecting the amphipod and other invertebrates in a 0.5 mm mesh bag. The samples were sieved through a 0.5 mm mesh and fixed in seawater formalin 4%, then transferred to 70% ethanol. Amphipod densities were expressed as mean (± SE) per m^2.

Caging experiments

Exclusion experiment

To test the top-down effects of predatory fishes on the amphipod assemblage structure, experimental reduction of predation intensity was carried out using cages from June 30th to September 1st 2009. This time period was chosen because significant caging artifacts were found to act on most species after 8 weeks (Kennelly 1991), and allowed enough time for several generations of amphipods to develop inside cages (Bellan-Santini 1999, Andersson et al. 2009). Because exclusion experiments are successful only if there are significant levels of predation in the uncaged treatment for comparison (Connell 1997), only the zone A was used. The experimental design consisted of 3 different treatments: uncaged areas, partial control cages and predator exclusion cages. Five replicates of the 3 experimental treatments were realized (for a total of 15 experimental plots). These treatment plots were randomly distributed in the *P. oceanica* meadow and separated spatially by 3 to 8 m. Exclusion cages measured 75 cm (L) x 75 cm (l) x 120 cm (h) and consisted of steel covered by plastic dark green mesh. A 13 mm mesh size was chosen to prevent the passage of predatory fishes and to avoid major caging artifacts (i.e. attenuation of light and water flow). Partial control cages were constructed from the same materials and in the same dimension as exclusion cages, except only half of each wall of the cage was covered by plastic mesh. Each partial control cage provides the structure of an exclusion cage but allows access to predators. Uncaged areas were unmanipulated plots of *P. oceanica* seagrass habitat.

Amphipod samples were collected with an airlift (see method above), 8 weeks after deployment of the exclusion cages. Amphipod processing was done like in the characterization section. Amphipod species abundance was quantified as density (individuals. m^{-2}) and biomass (mg dry weight. m^{-2}) after drying at 60°C for 48 h. The amphipod assemblage was also characterized according to total number of species (S) and Shannon-Wiener diversity index (H') = $-\sum_i p_i \log(p_i)$ where p_i is the proportion of the total count arising from the i^{th} species. One exclusion cage was not included for statistics because a fish *Symphodus rostratus* (effective predator of amphipods) entered inside the cage during the experiment.

Cages were scrubbed every 3 to 5 days during all the experiment to prevent algal fouling and to minimize light attenuation and cage effects on water flow. Cage effects on water flow were tested using 48 uniform plaster plots (method adapted from Gambi et al. 1989). These were set inside and outside 3 exclusion cages (n = 4 inside and outside each cage), at two different locations in the canopy: above the leaves and above the base of the rhizomes. The plots were collected 96 hours after deployment to allow enough plaster dissolution, dried and weighed and the percentage mass loss was recorded. In the middle of the experiment, the effects of light attenuation were tested by measuring light intensity (lux) inside and outside 3 exclusion cages. Measurements were made, using HOBO Pendant Temperature/Light Data Loggers, every hour during 17 days to integrate luminous temporal intra and interday variability. At the end of the experiment, 10 shoots of *P. oceanica* were collected in each treatment plots and plant descriptors were measured: shoot density (shoots.m^{-2}), leaf surface area (cm^2.shoot^{-1}), leaf and epiphyte biomass (g dw.shoot^{-1}), Coefficient A (percentage of leaves per shoot having alteration marks). In each plot, leaf litter material, together with the associated macrophytes debris, were collected inside plastic bags, and was quantified as litter biomass (g dw.m^{-2}) after drying at 60°C for 96 h.

Inclusion experiment

Inclusion experiment started the 29 August 2009 in the zone C because such experiment is successful only if there are significant levels of amphipod fauna availability. The experimental design consisted of 5 replicates of uncaged areas and inclusion cages, located at random in the *P. oceanica* meadow and separated spatially by 3 to 9 m. The structure and dimensions of inclusion cages were identical to the exclusion cages (see above). One adult fish *Coris julis* (132-146 mm length) was included in each cage. *Coris julis* is a small labrid fish widespread in the Mediterranean littoral and influenced by pressure from fishing (García-Rubies & Zabala 1990). This species lives also in seagrass meadows and is a daytime feeder preying upon small invertebrates such as molluscs, echinoderms and crustaceans. Amphipods are the second major food items (14% of the total number of food types), after molluscs (Bell & Harmelin-Vivien 1983). After 4 days of caging, individuals of *C. julis* were sampled using a net and amphipods were sampled using the airlift sampler (see method above) in each uncaged area and inclusion cage. Amphipod processing and assemblage characterization were done like in the previous section. In addition, amphipod size (distance along the dorsal side, from the distal end of the rostrum to the base of the telson) was recorded for amphipods > 3% of all specimens. Measures were done using images obtained with a DeltaPix camera connected to a stereomicroscope and the associated DpxView Pro Image Management Software (100 micron precision). One inclusion cage was not included for statistics because the *C. julis* was no more present inside the inclusion cage at the end of the experiment.

Statistical analyses

As the normality of distributions and homogeneity of variance were not encountered, non-parametric tests were performed. The exclusion experiment (including all amphipod and *P. oceanica* descriptors) was analyzed using Kruskal-Wallis ANOVA tests. When statistical testing showed significant differences, we used Dunn's post-hoc tests. Mann-Whitney tests were applied for the inclusion experiment and to test the effects of cages on water flow. Multivariate analyses were also performed in order to test the null hypothesis of an absence of difference in assemblage structure between treatments. We used one-way analyses of similarity test (ANOSIM). Similarity matrices were built by calculating the Bray-Curtis coefficients from square-root transformed abundance and biomass data. The data transformation was carried out to balance the contribution from the rarer species. The R statistics output of ANOSIM indicate the separation degree between groups of treatments with values close to 0 and 1 respectively showing complete similarity and dissimilarity (Clarke & Gorley 2006). Univariate and multivariate statistical analyses were respectively completed using Statistica v.9 and Primer v6 softwares. The significance level was set to 0.05.

Results

Characterization of HFD and LFD

We recorded 18 fish species at the 2 sampling sites (Table A1). The more speciose families were Labridae (8 species) and Sparidae (5 species). Based on the amphipod *IRI* values of each fish, 11 species were considered to have a predation impact on amphipods (10 species in HFD and 9 species in LFD, Table 1). The density of these fishes was 3.1 times higher at HFD (236.1 ± 62.4 ind.100 m^{-2}) compared with LFD (76.3 ± 16.4 ind.100 m^{-2}), while the Fish Predation Index was 1.9 times higher at HFD compared with LFD (Fig. 2).

Amphipods were presented with 471 individuals belonging to 24 species. The total amphipod density was 6.3 times higher at LFD (543.5 ± 83.6 ind. m^{-2}) compared with HFD (86.6 ± 24.5 ind. m^{-2}, Fig. 2). At the species level, *A. chiereghinii* (2.2 ± 1.3 ind. m^{-2} in HFD and 202.4 ± 31.4 ind. m^{-2} in LFD), *A. spinicornis* (17.3 ± 3.2 ind. m^{-2} in HFD and 127.7 ± 25.3 ind. m^{-2} in LFD) and *P. marina* (1.1 ± 1.1 ind. m^{-2} in HFD and 36.8 ± 11.3 ind. m^{-2} in LFD) explained a great part of the observed total amphipod density difference.

Table 1.

Mean (± SE) density (number of individuals 100 m^{-2}) of fishes presenting an amphipod index of relative importance (*IRI* from Bell & Harmelin-Vivien 1983, Kabasakal 2001), at the high fish density (HFD) site and the low fish density (LFD) site.

	Density		*IRI*
	HFD	LFD	
Labridae			
Coris julis	16.4 ± 6.5	23.4 ± 7.3	965
Symphodus doderleini	8.9 ± 4.5	8.4 ± 2.5	463
Symphodus mediterraneus	1.1 ± 0.7	0.6 ± 0.4	487
Symphodus melanocercus	0.0 ± 0.0	0.6 ± 0.4	167
Symphodus rostratus	0.4 ± 0.4	1.3 ± 0.7	829
Symphodus tinca	2.1 ± 1.8	0.6 ± 0.4	748
Thalassoma pavo	1.8 ± 0.9	0.0 ± 0.0	407
Mullidae			
Mullus surmuletus	0.7 ± 0.7	0.0 ± 0.0	105
Pomacentridae			
Chromis chromis	170.7 ± 53.4	34.4 ± 9.4	501
Sparidae			
Diplodus annularis	30.7 ± 7.6	5.6 ± 1.5	274
Diplodus vulgaris	3.2 ± 1.3	1.3 ± 0.8	299

Appendix. Table A1.

List of fish and amphipod taxa collected at the sampling sites during caging experiments (+, present; - , absent).

	HFD	LFD
Fishes		
Boops boops (Linnaeus, 1758)	+	+
Chromis chromis (Linnaeus, 1758)	+	+
Coris julis (Linnaeus, 1758)	+	+
Dincentrarchus labrax (Linnaeus, 1758)	+	-
Diplodus annularis (Linnaeus, 1758)	+	+
Diplodus vulgaris (Geoffroy Saint-Hilaire, 1817)	+	+
Labrus sp. (Linnaeus, 1758)	+	-
Mullus surmuletus (Linnaeus, 1758)	+	-
Oblada melanura (Linnaeus, 1758)	+	+
Sarpa salpa (Linnaeus, 1758)	+	+
Serranus cabrilla (Linnaeus, 1758)	+	+
Serranus scriba (Linnaeus, 1758)	+	+
Symphodus doderleini (Jordan, 1890)	+	+
Symphodus mediterraneus (Linnaeus, 1758)	+	+
Symphodus melanocercus (Risso, 1810)	-	+
Symphodus rostratus (Bloch, 1791)	+	+
Symphodus tinca (Linnaeus, 1758)	+	+
Thalassoma pavo (Linnaeus, 1758)	+	-

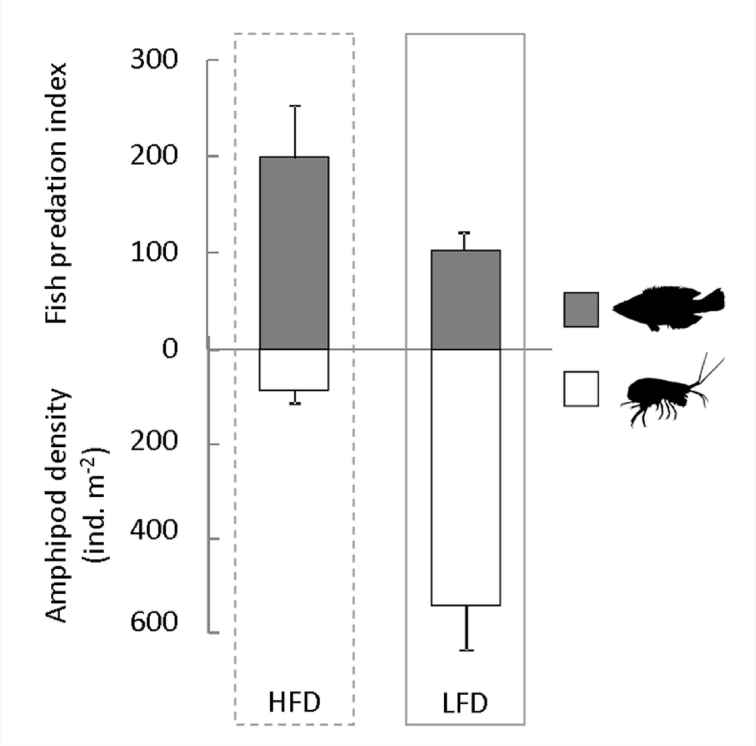
Appendix. Table A1. – (Cont'd)

	HFD	LFD
Amphipods		
Amphithoe helleri (Karaman, 1975)	-	+
Aora spinicornis (Afonso, 1976)	+	+
Apherusa chiereghinii (Giordani-Soika, 1950)	+	+
Apolochus neapolitanus (Della Valle, 1893)	+	+
Caprella acanthifera (Leach, 1814)	+	+
Caprella tavolarensis (Sturaro and Guerra-García, 2012)	+	+
Dexamine spiniventris (Costa, 1853)	+	+
Dexamine spinosa (Montagu, 1813)	-	+
Erichtonius punctatus (Bate, 1857)	+	-
Gammarella fucicola (Leach, 1814)	+	-
Gammaropsis palmata (Stebbing and Robertson, 1891)	-	+
Gitana sarsi (Boeck, 1871)	-	+
Guernea coalita (Norman, 1868)	+	-
Hyale camptonyx (Heller, 1866)	+	-
Iphimedia minuta (Sars, 1882)	+	+
Leptocheirus guttatus (Grube, 1864)	-	+
Leptocheirus sp	-	+
Liljeborgia dellavallei (Stebbing, 1906)	+	+
Lysianassina longicornis (Lucas, 1849)	+	+
Maera grossimana (Montagu, 1808)	+	-
Microdeutopus sp	-	+
Orchomene humilis (Costa, 1853)	+	+
Peltocoxa marioni (Catta, 1875)	-	+
Perioculodes aequimanus (Kossmann, 1880)	+	-
Phtisica marina (Slabber, 1769)	+	+
Podocerus variegatus (Leach, 1814)	-	+
Pseudoprotella phasma (Montagu, 1804)	+	+
Stenothoe dollfusi (Chevreux, 1887)	+	-
Synchelidium longidigitatum (Ruffo, 1947)	-	+
Tmetonyx nardonis (Heller, 1866)	-	+
Tritaeta gibbosa (Bate, 1862)	+	+

Fig. 2

Mean (± SE) Fish predation index (*FPI*) and total amphipod density (number of individuals m^{-2}) between the high fish density (HFD) site and the low fish density (LFD) site at the Tavolara-Punta Coda Cavallo Marine Protected Area.

Figure 2.


Fish predation index
Amphipod density (ind. m-2)
300
200
100
0
200
400
600
HFD
LFD

Caging experiments

Exclusion experiment

The effect of cages on water motion yielded to significant differences between exclusion cages and uncaged areas for plaster plots placed above the leaves (percentage of mass loss, mean ± SE; inside cages 54.3 ± 0.3% and outside cages 63.3 ± 0.5%, $p < 0.001$). No differences were observed inside the leaf stratum (inside cages 38.8 ± 0.8% and outside cages 41.5 ± 0.9%). For the effects of cages on light attenuation, we observed a mean decrease of luminous intensity within the cage of 26.2 ± 1.5%. Regarding the effects of cages on the meadow's characteristics, no differences were observed between uncaged areas, partial control cages and exclusion cages for shoot density, leaf surface area, leaf and epiphyte biomass, and litter biomass, except for Coefficient A ($p = 0.013$, Table A2).

A total of 331 individuals belonging to 15 families and 20 amphipod species were identified. For all samples, the assemblage was dominated by *A. spinicornis* (14.2% of the total number of individuals), *C. tavolarensis* (9.7%) and *Iphimedia minuta* (9.1%). Total amphipod exhibited a significant higher density ($p = 0.021$) in exclusion cages compared to partial control cages and uncaged areas after 8 weeks, while total amphipod biomass did not show significant differences between treatments ($p = 0.077$, Fig. 3, Table 2). At the suborder level, Gammaridea density increased in exclusion cages relative to uncaged areas and partial control cages ($p = 0.035$), while not biomass ($p = 0.077$). In the contrary, Caprellidea showed an increase in biomass in exclusion cages ($p = 0.036$) and no differences for density ($p = 0.097$, Table 2). At the species level, experimental treatment had a significant effect only on the Caprellidea *Caprella acanthifera* density ($p = 0.009$) and biomass ($p = 0.021$), and the Gammaridea *I. minuta* density ($p = 0.050$) (Table 2). A clear trend with near threshold level value was observed between treatments for number of species ($S = 5.8 \pm 1.4$ in uncaged area, $S = 5.2 \pm 1.3$ in partial control cage, $S = 10.5 \pm 1.3$ in exclusion cage, $p = 0.053$), but no significant differences was observed for diversity index

(*H′* = 1.49 ± 0.27 in uncaged area, *H′* = 1.30 ± 0.35 in partial control cage, *H′* = 2.08 ± 0.13 in exclusion cage, p = 0.087). There were no significant changes in amphipod assemblages among treatments for density (R = 0.02, p = 0.375) and biomass (R= -0.01, p = 0.442).

Appendix. Table A2.

Mean (± SE) density of *Posidonia oceanica* (shoots m^{-2}), leaf surface area (cm^2 $shoot^{-1}$), leaf and epiphyte biomass (g dw $shoot^{-1}$), litter biomass (g dw m^{-2}) and Coefficient A (%) between uncaged areas, partial control cages and exclusion cages in the exclusion experiment. Results of Kruskal-Wallis ANOVA between the treatments: significance level is reported and underlined when $p \leq 0.05$.

	Uncaged	Partial control	Exclusion	p
Shoot density	491.5 ± 17.9	534.8 ± 61.4	546.7 ± 47.7	0.685
Leaf surface area	311.03 ± 12.46	317.91 ± 13.34	298.41 ± 11.19	0.886
Leaf biomass	1.72 ± 0.17	1.74 ± 0.17	1.51 ± 0.09	0.755
Epiphyte biomass	0.08 ± 0.03	0.07 ± 0.01	0.08 ± 0.03	0.532
Litter biomass	39.55 ± 12.52	29.69 ± 7.22	19.97 ± 4.56	0.357
Coefficient A	38.75 ± 1.36	27.06 ± 1.63	28.18 ± 1.52	<u>0.013</u>

Figure 3.

Mean (± SE) density (number of individuals m-2) and biomass (mg dw m-2) of total amphipods (Gammaridea in open bars + Caprellidea in solid bars) between uncaged areas, partial control cages and exclusion cages.

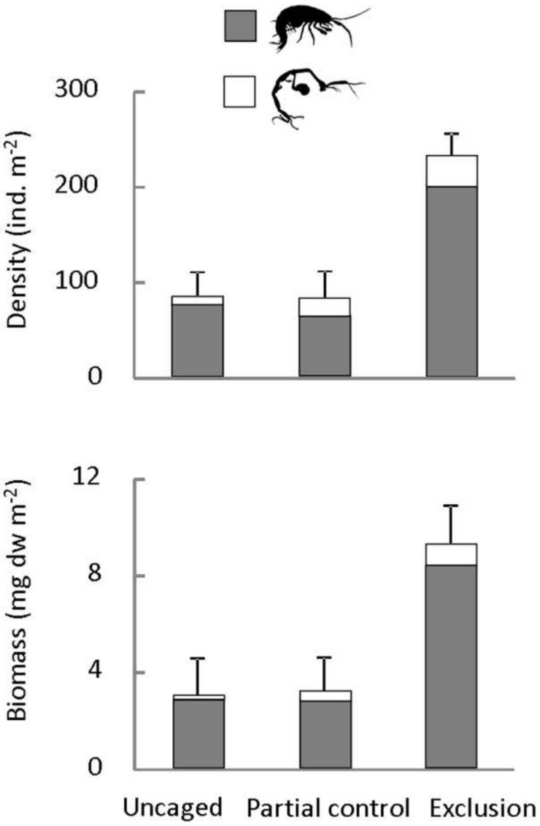


Table 2.

Mean (± SE) density (number of individuals m^{-2}) and biomass (mg dw m^{-2}), and proportion (%) of amphipod species (> 3% of all specimens) between uncaged areas, partial control cages and exclusion cages (n = 5, 5 and 4 respectively) of the exclusion experiment. Results of Kruskal-Wallis ANOVA between the treatments: significance level is reported and underlined when $p \leq 0.05$.

	Uncaged			Cage control			Exclusion cage			
	Mean	SE	%	Mean	SE	%	Mean	SE	%	p
Density (ind. m^{-2})										
Amphipods	86.6	24.5	100	84.4	27.7	100	234.1	22.4	100	0.021
Gammaridea	76.9	23.4	89	63.9	28.0	76	198.9	32.0	85	0.035
Caprellidea	9.7	2.7	11	20.6	10.0	24	35.2	10.0	15	0.097
Amphilochus neapolitanus	6.5	4.0	8	3.2	2.2	4	6.8	3.4	3	0.661
Aora spinicornis	17.3	3.2	20	21.7	6.2	26	14.9	5.6	6	0.913
Apherusa chiereghinii	2.2	1.3	3	4.3	4.3	5	23.0	11.8	10	0.185
Caprella acanthifera	0.0	0.0	0	3.2	2.2	4	14.9	3.4	6	0.009
Caprella tavolarensis	7.6	2.8	9	13.0	5.8	15	17.6	7.8	8	0.604
Dexamine spiniventris	6.5	4.3	8	3.2	3.2	4	20.3	9.5	9	0.139
Hyale camptonyx	4.3	3.2	5	3.2	3.2	4	16.2	5.8	7	0.082
Iphimedia minuta	5.4	3.4	6	7.6	3.2	9	24.4	6.8	10	0.050
Orchomene humilis	1.1	1.1	1	2.2	2.2	3	10.8	4.9	5	0.118
Other Gammaridea	33.6	8.8	39	18.4	9.2	22	83	10.5	35	0.016
Other Caprellidea	2.2	1.3	3	4.3	3.2	5	2.7	1.6	1	0.954
Biomass (mg dw m^{-2})										
Amphipods	3.12	1.50	100	3.29	1.36	100	9.42	1.56	100	0.077
Gammaridea	2.94	1.46	94	2.83	1.42	86	8.50	1.65	90	0.077
Caprellidea	0.17	0.06	6	0.47	0.21	14	0.92	0.15	10	0.036
Amphilochus neapolitanus	0.19	0.11	6	0.04	0.04	1	0.12	0.09	1	0.402
Aora spinicornis	0.31	0.10	10	0.63	0.18	19	0.68	0.42	7	0.519
Apherusa chiereghinii	0.05	0.03	2	0.27	0.27	8	0.47	0.17	5	0.257
Caprella acanthifera	0.00	0.00	0	0.19	0.12	6	0.47	0.11	5	0.021
Caprella tavolarensis	0.15	0.06	5	0.26	0.12	8	0.35	0.16	4	0.604
Dexamine spiniventris	0.15	0.12	5	0.44	0.44	13	1.06	0.53	11	0.103
Hyale camptonyx	0.37	0.26	12	0.19	0.19	6	1.19	0.49	13	0.068
Iphimedia minuta	0.19	0.14	6	0.22	0.09	7	1.53	0.87	16	0.084
Orchomene humilis	0.00	0.00	0	0.10	0.10	3	0.50	0.25	5	0.067
Other Gammaridea	1.67	1.11	53	0.93	0.48	28	2.95	1.19	31	0.175
Other Caprellidea	0.02	0.02	1	0.01	0.01	0	0.09	0.08	1	0.509

Inclusion experiment

A total of 741 individuals belonging to 14 families and 24 amphipod species were identified. For all samples, the assemblage was dominated by *A. chiereghinii* (39.4% of the total number of individuals), *A. spinicornis* (20%) and *P. marina* (5.5%). Total amphipod did not show significant differences in density and biomass between treatments, but are near the threshold level ($p = 0.063$ for density and biomass, Fig. 4, Table 3). This is also true at the suborder level for Gammaridea and Caprellidea ($p = 0.063$). At the species level, only Gammaridea *A. spinicornis* density ($p = 0.032$) and biomass ($p = 0.016$), and Caprellidea *P. marina* density ($p = 0.016$) exhibited a significant decrease within inclusion cages (Table 3). Uncaged areas and inclusion cages exhibited no significant differences for the number of species ($S = 9.3 \pm 1.0$ and $S = 9.2 \pm 0.8$, respectively) and diversity index ($H' = 1.45 \pm 0.07$ and $H' = 1.45 \pm 0.05$, respectively). There were no significant changes in amphipod assemblages between uncaged areas and inclusion cages, in terms of density ($R = 0.24$, $p = 0.056$) and biomass ($R = 0.06$, $p = 0.270$). A size-frequency analysis of major amphipod species and of the population structure of *A. spinicornis* and *A. chiereghinii* revealed that predator treatment mainly affected larger individuals, with an inversion of the first mode represented by individuals of 3-4 mm by amphipods of 2-3 mm in size (Fig. 5).

Table 3.
Mean (± SE) density (number of individuals m^{-2}) and biomass (mg dw m^{-2}), and proportion (%) of amphipod species (> 3% of all specimens) in uncaged areas and inclusion cages (n = 5 and 4 respectively) of the inclusion experiment. Results of the Mann-Whitney test between the treatments: significance level is reported and underlined when p ≤ 0.05.

	Uncaged			Inclusion			
	Mean	SE	%	Mean	SE	%	p
Density (ind. m^{-2})							
Total amphipod	543.5	83.6	100	323.4	66.3	100	0.063
Gammaridea	473.1	69.6	87	295.0	57.6	91	0.063
Aora spinicornis	127.7	25.3	24	40.6	12.0	13	0.032
Apherusa chiereghinii	202.4	31.4	37	142.1	27.3	44	0.190
Other Gammaridea	142.9	26.1	26	112.3	20.8	35	0.458
Caprellidea	70.4	17.1	13	28.4	8.9	9	0.063
Caprella acanthifera	15.2	7.9	3	14.9	6.0	5	1.000
Phtisica marina	36.8	11.3	7	9.5	4.1	3	0.016
Other Caprellidea	18.4	12.1	3	4.1	1.4	1	0.556
Biomass (mg dw m^{-2})							
Total amphipod	15.28	1.97	100	8.93	1.73	100	0.063
Gammaridea	14.07	1.84	92	8.25	1.67	92	0.063
Aora spinicornis	6.18	1.17	40	1.92	0.46	22	0.016
Apherusa chiereghinii	4.17	0.63	27	2.71	0.66	30	0.111
Other Gammaridea	3.72	0.74	24	3.63	0.83	41	1.000
Caprellidea	1.20	0.31	8	0.68	0.12	8	0.412
Caprella acanthifera	0.42	0.21	3	0.46	0.12	5	0.730
Phtisica marina	0.43	0.10	3	0.14	0.05	2	0.111
Other Caprellidea	0.35	0.15	2	0.08	0.08	1	0.286

Fig. 4

Mean (± SE) density (number of individuals m^{-2}) and biomass (mg dw m^{-2}) of total amphipods (Gammaridea in open bars + Caprellidea in solid bars) between uncaged areas and inclusion cages.

Figure 4.

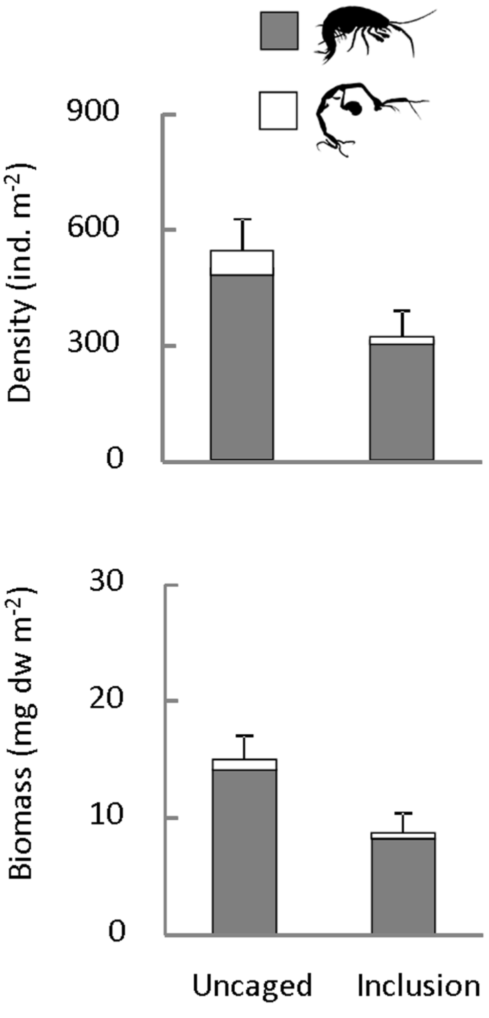
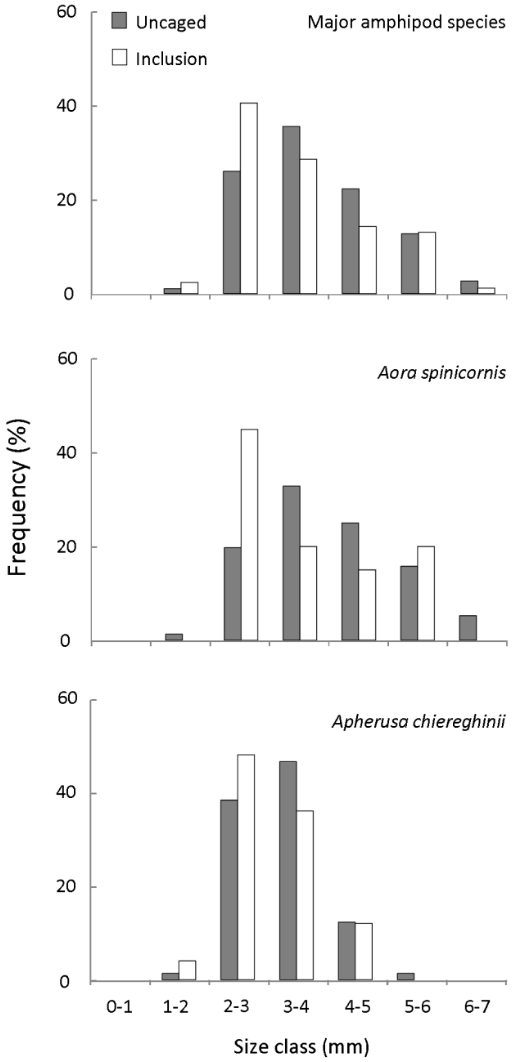

900
600
300
0
Density (ind. m-2)
30
20
10
0
Biomass (mg dw m-2)
Uncaged
Inclusion

Fig. 5

Size-frequency distributions of major amphipod species (> 3% of all specimens, n = 189 in uncaged areas and 84 in inclusion cages), Gammaridea *Aora spinicornis* (n = 76 in uncaged areas and 20 in inclusion cages) and Gammaridea *Apherusa chiereghinii* (n = 73 in uncaged areas and 50 in inclusion cages) for the inclusion experiment.

Figure 5.


Uncaged
Inclusion
Major amphipod species
Aora spinicornis
Apherusa chiereghinii
Frequency (%)
60
40
20
0
0-1
1-2
2-3
3-4
4-5
5-6
6-7
Size class (mm)

Discussion

The present study indicates distinct differences between the fully protected site and the partially protected site for density of fishes and amphipods. The higher densities of many fish species at the fully protected site is in agreement with the outcomes of previous studies. Results obtained at the TMPA for fish assemblages in sublittoral rocky reefs, showed higher values of biomass and size in fully protected sites compared to partially protected sites, and some fish responded also in terms of density (Di Franco et al. 2009, Guidetti et al. in press). In *P. oceanica* meadows, several authors have reported that large piscivorous and invertebrate-feeding fishes present generally greater abundances and number of species within fully protected sites compared to partially or unprotected sites (Francour 2000, Macpherson et al. 2002, Valle & Bayle-Sempere 2009). The lower densities of amphipods at the fully protected site relative to the partially protected site may be associated to the high fish predation intensity, which was 1.9 times higher. This is consistent with some studies suggesting that the spatial distribution of amphipod abundance associated with seagrasses, may be regulated by fish predation (Nelson 1979, Caine 1991).

Amphipod responses to fish predation

Caging experiments remain a valuable tool in predations studies in seagrasses (Edgar 1990, Hindell et al. 2001, Moksnes et al. 2008) and other marine ecosystems (Connell 1997, Sala 1997, Langlois et al. 2006). It is well known that it is complicate to eliminate the possibility that any effects detected might be due to caging artifacts. However, potential problems may be minimized or alleviated through careful planning, evaluation of potential artifacts and cautious interpretation (Kennelly 1991, Steele 1996, Connell 1997). In our study, major artifacts, such as reduced current speed inside cages did not appear to be important above the base of the rhizomes. This may be due the careful periodic scrubbing of cages to prevent the build-up of fouling (Sala & Boudouresque 1997), and phenological characteristics of the *Posidonia* canopy that play an important role in reducing water movement (Gambi et al. 1989). Attenuation of light was moderate inside cages and no differences were detected between treatments after 8 weeks for any measured biotic features of *P. oceanica*, except the percentage of leaves having alteration marks. This may be explained by higher grazing pressure of herbivores in uncaged areas (eg. the fish *Sarpa salpa*). We can therefore exclude major significant confounding effects due caging artifacts.

When predators are present, many caging studies have showed that invertebrate densities decrease (e.g. Edgar 1990, Kennelly 1991, Sala, 1997). This study provides caging experimental evidence that fish predators have strong effects on the structure of amphipod populations. Exclusion experiment showed that total amphipod density is positively affected by the exclusion of fish predation, and that a clear positive trend existed for the number of species. This coincided with other studies, which showed that amphipod densities became more abundant within fish exclusion cages (Sala 1997, Moksnes et al. 2008), and that the number of species of an invertebrate assemblage including amphipods, was greater in areas excluded from fish (Kennelly 1991). It has also been suggested that fish predation may determine seasonal changes in the number of amphipod species and diversity by selectively removing certain species (Nelson 1979). In our study, amphipod displayed species-specific patterns in response to fish exclusion which could be related to their varying life strategies and ecological behaviour. Species vulnerability to predation depends on its detectability and easiness to capture (Paloheimo 1979), which in turn are dependent on its morphology (e.g. pigmentation and body structure), behaviour and microhabitat distribution (Stein 1977). Our results showed that *C. acanthifera* seems to suffer from fish predation more than other amphipod species. This is probably because caprellids live more exposed on the surface and tips of the leaves (Virnstein et al. 1984), usually associated with epiphytes (Aoki 1999). They are important prey for many coastal fishes (Caine 1989, 1991, Woods 2009). Moreover, *C. acanthifera* can reach large size (13 mm) and spent time moving over the substratum in an inchworm-like movement (Guerra-García et al. 2002), which probably make it much more easily detected by a predator than other accompanying small size caprellids such as *C. tavolarensis* (2 to 6 mm) (Sturaro & Guerra-García 2012). Gammarids also responded to predation in terms of density and especially *I. minuta*, which was listed as a prey in the diet of *P. oceanica* fishes (Labropoulou & Plaitis 1995). Despite their motility, their habits to hide between seagrass blades (Virnstein et al. 1984) and/or the use of strategies such as burrowing and infaunal tube-dwelling, gammarids constitute one of the most abundant food items for *P. oceanica* fishes (Zupo & Stübing 2010). In the fully protected site, the relative availability of gammarid prey is much higher (89%) than caprellid prey (11%). This may influence their encounter probabilities with a predator and thus influence their susceptibility to predation.

In support to exclusion experiment, the results of inclusion cages also confirm top-down effects. The enclosed labrid fish predator reduced the density of the gammarid *Aora spinicornis* and the caprellid *Phtisica marina*, but a non-significant trend was observed for total amphipod density and biomass. These two amphipod species were abundant at the partially protected site, representing together more than 30% of the total number of amphipods and reaching almost 280 individuals m^{-2} in some areas. *Phtisica marina* is distributed on both sediment and blades in the seagrass meadow (González et al. 2008) and attaches the substrate in the“uprighy” position (Guerra-García et al. 2002), suggesting that this species can be easily detected by predators. Both species constituted preys found in the diet of several *P. oceanica* demersal fish species (Labropoulou & Plaitis 1995, Zupo & Stübing 2010). Surprisingly, *C. acanthifera* and *A. chiereghinii* did not show differences between treatments, while the former responded to fish predation in the exclusion experiment and the latter being one of the principal prey species in the diet of the *P. oceanica* fishes (Labropoulou & Plaitis 1995).

The fish *C. julis* is not an amphipod-feeder specialist. Nevertheless, inclusion treatment resulted in the decrease of larger individuals over small ones for major amphipods, suggesting that this fish preferentially consumed larger body length amphipod. Several studies in the marine environment focused on the mechanisms involved in prey selection by predatory fishes (Nelson 1979, 1981, Clements & Livingston 1984, Main 1985). It is suggested that the predator choice is primarily determined by prey size, but also by prey motion (Main 1985) and pigmentation (Clements & Livingston 1984). Predation by fishes causing a shift towards smaller amphipod individuals and/or species, appears to be common in marine ecosystems (Edgar & Aoki 1993, Moksnes et al. 2008). In a *Z. marina* seagrass meadow, treatment with a caged goby predator showed that size-specific predation reduced the abundance of adult gammarids by 93%, whereas juveniles were not reduced (Moksnes et al. 2008). Accordingly, our results indicated that fish predators can modify amphipod demography, which may have substantial consequences both for grazer effects on seagrass and epiphytic production, and energy transfer to higher trophic levels (Valentine & Duffy 2006).

Cascading effects?

A particular type of top-down control consists in trophic cascades that occur when a top predator indirectly affects lower trophic levels through interactions with intermediate trophic levels (Pinnegar et al. 2000). Removal of important fish predators by fishing or recovery in MPAs, can result in trophic cascades and indirect effects on marine benthic communities (Sala et al. 1998, Guidetti 2006, Claudet el al. 2011). Our study showed that predatory fishes can reduce amphipod densities in *P. oceanica* meadow at the fully protected site. In addition, it has been demonstrated that amphipod species can exert a top-down control on erected algal and animal epiphytes (Valentine & Duffy 2006), and can influence the whole *P. oceanica* epiphytic assemblage structure (Michel 2011). This may suggest a potential trophic cascade at the fully protected site of the TMPA, but further research is required to investigate this hypothesis. On the Swedish west coast, the presence of predators in eelgrass *Zostera marina*, reduced substantially the abundance of *Gammarus locusta* resulting in a leaf assemblage with high biomass of algae (Moksnes et al. 2008). However, it is difficult to demonstrate cascading effects as reactions down through the food webs progressively decrease (Planes et al. 2006), in particular in seagrass ecosystems where communities exhibit important degree of omnivory and feeding at different trophic levels (Bologna 2007). Trophic cascades do occur in high diversity MPAs (McClanahan 2005). Yet, evidence for assemblage wide trophic cascades are unusual and restricted to low diversity systems with simple trophic interactions (Strong 1992, Shurin et al. 2002). Thus, chances of detecting effects of trophic cascades in MPAs may be lower, as the number of species and/or trophic diversity may be greater than in unprotected areas (Planes et al. 2006).

In conclusion, this study provides evidence that predation by fish can be an important factor in structuring amphipod populations associated with *P. oceanica* seagrass. Fish predation can reduce density of amphipods at different levels (i.e. order, suborder and species) and in some cases biomass and size. Patterns observed at the species level suggest complex interactions which could be mainly related to behaviour of amphipod and fish species. This is a local study and it is unclear to what extent these results can be generalized to other areas at different times, where interactions between trophic levels may be weaker. Overall, these results give new piece of support to (1) the hypothesis that overexploitation of top predators contribute to important cascading effects, and (2) suggestions that effective management and conservation of seagrasses in MPAs requires detailed understanding of trophic interactions.

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Chapter 7

General discussion, conclusions and perspectives

Posidonia oceanica meadows, an amphipod biodiversity hotspot?

In the Mediterranean Sea, the seagrass *Posidonia oceanica* forms the most common and widespread meadows (Den Hartog 1970), whose extension has been estimated bewtween 2.5 and 5.5 million hectares (Buia et al. 2000, Procaccini et al. 2003). These meadows colonize mostly soft bottoms or rocky substrates, supporting a complex vagile fauna. Along with gasteropods and polychaetes, amphipods are one of the more abundant, rich and diverse group of *P. oceanica* meadows. Amphipods can represent 17 to 34 % of the numerical abundance of the vagile fauna and 22 to 31 % of the number of species (Mazzella et al. 1989, Gambi et al. 1992).

The presence of vegetation allows higher densities of animals and number of species when compared with unvegetated habitats, both soft bottoms (Heck & Orth 1980, Peterson et al. 1984, Heck et al 1989, Edgar et al. 1994, Irlandi 1994, Gray et al. 1996, Sánchez-Jerez et al. 1999b, Heck et al. 2003) and also shallow hard substrate biotopes (Sarda 1991). This pattern is usually attributed with an increase in habitat complexity (Stoner 1980, Edgar 1992, Ayala & Martín 2003, Taylor & Cole 1994) provided by the seagrass canopy and rhizome-root systems.

Previous studies showed that amphipods are also more abundant and/or more diverse in *P. oceanica* meadows, than in other macrophytes such as *Cymodocea nodosa* (Scipione et al. 1996, Sánchez-Jerez et al. 1999b, Como et al. 2008, Vazquez-Luis et al. 2009, Scipione & Zupo 2010), *Zostera marina* (Scipione & Zupo 2010), *Caulerpa prolifera* and *Caulerpa racemosa* (Vazquez-Luis et al. 2009). Moreover, the structure amphipod assemblages are different among these ecosystems (Sánchez-Jerez et al. 1999b, Vazquez et al. 2009).

Our research showed that amphipod assemblages of *P. oceanica* meadows of the Revellata Bay and the TMPA, are typified by high density, number of species and diversity, especially when compared with other studies from elsewhere in the Mediterranean Sea (Table 1). This study has allowed a total collection of 56 amphipod species (50 Gammaridea and 6 Caprellidea) in Corsican and Sardinian meadows. Among these species recorded, one recently described is new to science (*Caprella tavolarensis*), 11 species were collected for the first time in the Sardinian waters according to the check-list of the Amphipoda in Italian seas (Ruffo 2010), and 18 species were recorded for the first time in Corsican waters, in respect to the revision of Dauvin & Bellan-Santini (2002). In this latter case, a recent work of Michel (2011) take back the record to 6 species. Densities and number of species estimations are very variable and are essentially related to the method (Michel et al. 2010) and the sampling effort (Table 1).

The great amount of species firstly reported in the Corsican waters (50 % of the species collected) is due to the scarcity of investigation on amphipod sampling in this geographical area (Dauvin & Bellan-Santini 2002). The present study has recorded a high number of species, but for a limited coastal area (a few km), for homogeneous *P. oceanica* meadows between 10-15m depth, and only day sampling. Night sampling may potentially increase the number of species. This proves the need to significantly increase the prospection of the amphipods from seegrass meadows, notably *P. oceanica*. Therefore, based on our review of the literature, as well as our data, we expect that the total amphipod marine fauna of *P. oceanica* meadows should include more than 100 species.

In summary, the amphipod fauna of the Mediterranean Sea is well studied compared to other taxonomic groups (e.g. isopods). However, our results and data of the litterature suggested that the number of amphipod species is certainly underestimated due to general low sampling effort, still new species are discovered (see chapter 5). Therefore, *P. oceanica* meadows could be considered, perhaps, as a Mediterranean amphipod biodiversity hotspot. Yet, this complex and rich assemblage may present a high natural spatial variability.

Table 1.

Principal studies in which amphipod assemblages from *Posidonia oceanica* meadows has been assessed in the Western Mediterranean Sea. The table gives the total number of individuals collected in each study (N), number of species (S), estimated density (D), method used: airlift (A) or hand-towed net (H), and sampling depth (in m). * indicates density values estimatedusing the conversion factor of Russo & Vinci (1991). Data from this study are showed in grey. Numbers 1 to 19 refer to locations on Figure 1 page 277.

	Study area	N	S	D	Method	Depth	Reference
1	Alicante (S)	-	22	53	H	9-11	Sánchez-Jerez et al. 1999b
2	Alicante (S)	-	32	105	A	17	Sánchez-Jerez et al. 2000
3	Medes Islands (S)	29	9	1*	H	3-5	Scipione 1999
4	Revellata Bay (FR)	2563	28	7	H	10	Michel 2011
5	Revellata Bay (FR)	3337	36	220	A	11-13	This study
6	Porto Conte (IT)	407	25	18*	H	3-5	Scipione 1999
7	Oristano (IT)	-	17	-	A	2-8	Como et al. 2008
8	Tavolara (IT)	4512	51	218	A	10-15	This study
9	Marettimo (IT)	80	13	3*	H	3-5	Scipione 1999
10	Lampedusa (IT)	139	20	6*	H	3-5	Scipione 1999
11	Lacco Ameno, Ischia (IT)	6643	49	8*	H	1-30	Scipione & Fresi 1984
12	Lacco Ameno, Ischia (IT)	2093	47	6*	H	1-30	Mazzella et al. 1989
13	Lacco Ameno, Ischia (IT)	4217	77	5*	H	1-25	Gambi et al. 1992
14	Lacco Ameno, Ischia (IT)	112	13	5*	H	3-5	Scipione 1999
15	Castello Aragonese, Ischia (IT)	106	19	5*	H	3-5	Scipione 1999
16	San Domino (IT)	52	9	2*	H	3-5	Scipione 1999
17	Gallipoli (IT)	34	14	1*	H	3-5	Scipione 1999
18	Otranto (IT)	397	29	410	A	6.5	Scipione & Zupo 2010
19	Otranto (IT)	38	15	2*	H	6.5	Scipione & Zupo 2010

Amphipod natural variability is great at small and large scales

The multiscale spatial variability of amphipod assemblages in *P. oceanica* meadows may be driven by various processes operating at different scales, including biotic and abiotic interactions (chapters 1, 3 and 4). Hence, in order to distinguish between human interventions and natural patterns, an understanding of the basic patterns is required. This section summarizes and discusses spatial "natural" patterns observed in our research at the different scales, and focus our attention on the range of relevant processes that may be important to amphipod assemblages within *P. oceanica* meadows.

Small spatial scales

Our research revealed that amphipod natural variability in *P. oceanica* meadows is large at small spatial scales, particularly from ~1 m to ~10 m. This pattern is in relation to both total amphipod density and/or several species densities for both the Revellata Bay and the TMPA. As discussed in chapter 3 and 4, our results agree with studies on other taxa across a wide range of marine habitats, which found significant variation at small spatial scales (Morrisey et al. 1992, Underwood & Chapman 1996, 1998, Menconi et al. 1999, Benedetti-Cecchi 2001, Coleman 2002, Tanaka & Leite 2003, Fraschetti et al. 2005), notably in seagrasses (De Biasi et al. 2003, Sturaro 2007). This small scale variability seems to be one of the few common patterns between marine and terrestrial ecosystems (Fraschetti et al. 2005).

In marine environments, other authors find a high variability at small scales for amphipods associated to the macrophyte *Sargassum stenophyllum* (Tanaka & Leite 2003). They showed that the density of gammarid amphipods was patchy at small spatial scales (~1 m to ~10 m), while the same variability pattern was detected for caprellid amphipods (dominated by *Caprella scaura*), but was not consistent during the year. They suggested that various factors including habitat complexity, colonization by algal patches, reproductive and dispersal strategies may explain these patterns.

In our studies, several processes were mentioned in previous chapters to attempt to explain this pattern. At small spatial scales, variation of assemblages could result both from differences in habitat features and/or biological interactions (Thrush 1991, Underwood & Chapman 1996, Tanaka & Leite 2003). Several features of seagrass occurring at the scale of tens of centimetres to metres (e.g. shoot density, leaf, epiphyte and litter biomasses) may influence density and richness of organisms (Gillanders 2006, Hemminga & Duarte 2000). The meadow structure may enhance density of epifauna by reducing fish predation rates, but provide also a greater trophic resource availability, which is greater within dense seagrass (Bell & Westoby 1986, Duffy & Hay 1994, Jernakoff et al. 1996). Nevertheless, our results presented in chapters 3 and 4 emphasized two general points: (1) amphipod assemblages showed non or weak relationships with measured meadow features, and (2) among these features, litter biomass had the highest influence on the distribution and abundance of amphipod assemblages.

Relationships between faunal abundance and seagrass structural components such as shoot density and biomass are common (Heck & Orth 1980, Lewis & Stoner 1983, Bell & Westoby 1986, Attrill et al. 2000), but is far from being a generality. Many authors, found non or weak relationships between seagrass features and the structure of associated assemblages, notably for amphipods (Scipione et al. 1996, Turner et al. 1999, Vasapollo 2009), suggesting that other processes could influence faunal distribution.

Nevertheless, it is difficult with our data to decide what meadow variables (except litter biomass) are important for particular amphipod faunal variables (i.e. species denities or general descriptors). In fact, in certain cases, there was no consistency between statistical methods (i.e. multiple regression and distance-based redundancy analyses) and study areas (i.e. Revellata Bay and TMPA) for most variables. One explanation for this pattern is that the number of replicates at each area would not be enough (despite the important sampling effort) to capture the environmental spectrum, leading to biased amphipod-habitat relationships. Local-specific associations for amphipod species would be another explanation, suggesting a possible adaptation of amphipod species to resources available at each locality.

In addition, some methodological considerations should be emphasized here. First, sampling was done during a short period (i.e. July-August) at constant depth interval (i.e. 10-15m). The range of values of meadow features could be limited, compared to the potential values observed with seasonal and bathymetric variations. Nevertheless, at the Revellata Bay, range of values for density of the meadow (~90-680 shoots. m^{-2}), leaf biomass (~0.9-1.9 g dw. Shoot^{-1}) and epiphyte biomass (~0.1-0.5 g dw. Shoot^{-1}) was relatively high and close to the range of values observed with seasonal and bathymetric variations (Gobert 2002). Second, the finest sampling unit is based on the collection of 3 shoots, which could biased our estimations such as leaf and epiphyte biomasses, and masked potential relationship with amphipod assemblages. We estimated that a collection of ~30 shoots would be necessary to capture the "true variability" of epiphyte biomass within our sampling unit (data not showed). This was practically difficult. Third, study sites were chosen in the context of a comparative study, therefore with the initial supposition of comparable habitats. At last, only 5 variables were considered in our analyses. Other potential variables, as well as smaller and/or larger scales (e.g. meadow cover) may be important. Therefore, monitoring of other areas, with supplementary meadow variables and larger ranges would be necessary to better understand amphipod-habitat relationships in *P. oceanica* meadows (e.g. Vasapollo 2009). Nevertheless, because various factors may be involved in a complex manner, it is difficult to elucidate simple relationships between these variables and amphipod assemblages (Jernakoff et al. 1996).

Among all meadow features measured, litter biomass had the highest influence on the distribution and abundance of amphipod assemblages (chapters 3 and 4). Previous studies have already reported the importance of this compartment in structuring amphipod assemblages, but did not use a strong sampling design to support it (Scipione & Fresi 1984, Sánchez-Jerez et al. 2000, Michel 2011). *Posidonia oceanica* litter provides a complex structural habitat for an abundant animal community (Gallmetzer et al. 2005, Remy 2010) and is essential for various processes, including nutrient cycling and trophic web functioning (Boudouresque et al. 2006, Lepoint et al. 2006, Michel 2011). Across all samples collected in this study, litter biomass represented between 1 and 72% of live leaf biomass (data not showed), and can reach 200% in other meadows (Boudouresque et al. 2006). Therefore, the litter cover present within the meadow could be a vital microhabitat interconnected with the foliar stratum for amphipods (Michel 2011).

This study allows us to draw, for the first time, a picture of the multiscale spatial variability of litter biomass within *P. oceanica* meadow. Although litter biomass presented a high variability at the small scale (~ 1 m), the highest variability was observed between zones and regions; see chapters 3 and 4). Thus, the influence of litter biomass seems to be exerted mainly at large spatial scales. This pattern is not surprising, considering that the amount of litter is primarily under the control of large scale hydrodynamic forces (Pergent et al. 1997). Clearly, litter biomass is not sufficient to explain the small scale variability pattern of amphipod assemblages.

Fish predation may operate in patchy ways in heterogeneous seagrass ecosystems (as mentioned in chapter 3), creating gaps and localized differences in the abundance of prey species (Underwood 1997). In addition, the greater heterogeneity and dispersion in the structure of amphipod assemblages were observed where fish predation intensity was higher (i.e. fully protected area, FPA; chapters 4 and 6). At last, this hypothesis is likely to be plausible as we provide evidences that predation by fish can be an important factor in structuring *P. oceanica* amphipod populations (chapter 6). Predators can determine survival of prey species, but also modify interactions among them (Chase et al. 2002). In our experimental manipulations of predation intensity, patterns observed at the species level, suggest complex interactions, probably mainly related to behaviour of amphipod species.

Behavioural traits of species can influence their distribution, especially at smaller–scales (Underwood & Chapman 1996, Tanaka & Leite 2003). For instance, mobility differences among amphipods may influence variability at scales of 1 to 10s m. Free-living amphipods have generally good swimming ability, and may select distinct substrates in which to hide and forage (Tanaka & Leite 2003). Thus, for free-living gammarids such as *A. chiereghinii* and *D. spinosa*, distances up to 10s of metres may not be a barrier to dispersal (Virnstein & Curran 1986). In contrast, tube-builders such as *A. helleri* and *E. punctatus*, are more sedentary, selecting substrates where they can find both food and shelter (Buschmann 1990, Dixon & Moore 1997). At last, inquiline species live associated with invertebrates in a non-obligate relationship (Barnard and Karaman, 1991). *Tritaeta gibbosa* and *Leucothoe spinicarpa* are among the most frequent species to be find in association with sponges (Arndt 1933, Krapp-Schickel & Krapp 1975, Bellan-Santini 1999, Ruffo 1982). The latter species, called the ascidian-dwelling amphipod by Thiel (1999), has also been found living in Ascidiacea of the genus *Microcosmus* (Ruffo 1982), which are commoly encountered at the rhizome level of *P. oceanica* (Pers. Obs.). Thus, the high small scale variability of *L. spinicarpa* could be driven by the distribution of ascidians. Overall, it is expected that species with higher mobility may present less aggregative patterns at smaller spatial scales than species with lower mobility (Thrush 1991, Tanaka & Leite 2003). However, no distinct pattern was detected in our study between amphipods with these different lifestyles, probably because of other complex factors.

Reproductive behaviour of amphipods can influence their small-scale distribution. In fact, amphipods have a direct development and females carry their broods in a ventral pouch, which are released into the environment, as “small adults” (Barnard & karaman 1991). This often results in small-scale aggregations (Fenwick 1984, Thiel 1999a), where parental care can take place. For instances, observations showed that juveniles of *L. spinicarpa* remain with their parents and they relocate to larger, solitary ascidians only when they reach maturity (Thiel 1999a). In the genus *Leptocheirus*, maternal dwellings are important structures where juveniles find protection from predation (Thiel 1999b). Thus, it is possible to obtain a high-density patch through juveniles (Aoki 1999, Thiel 1999a). In our study, although, all samples presented ovigerous females, juveniles (essentialy from the genus *Apherusa*, *Dexamine*, *Aora* and *Caprella*) were very low in abundance representing only 0.7 and 1.4% of the total amphipod abundance, for respectively the Revellata Bay and the TMPA. These results may be probably largely underestimated and most juveniles certainly passed through the sampling net, and may not explain our patterns. In *P. oceanica* meadows, information on behavioural traits are very scarce and would be useful to understand the structure of amphipod assemblages and the high small spatial scale heterogeneity.

Landscape scale

The landscape approach (10s of m to km) has proven to be valuable in seagrass meadows (Robbins & Bell 1994, Boström et al. 2006). As mentioned in chapter 1, factors that operate at scales of 100 and 1000 of m include the meadow position within a bay and adjacent habitats (Gillanders 2006). For some authors, it seems clear that these scales may have significant effects on abundance and diversity of invertebrate populations, but also on predator-prey interactions (Bell et al. 1988, Bell et al. 2006, Gillanders 2006). For instance, non-vegetated habitats that surround a meadow allow an easy access to predators (Gillanders 2006).

Because the structure of seagrass meadows can vary throughout a bay, differences of meadow features may contribute to differences in faunal variables (Gillanders 2006). In addition, water circulation is likely to vary at large spatial scales, and may have an influence on amphipod assemblages of the *P. oceanica* meadow (Scipione & Fresi 1984). At the Revellata Bay, our results showed that amphipods assemblages (in terms of all general descriptors, most species densities/biomasses and structure) were not affected at the site (~100 m) and zone scale (~1000 m). Within the bay, information on the distribution of the different benthic ecosystems is available for the Revellata Bay (see chapter 3 Fig. 1). The apparent homogeneity of *P. oceanica* meadow at these large scales is also reflected in the results of all *P. oceanica* descriptors which were statistically similar. However, caution must be taken (e.g. for epiphyte biomass), since statistical test for the zone scale had a low number of degrees of freedom, which influenced negatively the power of the test. Thus, this probably reduced the detection of any significant response in some variables.

The proximity to adjacent habitats or matrix (e.g. rocky and sandy bottoms, other macrophytes) can play an important role on species associated to seagrass (Bell et al. 1988, Sogard 1989, Irlandi & Crawford 1997, Tanner 2006). Since, the nature of habitat and its distance from the seagrass meadow can affect the diversity of seagrass fauna (Sogard 1989), adjacent habitats may have a clear effect on the nature of epifaunal assemblage present in *Posidonia* seagrass, notably for amphipods (Tanner 2006). At the Revellata Bay, all sectors were selected at least at ~10 m from any other habitats, except for sectors 7 and 8 which presented at a few metres, small surfaces (~1-5 m^2) of rocky substrates (Pers. Obs.). Larger areas (>30 m^2) of sandy or rocky bottoms are far away (~50-500 m) and did not seem to have an influence, since no differences were detected in amphipod assemblages at these scales. Nevertheless, the landscape perspective in *P. oceanica* meadow is not well known (but sees Tanner 2006 about *Posidonia australis*). Further research is clearly required which consider the interaction of *P. oceanica* meadows with adjacent habitats, including the potential effect of varying distances between these habitats.

Regional scale

Investigations of amphipod assemblages within *P. oceanica* meadows over regional scales (10s to 100s of km and greater) are scarce (but see Scipione 1999, Katagan et al. 2001, Zakhama-Sraieb et al. 2011). Our data presented in chapters 3 and 4, as well as supplementary analyses integrating the regional scale (data not showed) showed that the structure of amphipod assemblages are significantly different between the Revellata Bay in Corsica and PPAs of the TMPA in Sardinia. Although, the three most abundant species are qualitatively the same (i.e. *A. chiereghinii*, *A. spinicornis* and *P. marina*), their relative abundances varied. Results also revealed that none of the general descriptors were different, but certain amphipod species (*A. neapolitanus*, *C. acanthifera* and *I. minuta*) showed marked differences in terms of density.

Differences in amphipod assemblages between our study areas may be explained by factors such as the meadow structure (Virnstein et al. 1984, Scipione 1999). However, as mentioned in our research, as well as in some previous studies (Scipione et al. 1996), amphipod faunal variables and meadow features are not or weakly correlated. At the regional scale, the only meadow feature who showed a significant difference was litter biomass, with higher significant values at the Revellata Bay (average of 38 g dw. m^{-2}) compared to the TMPA (average of 12 g dw. m^{-2}). The important litter biomass at the Revallata Bay compared to the TMPA is not due to its potential constant accumulation during July and August. Correlations between sampling times and litter biomasses were not significant at the Revellata Bay (r = -0.20, ns) and the TMPA (r = 0.24, ns). These differences are in the range of values obtained in other meadows separated by more than 100s of km on the Spanish coast at the same period (between 0.4 and 146 g dw. m^{-2} and an average (± SE) of 29 ± 9 g dw. m^{-2}; Cebrian & Duarte 2001). Yet, across all sampling sites, litter biomass explained only 13 % of the variation in amphipod assemblage structure, and consequently other factors may also be responsible.

The amount of litter can be considered as a tracer of water movement (Pergent et al. 1997). Thus, study zones at the Revellata Bay can be seen as litter accumulation zones characterized by low hydrodynamic forces. *Ampithoe helleri* and *A. spinicornis*, which prefer low hydrodynamic conditions (Scipione 1999), were among the main species contributing to dissimilarity between the two areas (data not showed). This seems to suggest that amphipod assemblages in *P. oceanica* meadows may be (at least in part) under the control of hydrodynamic forces (Scipione & Fresi 1984), especially at the regional scale.

Furthemore, our data compilation around the Western Mediterranean Sea (Fig. 1, Table 2) supports the idea that a common amphipod assemblage to all meadows cannot be recognized at the regional scale (Scipione 1999). This compilation shows the lack of common species to all meadows and the dominance of different species. Previous cited factors, as well as surrounding habitats and geographic location (e.g. latitude and/or basin in the Mediterranean Sea) may play a role in the differences between the meadows (Virnstein et al. 1984, Scipione 1999). Oristano in Western Sardinia is characterized by species generally found in fine sandy and/or muddy bottoms *Phoxocephalus aquosus* and *Ampelisca brevicornis*, and the mud tube-builder *Corophium sextonae* (Ruffo et al. 1982, 1993), indicating high sedimentation in this area. This meadow is certainly influenced by the Cabras lagoon at proximity (Como et al. 2007, 2008). *D. spinosa* are generally more abundant in sheltered meadows (Ledoyer 1962) present in Porto Conte, Ischia and San Domino (Mazzella et al. 1989, Gambi et al. 1992, Scipione 1999). For the two latter areas, this species is accompanied by *P. marina* and/or *A. helleri* and *A. ramondi*, which also prefer low hydrodynamics. Therefore, large within-study area differences may overwhelm potential latitudinal patterns (Virstein et al. 1984).

However, some common points emerge. Most of the abundant species belong to the same feeding categories (i.e. herbivores and omnivores). According to the literature more than 11 feeding guilds are described in *P. oceanica* meadows (Gambi et al. 1992). Recent data combining gut content examination and trophic tracers (fatty acids and stable isotope ratios) indicate that *A. chiereghinii*, *A. spincornis, A. helleri and D. spiniventris* consume preferentially epiphytes from the *P. oceanica* leaves, litter fragments and/or rhizomes (Michel 2011). Other studies classified *P. marina*, *C. acanthifera* and *P. phasma* as omnivores, while *H. schmidtii*, *C. crassicornis* and *D. spinosa* as herbivores or herbivores-deposit feeders (Gambi et al. 1992, Scipione 1999).

Another important point among study areas, is the importance of *A. chiereghinii,* the most frequent and abundant species collected in our research. *Apherusa chiereghinii* corresponds clearly to the most representative species of the assemblage in *P. oceanica* meadows of the Western Mediterranean Sea (Table 2). Yet, this species seems opportunist, as it is also found in other Mediterranean ecosystems such as *Cymodocea nodosa* (Scipione et al. 1996, Sanche-Jerez et al. 1999, Scipione & Zupo 2010), *Zostera* *marina* (Scipione & Zupo 2010), *Caulerpa prolifera* (Vazquez-Luis et al. 2009) and various photophilic algae (Bellan-Santini 1998).

Overall, discerning regional patterns between study areas (Fig. 1, Table 2) is likeky to be difficult due to differences in sampling designs (e.g. sampling depth and season) and methodologies across studies (e.g. hand-towed vs airlift) and the lack of studies in some regions. More comparative data are necessary to better characterize the amphipod assemblage of *P. oceanica* meadows throughout the Mediterranean Sea. A large scale comparative study using a similar design, method and protocol can be easily planned and organized among researchers from several countries.

Knowledge on the multiscale variation will inevitably influence the design of future comparative and experimental studies of amphipod assemblages. The variability detected at smaller scales (from ~1 m to ~10 m) may affect statistical power (Morrisey et al. 1992) in comparative studies where the examination of spatial patterns over large spatial scales is the basis of the principal hypothesis. For instance, the comparison of various areas with different protection levels is most often separated by the km scale. In such investigation, the detection of the large scale patterns is the principal aim. Thus, among replicate variation should minimal and small-scale samples should be of primary importance in order to represent adequately amphipod assemblages within sites. Samples collected over an area of ~1 m to ~10 m will include a range of patches and the “true variability” of the study site may be captured. This way is certainly better than examination of amphipods on several square metres, which is a very difficult task because of time and cost constraints. Using our multiscale approach, allowed to take into account the small scale variability of these populations and seems to be adequate for the assesment of amphipod responses to different protection levels in and outside a MPA, which depends primarily on our ability to separate the natural variability from potential effects of protection.

Figure 1.

Map of the Western Mediterranean Sea showing principal study areas where amphipod assemblages from *Posidonia oceanica* meadows has been assessed. See tables 1 and 2 for amphipod data details of each study area.

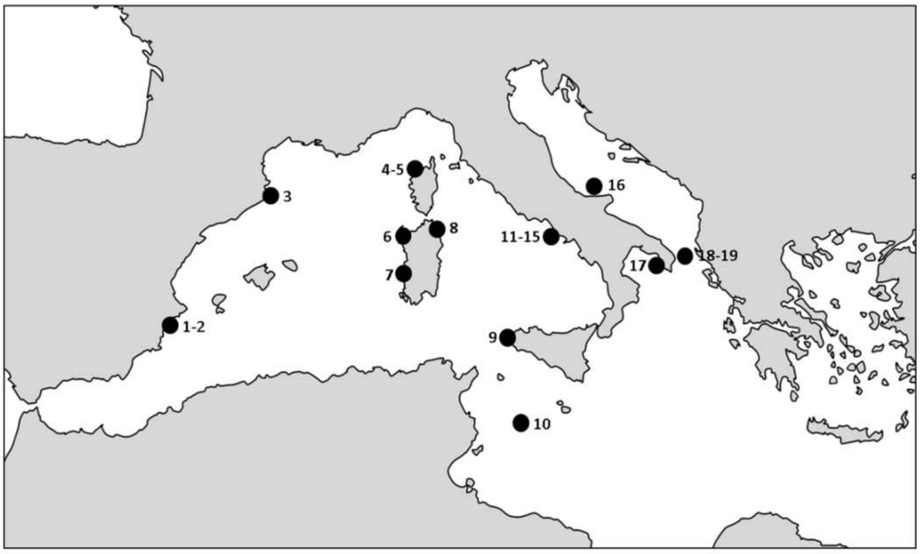


Table 2.

Principal studies in which amphipod assemblages from *Posidonia oceanica* meadows has been assessed in the Western Mediterranean Sea. The three most abundant species and their relative abundances (in percentage of the total number of individuals) and the method used (airlift: A or hand-towed net: H), are presented. Data from this study are showed in grey. Numbers 1 to 19 refer to locations on figure 1.

	Study area	Most abundant species	%					Method	Reference
1	Alicante (S)	*Dexamine spiniventris*	29	*Ischyrocerus inexpectatus*	18	*Hyale schmidtii*	15	H	Sánchez-Jerez et al. 1999b
2	Alicante (S)	*Apherusa chiereghinii*	-	*Siphonocetes* spp	-	*Lysianassina longicornis*	-	A	Sánchez-Jerez et al. 2000
3	Medes Islands (S)	*Aora spinicornis*	41	*Erichthonus punctatus*	21	*Apherusa chiereghinii*	14	H	Scipione 1999
4	Revellata Bay (FR)	*Apherusa chiereghinii*	64	*Aora spinicornis*	6	*Dexamine spiniventris*	5	H	Michel 2011
5	Revellata Bay (FR)	*Apherusa chiereghinii*	33	*Aora spinicornis*	20	*Phtisica marina*	12	A	This study
6	Porto Conte (IT)	*Dexamine spinosa*	34	*Apherusa chiereghinii*	21	*Caprella acanthifera*	15	H	Scipione 1999
7	Oristano (IT)	*Phoxocephalus aquosus*	-	*Ampelisca brevicornis*	-	*Corophium sextonae*	-	A	Como et al. 2008
8	Tavolara (IT)	*Apherusa chiereghinii*	29	*Phtisica marina*	16	*Aora spinicornis*	11	A	This study
9	Marettimo (IT)	*Cymadusa crassicornis*	30	*Ampithoe helleri*	25	*Aora spinicornis*	13	H	Scipione 1999
10	Lampedusa (IT)	*Apherusa chiereghinii*	32	*Hyale schmidtii*	27	*Apolochus neapolitanus*	19	H	Scipione 1999
11	Lacco Ameno, Ischia (IT)	*Apherusa chiereghinii*	59	*Phtisica marina*	10	*Pseudoprotella phasma*	6	H	Scipione & Fresi 1984
12	Lacco Ameno, Ischia (IT)	*Apherusa chiereghinii*	41	*Phtisica marina*	14	*Dexamine spinosa*	5	H	Mazzella et al. 1989
13	Lacco Ameno, Ischia (IT)	*Apherusa chiereghinii*	21	*Ampithoe helleri*	15	*Dexamine spinosa*	8	H	Gambi et al. 1992
14	Lacco Ameno, Ischia (IT)	*Ampithoe helleri*	30	*Phtisica marina*	21	*Dexamine spinosa*	21	H	Scipione 1999
15	Castello Aragonese, Ischia (IT)	*Ampithoe helleri*	30	*Ampithoe ramondi*	17	*Dexamine spinosa*	16	H	Scipione 1999
16	San Domino (IT)	*Aora spinicornis*	46	*Phtisica marina*	15	*Dexamine spinosa*	10	H	Scipione 1999
17	Gallipoli (IT)	*Hyale schmidtii*	29	*Dexamine spinosa*	15	*Apolochus neapolitanus*	12	H	Scipione 1999
18	Otranto (IT)	*Apolochus neapolitanus*	33	*Peltocoxa marioni*	12	*Apherusa chiereghinii*	10	A	Scipione & Zupo 2010
19	Otranto (IT)	*Apherusa chiereghinii*	22	*Peltocoxa marioni*	11	*Apolochus neapolitanus*	8	H	Scipione & Zupo 2010

Usefulness of hierarchical sampling design

Differences between sampling units distant from ~1 to 10s of metres from each other can be larger than differences between sites and areas separated by ~100s to 1000s metres (see previous section). Therefore, descriptions of amphipod assemblages obtained at only one sampling plot or sector within a site can lead to wrong conclusions about spatial patterns. In this context, it is essential to include more than one spatial scale in the sampling design of the assessment of the TMPA. This can help to elucidate potential effects of protection on amphipod assemblages.

The hierarchical sampling approach, is a powerful tool to investigate spatial (or temporal) patterns across scales (Fraschetti et al. 2005). Actually, this approach is advice in the view of the assessment of MPAs (Fraschetti et al. 2002, García-Charton et al. 2004, Ojeda-Martínez et al. 2011), and has been used in a number of studies on fish assemblages (García-Charton et al. 2004, Di Franco et al. 2009), but not on small macrozoobenthos. In several cases, sampling of small macrozoobenthos has been restricted to one site in FPA and one site outside (Boudouresque et al. 1992), and small sampling replication (Badalamenti et al. 1999, Milazzo et al. 2000). So that conclusions are not so defensible.

As any other methods, this approach is not completely free of problems (Underwood & Chapman 1996, Benedetti-Cecchi 2001). When using analysis of variance in this approach, the intensity of sampling and accordingly statistical power, decreases higher in the hierarchy. Hence, the capacity to detect significant varitions at the largest scales is lessened. In addition, if ecological information on the studied assemblages are scarce, the choice of the different scales becomes more intuitive, while it is essential that suitable scales are chosen (according to the taxa and ecosystem studied), for the evaluation of the effects of protection (Ojeda-Martínez et al. 2011).

Hierarchical sampling designs are one tool, and a range of methods exist to help to sort out spatial patterns (Pielou 1969, Underwood & Chapman 1996). One commonly used alternative is spatial autocorrelation (Legendre 1993). The latter requires very large sampling efforts, since data have to be collected continuously over the entire range of the study. In contrast, the hierarchical method allows to sample a much larger spatial extent than continuous one. Certainly, these methods can be complementary (Underwood & Chapman 1996), and a mix of several methods will generally provide the best way to examine spatial patterns (Underwood 1997). However, when larger scales are used such as in our study, the hierarchical sampling approach is the most precise way to obtain realistic interpretation of spatial patterns (García-Charton et al. 2004).

It is not possible to exclude that the variation in amphipod assemblages observed between the different protection levels at the TMPA may be preexisting and not related to the protection itself. No data describing amphipod assemblages or small macrozoobenthos are availbale before the TMPA was established. In addition, Mediterranean MPAs are usually, established in areas that already harbour healthy *P. oceanica* meadows, which may form favourable habitats for the development of a rich and abundant amphipod fauna. This emphasizes the fact that the choice of controls is crucial and and deserves careful consideration (Fraschetti et al. 2002). However, due to natural variability of ecosystems, it is alsmost impossible that any control is truly comparable with protected ones (Fraschetti et al. 2002).

Responses of amphipod assemblages to different protection levels

Amphipod assemblages are characterized by great small spatial scale variability in *P. oceanica* ecosystems. Patterns observed at the TMPA (in contrast to the Revellata Bay) showed also differences among zones, suggesting that protection might be superimposed upon natural variability. Nevertheless, several other potential factors operating at different scales (discussed in previous chapters and section) may not allow to separate such underlying variation from potential effects of protection. The most relevant natural factors, which are likely to be significant in regulating *P. oceanica* amphipod assemblages are summarized in figure 2. This simplified model of potential effects of protection (fully protected area) versus potential relevant natural factors integrate knowledge based on our results and our review of the literature on seagrass ecosystem.

In *P. oceanica* meadows, invertebrate feeding fish present generally greater abundance and number of species within fully protected sites compared to partially or unprotected sites (Francour 2000, Macpherson et al. 2002, Valle and Bayle-Sempere 2009). Futhermore, in this study, density of fishes and predation intensisty were higher in a fully protected site compared to a partially protected site. This explains the sign "+" associated to the interaction between "protection" and fish "predation".

Fish predation is typically considered as one of the principal factor promoting changes in amphipod assemblages and population decline in seagrasses (Nelson 1979, Stoner 1982, Caine 1991, Heck & Orth 2006, Moksnes et al. 2008). The present research provides evidence that predation by fish could be an important factor in structuring *P. oceanica* amphipod assemblages. Fish predation may reduce density of several amphipod species and in some cases biomass and size, explaining the "-" sign on this interaction. The indirect effect on amphipod assemblages is denoted by the green dotted arrow.

At the same time, protection can have contrasted effects on *P. oceanica* meadow features. In shallow zones, cessation of local human activities within FPAs may eliminate or reduce potential alteration on *P. oceanica* (Montefalcone et al. 2009), since the health of the meadow is generally related to the degree of local anthropization (Gobert et al. 2009). On the other hand, increasing grazing activity of herbivorous fishes (i.e. *Sarpa salpa*) in some protected sites can lead to an alteration of the structure of *P. oceanica* medows (Ferrari et al. 2008, Prado et al. 2008). This explains the sign "±" associated to the interaction "protection" and "habitat".

In this study, habitat features were relatively similar between zones and did not or only weakly (for litter biomass) influenced amphipod assemblages (chapters 3 and 4), which is in accordance with other studies (Scipione et al. 1996, Vasapollo 2009). Nevertheless, we should note that the influence of *P. oceanica* features on amphipod assemblages is controversial, explaining the sign "±" (see Zakhama-Sraieb et al. 2011, Sánchez-Jerez et al. 2000, Como et al. 2008). In contrast, amphipods feed on macroepiphytes and had an impact on epiphytic biomass (sign "-" in the direction of habitat which include food; Michel 2011), suggesting a potential trophic cascade at protected sites (chapter 6), but further research is required to investigate this hypothesis.

In our research, patterns observed at a range of scales, allowed us to focus our attention to other potential factors that may be important for amphipod assemblages (Fig. 2, round on the right), and generate explanatory hypotheses (previous section or chapters). These factors may contribute to the variability of amphipod assemblages at the different scales and superimpose the potential effects of protection (Fig. 2, round on the left).

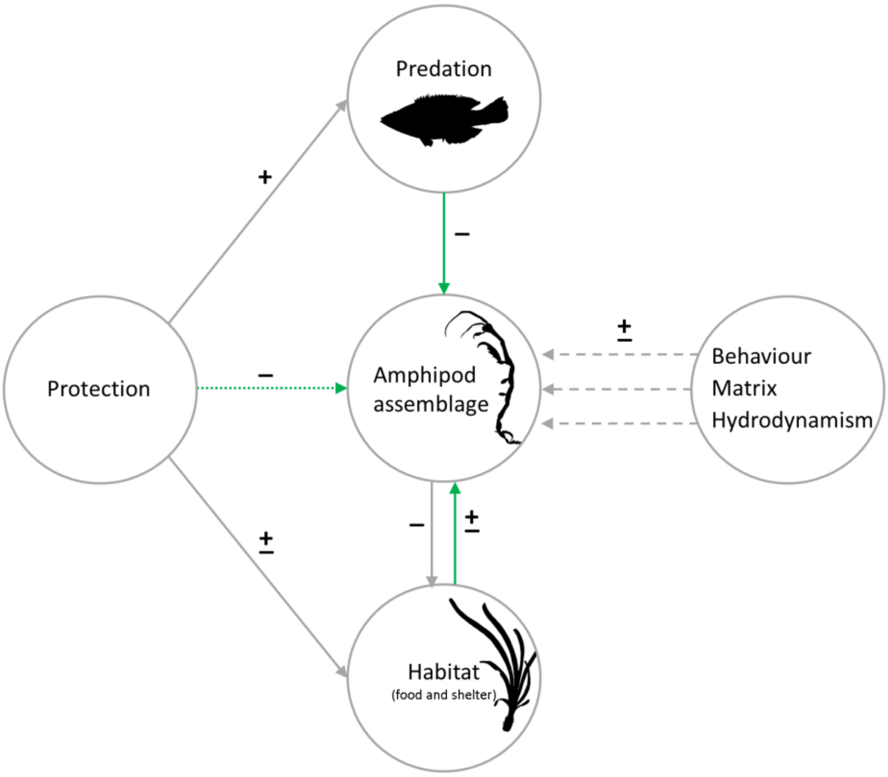
Within a single sector or site, amphipod assemblages showed a considerable small scale (from ~1 m to ~10 m) variability in terms of total amphipod density and/or several species densities. We suggest that this may primarily determined by behavioural processes including dispersion capabilities and reproduction. At the landscape scale (from ~10s of m to km), patterns may be determined by the matrix, or surrounding habitats. For instance, as mentioned in chapter 4, the *P. oceanica* meadow within the FPA of the TMPA is isolated (Navone 1992), and surrounded by sandy and rocky bottoms (Pers. Obs.). At the regional scale, amphipod assemblages may be under the control of hydrodynamic forces (see previous section).

This integrate overview of interacting factors in *P. oceanica* meadows is only partial. Several other factors, compartments and potential interactions are omitted. Nevertheless, it represents a general view of our understanding of the studied ecosystem, and emphasizes the important trophic place of amphipod assemblages. Of course, interactions are largely more complex and this figure is a simplified model.

So, based on all evidences developed in this thesis, our results suggest that protection in the FPA at the TMPA is likely to contribute partially (primarily via fish predation) to the observed variability patterns among zones. However, superimposed factors including behavioural traits of amphipod species and surrounding habitats (matrix) are likely to be also significant. Whether these changes are representative of all FPAs and whether those effects are positive or negative to the meadows, are still unknown.

Figure 2.

Simplified model of potential effects of protection (fully protected area) versus natural factors, regulating variability of amphipod assemblages in *Posidonia oceanica* meadows. Solid (direct effect) and dotted (indirect effect) green arrows indicate interactions evaluated and suggested by our results. Solid grey arrows indicate interactions confirmed by other studies. Broken grey arrows indicate hypothetical interactions suggested by our results and other studies. Positive (+) and negative (-) signs indicate that the factor enhance and diminishes, respectively, the importance of the other factor or assemblage, in the direction of the arrow. The combination "±" indicate ambivalent interactions (negative, positive or neutral) depending on the situation.



Gaps in knowledge and future directions

The general purpose of this research was to assess and better understand the potential responses of amphipod assemblages in *P. oceanica* meadows to different protection levels, in and outside a MPA. Two approaches were used. First, we examined variability patterns of amphipod assemblages at a range of spatial scales at the Revellata Bay and the TMPA. Our research highlighted a general pattern: amphipod natural variability in *P. oceanica* meadows is great at small and regional spatial scales. At small spatial scales, amphipod assemblages did not indicate or only weakly relationships with the meadow features. Alternative explanation of the high small scale variability include primarily behavioural processes of amphipod species.

There are more than 80 amphipod species described to date in *P. oceanica* meadows (Ruffo et al. 1998), but the ecology of most of them is little known. Based on the literature review of this thesis, there is currently no behavioural knowledge of amphipods living in *P. oceanica* meadows. For instance, no information related to topics such as mobility is available on the most representative species *A. chiereghinii*. Patterns observed at small scales won't be understood if researchers do not consider more studies on behavioural traits of species. Small scale variability should not be considered "noise", but involve a set of complex factors that determined species spatial and temporal positions (Coleman et al. 2004, Fraschetti et al. 2004). There are some indications (see previous section) that small-scale patchiness may also occur at even smaller scales (i.e. cm). More behavioural studies are necessary for most species inhabiting *P. oceanica* meadows and more generally seagrasses ecoystems.

At regional scale, a common structure of the amphipod assemblage between the Revellata Bay and the TMPA could not be recognized. Probably, hydrodynamic forces may explain this pattern. In a larger perspective, more comparative data is needed throughout the Mediterranean Sea to understand this pattern. A standardization of procedures including sampling design and methods must be carefully planned. Only then, processes responsible for this pattern could be determined. Small and regional scales variability is also certainly not restricted to amphipod species densities and biomass, or structure of the assemblages. Other variables might also show this pattern at similar scales.

At the TMPA, the structure of amphipod assemblages was patchy at all spatial scales, but differed clearly among protection levels. Furthermore, lower densities and/or biomasses of several frequent taxa were observed within the FPA compared to PPAs. We suggested that fish predation may be responsible for this pattern. In order to validate this hypothesis, the second approach of our research consisted to manipulate experimentaly predation intensity using exclusion and inclusion cages at the TMPA. Results provided evidence that predation by fish is likely to be an important factor in structuring amphipod assemblages. In fact, in this study, predatory fishes reduced certain amphipod densities at the fully protected site. At the same time, amphipod species can exert a top-down control on the whole *P. oceanica* epiphytic assemblage structure (Michel 2011). This may suggest a potential trophic cascade at the fully protected site of the TMPA. This topic clearly deserves further attention.

All along this study, results suggests that variability induced by protection is likely to contribute partially to the observed patterns among zones in a MPA. However, superimposed factors including behavioural traits of amphipod species and surrounding habitats (matrix) are likely to be also significant. Information needed to estimate the relative importance of those factors is scarce. It is a challenge to quantify each potential relevant factor. Therefore, long term multiscale spatial and temporal surveys of amphipod assemblages and more generally macrozoobenthos, combined with manipulative experiments, are needed to evaluate more precisely potential effects of protection in Mediterranean MPAs.

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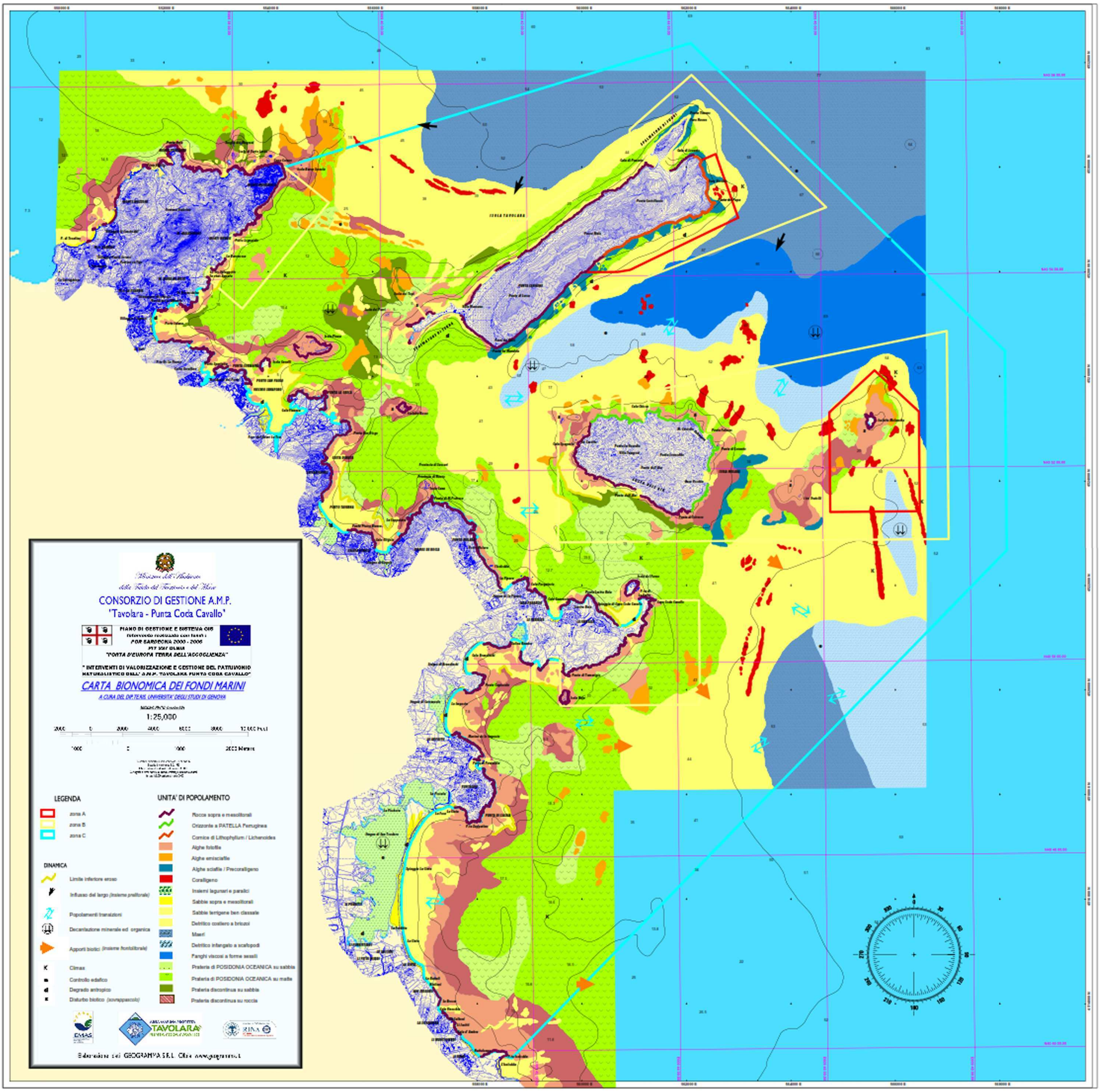
Annexes

I. Distribution of the different benthic ecosystems at the TMPA

II. Hierarchical designs used at the TMPA and the Revellata Bay


Ministero dell'Ambiente
della Tutela del Territorio e del Mare
CONSORZIO DI GESTIONE A.M.P.
'Tavolara - Punta Coda Cavallo'
PIANO DI GESTIONE E SISTEMA GIS
Intervento realizzato con fondi :
POR SARDEGNA 2000 - 2006
PIT SS7 OLBIA
"PORTA D'EUROPA TERRA DELL'ACCOGLIENZA"
" INTERVENTI DI VALORIZZAZIONE E GESTIONE DEL PATRIMONIO NATURALISTICO DELL' A.M.P. TAVOLARA PUNTA CODA CAVALLO"
CARTA BIONOMICA DEI FONDI MARINI
A CURA DEL DIP.TEAIS. UNIVERSITA' DEGLI STUDI DI GENOVA
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2000
0
2000
4000
6000
8000
10,000 Feet
1000
0
1000
2000 Meters
LEGENDA
zona A
zona B
zona C
DINAMICA
Limite inferiore eroso
Influsso del largo (insieme prelitorale)
Popolamenti transizioni
Decantazione minerale ed organica
Apporti biotici (insieme fontolitorale)
K
Climax
Controllo edafico
d
Degrado antropico
Disturbo biotico (sovrappascolo)
UNITA' DI POPOLAMENTO
Rocce sopra e mesolitorali
Orizzonte a PATELLA Ferruginea
Cornice di Lithophyllum / Lichenoides
Alghe fotofile
Alghe emisciafile
Alghe sciafile / Precoralligeno
Coralligeno
Insiemi lagunari e paralici
Sabbie sopra e mesolitorali
Sabbie terrigene ben classate
Detritico costiero a briozoi
Maerl
Detritico infangato a scafopodi
Fanghi viscosi a forme sessili
Prateria di POSIDONIA OCEANICA su sabbia
Prateria di POSIDONIA OCEANICA su matte
Prateria discontinua su sabbia
Prateria discontinua su roccia
EMAS
AREA MARINA PROTETTA TAVOLARA PUNTA CODA CAVALLO
RINA
Elaborazione del GEOGRAMMA S.R.L. Olbia www.geogramma.it
ISOLA TAVOLARA
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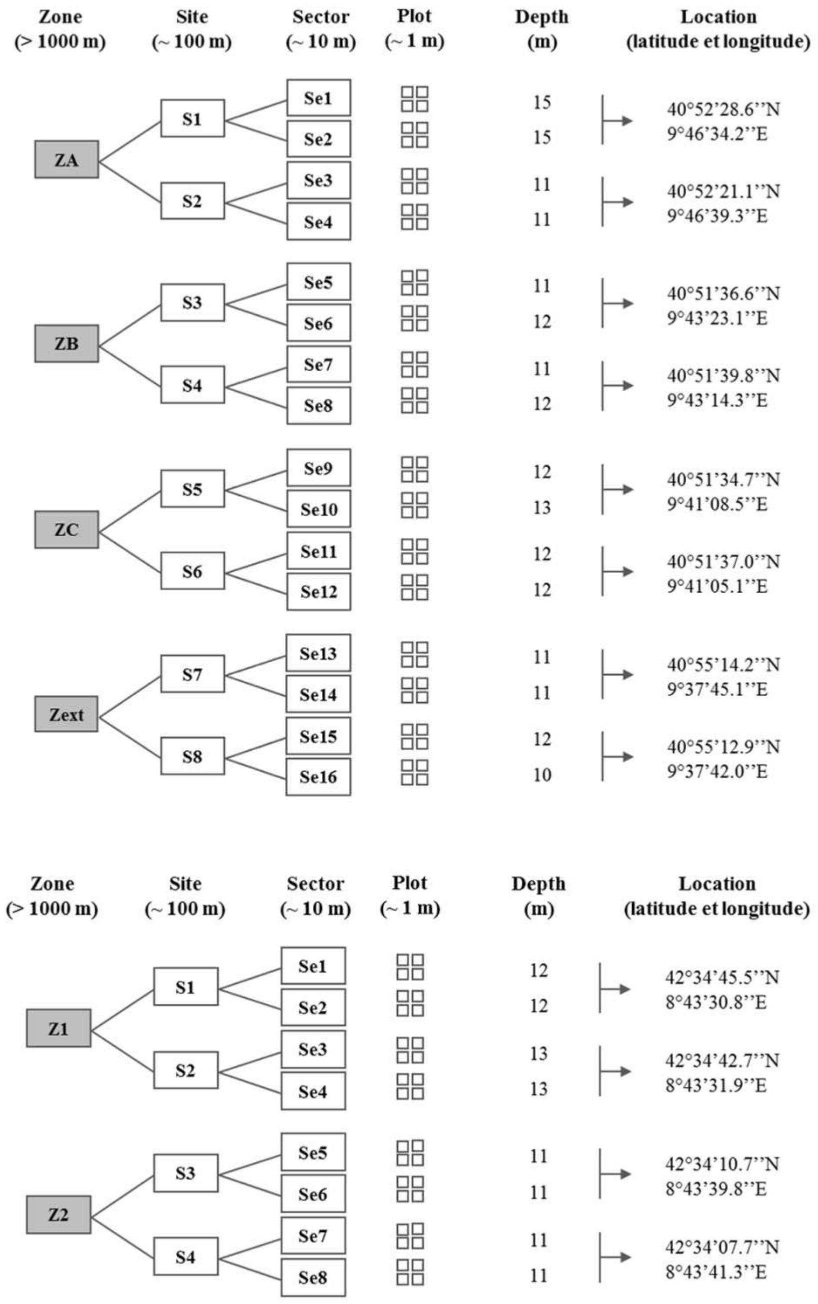
Hierarachical designs at the TMPA (top) and the Revellata Bay (bottom)



In the Mediterranean Sea, little research has been performed on the potential effects of protection on small macrozoobenthos. The difficult assessment of responses of marine populations to the establishment of marine protected areas, depends on the ability to separate the effects of management from other sources of variation. Thus, it is essential to quantify and understand the magnitude and range of natural variability of populations at different scales of observation, especially working in heterogeneous seagrass meadows.

The general purpose of this research is to assess and better understand the potential responses of amphipods in *Posidonia oceanica* meadows between different protection levels.

Two approaches were used. First, multiscale variability patterns of amphipod assemblages were investigated at the Revellata Bay (France) and the Tavolara-Punta Coda Cavallo Marine Protected Area (Italy), with a particular attention to the habitat-amphipod relationship. Second, the role of fish predation in affecting amphipod assemblages was evaluated using experimental manipulations of predation intensity. During the course of this research, a new amphipod, *Caprella tavolarensis* was discovered and described.

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University of Liege