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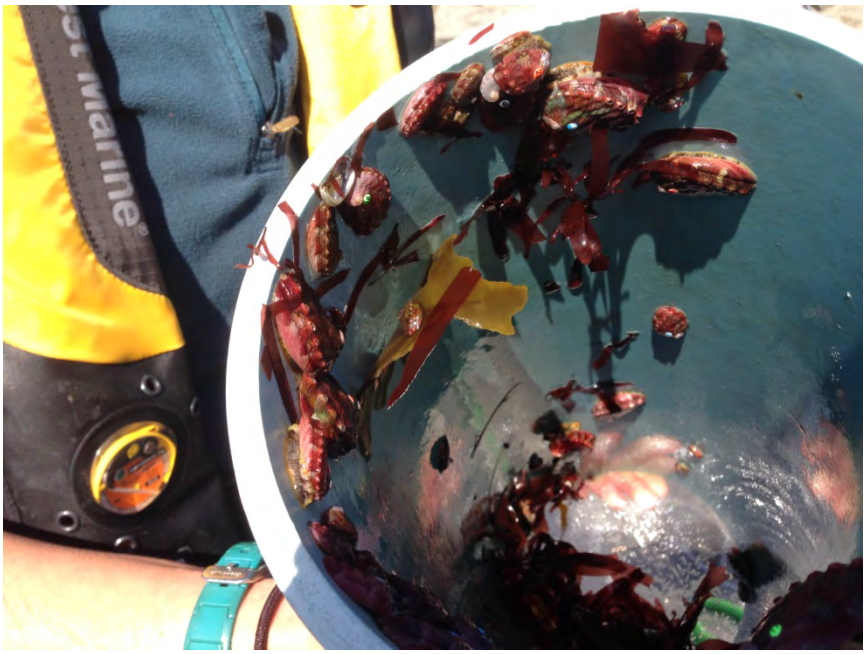

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Pinto Abalone Recovery Project
Final Report to the Skagit MRC
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September 4th, 2015

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 etg al. 2008, Bouma et al. 2012). Both of these measures indicate substantial trouble for pinto abalone in Puget Sound. 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 and cobbled substrates with plenty of cracks and crevices to hide in. Their habitat is primarily in the shallow subtidal zone, although they have been found in depths up to 100 m (NOAA 2007). The abalone diet changes during different life stages; these organisms are grazers and small juvenile abalones primarily graze on diatom and bacterial biofilms, 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/m^2 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 and Wildlife (WDFW), has developed a conservation aquaculture program designed to supplement wild depleted wild stocks. Adult broodstock abalone are being collected from the wild and brought to the hatchery located at the NOAA Mukilteo Research Station. These animals are being spawned in the laboratory to produce juvenile abalone for future out planting and to provide all early life stages for a variety of laboratory experiments. Over 6,000 of these healthy, genetically diverse hatchery produced juvenile abalone have been out planted to six rocky reef sites in Skagit County waters from 2009 to 2015 (plus an additional 2,500 abalone at two sites in San Juan County). A summary of the numbers of abalone (tagged and untagged) out planted to the Skagit County sites appears in Table 1. Surveys of these out plant sites to monitor survival, growth and movements were conducted from 2009 through 2015. Methods and results of surveys prior to 2015 can be found in annual WDFW and PSRF summary reports as well as in project reports by Shannon Point Marine Center (SPMC) (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 planted at Skagit County locations from 2009 through 2015 by site and by year.

Site	2009	2011	2014	2015[#]	Total by Site
Burrows West	304*	321	384	218	1,227
Burrows South	257	350*	384	218	1,209
Allan West	260	330*	384	218	1,192
Allan South	309*	305	384	218	1,216
South Cypress Reef	0	0	0	726**	726
Cypress Head (east)	0	0	0	726**	726
Total by Year	1,130	1,306	1,536	2,324	6,296

[#]This project.

*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, which has been

supplemented by additional support in 2014-2015 from the Washington Department of Natural Resources (WDNR), Aquatic Restoration Program. In 2015, Skagit County Marine Resources Committee (Skagit MRC) elected to adopt seeding and monitoring activities in the four previously seeded Skagit County sites (South and West Burrows Island and South and West Allan Island) and to sponsor seeding at two new county sites (South Cypress Reef and Cypress Head).

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 out planting 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 out planting at six sites in Skagit County. Hatchery responsibilities to produce abalone for out planting 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 out planting 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.

Spawning Success, Production and Grow Out

The primary production objective during the 2015 summer spawning season was 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 at the hatchery. In May and June, several induced spawns resulted in the production of almost 500,000 larvae competent for settlement and representing four genetically distinct families.

An estimated 3,500 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 out planting to Skagit County restoration sites in March 2016.

Juvenile Out Planting in Skagit County

In March 2015, the pinto abalone recovery team completed the fifth out plant of juvenile abalone within the last seven years in Washington State. Personnel for this out plant 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 out plant a genetically diverse and disease free cohort of abalone.

Development of two new juvenile out plant sites at Cypress Island

With collaboration from the WDFW shellfish dive team, PSRF was able to establish two new juvenile out plant restoration sites within the WDNR 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 and tagging of out planted families

More than 2300 juvenile pinto abalone were out planted to clean rocky reef habitat at six restoration sites within Skagit County (Table 1, Fig. 1). 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). Five of the 12 distinct families were also seeded onto the four previously established sites at Burrows and Allan Islands (March 3rd, 2015, 218 abalone per site).

At the new out plant sites at Cypress Island, approximately 400 juvenile abalone released at each site were uniquely tagged with numbered, colored bee tags. This tagging

effort will allow collection of mark/recapture data during the first year of the survey's post-out plant, 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 out planted abalone during the recent effort were from a 2013 hatchery cohort, while the Cypress Island sites were augmented with two hold-over families from a 2012 cohort. The mean shell length of abalone out planted to the two new Cypress Sites in 2015 was 25.9 mm and the mean shell length of abalone out planted to the other four sites was 21.5 mm, an optimal size range for out planting and achieving good survivorship. A total number of 6,296 individuals from 65 unique genetic families have now been introduced to six different juvenile out plant sites in Skagit County (Figs. 2 and 3).

A primary objective for the out plant sites is to conduct surveys for survival and growth 6-12 months post-out plant. 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.

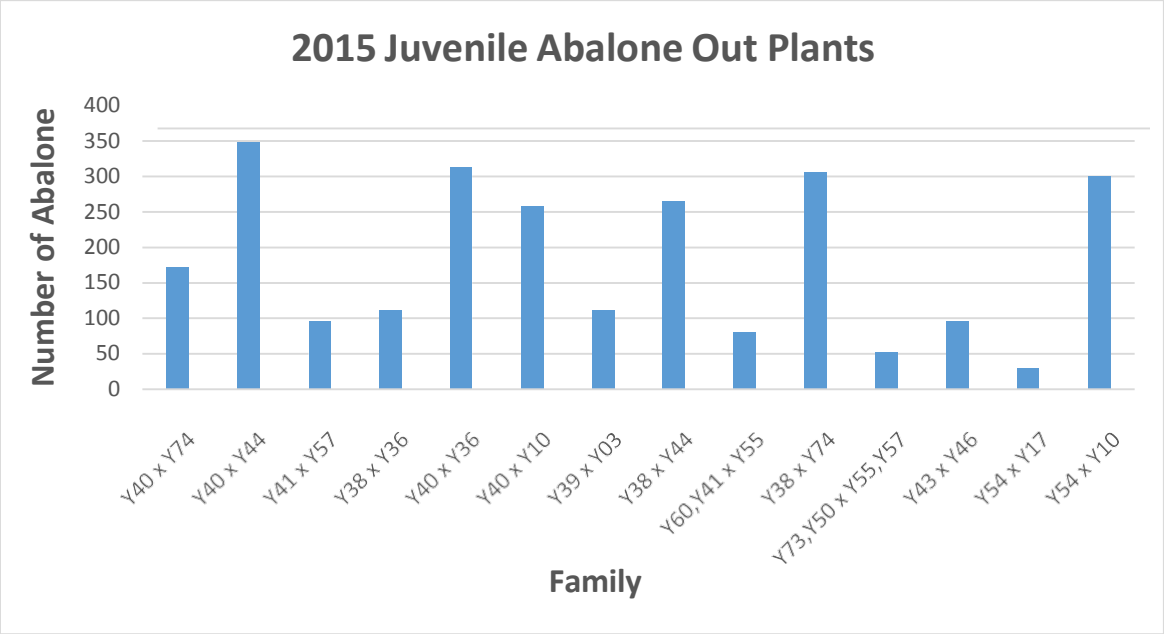

Figure 2. All juvenile abalone out planted in 2015 arranged by family. Family designation consists of female and male parent identification.

Juvenile Abalone Out Plant Site Monitoring 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 in Skagit County. All four of these restoration sites were surveyed prior to the 2015 out plant 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 m 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.

The monitoring surveys at the four Burrows and Allan Island sites in January and February 2015 found a total of 205 abalone of which 5% were found outside of the plot boundaries (Table 2). The largest number of abalone were found at the Allan West site. Mean shell length for all plots combined was 59.6 mm, with a range of 46.6 to 66.7 mm for the individual plots. The overall mean density of the observed abalone was 0.56/m^2, with individual plots ranging from 0.28 to 1.07 abalone/m^2.

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

Table 2. Juvenile abalone out plant survey data in Skagit County from Jan-Feb 2015.

Site	Area (m^2)	On Plot (n)	Off Plot (n)	Tagged (n)	Total Number	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

SL=maximum shell length measurement. Density calculation excludes abalone observed off plot.

Passive Integrated Transponder Survey

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 WDNR. The first reader was developed with advice from California Department of Fish and Wildlife 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 at 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 (Fig. 4).

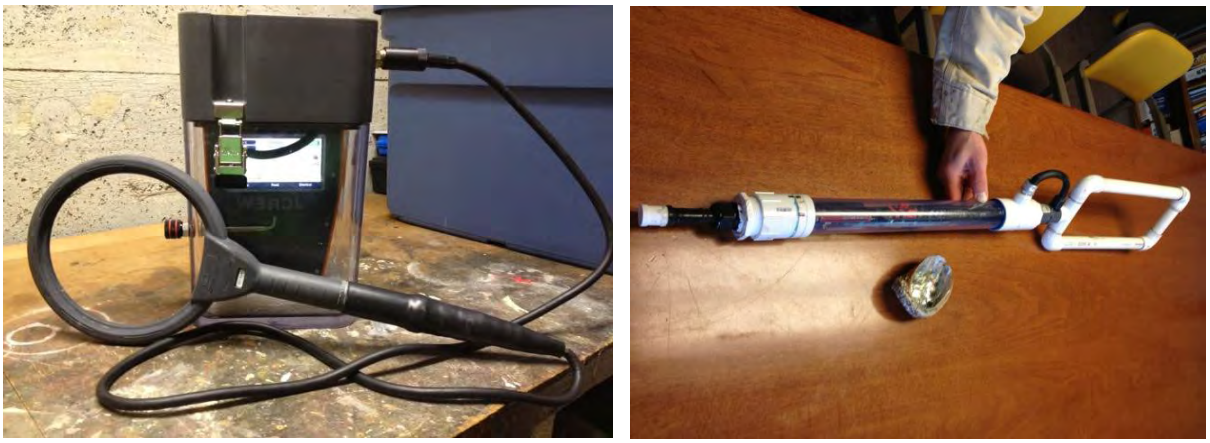

Figure 4. Biomark HPR Plus PIT tag reader contained in a custom Prevco Subsea dive housing, and underwater PIT tag reader developed and constructed by NOAA biologists.

Field trial: PIT tagged hatchery reared young adult abalone

A PIT tagging field trial was initiated in 2013 during a previous contract with WDNR 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 out plant. 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 out plant 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 out plant 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 out plant, 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 out planted, 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 out planted, again indicating that the abalone moved from module to module throughout the experiment.

Discussion

The concept of out planting 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) out planted 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). Out planted abalone survival rates associated with these projects have been highly variable (0-77%), depending on out plant size, location and species. Two early experimental projects in Puget Sound with juvenile pinto abalone seed found survival rates of 6.6-12% after one year (Rothaus, unpub. data, WDFW; Stevick 2010).

As of 2015, 6,296 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 and 2014, which total 3,972 animals. Of this total, 205 abalone were observed during early 2015 monitoring surveys at the four Burrows and Allan Island out plant sites. This translates to a survival rate of 5.2% 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. For instance, four repetitive SCUBA surveys at weekly intervals at four Skagit out plant sites in 2011 showed that there were at least 23.7% more abalone present compared to the first surveys of these sites (Hester et al. 2011). 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 Puget Sound 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 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 5.2% could actually be as high as 16.8%. Regardless of the actual survival rate, we do know that the current abalone densities in the four Burrows and Allan Island out plant plots now range from a low of 0.28 to a high of 1.07 abalone/m^2 (conservative estimates in this case, based on no application of a "show factor"). In all cases, the densities in these four out plant plots now exceed the postulated minimum density (0.15 abalone/m^2; Babcock and Kessing [1999]) needed to sustain successful spawning and egg fertilization.

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 out planted to the research site over the 26 month duration of the trial. More invasive survey methods covering habitat further afield from the out plant modules may have revealed additional PIT tagged abalone. Results from both the initial surveys and the recent survey indicate that tagging out planted 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.

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 out-plants at some of the existing 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 spawnings, 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).

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

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