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Original research article

Marine reserves help preserve genetic diversity after impacts derived from climate variability: Lessons from the pink abalone in Baja California

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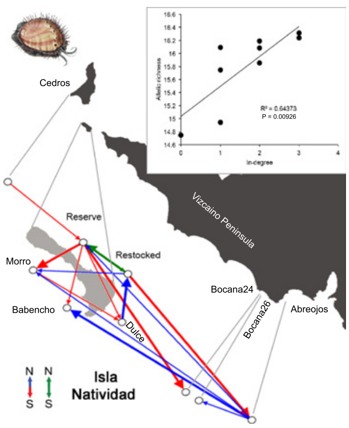
HIGHLIGHTS

- Genetic composition of abalone depended on history of exploitation and management.
- Reserve had high allelic diversity, effective size and no signs of bottlenecks.
- Collapsed population in California was isolated, had low diversity and high relatedness.
- Restocking increased frequency of related individuals and genetic differentiation.
- Reserve was the most important hub for larval dispersal facilitated by ocean currents.

GRAPHICAL ABSTRACT



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ABSTRACT

Genetic diversity is crucial for the adaptation of exploited species like the pink abalone (*Haliotis corrugata*), faced with threats from climate change, overfishing and impacts associated with aquaculture production. While marine reserves are commonly used to

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mitigate risks to marine populations, the duration, size, location and larval connectivity needed for a reserve to help conserve genetic resources is still poorly understood. Here, we examine the effects of fishing, reserves, and restocking on the genetic diversity of 10 populations from central Baja California, Mexico, and Southern California, USA. We demonstrate that each population shows characteristic genetic signatures according to recent management decisions. We found high allelic diversity, particularly rare alleles, a larger effective population size and a lack of a recent genetic bottleneck in pink abalones within a small (0.8 km^2), recently established (5 years) reserve in Baja California, compared to other fished sites after a climatic bottleneck. Higher diversity may result from the presence of older animals in the reserve. Due to its location, the reserve may also act as an important hub connecting distant populations via larval dispersal. In contrast, a population from California showed genetic isolation, loss of allelic diversity and high relatedness, consistent with the collapse of fisheries in the 1990s and their lack of recovery thereafter. In addition, a fished area in Baja California with a history of restocking for over a decade showed an increase in frequency of related individuals and high genetic differentiation from nearby sites that were consistent with the production of larvae from a few adults in the laboratory. A network of strategically placed small marine reserves that considers ocean circulation patterns could help to maintain genetic diversity and connectivity of exploited populations.

1. Introduction

Marine species around the world are under increasing pressure from multiple threats, including overfishing, ocean warming, acidification and hypoxia (Doney et al., 2012). Aquaculture production may release human pressure on natural populations, but it too is associated with negative impacts from pollution, using wild stocks to produce animal food, introduction of alien species and genetic alteration of natural populations (Klinger and Naylor, 2012). As we could be approaching a historical tipping point in the Earth's ecosystems resulting from human pressure (Barnosky et al., 2012), it is key to understand the individual and cumulative effects of multiple threats, and develop effective conservation and management tools to address these impacts.

An important, yet commonly overlooked aspect of biodiversity, is the conservation of genetic diversity (Laikre, 2010; Sgrò et al., 2011), which is closely associated to the effective size (*Ne*) of a population (Nei, 1987). The evolution needed to adapt in a rapidly changing environment and avoid extinction could occur relatively quickly based on standing genetic variation (as compared to new mutations) (Sgrò et al., 2011; Pespeni and Al, 2013). Within ecosystems, genetic variation provides key functions such as promoting recovery following a population collapse (Lotze et al., 2011) and enhancing ecosystem resistance and stability (Worm et al., 2006). Even small differences in genetic diversity could have important ecological changes in natural populations (Koh et al., 2012). Understanding the processes that maintain or erode genetic diversity is crucial for the conservation of exploited species. Overfishing can decrease genetic variation through bottlenecks, cause the loss of genetically unique stocks, select for early maturation and ultimately reduce the adaptability of wild populations (Hutchings and Reynolds, 2004). A recent meta-analysis across 140 species showed a 12% drop in allelic richness and 2% decrease in heterozygosity in overharvested populations compared to those that have not declined (Pinsky and Palumbi, 2014).

Globally, abalone fisheries are a classic example of overfishing. Most fisheries have collapsed and failed to recover for decades, including those in the USA, which were closed statewide in 1997, with the exception of a red abalone (*H. rufescens*) sport fishery north of San Francisco (Micheli et al., 2008, 2012). Populations along the Pacific coast of the Baja California Peninsula, Mexico, have experienced several cycles of collapse attributed to a combination of overfishing and the negative impacts of ENSO events (Morales-Bojorquez et al., 2008; Searcy-Bernal et al., 2010). However, abalones still sustain a vibrant economy in Mexico, albeit at levels an order of magnitude lower than the historical maximum (Micheli et al., 2012). Currently, ∼30% of catches are of pink (*Haliotis corrugata* Wood 1828) and ∼70% green (*Haliotis fulgens* Philippi 1845) abalones (Morales-Bojorquez et al., 2008; Searcy-Bernal et al., 2010).

Marine reserves have received increasing support at local, national and global scales, such as promising approaches for rebuilding depleted populations, conserving biodiversity and ensuring the continued flow of services from marine ecosystems, including climate change adaptation (Gaines et al., 2010; Green et al., 2014). The concept of networks of small coastal reserves for abalone dates back to the beginning of the last century (Edwards, 1913), and such a network was recently completed through the implementation of California's Marine Life Protection Act (Gleason et al., 2013). In Baja California, reserve establishment has instead occurred locally through the initiative of some coastal fishing communities (Micheli et al., 2012).

Another strategy aimed at promoting population recovery is augmentation through the release of captive-bred individuals into the wild, hereafter referred to as restocking. Restocking is a common practice to rebuild abalone fisheries or even avoid extinction (Hamasaki and Kitada, 2008; Micheli et al., 2008; Stierhoff et al., 2012). For example, during 2007 and 2008, over 130 million veliger larvae and 350,000 juveniles (10–15 mm in length) of pink and green abalone were released around central Baja California (Searcy-Bernal et al., 2010). Besides the intended effect of enhancing wild fisheries, restocking by using a few individuals to produce larvae could potentially reduce genetic diversity, increase genetic divergence among sites by genetic drift, and increase relatedness among individuals within sites (Roodt-Wilding, 2007; Lemer and Planes, 2012).

Despite the importance of genetic diversity in adaptation, few studies have attempted to determine whether marine reserves conserve genetic diversity within species (Miller and Ayre, 2008). Some studies have suggested reserves located around islands could be isolated and genetically depauperate (Bell and Okamura, 2005), limiting their efficacy for increasing larval export or population resilience (Bell, 2008). Large reserves are recommended for preserving genetic diversity (Almany et al., 2009), but in reality small reserves that are isolated represent the majority of reserves worldwide. Although small reserves maximize export of larvae across their boundaries, and thus could be optimal for increasing the benefits of protection to sedentary benthic abalone fisheries (Rossetto et al., in press), their efficacy for helping conservation and fisheries' goals remains controversial (Parnell et al., 2005; Lester et al., 2009).

Isla Natividad is a small island located ∼8 km off the coast from Punta Eugenia in the central region of the Baja California Peninsula, Mexico. In 2006, the local fishing community created two small (∼0.8 km^2) no-take reserves (Micheli et al., 2012). During 2009–2010, a widespread mass mortality of abalones and other benthic invertebrates took place around Isla Natividad (Micheli et al., 2012). Physical monitoring around the island associated this event with periods of low dissolved oxygen concentrations (Micheli et al., 2012). After the mortality event, pink abalone density significantly dropped (3.7 X reduction compared to 2006–2008) due to the combined mortality from fishing and the climatic event (Micheli et al., 2012). However, the study suggested the two small reserves maintained a higher fraction of large individuals (>14 cm) which are more fecund, helping to sustain levels of larval recruitment through the event compared to fished sites (Micheli et al., 2012; Rossetto et al., 2013). Here we evaluated current levels of genetic diversity and connectivity of pink abalone populations at Isla Natividad, central Baja California Mexico and Southern CA, USA, and the potential of marine reserves and restocking as tools for maintaining genetic diversity. In particular we aimed to: (1) examine the effects of fishing on genetic diversity and composition, and assess whether populations experience genetic bottlenecks associated with population declines; (2) compare levels of genetic diversity within the reserve to sites where fishing, restocking and long-term population collapse take place; (3) examine population structure and connectivity at different spatial scales (along the west coast of Baja California, southern California, USA, and sites around Isla Natividad) to investigate the potential of networks of reserves to maintain genetic diversity.

2. Methods

2.1. Densities

Pink abalones range from point Conception CA, USA to Magdalena Bay in Baja California Sur, Mexico, and are found at depths of between 8 and 22 m (Carballo and Mucino-Diaz, 1996). They produce millions of pelagic larvae that last between 5–10 days before settling to the seafloor, with a maximum duration of 15 days (Leighton, 1974). Size at sexual maturity (size at which 50% of individuals are mature) is ∼13 cm (Rossetto et al., 2013), or about 4 years of age (Shepherd et al., 1998). To monitor differences in population density among different populations since reserve establishment, we monitored *in-situ* densities during summer from 2006 to 2011 through SCUBA 30 X 2 m transects at four sites (11–30 transects/site) around Isla Natividad as detailed previously (Micheli et al., 2012). Surveyed sites (Fig. 1) included: one of the reserves established in 2006 and included in the Micheli et al. (2012) study, a fished site ∼2 km from the reserve, where restocking has occurred yearly during the last decade (Restocked), and two sites fished regularly (Babencho and Dulce). Estimates of densities from each site within each year were analyzed with analysis of variance (ANOVA).

2.2. Genetic sampling and genotyping

We collected a total of 414 genetic samples from pink abalone from 10 different populations (Table 1). The locations sampled included the four sites around Isla Natividad mentioned above (a no-take reserve and 3 fished sites, one restocked), and an additional fished population (Morro Prieto, hereafter "Morro") to examine genetic diversity, structure and connectivity at the local scale of continuous reef habitat (2–10 km), and where mass mortalities associated to hypoxia have been documented. We included another insular fished population located ∼50 km north of Isla Natividad (Isla Cedros, hereafter "Cedros"), and three fished populations located off the mainland of the central Baja Peninsula, ∼200 km to the South (Bocana24, Bocana26 and Punta Abreojos, hereafter "Abreojos", Fig. 1) to examine variation at regional scales of tens to hundreds of kilometers, among reefs separated by large expanses of open ocean and benthic habitat unsuitable for abalone (deep sandy bottoms). We included a population ∼700 km to the north in Point Loma, San Diego CA, USA, where all abalone fisheries have been closed since 1997, to contrast it with populations that are still supporting fisheries. Tissue samples (∼0.5 g of epipodium preserved in 95% ethanol) from the fished locations were collected from individuals landed during the commercial fishing season in spring 2009 and 2010 and were larger than the commercial size limit (≥130 mm). During 2009–2010 we sampled individuals larger than 100 mm within the reserve at Isla Natividad and off the coast of Point Loma using a non-lethal method through SCUBA, which involved carefully removing the snail from the substrate to take the epipodium sample, replacing the animal at the same spot and waiting for the animal to reattach to the substrate. Genomic

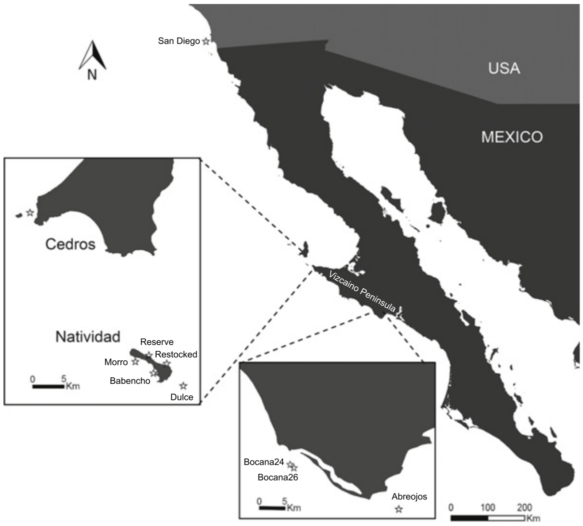

Fig. 1. Location of 10 pink abalone populations sampled in USA and Mexico. Exact locations are indicated by stars (⋆), including a marine reserve and a restocked site on the eastern side of Isla Natividad.

Table 1
Summary of genetic diversity for 10 pink abalone populations. Sample size (N), average number of alleles per locus (A), effective alleles (E_A), allelic richness using rarefaction (A_R), rare alleles (RA, frequency $\leq 5\%$), private alleles (P_A), observed heterozygosity (H_O). P values for BOTTLENECK (*P bottl.*) and MRATIO (*P Mratio*) tests of demographic reduction. Estimates of *Ne* based on linkage disequilibrium (LDNe) and sibship assignments (COL2Ne). Percentage of pairwise relatedness values equal or greater than a third-degree relative ($\% R \geq 0.12$). Eigenvector centrality estimates, in-degree and out-degree from a network describing first-generation migrants identified with GENECLASS2 (see Fig. 4). S.E. = Standard Error. C.I. = 95% Confidence Intervals. Note: for LDNe some populations showed low accuracy (∞).

	Isla Natividad									Average fished Natividad (±S.E.)	Reserve	% Change Reserve *vs.* fished Natividad
	San Diego	Cedros	Morro	Babencho	Re-stocked	Dulce	Bocana24	Bocana26	Abreojos			
N	38	39	39	40	47	38	38	40	40		55	
A	13.563	14.750	16.250	16.188	16.188	16.188	14.938	15.813	16.188	16.203	17.375	+7.229
±S.E.	1.033	0.990	1.195	1.148	1.242	1.054	0.910	1.259	1.170	0.013	1.207	
E_A	7.6967	9.017	10.353	9.775	10.205	10.150	9.611	10.095	9.983	10.120	10.350	+2.265
±S.E.	0.872	0.878	1.047	1.140	1.036	1.051	0.982	1.094	0.992	0.106	1.192	
A_R	13.56	14.75	16.24	16.08	15.85	16.19	14.94	15.75	16.09	16.090 (0.075)	16.31	+1.367
RA	6.813	7.313	8.688	8.063	9.250	8.688	7.125	7.563	8.625	8.672	10.063	+16.036
±S.E.	0.452	0.428	0.474	0.499	0.392	0.516	0.557	0.512	0.491	0.209	0.530	
P_A	0.313	0.250	0.375	0.313	0.125	0.250	0.188	0.250	0.375	0.265	0.250	−5.926
±S.E.	0.151	0.194	0.155	0.151	0.085	0.144	0.101	0.112	0.125	0.046	0.144	
H_O	0.729	0.785	0.764	0.767	0.791	0.751	0.674	0.697	0.713	0.768	0.732	−4.718
±S.E.	0.034	0.030	0.035	0.040	0.026	0.042	0.033	0.042	0.034	0.007	0.033	
H_E	0.830	0.864	0.877	0.858	0.869	0.869	0.866	0.861	0.872	0.868	0.865	−0.374
±S.E.	0.027	0.019	0.019	0.027	0.025	0.025	0.022	0.029	0.021	0.003	0.025	
P bottl.	0.177	0.019	0.003	0.021	0.004	0.021	0.022	0.019	0.000		0.075	
Mratio	0.776	0.858	0.848	0.832	0.875	0.859	0.823	0.858	0.816		0.875	
P Mratio	0.019	0.277	0.229	0.146	0.395	0.279	0.108	0.276	0.083		0.414	
LDNe	478.1	149.9	∞	195.8	682.9	309.4	∞	∞	∞	396.033	8560.2	+2061.484
Lower C.I.	166.6	102.7	544.5	141.8	305.2	178.1	286.2	488.9	664.2	104.039	624.3	
Upper C.I.	∞	265.6	∞	308.8	∞	1050.3	∞	∞	∞		∞	
COL2-Ne	128	106	165	240	131	134	201	240	142	167.500	180	+7.462
Lower C.I.	85	69	104	136	90	85	120	146	92	21.961	126	
Upper C.I.	239	184	322	598	206	228	460	580	263		281	
$\% R \geq 0.12$	11.66	9.71	7.82	8.61	11.0	9.38	10.81	9.61	10.12	9.20 (0.587)	10.37	+12.686
Centrality	–	0.047	0.155	0.086	0.155	0.124	0.038	0.047	0.155		0.191	
In-degree	–	0	3	2	2	2	1	1	1		3	
Out-degree	–	1	1	0	3	1	0	0	4		5	

DNA was extracted using Nucleospin columns (Macherey-Nagel). We measured genetic diversity and structure employing microsatellite loci because their high polymorphism and mutation rate could help to detect recent small changes in genetic composition. Although microsatellites are assumed to be neutral, and conclusions from genes under selection could vary, they are useful to set a benchmark for which markers under selection could be compared (Sgrò et al., 2011). From 18 perfect tetranucleotide microsatellite loci we had previously characterized (Greenley et al. 2012), we excluded two loci (HCOR03 & HCOR72) that had previously shown a high frequency of null alleles (≥21.3%) (Greenley et al., 2012) and consistently deviated from Hardy–Weinberg equilibrium (HWE) among all populations according to a probability test in GENEPOP 4.2 (Raymond and Rousset, 1995) after correcting for multiple tests (Rice, 1989). The remaining 16 unlinked loci were amplified and genotyped in all individuals as described previously (Greenley et al., 2012). Missing data among loci ranged from 2.41% to 9.1%, with an average of 5.5% across all 16 loci. Individuals included in analyses were genotyped at an average of 15 loci, without any trend of missing data within particular loci. The minimum number of loci successfully genotyped within any single individual was 13. From 160 possible combinations of 16 loci and 10 populations, 52 instances showed evidence of significant HWE deviations (corrected P value ≤0.0003), without any trend within loci or populations.

2.3. Genetic diversity, demographic bottlenecks and Ne

To test the hypothesis that the marine reserve could help to conserve genetic diversity compared to sites where overfishing and restocking take place, we used GENALEX 6.501 (Peakall and Smouse, 2006) to calculate the average number of observed alleles, rare alleles (frequency ≤5%), private alleles unique to one population and observed and expected heterozygosity. To corroborate that estimates of allelic diversity were independent of any disparities in the number of individuals sampled at each site, we calculated the effective number of alleles and allelic richness using rarefaction and the lowest sample size observed ($N = 38$) with the software HP-Rare 1.1 (Kalinowski, 2005).

To test the hypothesis that population reductions caused by fishing, climatic events and restocking leave genetic signatures that might be absent from marine reserves, we used the BOTTLENECK approach (Piry et al., 1999), using a sign test (Luikart and Cournet, 1998), alpha = 0.05 and the step-wise mutation model, which represents the gain or loss of exactly one microsatellite repeat unit, using a Bayesian approach for the estimation of parameters in the coalescent process. The software searches for an excess of heterozygote individuals based on the observed allele frequencies, a tell-tale sign that a population recently fluctuated and rare alleles were lost faster than heterozygosity (Luikart and Cournet, 1998). We calculated Mratio (Garza and Williamson, 2001) to test for a sustained bottleneck via a significant reduction in the ratio of the number of microsatellite alleles that are lost by drift to the range in allele sizes (10,000 simulations under one-step mutations, 10% of multistep mutations of average size 3.5, ancestral Ne of 500 and mutation rate 0.0005).

We tested the hypothesis that marine reserves could assist in increasing the number of adults contributing to reproduction (*Ne*) compared to sites where fishing and restocking take place. We estimated *Ne* using two different methods to assess robustness of results. Firstly, we used LDNE (Waples and Do, 2008) to estimate *Ne* based on linkage disequilibrium data using a bias correction, random mating, parametric confidence intervals and excluding rare alleles with a frequency below 0.01. Secondly, we calculated *Ne* based on sibship assignments under random mating, estimated 95% confidence intervals (C.I.) employing a full likelihood method, high accuracy, long simulations and no-prior probabilities with COLONY2 (Wang, 2009).

2.4. Relatedness and sibships

We investigated the hypothesis that overfishing and restocking could increase the degree of genetic relatedness within populations compared to the marine reserve. Related individuals in natural populations have been observed in the presence of local larval retention or self-recruitment (Christie et al., 2010), or in populations subject to restocking (Lemer and Planes, 2012). We used GENALEX to estimate pairwise individual relatedness values (Queller and Goodnight, 1989) assuming no previous inbreeding, and employing population-specific allele frequencies. For each population, we calculated average pairwise values and tested for statistical significance with 1000 permutations and 1000 bootstraps to estimate 95% C.I. We used COLONY (Jones and Wang, 2010) to identify pairs of individuals with a probability ≥95% of being full-sibs and half-sibs within each population, allowing us to confirm if first or second-degree relatives, respectively, are present. To test if the observed sib proportions deviate significantly from a random-mating population, we simulated 500 unrelated individual pairs with COLONY using observed population-specific allele frequencies, estimated sibs as for the empirical data, and tested for statistical differences with a binomial chi-square test.

2.5. Gene flow and genetic structure

We tested the prediction that bottlenecks caused by overfishing, climatic events and restocking could decrease connectivity and increase the levels of genetic structure among populations. We used GENODIVE 2.0b25 (Meirmans and Van Tienderen, 2004) to calculate equilibrium estimates of the level of allelic fixation via pairwise F_{ST}. F_{ST} values can underestimate genetic differentiation for highly variable markers like microsatellites because the maximum F_{ST} value possible decreases as the within-population heterozygosity increases (Meirmans and Hedrick, 2011). We calculated an index of genetic differentiation D (Jost, 2008) that is not affected by within-population diversity. The significance of pairwise F_{ST} values was assessed with an AMOVA test and 1000 permutations in GENODIVE. To assess the statistical power of our 16 markers to detect low F_{ST} values (e.g., ≤0.01), we used POWSIM 4.0 (Ryman et al., 2006) and a Chi-square test to compare simulations for 10 populations, employing observed sample sizes and allele frequencies and 1000 replicates. We did an

Analysis of Molecular Variance (AMOVA) in GENODIVE considering variation within individuals, among individuals and among populations and testing significance using 999 permutations. To test if dispersal among populations was limited by the magnitude of the geographic distance separating them, we performed an analysis of isolation by distance using a mantel test in GENALEX (1000 permutations, 1000 bootstraps resampling).

Allelic diversity is expected to increase with the number of sources supplying larvae to a site (Kool et al., 2011). We tested the hypothesis that, compared to fished and restocked locations, the marine reserve could act as a key hub site for marine connectivity by receiving and exporting larvae to multiple sites. To search for evidence of recent gene flow and explore the role of each site as a source or sink of larvae, we identified first-generation migrants using a Bayesian assignment (Rannala and Mountain, 1997) implemented in GENECLASS2 (Piry et al., 2004). For each individual, we assessed the likelihood ratio *L*-home/*L*-max using Monte Carlo resampling (Paetkau et al., 2004) and simulating 10,000 individuals. Individuals with a probability $\leq$0.01 of belonging to the population where they were sampled were identified as migrants. First-generation migrants were represented in a spatial directed network using the sites as nodes and the migrant individuals as edges employing the software NODEXL (Smith et al., 2010). The importance of each population within the network was estimated as the principle eigenvector centrality to identify 'hub' sites that are strongly connected as sources and sinks (Watson et al., 2011). We calculated out-degree and in-degree, or the number of edges leaving and entering a node, respectively, and used a linear regression analysis to explore if allelic richness corrected by rarefaction could be explained by eigenvector centrality, out-degree or in-degree. To provide a biological interpretation of the levels of genetic structure and gene flow present in our dataset, we used two methods to estimate the number of migrants in our global dataset under the infinite island model. First, we estimated the number of migrants from the global F_{ST} estimate with the formula $F_{ST} = 1/(4Nm + 1)$ (Wright, 1951) and then with the private allele method after correcting for sample sizes (Barton and Slatkin, 1986), as implemented in GENEPOP (Raymond and Rousset, 1995).

We tested for the existence of distinct genetic clusters (K) among sampled populations using two Bayesian assignment methods with spatial information that have high power to detect low differentiation (*e.g.*, $F_{ST} < 0.03$) (Guillot, 2008; Hubisz et al., 2009). We used GENELAND 3.1.4 (Guillot et al., 2008) and individual geographic coordinates and conducted analyses at two spatial scales: one with all 10 sites included, and another excluding the remote population of San Diego to increase the focus on smaller scales around central Baja. For each analysis, we generated 20 independent runs of 1×10^6 Markov chain Monte Carlo (MCMC) iterations, sampling every 1000th. We used the spatial model with null alleles, without uncertainty in coordinates and correlated allele frequencies. We discarded the first 50% of the run as the burn-in period and sampled the posterior distribution to estimate K mode. For comparison, we used STRUCTURE 2.3.1 (Hubisz et al., 2009) to perform 10 STRUCTURE runs for $K = 1$–10, and 10 runs for $K = 1$–9 excluding San Diego. In each run, we included a burn-in of 5×10^5 MCMC repetitions followed by the same number of iterations to sample the posterior, assuming admixture, correlated allele frequencies, and employing sampling location as a prior probability in the analysis (Hubisz et al., 2009). To estimate the most likely K with STRUCTURE, we used the ΔK and ΔF_{ST} methods implemented in CORRSIEVE (Campana et al., 2011).

3. Results

3.1. Densities

Pink abalone densities in 2006, when the reserves were established, ranged from 0.029 ± 0.010 S.E. (standard error) ind/m^2 in the reserve, to 0.060 ± 0.027 S.E. ind/m^2 (Dulce) among the four sites surveyed (Fig. A1). In 2007, densities at the four sites ranged from 0.023 ± 0.006 S.E. ind/m^2 (Dulce) to 0.040 ± 0.007 S.E. (restocked). Similar values were recorded in 2008, with densities ranging from 0.032 ± 0.011 S.E. ind/m^2 (restocked) to 0.043 ± 0.011 S.E. ind/m^2 (reserve). Analyses indicated no significant differences in abalone densities among populations during 2006 (ANOVA $F = 0.62$, $P = 0.54$, d.f. $= 2$), 2007 (ANOVA $F = 0.83$, $P = 0.48$, d.f. $= 3$) or 2008 (ANOVA $F = 0.26$, $P = 0.85$, d.f. $= 3$). During the 2009 mortality event, all populations exhibited a sharp drop in densities ranging from −71% (Dulce) to −43% (Babencho). In 2009, densities ranged from 0.009 ± 0.004 S.E. ind/m^2 (Dulce) to 0.019 ± 0.006 S.E. ind/m^2 (Babencho). During the 2010 mortality event, the collapse continued at the two fished sites ($\leq$0.004 ind/m^2) and the restocked site ($0.010 \pm$ S.E. 0.002 ind/m^2), while the reserve maintained slightly higher densities ($0.015 \pm$ S.E. 0.004 ind/m^2). Despite these trends, no significant differences in abalone densities were found among populations during 2009 (ANOVA $F = 0.83$, $P = 0.31$, d.f. $= 3$) and 2010 (ANOVA $F = 1.91$, $P = 0.13$, d.f. $= 3$). In 2011, the reserve and the restocked site showed significantly higher densities ($0.019 \pm$ S.E. 0.005 and $0.015 \pm$ S.E. 0.003 ind/m^2, respectively, Fig. S1) with respect to fished sites ($<$0.005 ind/m^2) (ANOVA $F = 7.09$, $P < 0.001$, d.f. $= 3$).

3.2. Genetic diversity, demographic bottlenecks and Ne

Pink abalones among 10 sampled populations showed average levels of observed heterozygosity ranging from 0.674 (Bocana24) to 0.791 (restocked), and effective alleles ranging from 7.69 (San Diego) to 10.35 (Reserve and Morro) (Table 1), indicating variation in genetic diversity across the range of the species. Allelic diversity as assessed by rarefaction was lowest at San Diego (13.56) and largest within the reserve (16.31). Rare alleles ranged from 6.813 (San Diego) to 10.063 (reserve). Private alleles varied from 0.125 (restocked) to 0.375 (Morro & Abreojos). Compared to fished sites around Isla Natividad

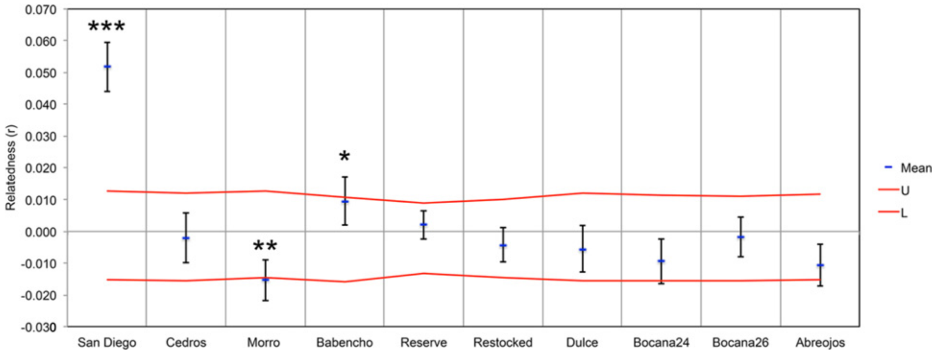

Fig. 2. Mean within population pairwise values of individual relatedness (r). Error bars represent 95% confidence intervals around r as estimated by bootstrap resampling. Upper (U) and lower (L) confidence limits bound the 95% confidence interval under random mating across sampled populations as determined by permutation. * $P = 0.040$, ** $P = 0.020$, *** $P = 0.001$.

(including restocked), the reserve showed a larger number of observed alleles and rare alleles (7.22% and 16.03% greater, respectively). After correcting for differences in sample size, the reserve had a larger number of effective alleles and greater allelic richness (an increase of 2.26% and 1.36%, respectively). In contrast, the reserve showed comparatively less private alleles and observed heterozygosity than the average detected at other sites around Isla Natividad (a decrease of 5.92% and 4.71%, respectively).

We detected a recent bottleneck at all sites in Mexico except within the reserve, and a prolonged bottleneck at San Diego. A significant excess of heterozygotes suggested a recent bottleneck in all fished populations including the restocked site (Sign-test all $P \leq 0.022$), but not within the reserve (Sign-test $P = 0.075$) or at San Diego (Sign-test $P = 0.177$). In contrast, a significant reduction in the ratio of alleles to allele range size consistent with a sustained bottleneck was detected only at San Diego (Mratio $= 0.776$, critical $M = 0.804$, $P = 0.019$), but not at any other site (M ratio range: 0.816 to 0.875, critical M range: 0.801 to 0.805, $P \geq 0.083$, Table 1).

A method used to estimate *Ne*, based on linkage disequilibrium, was unable to produce accurate calculations from the data on four fished populations (Morro, Bocana24, Bocana26 and Abreojos). In the remaining six populations, the lowest values were observed at Cedros (*Ne* = 149.9, 95% C.I. 102.7–265.6) and the largest in the reserve (*Ne* = 8560.2, 95% C.I. 624.3—infinitum) (Table 1). A distinct method based on sibship assignments also supported the lowest *Ne* at Cedros (*Ne* = 106, 95% C.I. 69–184) while the largest was found at Bocana26 and Babencho around Isla Natividad (*Ne* = 240). Overall, the reserve showed evidence of a larger *Ne* compared to the average observed at other sites around Isla Natividad (an increase of 2061.4% and 7.4% for the linkage disequilibrium and sibship methods, respectively, Table 1). Estimates of global *Ne* from all populations analyzed simultaneously ranged from an average of 6553.5 for the linkage disequilibrium method (C.I. 3411.9–64545.1) to 667 for the sibship method (C.I. 585–766).

3.3. Relatedness and sibships

Analyses of relatedness within sites (Fig. 2) showed individuals were significantly more related at San Diego ($r = 0.052$, Permutation test $P = 0.001$). Babencho showed some evidence of increased relatedness ($r = 0.01$, $P = 0.04$), while Morro displayed low average values ($r = -0.015$, $P = 0.02$). All other populations did not differ from random expectations (r range $= -0.011$ to 0.002, Permutation test all $P \geq 0.203$).

Simulations indicated that no first-degree relatives were expected under random mating in any of the populations (Fig. 3), despite a small percentage ($\leq$0.4%) being observed at four population sites (San Diego, Cedros, Babencho, Dulce). Babencho showed significantly less half sibs (0.25%) than predicted under random mating (1.81%, $X^2 = 10.47$, $P = 0.0012$). The restocked site showed significantly more second-degree relatives (2.12%) than expected from simulations (1.29%, $X^2 = 5.86$, $P = 0.0155$), supporting the presence of related individuals. All other comparisons were not significant (all $P \geq 0.145$). A detailed examination of the frequency distribution of individual pairwise values within sites (Fig. A2) revealed a bimodal distribution at San Diego, and the highest frequency of individuals with third-degree relatives or higher (11.66% with $R \geq 0.125$, Table 1), compared to the restocked site (11.0%), the marine reserve (10.37%) and the average observed among fished sites around Isla Natividad (9.20%). The two sites on the west side of Isla Natividad showed the lowest proportions of third-degree relatives (Morro = 7.82%, Babencho = 8.61).

3.4. Gene flow and genetic structure

A power analysis indicated our set of markers had a 100% chance to detect F_{ST} values as small as 0.005. For lower values ($F_{ST} = 0.002$ and 0.001), power decreased only slightly to 99.8% and 96.4%, respectively. Among all populations, the largest significant differences in allele frequencies were present between San Diego and all the other populations

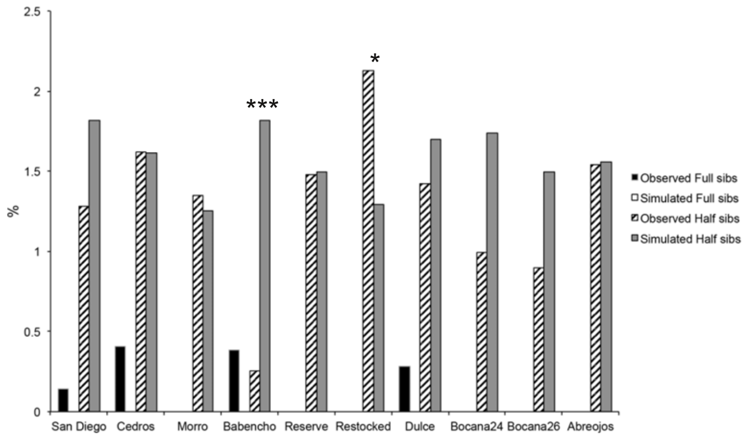

Fig. 3. Observed and simulated percentage of full-sibs and half-sibs identified within each population ($P \leq 0.05$) based on population-specific allele frequencies. *** $P = 0.001$, * $P = 0.015$.

Table 2
Genetic differentiation between populations. F_{ST} values (above diagonal) and Jost's D (below diagonal).

	San Diego	Cedros	Morro	Babencho	Reserve	Re-stocked	Dulce	Bocana24	Bocana26	Abreojos
San Diego	–	**0.012**[*]	**0.014**[*]	**0.014**[*]	**0.012**[*]	**0.013**[*]	**0.013**[*]	**0.011**[*]	**0.011**[*]	**0.013**[*]
Cedros	0.074	–	**0.003**	**0.005**	0	0.001	0.002	0.001	0.001	0.001
Morro	0.093	0.02	–	0.002	0.001	0.003	−0.001	−0.002	−0.002	−0.002
Babencho	0.088	0.033	0.012	–	**0.003**	**0.004**[*]	0.002	0.002	−0.001	0
Reserve	0.075	0.002	0.009	0.023	–	**0.004**[*]	−0.001	0	−0.002	−0.001
Re-stocked	0.081	0.005	0.02	0.026	0.028	–	0	−0.001	−0.001	0
Dulce	0.086	0.013	−0.005	0.016	−0.011	0.001	–	−0.001	−0.003	0.001
Bocana24	0.069	0.005	−0.017	0.017	0.002	−0.007	−0.008	–	−0.003	−0.001
Bocana26	0.07	0.006	−0.018	−0.009	−0.012	−0.006	−0.019	−0.022	–	−0.003
Abreojos	0.084	0.009	−0.015	−0.003	−0.005	0.002	0.007	−0.008	−0.02	–

Values in bold indicate $P < 0.05$.
[*] Shows significant differences in genotypic frequencies according to a Fisher exact test ($P \leq 0.0011$ after Bonferroni correction).

(F_{ST} range 0.011 to 0.014, Fisher exact test, $P < 0.001$ after Bonferroni correction) (D range 0.069 to 0.093) (Table 2). Differences in allele frequency were also present at Cedros when compared with two populations on the west side of Isla Natividad: Morro ($F_{ST} = 0.003$, $D = 0.02$) and Babencho ($F_{ST} = 0.005$, $D = 0.033$), although none of these were significant after correcting for multiple tests. Significant differentiation was also observed between the restocked site and the reserve ($F_{ST} = 0.004$, $D = 0.028$, Fisher exact test, $P < 0.001$) and between the restocked site and Babencho ($F_{ST} = 0.004$, $D = 0.026$, Fisher exact test, $P < 0.001$). All other pairwise comparisons were not significant, suggesting comparatively higher gene flow ($F_{ST} \leq 0.002$, $D \geq 0.017$). The range of values observed for the standardized measure ($D \leq 0.093$) supported that small but statistically significant differences could be biologically relevant (Jost, 2008). Similarly, the AMOVA showed small but significant differences among the 10 populations (0.2% of the variation, $F_{ST} = 0.002$, $P = 0.001$), compared to variation found within individuals (85.7%, $F_{IT} = 0.143$), and among individuals (14.1%, $F_{IS} = 0.141$, $P = 0.001$). Isolation by distance among all individuals was significant (Mantel test $P = 0.001$) but explained a small portion of the variance ($r^2 = 0.0148$) and was not significant after excluding San Diego ($P = 0.560$), indicating that geographic distance is an overall poor predictor of genetic differences in pink abalone.

We only identified 22 individuals as first-generation migrants (5.85% from all samples, excluding San Diego) ($P \leq 0.01$, Fig. 4). Symmetrical gene flow was only observed between the restocked site and the reserve on the east side of Isla Natividad, while all other connections were asymmetrical. According to the resulting network of migrants (Fig. 4), the reserve was identified as an important hub for connectivity among populations around the center of the peninsula (Eigenvector centrality $= 0.191$, In-degree $= 3$, Out-degree $= 5$) compared to the rest (Eigenvector centrality ≤ 0.155, Table 1). Linear regression analyses indicated allelic richness significantly increased at sites with high centralities ($R^2 = 0.613$, $P = 0.012$), and particularly at sites that receive larvae from multiple sources (i.e., higher in-degree) ($R^2 = 0.643$, $P = 0.009$, Fig. 4). Out-degree was not a significant predictor of allelic richness ($R^2 = 0.180$, $P = 0.253$). The global number of migrants supported by our data ranged from 124 based on the global F_{ST} method to 13.28 according to the private allele method (average frequency of private alleles = 0.0149). Based on global estimates of *Ne*, migrants likely represent less than 2% of the genetic effective size.

Two Bayesian assignment methods supported the presence of two genetic clusters separating San Diego from the rest of the populations from Mexico (Fig. 5, Fig. A3, Fig. A4). When San Diego was excluded, GENELAND further supported two clusters around the central portion of the peninsula, where Cedros was distinct from all other populations to the south.

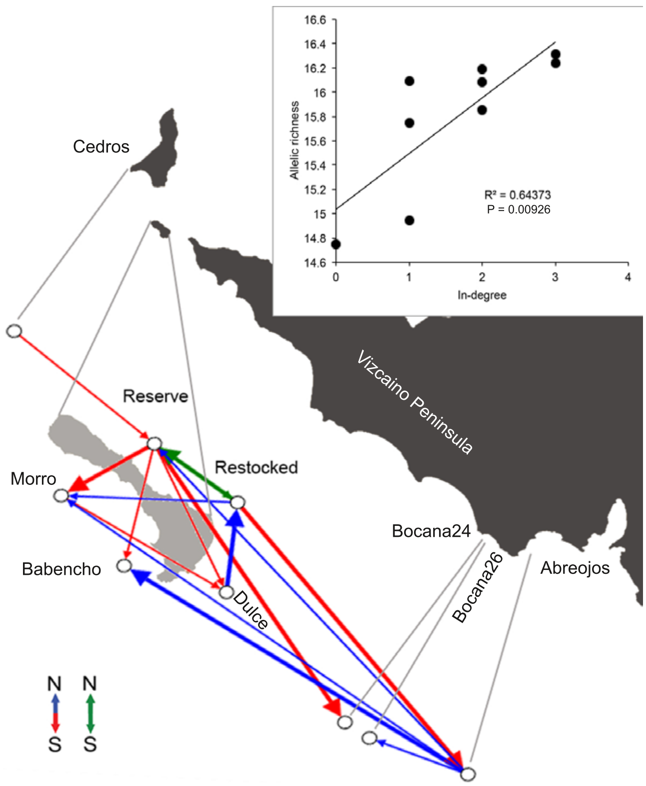

Fig. 4. Spatial directed network of first-generation migrants showing the location of each population in the central Baja California Peninsula (dark gray), with a zoom around Isla Natividad (light gray). Color-coded arrows indicate whether gene flow is asymmetrical (either North or South bound, respectively) or symmetrical. Thin lines represent a single migrant; bold lines indicate two migrants according to GENECLASS2. Insert graph shows a significant relationship between number of larval sources contributing to a site (in-degree) and allelic richness corrected by rarefaction. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Excluding San Diego, STRUCTURE suggested the presence of three clusters in central Baja California, but the first consisted of only three individuals (7.5%) from Babencho. The second cluster was present at Cedros, where 36% of individuals had assignment probabilities larger than 50%, including a few individuals from the restocked site and the reserve. The third cluster was prevalent at all sites but less frequent at Cedros.

4. Discussion

We were able to document some small but biologically relevant differences in the genetic composition of pink abalone populations subject to distinct management strategies and demographic trajectories, including populations open and closed to fishing, within recently established no-take marine reserves, and where larval restocking has taken place for a decade. Our study supports the hypothesis that management decisions can alter patterns of genetic diversity in relatively short periods, which could have implications for the evolutionary potential of valued marine resources and their long-term response to environmental change. Protection within a marine reserve increased allelic diversity for rare alleles and increased effective population size, compared to fished sites showing genetic bottlenecks. In particular, a sustained bottleneck and restocking both reduced allelic diversity and increased genetic differentiation and relatedness. Previous studies showed the small ($\sim$0.8 km^2) and recent (5 years) marine reserve can promote the recovery of abalone from a demographic bottleneck related to a hypoxic event by maintaining higher reproductive output and juvenile recruitment compared to fished sites (Micheli et al., 2012). Here we found that the reserve also shows higher allelic diversity compared to other fished sites around Isla Natividad. *In-situ* estimates of population density during the year the reserve was established (and the following year) were similar or even lower than at fished sites, indicating that the high levels of allelic diversity within the reserve were not associated with a healthy, dense population that predate the establishment of the reserve. On the contrary, these data suggest that higher allelic diversity might be due to the recovery of higher densities within the reserve. Although the lack of genetic samples before the reserve establishment prevents us from concluding a cause–effect relationship, several lines of evidence suggest a protective role within the reserve to conserve genetic diversity. Individual alleles evolve much more quickly than heterozygosity and are more sensitive to recent time frames (Luikart and Cournet, 1998). Rare alleles that are lost quickly during bottlenecks contributed significantly to diversity within the reserve, which lacked evidence of a recent genetic bottleneck. In contrast, the rest of the surveyed populations from Mexico exhibit evidence of recent bottlenecks.

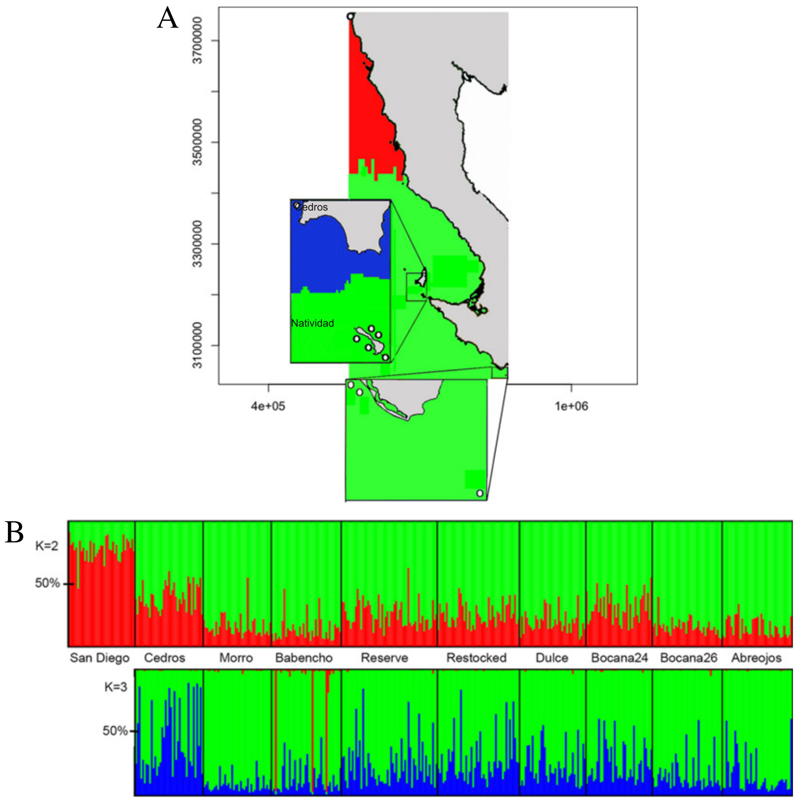

Fig. 5. Spatial Bayesian clustering results from GENELAND (A) and STRUCTURE (B). GENELAND results show the spatial distribution of distinct clusters (different colors) at two spatial scales: all populations, and all populations excluding San Diego (inserts). STRUCTURE output shows the probability (vertical axis) of individual membership to two clusters among all populations ($K = 2$, distinct colors, 414 individuals, upper panel) and excluding San Diego ($K = 3$, 376 individuals, lower panel). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

In line with our demographic surveys, the largest allelic diversity observed within the reserve was associated with a larger effective population size (*Ne)* when compared to other fished sites around Isla Natividad. The *Ne* estimates likely reflect the recent changes occurred after the reserve was established and the mortality event of 2009–2010. The linkage disequilibrium method depends on the effects of genetic drift on the generation that was sampled, such that the signal after the bottleneck was probably stronger than that of the pre-bottleneck (Waples, 2006). Estimates of full and half sibs from the sample also change quickly, for instance, if related individuals are removed by fishing or hypoxia. Larger allelic diversity within the reserve might be due to the presence of older animals, which are more abundant in the reserve (Micheli et al., 2012) and may provide additional cohorts not represented at fished sites leading to a better representation of allelic diversity. These older cohorts were likely recruited before reserve establishment but benefited from the protection from fisheries' activities. Based on the year the reserve was established (2006), the years when we sampled adults (2009–2010) and the fact that pink abalones reach sexual maturity at about 4 years of age, it is likely that our genetic sampling occurred too early to measure the positive effect that an increase in reproductive output within reserves had on maintaining local larval recruitment around Isla Natividad, as recorded using postlarvae collectors (Micheli et al., 2012; Rossetto et al., 2013).

Another mechanism that could help explain high allelic diversity within the reserve is its location, which appears to promote a role as a regional hub for larval dispersal. The network of migrant individuals showed the reserve as a sink of larvae coming from the two most distant populations sampled in central Baja California (Cedros and Abreojos), as well as from the nearby, restocked site. Only Morro, a population located on the west side of Isla Natividad and exposed to intense upwelling, showed similar evidence of migrants coming from three distinct sources, an observation aligned with high levels of allelic diversity observed at this location. The role of the reserve as a larval sink depends on the patterns of ocean circulation and is independent of the reserve establishment. However, the protection of cohorts within a reserve, even when their recruitment predates reserve establishment, has the potential to increase genetic diversity if the reserve is also a larval sink, compared to sites that are also larval sinks but where genetic variation contributed by migrant individuals is constantly removed by fishing. Unlike Morro, the reserve was identified as the most important source of larvae in our study (contributing to five other sites, four of them around Isla Natividad). Because adults from the commercial fishery were five to seven years old when sampled, these larval dispersal events also predate reserve establishment. However, this suggests that the reserve could act like a connectivity hub and capture and later export larvae to other distant sites (e.g., up to ∼200 km to the south), which could increase the adaptive potential of other populations and their chances to recover from perturbations.

It is important to highlight that the number of recruitment events could also explain genetic diversity within the reserve. As the number of recruitment events increases (even from few sources), genetic diversity could also increase.

The variation observed in patterns of genetic connectivity at different spatial scales suggest that local oceanographic patterns around central Baja California are more important than geographic distance in explaining genetic differences among nearby sites. For example, we found evidence that Cedros was relatively isolated from the rest of the sites, particularly from populations on the west side of Isla Natividad. Remarkably, no northward migration events were observed towards Cedros (In-degree $= 0$), and this site showed the lowest allelic diversity and *Ne* among the populations sampled in Mexico. This observation contrasts with the lack of genetic structure reported previously in this area for *H. corrugata* (Diaz-Viloria et al., 2009) and *H. fulgens* (Gutierrez-Gonzalez et al., 2007) using eight microsatellite markers or less. However, it is consistent with studies suggesting the channel between Cedros and Isla Natividad represents an oceanographic barrier where two coastal current systems converge. This oceanographic frontier is influenced by the presence of the Vizcaino Peninsula and by a large eddy that forms north of the Vizcaino Peninsula over the continental shelf, less than 100 m deep (Lynn, 1987; Diaz-Viloria et al., 2009). Other species with similar duration of pelagic larvae, including the wavy top snail (*Megastraea undosa*) and warty sea cucumber (*Parastichopus parvimensis*) show a genetic transition at the Vizcaino Peninsula (Haupt et al., 2013, submitted for publication). Additionally, we cannot discard the possibility that the genetic differentiation found at Cedros is also a consequence of recent population bottlenecks (either related to fisheries or climate). In contrast, high connectivity via larval dispersal seems present among some distant sites (e.g., Isla Natividad vs. La Bocana separated by ∼200 km). Larval dispersal at scales of several tens to a few hundred kilometers are reported in other abalone species based on oceanographic models (Miyake et al., 2010) and genetic studies (Park et al., 2012).

Genetic analyses of pink abalone from San Diego suggest a significant loss of genetic diversity consistent with the known history of collapse since the 1980s. San Diego showed the lowest levels of allelic diversity, particularly rare alleles, and the highest levels of relatedness. As this population has remained small for decades, it did not show evidence of a recent bottleneck, since the excess of heterozygosity after a bottleneck is detectable only for a short time, after which a new equilibrium between mutation and drift is reached (Luikart and Cournet, 1998). However, San Diego showed evidence of a sustained bottleneck supported by an Mratio that continued to decline (Garza and Williamson, 2001). We corroborated a previous study (Diaz-Viloria et al., 2009) showing that abalone from Southern California are genetically isolated from central Baja California. Given its geographic isolation, this population seems completely dependent upon self-recruitment compared to other sites, which can help explain the high frequency of related individuals. The fact that populations in southern California have failed to recover, highlights that self-recruitment might not be sufficient for the recovery of an isolated abalone population, and that larval dispersal from adjacent sites is key to maintaining genetic diversity and viability in the long-term. Under future climate change scenarios in the northern hemisphere, the local extinction of depleted populations with low genetic variation, such as San Diego, could be offset by range expansion (or translocation) of warm-adapted populations with high genetic variation to northern locations (Sgrò et al., 2011). This highlights that conserving genetic variation within reserves might benefit distant populations across the species range in the near future.

We found that the site where hatchery larvae and juveniles had been released over the last decade has a higher frequency of second-degree relatives and the largest proportion of third-degree relatives among all populations from Mexico. This is likely due to an unequal contribution of a few individuals used to produce larvae in the laboratory. Higher levels of relatedness among individuals could reduce fitness and further increase susceptibility to perturbations such as diseases (Keller and Waller, 2002; Roodt-Wilding, 2007). Other genetic signatures characteristic of the restocked population were the lowest proportion of private alleles and the largest observed heterozygosity, which could result from the artificial mixing of spawners taken from different populations, in each year of restocking. Unlike any other population south of Cedros, the restocked site also had small but significant F_{ST} values when compared to the adjacent reserve and at least one fishing site on the opposite side of Isla Natividad. The continuous release of larvae via restocking could have increased divergence in allele frequencies, which in extreme cases could cause outbreeding depression between hatchery and wild individuals (Roodt-Wilding, 2007; Lemer and Planes, 2012).

Our results suggest the location of the reserve at the boundary of two local oceanographic current systems is key in determining its potential role to preserve genetic diversity and supply larvae to other sites. A marine reserve network should include other important hubs of larval dispersal separated by tens to a few hundred kilometers to allow for larval connectivity. Compared to other species in California, our *Ne* estimates from *H. corrugata* (667–6553) were larger than the endangered white abalone *H. sorenseni* (census ∼2500, *Ne* near zero), similar to *H. fulgens*, which is listed as a species of concern (1100–3600), and several orders of magnitude lower than the more broadly distributed red abalone *H. rufescens* (350,000–3.5 million) (reviewed by Gruenthal et al., 2014). The relatively small *Ne* warns that moderate levels of genetic drift, selection and inbreeding driven by overfishing and restocking could have strong impacts on the genetic diversity of the species (Pinsky and Palumbi 2014).

4.1. *Conclusion*

Our study suggests that management decisions are capable of increasing or decreasing genetic diversity over relatively short time scales, highlighting that the fate of the genetic diversity of exploited species can depend on multiple management choices, sometimes with opposite effects. Such effects should be modeled and monitored over time and their collective impacts estimated, for example, through genetic monitoring (Schwartz et al., 2007), in order to know how to adapt management in order to maintain genetic diversity. If climate change acts in combination with overfishing and restocking to reduce local genetic diversity, then the evolutionary potential of populations to future selection pressures might be compromised in the long-term. Establishing a network of multiple small and interconnected reserves across the range of this and other depleted species could help to increase *Ne* and the resilience of natural populations, including conserving genetic diversity and promoting larval connectivity.

Acknowledgments

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Appendix A. Supplementary data

Supplementary material related to this article can be found online at http://dx.doi.org/10.1016/j.gecco.2015.07.005.

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