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Recruitment and recovery of pink abalone (*Haliotis corrugata*) in a historically overexploited kelp forest: Are local populations self-sustaining?

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ABSTRACT

After experiencing a reduction in density, many populations of benthic, broadcast spawning marine invertebrates have struggled to recover or have collapsed. Genetic techniques may help to distinguish populations that are self-sustaining from those at risk of further decline, and demographic interdependence among subpopulations. We tested the use of genetic data for identifying stable and self-sustaining abalone populations, as well as the efficacy of a restoration technique for use in those populations that are not. We created an artificial aggregation of wild adult pink abalone (*Haliotis corrugata*) in the Point Loma kelp forest near San Diego, CA, USA. We genetically analyzed those individuals and additional adults and juveniles in the broader region. A self-sustained population should not be demographically reliant upon immigration. Temporal variability in relatedness among juvenile cohorts, and a lack of fine-scale spatial structure in adult and juvenile relatedness indicated complex recruitment dynamics and/or long distance larval delivery. We estimated a low effective population size ($N_e = 188$) and a very low ratio of effective population to census population size ($N_e/N = 2.0 \times 10^{-3}$). These data are consistent with sweepstakes reproductive success, and suggest that the population is at risk of genetic diversity decline. Parentage assignment revealed that none of the juveniles sampled one year after aggregating adults had parents from this aggregation. Collectively, our results suggest that restoration efforts will need to achieve a greater density ($0.18\ m^{-2}$) and/or number of individuals (46) to improve local recruitment. Our results also suggest that the Point Loma kelp forest population of pink abalone is of insufficient density for long-term viability, may be reliant upon immigration, and cannot be defined as an independent "local" population unit.

1. Introduction

Many broadcast spawning marine invertebrates that are sessile or sedentary have experienced population collapse and failed to recover (Anderson et al., 2011; Hobday et al., 2000; Kalvass, 2000; Karpov et al., 2000; Peterson et al., 1996; Trimble et al., 2009). This is commonly attributed to reproductive Allee effects (spatial challenges to successful fertilization) that result from reduced density (Gascoigne and Lipcius, 2001). Pink abalone (*Haliotis corrugata*) abundance has declined precipitously throughout its range over the last 50 years (Rogers-Bennett, 2002), and the population has recovered little since the cessation of fishing in 1997. This suggests a low population growth rate at low density, and that recovery may require active restoration efforts. Successful recovery of abalone populations will require refinement of indicators for stable and self-sustaining local populations, as well as better understanding of the demographic interdependence among them.

Three ecological concepts are of high relevance to identifying self-sustaining abalone subpopulations. First, a population growth (λ) or replacement rate (R) equal to or greater than one is expected from a population that is stable or growing. Button (2008) estimated population growth rates for the pink abalone population in the Point Loma kelp forest off San Diego, CA to be 0.72–1.06 yr^{-1}, under an assumption of low fertilization success in a low density population. These growth rates suggest that the population may be stable or slowly declining. A growth rate consistently above one would be required to achieve recovery to a less vulnerable density level closer to those observed prior to over harvest.

Second, a self-sustained population should not be reliant upon immigration. Evidence of immigration to or emigration from a local population can aid in distinguishing when R is stable in isolation. In marine systems, the difficulty of observing larval dispersal often leads to reliance on genetic estimates of gene flow as a surrogate for individual movement. However, accuracy of estimating dispersal from gene flow can vary widely among species and analytic methods (Bohonak, 1999) and results vary for a variety of reasons among abalone species and populations. Many abalone species show low levels of genetic differentiation

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among populations (Gruenthal et al., 2007; Piggott et al., 2008; Will et al., 2011; Withler et al., 2003). However, significant small-scale structure does indicate low connectivity in some cases (Gruenthal and Burton, 2008; Temby et al., 2007). Apparent genetic panmixia can be a relict of past connectivity in such long-lived species when population declines are relatively recent. But even under a long-term equilibrium between drift and gene flow, only a small number of interpopulation dispersers each generation can homogenize allele frequencies. Therefore the addition of parentage analysis, intensive fine-scale sampling, and manipulative field experiments to frequency-based analyses of gene flow can provide a more complete picture of marine dispersal dynamics (Christie et al., 2010a; Selkoe et al., 2008, 2010).

Third, estimates of the minimum spawning density (MSD), below which populations are at risk of recruitment failure, are important for broadcast spawning marine invertebrates. Based on studies of recruitment failure in south Australian abalone (Shepherd and Brown, 1993) and the densities of red abalone in southern California before population declines (Karpov et al., 1998; Tegner et al., 1989), the California Department of Fish and Wildlife's Abalone Management and Recovery Plan suggests that a MSD of 0.2 m^{-2} is required for successful reproduction. This estimate is in need of refinement for application to other abalone species, like pink abalone, and improved understanding of the vulnerability of different density levels. Stock recruitment relationships are notoriously variable and particularly difficult to determine at low stock levels. Genetic tools may be applied to indicate the vulnerability of low density populations to further decline. For example, genetic estimates of effective population size (N_e) can describe a populations risk for genetic diversity loss. Genetic parentage assignment could also be used to directly measure the reproductive output of groups of adults at specific density levels.

The numerical and spatial scale of restoration or stock enhancement in abalone should be guided by these three concepts in order to assure that populations are self-sustaining indefinitely. In our study, we created an artificial aggregation of wild adult pink abalone in the Point Loma kelp forest near San Diego, CA, USA. We genetically analyzed those individuals and additional adults and juveniles in the broader San Diego region. These data were used to 1) identify reproductive output from the aggregation through parentage analysis and thereby test the efficacy of our methods for stock enhancement, 2) estimate the effective population size within the broader Point Loma population and it's risk for loss of genetic diversity at current densities, and 3) assess dispersal and the spatial definition of "local" through analysis of relatedness among juvenile cohorts and local adults and analysis of adult population structure using finely detailed location information.

2. Methods

2.1. Study site and artificial aggregation

Our study was conducted in the Point Loma kelp forest near San Diego, California, USA (Fig. 1) in typical pink abalone habitat consisting of giant kelp (*Macrocystis pyrifera*), understory kelps, and boulders on bedrock of claystone with shallow ledges undercutting the bottom. An artificial aggregation of wild pink abalone was created in September 2009, a time of year typically characterized by high water temperatures when abalone ripeness is near its highest level along the southern California and Baja California coastline (Button, 2008; Guzman-del Proo et al., 2000). The aggregation site was designated as a circle with a 9 m radius, creating a 254 m^2 area (Fig. 1). This area contained 23 adult pink abalone already residing in the site, representing a density of 0.09 abalone m^{-2}. On September 23, 2009, the 23 resident abalone were removed using an abalone iron, tagged, measured for shell length, sexed by visual inspection of the gonad, and tissue sampled (method described below) in situ before being returned to their initial position within 5 min. Tags consisted of both visual markers and acoustic transmitters for the purposes of a concurrent telemetry study (Coates et al., 2013). On the following day we tagged, measured, and sexed an additional 23 pink abalone from an area approximately 2.2 km to the north within the same kelp forest. We immediately placed these individuals haphazardly throughout the artificial aggregation in groups of

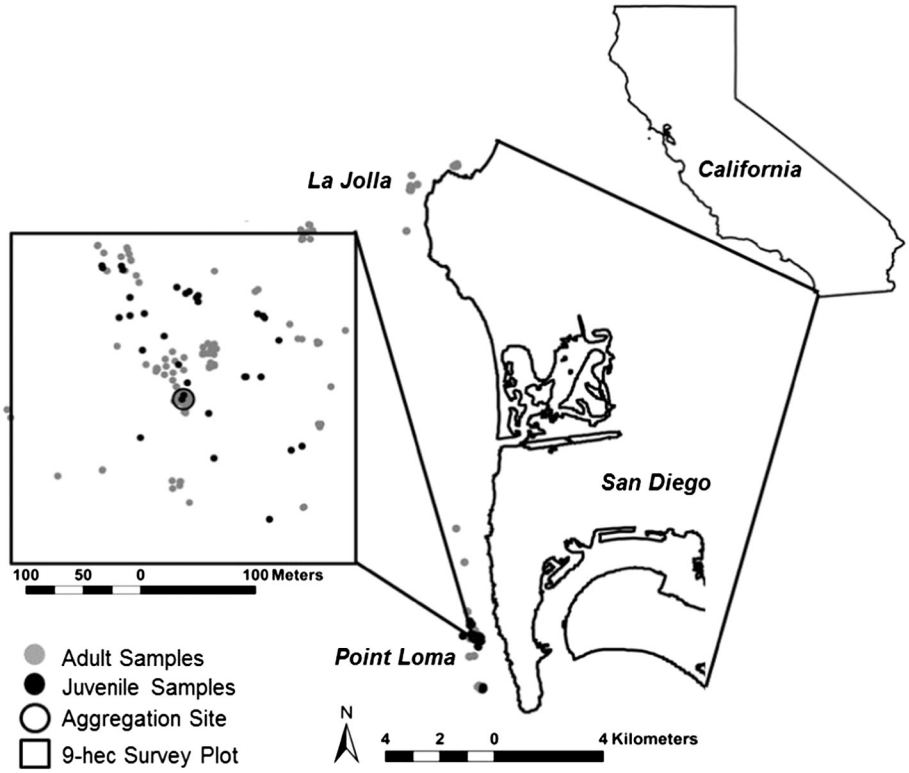

Fig. 1. Study location showing aggregation area, survey plot, and locations of tissue sampled individuals.

two to six, thereby creating a final density within the site of 0.18 m^2, close to the theoretical minimum spawning density required to sustain a viable population (0.2 m^{-2}) (California Department of Fish and Game, M.R., 2002).

2.2. Tissue sampling and survey methods

Tissue samples were collected from 386 individuals, consisting of one or two epipodial tentacles from each abalone. This is a standard method for abalone studies and is considered to be non-lethal and minimally disruptive (Slabbert and Roodt-Wilding, 2006). With the exception of juveniles and members of the aggregation, samples were taken without removing abalone from the substrate. Samples were immediately placed in 100% ethanol and stored at −80° until extraction.

During summer 2008, we collected tissue samples broadly throughout both the Point Loma and La Jolla kelp forests during haphazard searches for abalone. Most samples were taken from adults, but a few juveniles were opportunistically sampled as they were encountered (Table 1). We mapped the locations of samples either precisely by taking GPS positions on floats released by divers, or approximately within the trajectory of GPS positions taken at the beginning and end of dives. Detailed notes were taken on whether sampled abalone were alone or in groups with other sampled or unsampled individuals. Following sampling, divers marked each abalone with crayon (which is visible for a few days). New regions of the kelp forest were visited on each sampling date and pink abalone yearly movements are typically on a smaller spatial scale than these regions (Coates et al., 2013). Thus, we consider resampling to be very unlikely.

During summer 2009, we performed surveys to characterize the regional density and aggregation state of pink abalone in Point Loma. We conducted 20 belt transect surveys (4 m × 30 m) and 21 nearest neighbor distance surveys at randomly generated coordinates within a 9-hectare plot centered on the aggregation site. Across 19 of 21 surveys on which abalone could be found, populations were at low but aggregated densities (per-transect mean of 0.041 abalone $m^{-2} \pm 0.033$ SD; mean nearest neighbor distance of 3.5 m ± 4.1 SD). Abalone were tissue sampled during these surveys as well as during haphazard surveys similar to 2008, with effort focused within the 9-hectare plot.

During summer 2010 (one year following formation of the artificial aggregation), tissue sampling focused on juvenile abalone (<25 mm shell length (SL)), which would have been spawned within the past year and thus could have been offspring of the aggregated adults. Juveniles were located by overturning boulders of a size that is heavy but manageable for a diver. Approximately 40 diver hours were spent searching for juveniles within the 9-hectare area, and we spent another 40 h searching for juveniles outside of this area. When a juvenile outside the 9-hectare plot was located, we closely surveyed its surroundings and sampled all juveniles and adults in the immediate area. This was intended to produce even sampling effort for co-located adults and juveniles throughout the forest and to capitalize on the aggregated nature of the abalone.

Table 1
Number of tissue samples collected in different sampling years and locations.

Year	Stage	Point Loma			La Jolla	Total
		Aggregation site	9-hectare block	Other		
2008	Adult			67	34	101
	Juvenile			14		14
2009	Adult	49	96			145
2010	Adult			34		34
	Juvenile	3	30	33		66

2.3. DNA extraction, species identification and microsatellite typing

DNA was extracted from tentacle clippings using the DNeasy Blood and Tissue Kit (QIAGEN, Valencia, California, USA). All juvenile samples and a subset of 20 adult samples were amplified at the mtCOI gene using abalone-specific primers and PCR protocols from Metz et al. (1998). PCR product was cleaned using MO BIO Laboratories, Inc. UltraClean™ PCR Clean-up DNA Purification Kit and sent for sequencing to Genewiz, Inc. (La Jolla, California, USA). Sequences were compared to those published for abalone species on the west coast of the United States in Genbank to confirm species identity (Aguilera-Munoz et al., 2009, GenBank Direct Submission AY679080.1; Gruenthal and Burton, 2005).

A total of 17 microsatellite loci were tested for reliable amplification of product, compatibility in multiplexed reactions, and polymorphism. Nine were isolated from pink abalone (Hco15, Hco16, Hco19, Hco22, Hco47, Hco97, Hco194) or cross-amplified in pink abalone (Hka3, Hka56) by Diaz-Viloria et al. (2008). Another locus was isolated from *Haliotis discus hannai* (ca481) by Sun et al. (2007). Of three loci isolated from *Haliotis fulgens* and cross-amplified in pink abalone by Cruz et al. (2005), one (Hful603) did not amplify under any attempted conditions and two (Hful369 and Hful910) were found to be monomorphic. Four more loci were isolated from pink abalone (HCOR1, HCOR7, HCOR18, HCOR75) by Greenley et al. (2011). Therefore 14 loci were analyzed for all individuals after preliminary protocol development. Two multiplex PCR reactions were optimized for, 1) Hco22, Hco194, ca481, and 2) Hka3, Hco15, Hco16, Hco97, using the QIAGEN® Multiplex PCR Kit in a volume of 25 uL containing 12.5 uL master mix, 2.5 uL primer mix, 2 uL Q solution, and 2 uL DNA. PCR thermal conditions for these multiplex reactions were 15 min at 95 °C, followed by 32 cycles of 30 s at 94 °C, 30 s at 54°, and 30 s at 72 °C, and a final extension of 30 min at 60 °C. Three loci did not multiplex well at the PCR stage and required unique annealing temperatures (Hco19 at 65°, Hco47 at 57°, Hka56 at 51°). These PCR reactions were carried out in a volume of 20 uL containing 2 uL DNA, 0.75 uM of each primer, 0.2 mM dNTP, 1× Taq buffer (Invitrogen), 2 mM MgCl2, 0.1× BSA, and 0.09 U/uL Platinum Taq polymerase (Invitrogen). PCR thermal conditions for the three individual locus reactions were 2 min at 95 °C, followed by 32 cycles of 30 s at 94 °C, 30 s at the specific annealing temperature, and 30 s at 72 °C, and a final extension of 2 min at 72 °C. These loci were later pooled into a single sample for genotyping. Finally, another multiplex reaction (HCOR1, HCOR7, HCOR18, HCOR75) used the same QIAGEN Multiplex Kit reaction cocktail but a touchdown thermal protocol consisting of 94 °C for 5 min, 15 cycles of 94 °C for 30 s, 65–50 °C for 30 s (1 °C decrease each cycle), 72 °C for 30 s, followed by 40 cycles at 94 °C for 30 s, 55 °C for 30 s, 72 °C for 30 s, and a final extension of 72 °C for 5 min. Genotyping was performed on an *ABI* PRISM® 310 Genetic Analyzer at the SDSU Microchemical Core Facility. Chromatographs were scored automatically by GeneMapper® Software v4.0 and reviewed manually.

2.4. Data quality

PCR and genotyping were repeated for all samples with questionable allele calls and instances of single alleles for each locus. To assess error rates, a minimum of 20 randomly selected samples were also repeated through the PCR and genotyping stages at each locus, compared with original allele calls, and discrepancies were categorized as either allelic dropout or false alleles. The locus Hco47 was discarded prior to testing repeat samples due to a high proportion of samples producing no product or questionable product.

Data were tested for departure from expected Hardy–Weinberg genotype proportions (commonly called Hardy–Weinberg equilibrium: HWE) by locus across all samples and for separate groups consisting of 1) all adults from Point Loma regardless of collection year, 2) all adults from La Jolla, 3) juveniles from 2008, and 4) juveniles from 2010. HWE tests were performed using Arlequin v. 3.5 with 1,000,000

steps in the Markov chain and 100,000 dememorization steps (Excoffier and Lischer, 2010). Arlequin was also used to assess linkage disequilibrium for all samples with 10,000 permutations.

2.5. Parentage

We sought to identify parents of 58 juveniles collected in 2010 that were below 30 mm length using a method described by Christie (2010). The method is an R script which first defines a multi-locus exclusion probability based on Mendelian inheritance (i.e., the probability of two individuals sharing an allele at both gene copies for all loci). When multiplied by the number of pairwise comparisons, this probability gives the expected number of false parent–offspring pairs in the dataset. This expected number, and the genotypes of any actual putative pairs that could be true relatives, are used in a Bayesian framework to estimate the probability that each putative pair is true. Unlike the more commonly used Cervus parentage assignment software package, Christie's method does not require an estimate of the potential parent census population size, or the proportion of that population sampled. Therefore, results are not influenced by error in demographic estimates, making this method ideal for large wild populations in which only a small proportion of potential parents can be sampled. However, we confirmed our results using the categorical allocation likelihood approach implemented in Cervus v. 3.0 (Kalinowski et al., 2007).

Because Cervus does not accommodate large populations, we were required to make additional assumptions and procedural changes to accommodate our data (Kalinowski pers. comm.). We limited the potential parent pool to only those adults within the 9-hectare plot in Point Loma where transect surveys were performed and thus density information was reliable. This census population, estimated to be 3675 individuals, fell below the Cervus population size limit. Recruits may originate from areas outside the survey plot. However, including a wider potential parent pool encompassing all of the Point Loma and/or La Jolla kelp forests would require extrapolating our survey densities to a much broader spatial area that may not contain similar densities. This would also require using a relatively crude estimate of habitat area. Across all sampling years, a total of 147 adult samples were collected within the 9-hectare plot. The Cervus analysis was run as a single parent or "maternity" test because the sex of most of those individuals was unknown (number of candidate "mothers" was set to 3675; proportion of candidate mothers sampled was set to 4%). Simulations to calculate likelihood score critical values were run once using allele frequencies generated from all adults sampled throughout Point Loma and La Jolla, and a second time using only adults within the 9-hectare plot.

2.6. Effective population size in Point Loma kelp forest

We used both genetic (GoNe and Colony) and demographic (AgeNe) methods to estimate effective population size (N_e) for the Point Loma kelp forest (Coombs et al., 2012; Jones and Wang, 2010; Waples et al., 2011). GoNe and AgeNe require age-specific survival and birth rate data. Button and Rogers-Bennett (2011) reported parameters for a Richards growth function derived from mark-recapture data on pink abalone from the Point Loma kelp forest. We used these parameters to iteratively determine size at age up to 190 mm and 40 yrs. We then applied size stage-based survival and fecundities derived by Button (2008) to those ages. We assumed that mean survival and fecundity within a stage could apply to each size/age within that stage, and that parameters were equal for males and females (Table 2). This assumption is supported by the finding that sub-adult and adult male and female survival rates are not significantly different and do not change through time (Button and Rogers-Bennett, 2011).

GoNe v. 1.02 uses the temporal method for estimating N_e based on genetic drift (Coombs et al., 2012). We estimated drift with two separate analyses, first by comparing juveniles collected in 2008 with the first juvenile cohort of 2010, and second a replicate analysis comparing juveniles from 2008 and the second cohort of 2010. These two 2010 juvenile subcohorts were identified by separate size frequency modes (see Juvenile species identification and size distribution section). We truncated the size range of 2008 juveniles to 22–39 mm so that these individuals could reasonably be considered the product of one spawning event. Although our sampling scheme was not truly random, extensive preliminary analyses found no evidence for spatial autocorrelation in juvenile relatedness, even at the smallest spatial scale (see Results section). The program uses the Jorde and Ryman (1995) method to correct for the effect of overlapping generations, using age-based survival and fecundity data. This correction method is considered to be robust to uncertainty in the vital rate data (Jorde and Ryman, 1995). Other parameters used in GoNe were 40 age classes, 100 iterations for "C", Plan II sampling, 3 cohorts, and equal birth rate between the sexes.

Table 2
Vital rate parameters for GoNe and AgeNe analyses. These analyses require age-based survival & fecundity estimates. A matrix of 41 ages was used for both programs.

Size (mm)	Age (yrs)	Survival	Fecundity
0.01–51.02	0–3	0.51	0
66.58–102.43	4–8	0.51	0.1
109.56–153.41	9–18	0.77	0.84
156.66–189.64	19–39	0.77	1.625
190.36	40	0	1.625

Colony implements a single sample sibship method to calculate N_e (Wang, 2009). When this method is applied to samples from a single cohort, the result is an estimate of the effective number of breeders (N_b) rather than N_e. We considered only juveniles within the same size frequency mode or subcohort of 2010 to be likely siblings, and therefore ran separate Colony analyses using genotype data from each subcohort, yielding two estimates of N_b.

AgeNe calculates N_e, N_b and the ratio of N_e to census population size (N) in age structured populations (Waples et al., 2011). It requires only demographic information and is based on the assumption that age structure within a population will produce variance in reproductive success among individuals, and this will in turn affect N_e (Waples et al., 2011). We input the same age-based survival and fecundity data to AgeNe that was used for GoNe. Because AgeNe assumes that population size is at equilibrium, relative values among ages and sexes are more important than absolute values. We assumed an equal sex ratio, and a yearly cohort size (N1) of 618,000. This value of N1 was chosen because it led AgeNe to estimate an adult census population size N that matches our field estimate of N for Point Loma. The final parameter required by AgeNe is a Poisson scaling factor (α) that characterizes the variance to mean ratio of individual reproductive success. A value of $\alpha = 1$ equates to random variance in reproductive success among individuals of a given age, and $\alpha > 1$ produces over-dispersed variance. We analyzed the sensitivity of AgeNe output parameters to a range of α and juvenile survival values.

2.7. Cohort relatedness

We examined the pairwise relatedness of individuals within juvenile subcohorts from 2010 and between those subcohorts and local adults from the Point Loma kelp forest using Coancestry v. 1.0.1.0 (Wang, 2011). Using multilocus genotype data and locus-specific error rates, we calculated Queller and Goodnight relatedness values and confidence intervals for those values. We tested whether 1) within-cohort relatedness differed between the two juvenile subcohorts, and 2) pairwise relatedness between the first juvenile subcohort and local adults differed from pairwise relatedness between the second juvenile subcohort and local adults. Statistical significance was assessed using 1000 bootstraps in each analysis.

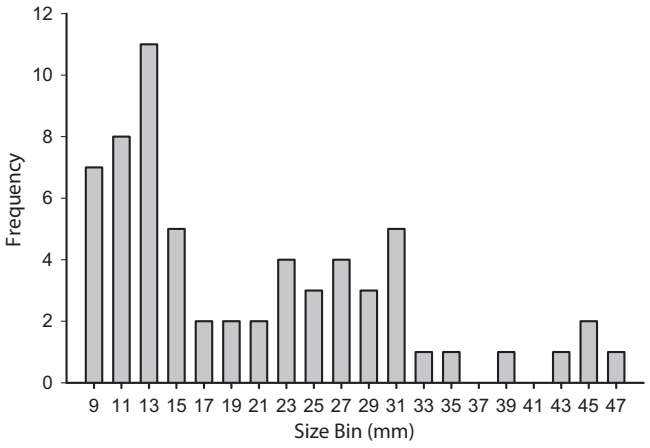

Fig. 2. Size frequency distribution of juvenile pink abalone collected in 2010.

2.8. Diversity and population structure

Standard genetic diversity indices (number of alleles, expected heterozygosity, observed heterozygosity) were estimated by Arlequin for all individuals, and for five groups (Point Loma adults, La Jolla adults, 2008 juveniles, 2010 juvenile cohort A, 2010 juvenile cohort B). An exact test of sample differentiation based on genotype frequencies was performed on these groups in a global test (30,000 Markov steps) and between all group pairs (100,000 Markov steps) using Arlequin. We assessed spatial autocorrelation in relatedness between all pairs of individuals within 500 m distance classes using GenAlEx v. 6.4. Spatial autocorrelation was also assessed separately for each of the five groups using 25 m distance classes.

3. Results

3.1. Juvenile species identification and size distribution

Of the 16 juvenile samples collected in 2008, 14 mtCOI sequences closely matched pink abalone sequences in our reference set of adults. One sequence was most similar to *Haliotis assimilis* and another to *Haliotis rufescens*. Of the total 90 juvenile samples collected in 2010, 66 sequences aligned with pink abalone. The other 24 sequences were made up of 15 *H. rufescens*, three *H. assimilis*, two *H. fulgens*, and four unassigned.

Juvenile sizes ranged from 19–44 and 9–47 mm SL in 2008 and 2010 respectively (n = 16. 66). The 2010 size frequency distribution showed two modes, indicating two separate spawning events (Fig. 2). We considered these modes to include individuals from 9–19 mm SL (mean 12 mm) and 21–35 mm (mean 27 mm) SL, respectively. Button (2008) reported the mean annual growth increment for abalone reared in the laboratory from settlement as 22 mm ($\sigma = 2.3$ mm). This suggests that juveniles within the smaller subcohort were spawned in the winter of 2009/2010 approximately 6 months prior to sampling, while the larger subcohort were spawned in late spring or early summer 2009 a year or more before sampling.

3.2. Analysis of Hardy–Weinberg genotype frequencies and diversity

For 10 of the 13 loci, there were relatively few departures from HWE, F_{IS} values were generally close to zero, and statistically significant departures were not concentrated in one particular group or gene (Table 3). When all samples were combined, F_{IS} values ranged from −0.068 to 0.073 (Table 3). One locus (COR1) was found to be significantly out of HWE in all sample groups. Additionally the loci Hka 56 and Hco194 were highly significantly out of HWE when all samples were grouped together and in one other sample group. These three loci were excluded, leaving a total of 10 microsatellite loci for all further analyses. Mean genetic diversity across all 10 loci and all samples combined was 20.23 for N_a, 0.65 for H_o, and 0.69 for H_e (Table 4). Mean observed and expected heterozygosity was similar among all sample groups except for juveniles collected in 2008, likely due to low sample size in that group.

3.3. Parentage

The Christie method for parentage assignment did not identify any parent–offspring relationships with a reasonable probability of being true. The Bayesian analysis identified three putative matches and each had a probability of being false equal to 1. The probability of any pair of individuals sharing an allele at each locus in their genotypes (Pr(δ)) was 0.00011. This is analogous to a probably of non-exclusion calculated with other methods, or the probability that a false parent cannot be excluded due to low data information content. Thus, we have high confidence that no parents of our 58 sampled juveniles from 2010 are in the set of 280 adults collected in 2008–2010. No reproductive benefit to the artificial aggregation was obvious in the local area, and no parent–offspring matches in the wider kelp forest could be used as a direct indicator of dispersal distance. Qualitatively similar results were obtained from Cervus (see Methods section).

3.4. Effective population size

From the genetic data, effective population size in the Point Loma kelp forest was estimated to be 188 individuals [95% CI: 146, 235]

Table 3
Significance levels of Hardy Weinberg Equilibrium tests and F_{IS} values per locus for all samples combined and separate sample groups.

Name	All (N = 360)			Pt Loma Adults (N = 246)			La Jolla Adults (N = 34)			Juveniles 2008 (N = 14)			Juveniles 2010 Cohort A (N = 35)			Juveniles 2010 Cohort B (N = 23)		
	p-val	S.E.	F_{IS} (W&C)	p-val	S.E.	F_{IS} (W&C)	p-val	S.E.	F_{IS} (W&C)	p-val	S.E.	F_{IS} (W&C)	p-val	S.E.	F_{IS} (W&C)	p-val	S.E.	F_{IS} (W&C)
Hka3	0.403	0.048	0.000	0.468	0.050	0.000	0.522	0.049	0.008	1.000	0.000	−0.031	0.152	0.000	0.025	1.000	0.000	−0.022
Hco15	0.141	0.029	−0.018	0.217	0.030	−0.039	0.349	0.015	−0.010	0.128	0.000	−0.130	**0.048**	0.000	0.165	0.871	0.000	0.079
Hco16	0.515	0.018	0.031	0.157	0.009	0.054	0.812	0.0130	0.005	0.554	0.000	−0.175	0.575	0.001	0.111	0.902	0.000	−0.095
Hco19	**0.005**	0.005	0.073	**0.000**	0.000	0.100	0.077	0.017	0.040	0.489	0.000	0.055	0.965	0.000	0.017	0.122	0.000	−0.029
Hco22	0.502	0.013	−0.018	0.323	0.012	0.041	0.992	0.001	−0.081	0.125	0.000	−0.079	0.174	0.000	−0.269	0.653	0.000	−0.173
Hco194	0.627	0.002	−0.062	0.610	0.002	−0.063	1.000	0.000	−0.049	1.000	0.000	−0.040	1.000	0.000	−0.030	1.000	0.000	−0.073
ca481	0.467	0.017	−0.002	0.514	0.012	0.031	0.183	0.007	−0.184	0.911	0.000	0.158	0.645	0.000	0.143	0.111	0.000	−0.310
HCOR7	0.182	0.028	0.030	0.518	0.040	0.030	0.722	0.037	−0.015	0.563	0.000	0.082	0.696	0.000	0.037	0.054	0.000	0.051
HCOR18	0.204	0.031	0.013	0.318	0.032	0.016	0.291	0.031	−0.028	0.939	0.000	−0.046	0.170	0.000	0.081	0.556	0.000	0.039
HCOR75	**0.012**	0.005	0.019	0.466	0.037	0.007	0.320	0.031	−0.001	0.433	0.000	0.063	**0.029**	0.000	0.112	0.364	0.000	0.029
Hka56	**0.000**	0.000	0.151	0.209	0.013	0.120	**0.005**	0.001	0.321	0.078	0.000	0.204	0.485	0.000	0.125	1.000	0.000	0.000
Hco97	**0.000**	0.000	0.136	**0.000**	0.000	0.153	0.600	0.002	0.115	1.000	0.000	-	0.492	0.001	0.174	1.000	0.000	−0.189
HCOR1	**0.000**	0.000	0.307	**0.000**	0.0000	0.312	**0.007**	0.003	0.195	**0.000**	0.000	0.529	**0.014**	0.000	0.252	**0.001**	0.000	0.338

Values in bold are significant to the α= 0.05 level.

Table 4
Diversity indices per locus including number of alleles (N_a), observed heterozygosity (H_o) and expected heterozygosity (H_e), shown for all samples combined and separate sample groups.

Name	All (N = 360)			Pt Loma adults (N = 246)			La Jolla adults (N = 34)			Juveniles 2008 (N = 14)			Juveniles 2010 Cohort A (N = 35)			Juveniles 2010 Cohort B (N = 23)		
	N_a	H_o	H_e	N_a	H_o	H_e	N_a	H_o	H_e	N_a	H_o	H_e	N_a	H_o	H_e	N_a	H_o	H_e
Hka3	90	0.975	0.975	83	0.975	0.976	40	0.971	0.978	21	1.000	0.971	38	0.941	0.965	28	1.000	0.979
Hco15	17	0.649	0.637	15	0.671	0.646	6	0.647	0.640	5	0.571	0.508	9	0.514	0.614	8	0.609	0.660
Hco16	6	0.557	0.575	5	0.545	0.576	6	0.529	0.532	3	0.571	0.489	5	0.514	0.578	5	0.696	0.637
Hco19	24	0.831	0.896	21	0.806	0.895	19	0.882	0.919	12	0.857	0.905	13	0.882	0.897	13	0.913	0.888
Hco22	5	0.722	0.710	5	0.683	0.712	5	0.794	0.735	4	0.714	0.664	5	0.914	0.723	5	0.783	0.700
Hco194	2	0.120	0.113	2	0.122	0.115	2	0.121	0.116	2	0.143	0.138	2	0.086	0.083	2	0.174	0.162
ca481	4	0.641	0.639	3	0.616	0.636	4	0.735	0.623	3	0.571	0.675	4	0.571	0.665	4	0.826	0.635
HCOR7	32	0.913	0.942	30	0.910	0.938	24	0.970	0.956	12	0.857	0.931	23	0.914	0.949	21	0.913	0.961
HCOR18	23	0.877	0.889	19	0.873	0.888	16	0.912	0.888	13	0.929	0.889	16	0.829	0.901	13	0.870	0.904
HCOR75	24	0.916	0.934	24	0.927	0.933	17	0.941	0.940	11	0.857	0.913	15	0.829	0.932	16	0.905	0.931
Mean	20.23	0.648	0.686	18.62	0.644	0.687	12.39	0.679	0.693	7.85	0.599	0.639	13.00	0.700	0.731	11.50	0.769	0.743

using GoNe and all loci combined. The generation time was estimated to be 10.1 years, close to the mean age at maturity of 8.2 yr for male and 8.7 yr for female pink abalone (Button and Rogers-Bennett, 2011). This results in a N_e/N ratio of approximately 2.0×10^{-3} for the Point Loma pink abalone population and 1.4×10^{-3} if La Jolla adults are considered potential parents as well. Colony estimated N_b for the two juvenile subcohorts of 2010 to be 66 individuals [95% CI: 43, 107] and 51 individuals [29, 108] respectively. The expected model for the relationship between N_e and N_b is $N_b < N_e < N_b(G)$ where G is generation time (Wang, 2009). Our results ($66 < 188 < 660$) are consistent with that relationship.

The demographic method of calculating N_e implemented in AgeNe required very large values of α (the Poisson scaling factor for reproductive success) to produce N_e and N_b values similar to those calculated by genetic methods. Although the demographic information we used in AgeNe was empirically derived, α is a parameter that may be adjusted by the user to examine the impact of individual variance in reproductive potential. Variance in reproductive success (V_k) generated only by age structure would be represented by $\alpha = 1$, while additional sources of variance or over-dispersion is represented by $\alpha > 1$. Given the vital rates and adult population size parameters appropriate for pink abalone in Point Loma, very high α values were required to produce N_e and N_b (309 and 62 respectively) values similar to those determined through genetic methods. This also indicated high variance in reproductive success among individuals ($V_k = 80{,}767$). Sensitivity of N_e to change in V_k was low for V_k values between 10,000 and 20,000 (Fig. 3). However, dramatic increase in N_e occurred with V_k values lower than 10,000.

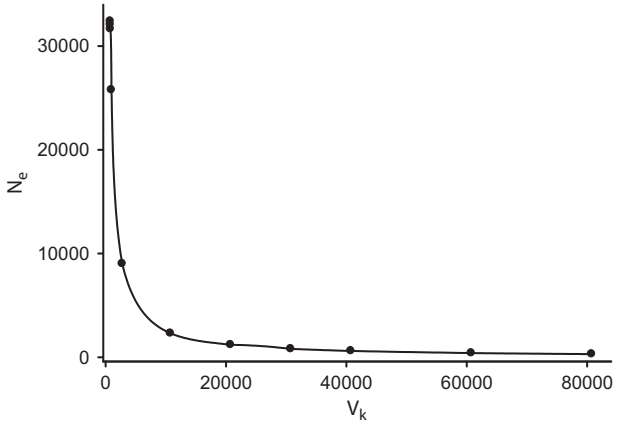

Fig. 3. Sensitivity of effective population size (N_e) to variance in reproductive success (V_k).

3.5. Cohort relatedness

Juveniles within the smaller 2010 subcohort were more related to one another (mean relatedness = −0.00063) than juveniles within the larger subcohort (mean = −0.01832), although the difference was not statistically significant (Table 5). Juveniles within the smaller subcohort were more related to neighboring adults in the Point Loma kelp forest (mean = 0.00285) than juveniles in the larger subcohort were to those same adults (mean = −0.01099) ($p < 0.01$, Table 5).

3.6. Population structure

There was no significant difference in genetic differentiation between adults sampled in Point Loma and La Jolla (contingency table test: $p = 0.20$), or between groups sampled at different times (p-values between 0.15 and 1.00). Our analyses of spatial autocorrelation took fine scale sample location coordinates into account and still failed to identify spatial structure. For all spatial autocorrelation analyses (combined data and each of the 5 groups separately), empirical relatedness statistics fell within the 95% CI for randomized data at every distance class. Preliminary analyses with several Bayesian clustering algorithms also failed to detect significant population structure. The consensus of these analyses is that fine scale genetic differentiation, if it exists, cannot be detected using these microsatellite markers.

4. Discussion

We investigated the use of genetic techniques for identifying self-sustaining abalone populations and their spatial scale using pink abalone within the Point Loma kelp forest. We also tested the efficacy of artificial aggregation of wild adults as a technique for stock enhancement and restoration. Self-sustained populations should not be demographically reliant on immigration. Low relatedness between separate juvenile cohorts, and between juveniles and local adults, indicated that recruitment is not a simple, locally controlled process within this kelp forest. Apparent genetic panmixia, even on a very fine scale, indicated that dispersal occurs regularly on a broader scale than the local kelp forest. Broad dispersal may also explain our inability to genetically match adults and juveniles as parents and offspring. Effective population size relative to census size ratios less than 0.01 indicate that the current natural density results in few successful reproducers that may not support a self-sustained population. Because no local recruits were offspring of adults aggregated to a density of 0.18 m^{-2}, stock enhancement strategies will likely need to use greater adult densities over larger spatial scales to achieved enhanced local recruitment.

Table 5
Results of cohort relatedness tests. Mean Queller & Goodnight relatedness among pairs of individuals from 2010 juvenile cohort A (Juv A) and 2010 juvenile cohort B (Juv B), and pairs of juveniles and adults from Point Loma (PL Adult). Significance level is indicated by placement of Difference in Means among the quantiles.

Groups compared	Mean relatedness	Variance	Difference in means	Quantiles					
				1%	2.5%	5%	95%	97.5%	99%
Juv A to Juv A	−0.001	0.023	0.018	−0.026	−0.021	−0.018	0.019	0.023	0.027
Juv B to Juv B	−0.018	0.021							
Juv A to PL Adult	0.003	0.024	0.014	−0.006	−0.005	−0.004	0.004	0.005	0.006
JuvB to PL Adult	−0.011	0.020							

4.1. Dispersal, gene flow, and genetic structure

Three of our analyses indicate that abalone are capable of long distance dispersal. We found no spatial genetic structure at the scale of the San Diego region, little relatedness between one juvenile cohort and local adults, and an absence of offspring neighboring artificially aggregated adults. This pattern of panmixia cannot confirm the level of reliance of the Point Loma population on demographic input from elsewhere but it does indicate that efforts to promote local populations may be frustrated by complex dispersal patterns.

Some aspects of abalone biology and habitat and suggest that short distance dispersal and the potential for low demographic connectivity despite genetic connectivity. Ecologically significant connectivity among marine organisms with a pelagic phase is thought to be at the scale of 50 to 100 km for most species (Cowen et al., 2006). However, the pelagic larval duration of abalone is at the low end of the spectrum at about 3 to 15 days (Leighton, 1974; Tegner and Butler, 1985) and abalone occupy complex reef habitat within kelp forests where currents are highly attenuated (Jackson, 1997). For example, hydrodynamic and Lagrangian particle-trajectory models have estimated the dispersal potential of abalone larvae to be <200 m in calm conditions (Stephens et al., 2006). While these factors may limit abalone larval dispersal distances, adult longevity and overlapping generations increase opportunities for even relatively rare successful long distance dispersal events to influence genetic patterns. Only a very small number of successful migrants across habitats each generation are required to prevent genetic differentiation (Slatkin, 1987), and many abalone spawning events occur within the span of a single generation.

Despite reasons to expect short distance dispersal from abalone, there are many examples of large-scale genetic panmixia in abalone species (Diaz-Viloria et al., 2009; Gruenthal et al., 2007; Gutierrez-Gonzalez et al., 2007; Will et al., 2011; Withler et al., 2003) and only a few examples of local scale differentiation (Bester-van der Merwe et al., 2011; Gruenthal and Burton, 2008; Temby et al., 2007). Findings of an absence of structure may have resulted from sampling resolutions that were limited to the scale of individual reefs. We collected detailed location information with accuracy within a few meters, and were still unable to identify spatial structure in pairwise genetic relatedness, adding confidence to our general findings of a lack of population structure.

Analysis of family relationships provides an alternative, sometimes successful, method for estimating dispersal. Parentage assignment has been successfully used as a direct measure of dispersal distance for a few marine species (Christie et al., 2010a,b; Jones et al., 2005; Planes et al., 2009). Because even threatened marine populations tend to be large, the technique has typically been limited to species with unique characteristics that allow large proportions of potential parents to be sampled (Jones et al., 2005; Planes et al., 2009). High fecundity, high larval and juvenile mortality, and the potential for long-distance dispersal also mean that even when sampling large numbers of recruits, one can expect to find very few offspring matches. For example, Christie (2010) was able to assign parentage in a highly fecund and abundant coral reef fish (low proportion of potential parents sampled) but identified only two parent–offspring matches among 437 adult and 314 juvenile samples.

We identified no parent–offspring matches for adults within our artificial aggregation or in the wider kelp forest. It is possible that abalone within our artificial aggregation did produce recruits that were broadly distributed. It is also possible that few if any aggregated individuals successfully reproduced given the very low N_b that we identified for the entire forest. However, we were able to confirm that recruitment in the local area of our aggregation was not produced by the aggregation itself and this provides evidence, using a non-frequency based technique, against local scale self-sustainability. Diaz-Viloria et al. (2013) aggregated pink abalone off the Baja California Peninsula of Mexico and also attempted identify the parentage of subsequent recruits in the area. None of the aggregated adults were identified as parents with high confidence and findings indicated low rates of self-recruitment.

We found that individuals from the juvenile cohort spawned in winter were both more related among themselves and more related to local adults than were individuals from the cohort spawned in summer, indicating that larval delivery to the Point Loma kelp forest is a complex process. Winter spawned juveniles appear to represent self-recruitment within the forest. Summer spawned juveniles were likely derived from a mix of locations, given the low relatedness within the cohort. The term “chaotic genetic patchiness” has been used to describe a frequently observed phenomenon in marine species of high temporal variation in allele frequencies among recruits (Hedgecock and Pudovkin, 2011; Hogan et al., 2010; Selkoe et al., 2010). The common assumption that abalone have relatively low dispersal potential does not appear to exclude them from this process. This pattern may arise from variation in larval sources due to variation in oceanographic flow, SRS, or post-settlement selection. We have no data relating to oceanographic patterns or post-settlement processes. However the low N_e/N ratio does indicate the population is experiencing SRS (Hedgecock and Pudovkin, 2011), and changes in oceanographic flow may vary seasonally with changes in kelp distribution (Jackson, 1997).

4.2. Consequences of current and augmented density

We assessed N_e and the N_e/N ratio for the Point Loma kelp forest population of pink abalone at their current natural density and spatial distribution because N_e influences population viability through links with future adaptive potential (Hare et al., 2011). It is defined as the size of an ideal population that would have the same rate of genetic drift as that observed in the natural population. A variety of factors can reduce N_e below N, including variance in reproductive success. In a review of variance in reproductive success among terrestrial species, Nunney (1996) found a mean standardized variance of 0.44 ± 0.32. Standardized variance was calculated as $(V_k/(\text{mean}^2))$. This and theoretical calculations lead to the conclusion that N_e/N should rarely be less than 0.5 and extremely unusual conditions would be required to achieve values as low as 0.1.

Processes acting on both adult and larval stages may act to increase variance in reproductive success (V_k) and decrease N_e/N. The SRS hypothesis for marine organisms supposes that adults have high fecundity, and that patchy dispersal, survival, and settlement success of larvae dictate a much larger variance in reproductive success than is observed in terrestrial species (Hedgecock and Pudovkin, 2011). Our estimate of Point Loma pink abalone $N_e/N = 2.9 \times 10^{-3}$ is consistent with SRS.

We hypothesize that while abalone meet criteria for species likely to show low N_e/N due to larval processes, SRS might also be produced or amplified by a reproductive Allee effect. Low density and patchy spatial distributions of adults result in tight clusters of some abalone and isolation of others, setting the stage for highly variable fertilization success and thus high V_k. Coates and Hovel (2014) demonstrate that high variance in zygote production results from spatial patterns of adults and reproductive asynchrony using an individual-based model. The sensitivity analysis in the present study showed that a substantial decrease in V_k would be needed to achieve biologically meaningful increase in N_e based on the vital rates in this pink abalone population. Realistic adult density increases modeled by Coates and Hovel (2014) resulted in decrease in variance of zygote production that is proportional to the required decrease in V_k. Therefore, without invoking processes acting on larval and juvenile stages, processes acting on adults are sufficient to explain observed low values of N_e.

Because joint estimates of N_e and N_b for iteroparous species are rare, an understanding of the relationship between these parameters may be useful in studies of other organisms with similar life histories (Waples et al., 2011). We estimated a 3-fold to 4-fold difference between N_e and N_b, depending on whether genetic or demographic approaches were used.

We tested artificial aggregation as a restoration technique by artificially manipulating density, creating a high density patch in the center of the kelp forest. Because non-exclusion probabilities were low, we conclude that there was no reproductive benefit to the aggregation in the local area after one year. The artificially produced density was 0.18 m^{-2}, just below the theoretically identified minimum for successful recruitment of 0.2 m^{-2}. Density within the aggregation site decreased over time due to mortality and emigration (Coates et al., 2013), and this may have also contributed to reduced reproductive output. Prior to winter when we estimate that the smaller 2010 juvenile cohort was spawned, five of the aggregated adults were confirmed mortalities. It is possible that densities of 0.2 m^{-2} or below are sufficient to produce self-sustaining local recruitment, but that the numerical and spatial scale of the aggregation effort was insufficient. In either case, our findings indicate that small scale restoration efforts may be unlikely to produce local benefits.

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