Microsatellite markers reveal shallow genetic differentiation between cohorts of the common sea urchin *Paracentrotus lividus* (Lamarck) in northwest Mediterranean

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Abstract

Temporal variability was studied in the common sea urchin *Paracentrotus lividus* through the analysis of the genetic composition of three yearly cohorts sampled over two consecutive springs in a locality in northwestern Mediterranean. Individuals were aged using growth ring patterns observed in tests and samples were genotyped for five microsatellite loci. No reduction of genetic diversity was observed relative to a sample of the adult population from the same location or within cohorts across years. *F*<sub>ST</sub> and *AMOVA* results indicated that the differentiation between cohorts is rather shallow and not significant, as most variability is found within cohorts and within individuals. This mild differentiation translated into estimates of effective population size of 90–100 individuals. When the observed excess of homozygotes was taken into account, the estimate of the average number of breeders increased to c. 300 individuals. Given our restricted sampling area and the known small-scale heterogeneity in recruitment in this species, our results suggest that at stretches of a few kilometres of shoreline, large numbers of progenitors are likely to contribute to the larval pool at each reproduction event. Intercohort variation in our samples is six times smaller than spatial variation between adults of four localities in the western Mediterranean. Our results indicate that, notwithstanding the stochastic events that take place during the long planktonic phase and during the settlement and recruitment processes, reproductive success in this species is high enough to produce cohorts genetically diverse and with little differentiation between them. Further research is needed before the link between genetic structure and underlying physical and biological processes can be well established.

Keywords: cohorts, effective population size, microsatellite loci, *Paracentrotus lividus*, sea urchins, temporal genetic structure

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Introduction

The study of both spatial and temporal variation of population structure is essential to fully understand factors that affect genetic variability and demographic processes within species. Spatial patterns have been extensively studied in the last years (reviewed in Avise 2000; Palumbi 2004; Cowen *et al*. 2006). For these studies, populations are often sampled without any regard for their cohort structure, thus mixing individuals of different ages collected at a given time. Comparatively, the temporal component of genetic variability has received considerably less attention. Most genetic studies show the net result, averaged over time, of dispersal patterns. Therefore, they yield little information concerning temporal structure of larval dispersal (Bossart & Prowell 1998), its impact on marine populations being still poorly understood (e.g. Caley *et al*. 1996).

The hypothesis of ‘sweepstake reproductive success’ suggests that chance determines how many and which adults actually contribute to the demographic continuity of marine species at each reproductive event (Hedgecock 1994a). High fecundity and juvenile mortality create potential for large variance in reproductive success. This random variation may generate, among other consequences,
chaotic patchiness in the genetic composition of each new generation arriving at a population, leading to genetic heterogeneity among local populations on a small spatial scale (Hedgecock 1994b; Edmands et al. 1996). These temporal changes in allele frequencies can be used to infer the effective population size ($N_e$) of natural and managed populations (Pollack 1983; Waples 1989; Jorde & Ryman 1995). Large stochastic variability in reproductive success may be explained by the small effective size frequently detected in marine organisms relative to census sizes, which are sometimes several orders of magnitude larger than $N_e$ (Hedgecock 1994a; Avise 2000; Turner et al. 2002; Hedgecock et al. 2007). If this is true, then recruits should have a reduced genetic diversity relative to the adult population. Besides, if only a subset of adults from a population contribute to reproduction at each spawning event, this may result in changes in allelic frequencies from one generation to the next, resulting in high differentiation among cohorts (even exceeding spatial differentiation among populations at broad geographical scales; Watts et al. 1990; Hedgecock 1994b; Edmands et al. 1996). Some studies on marine invertebrates confirm these predictions (e.g. Li & Hedgecock 1994b; Moberg & Burton 2000; Planes & Lenfant 2002; Pujolar et al. 2006; Hedgecock et al. 2007). On the contrary, other studies do not detect evidence of sweepstake reproduction when comparing adult and recruit genetic make-up in invertebrates and fish (Flowers et al. 2002; Bernal-Ramirez et al. 2003; Poulsen et al. 2006).

Sea urchins’ larvae can disperse over scales of hundreds of kilometres and therefore it is reasonable to think that their larval pool is well mixed over large spatial scales. However, echinoids feature high interannual variation in settlement and recruitment for reasons not fully understood (Ebert 1983; Schroeter et al. 1992; López et al. 1998; Hereu et al. 2004; Tomas et al. 2004), which may indicate heterogeneity in the larval pool at small scales. Indeed, many factors can determine the actual pool of larvae arriving at a given location, which will determine the genetic composition of adult populations. Hydrological features, phytoplankton availability, predator abundances or water temperature are among the multiple factors that can determine survival of larval batches. Since these factors vary in space and time, remarkable genetic variation between different groups of age has been observed in several studies concerning sea urchins. Edmands et al. (1996) found evidence for significant differentiation among subpopulations of recruits and between adults and recruits of Strongylocentrotus purpuratus from the same location based on allozymes, but Flowers et al. (2002) did not find a temporal structure in the same species using mitochondrial DNA. Similarly, Moberg & Burton (2000) acknowledged extensive between-year variation in the genetic structure of populations of Strongylocentrotus franciscanus, suggesting that larval pool is not well homogenized during the long planktonic larval phase. Other studies have suggested that selection upon larvae may cause differentiation in Echinometra mathaei and that forces causing genetic differentiation can act locally and occur in a single generation (Watts et al. 1990). A fine-scale patchiness in recruitment within localities has been detected in Paracentrotus lividus (Hereu et al. 2004; Tomas et al. 2004), reinforcing the idea of a nonhomogeneous pool of larvae in the water column.

Temporal genetic processes can be examined by sequential long-term sampling through time or by evaluating genetic data with respect to the age structure of the population sampled at a single point in time. The aim of the present study was to obtain the first insights of temporal genetic variation of the common sea urchin Paracentrotus lividus (Lamarck, 1816) in Tossa de Mar (northwestern Mediterranean; Fig. 1). Paracentrotus lividus is a keystone species in benthic sublittoral communities of the Mediterranean, as its browsing activity is one of the main factors regulating algal abundance (Palacín et al. 1998; Bouduresque & Verlaque 2001). In addition, this commercially important species is heavily harvested for its roe in some areas, which can lead to overfishing and population depletion in parts of its distribution range (e.g. Guidetti et al. 2004). Paracentrotus lividus is a long-lived free-spawning species with a long planktonic larval phase. Population dynamics of this species in the study area have been previously analysed by Lozano et al. (1995), Turon et al. (1995) and López et al. (1998). Despite the initial controversy on the subject, it now seems well established that a main spawning event occurs in spring and smaller events take place in autumn (López et al. 1998; Tomas et al. 2004). Taking this into account, we used microsatellite markers developed for this species (Calderón et al. 2009a) to analyse temporal genetic variability of cohorts of individuals arrived at this locality in three consecutive springs (2004, 2005 and 2006) sampled over 2 years (2006

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and 2007). Besides, this variability was compared with the variability observed between adults of spatially separated populations in the Iberian Mediterranean.

**Materials and methods**

**Sampling and age estimation**

In June 2006 and June 2007, samples of Paracentrotus lividus were collected by SCUBA in Tossa de Mar (41°43.26′N, 2°56.41′E; Fig. 1). The particular site sampled was very restricted spatially, comprising an area of c. 25 × 25 m of a bottom dominated by boulders at 15 m of depth. At this spot, small-sized sea urchins were abundant under the boulders. A total of 374 sea urchins of between 10 mm and 40 mm in diameter were sampled and kept in 96% ethanol at −20 °C until processed. Maximum diameter was measured to the nearest 0.1 mm in the laboratory described in Turon et al. (1995). In short, dried tests were immersed in xylene, which penetrates the calcite mesh (stereom) that constitutes the sea urchin test. Denser stereom corresponds to periods of active growth and appears as opaque rings, while looser stereom corresponds to periods of slow growth, visible as more translucent bands once embedded in xylene. The alternation of opaque and translucent bands was interpreted as yearly growth rings (see below). The pattern of these rings was examined with a stereomicroscope using the older plates, from the coronal (i.e. corresponding to the maximum diameter) to the perioral ones. A whole oral-aboral series of plates was also examined to discern true rings from smaller, supernumerary bands that may occur in some individuals due, for instance, to periods of stress. These supernumerary bands fade away when they approach the nucleus of the plates as younger plates are observed. The pattern of translucent/opaque rings was then transformed into age of individuals.

Turon et al. (1995) provided evidence of the annual formation of growth rings in P. lividus in this area, with a period of active growth occurring during winter–spring. To further validate the ageing method, 117 individuals were tagged with tetracycline in November 2005 and collected in January 2007 from the same location in Tossa de Mar. Tetracycline is an antibiotic that chelates with CaCO₃ and is thus incorporated into the tests. This tagging technique has proved to be an effective method to follow growth in sea urchins (Gage 1992). After 14 months, all sea urchins were collected at the particular spot where the tagging was conducted and 34% of the marked individuals could be recovered. Tests were cleaned and kept at −20 °C until observed under ultraviolet light. The position of the band of tetracycline (indicating the moment of tagging) was marked on the test with a scalpel. Growth during that period corresponds to the marginal deposition of calcite between the tetracycline mark and the distal end of the plates. Growth rings observed in that area were then examined with a stereomicroscope to confirm the annual formation of rings in P. lividus.

We use the term cohort to define the group of individuals that are assumed to have arrived at Tossa de Mar within a single recruitment event. Individuals belonging to cohorts arrived in spring 2004, 2005 and 2006 were used in this study, and these data were compared to an adult population (N = 27, larger than 50 mm in diameter) collected at the same location in 2005. It should be noted that recruits arrived on the same year of collection were too small for our sampling procedure and were not included in this study. Therefore, for the cohort recruited in 2004, we have samples collected in 2006 (when they were 2 years old) and 2007 (3 years old). For the cohort recruited in 2005, samples were collected at one (2006) and two (2007) years of age. Finally, for the cohort recruited in spring 2006 we have data of the 1-year-old juveniles (collected in 2007). These data can be pooled in different ways for analysis: as age-classes (1-, 2- and 3-year-old individuals), as cohorts (2004, 2005 and 2006), and as cohorts-by-year, referring to individuals of each cohort collected at each sampling year (cohorts-by-year will be designated with the cohort year first and the collection year second: Spring04–06, Spring04–07, Spring05–06, Spring05–07 and Spring06–07). Additionally and in order to compare temporal with spatial variation, we collected 44 adult individuals from three localities of the western Mediterranean (Fig. 1): Cabrera (Balearic Archipelago; 320 km from Tossa de Mar), Cabo de Gata (Almería; 750 km from Tossa de Mar) and Tarifa (Cádiz; c. 1000 km from Tossa de Mar).

**DNA extraction and genotyping**

DNA was extracted from gonads (or Aristotle’s lantern of small individuals) using REALPURE extraction kit (Durviz, Spain). Microsatellites are highly variable markers that have proven to be suitable for analyses of biogeographical processes operating over relatively localized spatial and short temporal scales (Estoup & Angers 1998). Thus, five polymorphic microsatellites previously developed for this species (Calderón et al. 2009a) were genotyped in this study: Pl-B, Pl-C, Pl-T, Pl-Hist and Pl-28F. Polymerase chain reactions were performed in a final volume of 25 μL containing 3 mM MgCl₂, 0.12 μM of each primer and 1 U of Taq polymerase (Promega). The forward primer for each locus was labelled with fluorescent dyes (6-FAM and HEX from Sigma-Genosys or NED from Applied Biosystems; Table 1).
Table 1 Number of alleles and allelic richness for each locus and each cohort-by-year as calculated by FSTAT.

| Locus (dye label) | Parameter     | Spring 04–06 (N = 31) | Spring 04–07 (N = 24) | Spring 05–06 (N = 15) | Spring 05–07 (N = 29) | Spring 06–07 (N = 22) | Adult population Tossa de Mar (N = 27) |
|-------------------|---------------|------------------------|------------------------|------------------------|------------------------|------------------------|---------------------------------------|
|                   |               | No. of alleles         | Allelic richness       |                        |                        |                        |                                       |
| PI-B (6–FAM)      | No. of alleles| 15                     | 13                     | 10                     | 16                     | 12                     | 15                                    |
|                   | Allelic richness| 12.558                 | 11.154                 | 10.891                 | 12.522                 | 11.135                 | 14.185                                |
|                   | H<sub>O</sub> | 0.355                  | 0.467                  | 0.4583                 | 0.586                  | 0.546                  | 0.259                                 |
|                   | H<sub>E</sub> | 0.919                  | 0.885                  | 0.901                  | 0.912                  | 0.908                  | 0.929                                 |
|                   | F<sub>TS</sub> | 0.618***               | 0.481***               | 0.497***               | 0.361***               | 0.405***               | 0.719***                              |
| PI-C (NED)        | No. of alleles| 18                     | 11                     | 12                     | 18                     | 16                     | 15                                    |
|                   | Allelic richness| 13.326                 | 13.419                 | 12.933                 | 13.546                 | 9.27                   | 13.973                                |
|                   | H<sub>O</sub> | 0.806                  | 0.543                  | 0.933                  | 0.759                  | 0.864                  | 0.704                                 |
|                   | H<sub>E</sub> | 0.924                  | 0.804                  | 0.903                  | 0.927                  | 0.889                  | 0.899                                 |
|                   | F<sub>TS</sub> | 0.129*                 | 0.331**                | –0.034                 | 0.185***               | 0.029                  | 0.218***                              |
| PI-T (HEX)        | No. of alleles| 19                     | 15                     | 15                     | 16                     | 13                     | 14                                    |
|                   | Allelic richness| 13.808                 | 13.420                 | 15.933                 | 11.748                 | 12.622                 | 13.524                                |
|                   | H<sub>O</sub> | 0.903                  | 0.750                  | 0.933                  | 0.897                  | 0.682                  | 0.630                                 |
|                   | H<sub>E</sub> | 0.916                  | 0.915                  | 0.931                  | 0.934                  | 0.906                  | 0.928                                 |
|                   | F<sub>TS</sub> | 0.015                  | 0.183***               | –0.003                 | 0.04                   | 0.252***               | 0.318***                              |
| PI-Hist (HEX)     | No. of alleles| 23                     | 19                     | 16                     | 21                     | 16                     | 25                                    |
|                   | Allelic richness| 16.308                 | 14.846                 | 16.956                 | 13.548                 | 15.761                 | 22.350                                |
|                   | H<sub>O</sub> | 0.581                  | 0.625                  | 0.956                  | 0.934                  | 0.905                  | 0.965                                 |
|                   | H<sub>E</sub> | 0.948                  | 0.944                  | 0.956                  | 0.934                  | 0.905                  | 0.965                                 |
|                   | F<sub>TS</sub> | 0.391***               | 0.343***               | 0.590***               | 0.377***               | 0.504***               | 0.464***                              |
| PI-28F (NED)      | No. of alleles| 22                     | 21                     | 16                     | 19                     | 19                     | 22                                    |
|                   | Allelic richness| 16.459                 | 15.492                 | 16.956                 | 16.214                 | 16.599                 | 20.183                                |
|                   | H<sub>O</sub> | 0.452                  | 0.751                  | 0.733                  | 0.655                  | 0.545                  | 0.518                                 |
|                   | H<sub>E</sub> | 0.951                  | 0.949                  | 0.956                  | 0.947                  | 0.947                  | 0.971                                 |
|                   | F<sub>TS</sub> | 0.529***               | 0.213***               | 0.423***               | 0.312***               | 0.433***               | 0.421***                              |
| All               | No. of alleles| 19.4                   | 15.8                   | 13.8                   | 18.0                   | 15.2                   | 15                                    |
|                   | Allelic richness| 14.492                 | 13.081                 | 13.8                   | 13.918                 | 13.238                 | 14.185 (± 4.12)                      |
|                   | H<sub>O</sub> | 0.626 (± 0.231)        | 0.625 (± 0.128)        | 0.693 (± 0.252)        | 0.697 (± 0.132)        | 0.618 (± 0.159)        | 0.533 (± 0.169)                      |
|                   | H<sub>E</sub> | 0.931 (± 0.016)        | 0.903 (± 0.059)        | 0.927 (± 0.033)        | 0.930 (± 0.013)        | 0.911 (± 0.022)        | 0.910 (± 0.027)                      |
|                   | F<sub>TS</sub> | 0.332***               | 0.312***               | 0.259***               | 0.255***               | 0.327***               | 0.429***                              |

H<sub>O</sub> and H<sub>E</sub> observed and expected heterozygosities, respectively; F<sub>TS</sub> inbreeding coefficients (Genetix). *: P < 0.05; **: P < 0.005; ***: P < 0.001.
Annealing temperatures followed Calderón et al. (2009a). Alleles were sized on an ABI 3700 automated sequencer relative to the internal standard ROX 70–500 (Ecogen) using PeakScanners software (Applied Biosystems).

Genetic and statistic analyses

Standard population genetic parameters were used to describe genetic variability within and among cohorts. The genetic diversity of each cohort-by-year was calculated as number of alleles and allelic richness per locus and combined over loci using the software FSTAT 2.9.3 (Goudet 2001). Genetix version 4.05.2 (Belkhir et al. 1996–2004) was used to calculate linkage disequilibrium among loci pooling all samples. This software was also used to estimate observed and expected heterozygosities, as well as to calculate Fst coefficients and test their significance (using 10 000 permutations). We used Micro-Checker version 2.2.3 (van Oosterhout et al. 2004) to further analyse potential causes of the deficit of heterozygotes observed.

Samples from the same cohort collected over the two consecutive sampling years were pooled together, and differentiation between cohorts was analysed. The program GenAlex (Peakall & Smouse 2006) was used to calculate FST between cohorts and relative to the adult population at Tossa de Mar (based on variance of allele frequencies, following Weir & Cockerham 1984). The significance of the observed values was tested with 9999 permutations of the data set. The same procedure was used to calculate spatial genetic differentiation, by computing FST between the adult populations of the four localities sampled. A further test of genetic differentiation among cohorts and among adult populations was carried out using the Fisher exact test on genotypic rather than allele frequencies available in GenePop (web version; Raymond & Rousset 1995). Genotypic rather than allele frequencies are used because, due to the deficit in heterozygotes observed in our samples (see below), the two alleles present in an individual were not independent. GenAlex was also used to perform analyses of molecular variance (AMOVA; Excoffier et al. 1992) including three hierarchical levels: within individuals, among individuals within cohorts and among cohorts. The significance of AMOVA was calculated with 9999 permutations of the original data.

Estimates of effective population sizes

We used two different methods for estimating effective population sizes (Ne) from changes in allelic frequencies among cohorts. The first method was analytical: the so-called temporal method (Nei & Tajima 1981; Pollack 1983; Waples 1989; Turner et al. 2001), as modified by Jorde & Ryman (1995, 1996) for overlapping generations. The explicit assumption in this method is that shifts in allele frequencies between consecutive cohorts are due to random genetic drift (plus sampling error). We further assumed that removal of some individuals for analyses had no effect on the allelic frequencies of the following cohorts and that the number of newborns in each generation was large (Jorde & Ryman 1996).

To measure changes in allele frequencies between consecutive cohorts we used the estimator Fs proposed by Jorde & Ryman (2007), which is unbiased for small sample sizes and skewed allele frequencies (as is characteristic of microsatellite data). The formula of the estimator is:

\[ F_s = \frac{\sum (x_i - y_i)}{\sum z_i (1 - z_i)} \]

where \( a \) is the number of alleles at a given locus; \( x_i \) is the observed frequency of the ith allele in the sample of individuals of the first cohort; \( y_i \) is the corresponding frequency in the sample drawn from the second cohort, and \( z_i \) is the mean of \( x_i \) and \( y_i \).

The estimator Fs was corrected for the expected effect of sampling as in Jorde & Ryman (2007). Our sampling scheme corresponds to Plan II (Waples 1989; Jorde & Ryman 2007):

\[ F_s = \frac{F_s(1 - 1/4\bar{n}) - 1/\bar{n}}{(1 + F_s/4)(1 - 1/2\bar{n})} \]

where \( \bar{n} \) is the harmonic mean of the sample sizes taken from the two cohorts (\( n_x \) and \( n_y \)). The \( F_s \) values were obtained for each locus and averaged using the program TempoFs (Jorde & Ryman 2007).

In the case of overlapping generations, the amount of temporal change in allele frequencies depends not only on \( N_e \) but also on demographic characteristics of the population. These are incorporated in the estimation of \( N_e \) through a correction factor (C) and an estimate of the generation interval (G; as defined in Jorde & Ryman 1995, 1996). For the computation of these correction factors, we need to estimate age-class specific survival rates (l) and birth rates (b). We used previous biological information on P. lividus (Lozano et al. 1995; Turon et al. 1995) to calculate C and G.

Finally, the effective population size \( N_e \) was calculated from the formula (Jorde & Ryman 1995, 1996):

\[ N_e = \frac{C}{2GF_s} \]

where \( F_s \) is the average of \( F_s \) values across loci. Data were pooled by cohort (Palm et al. 2003) to obtain a more robust estimate and pairs of consecutive cohorts were compared: 2004 with 2005, and 2005 with 2006. Confidence limits on \( F_s \) can be calculated on the basis of its standard error (assuming a normal distribution across loci; Jorde & Ryman 1996; Turner et al. 2001) and converted to confidence limits for \( N_e \).

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The assumptions of this simulation procedure are (i) that our pooled allele frequencies are representative of the allelic structure of the source metapopulation; (ii) that this allelic structure will not change appreciably, at least at the scale of a generation interval; (iii) that the number of progenitors was the same in the two cohorts compared; and (iv) that the relative contribution of these progenitors to the recruited individuals was the same. These assumptions may seem unrealistic, but the analytical method is also based on stringent assumptions. In particular, mating should be random and all changes in allele frequencies should be due to drift and sampling effects, neglecting for instance that many species of benthic invertebrates constitute open populations, and that migration can substantially bias population size estimates (Wang & Whitlock 2003). In this respect, the idea of a large metapopulation with unchanged allele frequencies over short periods of time may be more realistic to assess the number of progenitors than the idealized population concept implicit in the analytical method.

Results

Age assignments

Growth rings were successfully counted for every individual. Our tagging experiment confirmed the annual formation of growth rings, as suggested by Turon et al. (1995): all tagged individuals presented one opaque and one translucent band between the mark corresponding to the incorporation of tetracycline and the margin of the plates.

Based on this annual formation of growth rings, we classified all the sampled individuals in different age classes and we selected the subset of those individuals estimated to have recruited at Tossa de Mar in spring 2004, spring 2005 and spring 2006 (a total of 121 individuals). Grouping these samples in age classes, we have a representation of sea urchins estimated to be 1 year old: the cohort recruited in 2005 sampled in 2006 (N = 15) and the cohort recruited in 2006 sampled in 2007 (N = 22); 2 years old: the cohort recruited in 2004 sampled in 2006 (N = 31) and the cohort recruited in 2005 sampled in 2007 (N = 29); and 3 years old: the cohort recruited in 2004 sampled in 2007 (N = 24).

Figure 2 shows the size frequency distribution of these three age groups. It is apparent how size may vary considerably among individuals of the same age class. In particular, the size interval becomes wider as the individuals become older. We therefore confirm that band pattern is a better method for estimating age than size.

Genetic characteristics

The main genetic characteristics of the different cohorts-by-year studied, as well as those of the adult population from the same locality, are listed in Table 1. The alleles
found and their frequencies are presented in Appendix I. Linkage disequilibrium was not detected between any of the loci analysed, as previously observed by Calderón et al. (2009a).

A deficit of heterozygotes was detected for the five loci, with significant inbreeding coefficients for all loci and cohorts-by-year (Table 1), with the exception of locus C in two and locus T in three cohorts-by-year. According to Micro-Checker, null alleles may be present, as suggested by the general excess of homozygotes for most allele size classes. However, the lack of failed amplifications (homozygote individuals for null alleles) and the coincident result with other nuclear markers (Calderón et al. 2008) leave place for alternative explanations (see Discussion). Most alleles found in the adult population were recovered in the juveniles analysed, with the exception of 1 (out of 19) for locus B, 1 (out of 29) for locus C, four (out of 45) for locus Hist and 4 (out of 32) for locus 28F (see Appendix I).

There was no observable reduction in genetic diversity (in terms of allelic richness or expected heterozygosity) between successive years of a single cohort (compare in Table 1 the cohort recruited in 2004 sampled in 2006 and 2007, and the 2005 cohort sampled in 2006 and 2007) nor relative to the adult population from Tossa de Mar. Pooling data in age groups to increase our power, no pattern of decline was evident either (Fig. 3).

No significant differentiation was detected within cohorts collected over the two sampling years according to Fisher exact test on genotypic frequencies (results not shown). Therefore, samples were grouped by cohorts for further analyses, obtaining sample sizes of 55, 44 and 22 individuals for the cohorts of 2004, 2005 and 2006, respectively. The pairwise $F_{ST}$ values between the three cohorts analysed and the adult population at Tossa de Mar are shown in Table 2. None of these values were significant in a permutation test after Bonferroni correction. Similarly, Fisher exact test on genotypic frequencies also failed to show any significant difference among the samples (Table 2).

Concerning differentiation between four adult populations of the western Mediterranean (including Tossa de Mar), pairwise $F_{ST}$ values were globally higher than differentiation detected between cohorts. On average, $F_{ST}$ between the four adult Mediterranean populations was $0.023 \pm 0.007$ (mean $\pm$ SE), while the average values obtained in our between-cohort comparison (Table 2) were
0.0035 ± 0.001. Thus, spatial variation was more than sixfold the observed temporal variation among cohorts. The average \( F_{ST} \) value between Tossa de Mar and the other three Mediterranean populations was 0.010 ± 0.006, differentiation between cohorts from a single site being thus about three times smaller than differentiation between this site and other localities.

A hierarchical AMOVA analysis showed that most variation was found within individuals and among individuals within cohorts (c. 70% and 30%, respectively), with only a minor component (0.307%) associated with differentiation between cohorts, which was nevertheless marginally significant\( (P = 0.074; \text{Table 3}) \).

### Effective population sizes

We used previous demographic information gathered for *Paracentrotus lividus* in the locality studied to estimate the required parameters: age-class specific survival rates \( \lambda_i \) and birth rates \( b_i \); Lozano *et al.* 1995; Turon *et al.* 1995). Specifically, Turon *et al.* (1995) found, using growth ring counts, that the sea urchin population in Tossa de Mar comprised 11 age classes, corresponding to years of life, and instantaneous mortality rates were calculated to be 0.6 during the first two years of life, and 0.258 afterwards. These rates translate in finite rates of survival of 0.549 and 0.773 per year, respectively. We have used these figures to estimate \( \lambda_i \) rates.

Birth rates \( b_i \) are the number of offspring generated by an individual of age \( i \) that survive to age 1 (hence \( \lambda_i = 1 \) by definition). The birth rates were indirectly assessed from the relative gonad development in the different size classes studied in Lozano *et al.* (1995) and translated to age classes.

### Table 2

| Source                  | d.f. | MS    | Percentage of variance |
|-------------------------|------|-------|------------------------|
| Among cohorts           | 2    | 3.566 | 0.307                  |
| Among individuals within cohorts | 118  | 3.023 | 30.125**               |
| Within individuals      | 121  | 1.620 | 69.568**               |

Birth rates were then scaled so that \( \sum b_i \) equals 1, to meet the condition of constant population size. The parameters of interest are \( G \), the generation time (measured as \( \sum \lambda_i b_i \); Jorde & Ryman 1995), and \( C \), a correction factor defined by Equation (23) in Jorde & Ryman (1995) and calculated by iterating Equations (10) to (13) in Jorde & Ryman (1995).

The age-classes defined for the population of Tossa de Mar (Turon *et al.* 1995), and the age-specific estimates of survival and birth rates used in the estimation of \( G \) and \( C \) are given in Table 4. The estimate of \( G \) was 5.784 years, and the correction factor \( C \) converged to an estimated value of 14.785 after c. 30 iterations.

The observed average values of uncorrected \( F_{ST} \) and corrected \( F_{ST}^c \) allele frequency changes between cohorts and the results of the two approaches used to estimate effective sizes are summarized in Table 5. The analytical method provided an estimate of 132 individuals for the comparison between cohorts of 2004 and 2005, and 81 individuals when comparing the cohorts of 2005 and 2006. The simulation method provided estimates of \( F_{ST} \) that rapidly flattened out as the number of reproductive individuals \( (N_B) \) increased (Fig. 4). The asymptote of the curves corresponds to the residual allele differentiation expectable from the sampling effect alone. The number of progenitors \( (N_B) \) for which the mean \( F_{ST} \) value was the same as the observed value corresponded to 117 individuals (comparison 2004–2005) and 66 individuals (comparison 2005–2006). Confidence intervals based on a normal distribution of \( F_{ST} \) (analytical method) were calculated.
and $F_S$ (simulation method) were calculated. For the 2004–2005 comparison, the upper limit could not be estimated ($F_S^0$ was negative and $F_S$ was below the asymptotic part of the curve). We also calculated combined values of effective sizes for the three cohorts by averaging $F_S^0$ for all loci and pairs of cohorts in the analytical method and by comparing an average value of $F_{S}$ with a curve generated using the harmonic mean of the three cohort sample sizes in the simulation method. These combined estimates were of 100 and 90 individuals, respectively, with CI intervals ranging from 45 to 401.

The effect of nonrandom mating on the estimation of the number of progenitors could also be modelled with the simulation approach. In the above results, the program generated samples using only the allele frequencies of the progenitors. When we simulated samples of the same allele frequencies as the progenitors incorporating the level of homozygosis observed in our data set, the allele frequency change ($F_S$) between successive samples (cohorts) for a given number of breeders increased by c. 29%, resulting in an upwards shift of the curve. As a result, the observed average values of $F_S$ corresponded to a larger number of breeders ($N_b = 274$ for a combined estimate using the two pairs of cohorts). Thus, for an excess of homozygotes equivalent to the level found in our samples, the $N_e$ estimates (assuming random mating) are c. 3 times lower than the actual number of breeders.

A potential concern related to these results is the degree to which our sample sizes can hinder an accurate estimation of effective sizes when genetic differentiation between cohorts is small. In our case, calculations are possible only if the observed differentiation value falls above the asymptote of the curve, which represents the level of differentiation expected from stochastic sampling error. We generated curves for different sample sizes and plotted the generated $F_S$ value at which the slope of the curve is smaller than 10$^{-4}$ in logarithmic scale (as an arbitrary measure of having reached the plateau of the curve). Plotted these values against sample size, we can obtain an estimate of the power of the method (Fig. 5). For sample sizes corresponding to the harmonic mean of our cohorts, the observed $F_S$ values were clearly above the threshold detection line. In fact, we could reduce sample sizes by c. one-third and still be able to assess effective population sizes with the observed $F_S$ values.

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Table 5: Average temporal allele frequency shifts between consecutive cohorts expressed as $F_S^0$ and $F_S$ (means over loci) and associated effective size estimates for the two methods used. Results are given separately for each pair of cohorts and combined (see text for details). When possible, 95% confidence intervals are provided (n.a. not applicable; the corrected mean $F_S^0$ is negative or the lower bound for the mean $F_S$ is below the asymptotic part of the curve).

|                      | 2004 vs. 2005 | 2005 vs. 2006 | Combined   |
|----------------------|--------------|--------------|------------|
| **Analytical method**|              |              |            |
| Mean $F_S^0$         | 0.0097       | 0.0157       | 0.0126     |
| $N_e$                | 132          | 81           | 100        |
| 95% CI               | 66–n.a.      | 45–391       | 62–256     |
| **Simulation method**|              |              |            |
| Mean $F_S$           | 0.0303       | 0.0501       | 0.0401     |
| $N_b$                | 117          | 66           | 90         |
| 95% CI               | 53–n.a.      | 37–360       | 45–401     |

Fig. 4: Estimates of $F_S$ corresponding to comparisons between cohorts of the same size of those studied, obtained by simulating an increasing number of progenitors ($N_b$). Solid lines indicate the $N_b$ corresponding to the observed value of $F_S$ between the cohort of 2004 and 2005, and dashed lines indicate $N_b$ of the comparison between the cohort of 2005 and 2006. Error bars are standard deviations of 1000 replicates. Note log-log scale.

Fig. 5: Threshold $F_S$ values corresponding to the asymptote of the curves for increasing sample sizes (mean of 1000 replicates per sample size). The shaded area indicates the zone at which no estimation of the number of breeders is possible. Symbols correspond to the combinations of $F_S$ and sample sizes (harmonic means) of the 2004–2005 (solid symbol) and 2005–2006 (open symbol) comparisons.
Discussion

Only in recent years, the importance of temporal genetic structure in marine organisms has become widely acknowledged. Among other aspects, this structure has profound implications in species conservation (Turner et al. 2002; Palm et al. 2003). Unfortunately, temporal data are particularly scarce due to the difficulties in making reliable estimates of age, and our knowledge of temporal patterns of genetic structure lags much behind that of spatial patterns. This study provides the first insights on temporal genetic structure of populations of *Paracentrotus lividus*, an ecologically and commercially important species, showing a shallow variability between the cohorts analysed.

As already detected for other species of sea urchin (Gage 1991 and references therein), our results confirm that the use of growth rings on skeletal plates is a reliable method for estimating growth and thus, for inferring age in *P. lividus* (Turon et al. 1995). Indeed, the band pattern observed in the interambulacral plates corresponds to processes of seasonal growth. However, the appearance of supplementary translucent rings likely due to events of stress rendered the reading difficult, especially in older specimens that may have undergone several such episodes of stress. The reading of whole series of plates from the oral to the aboral end allowed us to discern true growth rings from smaller, artefactual rings.

No linkage disequilibrium was detected between any of our loci. These results are coincident with previous data available for a higher number of loci (Calderón et al. 2009a), confirming the validity of our results. Our data on microsatellite markers show a high genetic diversity within cohorts, as already detected with these same markers for adult populations of Tossa de Mar and Cabrera (Calderón et al. 2009a), and for geographically distant populations, based on nuclear and mitochondrial markers (Duran et al. 2004; Calderón et al. 2008). Additionally, our results did not show a reduction in diversity of cohorts relative to adult populations (Table 1). Furthermore, levels of differentiation detected between cohorts were more than six times lower than spatial differentiation found between localities located within 1000 km of shoreline. However, sample sizes for the analysed populations were relatively small, so the analysis of spatial structure should be taken with caution until more detailed studies can be performed.

Besides the high variability associated to settlement both at temporal and spatial scales, recruitment and other postsettlement events may also play a very important role in shaping genetic composition of cohorts in this species (Hereu et al. 2004; Tomas et al. 2004). No reduction in genetic diversity was observed in the cohorts for which data were available for the two sampling years (Table 1). There is no evidence therefore that high mortality and/or selection had a marked effect in the time course of genetic variability within cohorts. The annual mortality rate in *P. lividus* is high over the first year and lower as individuals grow older (Turon et al. 1995; López et al. 1998). In our case, individuals were at least 1 year old by the time we sampled them, so we could not assess the effects on genetic structure of mortality occurring during the critical early benthic phase (Gosselin & Qian 1997; Hunt & Scheibling 1997).

As in previous studies on this species, we observed a deficit of heterozygotes in all our cohorts and all our loci relative to what would be expected for populations at Hardy–Weinberg equilibrium (Table 1). The lack of heterozygotes for null alleles in our sample and the coincident results with other nuclear markers (Calderón et al. 2008, 2009a; authors’ unpublished research) suggest that null alleles are not the cause of this outcome. Deficits of heterozygotes are usually explained by selection against heterozygotes, Wahlund effects, inbreeding or a combination of these. Although none of these possibilities can be completely ruled out, our results on the gamete recognition protein bindin suggest that positive selection acting upon this protein may be responsible for some degree of assortative mating in *P. lividus* (Calderón et al. 2009b) that can contribute to an excess of homozygotes observed in our samples.

For marine invertebrates with sedentary adults, the genetic composition of populations is mainly influenced by the genetic composition of recruits, and not of migrating adults. Temporal variability in successive cohorts at a given locality is closely related (among other factors) to the effective population size, the smaller the *N*ₑ the larger the effect of genetic drift, leading to cohort differentiation. Although the unbiased estimator *F*_S used for the analytical method is particularly useful for microsatellite loci with a large number of alleles at low frequencies (Jorde & Ryman 2007), our estimates of effective population sizes should be taken with caution, considering that only three cohorts have been analysed and that sample sizes were relatively small. Indeed, this small sample size could be responsible for the large confidence intervals detected (Table 5; Appleyard & Ward 2006). However, the two independent methods used provided similar estimates of effective sizes of about 100 progenitors for the recruits arriving at our restricted sampling spot. Many studies have evidenced that nonrandom mating reduces estimations of *N*ₑ since inbreeding would cause faster coalescence of maternal and paternal alleles compared to random mating (Laporte & Charlesworth 2002; reviewed in Charlesworth 2009). It must be recalled that *N*ₑ refers to an idealized, Fisher–Wright population, and that deviations from these conditions result in discrepancies between *N*ₑ and the actual number of reproductive individuals. In agreement with these predictions, *N*ₑ estimates (assuming random mating) were c. three times smaller than the number of breeders *Nb* estimated when deviations of Hardy–Weinberg were incorporated in the analysis.
Although the estimated effective sizes may seem small at first sight, it should be noted that the meaning of \( N_e \) in sedentary species is necessarily linked to the sampling area covered. The marked spatial and temporal variability that characterizes recruitment of \( P. \) lividus at fine scales (Hereu et al. 2004; Tomas et al. 2004), coupled with the short movement range of this species, and particularly of juveniles that hide in cryptic habitats to avoid predation (Sala & Zabalza 1996; Palacín et al. 1997), likely result in a high demographic heterogeneity of recruits over small spatial scales, as found for other invertebrates (e.g. Johnson & Black 1982, 1984). Although this patchiness remains to be confirmed by genetic studies, our estimates of the number of individuals involved in the fathering of recruits from a spot of just several tens of square meters suggests that large \( N_e \) figures will be found when considering bigger spatial scales such as coastal stretches several kilometres across.

Two main signatures are left by strong sweepstake events: reduction of genetic diversity within cohorts relative to adult populations (which are a mixture of several cohorts) and high differentiation between cohorts arrived at a given location relative to differentiation found among spatially distant populations. None of these features characterize the samples of \( P. \) lividus analysed, so we can infer a relatively high reproductive success of adults in this species, with no significant sweepstake effect reducing genetic diversity of newly arrived cohorts and markedly altering allelic frequencies between them.

Pre- and postsettlement mortality are certainly taking place in this broadcast spawner, but our results imply that the larval pool that is able to successfully recruit and survive at this given location is sufficiently large to maintain high degrees of genetic diversity within populations. Further studies on small-scale genetic heterogeneity of recruits and studies using markers under selective pressures along a longer time frame may shed more light on the processes explaining temporal genetic structure in \( P. \) lividus.

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References

Appleyard SA, Ward RD (2006) Genetic diversity and effective population size in mass selection lines of Pacific oyster (Crassostrea gigas). Aquaculture, 254, 148–159.
Avise JC (2000) Phylogeography: The History and Formation of Species. Harvard University Press, Cambridge, Massachusetts.
Belkhir K, Borsa P, Chikhi L, Raufaste N, Bonhomme F (1996–2004) Genetix 4.05, logiciel sous Windows TM pour la génétique des populations. Laboratoire Génome, Populations, Interactions, CNRS UMR 5171, Université de Montpellier II, Montpellier, France.
Bernal-Ramírez JH, Adcock CJ, Hauser L, Carvalho GR, Smith PJ (2003) Temporal stability of genetic population structure in the New Zealand snapper, Pagrus auratus, and relationship to coastal currents. Marine Biology, 142, 567–574.
Bossart JL, Prowell DP (1998) Genetic estimates of population structure and gene flow: limitations, lessons and new directions. TREE, 13, 202–206.
Boudouresque CF, Verlaque M (2001) Ecology of Paracentrotus lividus. In: Edible Sea Urchins: Biology and Ecology (ed. Lawrence JM), pp. 177–512. Elsevier, Tampa, Florida.
Calderón I, Giribet G, Turon X (2008) Multilocus phylogeography of the edible common sea urchin Paracentrotus lividus in the Lusitanian region. Marine Biology, 154, 137–131.
Calderón I, Turon X, Pascual M (2009a) Isolation of 9 nuclear microsatellites in the common Mediterranean sea urchin Paracentrotus lividus (Lamark). Molecular Ecology Resources. DOI:10.1111/j.1755-0998.2009.02585.x.
Calderón I, Turon X, Lessios HA (2009b) Characterization of the sperm molecule bindin in the sea urchin genus Paracentrotus. Journal of Molecular Evolution. DOI: 10.1007/s00239-009-0219-4.
Caley MJ, Carr MH, Dixon MA, Hughes TP, Jones GP, Menge BA (1996) Recruitment and the local dynamics of open marine populations. Annual Review of Ecology and Systematics, 27, 477–500.
Charlesworth B (2009) Effective population size and patterns of molecular evolution and variation. Nature Reviews Genetics, 10, 195–205.
Cowen RK, Paris CB, Srinivasan A (2006) Scaling of connectivity in marine populations. Science, 311, 522–527.
Duran S, Palacín C, Becerro MA, Turon X, Giribet G (2004) Genetic diversity and population structure of the commercially harvested sea urchin Paracentrotus lividus (Echinodermata: Echinoidea). Molecular Ecology, 13, 3317–3328.
Ebert TA (1983) Recruitment in Echinoderms. In: Echinoderm Studies, Vol. 1 (eds Jangoux M, Lawrence JM), pp. 190–203. AA Balkema, Rotterdam, The Netherlands.
Edmands S, Moberg PE, Burton RS (1996) Allozyme and mitochondrial DNA evidence of population subdivision in the purple sea urchin Strongylocentrotus purpuratus. Marine Biology, 126, 443–450.
Estoup A, Angers B (1998) Microsatellites and minisatellites for molecular ecology: theoretical and empirical considerations. In: Advances in Molecular Ecology (ed. Carvalho G), pp. 59–88. NATO ASI series; IOS Press, Amsterdam, The Netherlands.
Excoffier L, Smouse PE, Quattro JM (1992) Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. Genetics, 131, 479–491.
Flowers JM, Schroeter SC, Burton RS (2002) The recruitment sweepstake has many winners: genetic evidence from the sea urchin Strongylocentrotus purpuratus. Evolution, 56, 1445–1453.
Gage JD (1991) Skeletal growth zones as age-markers in the sea urchin Psammechinus miliaris. Marine Biology, 110, 217–228.
Gage JD (1992) Natural growth bands and growth variability in the sea urchin Echinoidea. Advances in Molecular Ecology (ed. Carvalho G), pp. 126–131.
Gosselin LA, Qian PY (1997) Juvenile mortality in benthic marine invertebrates. Marine Ecology Progress Series, 146, 265–282.
Goudet J (2001) FSTAT, a program to estimate and test gene diversities and fixation indices (version 2.9.3). Available at http://www.unil.ch/izea/softwares/fstat.html.
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Guidetti P, Terlizzi A, Boero F (2004) Effects of the edible common sea urchin, *Paracentrotus lividus*, fishery along the Apulian rocky coast (SE Italy, Mediterranean Sea). *Fisheries Research*, 66, 287–297.

Hedgecock D (1994a) Does variance in reproductive success limit effective population size of marine organisms? In: *Genetics and Evolution of Aquatic Organisms* (ed. Beaumont A), pp. 122–134. Chapman & Hall, London.

Hedgecock D (1994b) Temporal and spatial genetic structure of marine animal populations in the California Current. *California Cooperative Oceanic Fisheries Reports*, 35, 73–81.

Hedgecock D, Launey S, Pudovkin AI, Naciri Y, Lapègue S, Bonhomme F (2007) Small effective number of parents (*N_e*) inferred for a naturally spawned cohort of juvenile European flat oysters *Ostrea edulis*. *Marine Biology*, 150, 1173–1182.

Hereu B, Zabala M, Linares C, Sala E (2004) Temporal and spatial variability in settlement of the sea urchin *Paracentrotus lividus* in the NW Mediterranean. *Marine Biology*, 144, 1011–1018.

Hunt HL, Scheibling RE (1997) The role of early post-settlement mortality in recruitment of benthic marine invertebrates: a review. *Marine Ecology Progress Series*, 155, 269–301.

Jensen M (1969) Age determination of echinoids. *Sarsia*, 37, 41–44.

Johnson MS, Black R (1984) Pattern beneath the chaos: the biology cycles and recruitment of *Paracentrotus lividus* (Lamarck) (Echinodermata: Echinoidea) in two contrasting habitats. *Marine Ecology Progress Series*, 179–191.

Jorde E, Ryman N (1996) Demographic genetics of brown trout (*Salmo trutta*) and estimation of effective population size from temporal change of allele frequencies. *Genetics*, 143, 1369–1381.

Jorde E, Ryman N (2007) Unbiased estimator for genetic drift and effective population size. *Genetics*, 177, 927–935.

Laporte V, Charlesworth B (2002) Effective population size and population subdivision in demographically structured populations. *Genetics*, 162, 501–519.

Li G, Hedgecock D (1998) Genetic heterogeneity, detected by PCR SSCP, among samples of larval Pacific oysters (*Crassostrea gigas*) supports the hypothesis of large variance in reproductive success. *Canadian Journal of Fisheries and Aquatic Sciences*, 55, 1025–1033.

López S, Turon X, Montero E, Palacín C, Duarte CM, Tarjuelo I (1998) Larval abundance, recruitment and early mortality in *Paracentrotus lividus* (Echinoidea). Interannual variability and plankton-benthos coupling. *Marine Ecology Progress Series*, 172, 239–251.

Lozano J, Galera J, López S, Turon X, Palacín C, Morera G (1995) Biological cycles and recruitment of *Paracentrotus lividus* (Lamarck) (Echinodermata: Echinoida) in two contrasting habitats. *Marine Ecology Progress Series*, 122, 179–191.

Moberg PE, Burton RS (2000) Genetic heterogeneity among adult and recruit red sea urchins, *Strongylocentrotus franciscanus*. *Marine Biology*, 136, 773–784.

Nei M, Tajima F (1981) Genetic drift and estimation of effective population size. *Genetics*, 98, 625–640.

van Oosterhout C, Hutchinson WF, Wills DPM, Shipley P (2004) Micro-Checker: software for identifying and correcting genotyping errors in microsatellite data. *Molecular Ecology Notes*, 4, 535–538.

Palacin G, Giribet G, Turon X (1997) Patch recolonization through migration by the echinoid *Paracentrotus lividus* in communities with high algal cover and low echinoid densities. *Cahiers de Biologie Marine*, 38, 267–271.

Palacin G, Giribet G, Carneró D, Dantart, L, Turon X (1998) Low density of sea urchins influence the structure of algal assemblages in the western Mediterranean. *Journal of Sea Research*, 39, 281–290.

Palm S, Laikre L, Jorde PE, Ryman N (2003) Effective population size and temporal genetic change in stream resident brown trout (*Salmo trutta* L.). *Conservation Genetics*, 4, 249–264.

Palumbi SR (2004) Marine reserves and ocean neighbourhoods: the spatial scale of marine populations and their management. *Annual Review of Environmental Resources*, 29, 31–68.

Peakall R, Smouse PE (2006) Genalex 6: genetic analysis in Excel. Population genetics software for teaching and research. *Molecular Ecology Notes*, 6, 288–295.

Planes S, Lenfant P (2002) Temporal change in the genetic structure between and within cohorts of a marine fish, *Diplodus sargus*, induced by a large variance in individual reproductive success. *Molecular Ecology*, 11, 1515–1524.

Pollack E (1983) A new method for estimating the effective population size from allele frequency changes. *Genetics*, 104, 531–548.

Poulsen NA, Nielsen EE, Schierup MH, Loeschcke V, Gronkjær P (2006) Long-term stability and effective population size in North Sea and Baltic Sea cod (*Gadus morhua*). *Molecular Ecology*, 15, 321–331.

Pujojar JM, Maes GE, Volckaer FAM (2006) Genetic patchiness among recruits in the European eel *Anguilla anguilla*. *Marine Ecology Progress Series*, 307, 209–217.

Raymond M, Rousset F (1995) GenePop (version 1.2): population genetics software for exact tests and ecumenicism. *Journal of Heredity*, 86, 248–249.

Sala E, Zabala M (1996) Fish predation and the structure of the sea urchin *Paracentrotus lividus* populations in the NW Mediterranean. *Marine Ecology Progress Series*, 140, 71–81.

Schroeter S, Dixon J, Ebert T, Richards J (1992) Urchins settlement patterns. In: *The Management and Enhancement of Sea Urchins and Other Kelp Bed Resources: A Pacific Rim Perspective* (ed. Dewees CM), p. 43. California Sea Grant, San Diego, California.

Tomas F, Romero J, Turon X (2004) Settlement and recruitment of the sea urchin *Paracentrotus lividus* in two contrasting habitats in the Mediterranean. *Marine Ecology Progress Series*, 282, 173–184.

Turner TF, Salter LA, Gold JR (2001) Temporal-method estimates of *Ne* from highly polymorphic loci. *Conservation Genetics*, 2, 297–308.

Turner TF, Wares JP, Gold JR (2002) Genetic effective size is three orders of magnitude smaller than adult census size in an abundant, estuarine-dependant marine fish (*Sciaenops ocellatus*). *Genetics*, 162, 1329–1339.

Turon X, Giribet G, López S, Palacín C (1995) Growth and population structure of *Paracentrotus lividus* (*Echinodermata: Echinoidea*) in two contrasting habitats. *Marine Ecology Progress Series*, 122, 193–204.

Wang J, Whitlock MC (2003) Estimating effective population size and migration rates from genetic samples over space and time. *Genetics*, 163, 429–446.

Waples RS (1989) A generalized approach for estimating effective population size from temporal changes in allele frequencies. *Genetics*, 121, 379–391.

Watts RJ, Johnson MS, Black R (1990) Effects of recruitment on genetic patchiness in the urchin *Echinometra mathaei* in Western Australia. *Marine Biology*, 105, 145–152.

Weir BS, Cockerham CC (1984) Estimating *F*-statistics for the analysis of population structure. *Evolution*, 38, 1358–1370.
Appendix I

Allele frequencies of the five microsatellite loci analysed for the cohorts and adult population of Tossa de Mar. Values in bold indicate alleles that are present in the adult population but that were not found in any of the cohorts studied.

| Allele | Locus B | Locus C | Locus T |
|--------|---------|---------|---------|
|        | Spring 2004 | Spring 2005 | Spring 2006 | Tossa Adults | Average | Spring 2004 | Spring 2005 | Spring 2006 | Tossa Adults | Average |
| 1      | 0.000 0.011 0.068 0.037 0.020 | 0.000 0.000 0.023 0.000 0.003 | 0.09 0.011 0.023 0.037 0.017 |
| 2      | 0.009 0.011 0.000 0.000 0.007 | 0.045 0.034 0.045 0.074 0.047 | 0.09 0.011 0.023 0.037 0.017 |
| 3      | 0.145 0.205 0.182 0.111 0.162 | 0.000 0.000 0.045 0.000 0.007 | 0.09 0.011 0.023 0.037 0.017 |
| 4      | 0.109 0.080 0.023 0.037 0.074 | 0.064 0.023 0.000 0.019 0.034 | 0.00 0.011 0.000 0.000 0.003 |
| 5      | 0.027 0.057 0.000 0.019 0.030 | 0.091 0.091 0.068 0.130 0.095 | 0.00 0.011 0.000 0.000 0.001 |
| 6      | 0.164 0.136 0.091 0.222 0.135 | 0.027 0.057 0.045 0.074 0.047 | 0.00 0.011 0.000 0.000 0.003 |
| 7      | 0.091 0.080 0.091 0.074 0.084 | 0.027 0.114 0.045 0.130 0.074 | 0.00 0.011 0.000 0.000 0.003 |
| 8      | 0.064 0.057 0.182 0.019 0.071 | 0.091 0.114 0.091 0.093 0.098 | 0.036 0.045 0.091 0.093 0.057 |
| 9      | 0.082 0.091 0.091 0.093 0.088 | 0.245 0.125 0.295 0.241 0.216 | 0.127 0.114 0.045 0.057 0.095 |
| 10     | 0.055 0.091 0.068 0.056 0.068 | 0.118 0.159 0.068 0.019 0.105 | 0.109 0.068 0.068 0.093 0.088 |
| 11     | 0.027 0.045 0.000 0.019 0.027 | 0.109 0.091 0.114 0.056 0.095 | 0.173 0.080 0.091 0.093 0.118 |
| 12     | 0.045 0.011 0.023 0.019 0.027 | 0.018 0.057 0.045 0.037 0.037 | 0.091 0.102 0.136 0.130 0.108 |
| 13     | 0.027 0.023 0.000 0.093 0.034 | 0.036 0.060 0.000 0.001 0.017 | 0.036 0.136 0.227 0.185 0.122 |
| 14     | 0.036 0.023 0.068 0.074 0.044 | 0.045 0.034 0.000 0.000 0.027 | 0.109 0.080 0.045 0.074 0.084 |
| 15     | 0.045 0.045 0.000 0.000 0.030 | 0.018 0.023 0.000 0.019 0.017 | 0.027 0.080 0.068 0.056 0.054 |
| 16     | 0.036 0.011 0.068 0.074 0.041 | 0.009 0.011 0.000 0.000 0.007 | 0.045 0.057 0.000 0.056 0.044 |
| 17     | 0.018 0.000 0.045 0.000 0.014 | 0.000 0.011 0.000 0.037 0.010 | 0.045 0.023 0.091 0.074 0.051 |
| 18     | 0.000 0.000 0.000 0.056 0.010 | 0.009 0.000 0.000 0.000 0.003 | 0.027 0.034 0.023 0.000 0.024 |
| 19     | 0.018 0.023 0.000 0.000 0.014 | 0.000 0.000 0.000 0.199 0.003 | 0.064 0.023 0.000 0.037 0.037 |

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## Appendix I

### Allelic Frequencies

| Allele | Locus Hist 2004 | Locus 28F 2004 | Locus Hist 2005 | Locus 28F 2005 | Locus Hist 2006 | Locus 28F 2006 | Locus 28F Adults Average |
|--------|----------------|----------------|----------------|----------------|----------------|----------------|--------------------------|
|        | Spring         | Spring         | Spring         | Spring         | Spring         | Spring         | Adults Average           |
| 1      | 0.000          | 0.011          | 0.000          | 0.000          | 0.000          | 0.000          | 0.003                    |
| 2      | 0.009          | 0.000          | 0.000          | 0.000          | 0.000          | 0.000          | 0.003                    |
| 3      | 0.000          | 0.011          | 0.045          | 0.000          | 0.000          | 0.000          | 0.010                    |
| 4      | 0.000          | 0.023          | 0.000          | 0.019          | 0.000          | 0.000          | 0.010                    |
| 5      | 0.018          | 0.011          | 0.000          | 0.000          | 0.000          | 0.000          | 0.007                    |
| 6      | 0.018          | 0.000          | 0.000          | 0.000          | 0.000          | 0.000          | 0.007                    |
| 7      | 0.009          | 0.000          | 0.000          | 0.000          | 0.000          | 0.000          | 0.003                    |
| 8      | 0.009          | 0.000          | 0.000          | 0.000          | 0.000          | 0.000          | 0.003                    |
| 9      | 0.009          | 0.091          | 0.000          | 0.000          | 0.000          | 0.000          | 0.030                    |
| 10     | 0.000          | 0.011          | 0.000          | 0.000          | 0.000          | 0.000          | 0.003                    |
| 11     | 0.027          | 0.011          | 0.023          | 0.056          | 0.027          | 0.000          | 0.003                    |
| 12     | 0.000          | 0.000          | 0.000          | 0.000          | 0.000          | 0.000          | 0.000                    |
| 13     | 0.027          | 0.080          | 0.227          | 0.000          | 0.068          | 0.000          | 0.068                    |
| 14     | 0.018          | 0.023          | 0.000          | 0.019          | 0.017          | 0.000          | 0.019                    |
| 15     | 0.127          | 0.057          | 0.182          | 0.093          | 0.108          | 0.000          | 0.030                    |
| 16     | 0.000          | 0.034          | 0.000          | 0.019          | 0.014          | 0.000          | 0.030                    |
| 17     | 0.082          | 0.080          | 0.023          | 0.019          | 0.061          | 0.000          | 0.030                    |
| 18     | 0.100          | 0.136          | 0.068          | 0.056          | 0.098          | 0.000          | 0.030                    |
| 19     | 0.018          | 0.000          | 0.000          | 0.000          | 0.007          | 0.000          | 0.030                    |
| 20     | 0.045          | 0.000          | 0.000          | 0.019          | 0.020          | 0.000          | 0.030                    |
| 21     | 0.009          | 0.057          | 0.000          | 0.056          | 0.030          | 0.000          | 0.030                    |
| 22     | 0.064          | 0.080          | 0.068          | 0.056          | 0.068          | 0.000          | 0.030                    |
| 23     | 0.045          | 0.000          | 0.000          | 0.019          | 0.020          | 0.000          | 0.030                    |
| 24     | 0.000          | 0.000          | 0.045          | 0.000          | 0.007          | 0.000          | 0.030                    |
| 25     | 0.064          | 0.023          | 0.223          | 0.000          | 0.034          | 0.000          | 0.030                    |
| 26     | 0.018          | 0.023          | 0.000          | 0.074          | 0.019          | 0.000          | 0.030                    |
| 27     | 0.018          | 0.023          | 0.091          | 0.074          | 0.041          | 0.000          | 0.030                    |
| 28     | 0.027          | 0.034          | 0.045          | 0.093          | 0.044          | 0.000          | 0.030                    |
| 29     | 0.009          | 0.011          | 0.023          | 0.056          | 0.020          | 0.000          | 0.030                    |
| 30     | 0.027          | 0.045          | 0.223          | 0.000          | 0.030          | 0.000          | 0.030                    |
| 31     | 0.000          | 0.000          | 0.000          | 0.019          | 0.003          | 0.000          | 0.030                    |
| 32     | 0.000          | 0.011          | 0.000          | 0.000          | 0.003          | 0.000          | 0.030                    |
| 33     | 0.045          | 0.011          | 0.045          | 0.093          | 0.044          | 0.000          | 0.030                    |
| 34     | 0.055          | 0.034          | 0.045          | 0.000          | 0.037          | 0.000          | 0.030                    |
| 35     | 0.045          | 0.034          | 0.023          | 0.019          | 0.034          | 0.000          | 0.030                    |
| 36     | 0.000          | 0.023          | 0.000          | 0.019          | 0.003          | 0.000          | 0.030                    |
| 37     | 0.018          | 0.000          | 0.000          | 0.019          | 0.010          | 0.000          | 0.030                    |
| 38     | 0.000          | 0.023          | 0.000          | 0.037          | 0.014          | 0.000          | 0.030                    |
| 39     | 0.000          | 0.000          | 0.000          | 0.019          | 0.003          | 0.000          | 0.030                    |
| 40     | 0.000          | 0.011          | 0.000          | 0.000          | 0.003          | 0.000          | 0.030                    |
| 41     | 0.000          | 0.011          | 0.000          | 0.000          | 0.003          | 0.000          | 0.030                    |
| 42     | 0.000          | 0.011          | 0.000          | 0.000          | 0.003          | 0.000          | 0.030                    |
| 43     | 0.000          | 0.011          | 0.000          | 0.000          | 0.003          | 0.000          | 0.030                    |
| 44     | 0.000          | 0.011          | 0.000          | 0.000          | 0.003          | 0.000          | 0.030                    |
| 45     | 0.018          | 0.000          | 0.000          | 0.000          | 0.007          | 0.000          | 0.030                    |