Assessing the impact of population depletion on mating system in the overexploited Mediterranean red coral

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Assessing the impact of population decline on mating system in the overexploited Mediterranean red coral

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ABSTRACT

1. Understanding the interactions among demographic parameters, mating system and population dynamics is key to predict the response of populations to global change. The Mediterranean red coral is a precious octocoral suffering from population decline due to overfishing and warming-driven mass mortality events.
2. While the demographic consequences of these two pressures are well characterized, little is known regarding their impact on population dynamics and evolution of red coral populations. The main objective of this study was to fill this gap focusing more particularly on mating pattern and genetic drift.
3. Combining sibship and progeny arrays analyses, a genetical characterization of the red coral mating system was conducted. In addition, a synchronic approach was developed comparing mating patterns in two populations with contrasted demographic patterns: a pristine-like population and a declining population.
4. The results show that polyandry is likely the norm in the red coral. The similar patterns of genetic diversity between adults and larvae combined to the lack of differential reproductive success among putative fathers did not support significant sweepstakes effects during larval production. While instantaneous biparental inbreeding was detected, no long-term inbreeding was observed even in the declining population. Mating patterns and effective population sizes in the two populations were not statistically different. Nevertheless, a trend towards a slightly higher inbreeding and a lower number of breeders was observed in the declining population.
5. Accordingly, we hypothesized that an increase in male gamete dispersal may buffer the increase of genetic drift expected in the declining population. This feedback between demographic decline and reproductive pattern may potentially takes part in the long-term persistence of red coral populations. However, the negative trend reported in the

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54 declining population unambiguously supports the need to maintain high densities of
55 reproductive colonies to promote the functioning of red coral populations.

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INTRODUCTION

The characterization of the interactions among demographic parameters, population dynamics and evolution of natural populations is key to define effective conservation policies (Ferrière, Dieckmann & Couvet 2004). In this context, the eco-evolutionary consequences of population decline induced by habitat fragmentation or overexploitation received particular attention in the past decades (e.g. Duminil et al. 2016; Dunlop, Eikeset & Stenseth 2015). Population decline is expected to cause the loss of genetic diversity due to an increase of genetic drift and inbreeding (Charlesworth & Willis 2009), which should, in turn, increase extinction risk (Spielman et al. 2004) and decrease adaptive potential (Frankham, Bradshaw & Brook 2014). The magnitude of the decrease of genetic diversity and the increase of drift and inbreeding are directly linked to the variation of effective population size (N_e) (Serbezov, Jorde, Bernatchez, Olsen & Vøllestad 2012; Wright 1931). N_e is a fundamental parameter for conservation biologists (Frankham 1995), which links population dynamics and underlying evolutionary processes (Hare et al. 2011). Indeed, N_e is related to different ecological drivers (e.g. population demographic parameters, life-history traits and mating systems; see Hedrick 2005; Nunney 1993; Waples 2006) and it gives insights into the evolutionary potential of a population (Hare et al. 2011). A drawback is that N_e is notoriously difficult to estimate in wild populations (Gilbert & Whitlock 2015) and the relationship between N_e and its ecological drivers is still a matter of debate (Karl 2008; Pearse & Anderson 2009).

In the marine realm, the majority of invertebrates are characterized by bentho-pelagic life cycles with a dispersive larval phase and a sessile adult phase. The reproduction usually involves long-lived, late-maturing and highly fecund individuals following a promiscuous mating strategy, in which any males mate with any females and vice versa (Karl 2008). As a by-product of promiscuity, multiple paternity (i.e. siring of a female clutch by more than one male) is likely to occur (e.g. Lasker, Gutiérrez-Rodríguez, Bala, Hannes & Bilewitch 2008; Mokthar-Jamai et

al. 2013; Warner et al. 2016). Marine invertebrates are also usually characterized by reduced N_e values compared to their high census population size (N_c) (Hauser & Carvalho 2008; Hedrick 2005). This reduction was explained by a differential reproductive success among individuals, the “sweepstakes reproductive success” hypothesis (Hedgcock 1994). Genetic imprints of sweepstake effects include a decrease in genetic diversity and an increase of linkage disequilibrium and relatedness from adults to recruits, which should result in a significant genetic differentiation between cohorts (Hedgcock & Pudovkin 2011). While multi-paternity and sweepstakes effect were demonstrated in different species (e.g. Christie, Johnson, Stallings & Hixon 2010; Selkoe, Gaines, Caselle & Warner 2006), their ubiquity in the marine realm still remains to be characterized (Hedgcock & Pudovkin 2011). Moreover, the impact of population decline on those particular mating patterns and the consequences for the evolutionary potential in marine invertebrates are still poorly understood (but see Plough, Moran & Marko 2014; Xue, Zhang & Liu 2016).

Here, the main objective was to gain insights into the putative feedbacks between mating patterns and demographic decline focusing on the Mediterranean red coral, *Corallium rubrum*. Distributed in the western Mediterranean and neighbouring Atlantic Ocean (Zibrowius, Monteiro-Marques & Grasshoff 1984), this precious octocoral with slow population dynamics, shows unambiguous signs of population decline due to overfishing for its use in jewelry and warming-induced mass mortality events (Garrabou et al. 2017; Montero-Serra, Linares, Doak, Ledoux & Garrabou 2018). The red coral is a gonochoric brooder with internal fertilization, balanced sex-ratio, annual reproductive cycle and late sexual maturity (i.e. 10 years of age) (Torrents & Garrabou 2011; Torrents, Garrabou, Marschal, & Harmelin 2005; Tsounis, Rossi, Aranguren, Gili, & Arntz 2006). Significant isolation by distance was demonstrated among colonies geo-referenced over half a square metre confirming a limited effective dispersal (Aurelle & Ledoux 2013; Costantini, Fauvelot, & Abbiati 2007a,b; Ledoux et al. 2010a) and

suggesting that reproduction units are restricted in space and composed of genetically related individuals (Ledoux et al. 2010b). The subtle temporal genetic structure demonstrated among adults and juveniles implied a low level of genetic drift at this spatial scale (Ledoux et al. 2010b). A lack of temporal genetic structure was reported among recruits and juveniles coming from two reproductive events in the same population (Costantini, Rugiu, Cerrano, & Abbiati 2018). While these studies shed new light on the reproduction of the red coral, a formal genetic characterization of the mating pattern is still essentially lacking. Moreover, the putative impacts of population decline on reproductive pattern remain to be tested.

Focused for the very first time on the larval phase in the red coral, this study aims to: i) genetically characterize the mating system with particular emphasis on multipaternity and inbreeding; ii) test for the sweepstakes reproductive success hypothesis by characterizing patterns of genetic diversity, relationships and temporal genetic structure among adults and larvae; and iii) estimate the strength of genetic drift based on different estimators of the number of breeders (N_b). To achieve these aims, a synchronic approach (i.e. the analyses of variations among contrasted populations at a given time) was developed by comparing the results of progeny arrays and sibship analyses in two red coral populations with highly contrasting demographic structure: a pristine-like (Garrahou et al. 2017) and a declining population (Linares et al. 2012).

MATERIALS AND METHODS

Sampling

Corallium rubrum colonies were sampled by scuba-diving at two sites with similar habitat features: Grotte Harmelin in Corsica (42.38° N, 8.54° E, France; HAR) and Pedra de Déu in Medes Islands (42.0461° N, 3.22477° E; Spain; DEU) (Figure 1). HAR is one of the last pristine-like shallow populations of red coral. It is characterized by the highest biomass of large-

sized colonies ($> 10\text{cm}$ in height) reported to date in the Mediterranean Sea with the co-existence of colonies of different sizes resulting in high density (201 colonies $\times \text{m}^{-2}$) (Garrabou et al. 2017). In contrast DEU, while situated in the “Parc naturel del Montgri, les Illes Medes i el Baix Ter”, shows signs of population decline with a shift in population size structure with the absence of large-size colonies, and lower biomass and density (49.4 colonies $\times \text{m}^{-2}$; Garrabou unpublished data; Linares et al. 2012). The sampling was conducted in June 2013 at the beginning of the annual reproduction period (Torrents & Garrabou 2011; Tsounis et al. 2006; Santangelo et al. 2003). Small fragments (1 to 2 cm) from adults with high reproductive potential (height $> 5\text{ cm}$; see Torrents, Garrabou, Marschal, & Harmelin, 2005) were randomly hand-collected over three square metres in the two populations ($N=30$ and $N=33$ in DEU and HAR, respectively). The fragments were preserved in 95% ethanol and stored at -80°C until sexing and DNA extraction.

Sexing was conducted by dissecting polyps under a binocular microscope. When a female colony was identified, 10 additional polyps were dissected and all the larvae were collected, preserved in 95% ethanol and stored at -80°C until DNA extraction.

DNA extraction, microsatellite genotyping, quality control and microsatellite characteristics

DNA extractions and genotyping of eight microsatellites previously developed in this species (Ledoux et al. 2010a) are detailed in Appendix A. MICRO-CHECKER 2.2.3 (Van Oosterhout et al. 2004) was used to test scoring errors. Because null alleles and genotyping errors can bias parentage analyses (Dakin & Avise 2004), we aimed to minimize their impact on the following analysis. First, all the individuals with more than two null genotypes (i.e. no amplification) were discarded. Then, a preliminary parentage analysis in COLONY 2.0.6.1 (Jones & Wang 2010) was conducted. Knowing the true maternal sibships between females and larvae, the objective of this analyses was to test whether the information contained in the genotypes was

sufficiently robust to reconstruct these relationships without specifying the maternal sibships. Only the larvae that were assigned with a probability equal to 1 to their true mother were retained. The final dataset comprised 204 samples coming from two populations (DEU and HAR) and assigned to two different stage-classes (adults [AD] and larvae [LAR]). In DEU, we analyzed 67 larvae (DEU-LAR) from eight mothers and 30 adults (DEU-AD; 10 males and 20 females including the eight mothers). In HAR, we analyzed 74 larvae (HAR-LAR) from 11 mothers and 33 adults (HAR-AD; 19 males and 14 females including the 11 mothers) (Table 1).

Genetic diversity analyses, long-term inbreeding and temporal genetic structure between adults and larvae

Frequencies of null alleles were estimated for each locus within each stage-class (i.e. adults and larvae) in FREENA (Chapuis & Estoup 2007). Tests for linkage equilibrium in each stage-class were conducted in GENETIX 4.05 (Belkhir et al. 2004) using 1000 permutations. The observed heterozygosity (H_o) and gene diversity (H_e ; Nei 1973) were computed for each stage-class in GENEPOP 4.1.4 (Rousset 2008). ADZE (Szpiech et al. 2008) was used to compute the allelic richness (Ar_g) using the rarefaction method (Petit et al. 1998) with g , the minimum number of genes at a locus in a population, equals to 56. The values of H_e and $Ar_{(56)}$ were compared between stage-classes in each population using Wilcoxon signed rank tests.

Because null alleles bias the estimation of F_{IS} , INEST (Chybicki & Burczyk 2009) was used under the Population Inbreeding Model to compute the F_{INEST} , an unbiased estimate of inbreeding coefficient. A first full model accounting for null alleles, inbreeding and genotyping failures was run for 200,000 iterations, a thinning of 100 and 50,000 burn-in. To test whether inbreeding was a significant component of the model, this model was compared with a model

without inbreeding based on the Deviance Information Criterion (DIC). The model with the lower DIC was retained.

Temporal genetic differentiation was estimated based on pairwise F_{ST} among adults and larvae within each population using θ , the Weir & Cockerham's (1984) estimator of F_{ST} . Genotypic differentiation was tested using an exact test with default parameters in GENEPOP.

Mating system analysis

Instantaneous biparental inbreeding and correlated paternity

Based on the maternal progeny arrays, the multilocus outcrossing rate (t_m) and the single outcrossing rate (t_s) were computed for each population (HAR: 11 families; DEU: 8 families) using the maximum likelihood method and Newton-Raphson iterations as implemented MLTR v.3.2 (Ritland 2002). A conservative approach was adopted excluding Mic25 and Mic27, which showed the highest level of null alleles and genotyping errors, respectively. The difference between t_m - t_s was used to estimate the level of instantaneous biparental inbreeding (mating among close relatives) in each population. In addition, the correlated paternity ($r_{(p)}$) defined as the proportion of full sibs within maternal progeny arrays was estimated for each population. The standard deviation and 95% confidence intervals (95% CI) of each parameter were estimated using a bootstrap procedure by resampling families 1000 times. These 95% CI were used to test whether the estimated values were different from 0 and for comparison among populations.

Characterization of the pattern of relationships

The maximum-likelihood method implemented in COLONY 2.0.6.1 (Jones & Wang 2009) was used to reconstruct the pattern of relationships among larvae and to assign paternity in each population accounting for genotyping errors (Wang 2004). The levels of allelic dropout were set to the frequencies of null alleles computed for each locus (see Wang 2004). For all loci, the

other type of genotyping errors was set to 0.01, an upward estimation of error rates based on the re-genotyping procedure (not shown). Known maternal sibs, the excluded maternity and the excluded maternal sibs were defined. Three runs with different seed numbers were conducted considering females and males as polygamous and using the Full-Likelihood method with very high and very long likelihood precision and length of run, respectively. The robustness of the results was tested using a reduced dataset of six microsatellites excluding Mic25 and Mic27. Because patterns of relationships were consistent and robust among runs, the results obtained for one run only are shown.

Sweepstakes reproductive success hypothesis

Test of reproductive skew among putative fathers

In case of sweepstake effects, differential reproductive success among individuals is expected (i.e. reproductive skew). The occurrence of differential reproductive success among the males inferred with COLONY in each population was tested using the Nonacs binomial skew index B implemented in SKEW CALCULATOR (Nonacs 2000, 2003). B ranges from -1 to +2. Random mating is observed for $B = 0$; negative values suggest that reproduction is more evenly distributed than expected while positive values correspond to a skew greater than expected suggesting differential reproductive success among individuals. The one-tailed p-value associated with B in each site was computed with 5000 simulations. The 95% confidence interval of B in each site was estimated.

Comparison of relatedness estimators among stage-classes

Sweepstake effects also induced an increase of genetic relatedness from reproductive adults to their descendants. The levels of relatedness for each stage-class in each population were computed using Lynch-Li moment-estimator (Li, Weeks & Chakravarti 1993; Lynch 1988) in

COANCESTRY (Wang 2011). The significance of the differences of the average relatedness between adults and larvae in each population was tested using a bootstraps procedure (1000).

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Estimation of effective population size (N_e) and relative impact of genetic drift

According to the challenges linked to estimation of effective population size (N_e) in long-lived and iteroparous species (Waples 2010; Wang 2016), such as the red coral, different estimators of N_e were computed (Hare et al. 2011). These computations, based on larvae coming from a single reproductive event, results in an estimation of effective number of breeders (N_b) instead of a “true” N_e . Empirical works suggested similar results between the two parameters (e.g. Beedee 2009; Duong, Scribner, Forsythe, Crossman, & Baker 2013).

Four estimators of N_b were computed: i) the sibship assignment method (Wang 2009); ii) the standard linkage disequilibrium method (Hill 1981) with Waples’ (2006) correction under the random-mating model, excluding rare alleles with frequencies of less than 0.02 and using a jackknife procedure to estimate confidence intervals (Waples & Do 2008, 2010); iii) the method based on molecular coancestry (Nomura et al. 2008). We also computed iv) moment-based F-statistics estimator (Jorde & Ryman 2007; Waples 1989) considering a sampling plan I (see Waples 2005 for details) and that the adults and the larvae in each population were separated by one generation. The last three estimators were computed using NeEstimator v.2 (Do et al. 2014). Following Waples & Do (2010) and Riquet et al. (2016), an unweighted harmonic mean of N_b in each population was computed to obtain an overall estimate of effective population size.

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RESULTS

Microsatellite characteristics, stage-class genetic diversity, long-term inbreeding and temporal genetic structure

No evidence of scoring errors due to stuttering or large allele dropout was found. In the two populations, all loci were polymorphic (p-value < 0.01). The frequency of null alleles (r) was between 0.09 for HAR-AD and HAR-LAR and 0.12 for DEU-AD and DEU-LAR (mean over populations $\pm$ SE = 0.1 ± 0.02). Observed heterozygosity values (H_o) were between 0.49 for DEU-LAR and 0.53 for HAR-AD (mean over populations $\pm$ SE = 0.51 ± 0.01). The gene diversity (H_e) ranged between 0.65 (HAR-LAR) and 0.72 (DEU-AD) (mean over populations $\pm$ SE = 0.68 ± 0.03). Regarding $Ar_{(56)}$, the highest and lowest values were observed for DEU-AD (11.1) and HAR-LAR (9) (mean $Ar_{(56)}$ over samples $\pm$ SE = 9.9 ± 1.03). None of the Wilcoxon signed ranked tests conducted to compare H_e and $Ar_{(56)}$ among adults and larvae in each population were significant after FDR correction for multiple tests (Table 1). F_{INEST} estimators were low but higher in adults compared to larvae (0.04 vs 0.02 in DEU and 0.03 vs. 0.01 in HAR). In all the cases, the model without inbreeding showed the lowest DIC suggesting a lack of inbreeding (results not shown).

The two temporal F_{ST} s were low (0.002 for DEU-AD vs. DEU-LAR and 0.005 for HAR-AD vs. HAR-LAR) but significant (p-values < 0.05).

Mating system analyses

Instantaneous biparental inbreeding and correlated paternity

The multilocus outcrossing rates (t_m) were close to 1 in both populations as expected in strictly outcrossing species. The single outcrossing rate (t_s) was 0.78 for DEU and 0.74 for HAR resulting in a higher signal of biparental inbreeding ($t_m - t_s$) in DEU (0.21; 95% CI = 0.13 - 0.45) than in HAR (0.17; 95% CI = 0.07 - 0.26). Based on the 95% CIs, the estimates of biparental inbreeding were significantly different from 0 in the two populations but the estimates were not significantly different between the two populations. Estimates of correlated paternity were 0.23

(95% CI = 0.09 - 0.46) for DEU and 0.12 for HAR (95% CI = -0.03 - 0.25). The two estimates were not significantly different based on their 95% CI (Table 2).

Pattern of relationships

Considering only relationships with a probability >0.8 , in DEU, six full-sib dyads and 246 half-sib dyads over the 2211 putative relationships were retained, corresponding to 0.27 % and 11.12 %, respectively. The total number of fathers was 32. In HAR, two full-sib dyads and 235 half-sib dyads over the 2701 putative relationships were observed, corresponding to 0.07 % and 8.7 %, respectively. The total number of fathers was 43. Evidence of multiple paternity was found in all the families from the two populations. The number of fathers for each family in DEU ranged from 6 to 10, with a mean value of 7.5 ± 1.5 fathers. In HAR, the number of fathers for each family varied from 2 to 10, with a mean value of 6.5 ± 2.4 fathers. The putative fathers were involved in multiple mating events producing 1 to 5 larvae, with a mean value of 2.1 ± 1.1 in DEU and a mean value of 1.9 ± 1 in HAR (Figure 2).

Sweepstakes reproductive success hypothesis

Reproductive skew among putative fathers

Although the number of larvae per putative father ranged from 1 to 5, the skew observed among males was not significantly different from random expectations in the two populations ($B = -0.0057$, p-value = 0.973 in DEU; $B = -0.008$ p-value=1 in HAR). This suggests a similar contribution of the different fathers to the pool of larvae.

Comparison of relatedness estimators among stage-classes

Mean estimates of relatedness based on the Lynch-Li moment-estimator ranged from -8.84×10^{-2} in DEU-AD to 7.65×10^{-2} in HAR-LAR. The differences of mean relatedness between adults and larvae were significant in the two populations (Table 3).

Estimation of effective population size (N_e)

Based on the sibship assignment method, N_b was 29 (95 %CI: 18-49) for DEU and 39 (95 %CI: 25-61) for HAR. The linkage disequilibrium and the molecular coalescent estimates of N_b were 38.3 (95 %CI: 30-50) and 4.7 (95 %CI: 1.9-8.7) in DEU and 35.9 (95 %CI: 29.1-45) and infinity in HAR, respectively. The estimates of N_b obtained with the temporal method were 25.5 (95 %CI: 17.4 - 35) in DEU and 34 (95 %CI: 22.6 - 47.7) in HAR. The resulting harmonic means of N_b were 12.8 for DEU and 48.2 for HAR (Table 4).

DISCUSSION

The impact of demographic decline on population dynamics and evolution is a central concern for conservation biologists. This study is the first genetic characterization of the mating system of an internal brooding octocoral. In spite of a lack of statistically significant differences in the mating patterns of the two red coral populations, a higher biparental inbreeding and correlated paternity combined with a lower number of breeders were reported in the declining population. While the results presented here may potentially be affected by the occurrence of null alleles, it is noteworthy that the dataset was filtered to decrease their impact (e.g. only mother/larvae pairs with assignment probability = 1 were retained). A broad set of complementary analyses relying on different statistical methods was also used and showed concordant results (i.e. sibship reconstruction vs. progeny array analyses, four different N_b estimators). Moreover, simulations considering different levels of genotyping errors demonstrated the robustness of the sibship reconstruction method implemented in COLONY (Wang 2004). The implications of these results for the basic ecological knowledge of the red coral and for its evolution in the context of population decline is discussed below.

Does multipaternity buffer long-term inbreeding?

Multipaternity is likely the norm in the red coral. Indeed, the 19 progeny arrays analysed were sired by more than one male as supported both by the sibship analyses in COLONY and by the correlation of paternity estimated in MLTR. Polyandry is expected in promiscuous mating species and was previously demonstrated in one red coral population based on kinship analyses among juveniles and adults (Ledoux et al. 2010b). The estimated mean number of larvae per father inferred in COLONY over the two populations studied here (1.88 ± 0.9 larvae / father) is in the same order as those previously reported in the same species (2.3 ± 0.8 offspring / parent; Ledoux et al. 2010b) and in another tropical octocoral *Antilloporgia elisabethae* (1.42 ± 0.7 larvae / father; Lasker, Gutiérrez-Rodríguez, Bala, Hannes, & Bilewitch 2008) and slightly lower than in the Mediterranean *Paramuricea clavata* (6.6 ± 4.7 larvae / father; Mokhtar-Jamaï et al. 2013). Variations in the level of multiple paternity among species are usual in marine invertebrates (e.g. Brante, Fernandez, & Viard 2011; Warner et al. 2016). While polyandry is a widespread strategy among organisms from sessile marine invertebrates to social mammals and including wind-pollinated plant and tree species (Simmons 2005), its evolution remains a controversial issue (Yasui et al. 2016). Focusing on sessile marine species in which polyandry is passive (i.e. females passively receive the gamete from different males), the adaptive value is questioned. Multiple mating may simply be correlated to the selective pressure increasing fertilization success when multiple individuals are present (Brante, Fernandez, & Viard 2011; Johnson & Yund 2007). Alternatively, the adaptive hypothesis lies on indirect genetic benefits for the females. Polyandry may increase offspring survival enhancing in turn female reproductive fitness by increasing the genetic diversity, and the viability or the competitiveness of the offspring through advantageous male genes (see Yasui & Garcia-Gonzales 2016; Zeh & Zeh 2000 for reviews). While it is notoriously challenging to test for indirect genetic effects (Yasui & Garcia-Gonzales 2016) and acknowledging the complexity of the interactions between mating system and

inbreeding (Frankham 1995), a decrease of inbreeding was also proposed as one of the indirect genetic benefits of multipaternity (Zeh & Zeh 2000). Here, the inbreeding varied in function of the temporal scale. The progeny array approach supports significant level of instantaneous biparental inbreeding (i.e. crossing between related mates). This result is in accordance with the view of red coral populations as mosaics of spatially restricted breeding units (< one square metre) composed by relatives (Ledoux et al. 2010b). In contrast, when considering the multilocus inbreeding coefficients unbiased by null alleles (F_{INEST}), which estimate the combined effect of inbreeding depression (i.e. the fitness decrease observed in related individuals) and biparental inbreeding over several generations, inbreeding was not statistically supported. This absence of long-term inbreeding may appear surprising considering the high and significant F_{IS} values reported in previous studies (e. g. Ledoux et al. 2010a). Nevertheless, these F_{IS} values were likely biased by null alleles and Wahlund effect (Ledoux et al. 2010a). Further analyses such as estimations of inbreeding coefficients on well-defined cohorts (from larvae to recruits, juveniles and adults) and reproduction experiments in aquaria should be envisaged to formally test a putative buffering effect of multipaternity on inbreeding in the red coral.

Soft sweepstakes effect and low genetic drift in the red coral.

Interestingly, larvae production does not seem to be impacted by strong sweepstakes effects in this species. None of the genetic diversity parameters tested were significantly different between larvae and adults in the two populations. No linkage disequilibrium was reported in the larvae although it is expected when a small number of individuals produced the next generation. Moreover, while a significant increase of genetic relatedness was reported from adults to larvae, the genetic differentiation between the two stage-classes in each population was only marginally significant. The high polymorphism of the microsatellites used here may

partially explain these results (Hedgecock & Pudovkin 2011). Nevertheless, the absence of sweepstake effect is also supported by the lack of reproductive skews among the fathers inferred with COLONY. Sweepstakes effect is expected in promiscuous sessile marine invertebrates with larval phase (Hedgecock & Pudovkin 2011) but the lack of sweepstake observed here is also supported by the null to low temporal differentiation previously reported among juveniles and adults (Ledoux et al. 2010b) or recruits and juveniles (Costantini, Rugiu, Cerrano, & Abbiati 2018). This can be related to red coral life-history traits and in particular, to the occurrence of mating among individuals from overlapping generations, which may buffer temporal changes of allelic frequencies (Riquet, Le Cam, Fonteneau, & Viard 2016). Comparing genetic characteristics among well-defined cohorts rather than stage-classes is needed to assess this hypothesis.

The impact of a promiscuous mating system on N_e and genetic drift has been previously debated (Karl 2008; Pearse & Anderson 2009). In species with a generation time greater than 10 years such as the red coral, multipaternity should only marginally impact N_e (Lotterhos 2011). While estimates of N_e must be considered cautiously and considering that similarity between N_b and N_e was assumed here, the results support moderate to high numbers of breeders in the two populations. These values are concordant with the high N_e previously reported over a similar spatial area in the long-lived and polyandrous octocoral *P. clavata* (Mokhtar-Jamāi et al. 2013). However, they contrast with the general trend in marine species in which N_e was shown to be orders of magnitude lower than the census population size (N_c) and genetic drift is usually stronger than expected (Hare et al. 2011; Hauser & Carvalho 2008). In accordance with the lack of sweepstake effect previously discussed, the estimations of N_b suggest genetic drift to be limited at the fine spatial and temporal scales under survey. This result supports recent studies questioning the ubiquity of low N_e s in marine species (Hare et al. 2011; Riquet, Le Cam, Fonteneau, & Viard 2016).

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427 Does population decline impact red coral mating system and effective population size?

428 The site “Grotte Harmelin” (HAR) is considered as one of the last pristine-like shallow
429 population of red coral offering the opportunity to characterize the baseline population
430 dynamics in this species (Garrahou et al. 2017). Contrasting the results obtained in this
431 population with the results obtained in a declining population (DEU) provides the first insights
432 into the consequences of demographic decline on mating system and effective population size
433 in the red coral.

434 None of the parameters (correlated paternity, biparental inbreeding and number of breeders)
435 estimated to compare the mating patterns between the two populations were significantly
436 different. This result contrasts with the positive correlation between population density and
437 mating pattern reported in copulating marine invertebrates (e.g., barnacles (Plough, Moran, &
438 Marko 2014); marine snails (Xue, Zhang, & Liu 2016)). In these species, the probability of
439 mate encounter increases with population density. The lack of differences among the two
440 populations is concordant with what was found between low and high-density populations in a
441 sperm-cast ascidian (Johnson & Yund 2007). In sperm-cast species such as the red coral, the
442 spermatozoa may cross greater distances in the water column in low density populations
443 buffering the influence of density on mate interactions (e.g. Duminil et al. 2016). Nevertheless,
444 in spite of this lack of statistically significant differences between the two populations, all the
445 analyses suggest some negative consequences of demographic decline. A trend toward higher
446 biparental inbreeding and correlated paternity combined with a lower number of breeders is
447 observed in the declining compared to the pristine-like population. Accordingly, we
448 hypothesized the expected increase in genetic drift, due the lower mate density in DEU
449 compared to HAR, to be balanced to some point by an increase of male gamete flow. While the
450 hypothesized buffering effect of male gamete flow remains to be formally tested in the red

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3 451 coral, it is likely subtle and limited as supported by the negative trend observed in the declining
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5 452 population. The characterization of spatial genetic structures in populations with contrasted
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7 453 demographic patterns and the inference of related demographic parameters (e.g. effective
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9 454 dispersal) is required to go further in this hypothesis and to improve our understanding of the
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12 455 feedbacks between demographic decline and mating patterns in the red coral.
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17 457 Overall, this study suggests the occurrence of subtle feedbacks between demographic and
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19 458 reproductive patterns in red coral populations. In complement to other processes (e.g. re-growth
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21 459 of damaged colonies; see Montero-Serra et al. 2015), these feedbacks may partially explain the
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23 460 long-term persistence of red coral populations in spite of the millennial fishing pressure. They
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25 461 are likely to provide a borrowed time (*sensu* Hughes, Linares, Dakos, van de Leemput, & van
26
27 462 Nes 2013) for the management and conservation of this threatened species. Nevertheless, the
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29 463 negative trend reported in the declining population unambiguously supports the maintenance
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31 464 of high density of reproductive colonies, and accordingly the diminution of direct
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33 465 anthropogenic pressures (e.g. fishing), as a prerequisite to maintain functional red coral
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Authors’ contributions:

JBL, CL and JG designed the study. CL, CCB and IMS performed sexing and larvae collection. JBL, PLS, SF and CCB performed laboratory work. JBL, SF and SC analyzed the data. JBL and SF wrote the manuscript with input from all authors. All authors approved the manuscript.

Data accessibility:

Microsatellite genotypes will be made available on Dryad.

Supporting information:

Additional information may be found in the online version of the article:

Appendix A: *DNA extraction and microsatellite genotyping*

Conflict of interest: No conflict of interest to declare.

Table 1: Genetic diversity of each stage-class in each site (DEU and HAR). N : number of individuals; r : frequency of null alleles; H_o : observed heterozygosity; H_e : gene diversity; $Ar_{(56)}$: rarefied allelic richness considering a minimum of 56 genes at a locus in a population; F_{INEST} : estimate of inbreeding coefficient unbiased by null alleles.

	N	r	H_o	H_e	Ar	$Ar_{(56)}$	F_{INEST}
DEU-AD	30	0.12	0.52	0.72	11.25	11.1	0.04
DEU-LAR	67	0.12	0.49	0.69	11	9.1	0.02
HAR-AD	33	0.09	0.53	0.66	10.62	10.3	0.03
HAR-LAR	74	0.09	0.52	0.65	11.62	9	0.01

Table 2: Mating system characteristics for each site. t_m : multilocus outcrossing rate; t_s : single outcrossing rate; t_m-t_s : level of biparental inbreeding; $r_{(p)}$: correlated paternity. The 95% confidence intervals (95% CI) of each parameter was estimated by resampling families 1000 times.

	t_m [95% CI]	t_s [95% CI]	t_m-t_s [95% CI]	$r_{(p)}$ [95% CI]
DEU	0.99 [0.95 ; 1.2]	0.78 [0.64 ; 0.89]	0.21 [0.13 ; 0.45]	0.23 [0.09 ; 0.46]
HAR	0.94 [0.86 ; 1]	0.77 [0.67 ; 0.88]	0.17 [0.07 ; 0.26]	0.12 [-0.03 ; 0.25]

Table 3: Estimates of relatedness for each stage-class in each population using the *LL*: Lynch-Li moment-estimator of relatedness. Observed value corresponds to the difference between adults and larvae in each population. The significance of the differences among the two stage-classes was tested using on 1000 bootstraps. Significant differences are shown in bold.

	<i>Lynch-Li</i>	Observed Value and 95% CI
DEU-AD	-8.84×10^{-2}	9×10^{-2} $[-2.5 \times 10^{-2} ; 2.51 \times 10^{-2}]$
DEU-LAR	2.31×10^{-3}	
HAR-AD	5.28×10^{-2}	2.37×10^{-2} $[-2.55 \times 10^{-2} ; 1.95 \times 10^{-2}]$
HAR-LAR	7.65×10^{-2}	

Table 4: Effective population size and 95 % confidence interval (between brackets) computed for each site using: i) the sibship assignment method; ii) the standard linkage disequilibrium method; iii) the method based on molecular coancestry; and iv) the moment based F-statistics methods.

	Sibship assignment method (Wang 2009)	Standard linkage disequilibrium method (Hill 1981)	Molecular coancestry method (Nomura et al. 2008)	Moment-based F-statistics estimator (Waples 1989; Jorde & Ryman 2007)	Unweighted harmonic mean
DEU	29 [18 - 49]	38.3 [30 - 50]	4.7 [1.9 - 8.7]	25.5 [17.4 - 35]	12.8
HAR	39 [25 - 61]	35.9 [29.1 - 45]	infinity	34 [22.6 - 47.7]	48.2

FIGURE LEGENDS:

Figure 1: Location of the two study sites: Pedra de Deu (DEU) in the Medes Islands within the Parc Natural del Montgrí, illes Medes i Baix Ter (Spain) and Grotte Harmelin (HAR) within the Réserve Naturelle de Scandola in Corsica (France). Pictures A and B show populations DEU and HAR, respectively. The same quadrat (20 x 20 cm) is used in the two pictures.

Figure 2: Kinship structure among larvae from each population (DEU and HAR) reconstructed based on their multilocus genotypes using COLONY. L/M: number of larvae per mother; L/F: number of larvae per putative father. Grey and blue squares correspond to one and two larvae, respectively, for the mother-putative father pair considered. * pair of larvae inferred as fullsib with a probability higher than 0.8.

Assessing the impact of population decline on mating system in the overexploited Mediterranean red coral

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Running title: Population decline, mating pattern and genetic drift in the red coral

Keywords: population decline; overfishing; mating system; inbreeding; genetic drift; Mediterranean octocoral

ABSTRACT

1. Understanding the interactions among demographic parameters, mating system and population dynamics is a critical step key to predict the response of populations to global change. The Mediterranean red coral is a precious octocoral suffering from population decline due to overfishing and warming-driven mass mortality events.
2. While the demographic consequences of these two pressures are well characterized, little is known regarding their impact on population dynamics and evolution of red coral populations. ~~Our~~ The main objective of this study was to fill this gap focusing more particularly on mating pattern and genetic drift.
- ~~3.~~ Combining sibship and progeny arrays analyses, we a genetically echaracterized characterization of the mating system of the red coral was conducted. and In addition, developed a synchronic approach was developed comparing mating patterns in two populations with contrasted demographic patterns: a pristine-like population and a declining population.
- ~~3.~~ The results show that We demonstrated that polyandry is likely the norm in this species red coral. Interestingly, the similar patterns of genetic diversity between adults and larvae combined to the lack of differential reproductive success among putative fathers did not support significant sweepstakes effects during larval production. While ~~we detected~~ instantaneous biparental inbreeding was detected, no long-term inbreeding was observed even in the declining population. Mating patterns and effective population sizes in the two populations were not statistically different. Nevertheless, a trend towards a slightly higher inbreeding and a lower number of breeders was observed in the declining population.
- ~~4.~~ Overall, no differences in mating patterns and similar estimates of effective population size were observed despite the contrasted demographic patterns of the two populations.

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5. Accordingly, we hypothesized that an increase in male gamete dispersal
may buffer the increase of genetic drift expected in the declining population. This
feedback between demographic decline and reproductive pattern may potentially takes
part in the long-term persistence of red coral populations. However, the negative trend
reported in the declining population unambiguously supports the need to maintain high
densities of reproductive colonies to promote the functioning of red coral populations.
~~we hypothesized that higher gamete dispersal may buffer to some point the potential~~
~~increase of genetic drift expected during population decline. Red coral populations may~~
~~thus be resilient to some levels of decline explaining potentially their long-term persistence~~
~~in spite of the millennial fishing pressure.~~

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5.

INTRODUCTION

The characterization of the interactions among demographic parameters, population dynamics and evolution of natural populations is key to define effective conservation policies (Ferrière, Dieckmann, & Couvet, 2004). In this context, the eco-evolutionary consequences of population decline induced by habitat fragmentation or overexploitation received particular attention in the past decades (e.g. [Duminil et al. 2016](#); Dunlop, Eikeset, & Stenseth, 2015; ~~Duminil et al. 2016~~). Population decline is expected to cause the loss of genetic diversity due to an increase of genetic drift and inbreeding (Charlesworth & Willis 2009), which should, in turn, increase extinction risk (Spielman et al. 2004) and decrease adaptive potential (Frankham, Bradshaw, & Brook, 2014). The magnitude of the decrease of genetic diversity and the increase of drift and inbreeding are directly linked to the variation of effective population size (N_e) (Serbezov, Jorde, Bernatchez, Olsen, & Vøllestad, 2012; Wright 1931). N_e is a fundamental parameter for conservation biologists (Frankham 1995), which links population dynamics and underlying evolutionary processes (Hare et al. 2011). Indeed, N_e is related to different ecological drivers (e.g. population demographic parameters, life-history traits and mating systems; see ~~Nunney 1993~~; Hedrick 2005; [Nunney 1993](#); Waples 2006) and it gives insights into the evolutionary potential of a population (Hare et al. 2011). A drawback is that N_e is notoriously difficult to

estimate in wild populations (Gilbert & Whitlock 2015) and the relationship between N_e and its ecological drivers is still a matter of debate (Karl 2008; Pearse & Anderson 2009).

In the marine realm, the majority of invertebrates are characterized by bentho-pelagic life cycles with a dispersive larval phase and a sessile adult phase. The reproduction usually involves long-lived, late-maturing and highly fecund individuals following a promiscuous mating strategy, in which any males mate with any females and vice versa (Karl 2008). As a by-product of promiscuity, multiple paternity (i.e. siring of a female clutch by more than one male) is likely to occur (e.g. Lasker, Gutiérrez-Rodríguez, Bala, Hannes, & Bilewitch, 2008; Mokhtar-Jamai et al. 2013; Warner et al. 2016). Marine invertebrates are also usually characterized by reduced N_e values compared to their high census population size (N_c) (Hauser & Carvalho 2008; Hedrick 2005; Hauser & Carvalho 2008). This reduction was explained by a differential reproductive success among individuals, the “sweepstakes reproductive success” hypothesis (Hedrick 1994). Genetic imprints of sweepstake effects include a decrease in genetic diversity and an increase of linkage disequilibrium and relatedness from adults to recruits, which should result in a significant genetic differentiation between cohorts (Hedrick & Pudovkin 2011). While multi-paternity and sweepstakes effect were demonstrated in different species (e.g. Christie, Johnson, Stallings & Hixon 2010; Selkoe, Gaines, Caselle, & Warner, 2006; Christie, Johnson, Stallings, & Hixon, 2010), their ubiquity in the marine realm still remains to be characterized (Hedrick & Pudovkin 2011). Moreover, From a broader perspective, the impact of population decline on those particular mating patterns and the consequences for the evolutionary potential in marine invertebrates is are still poorly understood (but see Plough, Moran, & Marko, 2014; Xue, Zhang, & Liu 2016). In the current environmental context, understanding how to enhance the resilience of populations facing demographic decline is urgently needed.

~~Our~~ The main objective here was to gain insights into the putative feedbacks between mating patterns and demographic decline focusing on the Mediterranean red coral, *Corallium rubrum*. Distributed in the western Mediterranean and neighbouring Atlantic Ocean (Zibrowius, Monteiro-Marques ~~&~~, Grasshoff, 1984), this precious octocoral with slow population dynamics, shows unambiguous signs of population decline due to overfishing for its use in jewelry and warming-induced mass mortality events (Garrabou et al. 2017; Montero-Serra, Linares, Doak, Ledoux, & Garrabou, 2018). The red coral is a gonochoric brooder with internal fertilization, balanced sex-ratio, annual reproductive cycle and late sexual maturity (i.e. 10 years of age) (Torrents & Garrabou 2011; Torrents, Garrabou, Marschal, & Harmelin, 2005; Tsounis, Rossi, Aranguren, Gili, & Arntz, 2006; ~~Garrabou & Torrents 2011~~). Significant isolation by distance was demonstrated among colonies geo-referenced over half a square meter ~~ere~~ confirming a limited effective dispersal (Aurelle & Ledoux 2013; Costantini, Fauvelot, & Abbiati, 2007a,b; Ledoux et al. 2010a; ~~Aurelle & Ledoux 2013~~) and suggesting that reproduction units are restricted in space and composed ~~by of~~ genetically related individuals (Ledoux et al. 2010b). The subtle temporal genetic structure demonstrated among adults and juveniles implied a low level of genetic drift at this spatial scale (Ledoux et al. 2010b). A lack of temporal genetic structure was reported among recruits and juveniles coming from two reproductive events in the same population (Costantini, ~~Ru~~giu, Cerrano, & Abbiati, 2018). While these studies shed new light on the reproduction of the red coral, a formal genetic characterization of the mating pattern is still essentially lacking. Moreover, the putative impacts of population decline ~~on on the reproduction~~ on the reproduction pattern remain to be tested. ~~Here, f~~ Focused for the very first time ~~in the~~ larval phase in the red coral ~~Corallium rubrum~~, ~~we focused on the larval phase to~~ this study aims to: i) genetically characterize the mating system with particular emphasis ~~size~~ on multipaternity and inbreeding; ii) test for the sweepstakes reproductive success hypothesis by characterizing patterns of genetic diversity, relationships

and temporal genetic structure among adults and larvae; and iii) estimate the strength of genetic drift based on different estimators of the number of breeders (N_b). To achieve these aims, ~~we developed~~ a synchronic approach (i.e. the analyses of variations among contrasted populations at a given time) was developed by comparing the results of progeny arrays and sibship analyses in two red coral populations with highly contrasting demographic structure: a pristine-like (Garrabou et al. 2017) and a declining population (Linares et al. 2012).

MATERIALS AND METHODS

Sampling

Corallium rubrum colonies were sampled by scuba-diving at two sites with similar habitat features: Grotte Harmelin in Corsica (42.38° N, 8.54° E, France; HAR) and Pedra de Déu in Medes Islands (42.0461° N, 3.22477° E; Spain; DEU) (Figure- 1). HAR is one of the last pristine-like shallow populations of red coral. It is characterized by the highest biomass of large-sized colonies (> 10cm in height) reported to date in the Mediterranean Sea with the co-existence of colonies of different sizes resulting in high density (201 colonies x m⁻²) (Garrabou et al. 2017). In contrast DEU, while situated in the “Parc naturel del Montgri, les Illes Medes i el Baix Ter”, shows signs of population decline with a shift in population size structure with the absence of large-size colonies, and lower biomass and density (49.4 colonies x m⁻²; Garrabou unpublished data; Linares et al. 2012). The sampling was conducted in June 2013 at the beginning of the annual reproduction period (Torrents & Garrabou 2011; Tsounis et al. 2006; Santangelo et al. 2003). Small fragments (1 to 2 cm) from ~~30 and 33~~ adults with high reproductive potential (height > 5 cm; see Torrents, Garrabou, Marschal, & Harmelin, 2005) were randomly hand-collected over three square meters in the two populations (N= 30 and N=33 in DEU and HAR, respectively). The fragments were preserved in 95% ethanol and stored at -80°C until sexing and DNA extraction.

Sexing was conducted by dissecting polyps under a binocular microscope. When a female colony was identified, 10 additional polyps were dissected and all the larvae were collected, preserved in 95% ethanol and stored at -80°C until DNA extraction.

DNA extraction, microsatellite genotyping, quality control and microsatellite characteristics

DNA extractions and genotyping of eight microsatellites previously developed in this species (Ledoux et al. 2010a) are detailed in Appendix A. MICRO-CHECKER 2.2.3 (Van Oosterhout et al. 2004) was used to test scoring errors. Because null alleles and genotyping errors can bias parentage analyses (Dakin & Avise 2004), we aimed to minimize their impact on the following analysis ~~(see below). We first, all the individuals with more than two null genotypes (i.e. no amplification) were~~ discarded ~~all the individuals with more than two null genotypes (i.e. no amplification).~~ Then, ~~we conducted~~ a preliminary parentage analysis in COLONY 2.0.6.1 (Jones & Wang 2010; ~~see below for details~~) was conducted. Knowing the true maternal sibships between females and larvae, ~~we the objective of this analyses was to~~ tested whether the information contained in the genotypes was sufficiently robust to reconstruct these relationships without specifying the maternal sibships. ~~We retained~~ Only the larvae that were assigned with a probability equal to 1 to their true mother were retained. The final dataset ~~was composed by~~ comprised 204 samples coming from two populations (DEU and HAR) and assigned to two different stage-classes (adults [AD] and larvae [LAR:-]). In DEU, we analyzed 67 larvae (DEU-LAR) from eight mothers and 30 adults (DEU-AD; 10 males and 20 females including the eight mothers). In HAR, we analyzed 74 larvae (HAR-LAR) from 11 mothers and 67 larvae ~~(DEU-LAR) from eight mothers in DEU~~ and 33 adults (HAR-AD; 19 males and 14 females including the 11 mothers) and 74 larvae (HAR-LAR) from 11 mothers in HAR (Table 1).

201 ***Genetic diversity analyses, long-term inbreeding and temporal genetic structure between***
 202 ***adults and larvae***

203 Frequencies of null alleles were estimated for each locus within each stage-class (i.e. adults and
 204 larvae) in FREENA (Chapuis & Estoup 2007). Tests for linkage equilibrium in each stage-class
 205 (~~i.e. adults and larvae~~) were conducted in GENETIX 4.05 (Belkhir et al. 2004) using 1000
 206 permutations. ~~We computed~~ The observed heterozygosity (H_o) and gene diversity (H_e ; Nei
 207 1973) were computed for each stage-class in GENEPOP 4.1.4 (Rousset 2008). ~~We used~~ ADZE
 208 (Szpiech et al. 2008) was used to compute the allelic richness (Ar_g) using the rarefaction method
 209 (Petit et al. 1998) with g , the minimum number of genes at a locus in a population, equals to
 210 56. The values of H_e and $Ar_{(56)}$ were compared between stage-classes in each population using
 211 Wilcoxon signed rank tests.

212 Because null alleles bias the estimation of F_{IS} , ~~we used~~ INEST (Chybicki & Burczyk 2009) was
 213 used under the Population Inbreeding Model to compute the F_{INEST} , an unbiased estimate of
 214 inbreeding coefficient. A first full model accounting for null alleles, inbreeding and genotyping
 215 failures was run for 200,000 iterations, a thinning of 100 and 50,000 burn-in. To test whether
 216 inbreeding was a significant component of the model, ~~we compared~~ this model was compared
 217 with a model without inbreeding based on the Deviance Information Criterion (DIC). The
 218 model with the lower DIC was retained.

219 Temporal genetic differentiation was estimated based on pairwise F_{ST} among adults and larvae
 220 within each population using θ , the Weir & Cockerham's (1984) estimator of F_{ST} . Genotypic
 221 differentiation was tested using an exact test with default parameters in GENEPOP.

222

223 ***Mating system analysis***

224 *Instantaneous biparental inbreeding and correlated paternity*

Based on the maternal progeny arrays, ~~we computed~~ the multilocus outcrossing rate (t_m) and the single outcrossing rate (t_s) were computed for each population (HAR: 11 families; DEU: 8 families) using the maximum likelihood method and Newton-Raphson iterations as implemented MLTR v.3.2 (Ritland 2002). ~~We adopted a~~ conservative approach was adopted ~~and excluded~~ Mic25 and Mic27, which showed the highest level of null alleles and genotyping errors, respectively. The difference between t_m - t_s was used to estimate the level of instantaneous biparental inbreeding (mating among close relatives) in each population. In addition, ~~we estimated~~ the correlated paternity ($r_{(p)}$) defined as the proportion of full sibs within maternal progeny arrays was estimated for each population (~~$r_{(p)}$ defined as the proportion of full sibs within maternal progeny arrays~~). The standard deviation and 95% confidence intervals (95% CI) of each parameter were estimated using a bootstrap procedure by resampling families 1000 times. These 95% CI were used to test whether the estimated values were different from 0 and for comparison among sitespopulations.

Characterization of the pattern of relationships

The maximum-likelihood method implemented in COLONY 2.0.6.1 (Jones & Wang 2009) was used to reconstruct the pattern of relationships among larvae and to assign paternity in each population accounting for genotyping errors (Wang 2004). ~~We set the~~ The levels of allelic dropout were set to the frequencies of null alleles computed for each locus (see Wang 2004). For all loci, the other type of genotyping errors was set to 0.01, an upward estimation of error rates based on the re-genotyping procedure (not shown). Known maternal sibs, the excluded maternity and the excluded maternal sibs were defined. ~~We conducted three~~ Three runs with different seed numbers were conducted considering females and males as polygamous and using the Full-Likelihood method with very high and very long likelihood precision and length of run, respectively. The robustness of the results was tested using a reduced dataset of six

microsatellites excluding Mic25 and Mic27. Because patterns of relationships were consistent and robust among runs, ~~we present~~ the results obtained for one run only are shown.

Sweepstakes reproductive success hypothesis

Test of reproductive skew among putative fathers

In case of sweepstake effects, differential reproductive success among individuals is expected (i.e. reproductive skew). ~~We tested the~~ The occurrence of differential reproductive success among the males inferred with COLONY in each population was tested using the Nonacs binomial skew index B implemented in SKEW CALCULATOR (Nonacs 2000, 2003). B ranges from -1 to +2. Random mating is observed for $B = 0$; negative values suggest that reproduction is more evenly distributed than expected while positive values correspond to a skew greater than expected suggesting differential reproductive success among individuals. The one-tailed p-value associated with B in each site was computed with 5000 simulations. ~~We then computed the~~ The 95% confidence interval of B in each site was estimated.

Comparison of relatedness estimators among stage-classes

Sweepstake effects also induced an increase of genetic relatedness from reproductive adults to their descendants. ~~We thus computed the~~ The levels of relatedness for each stage-class in each population were computed using Lynch-Li moment-estimator (~~Lynch 1988~~; Li, Weeks, & Chakravarti, 1993; Lynch 1988) in COANCESTRY (Wang 2011). ~~We tested the~~ The significance of the differences of the average relatedness between adults and larvae in each population was tested using a bootstraps procedure (1000).

Estimation of effective population size (N_e) and relative impact of genetic drift

According to the challenges linked to estimation of effective population size (N_e) in long-lived and iteroparous species (Waples 2010; Wang 2016), such as the red coral, ~~we compute different~~

different estimators of N_e were computed (Hare et al. 2011). These computations, based on larvae coming from a single reproductive event, results in an estimation of effective number of breeders (N_b) instead of a “true” N_e . Empirical works suggested similar results between the two parameters (e.g., Beedee 2009; Duong, Scribner, Forsythe, Crossman, & Baker, 2013).

We estimated four estimators of N_b were computed using: i) the sibship assignment method (Wang 2009); ii) the standard linkage disequilibrium method (Hill 1981) with Waples’ (2006) correction under the random-mating model, excluding rare alleles with frequencies of less than 0.02 and using a jackknife procedure to estimate confidence intervals (Waples & Do 2008, 2010); iii) the method based on molecular coancestry (Nomura et al. 2008). We also computed iv) moment-based F-statistics estimator (Waples 1989; Jorde & Ryman 2007; Waples 1989) considering a sampling plan I (see Waples 2005 for details) and that the adults and the larvae in each population were separated by one generation. The last three estimators were computed using NeEstimator v.2 (Do et al. 2014). Following Waples & Do (2010) and Riquet et al. (2016), an unweighted harmonic mean of N_b in each population was computed to obtain an overall estimate of effective population size.

RESULTS

Microsatellite characteristics, stage-class genetic diversity, long-term inbreeding and temporal genetic structure

No evidence of scoring errors due to stuttering or large allele dropout was found. In the two populations, all loci were polymorphic (p -value < 0.01). The frequency of null alleles (r) was between 0.09 for HAR-AD and HAR-LAR and 0.12 for DEU-AD and DEU-LAR (mean over populations $\pm$ SE = 0.1 ± 0.02). Observed heterozygosity values (H_o) were between 0.49 for DEU-LAR and 0.53 for HAR-AD (mean over populations $\pm$ SE = 0.51 ± 0.01). The gene diversity (H_e) ranged between 0.65 (HAR-LAR) and 0.72 (DEU-AD) (mean over populations

299 $\pm SE = 0.68 \pm 0.03$). Regarding $Ar_{(56)}$, the highest and lowest values were observed for DEU-
 300 AD (11.1) and HAR-LAR (9) (mean $Ar_{(56)}$ over samples $\pm SE = 9.9 \pm 1.03$). None of the
 301 Wilcoxon signed ranked tests conducted to compare H_e and $Ar_{(56)}$ among adults and larvae in
 302 each population ~~were~~ significant after FDR correction for multiple tests (Table 1). F_{INEST}
 303 estimators were low ~~and but~~ higher in adults compared to larvae (0.04 vs 0.02 in DEU and 0.03
 304 vs. 0.01 in HAR). In all the cases, the model without inbreeding showed the lowest DIC
 305 suggesting a lack of inbreeding (results not shown).
 306 The two temporal F_{ST} s were low (0.002 for DEU-AD vs. DEU-LAR and 0.005 for HAR-AD
 307 vs. HAR-LAR) but significant (p-values < 0.05).

309 *Mating system analyses*

310 *Instantaneous biparental inbreeding and correlated paternity*

311 The multilocus outcrossing rates (t_m) were close to 1 in both populations as expected in strictly
 312 outcrossing species. The single outcrossing rate (t_s) was 0.78 for DEU and 0.74 for HAR
 313 resulting in a higher signal of biparental inbreeding ($t_m - t_s$) in DEU (0.21; 95% CI = 0.13 - 0.45)
 314 than in HAR (0.17; 95% CI = 0.07 - 0.26). Based on the 95% CIs, The the estimates of
 315 biparental inbreeding were significantly different from 0 in the two populations (~~DEU: 95% CI~~
 316 ~~= 0.13 - 0.45; HAR: 95% CI = 0.07 - 0.26~~). No but the estimates were not significantly
 317 difference-different was observed between the two populations ~~as the two 95% CI overlapped~~.
 318 Estimates of correlated paternity were 0.23 (95% CI = 0.09 - 0.46) for DEU and 0.12 for HAR
 319 (95% CI = -0.03 - 0.25). The two estimates were not significantly different based on their 95%
 320 CI (Table 2).

321 *Pattern of relationships*

322 Considering only relationships with a probability > 0.8 , in DEU, ~~we obtained~~ six full-sib dyads
 323 and 246 half-sib dyads over the 2211 putative relationships were retained, corresponding to
 324 0.27 % and 11.12 %, respectively. The total number of fathers was 32. In HAR, ~~we observed~~

two full-sib dyads and 235 half-sib dyads over the 2701 putative relationships were observed, corresponding to 0.07 % and 8.7 %, respectively. The total number of fathers was 43. Evidence of multiple paternity was found in all the families from the two populations. The number of fathers for each family in DEU ranged from 6 to 10, with a mean value of 7.5 ± 1.5 fathers. In HAR, the number of fathers for each family varied from 2 to 10, with a mean value of 6.5 ± 2.4 fathers. The putative fathers were involved in multiple mating events producing 1 to 5 larvae, with a mean value of 2.1 ± 1.1 in DEU and a mean value of 1.9 ± 1 in HAR (Figure 2).

Sweepstakes reproductive success hypothesis

Reproductive skew among putative fathers

Although the number of larvae per putative father ranged from 1 to 5, the skew observed among males was not significantly different from random expectations in the two populations ($B = -0.0057$, $p\text{-value} = 0.973$ in DEU; $B = -0.008$, $p\text{-value} = 1$ in HAR). This suggests a similar contribution of the different fathers to the pool of larvae.

Comparison of relatedness estimators among stage-classes

Mean estimates of relatedness based on the Lynch-Li moment-estimator ranged from -8.84×10^{-2} in DEU-AD to 7.65×10^{-2} in HAR-LAR. The differences of mean relatedness between adults and larvae were significant in the two populations (Table 3).

Estimation of effective population size (N_b)

Based on the sibship assignment method, N_b was 29 (95 %CI: 18-49) for DEU and 39 (95 %CI: 25-61) for HAR. The linkage disequilibrium and the molecular coalescent estimates of N_b were 38.3 (95 %CI: 30-50) and 4.7 (95 %CI: 1.9-8.7) in DEU and 35.9 (95 %CI: 29.1-45) and infinity in HAR, respectively. The estimates of N_b obtained with the temporal method were 25.5 (95

349 %CI: 17.4 - 35) in DEU and 34 (95 %CI: 22.6 - 47.7) in HAR. The resulting harmonic means
 350 of N_b were 12.8 for DEU and 48.2 for HAR (Table 4).

351

352 DISCUSSION

353 The impact of demographic decline on population dynamics and evolution is a central concern
 354 for conservation biologists. This study, ~~which~~ is the first genetic characterization of the mating
 355 system of an internal brooding octocoral, ~~— In spite of a lack of statistically significant~~
 356 ~~differences in the mating patterns of the two populations, a higher biparental inbreeding and~~
 357 ~~correlated paternity combined with a lower number of breeders were reported in the declining~~
 358 ~~populations shows similar reproductive patterns between a pristine-like and a declining~~
 359 ~~population, suggesting that red coral may be relatively resilient to population decline.~~ While
 360 these results may potentially be affected by the occurrence of null alleles, it is noteworthy that
 361 the dataset was filtered to decrease their impact (e.g., only mother/larvae pairs with assignment
 362 probability = 1 were retained). ~~We also used a~~ broad set of complementary analyses relying
 363 on different statistical methods ~~was also used and, which~~ showed concordant results (~~ie.eg.~~
 364 sibship reconstruction vs. progeny array analyses, four different N_b estimators). Moreover,
 365 simulations considering different levels of genotyping errors demonstrated the robustness of
 366 the sibship reconstruction method implemented in COLONY (Wang 2004). ~~We thus consider~~
 367 ~~our results as relevant and we discuss below~~ ~~their~~ implications of these results for the basic
 368 ecological knowledge of the red coral and for its evolution in the context of population decline
 369 is discussed below.

370

371 *Does multipaternity buffer long-term inbreeding?*

372 ~~We demonstrate that~~ multipaternity is likely the norm in the red coral. Indeed, the 19 progeny
 373 arrays analyzed were sired by more than one male as supported both by the sibship analyses

in COLONY and by the correlation of paternity estimated in MLTR. Polyandry is expected in promiscuous mating species and was previously demonstrated in one red coral population based on kinship analyses among juveniles and adults (Ledoux et al. 2010b). The estimated mean number of larvae per father inferred in COLONY over the two populations studied here (1.88 ± 0.9 larvae / father) is in the same order as those previously reported in the same species (2.3 ± 0.8 offspring / parent; Ledoux et al. 2010b) and in another tropical octocoral *Antilloporia elisabethae* (1.42 ± 0.7 larvae / father; Lasker, Gutiérrez-Rodríguez, Bala, Hannes, & Bilewitch, 2008) and slightly lower than in the Mediterranean *Paramuricea clavata* (6.6 ± 4.7 larvae / father; Mokhtar-Jamaï et al. 2013). Variations in the level of multiple paternity among species are usual in marine invertebrates (e.g. Brante, Fernandez, & Viard, 2011; Warner et al. 2016). While polyandry is a widespread strategy among organisms from sessile marine invertebrates to social mammals and including wind-pollinated plant and tree species (Simmons 2005), its evolution remains a controversial issue (Yasui et al. 2016). Focusing on sessile marine species in which polyandry is passive (i.e. females passively receive the gamete from different males), the adaptive value is questioned. Multiple mating may simply be correlated to the selective pressure increasing fertilization success when multiple individuals are present (Johnson & Yund 2007; Brante, Fernandez, & Viard, 2011; Johnson & Yund 2007). Alternatively, the adaptive hypothesis lies on indirect genetic benefits for the females. Polyandry may increase offspring survival enhancing in turn female reproductive fitness by increasing the genetic diversity, and the viability or the competitiveness of the offspring through advantageous male genes (see Zeh & Zeh 2000; Yasui & Garcia-Gonzales 2016; Zeh & Zeh 2000 for reviews). While it is notoriously challenging to test for indirect genetic effects (Yasui & Garcia-Gonzales 2016) and acknowledging the complexity of the interactions between mating system and inbreeding (Frankham 1995), a decrease of inbreeding was also proposed as one of the indirect

genetic benefits of multipaternity (Zeh & Zeh 2000). Here, ~~we demonstrated that the~~ inbreeding varied in function of the temporal scale. The progeny array approach supports significant level of instantaneous biparental inbreeding (i.e. crossing between related mates). This result is in accordance with the view of red coral populations as mosaics of spatially restricted breeding units (< one square meter~~e~~) composed by relatives (Ledoux et al. 2010b). In contrast, when considering the multilocus inbreeding coefficients unbiased by null alleles (F_{INEST}), which estimate the combined effect of inbreeding depression (i.e. the fitness decrease observed in related individuals) and biparental inbreeding over several generations, inbreeding was not statistically supported. This absence of long-term inbreeding may appear surprising considering the high and significant F_{IS} values reported in previous studies (e. g. Ledoux et al. 2010a). Nevertheless, these F_{IS} values were likely biased by null alleles and Wahlund effect (Ledoux et al. 2010a). Further analyses such as estimations of inbreeding coefficients on well-defined cohorts (from larvae to recruits, juveniles and adults) and reproduction experiments in aquaria should be envisaged to formally test a putative buffering effect of multipaternity on inbreeding in the red coral.

Soft sweepstakes effect and low genetic drift in the red coral.

Interestingly, larvae production does not seem to be impacted by strong sweepstakes effects in this species. None of the genetic diversity parameters tested ~~were~~ significantly different between larvae and adults in the two populations. No linkage disequilibrium was reported in the larvae although it is expected when a small number of individuals produced the next generation. Moreover, while a significant increase of genetic relatedness was reported from adults to larvae, the genetic differentiation between the two stage-classes in each population was only marginally significant. The high polymorphism of the microsatellites used here may partially explain these results (Hedgecock & Pudovkin 2011). Nevertheless, ~~the~~ absence of

sweepstake effect is also supported by the lack of reproductive skews among the fathers inferred with COLONY. Sweepstakes effect is expected in promiscuous sessile marine invertebrates with larval phase (Hedgecock & Pudovkin 2011) but ~~our results~~ the lack of sweepstake observed here are-is also supported by the null to low temporal differentiation previously reported among juveniles and adults (Ledoux et al. 2010b) or recruits and juveniles (Costantini, ~~Rigiù~~ Rugiu, Cerrano, & Abbiati, 2018). This can be related to red coral life-history traits and in particular, to the occurrence of mating among individuals from overlapping generations, which may buffer temporal changes of allelic frequencies (Riquet, Le Cam, Fonteneau, & Viard, 2016). Comparing genetic characteristics among well-defined cohorts rather than stage-classes is needed to assess this hypothesis.

The impact of a promiscuous mating system on N_e and genetic drift has been previously debated (Karl 2008; Pearse & Anderson 2009). In species with a generation time ~~higher~~ greater than 10 years such as the red coral, multipaternity should only marginally impact N_e (Lotterhos 2011). While estimates of N_e must be considered cautiously and considering that ~~we assumed~~ similarity between N_b and N_e was assumed here, ~~our~~ the results support moderate to high numbers of breeders in the two populations. These values are concordant with the high N_e previously reported over a similar spatial area in the long-lived and polyandrous octocoral *P. clavata* (Mokhtar-Jamaï et al. 2013). However, they contrast with the general trend in marine species in which N_e was shown to be orders of magnitude lower than the census population size (N_c) ~~indueing and that~~ genetic drift is usually stronger than expected (~~Hauser & Carvalho 2008;~~ Hare et al. 2011; Hauser & Carvalho 2008). In accordance with the lack of sweepstake effect previously discussed, ~~our~~ the estimations of N_b suggest genetic drift to be limited at the fine spatial and temporal scales under survey. This result supports recent studies questioning the ubiquity of low N_e s in marine species (Hare et al. 2011; Riquet, Le Cam, Fonteneau, & Viard, 2016).

449
450 ***Does population decline impact red coral mating system and effective population size?***

451 The site “Grotte Harmelin” (HAR) is considered as one of the last pristine-like shallow
452 population of red coral offering the ~~priceless~~ opportunity to characterize the baseline population
453 dynamics in this species (Garrahou et al. 2017). Contrasting the results obtained in this
454 population with the results obtained in a declining population (DEU), ~~we~~ provides the first
455 insights into the consequences of demographic decline on mating system and effective
456 population size in the red coral.

457 None of the parameters (correlated paternity, biparental inbreeding and number of breeders)
458 estimated to compare the mating patterns between the two populations were significantly
459 different. This result contrasts with the positive correlation between population density and
460 mating pattern reported in copulating marine invertebrates (e.g., barnacles (Plough, Moran, &
461 Marko 2014); marine snails (Xue, Zhang, & Liu 2016)). In these species, the probability of
462 mate encounter increases with population density. The lack of differences among the two
463 populations is concordant with what was found between low and high-density populations in a
464 sperm-cast ascidian (Johnson & Yund 2007). In sperm-cast species such as the red coral, the
465 spermatozoa may cross greater distances in the water column in low density populations
466 buffering the influence of density on mate interactions (e.g. Duminil et al. 2016). Nevertheless,
467 in spite of this lack of statistically significant differences between the two populations, all the
468 analyses suggest some negative consequences of demographic decline. A trend toward higher
469 biparental inbreeding and correlated paternity combined with a lower number of breeders is
470 observed in the declining compared to the pristine-like population. Accordingly, we
471 hypothesized the expected increase in genetic drift, due the lower mate density in DEU
472 compared to HAR, to be balanced to some point by an increase of male gamete flow. While the
473 hypothesized buffering effect of male gamete flow remains to be formally tested in the red

coral, it is likely subtle and limited as supported by the negative trend observed in the declining population. The characterization of spatial genetic structures in populations with contrasted demographic patterns and the inference of related demographic parameters (e.g. effective dispersal) is required to go further in this hypothesis and to improve our understanding of the feedbacks between demographic decline and mating patterns in the red coral.

~~In spite of the trend toward higher inbreeding and genetic drift in the declining population (DEU), none of the parameters (correlated paternity, biparental inbreeding and number of breeders) estimated to compare the mating patterns between the two populations were significantly different. Contrasting with the positive correlation between population density and mating pattern reported in copulating marine invertebrates (e.g., barnacles in Plough, Moran, & Marko, 2014; marine snails in Xue, Zhang, & Liu 2016), this result is concordant with the lack of differences found between low and high density populations in a spermeast ascidian (Johnson & Yund 2007). In copulating species, the probability of mate encounter increases with population density. In spermeast species, the spermatozoa may cross higher distances in the water column in low density population buffering the influence of density on mate interactions. Such effect has been previously demonstrated in other long lived sessile species with similar reproductive strategies such as tropical trees in which pollen dispersal distance increases in declining population (e.g. Duminil et al. 2016). Based on these results, we propose that the expected increase in genetic drift due the lower mate density in DEU compared to HAR may be balanced by an increase of male gamete flow. The characterization of spatial genetic structures in populations with contrasted demographic patterns and the inference of related demographic parameters (e.g., effective dispersal) is thus a critical step to improve our understanding of the feedbacks between demographic decline and mating patterns in the red coral. This should also allow the identification of putative demographic thresholds beyond which the buffering effect of gamete dispersal will be inefficient.~~

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501 Overall, this study suggests the occurrence of subtle feedbacks between demographic and
502 reproductive patterns at-in red coral populations. In complement to other processes (e.g. re-
503 growth of damaged colonies; see Montero-Serra et al. 2015), these feedbacks may partially
504 explain the long-term persistence of red coral populations in spite of the millennial fishing
505 pressure. They are likely to provide a borrowed time (*sensu* Hughes, Linares, Dakos, van de
506 Leemput, & van Nes 2013) for the management and conservation of this threatened species.
507 Nevertheless, the negative trend reported in the declining population unambiguously supports
508 the maintenance of high density of reproductive colonies, and accordingly the diminution of
509 direct anthropogenic pressures (e.g. fishing), as a prerequisite to maintain functional red coral
510 populations. ~~are resilient to some levels of population decline potentially due to an increase of~~
511 ~~spermatozoa dispersal. This may potentially explain their long-term persistence in spite of the~~
512 ~~millennial fishing pressure and it provides a borrowed time (*sensu* Hughes, Linares, Dakos, van~~
513 ~~de Leemput, & van Nes, 2013) for the management and conservation of this threatened species.~~

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49 **Authors’ contributions:**
50 JBL, CL and JG designed the study. CL, CCB and IMS performed sexing and larvae
51 772 collection. JBL, PLS, SF and CCB performed laboratory work. JBL, SF and SC analyzed the
52 773 data. JBL and SF wrote the manuscript with input from all authors. All authors approved the
53 774 manuscript.
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57 **Data accessibility:**
58 778 Microsatellite genotypes will be made available on Dryad.
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Supporting information:

Additional information may be found in the online version of the article:

Appendix A: DNA extraction and microsatellite genotyping

Conflict of interest: No conflict of interest to declare.

Table 1: Genetic diversity of each stage-class in each site (DEU and HAR). N : number of individuals; r : frequency of null alleles; H_o : observed heterozygosity; H_e : gene diversity; $Ar_{(56)}$: rarefied allelic richness considering a minimum of 56 genes at a locus in a population; F_{INEST} : estimate of inbreeding coefficient unbiased by null alleles.

	N	r	H_o	H_e	Ar	$Ar_{(56)}$	F_{INEST}
DEU-AD	30	0.12	0.52	0.72	11.25	11.1	0.04
DEU-LAR	67	0.12	0.49	0.69	11	9.1	0.02
HAR-AD	33	0.09	0.53	0.66	10.62	10.3	0.03
HAR-LAR	74	0.09	0.52	0.65	11.62	9	0.01

Table 2: Mating system characteristics for each site. t_m : multilocus outcrossing rate; t_s : single outcrossing rate; t_m-t_s : level of biparental inbreeding; $r_{(p)}$: correlated paternity. The 95% confidence intervals (95% CI) of each parameter was estimated by resampling families 1000 times.

	Multilocus-outcrossing rate (t_m) and [95% CI]	Single-outcrossing rate (t_s) and [95% CI]	Biparental inbreeding (t_m-t_s) [and 95% CI]	Correlated paternity $r_{(p)}$ and [95% CI]
DEU	0.99 [0.95 ; 1.2]	0.78 [0.64 ; 0.89]	0.21 [0.13 ; 0.45]	0.23 [0.09 ; 0.46]
HAR	0.94 [0.86 ; 1]	0.77 [0.67 ; 0.88]	0.17 [0.07 ; 0.26]	0.12 [-0.03 ; 0.25]

Table 3: Estimates of relatedness for each stage-class in each population using the *LL*: Lynch-Li moment-estimator of relatedness. Observed value corresponds to the difference between adults and larvae in each population. The significance of the differences among the two stage-classes was tested using on 1000 bootstraps. Significant differences are shown in bold.

	<i>Lynch-Li</i>	Observed Value and 95% CI
DEU-AD	8.84×10^{-2}	9×10^{-2} $[-2.5 \times 10^{-2} ; 2.51 \times 10^{-2}]$
DEU-LAR	2.31×10^{-3}	
HAR-AD	5.28×10^{-2}	2.37×10^{-2} $[-2.55 \times 10^{-2} ; 1.95 \times 10^{-2}]$
HAR-LAR	7.65×10^{-2}	

Table 4: Effective population size and 95 % confidence interval (between brackets) computed for each site using: i) the sibship assignment method; ii) the standard linkage disequilibrium method; iii) the method based on molecular coancestry; and iv) the moment based F-statistics methods.

	Sibship assignment method (Wang 2009)	Standard linkage disequilibrium method (Hill 1981)	Molecular coancestry method (Nomura et al. 2008)	Moment-based F-statistics estimator (Waples 1989; Jorde & Ryman 2007)	Unweighted harmonic mean
DEU	29 [18 - 49]	38.3 [30 - 50]	4.7 [1.9 - 8.7]	25.5 [17.4 - 35]	12.8
HAR	39 [25 - 61]	35.9 [29.1 - 45]	infinity	34 [22.6 - 47.7]	48.2

FIGURE LEGENDS:

Figure 1: Location of the two study sites: Pedra de Deu (DEU) in the Medes Islands within the Parc Natural del Montgrí, illes Medes i Baix Ter (Spain) and Grotte Harmelin (HAR) within the Réserve Naturelle de Scandola in Corsica (France). Pictures A and B show populations DEU and HAR, respectively. The same quadrat (20 x 20 cm) is used in the two pictures.

Figure 2: Kinship structure among larvae from each population (DEU and HAR) reconstructed based on their multilocus genotypes using COLONY. L/M: number of larvae per mother; L/F: number of larvae per putative father. Grey and blue squares correspond to one and two larvae, respectively, for the mother-putative father pair considered. * pair of larvae inferred as fullsib with a probability higher than 0.8.

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Table 1: Characteristics and genetic diversity of each stage class in each site (DEU and HAR). N : number of individuals; r : frequency of null alleles; H_o : observed heterozygosity; H_e : gene diversity; $Ar_{(56)}$: rarefied allelic richness considering a minimum of 56 genes at a locus in a population; F_{INEST} : estimate of inbreeding coefficient unbiased by null alleles.

	N	r	H_o	H_e	Ar	$Ar_{(56)}$	F_{INEST}
DEU-AD	30	0.12	0.52	0.72	11.25	11.1	0.04
DEU-LAR	67	0.12	0.49	0.69	11	9.1	0.02
HAR-AD	33	0.09	0.53	0.66	10.62	10.3	0.03
HAR-LAR	74	0.09	0.52	0.65	11.62	9	0.01

Table 2: Mating system characteristics for each site. t_m : multilocus outcrossing rate; t_s : single outcrossing rate; t_m-t_s : level of biparental inbreeding; $r_{(p)}$: correlated paternity. The 95% confidence intervals (95% CI) of each parameter was estimated by resampling families 1000 times.

	Multilocus outcrossing rate (t_m) and 95% CI	Single outcrossing rate (t_s) and 95% CI	Biparental inbreeding (t_m-t_s) and 95% CI	Correlated paternity $r_{(p)}$ and 95% CI
DEU	0.99 [0.95 ; 1.2]	0.78 [0.64 ; 0.89]	0.21 [0.13 ; 0.45]	0.23 [0.09 ; 0.46]
HAR	0.94 [0.86 ; 1]	0.77 [0.67 ; 0.88]	0.17 [0.07 ; 0.26]	0.12 [-0.03 ; 0.25]

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Table 3: Estimates of relatedness for each stage-class in each population using the *LL*: Lynch-Li moment-estimator of relatedness. Observed value corresponds to the difference between adults and larvae in each population. The significance of the differences among the two stage-classes was tested using on 1000 bootstraps. Significant differences are shown in bold.

	<i>Lynch-Li</i>	Observed Value and 95% CI
DEU-AD	-8.84*10 ⁻²	9*10⁻²
DEU-LAR	2.31*10 ⁻³	[-2.5*10⁻² ; 2.51*10⁻²]
HAR-AD	5.28*10 ⁻²	2.37*10⁻²
HAR-LAR	7.65*10 ⁻²	[-2.55*10⁻² ; 1.95*10⁻²]

Table 4: Effective population size and 95 % confidence interval (between brackets) computed for each site using: i) the sibship assignment method; ii) the standard linkage disequilibrium method; iii) the method based on molecular coancestry; and iv) the moment based F-statistics methods.

	Sibship assignment method (Wang 2009)	Standard linkage disequilibrium method (Hill 1981)	Molecular coancestry method (Nomura et al. 2008)	Moment-based F-statistics estimator (Waples 1989; Jorde & Ryman 2007)	Unweighted harmonic mean
DEU	29 [18 - 49]	38.3 [30 - 50]	4.7 [1.9 - 8.7]	25.5 [17.4 - 35]	12.8
HAR	39 [25 - 61]	35.9 [29.1 - 45]	infinity	34 [22.6 - 47.7]	48.2