Pinto Abalone Recovery Project
Final Report to the Skagit MRC
Josh Bouma—Puget Sound Restoration Fund
September 4th, 2015

Introduction

The pinto abalone (*Haliotis kamtschatkana*) recovery project is a long-term collaboration between state and federal agencies, NGOs, universities, and tribes. This group includes researchers, technicians, managers, students and facilities support from the Washington Department of Fish & Wildlife (WDFW) Central Shellfish team; the Puget Sound Restoration Fund (PSRF); Western Washington University's Shannon Point Marine Center (SPMC); NOAA Mukilteo Research Station; the University of Washington, School of Aquatic & Fishery Sciences (UW) and others. Annual funding to PSRF from WDFW supporting consistent progress in abalone hatchery and restoration activities has been supplemented by additional support in 2014-2015 from both the Skagit Marine Resource Committee (MRC) and the Washington Department of Natural Resources, Aquatic Restoration Program (DNR).

In 2014-2015, PSRF continued management of pinto abalone conservation aquaculture facilities for development of restoration strategies. These hatchery, wet laboratory and nursery facilities are located at the NOAA Marine Fisheries Research Station in Mukilteo. PSRF also manages a new state-of-the-art shellfish conservation hatchery at the NOAA Manchester Research Station and PSRF will be expanding pinto abalone culture into this new shellfish hatchery during the fall of 2015 with the construction of a new fabric building for abalone nursery and grow-out.

The primary objective of the abalone recovery project is the production of genetically diverse disease-free hatchery raised larval and juvenile pinto abalone for supplementation and restoration of wild stocks, focusing on maintaining the genetic integrity and health of wild populations. In addition to managing hatchery efforts, PSRF collaborated with WDFW on all associated field efforts including surveys and juvenile outplanting at a number of restoration sites within the San Juan Archipelago, most of which are within Skagit County. The following report summarizes PSRF project accomplishments related to the Skagit MRC contract during the time period from September 2014-September 2015.

Hatchery Management

Juvenile pinto abalone were purchased by the Skagit MRC for outplanting activities in Skagit County. Hatchery responsibilities to produce abalone for outplanting projects included coordination, supervision and implementation of daily coverage, weekly maintenance and regular aquaculture activities at the NOAA Mukilteo Research Station:

- Tank cleaning & filter changes.

- Water quality monitoring—temperature, salinity, pH and dissolved oxygen. Seawater supply to the hatchery, nursery and grow-out greenhouse is buffered with sodium carbonate to elevate pH above 8.0. This requires regular probe calibration, controller/dosing pump maintenance and production of buffering solution.
- Animal health monitoring—mortalities and live juveniles sampled for histology and molecular diagnostics as part of comprehensive hatchery health screening.
- Abalone maintenance—inventory, measuring, weighing, tagging, genetic sampling, etc.
- Systems updates—plumbing, pump & heater maintenance, tank rack construction, etc.
- Supervision and direction over student, intern and technician research projects.
- Production—broodstock conditioning, induced spawning, larval rearing, nursery, juvenile grow-out, diatom and macroalgal culture, etc.

Spawning Success, Production and Grow Out

The primary production objective during the 2015 summer spawning season has been to conduct single-parent crosses with each spawning event. This optimizes the genetic input of our broodstock by producing as many distinct F1 families as possible, maximizing the effective population size within the hatchery. New broodstock were collected during the spring of 2015, and gonad maturation was evident upon arrival to the hatchery. In May and June, several induced spawns resulted in the production of almost 500,000 larvae competent for settlement representing four genetically distinct families. Spawning efforts will continue over the next few months until all available nursery tanks have been set with larvae.

An estimated 4000 juvenile abalone are currently being reared in the Mukilteo grow-out greenhouse ranging in shell length from 5-25 mm. These animals represent 11 unique families produced during 2014 spawning efforts. A portion of these abalone will be available for outplanting to Skagit County restoration sites in March 2016.

Juvenile Outplanting Efforts

Summary

In March 2015, the pinto abalone recovery team completed the fifth outplant of juvenile abalone within the last seven years here in Washington State. Personnel for this outplant consisted of researchers from WDFW, PSRF, and SPMC. The primary objective of the pinto abalone conservation aquaculture program is to “do no harm” to existing wild stocks of pinto abalone and therefore extreme care was taken during the restoration project described here to outplant a genetically diverse and disease free cohort of abalone.

Development of two new juvenile outplant sites at Cypress Island

With funding support from the Skagit MRC and DNR, and with collaboration from the WDFW shellfish dive team, PSRF was able to establish two new juvenile outplant restoration sites within the DNR aquatic reserve at Cypress Island. During the fall of 2014, reconnaissance dives

were conducted within appropriate habitat around Cypress Island and ideal locations were selected at both Cypress Head and along the southern shoreline in an area we refer to as the South Cypress Reef. At both sites, plot corners were permanently marked with pitons, all perimeter measurements and compass headings were recorded and accurate GPS coordinates were taken. Once each plot had been established, a thorough pre-survey of flora and fauna was conducted before abalone were introduced to the sites. There were no pre-existing abalone at either site.

Numbers, Proportions & Tagging of Outplanted Families

More than 2300 juvenile pinto abalone were outplanted to clean rocky reef habitat at six restoration sites within Skagit County. Twelve genetically distinct previously unrepresented families and an additional two families previously introduced to other sites were seeded onto the two new cites at Cypress Island (March 3rd, 2015, 726 abalone per site). Supported by MRC funding, five of the 12 distinct families were seeded onto the four sites at Burrows and Allan Islands in Burrows Bay (March 3rd, 2015, 218 abalone per site).

At the new outplant sites at Cypress Island, approximately 400 juvenile abalone released at each site were uniquely tagged with numbered, colored bee tags. This tagging effort will be an informative means of collecting mark/recapture data during the first year of surveys post-outplant including growth, survival by family and movement.

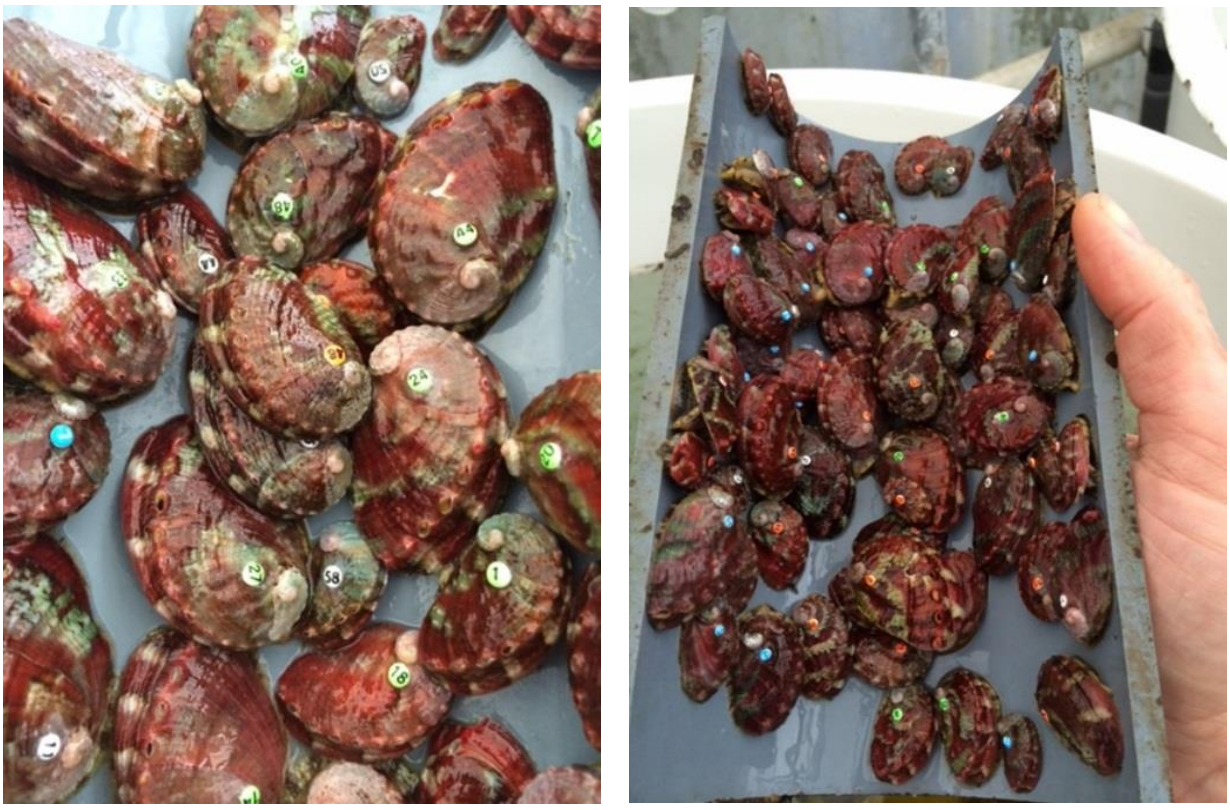

Figure 1. Tagged abalone prepared for release at Cypress Island.

Eight female and eight male broodstock were represented in the 12 new crosses. Most of the outplanted abalone during the recent effort were from the 2013 hatchery cohort, while the Cypress Island sites were augmented with two hold-over families from the 2012 cohort. The mean shell length of abalone outplanted to the two new Cypress Sites in 2015 was 25.9 mm and the mean shell length of abalone outplanted to the other four sites was 21.5 mm, an optimal size range for outplanting and achieving good survivorship.

A total number of 8512 individuals from 65 unique genetic families have now been introduced to eight different juvenile outplant sites throughout the San Juan Archipelago since 2009.

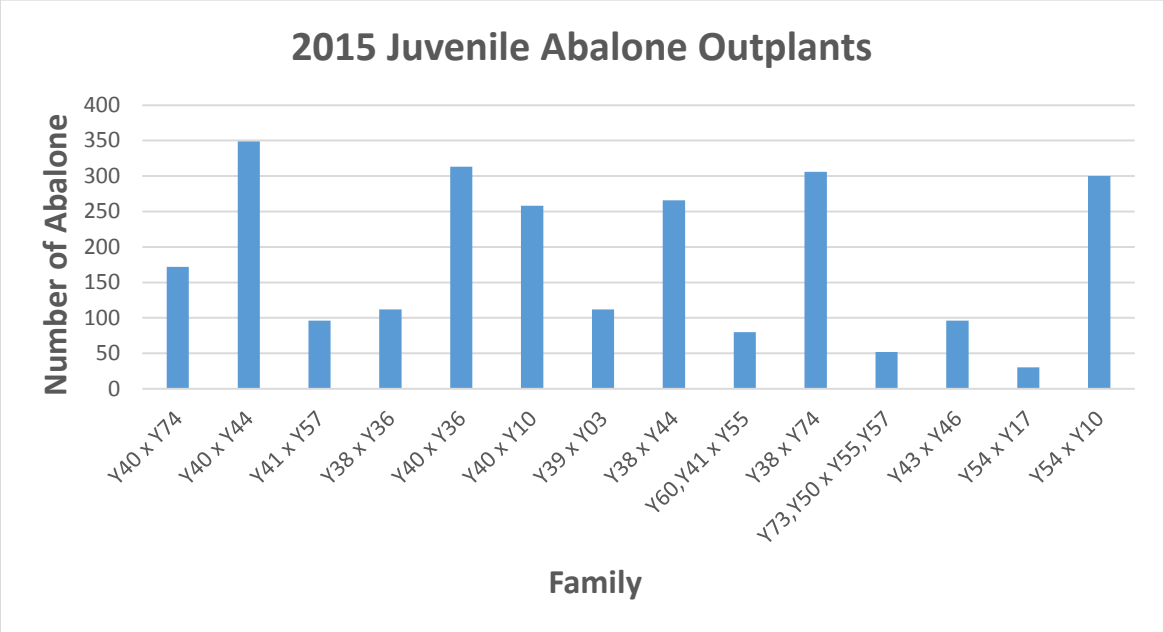

Figure 2. All juvenile abalone outplanted in 2015 arranged by family. Family designation consists of female and male parent identification.

Upcoming goals for the sites include conducting surveys for survival and growth 6-12 months post-outplant. With funding from Skagit MRC and others, this survey work will include high resolution temporal sampling (one survey per week for 6 weeks) at the two new Cypress Island sites to obtain mark-recapture growth, survival and movement data from the tagged abalone at these locations.

Figure 3. Outplant tubes filled with juvenile abalone are ready to be transported by divers to the restoration sites.

Juvenile Abalone Outplant Site Surveys

Between January and February, 2015, PSRF divers participated in dive surveys investigating survival, growth and emergence of hatchery reared pinto abalone introduced to the four existing restoration sites within Skagit County. All four of these restoration sites were surveyed prior to 2015 outplant activities. Survey set-up included locating the four plot corners, extending a survey tape measure around the plot to establish a perimeter, and installing weighted lines to distinguish 2 meter survey lanes across the plot. Divers meticulously conducted non-invasive surveys (boulders were not moved or flipped over) of each lane. Dive lights were used to investigate cracks, crevices and overhangs. Shell length and presence/absence of tags were recorded for all abalone observed (Table 1).

Site	Area (m^2)	On Plot (n)	Off Plot (n)	Tagged (n)	otal Numbe	Mean SL	Density (ab/m^2)
Burrows South	83.8	35	0	4	35	46.6	0.42
Burrows West	71.4	34	16	0	50	53.0	0.48
Allan South	101.7	28	1	0	29	64.3	0.28
Allan West	82.9	89	2	9	91	66.7	1.07

Table 1. Juvenile abalone outplant survey data in Skagit County from Jan-Feb 2015. SL=maximum shell length measurement. Density calculation excludes abalone observed off plot.

Passive Integrated Transponder Survey

Background

A reliable and robust tagging method is needed to track survival, growth and movement of abalone supplementation efforts. Common abalone tagging methods can be unsatisfactory due to tag loss, fouling and encrustation. Also, observing tag numbers of cryptically positioned abalone can be difficult. We have developed a reliable, low impact and long-term tagging method using passive integrated transponders (PIT). Small full-duplex PIT tags (8-9 mm in length) are glued to the leading interior edge of the abalone shell underneath the mantle tissue. Within 30 days, the tag is embedded in new nacre creating an inert, permanent identification. Abalone as small as 30 mm SL can be tagged with this method.

Two underwater tag readers were developed with previous funding from DNR. The first reader was developed with advice from CDFW biologists also pursuing the use of PIT tags on abalone. An HPR Plus reader and antenna were acquired from Biomark. Prevco Subsea Housings customized a housing for the Biomark HPR Plus including modifications to the antenna to ensure water resistance to depth. The second reader was developed by NOAA biologists with 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 all encased in PVC pipe.

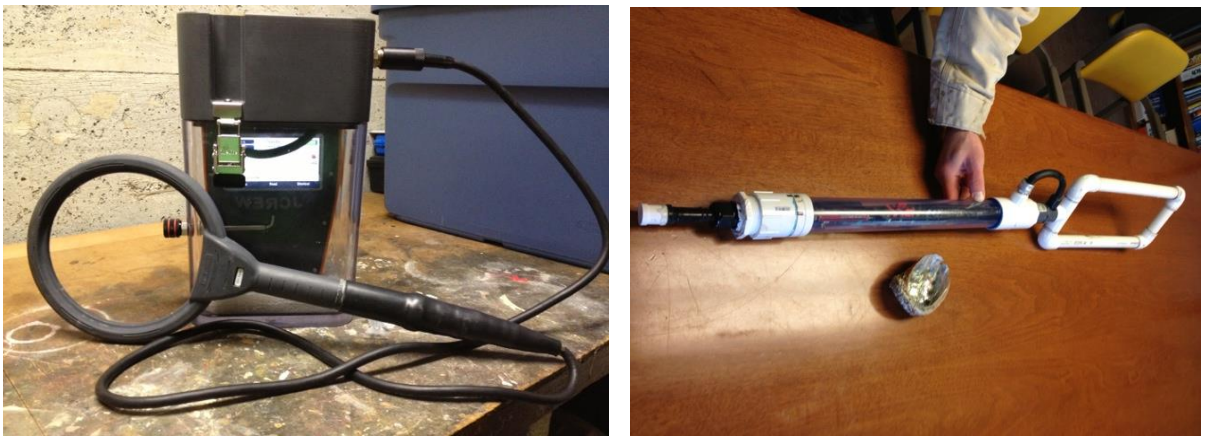

Figure 4. Biomark HPR Plus PIT tag reader contained in a custom Prevco Subsea dive housing, and underwater PIT tag reader developed and constructed by NOAA biologists.

Field Trial: PIT tagged hatchery reared young adult abalone

A PIT tagging field trial was established in 2013 during a previous contract with DNR 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 also estimates tag retention over time and survival of PIT tagged young abalone in wild.

PIT tags (9 mm HPT9 FDXB, Biomark Inc., Boise, ID) were secured on 40 juvenile or young adult abalone that ranged in size from 35-98 mm SL (Mean SL=68 mm) at the time of outplant. Each individual was also marked with a uniquely color coded and numbered bee tag (The Bee Works, Orillia, Ontario, CA) immediately prior to introduction to the study site.

In collaboration with SPMC, their seawater intake line reef was selected as an ideal site for this field trial. Four replicate ARMs (abalone recruitment modules-modified commercial crab pots with securable hinged lids) filled with coralline encrusted cobble were placed on top of the intake line concrete anchoring blocks, one ARM per block. 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 June, 2013 each of the four ARMs were seeded with 10 tagged abalone. Once per week over the following six weeks, divers conducted a full survey of the modules both visually for bee tags and with the dive PIT reader. Bee tag observations and positive PIT tag identifications were recorded on dive slates. During these initial short-term surveys, three mortalities were recorded and a majority of the abalone remained in the modules or were observed on the concrete anchoring blocks directly beneath the ARMs. Percentage of bee tag observations and positive PIT tag IDs was variable from ARM to ARM and survey to survey, and there was no significant difference in ability to record abalone IDs between the two tagging methods over the course of the six week short term surveys. In several instances, sweeping the ARM with the reader wand provided a positive PIT ID but no visual confirmation of the abalone.

Final PIT tag survey

With Skagit MRC funding, PSRF divers were able to conduct a final survey of the PIT tagged abalone on the SPMC intake reef, 26 months after the initial outplant occurred. The goals of this final survey were to determine the longevity of PIT tags in the field and to improve long term recovery of mark/recapture data from abalone from which the bee tag ID is no longer attainable. The SPMC research vessel Zoea was the dive platform for this survey. Divers used the Biomark reader and Prevco housing. Each of the four outplant modules and surrounding habitat were exhaustively examined, visually for bee tagged abalone by one diver and with the reader wand for PIT tag ID by the other diver.

Only two live abalone were observed during the survey for PIT tag identification, and a positive PIT tag ID was recorded for one of these two live abalone. This individual no longer possessed a bee tag and therefore would not have been identifiable without the PIT tag. In 26 months since outplant, this abalone grew 46 mm, increasing in shell length from 48 to 94 mm. We were also able to determine that this individual traveled almost 20 meters from where it was originally outplanted, passing through or around each of the four ARMs. The second live abalone

observed was deep in a crack beneath one of the concrete anchoring blocks and was only seen due to observation with a dive light. No bee tag was visible and it was not possible to insert the PIT tag reader antennae into the crack so no ID was obtained. Two empty shells were also recovered, both of which still had readable PIT tags embedded within the shell (Figure 5). Both of these mortalities were recovered within modules different from where they were outplanted, again indicating that the abalone moved from module to module throughout the experiment.

The low number of PIT tagged abalone observed during this final survey, while disappointing, is not surprising given the potential for predation and emigration of the abalone outplanted to the research site over the 26 month duration of the trial. More invasive survey methods covering habitat further afield from the outplant modules may have revealed additional PIT tagged abalone. Results from both the initial surveys and the recent survey indicate that tagging outplanted abalone with both the numbered/colored bee tags and with PIT tags provides the best potential for obtaining short term and long term data on survival, growth and movement post introduction from the hatchery to the field.

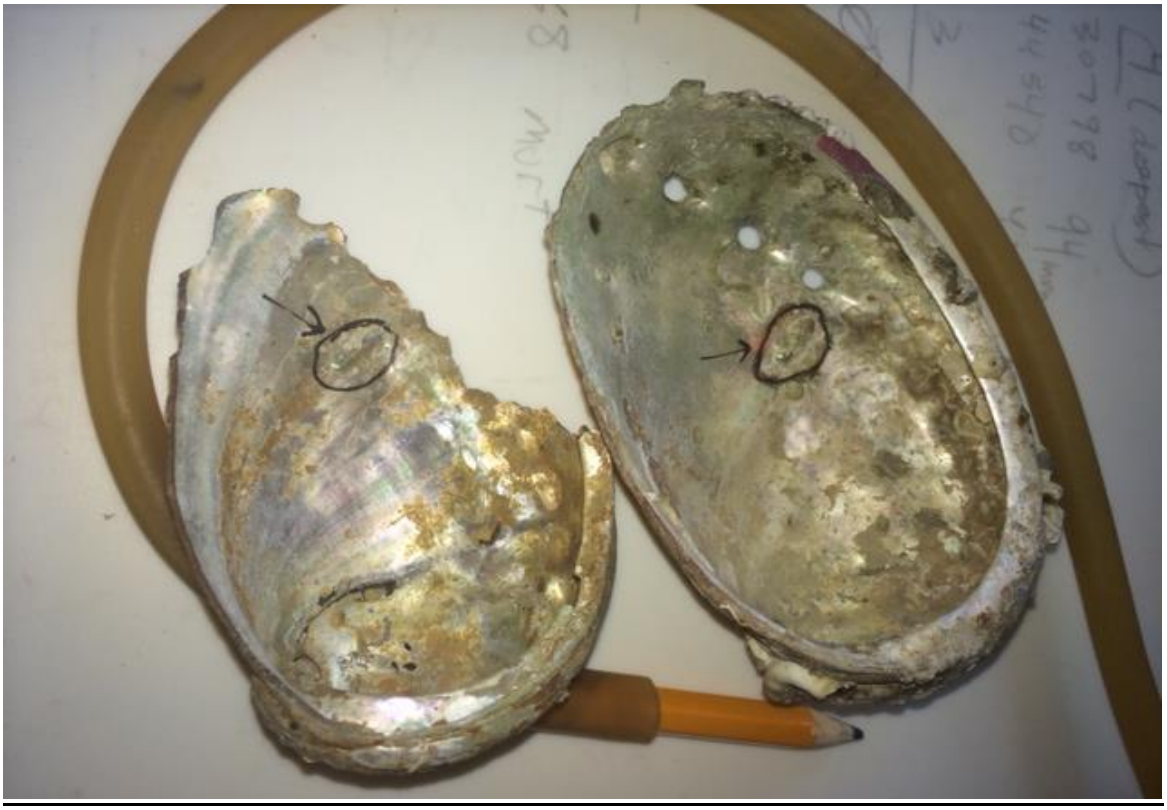

Figure 5. Abalone shells recovered during the final PIT tag survey two years post-outplant. Tags securely embedded in nacre (circled in black ink) are evident and readable.

Acknowledgments

This final report summarizes work accomplished through September 4th, 2015. Skagit County, a political subdivision of the State of Washington, is providing funding to Puget Sound Restoration Fund, a Washington non-profit corporation. Services to the County pertain to Task 8 Pinto Abalone Seeding and Monitoring under the Washington State Department of Ecology ("DOE") Grant SEANWS-2014-SkCoPW-00003 (Skagit County Contract Number C20140521), for the operations and projects of Skagit County as recommended by the Skagit County Marine Resources Committee. PSRF gratefully acknowledges the Skagit MRC and the Northwest Straits Commission for funding abalone recovery work in Skagit County in 2014-2015.