Mediterranean Marine Science

Vol 19, No 3 (2018)

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doi: 10.12681/mms.14184

Research Article

Mediterranean Marine Science
Indexed in WoS (Web of Science, ISI Thomson) and SCOPUS
The journal is available online at http://www.medit-mar-sc.net
DOI: http://dx.doi.org/10.12681/mms.14184

Spatio-temporal patterns based on demographic and genetic diversity of the purple sea urchin *Paracentrotus lividus* in the area around Corsica (Mediterranean Sea)

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Handling Editor: Xavier Turon

Received: 12 July 2017; Accepted: 12 September 2018; Published on line: 14 December 2018

Abstract

Sea urchins were harvested for decades in many areas throughout its distribution range, potentially leading to population collapse. In France, the purple sea urchin *Paracentrotus lividus* is intensively harvested. Yet, the demography and population dynamics remained under-documented, particularly in Corsica. In this context, we have characterized the fluctuations in density of several size classes at 8 sites around the island, and assessed the genetic diversity and structuring of the population. Densities recorded lie between 0 and 2.18 (± 0.41) individuals.m^{-2} and spatio-temporal variabilities have also been highlighted. The study of the influence of vegetation cover on the size classes suggests that small- and medium- sized individuals prefer substrates of intermediate heights, whereas individuals with a diameter ≥ 5 cm are more often observed on encrusting substrates, and may be responsible for the continuation of this type of benthic community. The genetic study indicates a high genetic diversity with a low genetic structuring. The N_e values obtained are similar to those described in previous papers. Due to estimates of local contemporary N_e and the homogeneous genetic diversity, our data tend to show that the Corsican population of *P. lividus* is not overexploited.

Keywords: *Paracentrotus lividus*; Abundances; Population dynamics; Vegetation cover; Substrates; Demographic history; Effective population size.

Introduction

The purple sea urchin *Paracentrotus lividus* (Lamarck, 1816) (Echinoidea: Parechinidae) is a species commonly observed in coastal ecosystems and subtidal zones (Boudouresque & Verlaque, 2001), at shallow depths between 0 and 30 m (Mortensen, 1927) but with decreasing abundances after 10 m (Chelazzi *et al*., 1997). In the Mediterranean Sea, it can be found on rocky reefs, in meadows of *Zostera marina* or in *Posidonia oceanica* seagrass beds (Verlaque, 1987), where it can find a suitable habitat and food resources (Prado *et al*., 2007). Sometimes sea urchins can be observed in coastal lagoons such as in Urbinu and Thau, where it lives on mud substrata or coarse sand (Fernandez *et al*., 2006, 2012). The complexity and dependence of sea urchins in relation to their habitat influence the population structure (Prado *et al*., 2012) and many factors like temperature and/or salinity can affect sea urchin abundances (Fernandez *et al*., 2006). Presence of other species such as the black sea urchin Arbacia lixula (Linnaeus, 1758) (Echinoidea: Arbaciidae) can affect the spatial repartition of *P. lividus* (Chelazzi *et al*., 1997; Bulleri *et al*., 1999). Anthropic factors such as the destruction of habitats (Prado *et al*., 2012) or harvesting (Andrew *et al*., 2002; Pais *et al*., 2007; Ceccherelli *et al*., 2011) can also impact population structure and especially size classes greater than 5 cm (minimum harvestable size). In particular, sea urchin fishing is common in many Mediterranean countries as part of both professional and recreational practices (Guidetti, 2004), and the impact of overfishing on *P. lividus* has been highlighted (Andrew *et al*., 2002; Pais *et al*., 2012; Bertocci *et al*., 2014). Finally, global warming and the ensuing increasing water temperature could lead to the collapse of P. lividus populations (Yeruham *et al*., 2015; Rilov, 2016) and promote the development of *A. lixula* (Privitera *et al*., 2011). As a result, in many areas, *P. lividus* populations have been extensively surveyed over several successive generations (see e.g. Hereu *et al*., 2012; Pais *et al*., 2012).

Sea urchins have a complex life cycle. During each

reproductive season, *P. lividus* benthic adults produce pelagic larvae, that can potentially disperse across long distances from their place of birth (Cowen *et al*., 2006). The new recruits that settle within a population, thus ensure the demographic maintenance of the recipient populations and influence its genetic diversity. As is the case for most marine species with a pelagic developmental life stages, *P. lividus* presents high variability in both the level of recruitment (Hereu *et al*., 2004) and the reproductive success (Hedgecock, 1994; Calderón *et al*., 2012), which may lead to a transient genetic structure within cohorts (Johnson & Black, 1982; Couvray & Coupé, 2018).

P. lividus populations are characterized by a high genetic diversity within both Atlantic and Mediterranean basins. Using either nuclear or mitochondrial markers, spatial structure has been evidenced between the Atlantic and the Mediterranean basins (Duran *et al*., 2004; Calderón *et al*., 2008), and between the Atlantic basin, the Adriatic basin and the western and eastern Mediterranean basins (Maltagliati *et al.*, 2010). More recently, Paterno *et al.* (2017), using more than 1 000 single nucleotide polymorphisms (SNPs), revealed a differentiation between the northern and southern regions of the Western Mediterranean basin. In addition, weak genetic structures, potentially below the larval dispersion range, could be observed within basins and even within regions, these being explained by both the variability of reproductive success and putative, relatively stable, hydrological features (Penant *et al*., 2013; Couvray & Coupé, 2018). In any case, to date, the genetic diversity across all analyzed regions has been distributed across and within populations or cohorts, rather than in mutually exclusive settings, suggesting that populations are quite well connected and regularly replenished by a sufficient amount of genetically diverse recruits. A similar pattern of genetic structure has been observed, overall, within the Mediterranean basin, in the related sea urchin *Arbacia lixula* (Pérez-Portela *et al*., 2018).

The measure of the effective population size N_e, which is defined as the size of an ideal population that has the same rate of change in allele frequencies or heterozygosity, as the observed population, also gives an interesting insight into the demographic history of a species. Fluctuations of N_e estimates actually provide information on variations of population sizes, across different time frames (i.e. ancient to contemporary times) and spatial scales (Wang, 2005). Thus, N_e estimates are interesting indicators for the management and conservation of natural populations (Frankham, 2010; Hare *et al*., 2011; Ruggeri *et al*., 2016). Indeed, as the genetic diversity accounts for the adaptive potential of a species, there are great concerns when consistent demographic decreases are observed. For instance, overharvesting has been shown to substantially reduce the genetic diversity of numerous marine species, and adversely affect their biology and natural sustainability. In the case of *P. lividus*, Calderón *et al*. (2009a) have estimated the local N_e in a site of the Iberian coast at around three hundred individuals.

In the end, although a growing number of works reports that N_e can reliably estimate census N_c population sizes (Ovenden *et al*., 2016), its use as a proxy of N_c remains unsuitable for many marine species with high fecundity and variance in reproductive success, and in which N_c are 2 to 6 orders of magnitude higher than N_e (Luikart *et al.,* 2010; Plough, 2016).

In Corsica island (western Mediterranean) (Fig. 1), *P. lividus* is an emblematic species and its harvesting is regulated. Professional and recreational fishermen must

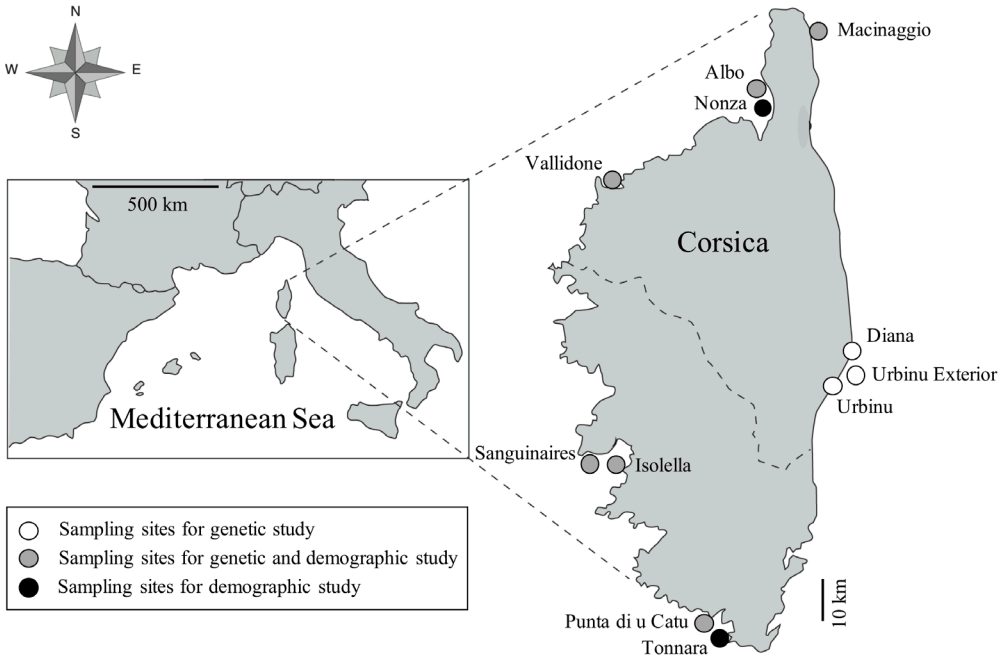

Fig. 1: Map showing the location of Corsica (NW Mediterranean Sea, France) and study sites.

respect quotas and capture periods. Only fishing by snorkeling is allowed and solely individual specimens with a test diameter greater than 5 cm (without spines) can be harvested. Despite these regulations, stakeholders are become concerned about the resource and little is known about either the demographic dynamics or the genetic diversity. Few previous data are available on the demographic structure and are focused on some specific areas of Corsica (see e.g. Boudouresque *et al.*, 1989; Fernandez *et al*., 2006). Moreover, the genetic structure of this species throughout Corsica is still unknown, although sporadic data on specific sites have been published (Calderón *et al*., 2008; Penant *et al*., 2013).

Considering that nowadays a better knowledge of the population structure and dynamics is required within the general context of resource sustainability, the aims of this study were (i) to determine the spatio-temporal dynamics of *P. lividus* demographically and genetically, and (ii) to estimate the effect of rocky substrate and vegetal cover on the abundance of *P. lividus* and *A. lixula.*

Materials and Methods

Sea urchin demographic structure

Data collection

Eight sites along the Corsican coastline were selected because of the representativeness of their location due to the fact that these sites are regularly frequented by professional sea urchin fishermen with the exception of Nonza, which is a regulated no-fishing area (Fig. 1). In Corsica, there are 4 fishing *prud'homies* (regulated fishing areas): the Bastia-Cap Corse *prud'homie* including the sites of Albo, Nonza and Macinaggio, the Balagne *prud'homie* including Vallidone, the Ajaccio *prud'homie* including Isolella and Sanguinaires and the Bonifacio *prud'homie* including Punta di u catu and Tonnara. Sites were chosen in collaboration with marine professionals in order to provide an overall view of the state of *P. lividus* stocks in the 4 *prud'homies*. There is no site in the South East as *P. lividus* is very scarce there because sand is the dominant substrate. The study was carried out in spring and autumn 2013 and 2014, i.e. after and before the sea urchin fishing period respectively. At each site, scuba divers used a non-permanent transect of 40 m^2 (20 x 2 m) at 3 depths: 3, 6 and 9 m. Each transect was divided into 4 areas of 5 m in length (10 m^2) to facilitate the obtention of subunits for statistical analyses. All *P. lividus* and *A. lixula* individuals present in the study areas were counted. The test diameters without spines of *P. lividus* (individuals above 2 cm) were recorded to the nearest cm with a plastic caliper. Individuals were listed in three size classes: small (2 cm < test diameter ≤ 3 cm), medium (3 cm < test diameter ≤ 5 cm) and large (5 cm < test diameter < 9 cm). Substrates and habitats were analyzed for each site using photo-quadrats. At each depth and within the transect, a quadrat of 1 m^2 was set up in each sub-area and a picture was taken of this quadrat. The proportion in terms of substrates and algal cover were estimated by processing the image using a predetermined scale. Based on our observations, the following classification was chosen for substrates: sloping and falling rocks, slab rocks, boulders, pebbles and cobbles, and sand. The same protocol was followed for algal cover and we used the following classification : encrusting stratum, turfy stratum, shrubby stratum and arborescent stratum (Ruitton *et al*., 2000). A fifth class was also added: "*Posidonia oceanica".*

Data analysis

When normality (Kolmogorov-Smirnov test) and heteroscedasticity (Cochran's test) were not respected, non-parametric tests were used. For the study of temporality, and given the small number of years sampled, the "year" and "season" factors were tested separately using the Mann-Whitney U Test. Spatial and temporal variabilities were tested with a non-parametric PERMANOVA. Abundances in sea urchins were subject to fourth-root transformation and a hierarchical design with three factors was applied: "season" as a fixed factor, "site" as a random factor and "depth" as a fixed factor. A pairwise tests process was employed for the "season", "site" and "depth" factors. *P*-values were obtained, for reach term of the model, with unrestricted permutations of raw data. To determine the preferential repartition of *P. lividus* by size classes in terms of rocky substrates and vegetation cover, we applied a Principal Components Analysis (PCA). The *A. lixula* abundances were also assessed using this method of analysis. PERMANOVAs were carried out using PRIMER software v. 6.1.16 and other tests were made with XLSTAT v. 19.01.

Genetic analysis

Samples collection

Fifty adults and fifty juveniles of purple sea urchins were harvested for genetic analysis per site via scuba diving during the springs of 2013 and 2014, at 9 localities of which 7 were considered for the study of sea urchin population densities (Fig. 1). Two localities of the eastern coast of the island, at the entrance of the Urbinu and Diana lagoons, which are known to have hosted sea urchins, were in fact included instead of Nonza and Tonnara. We considered 3 groups: a group of "Adults" (2013) with a test size was greater than 5 cm in diameter, and 2 cohorts of "Youngs" (2013 and 2014) with a test size of around 2 cm. The dataset has thus been ranked for 9 sites and 3 groups termed "Adults", "Young2013" and "Young2014" (Table 1, Annex).

A few spines were collected from each individual for DNA analyses on the boat using tweezers, then the sea urchins were returned to their local environment. Spines were kept in 96% ethanol and stored in the freezer at –80°C until the DNA extractions were carried out.

Genotyping

Genomic DNA was extracted and purified from spines as described in Coupé *et al*., (2011), and checked for concentration and purity by spectrophotometer analysis at 260 nm and 280 nm (Nanodrop-1000, Amersham). All individuals were genotyped at 5 microsatellite loci (Calderón *et al*., 2009b). Three duplex PCR were performed in a final volume of 25 µL containing 50 ng of DNA, 2.5 µL of 10X PCR buffer (5PRIME; Dutscher, France), 0.1 µM of each primer (Eurogentec), 1.5 mM MgCl2, 0.2 mM of each desoxynucleoside triphosphate (Amersham, Orsay, France) and 0.6 units of Taq DNA polymerase (5PRIME; Dutscher, France). The multiplexing processes for primer pairs were as follows (fluorescent label of the forward primer): Pl_28 (Dragonfly Orange), Pl_Hist (6-FAM) and Pl_T (VIC), Pl_B (PET) and Pl_C (NED). Quality-control measures included a positive control (that also served as the allelic standard) and a negative control (water) for each 96-well plate. Polymerase chain reactions were carried out with an initial denaturation step for 3 minutes at 94°C, followed by 35 cycles of denaturation at 94°C for 50 sec, hybridization for 50 sec at either 53°C (Pl_28 and Pl_B + Pl_C) or 58°C (Pl_Hist + Pl_T), elongation for 50 sec at 72°C, and a final elongation of 10 min at 72°C. Fragments were sized by capillary electrophoresis on a 3730XL genetic analyzer (Applied Biosystems) using the Genescan 600LIZ (Applied Biosystems) as an internal size standard. Peaks were assigned using STRand v. 2.2.30.

Genetic diversity and population genetic differentiation

Genotypes were checked for missing data using GenAIEx v. 6.5 (Peakall & Smouse, 2006, 2012). Individuals with less than 4 loci genotyped loci were removed from the dataset. At the end of the study missing data finally represented less than 5% of the full data for each locus, within each population. The dataset was then tested for null alleles, large allelic dropout and scoring errors with the software Micro-checker v. 2.2.3 (Van-Oosterhout *et al.*, 2004), and the potential influence of suspected null alleles on HWE and population differentiation was assessed with the program FreeNA (Chapuis & Estoup, 2007).

We calculated the genetic diversity for each locus in each population. FSTAT v. 2.9.3 (Goudet, 1995) was used to determine allele diversity (N_A), allelic richness (A_R) and the observed and expected heterozygosity (H_o ad H_e respectively). Linkage disequilibrium among loci were calculated within each population using ARLEQUIN v. 3.5.1.2 (Excoffier & Lischer, 2010). The corrected significance for multiple tests was set using the Benjamini & Hochberg Correction Procedure (Narum, 2006).

We calculated the Inbreeding Coefficient of Weir and Cockerham (F_{IS}) for the global population and within each group using FSTAT v. 2.9.3 (Goudet, 1995) by performing 1 000 genotype randomizations within the sample, and tested for significant deviations from the Hardy-Weinberg Equilibrium (HWE) on both these scales, using the randomization procedure (*P*-values of HWE were calculated through carrying out a precise test with 100 000 steps in the Markov Chain and 1 000 000 dememorization steps) implemented in ARLEQUIN v. 3.5.1.2 (Excoffier & Lischer, 2010).

The distribution of genetic diversity among population and temporal cohorts, was assessed by testing for allele-frequency heterogeneity using an AMOVA, under different scenarios of locality-clustering. Pairwise F_{ST} values were calculated using the Weir & Cockerham (1984) Estimator and the corresponding *P*-values with ARLEQUIN v.3.5.1.2.

Estimation of contemporary and long-term effective population sizes (N_e)

We assessed the effective population size (N_e) for the entire population, from the estimation of Theta using the Brownian microsatellite mutation model implemented in the MIGRATE v. 2.4.1 software (Beerli & Felsenstein, 2001). Each run of MIGRATE was performed with 10 short chains of 10 000 sampled and 500 recorded trees followed by a long chain of 100 000 sampled and 5 000 recorded trees. Theta values were then translated into an estimated effective size N_e, by dividing Theta by 4 and by the mutation rate for microsatellite loci, µ is expected to range between 10^{-2} or to 10^{-5} per locus and generation (Weber & Wong, 1993), though 10^{-3} and 10^{-4} generally account for high and average mutation rates, respectively (Jarne & Lagoda, 1996; Estoup & Angers, 1998). The long-term N_e was estimated at the island scale for the "Adults" and "Youngs" groups.

Contemporary effective population sizes were inferred from the linkage disequilibrium model implemented in NeEstimator v. 2 software, considering only alleles with a frequency were higher than 0.02. Contemporary N_e were assessed at the island and local spatial scales, for both "Adults" and "Youngs" groups. As recommended by Waples & Do (2010), we considered the lower bounds of the 95% CI as more informative N_e estimates.

Contemporary population size changes

To assess a putative impact of overharvesting, we tested for population bottlenecking using the method employed by Cornuet & Luikart (1996) which is based on the loss of rare alleles. Loss of rare alleles results in a reduction of allele diversity and an increase of heterozygosity excess within populations that have experienced a recent and/or low-magnitude bottleneck. Here, heterozygosity excess is defined as the Hardy-Weinberg heterozygosity H_e minus the heterozygosity at the mutation-drift equilibrium H_{eq}, and thus do not refers to the observed H_o. This method also yields a low type I error rate (falsely detecting a bottleneck when there is none). We tested for the ex-

cess of heterozygosity ($H_e > H_{eq}$) using BOTTLENECK v. 1.2.01 (Piry & Cornuet, 1999). Null distributions under mutation-drift equilibrium were determined considering either the Two-Phased Mutation (TPM) model (Di Rienzo *et al.,* 1994), with the proportion of single-step mutation events set at 90% and the variance set at 12% (Pujolar *et al*., 2011) or Single-step Stepwise (SMM) models; the TPM model is actually considered more appropriate for microsatellite loci (Shriver *et al.,* 1993; Di Rienzo *et al.,* 1994; Garza & Williamson, 2001). We used the Wilcoxon signed rank test to determine the significance of excess heterozygosity, as this test is more appropriate when using fewer than 20 microsatellite loci (Piry *et al*., 1999). These analyses were performed for each site for the group of "Adults", the group of pooled "Youngs", and for the entire population.

Results

Spatio-temporal variability of densities of P. lividus *around Corsica*

Average (± standard error [SE]) densities of *P. lividus* are presented by site and by season (Fig. 2). Whatever the season, the most abundant purple sea urchin sites were Sanguinaires, Punta di u catu and Tonnara at 3 m with densities between 0.90 ± 0.05 and 2.18 ± 0.41 individuals.m^{-2}. However, some sites showed very low densities, such as Macinaggio where the maximum density was observed during spring 2013 at 3 m with only 0.07 ± 0.02 individuals.m^{-2} (Fig. 2a). Regarding the depth, densities were very low or sometimes zero at 9 m, as was the case in Albo, Nonza, Isolella and Tonnara in spring 2014 (Fig. 2c). For seasons, maximum abundances in spring 2013 did not exceed 2 individuals.m^{-2}. Size class densities were variable, with the maximum of small and large classes found in autumn 2013 with 0.12 ± 0.01 and 0.74 ± 0.02 individuals.m^{-2} (Fig. 2b) respectively; the maximum medium size class density was observed in autumn 2014 with 0.69 ± 0.03 individuals.m^{-2} (Fig. 2d). Non-parametric Mann-Whitney Tests used to test the temporal variability with the factors "Year" and "Season" showed no significant difference in terms of abundance for any size classes (Mann-Whitney's U Test, $P > 0.05$).

The PERMANOVA showed a spatio-temporal variability in *P. lividus* abundances with significant differences for the factors "season[year]", "site" and "depth" (Table 1). The pairwise test revealed that for the "season*depth" factor, *P. lividus* abundances were significantly higher at 3 m and 6 m for springs 2013 and 2014 (Table 2). In autumn 2013 and 2014, the highest abundances were recorded at 6 m. For the "site*depth" factor, the pairwise test highlighted significantly lower abundances at a depth of 9 m for the Albo, Nonza, Punta di u catu and Tonnara sites whereas for the Sanguinaires site, abundances were significantly higher at 3 m (Table 3). No difference between the three depths was observed for Macinaggio and Vallidone. At depths of 3 m and 6 m Macinaggio had significantly lower abundances than the other sites, whereas, at 9 m, Vallidone and Sanguinaires had significantly higher abundances than the other sites. Significant differences were observed for the "season[year]" factor with the pairwise test for "spring 2013" being significantly lower than that of other seasons (Table 4). The paired-test carried out on the factor "sites" showed that the abundances recorded in Macinaggio were significantly lower than those of other sites. Finally, at 9 m, *P. lividus* abundances were significantly lower than at other depths (Table 4).

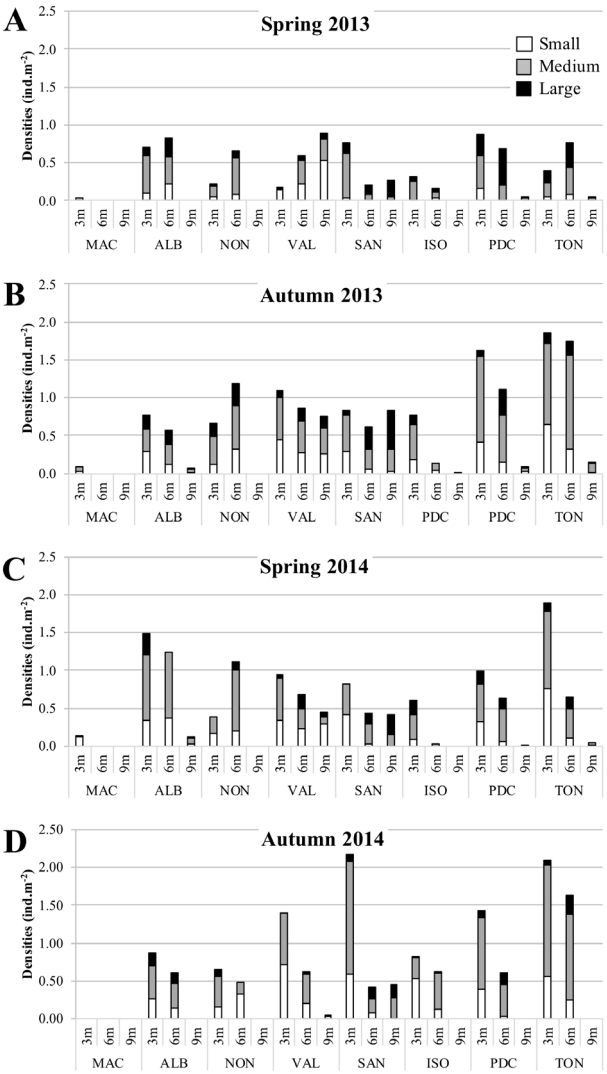

Fig. 2: Variations in the density of *P. lividus* (mean) across sites and depths. Sites are abbreviated as follows: MAC: Macinaggio; ALB: Albo; NON: Nonza; VAL: Vallidone; SAN: Sanguinaires; ISO: Isolella; PDC: Punta di u Catu; TON: Tonnara.

Influence of substrates, vegetation cover and abundances of A. lixula *on the size classes*

PCA was used to study the influence of substrate and

Table 1. PERMANOVA analyses based on Bray-Curtis dissimilarity for sea urchin abundances (significant values are in bold). Terms are abbreviated as follows: df: degree of freedom, MS: mean square.

Source of variation	df	MS	Pseudo-*F*	*P*-values
Season[Year]	3	1601.30	4.321	**0.001**
Site	7	6322.20	16.988	**0.001**
Depth	2	20650.00	11.882	**0.002**
Season*Site	21	370.61	0.996	0.490
Season*Depth	6	750.05	2.015	**0.012**
Site*Depth	14	1738.00	4.670	**0.001**
Residuals	42	372.15		
Total	95			

Table 2. Pairwise test following the PERMANOVA for the "Season*Depth" factor. The values are respectively t (*P*-value), (significant values are in bold).

Spring 2013	**3 m**	**6 m**	**9 m**
3 m			
6 m	1.018 (0.770)		
9 m	**3.518 (0.048)**	**4.536 (0.007)**	

Autumn 2013	**3 m**	**6 m**	**9 m**
3 m			
6 m	**7.179 (0.001)**		
9 m	3.643 (0.169)	**10.821 (<0.0001)**	

Spring 2014	**3 m**	**6 m**	**9 m**
3 m			
6 m	2.268 (0.429)		
9 m	**5.911 (0.004)**	**8.179 (<0.0001)**	

Autumn 2014	**3 m**	**6 m**	**9 m**
3 m			
6 m	**6.018 (0.029)**		
9 m	**5.714 (0.040)**	**11.732 (<0.0001)**	

3m	**Spring13**	**Autumn13**	**Spring14**	**Autumn14**
Spring13				
Autumn13	0.215 (0.632)			
Spring14	0.257 (0.692)	0.043 (0.855)		
Autumn14	0.059 (0.800)	0.198 (0.676)	0.155 (0.509)	

6m	**Spring13**	**Autumn13**	**Spring14**	**Autumn14**
Spring13				
Autumn13	**0.772 (0.014)**			
Spring14	0.353 (0.506)	0.419 (0.353)		
Autumn14	0.363 (0.483)	0.410 (0.374)	0.009 (1.000)	

9m	**Spring13**	**Autumn13**	**Spring14**	**Autumn14**
Spring13				
Autumn13	0.338 (0.142)			
Spring14	0.065 (0.714)	0.272 (0.127)		
Autumn14	0.023 (0.896)	0.361 (0.181)	0.089 (0.872)	

Table 3. Pairwise test following the PERMANOVA for the "Site*Depth" factor. The values are respectively t (*P*-value), (significant values are in bold).

MAC	3m	6m	9m
3m			
6m	2.143 (0.137)		
9m	2.173 (0.136)	2.321 (0.137)	

3m	MAC	ALB	NON	VAL	SAN	ISO	PDC	TON
MAC								
ALB	**1.866 (<0.0001)**							
NON	**1.156 (0.011)**	0.710 (0.365)						
VAL	**1.291 (0.002)**	0.574 (0.642)	0.135 (1.000)					
SAN	**1.548 (<0.0001)**	0.318 (0.977)	0.392 (0.930)	0.256 (0.994)				
ISO	**1.188 (0.008)**	0.678 (0.427)	0.032 (1.000)	0.104 (1.000)	0.360 (0.955)			
PDC	**1.935 (<0.0001)**	0.070 (1.000)	0.779 (0.247)	0.644 (0.496)	0.388 (0.933)	0.747 (0.298)		
TON	**1.808 (<0.0001)**	0.057 (1.000)	0.652 (0.479)	0.517 (0.755)	0.261 (0.993)	0.620 (0.546)	0.127 (1.000)	

ALB	3m	6m	9m
3m			
6m	0.439 (0.905)		
9m	**3.107 (0.027)**	**3.003 (0.033)**	

NON	3m	6 m	9 m
3m			
6m	1.017 (0.456)		
9m	**7.478 (0.022)**	**7.082 (0.030)**	

6m	MAC	ALB	NON	VAL	SAN	ISO	PDC	TON
MAC								
ALB	**1.885 (<0.0001)**							
NON	**1.774 (<0.0001)**	0.111 (1.000)						
VAL	**1.932 (<0.0001)**	0.047 (1.000)	**1.774 (<0.0001)**					
SAN	**1.433 (<0.0001)**	0.685 (0.277)	0.341 (0.941)	0.499 (0.684)				
ISO	0.748 (0.178)	**1.137 (0.003)**	**1.026 (0.013)**	**1.184 (0.002)**	0.685 (0.277)			
PDC	**1.784 (<0.0001)**	0.101 (1.000)	0.010 (1.000)	0.148 (1.000)	0.351 (0.931)	**1.036 (0.011)**		
TON	**2.124(<0.0001)**	0.239 (0.992)	0.350 (0.932)	0.192 (0.998)	0.691 (0.267)	**1.376 (0.000)**	0.340 (0.942)	

VAL	3 m	6 m	9 m
3m			
6m	1.052 (0.416)		
9m	0.821 (0.541)	1.012 (0.380)	

SAN	3m	6m	9m
3m			
6m	**2.473 (0.031)**		
9 m	**2.952 (0.034)**	0.893 (0.515)	

9m	MAC	ALB	NON	VAL	SAN	ISO	PDC	TON
MAC								
ALB	0.299 (0.729)							
NON	0.000 (1.000)	0.299 (0.729)						
VAL	**1.451 (<0.0001)**	**1.152 (<0.0001)**	**1.451 (<0.0001)**					
SAN	**1.358 (<0.0001)**	**1.059 (<0.0001)**	**1.358 (<0.0001)**	0.093 (1.000)				
ISO	0.025 (1.000)	0.274 (0.808)	0.025 (1.000)	**1.426 (<0.0001)**	**1.334 (<0.0001)**			
PDC	0.287 (0.769)	0.012 (1.000)	0.287 (0.769)	**1.164 (<0.0001)**	**1.072 (<0.0001)**	0.262 (0.842)		
TON	0.344 (0.567)	1.059 (<0.0001)	0.344 (0.567)	**1.108 (<0.0001)**	**1.015 (<0.0001)**	0.319 (0.659)	0.057 (1.000)	

ISO	3m	6m	9m
3m			
6m	1.576 (0.081)		
9m	**5.794 (0.031)**	2.412 (0.057)	

(continued)

Table 3 continued

PDC	3m	6m	9m
3m			
6m	2.061 (0.059)		
9m	**3.954 (0.032)**	**3.437 (0.043)**	

TON	3m	6m	9m
3m			
6m	1.414 (0.178)		
9m	**3.258 (0.031)**	**3.678 (0.025)**	

Table 4. Pairwise tests following the PERMANOVA for the "Season", "Site" and "Depth" factors. The values are respectively t (*P*-value), (significant values are in bold). Sites are abbreviated as follows: MAC: Macinaggio; ALB: Albo; NON: Nonza; VAL: Vallidone; SAN: Sanguinaires; ISO: Isolella; PDC: Punta di u Catu; TON: Tonnara.

Season	Spring13	Autumn13	Spring14	Autumn14
Spring13				
Autumn13	**2.123 (<0.018)**			
Spring14	**2.282 (0.013)**	1.310 (0.151)		
Autumn14	**2.607 (0.006)**	**2.431 (0.011)**	1.388 (0.156)	

Site	MAC	ALB	NON	VAL	SAN	ISO	PDC	TON
MAC								
ALB	**5.624 (0.002)**							
NON	**5.903 (0.001)**	1.336 (0.172)						
VAL	**6.536 (0.001)**	1.510 (0.087)	**2.787 (0.014)**					
SAN	**11.300 (0.001)**	**2.359 (0.030)**	**6.700 (0.001)**	**3.095 (0.004)**				
ISO	**4.663 (0.002)**	**2.955 (0.004)**	1.355 (0.193)	**3.650 (0.002)**	**4.713 (0.001)**			
PDC	**6.513 (0.001)**	0.944 (0.444)	**2.147 (0.043)**	**2.919 (0.004)**	1.840 (0.090)	**2.993 (0.008)**		
TON	**6.357 (0.001)**	1.090 (0.342)	**2.099 (0.037)**	**2.790 (0.005)**	**2.223 (0.012)**	**3.511 (0.006)**	1.154 (0.292)	

Depth	3m	6m	9m
3m			
6m	1.701 (0.053)		
9m	**3.967 (0.002)**	**3.388 (0.013)**	

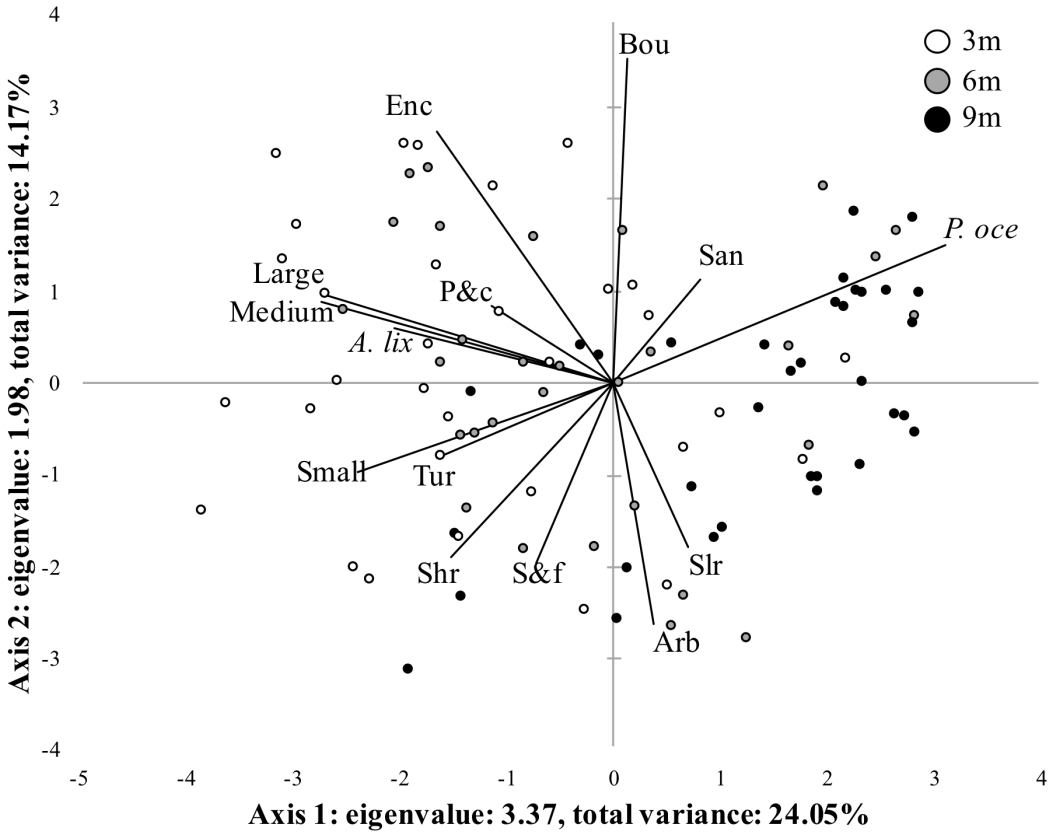

Fig. 3: PCA (Principal Component Analysis) Relationships between vegetal cover, rocky substrates, abundances of *A. lixula* and *P. lividus*. Factors are abbreviated as follows: Enc: encrusting vegetal cover; Tur: turfy vegetal cover; Shr: shrubby vegetal cover; Arb: arborescent vegetal cover; *P. oce*: *P. oceanica*; S&f: slopping and falling rocks; Slr: slab rocks; Bou: boulders; P&c: pebbles and cobbles; San: sand, *A. lix*: *A. lixula.*

vegetation cover on abundances of *A. lixula* and *P. lividus* by size classes (Fig. 3). The first 2 axes explain 38.2% of the total variability. The first axis separates the arborescent substrate, *P. oceanica* and sand from both sea urchin species (all size classes) and the other substrates. The second axis separates small *P. lividus* individuals from other sea urchins and intermediate substrates from encrusting substrate. Small *P. lividus* individuals have a strong positive correlation with the shrubby substratum (0.456; $P < 0.01$) (Table 2, 3, Annex). Medium and large individuals are positively correlated with the encrusting substratum (respectively: 0.408; $P < 0.01$ and 0.566; $P < 0.01$). The medium size class is also correlated with turfy substratum (0.300; $P < 0.01$). Finally, *A. lixula* is positively correlated with encrusting substratum (0.326; $P < 0.01$). In contrast, *P. oceanica* is negatively correlated with the presence of all sea urchins. No correlation was found between either sea urchin species and rocky substrates.

Genetic analysis

A total of 992 individuals were considered after we discarded the groups of "Adults" at the Macinaggio and Urbinu sites because we could not genotype a sufficient number of individuals (Table 1, Annex).

Analysis of the whole dataset did not show scoring errors due to large allele dropout or stuttering, but revealed significant values ($P < 0.05$) for potential null alleles. Indeed, high F_{IS} estimates were found to affect all loci and all groups, and were associated with significant deviations of the populations from the Hardy-Weinberg Equilibrium (Table 4, Annex). When considering null alleles, F_{IS} estimates were consistently reduced, with an overall $F_{IS} = 0.039$ (Table 5, Annex), indicating that HWD were likely the consequence of null alleles. Ten loci pairs out of 240 tested still displayed significant linkage disequilibrium after B-H correction, and we did not find similar patterns of LD among groups, suggesting that loci are independent.

All loci were found to be polymorphic, with the total number of alleles ranging from 13 to 31 (mean = 21.9; standard deviation SD = 3.6). The observed heterozygosity ranged from 0.379 to 1 (mean = 0.760; SD = 0.145) and was lower than the expected heterozygosity which

ranged from 0.886 to 0.961 (mean = 0.930; SD = 0.188). Genetic diversity indices were found to be highly homogeneous among groups as a one-way ANOVA did not reveal any difference among groups ($P > 0.5$), indicating that genetic diversity is maintained over generations (Table 4, Annex). Specifically, within groups of adults, the allelic richness ranged from 6.945 to 8.426 and the H_o and H_e ranged from 0.5 to 0.952 and from 0.890 to 0.961 respectively. Within groups of youngs, the allelic richness ranged from 6.553 to 8.413 and the H_o and H_e ranged from 0.442 to 0.979 and from 0.891 to 0.954 respectively.

Population genetic structure

The AMOVA analysis performed on all groups showed a weak but significant overall population structure (F_{ST} = 0.0037; P = 0.00098) and revealed that the genetic diversity was mostly distributed within the area (Table 6, Annex). The clustering of northern, southern and eastern areas into 3 groups did not result in more variation among groups.

After applying B-H correction for multiple comparisons, we observed a weak and non-significant pairwise genetic differentiation, within the group of Adults and the two groups of Youngs, and between Adults and Youngs for each population (Tables 7, 8, 9, Annex).

Demographic history inferences

Considering the absence of genetic structure across the island, long-term effective population sizes were estimated from the whole populations of Adults and Youngs. Based on the calculation of Theta (Table 10, Annex), we estimated the long-term N_e at 2 452 and 1 532 individuals for the populations of "Adults" or "Youngs", respectively, for a mutation rate fixed at 10^{-3}. Contemporary N_e estimates for the populations of "Adults" and "Youngs" across the island, were 15.620 and 6.538 individuals, respectively, with quite similar lower bound estimates of the 95% CI and infinite upper bounds (Table 10, Annex). Infinite upper bounds appeared consistent with the absence of significant linkage disequilibrium found by ARLEQUIN v. 3.5.1.2 (Waples & Do, 2010).

Local contemporary estimates of N_e were homogeneous for both populations of "Adults" (Mean = 302.9; CI: 197.7 – 408.0) and "Youngs" (Mean = 289.3; CI: 159.4 – 419.2). No difference was observed between the populations of "Youngs" and "Adults" at each site (Mann-Whitney Test: P = 0.966). Interestingly, populations of "Adults" and "Youngs" presenting the lowest N_e estimates are located at the extreme north and south of the Island (Table 10, Annex).

The bottleneck analysis performed did not reveal any significant excess in expected heterozygosity either on the island and local spatial scales, suggesting that the populations have not undergone a recent demographic erosion (Table 10, Annex). We could not find any relation between the contemporary N_e and the corresponding population densities.

Discussion

Spatio-temporal dynamics

Spatio-temporal variability of abundances was observed between seasons. For spring 2013, densities were significantly lower than in other seasons regardless of the size of *P. lividus* individuals (Fig. 2., Table 1). We nevertheless note that this difference is more pronounced in the medium size class (3 to 5 cm). Several authors have described fluctuations over short-term studies: the significant decrease in densities between 1979 and 1980 in Port Cross (France) (Azzolina, 1987) or the rapid increase between 1991 and 1992 in Italy (Benedetti-Cecchi & Cinelli, 1995). Moreover, Sala & Zabala (1996) showed that populations of purple sea urchins in the sublittoral zones in the Northwest of the Mediterranean Sea are very dynamic. Variations in *P. lividus* abundances were observed according to site and depth ("site*depth" factor) (Table 3). For the majority of sites, the lowest abundances were observed at 9 m and the vegetation cover at this depth is dominated by dense meadows or by *P. oceanica* (personal observation). This is consistent with the work of Romero *et al*., (1999) who did not observe *P. lividus* at 10 m depth. Tomas *et al.* (2004) also described a decrease in purple sea urchin abundances in seagrass meadows, they assumed that the low recruitment observed below 4 m depth combined with other factors could explain this phenomenon.

Our results highlighted a spatial variability of abundances in *P. lividus* populations within sites, and especially for the Macinaggio site which has the lowest densities (Fig. 2). In the past, this last site was known to be abundant in purple sea urchins and both professional and recreational fishing were developed there. It was difficult to assess the decline of *P. lividus* stocks in Macinaggio because there was no previous scientific data for this site. Nevertheless, according to professional fishermen and our personal observations, this site has no pollution and no habitat degradation has been observed. The most plausible hypothesis is that of overfishing combined with the fact that the area is not conducive to the arrival of new settlers. Indeed, smaller individuals are not even listed and the importance of settlement on the spatial distribution of *P. lividus* populations is well known (Tomas *et al*., 2004). Sea urchin populations in high pressure sites are highly dependent on abundances of individuals with test diameters between 3 and 5 cm (Loi *et al*., 2017). As fishing is prohibited for these individuals, their presence within a site probably reflects the existence of a larval supply. This was certainly the case in Macinaggio because the few *P. lividus* recorded were smaller than 5 cm (Fig. 1, Annex). Tonnara, Punta di u catu and Sanguinaires are familiar sites for fishermen yet it is within these sites that we have identified the highest abundances of *P. lividus*. Our results reveal a unimodal size-distribution with the dominant

mode corresponding to commercial size classes for these sites even after the fishing period. Hereu *et al*. (2012) also observed differences in densities on a small spatial scale, and advanced several hypotheses: the first indicates that the presence of various habitats favors the presence of sea urchins, the second advances that topography also plays a major role. In addition, the "medium" size class has the highest abundances at all sites and especially during autumn seasons (Fig. 1, Annex). Jacinto *et al*. (2013) have described a similar pattern in the Northwestern of Italy, they also affirm that the depth influences densities. Our data support this theory because the highest abundances were observed at 3 m and 6 m, while the lowest and zero rates were recorded at 9 m.

Although temporal variability was found in our study, it was difficult to conclude on the influence of fishing on purple sea urchin stocks (Tables 1, 4). However, in the two years sampled, we did not find evidence of the effect of fishing on *P.* lividus abundances. This impact has already been described in Sardinia (Pais *et al*., 2012) and in the North of Portugal (Bertocci *et al*., 2014). In Corsica, sea urchin harvesting is permitted from December to April and our sampling only took into account one fishing season (between autumn 2013 and spring 2014). It can be assumed that demographic maintenance through larval supply associated with fisheries regulation is effective in Corsica. Nonetheless, to verify this parameter, a long-term study should should be carried out and such data would be very important for the management of this species in Corsica. Some authors have indeed compared abundances of *P. lividus* in protected areas and in areas where fishing is authorized (Guidetti & Mori, 2005; Gianguzza *et al*., 2006; Pais *et al*., 2007; Ceccherelli *et al*., 2009, 2011; Pais *et al*., 2012).

Influence of substrate, vegetation cover and abundances of A. lixula

We did not observe any correlation between purple sea urchin abundance and rocky substrates whereas *P. lividus* is commonly found on boulders and solid rocks (Boudouresque & Verlaque, 2001) (Fig. 3, Table 2, Annex). In addition, wall height has little effect on the population structure of *P. lividus* and *A. lixula* (Davis *et al*., 2003). Regarding the vegetal cover, our results showed that this latter factor impacts the size class distribution of *P. lividus*. Small individuals seem to prefer macrophytes with an intermediate height, whereas medium and large size classes were found on substrates of low heights. We note that *P. lividus* was not or was very little observed in the *P. oceanica* seagrass. Furthermore, we recorded very low densities at 9 m where *P. oceanica* meadows dominated (data not shown). This is consistent with the work of Tomas *et al*. (2004), which showed that adult populations decrease significantly in *P. oceanica* as the depth increases. However, this is contrary to the findings of Boudouresque *et al*. (1992), carried out in Corsica at 3 m and 6 m in depth, where the presence of *P. lividus* is in fact described on this seagrass. Pinna *et al*. (2012) also showed a preference of *P. lividus* for *P. oceanica* in comparison with rocky substrate. It is well known that *P. lividus* acquires an adult diet at 1 cm (test diameter) with preferences for brown algae and for epiphytes present in *P. oceanica* meadows (Tomas *et al*., 2006). Knowing this information, it seems unlikely that in our study the distribution of sea urchins according to their size class on different vegetal covers is related to their diet. However, predation may explain this distribution. Guidetti & Mori (2005) have shown that small individuals were more subject to predation and that to compensate their low morpho-functional defenses, they seek shelters. In our case, these small individuals are found in shrubby substrate, which may indicate a cryptic behavior. The acquisition of a large size increase the defence of individuals against predators (Tegner & Dayton, 1981; Sala & Zabala, 1996; Sala, 1997 and references therein). These individuals are not then obliged to adopt an antipredator strategy, which could explain their presence in vegetal covers of low height.

Regarding *A. lixula*, we observed this species on encrusting substrate alongside medium and large classes of *P. lividus*. It is commonly accepted that these two species can coexist in the Mediterranean Sea, even if each has its own preferences in terms of geographical distribution, microhabitat (Boudouresque & Verlaque, 2001) and food (Privitera *et al*., 2008). It should be noted that *A. lixula* occupies a higher trophic level than *P. lividus* because it must be considered as an omnivore with a carnivorous tendency, whereas *P. lividus* is mainly herbivorous (Wangensteen *et al*., 2011; Agnetta *et al*., 2013). However both species can cause overgrazing and induce the transformation of macroalgal beds to coralline barrens (Bulleri *et al*., 1999; Bertocci *et al*., 2012; Bonaviri *et al*., 2012), and both species would be complementary in maintaining of barren grounds in the Mediterranean Sea (Privitera *et al*., 2008). Yet this seems unlikely to be the case in our study due to the low abundances observed. Indeed, in Corsica, overgrazing is observed when densities range between 7 to 17 individuals.m^{-2} (5 cm) (Verlaque, 1987). Our study showed the presence of *A. lixula* and *P. lividus* in encrusting substrates. When densities of these two species are high, they can transform macroalgae beds into bare rocks with encrusting algae (Tegner & Dayton, 1981; Sala *et al*., 1998). Palacin *et al*. (1998) showed that abundances of *P. lividus* < 5 individuals.m^{-2} can influence the evolution of macrophytic communities. In our study, the *P. lividus* abundances recorded are fewer than 5 individuals.m^{-2}. When *P. lividus* is present at low densities, it seems unable to transform benthic communities, however, associated with another herbivorous species, it may have the ability to maintain an encrusting substrate (Ruitton *et al*., 2000). Although *A. lixula* has a predominantly omnivorous diet with a carnivorous tendency (Wangensteen *et al*., 2011; Agnetta *et al*., 2013) it can graze encrusting coralline algae (Boudouresque & Verlaque, 2001). Therefore, the presence of *P. lividus* combined

with that of *A. lixula* would explain their presence on the encrusting substrate.

Genetic study

A genetic analysis has been performed to give additional insights into the natural *P. lividus* population across the Corsican island. For this purpose we considered 5 microsatellites described by Calderón *et al*. (2009a, b) and further used by Couvray *et al*. (2015, 2018).

In these previous studies, the distribution of genetic diversity was examined on both the spatial scales and across successive temporal cohorts along the Iberian and the south-eastern French coasts. Quite similar allelic diversity and inbreeding coefficients (F_{IS}) were evidenced for all microsatellites (Table 4, Annex). Deviations from the Hardy-Weinberg Equilibrium were explained by a likely assortative mating (Calderón *et al*., 2009a; Couvray & Coupé, 2018) and the presence of potential null alleles (Couvray & Coupé, 2018). In addition, these authors highlighted that most of the genetic diversity (> 99%) was found either within a population (i.e. on a spatial scale) or cohort (i.e. on a temporal scale), and that low of absent pairwise genetic differentiation (F_{ST}) could be observed, evidencing that populations remained highly genetically homogeneous across generations. Recently, quite similar results were obtained using ten microsatellites on the sea urchin *A. lixula* (Pérez-Portela *et al*., 2018). Our results are quite consistent with those previously reported, that is, demonstrating the absence of a genetic structure and the steady-state genetic diversity over time within populations, at each locality. Thus, the Corsican *P. lividus* population dynamics are rather like those of the *P. lividus* population in other regions of the NW Mediterranean Sea, and behaves, from a genetic point of view, as a panmictic population, consistent with the larval potential of dispersion and the continuum of populations along the coast.

From a demographic view, we found relatively large long-term and contemporary effective population sizes (Table 10, Annex). Indeed, contemporary estimates of N_e, across the island were 15 620 for the group of "Adults" and 6 538 for the group of "Youngs". The difference between the two groups is likely related to the number of temporal cohorts that compose the group of "Adults". Such estimates have not been reported yet across such a spatial scale in *P. lividus* species. Although many factors, such as the dimension of the sample and the number of loci used for the Wahlund effect, can make reliable effective population sizes difficult to assess (Allendorf *et al*., 2008; Hare *et al*., 2011; Macbeth *et al*., 2013; Ovenden *et al*., 2016), Calderón *et al*. (2009b) predicted that such high estimates should indeed be expected in a study based on sufficient populations of *P. lividus*, sampled over a large spatial scale. Moreover, our results are also similar to the contemporary N_e inferred in the *Tripneustes gratilla* sea urchin (Casilagan *et al*., 2013). At local spatial scales, contemporary N_e estimates are in accordance with the ones calculated by Calderon *et al*. (2009b), and more recently for *A. lixula* (Pérez-Portela *et al*., 2018), in Iberic local populations.

Long-term N_e estimates were consistent with contemporary ones, considering mutation rates that would range from 10^{-3} to 10^{-4}, with a difference between the groups of "Adults" and "Youngs" similar to the one found for contemporary estimates (Table 10, Annex). The long-term N_e represents the harmonic mean of N_e, over a period of around $4N_e$ generations (Beerli, 2009). Thus, depending on the mutation rates, long-term N_e reflects the averaged N_e from 9 800 to 98 000 generations ago, likely after the expansion of the species into the Mediterranean Sea, estimated 46 000 and 101 000 generations ago, based on COI mitochondrial markers (Duran *et al*., 2004).

Hence, the consistency between long-term and contemporary N_e estimates, the absence of a bottleneck on an island scale that would have occured 0.4 to $4N_e$ generations ago (Cornuet & Luikart, 1996) indicate that the Corsican population of *P. lividus* likely have not experienced significant demographic changes during contemporary times.

Moreover, the similar estimates of the local contemporary N_e and constant genetic diversity, observed between the groups of "Adults" and "Youngs", at each site over the course of this study suggests that either the species is actually not overharvested (Allendorf *et al*., 2008; Pinsky & Palumbi, 2013), or that the current level of harvesting, at the metapopulation scale encompassing the Corsican island, has no impact on the genetic diversity.

The calculated N_e estimates likely do not reflect the census size, which is expected to be several orders of magnitude higher in highly fecund and abundant marine species (Plough *et al*., 2016), such as the prawn *Penaeus esculantus* (Ovenden *et al*., 2007) for instance. Nor do they reflect the relative abundances observed between sites. However, it is noteworthy that the lowest N_e values were found at Macinaggio, were abundances were the lowest. In a more general view, the lowest N_e estimates were situated in the North and the South of the island, potentially resulting from specific hydrodynamic features that would potentially reduce the occurrence of recruitment at those places.

This work was mainly aimed at establishing a first inventory of the abundances of *P. lividus* around Corsica. It has revealed spatial and temporal variabilities and the abundances listed are similar to those described in previous works, with the exception of the Macinaggio site which has very low abundances. From a resource conservation point, we recommend the continuation of this monitoring to better identify the factors responsible for the evolution of abundances and to observe the real impact of the harvesting on wild stocks. The genetic data rather indicate a healthy population on the island spatial scale, as already suggested in other Mediterranean regions (Duran *et al*. 2004), and a limited, if any, impact of harvesting on population. What is of note is the fact that the difference in N_e estimates could be an indication of

contrasted population dynamics occurring on a fine scale that should be further studied through genetic and physical approaches.

Acknowledgements

This research was funded by the "Fonds Européen de Développement Economique et Régional" (FEDER), the "Collectivité Territoriale de Corse" (CTC) and the "Université de Corse Pasquale Paoli" (UCPP). The authors are grateful to the team of the "Réserve Naturelle des Bouches de Bonifacio" (RNBB) and the "Comité Régional des Pêches Maritimes et des Elevages Marins de Corse" (CRPMEM) for their collaboration. We would like to thank Dr. Sandrine Gaillard (PFT Biotechservices, France) for carrying out genotyping. Special thanks to team of scuba divers of the UMS 3514 Stella Mare for their efforts during the underwater surveys and especially Romain Bastien for field management.

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ANNEX

Table 1. Summary table of site abbreviations, temporal-cohorts and populations.

Site	Abbreviation	Temporal-cohort	Population abbreviation
Macinaggio	MAC	Young 2013	MAC13Y
		Young 2014	MAC14Y
Albo	ALB	Adult	ALBA
		Young 2013	ALB13Y
		Young 2014	ALB14Y
Vallidone	VAL	Adult	VALA
		Young 2013	VAL13Y
		Young 2014	VAL14Y
Sanguinaires	SAN	Adult	SANA
		Young 2013	SAN13Y
		Young 2014	SAN14Y
Isolella	ISO	Adult	ISOA
		Young 2013	ISO13Y
		Young 2014	ISO14Y
Punta di u catu	PDC	Adult	PDCA
		Young 2013	PDC13Y
		Young 2014	PDC14Y
Diana	DIA	Adult	DIA14A
		Young 2014	DIA14Y
Urbinu	URB	Adult	URB14A
		Young 2014	URB14Y
Outer Urbinu	URBO	Adult	URBO14A
		Young 2014	URBO14Y

Table 2. PCA (Pearson) Correlation matrix. Significant values are in bold, for α set to 0.05. Factors are abbreviated as follows: *A. lix*: *A. lixula*, Enc: encrusting vegetal cover; Tur: turfy vegetal cover; Shr: shrubby vegetal cover; Arb: arborescent vegetal cover; *P. oce*: *P. oceanica*; S&f: slopping and falling rocks; Slr: slab rocks; Bou: boulders; P&c: pebbles and cobbles; San: sand.

	Small	**Medium**	**Large**	***A. lix***	**Enc**	**Tur**	**Shr**	**Arb**	***P. oce***	**S&f**	**Slr**	**Bou**	**P&c**	**San**
Small														
Medium	**0.526**													
Large	**0.245**	**0.547**												
A. lix	**0.236**	**0.227**	**0.449**											
Enc	0.096	**0.408**	**0.566**	**0.326**										
Tur	**0.294**	**0.300**	0.198	0.089	**-0.238**									
Shr	**0.456**	0.126	-0.007	0.190	-0.162	0.06								
Arb	-0.119	-0.147	0.032	-0.031	**-0.281**	-0.155	-0.126							
P.oce	**-0.489**	**-0.488**	**-0.561**	**-0.397**	**-0.269**	**-0.465**	**-0.487**	**-0.259**						
S&f	0.085	0.008	0.174	0.076	-0.071	0.131	0.067	0.091	-0.142					
Slr	0.027	-0.073	-0.172	-0.097	-0.034	**-0.219**	0.03	**0.239**	0.006	**-0.412**				
Bou	-0.083	0.072	-0.011	0.089	0.109	0.009	-0.156	**-0.281**	0.195	**-0.464**	**-0.441**			
P&c	0.053	0.187	0.169	0.022	0.174	0.070	**0.220**	-0.093	**-0.254**	-0.174	-0.114	0.054		
San	-0.05	-0.088	-0.079	-0.114	0.108	-0.169	-0.165	-0.103	**0.202**	-0.107	-0.003	-0.060	-0.061	

Table 3. Contribution of variables on axes (%). Factors are abbreviated as follows: *A. lix*: *A. lixula*; Enc: encrusting vegetal cover; Tur: turfy vegetal cover; Shr: shrubby vegetal cover; Arb: arborescent vegetal cover; *P. oce*: *P. oceanica*; S&f: slopping and falling rocks; Slr: slab rocks; Bou: boulders; P&c: pebbles and cobbles; San: sand.

	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11	F12	F13	F14
Small	12.559	2.059	0.330	6.216	10.812	4.523	2.807	0.069	13.180	13.973	27.190	6.028	0.254	0.000
Medium	16.511	1.706	0.437	0.356	0.700	9.736	2.138	4.503	11.656	3.913	37.002	11.251	0.092	0.000
Large	16.541	2.095	3.950	9.201	1.543	0.139	0.892	0.000	0.102	3.381	3.305	58.828	0.022	0.000
A. lix	9.297	0.764	1.116	1.697	1.706	2.258	30.775	9.721	18.145	20.750	1.626	1.968	0.176	0.000
Enc	6.081	16.511	16.147	2.834	1.511	1.518	0.002	5.165	0.048	6.189	15.506	10.129	0.027	18.332
Tur	6.001	1.488	19.126	0.182	0.870	22.093	7.111	0.617	23.334	0.420	0.945	0.367	0.317	17.130
Shr	5.172	7.935	1.864	16.761	4.014	18.956	5.945	1.019	0.698	14.535	8.061	0.339	0.109	14.592
Arb	0.296	15.219	7.788	3.819	23.530	0.243	1.133	14.849	16.176	0.900	0.222	0.799	0.023	15.002
P. oce	21.010	5.021	0.616	0.195	2.541	0.006	0.947	4.717	0.119	16.986	4.017	8.710	0.177	34.938
S&f	1.143	8.351	6.613	34.706	5.970	5.150	0.017	3.061	0.718	1.245	0.020	0.940	32.065	0.000
Slr	1.072	7.030	31.174	11.084	0.010	5.963	0.516	4.490	6.760	0.185	0.218	0.463	31.034	0.000
Bou	0.037	27.516	7.316	3.417	10.134	0.879	4.626	4.589	7.848	1.168	0.284	0.016	32.170	0.001
P&c	2.869	1.520	0.004	9.146	2.876	27.755	36.035	0.000	1.054	16.287	0.469	0.123	1.862	0.000
San	1.410	2.785	3.519	0.385	33.782	0.781	7.057	47.200	0.161	0.069	1.133	0.038	1.674	0.004

Table 5. Overall F_{IS} estimates considering null alleles or not.

Locus	With NA	Without NA
Pl_28	0.245	0.035
Pl_Hist	0.071	n.d.
Pl_T	0.149	0.017
Pl_B	0.408	0.029
Pl_C	-0.004	n.d.
All	0.256	0.039

Table 6. AMOVA analysis for all combined populations and by group, with all loci (significant values are in bold).

Analysis of all populations combined				
	Source of variation	Sum of squares	Variance components	Percentage variation
F_{ST}	Among populations	28.768	0.00309	0.13204
	Within population	4652.018	2.33453	**99.86796**
	Total	4680.786	2.33762	
		F_{ST} = 0.00132; *P*-value = 0.00000; 1023 perm.		

Analysis by group				
	Source of variation	Sum of squares	Variance components	Percentage variation
F_{ST}	Among groups	28.768	-0.00213	-0.09108
	Among populations within groups	41.476	0.01076	0.4605
	Within populations	4610.542	2.3289	**99.63058**
	Total	4680.786	2.33753	
		F_{ST} = 0.00369; *P*-value = 0.00098; 1023 perm.		

Table 4. Summary of genetic characteristics by site and by temporal-cohorts. H_o and H_e: observed and expected heterozygosity; F_{IS}: deficit of heterozygotes within populations; HW (*P*-value): deviance from Hardy-Weinberg Equilibrium (significant values are in bold). **P*-value < 0.05, ***P*-value < 0.01.

		Location /cohort																					
		ALBA	ALB13Y	ALB14Y	VALA	VAL13Y	VAL14Y	SANA	SAN13Y	SAN14Y	ISOA	ISO13Y	ISO14Y	PDCA	PDC13Y	PDC14Y	URB14Y	URBOA	URBO14Y	DIA14A	DIA14Y	MA-C14Y	All
Locus	Parameter	(N=24)	(N=44)	(N=32)	(N=86)	(N=44)	(N=48)	(N=76)	(N=45)	(N=48)	(N=86)	(N=35)	(N=46)	(N=37)	(N=46)	(N=48)	(N=46)	(N=41)	(N=44)	(N=46)	(N=45)	(N=25)	(N=992)
Pl_28	**No. of alleles**	23	26	20	25	22	23	26	24	27	31	24	22	24	23	24	22	24	23	26	23	22	39
	Allelic richness	8.185	8.413	7.625	7.807	8.102	8.126	8.057	7.942	8.168	8.026	8.350	8.000	7.624	8.083	7.924	7.981	7.887	7.825	8.193	7.803	8.053	38.987
	H_o	0.750	0.90909	0.50000	0.634	0.698	0.761	0.747	0.659	0.729	0.65854	0.73333	0.75556	0.595	0.62222	0.54167	0.674	0.829	0.841	0.81818	0.77778	0.65517	0.70700
	H_e	0.953	0.96082	0.93552	0.942	0.952	0.953	0.949	0.946	0.954	0.94688	0.95876	0.94906	0.919	0.95106	0.94474	0.948	0.944	0.941	0.95324	0.94107	0.94979	0.95245
	F_{IS}	**0.217****	0.054	**0.470****	**0.328****	**0.27****	**0.203****	**0.215****	**0.306****	**0.237****	**0.306****	**0.238****	**0.206****	**0.353****	**0.348****	**0.429****	**0.291****	0.123	**0.108****	**0.143****	0.175*	**0.314****	0.245
Pl_Hist	**No. of alleles**	22	24	21	30	25	26	26	26	26	27	24	25	20	25	26	24	20	25	27	22	23	37
	Allelic richness	8.426	8.133	8.109	7.998	8.147	8.112	7.739	7.987	8.008	7.914	8.348	8.056	7.285	8.191	8.247	8.084	7.956	8.069	8.123	7.707	8.163	36.920
	H_o	0.78261	0.93182	0.93750	0.817	0.909	0.833	0.808	0.909	0.854	0.83529	0.90000	0.84783	0.848	0.91304	0.89362	0.891	0.850	0.884	0.76087	0.81818	0.93333	0.86395
	H_e	0.96135	0.95246	0.95188	0.947	0.952	0.952	0.939	0.947	0.946	0.94347	0.95876	0.94935	0.914	0.95318	0.95585	0.951	0.948	0.950	0.95031	0.93730	0.95311	0.95030
	F_{IS}	**0.189****	0.022	0.015	**0.138****	0.046	**0.126***	**0.14****	**0.04***	**0.098***	**0.115****	0.062	**0.108****	0.071	0.043	0.066	**0.063***	**0.104****	0.071	**0.201****	**0.128***	0.021	0.071
Pl_T	**No. of alleles**	16	23	21	24	21	23	24	19	22	30	17	24	22	20	21	18	19	24	23	20	19	50
	Allelic richness	7.607	7.659	7.661	7.435	7.484	7.514	7.420	7.556	7.642	7.502	7.332	7.908	7.052	7.676	7.446	7.430	7.831	7.666	7.639	7.574	7.745	49.800
	H_o	0.91667	0.83333	0.81250	0.741	0.841	0.750	0.760	0.818	0.729	0.81176	0.86667	0.72727	0.743	0.82609	0.76596	0.667	0.878	0.744	0.82609	0.65909	0.66667	0.77800
	H_e	0.93528	0.93718	0.93601	0.930	0.929	0.929	0.929	0.934	0.935	0.92802	0.92599	0.94514	0.920	0.93837	0.92954	0.929	0.943	0.934	0.93669	0.93469	0.93955	0.93518
	F_{IS}	0.020	0.112	**0.134***	**0.204****	0.096	**0.194****	**0.183****	**0.125****	**0.222****	**0.126****	0.065	**0.233****	0.193	**0.121***	**0.178***	**0.285****	0.070	**0.205****	**0.119***	**0.297****	**0.294****	0.149
Pl_B	**No. of alleles**	13	18	16	21	19	19	19	19	17	23	16	18	16	18	17	16	13	17	17	22	16	31
	Allelic richness	7.034	7.349	6.809	7.125	7.197	6.996	6.945	6.803	6.890	7.402	7.010	7.318	7.077	7.230	6.916	6.937	6.561	6.886	7.526	7.162	7.327	31.000
	H_o	0.54167	0.57143	0.70968	0.549	0.442	0.681	0.528	0.591	0.532	0.57317	0.41379	0.47727	0.500	0.52273	0.69565	0.545	0.610	0.535	0.65909	0.58140	0.37931	0.56028
	H_e	0.91578	0.92685	0.90481	0.916	0.917	0.910	0.904	0.904	0.908	0.92735	0.90623	0.92241	0.917	0.92137	0.90444	0.907	0.896	0.900	0.93339	0.91628	0.92377	0.91957
	F_{IS}	0.414**	**0.386****	**0.218***	**0.403****	**0.521****	**0.254****	**0.418****	**0.349****	**0.417****	**0.383****	**0.548****	**0.485****	**0.454****	**0.436****	**0.233****	**0.401****	**0.322****	**0.409****	**0.296****	**0.368****	**0.594****	0.408
Pl_C	**No. of alleles**	16	20	20	26	20	25	25	20	21	30	19	24	18	19	19	18	24	23	22	23	18	47
	Allelic richness	7.087	6.553	7.659	7.302	6.651	7.586	7.378	6.883	7.619	7.395	7.531	6.920	6.871	6.817	6.670	7.232	7.449	7.357	6.890	6.917	7.181	46.829
	H_o	0.87500	0.79070	1.00000	0.952	0.886	0.979	0.945	0.711	0.979	0.90244	0.90000	0.88636	0.839	0.80000	0.85106	0.957	0.951	0.864	0.84444	0.86667	1.00000	0.89662
	H_e	0.91312	0.88673	0.93651	0.923	0.891	0.928	0.923	0.900	0.936	0.92189	0.93220	0.89394	0.890	0.90287	0.89659	0.920	0.926	0.920	0.90062	0.89988	0.91864	0.91701
	F_{IS}	0.043	0.109**	-0.069	-0.032	0.005	-0.055	-0.024	**0.212****	-0.047	0.021	0.035	0.009	0.058	**0.115****	0.051	**-0.04***	-0.027	0.062	0.063	0.037	-0.090	-0.004
All	**No. of alleles**	19	24	19	26	22	22	25	22	23	28	20	22	20	21	21	19	20	22	23	21	19	20.688
	+/-	1.713	1.688	0.919	1.537	0.931	1.308	1.256	1.238	1.493	1.308	1.579	1.571	1.202	1.078	1.520	1.276	1.662	1.145	1.520	1.249	1.606	0.445
	H_o	0.711	0.752	0.715	0.678	0.676	0.717	0.708	0.664	0.693	0.667	0.671	0.663	0.625	0.660	0.656	0.676	0.764	0.735	0.673	0.649	0.645	0.682
	+/-	0.068	0.078	0.110	0.083	0.101	0.094	0.074	0.087	0.094	0.102	0.112	0.093	0.098	0.097	0.107	0.094	0.076	0.065	0.096	0.101	0.128	0.019
	H_e	0.919	0.926	0.918	0.929	0.921	0.922	0.926	0.919	0.928	0.929	0.921	0.918	0.915	0.925	0.917	0.922	0.922	0.920	0.924	0.914	0.916	0.915
	+/-	0.008	0.012	0.007	0.006	0.010	0.007	0.007	0.009	0.007	0.004	0.008	0.010	0.006	0.008	0.009	0.007	0.008	0.007	0.008	0.007	0.009	0.003
	F_{IS}	0.226	0.187	0.222	0.269	0.264	0.224	0.235	0.278	0.254	0.281	0.273	0.279	0.315	0.286	0.284	0.267	0.172	0.203	0.270	0.290	0.300	0.256

Table 7. Pairwise F_{ST} between adult cohorts. No significant values were retrieved after B-H correction for multiple comparisons.

	ALBA	VALA	SANA	ISOA	PDCA	URB14A	URBO14A	DIA14A
ALBA								
VALA	0.00168							
SANA	0.00655	0.00214						
ISOA	0.00250	0.00420	0.00261					
PDCA	0.00507	0.00138	0.00253	0.00152				
URB14A	0.00467	0.00724	0.00826	0.01000	0.01003			
URBO14A	0.00394	0.00092	0.00164	0.00264	-0.00060	0.00550		
DIA14A	0.00071	0.00656	0.00625	0.00298	-0.00064	0.00943	0.00638	

Table 8. Pairwise F_{ST} between the 2013 young cohorts. No significant values were retrieved after B-H correction for multiple comparisons.

	ALB13Y	VAL13Y	SAN13Y	ISO13Y	PDC13Y
ALB13Y					
VAL13Y	0.00240				
SAN13Y	0.00180	0.00229			
ISO13Y	0.00792	0.00687	0.00426		
PDC13Y	0.00194	0.00335	0.00100	0.00507	

Table 9. Pairwise F_{ST} between the 2014 young cohorts. No significant values were retrieved after B-H correction for multiple comparisons.

	ALB14Y	VAL14Y	SAN14Y	ISO14Y	PDC14Y	URB14Y	URBO14Y	DIA14Y	MAC14Y
ALB14Y									
VAL14Y	0.00213								
SAN14Y	0.00022	0.00497							
ISO14Y	0.00362	0.00126	0.00351						
PDC14Y	0.00517	0.00452	0.00674	0.00599					
URB14Y	0.00175	0.00186	0.00371	0.00166	-0.00175				
URBO14Y	0.00749	0.00347	0.00741	0.00155	0.00752	0.00655			
DIA14Y	0.00082	0.00192	0.00607	0.00196	0.00340	0.00063	0.00354		
MAC14Y	0.00101	-0.00195	0.00052	0.00145	-0.00159	-0.00280	0.00094	0.00083	

Table 10. Estimates of effective population size (N_e) and population bottleneck. ° not determined due to insufficient number of individuals; * the 2013 and 2014 cohorts have been combined, n.d.: not determined due to insufficient number of individuals.

		NeEstimator	**MIGRATE**	**One-tailed Wilcoxon sign rank test for heterozygosity excess**	
Population	**Locality**	**95% CI of N_e (N_e estimates)**	**Theta (95% CI)**	**T.P.M***	**S.M.M**
All	**All**			**0.92188**	**1**
Youngs	ALB	146.4 – ∞		0.9531	0.9531
	VAL	420.6 – ∞		0.8906	0.8906
	SAN	232.6 – ∞		0.9531	0.9687
	ISO	362.2 – ∞		0.9218	0.6875
	PDC	192.2 – ∞		0.9218	0.8906
	URB	506.5 – ∞		0.4062	0.8906
	DIA	164.7 – ∞		0.8906	0.9687
	MAC	97.3 – ∞		0.8906	0.3125
	All	**1 754.6 –** ∞	**6.13 (5.974 – 6.3042)**	**0.9843**	**0.9843**
Adults	ALB	117.0 – ∞		0.9218	0.9218
	VAL	289.8 – ∞		0.9531	0.9843
	SAN	335.7 – ∞		0.9531	0.9687
	ISO	412.2 – ∞		0.9687	1.0000
	PDC	187.6 – ∞		0.6875	0.9687
	URB	418.3 – ∞		0.9218	0.4062
	DIA	359.5 – ∞		0.8906	0.8906
	MAC°	n.d.		n.d.	n.d.
	All	**1 573.8 –** ∞	**9.81 (9.417 – 10.212)**	**0.9531**	**0.9843**

***** The proportion of S.M.M. in T.P.M. is 90% with a variance fixed at 12%

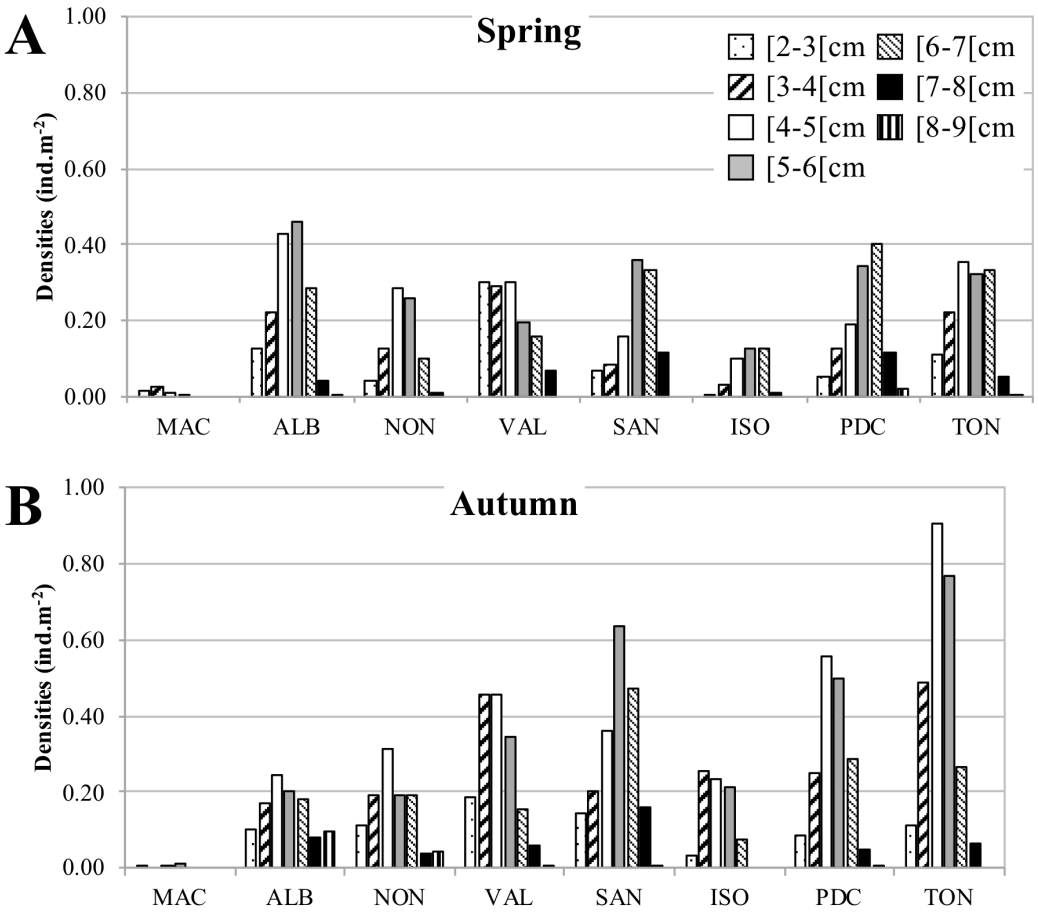

Fig. 1: Densities of *P. lividus* (mean) by size class across sites, pooled by season. Sites are abbreviated as follows: MAC: Macinaggio; ALB: Albo; NON: Nonza; VAL: Vallidone; SAN: Sanguinaires; ISO: Isolella; PDC: Punta di u Catu; TON: Tonnara.