Title
Conservation Genetics of California Abalone: Developing Tools for Management

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Final Technical Narrative

Introduction: Over the past three decades, we have witnessed dramatic declines of abalone stocks along the southern California coast. What was once an important fisheries resource (commercial and recreational) has completely disappeared and there is now a moratorium on all abalone harvesting in the region (California Department of Fish and Game 2002). Populations of white, red, black, pink and green abalone have all been impacted; the former species, *Haliotis sorenseni*, has become the first marine invertebrate to be listed as an endangered species. Both anthropogenic (overexploitation and habitat destruction) and natural (disease and predation) processes have contributed to the decline in abalone abundance. Attempts to develop effective conservation and recovery strategies for abalone require understanding many aspects of the biology of these species. The primary question is: can reproduction in remnant populations provide the recruits necessary for recovery of abalone populations that have gone locally extinct? Two factors are important in answering this question. First, there is the question of fertilization success in low abundance populations of broadcast spawning invertebrates; can males and females locate one another and successfully produce fertilized eggs? The second issue regards the level of "connectivity" of abalone populations; can success in one part of the species range provide a natural reseeding in other parts of the range? This project used population genetic methods to address the latter question.

Methods: Tissue from wild red abalone (N = 473) collected between the years 1998 and 2000 at nine locations was obtained from the California Department of Fish & Game. Wild black abalone tissue (N = 588) was collected from 11 locations by either the California Department of Fish & Game (coastal samples) or Glenn VanBlaricom and Melinda Chambers at the University of Washington (island samples). Epipodial tentacle or foot clippings were preserved in ethanol or water and stored at -80ºC. From north to south along the California coast, red abalone collection sites included Crescent City (CC, N = 31), Trinidad Head (TR, N = 38), Shelter Cove (SH, N = 59), 7 House Cove near Caspar (7HC, N = 58), Van Damme State Park (VD, N = 60), Salt Point State Park (SP, N = 61), Horseshoe Cove (HSC, N = 60), Monterey (MYR, N = 24), and San Miguel Island (SMIR, N = 46). Clippings from a second cohort of animals in a smaller size class (Van Damme juveniles, VDJ, N = 36) were obtained in 2000 from Van Damme State Park to preliminarily examine possible temporal changes in population structure. Similarly, black abalone collection sites included Scotts Creek (SC, N = 63), Asilomar (ASL, N = 56), Monterey (MYB, N = 54), Carmel Point (CP, N = 23), Big Creek (BC, N = 14), Cambria (CA, N = 58), and Vandenberg Air Force Base (VA, N = 48) along the

central coast of mainland California; and San Miguel Island (SMIB, N = 101), Santa Rosa Island (SRI, N = 55), Santa Cruz Island (SCI, N = 29), and San Nicolas Island (SNI, N = 87) in the Southern California Bight (SCB).

DNA was extracted from approximately 25 milligrams of tissue using a DNeasy Tissue Kit (Qiagen Inc., Valencia, CA) and eluted in 200 μl of TE buffer (10 mM Tris, 1 mM EDTA). A 5 µL aliquot of the stock DNA was diluted with 45 µL of DNA-grade water, placed in 96-well trays, and stored at 4ºC for use in the polymerase chain reaction (PCR). Reactions were carried out in 25 µL volumes, including 1 µL of template DNA, 0.5 U REDTaq DNA polymerase (Sigma-Aldrich Corporation, St. Louis, MO), 2.5 µL 10X PCR buffer, 2.5 mM $MgCl_2$ final concentration, 2 µM each dNTP final concentration, and 5 pmoles each primer. An approximately 580 basepair (bp) fragment of the mitochondrial cytochrome oxidase subunit one (COI) gene was amplified in subsets of 309 red and 238 black abalone. PCR primers for COI are taken from Metz *et al*. 1998: AB-COIF (forward: 5'-TGATCCGGCTTAGTCGGACTGC) and AB-COIR (reverse: 5'-GATGTCTTGAAATTACGGTCGGT). PCR cycling conditions included an initial two minute 95ºC denaturation followed by 35 cycles of 95ºC for 30 s, 50ºC for 30 s, and 72ºC for 30 s. One compound tri-/tetranucleotide (*Hka3*) and four dinucleotide (*Hka28*, *Hka40*, *Hka56*, and *Hka80*) microsatellite loci were amplified in 473 red abalone and 588 black abalone. Primers used were originally developed for a closely-related species, the pinto abalone *H. kamtschatkana* (Miller *et al*. 2001). The forward primers were 5' end-labeled with one of three dyes HEX, TET, or 6-FAM for fluorescent visualization.

Microsatellite Genotyping. Fifteen µL of a denaturing solution containing xylene cyanol and bromophenol blue dyes in 99% formamide were added to each 25 µL PCR product. Samples were then loaded onto vertical 0.4mm thick large format (33 x 39 cm) 6% polyacrylamide denaturing gels containing 33% formamide and 7M urea. ROX-labeled MegaBACE™ ET900-R Size Standard (Amersham Pharmacia Inc., Piscataway, NJ) or Genemark 350 Fluorescent DNA Ladder (Northern Biotechnology, Schofield, WI) was run concurrently as a size standard. Gels were electrophoresed at 60 Watts for two to three hours and scanned on a Typhoon 9410 Variable Mode Imager (Molecular Dynamics Inc., Sunnyvale, CA). The data were analyzed with GENEPOP v3.4 (Raymond and Rousset 1995, 2003).

COI Sequencing. The amplified fragment was sequenced on a MegaBACE™ 1000 (Molecular Dynamics Inc., Sunnyvale, CA) DNA sequencer using the forward primer AB-COIF and a DYEnamic™ ET dye terminator sequencing kit (Amersham Pharmacia Inc., Piscataway, NJ). Where direct comparisons were made between black and red abalone, red abalone sequences were edited to an equivalent 403 bp.

Results: Data from the microsatellite loci were used in determining population genetic structure. The compound repeat *Hka*3 locus contained 75 alleles in our sample of red abalone (this locus could not be amplified in the black abalone sample). The remaining four dinucleotide repeat loci contained between 26 and 36 alleles in both species. Summary statistics and estimates of population genetic parameters were generated using GENEPOP v3.4.

Genetic connectivity. Estimates of the parameters θ_{ST} and ρ_{ST} (F_{ST} analogues, Wright 1978) were generated to evaluate levels of genetic structure in both abalone species. An hierarchical analysis of molecular variance (AMOVA) was performed on the haploid COI sequence data to estimate θ_{ST} (Schneider *et al.* 2000), whereas estimates of ρ_{ST} were calculated using the standardized variance in allele frequencies of the microsatellite loci (Weir and Cockerham 1984; Slatkin 1995; Michalakis and Excoffier 1996; Raymond and Rousset 1995, 2003). The basic finding, consistent with previous population genetic studies on the red and black abalone (Burton and Tegner 2000; Hamm and Burton 2000) is that there is more genetic structure in the black abalone than in the red abalone at both mitochondrial and nuclear loci.

***Haliotis rufescens*:** Only one microsatellite locus, *Hka*28 showed a signal of population divergence in red abalone. When all loci were combined, only the pairwise comparison between VDJ and HSC was significant ($P < 0.05$). COI data also showed little evidence for divergence among red abalone populations. Regardless of grouping, ≥ 99% of the variation in the AMOVA was within populations (global $F_{ST} = 0.00688$, $X^2 = 3.27$, df = 1, $P > 0.05$).

Haliotis cracherodii: When all of the microsatellite data were combined in analysis, pairwise levels of genetic connectivity appeared lowest between mainland populations; ρ_{ST} tended to be highly significant ($P < 0.001$). Populations from the Channel Islands not significantly diverged from one another or from SC and ASL in the northern portion of the sample range. Values of ρ_{ST} were significant between the four northern mainland populations and those at CA, VD, and in the SCB at *Hka*28. There appears to be a transition zone near the central California populations of BC and CA. BC is moderately to highly diverged from populations south of CA (VD and in the SCB) and CA is moderately diverged from the mainland populations north of BC. However, CA and BC are not significantly diverged from one another. Generally, populations north and south of the Big Sur area on the California coast may not exchange frequent migrants, but admixture is common within the regions above and below. However, a different pattern emerges from the COI data. In this case, it appears that BC is significantly divergent from six of ten populations ($P < 0.05$), and there is sporadic divergence between three other populations (ASL, CP, and SCI). In the AMOVA, groups were formed based on the levels of genetic divergence. The highest among group variation (5.97%) was between three groups, including Big Creek, Santa Cruz Island, and the remaining populations.

Discussion. Despite similar life histories, the red abalone populations sampled exhibit less genetic divergence than the black abalone populations sampled, while spread over a broader geographic distance. The pattern may depend on the ocean currents abalone larvae experience during their pelagic phase (Hamm and Burton 2000). Data from drifters released in the Santa Barbara Channel and Santa Maria Basin off southern California reveal local eddies and long-range currents that may retain abalone larvae or transport them great distances depending on coastal location and season. In winter months, currents off the California coast are more unidirectional in flow, while upwelling

and depth stratification occur during summer months. Red abalone may take advantage of both winter and summer regimes due to the variability in their spawning season, providing populations with both local recruitment (summer) and pulses of recruits from distant sources (winter). Black abalone, however, have a more restricted breeding season (late spring and summer) when flow patterns display coastal eddying, upwelling, and seaward jets that may cause local retention and/or offshore movement of larvae.

Since black abalone are thought to be ancestral to Eastern Pacific abalone species, it is unusual that their mtDNA show a signal of reduced diversity in comparison to red abalone; haplotype divergence is much deeper in the reds where there is more than one common haplotype. It is possible that the black abalone mitochondrion has undergone a selective sweep and variation is recently being reintroduced into the pool. A possible cause of a selective sweep of the mitochondrion (disease) is consistent with a recent outbreak of Withering Syndrome (WS) in populations of black abalone along the mainland coast of California and in the islands in the Southern California Bight. WS is caused by a Rickettsiales Like Prokaryote (RLP) that forms bacterial inclusions in the digestive glands and postoesophagus of California *Haliotis* species and causes contraction and atrophy of the foot (Friedman et al. 2000; 2002). Both red and black abalone are susceptible to the disease. However, only black abalone populations are being virtually wiped out as the disease moves northward along the coast. The contraction of WS due to the RLP is temperature dependent, and the black abalone range is shifted southward of red abalone (warmer waters) and the animal lives in the high intertidal (greater temperature shifts at low tide due to exposure). A situation such as this may have occurred in the recent past, creating a severe population bottleneck, and a selective sweep of the mitochondrion.

Full description of the results of this study are in preparation for publication.

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