Research Article

The exploitation of limpets in a Mediterranean Marine Protected Area: assessing the effectiveness of protection in the intertidal zone

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Abstract

Limpets are intertidal keystone grazers and their overexploitation could have significant consequences for intertidal communities. Limpets are harvested around the Sinis Peninsula (Sardinia, Italy) but harvesting is prohibited within the "Penisola del Sinis - Isola di Mal di Ventre" Marine Protected Area (MPA). This work assesses the effects of human harvesting on the population dynamics of three common Mediterranean species of *Patella*, namely, *P. rustica*, *P. caerulea* and *P. ulyssiponensis*, testing the effectiveness of the MPA and the role of site accessibility in limiting the intensity of such harvesting pressure. In the period between June 2015 and August 2016, limpet abundance and size were recorded on a monthly basis by means of photographic frames within linear transects at ten sites spread out along the coastline of the Sinis Peninsula to assess growth and temporal patterns. Limpets older than two years are extremely rare in the study area. Limpets are more abundant within the MPA in comparison with non-protected areas and within less accessible sites in comparison with areas where the intertidal zone is easily accessible from land. Despite this, overall human-induced mortality in the area is high, indeed the pool of limpets observed with a mean density of 104.3 ± 9.7 limpets/m² during the first survey was reduced by 99.2% in less than one year. This work demonstrates that human harvesting strongly affects the population dynamics of *Patella* species in the area and that within the MPA this stressor is not efficiently reduced.

Keywords: *Patella*; Mediterranean, human harvesting, intertidal, accessibility, marine protected area.

Introduction

Worldwide, with most of the human population concentrating near the sea, coastal areas are strongly exposed to anthropogenic disturbance such as urbanization, pollution, non-native species introduction, habitat degradation and the exploitation of marine organisms (Worm *et al.*, 2006; Airoldi & Beck, 2007; Halpern *et al.*, 2015). For this reason, coastal environments are considered the most degraded habitats on the planet (Millennium Ecosystem Assessment, 2005). The biodiversity of the intertidal zone, especially in densely human-populated areas, can be affected strongly by harvesting and other impacts connected with human frequentation on the coast (Crowe *et al.*, 2000; Thompson *et al.*, 2002). Intertidal invertebrates are collected by humans mainly as a source of food, although this activity is also carried out by different types of coastal users, e.g. fishing bait collectors. In Mediterranean countries, shore invertebrates are collected frequently not only by professional but also by recreational harvesters (Diogo *et al.*, 2016).

Intense harvesting pressures could affect negatively both target and non-target species (Lasiak, 1998) and provoke shifts in intertidal community structure when keystone species are the object of collection (Ebenman & Jonsson, 2005). Furthermore, as humans tend to exploit mainly large individuals, harvesting can reduce the mean size in exploited populations and thereby negatively affect their reproductive potential, especially if the proportion of males and females is a function of size (Fenberg & Roy, 2008; Espinosa *et al.*, 2009). The assessment of the collection rate of intertidal organisms deserves attention, as some species collected frequently are extremely important for the maintenance of shore biodiversity. Limpets are common intertidal gastropods (Branch, 1981) that in some regions are intensively collected, mainly for human consumption. These organisms are considered keystone species because their grazing activities can limit algal cover on rocks and thus influence the characteristics and the dynamics of the resident intertidal community (Boaventura *et al.*, 2002; Jenkins *et al.*, 2005; Martins *et al.*, 2008; Borges *et al.*, 2015).

Several case studies have demonstrated declines in limpet populations as a consequence of human harvesting (Ferraz *et al.*, 2001; Weber & Hawkins, 2002; Navarro *et al.*, 2005; Sagarin *et al.*, 2007; Fenberg & Roy, 2012), especially in populated areas. Harvesting intensity is often limited by the physical features of an area, which can reduce the number of collectors visiting a site (Garcia & Smith, 2013). Abundance and size structure

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of both sessile and sedentary exploited species is indeed often related to the accessibility by people of harvesting sites (Oliva & Castilla, 1986; Keough & Quinn, 2000; Moreno, 2001; Thompson *et al.*, 2002; Paracuellos *et al.*, 2003; Ceccherelli *et al.*, 2011).

To counteract overexploitation of marine biological resources, an increasing number of Marine Protected Areas (MPAs) have been established around the globe during the past decades. MPAs can ensure the protection of the entire marine community and are thus considered useful tools for an ecosystem-based approach to conservation. The mitigation of human pressures within well-managed and enforced MPAs can enhance the rebuilding of exploited stocks, equilibrate perturbed interspecies interactions and increase species and habitat conservation, thereby promoting biodiversity (Fenberg *et al.*, 2012). Many MPAs, however, fail to meet their conservation goals and are considered "paper parks" that provide limited protection to their marine fauna (Guidetti *et al.*, 2008; De Santo, 2013; Rife *et al.*, 2013; Katsanevakis *et al.*, 2015; Plumeridge & Roberts, 2017). Assessing how existing management measures provide benefits for biological resources is therefore crucial. Despite the fact that intertidal shellfish collection is a common activity in many Mediterranean regions, a minority of studies have focused on the effectiveness of MPAs in promoting the conservation of intertidal biological resources, with the main emphasis on large endangered species (Espinosa & Rivera-Ingraham, 2016 and literature therein). Four species of *Patella* are present around the Sinis Peninsula (Italy, W Mediterranean): *P. ferruginea*, *P. caerulea*, *P. rustica* and *P. ulyssiponensis* (Coppa *et al.*, 2016). The geographical distribution of *P. ferruginea* is restricted to a few spots in the Western Mediterranean basin (Espinosa *et al.*, 2014). On the contrary, *P. caerulea*, *P. rustica* and *P. ulyssiponensis* are very common in the rocky shores of the whole Mediterranean Sea (Palomares & Pauly, 2017). These species have also differential vertical distributions along the shore: *P. rustica* is present in the upper intertidal and in the low splash zone, *P. ferruginea* and *P. ulyssiponensis* in the low intertidal. Finally, *P. caerulea* has the widest vertical distribution, ranging from the low intertidal to the upper subtidal (up to 10 m depth) (Belkhodja & Romdhane, 2012). *P. ferruginea* is protected under the Bern Convention (listed as 'strictly protected' in the Annex II), the Habitats Directive (listed as 'species in need of strict protection' in the Annex IV) and under the Barcelona Convention (listed as 'endangered or threatened species' in the Annex II). This species is considered particularly vulnerable to human pressures as it reaches a larger size (>100 mm of shell length), has a slower growth rate and an older age at maturity compared to the other species (Espinosa *et al.*, 2006; 2014).

The exploitation of limpets around the Sinis Peninsula is a common activity and is formally limited by the local MPA (The "Penisola del Sinis - Isola di Mal di Ventre" MPA) that was established in 1997 and is the second largest MPA in Italy (covering about 25,000 ha). Previous biological and socio-legal studies conducted in the area highlighted that the Sinis MPA has a low effectiveness for the conservation of marine species. Different factors contribute to hinder its effectiveness, such as the diffuse lack of compliance with management rules (Pieraccini *et al.*, 2017), the lack of adequate enforcement (Guidetti *et al.*, 2008) and the high professional and recreational fishing pressure (Casola *et al.*, 2014). Low protection effects were indeed observed for coastal fish assemblages (Marra *et al.*, 2016) and for commercial benthic species (Pieraccini *et al.*, 2017).

The collection of intertidal invertebrates is forbidden within the MPA. Despite this, illegal harvesting was often observed in protected sites. Two studies on the effectiveness of the local MPA in controlling limpet harvesting have been conducted for *P. ferruginea* in a remote area of the Sinis Peninsula (Coppa *et al.*, 2012; 2016). These works showed that the species is mainly present on hardly accessible shores and within the no-take/no-entry zone of the MPA, highlighting an ongoing temporal contraction of the local population. As *P. ferruginea* is a giant limpet species, it could be a preferential target of collection. To better understand whether human harvesting is a primary and diffuse source of disturbance for intertidal populations in the area, the quantification of mortality rates of small limpet species is crucial.

This study analysed temporal patterns of abundance and size structure of the small species *P. caerulea*, *P. rustica* and *P. ulyssiponensis* in relation to legal protection and site accessibility. The study was conducted along the coastline of the Sinis Peninsula, where no data were yet available regarding the intensity of harvesting and the effectiveness of the MPA to counteract it. The primary aim of the study was to identify and follow through time the same pool of limpet and to test if mortality rates were lower within the MPA, next to testing if within the MPA limpets were present at higher abundances and larger sizes in comparison with unprotected sites.

Material and Methods

Study area

The Sinis Peninsula is located in central-western Sardinia (Italy) (39.942328° N; 8.430219° E) (Fig. 1). Here, the coastline is about 35 km long and is characterised by an alternation of sandy beaches and rocky shores. The rocky shore consists mainly of a sandstone substratum, with the exception of Cape San Marco in the south of the Peninsula, that is basaltic, and of Su Tingiosu, in the north, which is mainly calcareous. There are four cliff areas: Cape Mannu, Su Tingiosu, Torre Seu and Cape San Marco. In these areas, access to the intertidal zone is limited by the absence of direct paths to the shore and access is only possible from the sea. In contrast, in Su Pallosu, S'Arena Scoada, Is Arutas, San Giovanni, La

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Caletta and Tharros, the rocky intertidal zone is delimited by sandy beaches and is therefore easily accessible from both the land and the sea.

Data collection

Ten sites were selected along the Sinis Peninsula (Fig. 1) and within each site three permanent 2 m long transects were established. Within transects, all limpets were counted taking into account their maximal vertical distribution thus obtaining a sampling area of 2 m² for each replicate (that included the whole intertidal zone – mean tidal excursion

18.5 ± 0.86 cm – and the low littoral portion of the shore – mean width 32.33 ± 3.96 cm). The sampling sites included both easily accessible sites (i.e. directly reachable by walking) and hardly accessible ones (i.e. cliff areas that cannot be reached directly from the land). Six of the ten sites are located within the MPA. Site selection also aimed at including areas both exposed and non-exposed to the main wind, and thus to wave action (Mistral, NW wind) (Table 1).

Data on shell length measurements and limpet abundance were collected monthly during low tide by means of photographic frames over a period of one year, from June 2015 until August 2016. Due to bad weather
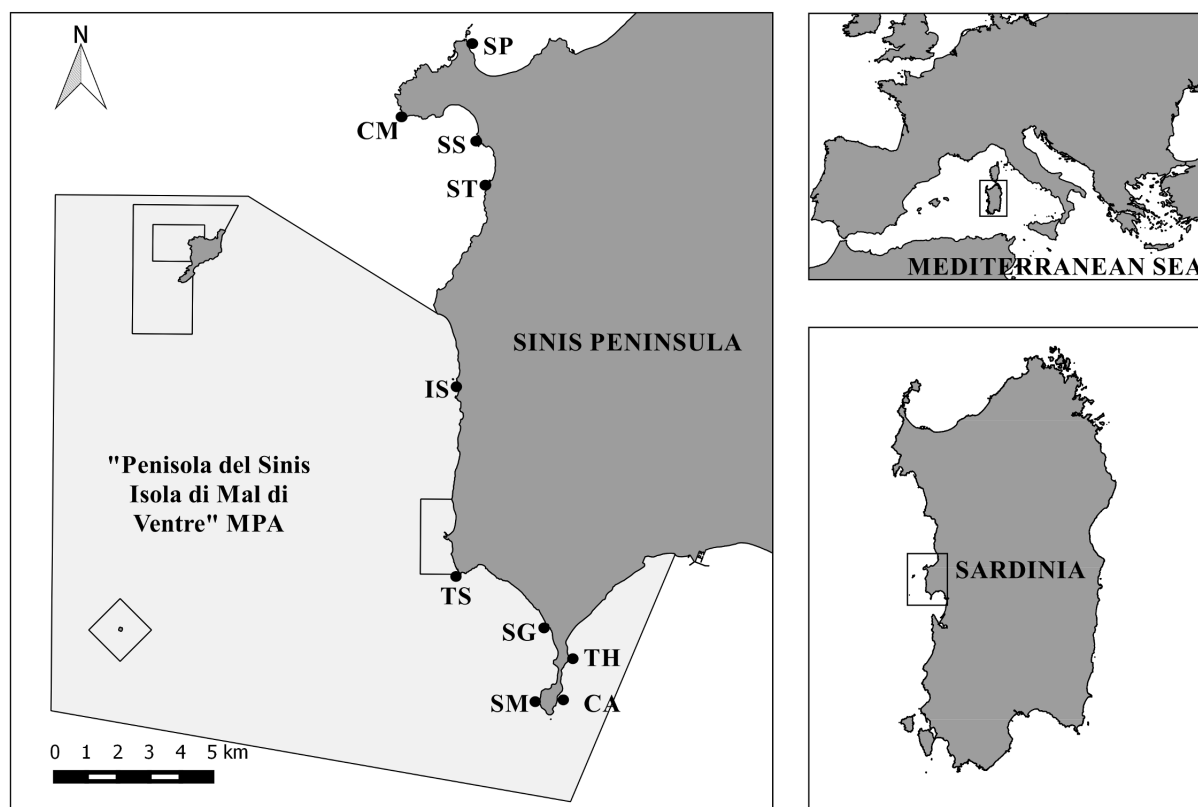

Fig.1: Study area. Sampling sites and "Penisola del Sinis - Isola di Mal di Ventre MPA" zonation. SP = Su Pallosu; CM = Cape Mannu; SS = S' Arena Scoada; ST = Su Tingiosu; IS = Is Arutas; TS = Torre Seu; SG = San Giovanni; SM = Cape San Marco; CA = La Caletta; TH = Tharros.

Table 1. Characteristics of the sampling sites.

Site name	Abbreviation	Protection level	Physical accessibility	NW Exposure	Mean abundance (N limpets/m2 ± st. err.)
Torre Seu	TS	MPA	low	Exposed	132.1 ± 10.7
Cape San Marco	SM	MPA	low	Exposed	74.2 ± 6.5
Cape Mannu	CM	OUT	low	Exposed	89.0 ± 5.0
Su Tingiosu	ST	OUT	low	Exposed	108.5 ± 10.0
San Giovanni	SG	MPA	high	Exposed	156.9 ± 14.3
Is Arutas	IS	MPA	high	Exposed	100.5 ± 15.0
Tharros	TH	MPA	high	Not exposed	59.8 ± 4.5
La Caletta	CA	MPA	high	Not exposed	48.2 ± 4.2
S'Arena Scoada	SS	OUT	high	Exposed	66.2 ± 3.2
Su Pallosu	SP	OUT	high	Not exposed	55.7 ± 4 .8

conditions, data were not collected in November 2015, February and April 2016. The decision to collect data on a monthly basis was due to the need of following the same pool of limpets through time, by removing from the dataset those new recruits that settled after the first survey. Such pool was identified on the basis of growth rates (see "Data Analysis" section for details). A monthly-based collection of length/frequency data is indeed recommended for the assessment of growth rates of species with a life span of few years and with multiple spawning events per year (Sparre & Venema, 1998). Furthermore, a monthly-based data collection, rather than a wider one, permitted to better estimate whether the observed dynamics could be related to harvesting rates connected with human frequentation of the coast.

Pictures were analysed using *ZEN 2012 (blue edition)* software to obtain data on limpet density and size (total shell length). The length measurements obtained from each photographic frame were calibrated by means of a reference length that was placed on the rock before taking photos. Length measurements were grouped into 5mm size classes and the frequency of each class was calculated every month. The abundance of the natural predators of limpets was also recorded during every survey by means of 5-metre radius searches around each transect. The species considered where the crabs *Pachygrapsus marmoratus* and *Eriphia verrucosa*, and the whelk *Stramonita haemastoma,* which are the main predators of limpets acting in the intertidal shores of the study area (Coppa *et al.*, 2012). Previous observations did not revealed any encounters between limpets and other kind of predators.

Data analyses

In order to follow the same pool of limpets across time, new recruits were removed from the dataset during each survey. The growth rates of the different species were estimated from length/frequency data and averaged to determine the growth of a generic "*Patella* sp." species. Once the growth curve was obtained, the length-at-age of "*Patella* sp." was calculated and used to identify small individuals that settled after the first survey (June 2015). These individuals were not considered in subsequent statistical analyses. Length frequency data were analysed using the FISAT II (FAO-ICLARM stock assessment tools II) software package. The growth of limpets was estimated using the von Bertalanffy growth function (VBGF): $Lt = L\infty [1-e^{-Kt}]$. The parameters of the growth curves were assessed from length/frequency data belonging to monospecific transects, using different routines: ELEFAN I (K-scanning and response surface analysis) and Modal Progression Analysis (Gayanilo *et al.*, 2005) that is based on the separation of age cohorts from length/frequency data according to the Bhattacharya method (Sparre & Venema, 1998). As the K-scanning analysis required an initial guess for the $L\infty$ for every species, this parameter was set at the value obtained for

the biggest limpet observed in the study area. To validate the models, a sample of at least 45 limpets from different replicates was selected for each species. Each specimen was identified in the photographs and followed every month in order to assess its increment in shell length. Data on growth increments from this sample were then plotted with the different VBGFs, in order to assess which of the growth functions obtained best fit the data.

Repeated measures permutational analysis of variance based on binomial distance dissimilarity was run on square root transformed data with the PRIMER 6 and PERMANOVA+ statistical software (Anderson *et al.*, 2008) to test if "Time" (12 levels), "Accessibility" (two levels: high, low), "Protection" (two levels: within MPA, Out) and "Site" (nested in "Protection" and in "Accessibility") significantly affected limpet abundance. Each factor was treated as fixed in the statistical design. Every month, the size structure of the original pool of limpets was compared among levels of legal protection and site accessibility with the Kolmogorov-Smirnoff test using the Bonferroni Correction for multiple comparisons.

Results

The shell length of the limpet individuals observed during this study ranged from 1 to 53 mm. Limpet density differed among sites: considering the whole study period, the highest mean values were obtained from SG (156.9 ± 14.3 limpets/m²) and TS (132.1 ± 10.7 limpets/m²), with the former site being characterized by high variability among replicates. On the contrary, the lowest mean densities were observed in CA (48.2 ± 4.2 limpets/m²). Not exposed sites showed lower density values in comparison with sites exposed to the main wind (Table 1).

Temporal oscillations in the mean number of limpets were obtained for all sites: a decreasing trend is usually observed between June and October 2015, while in winter abundance trends tended to increase (Fig. 2). Increase in total abundance was associated with an increase in the number of small individuals (≤10 mm), which can be explained by the arrival of new recruits on the shore. Within the MPA, the strongest intermonth reduction in the mean number of limpets was observed between July and August 2016 at low accessible sites (-41.5%) and between June and July 2015 at highly accessible sites (-26.6%). In unprotected sites, the strongest intermonth reduction was observed between July and August 2016 at low accessible sites (-30.7%) and between September and October 2015 at highly accessible sites (-32.9%) (Fig. 2).

The size structure detected initially (June 2015) was dominated by individuals with shell length between 11 and 15 mm (Fig. 3). Limpets larger than 35 mm were extremely rare: the maximum number was detected at CM (6 individuals), 5 were observed at SM and TS, 4 at SP, 3 at SS and 2 each at ST, TH, and CA. No limpets >35 mm in shell length were detected at SG and IS.
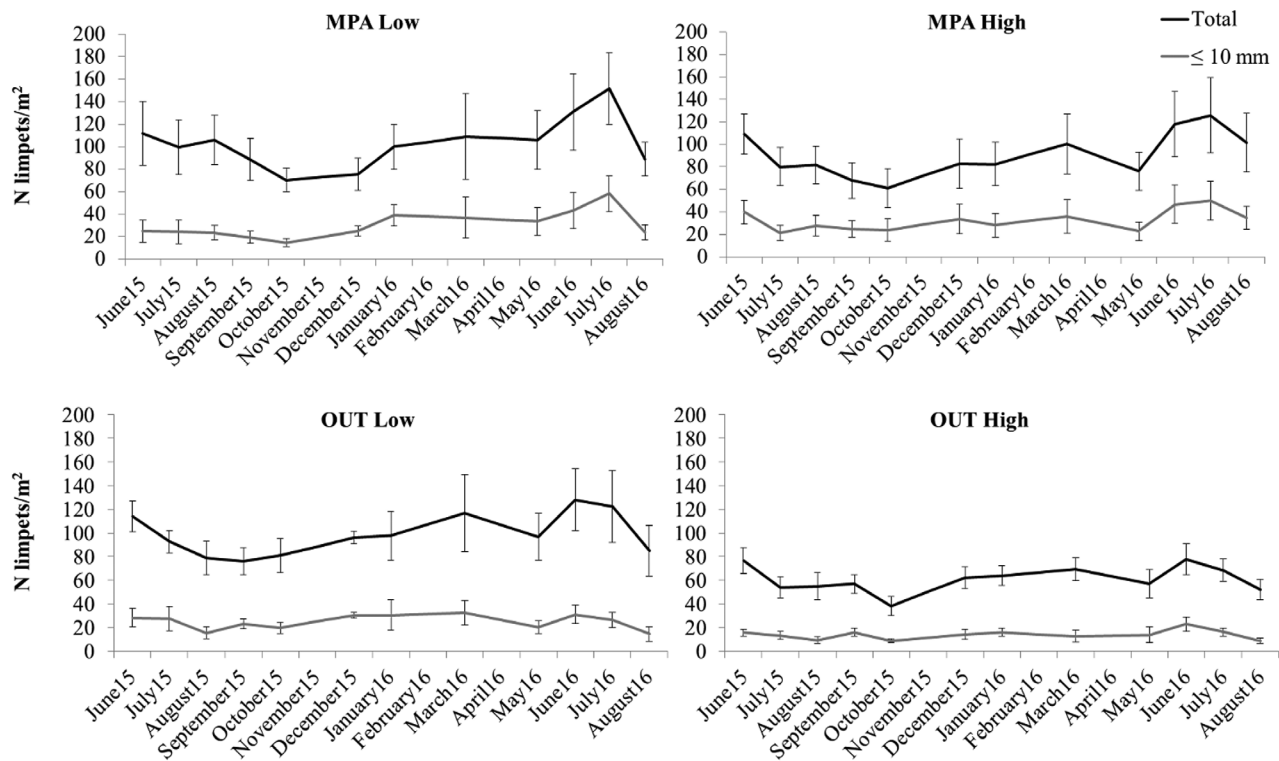
Fig. 2: Seasonal variations of mean abundance (N limpets/m² ± st. err.) for the whole population and individuals ≤10 mm among levels of protection and accessibility.
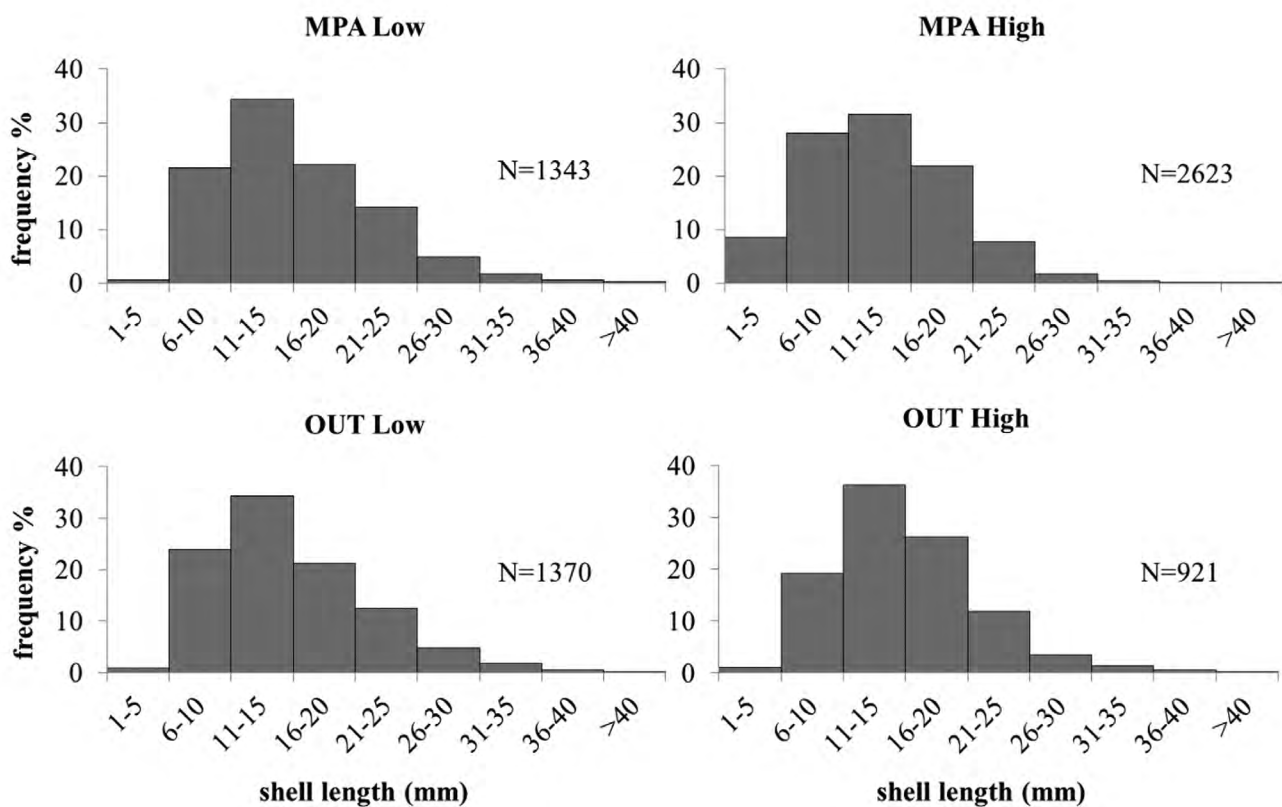

Fig. 3: Size frequency distribution (mean % ± st. err.) detected during the first survey (June 2015) among levels of protection and accessibility.

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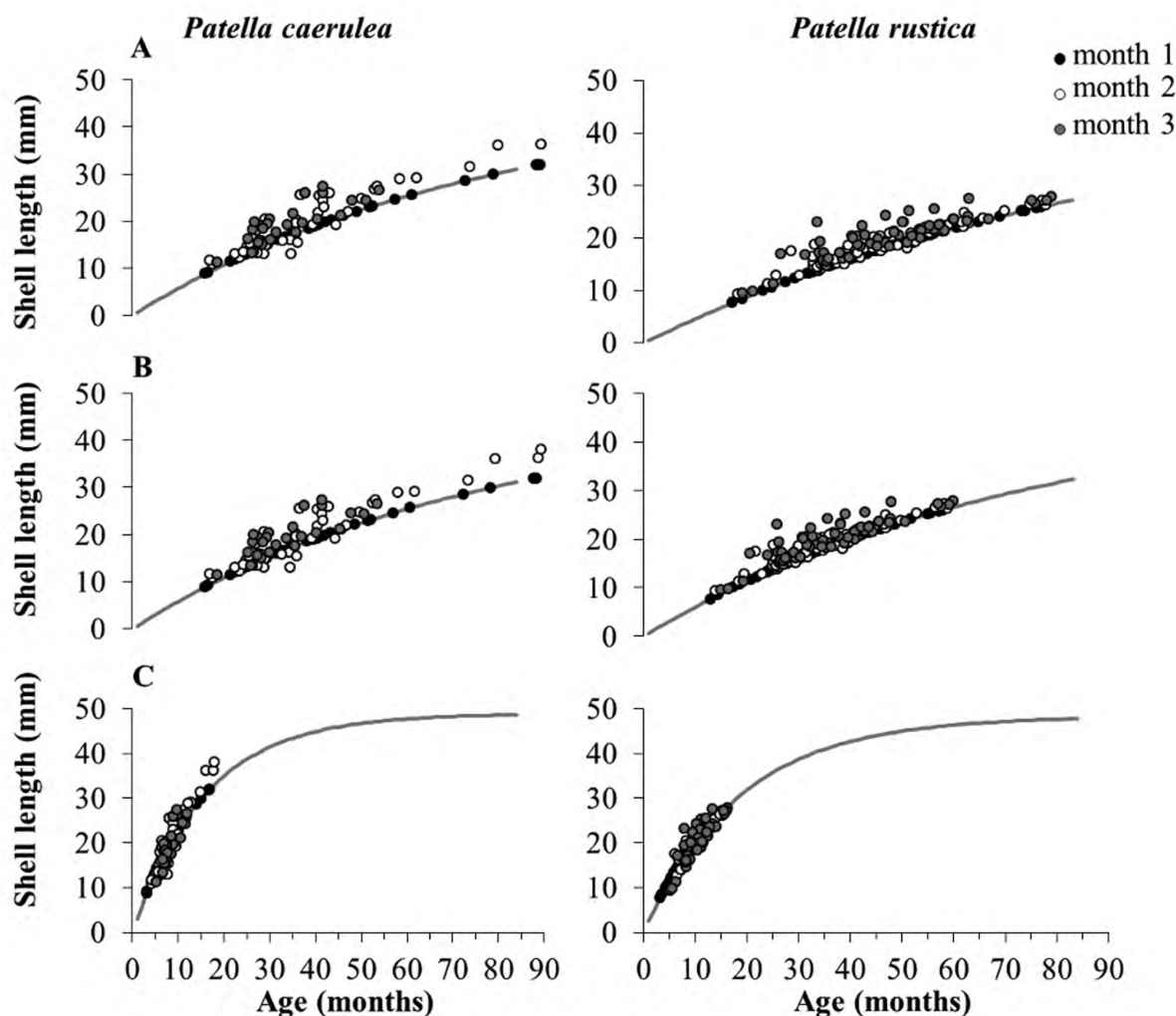*Medit. Mar. Sci., 18/3, 2017, 406-423*

Fig. 4: Validation of the growth models obtained with different FISAT II routines obtained by following through time a sample of specimens of *Patella caerulea* and *P. rustica*. a) K-scanning method; b) Response surface analysis; c) Modal Progression Analysis.

Data from one survey (December 2015) were used to estimate the relative abundance of the different species. With a total N of 4,797 individuals, the most common species was *P. caerulea* (60.3%) followed by *P. rustica* (32.8%). *Patella ulyssiponensis* was observed in low numbers (2.3%) and was not, therefore, considered for growth curve analysis. In 4.6% of the cases, it was not possible to assign species names accurately. A total of 45 individuals of *P. caerulea* and 64 of *P. rustica* were identified and followed in the photo frames (in most cases, the selected limpets were not detected again after three months). For each limpet, the length recorded during every survey was compared with the theoretical length that an individual of the same starting length should have reached after the same period of time according to the different growth models. For both *P. caerulea* and *P. rustica,* the growth models obtained with the K-scanning and Response Surface Analysis underestimated the growth of the limpets, while the Modal Progression Analysis produced the model that best fitted the data (Fig. 4). The parameters detected were $L\infty = 48.9$ mm; $K = 0.7$

($P. caerulea$) and $L\infty = 48.3$ mm; $K = 0.6$ (*P. rustica*).

Lengths at age of the two species were averaged to obtain the growth curve for a generic "*Patella* sp." species. Individuals that during each survey were smaller than the minimal shell length that the original pool (detected in June 2015) was expected to have reached were removed from the dataset. The permanova analysis on abundance of limpets showed that the factors "Time", "Protection", "Accessibility" and "Site" were significant. Similarly, the interaction between "Protection x Accessibility" and "Time x Site" were also significant (Table 2).

In particular, abundance tended to decrease through time: the level for June 2015 (104.3 ± 9.7 limpets/m²) being significantly higher than the levels detected in the following months, for example. Abundance in the three following surveys (July, August and September) did not decrease significantly. A second significant decrease was observed in October 2015 (Fig. 5A). The abundance values recorded during the December survey did not differ from the ones observed in the previous survey, but were significantly higher than in the following months. Mean

Table 2. Results of the Repeated Measures Permanova on abundance of the original pool of limpets. n.s. = not significant.

Source	df	SS	MS	Pseudo-F	p	Unique perm
Ti	11	684.6	62.2	652.4	**<0.001**	9974
Pr	1	5.1	5.1	53.1	**<0.001**	9984
Ac	1	37.7	37.7	395.5	**<0.001**	9976
TixPr	11	-5.5	-0.5	-5.3	n.s.	9961
TixAc	11	-26.0	-2.4	-24.8	n.s.	9959
PrxAc	1	1.8	1.8	18.9	**<0.01**	9978
Si(AcxPr)	6	6.1	1.0	10.7	**<0.001**	9967
TixPrxAc	11	-1.8	-0.2	-1.7	n.s	9957
TixSi(AcxPr)	66	16.6	0.2	2.6	**<0.001**	9921
Res	239	22.8	0.1			
Total	358	818.3				

abundance in January 2016 (20.9 ± 2.1) was higher than in March 2016 (11.0 ± 1.6) and values of March were higher than in May 2016 (5.0 ± 0.7). Values for May 2016 did not differ from the June 2016 values and values of June were higher than in July 2016 (2.5 ± 0.6). Finally, values for July 2016 did not differ from the August 2016 values. The lowest mean level was observed during the August 2016 survey (0.8 ± 0.2 limpets/m²) (Fig 5A; Appendix 1).

Within the MPA, the mean number of limpets was higher than at unprotected sites (38.3 ± 3.4 and 34.3 ± 3.0 limpets/m², respectively). Furthermore, higher abundances were found at low accessibility sites (MPA: 44.6 ± 6.2; OUT: 42.0 ± 4.9) than at highly accessible sites (MPA: 35.2 ± 4.0; OUT: 26.7 ± 3.4). Significant differences between levels of accessibility were observed both within the MPA and the unprotected sites. Conversely, when abundance values were tested within the same level of physical accessibility, significant differences were observed only between the highly accessible sites of the MPA and the highly accessible sites of OUT (Fig 5B; Appendix 1). No significant differences were observed between low accessible sites. Conversely, differences in

mean abundance sporadically arose among sites of high accessibility both within the MPA and in unprotected sites but the patterns observed were not consistent across time (Appendix 1).

Temporal variations in the number of limpets belonging to the original pool were detected within sites (Appendix 2). In particular, the pool of limpets detected in the first survey decreased at all the sampling sites during the study period and the number of limpets almost completely disappeared at every combination of protection and accessibility (Fig. 6).

The abundance of predators (*Pachygrapsus marmoratus*, *Eriphia verrucosa*, *Stramonita haemastoma*) did not differ significantly in relation with the level of protection or site accessibility. Conversely, "Time" and its interaction with "Protection", "Accessibility" and "Site" were significant. The interaction "Time x Protection x Accessibility" was also significant (Table 3). Differences in the abundance of limpet predators were not constant and arose only occasionally (Fig.7; Appendix 1). Spatial variability in predator abundances was not detected during every survey (Appendix 1).
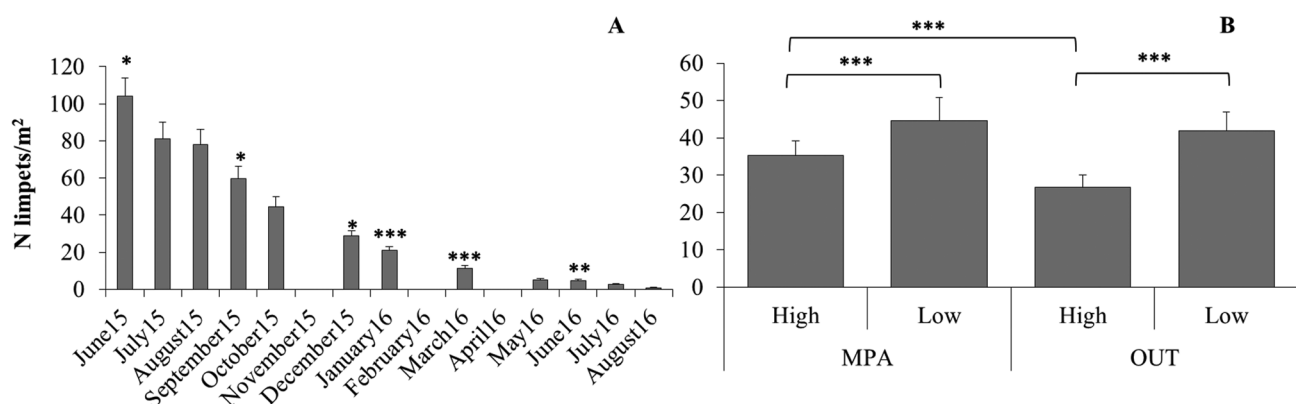

Fig. 5: Mean abundance (N limpets/m² ± st. err.) of the original pool: A) for levels of factor "Time" and B) for levels of "Protection" and "Accessibility of the site". In Fig. 5A asterisks indicate significantly higher values in comparison with the following month. * = p < 0.05; ** = p < 0.01; *** = p < 0.001.

During the first survey, the size structure of limpets detected in MPA High was significantly different from the one observed in the other levels of protection and accessibility (Table 4). In particular, it was characterized by a high abundance of small individuals (shell length <10 mm) that constituted 36.6% of the population, whilst at the other sites these size classes constituted around 20% (Fig. 8). In July 2015, size composition among the different sites differed as regards the percentage of individuals <20 mm, which was higher at highly accessible sites (MPA High: 90.5%; OUT High: 90.0%; MPA Low: 80.5%; OUT Low: 80.4%). In August 2015, MPA High differed from all the other sites due to limpets <10 mm (29.1%) with respect to MPA Low (21.5%) and OUT High (15.4%), and to individuals <15 mm (62.6%) with respect to OUT Low (51.6%). In September 2015, MPA High differed from MPA Low and OUT Low. This difference was due to limpets <20 mm comprising 90.8%

of the population in MPA High, 79.7% in MPA Low and 82.0% in OUT low. Such differences in size structure were also observed between MPA Low (79.7%) and OUT High (90.0%). In October 2015, MPA High differed as regards the percentage of limpets <20 mm (88.7%) with respect to Low accessible sites both within the MPA (75.0%) and in OUT (78.6%). A difference in the size structure was also observed between MPA Low and OUT High (87.0%). In MPA High, in December 2015, shell lengths <20 mm represented 80.0% of the population, a percentage that differs significantly with respect to the low accessible sites, both within the MPA (66.8%) and in OUT (68.8%). Limpets <20 mm during the January 2016 survey, were more important in the population of OUT High (76.7%) in comparison with MPA Low (55.9%) and OUT Low (58.3%). In the following surveys, no differences in size structures were found among levels of protection and accessibility
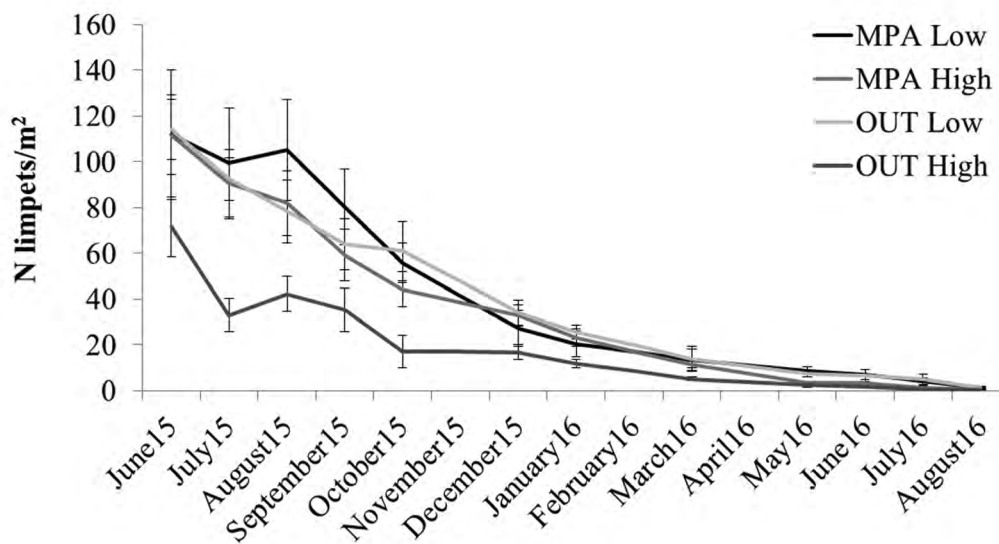
Fig. 6: Abundance trend of the original pool among levels of protection and accessibility (N limpets/m² ± st. err.).

Table 3. Results of the Repeated Measures Permanova on abundance of limpet predators. n.s. = not significant.

Source	df	SS	MS	Pseudo-F	p	Unique perm
Ti	11	21.069	1.9153	12.548	**<0.001**	9945
Pr	1	-3.79E-03	-3.79E-03	-2.48E-02	n.s.	9958
Ac	1	0.13295	0.13295	0.87098	n.s.	9952
TixPr	11	5.8094	0.52813	3.4599	**<0.01**	9951
TixAc	11	4.0764	0.37058	2.4277	**<0.05**	9941
PrxAc	1	-4.42E-02	-4.42E-02	-0.28948	n.s.	9955
Si(AcxPr)	6	1.2381	0.20636	1.3519	n.s.	9964
TixPrxAc	11	5.8751	0.5341	3.499	**<0.01**	9949
TixSi(AcxPr)	66	27.224	0.41248	2.7023	**<0.001**	9891
Res	240	36.634	0.15264			
Total	359	105.76				

Fig. 7: Seasonal variations of the mean abundance of limpet predators (N predators/search ± st. err.) among levels of protection and accessibility.

Table 4. Pairwise comparisons of size structures among levels of protection (MPA, OUT) and accessibility (High, Low). The Kolmogorov-Smirnoff test with Bonferroni Correction for multiple comparisons was used. D value in August 2016 was calculated with the formula for small samples "max. diff.*n_1*n_2" (with $n_1, n_2 < 25$). n.s. = not significant.

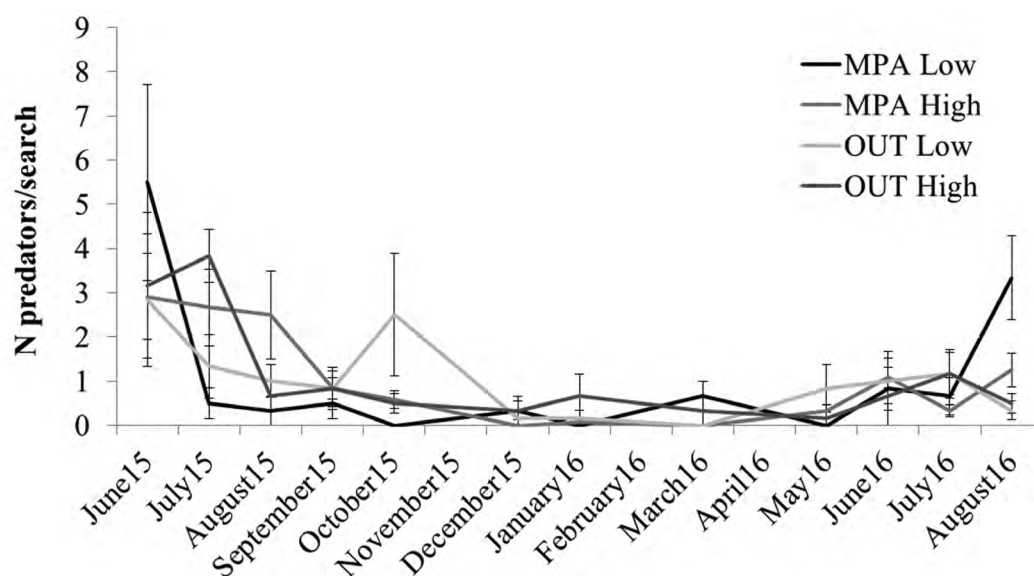|	MPA High MPA Low		MPA High OUT High		MPA High OUT Low		MPA Low OUT High		MPA Low OUT Low		OUT High OUT Low	
	D	**p**	**D**	**p**	**D**	**p**	**D**	**p**	**D**	**p**	**D**	**p**
June15	0.145	0.001	0.164	0.001	0.119	0.001	0.041	n.s	0.026	n.s	0.045	n.s
July15	0.100	0.001	0.024	n.s	0.101	0.001	0.095	0.001	0.054	n.s	0.096	0.001
August15	0.077	0.001	0.137	0.001	0.105	0.001	0.061	n.s	0.067	n.s	0.060	n.s
September15	0.111	0.001	0.022	n.s	0.088	0.001	0.103	0.001	0.034	n.s	0.080	n.s
October15	0.138	0.001	0.017	n.s	0.101	0.001	0.120	0.01	0.036	n.s	0.084	n.s
December15	0.133	0.001	0.012	n.s	0.113	0.01	0.121	n.s	0.022	n.s	0.101	n.s
January16	0.121	n.s	0.087	n.s	0.097	n.s	0.208	0.001	0.024	n.s	0.184	0.001
March16	0.101	n.s	0.031	n.s	0.035	n.s	0.120	n.s	0.066	n.s	0.054	n.s
May16	0.066	n.s	0.033	n.s	0.028	n.s	0.077	n.s	0.094	n.s	0.024	n.s
June16	0.100	n.s	0.099	n.s	0.038	n.s	0.178	n.s	0.064	n.s	0.114	n.s
July16	0.101	n.s	0.103	n.s	0.086	n.s	0.077	n.s	0.021	n.s	0.086	n.s
August16	76	n.s	8	n.s	25	n.s	36	n.s	65	n.s	5	n.s

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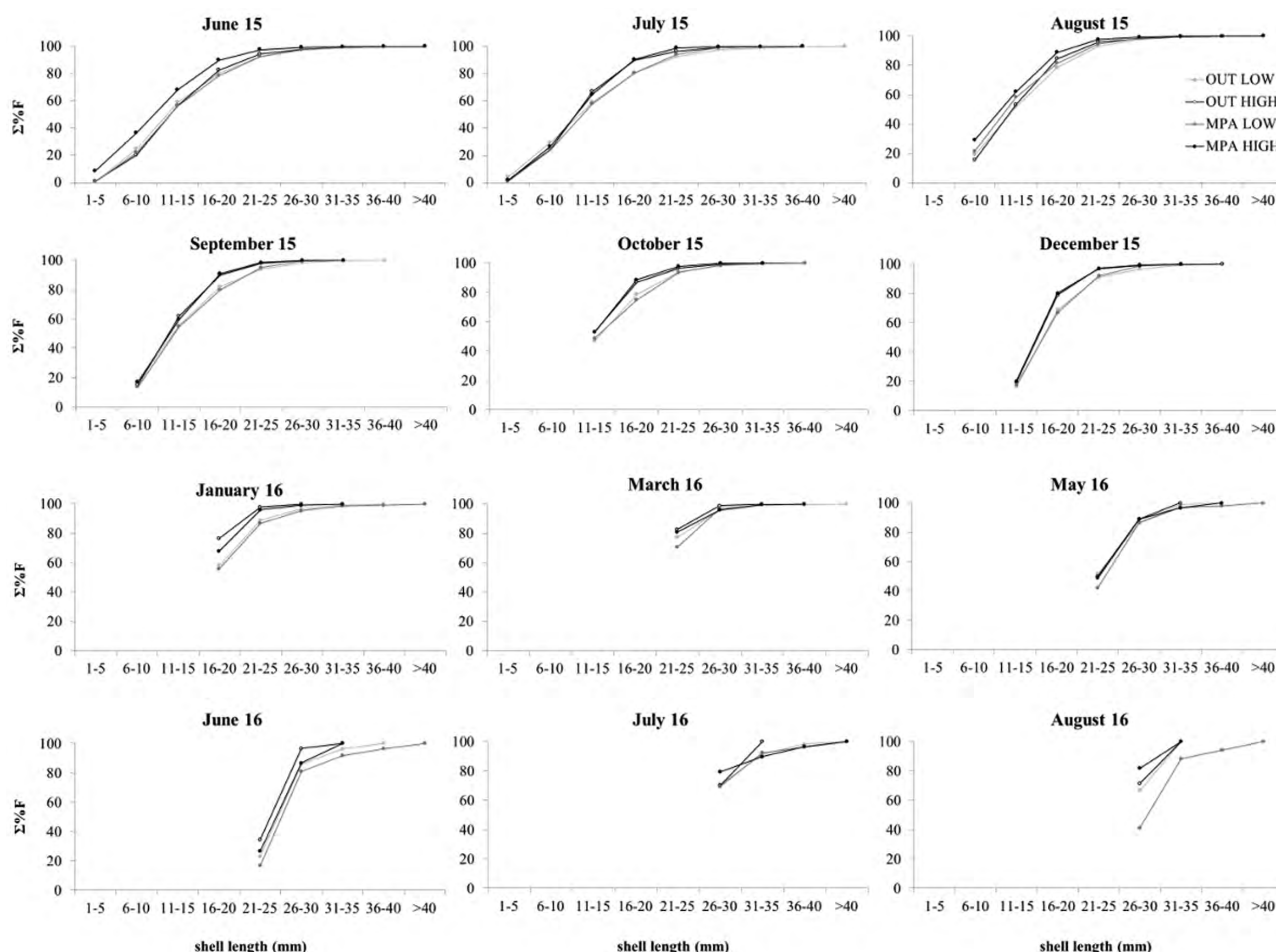*Medit. Mar. Sci., 18/3, 2017, 406-423*

Fig. 8: Seasonal cumulative size frequency curves of *Patella* sp. among levels of protection and accessibility. Only the original pool of limpets detected during the first survey is shown.

Discussion

Abundance trend

Marine Protected Areas could represent a precious tool for limiting overexploitation and favouring sustainable management of biological resources. However, recent studies have shown that many protected areas are poorly effective (Edgar *et al.*, 2014). The analysis of temporal trends in abundance and size of target species allows us to determine to what extent the actual exploitation rate is affecting local populations and whether existing MPAs are providing the protection they were established to achieve. This study is the first to provide data on the distribution and mortality rates of limpets along the coastline of the Sinis Peninsula and has assessed the effect of protection on the intertidal zone in the "Penisola del Sinis - Isola di Mal di Ventre" MPA.

Overall, the study area displays strong spatial and temporal variability in the number of limpets found on the shore. Variability within sites was also high, in particular for SG and IS, both hosting one replicate where *P. rustica* individuals were abundant during the study period. Sites exposed to the prevailing wind (the north-western Mistral)

acting on the area and, consequently, more exposed to wave action, displayed, on average, values >50 limpets/m^2. Conversely, non-exposed sites showed lower mean abundance values. Intertidal assemblages are influenced strongly by hydrodynamics (Bustamante & Branch, 1996) and many studies have shown that limpet species tend to be more abundant on rocks exposed to medium-strong wave action (Branch & Marsh, 1978; Branch, 1981; Denny, 2000; Denny & Blanchette, 2000; Tlig-Zouari *et al.*, 2010). This pattern was confirmed also for the species considered in this study, as also observed around the Sinis Peninsula for *P. ferruginea* (Coppa *et al.*, 2012).

Growth and mortality

Few data are available in the literature on longevity and growth of the study species. Prusina *et al.* (2015) compared the growth of *P. rustica* in the Adriatic Sea with that of other limpet species taken from the literature. The authors detected a growth performance index value (Ø = logK + 2logL∞) of 2.6, that was among the lowest reported for limpets in the literature (Prusina *et al.*, 2015, table 2) and an L∞ = 38.2 mm. In the sample used for that study (max shell length = 33.6 mm), the estimated mean age was

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2.9 years with some individuals aged more than 7 years. The growth performance index values obtained during this study were higher (3.2 for *P. rustica*; 3.3 for *P. caerulea*) and similar to the ones obtained for other species such as *P. ferruginea* (Espinosa *et al.*, 2008) *Cellana testudinaria* (Khow, 2007), *Fissurella crassa* (Bretos, 1980) and *Scrutellastra longicosta* (Branch, 1974). According to the obtained models, the size structure observed during the first survey in the study area was dominated by approximately 6-month old (11-15 mm) limpets, with few individuals older than 2 years. Limpets >35 mm were extremely rare and found mainly at less accessible sites. In comparison with other areas where these species can easily exceed 40 mm in length (Christiaens, 1973), smaller sizes were found around the Sinis Peninsula, thus suggesting a higher mortality rate. This hypothesis is also confirmed by the fact that the pool of limpets detected during the first survey was depleted rapidly during the study period, being close to extirpation after only one year.

The role of natural predators

Natural predation can be a strong regulating force affecting intertidal assemblages (Menge, 2000; Silva *et al.*, 2008, 2010a). On Atlantic and Pacific shores, limpets are predated by birds, sea-stars, fish, gastropods and crabs (Marsh, 1986; Cannicci *et al.*, 2007; Silva *et al.*, 2008; Flores *et al.*, 2001, Silva *et al.*, 2010b) but in the rocky intertidal zone of the study area the most common limpet predators are crabs (*Pachygrapsus marmoratus* and *Eriphia verrucosa*), and whelks (*Stramonita haemastoma*) (Coppa *et al.*, 2012). The intertidal surveys conducted during the study period revealed low numbers of natural predators in comparison with other areas such as central Portugal (Flores *et al.*, 2001), southwest Britain (Silva *et al.*, 2010a) and the Israeli coast (Rilov *et al.*, 2004). It must be taken into account, however, that the abundance of predators could have been underestimated as the surveys were only conducted during periods of day light. Some studies have indeed reported that the above predators are more active at night (Rilov *et al.*, 2005; Silva *et al.*, 2010a) although this behaviour is not common at all latitudes (Canicci *et al.* 1999). Around the Sinis Peninula, the abundance of predators was not consistently associated with the abundance of limpet prey: significant differences in the abundance of predators arose only sporadically between levels of protection and accessibility, and not always with the same pattern. Therefore, natural mortality appears to play a minor role in determining the observed fluctuations in limpet abundance and size. This observation is in line with other studies that did not detect a strong influence of natural predation on limpet population sizes, as observed for instance for *P. depressa* along the coast of Portugal (Brazão *et al.*, 2009; Silva *et al.*, 2004). Further studies specifically designed to assess the intensity of natural predation in the study area would be useful to confirm this hypothesis.

The role of the MPA and of physical accessibility

Marine Protected Areas are considered important tools for the conservation of species and habitats, and their role in enhancing the biomass of exploited limpets has been assessed by several extra-Mediterranean studies (e.g. Kido & Murray, 2003; Branch & Odendaal, 2003; Martins *et al.*, 2011; López *et al.*, 2012). Highly vulnerable species are more likely to benefit from the establishment of protected areas, as demonstrated by case studies on large limpets that are often present within MPAs and other protected areas (Fenberg & Roy, 2012; Espinosa *et al.*, 2014; García-Gómez *et al.*, 2015). The Sinis MPA was established more than fifteen years ago in an area where human pressure on marine resources was intense (Casola *et al.*, 2014; Pieraccini *et al.*, 2017) and where the gathering of intertidal organisms was frequently performed as a recreational activity by local communities (Coppa *et al.*, 2012). Despite the fact that the MPA hosts one of the last populations of *P. ferruginea* in the Mediterranean (Coppa *et al.*, 2012), a recent study has highlighted that the protection measures implemented have not been very effective as regards the conservation of this species, whose population is actually in decline (Coppa *et al.*, 2016). This study suggests that conservation of this intertidal species is influenced strongly by the physical accessibility of sites, rather than by the protection measures of the MPA only. This work further explores the role of legal and physical protection in limiting the harvesting of limpets in the study area. The abundance of *P. caerulea*, *P. rustica* and *P. ulyssiponensis* was higher within the MPA and within sites with low accessibility. This suggests that both the legal and the physical characteristics of a site can provide protection. Moreover, the differences between low accessible sites are not pronounced, whilst those between highly accessible sites are significant, i.e. there are more limpets at sites that are easily reached within the MPA than in OUT. These results reinforce the argument that the presence of the MPA is important in supporting limpet populations, and that when legal protection is not implemented, limpet mortality is particularly high in highly accessible sites. The size structure of the original pool of limpets differed among levels of protection and accessibility during the period between June 2015 and January 2016. From March 2016, when all limpets reached a minimum shell length of 20 mm, no more differences in size structure were observed. In particular, highly accessible sites tended to be characterized by smaller sizes compared to sites of low accessibility. These results highlighted the fact that highly accessible sites not only showed lower abundance values, but also smaller sizes. At highly accessible sites, mean abundance levels were higher within the MPA than outside, but in the MPA sizes tended to be smaller. Following the same pool of limpet thought time permitted to determine that in the area the mortality rates are extremely high. Despite the fact that the species considered in this study have a multi-annual life span, the analysis of

temporal trends revealed that limpets can unlikely survive more than one year even within the MPA and in hardly accessible sites. Considering the fact that collection of intertidal invertebrates is strictly forbidden in all the MPA, the decreasing trends observed clearly highlight a lack of effectiveness of management rules.

Conclusions

The establishment of MPAs could reduce human pressure in areas where overexploitation is threating marine resources. This work demonstrates that gathering around the Sinis Peninsula is a strong and diffuse stressor for *Patella* species, affecting not only the very large *P. ferruginea* but also the smaller species *P. caerulea*, *P. rustica* and *P. ulyssiponensis*. Even if the total abundance of limpets could rapidly increase across time, as a consequence of the continuous arrival of new recruits on the shore, the human-induced mortality rate is not negligible and appears to play a major role in the observed fluctuations in the number of individuals. The MPA's formal ban on the gathering of shellfish in the intertidal zone provides some benefits for the limpet population. Abundance is indeed higher within the MPA compared to outside. Nevertheless, there is evidence that protection rules do not efficiently prevent harvesting. Even within the MPA, the mortality rate of limpets is high and a decreasing trend in abundance has been recorded herein for each site. Physical accessibility strongly influences the rate of harvesting around the Sinis Peninsula. Dynamics observed for small *Patella* species are in line with previous studies conducted in the same area on the endangered *P. ferruginea,* whose distribution is limited to hardly accessible sites and remote areas, such as the island of Mal di Ventre and Cape San Marco on the mainland (Coppa *et al.*, 2012, 2016; Marra *et al.*, 2016). The physical characteristics of an area, such as the presence of cliffs, slippery rocks, wave exposure, could limit the intensity of human collection pressures and provide protection to sessile and sedentary species, as reported in many case studies (Keough & Quinn, 2000; Paracuellos *et al.*, 2003; Ceccherelli *et al.*, 2005). Such studies are important for determining whether existing MPAs are actually reducing human pressure on marine biodiversity, and contribute to highlight gaps in the effectiveness of conservation measures.

Acknowledgements

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Pairwise comparisons of abundance of the original pool of limpets and of abundance of limpet predators. See Appendix 2 and 3 for remaining comparisons. p values are shown. n.s. = not significant.

Limpets abundance				
Si	**Ti**		**TixSi**	
SM=TS	June15≠July15, August15 **p<0.05**; ≠September15 **p<0.01**	June15	SG≠CA **p<0.05**	
CA≠(TH=SG=IS) **p<0.001**	June15, July15, August15 ≠ October15-August16 **p<0.001**	July15	SG≠TH,CA,IS; TH≠CA **p<0.05**	
CM=ST	September15≠October15 **p<0.05**	August15	SG≠TH,CA **p<0.05**	
SP≠SS **p<0.001**	September15≠December15-August16 **p<0.001**	September15	CA=IS=SG=TH=SP=SS=ST=CM=SM=TS	
	October15≠January16 **p<0.01**	October15	CA≠IS,SG,TH; TH≠SG **p<0.05**	
PrxAc	October15, December15, January16 ≠March16-August16 **p<0.001**	December15	SP≠SS **p<0.05**	
MPA Low≠MPA High **p<0.001**	December15≠January16 **p<0.05**	January16	SP≠SS **p<0.01**	
OUT Low≠OUT High **p<0.001**	July15=August15=September15=October15=December15	March16	CA≠IS,TH **p<0.05**	
MPA High≠OUT High **p<0.001**	March16≠May16-August16 **p<0.001**	May16	CA=IS=SG=TH=SP=SS=ST=CM=SM=TS	
MPA Low=OUT Low	May16 =June16	June16	SP≠SS **p<0.05**	
	May16≠July16-August16 **p<0.001**	July16	CA=IS=SG=TH=SP=SS=ST=CM=SM=TS	
	June16≠July16 **p<0.01**	August16	CA=IS=SG=TH=SP=SS=ST=CM=SM=TS	
	June16≠August16			
	July16=August16 **p<0.001**			

Predators abundance	
Ti	**TixPr**
June15=July15=August16	MPA≠OUT: October15, August16 **p<0.01**
June15≠August15=September15=July16 **p<0.05**	**TixAc**
June15≠October15 **p<0.01**	High≠Low: July15 **p<0.001**
June15, July15≠December15-May16 **p<0.001**	**TixPrxAc**
July15, October15=August15	MPA High≠OUT High: August15, December15 **p<0.05**
July15≠September15=June16 **p<0.05**	MPA Low≠OUT Low: October15, August16 **p<0.01**
July15≠October15=July16 **p<0.01**	MPA High≠ MPA Low: July15, August15, October15, March16, May16 **p<0.05**
August15≠December15-March16 **p<0.001**	OUT High≠ OUT Low: July15 **p<0.05**
August15 ≠May16 **p<0.01**	**TixSi**
August15=June16=July16= August16	June15: SM≠TS; SP≠SS **p<0.05**
September15=October15≠December15,January16 **p<0.01**	July15: SG≠CA, IS **p<0.05**
September15=October15=June16=August16	August15: SP≠SS **p<0.001**
October15≠March16 **p<0.01**	September15: n.s.
October15≠May16 **p<0.05**	October15: CA≠SG **p<0.001**; CM≠ST **p<0.05**
December15=January16 =March16=May16	December15: n.s.
December15,January16, March16≠June16, July16 **p<0.01**	January16: n.s.
December15,January16, March16, May ≠ August16 **p<0.001**	March16: n.s.
March16=May16	May16: n.s.
May16≠June16 **p<0.05** ≠July16 **p<0.01**	June16: n.s.
	July16: TH≠SG; SP≠SS **p<0.001**
	August16: SM≠TS **p<0.05**

Pairwise comparisons of abundance of the original limpet pool among time levels within each site. p values are shown. n.s. = not significant.

Groups	SM	TS	TH	SG	IS	CA	CM	ST	SS	SP
June15, July15	n.s	n.s	n.s	n.s	n.s	< 0.05	n.s	n.s	n.s	n.s
June15, August15	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s
June15, September15	n.s	n.s	n.s	n.s	n.s	< 0.05	< 0.05	n.s	< 0.05	n.s
June15, October15	n.s	< 0.05	< 0.05	< 0.05	n.s	< 0.01	< 0.05	n.s	< 0.01	n.s
June15, December15	< 0.05	< 0.01	< 0.05	< 0.05	n.s	< 0.01	< 0.001	< 0.05	< 0.01	< 0.05
June15, January16	< 0.05	< 0.01	< 0.05	< 0.001	n.s	< 0.01	< 0.001	< 0.01	< 0.01	< 0.01
June15, March16	< 0.01	< 0.01	< 0.01	< 0.001	< 0.05	< 0.01	< 0.001	< 0.01	< 0.001	< 0.01
June15, May16	< 0.01	< 0.01	< 0.01	< 0.001	< 0.05	< 0.01	< 0.001	< 0.01	< 0.001	< 0.001
June15, June16	< 0.01	< 0.01	< 0.001	< 0.001	< 0.05	< 0.001	< 0.001	< 0.01	< 0.001	< 0.001
June15, July16	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.001	< 0.01	< 0.01	< 0.01	< 0.001
June15, August16	< 0.01	< 0.001	< 0.001	< 0.001	< 0.01	< 0.001	< 0.001	< 0.001	< 0.01	< 0.001
July15, August15	n.s	n.s	n.s	n.s	n.s	< 0.05	n.s	n.s	n.s	n.s
July15, September15	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	< 0.01	n.s
July15, October15	n.s	n.s	n.s	< 0.05	n.s	n.s	n.s	n.s	< 0.05	n.s
July15, December15	n.s	< 0.05	< 0.05	< 0.01	n.s	n.s	< 0.01	< 0.05	< 0.01	n.s
July15, January16	< 0.05	< 0.05	< 0.05	< 0.01	n.s	< 0.05	< 0.01	< 0.001	< 0.001	< 0.05
July15, March16	< 0.05	< 0.01	< 0.01	< 0.001	< 0.05	< 0.01	< 0.001	< 0.05	< 0.001	< 0.05
July15, May16	< 0.05	< 0.01	< 0.01	< 0.001	< 0.01	< 0.001	< 0.001	< 0.01	< 0.001	< 0.01
July15, June16	< 0.05	< 0.01	< 0.01	< 0.001	< 0.01	< 0.001	< 0.001	< 0.01	< 0.001	< 0.01
July15, July16	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
July15, August16	< 0.01	< 0.01	< 0.01	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.01	< 0.01
August15, September15	n.s	n.s	n.s	n.s	n.s	< 0.05	n.s	n.s	< 0.05	n.s
August15, October15	n.s	< 0.05	n.s	n.s	n.s	< 0.05	n.s	n.s	< 0.05	n.s
August15, December15	n.s	< 0.05	n.s	< 0.05	n.s	< 0.05	< 0.05	< 0.05	< 0.05	n.s
August15, January16	n.s	< 0.01	n.s	< 0.01	< 0.05	< 0.01	< 0.05	< 0.05	< 0.01	n.s
August15, March16	< 0.05	< 0.01	< 0.05	< 0.001	< 0.05	< 0.001	< 0.01	< 0.05	< 0.01	< 0.05
August15, May16	< 0.05	< 0.001	< 0.05	< 0.001	< 0.01	< 0.001	< 0.01	< 0.01	< 0.001	< 0.01
August15, June16	< 0.05	< 0.01	< 0.05	< 0.001	< 0.01	< 0.001	< 0.01	< 0.05	< 0.001	< 0.01
August15, July16	< 0.05	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
August15, August16	< 0.05	< 0.01	< 0.01	< 0.001	< 0.001	< 0.001	< 0.01	< 0.001	< 0.01	< 0.01
September15, October15	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s
September15, December15	< 0.05	< 0.05	n.s	n.s	n.s	n.s	n.s	n.s	n.s	< 0.05
September15, January16	< 0.05	< 0.05	n.s	n.s	n.s	n.s	< 0.05	n.s	< 0.01	< 0.05
September15, March16	< 0.01	< 0.05	< 0.05	< 0.05	< 0.05	< 0.01	< 0.01	< 0.05	< 0.01	< 0.01
September15, May16	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.001	< 0.01
September15, June16	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.05	< 0.001	< 0.001
September15, July16	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.05	< 0.01	< 0.001
September15, August16	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.001	< 0.01	< 0.001	< 0.01	< 0.001
October15, December15	< 0.05	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s
October15, January16	< 0.01	n.s	n.s	< 0.01	n.s	n.s	n.s	n.s	n.s	n.s
October15, March16	< 0.01	< 0.05	< 0.01	< 0.01	< 0.05	n.s	< 0.05	< 0.05	< 0.05	n.s
October15, May16	< 0.01	< 0.01	< 0.01	< 0.001	< 0.01	n.s	< 0.05	< 0.01	< 0.001	< 0.05
October15, June16	< 0.01	< 0.05	< 0.001	< 0.001	< 0.01	n.s	< 0.01	< 0.05	< 0.01	< 0.05
October15, July16	< 0.001	< 0.01	< 0.01	< 0.01	< 0.001	n.s	< 0.01	< 0.05	< 0.05	< 0.01
October15, August16	< 0.001	< 0.01	< 0.01	< 0.01	< 0.01	n.s	< 0.01	< 0.01	< 0.01	< 0.01
December15, January16	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	< 0.01	n.s
December15, March16	< 0.05	n.s	< 0.05	n.s	< 0.05	< 0.05	< 0.01	n.s	< 0.01	< 0.05
December15, May16	< 0.05	< 0.05	< 0.05	< 0.05	< 0.01	< 0.05	< 0.01	< 0.05	< 0.001	< 0.01
December15, June16	< 0.05	< 0.05	< 0.001	< 0.05	< 0.01	< 0.01	< 0.01	< 0.05	< 0.001	< 0.01
December15, July16	< 0.01	< 0.05	< 0.01	< 0.05	< 0.01	< 0.01	< 0.05	< 0.05	< 0.01	< 0.01
December15, August16	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
January16, March16	< 0.001	n.s	n.s	n.s	n.s	< 0.05	< 0.05	n.s	< 0.05	< 0.05
January16, May16	< 0.05	n.s	< 0.05	< 0.01	< 0.05	< 0.01	< 0.05	< 0.05	< 0.001	< 0.01
January16, June16	n.s	n.s	< 0.05	< 0.01	< 0.05	< 0.01	< 0.01	n.s	< 0.001	< 0.01
January16, July16	< 0.01	< 0.05	< 0.01	< 0.05	< 0.01	< 0.01	< 0.05	< 0.05	< 0.05	< 0.01
January16, August16	< 0.001	< 0.01	< 0.01	< 0.01	< 0.01	< 0.001	< 0.01	< 0.001	< 0.01	< 0.01
March16, May16	n.s	n.s	n.s	< 0.01	< 0.05	n.s	n.s	n.s	< 0.01	< 0.05
March16, June16	n.s	n.s	< 0.01	< 0.01	< 0.05	n.s	n.s	n.s	< 0.05	< 0.01
March16, July16	< 0.05	n.s	< 0.05	n.s	< 0.01	< 0.05	n.s	n.s	< 0.05	< 0.01
March16, August16	< 0.001	< 0.05	< 0.05	< 0.05	< 0.01	< 0.01	< 0.05	< 0.05	< 0.05	< 0.01
May16, June16	n.s	n.s	n.s	n.s	n.s	n.s	< 0.05	n.s	< 0.05	n.s
May16, July16	< 0.05	n.s	< 0.05	n.s	< 0.01	n.s	n.s	n.s	n.s	< 0.05
May16, August16	< 0.01	< 0.05	< 0.05	n.s	< 0.01	< 0.01	< 0.05	n.s	n.s	< 0.05
June16, July16	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s
June16, August16	n.s	n.s	< 0.05	n.s	< 0.01	< 0.05	n.s	n.s	n.s	n.s
July16, August16	n.s	n.s	n.s	n.s	< 0.05	n.s	n.s	n.s	n.s	n.s

APPENDIX 3

Pairwise comparisons of abundance of limpet predators among time levels. p values are shown. n.s. = not significant.

id	AMP	OUT	HIGH	LOW	AMP HIGH	AMP Low	OUT High	OUT Low	TH	CA	SG	IS	SM	TS	SP	SS	CM	ST
June15, July15	n.s.	n.s.	n.s.	n.s.	n.s.	<0.05	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	<0.001	n.s.	n.s.	n.s.	n.s.	n.s.
June15, August15	<0.05	n.s.	n.s.	n.s.	n.s.	<0.01	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	<0.001	n.s.	<0.01	n.s.	n.s.	n.s.
June15, September15	<0.05	n.s.	n.s.	n.s.	n.s.	<0.05	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	<0.001	n.s.	n.s.	n.s.	n.s.	n.s.
June15, October15	<0.001	n.s.	<0.05	<0.05	n.s.	<0.001	n.s.	n.s.	<0.05	n.s.	n.s.	n.s.	<0.001	n.s.	<0.05	n.s.	n.s.	n.s.
June15, December15	<0.001	<0.05	<0.001	<0.01	<0.01	<0.01	<0.05	n.s.	<0.01	n.s.	n.s.	n.s.	<0.05	n.s.	<0.01	n.s.	n.s.	n.s.
June15, January16	<0.001	<0.05	<0.01	<0.001	<0.01	<0.001	n.s.	n.s.	<0.05	n.s.	n.s.	n.s.	<0.001	n.s.	<0.01	n.s.	n.s.	n.s.
June15, March16	<0.001	<0.01	<0.001	<0.01	<0.01	n.s.	<0.05	n.s.	<0.01	n.s.	n.s.	n.s.	<0.05	n.s.	<0.01	n.s.	n.s.	n.s.
June15, May16	<0.001	<0.05	<0.01	<0.01	<0.05	<0.001	<0.05	n.s.	<0.01	n.s.	n.s.	n.s.	<0.001	n.s.	<0.01	n.s.	n.s.	n.s.
June15, June16	<0.05	n.s.	n.s.	<0.05	n.s.	<0.05	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	<0.05	n.s.	n.s.	n.s.	n.s.	n.s.
June15, July16	<0.01	n.s.	<0.05	n.s.	<0.05	<0.05	n.s.	n.s.	<0.05	n.s.	n.s.	n.s.	<0.05	n.s.	<0.01	n.s.	n.s.	n.s.
June15, August16	n.s	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	<0.05	n.s.	<0.05	n.s.	n.s.	n.s.
July15, August15	n.s.	<0.05	<0.01	n.s.	n.s.	n.s.	<0.001	n.s.	n.s.	<0.001	n.s.	n.s.	n.s.	n.s.	<0.001	<0.05	n.s.	n.s.
July15, September15	n.s.	<0.05	<0.01	n.s.	n.s.	n.s.	<0.05	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	<0.05	n.s.	n.s.	n.s.
July15, October15	<0.01	n.s.	<0.001	n.s.	<0.05	n.s.	<0.01	n.s.	<0.05	n.s.	<0.05	n.s.	n.s.	n.s.	<0.05	n.s.	n.s.	n.s.
July15, December15	<0.001	<0.001	<0.001	n.s.	<0.001	n.s.	<0.01	n.s.	<0.001	n.s.	<0.001	n.s.	n.s.	n.s.	<0.001	n.s.	n.s.	n.s.
July15, January16	<0.001	<0.01	<0.001	<0.05	<0.001	n.s.	<0.01	n.s.	<0.05	n.s.	<0.001	n.s.	n.s.	n.s.	<0.001	n.s.	n.s.	n.s.
July15, March16	<0.01	<0.001	<0.001	n.s.	<0.001	n.s.	<0.001	n.s.	<0.001	n.s.	<0.001	n.s.	n.s.	n.s.	<0.001	n.s.	n.s.	n.s.
July15, May16	<0.001	<0.01	<0.001	n.s.	<0.001	n.s.	<0.001	n.s.	<0.001	n.s.	<0.05	n.s.	n.s.	n.s.	<0.001	<0.05	n.s.	n.s.
July15, June16	n.s.	n.s.	<0.01	n.s.	n.s.	n.s.	<0.05	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	<0.05	n.s.	n.s.	n.s.
July15, July16	<0.05	n.s.	<0.05	n.s.	<0.01	n.s.	<0.001	n.s.	<0.05	n.s.	<0.05	n.s.	n.s.	n.s.	<0.01	n.s.	n.s.	n.s.
July15, August16	n.s.	<0.01	<0.01	n.s.	n.s.	<0.01	<0.01	n.s.	n.s.	n.s.	n.s.	n.s.	<0.001	n.s.	<0.05	n.s.	n.s.	n.s.
August15, September15	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	<0.05	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
August15, October15	<0.05	n.s.	n.s.	n.s.	<0.05	n.s.	n.s.	n.s.	n.s.	n.s.	<0.05	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
August15, December15	<0.01	<0.05	<0.001	n.s.	<0.001	n.s.	n.s.	n.s.	n.s.	n.s.	<0.01	<0.001	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
August15, January16	<0.001	n.s.	<0.001	n.s.	<0.01	n.s.	n.s.	n.s.	n.s.	n.s.	<0.01	<0.001	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
August15, March16	<0.05	<0.01	<0.001	n.s.	<0.001	n.s.	n.s.	<0.05	n.s.	n.s.	<0.01	<0.001	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
August15, May16	<0.01	n.s.	<0.001	n.s.	<0.01	n.s.	<0.05	n.s.	n.s.	n.s.	<0.05	<0.001	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
August15, June16	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
August15, July16	n.s.	<0.05	n.s.	n.s.	<0.01	n.s.	<0.01	n.s.	n.s.	n.s.	<0.05	n.s.	n.s.	n.s.	<0.05	n.s.	n.s.	n.s.
August15, August16	<0.05	n.s.	n.s.	<0.05	n.s.	<0.01	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	<0.001	n.s.	n.s.	n.s.	n.s.	<0.001
September15, October15	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
September15, December15	<0.05	n.s.	<0.05	n.s.	<0.01	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
September15, January16	<0.01	n.s.	n.s.	n.s.	<0.05	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
September15, March16	n.s.	<0.05	<0.01	n.s.	<0.01	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
September15, May16	<0.05	n.s.	<0.05	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
September15, June16	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
September15, July16	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	<0.05	n.s.	n.s.
September15, August16	<0.01	n.s.	n.s.	<0.05	n.s.	<0.01	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	<0.001	n.s.	n.s.	n.s.	n.s.	n.s.
October15, December15	<0.05	<0.05	<0.05	<0.05	<0.01	n.s.	n.s.	<0.05	n.s.	n.s.	n.s.	<0.001	n.s.	n.s.	n.s.	n.s.	<0.001	n.s.
October15, January16	n.s.	<0.05	n.s.	<0.01	<0.05	n.s.	n.s.	<0.05	<0.001	n.s.	n.s.	<0.001	n.s.	n.s.	n.s.	n.s.	<0.001	n.s.
October15, March16	n.s.	<0.01	<0.05	n.s.	<0.01	n.s.	n.s.	<0.01	n.s.	n.s.	n.s.	<0.001	n.s.	n.s.	n.s.	n.s.	<0.001	n.s.
October15, May16	n.s.	<0.05	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	<0.001	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
October15, June16	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
October15, July16	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	<0.001	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
October15, August16	<0.001	n.s.	n.s.	<0.01	n.s.	<0.001	n.s.	<0.05	n.s.	n.s.	n.s.	<0.001	<0.001	<0.001	n.s.	<0.01	n.s.	n.s.
December15, January16	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	<0.001
December15, March16	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
December15, May16	n.s.	n.s.	n.s.	n.s.	<0.01	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
December15, June16	<0.05	n.s.	<0.01	n.s.	<0.001	n.s.	n.s.	n.s.	n.s.	n.s.	<0.001	<0.001	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
December15, July16	n.s.	<0.05	<0.01	n.s.	<0.05	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
December15, August16	<0.001	n.s.	<0.05	<0.001	<0.01	<0.01	n.s.	n.s.	n.s.	<0.001	n.s.	n.s.	<0.05	<0.001	n.s.	n.s.	n.s.	n.s.
January16, March16	<0.05	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
January16, May16	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
January16, June16	<0.01	n.s.	<0.05	n.s.	<0.001	n.s.	n.s.	n.s.	n.s.	n.s.	<0.001	<0.001	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
January16, July16	<0.05	<0.05	n.s.	<0.05	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
January16, August16	<0.001	n.s.	n.s.	<0.001	<0.01	<0.001	n.s.	n.s.	n.s.	<0.001	n.s.	n.s.	<0.001	<0.001	n.s.	n.s.	n.s.	n.s.
March16, May16	n.s.	n.s.	n.s.	n.s.	<0.01	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
March16, June16	n.s.	n.s.	<0.01	n.s.	<0.001	n.s.	n.s.	n.s.	n.s.	n.s.	<0.001	<0.001	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
March16, July16	n.s.	<0.01	<0.01	n.s.	<0.05	n.s.	n.s.	<0.05	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
March16, August16	<0.001	n.s.	<0.01	<0.01	<0.01	<0.05	n.s.	n.s.	n.s.	<0.001	n.s.	n.s.	<0.05	n.s.	n.s.	n.s.	n.s.	n.s.
May16, June16	<0.05	n.s.	<0.01	n.s.	<0.01	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	<0.001	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
May16, July16	n.s.	n.s.	<0.05	n.s.	n.s.	n.s.	<0.05	n.s.	n.s.	n.s.	n.s.	<0.001	n.s.	n.s.	n.s.	<0.05	n.s.	n.s.
May16, August16	<0.001	n.s.	<0.05	<0.001	<0.05	<0.001	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	<0.001	<0.001	n.s.	n.s.	n.s.	n.s.
June16, July16	n.s.	n.s.	n.s.	n.s.	<0.05	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
June16, August16	<0.05	n.s.	n.s.	n.s.	n.s.	<0.05	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	<0.001	n.s.	n.s.	n.s.	n.s.	n.s.
July16, August16	<0.001	n.s.	n.s.	n.s.	n.s.	<0.01	n.s.	n.s.	n.s.	<0.001	n.s.	n.s.	<0.001	n.s.	n.s.	n.s.	n.s.	n.s.