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Pinto Abalone Recovery Project
2024 Final Report to the Skagit MRC
Josh Bouma, Puget Sound Restoration Fund
Paul Dinnel, Skagit MRC
September 30th, 2024

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in the San Juan Archipelago (SJA) even after the closure of the recreational fishery in 1994 (Rothaus et al. 2008, WDFW unpublished data).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have been observed and the average size of abalone continues to increase (Rothaus et al. 2008, Bouma et al. 2012, WDFW unpublished data).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 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 (Sowul et al. 2021). They 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, and 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). This 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 Keesing (1999) estimated that the minimum density for successful reproduction to occur is 0.15

abalone/m^2. Extensive sampling has shown that the remaining San Juan Archipelago (SJA) pinto abalone population is well below this threshold and unable to facilitate necessary reproduction for natural population recovery.

Steps are being taken 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, resource managers, students, technicians and facilities support from Washington Department of Fish & Wildlife (WDFW); Puget Sound Restoration Fund (PSRF); Western Washington University's Shannon Point Marine Center (WWU SPMC); Skagit County Marine Resources Committee (Skagit MRC); the NOAA Manchester Research Station; Port Townsend Marine Science Center (PTMSC); University of Washington, School of Aquatic & Fishery Sciences (UW); the Seattle Aquarium; Samish Indian Nation; Lummi Nation; Makah Tribe and others.

History of Pinto Abalone Restoration Efforts in Skagit County

Efforts to restore pinto abalone in Skagit County waters began in 2005 when a survey was conducted at six county sites by Goergen and Dinnel (2005). Abalone densities at these sites were found to be substantially less than previous surveys by Washington Department of Fish and Wildlife (WDFW). During the period of 2009 through 2013 Western Washington University's Shannon Point Marine Center (SPMC) research staff and students teamed with Puget Sound Restoration Fund (PSRF) to assist with juvenile abalone outplants, monitoring of the outplanted abalone, and the conduct of various laboratory feeding and predation studies (Bergman 2009, Pratt 2010, Hester et al. 2011, Benolkin et al. 2012, Walker et al. 2013). In 2013, the lead abalone researcher and student mentor, Dr. Paul Dinnel, retired from WWU and the SPMC/PSRF team effort ended. However, Dr, Dinnel was an appointed member of the Skagit County Marine Resources Committee (Skagit MRC) dating back to 2000. His continuation with Skagit MRC post-retirement lead to the MRC's adoption of pinto abalone restoration efforts and the teaming with PSRF. The MRC's adoption of the abalone restoration project in 2014 fit very well with the stated purpose of the MRC: "To protect and restore marine populations to healthy sustainable levels" (www.skagitmrc.org/about-us/) and "To protect and restore marine, coastal and nearshore habitats, prevent loss and achieve a net gain of healthy habitat" (www.skagitmrc.org/about-us/background/).

Additionally, teaming with PSRF was necessary as the MRC did not have the necessary facilities to raise juvenile abalone, nor did it have the boats and certified scientific divers required for abalone outplanting and monitoring. Since 2014 Dr. Dinnel has worked with PSRF personnel in non-hatchery and non-diving activities. He has lent his expertise to the project through team coordination, planning, contractual and reporting duties, occasional team meetings, and

observations on selected outplanting/monitoring cruises. During this time approximately 26,000 juvenile abalone have been outplanted at eleven Skagit County restoration sites. For a year-by-year summary of abalone restoration activities see copies of our annual reports (Bouma and Dinnel, 2017, 2018, 2019, 2020, 2021, 2022, 2023).

Kenneth K. Chew Center: Abalone Hatchery Production & Nursery Husbandry

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 (Chew Center), is devoted to restoration and conservation of native shellfish and other marine species; work is ongoing on species such as pinto abalone, Olympia oysters, giant sea cucumbers, basket cockles, Dungeness crab, 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 the Chew Center, 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. More than 60,000 of these healthy, genetically diverse hatchery produced juvenile abalone have now been outplanted to 37 rocky reef sites in Skagit, San Juan and Island County waters since 2009. Approximately 26,000 of these abalone have been outplanted at 11 sites in Skagit 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, which provide estimates of survival and growth of abalone populations released into the wild (Carson et al. 2019). Methods and results of surveys prior to 2024 can be found in annual WDFW, PSRF and Skagit MRC summary reports (Bouma and Dinnel 2017, 2018, 2019, 2020, 2021, 2022 and 2023) 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, Garden and Hoffman 2024). The following report summarizes PSRF project accomplishments related to the contract listed above during the period from October 1st, 2023-September 30th, 2024.

As in the previous year, the 2024 abalone conservation hatchery season was successful in producing enough healthy, genetically diverse larvae to fill all available nursery tanks at both

our main NOAA Manchester Chew Center location (MCR) and at the Seattle Aquarium Off-Site Animal Care Center (SA ACC). Spawning efforts began in June and were completed by late July and resulted in 19 new families produced from 9 female and 7 male broodstock (Table 1). The hatchery team conducted the first two spawns on subsequent days in June resulting in 797K eggs from three females crossed with different males, creating three distinct families. Healthy, competent larvae were settled into 20 tanks at MCR and 9 tanks at SA ACC one-week post-spawn. A third successful spawn was induced in early July. The July spawn produced 6.4M eggs fertilized from six females, crossed with four different males, and resulting in 16 genetically unique families. From this cohort, 724K healthy larvae were settled into nursery tanks at MCR and the SA ACC. In total, 77 available nursery tanks at both facilities were filled with post-set this year (Table 2). The other 37 nursery tanks at MCR and SA ACC were not available for settlement as they are in use for rearing older juveniles from the 2023 cohort that were not outplanted in 2024.

Table 1. Annual abalone spawn and settlement summary, 2023 & 2024.

Year	# Males used	# Males crossed	# Females used	# Females crossed	Larvae produced	Larvae set	Larvae culled
2023	27	11	11	4	3,997,400	1,156,800	2,840,600
2024	21	7	24	9	4,188,600	724,200	3,464,400

Table 2. Annual abalone production by spawn date, 2023 & 2024.

Spawn Date	Settlement Date	# Larvae Produced	# Tanks set at MCR	# Tanks set at SA
5/31/23	6/7/23	111,600	12	8
7/12/23	7/19/23	15,200	2	2
8/16/23	8/23/23	3,706,600	81	22
6/18/24	6/26/24	636,000	19	6
6/19/24	6/26/24	25,200	1	3
7/3/24	7/11/24	3,527,400	49	15

Maintenance and husbandry of cohort 2023 post-set as they grew into juveniles was one of the primary activities within the abalone nursery during the fall, winter, and spring. Weekly care of these populations in the nursery included gentle tank cleaning and feeding with three diatom species cultured at the Chew Center (*Amphora salina, Navicula incerta, Cylindrotheca*

closterium). Once post-set abalone from 2023 spawns reached 5 mm in shell length, they were weaned onto dulse (*Palmaria mollis)* by blending it into small flakes and introducing it into the grow-out tanks. At six months post-settlement, hatchery staff continued to size sort and consolidate within families to keep similar-sized juvenile animals within a tank, aiming to decrease within-tank competition. Inventory and shell length data were recorded to track growth and survival.

For the 2023 cohort, juvenile mortality was tracked from 4-19 weeks after settlement in nine tanks at the Chew Center (Figure 1). Tanks were chosen to represent unique families, settlement dates, and tank locations throughout the nursery. During the weekly cleanings, shells were collected then counted under a stereo microscope. Since the settlement density was different for multiple tanks, the number of shells collected was divided by the larvae settlement number, giving a "percent dead from set density". Figure 1 shows the declining trend in the number of shells collected over time.

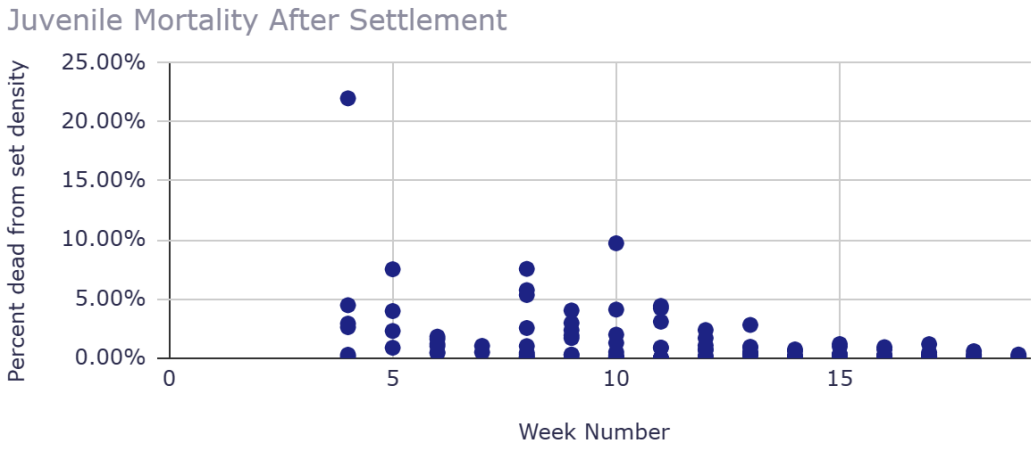

Figure 1. Juvenile mortality after settlement.

Inventories of juvenile abalone at MCR and SA ACC were conducted monthly beginning 4-5 months after settlement. Figure 2 represents total juvenile counts from MCR and SA ACC 4-11 months post-settlement. The first inventory was in December 2023 and the last inventory was in June 2024 before the June outplant effort.

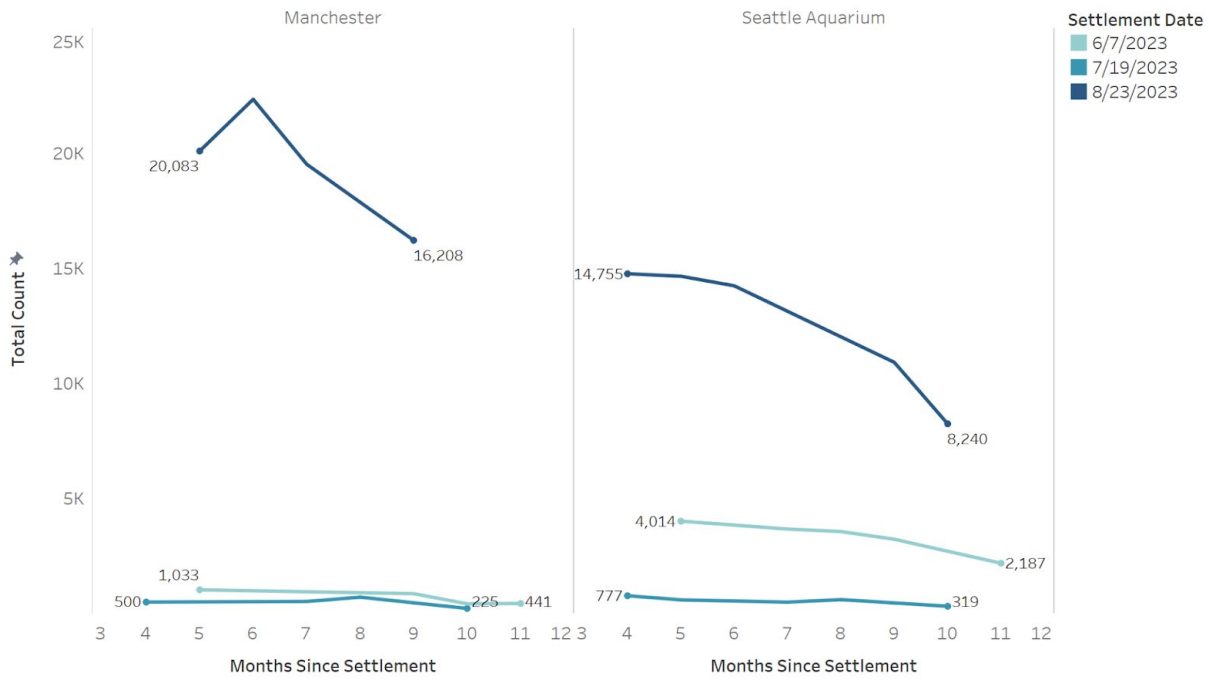

Figure 2. Total counts of juvenile pinto abalone from 2023 cohort (December 2023 - June 2024).

General maintenance of hatchery and nursery systems includes regular filter changes, pump replacement, buffering system management, seawater chemistry probe cleaning/calibration/replacement and water quality data monitoring. PSRF staff regularly monitors incoming seawater chemistry and quality, and also examines for potentially problematic nuisance species in the nursery water systems.

Abalone Health & Disease Screening

To confirm no disease-causing pathogens were present in hatchery-reared animals prior to moving abalone between facilities and outplanting animals into the wild, an annual hatchery health assessment was conducted by pathologist Dr. Ralph Elston, AquaTechnics Inc. Live samples were sent for histology to detect known infectious diseases and PCR analysis to determine if withering syndrome was present. On February 6th, 2024, health screening results from all three facilities (PSRF Chew Center at NOAA Manchester, Seattle Aquarium, PTMSC) showed no disease-causing pathogens present. A total of 360 live abalone were sent for sampling, with each facility sending 60 live juveniles for histology and 60 live juveniles for PCR. Upon this confirmation of abalone hatchery health, new transfer permits were received from WDFW to move abalone from the nursery facilities for outplanting.

Abalone Broodstock

At the end of the 2024 spawning season, PSRF housed a total of 59 broodstock: 39 males and 20 females. In April, prior to the hatchery season, PSRF and WDFW divers collected 22 new broodstock from the San Juan Islands: 12 females and 10 males (Figure 3). All new broodstock

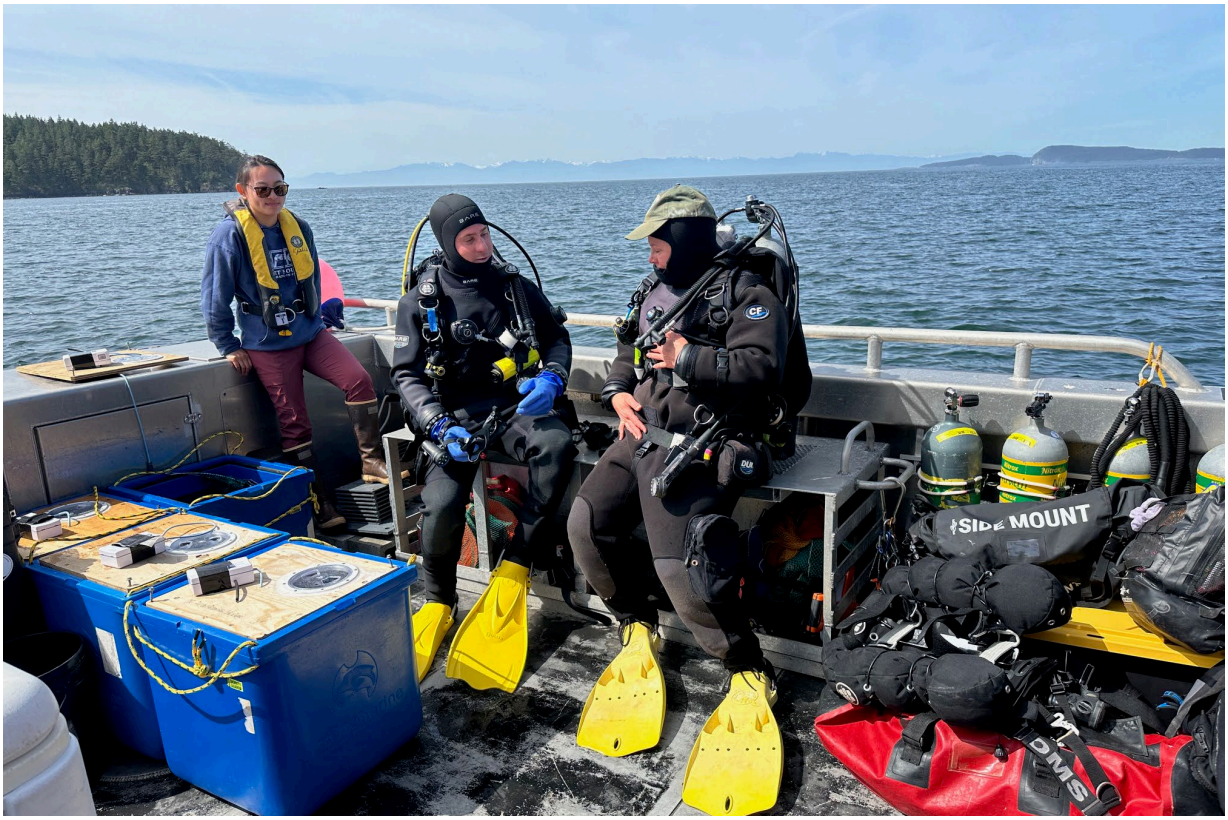

Figure 3. Divers prepare for a San Juan Islands broodstock collection dive in 2024. Insulated blue containers with aerators are ready to transport animals to the hatchery.

underwent the standard intake protocol: they were measured, weighed, ranked by gonad index, fouling sponges removed from the shell, animals were tagged with vinyl disc tags and PIT tags, non-lethal genetic samples were taken and archived using epipodial tentacle clips, and general observation of overall health was noted. Regular inventory, health and maintenance checks were conducted on all broodstock; tanks were fed and cleaned weekly, and animals were measured, weighed and re-tagged as necessary. Broodstock abalone were fed two types of macroalgae species: dulse *(Pamaria mollis)* and bull kelp (*Nereocystis luetkeana).* PSRF staff produce dulse in tumble culture and bull kelp is wild collected as needed to sustain hatchery needs. Broodstock mortality was much lower during this reporting period than in previous years, possibly due to changes in how temperature stress was used in the induced spawning protocol.

Juvenile Abalone Outplant Site Monitoring

Between February-April 2024, PSRF, WDFW and SA ACC divers surveyed for survival, growth and emergence of hatchery reared pinto abalone at two Skagit County sites outplanted in 2023. The sites outplanted in 2023 were newly established last year (code names Torch and Goodenough). Following guidelines adopted by WDFW and described in the Recovery Plan, when restoration sites are established, they are outplanted in the first year and if successful then subsequently overseeded every three years to maintain aggregations and boost genetic diversity.

Survey set-up in 2024 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. Surveys also included the addition of a 2 m perimeter lane around the entire outside of each plot. This additional perimeter lane roughly doubles the amount of area surveyed at each site during a standard survey and provides informative emigration data. Divers meticulously conducted non-invasive surveys 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, if identifiable, were recorded for all abalone observed. The 2023 outplants included mostly one-year-old cohorts, with the addition of a small number of 2-year-olds, the older of which were marked with orange glue dots easily visible underwater to divers. 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). If found, empty abalone shells from mortalities were collected, measured and observed for tags when encountered and then removed from the plot.

The monitoring surveys at Torch and Goodenough only found a total of 27 abalone during this one-year post outplant survey, and none were found outside of the plot boundaries. At Torch, 11 abalone were observed including two juveniles with orange tags, eight untagged juveniles and one large wild adult. At Goodenough, 16 abalone were observed including four juveniles with orange tags and 12 untagged juveniles. Survival (observation rate of outplants at the one-year mark) at Torch was 2.3% for 2-yr-olds and 1.1% for 1-yr-olds, and at Goodenough it was 4.5% for 2-yr-olds and 1.7% for 1-yr-olds. Mean shell length was 32.3 mm at Torch and 36.8 mm at Goodenough (Table 3). Observations at both plots, excluding the one wild adult, ranged in size from 10-60 mm shell length.

Both sites experienced a significant amount of siltation, likely deposited during rapid tidal exchange currents moving sediment through Deception Pass. There was also evidence of strong surge activity (likely from windstorms) as several corner pitons were loose or missing and an

oceanographic sensor array at one of the sites was moved and damaged. The low survival of both age cohorts places Torch in the category of sites not to be re-seeded, and although Goodenough was marginal, it was kept in the category of sites to receive an overseeding of juvenile abalone for a second year.

Table 3. Juvenile abalone outplant survey data collected at two sites in Skagit County in 2024. SL=maximum shell length measurement.

Site	Observed 1-yr-old (n)	Observed 2 yr-old (n)	Wild Adult (n)	Mean SL (mm)	Outplanted 1-yr-old (n)	Outplanted 2-yr-old (n)	1-yr-old survival	2-yr-old survival
Torch	8	2	1	32.3	746	87	1.1%	2.3%
Goodenough	12	4	0	36.8	708	89	1.7%	4.5%
Total	**20**	**6**	**1**	**35.0**	**1454**	**176**	**1.4%**	**3.4%**

Juvenile Abalone Outplanting

Between April and June 2024, in collaboration with divers from the WDFW subtidal shellfish team, Samish Indian Nation, Seattle Aquarium and the SPMC REU program, the PSRF team completed the 12th juvenile abalone outplant over the past 16 years at recovery sites in Skagit County. 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. Since 2009, more than 26,000 pinto abalone from over 250 families have been outplanted to 11 different restoration sites in Skagit County; two of which were newly established in 2023 and another two newly established in 2024.

In 2024, 6344 juvenile abalone were outplanted to 9 sites in Skagit County, representing 22 genetically distinct families produced at the Chew Center in 2022 and 2023 and reared at the Chew Center, Seattle Aquarium and PTMSC (Table 4). The number of abalone outplanted ranged from approximately 525-807 per site (Figure 4) and the mean SL by site was between 8-14 mm.

In partnership with the Samish Indian Nation natural resources team, reconnaissance dives were conducted in Skagit County this year to establish a new outplant site on Sinclair Island, given the name Kwo´sen (Star). This site was outplanted by Samish divers and will be monitored and maintained by the Samish dive team next year. With guidance from PSRF, a second new site in Skagit County was set up and named King, outplanted by SPMC REU divers as part of a study examining dispersal rates of juvenile abalone after release. Both new sites were set up with permanent corner markers; site maps were created including site features, depths, compass headings between corner markers and GPS coordinates.

Table 4. 2024 Skagit County abalone outplant summary.

Juveniles Outplanted (n)	Unique Families (n)	Sites (n)	Mean SL 1-yr-olds (mm)
6,344	22	9	9.3

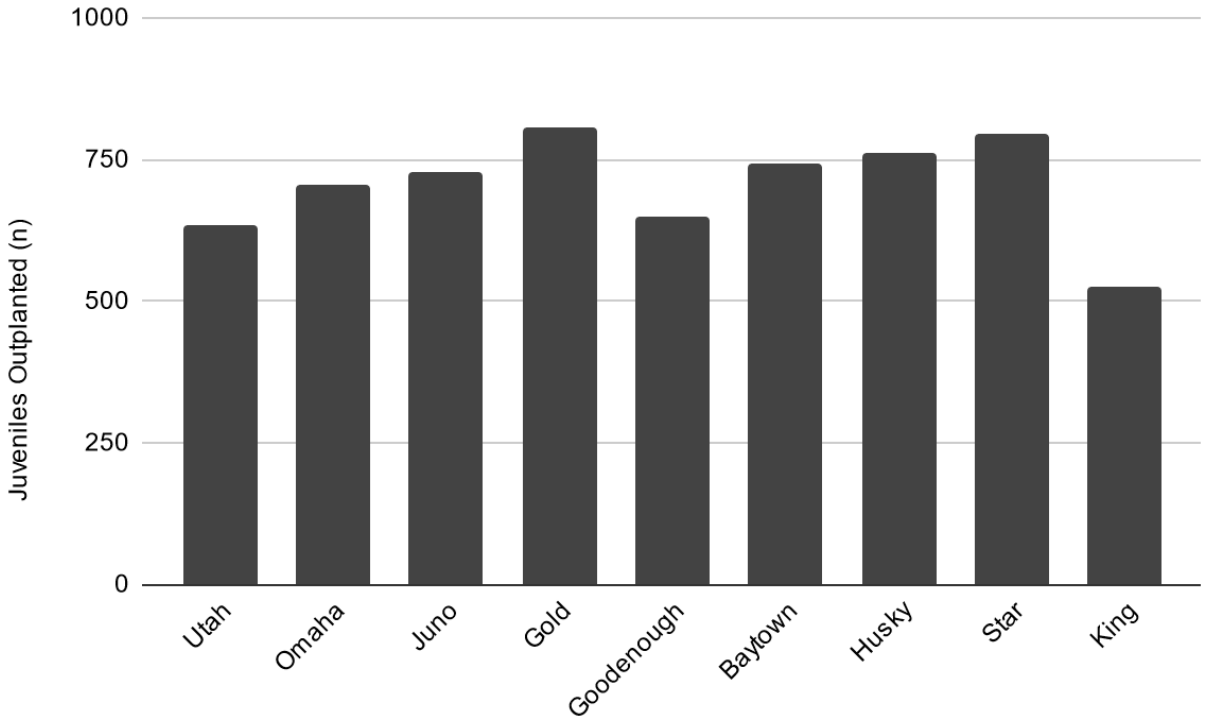

Figure 4. Skagit County abalone outplanted (n) by site in 2024. Target n per site was 700.

In preparation for outplanting, PSRF and SA ACC staff conducted inventory, collected shell length data, and sorted families into outplant groups (Figure 5). Additionally, all juveniles from the 2022 cohort were marked with a purple-colored glue dot (Coraffix cyanoacrylate adhesive mixed with Eye Candy mica powder pigment) before outplanting to identify them from the younger group using the same methods as the previous five years (Figure 6). The mean shell length of outplanted juveniles across all 9 Skagit County sites in 2024 was 10.1 mm for the younger cohort and 18.5 mm for the older cohort.

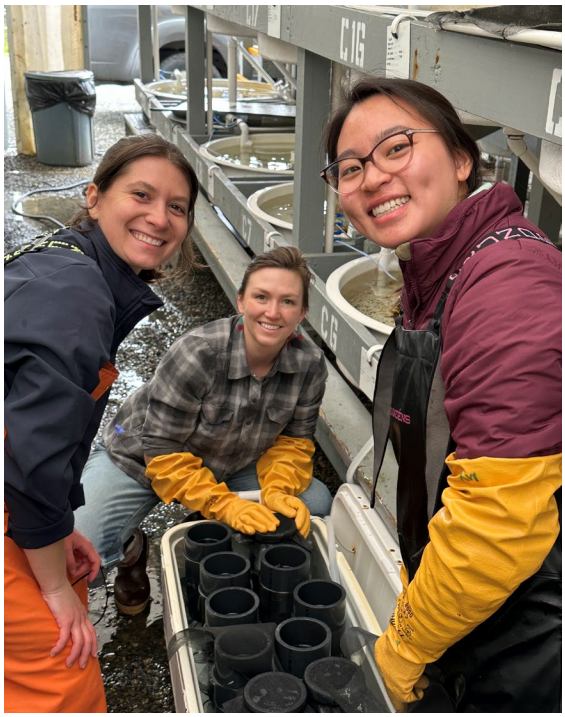

Figure 5. PSRF Hatchery technicians and Evergreen College Shewmaker scholar sort and prepare abalone for outplant.

Two oceanographic sensor arrays that were installed at Torch and Goodenough in April 2023, were removed from outplant sites during 2024 field work. These arrays, constructed by UW PhD candidate Eileen Bates, gathered data on pH, temperature, salinity, dissolved oxygen, light penetration and current flow, had been installed on 18 outplant sites over the past three years. Sensor retrieval, data downloads, probe calibration and redeployment by PSRF and WDFW divers occurred every two months for the past three years. No correlation was made between any of these oceanographic parameters and low or high survival at outplant sites.

Collaboration Building & Outreach

In March 2024, Josh Bouma attended the White Abalone Recovery Program outplanting workshop, hosted by the NOAA Long Beach office and the Aquarium of the Pacific, along with Katie Sowul, Lead Abalone Biologist at WDFW. Josh presented a talk summarizing the progress of pinto abalone conservation aquaculture and outplanting in Washington, focusing on some of the lessons learned over the 15-year duration of the project. The workshop offered a unique opportunity to share information between recovery programs in WA and CA.

The Samish Indian Nation, Department of Natural Resources, received a grant from WDFW to expand pinto abalone recovery efforts. Josh Bouma spent two days in early May 2024 training their dive team in the water (Figure 7). This work included finding and establishing a new outplant site at Sinclair Island in Skagit County, installing corner pitons and creating a map with perimeter distances, compass headings and depths. The new outplant site was given the name Kwo´sen (Star). The training dives also included finding, setting up and surveying an existing site (Gold) for the presence and size of outplanted abalone. The Samish dive team outplanted the new site with juvenile abalone in June; moving forward Samish will take on annual monitoring and reseeding of both Kwo´sen and Gold.

Figure 6. Two-yr-old juvenile abalone marked with a purple glue alongside unmarked one-yr-olds in preparation for outplanting.

PSRF also strengthened a long-running collaboration with SPMC by guiding a novel abalone outplant student research project. Making the most of the abundant 2023 abalone hatchery cohort, 525 juvenile abalone were provided to a team of SCUBA-focused Research Experience for Undergraduates (REU) students and Our World Underwater (OWU) scholars from Western Washington University's Shannon Point Marine Lab. They tagged the abalone, released them at different densities to a new outplant site in Burrows Bay named King, student divers monitored subsequent movement and dispersal from outplant modules. The students learned that outplanting juveniles at three different densities had no significant impact on how quickly they left the outplant modules to find protection in surrounding habitat (Garden and Hoffman 2024). This project provided an opportunity for students to learn about pinto abalone restoration strategies, gain experience doing scientific diving, and help us understand more about abalone behavior. This effort and new partnership will also provide a long-term opportunity for SPMC scientific diving students and future REUs to overseed, monitor and study a SPMC managed abalone restoration outplant site.

Figure 7. Samish Indian Nation and PSRF divers return from abalone research dives in 2024.

Acknowledgments

This final report summarizes work accomplished by PSRF in coordination with Paul Dinnel, Skagit MRC's Project Lead, and Tracy Alker, Skagit MRC Administrative Coordinator, through September 30th, 2024. This project has been funded wholly or in part by the United States Environmental Protection Agency via the Puget Sound Partnership and the Northwest Straits Commission under assistance agreement Grant SEANWS-2023-SkCoPW-00003 to Skagit County. The contents of this document do not necessarily reflect the views and policies of the United States Environmental Protection Agency, nor does mention of trade names or commercial products constitute endorsement or recommendation for use.

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