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"The role of the seagrass Posidonia oceanica *on the sea urchin* Paracentrotus lividus*"*

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"Per sempre me ne andrò per questi lidi,
Tra la sabbia e la schiuma del mare.
L'alta marea cancellerà le mie impronte,
E il vento disperderà la schiuma.
Ma il mare e la spiaggia dureranno
In eterno."

(Gibran Kahlil Gibran, 1926)

" Tirato dalla mia bramosa voglia, vago di vedere la gran confusione delle varie e strane forme fatte dall'artifiziosa Natura…"

Leonardo Da Vinci

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INTRODUCTION

Seagrass meadows form extremely complex ecosystems that function through detritus-based food webs as well as herbivore webs. In the latter living seagrass plants as well as epiphytes on the plants are grazed. Seagrass meadows have recently been recognized as an important marine resource. The major function of seagrasses were enumerated by Wood, Odum, and Zieman (1969): (1) the plants stabilizes and hold bottom sediments even through the enormous stresses of hurricanes and temperate storms; (2) the leaves slow and retard water currents and waves, promoting sedimentation of particulate matter inhibiting resuspension of organic and inorganic matter; (3) the meadow serves as a shelter and refuge for resident and transient adult and juvenile animals, many of which are of commercial and recreational importance; (4) the feeding pathways consist of both direct grazing on the leaves or epiphytes and detrital pathways; (5) the plants attain a high production and growth (leaves of some species can grow 5-10 mm per day); (6) the plants produce and trap detritus and secrete dissolved organic matter that tends to internalize nutrient cycles within the ecosystem.

Seagrass meadows serve as "nursery habitat" for a variety of economically important finfish and shellfish. While the importance of grazing in algal-dominated communities is well documented (Strong, 1992), the premise that the ingestion of living seagrass biomass is infrequent and inconsequential remains one of the central tenets of current food web theory. One of the most interesting aspects of seagrass beds is there apparent unpalatability to grazers. Based on studies in other ecosystem, we know that plants can compensate for losses to grazers either by increasing nutrient uptake from the surrounding environment or by translocating nutrients among physiologically integrated ramets (Heck and Valentine 2006, and reference therein).

In the Mediterranean Sea, **Posidonia oceanica** (L.) Delile forms monospecific meadows with different types of coverage pattern (continuous to patchy with leopard-skin, in row distributions

with shoot densities ranging from 150-300 shoots m^{-2} (very sparse bed) to more than 700 shoots m^{-2} (very sparse bed) (Giraud, 1977)). The shoots are borne by rhizomes growing either vertically (orthotrophic rhizome) avoiding burial, or horizontally (plagiotropic rhizome), enabling colonization. The leaves act as sediment-traps accumulating inorganic and organic particulate matter. The progressive silting and the two types of rhizome growth result in a typical terraced formation called 'matte' consisting of the intertwing of various strata of rhizomes, roots, and sediment. In shallow waters, such an accumulation of material raises the bed to the surface. The rhizomes grow horizontally until space has been completely colonized. The beds have wide spacing between many vertical shoots with few horizontal apices (Boudouresque and Meisnez 1982; Gobert et al. 2006 and reference therein).

The *Posidonia oceanica* beds is the climax community of a successional process (Gobert et al. 2006 and reference therein). The development of the bed seems to need a substratum rich in organic material. Pioneer species such as *Caulerpa prolifera* settle, together with small phanerogams of the genera *Cymodocea* and *Zostera* can produce suitable sediments for seed germination or shoot growth. Settlement, growth, and multiplication of *P. oceanica* shoots reduce the light intensity at the seafloor which causes the mortality and the disappearance of the pioneer species (Boudouresque and Meisnez 1982).

In the Mediterranean Sea, the sea urchin **Paracentrotus lividus** (Lamarck) is commonly found in shallow subtidal reefs and in *Posidonia oceanica* (L.) Delile meadows (Tortonese 1965). This echinoid plays a key role in controlling macrophyte communities on rocky habitats as it is capable of depleting erect algae (*e.g.* Benedetti-Cecchi et al. 1998; Bulleri et al. 1999; Hereu 2006), whereas in *P. oceanica* beds it has been reported to feed preferentially on epiphytes rather than on plant material (Nédelec and Verlaque 1984; Sheperd 1987; Tomas et al. 2005, 2006). Under natural conditions, adult density of *P. lividus* is higher on rocky walls than in *P. oceanica* (only up to 6 individuals m^{-2}, Boudouresque and Verlaque 2001), and no appreciable successful recruitment takes

place in seagrass meadows, where adult population cannot be sustained by local recruitment (Tomas et al. 2004).

The sea urchin *Paracentrotus lividus* is one of the benthic invertebrate species been intensely harvested for commercial and recreational purposes. Sea urchin fishery has been differently practised through years among the geographical areas of the Basin (mainly in the southern regions), but lately populations of *P. lividus* are exploited either by authorized fishermen and poachers at very extended coastal areas throughout the year. Human predation of *P. lividus* is known to occur either in rocky reef habitat and *Posidonia oceanica* (personal observations) on specimens larger than about 50 mm in test diameter. Only recently researchers have focused attention on the effect of protection on this sea urchin species (*i.e.* Guidetti 2006a; Guidetti et al. 2004; Gianguzza et al. 2006; Pais et al. 2007). Although protection of coastal areas from fishing is generally meant a tool for increasing abundance of harvested species, it can theoretically have contrasting effects on *P. lividus*, generally being restrictions on fishing of both this sea urchin and its natural fish predators, which have been indicated in *Diplodus sargus* (L.)*, Diplodus vulgaris* (Geoffr.)*, Sparus aurata* (L.)*, Coris julis* (L.) and *Thalassoma pavo* (L.). These latter are generally known to show a predatory behaviour at rocky habitats on medium and large-sized specimens (Sala and Zabala 1996; Sala 1997; Guidetti 2004, 2006b), although some other authors suggest that predation can occur also on juveniles (Hereu et al. 2005).

In this research several aspects of the interaction between the seagrass *Posidonia oceanica* and the sea urchin *Paracentrotus lividus* were investigated.

The **first** experiment was a descriptive study that had the aim to investigate whether protection has the same effect on the population structure of *Paracentrotus lividus* occurring on rocky reef habitat and in *Posidonia oceanica* beds. It consisted in an extensive sampling program conducted at the two habitats at several locations of Capo Caccia – Isola Piana MPA under different protection level. Results were needed to generate hypotheses about the influence of human harvesting, predatory pressure and migration processes on *P. lividus* at the two habitats.

The **second** is also a descriptive study that had the aim to estimate herbivory pressure of *Paracentrotus lividus* on *Posidonia oceanica* through indirect measures. More specifically, it was asked what portion of the seagrass leaf is attacked by the sea urchins, whether the abundance of grazing marks depend on the number of sea urchins and whether herbivory of *P. lividus* depends on shoot density of the seagrass.

The **third** experiment was done to estimate the daily migrations of *Paracentrotus lividus* from a rocky habitat to *Posidonia oceanica* patches and particularly if they depend on the size of sea urchins and the distance from the patch edge. It was postulated that *P. lividus* moves randomly to the seagrass patch and that the probability of reaching it could decrease with the distance.

The **forth** experiment had the purpose to investigate the role of *Posidonia oceanica* structure on *Paracentrotus lividus* distribution. At this aim, the manipulation of the seagrass structure has been achieved by modifying the shoot density and leaf high. Also, in this experiment artificial (plastic) seagrass units mimicking natural seagrass have been used so that different combinations of shelter and food were provided.

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CHAPTER 1

Evaluating the effects of *Posidonia oceanica* vs. rocky reef habitat on *Paracentrotus lividus* distribution at a north western Mediterranean Marine Protected Area*

Chapter 1. <u>Evaluating the effects of *Posidonia oceanica* vs. rocky reef habitat on *Paracentrotus lividus* distribution at a NW Mediterranean MPA</u>

ABSTRACT

The sea urchin *Paracentrotus lividus* in the Mediterranean is common on shallow subtidal rocky habitats and in *Posidonia oceanica* beds. This study has the aim to investigate whether protection has the same effect on the population structure of *P. lividus* occurring on rocky reef habitat and in *P. oceanica* beds. Results are needed to generate hypotheses about the influence of human harvesting, predatory pressure and migration processes on *P. lividus* at the two habitats. At this aim, we have sampled *P. lividus* at seven locations at the Gulf of Alghero (North West Sardinia) where Capo Caccia-Isola Piana MPA occurs: 1 location is placed in a A zone, where no harvest of *P. lividus* is allowed (NH), 3 locations are placed in B zones, where harvest is restricted (RH), and the other 3 are located outside the MPA where sea urchins are harvested without restrictions (UH). Density of *P. lividus* was assessed in 10 replicates per location using quadrats of 1×1 m. The size of 20 individual (test diameter without spines) per location was measured by means of a calliper. Sea urchins, finally, were grouped into size classes to examine frequency distributions. Sampling was performed at the end of a harvesting period (April-May 2006). Analyses of data have highlighted a significant variability for both response variables among locations. No differences were found among levels of protection (NH vs. RH vs. UH), while in *P. oceanica* habitat a significant lower density and a higher size were found rather than in rocky habitat, independently on the protection. This finding suggests that in rocky habitat settlement and recruitment could be higher successful events and that in *P. oceanica* meadows large-sized immigrants coming from the rocky habitat contribute to the population structure in the seagrass habitat. The need to define the role of *P. oceanica* habitat identifying the mechanisms of influence on *P. lividus* individuals is also discussed.

Key words: Marine Protected Area, *Paracentrotus lividus*, *Posidonia oceanica,* spatial distribution, test diameter.

Chapter 1. <u>Evaluating the effects of *Posidonia oceanica* vs. rocky reef habitat on *Paracentrotus lividus* distribution at a NW Mediterranean MPA</u>

INTRODUCTION

Estimation of the direct and indirect effects of protection on benthic species has become a very important goal of marine biological conservation. In fact, benthic assemblages and habitat are deeply altered or damaged through both direct removal (Dayton et al. 1995; Turner et al.1999; Link 2002) and indirect cascading community effects (Sala et al. 1998; Menge 2000; Micheli et al. 2005; Tegner and Dayton 2000; Guidetti 2006a). In fact, fishing has the potential to directly affect target species and, on the other hand, influence the structure of whole assemblages and ecosystem-functioning mainly through indirect cascading effects (Micheli et al. 2001). However, besides high level predatory fishes removal, fishing can also involve the harvesting of several benthic invertebrates that, although at a lower level in the food chain, are directly involved in the trophic cascade. Edible sea urchins are a good example of such benthic invertebrates, being at the same time prey of predatory fishes and active grazers.

In the Mediterranean the sea urchin *Paracentrotus lividus* (Lamarck) is common on shallow subtidal rocky habitats and in *Posidonia oceanica* (L.) Delile beds where it occurs on rhizomes among shoots. On rocky habitats, at high densities, it overgrazes complex algal assemblages turning them into barren areas dominated by a few species of encrusting algae (e.g. Benedetti-Cecchi et al. 1998; Hereu 2006), whereas in *P. oceanica* beds it has been reported to feed preferentially on epiphytes rather than on plant material (Nédelec and Verlaque 1984; Shepherd 1987; Tomas et al. 2005; Tomas et al. 2006). Similarly, its population structure differs between these two habitats: indeed, adult density of *P. lividus* is higher on rocky reefs than in *P. oceanica* (only up to 6 individuals m^{-2}, Boudouresque and Verlaque 2001).

Overall, the spatial distribution of *Paracentrotus lividus* is influenced by many processes such as predation (Sala and Zabala 1996; Guidetti 2004; Hereu et al. 2005), recruitment (Tomas et al. 2004), migration (Palacín et al. 1997; Crook et al. 2000) and competition (Guidetti et al. 2004; but see Gianguzza et al. 2006). In addition, especially where predation pressure is high, heterogeneity of the substratum plays a significant role providing shelters to individuals of *P. lividus*, thus

allowing the structuring of populations (Hereu et al. 2005; Bonaviri et al. 2005). Although there is an extensive literature on this species devoted to many of these features on rocky habitats, processes operating on populations occurring in *Posidonia oceanica* habitats are still mostly neglected. Information collected by Tomas et al. (2004) suggest that no appreciable successful recruitment of *P. lividus* seems to take place in seagrass meadows, where adult abundance should not be sustained by local recruitment, but immigration. However, experimental investigations about the effect of predation, migration and competition on population structure of *P. lividus* in this habitat still need to be properly done.

In the Mediterranean, the sea urchin *Paracentrotus lividus* is one of the benthic invertebrate species been intensely harvested for commercial and recreational purposes. Sea urchin fishery has been differently practised through years among the geographical areas of the Basin (mainly in the southern regions), but lately populations of *P. lividus* are exploited either by authorized fishermen and poachers at very extended coastal areas throughout the year. Human predation of *P. lividus* is known to occur either in rocky reef habitat and *Posidonia oceanica* (personal observations) on specimens larger than about 50 mm in test diameter. Only recently researchers have focused attention on the effect of protection on this sea urchin species (*i.e.* Guidetti 2006b, Guidetti et al. 2005; Gianguzza et al. 2006; Pais et al. 2007). Although protection of coastal areas from fishing is generally meant a tool for increasing abundance of harvested species, it can theoretically have contrasting effects on *P. lividus*, generally being restrictions on fishing of both this sea urchin and its natural fish predators, which have been indicated in *Diplodus sargus* (L.)*, Diplodus vulgaris* (Geoffr.)*, Sparus aurata* (L.)*, Coris julis* (L.) and *Thalassoma pavo* (L.). These latter are generally known to show a predatory behaviour at rocky habitats on medium and large-sized specimens (Sala and Zabala 1996; Sala 1997; Guidetti 2004, 2006c), although some other authors suggest that predation can occur also on juveniles (Hereu et al. 2005).

Possibly because of the differential contribution of effects of protection, a complete portfolio of distribution patterns for *Paracentrotus lividus* have been evidenced on rocky habitats of many

Mediterranean marine reserves: Guidetti et al. (2005) have found no cascading effects of predator removal on prey despite a significant differences in predator density; Micheli et al. (2005) have evidenced significant indirect effects on benthic assemblages only at low wave exposure sites; Guidetti (2006a) has found evidence of significant predatory interactions at protected sites; Gianguzza et al. (2006) have found an higher abundance at the protected location rather than outside. Recently, Pais et al. (2007) indicated that higher abundance of sea urchins found inside protected locations, was attributable to a low fish recovery since protection was established. Furthermore, whether the effect of protection on the population structure of *P. lividus* in *Posidonia oceanica* habitat should not be neglected and to what extent it influences the abundance of this resource, it is presently unknown. For example, if in the seagrass, *P. lividus* is not affected by natural predation pressure, the indirect influence of predators would only be present on the rocky habitat.

The implementation of a Marine protected Area (MPA) within a geographical area where exploitation of *Paracentrotus lividus* has been intensely conducted for decades may thus represent a precious opportunity to evaluate the effect of different intensity of human harvest on spatial distribution of this echinoderm. This study has the aim to investigate whether protection has the same effect on the population structure of *P. lividus* occurring on rocky reef habitat and in *Posidonia oceanica* beds. Results are needed to generate hypotheses about the influence of human harvesting, predatory pressure and migration processes on *P. lividus* at the two habitats.

MATERIALS AND METHODS

This study was carried out in the Gulf of Alghero (North West Sardinia, Italy), where Capo Caccia-Isola Piana MPA has been established since 2002. Sampling was done at 7 locations (Fig. 1) each about 200 m^2 in size: 1 location was located in the A zone (integral protection), where no harvest of *Paracentrotus lividus* is allowed (NH), 3 locations were located in the B zone, where from 2006, harvest is restricted (RH), and the other 3 were located outside the MPA, where sea

urchins are intensively harvested without restrictions (UH). At the MPA, restricted harvest is performed by 4 authorized professional collectors who can harvest up to 1000 individuals/day for a total of 20 fishing days/year from 1st November to 31st March. At each location rocky reefs and *Posidonia oceanica* habitat at 6-10 m deep were considered. Density of *P. lividus* was assessed using quadrats of 1×1 m (at least 3 m apart) while size of individuals (test diameter without spines) was measured by means of a calliper (± 0.1 mm). For each combination 'location × habitat' 10 and 20 replicates were considered randomly for density and size, respectively. Sampling was performed after the end of the harvesting period (April-May 2006).
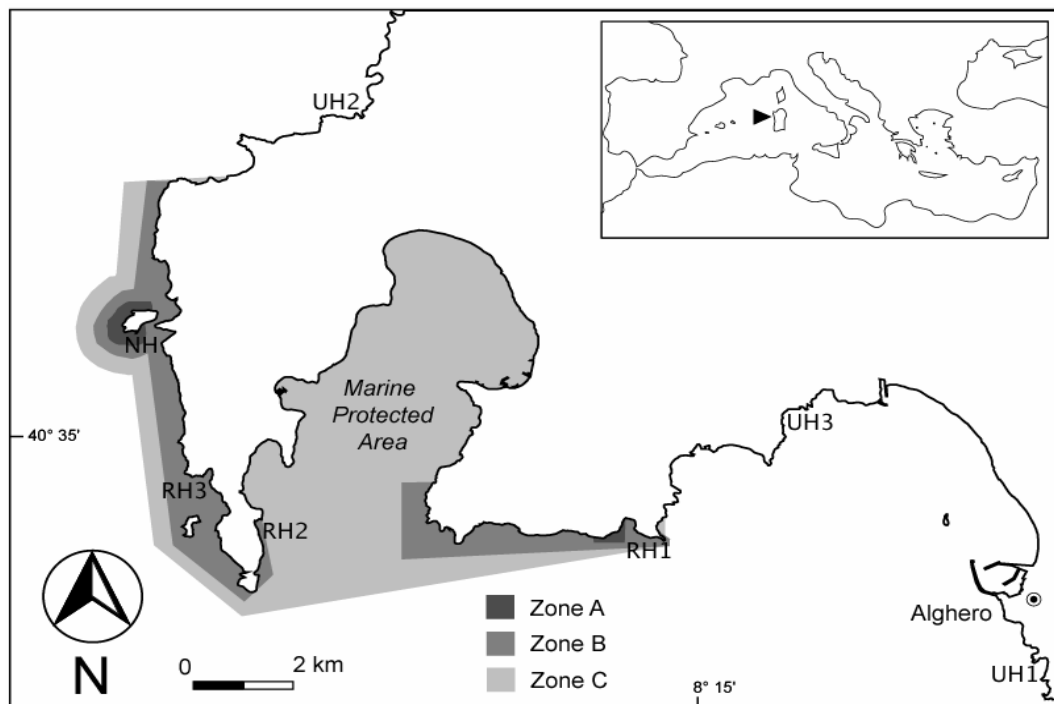

Fig. 1. Study locations inside and outside Capo Caccia – Isola Piana MPA. Locations where unlimited harvest of *Paracentrotus lividus* occurs: Cala Bona (UH1), Torre Porticciolo (UH2) and Punta Negra (UH3). Locations where restricted harvest of *P. lividus* occurs: Polt'Agra (RH1), Cala Bollo (RH2) and Cala Inferno (RH3). Location where no harvest of *P. lividus* occurs: Isola Piana (NH).

Analyses of variance were used to test hypotheses about protection and habitat effects on the abundance and size of *Paracentrotus lividus*. Protection was treated as a fixed factor with 3 levels in analyses: No Harvest (NH), Restricted Harvest (RH) and Unlimited Harvest (UH). Locations

were treated as a random factor nested in Protection. There was no specific *a priori* ecological reason for including any of these locations in the study. The only purpose of having 3 locations at B protection and 3 locations as controls was to provide a minimum degree of replication within each level of protection, in order to construct an appropriate test for the main comparisons of interest: 'NH' vs. 'RH' and 'UH' protection. Random selection of locations was, however, not possible due to the zoning plan of the MPA. Factors in analyses were 3: Protection ('NH', 'RH' and 'UH' levels), Location (three levels, nested within Protection), and Habitat (fixed, rocky and *Posidonia oceanica* levels, orthogonal to Location). Only 1 location at level 'NH' protection was sampled and, therefore, location was not completely orthogonal to levels of protection. Asymmetrical mixed-model ANOVA was used: between 'NH' protection vs. the others, and among the others, between 'RH' and 'UH' protection. The required sums of squares for the asymmetrical components of these analyses were calculated following Underwood (1997). Cochran's C test was used to check the assumption of homogeneity of variances and, when necessary, data were log-transformed to remove heterogeneous variances (Underwood 1997).

RESULTS

The number of individuals of *Paracentrotus lividus* was highly variable either among locations and between habitats: the mean density was over 10 ind m^{-2} at rocky habitats even at locations where harvest is unlimited and at one location where harvest is restricted (Fig 2a). At each location, mean test diameter was always higher at *Posidonia oceanica* rather than at rocky habitat, reaching the highest values of about 60 mm in zone A at both habitats (Fig. 2b).
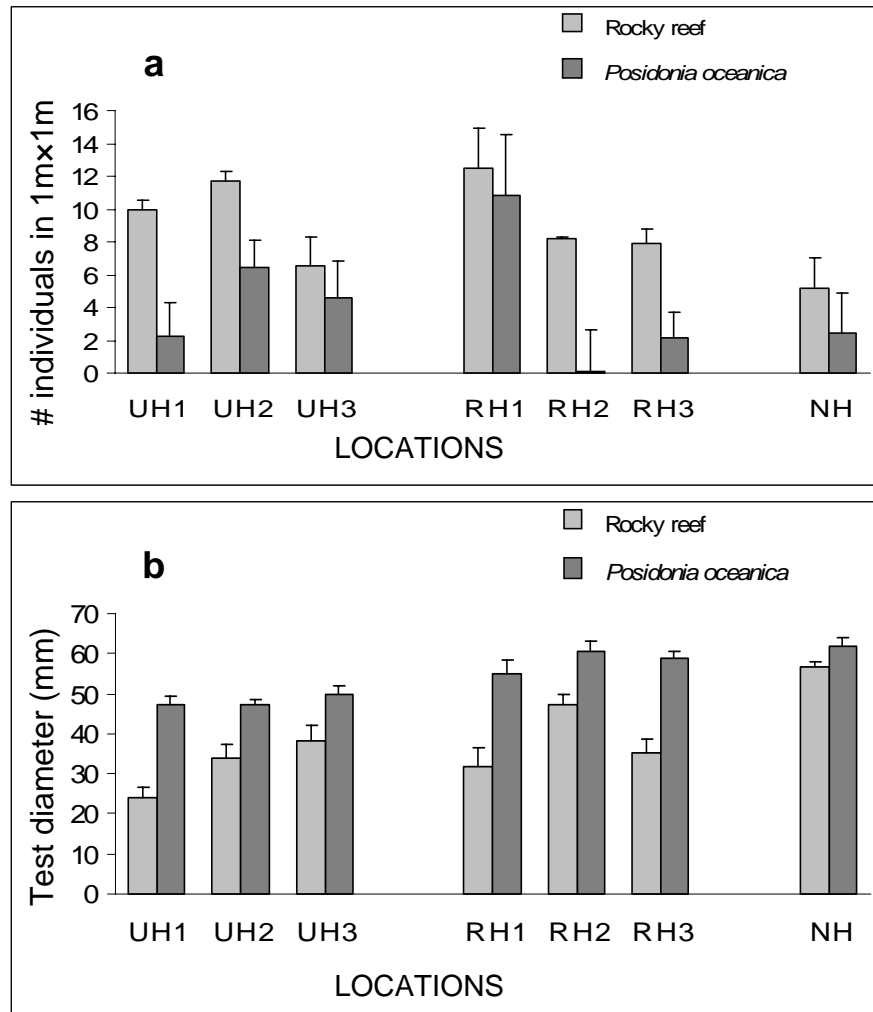
Fig. 2. Mean number (+SE) of individuals (**a**) and test diameter (**b**) of *Paracentrotus lividus* at *Posidonia oceanica* and rocky reef habitat at each location. UH unlimited harvest of *P. lividus* locations, RH restricted harvest of *P. lividus* locations and NH no harvest of *P. lividus* location.

The analyses of data for both response variables highlighted that no significant differences exist among the three levels of protection, either in the contrast between NH and the other levels and in the contrast between RH and UH levels (Table 1), suggesting that both types of restrictions on harvest did produce the same effect on population structure of *Paracentrotus lividus*. The effect of habitat was evidenced for both abundance and size of the sea urchins: in *Posidonia oceanica* habitat, density was significantly lower and size higher than in rocky habitat, consistently among the different levels of protection. In fact, no significant interaction between habitat and contrasts of protection was found significant for either variables. A significant variability among locations for

both density and size of *P. lividus* was highlighted within RH and UH protection levels. Also, at these levels the effect of habitat was different depending on the location, as clearly evidenced in Figs. 2a and 2b.

Table 1 Asymmetrical ANOVAs on *Paracentrotus lividus* abundance and size at Capo Caccia - Isola Piana MPA. Degrees of freedom of Residual and Residual others refer to both ANOVAs (abundance/size).

Source of variation	df	abundance SS	MS	F	p	Size SS	MS	F	P	F versus
Location =L	6	35.97	5.99	12.06	**0.000**	10.11	1.69	11.54	**0.000**	Residual
NH vs others	1	6.45	6.45	0.88	0.401	4.82	4.82	5.28	0.083	L(RH vs UH)
Among others	5	29.52				5.29				
RH vs UH	1	0.23	0.23	0.03	0.868	1.63	1.63	1.79	0.252	L(RH vs UH)
L(RH vs UH)	4	29.29	7.32	15.46	**0.000**	3.65	0.91	5.48	**0.020**	Residual$_{others}$
Habitat =H	1	39.46	39.46	79.45	**0.000**	14.13	14.13	96.77	**0.000**	Residual
HXL	6	9.52	1.59	3.19	**0.006**	3.40	0.57	3.88	**0.001**	Residual
HXNH vs others	1	0.17	0.17	0.08	0.797	1.60	1.60	3.58	0.132	HXL(RH vs UH)
HXAmong others	5	9.35	1.87			1.80	0.36			
HXRH vs UH	1	0.33	0.33	0.15	0.722	0.01	0.01	0.03	0.873	HXL(RH vs UH)
HXL(RH vs UH)	4	9.02	2.26	4.76	**0.001**	1.79	0.45	2.69	**0.032**	Residual
Residual	266/126	62.59	0.50			38.84	0.15			
Residual $_{others}$	228/108	51.16	0.47			37.95	0.17			
transformation		sqrt(x+1)				ln(x+1)				
Cochran's test		0.2057 ns				0.2821 ns				

Test size-frequency distribution of *Paracentrotus lividus* was quite different among levels of protection and between habitats (Fig. 3). In particular, at the NH location we detected individuals from 60 to 80 mm and from 40 to 90 mm at the rocky and *Posidonia oceanica* habitat, respectively. Overall, at this location similar distributions were observed at the two habitats. No specimens belonging to the size-classes of 20 and 30 mm were found at both habitats at this location. Conversely, at the other locations (RH and UH levels) frequency distribution patterns were wider across classes and distinguishable between habitats. At some of the locations (for example RH1,

RH3, UH1), two different modal size-classes were evidenced for both the habitats, showing a higher mode for *P. oceanica*. However, at each location larger individuals were always found in the seagrass habitat except for UH3 location: the largest size-class ranged from 60 to 90 mm at UH3 and RH2, respectively. *P. lividus* specimens of the 90 mm size-class were not found at the rocky habitat at any of the locations considered.
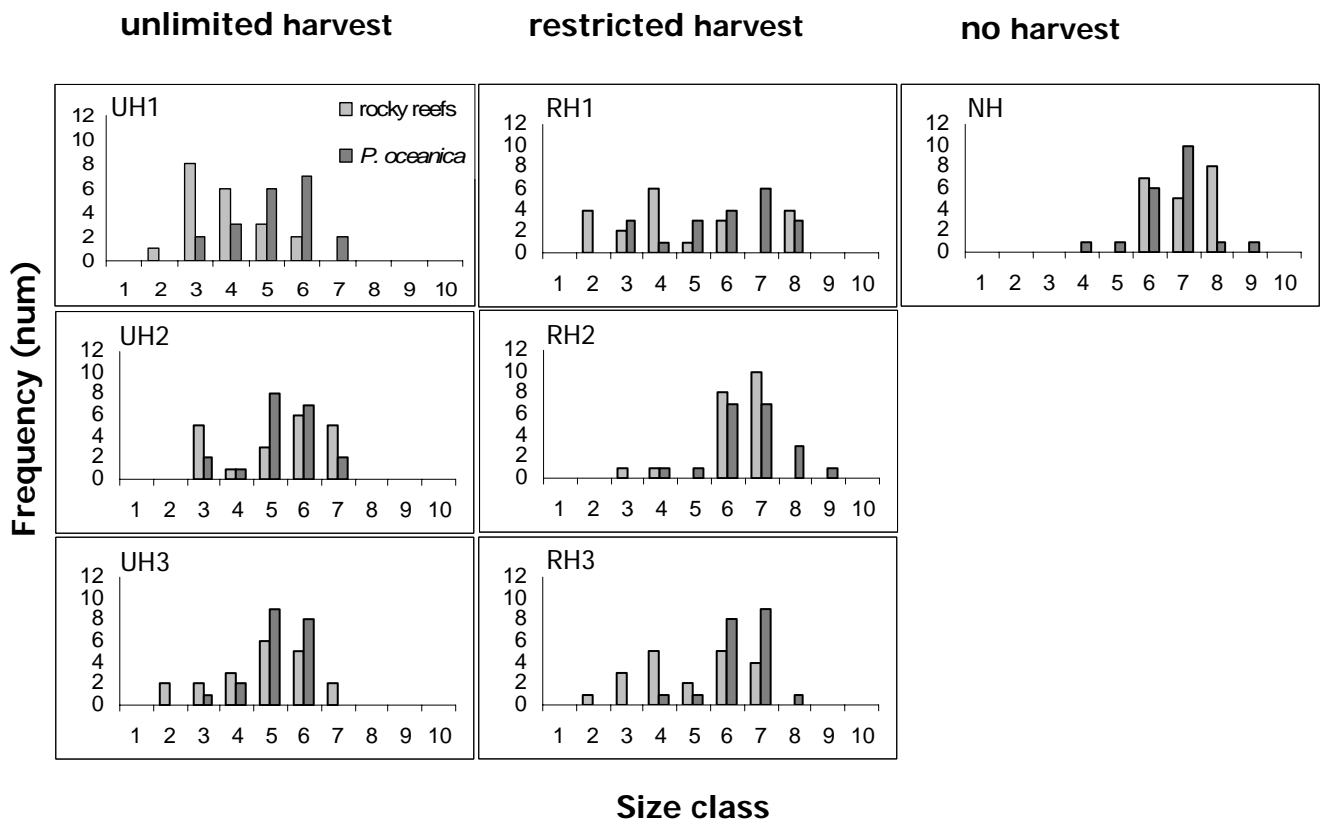

Fig. 3. Sea urchin test size frequency at each location at the two habitats: *Posidonia oceanica* and rocky reef habitat. Size class 1= 0-1 cm, 2= 1-2 cm, 3= 2-3 cm, 4= 3-4 cm, 5= 4-5 cm, 6= 5-6 cm, 7= 6-7 cm, 8= 7-8 cm, 9= 8-9 cm. UH unlimited harvest of *Paracentrotus lividus* locations, RH restricted harvest of *P. lividus* locations and NH no harvest of *P. lividus* location.

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Chapter 1. Evaluating the effects of *Posidonia oceanica* vs. rocky reef habitat on *Paracentrotus lividus* distribution at a NW Mediterranean MPA

DISCUSSION

Overall, we found that at Capo Caccia - Isola Piana MPA no significant differences occur either in *Paracentrotus lividus* abundance and size among different levels of protection suggesting that harvesting restrictions (zone A and B) on sea urchin harvest would have no effect on the population structure of this species. The lack of protection effect on this species could be ascertained to the lack of the re-establishment of trophic interactions due to the small time elapsed since the MPA was declared. Actually, fishing prohibitions in Mediterranean marine reserves may re-establish lost interactions among strongly interactive species with potential community-wide effects (Guidetti and Sala 2007). However, at Capo Caccia - Isola Piana MPA a relative scarcity of *P. lividus* predators is still perceived (personal observation) and probably protection has not been long enough to encompass the life span of some predator fish species.

However, even if the effects of protection were not significant on both response variables, it is worth nothing that our sampling program revealed a general lack of small-sized *Paracentrotus lividus* individuals (*i.e.* <30 mm in test diameter) at the zone A (no-harvest location), while small specimens (even 20 mm in size) were found at several of the unlimited and restricted harvest locations. This finding could lead to generate the hypothesis that at the NH location a higher fish predation rate is exerted on small–sized individuals and that *P. lividus* recruitment is consequently more influenced by natural predation pressure.

Nevertheless, similar population structures of *Paracentrotus lividus* could be produced by the negative effects of human harvest and predatory pressure which covary at the locations: in fact, where lower is the human harvest of sea urchin, higher is the natural predatory pressure, and vice versa (*i.e.* Guidetti 2006a). This can clearly happen since restrictions generally involve both the fishing of *P. lividus* and its natural predators.

Unfortunately, the lack of a significant effect of protection on this species did not really allow to detect the eventual interactive effect of the habitat and protection that still remains an interesting hypothesis that needs to be tested. However, the significant effect of the habitat on population

structure of *Paracentrotus lividus* revealed that larger individuals inhabits *Posidonia oceanica* meadow but that in this habitat a lower population density occurs consistently at all the locations investigated, independently on the protection level. This finding suggests two possible hypotheses: (1) in rocky habitat settlement and recruitment are higher successful events; (2) in *P. oceanica* meadows large-sized immigrants coming from the rocky habitat contribute to the population structure of the seagrass habitat.

The first hypothesis is consistent with Tomas et al. (2004), who indicated a lower abundance of settlers and recruits in *Posidonia oceanica* rather than at the rocky habitat. However, no data about the population dynamics of *Paracentrotus lividus* in this habitat have ever yet been estimated, and thus no information about the mortality of small-sized individuals is still available. Further, feeding of recruits vs. juvenile and adult specimens is not known and whether a change in food preference occurs during *P. lividus* life cycle has not been investigated. Indeed, very different resources are available at the two habitats: in *P. oceanica* beds, *P. lividus* is likely to feed on leaves of the seagrass and on the few algae understoried by the seagrass canopy (that mostly grow on rhizomes), while at rocky habitat more diversified macroalgal assemblages (*i.e.* composed by several turf and erect species) occur (Ruitton et al. 2000). Therefore, it is probable that recruits of *P. lividus* could have different growth and mortality rate between habitats merely based on differences in food availability.

The second hypothesis (*i.e.* the possible immigration of large-sized individuals of *Paracentrotus lividus* from the rock to the seagrass meadow) should deserve particular consideration. The few available data about sea urchin density in different *Posidonia oceanica* meadows at Capo Caccia – Isola Piana MPA indicated that abundance would decrease with the distance from the edge of the meadow and that it would be higher where the seagrass bed is next to the rocky habitat (0.7 ± 0.35, mean number of individuals per $m^2 \pm$ SE). In fact, where sand is accumulated at the margin of the seagrass, density of *P. lividus* is similar to that found inside the meadow (0.83 ± 0.33, mean number of individuals per $m^2 \pm$ SE). These findings suggest that

migration could have an important influence on the population structure of *P. lividus* at *P. oceanica* habitat.

However, description of migration by aggregation fronts of *Strongylocentrotus droebachiensis* (Müller) seems recurrent (Vadas et al. 1986; Schiebling et al. 1999; Alcoverro and Mariani 2002; Dumont et al. 2006) but no similar phenomenon has been described for *P. lividus* (but see Chelazzi et al. 1997), although several papers have been addressed to identify factors that influence sea urchin movements at a lower scale. Among the important determinants for *P. lividus* behaviour investigated, the presence of predatory fish certainly mediates the sea urchin behaviour by causing urchins to shelter and thus diminishing their diel foraging pattern (Carpenter 1984; Scheibling and Hamm 1991; Sala 1996). In fact, *P. lividus* shows a circadian pattern of activity with more active specimens during the night as a defence against diurnal predators (Hereu 2005). However, as no experiments on *P. lividus* have been conducted yet, its movement pattern and perception of the habitat landscape still remain unknown, so that whether the search for food and shelter relative to the distribution of food patches and shelter availability occurs at random, needs to be cleared.

The high spatial variability of *Paracentrotus lividus* abundance and size at the scale of location detected in this study is absolutely consistent with many other studies that identified spatial heterogeneity as one the most important determinants for its population structure. In fact, for example, different substrate heterogeneity (thus availability of refuges) can produce different structure of populations, especially in marine reserves where juvenile mortality due to predation is minimized by shelters (Benedetti-Cecchi and Cinelli 1995; Sala et al. 1998; Ruitton et al. 2000; Barnes and Crook 2001). Then, sampled locations in this study were selected as replicates within each harvest level and, although they were similar in geomorphology, seascape of rocky substrates appeared quite different among them. Also, the different exposition of locations sampled in this study is likely to have influenced the spatial variability since shore exposition has already been indicated as an important determinant on the structure of *P. lividus* populations (*i.e.* Chelazzi et al.

1997). However, this result suggests that a high spatial replication at the scale of location is needed when *P. lividus* population structure needs to be estimated.

Furthermore, whether the effect of both protection and habitat on *Paracentrotus lividus* population structure varies among different periods of the year, still remains unknown. Seasonality would deserve further attention since many features regulating sea urchin distribution undergo variations through time. For example, human harvest is focused in the winter season when the reproductive effort of the sea urchin is maximized and, on the other hand, the phenology of algal assemblages on rocky reefs as well as the behaviour of predators are linked to the season (*e.g.* Sala and Zabala 1996; Barnes and Crook 2001). Thus, the behaviour of *P. lividus* could also greatly change through time. In this contest, it would be crucial to define the role of *Posidonia oceanica* habitat for *P. lividus* individuals testing specific hypotheses on temporal variability of the mechanisms of influence.

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CHAPTER 2

Investigating the variability in *Paracentrotus lividus* herbivory on *Posidonia oceanica**

ABSTRACT

This mensurative study is a contribution to understanding aspects of the interaction between *Posidonia oceanica* and *Paracentrotus lividus*. Specifically, we examined the magnitude and variability of leaf herbivory due to this sea urchin by analysing the dependence of the number of grazing marks attributable to *P. lividus* on sea urchin density, the dependence of *P. lividus* density on *P. oceanica* shoot density. Also, the variability of *P. lividus* grazing was investigated at the scale of *P. oceanica* shoots, estimating whether it interests consistently adult, intermediate and juvenile leaves and different portions of the leaf (distance from the leaf base).

The number of individuals of *Paracentrotus lividus* was highly variable among locations and between areas. No significant linear dependence of *P. lividus* density on *Posidonia oceanica* shoot density was detected. A sigmoid function was found to better describe the relation between the *P. lividus* density and the number of its grazing marks. The distance class - frequency distribution of *P. lividus* grazing marks on pooled data revealed that this herbivore affected the length of *P. oceanica* leaf until about 700 mm from the base and the attacks were concentrated to the lower portion, near the base. We finally discussed the implications of our results on the general importance of *P. oceanica* as a food resource for this herbivore.

Key words: Epiphytes, grazing, herbivory, *Paracentrotus lividus*, plant-herbivore interaction, *Posidonia oceanica*.

INTRODUCTION

Seagrass ecology has traditionally been dominated by classical investigations on plant physiology and the impacts of eutrophication on seagrass growth, with little attention to the community-oriented approaches employed effectively in other branches of marine ecology (Valentine and Duffy 2006). In fact, there is increasing worldwide evidence that pressure exerted by marine herbivores, other than large vertebrates, may also play an important role in the energetics and interaction network of seagrass ecosystem (*e.g.* Tomas et al. 2005a). In fact, whereas the central role of grazers in structuring macroalgal assemblages on rocky habitats is well documented and widely recognized (Benedetti-Cecchi et al. 1998; Sala et al. 1998; Bulleri et al. 2002; Guidetti and Dulčic 2007), we know less about the population and community-level impacts of herbivores on seagrass ecosystems (but see review by Valentine and Duffy 2006). However, recently, it has become more evident that herbivory on seagrasses, which includes estimates of consumption of seagrass themselves as well as epiphytic organisms (Valentine and Heck 1999; Heck and Valentine 2006; Prado et al. 2007a), plays an important role in the dynamics of shallow nearshore seagrass habitat (Tomas et al. 2005a; Heck and Valentine 2006).

Herbivory on seagrasses can be very variable (Cebrián and Duarte 1998): in tropical seagrasses overgrazing is more frequent (Mariani and Alcoverro 1999; Alcoverro and Mariani 2002) than in temperate seagrasses, in which herbivores pressure is lower (Cebrián et al. 1996; Cebrián and Duarte 1998), although a lower number of investigations have been conducted on the latter plants. In general the interaction between herbivores and seagrass can be mediated by epiphytes (Williams and Heck 2001; Tomas et al. 2005a; Young et al. 2005), at least in part, also because seagrasses do not appear to be an attractive food source (Frantzis and Grémare 1992; Benedetti-Cecchi et al. 1998; Bulleri et al. 1999; Hereu 2004, 2006). Valentine and Heck (2001) suggested that variability in the grazing of seagrasses can be due to the leaf nutritive quality, and further Lyons and Scheibling (2007) suggested that the feeding strategies of herbivores reflect the

need to optimize nutrition, and that energy or nutrient food are sometimes preferred to lower quality foods.

In the Mediterranean, herbivory appears to be a minor factor controlling production of the endemic species _Posidonia oceanica_ (L.) Delile, in which it seems to be limited by others factors (Tomas et al. 2005b; Alcoverro 1995). On this species, herbivory is due to two main macroherbivores: the sea urchin _Paracentrotus lividus_ (Lamarck) and the sparid fish _Sarpa salpa_ (Linné) (Verlaque 1987, 1990). They both have a distribution markedly skewed with depth, and exert the maximal pressure in shallow beds (0-10m; Boudouresque and Velarque 2001; Tomas et al. 2005a). Other small organisms, such as the sea urchin _Psammechinus microtuberculatus_ (Blainville) (Paul et al. 1984), some decapods and isopods (for ex. _Idotea_ spp.) feed on _P. oceanica_, but negligibly (Zupo and Fresi 1985; Mazzella et al. 1992; Cebrián et al. 1996; Tomas et al. 2005b).

Whereas schools of _Sarpa salpa_ are common in shallow Mediterranean waters (seagrass meadows and rocky bottoms (Velarque 1990; Tomas et al 2005a; Prado et al. 2007a), _Paracentrotus lividus_ is generally found in low densities in such meadows (0 to 6 ind./m^2; see review of Boudouresque and Velarque 2001 and Ceccherelli et al. submitted). On the whole, most of _Posidonia oceanica_ consumption (approximately 75%) has been attributed to _S. salpa_ (Cebrián et al., 1996), although the relative importance of fish grazing varies strongly both spatially and temporally (Alcoverro et al. 1997; Peirano et al. 2001; Tomas et al. 2005a). Further, as sea urchin populations are stable (Tomas et al. 2004), others sea urchins exhibit a strong seasonal feeding pattern (Lozano et al. 1995; Peirano et al. 2001).

Some authors have already investigated that the interaction between _Posidonia oceanica_ and _Paracentrotus lividus_ appears to be mediated by epiphytes, at least in part, that seem to make the leaves more palatable for the herbivore (Alcoverro et al. 1997; Tomas et al. 2005b, 2006; Prado et al. 2007b). In fact, it has been reported that _P. lividus_ feeds preferentially on epiphytes rather than on plant material (Alcoverro et al. 1997; Nédelec and Verlaque 1984; Sheperd 1987; Tomas et al. 2005b, 2006), grazing on the final portion of the seagrass leaves for the higher abundance of

epiphytes (Ott and Maurer 1976; Traer 1980; Verlaque and Nedelec 1983; Nedelec and Verlaque 1984; Sheperd 1987). The *P. lividus* preference of epiphytes could be attributed to their higher nutritional quality (*i.e.* lower C/N ratios) in comparison to the seagrass (Duarte 1990; Mazzella et al. 1992; Alcoverro et al. 1997, 2000). This has been evidenced using stable isotopes which have showed that *P. lividus* obtains most of the nitrogen from epiphytes (Tomas 2004; Tomas et al. 2006). Further, since sea urchins feed on the tips of the leaves (Boudouresque and Velarque 2001), grazing marks left by this herbivore could be lost when for any reason the leaf breaks. This is what Tomas et al. (2005a) have suggested at high grazing pressure of *Sarpa salpa,* able to cut away the leaves with their bites.

Overall, it is likely that effects of grazers, both *Paracentrotus lividus* and *Sarpa salpa*, on *Posidonia oceanica* biomass and production are considerable (*e.g.* Prado et al. 2007a). At this regard, variability in herbivory needs to be properly estimated since it could be influenced by possible interactions between herbivore species. This mensurative study is a contribution to understanding aspects of the interaction between *P. oceanica* and *P. lividus*. Specifically, we examined the magnitude and variability of leaf herbivory due to this sea urchin by analysing the dependence of the number of grazing marks attributable to *P. lividus* on sea urchin density, the dependence of *P. lividus* density on *P. oceanica* shoot density. Also, the variability of *P. lividus* grazing was investigated at the scale of *P. oceanica* shoots, estimating whether it interests consistently adult, intermediate and juvenile leaves and different portions of the leaf (distance from the leaf base). We finally discussed the implications of our results on the general importance of *P. oceanica* as a food resource for this herbivore.

MATERIAL AND METHODS

This study was carried out in *Posidonia oceanica* meadows around the Gulf of Alghero (North West Sardinia, Italy), where Capo Caccia-Isola Piana MPA has been established since 2002. Sampling was done at 6 locations (about 2 kms apart), randomly chosen, where shallow *P. oceanica*

meadows (about 7 m deep) were continuous and next to rocky habitat or sandy bottom. Four of
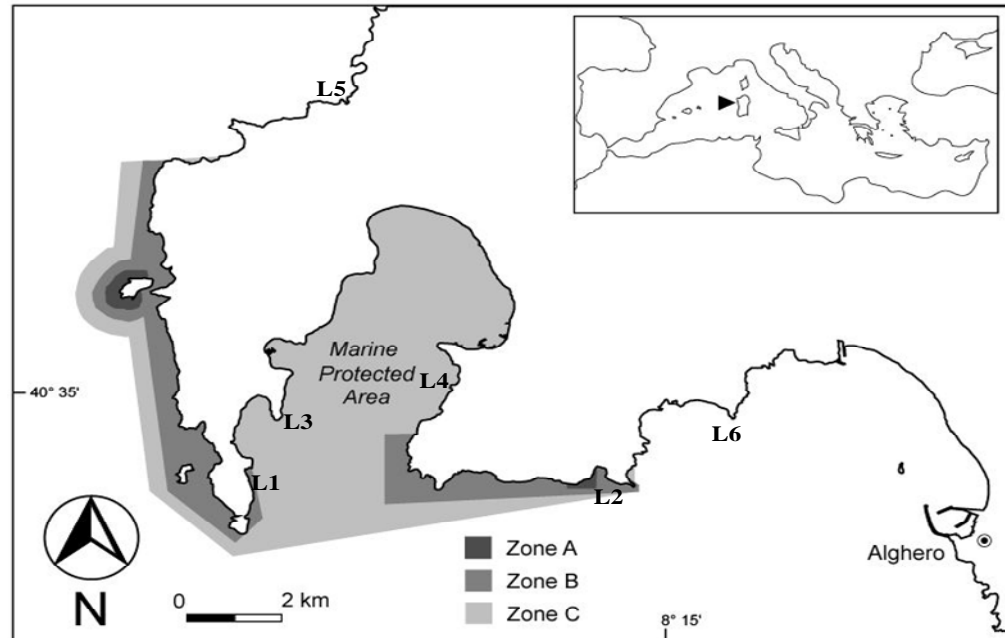
them were located inside the MPA (Fig.1).

Fig. 1 Study locations inside and outside Capo Caccia – Isola Piana MPA. Sampling locations were: Torre del Bollo (L1), Polt Agra (L2), Dragunara (L3), Bramassa (L4), Torre Porticciolo (L5) and Punta Negra (L6).

Field sampling was conducted from July to October 2007. At each location, two areas about

200 m apart were selected. Within each area, ten quadrats 1×1 m were randomly positioned in the

meadow to gain data about *Paracentrotus lividus* abundance. *Posidonia oceanica* shoot density was

measured in a 40×40 cm plot positioned inside each 1×1 m quadrat; two shoots at random were

harvested within each plot and taken to the laboratory so that a total of 240 shoots were collected

for the whole experiment.

Once in laboratory, phenological analyses were performed so that juvenile, intermediate and

adult leaves were counted and measured (length and width) for each shoot. Further grazing marks

left by herbivores on each of these leaves were identified, counted and the distance from the leaf

base was measured. The herbivores on *Posidonia oceanica* leave marks easily distinguishable from

the shape as indicated by Boudouresque and Meinesz (1982). Other authors have already used these

marks to indirectly estimate grazing pressure of various herbivores (Prado et al. 2007a, 2007b;

Tomas et al. 2005a). The relationship among *P. oceanica* phenology, *Paracentrotus lividus* density and grazing marks was examined.

Analyses of variance were used to estimate spatial variability of *Paracentrotus lividus* abundance, number of grazing marks either of *P. lividus* and *Sarpa salpa*. Two-way ANOVAs were performed to test the hypothesis that there were no differences at the scale of the locations and areas: both Locations and Area (nested in Location) were treated as a random factors.

The number of grazing marks left by the two herbivores on the leaves analysed were used to construct a distance class - frequency distribution and to test the hypothesis that *Paracentrotus lividus* and *Sarpa salpa* grazed at different height of *Posidonia oceanica* canopy a three-way ANOVA was performed: both species (*P. lividus* and *S. salpa*) and distance classes of grazing marks from the base (0-100, 101-200, 201-300, 301-400, 401-500, 501-600, 601-700, 701-800, 801-900, 901-1000 and 1001-1100 mm) were treated as fixed and orthogonal while Location (six levels) was treated random and orthogonal. The total number of grazing marks found on the leaves of each area was used as replicate (n=2). SNK test was used to compare means of significant factors and Cochran's C test was used to check the assumption of homogeneity of variances (Underwood 1997).

Further, to test the hypothesis that variables were correlated, linear and non linear equations were searched to describe such relations (SAS).

RESULTS

The number of individuals of *Paracentrotus lividus* was highly variable among locations and between areas (Table 1): the highest density was recorded at L1 within the MPA, while at L4 no urchin were found (Fig. 2). No significant linear dependence of *P. lividus* density on *Posidonia oceanica* shoot density was found either using raw data and averaged values per experimental area (Fig. 3).

Table 1. ANOVAs on *Paracentrotus lividus* density and grazing marks and *Sarpa salpa* grazing marks. *= significant p

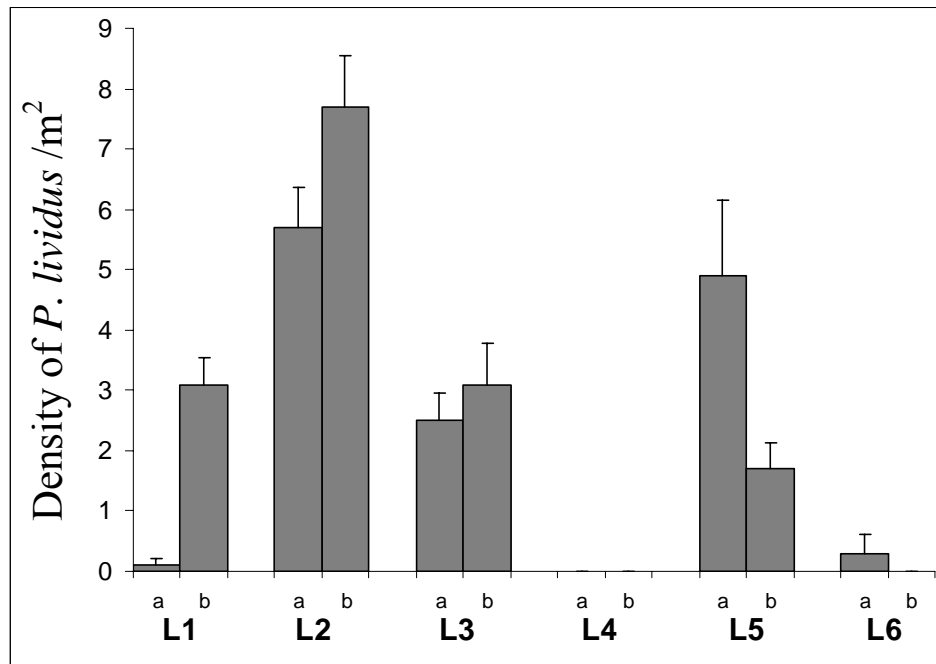| Source of variation	*P. lividus* density			*P. lividus* grazing marks			*S. salpa* grazing marks		
	df	MS	F	df	MS	F	df	MS	F
Location =L	5	11.83	6.31*	5	37.55	21.67 *	5	11.29	11.42
Area =A(L)	6	1.88	13.36*	6	1.73	3.19 *	6	0.99	1.67
Plot(LXA) =P	-			108	0.54	4.26 *	108	0.59	1.40
Residual	108	0.14		120	0.13		120	0.42	
transformation	ln (x+1)			ln (x+1)			ln (x+1)		
Cochran's test	0.1840			0.1575			0.0725		

Fig. 2. *Paracentrotus lividus*. Mean number (+SE) of sea urchin density at quadrats (1×1 m) in *Posidonia oceanica* at each location.
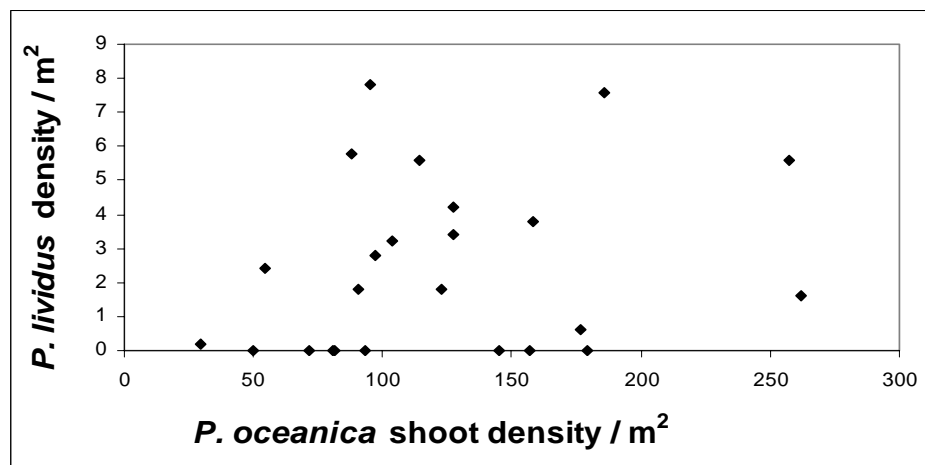

Fig. 3. Correlation between *Paracentrotus lividus* density and *Posidonia oceanica* shoot density.

Grazing marks on *Posidonia oceanica* leaves were mostly due to the 2 macroherbivores, while attacks by other herbivores were extremely rare (5 marks of isopods out of all leaves analysed). For *Paracentrotus lividus* grazing marks the analyses of data highlighted that there are significant variability at the three spatial scales considered (plot, area, location) as evidenced in Table 1 and Fig. 4. Grazing marks left by sea urchins were mainly observed at L2, within the MPA. Phenological analyses have revealed that the most affected leaves from *P. lividus* grazing were adult (91.8%) whereas no grazing marks were found on juvenile leaves.
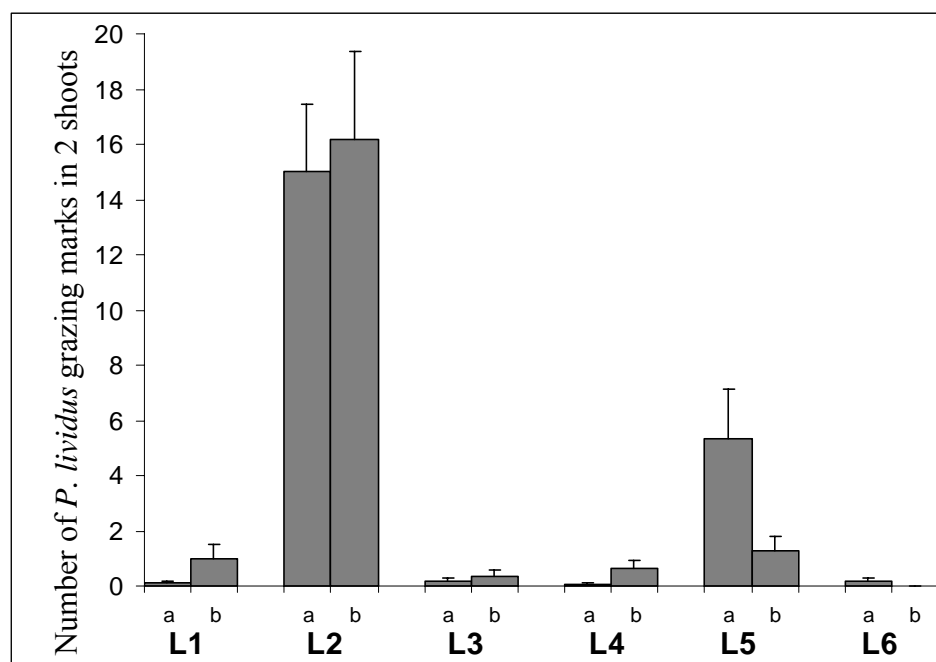
Fig. 4. *Paracentrotus lividus*. Mean number (+SE) of grazing marks
in *Posidonia oceanica* for area of each location.

Sarpa salpa grazing marks were quite abundant at all locations (Fig. 5). The analyses of data highlighted that there are significant variability at all scales considered (Table 1). Similarly to sea urchins, the most abundant grazing marks were found on adult *Posidonia oceanica* leaves (84.4%) while blades of the juveniles were always found intact. It is worth noting that low abundance of fish bites correspond to a high number of *Paracentrotus lividus* grazing marks (fig. 4 and 5).
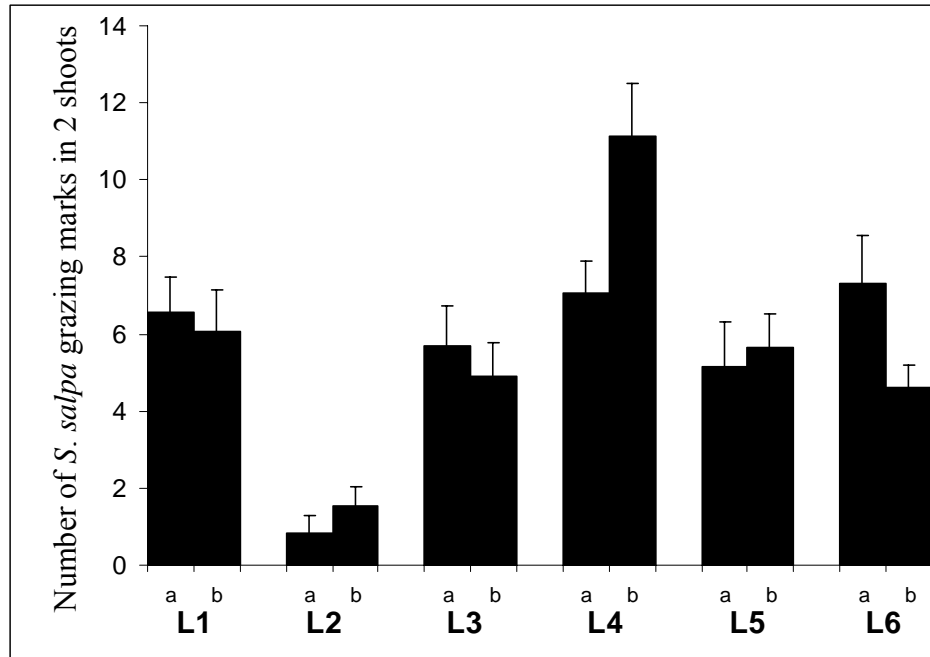
Fig. 5. *Sarpa salpa*. Mean number (+SE) of grazing marks in *Posidonia oceanica* for area of each location

A significant dependence of the number of *Paracentrotus lividus* grazing marks on sea urchin density (R^2=0.7160, p=0.0003) has been found considering averaged values per experimental areas where *P. lividus* occurred (Fig. 6). A sigmoid function, $y = \dfrac{L}{1+ce^{ax}}$, was found to better describe the relation between the two variables where *L* is the carrying capacity (the maximum number of grazing marks per area) (Sokal and Rohlf, 1969). Estimates of parameters, $c=e^{5.61214}=273.7294$ and $a =-1.04725$, have allowed to detect coordinates (5.36, 17.5) of the flexure point.
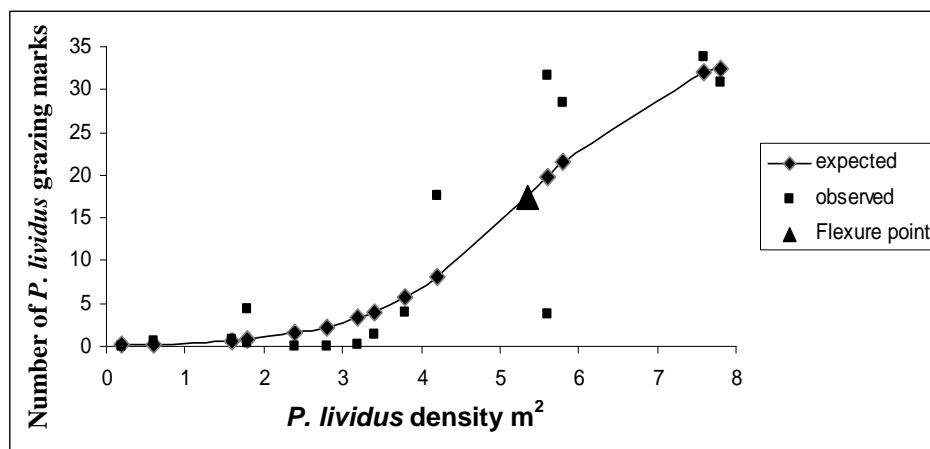
Fig.6. Sigmoid function of dependence of the number of *Paracentrotus lividus* grazing marks on sea urchin density.

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The distance class - frequency distribution of *Paracentrotus lividus* grazing marks on pooled data (Fig. 7) revealed that this herbivore affected the length of *Posidonia oceanica* leaf until about 700 mm from the base and attacks were concentrated to the lower portion (*i.e.* near the base). Conversely, the higher number of *Sarpa salpa* grazing marks have been recorded at higher portions of the leaf, although attacks have been found on the whole length.
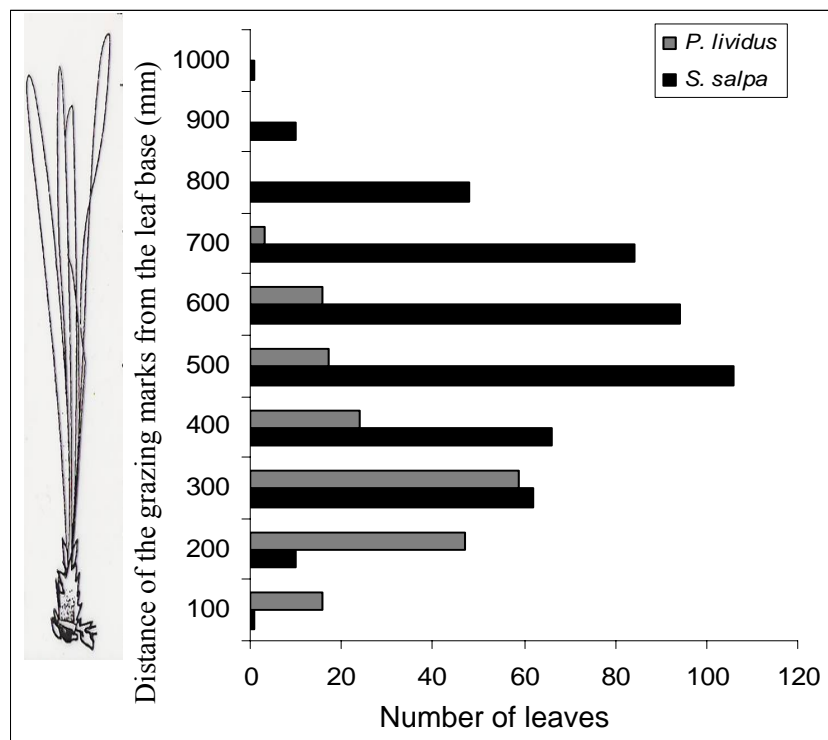
Fig. 7. The distance class - frequency distribution of *Paracentrotus lividus* grazing marks on pooled data of total leaves (0-100, 101-200, 201-300, 301-400, 401-500, 501-600, 601-700, 701-800, 801-900, 901-1000 and 1001-1100 mm).

The three way-ANOVA highlighted that there is a significant variability between the distance from the leaf base of marks left by the two herbivore species, *Paracentrotus lividus* and *Sarpa salpa* (Table 2).

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Table 2. ANOVA on relation between species (*P. lividus* and *Sarpa salpa*) and distance from the leaf base.

Source of variation		df	SS	MS	F	P
Species	=Sp	1	23.79	23.79	1.69	0.2503
Distance	=D	10	110.41	11.04	5.66	**0.0000**
Location	=L	5	16.72	3.34	7.67	**0.0000**
SpXD		10	55.95	5.59	3.97	**0.0005**
SpXL		5	70.41	14.08	32.30	**0.0000**
DXL		50	97.51	1.95	4.47	**0.0000**
SpXDXL		50	70.39	1.40	3.23	**0.0000**
Residual		132	55.55	0.43		
transformation		ln (x+1)				
Cochran's test		0.0965				

Although, a very high number of grazing marks were detected at about 300 mm from the leaf base, SNK test (SE=0.342) identified alternative hypotheses only for five distance classes (from 301 to 800 mm): grazing marks left by *Paracentrotus lividus* were significantly lower than those left by the herbivore fish. Also, this interaction seems to depend on the scale of the location (Table 2) although sensible variations were also evidenced by graphical inspection (Fig. 8).
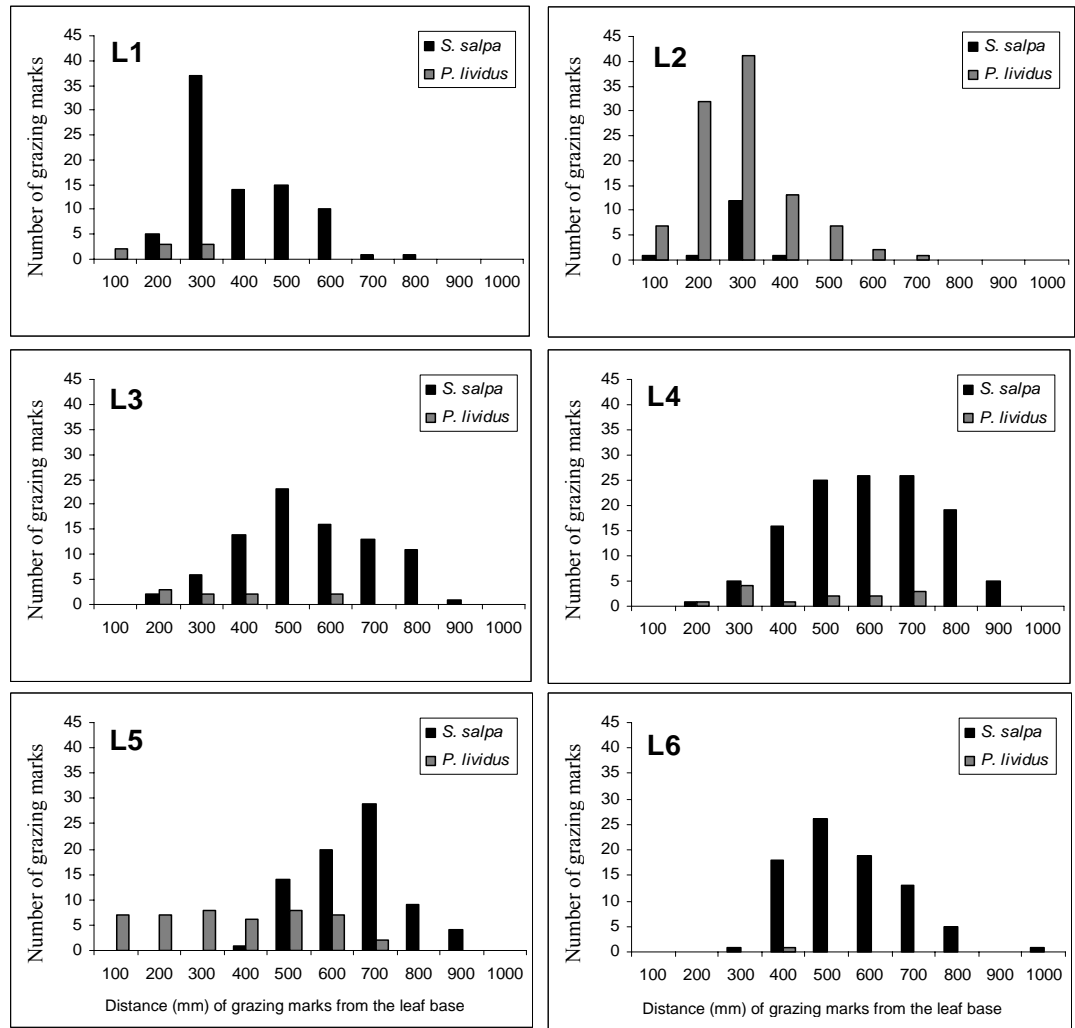
Fig. 8. Relation among distance from the leaf base of marks left by the two herbivore species, *Paracentrotus lividus* and *Sarpa salpa* at each location.

In particular, at the L2 an evident higher abundance of *Paracentrotus lividus* marks rather than *Sarpa salpa* was found at the lower half of the leaf and this is the only location where no marks left by the fish were found higher than 500 mm from the base. Further, for location L4 it is worth nothing that few *P. lividus* grazing marks have been found on leaf shoots even if no urchins were detected during counts in the field.

DISCUSSION

The results have evidenced the existence of a high variability in *Paracentrotus lividus* density at all spatial scales considered. At the scale of the location and area, this result is absolutely consistent with other studies (Ceccherelli et al. submit.) that have already suggested the important role of environmental conditions, such as substrate heterogeneity (Guidetti 2000; Ruitton et al. 2000; Hereu et al. 2005) and exposition (Chelazzi et al. 1997) on sea urchin distribution. However, in *Posidonia oceanica* habitat *P. lividus* density could be due to the amount of sediment accumulation at the border of the seagrass meadow: the lack and the very low abundance of sea urchins at some locations has been found either in seagrass beds where sandy bottoms occurred between the rocky habitat and the upper margin of the meadow and where samplings have been conducted inside the meadow, far from the margin (personal observation). Conversely, where the upper edge of the meadow was next to the rocky habitat the density of *P. lividus* individuals was higher. Further, although the higher abundance of sea urchins was found at a location (L2) within the B zone of the MPA, no evident relation was found between protection and *P. lividus* density. Nevertheless the high variability in *P. lividus* abundance in *P. oceanica* habitat cannot be explained by shoot density since no significant relation was found between these two variables. Overall, almost all *P. lividus* individuals sampled in this habitat was larger than 35 mm in test diameter and this supports the hypothesis that sea urchins migration, whether intentional or not (Abraham 2007; Pinna et al. submit.) from the rocky reefs is a major determinant on its distribution in *P. oceanica*, as already suggested by Tomas et al. (2004) and Ceccherelli et al. (submit.).

Further, our results, accordingly with Prado et al. (2007a), highlighted that the herbivory pressure by *Paracentrotus lividus* on *Posidonia oceanica* estimated indirectly by means of grazing marks counts, was lower than by *Sarpa salpa*. However, the grazing impact due to the former species may be underestimated due to the fact that lateral bites exerted by large *S. salpa* individuals may enhance leaf break, removing portions of the blade with other grazing marks (Tomas et al. 2005a). As suggest by some authors (Velarque 1990; Peirano et al. 2001; Tomas et al. 2005b; Prado

et al. 2007a) intense grazing on *P. oceanica* by *S. salpa* is a common phenomenon in shallow waters during summer months, before descending to spawn to deeper waters (Velarque 1990). In this period the occurrence of massive schools of this species can result in repeated attacks to the same leaves causing a biomass depletion that can mask marks of previous attacks. At the same time, other studies indicated that sea urchins, and in general herbivores, prefer the oldest part of the leaves for the presence of epiphytes (Cebrián et al. 1996; Alcoverro et al. 1997; Boudouresque and Velarque 2001; Peirano et al. 2001; Prado et al. 2007b). Also, some other suggest that although young sea urchins feed on both rhizomes and leaves, adults have a low-specialised diet and graze preferentially on the highly epiphytized distal part of the leaves (Nedelec and Velarque 1984; Zupo and Fresi 1984).

However, this is in contrast to our results which have suggested *Paracentrotus lividus* to graze on lower portion of the leaves, whereas the attacks of *Sarpa salpa* have been recorded at higher portions. If at the study locations sea urchins feed at lower heights of the seagrass canopy the prediction would be that *S. salpa* herbivory can be underestimated. Moreover, although in some studies it has been evidenced that the frequency of bitten shoots does not always correlate well with herbivore population measurements (Tomas et al. 2005a), in this experiment a significant dependence of the number of *P. lividus* grazing marks on sea urchin density has been found. In fact, the sigmoid function has suggested that, although *P. lividus* density increases until 5.36 individuals/m^2 correspond exponential increase of the number of grazing marks, at higher densities this relation seems to stabilize to a plateau value. This finding can be explained by an overall defoliation of shoots at higher sea urchin density and this would indicate that indirect estimates of herbivory would be unreliable. On the other hand, at one location (L4) no *P. lividus* individuals have been found during sampling, despite the presence of sea urchin grazing marks. Whether this could depend on the spatial variability of a low dense population of *P. lividus* or on a previous removal of individuals by natural predators or humans (since harvest was possible at the location) it is not known.

Further, phenological analyses confirmed that the most affected leaves from *Paracentrotus lividus* and *Sarpa salpa* grazing were adult, whereas no grazing marks were found on juvenile leaves. However, Cebrián et al. (1996) sustained that *S. salpa* feeds preferentially the intermediate (*i.e.* mid-aged) leaves on the shoot, whereas that *P. lividus* attacks the leaves that are trapped by its spines showing a low selective strategy. This could also be in accordance with our data, since adult leaves correspond to higher exposure because of the external position within the shoot.

Nevertheless, whether significant variability in feeding activity of *Paracentrotus lividus* exists either across seasons and locations still needs to be properly estimated. In fact, although authors indicate that *P. lividus* populations are very stable throughout the year (Tomas et al. 2004), others sustain that sea urchins show a strong seasonal feeding pattern (Lozano et al. 1995). Moreover, many other factors seem to influence feeding behaviour of *P. lividus* (Fernandez and Boudouresque 2000; Boudouresque and Velarque 2001; Abraham 2007) and manipulative investigations should be conducted.

Nevertheless, several authors suggest that indirect measures may not accurately represent herbivore consumption and thus could lead to misleading notions on the real importance of grazing in seagrass food webs (Tomas et al. 2005a), while direct quantification of seagrass biomass removal by herbivores has only rarely been attempted (Valentine and Heck 1999; Heck and Valentine 2006). Heck and Valentine (2006) suggested that many, if not most, prior estimates of grazing on seagrasses developed using indirect methods were far too low. However, indirect measures of herbivory pressure allow to arise hypotheses that need specific manipulative experiments to be tested. Such studies will provide estimates of the amount of seagrass production directly entering nearshore food webs, and they will improve our understanding of the factors that control spatial and temporal variability of seagrass herbivory.

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CHAPTER 3

**Investigating the effect of *Posidonia oceanica* patches
on the distribution patterns of *Paracentrotus lividus*
in a central western Mediterranean Marine
Protected Area ***

Chapter 3. Investigating the effect of *Posidonia oceanica* patches on the distribution patterns of *Paracentrotus lividus* in a central western Mediterranean MPA

ABSTRACT

The sea urchin *Paracentrotus lividus* (Lamarck) is the most common grazer in the Mediterranean infralittoral. In this study, we investigated the movement of this echinoid from a rocky habitat to *Posidonia oceanica* (L.) Delile patches depending on both the size of sea urchins and their distance from the patch edge. We postulated that *P. lividus* would move randomly to a seagrass patch and that the probability of reaching it should decrease with distance. At this aim, we conducted a manipulative experiment (in four times from October 2006 to June 2007) at a location within a Mediterranean MPA, where several *P. oceanica* patches were intersperse on rocky platforms. On each time, after an accurate removal of sea urchins living inside them, 15 of these patches were randomly attributed in sets of 3 to 5 different addition treatments with groups of 10 large and small *P. lividus* specimens (test diameter >50 mm and <30 mm, respectively) at close and far distances (25 cm and 100 cm, respectively) from the edge of the patches as follows: large-close (LC), large-far (LF), small-close (SC), small-far (SF), and controls (Cs) where no urchins were added. The abundance of sea urchins inside the patches was counted after 24 hours. Asymmetrical ANOVA highlighted significant variability among treatments, while no significant differences were detected among times. Moreover, significant differences were found for distance from the patches, but no significant effect for sea urchin size was evidenced. These results suggested that *P. lividus* specimens close to *P. oceanica* patches may have a higher probability to reach them, and that sea urchins are likely to move randomly to the seagrass patches because no clear preference for the seagrass was exhibited. Further, similar ability to move towards the patches was highlighted for different sized specimens, indicating that migration from one habitat to the other is possible also for small individuals. Overall, the results from this study open new views towards the general understanding of the importance of spatial arrangement of *P. oceanica* and rocky reef habitats on the spatial distribution of *P. lividus*.

Key words: Home range, migration, *Paracentrotus lividus*, *Posidonia oceanica*.

Chapter 3. <u>Investigating the effect of *Posidonia oceanica* patches on the distribution patterns of *Paracentrotus lividus* in a central western Mediterranean MPA</u>

INTRODUCTION

In the Mediterranean Sea, the sea urchin *Paracentrotus lividus* (Lamarck) is commonly found in shallow subtidal rocky reefs and in the seagrass *Posidonia oceanica* (L.) Delile meadows (Tortonese 1965). This echinoid plays a key role in controlling macrophyte communities on rocky habitats as it is capable of depleting erect algae (*e.g.* Benedetti-Cecchi et al. 1998; Hereu 2006), whereas in *P. oceanica* beds it has been reported to feed preferentially on epiphytes rather than on plant material (Nédelec and Verlaque 1984; Sheperd 1987; Tomas et al. 2005, 2006). Under natural conditions, adult density of *P. lividus* is higher on rocky walls than in *P. oceanica* (only up to 6 individuals m^{-2}, Boudouresque and Verlaque 2001), and no appreciable successful recruitment takes place in seagrass meadows, where adult population cannot be sustained by local recruitment (Tomas et al. 2004).

Processes such as predation, recruitment, migration, and disease exert a crucial role in sea urchin population dynamics and consequently they may influence the spatial distribution of individuals. Although there is an extensive literature on *Paracentrotus lividus* devoted to understand the importance of many of these features (Crook et al. 2000; Fernandez et al. 2001; Guidetti 2004; Tomas et al. 2004; Hereu et al. 2005), the role of migration from a habitat to another has been neglected for years. Barnes and Crook (2001) suggested that sea urchin size, covering and season can be important determinants of migratory behaviour that should deserve appropriate experimental investigations.

Although description of migration by aggregation fronts of sea urchins such as *Strongylocentrotus droebachiensis* seems recurrent (Vadas et al. 1986; Schiebling et al. 1999; Dumont et al. 2006), no similar phenomenon has been described for *Paracentrotus lividus* (but see Chelazzi et al. 1997) as several papers have only been addressed to identify factors that influence sea urchin movements at a lower scale. For example, food availability (Andrew and Stocker 1986) and water flow (Kawamata 1998) are known to be important determinants of *P. lividus* behaviour. However, the presence of predatory fish certainly mediates the sea urchin behaviour by causing

urchins to shelter and thus diminishing their diel foraging activity (Carpenter 1984; Scheibling and Hamm 1991; Sala 1996). *P. lividus* shows a circadian pattern of activity with more active specimens during the night as a defence against diurnal predators. Previous evaluations of the movement patterns of this species in a NW Mediterranean marine reserve estimated the linear distance travelled in a 24 h period up to 220 cm and 130 cm in unprotected and protected areas, respectively, with a mean home range of 51 cm (Hereu 2005). Specifically, after 24 hours about 85% of the individuals travelled less than 100 cm.

Most animals are on the move searching for food and shelter or to avoid predation and environmental stress (Swingland and Greenwood 1982). Movement patterns of sea urchins such as *Strongylocentrotus droebachiensis* can be adequately described by a random walk model, with frequent reverses in direction and occurrence of turning angles, and that deviations likely indicate that local environmental factors such as patches of food or physical conditions may influence the movement (Dumont et al. 2007). As no experiments on *Paracentrotus lividus* have been conducted in the field, its movement patterns and perception of the habitat landscape still remain unknown. Thus, whether the search for food and shelter relative to the distribution of food patches and shelter availability occurs at random needs to be cleared.

In this study, we estimated the daily migrations of *Paracentrotus lividus* from a rocky habitat to *Posidonia oceanica* patches depending on the size of sea urchins and the distance from the patch edge. We postulate that *P. lividus* would move randomly to the seagrass patch, and that the probability of reaching it should decrease with distance. At this aim, we conducted a manipulative experiment at a location within a central western Mediterranean Marine Protected Area (MPA), where several *P. oceanica* patches were intersperse on rocky platforms.

MATERIAL AND METHODS

The experiment was conducted within the Capo Caccia-Isola Piana MPA (NW Sardinia, Italy) at Polt Agra Bay (Fig. 1), where numbered patches of *Posidonia oceanica* (about 2 m^2 in size, 100

shoots m^{-2} in density, and 35 cm in canopy height) occurring on rocky platforms at a depth of about 5-8 m were considered. Four replicated times (9-10 October 2006, 13-14 November 2006, 20-21 April 2007, 5-6 June 2007, hereafter T1, T2, T3 and T4, respectively) were chosen and for each time two days (hereafter D1 and D2) were spent in the field.
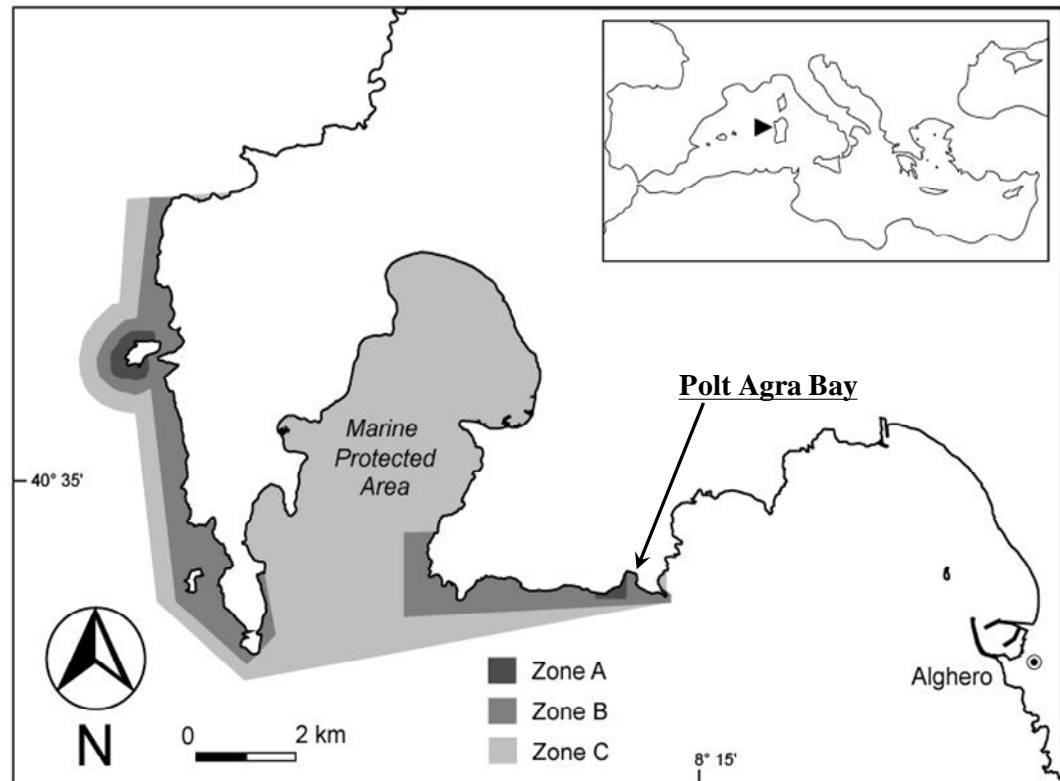

Fig. 1. Study locations inside Capo Caccia – Isola Piana MPA (B zone).

During D1, 15 patches have been accurately cleared from *Paracentrotus lividus* by SCUBA divers, paying attention to remove all the individuals inside each patch and all around it for about 2 m from its edge. These patches were randomly attributed in sets of 3 to 5 different urchin addition treatments with groups of large and small *P. lividus* specimens (test diameter larger than 50 mm and smaller than 30 mm, respectively) at close and far distances (25 cm and 100 cm, respectively) from the edge of the patches as follows: large-close (LC), large-far (LF), small-close (SC), small-far (SF), and controls (Cs) where no urchins were added. Close and far positions refer to 25 cm and 100 cm from the edge of each patch, which were judged to be long enough to be covered in 24 hours

(Hereu 2005). Depending on the treatment (*i.e.*, size-distance, Fig. 2), 10 *P. lividus* individuals were homogeneously positioned around each patch.

Controls (Cs) were used to estimate possible migration of *Paracentrotus lividus* from reefs farer than 200 cm, as well as the number of individuals missed during removal. In this study, no tagging methods have been used, so that no artefacts due to this procedure could affect results. In fact, even if several workers described different sea urchin tagging techniques (see Duggan and Miller 2001 for a review), most of these methods are intrusive for the perforation of the test and, although in some cases estimates of survival were given, no data of tagging effect on the sea urchin behaviour are available.

During D2 the number of sea urchins found inside the *Posidonia oceanica* patch was counted and test diameter was measured for each individuals. Pictures of benthic assemblages around the seagrass patches were taken to give estimates of macroalgal abundance for each taxa.

Data obtained were analysed by asymmetrical ANOVA, where 'Treatment' was treated as fixed and orthogonal to 'Time' that was considered as random. Among addition treatments 'Size' and 'Distance' were treated as fixed and orthogonal. The contrast between addition treatments and controls was tested following calculation indicated by Underwood (1997)

RESULTS AND DISCUSSION

For all sampling times, specimens of *Paracentrotus lividus* were not found inside all the patches with addition treatments (*i.e.*, LC, SC, LF and SF), nor inside Cs. The total number of *P. lividus* found in each *Posidonia oceanica* patch reached 9 in LF, 11 in LC, 8 in SF, 12 in SC and 6 in Cs, respectively. Although a higher number of sea urchins was observed in addition treatments rather than in Cs, no significant difference was evidenced by ANOVA (Tab. 1). Moreover, no significant effect was found for the size among addition treatments, while significant differences were detected for the distance. In fact, a lower number of *P. lividus* was found for both far treatments (*i.e*, LF and

SF). Furthermore, consistent results were found through times, suggesting that no significant temporal variability occurred during the study period.

| Table 1 Asymmetrical ANOVA on the number of *Paracentrotus lividus* in each *Posidonia oceanica* patch.						
Source of variation	SS	DF	MS	*F*	*P*	*F* versus
Treatment =Tr	95.6	4	23.90	3.91	0.0293	TrXTi
Cs vs Addition	63.04	1	63.04	6.15	0.0893	TiXCs vs Additino
among Addition	32.56	3	10.85	2.30	0.1462	TiXAddition
Size=S	6.02	1	6.02	1.96	0.2563	TiXS
Distance=D	25.52	1	25.52	11.38	**0.0433**	TiXD
SXD	1.02	1	1.02	0.12	0.7566	TiXSXD
Time=Ti	42.05	3	14.02	1.40	0.2583	Res
TrXTi	73.27	12	6.11	0.61	0.8226	Res
TiXCs vs Addition	30.75	3	10.25	5.93	**0.0197**	Res Cs vs additino
TiXAddition	42.52	9	4.72	0.39	0.9311	Res among additino
TiXS	9.22	3	3.07	0.25	0.8581	Res among additino
TiXD	6.72	3	2.24	0.18	0.9058	Res among additino
TiXSXD	26.56	3	8.85	0.73	0.5416	Res among additino
Res	401.83	40	10.05			
Res among addition	388.00	32	12.13			
Res Cs vs addition	13.83	8	1.73			
SNK Test						
Distance						
SE=0.3057	Far<Close					
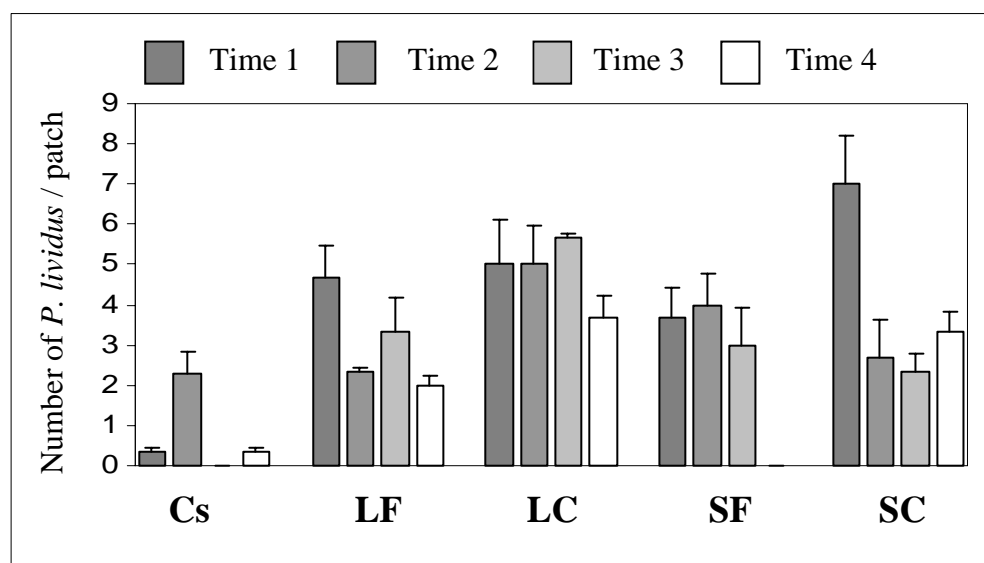
Fig. 2. Mean number (+SE) of *Paracentrotus lividus* density at each treatment patches in each sampling time.

This is in contrast with the observations of Barnes and Crook (2001), that indicated size-specific migration patterns for this species on rocky substrates. Based on our results, we can also hypothesize that sea urchins are likely to move randomly to the seagrass patches because no clear preference for the seagrass was exhibited. In fact, if for any reason *Paracentrotus lividus* had preferred *Posidonia oceanica* habitat to the rocky habitat, a similar number of specimens would have been found at patches regardless the distance from the edge of the seagrass patch, since both trails from the far and the close position should be coverable for this species during each experimental time (*i.e.* 24 h). Overall, although *P. lividus* is one of the most important grazer of *P. oceanica* (*e.g.* Nedelec and Velarque 1984; Boudoresque and Velarque 2001), the role of this seagrass on the distribution patterns of this echinoid still needs to be clearly defined. In fact, the results of this study support the hypothesis that *P. lividus* moves stochastically since no evidence of intentional changes of habitat (*i.e.* from rocks to *P. oceanica* patches) has been provided. This could suggest that *P. lividus* specimens found in *P. oceanica* patches are the result of those individuals that encountered the seagrass patch and remained in this habitat. This would also explain why in such seagrass patches we generally find higher abundance of *P. lividus* than on the next rocky reef (9.2 and 6.5 average abundance m^{-2}, respectively). Altogether, this would be in accordance with the observations reported by other authors (Domenici et al. 2003; Dumont et al. 2007), who suggested that reaching a shelter may correspond to a strategy of random searching behaviour.

Further, the fact that this experiment has been performed through times of different seasons and that no significant effect of the time has been detected, suggests several hypotheses. First, that the structure of algal assemblages on rocky reefs does not influence significantly the movement of *Paracentrotus lividus* between the habitats. In fact, at all sampling times macroalgal assemblages, although different through seasons, were composed of several palatable species (Velarque and Nedelec 1983; Velarque 1987; Benedetti-Cecchi et al. 1998), such as *Padina pavonica*, *Laurencia* spp., Dictyotales, Sphacelariales and algal turfs, that occurred abundantly at the site. Moreover, during the whole experiment neither large cover of encrusting calcified rhodophytes occurred nor

bare rock were detected around the seagrass patches, indicating that on the rocks there were no signs of *P. lividus* overgrazing and that, conversely, the availability of possible food resources was high. Nevertheless, further manipulative research is needed to investigate the importance of the structure of macroalgal assemblages on rocky habitat on *P. lividus* movement towards *Posidonia oceanica* patches.

The second hypothesis is that the temporal variability of foraging activity of *Paracentrotus lividus* through the seasons already suggested by some authors (Lozano et al. 1995; Peirano et al. 2001) did not significantly influence the results of this study. Thus, although the feeding preferences of this herbivore would probably depend on the season, our findings suggest that some other stronger determinants could affect its behaviour. Indeed, the hypothesis that the movement of *P. lividus* individuals towards the seagrass patches could be greatly affected by an 'escape behaviour' (Hereu et al. 2004; Hereu 2004, 2005) regardless of the season, it is strongly supported. Nevertheless, although during the experiment no signs of attacks on sea urchins (*i.e.* test fragments or broken individuals) were observed around the patches, and the relative recent establishment of this MPA (dated December 2002) has not determined so far significant benefits on fish assemblages structure (Pais et al. 2007), it is highly reliable that predators could control sea urchin population density and distribution (Guidetti 2007).

Another important issue that is worth considering is about the lack of significant differences among addition treatments and controls. We believe that this result could be due to the low power of the test and, thus, we suppose that a higher number of sampling times would detect statistical differences. However, to detect whether the few *Paracentrotus lividus* individuals found in control patches derive from very large migrations or they are simply individuals missed during the removal on D1, tagged individuals would have been required. As already mentioned, we wanted to take particular care to avoid any procedural artefacts and no tagging technique considered prior to the experiment was evaluated sufficiently acceptable. At this regard it is ignored what influence *P. lividus* individuals translocation, when positioned around the seagrass patches, could have had on

their behaviour: whether this procedure stresses the sea urchins and affects their need to search for a shelter still needs to be investigated. For this reason, in this experiment the removed urchins from patches on each D1 were purposely transplanted far away from the next rocky habitat so that they were not used for the experimental additions. In fact, the little literature available on transplant experiments with other echinoids produced contrasting results since Tertschnig (1989) and Yusa and Yamamoto (1994) did not show any effect of the habitat of origin, while Dumont et al. (2006) suggested that transplants led to less actions.

Several questions can arise about the influence of environmental physical conditions of the environment on *Paracentrotus lividus* behaviour. For instance, this experiment was performed during only days of calm sea conditions, merely for our logistic convenience. However, wave action seems to influence the spatial distribution of sea urchins (Chelazzi et al. 1997; Kawamata 1998) and whether other results would have been obtained performing the experiment during, immediately before or after storms it is not very predictable (but see Chelazzi et al. 1997). Also, the importance of the structure complexity of *Posidonia oceanica* canopy (density of shoots and leaf length) could have affected the environment, for example by decreasing irradiance and water motion (*e.g.* Boudoresque and Meisnesz 1982; Enríquez et al. 1992), and thus have provided shelters of different suitability. At this regard, further manipulative research is needed to investigate what are the factors regulating the spatial distribution of *P. lividus* in *P. oceanica* meadows and specifically, how the structure of seagrass canopy influence *P. lividus* abundance. Overall, the results from this study open new views towards the general understanding of the importance of spatial arrangement of *P. oceanica* and rocky reef habitats on the distribution patterns of the Mediterranean sea urchin *P. lividus*.

ACKNOWLEDGEMENTS

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CHAPTER 4

The effect of *Posidonia oceanica* canopy structure on *Paracentrotus lividus* distribution pattern*

ABSTRACT

Seagrasses influence the surrounding environment by modifying the substrate, light conditions and water motion and provide a physical habitat used as predation refuge and that thus can have a great influence on composition and distribution on the organisms that inhabit within the meadow. In the Mediterranean, *Paracentrotus lividus* is one of the main herbivore on the seagrass *Posidonia oceanica*, where it occurs on rhizomes among shoots. Under natural conditions, adult density of *P. lividus* is higher on rocky walls than in *P. oceanica*, and no appreciable successful recruitment takes place in seagrass meadows, where adult population cannot be sustained by local recruitment. Nevertheless, in *P. oceanica*, *P. lividus* can likely find a suitable habitat, considering the high complexity structure and the availability of food. At this regard, the purpose of this study was to investigate the role of *P. oceanica* canopy structure on *P. lividus* distribution. The experiment consists in a manipulation of the seagrass structure achieved by modifying the shoot density (20%, 50% and 100%) and leaf high (natural and halved). For each treatment combination three types of *P. oceanica* were considered: natural seagrass (NS), artificial seagrass + frame (AS+F) and natural seagrass + frame (NS+F). The structure of *P. oceanica* of artificial (plastic) seagrass (AS) units, mimicking natural seagrass (NS), was reproduced so that shelter but no food was provided. NS+F *P. oceanica* was used to test the effect of the frame used to hold the plastic stripes in AS+F units. An higher abundance of *P. lividus* individuals at NS units rather than at AS units would suggest that the food provided by *P. oceanica* is a significant determinant on the spatial distribution of the sea urchin in this habitat. Further, to comprehend mechanisms of interaction, water motion and light measurements were taken: these data would highlight both whether pruning the seagrass shoots for each canopy type and using AS+F units altered the effect of reducing bottom current regime and irradiance. Results have highlighted that *P. lividus* distribution is affected by *P. oceanica* shoot density in different ways, as highlighted by the significant '*Posidonia* × Density' interaction. In fact, no significant differences in *P. lividus* abundance among NS, AS+F and NS+F *Posidonia* type treatments were found at low shoot densities (20% and 50%), while at natural density (100%) a

higher abundance was found in NS rather than in AS+F and NS+F units. This suggests that at lower densities the artefact effect of frame did not significantly contribute to the observed variability which is entirely due to the canopy material. Further, the similar *P. lividus* abundance between AS+F and NS+F types at all densities suggests that *P. oceanica* mainly plays a refuge role rather than food resource. Also, the significantly higher irradiance found at the lowered shoot densities of *P. oceanica* could explain the higher abundance of *P. lividus* at the highest density of the seagrass at NS units, while water motion cannot be invoked as a factor responsible of the distribution since no significant differences were found among treatments' combinations. However, the hypothesis that *P. lividus* individuals primarily use *P. oceanica* to find a shelter and then they feed on it only for opportunity, still needs to be clarify. Overall, although many questions still remain unanswered, these findings add important knowledge to the understanding of *P. oceanica – P. lividus* interaction.

Key words: Canopy, *Paracentrotus lividus*, plant-herbivore interaction, *Posidonia oceanica,* sea urchins, spatial distribution.

INTRODUCTION

Seagrass meadows are extremely high productive systems and support a great abundance and diversity of species. Further, a number of commercially and recreationally important species, including both fish and invertebrates, have been linked to seagrasses for some stage of their life cycle, although few of them use seagrasses throughout their lifespan (Bronwyn 2006).

Seagrass meadows provide shelter for species assemblages (Carpenter 1984; McClanahan and Muthiga 1989) mainly due to the structural complexity that shades (Verweij et al. 2006) and baffles currents (Guidetti 2000) as, for example, already highlighted for juvenile reef fish (Verweij et al. 2006; Beck et al. 2001; Guidetti 2000). Variation in canopy height and shoot density on wide spatial scale of seagrass meadows can have substantial effect on abundance of common fish and decapods (Bell and Westoby 1986; Kennelly 1989). Coupled with this, higher complexity habitats have both producing and sediment trapping potential, as a result of attenuation of hydrodynamic conditions within their canopies (Papadimitriou et al. 2005; Hauser et al. 2006; Koch 1993; de Boer 2007). Furthermore, seagrasses influence the surrounding environment by modifying the substrate and light conditions (Jones et al. 1994; Koch 2001; Williams 1987). All the factors above can have a great influence on composition and distribution on the organisms that inhabit within the meadow (Gambi et al. 1990; Hovel et al. 2002).

Thus, besides the protection from predators seagrass meadows provide an increased food availability and both are often cited as reasons to explain why such large number of organisms are associated with seagrasses (Valentine and Duffy 2006). Although studies that evaluated whether more food is available in these habitats are scarce (but see Williams and Ruckelshaus 1993), there is increasing evidence that the consumption of seagrasses is more important than previously thought. In fact, Konar (2000, also references therein) suggests that seagrass species may structure communities mainly through trophic interactions with herbivores. Also, there is enough evidence to support the hypothesis that this plant-herbivore relationship seems largely mediated by the presence

of epiphytes (Alcoverro et al. 1997; Zimmerman et al. 2001; Tomas et al. 2005; Borowitzka et al. 2006; Heck and Valentine 2006; Prado et al. 2007a).

In the Mediterranean, *Posidonia oceanica* L. Delile is the dominant seagrass species, which forms extensive meadows from the shallow subtidal to about 30 to 40 m of depth. This seagrass is considered to structure habitats highly complex for the high shoot density, length of the leaves, leaf longevity and low shoot turnover compared to the other seagrasses (*e.g.* Duarte 1991; Marbà and Duarte 1996; Hemminga and Duarte 2000). Its structural complexity affect food webs by modifying ecosystem structure, providing habitat, as well as producing organic matter (Gobert et al. 2006).

One of the main herbivore of *Posidonia oceanica* is the sea urchin *Paracentrotus lividus* (Lamarck). Commonly, *P. lividus* occurs on shallow subtidal rocky habitats where, at high densities, it overgrazes complex algal assemblages turning them into barren areas dominated by a few species of encrusting algae (*e.g.* Benedetti-Cecchi et al. 1998; Hereu 2006). Most of ecological research on *P. lividus* has been addressed to understand causes underlying the distribution patterns on rocky habitat. For example, structural complexity of the rocky habitat seems to greatly affect the spatial variability of *P. lividus*, being particularly important for juveniles survivor and when predation pressure is high (Guidetti et al. 2003; Bonaviri et al. 2005; Hereu et al. 2005). Moreover, the spatial distribution of *P. lividus* seems influenced by many processes such as predation (Sala and Zabala 1996; Guidetti 2004; Hereu et al. 2005), recruitment (Tomas et al. 2004), migration (Palacín et al. 1997; Crook et al. 2000) and competition (Guidetti et al. 2004; but see Gianguzza et al. 2006).

Paracentrotus lividus in the Mediterranean is commonly found in *Posidonia oceanica* meadows, where it occurs on rhizomes among shoots. Population structure of *P. lividus* seems quite different at the two habitats being adult density of *P. lividus* higher on rocky reefs than in *P. oceanica*, as suggested by Boudouresque and Verlaque (2001) and Ceccherelli et al. (submitted). Likely, in this habitat, *P. lividus* can find a suitable habitat, considering the high complexity structure and the availability of food. In fact, authors have already investigated on the consumption

of *P. oceanica* and has been evidenced that the presence of epiphytes on seagrass makes the leaves more palatable for *P. lividus* (Tomas et al. 2005; Tomas et al. 2006). In fact, *P. oceanica* beds it has been reported to feed preferentially on epiphytes rather than on plant material (Nédelec and Verlaque 1984; Sheperd 1987; Alcoverro et al. 1997; Tomas et al. 2005, 2006).

Furthermore, recent studies have been addressed to understand which factors influence the movement patterns of *Paracentrotus lividus* on rocky habitats (Crook et al. 2000; Barnes and Crook 2001; Verling et al. 2002; Hereu 2005). Some authors suggest that migration could be mainly driven by predation and also due to avoid light (Barnes and Crook 2001). Whether the movement of *P. lividus* from the rocky habitat to *Posidonia oceanica* meadows occurs at random as a preference and what are the factors regulating the spatial distribution in this habitat are questions that still need to be answered. The higher number of *P. lividus* individuals at the margin of *P. oceanica* meadows (especially where the seagrass is directly next to rocky reefs) rather than inside (personal observation), and the higher frequency of large size classes individuals inside the meadow compared to outside, support the hypothesis that a considerable abundance of individuals migrate from the rocky reef habitat.

The purpose of this study was to investigate the role of *Posidonia oceanica* structure on *Paracentrotus lividus* distribution. At this aim, the manipulation of the seagrass structure has been achieved by modifying the shoot density and leaf high. Also, in this experiment artificial (plastic) seagrass (AS) units mimicking natural seagrass (NS) units have been used: at these treatments, the structure of *P. oceanica* was reproduced so that shelter but no food was provided. An higher abundance of *P. lividus* individuals at NS units rather than at AS units would suggest that the food provided by *P. oceanica* is a significant determinant on the spatial distribution of the sea urchin in this habitat. Also, whether the abundance of *P. lividus* depends on the structure of *P. oceanica* canopy would be evidenced by comparing treatments.

MATERIALS AND METHODS

This study was carried out in the Gulf of Alghero (North West Sardinia, Italy), near Capo Caccia-Isola Piana MPA (40° 34' N - 8° 13' E), at a small bay near a shore with accommodation facilities and living units (Fig.1). The experiment was done during winter, from November 2006 to March 2007, when tourist visitation is extremely low. Sampling was done at the edge of a *Posidonia oceanica* continuous meadow: a location of about 500 m^2 wide at about 5 m deep was chosen. The seagrass edge, which corresponds to the upper limit of the meadow, was next to a rocky habitat and this would enhance sea urchins' supply.
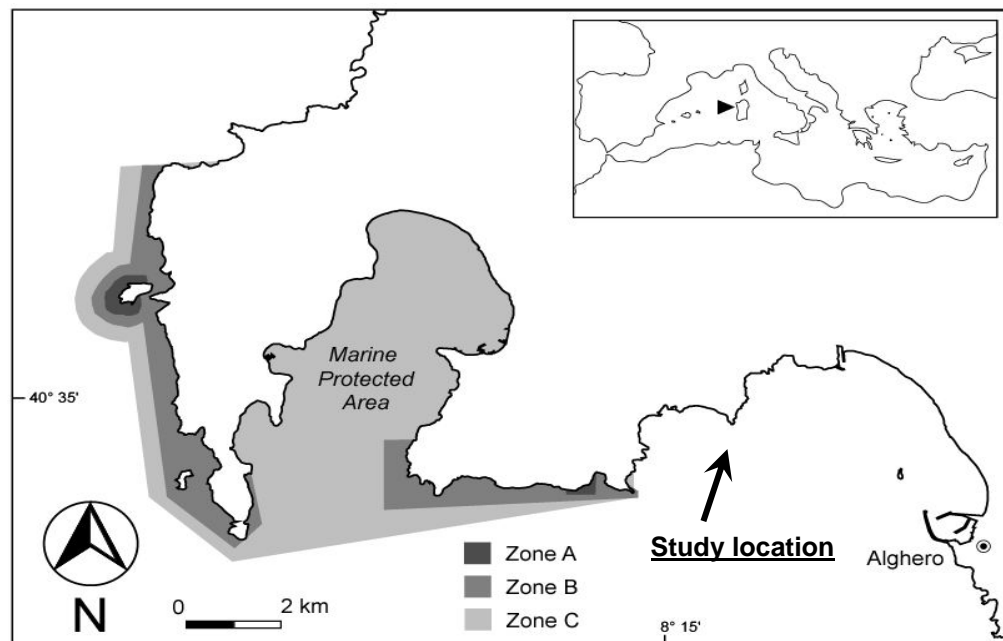
Fig. 1 Study location outside Capo Caccia – Isola Piana MPA at Punta Negra.

The rocky habitat consists of gently sloping platforms covered mainly by: algal turfs, *Codium bursa* (Agardh 1822), *Halimeda tuna* (Ellis & Sol.) J. V. Lamour, *Dasycladus vermicularis* (Mayhoub 1976), *Udotea petiolata* (Turra) Borgesen.

To test one of the potential mechanisms of influence of *Posidonia oceanica* canopy on *Paracentrotus lividus* distribution, artificial structures were made which could reduce light and velocity of near-bottom water currents as *P. oceanica* canopy, but could not provide any food resource.

To reproduce the broad characteristics of the seagrass habitat, the natural shoot density, leaf height and number of leaves per shoot were estimated in the field at the beginning of the experiment. *Posidonia oceanica* mean shoot density in 40×40 cm was 75.67 cm (± 4.67 SD), mean leaf height was 45.33 cm (±7.03 SD), while the mean number of leaves per shoot was 5.81 cm (± 0.63 SD). Artificial structures (AS+F) were constructed from a 40×80 cm frame of plastic coated iron with a 5×10 cm mesh to which mimics of *P. oceanica* leaves were tied. Artificial leaves were made of green plastic stripes and organized to form a density of shoot and length of leaves that were similar to natural *P. oceanica* at that site: each shoot was made of 3 folded stripes of 100 cm (that corresponds to six leaves of 50 cm) and a total of 150 shoots were tied to each frame.

In the experiment, a total of three types of *Posidonia oceanica* canopy (treatment) were established: natural seagrass (NS), artificial seagrass + frame (AS+F) and natural seagrass + frame (NS+F). In fact, to compare the effects of the NS with AS+F another type of canopy treatment was included in the experimental design so that the effect of undesirable factors such as those due to the frame, besides lower food availability, could be excluded. Indeed, at each NS+F unit the same plastic coated iron frame without leaf mimics was secured in 40×80 cm units where natural leaves were present. Eventual differences between NS and NS+F units would have highlighted the intruding influence of the frame. Conversely, each AS+F unit was placed on 40×80 cm areas, where natural seagrass vegetation was removed, in which the frame with leaf mimic was secured at the corners with two 35 cm iron dowels driven below the substratum using a hammer.

Experimental units (replicates) 40×80 cm sized quadrats were prepared at the edge of the seagrass meadow, the position within the meadow where *Paracentrotus lividus* seems more abundant (personal observation). Overall, the three types of *Posidonia oceanica* were deployed at two different times so that a total of six times were necessary to perform the entire experiment. In fact to handle an experimental design in which the three types of seagrass canopy were used would have been a too complex work, and it would have been necessary to deal with a very wide *P. oceanica* edge surface. For this reason, it was preferred to replicate times (twice) within each type

of *P. oceanica* canopy treatment. At each time, a total of six combinations of 'shoot density × leaf length' were established by manipulating canopy height (natural and reduced to half), by cutting leaves, and shoot density (20%, 50% and 100%), by clipping shoots using common scissors. Each treatment combination was three-replicated (randomly assignment) so that 18 experimental units were obtained for each time. For AS+F units, the reduce canopy height was obtained by tying 50 cm plastic stripes to the frame, while 75 and 30 bundles of stripes were used to reproduced the 50% and 20% shoot density, respectively.

At each experimental time, once experimental units were established, five *Paracentrotus lividus* adult individuals (larger than 50 mm in test diameter) were positioned in every experimental unit: sea urchins were collected from the near rocky reef, 20 m distant from the seagrass edge. After a week from positioning, sea urchins abundance was recorded in each unit.

A four-way ANOVA of *Paracentrotus lividus* abundance was performed: '*Posidonia*' type (NS, AS+F and NS+F), shoot 'density' (100%, 50% and 20%) and canopy 'height' (Natural and Reduced) were treated as fixed and orthogonal, while the factor 'time' as random (2 levels) and nested in *Posidonia* type. Cochran's test was performed to check for homogeneity of variances, while SNK test was used to make *a posteriori* comparisons of means (Underwood 1997).

To provide information on how pruning the seagrass shoots altered the effect of reducing bottom current regime at the experimental units we used pre-weighed plaster balls that were exposed to the water flow for 24 h at calm conditions. In order to avoid the effect of abrasion, care was taken to place the balls so that they did not contact plants. Weight loss during field exposure gave estimates of the water movement because it controlled dissolution rates (Gambi et al. 1989). For each of the six combinations 'density × length' two replicate plaster balls were positioned and to calibrate weight loss 6 balls were kept in still sea water for the same exposure time. After recovery, balls were rinsed lightly in freshwater to remove salts, dried at 60°C for 24 h and weighed. The dissolution (weight loss) of each ball was converted to an estimate of flux (cm/s) according to the following function obtained by Bailey-Brock (1979),

Flux = 3.65(Me/Mc-1)

where Me is the weight loss of each plaster ball during field exposure and Mc is the weight loss of calibration balls.

Furthermore, to provide estimates of the effect of AS+F units on irradiance compared to NS units, light measurements were taken under the two types of seagrass canopy using a quantum photometer. Two replicate readings were done for each of the six combinations 'density × length'. Then, data collected for flux and irradiance were analyse by three-way ANOVA excluding the factor 'time' to the design previously described. Eventual differences between NS and AS+F units would have highlighted the intruding influence of plastic material rather than only food depletion.

RESULTS

Overall, the abundance of *Paracentrotus lividus* in the *Posidonia oceanica* experimental units was quite consistent to natural abundance in this seagrass meadows. In particular, from 1 up to 7 individuals of *P. lividus* occurred in most of the units while the lack of them was recorded only very rarely (Fig. 2). Analysis of data has shown a significant '*Posidonia* × Density' interaction (Po × De) effect on the abundance of *P. lividus* (Table 1) and the SNK test on this interaction has highlighted that there were no differences in mean abundance of *P. lividus* among *P. oceanica* types at 20% and 50% shoot density, while at natural shoot density (100%) an higher abundance was detected at natural *P. oceanica* (NS) units, rather than at AS units and NS+F units (Table 1). Furthermore, at AS and NS+F units there were not differences among the three levels of shoot density, while in NS units *P. lividus* abundance increased with the increase in shoot density, at both canopy heights, as highlighted by significant ranking among levels (Table 1). On the contrary, no significant effect of the canopy height was detected by the analysis, at none of the canopy type. Finally, differences between times on the *P. lividus* abundance were no significant, as revealed by ANOVA results.
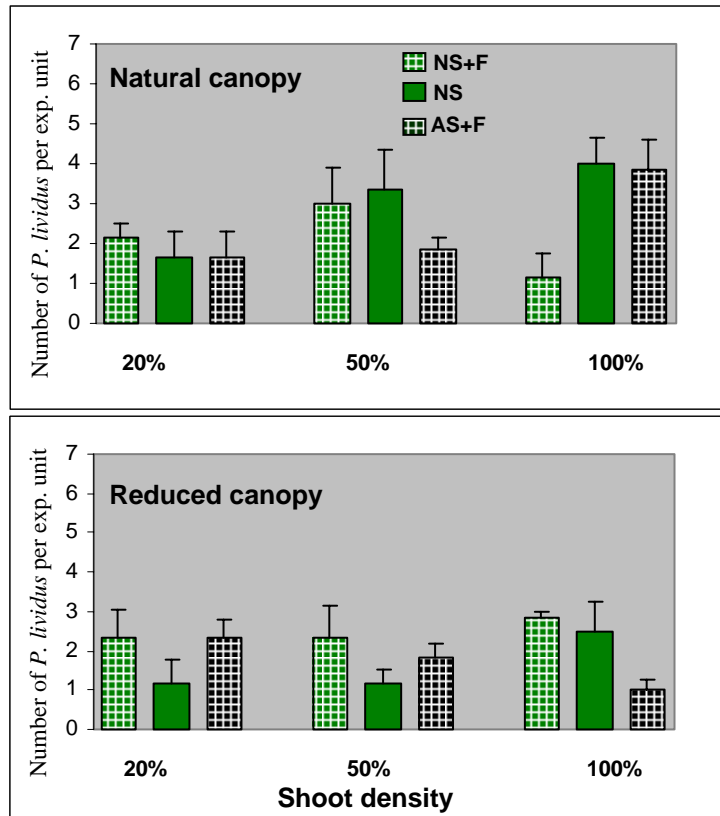
Fig. 2. Mean number (+SE) of *Paracentrotus lividus* abundance at different canopy height (N=natural, R=reduced).

Table 1. Results four-way ANOVA comparing the number of *Paracentrotus lividus* among *Posidonia* types (NS, AS+F, NS+F), times, canopy height (Natural and Reduced) and shoot density (100%, 50%, 20%).

Source of variation	df	MS	F	p
Posidonia = P	2	0.5926	0.12	0.8905
Time = T(P)	3	4.9167	2.59	0.0594
Height = H	1	8.8981	5.62	0.0984
Density = D	2	4.0093	6.01	**0.0369**
P×H	2	7.2593	4.58	0.1224
P×D	4	4.2731	6.41	**0.0234**
H×T (P)	3	1.5833	0.83	0.4795
D×T (P)	6	0.6667	0.35	0.9070
H×D	2	3.1759	0.91	0.2287
P×H×D	4	6.7454	4.05	0.0630
D×H×T(P)	6	1.6667	0.88	0.5155
Residual	72	1.8981		
Cochran test	C = 0.1024 ns			

SNK TEST of interaction P×D		
SE = 0.235		
20%	N = N + G = A + G	
50%	N = N + G = A + G	
100%	N > N + G = A + G	
N	20% < 50% < 100%	
N+G	20% = 50% = 100%	
A+G	20% = 50% = 100%	

Differences in irradiance were highly dependent on *Posidonia oceanica* structure of the canopy and type (Fig. 3a). In fact, a significant 'type × height' interaction indicated that greater light intensity occurred at reduced rather than natural height, both at NS and AS units, and that at reduced canopy height a higher light intensity was recorded at AS units rather than NS units (Table 2, SNK test). Furthermore, independently on *P. oceanica* canopy height and types, SNK test detected a significant lower irradiance at 100% compared to 50% and 20% shoot density (Table 2, SNK test).
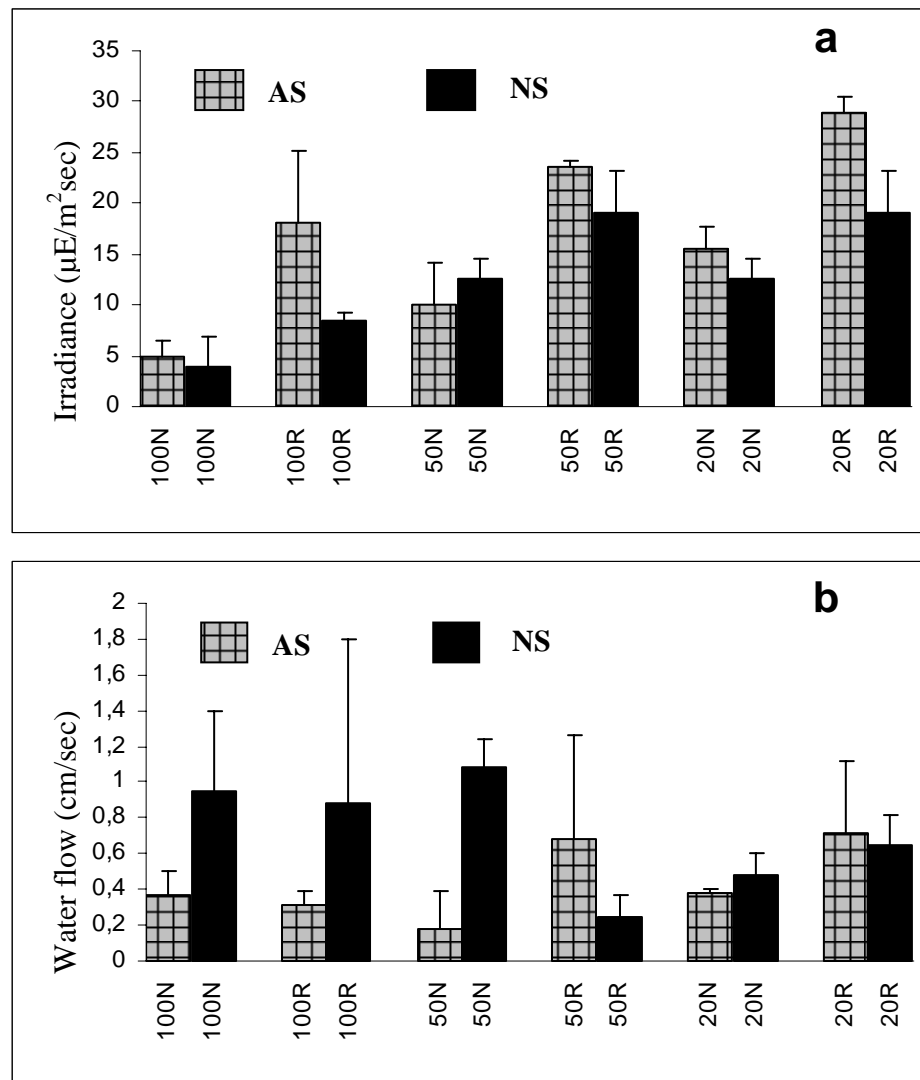

Fig. 3. Mean (+SE) irradiance (a) and water flow (b) at different *Posidonia* types, shoot density and canopy height.

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Furthermore, although water flow was quite variable among units, analysis did not detect any significant differences in current velocity suggesting that there were not different influences on current velocity between AS and NS units (Fig. 3b and Table 2). Surprisingly, significant variations in water flow among levels of *Posidonia oceanica* shoot density and canopy height were not evidenced by the analysis. However, a higher water movement at NS units was evident rather than at AS units, although greater plaster depletion at 100% and 50% shoot density could also be due to abrasion by direct leaf movement during the exposure (personal observation).

Table 2. Results three-way ANOVA comparing water flow and irradiance among *Posidonia* types (NS, AS+F, NS+F), canopy height (Natural and Reduced) and shoot density (100%, 50%, 20%).

Source of variation	df	Water flow			Irradiance		
		MS	F	p	MS	F	P
Posidonia = P	1	0.4496	3.19	0.0993	0.0308	30.56	0.0001
Density = D	2	0.0150	0.11	0.8998	0.0120	11.91	0.0014
Height = H	1	0.0004	0.00	0.9577	0.0486	48.20	0.0000
P×D	2	0.1570	1.11	0.3597	0.0107	10.62	0.0022
P×H	1	0.3799	2.70	0.1265	0.0113	11.17	0.0059
D×H	2	0.0926	0.66	0.5359	0.0002	0.16	0.8530
P×D×H	2	0.2574	1.83	0.2029	0.0002	0.15	0.8599
Residual	12	0.1409			0.0010		
Cochran test		C = 0.4953 ns			C = 0.4132 ns		

DISCUSSION

Results have highlighted that *Paracentrotus lividus* distribution is affected by *Posidonia oceanica* shoot density in different ways, as highlighted by the significant '*Posidonia* × Density' interaction. In fact, no significant differences in *P. lividus* abundance among NS, AS+F and NS+F *Posidonia* type treatments were found at low shoot densities (20% and 50%), while at natural density (100%) a higher abundance was found in NS rather than in AS+F and NS+F units. This suggests that at lower densities the artefact effect of frame did not significantly contribute to the observed variability which is entirely due to the canopy material. Further, the similar *P. lividus* abundance between AS+F and NS+F types at all densities suggests that *P. oceanica* mainly plays a

refuge role rather than food resource. However, at 100% shoot density the analysis has revealed the artefact effect of the frame; particularly, at natural shoot density, the frame had a negative effect on *P. lividus* abundance so that estimates of artificial habitat effect could lead to wrong conclusions for the impossibility to distinguish between frame and plastic effect. Even if results have highlighted an artefact effect of the frame, this not excludes that *P. lividus* can utilize the densest *P. oceanica* as refuge, just as it has emerged for the other shoot densities. However, we ignore the mechanism that could have led to these results since we cannot think of an influence of the frame that could be relevant at the natural seagrass density and not at the lower densities. Nevertheless, differences in *P. lividus* abundance between NS and AS+F could not be ascertained to a different hydrodynamics. Indeed, our results have highlighted that there were no significant differences in current velocity between canopy types. Further, no significant variations in water flow among levels of *P. oceanica* shoot density and canopy height were evidenced.

However, the increased abundance of *Paracentrotus lividus* at NS canopy type with the increase shoot density suggests that different combinations of environmental conditions occur at the three density levels. The changes in reducing bottom current regime and in light regime, are mechanisms by which *Posidonia oceanica* has been shown to affect the understory assemblages (Ceccherelli and Cinelli 1999). As suggested by some authors, shade may be an important factor affecting *P. lividus* distribution (Barnes and Crook 2001; Verling et al. 2002) and dense shoots of seagrasses could provide such condition (Ceccherelli and Cinelli 1999; Verweij et al. 2006). Consistently, in this study reduced irradiance have been found at the lowered densities of *P. oceanica* and this could explain the dependence of the abundance of *P. lividus* on the density of the seagrass.

For this experiment, we assume that *Paracentrotus lividus* individuals that are found in the units are the result of those moving from the rocky areas around the seagrass edge and those from the meadow, although we believe that those coming from the rocks could contribute more because of their overall higher abundance at this habitat. Probably, the suggestion given by our results that

P. lividus can utilize *Posidonia oceanica* primarily as refuge together with the fact that *P. lividus* grazing does occur on *P. oceanica*, can be explained by the hypothesis that this sea urchin is attracted by the seagrass where it can find a refuge from predators and water motion and, once it finds the shelter, it grazes on it. Some other authors had suggested that *P. lividus* behaves fundamentally as an important feeder able widely to adapt to availability of food resources (Zupo and Fresi 1984). This would also be supported by studies on *P. lividus* food preference between palatable algae and *P. oceanica*. In fact, the algal species that are depleted by *P. lividus* grazing (Benedetti-Cecchi et al. 1998; Bulleri et al. 1999; Hereu 2006) and the importance of *P. lividus* feeding on *P. oceanica* leaves (Alcoverro et al. 1997; Prado et al. 2007a, 2007b) have both already been evidenced by field experiments. Further, a very recent work by Vergés et al. (2007) has highlighted the chemical deterrence of *P. oceanica* on *P. lividus* and several other predators. However, whether *P. lividus* has a feeding preference on macroalgae rather than on the seagrass still needs further insights.

Furthermore, to demonstrate that *Paracentrotus lividus* primarily use *Posidonia oceanica* to find the shelter and that it feeds on it only for opportunity, the use of artificial seagrass structures of different dimensions is probably needed. In fact, in this experiment, the small patch structures of artificial seagrass used could have served as suitable shelters as the grazing could have been performed in the nearby algae on rocky reefs and in natural *P. oceanica*. In this way, artificial structures could have functioned to *P. lividus* just as rocky crevices. Conversely, patches of artificial seagrass larger than *P. lividus* calculated home range (several square metres, Hereu 2005) are expected to host most of the sea urchins at the margin so that grazing could happen at the next habitat. However, to estimate the influence of seagrass patch arrangement on the sea urchin distribution, *P. lividus* home range should be estimated at artificial seagrass units of different patch size fixed at rocky substratum with different levels of crevices availability.

In fact, as highlighted by many studies, structural complexity may be an important determinant for fish and invertebrate communities. In particular, as suggested by Ruitton et al.

(2000), the size of shelter must be proportional to the invertebrate size, to be effective and, further, that the abundance increases with increasing structural complexity (Bonaviri et al. 2005). In Mediterranean rocky habitat this has also been shown for juveniles of _Paracentrotus lividus_ (Hereu et al. 2005). Indeed, in presence of high predator pressure the availability of shelters determines a cryptic behaviour on _P. lividus_ (Guidetti et al. 2003) and a successful reduction in predation rate (Carpenter 1984; McClanahm and Muthiga 1989; Sala et al. 1998). In this Basin, it has been suggested that _P. lividus_ could have a nictemeral behaviour, mainly due to the search of food: at night it grazes on the rocks and during the day it shelters to avoid predators (Dance 1987; Hereu 2004). However, other studies conducted at Lock Hyne (Ireland), have shown grazing activity by _P. lividus_ during the day, in order to avoid nocturnal predators (Ebling et al. 1966; Barnes and Crook 2001).

At the study site, although the rocky platforms have not likely provided good shelters, since irregularities of the rocks were small and rare, they were covered by algal turfs that have been shown to be highly palatable for _Paracentrotus lividus_ (Benedetti-Cecchi et al. 1998; Bulleri et al. 1999; Hereu 2006). The conformation of this rocky habitat supports the hypothesis that _P. lividus_ could firstly consider _Posidonia oceanica_ meadow as a refuge and that the abundance of this sea urchin species in this seagrass could mostly be due to the migration from the rocky habitat.

Further relevance should be given to protection from human activity, as another factor that potentially affects _Paracentrotus lividus_ behaviour and, consequently, distribution. In fact, in MPAs, where there is a high density of fish predators, such as _Diplodus sargus_ (L.)_, Diplodus vulgaris_ (Geoffr.)_, Sparus aurata_ (L.)_, Coris julis_ (L.) and _Thalassoma pavo_ (L.), sea urchins movement is lower than at unprotected areas where low predator pressure occurs (Hereu 2005). At this regard, it is worth highlighting that this experiment was carried out in an unprotected area and that at this site the search for _P. lividus_ of a shelter should have not been greatly influenced by the predator pressure. However, at the moment we don't know whether the results obtained in this study have been affected by the low density of predators and only a high spatial replication at the

scale of locations at both high and low predation pressure would be needed to properly define the influence of predation on the role of *Posidonia oceanica* habitat on *P. lividus* distribution. However, if the importance of the refuge role of *P. oceanica* has been evidenced outside a MPA (this study), this should only be augmented inside and absolutely more probable at reserve areas.

Furthermore, whether the effect of *Posidonia oceanica* habitat on *Paracentrotus lividus* distribution could vary among different periods of the year, still remains unknown. Seasonality would deserve further attention since many features regulating sea urchin distribution undergo variations through time. Besides human harvest, which is focused in the winter season when the reproductive effort of the sea urchin is maximized, phenology of algal assemblages on rocky reefs as well as the behaviour of predators are likely linked to the season (Sala and Zabala 1996; Barnes and Crook 2001; Tomas et al. 2005). Thus, the behaviour of *P. lividus* could also greatly change through time. In this contest, it would be crucial to define the role of *P. oceanica* habitat for *P. lividus* individuals testing specific hypotheses on temporal variability at the scale of the season. Although many questions still remain unanswered, these findings add important knowledge to the understanding of *P. oceanica* – *P. lividus* interaction and arose some more specific hypotheses that need to be tested to identify the overall role of this seagrass on this sea urchin.

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