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RESEARCH ARTICLE

Development and application of genomic tools to the restoration of green abalone in southern California

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Abstract Due to severe declines in abundance throughout southern California, the green abalone (*Haliotis fulgens* Philippi 1845) became protected under a state-sponsored fishery moratorium in 1997 and was declared a NOAA NMFS Species of Concern in 2004. Recently, *H. fulgens* was chosen for possible stock restoration via translocation of wild adults to depleted habitat and supplementation through releasing cultured individuals. Before a management plan could be developed, however, an understanding of the species' natural population genetic structure was needed. We used a genomic technique called restriction site associated DNA sequencing (RADSeq) to address the issue. RADSeq enabled discovery of 1,209 single nucleotide polymorphisms theoretically spread genome-wide in *H. fulgens*. Analyses suggested the species may be panmictic throughout our sampled range, with an effective population size (N_e) of 1,100–3,600. Hence, limitations to management, such as requiring local broodstock and restricting translocation potential, might be unnecessary. Sites with larger populations may be suitable sources for restoration of depleted sites (e.g. the Palos Verdes Peninsula), although the extent of local adaptation remains

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unknown. Despite this potential for restoration, results gathered on a sample of cultured *H. fulgens* illustrated how quickly genetic diversity can be lost through captive breeding. To help mitigate a drop in N_e due to hatchery supplementation, we recommend collection and replacement of $\geq$100 wild abalone per generation for broodstock and close management of the proportion of cultured individuals in the wild. Successful implementation will depend on operational capacity and the resilience of the source populations to broodstock collection.

Keywords Abalone · Genomics · Population genetics · Restriction site associated DNA sequencing · Single nucleotide polymorphism · Stock enhancement

Introduction

Abalone are large, relatively long-lived marine gastropods in the genus *Haliotis* found in temperate and tropical macroalgal communities worldwide (Geiger 2000). Abalone populations in the state of California, USA, once supported valuable commercial and recreational fisheries, but serial crashes in all commercially-fished species prompted the California Department of Fish and Wildlife (CDFW, formerly Department of Fish and Game) to declare a moratorium on the state's abalone fishery in 1997 (CDFG 2005). Crashes were extremely severe for the white (*Haliotis sorenseni*) and the black (*H. cracherodii*) abalone, which were declared endangered in 2001 and 2009, respectively, under the Endangered Species Act of 1973. Two additional species, the pink (*H. corrugata*) and the green (*H. fulgens* Philippi 1845), were the subject of short-lived but intense fisheries in southern California. Catch totals peaked in 1969 and 1971, respectively, but quickly dropped (CDFG 2005). Moreover, survey cruises undertaken approximately 25 years later for the once common *H. fulgens* found densities of only 0–40 individuals per hectare in former Channel Islands habitat (CDFG 2005). Due to the extensive depletion and an apparent lack of recovery, the NOAA National Marine Fisheries Service (NMFS) listed *H. fulgens* (and *H. corrugata*) as a Species of Concern in 2004. Only the red abalone (*H. rufescens*) continues to support a limited recreational fishery north of San Francisco Bay, despite its near extirpation, as well, in central and southern California.

Under the 2005 CDFW Abalone Recovery and Management Plan, a variety of potential restoration activities exist to augment natural abalone stocks, including translocation of adults from healthier populations to depleted regions with suitable habitat, artificial aggregation, and stock enhancement by outplanting (releasing) various life stages of cultured (hatchery-bred) individuals into the environment (Travis et al. 1998; CDFG 2005; NMFS 2008). Translocation and aggregation have not been well-researched in abalone, while stock enhancement has admittedly met with limited success in California and Mexico (e.g. low apparent survival of outplants; Burton and Tegner 2000; Lapota et al. 2000; Gutiérrez-Gonzalez and Perez-Enriquez 2005).

Nevertheless, the continuing fishery moratorium may be an insufficient measure for ensuring abalone recovery and persistence in California. Abalone are semi-sedentary broadcast spawners that rely on a planktonic larval phase for dispersal (Leighton 2000), but hydrographic and genetic research have indicated several species in California and elsewhere experience self-recruitment (Tegner and Butler 1985; Prince et al. 1987; Evans et al. 2004b; Tang et al. 2005; Gruenthal et al. 2007; Gruenthal and Burton 2008). Local recruitment coupled with diminished abundances and low adult densities means extant populations may provide insufficient numbers of migrants (larvae) to replenish depleted and former habitat in the near term, if at all (Gruenthal and Burton 2008). Indeed, evidence suggests abalone have experienced recruitment failure in southern California (Rogers-Bennett et al. 2004; Miner et al. 2007). Depletion of the breeding stock, resulting in mate limitation and the Allee effect, and the alteration or destruction of habitat that historically supported healthy populations played significant roles. Functionally and locally extinct populations might only recover under active stock restoration (optimally, in conjunction with ecosystem management methods; Miner et al. 2007).

Haliotis fulgens is the only California abalone species after *H. sorenseni* under consideration for stock restoration, and various implementation options are being explored by cooperating state and federal agencies, academic institutions, and non-profit organizations. Central to developing and executing a responsible restoration program is knowledge of the target species' population genetic structure (Waples and Naish 2009). Efforts should be made to match the genetic background of outplanted progeny or translocated individuals with that of the supplemented population (Taniguchi 2003; FWC 2007). Prior research by Gutiérrez-Gonzalez et al. (2007) indicated *H. fulgens* populations along coastal Baja California Sur, Mexico, were undifferentiated, but a spatial genetic assessment of the species in southern California has never been conducted. Studies on other haliotids have yielded variable and occasionally contradictory results, depending on the target species; its habitat, spawning season, or geographic range; hydrography; the scale of sampling; or the genetic marker type used (Withler et al. 2003; Evans et al. 2004b; Tang et al. 2005; Li and Kijima 2006; Gruenthal et al. 2007; Temby et al. 2007; Gruenthal and Burton 2008; Díaz-Viloria et al. 2009). Hence, it was difficult to evaluate the consequences of translocating or breeding *H. fulgens* from

geographically distant areas or determine the appropriate spatial scale on which to act. Without genetic information, the most conservative approach would be necessary (i.e. limited translocation potential and site-specific broodstock collection and maintenance), increasing captive rearing facility requirements and decreasing restoration options.

We assessed the population genetic structure of *H. fulgens* in the southern half of the southern California Bight, where initial stock restoration may be conducted, using restriction site associated DNA sequencing (RADSeq). Genomic tools like RADSeq have the ability to transform the field of conservation genetics by significantly improving estimates of neutral population structure and facilitating the discovery of markers under selection (Allendorf et al. 2010). As opposed to standard marker isolation methods that typically develop in the ones to tens of markers (e.g. microsatellite loci or single gene sequencing), RADSeq produces millions of short-read sequences containing thousands of single nucleotide polymorphisms (SNPs) theoretically spread throughout the target genome. This expanded representation provides the opportunity for holistic estimation of within and among individual- and population-level variation in non-model organisms with little or no genomic resources (Emerson et al. 2010; Davey and Blaxter 2010; Rubin et al. 2012; Reitzel et al. 2013), like abalone. We applied our results toward the design of future restoration efforts for *H. fulgens* in southern California, defining the spatial scale for managing populations and making recommendations for broodstock management.

Materials and methods

Sample collection, DNA extraction, and RADSeq

The geographic range of *H. fulgens* extends from Point Conception in California south along the Pacific coast of Baja California, Mexico, but sampling efforts were limited to the area proposed for restoration in the southern half of the southern California Bight. During the fall of 2011 and spring of 2012, epipodial tentacles were clipped from wild abalone in five southern California regions (Fig. 1), including the Palos Verdes Peninsula (PV; N = 5); Corona Del Mar, Laguna Beach, and Dana Point in Orange County (OC; N = 26); Swami's and Bird Rock in San Diego County (SD; N = 30); various sites around Santa Catalina Island (SCAT; N = 25); and Pyramid Cove at San Clemente Island (SCLE; N = 1). Also obtained was a small sample from F_{3+} cultured abalone (true pedigree unknown) held at the LA Conservation Corps' The SEA Lab in Redondo Beach, CA (SL; N = 5). See Table 1 for basic sampling information and Supplementary Table S1 for more site-specific details.

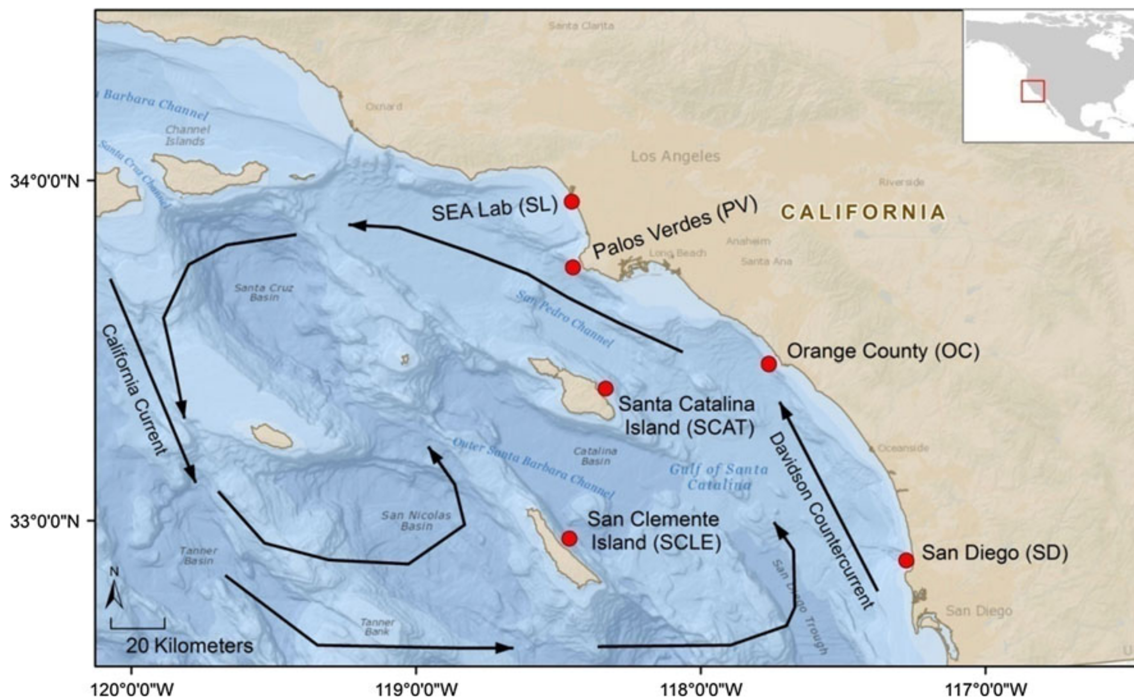

Fig. 1 Map of *H. fulgens* collection sites in southern California. Tissue samples from cultured abalone provided by The SEA Lab (SL) facility in Redondo Beach. Wild abalone sampled from Palos Verdes (PV), Orange County (OC), San Diego County (SD), Santa Catalina Island (SCAT), and San Clemente Island (SCLE). Primary water flow patterns present during *H. fulgens*' spawning season in the southern California Bight marked by *black arrows*. *Scale bar* at lower left. *Inset* shows location on the North American continent

Table 1 Basic sampling information for *H. fulgens* collection sites in southern California

No.	Population	Region	$N_{collected}$	N_{final}
1	SL	The SEA Lab	5	5
2	PV	Palos Verdes	5	5
3	OC	Orange County	26	23
4	SD	San Diego County	30	29
5	SCAT	Santa Catalina Island	25	15
6	SCLE	San Clemente Island	1	1
Total			92	78

Number of abalone sampled per region ($N_{collected}$) and final sample size used in analyses (N_{final}) included. N_{final} excludes individuals failing SNP development (n = 13) or repeat sampled (n = 1). See Supplementary Table S1 for detailed information

Tissues were preserved in 95–100 % undenatured ethanol and stored at room temperature.

DNA was extracted from up to one whole epipodial tentacle from each abalone. Extractions were performed in 96-well format using a DNeasy 96 Blood & Tissue Kit (Qiagen, Inc., Valencia, CA). Preparation of pooled RAD tagged DNA fragments, including SbfI restriction enzyme digestion, adapter ligation, shearing, and PCR, was conducted according to Baird et al. (2008) and Hohenlohe et al. (2011). Library size [350–1,000 basepair (bp) target length], concentration, and quality were assessed with a 2100 Bioanalyzer and DNA 1000 Kit (Agilent Technologies, Santa Clara, CA). Libraries were pooled according to University of Oregon Genomics Core Facility (UOGCF) specifications. Next-generation DNA sequencing (100 bp target length) was carried out by the UOGCF on a HiSeq 2000 (Illumina, Inc., San Diego, CA).

SNP filtering

Compressed data were downloaded from the UOGCF website and filtered to retain successful sequencing clusters. Raw reads were quality filtered and demultiplexed using the subprogram `process-radtags` (flags: -e sbfI -c -q -E phred33) in Stacks v0.9999 (Catchen et al. 2011, 2013). Sequence alignment, SNP discovery, catalog construction, and genotyping were performed using a subset of the core Stacks pipeline subprograms, including `ustacks` (flags: -d -r -i 1 -p 4 –alpha .1 –model_type bounded –bound_low .001 –bound_high .01), `cstacks`, `sstacks`, and `populations` (flags: -r .5 -G -V). A genotype file containing putative biallelic SNPs present in ≥50 % of individuals was output from `populations` in GENEPOP format.

Further SNP filtering was performed in Excel 2010 (Microsoft Corp., Redmond, WA). SNPs present in <70 % of individuals were removed. SNPs beyond base pair 87 of the RAD tag were also removed, since they were likely sequencing errors as signified by a marked increase in the number of apparent SNPs from an average of 78–117 per RAD tag position. To further minimize the retention of SNPs due to sequencing errors, allele frequencies were generated in GENEPOP v4.2 (Rousset 2008), and SNPs with minor allele frequencies (MAFs) <0.10 were removed (e.g. Roesti et al. 2012). For RAD tags containing ≥2 SNPs, one SNP was retained per tag to minimize physical linkage. If MAFs were equal among SNPs within a tag (fully linked), all but one SNP were removed through simple elimination. If MAFs were unequal, the SNP with the highest average MAF (signifying greater variability) was retained.

Paired-end (PE) assembly and BLAST annotation

PE assembly was conducted for each locus using CAP3 (Huang and Madan 1999) as recommended by Hohenlohe et al. (2013) and the methods of Etter et al. (2011) and Everett et al. (2012) to increase query lengths for BLAST annotation. Consensus sequences were aligned to the UniProtKB/Swiss-Prot database using the BLASTX search algorithm. Alignments with E-values $<10^{-4}$ were retained. If multiple alignments generated E-values $<10^{-4}$ for the same locus, the alignment with the lowest E-value was retained.

Statistical analyses

Coefficients of relatedness (*r*, percentage of genes two individuals theoretically share by common descent) and putative relationships (e.g. unrelated, siblings, parent/offspring; 95 % confidence level, 10^4 simulations) for individual pairwise comparisons were estimated under the maximum likelihood framework in ML-Relate (Kalinowski and Taper 2006). The genetically effective population size (N_e) was estimated for the wild sample under the random mating model using the linkage disequilibrium method (Waples and Do 2008). Values of R^2 were first generated in NeEstimator v2b (NeEstimator Group, unpublished software modified from Ovenden et al. 2007). N_e was then estimated in R according to the methods of Waples (2006) and Peel et al. (2012), with MAF cutoffs of 0.01, 0.02 and 0.05 (scripts available on request). To test the effects of physical linkage on the N_e estimates, we used two datasets, including (1) all pairwise locus comparisons and (2) pairwise comparisons with $R^2 \leq 0.5$.

The Bayesian cluster analysis program *Structure* v3.4.1 (Pritchard et al. 2000; Falush et al. 2003, 2007; Hubisz et al. 2009) was used with the default settings (admixture; inferred initial $\alpha = 1.0$, with a uniform prior across populations; correlation of allele frequencies within populations) to identify unique genomic signatures. Five exploratory runs consisted of an initial burn-in of 10^3

Markov Chain Monte Carlo (MCMC) iterations followed by an additional 10^4 MCMC iterations for each of inferred clusters (K) numbering one to 10. The results package was input into Structure Harvester v0.6.93 (Earl and von Holdt 2012), which uses the Evanno et al. (2005) method to calculate the rate of change in the likelihood distribution among Ks to estimate the true number of clusters. Based on the results and apparent stabilization of the summary statistics (α, F_{ST}, lnL) at <5,000 iterations, a final five *Structure* runs consisted of an initial burn-in of 10^4 MCMC iterations followed by 10^5 MCMC iterations for each of K numbering one to five. A second set of runs mirroring those above but excluding any outlier samples was performed to assess whether clustering in the remaining samples may have been masked by the signal from the outlier sample(s).

For an alternate visualization of the data, a pairwise allele sharing distance (ASD) matrix containing all individuals was generated with the non-parametric R package AWclust (Gao and Starmer 2007, 2008; Gao and Martin 2009). AWclust measures ASD by calculating the average number of shared alleles across loci between each pair of individuals. The covariance-standardized principal coordinates analysis (PCoA) method in GenAlEx v.6.5 (Peakall and Smouse 2006, 2012) was then used to partition the ASDs among individuals in multidimensional coordinate space.

Standard locus-specific, global, and pairwise population genetic analyses were performed to anchor the research in the context of typical population genetic studies. Allele frequencies, Hardy–Weinberg equilibrium (HWE), F_{IS}, F_{ST}, and pairwise genic differentiation were estimated with Genepop using the default parameters. The percentage of polymorphic loci and expected and observed heterozygosities (H_O and H_E, respectively) were calculated in GenAlEx.

Outlier tests of selection were conducted with BayeScan v2.1 (Foll and Gaggiotti 2008; Foll et al. 2010; Fischer et al. 2011) using the default settings, with 20,000 iterations and a false discovery rate of 0.05, to determine whether locus-specific divergence estimates were consistent with neutral variation. BayeScan employs a Bayesian approach to identify candidate loci under selection, based on allele frequency differences among populations (Foll and Gaggiotti 2008). To account for false positives due to uneven sample sizes, runs were performed on three population groupings: (1) all populations, (2) all wild populations, and (3) the OC and SD populations.

Results

RADSeq, SNP discovery, and filtering

At least 1 µg of DNA was extracted from 74, 0.75 µg from 7, and 0.5 µg from 5 of the 92 total individuals. Six (four from SCAT and two from OC) failed to provide sufficient DNA and were excluded from further processing. During RAD library preparation, DNAs from four more individuals (three from SCAT and one from OC) from the 0.5 µg DNA extraction group failed and were excluded from further processing. See Table S1 "Fail" for a listing of these and all other excluded samples (see below). The remaining libraries were combined for Illumina sequencing, with two single-read lanes consisting of product from 28 individuals each and one PE lane consisting of 26 individuals (10 µM DNA equalized among pools; 82 total individuals).

Approximately 5.23×10^8 successful sequencing reads were retained for input into the Stacks pipeline. To minimize false positives when discovering SNPs, catalogs created by `cstacks` were generated from the two most data-rich individuals from each population (Table S1 "Cat"). Processing through Stacks resulted in the initial discovery of 1.18×10^5 loci and final retention of 7,232 putative biallelic SNPs. Three SCAT individuals failed to genotype at the vast majority ($\geq$84 %) of SNPs and were excluded from further analyses. SNP filtering for coverage, potential sequencing errors, and physical linkage within RAD tags resulted in a final data set containing 1,209 SNPs and 79 individuals (five from SL, five from PV, 23 from OC, 30 from SD, 15 from SCAT, and one from SCLE). NCBI dbSNP Submitter SNP accession numbers for all 1,209 SNPs are presented in Supplementary Table S2.

PE assembly and BLAST annotation

PE assemblies produced 1,209 contigs, with an average length of 583 bp (range of 102–1,053 bp). BLAST annotation of these contigs yielded significant hits for 87 SNPs (~7 %; Supplementary Table S3). Common functional groups included zinc finger proteins, dyneins, transposable elements, and proteins involved in respiration.

Individual-based analyses

Pairwise coefficients of relatedness (*r*) for the full data matrix ranged from 0.00 to 0.98. The *r* value of 0.98 between SD_0130 and SD_0131 was consistent with the same animal being sampled twice, and SD_0131 was removed from further analyses. The mean *r* value (reported as $\bar{x} \pm s$) for the remaining 78 individuals was low at 0.02 ± 0.037. Relatedness among the five SL individuals ($0.38 \leq r \leq 0.67$, $\bar{x} = 0.46 \pm 0.088$) was significantly higher than the overall mean and consistent with full sibship at the 95 % confidence level. The *r* values for the wild sample, excluding SL, ranged from 0.00 to 0.44 ($\bar{x} = 0.02 \pm 0.026$). The vast majority of pairwise comparisons (91 %) were deemed unrelated at the 95 % confidence level, and "unrelated" was the most likely

Table 2 N_e for *H. fulgens* in southern California

R^2 cutoff	MAF cutoff		
	0.05	0.02	0.01
1.0	1,284 (972–1,884)	1,387 (1,045–2,058)	1,110 (882–1,497)
0.5	3,089 (1,750–12,883)	3,579 (1,946–21,519)	2,922 (1,743–8,920)

Estimations based on various MAF and R^2 cutoff values. R^2 cutoffs exclude all pairwise locus comparisons with R^2 values in excess of the cutoff value (e.g. $R^2 = 1.0$ includes all pairwise comparisons and $R^2 = 0.5$ excludes those with $R^2 > 0.5$). Parametric 95 % CIs in parentheses

relationship for another 8.8 % of comparisons, as well. Higher order relationships were sporadic and did not correlate with population of origin.

N_e estimates for wild *H. fulgens* are reported in Table 2. Exclusion of pairwise locus comparisons according to R^2 did affect N_e, indicating moderate linkage among loci. Estimates ranged from a low of 1,110 (95 % CI of 882–1,497 at MAF = 0.01, $R^2 = 1.0$) to a high of 3,579 (95 % CI of 1,946–21,519 at MAF = 0.02, $R^2 = 0.5$).

Exploratory [mean lnP(K) = −69,090 ± 18.7, δK = 71.7] and final [mean lnP(K) = −69,117 ± 22.6, δK = 54.9] analyses on the full dataset with *Structure* and Structure Harvester suggested K = 2 clusters, with the SL group forming a cluster isolated from the 73 wild individuals. Analysis of the wild dataset, excluding the SL outlier samples, using Structure Harvester suggested a further K = 2 clusters [exploratory mean lnP(K) = −65,948 ± 317.4; δK = 5.4 and final mean lnP(K) = −65,641 ± 24.0; δK = 1.7], with no clear pattern of isolation. The Evanno method cannot address K = 1, however, at which the likelihood was marginally higher [mean lnP(K) = −65,542 ± 2.2].

In PCoA1 (Fig. 2a), Coordinate 1 accounted for ~26 % of variation in ASD, with most individuals falling into two large, closely-spaced groups, but the clustering was not correlated with population of origin. Only two individuals (PV_0097 and SCAT_0076) were visually identified as outlier samples along Coordinate 1. The five SL individuals, PV_0097, SCAT_0037, and SCAT_0076 were partitioned out along Coordinate 2 and SD_0128, SCAT_0028, SCAT_0037, SCAT_0067, and (marginally) OC_0110 fell along Coordinate 3 (Fig. 2b), helping to account for an additional 21 and 15 % of variation, respectively. Less than 40 % of variation was partitioned among the remaining axes. Excluding the 12 outlier samples above, PCoA2 partitioned out 18, 17, and 17 % of variation along Coordinates 1–3, respectively, but the patterning was again not correlated with population of origin (Fig. 2c). Only SCAT_0038 might be considered an outlier sample along Coordinate 3.

Population-level analyses

SCAT and the single SCLE individual (N = 16) were combined into an "island" grouping (SCAT). Results from most of the population-level statistical analyses were consolidated into Table S2. Briefly, the percentages of polymorphic loci were 48.5 % in SL, 80.2 % in PV, 94.0 % in OC, 94.6 % in SD, and 90.3 % in SCAT, with a mean of 81.5 % (SE of 8.6 %). Overall H_O and H_E were 0.263 and 0.270, respectively. Within populations, H_O ranged from a low of 0.222 in SL to a high of 0.280 in PV, while H_E ranged from 0.180 in SL to 0.303 in SD. Sporadic deviations from HWE were seen among loci and populations ($P < 0.001$). F_{IS} was −0.102 for SL, 0.125 for PV, 0.105 for OC, 0.123 for SD, and 0.139 for SCAT. F_{ST} was 0.029 across all populations and 0.003 when excluding SL. Pairwise F_{ST} ranged from −0.001 to 0.174, with comparisons involving SL resulting in F_{ST} values one to two orders of magnitude larger than any other comparison. Similarly, all comparisons involving SL were deemed highly significant ($P < 0.001$) in exact tests of pairwise population differentiation. Pairwise F_{ST} and χ^2 values for exact tests are reported in Table 3. Outlier tests for selection revealed no significant loci in any of the three population groupings.

Discussion

Spatial population genetic structure

We discovered 7,232 putative biallelic SNPs and retained 1,209 to assess the population genetic structure of *H. fulgens* from the southern half of the southern California Bight, the site of proposed restoration efforts, but found no evidence of differentiation. This result was largely consistent with prior research conducted by Gutiérrez-Gonzalez et al. (2007) along 500–600 km of Baja California Sur, Mexico, where peninsular samples were genetically homogenous. In that study, significant divergence in microsatellite allele frequencies was only evident in pairwise comparisons involving Isla Guadalupe, nearly 250 km off the west coast of Baja.

Abalone release gametes into the water column in spatially and temporally synchronized group events (broadcast spawn). Fertilized eggs are negatively buoyant and the pelagic larval phase is short in terms of other marine

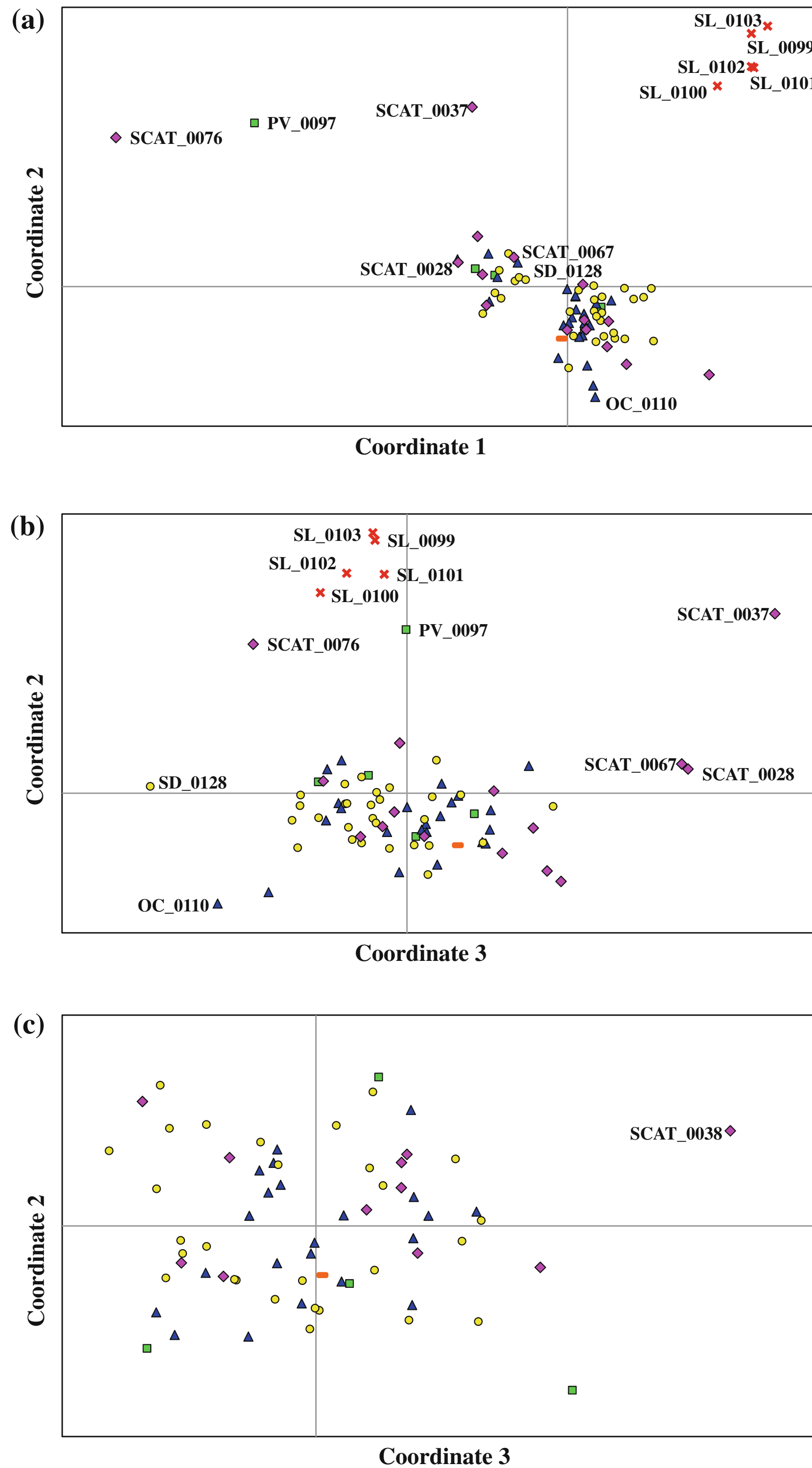

Fig. 2 Principal coordinates analyses (PCoAs) based on pairwise ASD. ASD is a genetic distance measure calculated from the average number of shared alleles between each pair of individuals. PCoA1 includes all 78 individuals and partitions ASD along coordinate axes 1 and 2 in **a** and 2 and 3 in **b**. Coordinates *1–3* represent 26, 21, and 15 % of variation, respectively. Outlier samples in first three axes labeled with ID coded by collection site: The SEA Lab (SL), Palos Verdes (PV), Orange County (OC), San Diego County (SD) Santa Catalina Island (SCAT), and San Clemente Island (SCLE). Collection site coded by color and shape: SL (*red crosses*), PV (*green squares*), OC (*blue triangles*), SD (*yellow circles*), SCAT (*pink diamonds*), and SCLE (*orange dash*). PCoA2 in **c** excludes labeled outlier samples from PCoA1. Coordinates *2* and *3* each represent 17 % of variation. (Color figure online)

Table 3 Pairwise tests of population divergence

	SL	PV	OC	SD	SCAT
SL	–	0.174	0.134	0.125	0.142
PV	2426*	–	0.001	0.007	−0.001
OC	∞*	1783	–	0.003	0.003
SD	∞*	1919	2195	–	0.003
SCAT	∞*	1704	2026	2079	–

F_{ST} reported above diagonal and χ^2-values for exact tests of genic differentiation below diagonal. Significant χ^2-values denoted by asterisk (*) at $P < 0.001$. Collection sites include The SEA Lab (SL), Palos Verdes (PV), Orange County (OC), San Diego County (SD), and Santa Catalina Island [SCAT; includes San Clemente Island (SCLE)]

invertebrates at 5–14 days (Leighton 2000). These characteristics, in conjunction with the attenuated water flow typical to kelp forests (Gaylord et al. 2004), suggest abalone larvae settle close to natal grounds (e.g. Prince et al. 1987). Yet, both population-level and individual-based analyses indicated *H. fulgens* lacks spatial genetic structuring, which is more exhibitive of panmixia than self-recruitment, within the confines of our sampled range.

General water circulation patterns in the southern California Bight are counterclockwise during *H. fulgens*' spawning season in early summer through early fall (CDFG 2005), with a smaller semi-permanent cyclonic gyre in the Santa Barbara Basin and a second, wind-driven surface current called the Southern California Eddy (SCE) extending from the northern Channel Islands to the border with Mexico (Fig. 1; Bray et al. 1999). Two larger, linear currents sandwich the SCE, including the southward flowing California Current and the coastal, seasonally northward flowing Davidson Countercurrent. Larvae entrained in circulation further offshore have the potential to reach sites throughout the study area, which could prevent fixation of alleles, particularly over successive spawning seasons and/or generations. Nevertheless, this pattern does not preclude predominantly local recruitment as ≤10 migrants per generation can be sufficient for maintaining genetic diversity across broader geographic ranges (Franklin 1980; Allendorf and Phelps 1981; Mills and Allendorf 1996). Given the rapid and relatively recent fishery crash, the current lack of structure may also be a remnant of dynamics no longer present in the species. We cannot explore this potentiality further because the generation time (average age of spawning individuals in a population) is unknown.

Any genetic patchiness in *H. fulgens* was evident in the PCoAs within rather than among regions, possibly consistent with extrinsic episodic recruitment. These localizations do not detract from hypothetical panmixia within our sampled range but rather enhance it, especially if indicative of recruitment from uncharacterized (potentially divergent) sources, such as the northern Channel Islands or Mexico. It is unlikely these aberrant individuals were other than *H. fulgens*, since none were more unusual than the captive-bred SL group. The remaining wild individuals were split into two well-mixed groups along Coordinate 1 in PCoA1, a pattern which could be influenced by some unmeasured or unknown environmental variable, although outlier testing was consistent with neutrality and the clustering disappeared in the PCoA2 reanalysis.

Our results (and those of Gutiérrez-Gonzalez et al. 2007) contrast with research on two other abalone species with populations in southern California, *H. rufescens* and *H. cracherodii*, which exhibited significant structuring across their respective ranges (Tegner and Butler 1985; Gruenthal et al. 2007; Gruenthal and Burton 2008). *H. fulgens* inhabits rocky reefs from the low intertidal to potentially as deep as 18 m, whereas *H. cracherodii* is often found clustered in crevices from the high intertidal to 6 m deep (Neuman et al. 2010). As such, *H. cracherodii* experiences unique, unstable, and localized environmental pressures and water circulation regimes, which can intensify self-recruitment and local adaptation and drive population divergence. Despite somewhat disjunct geographic ranges, the habitat and behavioral characteristics of *H. fulgens* are more closely aligned with *H. rufescens*, but our spatial scale for sampling may not have been large enough to detect regional differences. The maximum inter-population distance studied for *H. fulgens* was 160–200 km (SD to PV), equivalent to the minimum distance reported in Gruenthal et al. (2007) for *H. rufescens*, at which no significant differentiation was seen. Divergence was apparent in *H. rufescens* at >300 km but only when using over 150 amplified fragment length polymorphism (AFLP) loci.

We found no evidence of selection across SNP loci, indicating our results were consistent with neutral variation (e.g. Reitzel et al. 2013), although our ability to identify non-neutral loci was diminished by our sample characteristics. Specifically, BayeScan produces lower Type I and II error rates than similar programs (Narum and Hess 2011) and can be used on very small sample sizes without the risk of any particular bias (Foll et al. 2010), but unequal sample sizes (Groupings 1 and 2) and the small number of populations (Grouping 3) may have reduced our power of detection. Moreover, the PV sample might represent a significant portion of all *H. fulgens* remaining at that site, whereas SCAT exhibited a markedly high failure rate during the SNP development process. 70 % of the failed SCAT individuals were collected from Iron Bound Cove (Table S1), and allele frequency distributions may not have been well-characterized for that population. Yet, Hale et al. (2012) determined that the accuracy and precision of certain metrics (e.g. H_E) stabilized at sample sizes of ≤20, and

Willing et al. (2012) concluded that as few as 4–6 individuals per population can be sufficient for detecting differentiation when using ≥1,000 SNPs and an unbiased F_{ST} estimator, like the weighted ANOVA we used in Genepop (per Weir and Cockerham 1984). The fact that small sample sizes will still provide useful population genetic information is particularly relevant when obtaining larger samples can be difficult (Willing et al. 2012), such as for heavily depleted species and species for which sampling is labor intensive or potentially dangerous, like abalone.

Implications for stock restoration

Despite the 1997 fishery moratorium, *H. fulgens* abundances have not rebounded in southern California (CDFG 2005), nor are there indications that heavily depleted or locally extinct populations will recover naturally in the near future (e.g. Rogers-Bennett et al. 2004; Miner et al. 2007). Active stock restoration may be necessary, and understanding the population genetic structure of *H. fulgens* will be key to developing and implementing restoration efforts for the species. Since spatial structure was discovered in other California abalone, it was suggested that efforts may be necessary to preserve potential population genetic differences in *H. fulgens*, particularly between the mainland coast and islands (Chambers et al. 2006; Gruenthal et al. 2007; Gruenthal and Burton 2008). This action would present significant procedural limitations to restoring the species in southern California. Because we did not find evidence of population differentiation, sites within our sampled range at a few of the southern Channel Islands (e.g. Santa Catalina) showing signs of stability or recovery may be excellent sources of adults for translocation or broodstock development to replenish heavily depleted areas (e.g. the Palos Verdes Peninsula). However, it is also notable that several recent studies have documented local adaptation in wild populations, including *H. rufescens*, despite high gene flow throughout much of the genome (Via 2009; De Wit and Palumbi 2013; Gagnaire et al. 2013; Hemmer-Hansen et al. 2013). Future research on *H. fulgens* should focus on obtaining a higher density of SNP loci and constructing a RAD-based linkage map to more thoroughly test for local adaptation.

Today, enhancement hatcheries must be implemented with caution (Camara and Vadopalas 2009). Possible concerns regarding artificial stocking include a high rate of mortality for cultured animals in the wild, as well as loss of genetic diversity, swamping the native gene pool with hatchery-based alleles, the introduction of non-native alleles resulting in outbreeding depression (also relevant for translocation), and the introduction of deleterious alleles in frequencies higher than would naturally occur (Utter 1998; LeVay et al. 2007; Araki and Schmid 2010; Waples et al. 2012). These various negative genetic effects have been documented in multiple hatchery-propagated species, including abalone (e.g. Cross and King 1983; Smith and Conroy 1992; Waples and Do 1994; Evans et al. 2004a; Sekino et al. 2005; Lemay and Boulding 2009).

Genetic variation is a means by which a population can persist in the face of stochastic environmental forcing brought about anthropogenically (e.g. habitat destruction, climate change), through natural disasters (e.g. 100-year storms), and by competition for resources (e.g. food, shelter). Estimates of N_e for the wild *H. fulgens* ranged from approximately 1,100–3,600, indicating a moderate amount of genetic diversity is still present in the natural population, despite extensive depletion. In comparison, the total census size of the endangered *H. sorenseni* was estimated at ~2,500, with N_e near zero, while N_e was between 350,000 and 3.5 million for the more abundant *H. rufescens* and near 420,000 for *H. kamtschatkana* (Hobday et al. 2001; Withler et al. 2001; De Wit and Palumbi 2013). How this bodes for *H. fulgens*' resiliency in the face of environmental change is unknown, but it does suggest any restoration efforts for the southern California stock should be conducted with discretion.

Knowledge of N_e can also help researchers estimate a sufficient broodstock census size (N_c) and map mating scenarios that maximize genetic diversity and manage the proportion of cultured individuals in the wild (ideally, at <10 %; Waples et al. 2012). Because hatchery stocking can artificially magnify inbreeding (Ryman and Laikre 1991; Waples and Do 1994), it is extremely important to monitor N_c when evaluating the inbreeding potential of supplemented populations (Duchesne and Bernatchez 2002). Previous research suggested collecting as few as 50 individuals from each source population (Frankel and Soulé 1981; Miller and Kapuscinski 2003; Moyle et al. 2008), whereas others recommended 50–100 breeding pairs (Kincaid 1983; Allendorf and Ryman 1987). For N_e in the 1,000–5,000 range, as here, supportive breeding could significantly reduce wild genetic diversity even with $N_c > 100$ (Ryman et al. 1995; Waples et al. 2012).

Managing genetic diversity in the hatchery and wild, however, involves tradeoffs between program capacity, broodstock diversity, and the resilience of the source population to broodstock collection (SJRRP 2010). We recommend ≥100 (absolute minimum of 50) wild adult *H. fulgens* be collected for captive breeding purposes and replaced per generation. This broodstock will have significantly less genetic diversity than the natural stock, but the populations proposed for restoration have been decimated or extirpated and broodstock holding and larval and juvenile rearing capacities are limited. However, because we advocate using wild abalone for broodstock, care must be taken to avoid broodstock mining of the source populations

(e.g. Remshardt et al. 2009), which can result in reduced genetic variability, depensation (e.g. the Allee effect), and even local extinction.

Characterizing the genetic diversity of the broodstock and developing breeding schemes to mitigate the loss of that diversity, while outside of the scope of this research, will be necessary next steps (Fisch et al. 2013). The genomic data gathered on cultured *H. fulgens*, in conjunction with extensive research conducted worldwide on cultured abalone (e.g. Smith and Conroy 1992; Evans et al. 2004a; Hara and Sekino 2007; Lemay and Boulding 2009; Slabbert et al. 2009), demonstrated how quickly (within one to a few generations) hatcheries can purge genetic diversity and/or shift the genetic signature relative to a target population (Araki and Schmid 2010). *H. fulgens* sampled from The SEA Lab facility in Redondo Beach, CA, were consistently different from the wild group. Although the SL sample pedigree is unknown, they are thought to be from an F_{3+} generation bred in captivity, with original source broodstock from PV (B. Scheiwe, The SEA Lab, personal communication). The proportion of polymorphic loci and overall heterozygosity were substantially lower in SL than any wild sample, even though F_{IS} was negative, indicating excess heterozygosity relative to expectations (Allendorf 1986). Along with the high level of relatedness, it is conceivable the SL sample was reared from a single, induced spawning event produced through a cross between two adults from somewhat divergent culture lineages. Similar results were seen for *H. fulgens* broodstock at the Space and Naval Warfare Systems Command (SPAWAR) in San Diego, CA (Gruenthal 2007). The SPAWAR abalone, originally sourced from a local culture facility to produce larvae for toxicity studies (D. Lapota, SPAWAR, personal communication), exhibited high heterozygosity but significantly less allelic diversity at several microsatellite loci when compared to a small wild sample.

Despite the likelihood of low genetic diversity, multiple instances of outplanting F_{2+} cultured *H. fulgens* have occurred on an experimental level off southern California since 2000 (Lapota et al. 2000; N. Caruso, Get Inspired!, personal communication). Although the genetic and ecological effects of releasing individuals such as these into the environment can be serious, as described above, these releases do offer an opportunity to assess the potential success of outplanting *H. fulgens* on a larger scale. In addition to traditional physical tagging, unique combinations of alleles at multiple genetic loci or alleles common in outplants but rare in the native population can be exploited to track outplant survivorship and even reproduction (Pella and Milner 1987; Letcher and King 1999; Tringali 2006). Nevertheless, this capability is not justification for the practice, given the risks, as the use of wild broodstock coupled with significant changes to breeding protocols to maintain genetic diversity and regular monitoring are strongly recommended for future restoration efforts. With good experimental design implemented from the start, the uncertainties associated with captive propagation and outplanting will be addressed as best possible for *H. fulgens*, while the potential ecological, social, and economic benefits of restoring depleted abalone populations can be investigated more fully.

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