Abalone Restoration in the Pacific Northwest

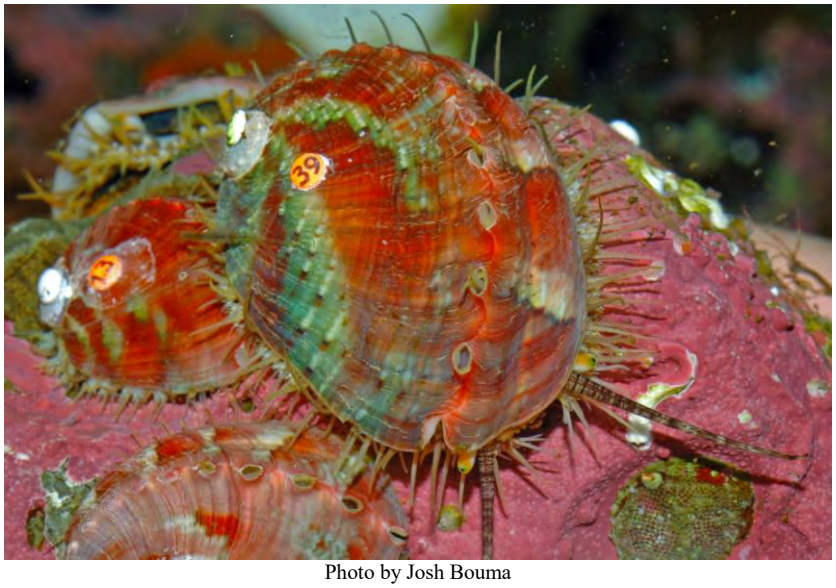

Photo by Josh Bouma

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Collaborators:
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Washington Department of Fish and Wildlife
Washington Department of Natural Resources
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Skagit County Marine Resource Committee
Port Townsend Marine Science Center
Jamestown S'Klallam Tribe

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GRANT DETAILS

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PINTO ABALONE STATUS

The pinto abalone (*Haliotis kamtschatkana* Jonas 1845) was added to the National Marine Fisheries Service's (NMFS') "Species of Concern" list on April 15, 2004 (69 FR 19975). On July 1, 2013, the National Marine Fisheries Service (NMFS) received a petition from the Natural Resources Defense Council (NRDC) requesting that the pinto abalone (*Haliotis kamtschatkana*) be listed as threatened or endangered under the Endangered Species Act (ESA) and that critical habitat be designated for the species. On August 5, 2013, NMFS received a second petition, filed by the Center for Biological Diversity (CBD) to list the pinto abalone under the ESA and designate critical habitat. The species is currently listed under the "Endangered A2abd" category on the International Union for Conservation of Nature (IUCN) Red List of Threatened Species (McDougall et al. 2006). On November 18, 2013, NMFS determined that the petitions presented substantial information indicating that the petitioned action may be warranted for pinto abalone (a "positive 90-day finding") and published the finding in the Federal Register (78 FR 69033), pursuant to 50 CFR 424.14.

In the fall of 2013, NMFS assembled a Status Review Team (SRT) to compile and review the best available information regarding the status of the species and to assess the extinction risk and threats facing the species. As of this writing, there has been no decision regarding a recommendation to list pinto abalone as a threatened or endangered species.

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EXECUTIVE SUMMARY

Introduction

Pinto abalone (*Haliotis kamtschatkana* Jonas 1845) populations along the west coast of North America are in serious decline and in Washington State are at risk of collapse. A collaboration of universities, state agencies, tribes, federal agencies and the private aquaculture industry was formed to develop remote propagation methods at the NOAA Mukilteo Research facility with the goal of supplementing abalone stocks to reverse the decadal trend of declining abundance. In 2011, WDFW applied for a NOAA grant to enhance and improve restoration efforts for pinto abalone. A NOAA grant (#NA11NMF4720277) was awarded, through the Proactive Conservation Program, and funded Washington pinto abalone restoration efforts at a critical time when seed money from other grant sources was shrinking or no longer available and state funds for natural resource agency management were being reduced or eliminated due to the Great Recession. The original 3 year NOAA grant was budgeted at a total level of $635,646 of which $561,111 was federal sourcing and $74,530 was non-federal. The grant was fully funded in the first year and then federal funding was reduced to $60,000 in the second year and to zero in the third year. The actual total federal funding level for the 3 year grant was $241,988. Seven recovery objectives were established for this grant and the results are described below.

Captive rearing

Captive rearing of pinto abalone is a key restoration strategy in Washington. This is because the natural population is collapsing, with no sign of successful recruitment, and supplementation may be the only way to create reproductive densities in the wild. NOAA continues to provide generous in-kind contributions to the pinto abalone restoration effort by collaborating with restoration partners and providing necessary hatchery and nursery space at their Mukilteo Research facility. NOAA grant funds have greatly improved captive rearing of pinto abalone in a hatchery/nursery complex at Mukilteo, Washington through better water quality monitoring and treatment of intake waters to buffer against low pH (high acidity), development of improved diets for rearing abalone, and optimization of larval settlement by testing GABA concentrations under various exposure durations. This grant also supported genotyping work that resulted in identification of four microsatellite loci that used to determine sibling relationships of broodstock and also to infer parentage in hatchery reared abalone, preventing inbreeding of siblings and providing a tool for identifying hatchery reared abalone in the wild. Genetic samples were taken from each abalone that were collected in the wild and used as hatchery broodstock. Pedigrees were tracked to optimize spawning and maximize the genetic diversity of hatchery reared abalone. A total of 51 abalone broodstock were collected from the wild during the grant period and some of these broodstock contributed to producing 47 genetically distinct families and 4.16 million settled larvae in the hatchery.

*Tagging method*s

Identification of individual abalone over time is essential to improve estimates of abalone survival and growth and to track broodstock in the hatchery. Individual identification makes it possible to measure individual growth and survival, to distinguish between wild stocks and hatchery reared stocks, and to easily discern between broodstock during spawning events. Stainless steel tags, bee tags, and plastic disc tags wired through the respiratory pores or adhered to the shell have proved to be unreliable over time (unpublished, WDFW). We have tried various methods of injecting Passive Integrated Transponder (PIT) tags and adhering PIT tags, and found that adhering PIT tags to the ventral anterior of the shell had high retention rates. Adult abalone formed nacre over the PIT tags, resulting in permanent individual identification that could be read by standard and experimental PIT tag readers employed. Adhering PIT tags in this manner had no significant effect on growth or survival. The results of this study have been published by Hale *et al*. (2012). Long-term results may be available from PIT tagged adults which were aggregated at two sites along San Juan Island and from PIT tagged juveniles which were outplanted on the Shannon Point Marine Center seawater intake line reef near Anacortes, Washington. A follow-up study has been funded by the Skagit County Marine Resource Committee.

Evaluate Efficacy of Aggregating Abalone

It has been postulated that reproductively viable adult abalone can be aggregated to increase density to a critical threshold where natural reproduction can occur. We attempted to test this by aggregating adults from two sources: 1) hatchery broodstock that had already made sufficient genetic contribution toward hatchery spawning events, and 2) wild single abalone that are presumed to make little or no contribution to natural reproduction. We collected a total of 60 adult abalone, originating as hatchery broodstock or singleton wild abalone, and placed these abalone within two well-defined plots along the western shoreline of San Juan Island in 2008. The plots already contained 30 resident abalone, and the total population on the two plots following aggregation was 90. The two plots were re-surveyed during the first year of this grant in 2012 and confirmed mortality of aggregated tagged abalone over 4 years was estimated at 57% for one plot and 37% percent for the other. We found similar results when 21 PIT tagged adult abalone were added to a plot containing 26 resident abalone in September of 2012 (n=47 post-aggregation). In March 2014 this site was re-surveyed and the census within the plot was only 14 live animals, a 70% reduction over a 16 month time frame. A number of factors were identified that may have contributed to apparent low survival including emigration from the plots, domestication of hatchery broodstock, age of aggregated abalone, handling mortality, natural predation, and change in ocean conditions. A second objective was to determine if aggregation resulted in local recruitment. There was no observed evidence on any of these sites of local recruitment that could be attributable to aggregating adults. This should not be

surprising since determining local recruitment from a broadcast spawner, such as pinto abalone, is a very challenging problem and is beyond the scope of this grant. Aggregation may not be a viable strategy for abalone restoration until factors affecting survival and recruitment are better understood. Further study is recommended.

Juvenile abalone outplanting

If hatchery-raised juvenile abalone can survive in the wild, mature and achieve a sufficient density to successfully reproduce, then this strategy could be a cornerstone to reversing the decline of pinto abalone populations. Prior experimental outplanting of juvenile abalone demonstrated that hatchery-reared abalone can survive in the wild and the results further informed us of an optimal size range to maximize survival. An over-arching goal of outplanting juvenile abalone is to maximize genetic diversity by adding as many distinct genetic families as possible, with equal representation of each family (same number of individuals in each distinct family). This NOAA grant allowed us to outplant a total of 2,721 juvenile pinto abalone on 6 restoration sites within the San Juan Archipelago (SJA) during the grant time frame. These sites had been previously seeded with juvenile pinto abalone which represented 25 distinct families. The outplanting completed during the grant represents an additional 28 distinct families added to these 6 sites, and this effort brings the total number of distinct genetic families to 53. The mean shell length of untagged abalone outplanted in 2013 was 30.1 mm and in 2014 was 19.3 mm. An additional 3,000 juvenile abalone, representing 16 families and ranging in size from 5-30 mm, remain in the hatchery and are scheduled for outplanting in the SJA in early spring of 2015. Care was taken to optimize the genetic diversity of outplanted juvenile abalone and representative abalone were screened to assure that these animals were disease free. A portion of the abalone outplanted were tagged and all abalone were placed into predator exclusion modules for the first 24 hours *in situ*.

Surveys of 4 outplant sites at BIS, BIW, AIS & AIW* that were done prior to the 2014 outplanting showed that abalone densities were 0.21, 0.21, 0.40 and 0.69 abalone/m^2 respectively. All of these densities are within or exceed the range densities identified by Babcock and Keesing (1999) to prevent population collapse. It is encouraging that isolated areas of restoration have been achieved and these results will inform future restoration strategies.

High Resolution Temporal Sampling

Pinto abalone are typically cryptic in their preferred habitat and small abalone often occupy rocky crevices and underneath cobble and boulders (presumably to avoid predation). We proposed that estimates of abundance, survival and growth can be improved by accurately identifying individual animals and developing a sampling strategy to optimize recapture. To identify individual juvenile abalone, we adhered distinctly numbered and colored plastic disc

tags to their shells prior to outplanting on two sites (BIS & AIW) in the SJA in 2011. Abalone outplanted in 2009 were not tagged, but were distinguished from the 2011 outplanted abalone by the difference in size structure. A total of 1197 abalone were outplanted in 2009 and 2011. To determine recapture or re-observation rates we conducted a series of diver surveys within a narrow range of time.

During July and August 2012 five repetitive dive surveys using were made on these two sites (BIS & AIW) in consecutive weeks. The surveys were non-invasive, meaning that lights and mirrors were implements used by divers to detect abalone, but there was no significant disruption to the substrate such as removing cobble to search underneath for cryptic abalone. A total of 137 juvenile and young adult abalone were observed in 2012 at these two sites, which is an approximate survival rate of 11.4%.

An intriguing finding of this high resolution sampling strategy is that only two tagged abalone were observed at each site more than once (total of 4 tagged abalone at both sites) during the 5 surveys. The sample size of abalone with readable tags was 26, which indicates a refind rate of about 15.4%. This means that for a single survey, most juvenile abalone were not visible to divers in spite of intensive searching using non-destructive methods. Further, if additional site surveys had been conducted then it is likely that more abalone would have been identified. To obtain better information about *in situ* survival and growth, we conclude that high resolution temporal sampling may be a valuable strategy.

This study also indicates that tag retention from the 2 outplant years may be as low as 3.8% between March 2011 and August 2012 (about 17 months). The number of tagged abalone was 680 and only 26 were observed 17 months later. Improvements to tag methods are described above and in greater detail in the body of this report.

Mean growth of tagged abalone was 9.0 (+/- 8.7 SD) mm shell length (SL) at BIS and 19.9 (+/- 12.8 SD) mm SL at (AIW) over 17 months. The variance for this data is high and we recommend increasing the sample size of tagged abalone for future *in situ* growth estimate studies.

Collectively these findings inform managers of a need to develop persistent tagging methods (perhaps PIT tags that are smaller than what is currently available) and the value of repetitive sampling of behaviorally cryptic young abalone to make improved estimates of abundance, survival and growth.

Develop and deploy pilot field nurseries

Development of field nurseries to grow small abalone presents several potential benefits by expanding limited nursery space at the Mukilteo Research facility, acclimating abalone to natural conditions while at the same time excluding predators and by involving the local community in restoration activities. The first abalone field nursery modules that we used were developed by Bob Selzler, a retired mechanical engineer and a Puget Sound Restoration Fund (PSRF) volunteer. PSRF partnered with SeaDoc Society to find shoreline owners with docks to host field nurseries. Sites were selected and eight nursery modules were installed in 2013 with 100 abalone (mean SL=18 mm) seeded into each module, a total of 800 abalone. The abalone held by the Port Townsend Marine Science Center (PTMSC) were closely monitored and maintained by volunteers for 9 months. Only one mortality (death) occurred in a module held in a PTMSC lab and four animals died in a module held suspended below the PTMC dock. Mean SL increased from 25.2 to 33.8 mm in the dock module and 24.4 to 32.2 mm in the lab module. The abalone nursery modules were a popular attraction at the PTMSC, volunteers used the exhibit to educate and inform visitors of broader abalone recovery efforts. Given the tremendous positive feedback from the public, maintaining abalone at the PTMSC proved to be a successful pinto abalone outreach tool. Growth rates were lower in modules held at private dock locations in the SJA, possibly due to inconsistent attention to feeding and cleaning. The pilot field nursery design proved to be very successful in terms of abalone survival and growth. With a stronger commitment of citizens to this type of project the number of outplant modules could be increased, giving this strategy a potential to make a significant contribution toward abalone recovery in the future.

Assessing abundance at 10 index sites in the San Juan Archipelago (SJA)

Ten permanent abalone index sites were established in 1992 in the SJA to monitor log-term trends of the SJA pinto abalone population. Seven non-destructive surveys were done from 1992 to 2009. Prior to this grant, these index sites showed a continued downward trend in abundance along with a trend of increasing mean size (shell length, SL). Two sites were extirpated in 2009, no abalone were observed within the index sites. With NOAA grant funding we were able to update this information by surveying all ten index sites in 2013. We found a 92% decline in index station abundance between 1992 and 2013 and 5 out of 10 stations were extirpated. The mean density of all stations was 0.012 abalone m^{-2} and all station densities were below the critical density range needed to prevent population collapse. The mean size of pinto abalone increased from 105.3 mm shell length (SL) in 1992 to 118.4 mm SL in 2013. Only 1 abalone observed during 2013 surveys of index stations was considered to be an emergent juvenile recruit (defined as SL<90 mm). The percent of emergent juvenile recruits compared to the total number of pinto abalone between survey years 1979 (from timed swim surveys), 1992 (index station survey) and 2013 (index station survey) has declined from 31.8% to 17.4% to 7.1%, respectively.

The trend data suggests that pinto abalone populations in Washington continue to decline and recruitment has failed over the last several decades. This information has helped to inform stakeholders of the urgency to respond to and improve interventions and has also informed a NOAA biological review team that is considering petitions to list pinto abalone as either threatened or endangered under the Endangered Species Act.

Synergies and leveraging of funds

In addition to furthering abalone restoration efforts in Washington, NOAA funding support under this grant and in-kind use of the NOAA Mukilteo Research facility has provided a stable infra-structure for collaboration of professional researchers, opportunities for student research, hosting of interns, providing education opportunities for students attending Western Washington University and the University of Washington, providing employment opportunities for biological staff and hatchery technicians, and developing community outreach. Local TV and radio stations have elevated public awareness by broadcasting news coverage of Washington restoration outplants and KCTS public TV and local NPR station KUOW have developed program content for abalone restoration outplants. The progress made under this grant, combined with an elevated public awareness, has helped PSRF and WDFW obtain additional funding from the Washington Department of Natural Resources to continue abalone restoration work following completion of this NOAA grant. The Washington legislature made a specific line item budget allocation of $100K for the 2013-15 biennium under the Shellfish Initiative to develop new shellfish hatchery facilities at the NOAA Manchester Research facility. The established hatchery and developed methods, plus the relocation of a NOAA project working on ocean acidification questions to the Mukilteo Research facility, has inspired the Tulalip Tribe to submit a proposal to NOAA to continue and expand hatchery production, increase pinto abalone assessment locations and depth ranges, and to answer key questions about *in situ* ocean conditions at outplant sites. The 2013 index station survey, funded by this grant, was used to provide the NOAA biological review team with updated pinto abalone stock status information, which may be used as part of the consideration to list this species as threatened or endangered. As a result of this grant, a manuscript describing optimization of tagging trials was published (Hale *et al*. 2012); a manuscript describing GABA trials to optimize abalone settlement will be submitted to a peer reviewed journal in December, 2014; and a manuscript of juvenile abalone PIT tagging trials at the SPMC intake line reef will be submitted following a final survey in the spring of 2015.

*Throughout this report acronyms are used for abalone locations to comply with WDFW policy #5210 for disseminating information about sensitive species and habitats.

PROJECT GOALS AND OBJECTIVES

The main goal of the Washington State pinto abalone recovery team is to reverse the decline of abalone stocks in the Pacific Northwest, via supplementation, and to prevent abalone extirpation. The strategic goal of the recovery team is to return the abalone population to a self-sustainable level. In February 2011, the recovery team was awarded a three-year NOAA Species of Concern (SOC) Grant funding a project focused on species restoration methods for the pinto abalone (*Haliotis kamtschatkana*). Prior to the award, Washington state restoration efforts had included development of hatchery and nursery programs for captive propagation and rearing of abalone. The project focus was further development and optimization of abalone husbandry protocols and restoration methods that would achieve Washington State recovery goals and also inform other West Coast abalone restoration efforts. The specific objectives of the project, "Abalone Restoration in the Pacific Northwest" were to:

1) Optimize captive spawning and rearing methods at the Mukilteo and Port Gamble facilities.

2) Develop additional tagging methods to improve estimates of outplanted juvenile and aggregated adult abalone survival and growth.

3) Evaluate the efficacy of aggregating abalone in the wild as a method of enhancing natural recruitment.

4) Outplant additional juvenile abalone in the San Juan Archipelago to attain localized minimum reproductive densities and to increase the number of discrete families at outplant sites.

5) Conduct high resolution temporal sampling of outplant sites to improve recent, post-outplant survival estimates.

6) Develop and deploy pilot field nurseries to help engage and involve dock and marine facility partners, thereby building greater community support for abalone recovery efforts. Conduct outreach to involve volunteers in tagging abalone and preparing transport tubes prior to outplants.

7) Assess 10 index sites in the San Juan Archipelago to evaluate abundance trends and size structure of the wild population.

Funding of this project provided opportunities to test novel approaches to hatchery optimization and field outplant strategies, advancing the overall knowledge base for abalone restoration efforts coast wide. Additionally, fulfillment of the stated project goals allowed for practical implementation of several key recommendations of the abalone recovery plan for Washington State. The body of work represented within the scope of this grant provides a foundation and is

an essential step toward restoring Washington State Pinto abalone populations to self-sustaining levels. Specific project activities are described below in order of the seven stated objectives.

Grant Objective 1

Optimize captive spawning and rearing methods at the Mukilteo and Port Gamble facilities.

Hatchery management & maintenance

The Puget Sound Restoration Fund (PSRF) coordinated and managed all activities at the pinto abalone restoration aquaculture hatchery, located at the NOAA Mukilteo Research Station. A number of abalone recovery collaborators were involved in conservation aquaculture activities at the Mukilteo facility during the extent of the SOC grant period including PSRF, NOAA, Washington Department of Fish and Wildlife (WDFW) and University of Washington (UW). Hatchery management responsibilities during this time period included coordination and supervision of daily coverage, weekly maintenance and other regular activities:

- Tank cleaning & filter changes.
- Water quality monitoring - temperature, salinity, pH, dissolved oxygen and total gas saturation.
- Animal health monitoring - mortalities and live juveniles sampled for histology and molecular diagnostics as part of annual comprehensive hatchery health screening.
- Abalone maintenance - inventory, measuring, weighing, feeding, tagging, genetic sampling.
- Systems updates - plumbing, pump & heater maintenance, tank rack construction.
- Supervision, training and direction over student, intern and technician research projects.
- Production - broodstock conditioning, induced spawning, larval rearing, nursery, juvenile grow-out, diatom and macroalgal culture.

Hatchery health assessments, completed by Dr. Carolyn Friedman of the UW, were conducted annually. No notifiable pathogens were detected during the assessments. Hatchery broodstock mortality was higher than expected over the course of the grant period and was attributed to aging combined with reproductive stress. Individuals that died were more frequently female, although a number of ripe males also died. Losses were attributed to gonad maturation coupled with lack of spawning leading to physiological stress and mortality.

PSRF hatchery staff members collaborated with University of Washington scientists to measure pH more accurately by using spectrophotometry and preserving samples for total alkalinity and total CO_2 (on selected samples, see Table 1 below). Spectrophotometric pH measurements were as low as 7.55 at times. This infrequent low pH likely impacted shell development, health and survival of hatchery raised abalone during larval and juvenile life stages. Closer examination of

these impacts will be necessary to determine more detailed impacts on gonad maturation, egg quality, fertilization success, settlement competency, metamorphosis and early growth and survival. It was determined that implementation of seawater buffering systems at the hatchery to ameliorate low pH were necessary. Beginning in November 2013, seawater supply to the hatchery, nursery and grow-out greenhouse has been buffered with sodium carbonate to elevate pH above 8.0. This requires regular probe calibration, controller/dosing pump maintenance and production of buffering solution.

TABLE 1.

Mean (± standard deviation) of pH, pCO_2, and aragonite saturation state (Ω) of seawater samples from the Mukilteo Field Station collected in 2013 (n=15).

	pH	**pCO_2**	**Ω Aragonite**
Mean	7.81	694.48	0.98
SD	0.06	104.55	0.12

Due to recent difficulties in spawning pinto abalone during their historic reproductive period, combined with our observations of low pH waters, the effects of ocean acidification on abalone reproduction and larval survival were examined. The effects of reduced pH on pinto abalone broodstock conditioning and larval survival were tested by UW and PSRF staff at the UW Friday Harbor Marine Labs. Although broodstock conditioning pH (reduced or ambient) did not influence larval survival ($F=1.835$, $df=1$, $P=0.185$), fertilization and larval rearing condition were influenced. Larvae held at pH 8.0 had significantly higher survival than at 7.6 ($F=14.842$, $df=1$, $P=0.001$). In addition, an interaction between conditioning pH and fertilization/larval rearing was observed ($F=8.882$, $df=1$, $P=0.006$). Larvae whose fathers were conditioned at pH 8.0 but were fertilized and maintained in pH 7.6 seawater survived worse (59%) than those in all other treatments combined (71-79%). These data illustrate that seawater pH may influence pinto abalone fitness and that the influence of ocean acidification on the ability of pinto abalone to maintain populations warrants further study.

Microalgae culture systems were expanded during this grant and two strains of benthic diatoms were purchased to enhance post-larval feeding and growth. *Amphora* sp. and *Navicula* sp. have been used successfully in abalone aquaculture worldwide. PSRF hatchery staff have now cultured *A. salina* and *N. incerta* at the Mukilteo, Port Gamble and Manchester facilities. Various diatom culture techniques have been tested and when our diatom production with these two strains is consistent, post-larval abalone feeding trials will begin to optimize nursery diets.

Hatchery manager, Josh Bouma, visited commercial abalone aquaculture facilities in California in October 2012 (partially funded through the NOAA SOC internal and a grant to Friedman, UW) to gain insight into efficient and productive abalone hatchery methods. Managers at The Abalone Farm in Cayucos and The Cultured Abalone in Goleta shared information on broodstock conditioning and selection, induced spawning methods, larval culture and health management, settlement and post-larval nursery, early feeding strategies and general grow out.

Broodstock Collections

To increase the genetic diversity of the broodstock population, offset hatchery mortalities and meet program management goals for increased production potential, additional abalone were collected from the San Juan Archipelago (SJA) annually. Collection objectives were to obtain both male and female abalone, to collect animals that displayed a gonad index of 2-3 (i.e. "ripe" animals), and to collect from multiple locations. Abalone were collected within the SJA across a wide geographic range. The primary collection goal was to target reproductively isolated individual animals and make every effort to avoid disturbance of existing natural aggregations. Divers searched collection sites to ensure that each collected individual singleton abalone was separated by at least 15 meters linear distance from local conspecifics. Divers from WDFW, PSRF, Western Washington University's Shannon Point Marine Center (SPMC) and the Jamestown S'Klallam Tribe participated in broodstock collections.

During the course of the SOC funding period, a total of 51 new abalone were collected from the wild including 37 females and 14 males. All new broodstock abalone were tagged, measured, weighed and delivered to the Mukilteo hatchery. Tags included both disc and bee tags affixed to the exterior shell. These abalone were also PIT tagged to provide efficient and permanent identification. Genetic tissue samples (epipodial tentacle clips) were taken from each individual for genotyping and samples were archived at the UW Friedman Lab.

Hatchery production

The primary production objective during the 2011-2014 spawning seasons was to conduct either single-parent crosses or partial factorial matrix crosses with each induced spawning event to maximize the genetic input of our broodstock and produce as many discrete F1 families as possible. In Grant Year 3 (GY3), the abalone hatchery upgraded from six egg hatching and larval rearing systems to eight, increasing our ability during each spawning event to produce eight distinct crosses. The decision to conduct single parent vs. partial factorial matrix crosses was based on how many broodstock were successfully induced to release gametes and maximizing the contribution of as many parents as possible within the eight culture systems available.

In GY1, five spawning events between September and November 2011 produced usable quantities of gametes from both sexes that resulted in healthy larvae competent for settlement.

Several other spawns produced gametes but in insufficient numbers for fertilization. Fourteen of our broodstock abalone (7 females and 7 males) contributed gametes to an F1 batch of offspring. Eight distinct crosses were produced, all of which were single parent crosses. An estimated 957,000 healthy larvae from these 8 distinct crosses were settled into nursery tanks in the Mukilteo wet laboratory and greenhouse. Additionally, several spawning attempts with F1 abalone were successful and a number of F2 families were produced. An estimated 184,000 and 95,000 F2 larvae and were settled in Mukilteo and at the Port Gamble nursery respectively. In keeping with our conservation genetics protocols, F2 families were only used for research and not for restoration activities in the field.

Broodstock conditioning (primarily for females) during the latter half of GY1 (early 2012) in preparation for reproduction was slow and abalone during this period generally did not show evidence of ripening. All six spawning attempts between May-August 2012 produced sperm but no eggs.

In GY2, broodstock conditioning in preparation for induced spawning occurred later in the season than expected and two spawns were successful in November, 2012. During these two spawning events, 12 broodstock released gametes (7 females and 5 males) and 12 unique single-parent crosses were produced. A total of 911,000 healthy larvae were settled into nursery tanks in Mukilteo. Survival was high for three of these families and low for the remaining nine.

In contrast to 2012, broodstock abalone during the latter half of GY2 displayed early gonad ripening and were successfully induced to spawn beginning in June 2013. Between June and August, 2013, four spawning events from four attempts were successful in producing usable quantities of gametes from both sexes that resulted in healthy larvae competent for settlement. Sixteen of our broodstock abalone (8 females and 8 males) contributed gametes to an F1 batch of offspring. Fifteen distinct crosses were produced, all of which were single parent crosses. An estimated 1.19 million healthy larvae from these 15 distinct crosses were settled into nursery tanks in the Mukilteo wet laboratory and greenhouse.

In GY3 (early 2014), broodstock conditioning in preparation for induced spawning occurred rapidly. This is possibly due to broodstock females having been newly collected from the wild in March and April with some gonad maturation already underway upon arrival at the hatchery. From May through July, gametes were captured from four induced spawns and one volitional spawn. Fourteen broodstock released gametes during this time (5 females, 9 males) and 12 unique single-parent crosses were produced. A total of nearly 1.1 million healthy larvae were settled into nursery tanks in Mukilteo. Post-set abalone from all 12 2014 crosses are visible to the naked eye and early estimates indicate that survival has been high in several, but not all, of these families.

Broodstock abalone conditioning trials, with supplemental funding by a NOAA SOC internal grant program, were conducted in GY1. In summary, it appears that female broodstock were

better conditioned when held outdoors in a greenhouse than those held indoors under artificial light. This could be a response to natural vs. artificial light, natural vs. artificial day length, better accumulation of the diatom biofilm or seawater temperature. Conditioning was not enhanced by feeding broodstock an artificial diet used in commercial abalone aquaculture. Our recipe was supplied by The Abalone Farm in Cayucos, CA and produced at the Mukilteo hatchery. The artificial feed was made by combining 32.2% yellow corn flour; 20.1% nutribinder superpowder; 14.7% fish meal; 12.1% vital wheat gluten; 12.1% whole wheat flour; 4.0% kelp flour; 4.0% soy flour; and 0.8% manucol HV. The dry ingredients were combined with water to make a pasta like paste and were processed through a pasta maker. After the feed was made, it was dried under a fan and kept in a refrigerator for storage. This artificial diet fed to abalone during a three month conditioning period did not result in increased gonad index, higher induced spawning success rates or greater numbers of eggs released from females during induced spawning attempts when compared to abalone conditioned on the standard diet of fresh macroalgae.

In total over the duration of this funding period, which included four spawning seasons within the hatchery, 47 genetically distinct F1 families were produced, mostly from single-parent crosses. More than 4.16 million larvae were successfully reared through the larval period and induced to settle into nursery aquaculture tanks. Our bottleneck within the hatchery continues to be survival through the early post-larval life stage. Mortality rates are high during the first three months post-settlement and this is likely due to a combination of competition from high density settlement rates and a failure to provide an optimal diet. Survival rates for abalone in the hatchery from larval competency to juvenile outplant size 18 months later (shell length 20mm) remain below 1%. While the number of larvae reared and settled is not a good indicator of whole hatchery production success, this number does indicate that our ability to condition broodstock, induce spawning from multiple males and females and rear genetically diverse larvae to settlement competency has improved.

Hatchery inventory of 2012-2013 cohorts was recently conducted and more than 3000 juvenile abalone from 16 families ranging in size from 5-30 mm in shell length from these families are being reared successfully at the Mukilteo facility. A majority of these animals will be used in supplementation activities scheduled for early spring 2015 in the SJA.

GABA optimization for larval settlement

Larval settlement and metamorphosis are key stages in successful culture of abalone species for both commercial and restoration efforts. Gamma-aminobutyric acid (GABA) is widely used in hatcheries to induce settlement. Larval pinto abalone were reared to competency and induced to settle with 0, 5, 10, 20, 40, 60, 80 and 100 μM GABA concentrations at durations of 0.5, 3, and 15.5 hrs. At sixty hours following GABA exposure, survival and metamorphosis were evaluated using light microscopy. Metamorphosis and survival were observed 14 days post settlement. Metamorphosis and survival in the 15 hour groups were limited due to high GABA

concentrations. There were no differences in initial survival proportions within or between the 30 min and 3 hour treatment groups. There were also no differences in initial metamorphosis proportions in the 30 min treatment groups, excluding the controls.

A second trial was conducted examining more specific concentrations at a single, shortened exposure duration. GABA concentrations of 0, 5, 10, 15, 20, and 25 μM were used for a duration of 45 min. Metamorphosis and survival were observed at sixty hours and 14 days post settlement. Initial review of results from the second trial indicates there may be no differences in metamorphosis and survival, excluding controls, across the five treatments, although survival was highest at the end of the trial within the 15 μM treatment group. Shorter exposure duration to GABA and therefore shorter static water times, could lead to better hatchery metamorphosis and survival rates of abalone. Determining optimal usage of this chemical cue by increasing concentrations and shortening exposure durations may be used to great effect in larval seeding abalone restoration strategies.

Preparation of a manuscript describing all results from our GABA optimization experiments at the hatchery is nearly complete and will be submitted for peer reviewed publication in the Journal of Shellfish Research by the end of December, 2014.

Pinto abalone genotyping

Until recently, pinto abalone genetics were poorly understood. Partially through the SOC grant, genotype work at the UW Friedman Laboratory yielded four microsatellite loci that were sufficient to infer parentage in hatchery reared pinto abalone individuals. Progeny abalone were successfully typed back to parents. The four microsatellite loci were also used to genotype wild broodstock and demonstrated that, while most individuals were unrelated, some individuals had a high probability of a sibling relationship. A fifth microsatellite locus was optimized, and used to test relatedness and inbreeding coefficients on wild caught individuals. Abalone collected through 2013 were analyzed with 4 loci (Figures 1 and 2) and all abalone samples, including those collected in 2014 (n=19) were analyzed with 5 loci (Figures 3 and 4). As some loci did not amplify from individuals, these animals were omitted from analysis.

Using 4 loci, full sibship was estimated by the program COLONY (Wang, 2004) for three pairs of individuals of 65 broodstock tested: FY41 and MY16, FY50 and MY29, and MY75 and FY32. For these pairs of individuals, COANCESTRY (Wang, 2011) estimated the following relatedness:

a. FY41 and MY16 (fullsibs by Colony) with relatedness probability of 0.5817 (95%CI = 0.011-0.909) - 0.7498 (95%CI = 0.250 -1.00) using the estimators of Wang and TrioML from Coancestry 1.0.1.2, respectively.

b. FY50 and MY29 with a relatedness probability of 0.225 (95%CI = 0.000-0.500) - 0.292 (95%CI =-0.053-0.537) using the estimators of Wang and TrioML from Coancestry 1.0.1.2, respectively.
c. MY75 and FY32 (Colony Program) with a relatedness probability of 0.3633 (95%CI = -0.075-0.7276) – 0.2866 (95%CI = 0.000 – 0.750) using the estimators of Wang and TrioML from Coancestry 1.0.1.2, respectively.

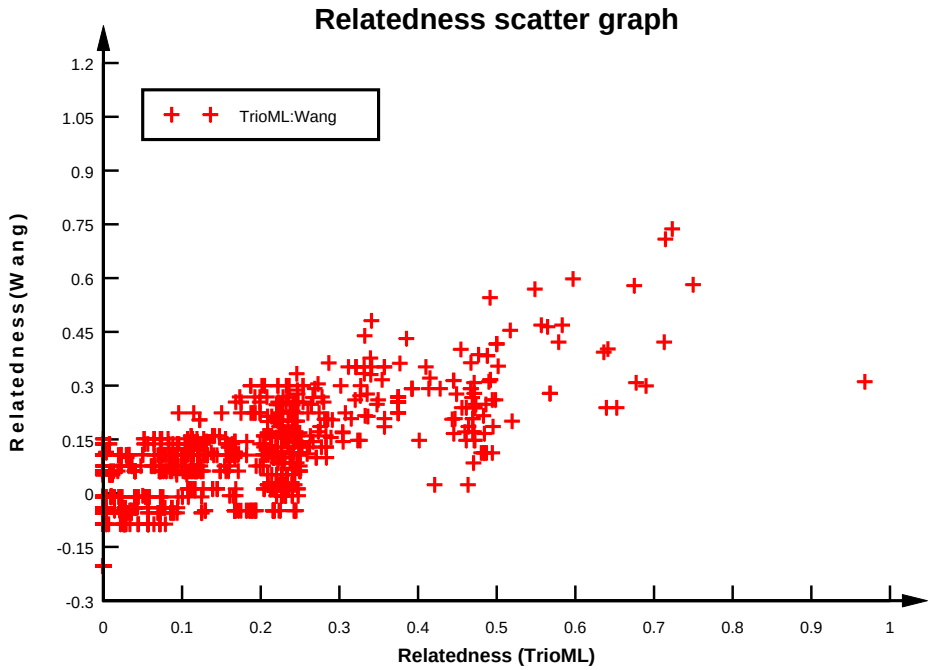

Figure 1: Relatedness scatter graph showing concordance of relatedness of three pairs of tested hatchery pinto abalone broodstock.

Coancestry was also used to estimate inbreeding. Figure 2 below illustrates that using the four microsatellite loci for pinto abalone, that only two individuals had an inbreeding coefficient over 0.5 (denoted by dark red individual tag number on X axis) and another six individuals had inbreeding coefficients between 0.2 and 0.47 (orange individual tag number on X axis). These data also support our finding that most individuals collected for captive rearing are unrelated or not closely related and that inbreeding depression is not a significant threat to the captive broodstock program.

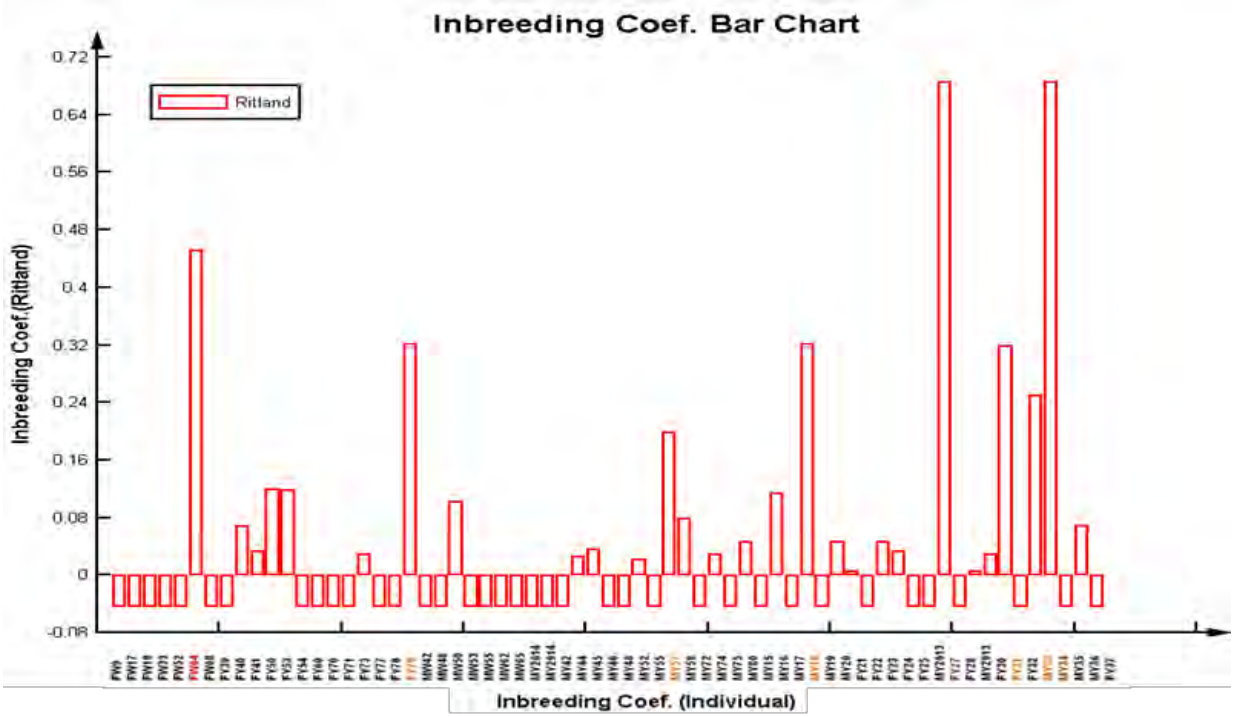

Figure 2: Bar chart showing inbreeding coefficients for tested hatchery pinto abalone broodstock estimated using 4 microsatellite loci.

Using 5 microsatellite loci, we observed a higher degree of inbreeding using two separate methods (TrioML and Ritland), illustrating variation in estimates of inbreeding (Figure 3). The Ritland estimator is more conservative than those of TrioML (Figure 4).

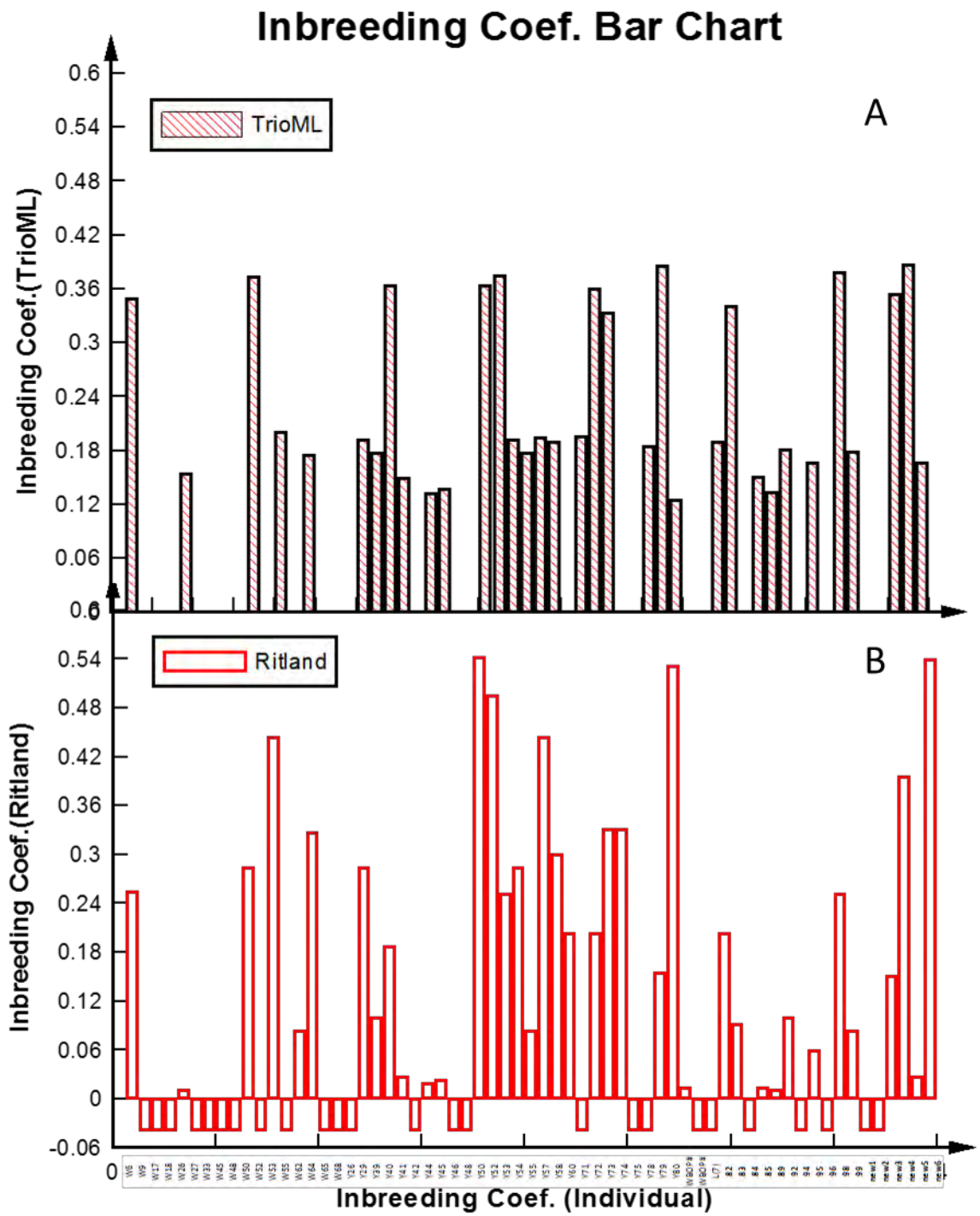

Figure 3: Bar chart showing inbreeding coefficients for tested hatchery pinto abalone broodstock estimated using 5 microsatellite loci.

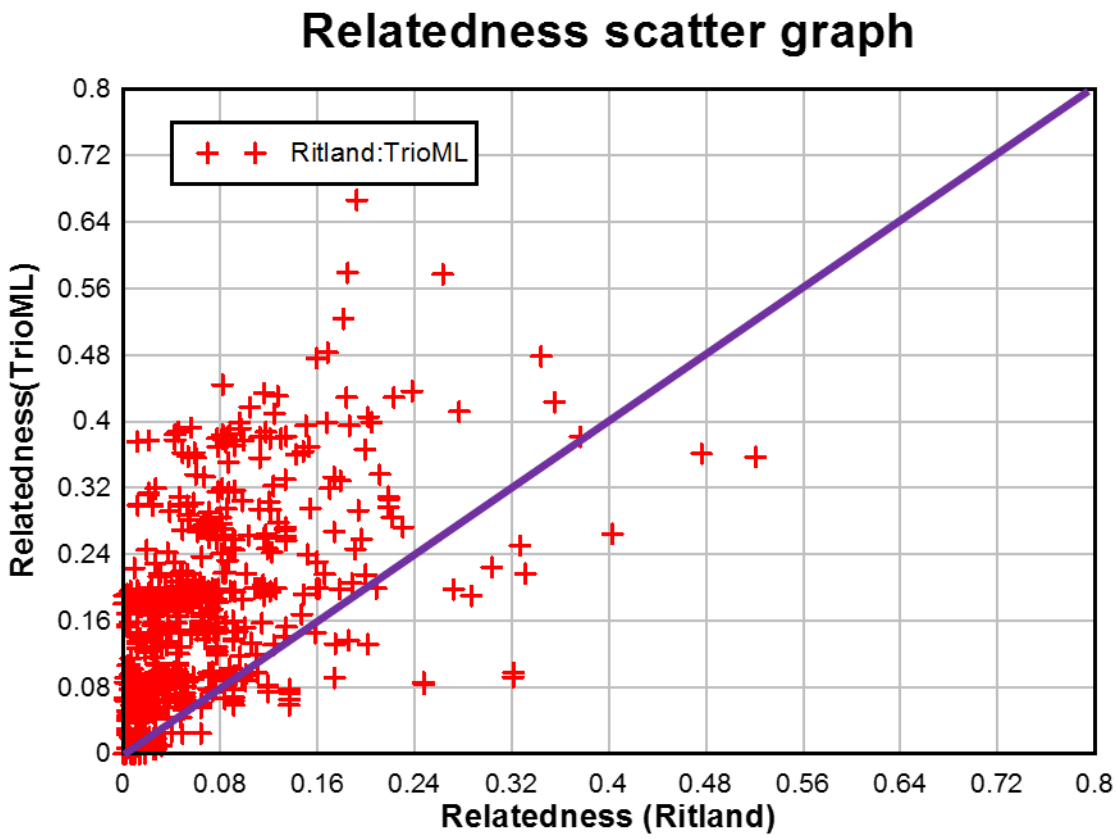

Figure 4. **Scatter plot illustrating the degree of concordance between inbreeding estimators Ritland and TrioML. The diagonal line indicates complete concordance and demonstrates that TrioML estimates a higher inbreeding coefficient than does the Ritland estimator.**

Projected relationships (using the freeware ML Relate [Kalinowski *et al*. 2006]) indicated that, while many individuals were unrelated to one another, a number of individuals were estimated as half-sibs (HS, share one parent). A few individuals were estimated as full-sibs (FS, sharing both parents), and one potential parent-offspring (PO) relationship was estimated (Figure 5). The small pinto abalone meta-population size highlights the need to genotype individuals in a conservation breeding program to reduce the potential mating of closely related individuals.

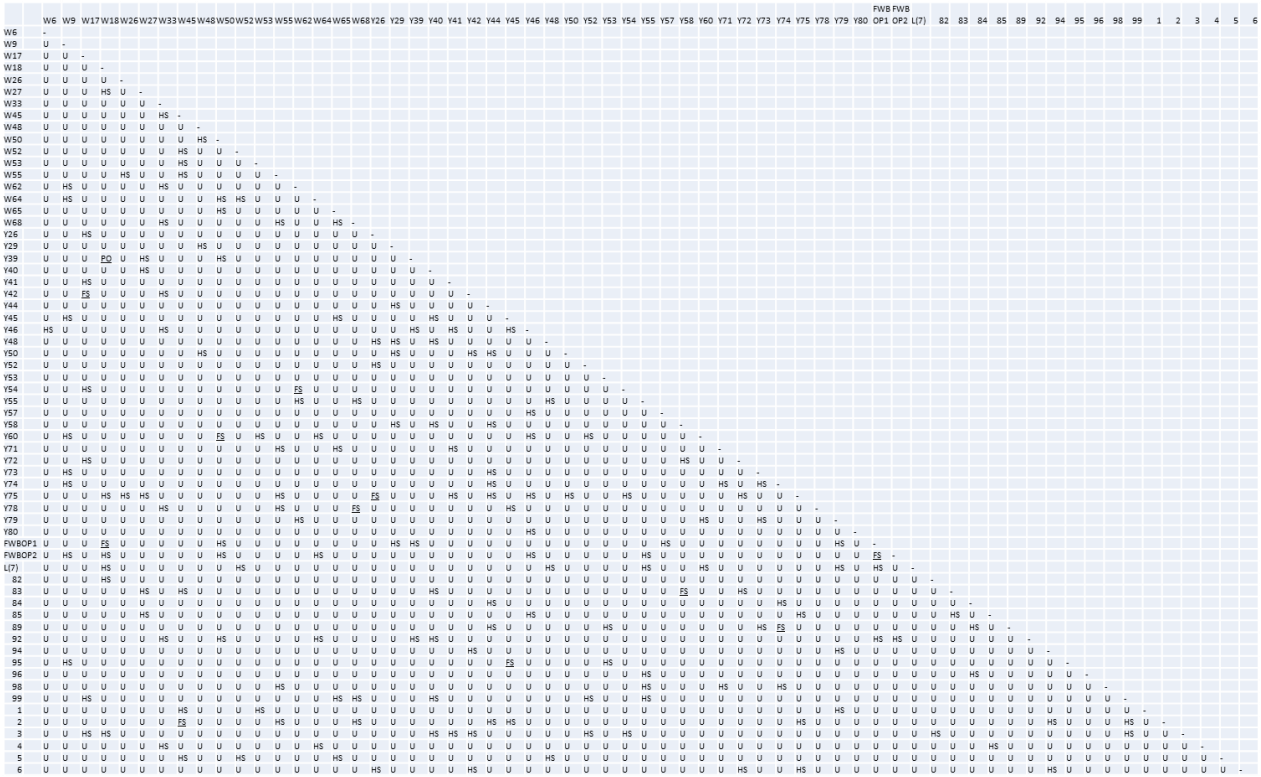

Figure 5. **Projected relationships among wild caught pinto abalone from San Juan Archipelago. Individuals were estimated as unrelated (U), half-sibs (HS), full-sibs (FS), and a single parent-offspring (*PO*). Note that most estimated relatedness among individuals is at the half-sib level (sharing one parent).**

Grant Objective 2

Develop additional tagging methods to improve estimates of outplanted juvenile and aggregated adult abalone survival and growth.

Laboratory studies

Tagging methods for pinto abalone have included attaching stainless steel tags wired through respiratory pores and adhesion of bee tags and plastic disc tags near the spire of the abalone. These methods are unreliable due to tag loss, shell erosion and encrustations that obscure the tag identification. A controlled study was completed at the Mukilteo Research facility testing Passive Integrated Transponder (PIT) tags as a tagging method for abalone. In trial one, 9 mm PIT tags were attached to small adults at the dorsal exterior and ventral interior of the shell using cyanoacrylate glue and by injection into the foot muscle. Growth, survival and tag retention were tracked for 15 months. PIT tags did not appear to influence animal growth as evidenced by similar growth rates among tagged (inner shell, outer shell and inter-muscular) and untagged individuals (ANOVA, F=1.40, 3 df, P = 0.262). Survival was similarly unaffected by tagging

(x^2=0.57, 1 df, P=0.45). Tag retention by adhesion to the shell internally and externally was very good (90 and 80% respectively) as compared to injection into the foot (10% retention).

In a second trial, 8.4 mm PIT tags were applied to the ventral anterior of the shell of juvenile abalone (<25 mm SL) and growth, survival and tag retention were monitored for 6 months. While no differences in survival between control and tagged animals were found and tag retention was high, this tagging method for small abalone needs refinement. Abnormal epipodial tissue near the tagging site was observed in eight juveniles, seven abalone displayed abnormal shell growth and a majority of tagged juveniles exhibited receded mantle tissue that had failed to cover the tag. In small abalone, the relatively large tag size combined with insufficient mantle tissue to cover the PIT tag may preclude tag assimilation within the nacreous shell layer. Only one juvenile tagged abalone in the second trial had mantle tissue covering the tag with deposited nacre over the PIT tag.

Overall, adhering PIT tags to the ventral anterior of the shell had the highest retention rates, and adult abalone incorporated the tags into their shell by forming nacre over them. PIT readers were still able to detect the tags even when the tag was incorporated into the shell. Results of this study have been published in Hale *et al.* (2012).

PIT tag reader development & field trials

Due to an unexpected reduction of SOC funding in GY2, the next phases of abalone PIT tagging development were supplemented with funding from the Washington Department of Natural Resources (WDNR) and allowed PIT tagging work to continue. This included development of two different underwater tag readers rated to dive depths, and field trials using these readers to survey sites outplanted with PIT tagged reintroduced broodstock and hatchery reared young adult abalone.

The first reader was developed with advice from California Department of Fish & Wildlife personnel Ian Taniguchi, Derek Stein and Kai Lampson. These biologists have successfully marked and tracked adult abalone using PIT tags adhered to the shell exterior. An HPR Plus reader and antenna were acquired from Biomark, a leading developer of PIT tags, readers and applications of tagging in various fish & wildlife research and conservation projects. Prevco Subsea Housings customized a housing for the Biomark HPR Plus, including modifications to the antenna to ensure water resistance to depth (Figure 6).

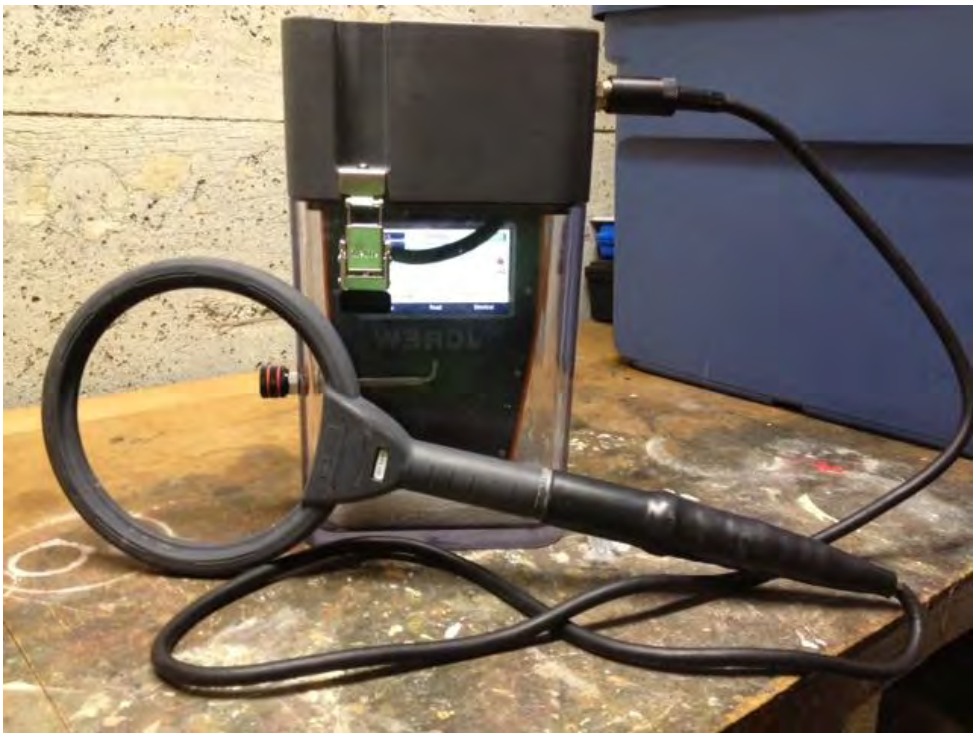

Figure 6. Biomark HPR Plus PIT tag reader contained in a custom Prevco Subsea dive housing.

The second reader was developed by NOAA biologists Todd Bennett and Gabriel Brooks, both of whom have extensive experience in fish tagging projects and PIT reader construction. This reader was assembled from recycled and inexpensive parts including the antenna coil, reader board, LCD display, rechargeable battery pack, power switch and data download port. The antenna and reader body were made diveable by encasing all components in ¾" (antenna) or 2" (reader body) PVC pipe (Figure 7).

Figure 7. Underwater PIT tag reader developed and constructed by NOAA biologists.

Field Trial 1: PIT tagged reintroduced hatchery broodstock

In 2012, pinto abalone broodstock were reintroduced to the wild. This batch of broodstock had been in the hatchery since 2008 and had been collected from the waters of the San Juan Archipelago as part of a broodstock aggregation study and hatchery restocking. These abalone had been part of the hatchery spawning rotation each season from 2008-2012 and many contributed gametes to various F1 generation crosses used in juvenile outplanting restoration efforts. Before removal from the hatchery, a full disease and health screening was conducted.

Twenty one broodstock, (11 females, 10 males) were measured and weighed, and marked with a white plastic disc tag (Floy Tag & Mfg. Inc., Seattle, WA) glued immediately forward of the shell apex, a white bee tag (The Bee Works, Orillia, Ontario, CA) glued to the rear of the shell apex, and a 9 mm full-duplex PIT tag (HPT9, Biomark Inc., Boise, ID) glued to the ventral surface of the shell beneath the mantle tissue. PIT tagging occurred 18 months prior to reintroduction and all tags had been fully embedded in nacre. Abalone were transported to a west San Juan Island aggregation site that last received hatchery broodstock and aggregated

abalone in early 2008. The most recent previous survey of this site occurred in February 2012. A central area was identified within the aggregation plot and all 21 abalone were located onto this roughly 9 m^2 area. A brief examination of the surrounding habitat was conducted and several resident abalone were sighted. Several large *Pycnopodia* sea stars were removed from the area to reduce predation pressure at a time that abalone might have been experiencing handling stress.

Seven months later, the study site was revisited and exhaustively surveyed with the NOAA PIT tag dive reader to collect mark-recapture data on the adult abalone reintroduced to this site. Personnel, equipment, boat and travel expenses for this survey were paid for with funding from both the WDNR contract and from the NOAA SOC grant. All four plot corners were located, the perimeter was delineated and the plot was divided into seven lanes, each 3 m wide. Divers surveyed side by side within each lane carefully examining all substrate using dive lights and mirrors to investigate crack, crevice and overhang habitat. All abalone observed were measured for maximum shell length and examined for disc tags, bee tags, and swept with the underwater reader for PIT tag ID recovery. While the primary objective was to recover PIT tag identification from reintroduced abalone, data were collected from all abalone observed including those without any numbered tag or PIT tag ID, abalone that were either wild or potentially from a previous adult aggregation project.

Twenty-four live abalone were observed and eight abalone mortalities were collected during this survey. Of these observations, eleven live abalone and four empty (mort) shells were positively identified with the underwater PIT tag reader. When combining PIT tag identification of live and dead abalone, more than 71% of the PIT tagged reintroduced adults were recaptured after seven months in the field during this single survey. Performance of the underwater reader developed by NOAA exceeded expectations. As determined in lab trials, read range is limited when using small full-duplex tags in seawater and it was necessary to position the reader antenna within 6” of an observed abalone to acquire a tag ID. The reader performed well when identifying multiple abalone in an aggregation, and when sweeping deep within crevices and underneath boulders.

A notable observation from this survey was the high occurrence of recovering a PIT tag ID from an animal whose disc or bee tags were either missing or unreadable due to numbers scratched off the tag, biofouling over the tag, or positioning of the abalone prohibiting visual tag ID. This observation confirms the efficacy of PIT tags over other commonly used methods for tagging abalone. Our previous estimates of disc and bee tag longevity from outplant and aggregation work assumed tag retention for about one year. This study demonstrates that visual tags on abalone can be entirely useless within seven months of introduction to the wild.

Field Trial 2: PIT tagged hatchery reared young adult abalone

A second PIT tagging field trial was initiated to determine whether PIT tags are more identifiable than commonly used bee tags for marking juvenile or young adult abalone in the field. This study will also estimate tag retention over time and survival of PIT tagged young abalone in wild. This study is ongoing and will be finalized in 2015 with funding from the Skagit County Marine Resources Committee. This study will also compare performance of the two underwater readers that have been developed.

For the second tagging field trial, PIT tags (9 mm HPT9 FDXB, Biomark Inc., Boise, ID) were secured as described above on 40 juvenile to young adult abalone produced at the Mukilteo hatchery at least 18 months prior to initiating the field trial. Abalone ranged in size from 35-98 mm shell length (SL) (Mean SL=68 mm) at the time of outplant. Each individual was also marked with a uniquely color coded and numbered bee tag immediately prior to introduction to the study site.

In collaboration with SPMC personnel, the SPMC seawater intake line reef was selected as an ideal site for this field trial. Intake lines run several hundred feet offshore from the beach and are secured to the bottom by 3'x 3'x 2' concrete anchoring blocks. Each block is surrounded by sand, gravel and some cobble. Four replicate ARMs (abalone recruitment modules-modified commercial crab pots with securable hinged lids) were placed on top of the intake line concrete anchoring blocks, one ARM per block. ARMs were filled with fist to head sized coralline encrusted cobble. While the wire mesh enclosing each unit is large enough to allow abalone to move from the module, ideal substrate within each unit acts as an isolated island habitat surrounded by less desirable substrate intended to reduce emigration of abalone away from the module.

In 2013 each of the four ARMs were seeded with ten tagged abalone. Six weekly surveys were conducted during which each diver conducted observation of the modules both visually for bee tags and with the submersible PIT reader. Bee tag observations and positive PIT tag identifications were recorded on dive slates. One final dive survey will be conducted to compare reader performance and tag longevity one year after introduction.

Only three mortalities were recorded and it appeared a majority of the abalone either remained in the modules or were residing on the concrete anchoring blocks directly beneath the ARMs. Percentage of bee tag observations and positive PIT tag IDs were variable from ARM to ARM and survey to survey, but there was no significant difference in ability to record abalone IDs between the two tagging methods or between the two readers. There were several instances where sweeping the ARM with the reader provided a positive PIT ID but no visual confirmation of the abalone. In these occurrences it was difficult to confirm if this was a live abalone or empty shell hidden within the cobble or underneath the concrete block. Results from this study

will be submitted for publication in a peer reviewed journal following our final survey by spring 2015.

Grant Objective 3

Evaluate the efficacy of aggregating abalone in the wild as a method of enhancing natural recruitment.

Aggregation of adult abalone in the wild has been proposed as a high priority restoration strategy in multiple Pacific Northwest abalone restoration plans. In 2008, the pinto abalone recovery team initiated a pilot aggregation study at two sites in the SJA where 30 total adult abalone were reintroduced and aggregated at each of the two sites. The source of the aggregated abalone were broodstock previously held at the Mukilteo hatchery (n=27) and singleton abalone (perceived as reproductively isolated), collected specifically for the aggregation study and originating from the southern SJA (n=33), total n=60. Hatchery broodstock and collected wildstock destined for the aggregation experimental site were tagged (numbered vinyl disk tag and bee tag affixed to exterior shell surface of each individual) for later identification. When combined with existing resident abalone observed on the sites (therefore untagged), minimum surveyed densities of 0.12 (Site SE) and 0.13 abalone/m^2 (Site TG) were estimated for the initial aggregation. Two follow up surveys were completed at 12 and 18 months post-aggregation to evaluate the persistence of aggregations. Post-aggregation, the respective observed densities at the aggregation sites were 0.10 and 0.09 (March 2009) and 0.09 and 0.08 abalone/m^2 (October 2009). Results of the initial aggregation and three subsequent post-aggregation surveys are reported in Friedman *et al*. (2011).

Within the scope of the present grant, objectives pertaining to aggregation as a restoration strategy were to: 1) Quantify the persistence of abalone aggregations for use in evaluating the long-term efficacy of this strategy; and 2) Quantify recruitment resulting from aggregations. To discern between aggregated abalone and recruits, genetic material was collected from aggregated abalone in 2008 and archived. Surveys of the existing aggregation sites were initially proposed for grant years 1 and 3 (GY1 and GY3), however, loss of grant funding during GY3 precluded a second full scale survey at both aggregation sites.

On February 7 and 8, 2012 (GY1), PSRF and WDFW biologists resurveyed each of the two abalone aggregation sites established in 2008. Resulting density estimates of the two surveyed sites for the all survey visits are shown below in Figure 8. Overall density of live observed abalone declined 41% (2008 initial n=44, 2012 n=26) at Site TG and 72% (2008 initial n=46, 2012 n=13) at Site SE over the four year period.

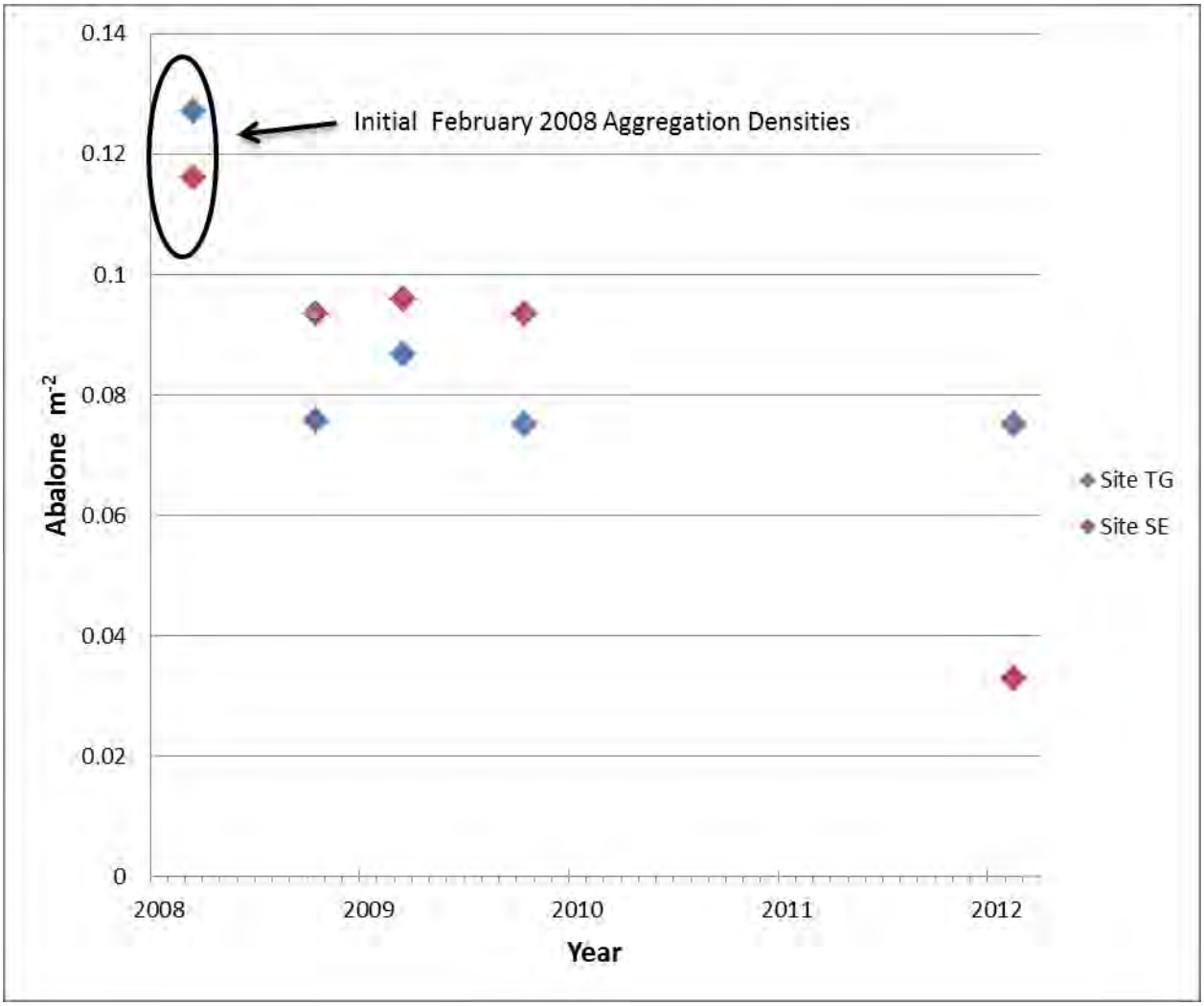

Figure 8: **Observed abalone densities from diver surveys of two aggregation sites in the San Juan Archipelago.**

Abundance of abalone observed during site surveys for each of the two sites is shown below in Figures 9 and 10. Total abalone counts are further differentiated for observed tagged and untagged animals for each survey period. Observed tagged abalone observations decline over the course of the study; no live tagged abalone were observed for either site during the final 2012 survey.

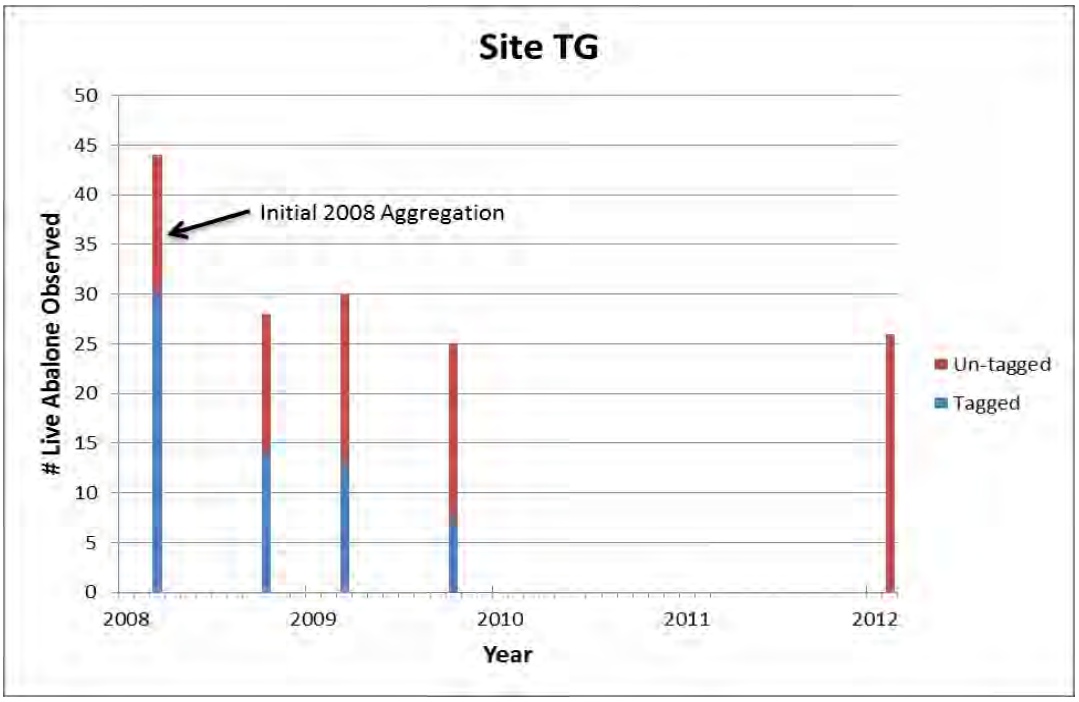

Figure 9: **Live abalone counts from diver surveys of aggregation study Site TG. Blue bars indicate tagged abalone, red bars indicate non-tagged abalone.**

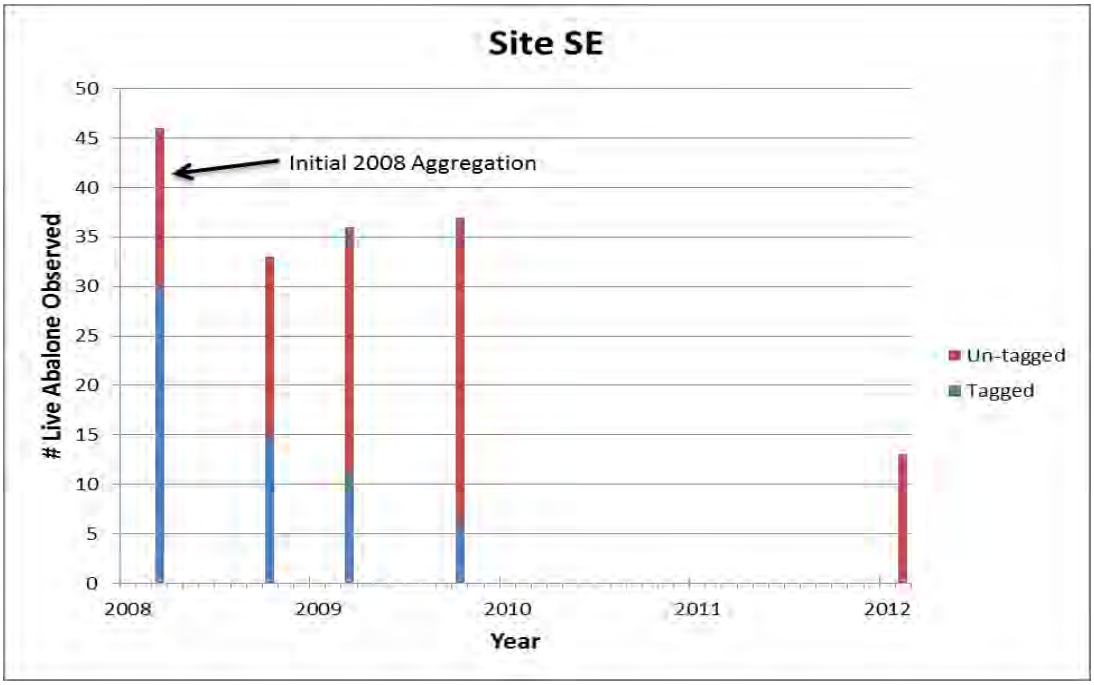

Figure 10: **Live abalone counts from diver surveys of aggregation study Site SE. Blue bars indicate tagged abalone, red bars indicate non-tagged abalone.**

Confirmed mortalities of initial aggregates (tagged animals, 30 per site) were 17 at Site TG and 11 at Site SE over the course of the four year study period. This represents a 57% and 37% confirmed loss of initial aggregates, for the respective sites, over the study period. Overall observed mortality (tagged and untagged mort shells observed within the sites during all surveys) were 29 and 30, for the respective sites.

Tag loss, or tag encrustation to the point that tags are completely obscured, is likely to have occurred with aggregated abalone during the course of the study. Friedman *et al*. (2011) reports that survey divers found partially encrusted abalone tags as early as 6 months post-aggregation. Crustose coralline algae is the primary encrusting organism observed obscuring both the disk and bee tags originally placed on that shell. Tags were occasionally discovered on collected mortalities (shells) when encrusting organisms were scraped off from the shell location where tags were affixed. Thus, it is apparent that the change observed in the abundance of tagged (aggregated) vs. untagged animals could likely be attributed in large part to tag loss rather than recruitment or immigration.

The two primary objectives for evaluation of aggregating animals as a restoration strategy for pinto abalone were to quantify the persistence of abalone aggregations and to quantify any recruitment resulting from those aggregations. The reported density necessary for recruitment in broadcast spawning marine invertebrates (Shepherd and Partington, 1995; Babcock and Keesing, 1999) is above 0.15 abalone/m^2. The initial 2008 aggregation elevated overall abalone density levels to 0.12 (Site SE) and 0.13 abalone/m^2 (Site TG). These aggregations were intended to establish pinto abalone densities simulating naturally occurring, reproductively successful, aggregations. Observed declines in site densities over the period of the study suggest that while aggregates did persist within boundaries of the study sites, subsequent observed mortalities have reduced site density to well below successful spawning threshold levels. Further, there was no observed evidence of recruitment attributable to the aggregations during the four year study. Proper analysis of persistence of aggregates was confounded by the complete loss of tag data.

In Friedman *et al*. (2011), analysis of mortalities of tagged animals suggest that reintroduced hatchery broodstock had a lower survival rate than wild aggregates (37% vs. 18% for both sites combined). The majority of re-introduced broodstock had been held in captivity for five to six years and may have been habituated to hatchery conditions and may have been older individuals nearing the end of expected life-span. Additionally, handling stress associated with the re-introduction may have weakened or injured animals, possibly attracting predators, and contributing to mortality observed during the study period. A potential concurrent factor that should also be considered is that significant reductions of wild stock densities were observed at two index stations located within the general vicinity of the aggregations during the same relative time frame. These two particular stations happened to be the highest observed densities of the ten index stations when surveyed in 2009 (Station DB 0.058 abalone/m^2, Station OEP 0.074

abalone/m^2). These stations were locally extirpated when surveyed in 2013 (See Grant Objective 7 discussion below for details).

In an attempt to address issues related to tag loss in assessing aggregation as a restoration strategy, PSRF and WDFW biologists reintroduced 21 PIT tagged pinto abalone broodstock to the TG aggregation site in September 2012 (additional PIT tagged abalone are not included in above figures). Details of the PIT tagging trials associated with this introduction are detailed above in the Grant Objective 2 discussion. Following the reintroduction of the 21 PIT tagged abalone to the TG site, overall site density (tagged and observed existing untagged animals) was estimated to have increased to 0.14 abalone/m^2. Subsequent surveys of the TG site in April 2013 (GY2) and March 2014 (GY3) using underwater PIT tag reader technology (see Grant Objective 2 discussion above) were successful in relocating PIT tags. Unfortunately, trends of abalone density and mortality followed those previously observed at the site. Overall density of live observed abalone declined 70% (2012 initial n=47, 2014 n=14) at Site TG over the 1.3 year period. There were eight confirmed mortalities of PIT tagged animals and 14 untagged mortalities over this same time. The overall observed density of the TG site was 0.04 abalone/m^2 at the March 2014 survey. This density is half the level observed during the February 2014 TG site survey (0.08 abalone/m^2) in spite of the addition of the 21 PIT tagged animals in September 2012.

Aggregation densities may have been too low to allow any significant local recruitment. However, since the experimental plot size is very small compared to available habitat in the area, it is possible that successful reproduction did occur and nearby recruits were not detected. A more expansive survey of the nearby habitat would be necessary to test this hypothesis. Mortality on the aggregation sites was very high and could be attributed to several factors including domestication in the hatchery, age of aggregated abalone, injury during handling, natural predation and unfavorable oceanic conditions. We do not have sufficient information to identify any single factor as causal. Further investigation is warranted to determine if aggregation could be a viable pinto abalone restoration strategy in Washington. Given the continuing negative trend for Washington's wild pinto abalone populations, coupled with the critical need for broodstock augmentation for continuance of the hatchery restoration program, it is doubtful that sufficient numbers of animals are available for continued aggregation work. Additionally, until factors contributing to the high levels of mortality observed during this study are identified and mitigated, aggregation as a restoration strategy appears to be a futile pursuit.

Grant Objective 4

Outplant additional juvenile abalone in the San Juan Archipelago to attain localized minimum reproductive densities and to increase the number of discrete families at outplant sites.

Summary

With NOAA SOC funding, the pinto abalone recovery team completed juvenile abalone outplants in both 2013 and 2014 at six established restoration sites within the SJA. Personnel for this outplant consisted of researchers from the WDFW, PSRF, SPMC, UW as well as media crews from both KUOW and KCTS. The primary objective of the pinto abalone conservation aquaculture program is to "do no harm" to existing wild stocks of abalone and therefore extreme care was taken during restoration efforts described here to outplant genetically diverse and disease free cohorts of abalone.

Disease screening & permitting

A complete disease screening of the hatchery population was completed prior to conducting each abalone outplant. Live juvenile abalone (n=60 per year) representing multiple families were sampled from the Mukilteo hatchery and dissected for pathology. Histology slides were screened by Dr. Carolyn Friedman, UW. All available pathology slides from broodstock and juvenile abalone mortalities in Mukilteo from that year were also reviewed. Results indicated no presence of known pathogens or infectious disease. After confirmation that hatchery stocks were free of known infectious pathogens, a shellfish transfer permit was obtained from Brady Blake, WDFW, for moving abalone from the Mukilteo facility into the field.

Numbers & proportions of outplanted families

In March 2013, juvenile abalone (n=800) were outplanted to clean rocky reef habitat at two of our six restoration sites, located in the central SJA. Fifteen genetically distinct previously unrepresented families were overseeded (sites had previously been outplanted) onto the two sites. Fourteen female and 14 male broodstock were represented in these 15 crosses (Figure 11). Most of the outplanted abalone during this effort were from 2010 and 2011 hatchery cohorts. The mean shell length of all tagged abalone outplanted in 2013 was 51.5 mm while the mean shell length of all untagged abalone outplanted was 30.1 mm. No single family represented more than 19% of the entire outplant population in 2013.

In 2014, juvenile pinto abalone (n=1921) were outplanted at five of our six restoration sites. Thirteen genetically distinct previously unrepresented families were outplanted onto the four sites located in the eastern SJA (Sites BIW, BIS, AIW, and AIS; March 2014) and to one site (LIW) in the central SJA (June 2014). Nine female and six male broodstock were represented in these 13 crosses (Figure 12). A total number of 53 unique genetic families have now been introduced to the six juvenile outplant sites in the SJA since 2009. Most of the outplanted abalone during the recent effort were from the 2012 hatchery cohort, augmented with two fast growing families from the 2013 cohort. The mean shell length of all abalone outplanted in 2014 was 19.3 mm and no abalone were tagged for this outplant.

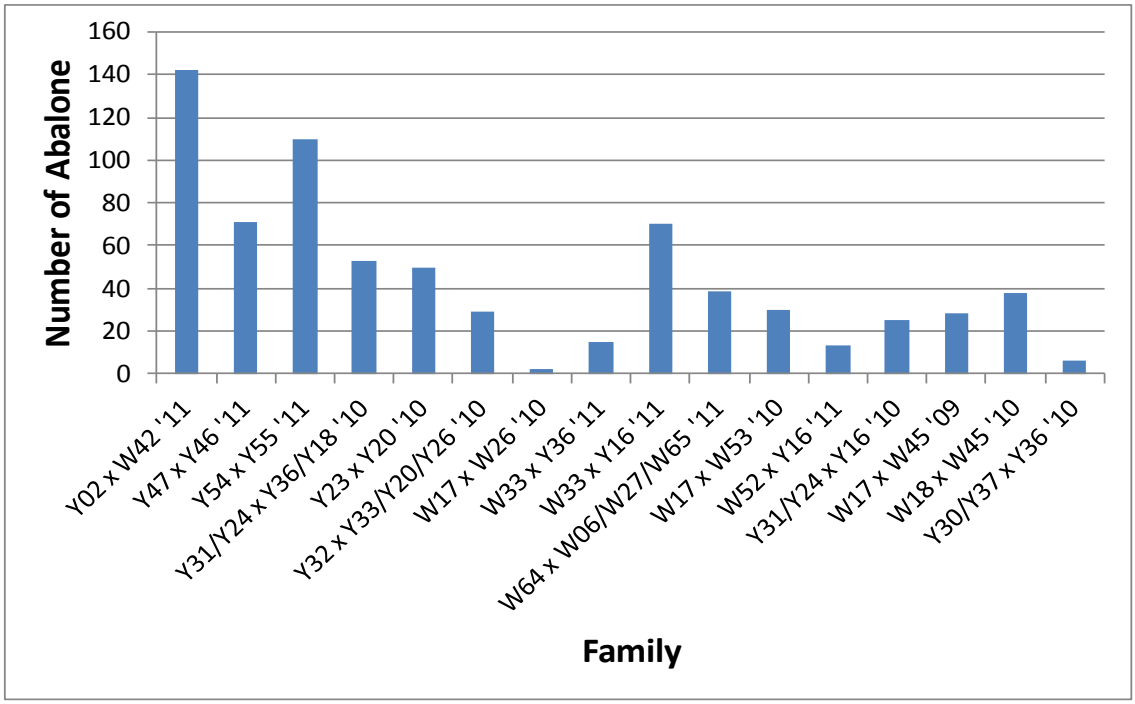

Figure 11. All juvenile abalone outplanted in 2013 arranged by family. Family designation consists of female and male parent ID as well as year class.

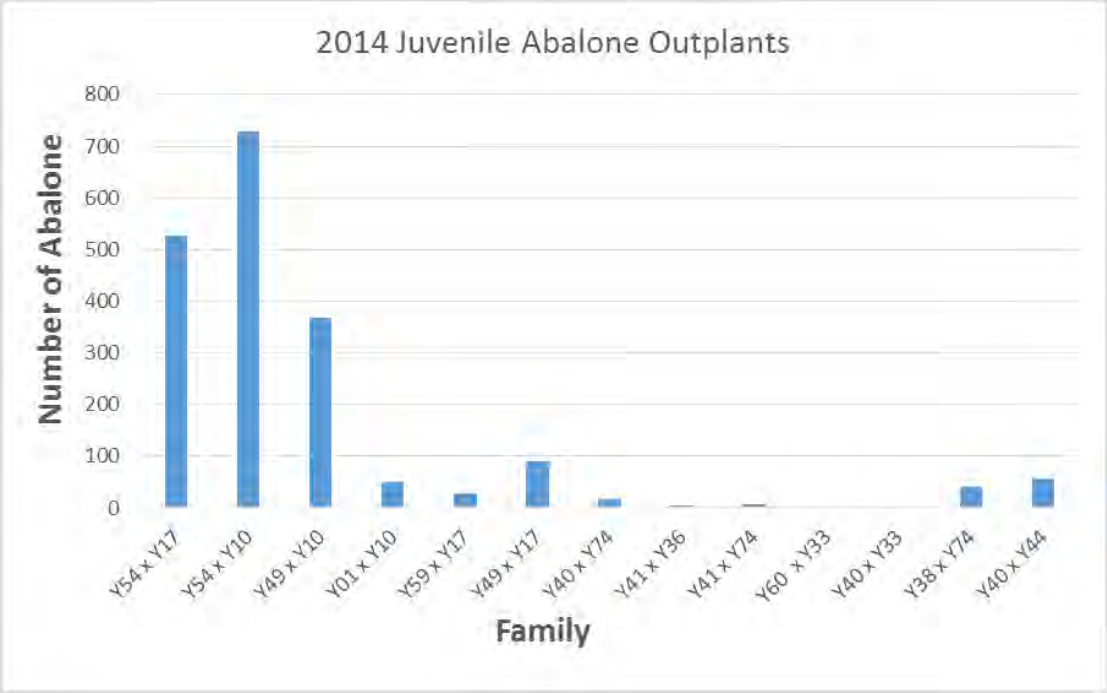

Figure 12. All juvenile abalone outplanted in 2014 arranged by family. Family designation consists of female and male parent identification.

Tagging

Unique tagging of outplanted juvenile abalone provided an opportunity for monitoring survival and growth by individual, family and year class. Due to cost, time restraints and inconclusive evidence on health impacts of tagging, only a portion of outplanted abalone were tagged in 2013. White oval-shaped vinyl tags with three digit numbers purchased from Floy Tag were utilized for this outplant effort. Bee tags were not used as all bee tag colors and numbers are already represented on the two LI (LIW and LIE) sites. Tags were affixed to the abalone shell immediately forward of the apex using Zap-a-Gap model glue.

Outplant modules

PVC pipe (6" ID) was acquired from the UW Bothell campus (left over from a wetlands project). As in previous outplants, this pipe was cut into approximately 18" long sections. Tubes recovered from previous outplants were also reused. A large diameter chop saw was rented for cutting due to the large diameter of the pipe. Each section of tube was power washed and scrubbed. Tubes were labelled by destined site for convenience once in the field. All tubes were conditioned in flow-through tanks at the Mukilteo lab for several weeks prior to being loaded with animals.

Module loading

One day prior to the outplant day, abalone were transferred from their holding tanks into the PVC outplant tubes. All the tubes destined for one particular site were positioned upright in a large seawater-filled cooler with one layer of fiberglass window screen (2 mm mesh size) secured with rubber bands covering the bottom end. Approximately 35-48 abalone were loaded into each tube based on the total density for a particular site. The open end of the tube was then closed with one layer of window screen and secured with a rubber band. All tubes were housed overnight in flow through tanks in the Mukilteo greenhouse.

Abalone transport and outplant

The outplant tubes were transported from Mukilteo directly to Anacortes in a large fish tote filled with seawater in the back of a truck. To aerate the tote during transport, a 12V battery with a DC to AC inverter was used to power an aquarium pump with two large air stones placed in the tote. The fish tote, batteries, inverters, air pumps, tubing and large air stones came from the Mukilteo lab.

Fish totes were drained at the Skyline Marina and loaded onto either the WDFW RV Clamdestine or the SPMC RV Zoea, driven into Burrows Channel and refilled with seawater and transported to the outplant sites (Figure 13). Once on site, tubes were carried to depth in bungee-corded bundles of three or four by divers and placed within the delineated outplant plots in areas that appeared to have suitable substrate and cryptic habitat onto which juvenile abalone could exit. Tubes were wedged amongst cobble and boulders to secure them against current and surge. Between 3-24 hours after the tubes were delivered to the sites, divers removed the mesh from the tubes and the abalone were free to move from the modules out onto the surrounding substrate.

Most PVC outplant modules were removed approximately three months after the introduction. No follow-up work has been done on the LIW site since the June 2014 outplant. Upcoming goals for all sites include removing any remaining empty PVC modules and also conducting surveys for survival and growth one year post-outplant. This work will be conducted in early 2015 with funding from the Skagit County Marine Resources Committee.

Figure 13. Outplant tubes filled with juvenile abalone are ready to be transported by divers to the reef at one of the restoration sites.

Grant Objective 5

Conduct high resolution temporal sampling of outplant sites to improve recent, post-outplant survival estimates.

Pinto abalone are typically cryptic in their preferred habitat and small abalone often occupy rocky crevices and underneath cobble and boulders (presumably to avoid predation). Calculating estimates of abundance, survival and growth can be improved by accurately identifying individual animals and developing a sampling strategy to optimize recapture. To identify individual juvenile abalone, we adhered distinctly numbered and colored plastic disc tags to their shells prior to outplanting on two sites (BIS & AIW) in the SJA in 2011. Abalone outplanted in 2009 were not tagged, but were distinguished from the 2011 outplanted abalone by the difference in size structure. To determine recapture or re-observation rates we conducted a series of diver surveys within a narrow range of time.

During July and August 2012 five repetitive dive surveys using were made on these two sites (BIS & AIW). The two sites are 82.7 m^2 and 69.7 m^2 in size respectively and they were divided into lanes 2 m wide using lead lines. Divers performed non-invasive surveys by diving each lane at a rate of approximately 0.5 m/min; rates were variable depending on complexity of the substrate. Mirrors and lights were used to help find abalone. Shell length, cryptic status, and tag

color and number were recorded. If an abalone was located in a way that could not be accurately measured, size was estimated.

We were able to confidently identify which organisms surveyed were from the 2009 out plant and which were from the 2011 out plant by using shell length frequency data. From the length frequency data we were able to discern that abalone with shell lengths <90 mm were likely out planted in 2011. The largest tagged abalone observed in July/August 2012 (from the 2011 out plant – the 2009 out planted abalone were not tagged) was 85 mm shell length (SL). Abalone between 90 mm and 115 mm SL were likely survivors of the 2009 out plant. Abalone with SL> 115 mm were assumed to be wild adult abalone.

A total of 1197 abalone were outplanted in 2009 and 2011 and a total of 137 juvenile and young adult abalone were observed in 2012 at these two sites, which is an approximate overall survival of 11.4%. Considering just the 2009 outplant (untagged), the survival estimates were based entirely on the number of abalone observed in the appropriate size range (90-115 mm). Survival from the 2009 outplant was very low at 0.4% for the BIS site and 2.6% for the AIW site.

Only two tagged abalone were observed at each site more than once (n=4 refind observations for both sites). The sample size of abalone with readable tags was only 26 (originally tagged abalone=680), which indicates a refind rate of about 15.4% for the five surveys.

Tag retention from the 2011 outplant may be as low as 3.8% between March 2011 and August 2012 (about 17 months). If all abalone were accounted for, including within plot and off-plot deaths and emigrant abalone, then the tag retention rate could be more accurately estimated. On a practical level, this level of information is very difficult to obtain, therefore our estimate of tag retention is likely low.

Mean growth of tagged abalone was 9.0 (+/- 8.7 SD) mm shell length (SL) at BIS and 19.9 (+/- 12.8 SD) mm SL at (AIW), (Table 2). The variance for this data is high and we recommend increasing the sample size of tagged abalone for future *in situ* growth estimates.

Continuing the choice to make repetitive surveys at only two of the six out plant sites lead to the discovery of more individual organisms at these sites than single surveys alone. Because of the reclusive nature of juvenile abalone, actual survival rates are very difficult to determine using visual survey methods. Based upon the fact that there were very few re-finds of tagged abalone, it can be reasonably assumed that if additional site surveys had been conducted then additional abalone would have been located.

Out plant efforts will likely be expanded in future years, most likely with a continuation of the current out plant procedures as well as developing new methods of surveying and out planting. One method that has been tested is the use of passive integrated transponder (PIT) tags instead of

colored bee tags (Hale *et al*. 2012). PIT tag readers can electronically scan reef areas to detect signals from tags attached to abalone, including those hidden from diver's view. The benefits of using a tagging system like this could greatly improve encounter rates. The tags will theoretically last the entire life of the abalone instead of the 1-2 years estimated life span for the bee tags or disc tags.

These findings inform managers of a need to develop persistent tagging methods (perhaps PIT tags that are smaller than what is currently available for juvenile abalone) and the value of repetitive sampling of behaviorally cryptic young abalone to make improved estimates of abundance, survival and growth.

TABLE 2.

Initial (March 2011) and summer 2012 shell lengths (mm) of individual tagged abalone out planted at BIS and AIW sites in March 2011 and recovered in July/August 2012. (Modified from Benolkin *et al*., 2013).

BIS

Tag Color/Number	Initial Size (2011)	Recovery Size (2012)	Size Increase
Orange 63	22.2	42	19.8
Blue 26	21.3	33	11.7
Orange 90	17.9	30	12.1
Orange 77	20.4	28	7.6
Green 78	21.4	20	-1.4
Orange 71	29.1	25	-4.1
Green 35	16.1	20	3.9
Orange 52	24.1	41	16.9
Orange 87	21.7	43	21.3
Orange 99	14.1	26	11.9
Orange 41	19.3	19	-0.3
Average ± SD =	20.7 ± 4.0	29.7 ± 9.0	9.0 ± 8.7

AIW

Tag Color/Number	Initial Size (2011)	Recovery Size (2012)	Size Increase
Blue 10	30.8	42	11.2
Orange 63	26.1	33	6.9
White 30	14.7	30	15.3
Blue 35	21.0	50	29.0
Green 59	15.4	33	17.6
Blue 49	39.0	76	37.0
Blue 43	18.9	40	21.1
Blue 74	34.3	45	10.7
Blue 40	35.1	85	49.9
Blue 11	29.4	48	18.6
Orange 21	15.7	24	8.3
Green 97	20.7	57	36.3
Blue 85	38.3	44	5.7
Green 45	17.9	35	17.1
Orange 57	18.6	32	13.4
Average ± SD =	25.1 ± 8.7	44.9 ± 16.9	19.9 ± 12.8

Grant Objective 6

Develop and deploy pilot field nurseries to help engage and involve dock and marine facility partners, thereby building greater community support for abalone recovery efforts. Conduct outreach to involve volunteers in tagging abalone and preparing transport tubes prior to outplants.

Background

The Puget Sound Restoration Fund tested and deployed experimental field nurseries in the San Juan Islands and Strait of Juan de Fuca to help expand pinto abalone recovery efforts in Washington. Pilot-scale abalone nurseries are part of a larger recovery effort to halt the decline of abalone stocks in the Pacific Northwest and to rebuild abalone populations to a self-sustaining level via supplementation. Remote field nurseries may benefit the program by increasing grow-out space for juveniles not quite ready for outplanting to the wild, reserving limited hatchery space for spawning, larval settlement and early nursery. Field nurseries have also been developed to help engage and involve shoreline property owners and marine facility partners, thereby building greater community support and exposure for abalone recovery efforts. Field nurseries were originally proposed in the NOAA SOC scope of work, but this project was postponed when SOC funds were reduced. Washington Department of Natural Resources (WDNR) funding for abalone recovery work from 2012-2013 restored field nursery testing on a smaller scale than initially proposed.

Remote field nursery module design

The field nurseries consist of cage-like structures fabricated from milk crates that can be filled with dime-sized abalone and suspended from a dock. PSRF volunteer and retired mechanical engineer Bob Selzler committed 60 hours to design and construct the nursery cages. In addition to the main housing built from large milk crates, materials for assembly included oyster bag fabric to line the housing, aluminum angle, perforated sheet and hinge for the cage lid, a boat hatch to serve as a feeding port on the lid, plate racks within the cage made of wavy fiberglass sheeting to increase grazing surface area for abalone within the module, a poly line bridle for dock suspension and various other fasteners and cable ties (Figure 14).

Figure 14. Materials used to construct the abalone field nurseries.

Nursery module installation and outreach

PSRF partnered with the SeaDoc Society to solicit dock owners and shoreline residents to host these remote abalone field nurseries. SeaDoc Society posted an announcement in their quarterly newsletter advertising the opportunity and response from abalone recovery supporters was significant. Three hosts were selected to test the first round of nurseries based on their location in areas with good water quality, high flow and access to macroalgal food sources. The three sites selected include the Port Townsend Marine Science Center (PTMSC), a private residence on Bell Island at the eastern edge of Wasp Passage, SJA, and a private residence in Grindstone Harbor on the south shore of Orcas Island. Reconnaissance trips were taken to all three sites prior to installation. A fourth site was added when 200 abalone from the Port Gamble nursery facility were moved from lab tanks into several nursery modules hanging from the Port Gamble dock.

Nurseries were installed at the PTMSC in 2013. Two cages were delivered, one was hung beneath the pier while the other was hung in a wet lab tank inside the building. One hundred juvenile abalone reared at the Mukilteo abalone hatchery were seeded into each module (mean SL=18 mm). PTMSC recruited a team of volunteers to monitor the project, these volunteers measured, weighed and bee tagged a subset of abalone from each module and collected growth and survival data throughout the nursery trial (Figure 15). Maintenance included a weekly kelp feeding regimen and scrubbing the modules to remove biofouling to promote water flow through the module. The cages were checked routinely for mortality and growth. The abalone nursery

modules were a popular attraction at the PTMSC, volunteers used the exhibit to educate and inform visitors of broader abalone recovery efforts. Given the tremendous positive feedback from the public, maintaining abalone at the PTMSC proved to be a successful pinto abalone outreach tool.

Figure 15. Abalone are measured before being stocked into the PTMSC nursery modules.

The second and third remote field nurseries were installed in 2013 with the help of SeaDoc personnel Jean Spalti and intern Jacquelyn, a reporter from the Islands Sounder named Cali Bagby, PSRF personnel as well as the dedicated hosts and dock owners. Two cages, each stocked with 100 juvenile abalone were deployed at each of the two San Juan Islands locations (Figures 16 and 17). In total, eight nursery cages were operational at four locations rearing 800 small pinto abalone.

Figure 16. Hatchery cultured juvenile pinto abalone ready to be stocked in a remote field nursery.

Figure 17. Remote field nurseries are lowered into the water beneath the host's dock.

Abalone Field Nursery Results

Initial lab testing of a prototype nursery module for remote rearing was finished at the Mukilteo Research facility early in GY1. Twenty five juvenile abalone, mean SL 18.1 mm, were stocked into the cage and the cage was suspended in a high flow 800 L culture tank. Growth, survival, feeding behavior and structure usage within the cage were observed every one-two weeks for five months. Five of 25 abalone died during this time. Mean SL at conclusion was 22.4 mm, a growth rate similar to abalone in standard aquaculture tanks within the hatchery. Abalone used sidewalls and corners of cage almost exclusively, use of plate structure within cage was limited. The module size was increased for field testing and the module design was modified to provide more complex structure within.

Abalone reared for nine months in the two Port Townsend Marine Science Center field nurseries, one hung from the dock and the other maintained within an aquarium tank, revealed no significant difference in shell growth ($t = 1.9246$, $df = 51.941$, p-value = 0.05976, Table 3) or weight gain ($t = 1.3504$, $df = 51.53$, p-value = 0.1828, Table 4). Initially stocked with 100

abalone each, only one animal died within the lab module and four animals died within the dock module during a nine month culture period.

TABLE 3.

Shell length information for dock and lab abalone nursery modules at PTMSC.

	Start SL (mm)	End SL (mm)	SD	Avg SL gain (mm)
Dock	25.2	33.8	13.6	9.9
Lab	24.4	32.2	8.5	7.8

TABLE 4.

Weight information for dock and lab abalone nursery modules at PTMSC.

	Start Weight (g)	End Weight (g)	SD	Avg Weight gain (g)
Dock	2.88	6.98	3.77	4.10
Lab	2.64	5.88	3.21	3.24

Abalone were reared in nurseries at two San Juan County sites; an Orcas Island private dock for nearly four months and a Bell Island private dock for more than five months. Survival was high in three of the four modules deployed (Table 5). Lower survival evident in the fourth module was likely escapement due to a broken lid that went undetected for several days. These San Juan County nursery sites maintained by local dock owners did not receive as consistent attention (feeding and module cleaning to remove fouling) as necessary resulting in lower growth rates. Nursery caretakers originally committed to a minimum of six months maintaining the modules, but both sites were discontinued when the dock owners decided to leave the islands for the winter.

TABLE 5.

Survival and growth data from two San Juan Islands field nursery sites.

Location	Start Date	End Date	Start N	End N	Start Avg SL (mm)	End Avg SL (mm)	Avg SL gain (mm)
Orcas Is Crate 3	6/25/13	10/18/13	100	87	20.2	26.1	5.9
Orcas Is Crate 4	6/25/13	10/18/13	100	89	21.0	24.2	3.2
Bell Is Crate 5	6/25/13	12/2/13	100	87	20.5	26.4	5.9
Bell Is Crate 6	6/25/13	12/2/13	100	63	18.4	21.2	2.8

Grant Objective 7

Assess 10 index sites in the San Juan Archipelago to evaluate abundance trends and size structure of the wild population.

Beginning in the late 1980's, information from diver surveys, Enforcement reports, creel data and reports from the UW's Friday Harbor Marine Laboratories all indicated trends of serious decline of Washington State pinto abalone stocks. This prompted regulatory restrictions to recreational harvest and ultimately resulted in the closure of the Washington recreational fishery in 1994. In 1992, WDFW established ten survey stations throughout the SJA, in areas known to have high quantities of pinto abalone, as an abundance index for the pinto abalone population in this region. Surveys of the ten index stations have provided evidence of a continuing trend of decline in pinto abalone abundance. Additionally, abalone size data have shown a lack of recent significant recruitment coupled with a trend of increasing shell lengths, indicating an aging population without replacement. Rothaus *et al*. (2008) reports on pinto abalone index station survey results with data through 2006. A subsequent survey was completed in 2009 followed by 2013 survey that was completed with NOAA SOC grant funding.

Abundance Trends

PSRF and WDFW divers completed surveys at each of the ten abalone index stations in the winter of 2013 (GY2). The total number of live abalone observed within the ten surveyed sites in 2013 was twenty-seven. Abalone abundance for all index station survey years is shown below in Table 6. Highlighted in yellow, five of the ten index sites surveyed in 2013 are now locally extirpated (i.e., the number of abalone observed was zero). Total site abundance fell to roughly half that observed in the previous 2009 survey. Comparing the site counts from the original index station surveys in 1992 to the 2013 survey, abalone abundance has declined 92%. The pinto abalone population was already considered to be overharvested and depressed prior to 1992 and the total abundance decline of the population is likely much greater than the index station data suggests.

TABLE 6.

Abundance of pinto abalone at ten index stations in the SJA between 1992 and 2013. Extirpated stations in 2013 are highlighted in yellow.

Location	1992	1994	1996	2003	2004/2005	2006	2009	2013
LIW	48	44	36	7	8	2	4	1
WR	20	34	32	7	9	10	9	7
CI	46	21	8	0	1	0	1	0
PR	45	19	19	2	0	0	0	0
RI	41	34	23	3	1	1	0	0
BCI	41	41	74	39	14	13	6	12
SI	49	16	30	18	17	10	5	1
DB	22	24	31	19	15	11	9	0
OEP	22	29	14	18	11	12	17	0
LIE	17	26	30	24	27	13	9	6
Total	**351**	**288**	**297**	**137**	**103**	**72**	**60**	**27**

Density of pinto abalone on index sites for all survey years is shown in Table 7. Mean density for all stations, for all survey years, is shown in Figure 18.

TABLE 7.

Density of pinto abalone at ten index stations in the SJA between 1992 and 2013. Mean density equals total index station counts divided by total index station area.

Location	Area m^2	1992	1994	1996	2003	2004/2005	2006	2009	2013
LIW	**152.0**	0.32	0.29	0.24	0.046	0.053	0.013	0.026	0.007
WR	**190.0**	0.11	0.18	0.17	0.037	0.047	0.053	0.047	0.037
CI	**316.0**	0.15	0.07	0.03	0.000	0.003	0.000	0.003	0.000
PR	**375.0**	0.12	0.05	0.05	0.005	0.000	0.000	0.000	0.000
RI	**135.0**	0.30	0.25	0.17	0.022	0.007	0.007	0.000	0.000
BCI	**158.0**	0.26	0.26	0.47	0.247	0.089	0.082	0.038	0.076
SI	**176.0**	0.28	0.09	0.17	0.102	0.097	0.057	0.028	0.006
DB	**155.0**	0.14	0.15	0.20	0.123	0.097	0.071	0.058	0.000
OEP	**229.0**	0.10	0.13	0.06	0.079	0.048	0.052	0.074	0.000
LIE	**356.0**	0.05	0.07	0.08	0.067	0.076	0.037	0.025	0.017
Mean Density		0.157	0.128	0.132	0.061	0.046	0.032	0.027	0.012

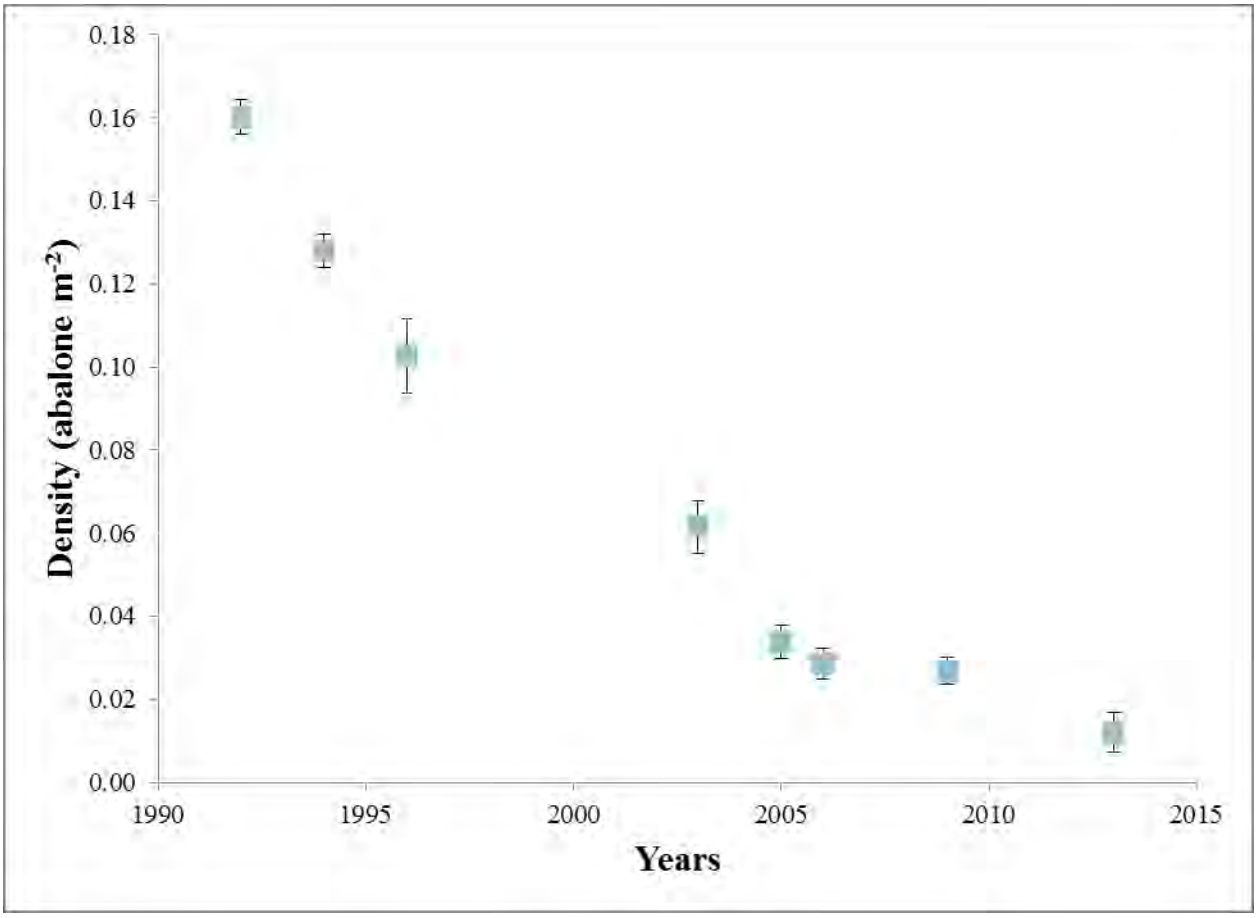

Figure 18. Change in mean density of pinto abalone at 10 index sites established in the San Juan Archipelago. Points are mean density of abalone m^{-2} with bars representing the standard error of the mean.

Adult pinto abalone aggregations have been rarely noted during recent Washington pinto abalone surveys. The reported density necessary for successful recruitment in broadcast spawning marine invertebrates (Shepherd and Partington, 1995; Babcock and Keesing, 1999) is above 0.15 abalone/m^2. Lacking an analysis of nearest neighbor data for pinto abalone in Washington, we can only compare mean densities as a factor affecting fertilization and recruitment success. Observed pinto abalone densities for surveys of the ten index stations at the beginning of the time series in 1992 indicate that half were below the reported density threshold of 0.15 abalone/m^2. By 2005, all ten stations were below the threshold. We do not know if these reported critical density thresholds hold true for pinto abalone, but based on the trend of population decline and lack of significant recruitment it is reasonable to conclude that pinto abalone spawning and recruitment are affected by a very low abundance of adults.

Size Structure

Pinto abalone shell length (SL) measurements are taken from the margin near the apex to the opposite margin location that gives the greatest total length. The range of SL of live abalone

observed during the 2013 survey is 55-152 mm (n=56 live abalone), with a mean shell length of 118.4 mm. These are abalone that were observed within the index stations and those that were measured opportunistically when they were encountered within the immediate vicinity of these sites. Pinto abalone SL measurements for all index site surveys from all years are shown below in Table 8.

TABLE 8.

Summary of pinto abalone shell length (SL) measurements from 10 index sites in the San Juan Archipelago.

Year	n	Mean SL (mm)	95% C.I.	Minimum SL (mm)	Maximum SL (mm)
1992	340	105.3	1.73	42	142
1994	281	107.8	2.07	53	145
1996	268	107.5	2.18	41	145
2003	136	114.8	2.70	46	146
2004/05	103	113.7	2.83	56	141
2006	104	115.4	2.42	80	139
2009	60	115.5	4.37	71	147
2013	56	118.4	4.97	55	152

For temporal contrast, we compared size structure between data collected in 1979-1981 to size data collected in 2013. As a baseline size structure for this population, the size frequency of pinto abalone encountered during timed swim surveys completed in 1979 (n= 754) is shown in Figure 19 and has an arithmetic mean SL of 97.6 mm (95% C.I. = +/-1.36 mm). The mean SL of abalone surveyed on index sites in 2013 was 118.4 mm (95% C.I.=+/-4.97 mm, Figure 20). Though there are differences in sampling protocols (e.g. sample size, number of sites, location of

sites) between early pinto abalone survey data and current index station survey methods, these data may be useful when comparing shifts in size frequency over time.

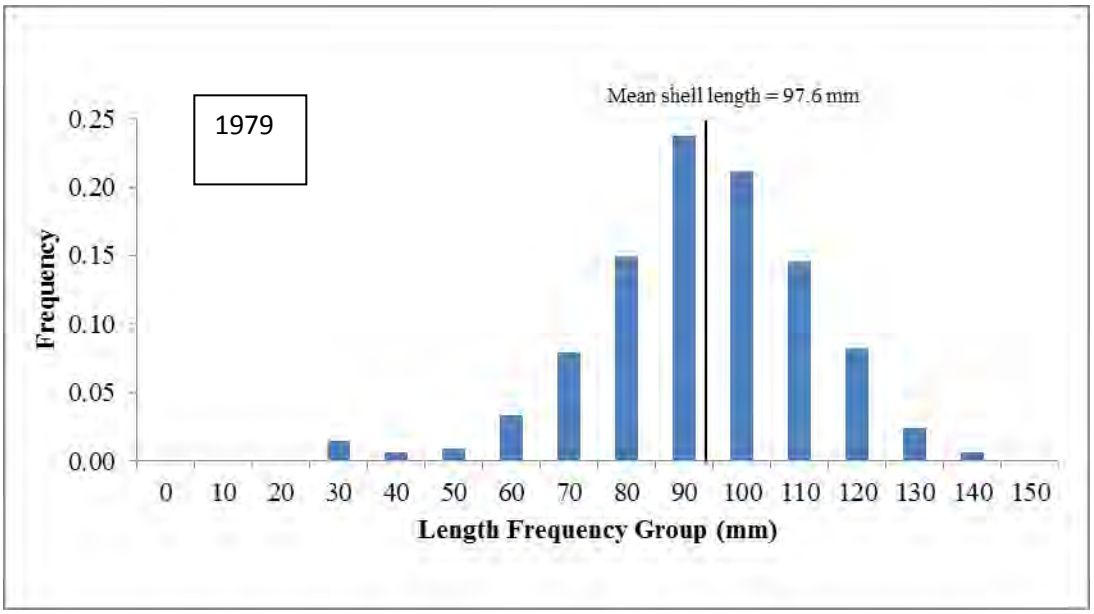

Figure 19. Shell length frequency distribution of pinto abalone (n=754 abalone), grouped in bins of 10mm, encountered during timed swim surveys by WDFW in 1979. Solid vertical line represents the mean shell length from 1979 (baseline).

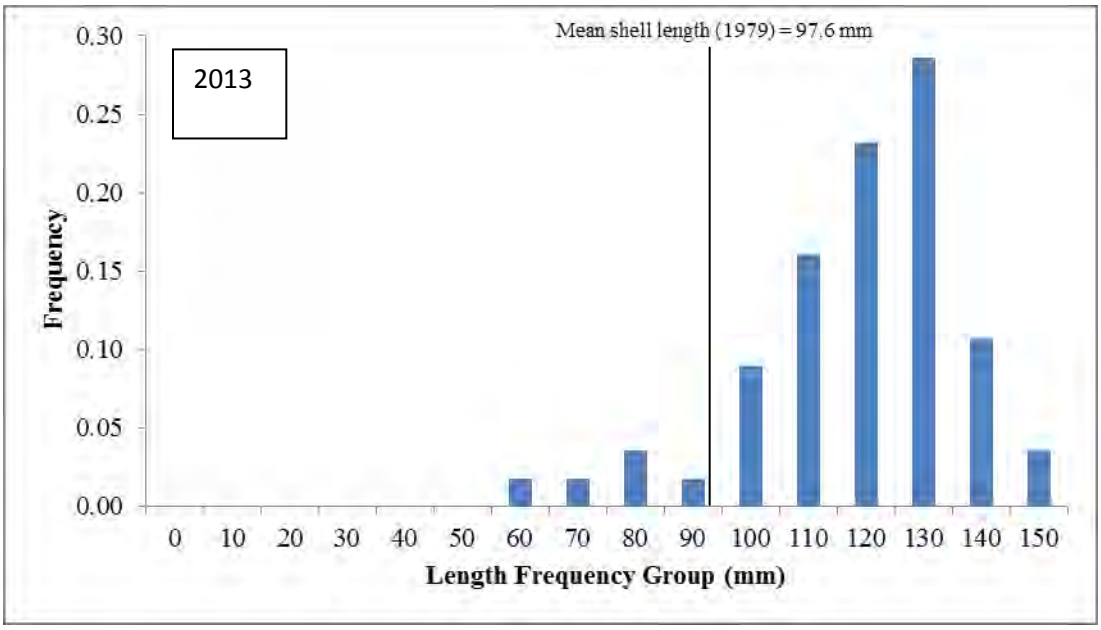

Figure 20. Shell length frequency distribution of pinto abalone (n=56), grouped in bins of 10mm, encountered during index station surveys by WDFW in 2013. Solid vertical line represents the mean shell length from a 1979 (baseline) timed swim survey.

For all Washington State pinto abalone SL data collected in the SJA, there is a significant shift of size frequency distributions from 1979 to 2013. From early pinto abalone surveys (1979-1981) and index station surveys from 1992 to 2013 a total of 2581 pinto abalone SLs were taken. The maximum SL observed was 159 mm from the 1980 survey. The trend of mean SL is toward larger, presumably older animals, and using a linear best fit to the SL data, the mean SL is increasing at about 0.5 mm per year on average (Figure 21).

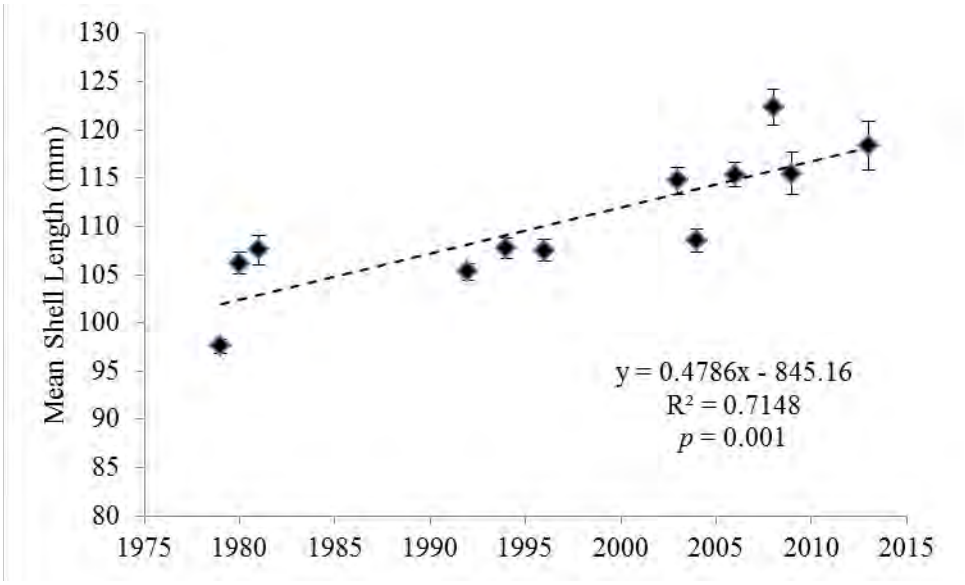

Figure 21: Change in pinto abalone mean shell length over time on timed survey transects and index station surveys in the SJA. Points are mean shell length with bars representing the standard error of the mean. The linear best fit line has a positive slope of ~0.5 mm/year.

Collective SL data from surveys, when clustered into three logical chronologic groups, show distinctly different distributions (Figure 22). A pairwise Kolgomorov-Smirnov test shows that all three sample distributions depicted were not drawn from the same overall distribution ($p < 0.001$).

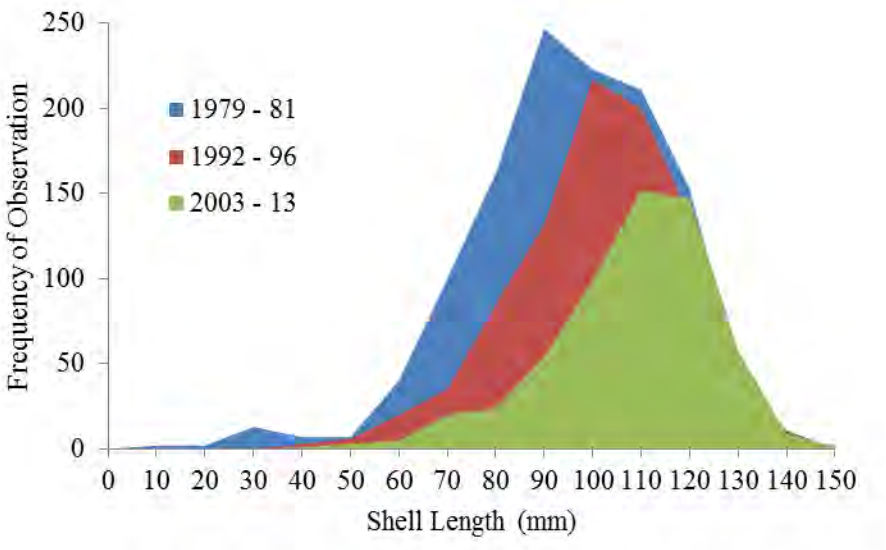

Figure 22: Change in pinto abalone shell length frequency distribution over time on timed survey transects and index station surveys in the SJA.

Along with this shift in the size structure toward presumably older individuals, there is very little evidence of recent recruitment. Emergent juvenile recruits of pinto abalone have been defined as those animals with SL of less than 90 mm (Rothaus *et al*. 2008). During the 2013 survey, only one emergent abalone (emergent defined as <90 mm,) was observed with a 55 mm SL and 78% of the animals observed had SL over 100 mm, providing additional evidence of low recruitment or recruitment failure. The percent of emergent juvenile pinto abalone recruits between survey years 1979, 1992 and 2013 has declined from 31.8% to 17.4% to 7.1%, respectively. This suggests a serious decadal decline in juvenile pinto abalone recruitment and survival.

BUDGET AND EXPENDITURES

The NOAA grant (#NA11NMF4720277) through the Proactive Conservation Program funded Washington pinto abalone restoration efforts at a critical time when seed money from other grant sources were no longer available and state funds for natural resource agency management were being reduced or eliminated due to the Great Recession. The original 3 year grant was budgeted at a total level of $635,646 of which $561,111 was federal sourcing and $74,530 was non-federal. The grant was fully funded in the first year (grant year one - GY1) and then federal funding was reduced to $60,000 in GY2 and to zero in GY3. The total federal funding level for the 3 year grant was $241,988 (Table 9).

TABLE 9.

NOAA SOC Grant budget by grant year.

Budget for NOAA grant – Abalone Restoration in the Pacific Northwest			
Year	Federal Share of Cost	Recipient Share of Cost	Totals
GY1 (9/2011 – 8/2012)	181,988	24,843	206,831
GY2 (9/2012 – 8/2013)	60,000	0	60,000
GY3 (9/2013 – 8/2014)	0	0	0
TOTALS	241,988	24,843	266,831

The majority of expenditures were made by the Puget Sound Restoration Fund (PSRF) on staff, equipment and supplies to support remote propagation of pinto abalone at the NOAA Mukilteo Research facility. In conjunction with propagation activity, expenditures were made on pinto abalone hatchery health management and disease screening and genetic management by the University of Washington (UW). PSRF also had primary responsibilities for outreach and education and developing and deploying field nursery systems. Western Washington University - Shannon Point Marine Science Center (WWU) conducted high resolution temporal sampling at pinto abalone outplant sites and provided support for field operations including holding facilities for broodstock and juvenile abalone outplants. The Jamestown S'Klallam Tribe (JST) provided dive assistance to PSRF and the WA Department of Fish and Wildlife (WDFW) in broodstock collection activities. WDFW was the lead agency responsible for contract administration and all field related activities. Total federally funded expenditures were $240,256 (Table 10).

Table 10.

NOAA SOC Grant expenditures by budget item.

Expenditures for NOAA grant – Abalone Restoration in the Pacific Northwest	
Budget item	Expenditures for 3 year grant period
PSRF contract	135,514.00
UW contract	45,872.80
WWU contract	7,668.00
JST contract	739.50
WDFW (direct)	41,774.70
WDFW (indirect)	8,687.00
TOTAL	240,256.00

Note: Data in this table is summarized from WDFW financial reports through 10/20/2014 and final expenditures may vary slightly.

In-kind contributions, or recipient share of the project costs, include $34,925 by PSRF for hatchery manager and hatchery technician staff time (188 hours and 901 hours, respectively) to support outplant experiments, PIT tagging trials and development of field nursery modules. WDFW contributed $11,434.04 in staff time and dive hours (313.5 hours and 128 hours, respectively) to support a variety of restoration activities including broodstock collection, index site surveys, juvenile abalone outplanting, and survey of an abalone aggregation site.

LITERATURE CITED

Babcock, R. and Keesing J. 1999. Fertilization biology of the abalone *Haliotis laevigata*: Laboratory and field studies. Can. J. Fish. Aquat. Sci. 56(9):1668-1678

Benolkin, A., A. Thomson, P. Dinnel and N. Schwarck. Survey of previously out planted pinto abalone (*Haliotis kamtschatkana*) and an exploration of an optimal weaning diet. Final Report for the 2012 Research Experience for Undergraduates program, Shannon Point Marine Center, Western Washington University. 20 pp.

Bouma, J.V. 2007. Early life history dynamics of pinto abalone (*Haliotis kamtschatkana*) and implications for recovery in the San Juan Archipelago, Washington State. MS Thesis, School of Aquatic and Fishery Sciences, Univ. Wash., Seattle, WA.

Friedman, C., D. Rothaus and B. Vadopalas. 2011. The Efficacy of Aggregation as an In-situ Restoration Technique for the Recovery of Pinto Abalone (*Haliotis kamtschatkana*) in the San Juan Archipelago. Final Grant Report to the SeaDoc Society, unpublished.

Hale, J.R., Bouma, J.V., Vadopalas, B. & C.S. Friedman. 2012. Evaluation of passive integrated transponders for abalone: Tag placement, retention, and effect on survival. Journal of Shellfish Research 31(3): 789-794.

Hester, J.B., J.M. Walker, P.A. Dinnel and N.T. Schwarck. 2011. Survey of previously out planted pinto (northern) abalone (*Haliotis kamtschatkana*) in the San Juan Island Archipelago, Washington State. Final Report for the 2011 Research Experience for Undergraduates program, Shannon Point Marine Center, Western Washington University. 13 pp.

Kalinowski S.T., A.P. Wagner, M.L. Taper. 2006. ML-Relate: a computer program for maximum likelihood estimation of relatedness and relationship. *Molecular Ecology Notes* 6:576-579.

Pratt, P. 2010. Survey of previously out planted abalone, *Haliotis kamtschatkana*, and effects of weaning diets on growth rates of hatchery individuals. Final Report for the 2010 Research Experience for Undergraduates program, Shannon Point Marine Center, Western Washington University. 19 pp.

Rothaus, D., B. Vadopalas, and C. Friedman. 2008. Precipitous declines in pinto abalone (*Haliotis kamtschatkana kamtschatkana*) abundance in the San Juan Archipelago, Washington, USA, despite statewide fishery closure. Canadian Journal of Fisheries and Aquatic Sciences 65: 2703-2711.

Shepherd, S. A., and Partington, D. 1995. Studies on southern Australian abalone (Genus *Haliotis*). XVI. Recruitment, habitat and stock relations. Mar Freshwater Res. 46:669-680.

Walker, J, N. Schwarck, V. Hodges, T. Tymon, A. Thomson, K. Gabrian-Voorhees and P. Dinnel. 2013. Final Report by Shannon Point Marine Center, Western Washington University. 10 pp.

Wang, J. 2004. Sibship reconstruction from genetic data with typing errors. *Genetics* 166: 1963-1979.

Wang, J. 2011. COANCESTRY**:** A program for simulating, estimating and analyzing relatedness and inbreeding coefficients. *Molecular Ecology Resources* 11: 141–145.