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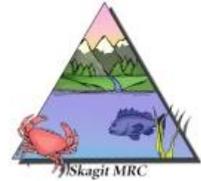
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Pinto Abalone Recovery Project
2016 Final Report to the Skagit MRC
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Introduction

Since 1969, worldwide abalone populations have declined by more than 50% with many species now recognized as threatened, endangered, or a species of concern (Hale et al. 2012). Abalone are mollusks belonging to the order Gastropoda and are closely related to slugs and snails. The pinto (northern) abalone, *Haliotis kamtschatkana*, is a species indigenous to Washington waters; they are considered functionally extinct by the state of Washington because the current number and distribution of reproductive wild abalone is too low and too widely distributed to maintain a sustainable population. The current threatened state of the Washington pinto abalone population is largely due to anthropogenic factors, primarily overharvesting (Bouma 2007).

The densities of pinto abalone in Puget Sound have been declining for over two decades, even after the closure of the recreational fishery in 1994 (populations in Washington state were never high enough to support a commercial fishery). Currently, no significant numbers of juvenile recruits have been found and the average size of abalone continues to increase (Rothaus et al. 2008, Bouma et al. 2012). Both of these measures indicate likely recruitment failure of pinto abalone in areas of historical presence such as the San Juan Archipelago. They are now listed as a U.S. Federal Species of Concern, a Washington State Candidate Species and Species of Greatest Conservation Need and as a Canadian Endangered Species (PSRF 2014).

The abalone life cycle begins with a broadcast spawning event with external fertilization of the eggs in the water column, which leads to planktonic larvae. After 7-10 days, the larvae go through metamorphosis and settle onto rocks coated in pink crustose coralline algae. Juveniles prefer rocky reef and cobbled substrates with crack and crevice habitat to hide in. They occur primarily in the shallow subtidal zone, although they have been found in depths up to 100 ft (NOAA 2007). The abalone diet changes during different life stages; these organisms are grazers and small juvenile abalones primarily graze on the diatom and bacterial biofilm, while the adults feed on various species of macroalgae.

The apparent recruitment failure and complete lack of recovery for this species is thought to be largely due to the Allee effect (Allee et al. 1949). The Allee effect can occur when existing animals are not able to find each other and reproduce successfully; a low population density means less successful reproduction and a positive feedback loop that leads to eventual population extinction. Abalone are broadcast spawners, meaning that they release sperm and eggs into the water column where fertilization occurs. Since abalone typically have a small home range, there must be other abalone present for fertilization to be successful. Babcock

and Kessing (1999) estimated that the minimum density is 0.15 abalone/m² in order for successful reproduction to occur. Extensive sampling has shown that the remaining Puget Sound pinto abalone population is likely not dense enough to facilitate the reproduction necessary for the population to recover.

Steps are being taken in an effort to help restore the pinto abalone population in northern Puget Sound waters. Puget Sound Restoration Fund (PSRF), with oversight from the Washington Department of Fish & Wildlife (WDFW), has developed a conservation aquaculture program designed to supplement depleted wild stocks. Adult broodstock abalone are collected from the wild and brought into the hatchery located at the NOAA Mukilteo Research Station. These animals are spawned in the laboratory to produce larval and juvenile abalone for future outplanting and to provide all early life stages for a variety of laboratory experiments. Over 7300 of these healthy, genetically diverse hatchery produced juvenile abalone have been outplanted to six rocky reef sites in Skagit County waters from 2009 to 2016 (plus an additional 3600 abalone at four sites in San Juan County). A summary of the numbers of abalone (tagged and untagged) outplanted to the Skagit County sites appears in Table 1. Surveys of these outplant sites to monitor survival, growth and movement have been conducted at least annually from 2009 through 2016 to provide estimates of survival and growth of abalone released into the wild. Methods and results of surveys prior to 2016 can be found in annual WDFW, PSRF and Skagit MRC summary reports as well as project reports by Shannon Point Marine Center (SPMC) students (Bergman 2009, Pratt and Dinnel 2010, Hester et al. 2011, Benolkin et al. 2012, Walker et al. 2013).

Table 1. Number of juvenile pinto abalone outplanted at Skagit County locations from 2009 through 2016 by site and year.

Site	2009	2011	2014	2015	2016 [#]	Total by Site
Burrows Island West	304*	321	358	218	0	1201
Burrows Island South	257	350*	358	218	0	1183
Allan Island West	260	330*	358	218	0	1166
Allan Island South	309*	305	358	218	0	1190
South Cypress Reef	0	0	0	726**	600	1326
Cypress Head	0	0	0	726**	601	1327
Total by Year	1130	1306	1432	2324	1201	7393

This project.

* These juvenile abalone were tagged.

**Approximately 400 of the 726 juvenile abalone were tagged at each of these two sites.

The pinto abalone recovery project in Washington State is a long-term collaboration between county, state and federal agencies, NGOs, universities, and tribes. This group includes researchers, technicians, managers, students and facilities support from the WDFW Central Shellfish team; PSRF; Western Washington University's Shannon Point Marine Center (SPMC); the NOAA Mukilteo Research Station; the University of Washington, School of

Aquatic & Fishery Sciences (UW) and others. Annual funding to PSRF from WDFW supports consistent progress in abalone hatchery and restoration activities. This support has been supplemented by additional funding in 2014-2016 from the Washington Department of Natural Resources Aquatic Restoration Program (WDNR) and the NOAA Protected Resources Division that increases hatchery capacities and field efficiencies. In 2016, Skagit County Marine Resources Committee (Skagit MRC) elected to continue supporting monitoring activities in the four previously seeded Skagit County sites (South and West Burrows Island, South and West Allan Island), to sponsor another round of seeding at the two newer outplant sites (South Cypress Reef and Cypress Head) and to fund high resolution repetitive surveys at the South Cypress Reef and Cypress Head sites.

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 collaborates 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 project accomplishments related to Skagit MRC's grant agreement #SEANWS-2015-SkCoPW-00001 Task 4 during the time period from January 2016-September 2016.

Hatchery Management

Hatchery produced juvenile pinto abalone reared with Skagit MRC support were designated for introduction at two sites 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. Specific tasks necessary to produce juvenile abalone for outplanting include:

- 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 and genetic sampling.
- 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, juvenile grow-out and diatom and macroalgal culture.

In addition to the 2014 juvenile cohort used for the outplanting project described in the next section of this report, the Mukilteo abalone hatchery also currently holds approximately 6500 small juveniles from the 2015 production year class. This batch is composed of 18 genetically distinct and previously unrepresented families produced from 7 female and 13 male broodstock parents. Most of these juveniles are still less than 10 mm in shell length. These animals will be reared and maintained by PSRF hatchery staff in the Mukilteo system in preparation for outplanting projects in Skagit and San Juan Counties in 2017.

Juvenile Outplanting in Skagit County

In April 2016, the Washington state pinto abalone recovery team completed the sixth outplant of juvenile abalone from our conservation aquaculture program since 2009. 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 outplants described here to introduce a genetically diverse and disease free cohort of abalone.

More than 1200 juvenile pinto abalone were outplanted to clean rocky reef habitat on April 19th, 2016 at Cypress Head and South Cypress Reef, two of the six restoration sites within Skagit County (Table 1, Fig. 1). Established in 2015, these two Cypress Island sites have now been seeded twice with funding support from the Skagit MRC with a total of 2653 juvenile abalone to date. Abalone destined for Cypress Island this year were not tagged as in the previous year as all possible tag number and color combinations were used on the animals outplanted there in 2015. Monitoring surveys, described in the following sections of this report were completed before new abalone were released onto the sites. The remaining four restoration sites in Skagit County (at Burrows and Allan Islands) were not overseeded in 2016 due to limited time, funding and other commitments to seed areas outside of Skagit County.



Figure 1. Untagged abalone prepared for release at Cypress Island in 2016.

The 2016 introduced juvenile cohort represented 14 new, genetically distinct families, produced from nine female and 13 male broodstock parents (Table 2). Most of the introduced abalone during the recent effort were from the 2014 hatchery cohort, supplemented with several fast growing families from 2015 hatchery production efforts. The mean shell length (SL) of abalone released to these two sites in 2016 was 27.6 mm, from an optimal size range for outplanting and achieving good survivorship. A total number of 7393 individuals from 79 unique genetic families have now been introduced to six different juvenile out plant sites in Skagit County (an additional 3600 juvenile abalone have been seeded to four other sites in San Juan County).

Table 2. All juvenile abalone outplanted in 2016 arranged by family. Family designation consists of female and male parent identification.

Family	Number	Mean Shell Length (mm)
OR06 x Y03 '14	58	28.3
OR05 x Y44 '14	132	24.1
Y82 x OR02 '14	41	32.7
Y92 x Y80, Y55, Y46 '14	46	34.6
OR06 x Y16 '14	280	22.1
OR06 x OR01 '14	280	27.1
Y81 x Y55 '14	48	32.4
OR06 x Y08 '14	98	28.7
Y87 x Y74, Y44, '14	12	30.8
Y81 x Y80, Y46 '14	28	39.2
Y95, Y85 x OR10 '15	24	20.3
OR13 x OR08 '15	18	24.4
Y95 x OR08 '15	21	27.5
OR13 x OR07 '15	115	15.1
Total & Mean SL:	1201	27.6

Juvenile Abalone Outplant Site Monitoring-Burrows and Allan Islands

Between March-April, 2016, PSRF divers participated with WDFW in dive surveys investigating survival, growth and emergence of hatchery reared pinto abalone introduced to the four original restoration sites in Skagit County, located along the shorelines of Burrows and Allan Islands. Survey set-up included locating the four permanently marked plot corners, extending a survey tape measure around the plot to establish a perimeter, and installing weighted lines to distinguish 2 m survey lanes across the plot. To investigate possible emigration beyond the outplant plot, 2016 surveys included the addition of a 2 m lane around the outside perimeter of the each plot. This additional perimeter lane effectively doubled the amount of area surveyed at each site. Monitoring efforts in previous years had opportunistically surveyed outside the plot when there was available dive time, but the measured, consistent and complete perimeter surveys this year produced a tremendous amount of informative emigration data not acquired previously.

Divers meticulously conducted non-invasive surveys (boulders were not moved or flipped over) of each lane, including the full perimeter sweep. Dive lights were used to investigate cracks, crevices and overhangs. The shell length and presence/absence of tags including tag number and color if identifiable were recorded for all abalone

observed. Empty abalone shells from mortalities were collected, measured and observed for tags when encountered and removed from the plot.

The monitoring surveys at the four Burrows and Allan Island sites in 2016 found a total of 296 abalone of which 17% were found outside of the plot boundaries (Table 3). The largest number of abalone (n=142 combined on and off plot) were found at the Allan West site and the plot density at this site was 1.34 abalone/m². Mean shell length of observed abalone for all four plots combined was 72.5 mm. Abalone as small as 20 mm and as large as 131 mm were observed during the surveys. The overall mean density of the observed abalone was 0.67/m², with individual plots ranging from 0.27 to 1.34 abalone/m².



Figure 2. Outplant tubes filled with juvenile abalone are ready to be transported by divers to the Cypress Island restoration sites in April 2016.

Table 3. Juvenile abalone outplant survey data at four sites in Skagit County from March-April 2016. SL=maximum shell length measurement.

Site	Plot Area (m ²)	On Plot (n)	Plot Density (Ab/m ²)	Perimeter Area (m ²)	Off Plot (n)	Perimeter Density (Ab/m ²)	Mean SL (mm)
Burrows South	98.2	57	0.58	96.8	2	0.02	64.4
Burrows West	75.3	20	0.27	84.8	8	0.09	68.2
Allan South	102.5	48	0.47	97.2	19	0.20	77.6
Allan West	90.0	121	1.34	93.2	21	0.23	74.4
Total	366	246	0.67	372	50	0.13	72.5

Repetitive Surveys at Two Cypress Island Sites

Standard monitoring of pinto abalone restoration locations in the San Juan Archipelago typically involves one annual dive survey per site, as described in the previous section of this report. Results from a single survey can be misleading though as abalone are cryptic by nature, particularly during the first few years following an outplant when the juveniles remain hidden in rocky reef crevices and under boulders to avoid predation. Pinto abalone will also move while foraging and may be exposed or visible by divers at one time but hidden 24 hrs later. A single survey is therefore likely to reveal only a portion of the abalone that are actually present. Repetitive surveys at locations with tagged abalone can significantly increase the estimate of survival on an outplant site as unique tag identifications occur with each survey resulting in a higher cumulative encounter rate.

In March and April, 2016 divers from PSRF and WDFW conducted four repetitive surveys over a one month period at the two juvenile abalone outplant sites at Cypress Island. These two sites were established in early 2015 and each seeded with 726 abalone, of which 369 were marked with uniquely colored and numbered tags during the initial seeding. These repetitive surveys occurred before any overseeding occurred in 2016. Survey methods were as described above and included the additional 2 m off-plot perimeter survey. Based on anecdotal observations during the first survey at Cypress Head showing that many abalone had emigrated deeper than the plot, the remaining surveys also included four additional 2 m horizontal lanes along the deep perimeter of the plot (beyond the established perimeter lane).

At the South Cypress Reef site, as many as 17 tagged and 25 untagged abalone were observed in a single dive, but the cumulative number of tagged abalone observed over four surveys was 38. At the Cypress Head site, as many as 21 tagged and 36 untagged abalone were observed in a single dive, but the cumulative number of tagged abalone observed over four surveys was 46 (Table 4).

Table 4. Results from four repetitive surveys in 2016 over a one month period at the two Cypress Island juvenile abalone outplant sites.

South Cypress Reef	24-Mar	29-Mar	13-Apr	20-Apr
Tagged	17	11	14	14
New tag observations	-	8	6	7
Untagged	25	16	19	14
Total (Tag and no tag)	42	27	33	28
<i>Cumulative Tagged</i>	<i>17</i>	<i>25</i>	<i>31</i>	<i>38</i>

Cypress Head	22-Mar	30-Mar	14-Apr	19-Apr
Tagged	18	21	20	14
New tag observations	-	7	12	9
Untagged	23	30	36	17
Total (Tag and no tag)	41	51	56	31
<i>Cumulative Tagged</i>	<i>18</i>	<i>25</i>	<i>37</i>	<i>46</i>

Discussion

The concept of outplanting hatchery raised juvenile abalone is not a new one. Work to enhance natural fisheries for abalone by seeding juveniles began about 3 decades ago in Japan (Uki 1981). Tateishi et al. (1978) found a 9-month survival rate of 48.6% for small (14 mm) outplanted abalone. Saito (1984) determined that 2-3 year survival of hatchery seed was 5-10% (versus 20-25% for naturally set seed). Kojima (1995) found survival rates ranging from 12-51% over a 2-6 year period for 15-40 mm seed. In addition to work in Japan, there have been other seeding projects in Australia, Taiwan, New Zealand and along the coast of California (reviewed by Tegner and Butler 1989; see Table 3 in PSRF 2014). Outplanted abalone survival rates associated with these projects have been highly variable (0-77%), depending on outplant size, location and species. Two early experimental projects in the Strait of Juan de Fuca with juvenile pinto abalone seed found survival rates of 6.6-12% after one year (Rothaus, unpub. data, WDFW; Stevick 2010).

As of 2016, 7393 juvenile pinto abalone have been out planted at six Skagit County sites. Of these, we now have survival estimates for abalone planted in 2009, 2011, 2014 and 2015, which total 6192 animals. Of this total, 441 abalone were observed during early 2016 monitoring surveys at the Burrows, Allan and Cypress Island outplant sites. This translates to a survival rate of 7.1% for those animals. However, this is a very conservative estimate of survival due to the fact that juvenile abalone are very cryptic and are often hidden by the complex nature of their habitat. This is confirmed by the four repetitive SCUBA surveys over

the course of one month at the South Cypress Reef and Cypress Head outplant sites in 2016. These surveys showed that there were at least 50% and 60.8% more abalone present respectively when compared to the first survey at S. Cypress Reef and the second survey at Cypress Head (given that beginning with the second survey at Cypress Head there was more habitat surveyed due to the addition of the four deep perimeter lanes). Further, all of the surveys in Skagit County have been non-invasive (i.e., no rocks were moved to reveal hidden abalone). Two previous studies in Washington state compared non-invasive with invasive (rocks moved to find hidden abalone) surveys at the same sites and found that the non-invasive surveys found only about 31% of those pinto abalone actually present (Rothaus, unpub. data, WDFW; Stevick 2010). The authors of those studies suggested that this "show factor" of 31% can be used to adjust the results of non-invasive surveys (at least for smaller abalone).

If we apply this "show factor" to the most recent survey of Skagit County plots, the estimated survival rate of 7.1% could actually be as high as 22.9%. Regardless of the actual survival rate, we do know that the current abalone densities in the four Burrows and Allan Island outplant plots now range from a low of 0.27 to a high of 1.34 abalone/m² (conservative estimates in this case, based on no application of a "show factor"). In all cases, the densities in these four outplant plots now exceed the postulated minimum density (0.15 abalone/m²; Babcock and Kessing [1999]) needed to sustain successful spawning and egg fertilization. Pinto abalone become reproductive at a size of 50 mm SL, and given that the mean SL of abalone observed at the Burrows and Allan sites is 72.5 mm, it is likely there is successful spawning occurring there.

Future abalone enhancement work envisioned by Skagit MRC and PSRF in Skagit County waters include: 1) continued occasional monitoring surveys of the plots already seeded to optimize future seeding location selection, 2) continued outplants at some of the extant plots plus creation of new plots in promising locations, 3) off-plot surveys at various distances to assess abalone migration patterns and to monitor for settlement of abalone from natural spawning, and 4) possible further exploration of "larval seeding", which deploys late stage larvae that have been conditioned to settle into complex natural habitats (or into special rock-containing modules in which larvae can settle, grow and emigrate from).

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