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Pinto Abalone Recovery Project
2018 Final Report to the Skagit MRC
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September 30th, 2018

Introduction

The pinto (northern) abalone, *Haliotis kamtschatkana*, is the primary abalone species indigenous to Washington waters. Populations are severely depleted and considered functionally extinct 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, including overharvesting during the legal recreational fishery and poaching during the 1980-90s (Bouma 2007). Populations in Washington state never supported a commercial fishery for abalone. Between 1992 and 2017, the density of pinto abalone declined by 98% at 10 index sites in the San Juan Archipelago (SJA) even after the closure of the recreational fishery in 1994 (Rothaus et al. 2008, WDFW unpublished data). Insignificant numbers of juvenile recruits have been observed and the average size of abalone continues to increase (Rothaus et al. 2008, Bouma et al. 2012, WDFW unpublished data). Both of these measures indicate likely recruitment failure of pinto abalone in areas of historical presence. 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).

Abalone are broadcast spawning invertebrates, gametes undergo fertilization in the water column. After a 7-10 day planktonic larval phase, the larvae go through metamorphosis and settle onto rocks encrusted with pink coralline algae. Juveniles prefer rocky reef and cobbled substrates with crack and crevice habitat to hide in. This large marine snail occurs 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; larval abalone are lecithotrophic while planktonic, small juvenile abalone 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. 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 San Juan Archipelago pinto abalone population is well below this threshold, unable to facilitate necessary reproduction for natural population recovery.

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 a hatchery located at the NOAA Manchester Research Station. These animals are spawned in the laboratory to produce larval and juvenile abalone for future outplanting and to provide early life stages for a variety of laboratory experiments. Almost 9200 of these healthy, genetically diverse hatchery produced juvenile abalone have been outplanted to six rocky reef sites in Skagit County waters from 2009 to 2017 (plus an additional 5800 abalone at six sites in San Juan County). A summary of the numbers of abalone 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 2018 to provide estimates of survival and growth of abalone released into the wild. Methods and results of surveys prior to 2018 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 2017 by site and year.

Site	2009	2011	2013	2014	2015	2016	2017#	Total by Site
Burrows Island West	304	321	0	358	218	0	0	**1201**
Burrows Island South	257	350	0	358	218	0	456	**1639**
Allan Island West	260	330	0	358	218	0	431	**1597**
Allan Island South	309	305	0	358	218	0	454	**1644**
South Cypress Reef	0	0	0	0	726	600	0	**1326**
Cypress Head	0	0	0	0	726	601	458	**1785**
Total by Year	**1130**	**1306**	**0**	**1432**	**2324**	**1201**	**1799**	**9192**

Most recent outplant effort, no juvenile abalone were outplanted in 2018.

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; Puget Sound Restoration Fund; Western Washington University's

Shannon Point Marine Center (SPMC); the NOAA Mukilteo & Manchester Research Stations; the University of Washington, School of Aquatic & Fishery Sciences (UW); outreach facilities such as the Port Townsend Marine Science Center, WWU's Sea Discovery Center in Poulsbo, Bellingham Marine Life Center 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 2017-2018 from the NOAA Protected Resources Division that increases hatchery capacities and field efficiencies. In 2017-2018, the Skagit County Marine Resources Committee (Skagit MRC) elected to continue supporting monitoring activities at the previously seeded Skagit County sites (South and West Burrows Island, South and West Allan Island, South Cypress Reef and Cypress Head) and additional surveys at a younger juvenile outplant experimental site at Allen Island. MRC funding also allowed PSRF to collaborate with graduate student research being conducted at SPMC investigating the efficacy of larval outplanting as a recovery strategy.

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 the abalone conservation aquaculture program, PSRF collaborates with WDFW on all associated field efforts including outplant site surveys, restoration strategy field trials and juvenile outplanting. The following report summarizes project accomplishments related to Skagit MRC's grant agreement #SEANWS-2017-SkCoPW-00002 Task 5 during the time period from October 2017-September 2018.

Hatchery Management

The conservation aquaculture program managed by PSRF and operated at the NOAA Manchester Research Station produces larval and juvenile pinto abalone for research and outplanting. Hatchery responsibilities to produce abalone for future outplanting projects includes coordination, supervision and implementation of daily coverage, weekly maintenance and regular aquaculture activities at the Ken Chew Center for Shellfish Research & Restoration at NOAA Manchester. Specific tasks necessary to produce hatchery reared abalone include:

- Tank cleaning & filter maintenance.
- Water quality monitoring—temperature, salinity, pH, dissolved oxygen, total gas pressure and flow rates.
- 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 are 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/macroalgal culture.

Maintenance and husbandry of post-set animals from the 2017 cohort was one of the primary activities within the abalone nursery during this past fall/winter/spring. PSRF hatchery staff trained a new technician at the end of 2017 to manage the maintenance tasks listed above. Weekly care of this post-set population in the nursery included gentle tank cleaning, feeding with three diatom species and the red macroalgae dulse (*Palmaria mollis*) cultured at the hatchery. Once post-set abalone reached 5 mm in shell length, they were weaned onto the dulse by blending it into small flakes and introducing it into the grow-out tanks.

While spawning and settlement success had been high during the summer and early fall 2017, post-set tanks from most families and spawning cohorts experienced high mortality in early November 2017. It is possible this mortality was connected to low flow issues at the facility during main intake system maintenance and pump replacement. Seawater temperature control was also difficult during this time. Growth rates of survivors continued to be slow through the winter.

In response to this low survival and slow growth rates possibly connected to flow issues at the facility, seawater temperature control difficulties and low pH, PSRF developed a plan for upgrades to the nursery building seawater supply. A buffering system using sodium carbonate was installed in the nursery in March and in the hatchery in May to elevate pH of incoming seawater from 7.75 to 8.0. This buffered seawater currently supplies all abalone grow-out and broodstock tanks in the nursery and the larvaculture system in the hatchery. The seawater reservoir in the nursery was replaced in June and by tripling its size has made possible better temperature and pH control. The glycol system that heats seawater to the hatchery will be upgraded to also support heated seawater to the nursery building. A new heat exchanger, glycol reservoir and all associated pumps, controllers and plumbing have been installed and the system will be heating seawater to the abalone nursery by the end of October 2018.

PSRF collected the annual hatchery health sample of 60 live juveniles representative of all the families produced during the 2017 spawning season for disease screening on February 5th,

2018. A pathology report from Dr. Ralph Elston, Aquatechnics Inc. concluded that no disease causing pathogens were present in the samples and gave the current hatchery seed group a clean bill of health. Inventory and shell length data were collected in June 2018 by the PSRF abalone technician for all families produced in 2017. 2500 juvenile abalone from 14 families will be cultured through the winter and available for restoration outplants in both Skagit and San Juan Counties in March 2019.

Annual broodstock collections were conducted in the San Juan Islands in March 2018 and 15 new animals (8 females, 7 males) were brought to the Manchester hatchery. All new broodstock were measured and weighed, ranked by gonad index, fouling sponges were removed from the shell, animals were tagged with vinyl disc and PIT tags, and genetic samples were taken using epipodial tentacle clips and archived for later analysis. General observation of overall health was also noted. The 2018 production season concluded in September and 21 new genetically distinct families were produced. This included 1.8 million competent larvae from 7 females and 18 males settled into 68 culture tanks at the station.

Juvenile Outplanting in Skagit County

The Washington state pinto abalone recovery team did not do any juvenile outplanting to restoration sites in either Skagit or San Juan County in 2018. This was primarily due to priority being given to the young abalone outplant experiment described in a later section of this report. In order to conduct that experiment successfully, the hatchery-reared cohort that would have typically been outplanted in 2018 at an age of approximately 18 months and 20 mm in shell length was instead outplanted at earlier juvenile life stages in 2017. These animals were necessary to test the hypothesis that the conservation aquaculture program can be maximized by instead releasing abalone to the wild at an age of 9-14 months old.

The primary objective of the pinto abalone conservation aquaculture program aims to “do no harm” to existing wild stocks of pinto abalone and therefore extreme care is always taken during restoration outplants to introduce a genetically diverse, healthy cohort of abalone. If future outplant cohorts consist of younger, smaller abalone, this could prevent both hatchery acclimation and hatchery selection concerns.

Established in 2009 and 2015 respectively, the Burrows Bay (Burrows and Allan Islands) and Cypress Island restoration sites, with funding support in part from the Skagit MRC, have been seeded in 2009, 2011, 2013, 2014, 2015, 2016 and 2017. A total number of 9192 individuals from 88 unique genetic families have been introduced to these six different juvenile outplant sites in Skagit County. As a whole, the pinto abalone recovery program has introduced more

than 15,000 juvenile abalone from 96 families to 12 sites in both Skagit and San Juan Counties.

Figure 1. Divers spend their surface interval between dives traversing from one site to the next during abalone work in February 2018.

Juvenile Abalone Outplant Site Monitoring-Cypress, Burrows and Allan Islands

Between February-March, 2018, PSRF divers participated with WDFW in dive surveys investigating survival, growth and emergence of hatchery reared pinto abalone introduced to the six restoration sites in Skagit County, located along the shorelines of Cypress, 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. Replicating similar perimeter sweeps initiated in 2016 and continued in 2017, surveys this year included the addition of the 2 m survey around the entire outside perimeter of each plot. This additional perimeter lane doubles the amount of area surveyed at each site during a standard survey and provides informative emigration data.

Divers meticulously conducted non-invasive surveys (boulders were not moved or flipped over) of each lane, including the full perimeter sweep. Dive lights and small mirrors 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. Notes were also taken on where each observation was made within the plot lane (deep, mid or shallow) and how the animal was oriented within the substrate (cryptic, semi-cryptic or emergent). Empty abalone shells from mortalities were collected, measured and observed for tags when encountered and then removed from the plot. A team that included six different divers conducted 31 dives to complete the site surveys at Burrows, Allan and Cypress Islands (Figure 2).

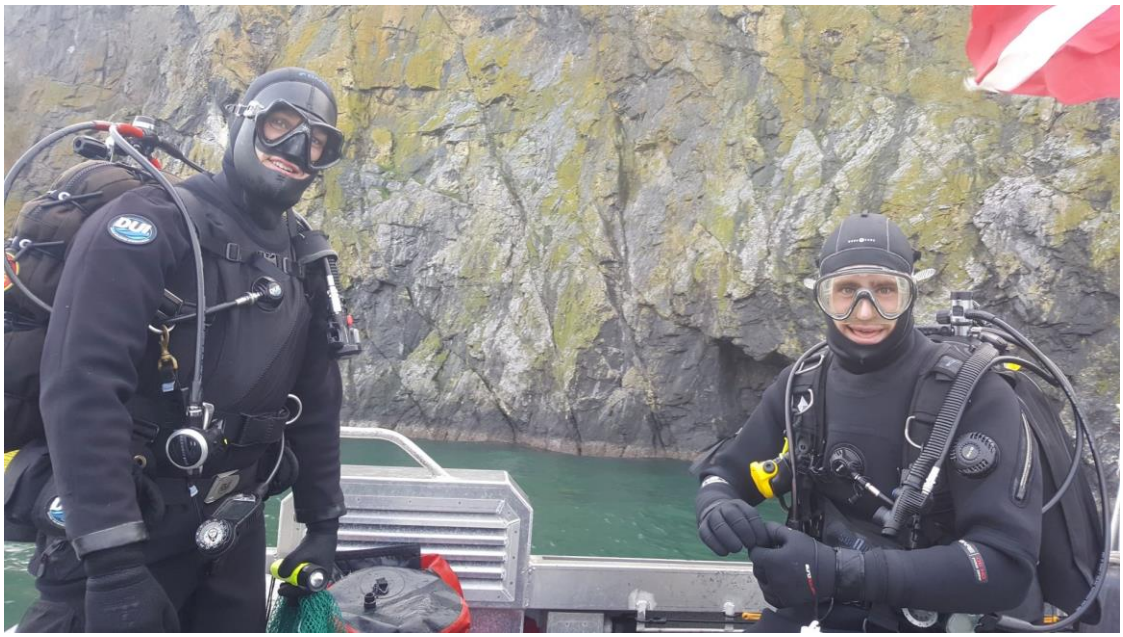

Figure 2. PSRF divers prepare for abalone work in Skagit County in March 2018.

Figure 3. Adult pinto abalone observed on a recovery plot likely to be from an original outplant cohort.

The monitoring surveys at the six Cypress, Burrows and Allan Island sites in 2018 found a total of 537 abalone (an increase from 372 in 2017) of which 33% were found outside of the plot boundaries (Table 2). The largest number of abalone (n=185 combined on and off plot) were found at the Allan West site and the plot density at this site was 1.53 abalone/m^2. Mean shell length of observed abalone for all six plots combined was 77.2 mm. Abalone as small as 15 mm and as large a 134 mm were observed during the surveys (Figure 3). The overall mean density of observed abalone on-plot was 0.69/m^2 (an increase from 0.50/m^2 in 2017) with individual plots ranging from 0.13 to 1.53 abalone/m^2 (Table 2).

Table 2. Juvenile abalone outplant survey data at six sites in Skagit County from February-March 2018. 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	75	0.76	96.8	33	0.34	77.8
Burrows West	75.3	10	0.13	84.8	16	0.19	102.7
Allan South	102.5	55	0.54	97.2	17	0.17	88.2
Allan West	90.0	138	1.53	93.2	47	0.50	79.5
South Cypress Reef	77.9	16	0.21	131.1	30	0.23	66.8
Cypress Head	79.3	66	0.83	162.6	34	0.21	64.2
Total	**523.2**	**360**	**0.69**	**665.7**	**177**	**0.27**	**77.2**

*Perimeter survey areas at South Cypress Reef and Cypress Head were expanded in 2018 by adding additional 2 m survey lanes beyond the standard perimeter due to a number of abalone observations beyond the perimeter.

**Mean SL at each site includes animals observed both on and off plot.

Table 3. Outplant success defined at the six restoration sites in Skagit County. SL=maximum shell length measurement.

Site	Total Outplanted	Current # >50 mm SL	% Outplant Success*	Continue Overseeding
Burrows South	1639	76	4.6%	Yes
Burrows West	1202	26	2.2%	No
Allan South	1644	58	3.5%	Yes
Allan West	1597	159	10.0%	Yes
South Cypress Reef	1326	38	2.9%	No
Cypress Head	1786	77	4.3%	Yes
Total	**9194**	**434**	4.7%	

*Outplant success over time is defined by the percentage of animals over 50 mm SL at each site divided by the number of abalone outplanted to that site at least a year prior to the survey.

Analysis of outplant monitoring data provides evidence that some sites should not receive continued overseeding due to poor survival (Table 3). Metrics for this determination include the percentage survival at one year post-outplant and the percentage of outplant success (the percentage of animals that have reached reproductive maturity, 50 mm shell length, divided by the number outplanted to the site at least a year prior to the survey). These discontinued sites will continue to be surveyed each year along with the overseeded sites to confirm proper designation as non-productive.

Younger Abalone Outplant Experiment-Allan Island

With funding support from WDFW, Skagit MRC, NOAA and the Hall Family Foundation, an outplant experiment to test the hypothesis that hatchery-reared abalone can be successfully introduced at a younger age and smaller size was initiated in early 2017. Background for this project, description of methods, timeline and results to date were described in detail in the PSRF annual abalone report to Skagit MRC in last year's report, September 2017 (Bouma and Dinnel 2017). In summary, restoration outplants could be maximized by shifting away from our current strategy of seeding hatchery reared juveniles at an 18 month age and 20 mm shell length. Instead, seeding would occur to recovery sites with robust animals from the hatchery as early as 9 months post-settlement. This potential new outplant strategy reduces significant demands on time and resources within the hatchery (technician labor, equipment and supplies, pump maintenance, utility costs for heating and lighting, feed requirements etc.). It also protects against some of the drawbacks of rearing juveniles in the hatchery for extended periods prior to release such as hatchery acclimation and hatchery selection.

Experimental modules were filled with coralline algae encrusted cobble, spaced 30 m apart and placed at Allan Island along the shoreline contour and at the same depth elevation. Three replicate young abalone modules (YAMs 1, 3 & 5) were seeded with 9 month-old abalone (mean SL=8 mm) in April 2017. Three additional replicates (YAMs 2, 4 & 6) were seeded with abalone as the second age treatment; 14 month-old juveniles in September 2017. This second batch of animals had remained in nursery tanks at the hatchery for an additional five months, and were outplanted (mean SL=14 mm) with identical methods. Additionally, two control modules (crab trap and cobble included) were set up in culture tanks at the Mukilteo hatchery and seeded with 9 month and 14 month juveniles in April and September 2017, respectively, with similar methods to the field modules.

The initial survey plan was to observe one replicate from each age treatment on six month intervals. Divers determine survival by deconstructing modules *in situ* (Figure 4). Each rock is carefully removed and inspected for the presence of juvenile abalone, which are measured

using calipers. Afterward, the module is carefully reconstructed. A three meter perimeter of surrounding substrate is also invasively sampled by divers, lifting, turning and examining each piece of cobble, to locate individuals that have left the YAMs. The hatchery control modules are also surveyed for survival and growth at the same time intervals as experimental field modules.

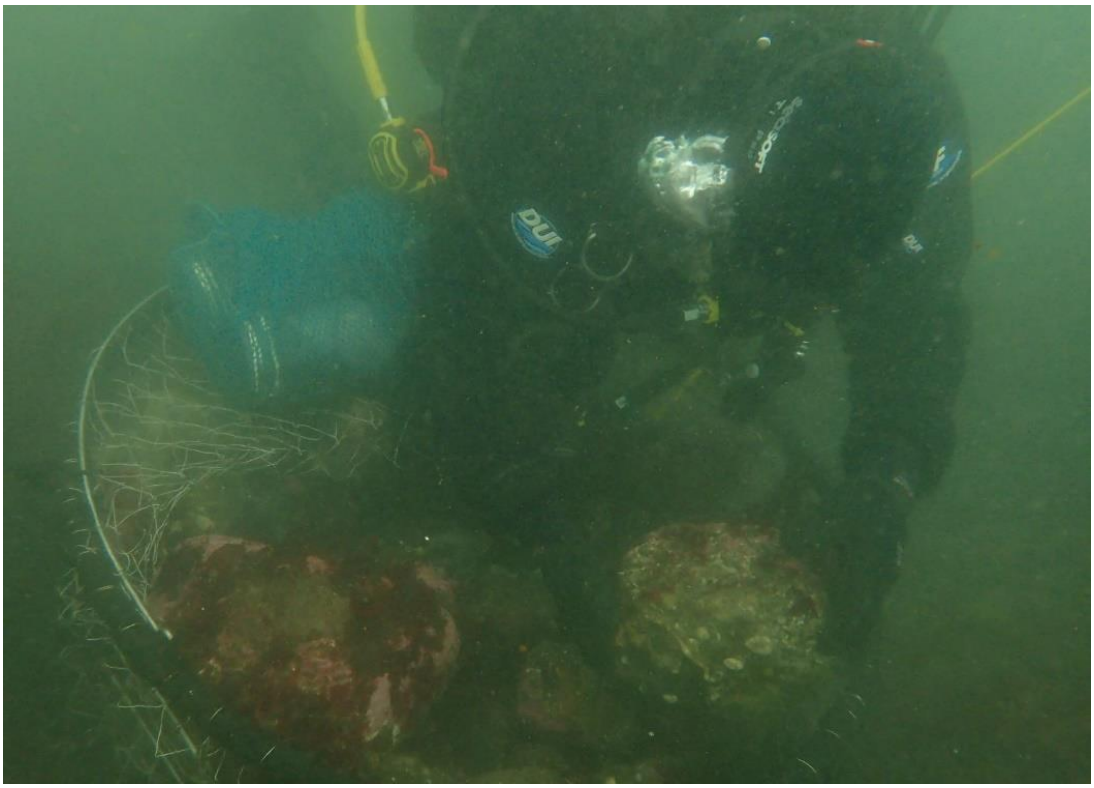

Figure 4. A diver surveys a young abalone outplant module (YAM) for juvenile abalone.

This pilot-scale outplant trial of earlier stage juvenile abalone follows the timeline below. Completed objectives are in black, objectives covered in this report are in blue and upcoming objectives are in red.

- June-September 2016, induced spawning at Mukilteo hatchery to produce young cohort.
- October 2016-March 2017, post-larval/early juvenile nursery culture at Mukilteo hatchery.
- March 2017, divers investigate & confirm northwest Allan Island experiment site.
- April 2017, outplant of 9 month-old juveniles (~8mm shell length) to YAMs 1, 3, 5.
- September 2017, outplant of 14 month-old juveniles (~14mm shell length) to YAMs 2, 4 & 6. Survey #1 at ~6 months post-outplant (younger age treatment only, lab control included).
- March 2018, survey #2 at 12 months and 6 months, respectively, post-outplant (both age treatments, lab controls included).

- October 2018, survey #3 at 19 months and 13 months, respectively, post-outplant (both age treatments, lab controls included).
- March 2019, survey #4 at 24 months and 18 months respectively, post-outplant (both age treatments).
- April 2019, final analysis and summary of survey results to inform all 2019 outplant efforts.

Survey results from March 2018 include thorough surveys of the 9 mo age treatment modules (YAM #1, 3, 5) marking one year post-outplant, and the 14 mo age treatment (YAM #4) marking 6 months post-outplant. This included deconstruction of the module and invasive observation of all substrate up to 3 m from the YAM.

No abalone were observed within or surrounding YAM 1, which was not surprising given that only 2 abalone were observed at this site during the previous survey. However, in YAMs 3 and 5, there were 16 and 13 abalone observed respectively. This is an average survival rate across all three modules (9 mo age at outplant) of 9.7% for the 1 yr period following outplant. The experimental design calls for observation of one replicate every 6 months, therefore only one module was surveyed from the 14 mo age treatment in March 2018. During deconstruction of the module and invasive observation of all substrate up to 3 m away from YAM 4, a total of 10 abalone were counted. This is a survival rate for juveniles 14 months-old at outplant of 12.3% for the 6 month period post-outplant. Most of the abalone seen during these two surveys were located in the top third of substrate within the YAMs and were also oriented on clean, coralline encrusted portions of the cobble.

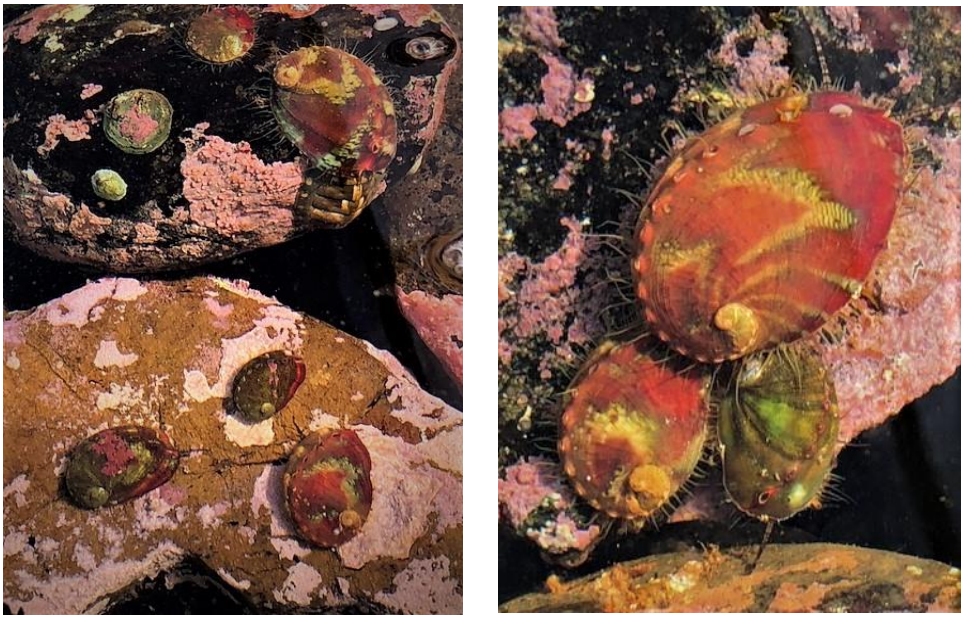

Figure 5. Juvenile pinto abalone from the YAM experiment control modules.

The hatchery control modules, set up with a similar crab trap cobble complex and seeded with the same group of 9 and 14 month-old abalone were both surveyed in March 2018 (Figure 5). Each piece of substrate was removed from the tank and observed closely for survival. 49 abalone were counted and measured in the 9 mo age control, a survival rate of 48% over 1 yr since seeding. In comparison, 48 abalone were counted in the 14 mo-old control, a survival rate of 62% after 6 months post-seeding (Table 4).

Table 4. March 2018 survey results from the younger juvenile abalone outplant experiment at Allan Island, Skagit County.

Treatment	Module	# Abalone Observed	% Survival	Mean SL (mm)
9 mo-age*	YAM 1	0	0	0
	YAM 3	16	15	22.3
	YAM 5	13	12	20.5
	Avg	10	9	21.4
	Lab Control	49	48	28.2
14 mo-age**	YAM 4	10	12	13.4
	Lab Control	48	62	26.6

*At time of survey, 9 mo-age treatment group was 12 months post-seeding.

**At time of survey, 14 mo-age treatment group was 6 months post-seeding.

Two survey periods remain in this experiment. In October 2018, all three YAMs (1, 3 and 5) from the younger age treatment will be resurveyed and two modules from the older age treatment will be surveyed (YAM 4 resurveyed, YAM 2 first survey). In March 2019 all six modules will be surveyed one final time for survival and growth.

Larval Outplant Experiment-WWU/SPMC Research

To investigate if larval outplanting may be an alternate recovery strategy, PSRF is collaborating on a research project with a graduate student, Katie Mills-Orcutt, from Dr. Deborah Donovan's lab at WWU. Katie is conducting a small-scale field experiment using two replicate sites in Skagit County, one at Young Island in Burrows Bay and the other near the intake pipeline at SPMC. Settlement modules were constructed from plastic milk crates and filled with stacks of polycarbonate plates for sampling. Each site consists of three modules that are tented for larval retention, three that are open (no Nitex), and three control modules. Control modules consist of open modules with no hatchery-reared larvae added. These controls will determine if modules have been placed far enough apart by showing whether larvae traveled between

modules at the time of outplanting. There are 24 modules in total, nine each at both field sites and six laboratory controls (three tented and three open modules).

Abalone larvae from an induced spawn with wild-collected broodstock at the NOAA Manchester station were reared both at the NOAA Manchester and at SPMC. Competent larvae were then exposed to a chemical settlement cue (GABA) and introduced into experimental modules (n=7000 larvae/module) in July 2018 (Figure 6). The modules are now being sampled each month from August-November 2018. This is accomplished by divers opening each module and collecting one set of settlement plates during each sample collection. Plates are transported in seawater filled containers back to the lab and analyzed via light microscopy. Katie will be calculating density and size of settled abalone from each sampling. Laboratory controls will be used to compare site survival and growth rates to a controlled setting. PSRF advised on experimental design and has participated in diving activities to conduct site reconnaissance, set-up, larval seeding and sample collection. Experimental results will be communicated in spring 2019.

Figure 6. Polycarbonate plate racks are constructed and placed into larval seeding modules. PSRF and SPMC personnel prepare larvae-filled syringes for divers to inject into the modules on settlement day.

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 7-12% after one year (Rothaus, unpub. data, WDFW; Stevick 2010).

Between the years 2009-2017, 9192 juvenile pinto abalone have been outplanted at six Skagit County sites. Survival estimates are based on annual monitoring at all six of these sites. Of the hatchery-reared outplant total, 537 abalone were observed during 2018 monitoring surveys at the Burrows, Allan and Cypress Island sites. This translates to a survival rate of 6% 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 has been confirmed by repetitive SCUBA surveys at several outplant sites in previous years, including South Cypress Reef, Cypress Head, Burrows South and Allan West. These repetitive surveys revealed that there were as many as 61% more abalone present when compared to a single survey. 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). Even this show factor is conservative as it does not account for the abalone that have emigrated off the plot and been observed within the plot perimeter. If we apply this "show factor" to the most recent survey of Skagit County plots, the estimated survival rate of 6% could be much higher.

Regardless of the actual survival rate, we do know that the current abalone densities at five of the six Skagit County outplant plots exceeds the postulated minimum density (0.15 abalone/m^2; Babcock and Kessing [1999]) needed to sustain successful spawning and egg fertilization. Pinto abalone become reproductive at a size of 50 mm SL. Given that the mean SL of abalone observed at these sites is 77.2 mm and that we observe emergence and aggregation of adults on many of the sites, it is safely assumed that there is successful spawning occurring there. Recent analysis has focused on determining when a site can be declared unsuccessful and therefore seeding at that site discontinued. Another current focus is on determining when a site has reached a favorable density for maintained successful

spawning activity and seeding at that site can therefore be suspended for several years. These focus areas will optimize use of the juvenile abalone that leave the hatchery for restoration efforts.

Our studies examining the possibility of outplanting abalone to restoration sites at earlier life stages (larval or younger juvenile) will inform future restoration strategies, direct hatchery production and potentially increase our capacity to scale up our recovery efforts. Survey results from the exhaustive examination of the 9 month age group one year post-outplant (average survival of 9.7% from the three replicates) are very similar to our typical one year survival rate of around 10% of 18 month outplanted juveniles. At 12% survival after six months, the one replicate surveyed from the 14 month age group also indicates promising results. The next set of surveys, scheduled for October 2018, will provide a more thorough opportunity to exhaustively examine at least two of the three replicates from the second age treatment at the one year post-outplant time period. With continued support, PSRF will fine-tune methodologies for outplanting success and long term recovery of the species within the San Juan Archipelago.

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) continued monitoring and analysis of both the larval and younger juvenile abalone outplant experiments.

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