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Pinto Abalone Recovery Project
2019 Final Report to the Skagit MRC
Josh Bouma, Puget Sound Restoration Fund
Paul Dinnel, Skagit MRC
September 30th, 2019

Introduction

The pinto (northern) abalone, *Haliotis kamtschatkana*, is the primary abalone species indigenous to Washington waters, but populations are severely depleted and considered functionally extinct. The current number and distribution of reproductive wild abalone is too low and too widely distributed to maintain a sustainable population. The precipitous decline of abalone in Washington is largely due to anthropogenic factors, including overharvesting during the legal recreational fishery and poaching during the 1980-90s (Bouma 2007). Numbers in Washington state never supported a commercial fishery for abalone. Between 1992 and 2017, the density of pinto abalone declined by 97% 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in the San Juan Archipelago (SJA) even after the closure of the recreational fishery in 1994 (Rothaus et al. 2008, WDFW unpublished data).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have been observed and the average size of abalone continues to increase (Rothaus et al. 2008, Bouma et al. 2012, WDFW unpublished data).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 Washington State Endangered Species as of May 2019, and are also listed as a U.S. Federal Species of Concern and 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) which can occur when existing animals, particularly broadcast spawning invertebrates like abalone, 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. The recovery project is a long-term collaboration between government agencies, NGOs, universities, tribes and more. This group includes researchers, managers, students and facilities support from the Washington Department of Fish & Wildlife (WDFW); the Puget Sound Restoration Fund (PSRF); Western Washington University's Shannon Point Marine Center (WWU SPMC); the NOAA Manchester Research Station; the Port Townsend Marine Science Center (PTMSC); the WWU SEA Discovery Center in Poulsbo; the University of Washington, School of Aquatic & Fishery Sciences (UW) and others. Annual funding to PSRF from the Skagit County Marine Resource Committee (Skagit MRC-Skagit County contract #C20180471) supports abalone conservation aquaculture and restoration activities in Skagit County. This funding was supplemented by additional support in 2018-2019 for abalone recovery work throughout Washington waters from WDFW, the SeaDoc Society, the Benjamin & Margaret Hall Family Foundation and the Charlotte Martin Foundation.

Puget Sound Restoration Fund, with oversight from WDFW, has developed a conservation aquaculture program designed to supplement depleted wild stocks. The hatchery, wet laboratory and nursery facilities are located at the NOAA Marine Fisheries Research Station in Manchester, WA. This shellfish hatchery, named the Kenneth K. Chew Center for Shellfish Research and Restoration is devoted to native shellfish and other marine species; work is ongoing on species such as pinto abalone, Olympia oysters, giant sea cucumbers, basket cockles, bull kelp and sugar kelp. The Chew Center has been operated by PSRF since 2013 through a Cooperative Research and Development Agreement (CRADA) with NOAA. PSRF began moving the abalone aquaculture program from the NOAA Mukilteo Research station to the Chew Center in 2016 and all pinto abalone hatchery production and research activities are now conducted at the NOAA Manchester Station.

Adult broodstock abalone are collected from the wild and brought into a hatchery, 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 11,400 of these healthy, genetically diverse hatchery produced juvenile abalone have been outplanted to eight rocky reef sites in Skagit County waters since 2009 (two of these sites are new in 2019). An additional 10,200 abalone have also been outplanted at ten sites in San Juan County, creating a recovery network across a significant portion of their geographical range in Washington. Surveys of some of these outplant sites are conducted each year to monitor

survival, growth and movement providing estimates of survival and growth of abalone released into the wild (Carson et al. 2019). Methods and results of surveys prior to 2019 can be found in annual WDFW, PSRF and Skagit MRC summary reports (Bouma and Dinnel 2017, Bouma and Dinnel 2018) 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). The following report summarizes PSRF project accomplishments related to the contract listed above during the time period from October 1st, 2018-September 30th, 2019.

Nursery Husbandry & Hatchery Production

Culture and husbandry of the 2017 juvenile and 2018 post-set cohorts was the primary activity within the abalone nursery from fall of 2018 to early spring 2019 in preparation for spring restoration outplants. Weekly care of these two yearclass populations in the nursery included gentle tank cleaning, feeding post-set with three diatom species cultured in the hatchery and feeding juveniles macroalgae (*Palmaria mollis* cultured at the lab and *Nereocystis luetkeana* collected in the wild). Regular inventory data of nursery abalone were taken (numbers and shell lengths) to track survival and growth during the culture process.

While the entire 2017 hatchery cohort was outplanted in 2019, a portion of the 2018 cohort of juvenile abalone had not reached an appropriate size for release, particularly from the families produced at the end of the 2018 spawning season in September. From these spawns, 684 juveniles from 4 families (mean SL 5.5 mm), continue to be reared in the PSRF nursery to eventually be combined with the new 2019 cohort for outplanting in spring 2020. Additionally, and similar to efforts in the previous year, PSRF delivered a batch of 2018s (n=1100) that will be reared until outplant at the Port Townsend Marine Science Center.

The 2019 pinto abalone spawning production season at the Chew Center came to a conclusion shortly before the writing of this report. Beginning May 22nd and concluding with the last batch of larval settlement on September 10th, 2019 six of the eight attempted spawns were successful and produced larvae for settlement in the nursery. Twenty-six distinct novel families were created from 12 females and 15 males and 2.09 million larvae were settled into 72 culture tanks (Table 1). An additional 1.931 million competent larvae from 2019 spawning events were not utilized for settlement and grow-out to avoid flooding the nursery with genetically homogeneous F1s. PSRF aimed to produce as many new families as possible early in the 2019 season to ensure that nursery grow-out occurred successfully during optimal summer growing conditions with the end goal of having this cohort ready for outplanting in spring 2020 at an age under one year old.

MALE BROODSTOCK ID	FEMALE BROODSTOCK ID											
	OR62	**OR65**	**OR67**	**OR68**	**OR88**	**OR92**	**OR93**	**OR94**	**OR96**	**OR98**	**GR03**	**GR14**
OR29												250
OR45				52								52
OR50												250
OR55												250
OR57				52								52
OR74								53				
OR85								182				
OR87								26		9		
OR95										10		
OR97								115				
GR05	11/3	11/3	11/3		13					3		
GR08		57	127			30			25		68	
GR09								169	150			
GR11							10				46	
GR12								29				

Table 1. Pinto abalone families produced at the Chew Center during the 2019 spawning season. Female broodstock ID on horizontal axis; male broodstock ID on vertical axis. Number of competent larvae (x1000) per cross settled into nursery culture tanks is shown within each square. Numbers with a slash represent pooled groups of multiple crosses (half-sibling crosses).

Nursery Management

In addition to the general abalone husbandry routine, nursery system management for optimal animal health and grow-out also included regular filter changes, pump replacement, buffering system management, water chemistry probe cleaning/calibration/replacement and water quality data monitoring. Installation of a glycol system for heating seawater to the nursery building was accomplished during the fall of 2018. The new heat exchanger, glycol reservoir and all associated pumps, controllers and plumbing significantly upgraded the capacity to bring heated seawater to abalone grow-out tanks and temperature in these culture tanks was controlled successfully and consistently through the colder winter months. The heat pump previously relied upon for both heating and chilling is now dedicated to chilling during summer months. Between the new glycol heat exchanger and existing chilling unit, nursery culture tanks are now consistently held at 13 C +/- 2 C throughout the year.

Health & Disease

On January 15th, 2019 PSRF collected the annual hatchery health sample of juvenile abalone representative of all the families produced during both the 2017 and 2018 spawning seasons for disease screening. This sample included 80 live individuals for histology and 80 live individuals for PCR analysis for withering syndrome. A pathology report from Dr. Ralph Elston, Aquatechnics Inc. was finalized in February. Upon histological examination and PCR analysis, Dr. Elston concluded that no disease causing pathogens were present in the samples and he

gave the hatchery abalone seed group a clean bill of health for introduction to outplant sites in the wild.

Abalone Broodstock

New broodstock abalone for the captive breeding program were collected by PSRF and WDFW divers in the San Juan Islands during the months of March-May 2019. In total, 14 adult abalone (6 females, 8 males) were brought to the Manchester hatchery this year. All new broodstock underwent the standard intake protocol: they were measured, weighed, ranked by gonad index, fouling sponges were removed from the shell, animals were tagged with vinyl disc tags and PIT tags, and non-lethal genetic samples were taken using epipodial tentacle clips. General observation of overall health was also noted. Upon arrival to the Manchester hatchery, new broodstock were isolated into their own tanks, separated by sex and fed kelp *ad libitum* in preparation for the 2019 spawning season.

Juvenile Outplanting

In collaboration with WDFW subtidal shellfish biologists, the PSRF team completed the largest outplant to date in April, 2019 (Figure 1). A fundamental objective of the pinto abalone conservation aquaculture program is to "do no harm" to existing wild stocks of abalone and therefore extreme care was taken during the restoration effort described here to outplant a genetically diverse and disease free cohort of abalone.

Figure 1: Divers prepare to release juvenile abalone to a new outplant site in 2019.

Development of new outplant sites

Two new outplant sites were created in Skagit County in 2019, both located near Fidalgo Head. An additional four sites were added in San Juan County (James Island and Spieden Channel)

with funding support from other sources. Thorough reconnaissance dives were conducted to confirm each site matched criteria for juvenile outplanting: contiguous clean rocky reef, boulder or cobble habitat (10 m longshore x 8 m inshore) at depths between 10-40' MLLW, significant coverage with crustose coralline algae, presence of canopy forming kelp (*N. luetkeana*) and moderate presence of understory and drift algae, high tidal current flow and presence/absence of appropriate competitors/predators. Historic or observed presence of wild adult pinto abalone was also considered. Outplant plots were marked with permanent corner markers (pitons driven into rock crevices) and yellow floating poly line and all perimeter measurements, compass headings, corner depths and precise GPS coordinates were recorded. Field notes, site maps and associated data for new sites have been archived by both PSRF and the WDFW subtidal shellfish group. No pre-outplant survey of flora and fauna was conducted at these new sites before abalone were introduced.

Description of juvenile outplant groups

2161 juvenile abalone (6559 abalone total if including all six new sites) representing 26 genetically distinct families from the 2017 and 2018 Manchester hatchery cohorts were seeded to the two new Skagit County outplant sites (Table 2). Eleven different females and 21 different males contributed to these 26 families. The actual number introduced to each site ranged from 1044-1117 abalone (average 1093/site). No new abalone were overseeded onto the six pre-existing outplant sites in Skagit County (or the six previously seeded sites in San Juan County). Since 2009, nearly 22,000 pinto abalone have been outplanted to 18 different restorations sites in the San Juan Archipelago, with 11,400 of these animals introduced to the 8 sites in Skagit County.

The outplant effort this year was unique in that both a younger and older year class were overseeded together onto the same sites. Mean shell length of the 2017 and 2018 cohorts at outplant time was 19.3 mm and 9.2 mm respectively. Following a 2.5 year study conducted by PSRF and WDFW investigating survival and growth of younger-aged outplants completed in February 2019, outplant strategies will now shift towards releasing hatchery juveniles before they reach one year in age. This will significantly improve annual production cycles in the hatchery. The 2019 outplants represent an opportunity to monitor the two cohorts over the next year to confirm survivorship of the younger group is similar to the older group. In preparation for outplanting, PSRF hatchery staff conducted inventory, collected shell length data and sorted families into

Figure 2: Abalone are marked with a colored glue dot to mark them as the older of two ouplant cohorts.

outplant groups. Additionally, all juveniles from the 2017 cohort were marked with a colored glue dot (Coraffix cyanoacrylate adhesive mixed with Eye Candy mica powder "Baku Red" pigment) before outplanting to identify them from the younger group (Figure 2).

MALE BROODSTOCK ID	FEMALE BROODSTOCK ID										
	OR35	OR51	OR59	OR62	OR65	OR66	OR67	OR68	OR70	OR76	OR79
OR03	28/4	84/2									
OR08	28/4	98/2			80						
OR29					134						
OR46									82/3		
OR47	28/4	84/2									
OR57				287							41
OR60							600				
OR71					54					81/2	
OR74						1002	822		82/3		
OR75							750				
OR77				360							234
OR78							716			81/2	
OR81									82/3		
OR82				424	350						166
OR84					22						
OR85			62								
OR86								13			
OR87					68						
OR95					248						
OR97			162								
Y36	28/4	98/2									

Table 2. Pinto abalone families produced at the Chew Center in 2017/2018 and outplanted in 2019. Female broodstock ID on horizontal axis; males on vertical axis. Number of individuals outplanted per cross is shown within each square (yellow = 2017 progeny; green = 2018 progeny). Numbers with a slash represent pooled groups of multiple crosses. Total number of seed outplanted: 6,559 (6,968 – 409 mortalities post sort).

Outplant Modules, Loading and Transport

Outplant modules were made from PVC pipe (6" ID) originally acquired from the UW Bothell campus (repurposed from a wetlands project) cut into approximately 18" long sections. Most of the tubes this year were recovered from previous outplants and reused. Each section of tube was power washed and scrubbed. Tubes were numbered by site destination for convenience once in the field. All tubes were conditioned in flow-through tanks at the Manchester lab for several weeks prior to being loaded with animals.

One day prior to outplant, abalone were transferred from their holding tanks at Manchester into the PVC outplant tubes (Figure 3). All the tubes destined for a particular site were positioned upright in a large seawater-filled fish cooler with one layer of fiberglass window screen (2 mm mesh size) secured with rubber bands covering the bottom end. Approximately 120 abalone were loaded into each tube. The open end of the tube was then closed with one layer of window screen and secured with a rubber band. Modules were then loaded into a seawater filled 4'x 4' fish tote in the back of a 5'x 9' U-Haul towable trailer (Figure 4).

Abalone in the U-Haul trailer were transported that same day from Manchester to Anacortes via the Port Townsend ferry. The large fish tote in the trailer was aerated during transport using a 12V battery, DC to AC inverter, an aquarium pump and two large air stones placed in the tote. The trailer was driven directly to SPMC where it was disconnected, and the tote was supplied with flow-through seawater and aeration overnight.

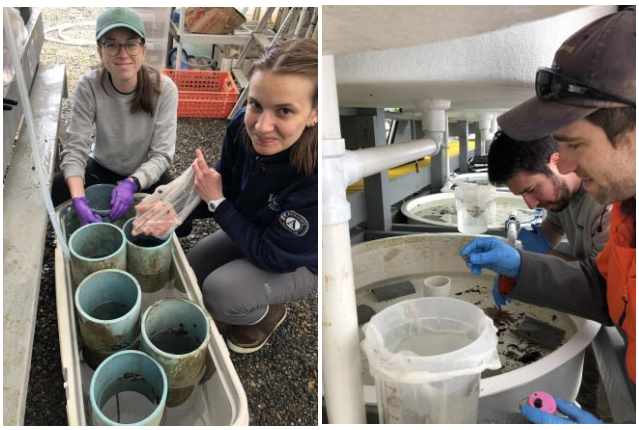

Figure 3: PSRF staff count, organize and load juvenile abalone in preparation for outplanting in 2019.

The following morning, the trailer was then towed to Cap Sante Marina where it was drained and abalone modules were transferred into a waiting seawater filled, aerated fish tote on board the WDFW R/V Enumerator. Once at outplant sites, tubes were bound together with webbing straps and quick release buckles in bundles of three and carried to depth by divers (Figure 5). Tubes were placed within the delineated outplant plots in areas that appeared to have suitable substrate and cryptic habitat onto which juvenile abalone could exit. Tubes were wedged amongst cobble and boulders to secure them against current and surge. Once the tubes were secured within the substrate, divers immediately removed the mesh from the tubes and the abalone were free to move from the modules out onto the surrounding substrate. PVC outplant modules will be collected during site surveys in early 2020, cleaned and reused during future outplant efforts.

Figure 4: PSRF crew send abalone on their way to Anacortes.

Genetic Assessment of Wild and Hatchery Pinto Abalone

Lead by NOAA scientist Dr. Rick Goetz, genetic assessment of PSRF's pinto abalone restoration aquaculture programs is underway. This project will develop new genetic markers for abalone in the form of SNPs using Next Gen RAD-sequencing. SNPs will provide information on genetic variation and allow for analysis of population genetics. Questions specific to the abalone restoration program that will be answered with development of new markers include understanding how related are the broodstocks that have been used for restoration aquaculture, how different are hatchery progeny in comparison to wild stocks and also how closely does the genetic structure of surviving outplant populations match that of wild stocks.

Figure 5: PSRF staff and Skagit MRC volunteer prepare to release juvenile abalone from the WDFW research vessel at a new outplant site near Fidalgo Head.

To sample the wild population in Washington, PSRF and WDFW divers collected tissue in April/May 2019 from locations throughout the SJAs. Genetic samples were taken from the 14 newly acquired abalone broodstock before they were brought to the hatchery, and also from an additional 22 wild abalone that were found in aggregations, brought to the boat for sampling and then returned to the location where they were collected.

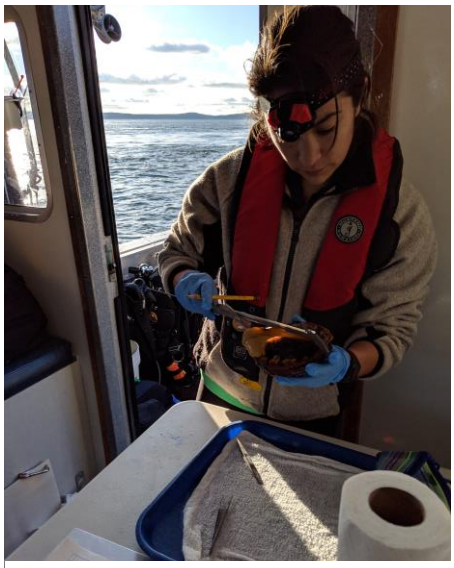

Figure 6: Tissue samples and other metrics are collected from abalone on outplant sites.

Divers also collected 277 abalone from the four most productive outplant sites (three of which are in Skagit County): Burrows South, Allan West, Cypress Head and Sunken Rock Reef (Table 3). These four sites were surveyed exhaustively according to standard outplant site protocols. Observed abalone were collected using *Pycnopodia helianthoides* sea stars to remove without injuring and brought to the surface in individual mesh pouches. On board the research vessel, sampling was accomplished by taking a non-lethal epipodial tentacle clip preserved in molecular grade ethanol. Other metrics were taken including shell length, sex and notation of tag identification numbers/colors when present (Figure 6). Abalone were

then returned to the site as close to where they were collected as possible. Genetic sampling surveys replaced the standard monitoring surveys this year and due to the extensive effort required, only three of the six existing Skagit County restoration sites were surveyed.

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	37	0.38	96.8	0	0.00	88.7
Allan West	90.0	96	1.07	93.2	0	0.00	99.3
Cypress Head	79.3	68	0.86	87	4	0.05	81.4
Sunken Rock Reef	69.8	51	0.73	85	21	0.25	66.5
Total	**337.3**	**252**	**0.75**	**362.0**	**25**	**0.07**	**84.8**

Table 3. Pinto abalone outplant site survey data from genetic sampling efforts at four sites in 2019. SL=maximum shell length measurements.

Another 130 juvenile, hatchery-raised abalone representing all the families produced at the Chew Center in 2017 and 2018 for restoration outplants were sampled lethally, the foot and epipodium were dissected out and preserved. Other groups of tissue samples that will be included in the analysis include nearly 100 archived broodstock abalone from SJA collections between 2011-2018 and an outgroup of pinto abalone samples (n=40) collected by collaborators in Ketchikan, Alaska. DNA extractions are underway, the first group of libraries will be sent to Novogene for sequencing in October 2019 and bioinformatics analysis of wild vs. hatchery progeny population structures will begin during the winter 2019/2020.

PSRF Supported Abalone Research

1. Caitlin O'Brien (WWU; advisor - Deborah Donovan): Caitlin conducted experiments at the Chew Center to determine the efficacy of sperm cryopreservation - a strategy by which hatchery managers can build a genetic library to improve production and maximize genetic diversity. She evaluated three commonly used cryoprotectants, a series of freeze/thaw temperatures, and developed methods of quality assessment specific to pinto abalone. She found that cryopreserved sperm successfully fertilized eggs, but with lower success than with untreated sperm. Her work is the first attempt into cryopreservation of pinto abalone sperm and provides a foundation for future research to optimize the methods developed. Caitlin completed her Master's degree in 2019.

2. Katie Mills-Orcutt (WWU; advisor - Deborah Donovan): Katie tested the effectiveness of outplanting to field sites during the abalone larval life phase to examine if the strategy can be used for rebuilding wild populations. PSRF provided Katie with 536,000 abalone larvae from the Chew Center, of which more than 100,000 larvae were seeded into pre-constructed larval

abalone modules (LAMs) at her two research sites in Skagit County, one along the southwestern shoreline of Young Island in Burrows Bay and the other near the SPMC intake reef on Fidalgo Head. The LAMs were sampled over four months, to assess seeding and retention strategies. Four months after outplanting, abundance of juvenile pinto abalone was significantly greater in LAMs that had screen tenting over the module for 24 hours after seeding, compared to open and control LAMs held at SPMC. Her findings indicate that outplanting pinto abalone as larvae into tented structures may be a cost-effective alternative to traditional juvenile outplanting. Katie completed her Master's degree in 2019.

3. Eileen Bates (UW; advisor – Jacqueline Padilla-Gamiño): Eileen has begun an experiment to study the interactive effects of ocean acidification and temperature on pinto abalone larvae. She has completed one round of the experiment, which she plans to repeat in April 2020. Early results indicate that Pinto abalone larvae are quite sensitive to both elevated temperatures and ocean acidification with significant negative effects observed on hatching rates, larval development and survival and settlement rates.

Outreach

PSRF supports pinto abalone recovery outreach activities at the Port Townsend Marine Science Center. As part of an ALEA grant awarded to the PTMSC, PSRF helped create a conservation aquaculture display that replicates PSRF's abalone grow-out system. PSRF produced and delivered 1000 juvenile abalone to the PTMSC in June 2018, these animals were reared by PTMSC staff and volunteers and more than 800 were then included in the 2019 outplant group (Figure 7). An additional 1100 juveniles from nine different 2018 families were delivered to the PTMSC in May 2019 and will be reared there until 2020 outplant efforts. Josh Bouma gave an abalone restoration presentation to a group of staff, volunteers and guests at the monthly PTMSC brown bag lunch seminar on June 18th, 2019.

Figure 7: Staff and volunteers at the PTMSC tag and measure abalone destined for outplanting in 2019.

PSRF collaborated with Dr. Sylvia Yang, the Directory of WWU's SEA Discovery Center in Poulsbo to design and develop an aquarium display and outreach materials, part of an education program highlighting PSRF projects such as abalone recovery. PSRF collected and delivered coralline encrusted rock for the display. The display tank was constructed during the late summer, 2018 and included other subtidal rocky reef animals found in similar habitats to

abalone. 25 young adult abalone (mean SL 65 mm) from PSRF's hatchery were added to the tanks in September and December, 2018.

Pinto abalone were listed by WDFW as a state endangered species on May 31st, 2019. To bring attention to this listing, PSRF hosted a media day at the Ken Chew Center for Shellfish Research & Restoration at NOAA Manchester on June 4th, 2019 that included a tour of the abalone program facilities and photo/video/interview opportunities. The event was well attended by writers and reporters and the following list summarizes where the resulting articles were published or aired:

Skagit Valley Herald
CrossCut
Kitsap Sun
Island's Sounder
KUOW
Centralia Chronicle
High Country News
Islands Weekly
Peninsula Daily News
North Kitsap Herald
Journal of the San Juans
Port Orchard Independent
WDFW social media, Instagram/Twitter/Facebook Live, etc.
WDFW Director's Bulletin

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