Living upside down: patterns of red coral settlement in a cave (#21242)

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Living upside down: patterns of red coral settlement in a cave

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Background. Larval settlement and intra-specific interactions during the recruitment phase are crucial in determining the distribution and density of sessile marine populations. Marine caves are confined and stable habitats. As such, they provide natural laboratory to study the settlement and recruitment processes in sessile invertebrates, including the valuable Mediterranean red coral *Corallium rubrum*. Here, the spatial and temporal variability of red coral settlers in an underwater cave was investigated using demographic and genetic approaches.

Methods. Sixteen PVC tiles were placed on the walls and ceiling of the cave and recovered after twenty months. A total of 372 individuals belonging to two different reproductive events were recorded. Basal diameter, height, and number of polyps were measured, and 7 microsatellites loci were used to genotype them.

Results. Significant differences in the colonization rate were observed both between the two temporal cohorts and between ceiling and walls. No genetic structuring was observed between cohorts. Overall, we found high levels of relatedness among individuals.

Discussion. The results suggest that in the cave the *C. rubrum* population is closed and self-recruiting and that a small proportion of individuals contribute to the recruitment.

LIVING UPSIDE DOWN: PATTERNS OF RED CORAL SETTLEMENT IN A CAVE

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Abstract

Background. Larval settlement and intra-specific interactions during the recruitment phase are crucial in determining the distribution and density of sessile marine populations. Marine caves are confined and stable habitats. As such, they provide natural laboratory to study the settlement and recruitment processes in sessile invertebrates, including the valuable Mediterranean red coral *Corallium rubrum*. Here, the spatial and temporal variability of red coral settlers in an underwater cave was investigated using demographic and genetic approaches.

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Results. Significant differences in the colonization rate were observed both between the two temporal cohorts and between ceiling and walls. No genetic structuring was observed between cohorts. Overall, we found high levels of relatedness among individuals.

Conclusion. The results suggest that in the cave the *C. rubrum* population is closed and self-recruiting and that a small proportion of individuals contribute to the recruitment.

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37 **Introduction**

38 Recovery and resilience of benthic species mostly depend on their early life history stages
39 such as dispersal, settlement and recruitment. The settlement is influenced by events occurring
40 during the planktonic stage (Babcock & Mundy, 1996). It has been shown that abiotic (Keough
41 & Downes, 1982; Torrents & Garrabou, 2011) and biotic (Lindsay, Wethey & Woodin, 1997)
42 factors, as well as larval behaviour (Martínez-Quintana et al., 2014) may influence dispersal and
43 larval mortality during the pre-settlement period. After the settlement, other sources of mortality
44 (e.g. intraspecific competition, predation, detachment from the substrate) can affect the
45 recruitment process (Perkol-Finkel et al., 2008; Santangelo et al., 2012). Larval dispersal and
46 recruitment play a primary role in maintaining genetic diversity and populating new areas. In fact
47 recruitment coming only or mostly from local populations (self-recruitment) generally leads to
48 an impoverishment of the genetic variability and thus decreasing the population resilience to
49 stressors (Brazeau, Sammarco & Atchison, 2011; Lasker, 2013).

50 Sessile marine invertebrates such as corals, ascidians and sponges, release very small larvae
51 and have patchily distributed populations. For these marine organisms, the application of direct
52 larval observation or tracking is very difficult (Levin, 1990). Early life stages (from larval release
53 to recruitment) have been investigated using different tools such as larval behaviour in the lab
54 (Guizien et al. 2012; Martinez-Quintana et al 2014), field experimental studies on settlement and
55 post-settlement processes (Underwood & Fairweather, 1989), biophysical circulation modelling
56 (Guizien et al., 2006) and population genetics (Jones, Planes & Thorrold, 2005). Moreover,
57 recruitment can be indirectly estimated from demographic parameters (e.g. age structure,

population density, growth rates) as well as from the genetic makeup of populations (e.g. genetic variability, relatedness and structuring) (Brazeau, Sammarco & Atchison, 2011; Bramanti & Edmunds, 2016).

Settlement tiles have been also used to study the demography of sessile organisms and provided reliable measures of recruitment variability and growth rates of coral populations (Bramanti et al., 2007; Bramanti & Edmunds, 2016) at different spatial and temporal scales (Green and Edmunds 2011; Santangelo et al. 2012). Further, the analysis of the genetic structure of recruits gives information on the relative contribution of external versus local larvae. The strong spatial genetic structuring (SGS) observed in several sessile invertebrates suggested that recruitment is often local, probably as a result of the short effective dispersal of larvae and their tendency to be philopatric (Costantini, Fauvelot & Abbiati, 2007a; Ledoux et al., 2010a). However, according to Padron et al. (submitted), genetic structuring can be affected by the demographic history and therefore, especially for long-living species, SGS of adult individuals cannot inform on the dispersal capabilities of larvae. However, to date, only few studies have analysed the SGS in the first years of life in marine invertebrates (Brazeau, Sammarco & Atchison, 2011; Torda et al., 2013; Smilansky & Lasker, 2014).

Underwater caves (sensu Rastorgueff et al. 2015) represent an excellent natural mesocosm to study the role of new propagules on population dynamics and connectivity of benthic invertebrates. Caves are confined and not exposed to the dominant currents, hence they are more sheltered than cliffs, and less exposed to changes in water conditions, pollutants or potential pathogens carried by currents (Harmelin et al. 1985; Garrabou et al. 2001), thus providing a natural protection from disturbances associated with waterborne substances (Garrabou & Harmelin, 2002). Nevertheless, underwater caves can be affected by several threats such as

mechanical disturbance (SCUBA divers), temperature increase, sediment deposit and waste outflows (Chevaldonné & Lejeusne, 2003; Parravicini et al., 2010; Giakoumi et al., 2013). Nowadays, due to their high species richness, underwater caves are considered a Mediterranean biodiversity reservoir and they have been included in two Action Plans by UNEP/MAP-RAC/SPA (2008; 2013).

Among the species inhabiting caves in the Mediterranean Sea, the red coral (*Corallium rubrum* L. 1758) is one of the ~~more~~ abundant in the shallower part of its bathymetrical distribution due to its preference for dim-light conditions and down-facing surfaces (Laborel & Vacelet, 1961; Virgilio, Airoldi & Abbiati, 2006) and to the effects of hundreds of years of harvesting (Cau et al., 2016; Garrabou et al., 2017). Moreover, due to its high economic value (Bussoletti et al., 2010; Tsounis et al., 2013), red coral colonies have been heavily harvested and most of the coastal populations are currently depleted (Tsounis et al., 2013). Several studies carried out on shallow-water red coral populations inhabiting vertical cliffs or small crevices using artificial substrates (Bramanti, Magagnini & Santangelo, 2003; Bramanti et al., 2007; Santangelo et al., 2012) showed a huge variability in the density of recruitment between different sites that were attributed to several environmental factors (e.g. competition for space, predation, overgrowth). Population genetic studies, conducted only on adult colonies, have shown large heterozygosity deficiencies, suggesting the occurrence of inbreeding (non-random mating) within populations (Costantini, Fauvelot & Abbiati, 2007a,b, Ledoux et al., 2010b,a; Aurelle et al., 2011) or demographic instability (Padron & Guizien, 2015) and a strong genetic structuring at distances of less than one meter (Costantini, Fauvelot & Abbiati, 2007a; Ledoux et al., 2010a). In the present study demography and genetic structure of recruits on experimental tiles were

analysed to investigate the processes acting during the early phases of the red coral life cycle and to highlight the spatial and temporal variability of settlement within a submarine cave.

Materials and methods

Study area and experimental design

The study was conducted on the Colombara cave (Lat 44° 18' 35", Long 9° 10' 37") located on the east coast of the Gulf of San Fruttuoso (Italy). The cave, 10 meters long, stands 29 to 39 m depth on a rocky cliff oriented from northeast to southwest. The cave walls harbor an assemblage of sessile invertebrates typical of the sublittoral caves of the North-West Mediterranean Sea (Morri et al., 1986).

Field experiments were approved by the Director of the Portofino MPA and by the Università Politecnica delle Marche (Authorization n. 3/2011 (n. prot. 449/2-1-5.) and authorization n° 4/2012 (Protoc. N° 409/2-1-1)).

In June 2010, about 3 weeks before red coral spawning (Santangelo et al., 2003), sixteen 20 x 20 cm white PVC tiles were fixed inside the cave by a steel screw. In order to test if recruitment is influenced by orientation, eight tiles were located on the vertical walls and eight on the ceiling of the cave (Fig. 1). Ten PVC tiles were also fixed in a vertical wall outside the cave and at the same depth (Faro Point) where the red coral population has an adult density of 425 ± 100 colonies x m^2 (Bavestrello et al., 2015). In summer 2011 ~~the tiles were removed a no a~~ single recruit was found.

Fourteen tiles (two tiles located on the vertical wall were lost (Fig. 1)) within the cave were removed in February 2012, after 2 reproductive events (summer 2010 and summer 2011). Recovered tiles were stored with 80% ethanol and transferred to the laboratory. A picture of each tile was taken with a NIKON camera fixed to a stereomicroscope NIKON SMZ 1500 and

analysed with IMAGE J software (http://imagej.nihgov/ij) in order to measure size (diameter) and to study the spatial distribution of the new individuals. The position of each individual was marked and size (diameter) was measured by averaging the minimum and maximum width. All the individuals were then removed from the tiles and for each of them, the number of polyps was counted under the dissecting microscope. Polyps were removed from each individual and stored in plastic tubes with 80% ethanol at 4°C for the upcoming DNA extraction.

Based on the literature on red coral early life stages (Bramanti et al., 2005) the age of each individual was estimated on the basis of its height: individuals with an encrusting button shape were assigned to the cohort 2011 (hereafter recruits); individuals that developed in height forming a small branch were assigned to the cohort 2010 (hereafter juveniles) (Fig. 2).

Measuring growth performances

The size structure of red coral individuals was obtained by analysing the frequency distributions of the basal diameter (for both recruits and juveniles) and of colony height (only for juveniles). The correlations between the parameters (diameter vs. height; diameter vs. number of polyps; height vs. number of polyps) were ~~checked by~~ Pearson's coefficient. Moreover, we tested the temporal and spatial variations of the abundance of individuals by two separate one-way ANOVAs with Cohort (two levels; fixed; recruits and juveniles) and Position (two levels; fixed; ceiling and walls) as factors.

Measuring genetic variability and structuring

Due to the small number of *C. rubrum* individuals found on the tiles deployed on the walls, the molecular analysis was carried out only on the individuals occurring on the tiles deployed on the ceiling of the cave.

Total genomic DNA was extracted from each individual (1 to 4 polyps per colony) following the CTAB protocol and purified by standard chloroform procedure. Seven microsatellite loci COR9, COR46, L7, COR58, MIC20, MIC24, MIC26 (Costantini & Abbiati, 2006; Ledoux et al., 2010b) were amplified either as single locus or in multiplex using a QIAGEN Multiplex PCR Kit. Genotyping was performed by MACROGEN INC. Allele sizing was determined with Peak Scanner v1.0 software. Scoring errors due to stuttering, large allele dropout and null alleles were controlled with MICROCHECKER version 2.2.3 (Van Oosterhout et al., 2004). Estimated frequencies of putative null alleles were subsequently calculated for each locus using the expectation maximization (EM) algorithm of Dempster et al. (1977) implemented in FREENA (Chapuis & Estoup, 2007). Red corals settlers sharing the same multilocus genotype (MLG) were identified using GENALEX version 6.1 (Peakall & Smouse, 2006). Identical MLGs can result from two distinct sexual reproduction events. To test this, the unbiased probability of identity (P_{ID}) that two sampled individuals share identical MLG by chance (Kendall & Stewart, 1977) was computed. The total number of alleles (N_a), observed (H_o), and unbiased expected (H_e) heterozygosities (Nei, 1973) were calculated for each locus and for each cohort of settlers (either recruits and juveniles) using GENETIX software package version 4.03 (Belkhir et al., 2000). The *f* estimator of F_{IS} (Weir & Cockerham, 1984) was computed, and significant departures from the Hardy–Weinberg equilibrium were tested using Fisher's exact test in GENEPOP version 3.4 (http://genepop.curtin.edu.au/; (Raymond & Rousset, 1995), with the

level of significance determinate by a Markov chain randomization. Significant differences in genetic diversity (H_o, H_e and F_{IS}) between recruits and juveniles were tested using a Student's t-test.

To determine whether individuals were more related than expected under panmixia, the R_{XY} pairwise relatedness coefficient (Queller & Goodnight, 1989) was computed separately for recruits and for juveniles. This index varies between 0 and 1 with R_{XY} = 1.0 for full-sib relationships, R_{XY} = 0.5 for half-sibs and R_{XY}= 0 for unrelated individuals in an infinitely large panmictic population (Peakall & Smouse, 2006). The observed mean and variance of R_{XY} were compared with their expected distribution under the null hypothesis of panmixia using 1000 permutations of alleles as implemented in IDENTIX (Belkhir, Castric & Bonhomme, 2002). The null distribution was obtained by a conventional Monte Carlo resampling procedure, which randomly selected 10,000 genotypes without replacement and then recalculating the statistic. Null hypothesis could be rejected with a significance level of 5%, given that the observed value of the statistic was above the 95% level of the resampled statistics.

An exclusion test, performed in GENECLASS 2.0 (Piry et al., 2004) using the simulation method by Rannala and Mountain (1997), was used to test whether each individual originated from its reference tile. The probability of individual genotypes coming from each tile was calculated by comparing individual genotypes to 10000 simulated settlers per tile. GENECLASS 2.0 was also used to identify putative first-generation migrants among tiles. Red coral individuals were excluded from a reference tile if the probability of exclusion was greater than 0.99 ($a \leq 0.01$) and assigned to another tile as a potential source if assignment probabilities were greater than 0.1.

The contemporary effective population size (*N*e) for each cohort was inferred using the *sibship* assignment method (Wang et al., 2009), which does not assume random mating (Luikart et al. 2010), and available in the software COLONY (Jones & Wang, 2010).

Genotypic differentiation between the recruits and juveniles was tested with an exact test (Markov chain parameters: 1,000 dememorizations, followed by 1,000 batches of 1,000 iterations per batch), and the P value of the log-likelihood (G)-based exact test (Goudet et al., 1996) was estimated in GENEPOP. To perform the analysis a subset of recruits, comparable to the number of juveniles, ~~were~~ randomly selected.

The number of genetic clusters, K, was estimated by a Bayesian approach implemented in the program STRUCTURE version 2.2 (Pritchard, Matthew & Donnelly, 2000; Falush, Stephens & Pritchard, 2007). Mean and variance of log likelihoods of the number of clusters for $K = 1$ to $K = 10$ were inferred by running structure ten times with 1000000 repetitions each (burn-in = 100000 iterations) under the admixture ancestry model and the assumption of correlated allele frequencies among samples. Due to the presence of null alleles, the clustering analysis was conducted on the original data set, using the option of null alleles coded as recessive alleles described in Falush et al. (2007). Mean likelihoods of K from ten runs were plotted using STRUCTURE HARVESTER 0.56.3 (available at http://taylor0.biology.ucla.edu/struct_harvest/).

Spatial autocorrelation analyses among individuals were performed with SPAGEDI (Hardy & Vekemans, 2002). We used as statistics the Loiselle's kinship coefficient (ϕij; Loiselle et al. 1995) which is not dependent on Hardy–Weinberg (HW) equilibrium conditions (Hardy, 1999) and it has been proved to be very effective in determining spatial genetic structure (Vekemans & Hardy, 2004). Two different analyses were performed. For the first one aimed to

test spatial autocorrelation within the cave, distance categories were set based on the distance between ~~two~~ tiles ~~(20 cm)~~ and considering the distance between two individuals found within the same tile as zero. For the second one, a spatial autocorrelation analyses within those tiles containing more individuals (T4, T5, T7, T8, T9 and T10; see results) were carried out. Taking into account the distance between individuals, the distance categories were set in such a way that the number of pairwise comparisons within each distance category was approximately constant. Statistical significance was based on permuting individual locations among all individuals 10000 times and calculating upper and lower 95% confidence interval for each distance class.

Results

Red coral recruitment

Overall 372 individuals were observed on the 14 tiles. ~~*C.*~~ *rubrum* settled on every tile deployed on the ceiling of the cave, but, recruitment failed on three out of eight tiles on the walls (T1, T13, T14). In fact, of the 372 individuals, 350 were found on the ceiling tiles, and only 22 on the tiles deployed on the walls, corresponding to a significantly higher density on the ceiling than on walls (ANOVA: $F_{1,14} = 11.45$; $P < 0.01$, Fig. 2). On tiles located on the ceiling of the cave both recruits and juveniles were found, while on walls only recruits occurred (Fig. 3). Of the 350 individuals on the ceiling, 301 were recruits and 49 were juveniles, corresponding to a density of 26.5 ± 23.5 /400 cm^2 and of 5 ± 7.52 / 400 cm^2, respectively (ANOVA: $F_{1,26} = 5.21$; $P < 0.01$, Fig. 2).

Growth performances

The size structure of the recruits presented a decreasing and monotonous trend with 126 recruits (43.2%) with a diameter <1.5 mm. Only five recruits (1.6%) had a diameter > 6 mm and as also previously observed (Cerrano C, pers. comm.) these recruits seem formed by two or more planulae merged together (Fig. 2). The number of polyps ranged from 1 to 23, with ~~the~~ 70% of the recruits presenting 1-2 polyps, 21.3% between 3-4 polyps and ~~the~~ 8.7% more than four polyps (mean ± SD = 2.34 ± 2.16 polyps/recruit). Pearson's coefficient showed a low correlation (C.I. = 0.4) between ~~diameter and number of polyps~~ (Fig. 4).

The size structure of juveniles showed a more variable trend, with diameter values ranging from 0.14 mm to 8.9 mm with an average value of 2.6 ± 1.5 mm. The number of polyps ranged from 3 to 22, with an average number of 9.23 ± 4.6. ~~9.7%~~ of the juveniles had 3-4 polyps while 90.3% of them had more than five polyps. The height of the juveniles ranged from 0.5 to 9.15 mm, with a mean of 3.3 ± 1.8 mm. Pearson's correlation coefficients showed no correlation between diameter and number of polyps (C.I. = 0.06, Fig. 5a) ~~and~~ diameter and height (C.I. = 0.04, Fig. 5b). A weak correlation was observed between height and number of polyps (C.I. = 0.4, Fig. 5c).

Genetic variability

A total of 290 red coral individuals were genotyped. No evidence of scoring errors due to stuttering or large allele dropout were found, according to MICROCHECKER. An excess of homozygotes was detected at all loci due to the presence of null alleles. Null allele frequencies ranged from 0.09 (Mic24) to 0.25 (Cor9, Mic26 and Cor58). Number of alleles ranged from 10 (Mic20) to 23 (Cor9 and Mic26). The expected and observed heterozygosity ranged from 0.29

(Cor46) to 0.81(Cor9) and from 0.13 (Mic26) to 0.64 (Mic20), respectively. The estimators of F_{IS} (*f*) were positive and ranged from 0.21 (Mic20) to 0.86 (Cor48) (Table 1).

We found a low genetic ~~variability~~ within individuals (H_e= 0.59 ± 0.16 and H_o= 0.29±0.2; mean ± SD). Out of the 290 individuals analysed, 281 different multilocus genotypes (MLGs) were identified. Four MLGs were found twice; one MLG was encountered three times and one four times. Individuals sharing the same MLG always came from the same settlement tile. The tiles where identical MLGs were found were T7, T8 and T10. The individuals sharing the same MLG were between 0.2 cm and 13.65 cm away (in T7 and T10, respectively). In T8 and T10, one recruit and one juvenile shared a MLG. The unbiased probability of identity (P_{ID}) that two sampled individuals share identical MLG by chance was $1.5e^{-04}$.

No significant genetic variability was observed between juveniles and recruits in terms of H_e and H_o (P values associated with the permutation procedure: P_{Ho}=0.65 and P_{He}=0.71); ~~while~~ recruits showed a significantly higher allelic richness than juveniles (P_{Ar}=0.001).

Relatedness

We found a high degree of genetic relatedness among individuals: a t-test performed on the mean relatedness observed and those obtained through a permutation procedure (expected under panmixia) revealed significant differences between them (observed mean rxy=0.027, resampled mean rxy=0.014, t=1173, P = 0.001). Pairwise relatedness based on the Queller and Goodnight (1989) coefficient revealed that 25.98% of the pairwise comparisons were involved in one or more parentage relationships, with 8.57% and 17.41% of individuals involved in a full-sib and half-sib relationship, respectively. These percentages were numerically similar considering the relatedness within the two temporal cohorts separately.

The percentage of individuals correctly assigned to their source by the classical individual assignments method using GENECLASS was around 50%. Exclusion tests identified 81 putative first-generation migrants within the cave corresponding to ~28% of all individuals. All the 81 putative migrants have been assigned to other tiles: 37 individuals were assigned to the closest tile, while 44 of them were assigned to more distant tiles. The effective population size (Ne) was estimated in 30 for recruits (95% CI: 18-49) and 20 for juveniles (95% CI: 11-39).

Spatial and temporal genetic structure

No genetic structuring was observed between recruits and juveniles (F_{ST} = 0.008, P = 0.16). The clustering method did not find spatial genetic discontinuity among all the individuals as only one cluster was detected (K=1, Mean LnP (K) = -5679.455).

Significantly positive kinship coefficients were detected ~~between all the individuals within~~ the single tile (φij = 0.066, $p < 0.001$) indicating that individuals within tiles had a higher genetic relatedness than random pairs of individuals. ~~The~~ autocorrelogram suggested an estimated patch size of less than 40 cm (Fig. 6). The spatial autocorrelation between individuals within each tile showed a significant positive value within a range from 3 to 10 cm.

Discussion

In the present study population structure and population genetic approaches were used to investigate the early life history traits of the Mediterranean red coral, through settlement tiles deployed inside a submarine cave. In the submarine cave, red coral recruited every year with significant differences in abundance at both temporal and spatial scales. Moreover, no significant genetic differences at both scales were detected.

303 During summers 2010 and 2011 *C. rubrum* successfully recruited on artificial tiles inside
304 the cave. The density of settlement was higher than that observed by Bramanti et al. (2014) for
305 populations of the Cap de Creus marine reserve (Costa Brava: 42°29.21' N; 03°30.18'E, Spain,
306 5.6 ± 2.8 individuals / 400 cm^2) and on vertical cliffs in Portofino (Eastern Ligurian Sea:
307 44°18.18' N; 09°12.83'E, Italy. 17.5 ± 4.7 individuals / 400 cm^2). A significant difference in
308 abundance of individuals was detected between walls and ceiling, with red coral recruitment
309 more successful on the ceiling of the cave. The only three tiles (T1, T13, and T14) where no
310 recruitment was recorded were positioned inside the red coral population growing on the walls of
311 the cave. The mechanisms at the base of these differences are not fully understood yet, but they
312 could be related to larval behavior (Martínez-Quintana et al., 2014), habitat characteristics (Cau
313 et al., 2016), or different abiotic and biotic factors, such as water flow, shade from light and/or
314 sedimentation (Virgilio, Airoldi & Abbiati, 2006). Even if *C. rubrum* larvae could survive at
315 least 16 days (potentially up to 42 days) in the plankton (Martínez-Quintana et al., 2014) inside
316 caves and/or overhangs they probably remain trapped in the ceiling due to their active upward
317 swimming behavior (Weinberg, 1979; Martínez-Quintana et al., 2014). Moreover, heterogeneity
318 of the substrate (e.g. small protuberances, hollows, flats, silt deposits on walls) may lead to a
319 significant microhabitat complexity, which might positively affect the recruitment and the
320 interactions among established organisms (Airoldi & Cinelli, 1996; Benedetti-Cecchi et al.,
321 1996).

322 Due to the difficulties in identifying tiny individuals (Bramanti, Magagnini & Santangelo,
323 2003), the early life stage of this species has been studied only in a few *C. rubrum* populations
324 (Garrabou & Harmelin, 2002; Bramanti et al., 2005, 2007; Santangelo & Cupido, 2012). Here for
325 the first time it has been possible to subdivide the *C. rubrum* individuals into two subclasses:

recruits and juveniles. Significant differences in the abundance of recruits and juveniles were observed, suggesting high post-settlement mortality. This high mortality could be related to the intra- and inter-specific interactions mediated by chemical cues release, bioturbation and food limitation, local flow patterns, predation and competition for space (Fraschetti et al., 2002). Competition for space, together with grazing intensity, is known to produce variations in coral recruitment at this spatial scale (Babcock et al. 1985). Moreover, also post-settlement processes are known to induce high mortality rates during the first months of life greatly influencing early population dynamics and recruitment rates of gorgonians (Caley et al., 1996; Perkol-Finkel et al., 2008) and of *C. rubrum* in particular (Garrabou & Harmelin, 2002; Bramanti, Magagnini & Santangelo, 2003). It is also possible that observed differences arose from ~~to~~ inter-annual variability in larval supply.

The number of polyps per individuals found was consistent to those measured by Bramanti et al. (2005), with a higher number of polyps in juveniles compared to recruits, and a significant correlation between number of polyps and height in the juveniles. Moreover, individuals settling on tiles showed a considerable variability in diameter and height, suggesting that growth rate in early red coral stages may be extremely variable and possibly much higher than the growth rates estimated in adult colonies (Bramanti et al. 2005; but see Table S2 in Cerrano et al. (2013)).

The level of genetic variability found was lower than values reported previously in other red coral populations (Costantini, Fauvelot & Abbiati, 2007a; Ledoux et al., 2010a). Lower genetic variability could be due to the small population size, and/or to a low effective population size with only a small proportion of the adults that contribute to maintain the populations. Based on the high density of individuals observed and the high density of the adults within the cave (Cerrano C. pers. comm.) and on the vertical walls outside the cave (the mean density of colonies

in the Portofino Promontory was 227 ± 37 colonies x m^2, Bavestrello et al., (2015)) the first hypothesis seems unlikely. A small effective population size is also confirmed by the values estimated from sibship assignment (30% of individuals related at some degree).

The significant deviations from Hardy–Weinberg equilibrium, emphasized in the high positive F_{IS} estimates, suggest high inbreeding rates (e.g. mating between relatives) within the Colombara cave (but see also Costantini et al. 2007b). In the data set, null alleles were found for some loci and tiles, but given their frequency, and previous observations in other red coral populations (Costantini, Fauvelot & Abbiati, 2007b), it is unlikely that the observed positive F_{IS} estimates were caused only by the presence of null alleles. As observed ~~before,~~ larval behaviour more than larval dispersal and processes affecting the intra-population gene flow might have contributed to the observed heterozygote deficits. The low genetic variability and high inbreeding rates are considered to affect populations negatively. Despite this, there are no indications of decreasing fitness due to inbreeding depression in the shallow red coral populations (Bramanti, Iannelli & Santangelo, 2009). However, further restrictions on the genetic diversity, and hence reduction of population size due to, for example, environmental changes, could increase inbreeding above the 'inbreeding threshold,' resulting in loss of fitness and risk of extinction (Frankham, 1995).

Conclusion

The Colombara cave seems to host a closed and self-recruiting population of *C. rubrum*. All genotyped individuals suggest that they belong to a single homogeneous population. On the other hand, the genetic similarity observed together with the high number of putative migrants, indicates that larvae can disperse to a certain extent within the cave. The detail of small-scale spatial distribution provided by our experimental design revealed a fine genetic structure within

the apparently homogeneous cave. A spatial autocorrelation was observed within each tile. Since patterns of spatial autocorrelation are dependent on both sample size and distance between samples, a patch size of less than 8 cm could be observed.

No genetic structure was observed between recruits and juveniles suggesting that the two reproductive events interested individuals belong to the same genetic pool. All these aspects together with the high relatedness among settlers (full and half-sib relationship), suggest that in the two years of the study a low and genetically similar number of progenitors have contributed to the larval pool in both reproductive events.

To conclude, our data represent another brick on the wall of the knowledge on the fundamental ecological processes ruling the early life history of red coral, involving the environmental factors (e.g. light, temperature, etc.), and biological processes (e.g. reproductive biology, recruitment, connectivity) which regulate their survival and distribution.

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References

Airoldi L, Cinelli F 1996. Variability of fluxes of particulate material in a submarine cave with chemolithoautotrophic inputs of organic-carbon. *Marine Ecology Progress Series* 139:205–217. DOI:10.3354/meps139205

Aurelle D, Ledoux J-B, Rocher C, Borsa P, Chenuil A, Féral J-P. 2011. Phylogeography of the

red coral (*Corallium rubrum*): inferences on the evolutionary history of a temperate gorgonian. *Genetica* 139:855–869. DOI: 10.1007/s10709-011-9589-6.

Babcock R, Mundy C. 1996. Coral recruitment: consequences of settlement choice for early growth and survivorship in two scleractinians. *Journal of Experimental Marine Biology and Ecology* 206:179–201. DOI: https://doi.org/10.1016/S0022-0981(96)02622-6

Bavestrello G, Bo M, Bertolino M, Betti F, Cattaneo-Vietti R. 2015. Long-term comparison of structure and dynamics of the red coral metapopulation of the Portofino Promontory (Ligurian Sea): a case-study for a Marine Protected Area in the Mediterranean Sea. 36:1354–1363. DOI: 10.1111/maec.12235.

Belkhir K, Borsa P, Chikhi L, Raufaste N, Bonhomme F. 2000. GENETIX 4.05, Logiciel sous Windows pour la Génétique des Populations. Montpellier: Laboratoire Génome, Université Montpellier.

Belkhir K, Castric V, Bonhomme F. 2002. Identix, a software to test for relatedness in a population using permutation methods. *Molecular Ecology Notes* 2:611–614. DOI: 10.1046/j.1471-8286.2002.00273.x.

Benedetti-Cecchi L, Airoldi L, Abbiati M, Cinelli F. 1996. Estimating the abundance of benthic invertebrates: a comparison of procedures and variability between observers. *Marine Ecology Progress Series* 138:93–101.

Benedetti-Cecchi L, Airoldi L, Abbiati M, Cinelli F. 1998. Spatial variability in the distribution of sponges and cnidarians in a sublittoral marine cave with sulphur-water springs. *Journal of the Marine Biological Association of the United Kingdom* 78:43–58.

Bramanti L, Edmunds P. 2016. Density-dependent recruitment mediates coral population dynamics on a coral reef. *Coral Reefs* 35:543–553. DOI: 10.1007/s00338-016-1413-4.

Bramanti L, Iannelli M, Santangelo G. 2009. Mathematical modelling for conservation and management of gorgonians corals: youngs and olds, could they coexist? *Ecological Modelling* 220:2851–2856. DOI: 10.1016/j.ecolmodel.2009.01.031.

Bramanti L, Magagnini G, De Maio L, Santangelo G. 2005. Recruitment, early survival and growth of the Mediterranean red coral *Corallium rubrum* (L 1758), a 4-year study. *Journal of Experimental Marine Biology and Ecology* 314:69–78. DOI: 10.1016/j.jembe.2004.08.029.

Bramanti L, Magagnini G, Santangelo G. 2003. Settlement and recruitment: the first stages in the life cycle of two epibenthic suspension feeders (*Corallium rubrum* and *Anomia ephippium*). *Italian Journal of Zoology*:1–14.

Bramanti L, Rossi S, Tsounis G, Gili JM, Santangelo G. 2007. Settlement and early survival of red coral on artificial substrates in different geographic areas: some clues for demography

and restoration. *Hydrobiologia* 580:219–224. DOI: 10.1007/s10750-006-0452-1.

Bramanti L, Vielmini I, Rossi S, Tsounis G, Iannelli M, Cattaneo-Vietti R, Priori C, Santangelo G. 2014. Demographic parameters of two populations of red coral (*Corallium rubrum* L. 1758) in the North Western Mediterranean. *Marine Biology* 161:1015–1026. DOI: 10.1007/s00227-013-2383-5.

Brazeau D, Sammarco P, Atchison A. 2011. Micro-scale genetic heterogeneity and structure in coral recruitment: fine-scale patchiness. *Aquatic Biology* 12:55–67. DOI: 10.3354/ab00313.

Bussoletti E, Cottingham D, Bruckner A, Roberts G, Sandulli R. 2010. Proceedings of the international workshop on red coral science, management, and trade: lessons from the Mediterranean. Naples, Italy, September 23-26, 2009.

Caley J, Carr M, Hixon M, Hughes T, Jones G, Menge B. 1996. Recruitment and the local dynamics of open marine populations. *Annual Revue in Ecology and Systematic* 27:477–500.

Cau A, Bramanti L, Cannas R, Follesa MC, Angiolillo M, Canese S, Bo M, Cuccu D, Guizien K. 2016. Habitat constraints and self-thinning shape Mediterranean red coral deep population structure: implications for conservation practice. *Scientific Reports* 6:23322. DOI:10.1038/srep23322

Cerrano C, Cardini U, Bianchelli S, Corinaldesi C, Pusceddu A, Danovaro R. 2013. Red coral extinction risk enhanced by ocean acidification. *Scientific Reports* 3. DOI: 10.1038/srep01457.

Chapuis M, Estoup A. 2007. Microsatellite null alleles and estimation of population differentiation. *Molecular Biology and Evolution* 24:621–631. DOI: 10.1093/molbev/msl191.

Chevaldonné P., Lejeusne C. 2003. Regional warming-induced species shift in north-west Mediterranean marine caves. E. *Ecology Letters* 6:371–379.

Costantini F, Abbiati M. 2006. Development of microsatellite markers for the Mediterranean gorgonian coral *Corallium rubrum. Molecular Ecology Notes* 6:521–523. DOI: 10.1111/j.1471-8286.2006.01305.x.

Costantini F, Fauvelot C, Abbiati M. 2007a. Fine-scale genetic structuring in *Corallium rubrum:* evidence of inbreeding and limited effective larval dispersal. *Marine Ecology Progress Series* 340:109–119. DOI: 10.3354/meps340109.

Costantini F, Fauvelot C, Abbiati M. 2007b. Genetic structuring of the temperate gorgonian coral (*Corallium rubrum*) across the western Mediterranean Sea revealed by microsatellites and nuclear sequences. *Molecular Ecology* 16:5168–82. DOI: 10.1111/j.1365-294X.2007.03579.x.

Dempster A, Laird N, Rubin D. 1977. Maximum likelihood from incomplete data via the EM algorithm. *Journal of the Royal Statistical Society* 39:1–38.

Falush D, Stephens M, Pritchard J. 2007. Inference of population structure using multilocus genotype data: dominant markers and null alleles. *Molecular Ecology Notes* 7:574–578. DOI: 10.1111/j.1471-8286.2007.01758.x.

Frankham R. 1995. Inbreeding and Extinction: A Threshold Effect. *Conservation Biology* 9:792–799. DOI: 10.1046/j.1523-1739.1995.09040792.x.

Fraschetti S, Giangrande A, Terlizzi A, Boero F. 2002. Pre- and post-settlement events in benthic community dynamics. *Oceanologica Acta* 25:285–295. DOI: 10.1016/S0399-1784(02)01194-5.

Garrabou J, Harmelin J. 2002. A 20-year study on life-history traits of a harvested long-lived temperate coral in the NW Mediterranean: insights into conservation and management needs. *Journal of Animal Ecology* 71:966–978. DOI: 10.1046/j.1365-2656.2002.00661.x.

Garrabou J, Perez T, Sartoretto S, Harmelin J. 2001. Mass mortality event in red coral *Corallium rubrum* populations in the Provence region (France, NW Mediterranean). *Marine Ecology Progress Series* 217:263–272. DOI: 10.3354/meps217263.

Garrabou J, Sala E, Linares C, Ledoux JB, Dominici JM. 2017. Re-shifting the ecological baseline for the overexploited Mediterranean red coral. *Nature Publishing Group*:1–6. DOI: 10.1038/srep42404.

Giakoumi S, Sini M, Gerovasileiou V, Mazor T, Beher J, Possingham HP, Abdulla A, Ã‡Inar ME, Dendrinos P, Gucu AC, Karamanlidis AA, Rodic P, Panayotidis P, Taskin E, Jaklin A, Voultsiadou E, Webster C, Zenetos A, Katsanevakis S. 2013. Ecoregion-Based Conservation Planning in the Mediterranean: dealing with large-scale heterogeneity. *PLoS ONE* 8:e76449. DOI: 10.1371/journal.pone.0076449.

Goudet J, Raymond M, De-Meeus T, Rousset F. 1996. Testing differentiation in diploid populations. *Genetics* 144:1933–1940.

Guizien K, Belharet M, Guarini J, Marsaleix P. 2012. Using larval dispersal simulations for marine protected area design: application to the Gulf of Lions (northwest Mediterranean). *Limnoogy and Oceanography* 57:1099–1112. DOI: 10.4319/lo.2012.57.4.1099

Guizien K, Brochier T, Duchêne JC, Koh BS, Marsaleix P. 2006. Dispersal of *Owenia fusiformis* larvae by wind-driven currents: turbulence, swimming behaviour and mortality in a threedimensional stochastic model. *Marine Ecology Progress Series* 311:47–66. DOI: 10.3354/meps311047

Hardy OJ. 1999. Isolation by distance in a continuous population: reconciliation between spatial autocorrelation analysis and population genetics models. *Heredity* 83:145. DOI:

10.1046/j.1365-2540.1999.00558.x.

Hardy OJ, Vekemans X. 2002. Spagedi: a versatile computer program to analyse spatial genetic structure at the individual or population levels. *Molecular Ecology Notes* 2:618–620. DOI: 10.1046/j.1471-8286.2002.00305.x.

Harmelin J-G, Vacelet J, Vasseur P. 1985. Les grottes sous-marines obscures: un mileu extrème et remarquable biotope refuge. *Tethys* 1985:214–219.

Jones GP, Planes S, Thorrold SR. 2005. Coral reef fish larvae settle close to home. *Current Biology* 15:1314–8. DOI: 10.1016/j.cub.2005.06.061.

Jones OR, Wang J. 2010. COLONY: a program for parentage and sibship inference from multilocus genotype data. *Molecular Ecology Resources* 10:551–5. DOI: 10.1111/j.1755-0998.2009.02787.x.

Kendall M, Stewart A. 1977. *The advanced theory of statistics. Vol. 1*. New York: Macmillan.

Keough MJ, Downes BJ. 1982. Recruitment of marine invertebrates : the role of active larval choices and early mortality. *Oecologia* 54:348–352.

Laborel J, Vacelet J. 1961. Répartition bionomique du Corallium rubrum LMCK dans les grottes et falaises sous-marines. *Rapports et Proces-Verbaux des Réunions de la Commission Internationale pour l'Exploration Scientifique de la Mer Méditerranée* 16:464–469.

Lasker HR. 2013. Recruitment and resilience of a harvested Caribbean octocoral. *PloS one* 8:e74587. DOI: 10.1371/journal.pone.0074587.

Ledoux J-B, Garrabou J, Bianchimani O, Drap P, Féral J-P, Aurelle D. 2010a. Fine-scale genetic structure and inferences on population biology in the threatened Mediterranean red coral, *Corallium rubrum*. *Molecular ecology*:4204–4216. DOI: 10.1111/j.1365-294X.2010.04814.x.

Ledoux J-B, Mokhtar-Jamaï K, Roby C, Féral J-P, Garrabou J, Aurelle D. 2010b. Genetic survey of shallow populations of the Mediterranean red coral [*Corallium rubrum* (Linnaeus, 1758)]: new insights into evolutionary processes shaping nuclear diversity and implications for conservation. *Molecular Ecology* 19:675–90. DOI: 10.1111/j.1365-294X.2009.04516.x.

Levin L. 1990. A review of methods for labeling and tracking marine invertebrate larvae. *Ophelia* 32:115–144.

Lindsay SM, Wethey DS, Woodin SA. 1997. Modeling interactions of browsing predation, infaunal activity, and recruitment in marine soft-sediment habitats. *Oceanographic Literature Review* 44.

Loiselle BA, Sork VL, Nason J, Graham C. 1995. Spatial genetic structure of a tropical

534 understory Shrub, *Psychotria officinalis* (Rubiaceae). *American Journal of Botany* 82:1420.
535 DOI: 10.2307/2445869.

536 Martínez-Quintana A, Bramanti L, Viladrich N, Rossi S, Guizien K. 2014. Quantification of
537 larval traits driving connectivity: the case of *Corallium rubrum* (L. 1758). *Marine Biology*.
538 DOI: 10.1007/s00227-014-2599-z.

539 Morri C, Bianchi CN, Damiani V, Peirano A, Romeo G, Tunesi L. 1986. L'ambiente marino tra
540 Punta della Chiappa e Sestri Levante (Mar Ligure): profilo ecotipologico e proposta di carta
541 bionómica. *Bollettino dei Musei e Degli Istituti Biologici dell'Università di Genova* 52:213–
542 231.

543 Nei M. 1973. Analysis of fene diversity in subdivided populations. *Proceedings of the National*
544 *Academy of Sciences* 70:3321–3323. DOI: 10.1073/pnas.70.12.3321.

545 Van Oosterhout C, Hutchinson WF, Wills DPM, Shipley P. 2004. Micro-checker: software for
546 identifying and correcting genotyping errors in microsatellite data. *Molecular Ecology*
547 *Notes* 4:535–538. DOI: 10.1111/j.1471-8286.2004.00684.x.

548 Padron M, Guizien K. 2015. Modelling the effect of demographic traits and connectivity on the
549 genetic structuration of marine metapopulations of sedentary benthic invertebrates. *ICES*
550 *Journal of Marine Science*:fsv158. DOI: 10.1093/icesjms/fst034.

551 Padrón M, Costantini F, Baksay S, Bramanti L, Guizien K (submitted) Larval transport explains
552 contemporary gene flow in a Mediterranean gorgonian. *Coral Reefs*

553 Parravicini V, Guidetti P, Morri C, Montefalcone M, Donato M, Bianchi CN. 2010.
554 Consequences of sea water temperature anomalies on a Mediterranean submarine cave
555 ecosystem. *Estuarine, Coastal and Shelf Science* 86:276–282. DOI:
556 10.1016/j.ecss.2009.11.004.

557 Peakall R, Smouse PE. 2006. Genalex 6: genetic analysis in Excel. Population genetic software
558 for teaching and research. *Molecular Ecology Notes* 6:288–295. DOI: 10.1111/j.1471-
559 8286.2005.01155.x.

560 Perkol-Finkel S, Zilman G, Sella I, Miloh T, Benayahu Y. 2008. Floating and fixed artificial
561 habitats:spatial and temporal patterns of benthic communities in a coral reef environment.
562 *Estuarine, Coastal and Shelf Science* 77:491–500. DOI: 10.1016/j.ecss.2007.10.005.

563 Piry S, Alapetite A, Cornuet J-M, Paetkau D, Baudouin L, Estoup A. 2004. GENECLASS2: a
564 software for genetic assignment and first-generation migrant detection. *The Journal of*
565 *Heredity* 95:536–9. DOI: 10.1093/jhered/esh074.

566 Pritchard J, Matthew S, Donnelly P. 2000. Inference of population structure using multilocus
567 genotype data. *Genetics* 155:945–959.

568 Queller D., Goodnight K. 1989. Estimating relatedness using genetic markers. *Evolution* 43:258–
569 275.

570 Rannala B, Mountain JL. 1997. Detecting immigration by using multilocus genotypes.
571 *Proceedings of the National Academy of Sciences* 94:9197–9201. DOI:
572 10.1073/pnas.94.17.9197.

573 Rastorgueff PA, Bellan-Santini D, Bianchi CN, Bussotti S, Chevaldonné ., Guidetti ., Harmelin
574 J.G., Montefalcone M, Morri C, Perez T, Ruitton S, Vacelet J. PS. 2015. An ecosystem-
575 based approach to evaluate the ecological quality of Mediterranean undersea caves.
576 *Ecological Indicators* 54:137–152.

577 Raymond M., Rousset F. 1995. An exact test for population differentiation. *Evolution* 49:1280–
578 1283. DOI: 10.2307/2410454.

579 Santangelo G, Bramanti L, Rossi S, Tsounis G, Vielmini I, Lott C, Gili JM. 2012. Patterns of
580 variation in recruitment and post-recruitment processes of the Mediterranean precious
581 gorgonian coral *Corallium rubrum*. *Journal of Experimental Marine Biology and Ecology*
582 411:7–13. DOI: 10.1016/j.jembe.2011.10.030.

583 Santangelo G, Carletti E, Maggi E, Bramanti L. 2003. Reproduction and population sexual
584 structure of the overexploited Mediterranean red coral Corallium rubrum. *Marine Ecology*
585 *Progress Series* 248:99–108. DOI: 10.3354/meps248099.

586 Santangelo G, Cupido R. 2012. Demography of long-lived octocorals: survival and local
587 extinction. *Proceedings of the 12th International Coral Reef Symposium*:9–13.

588 Smilansky V, Lasker HR. 2014. Fine-scale genetic structure in the surface brooding Caribbean
589 octocoral, *Antillogorgia elisabethae*. *Marine Biology* 161:853–861. DOI: 10.1007/s00227-
590 013-2385-3.

591 Torda G, Lundgren P, Willis BL, van Oppen MJH. 2013. Genetic assignment of recruits reveals
592 short- and long-distance larval dispersal in *Pocillopora damicornis* on the Great Barrier
593 Reef. *Molecular Ecology* 22:5821–34. DOI: 10.1111/mec.12539.

594 Torrents O, Garrabou J. 2011. Fecundity of red coral *Corallium rubrum* (L.) populations
595 inhabiting in contrasting environmental conditions in the NW Mediterranean. *Marine*
596 *Biology* 158:1019–1028. DOI: 10.1007/s00227-011-1627-5.

597 Tsounis G, Rossi S, Bramanti L, Santangelo G. 2013. Management hurdles for sustainable
598 harvesting of *Corallium rubrum*. *Marine Policy* 39:361–364. DOI:
599 10.1016/j.marpol.2012.12.010.

600 Underwood A, Fairweather P. 1989. Supply-side ecology and benthic marine assemblages.
601 *TREE* 4:16–19.

602 Vekemans X, Hardy OJ. 2004. New insights from fine-scale spatial genetic structure analyses in
603 plant populations. *Molecular Ecology* 13:921–935. DOI: 10.1046/j.1365-
604 294X.2004.02076.x.

605 Virgilio M, Airoldi L, Abbiati M. 2006. Spatial and temporal variations of assemblages in a
606 Mediterranean coralligenous reef and relationships with surface orientation. *Coral Reefs*
607 25:265–272. DOI: 10.1007/s00338-006-0100-2.

608 Wang S, Zhang L, Meyer E, Matz MV. 2009. Construction of a high-resolution genetic linkage
609 map and comparative genome analysis for the reef-building coral *Acropora millepora*.
610 *Genome Biology* 10:R126. DOI: 10.1186/gb-2009-10-11-r126.

611 Weinberg S. 1979. The light-dependent behaiviour of planulae larvae of *Eunicella singularis* and
612 *Corallium rubrum* and its implications for for octocoral ecology. *Bijds. Dijerkd* 49:16–30.

613 Weir B, Cockerham C. 1984. Estimating F-statistics for the analysis of population structure.
614 *Evolution* 38:1358–1370. DOI: 10.2307/2408641.

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617

618

Figure 1

Scheme of the tiles deployed.

Draft of the underwater cave and scheme of the tiles deployed for this experiment. White rectangles represent the tiles.

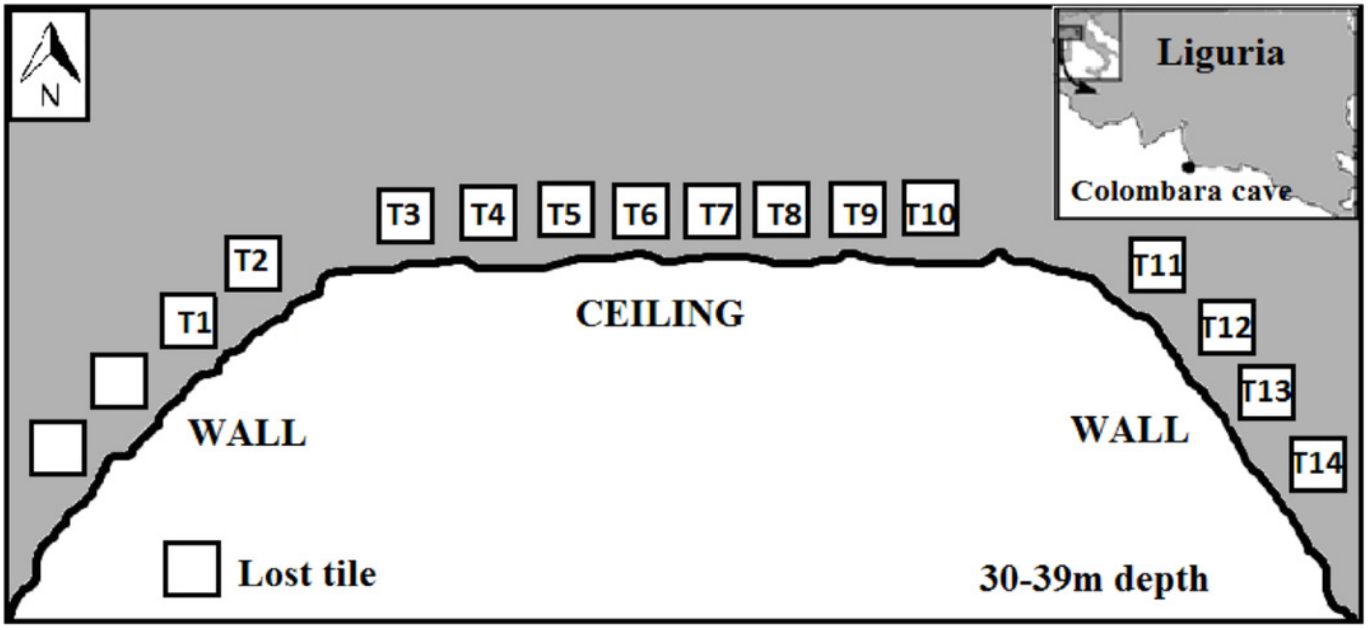


Figure 2

Example of a PVC tile recovered from the Colombara cave after two years.

(A) tile T7; (B) zoom of a recruit; (C) a juvenile and (D) a recruit probably derived by two merged planulae .

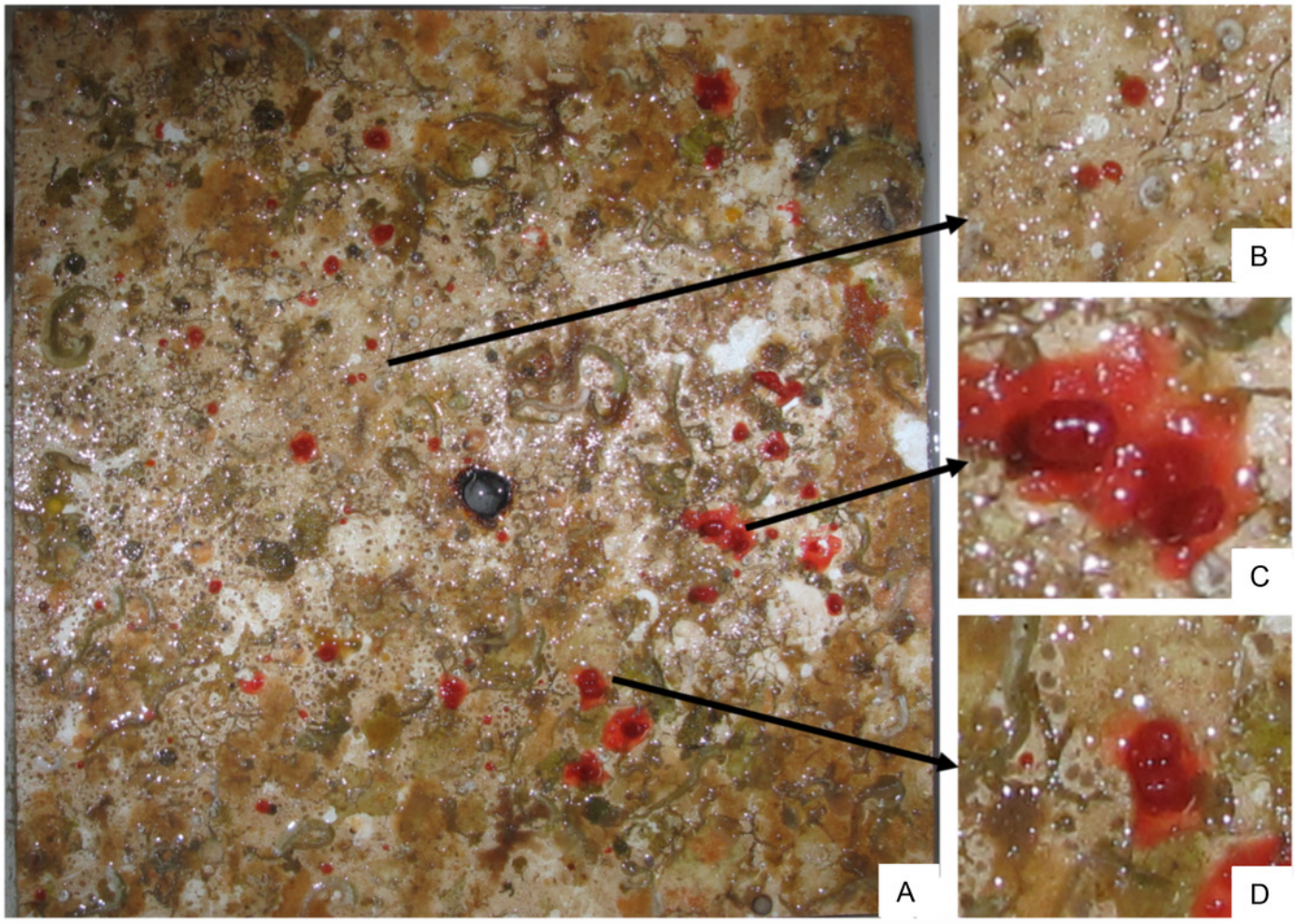


Figure 3

Number of settlers on the 14 tiles deployed in the Colombara cave.

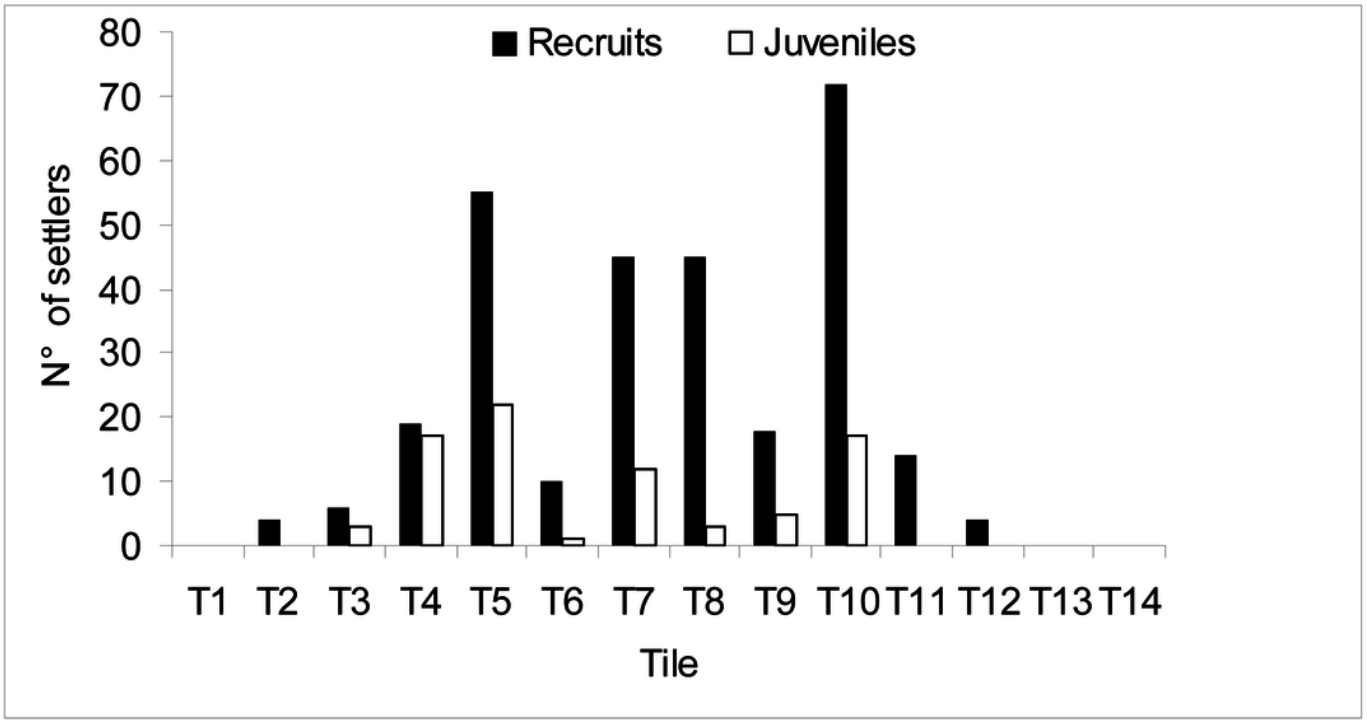


Figure 4

Distribution of red coral recruits.

A) diameter classes; B) n. of polyp’s classes; C) Relationship between n. of polyps and diameter. C.I.: Pearson coefficient.

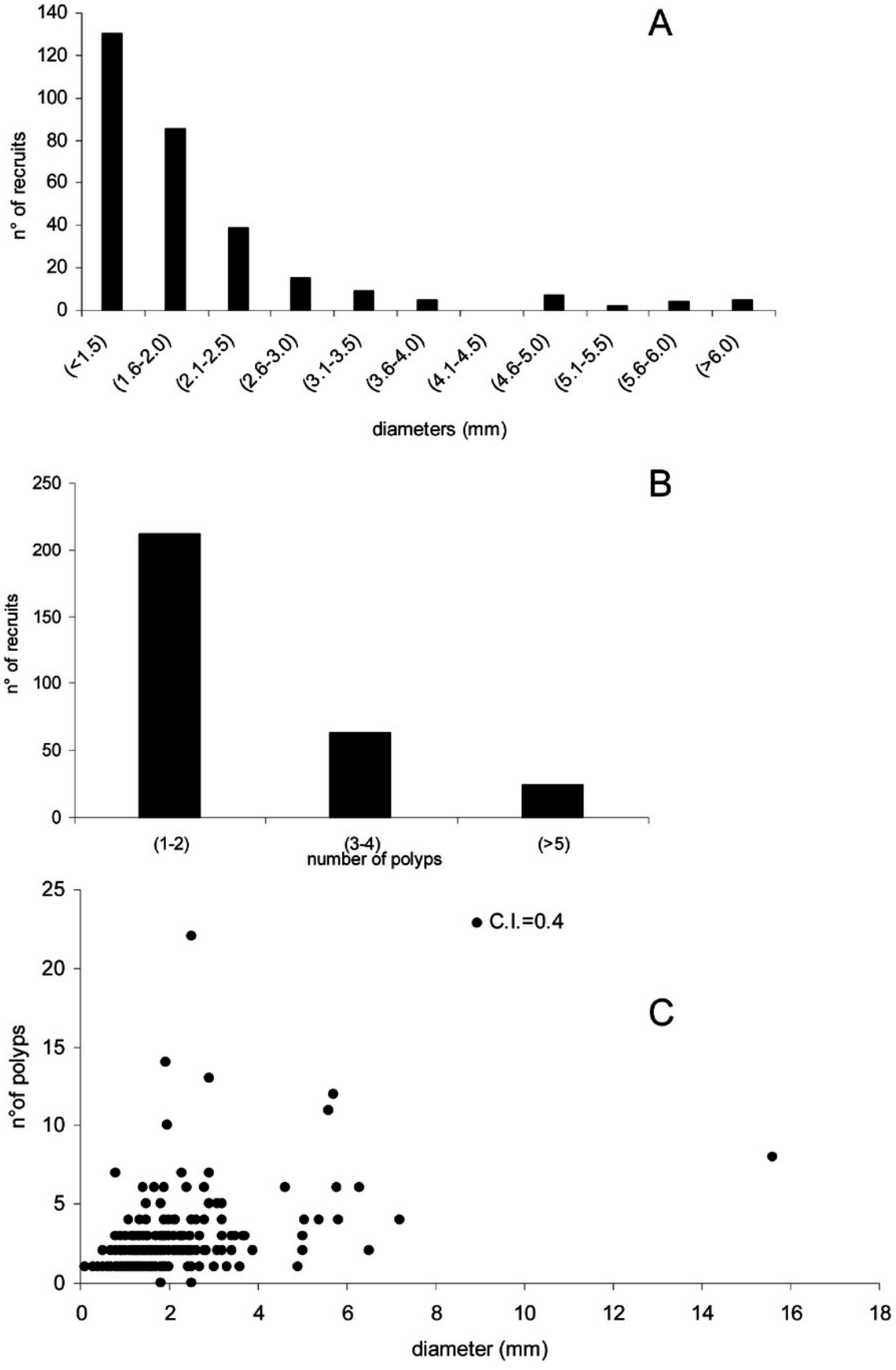
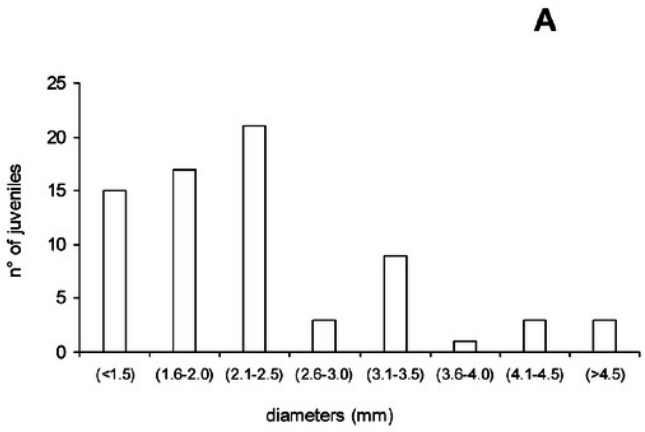
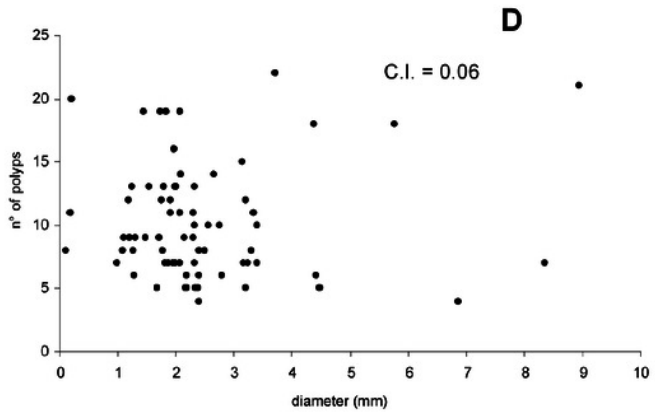

A
n° of recruits
140
120
100
80
60
40
20
0
(<1.5)
(1.6-2.0)
(2.1-2.5)
(2.6-3.0)
(3.1-3.5)
(3.6-4.0)
(4.1-4.5)
(4.6-5.0)
(5.1-5.5)
(5.6-6.0)
(>6.0)
diameters (mm)
B
n° of recruits
250
200
150
100
50
0
(1-2)
(3-4)
(>5)
number of polyps
C
C.I.=0.4
n°of polyps
25
20
15
10
5
0
0
2
4
6
8
10
12
14
16
18
diameter (mm)

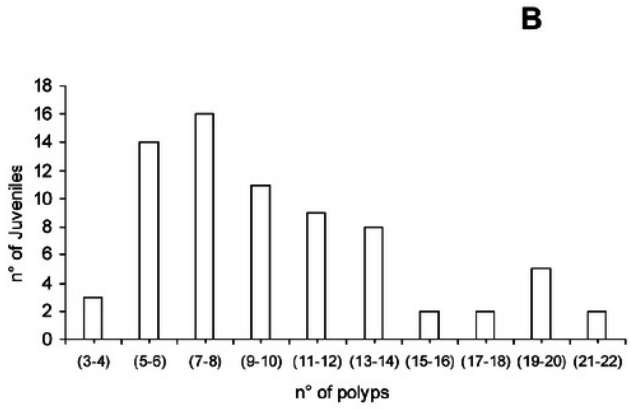
Figure 5

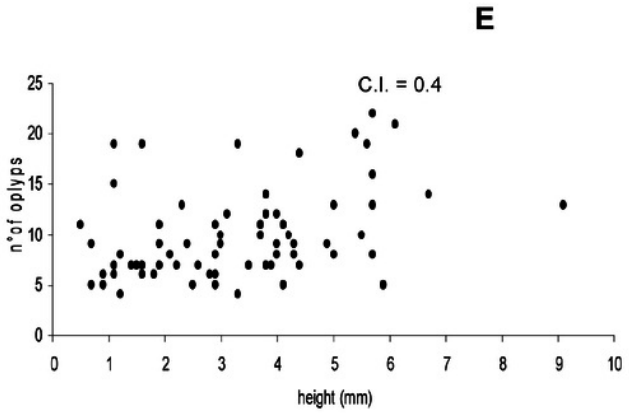
Distribution of red coral juveniles.

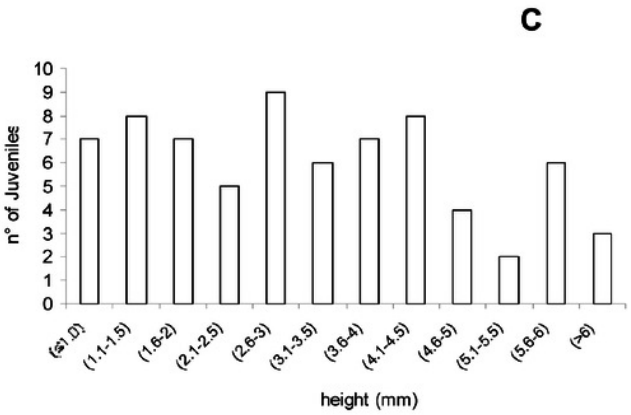
A) diameter classes; B) n. of polyps classes; C) height classes. Relationship between: D) n of polyps and diameter; E) height and diameter; F) n. of polyps and height. C.I.: Pearson coefficient.


A
n° of juveniles
25
20
15
10
5
0
(<1.5)
(1.6-2.0)
(2.1-2.5)
(2.6-3.0)
(3.1-3.5)
(3.6-4.0)
(4.1-4.5)
(>4.5)
diameters (mm)


D
C.I. = 0.06
n° of polyps
25
20
15
10
5
0
0
1
2
3
4
5
6
7
8
9
10
diameter (mm)


B
n° of Juveniles
18
16
14
12
10
8
6
4
2
0
(3-4)
(5-6)
(7-8)
(9-10)
(11-12)
(13-14)
(15-16)
(17-18)
(19-20)
(21-22)
n° of polyps


E
C.I. = 0.4
n° of oplyps
25
20
15
10
5
0
0
1
2
3
4
5
6
7
8
9
10
height (mm)


C
n° of Juveniles
10
9
8
7
6
5
4
3
2
1
0
(≤1.0)
(1.1-1.5)
(1.6-2)
(2.1-2.5)
(2.6-3)
(3.1-3.5)
(3.6-4)
(4.1-4.5)
(4.6-5)
(5.1-5.5)
(5.6-6)
(>6)
height (mm)

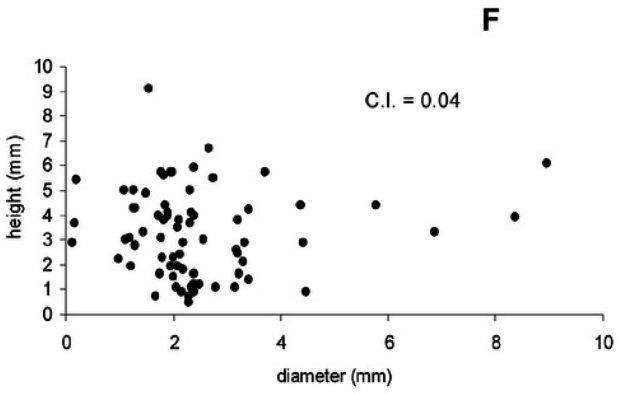
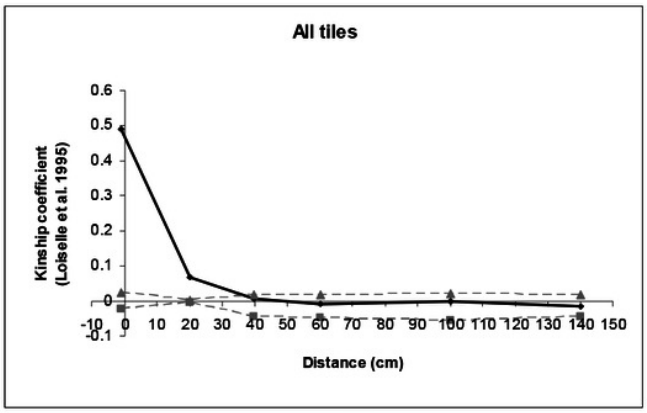
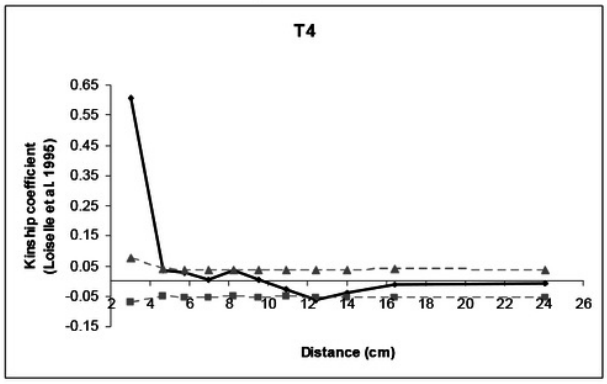

F
C.I. = 0.04
height (mm)
10
9
8
7
6
5
4
3
2
1
0
0
2
4
6
8
10
diameter (mm)

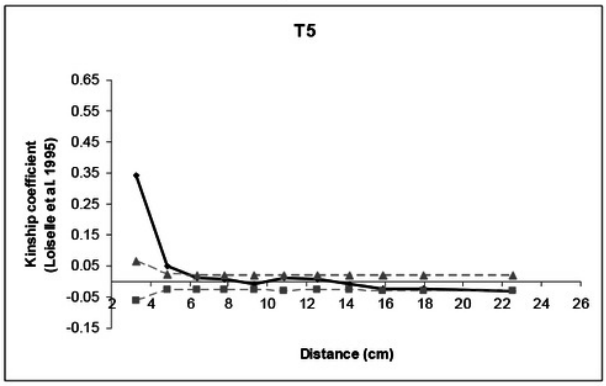
Figure 6

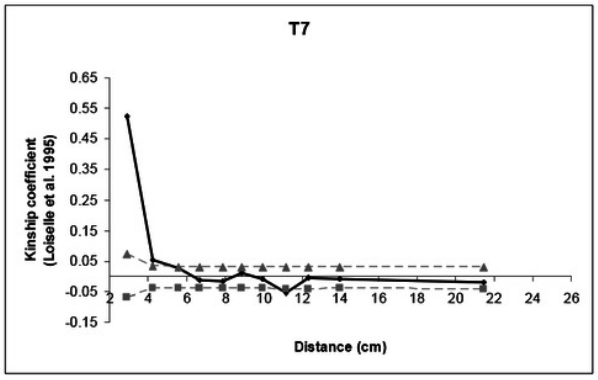
Spatial autocorrelation analysis of Loiselle kinship coefficient (Loiselle et al. 1995).

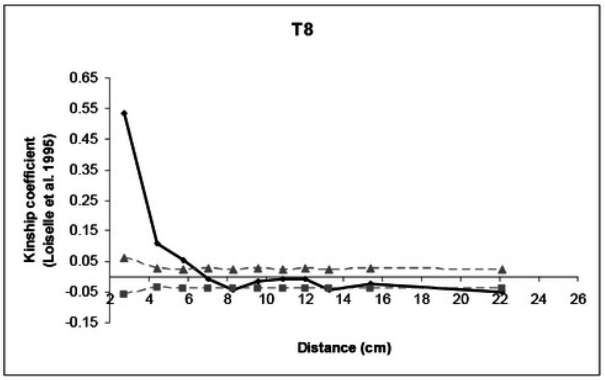
All tiles: correlogram performed for all the individuals within the cave and for each tiles with enough numbers of settlers. Grey lines represent 95% confidence intervals.

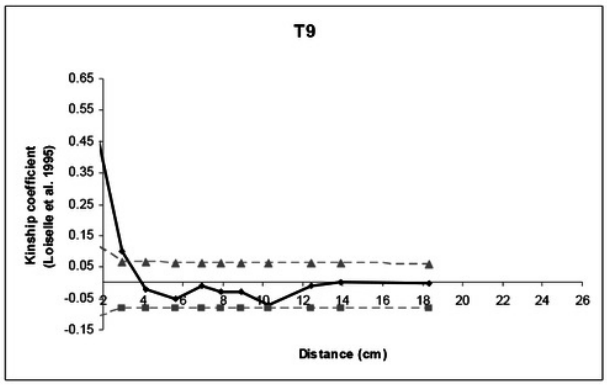

All tiles
Kinship coefficient (Loiselle et al. 1995)
Distance (cm)


T4
Kinship coefficient (Loiselle et al. 1995)
Distance (cm)


T5
Kinship coefficient (Loiselle et al. 1995)
Distance (cm)


T7
Kinship coefficient (Loiselle et al. 1995)
Distance (cm)


T8
Kinship coefficient (Loiselle et al. 1995)
Distance (cm)


T9
Kinship coefficient (Loiselle et al. 1995)
Distance (cm)

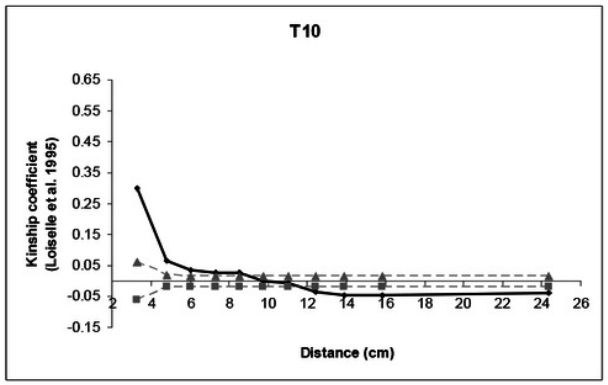

T10
Kinship coefficient (Loiselle et al. 1995)
Distance (cm)

Table 1(on next page)

Locus characteristics for all the individuals

Locus characteristics for all the individuals: number of alleles per locus (Na); null allele frequency (r); gene diversity (H_e, Nei 1973); observed heterozygosity (H_o); Weir & Cockerham (1984) estimator of F_{IS} (*f*). * Significant deviation from panmixia after false discovery rate correction at 0.05.

Table 1: Locus characteristics for all the settlers: number of alleles per locus (Na); null allele frequency (r); gene diversity (H_e, Nei 1973); observed heterozygosity (H_o); Weir & Cockerham (1984) estimator of F_{IS} (*f*). * Significant deviation from panmixia after false discovery rate correction at 0.05.

	N	Na	R	H_e	H_o	*f*
Cor9	286	23	0.22	0.81	0.31	0.61*
Mic20	289	10	0.1	0.6	0.64	0.21*
Mic24	283	18	0.09	0.67	0.43	0.37*
Mic26	283	23	0.25	0.6	0.13	0.79*
Cor46	218	19	0.19	0.29	0.04	0.86*
L7	283	20	0.1	0.56	0.32	0.44*
Cor58	235	16	0.25	0.66	0.17	0.75*