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Pinto Abalone Recovery Project
2021 Final Report to the Skagit MRC
Josh Bouma, Puget Sound Restoration Fund
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October 8th, 2021

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in the San Juan Archipelago (SJA) even after the closure of the recreational fishery in 1994 (Rothaus et al. 2008, WDFW unpublished data).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have been observed and the average size of abalone continues to increase (Rothaus et al. 2008, Bouma et al. 2012, WDFW unpublished data).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 Kessing (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 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 #C20190432, A20200039) supports abalone conservation aquaculture and restoration activities in Skagit County. This funding support for abalone restoration in 2020-2021 was supplemented by funding from WDFW, NOAA and the Benjamin & Margaret Hall Family 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 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 22,000 abalone have also been outplanted at 12 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, 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 2021 can be found in annual WDFW, PSRF and Skagit MRC summary reports (Bouma and Dinnel 2017, 2018, 2019 and 2020) 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, 2020-September 30th, 2021.

Hatchery Production & Nursery Husbandry

During the 2021 spawning season that began in May and concluded in July 2021, PSRF successfully induced five out of five attempted spawns and produced 27 unique single-parent families using eight females and 16 males. Seventy-seven grow-out tanks were settled with nearly 1.12 million larvae. In addition to the larvae set at the Chew Center, 2021 marks the first year PSRF has settled larvae into culture tanks at our newest partner facility, the Seattle Aquarium. By the end of July, of the 38 grow-out tanks available at the Seattle Aquarium, 18 tanks were settled with 270,000 larvae from nine genetically distinct families (Figure 1).

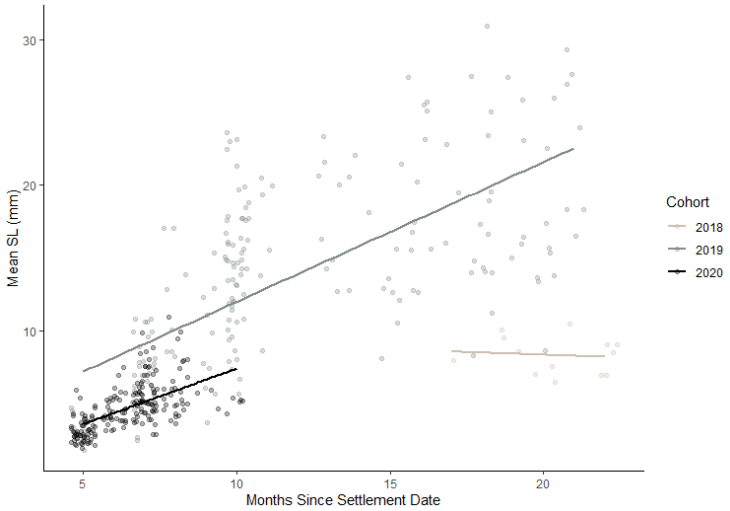
Figure 1. PSRF Chew Center staff prepare larvae to be transported to the Seattle Aquarium, which were settled into culture tanks at the Aquarium replicating the Chew Center nursery system.

Maintenance and husbandry of post-set animals is one of the primary activities within the abalone nursery during the fall, winter, and spring. Weekly care of this post-set population in the nursery includes gentle tank cleaning and feeding with three diatom species. 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 continue 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 are recorded bi-monthly to track growth and survival (Figure 2).

In addition to post-set culture, rearing juveniles that did not get outplanted at the one-year mark continues to be an important part of the Chew Center nursery program. Only a third of the available juvenile abalone cohort produced in 2020 were above 5 mm and therefore released during the 2021 outplant season. The remaining 15,000 animals are currently overwintering at the Chew Center (~7900 juvenile abalone), PTMSC (~1,300 juvenile abalone), and in 20 tanks at the newly established satellite facility at the Seattle Aquarium (~6000 juvenile abalone). The remainder of the 2020 cohort animals will be outplanted to recovery sites in Skagit, San Juan and possibly Island or Jefferson counties in spring of 2022.

Figure 2. Shell length (SL) of hatchery-reared juvenile abalone: July 1, 2019 to June 30, 2021. Note: 2018 cohort data only includes shell length data of overwintered, two-year old animals not selected for the 2019 outplant season.



Health & Disease

To confirm no disease-causing pathogens are present in hatchery-reared animals prior to outplanting animals into the wild, an annual hatchery health assessment is conducted by Dr. Ralph Elston of AquaTechnics Inc. Live samples are sent for histology to detect known infectious diseases and PCR analysis to determine if withering syndrome is present. In December 2020, results from 120 live juveniles (60 histology and 60 PCR) representing families produced during the 2019 and2020 spawning seasons and destined for restoration outplant sites showed no disease-causing pathogens were present. Similar hatchery health screenings were conducted at the Port Townsend Marine Science Center in 2020 with clean pathology

results reported. Upon this confirmation of abalone hatchery health, new transfer permits were received from WDFW to move abalone from the nursey facilities for outplanting in 2021.

Abalone Broodstock

As of June 2021, PSRF had 55 broodstock animals (15 females, 40 males) at the Chew Center. Regular inventory, health and maintenance checks are conducted on all broodstock, tanks are fed and cleaned weekly, and twice-yearly animals are measured, weighed and re-tagged as necessary. Annual broodstock collections were conducted by PSRF and WDFW divers in the San Juan Islands (Figure 3) as the spring field season concluded in May, prior to the 2021 spawning season beginning. Twenty-six wild collected adults were brought into the Chew Center during these collection efforts in 2021 (14 females, 12 males). 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, non-lethal genetic samples were taken using epipodial tentacle clips, and general observation of overall health was noted.

Figure 3. A WDFW diver finds a wild pinto abalone and contemplates collection for broodstock.

Photo: K. Sowul, WDFW

Juvenile Abalone Outplant Site Monitoring

Between February-April, 2021, PSRF divers participated with WDFW in surveys investigating survival, growth and emergence of hatchery reared pinto abalone at seven of the eight restoration sites in Skagit County. Code names will be used to define these sites in place of more geographically descriptive site names to maintain anonymity of outplant locations. Four of these sites had been outplanted with an overseeding of juvenile abalone in 2020. Two of the original Skagit County outplant sites that were not surveyed or outplanted last year due to apparent low survival over multiple outplant years were revisited again in 2021; a full survey was conducted on one while a partial look at the other was all that was possible because monitoring days in the field schedule had been exhausted. Additionally, two sites in Skagit County that were outplanted for the first time in 2019 and monitored in 2020 (resulting in observed low survival) were surveyed again in 2021 to determine if they would be overseeded this year.

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. 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. 2020 outplants included one- and two-year-old cohorts, the older of which were marked with pink 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 seven Skagit County sites in 2021 found a total of 523 abalone, of which 31% were found outside of the plot boundaries (Table 1). The largest number of abalone (n=181 combined on and off plot) were found at Omaha and the plot density at this site was 1.41 abalone/m^2. The density of abalone at Omaha when areas of both the plot and surrounding perimeter were combined was 1.01 abalone/m^2. Mean shell length of observed abalone for all seven plots combined was 88.2 mm. The overall mean density of observed abalone on-plot for the seven sites was 0.55/m^2 with individual plots ranging from 0.09 to 1.41 abalone/m^2 (Table 1). Of the four longer-term sites (Gold, Utah, Omaha and Baytown) that were overseeded in 2020, Omaha had the best post-outplant observation rate of juvenile abalone with 42 abalone counted that were estimated to be from 2020 outplant efforts. This represents an observed recovery rate of 7% after one year on the plot, slightly below the target one year survival rate of 10%.

Husky and Juno, two of the original Skagit County outplant sites that had not been surveyed or outplanted for several years due to apparent low survival over multiple outplant years, revealed surprising numbers of abalone during 2021 observations. During a complete survey of Husky, including the plot perimeter, 44 adult abalone were counted, and mean shell length was 110.5 mm (Table 1). While time did not allow a full survey of Juno, divers examined part of the plot and several meters of perimeter habitat off plot and observed several dozen adult abalone. At both locations, abalone have emigrated off plot but remained aggregated nearby within ideal rocky reef habitat. The number of abalone observed at Switchback and Vitality (newly established and seeded for the first time in 2019, not overseeded in 2020) was similar to what was observed during monitoring in 2020. It is possible that low counts during previous and recent surveys are due to surviving juveniles being cryptic within the complex habitat on these

sites, or due to emigration off the plot to more suitable habitat. These sites will continue to be monitored next year.

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)
Gold	98.2	107	1.09	96.8	29	0.30	91.5
Juno*	75.3	n/a	n/a	84.8	n/a	n/a	n/a
Utah	102.5	59	0.58	97.2	18	0.19	93.2
Omaha	90.0	127	1.41	93.2	54	0.58	88.4
Baytown	79.3	35	0.44	81.1	28	0.35	90.7
Husky	77.9	16	0.21	87.4	28	0.32	110.5
Switchback	73.0	11	0.15	73.0	4	0.05	74.1
Vitality	64.0	6	0.09	67.0	1	0.01	69.7
Total	**660.2**	**361**	**0.55**	**680.5**	**162**	**0.24**	**88.2**

Table 1. Juvenile abalone outplant survey data collected at seven sites in Skagit County from February-April 2021. SL=maximum shell length measurement, mean includes all on and off plot observations. *Juno was visited and examined but a survey was not completed due to limited remaining survey time in the field schedule. Gold, Utah, Omaha and Baytown had been overseeded with another round of juveniles in 2020. Switchback and Vitality were newly established sites outplanted for the first time in 2019 and not overseeded in 2020.

Juvenile Abalone Outplanting

In April and May 2021, in collaboration with WDFW subtidal shellfish biologists, the PSRF team completed the 11th juvenile abalone outplant over the past 13 years at recovery sites in the San Juan Archipelago. 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 40,000 pinto abalone have been outplanted to 21 different restoration sites in the San Juan Islands; 18,000 of these healthy, genetically diverse hatchery produced juvenile abalone have been outplanted to eight rocky reef sites in Skagit County waters.

Figure 4. A two-year-old juvenile abalone with several one-year-old hitchhikers exiting the outplant tube onto surrounding rocky reef substrate.

Description of juvenile outplant groups

10,693 juvenile abalone representing 38 genetically distinct families from the 2019 and 2020 Chew Center cohorts were seeded to ten sites in the San Juan Islands (seven existing, three new); this included 4298 abalone that were seeded to four existing sites in Skagit County. The Skagit County restoration sites seeded in 2021 included Husky, Juno, Vitality and Switchback. The average number of abalone outplanted per site at these four Skagit County sites was 1075 animals. Switchback and Vitality, the two newest sites created in Skagit County in 2019 that were not overseeded in 2020 due to disappointing yet inconclusive survival results observed during two years of monitoring efforts, were outplanted again this year as it was determined to be worth another seeding effort given the observed habitat/substrate quality and presence of several wild abalone observed during recent surveys.

In preparation for outplanting, PSRF staff conducted an inventory, collected shell length data and sorted families into outplant groups. Additionally, all juveniles from the 2019 cohort were marked with a colored glue dot (Coraffix cyanoacrylate adhesive mixed with Eye Candy mica powder blue pigment) before outplanting to identify them from the younger group using the same methods as the previous two years. The 2021 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 (Figure 4). Mean shell length of the 2019 and 2020 cohorts at all four Skagit County sites were 19.5 mm and 9.9 mm respectively. Following a 2.5-year study completed in February 2019 by PSRF and WDFW to investigate survival and growth of younger-aged outplants, outplant strategies have shifted towards releasing many hatchery juveniles before they reach one year in age. This has substantially increased annual production cycles in the Chew Center. The 2021 outplants represent the third and largest opportunity to test and monitor the two age cohorts over the next year to confirm that survivorship of the younger group is similar to the older group.

Outplant Modules, Loading and Transport

Outplant modules were made from PVC pipe (6" ID, 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. 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 125 abalone from both age cohorts combined were loaded into each tube (Figure 5). The open end of the tube was then closed with one layer of window screen and secured with a rubber band. Modules were then placed back into a flow-through seawater tank at the Chew Center for holding overnight.

Figure 5. An outplant module is placed into rocky substrate on an outplant site, after screens are removed the juvenile abalone quickly move onto the reef.

The following morning, modules were transferred into a seawater filled 4'x 4' fish tote in a U-Haul box truck and transported from Manchester to Anacortes. The large fish tote in the truck 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 tote was drained at the marina and abalone modules were transferred into a waiting seawater filled, aerated fish tote on board the WDFW R/V Enumerator during the first week of outplanting and the WDFW Enforcement vessel during the second week of outplanting (Figure 6).

Figure 6. PSRF/WDFW personnel load abalone from the Chew Center onto the research vessel and enforcement vessel in Anacortes. Divers prepare to jump into the water with outplant tubes.

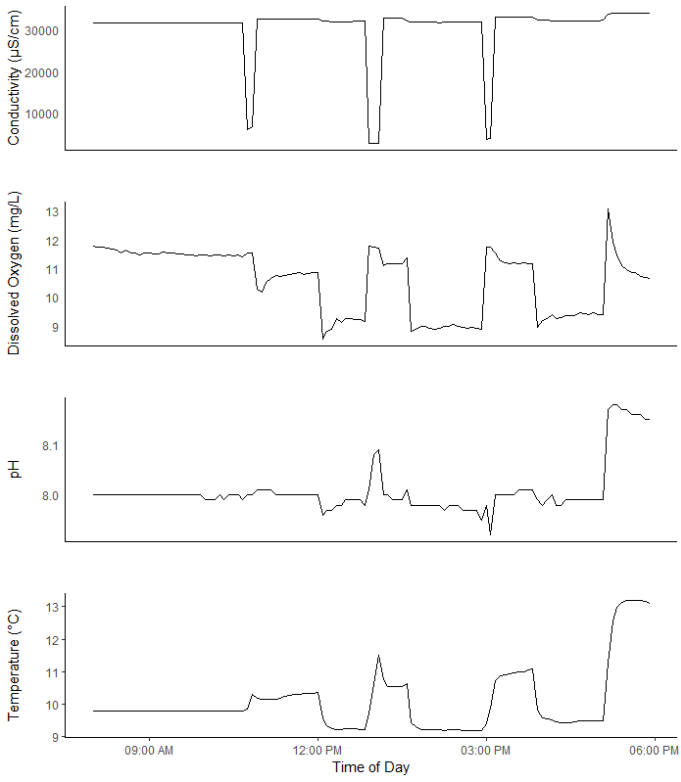
Once at outplant sites, tubes were bound together with webbing straps and quick release buckles in bundles of four and carried to depth by divers. Tubes were placed within the delineated outplant plots in areas that appeared to have suitable substrate and cryptic habitat onto which juvenile abalone could 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 2022, cleaned and reused during future outplant efforts.

Similar to a study conducted in 2020 looking at the stress to abalone of being handled and transported to restoration sites, during the first outplant of 2021, a group of 41 animals from the 2019 and 2020 cohorts were exposed to all the same stressors of the outplanting process in

a separate module. This included sorting, transport via the fish tote in a U-haul, boat ride and even being carried down to depth by divers. This test module was then returned to the Chew Center nursery at the end of the outplant day to be monitored for any deleterious effects. No mortalities were immediately observed from this handling and transport. However, after one month of monitoring in a nursery tank at the Chew Center post-outplant, a single mortality was reported from this control group.

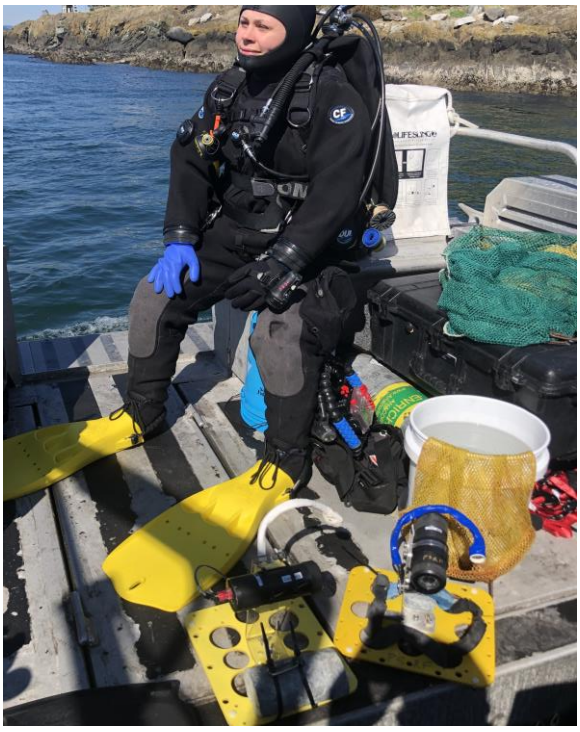
During outplant efforts in 2021, water quality loggers tracked temperature, pH, dissolved oxygen and salinity for the duration of the outplant day. Logger data from outplant efforts in 2021 (Figure 7) show that juvenile abalone experienced consistent temperatures during loading and transport from the Chew Center to the field, and only a 1^{o} C temperature drop when the modules were secured within the rocky reef substrate at an outplant site. Minor, temporary water quality changes occurred when the abalone were transferred from the U-haul tote into fresh seawater in the dive vessel tote and also when the abalone were moved from the tote and into the ocean by divers. These stressors do not appear to be detrimental to abalone health during the outplant process.

Figure 7. Water quality data during 2021 outplant from data loggers during the first day of outplanting. Probes started collecting data at the Chew Center Hatchery and were transported via U-Haul, in the same tote as the abalone, to the WDFW boat for outplant around 10:00 AM. All probes went down on three outplant dives from noon to 1:00 PM, 1:30 PM to 3:00 PM, and 4:00 to 5:00 PM. Sometime after 5:00 PM the probes were brought to the surface and placed in a bucket of fresh seawater to conclude the day's events.



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 (Figure 8). These cameras were first used during outplant effortsin 2019, but only three sites, one camera each, were monitored during that effort. Analyses revealed a significant presence of kelp greenling but no clear evidence of active predation. This year, two cameras were installed on each of six sites to increase the opportunity to capture images of ongoing predation during the 48 hours following release of abalone. More than 46,000 images were captured from these cameras. PSRF and WDFW are in the process of setting up an internship for a UW student who will analyze these images and summarize relevant predation activities.

Figure 8. A diver prepares to descend with time lapse cameras.



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 will be guided by a Pinto Abalone Recovery Plan approved by state, tribal and NGO researchers. The Plan is open to public comment and will be presented to the Washington Fish & Wildlife Commission. WDFW, PSRF and Northwest Indian Fisheries Commission biologists have drafted the Recovery Plan over the past year (Sowul et al. 2021). It can be found on both the WDFW and PSRF websites listed below. The Public Comment period is open until January 6th, 2022, and comments regarding the Pinto Abalone Recovery Plan for Washington State are encouraged and can be provided at the third link below.

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

Acknowledgments

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