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Pinto Abalone Recovery Project
2022 Final Report to the Skagit MRC
Josh Bouma, Puget Sound Restoration Fund
Paul Dinnel, Skagit MRC
September 30th, 2022

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in the San Juan Archipelago (SJA) even after the closure of the recreational fishery in 1994 (Rothaus et al. 2008, WDFW unpublished data).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have been observed and the average size of abalone continues to increase (Rothaus et al. 2008, Bouma et al. 2012, WDFW unpublished data).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. 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, 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 Keesing (1999) estimated that the minimum density is 0.15 abalone/m^2 for successful reproduction to

occur. Extensive sampling has shown that the remaining San Juan Archipelago (SJA) pinto abalone population is well below this threshold, 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, 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 University of Washington, School of Aquatic & Fishery Sciences (UW); the Seattle Aquarium and others. Annual funding to PSRF from the Skagit County Marine Resources Committee (Skagit MRC-Skagit County contract #C20210470) supports abalone conservation aquaculture and restoration activities in Skagit County. This funding support for abalone restoration in 2021-2022 was supplemented by funding from WDFW, PSRF, the Benjamin & Margaret Hall Family Foundation and other anonymous private donors.

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 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 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. Almost 18,000 of these healthy, genetically diverse hatchery produced juvenile abalone have now been outplanted to eight rocky reef sites in Skagit County waters since 2009. Approximately another 27,000 abalone have also been outplanted at 17 sites in San Juan County and two sites in Island 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 2022 can be found in annual WDFW, PSRF and Skagit MRC summary reports (Bouma and Dinnel 2017, 2018, 2019, 2020 and 2021) 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 period from October 1st, 2021-September 30th, 2022.

Chew Center: Hatchery Production & Nursery Husbandry

During the 2022 induced spawning season, which began in June and concluded in August, PSRF successfully induced three out of six attempted spawns and produced 20 unique single-parent families using six females and 11 males. A total of 1.8 million abalone larvae were cultured to competence and 1.27 million of these larvae were settled into nursery grow-out tanks (558,000 healthy larvae were culled due to lack of settlement space). Ninety-six grow-out tanks were settled with 1.04 million larvae at the NOAA Manchester Chew Center at a density of nearly 11,000 larvae per tank. Additionally, 2022 was the second consecutive year PSRF has settled larvae into culture tanks at our newest partner facility, the Seattle Aquarium. By the end of August, 37 grow-out tanks available at the Seattle Aquarium were settled with 228,000 larvae from all the genetically distinct families produced during the spawning season at the Chew Center, at a density of roughly 6000 larvae per tank. These cohorts will be reared for outplanting during spring field seasons in 2023 and 2024 depending on growth rates and survival.

Maintenance and husbandry of post-set and juvenile animals is one of the primary activities within the abalone nursery. Weekly care of these populations in the nursery included gentle tank cleaning and feeding with three diatom species cultured on site. Once post-set abalone reached 5 mm in shell length, they were weaned onto dulse, *Palmaria mollis*, 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 bi-monthly to track growth and survival.

Since 2019, the nursery has seen the reoccurrence of three nuisance species in summer months, *Askashiwo sanguinea*, *Ctenodrilus serratus*, and *Serratia* (Figure 1). This annual reporting period only saw a short, weeklong bloom of the large dinoflagellate, *A. sanguinea*. It is unlikely that the short residence time had an impact on survival of post-settled abalone. *C. serratus,* a 1-2 mm polychaete worm recently identified in the Chew Center nursery culture tanks but common to aquaculture systems generally, was widespread in its growth throughout nursery settlement tanks from July to December 2021. A concerted effort was made by PSRF

staff to continually siphon out the small ubiquitous polychaete worm to minimize and control the population. To further reduce *C. serratus* populations, post-set abalone that reached 5 mm were moved to tanks with newly established, worm-free benthic biofilm. This transition allowed staff to clean and sterilize tanks heavily inundated with *C. serratus.* To date, *C. serratus* has no known negative association with shellfish. In addition, the common bacterium*, Serratia*, had a widespread growth during the Spring 2021 settlement and persisted until fall and winter months. The decrease in growth was likely in response to the changes in water quality during fall and winter months. It is unclear if the widespread growth of *Serratia* had a negative impact on post-settled abalone survival during this reporting period.

To increase biosecurity in the nursery and hatchery, PSRF staff implemented an overnight bleach bath for rinsed filters to further limit the entrance of unwanted particles into seawater systems and continuously monitor for presence of *A. sanguinea* and/or *Ctenodrilus*. If either were seen, staff drained, cleaned and sterilized the nursery header tank, replaced filters, sterilized filter canisters, flushed and drained seawater manifolds, and siphoned culture tanks. PSRF staff repeated this regimen when necessary until the bloom and/or presence of the worms dissipated.

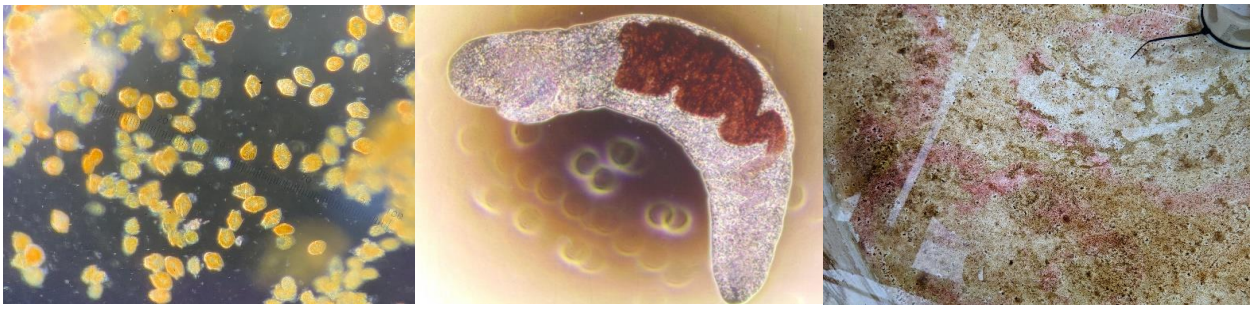

Figure 1. Unwanted species found in nursery settlement tanks during past production and rearing seasons. Images left to right: *Askashiwo sanguinea*, *Ctenodrilus serratus*, and *Serratia* sp.

An additional step was taken this year to reduce the impact of nuisance/fouling species in the nursery seawater system. In May 2022 when the density of juvenile abalone in the nursery was minimal following spring outplants and remaining animals could be moved into other tanks, PSRF staff undertook a complete sterilization of the nursery seawater system. This included draining the system (main reservoir, supply lines to 4-tiered culture tank rack, head pipes, seawater return/degassing column and all associated manifolds), flushing it with fresh water, then refilling it with a freshwater/hydrochloric acid dilution. The system was left static for 24 hrs then flushed with freshwater and then repeated a second time. After the second flush, seawater was run through the system for several days before animals were returned to the nursery tanks.

Following last year's largest outplant to date in Spring 2021, all animals less than 5 mm at the time of outplant that year were held in culture to be outplanted Spring 2022. Of the 26,500 animals that remained from the 2020 cohort in summer 2021, roughly 15,000 juvenile abalone were held at the Chew Center, 1,500 were sent to Port Townsend Marine Science Center, and 9,600 abalone were sent to the Seattle Aquarium. At the time of outplant in April 2022, just under 3,500 two-year-old abalone remained across all facilities with an average mean maximum length of 14 mm.

Both cohorts at the Chew Center and Seattle Aquarium experienced high levels of mortality during this reporting period. Currently, it is unclear if the high presence of worms, *C. serratus,* and/or *Serratia* at the Chew Center affected survival. It is likely that some juvenile mortality during this period can be attributed to delayed impacts from summer heat waves and associated water quality at both facilities. In addition, the overwintered 2020 cohort animals were all slow growing animals, likely making them more susceptible to changing temperatures and water quality.

Health & Disease

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 Incorporated. Live samples were sent for histology to detect known infectious diseases and PCR analysis to determine if withering syndrome was present. On February 4th, 2022, health screening results from all three-facilities (PSRF Chew Center, 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 nursey facilities for outplanting.

Abalone Broodstock

At the end of this reporting period, PSRF had 55 broodstock abalone (17 females, 38 males) at the Chew Center. A total of 27 broodstock were collected from the San Juan Islands during Spring 2022 collection dives (15 females, 12 males)(Figure 2). In addition, eight male broodstock were selected from existing hatchery stock for relocation to the Seattle Aquarium in May 2022. These animals were selected based on high contribution to outplanted hatchery progeny or repeated poor gonad maturation. These abalone will remain at the Seattle Aquarium for display use only.

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. All new broodstock 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 using epipodial tentacle clips, and general observation of overall health was noted. Broodstock abalone were fed two types of macroalgae species: dulse, *Palmaria mollis,* and bull kelp, *Nereocystis luetkeana.* PSRF staff co-culture dulse and bull kelp to help sustain hatchery needs.

Figure 2. Adult pinto abalone observed during broodstock dives.

Juvenile Abalone Outplant Site Monitoring

Between February-April 2022, PSRF divers participated with WDFW in surveys investigating survival, growth and emergence of hatchery reared pinto abalone at sites outplanted in 2021 including four of the eight restoration sites in Skagit County: Husky, Juno, Switchback and Vitality. These code names are used to define sites in place of more geographically descriptive site names to maintain anonymity of outplant locations.

Switchback and Vitality were newly established sites outplanted with juvenile abalone for the first time in 2019 and overseeded in 2021. Husky and Juno are two of the original Skagit County

outplant sites established in 2009. They had not been surveyed or outplanted for several years due to apparent low survival over multiple outplant years, but 2021 surveys revealed surprising numbers of abalone, particularly during perimeter surveys. At both locations, abalone had emigrated off plot but remained aggregated nearby within ideal rocky reef habitat. Additional plot area was added to Juno, and both sites were returned to the outplant rotation and overseeded with juvenile abalone in 2021.

Survey set-up in 2022 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. 2021 outplants included one- and two-year-old cohorts, the older of which were marked with blue 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). Empty abalone shells from mortalities were collected, measured and observed for tags when encountered and then removed from the plot.

The monitoring surveys at the four Skagit County sites in 2022 found a total of 275 abalone, of which 20% were found outside of the plot boundaries (Table 1). The largest number of abalone (n=102 combined on and off plot) were found at Juno and the plot density at this site was 0.61 abalone/m^2. Juno is a unique site as new plot area was added to the original site to incorporate ideal habitat to which many outplanted abalone had emigrated over time. While the entire plot (existing and new area) was surveyed, density was much greater in the three survey lanes within the new plot. Density here was 1.14 abalone/m^2, 64 abalone were observed in the new plot ranging in size from 9-142 mm shell length.

Mean shell length of observed abalone for all four plots combined was 54 mm. The overall mean density of observed abalone on-plot for the four sites was 0.63/m^2 with individual plots ranging from 0.23 to 1.03 abalone/m^2 (Table 1). When comparing the 2-yo and 1-yo cohorts, identifiable by the blue glue dots on the shell of the older cohort, survival was estimated using the number outplanted compared to the number observed, and survival was greater for the older cohort as expected. Survival of the older cohort was estimated as high as 9.4% for Juno

and as low as 2.7% for Husky. Survival of the younger cohort was 6.8% at Switchback and 0.2% at Husky.

Table 1. Juvenile abalone outplant survey data collected at four sites in Skagit County from February-April 2022. SL=maximum shell length measurement, mean includes all on and off plot observations. *Plot area at Juno increased by more than 56m^2 in 2021 when the site was expanded to incorporate nearby ideal outplant habitat.

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)
Juno*	131.4	80	0.61	104.8	22	0.21	67
Husky	77.9	18	0.23	87.4	22	0.25	100
Switchback	73.0	75	1.03	73.0	8	0.11	28
Vitality	64.0	46	0.72	67.0	4	0.06	35
Total	**346.3**	**219**	**0.63**	**332.2**	**56**	**0.17**	**54**

Juvenile Abalone Outplanting

In April and May 2022, in collaboration with WDFW subtidal shellfish biologists, the PSRF team completed the 12th juvenile abalone outplant over the past 14 years at recovery sites in the San Juan Archipelago and surrounding areas. 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, nearly 45,000 pinto abalone have been outplanted to 28 different restoration sites in Skagit, San Juan and Island counties; 18,000 of these hatchery produced juvenile abalone have been outplanted to eight rocky reef sites in Skagit County waters.

In 2022, 7283 juvenile abalone representing 41 genetically distinct families produced at the Chew Center in 2020 and 2021 and reared at the Chew Center, Seattle Aquarium and PTMSC were seeded to eight sites in the San Juan Islands (two existing, six new); mean shell length across both age cohorts was 14.5 mm (Table 2). Unfortunately, no Skagit County restoration sites were created or overseeded in 2022. Following guidelines adopted by WDFW and described in the Recovery Plan, when restoration sites are established, they are outplanted in the first and second year and then subsequently overseeded every three to four years to maintain aggregations and boost genetic diversity. The eight existing Skagit County sites were not due to receive juvenile abalone in 2022 according to this rotation. The abalone recovery team decided to prioritize development of new sites in northwestern areas of the SJA and at Smith Island. Reconnaissance dives were conducted in Skagit County early in the monitoring season and during broodstock collection; several sites for future outplants were identified in

areas surrounding Rosario Beach/Deception Pass. These sites will be revisited early during the 2023 dive season and evaluated further.

In preparation for outplanting, PSRF staff conducted an inventory, collected shell length data, and sorted families into outplant groups. Additionally, all juveniles from the 2020 cohort were marked with a colored glue dot (Coraffix cyanoacrylate adhesive mixed with Eye Candy mica powder green pigment) before outplanting to identify them from the younger group using the same methods as the previous two years (Figure 3). The 2022 outplant effort was similar to the previous two years in that both a younger and older year class were overseeded together onto the same sites (Table 2). The 2022 outplants represent the fourth opportunity to test and monitor the two age cohorts.

Table 2. Summary of pinto abalone outplanted in 2022.

		Outplant Site										
Cohort	Number of Families	Iceberg	Dracula	Landcrab	Cottage	Cherry Blossom	Cartwheel	Watchtower	Brushwood	N outplanted per cohort	Mean maximum length (mm)	Animals remaining in rearing facility
2020	23	534	552	550	555	581	516	513	524	4325	14.64	0
2021	18	380	372	364	369	369	360	376	368	2958	14.30	1206
2020 and 2021 Total	41	914	924	914	924	950	876	889	892	7283	14.52	1206*

*Of the 1,206 2021 cohort abalone remaining, an estimated 165 are overwintering at Seattle Aquarium and just under 1,000 are overwintering at Port Townsend Marine Science Center to be outplanted Spring 2023. An additional 300 2021 cohort animals were sent to Rosario Marine Research Station for experimental use, PI: Dr. Kirt Onthank, Director, Rosario Beach Marine Laboratory, Walla Walla University.

Time lapse cameras (Subaqua Imaging Systems, Model 24VDCFL) were installed on outplant sites to capture abalone movement from the outplant modules and to inform researchers on what predation may be occurring following release of juvenile abalone. These cameras were first used during outplant efforts in 2019 and each year since. Analyses continue to reveal a significant presence of kelp greenling but no clear evidence of active predation. Through an internship program at UW, PSRF and WDFW collaborate with a UW student to analyze these images and summarize relevant predation activities.

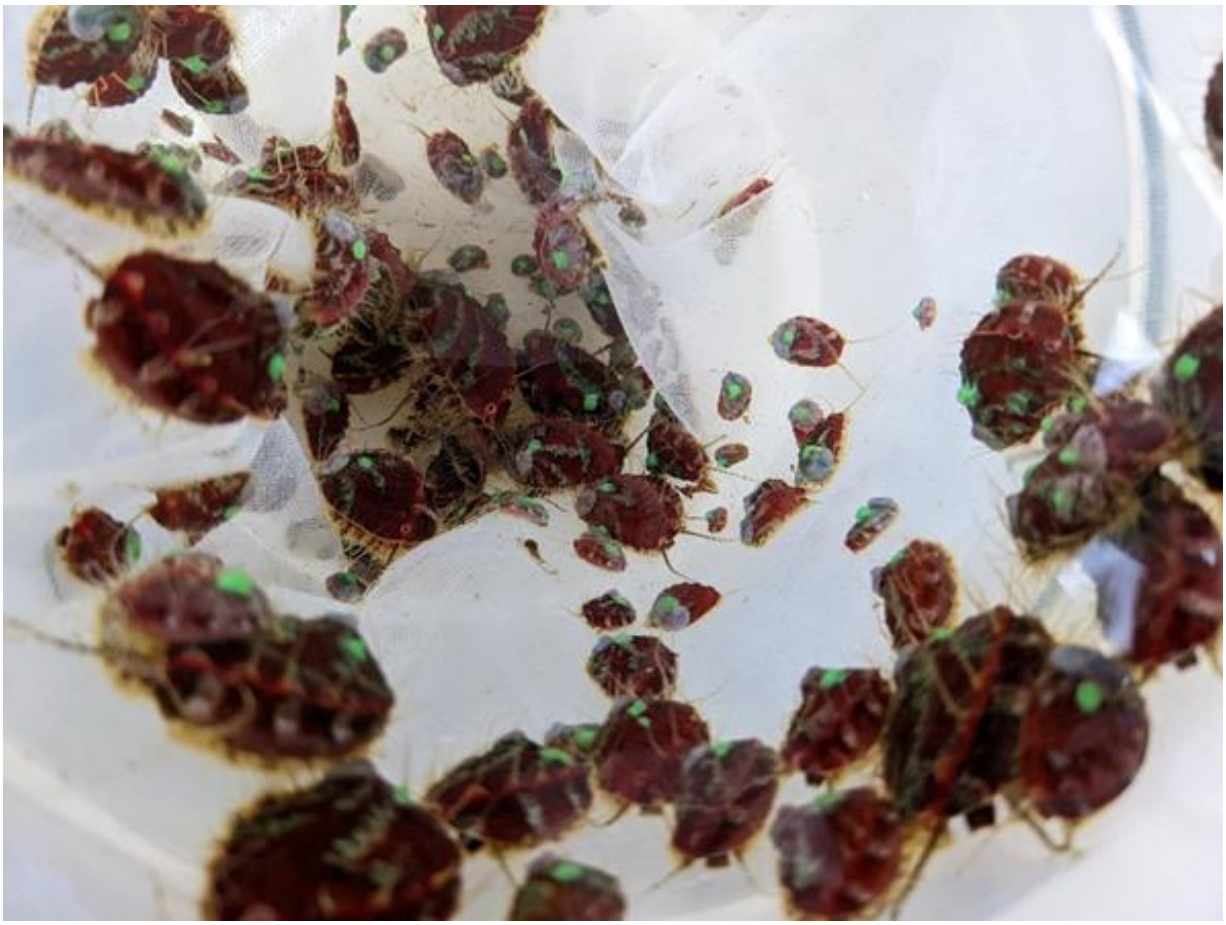

Figure 3. Juvenile abalone tagged and ready to be loaded into outplant tubes in April 2022. Green dots identify the older hatchery cohort (2-yo produced in 2020).

Outreach & the Pinto Abalone Recovery Plan

As a state listed endangered species, conservation aquaculture and field restoration activities to bring abalone back from the brink of local extirpation is guided by a Pinto Abalone Recovery Plan approved by state, tribal and NGO researchers. In addition to thorough review from collaborators, the Plan was open to public comment and was also presented to the Washington Fish & Wildlife Commission. WDFW, PSRF and Northwest Indian Fisheries Commission biologists finalized the Recovery Plan over the past two years (Sowul et al. 2021). It can be found on both the WDFW and PSRF websites listed below.

https://wdfw.wa.gov/publications/02284
https://restorationfund.org/programs/pintoabalone
https://publicinput.com/PintoAbalone

Acknowledgments

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