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ARTICLE

Mixed-Age Releases of the Endangered Pinto Abalone (*Haliotis kamtschatkana*) to Maximize Hatchery Production and Survival in the Wild

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ABSTRACT

The Washington State recovery programme for endangered pinto abalone (*Haliotis kamtschatkana*) relies on captive breeding. From 2009 to 2017, juveniles from wild parent crosses were released after an average of 20 months in the hatchery (average size 24 mm), and the results of a mark-recapture experiment suggested that size-at-release was not important to survival. The results of a pilot study suggested that abalone released at 9-month age survived at similar rates to previous releases at 20 months but that there was a significant cost to growth and survival for the 14-month releases. From 2019 to 2022, an average of 7000 mixed-age juveniles were released on a different subset of 24 restoration sites each year. Larger individuals (> 5 mm) from each family were released at 9 months (first years), with the remainder held in the hatchery to be released at 20 months (second years). The resulting survival at successful sites from 9- to 32-month age was 0.6%–6.1% for first years and 0.6%–4.5% for second years. In two out of three trials, there was little or no evidence of reduced survival or growth as a result of releasing almost a year early. When combined with the cost savings of rearing animals for a shorter duration, possible benefits to growth, reduction in hatchery acclimatization or selection, better rotation of hatchery resources and culture space and mixing 2 years of genetic crosses into one release, we suggest that mixed-age releases are the most efficient way forward for the conservation of pinto abalone.

1 | Introduction

Conservation practitioners usually wish to maximize the impact of their actions given a fixed and often limited budget. In captive breeding of threatened or endangered species, the goal is to maximize the numbers of individuals that survive beyond reintroduction to reproduce in the wild, perpetuating the species. The fixed budget, space or other resources for rearing requires economization of the investment devoted to each potential future adult. A key decision is when to cease investing time and hatchery resources into rearing and instead release individuals into the wild. Often, the question is whether to produce fewer older individuals who are either at or likely to survive to reproductive age versus producing more younger individuals who may have a lower chance of surviving to maturity. This question has been considered for many species in both terrestrial (e.g., Sarrazin and

Legendre 2000) and marine environments, including fish (e.g., Gil et al. 2015; Leber, Cantrell, and Leung 2011) and invertebrates (e.g., Stoner 2019). Of course, this decision must consider the life history attributes and limitations of the species.

For marine invertebrates, there is some evidence of the survival benefit to rearing larger/older individuals prior to release (e.g., Purcell and Simutoga 2008), including for abalone (De Waal et al. 2013). However, rearing to larger size may not always be the most cost effective (Roberts et al. 2007), and the dependence of survival on size can vary seasonally (Johnson et al. 2008). There are additional behavioural costs to longer rearing times, including potential acclimatization to the artificial environment (Hansen and Gosselin 2016), or 'domestication selection' of certain individuals (Nascimento-Shulze et al. 2021), which may reduce fitness in the wild.

The pinto abalone (*Haliotis kamtschatkana*) is a large marine mollusc in the family Haliotidae and is listed as endangered in the state of Washington, USA (Sowul et al. 2022) because it is not likely to persist without intervention. It is the only species of abalone in the state. Pinto abalone can grow to a shell length of approximately 16.5 cm, and though their true life span is unknown, they are believed to live at least 15–20 years. It inhabits shallow rocky reefs and feeds on diatoms and macroalgae. Harvested since time immemorial by indigenous people and later by other inhabitants of the state, it is prized as a food source, as cultural resource and for its iridescent shell. The Washington Department of Fish and Wildlife authorized recreational fishing from 1959 through 1994; no commercial fisheries were ever authorized. Concern for the depletion of pinto abalone triggered the closure of the fishery in 1994 (Sowul et al. 2022). Illegal harvest occurred during the authorized fishery and may have been commercial in scale.

Legal and illegal harvest likely reduced the density of pinto abalone in Washington to below an Allee Threshold (Allee 1938). At monitored sites in the San Juan Islands from 1992 to 2006, combined observations of dwindling remnant populations, increases in average size of individuals and low numbers of observed juveniles (abalone < 50 mm in shell length) suggested population-wide reproductive failure (Rothaus, Vadopalas, and Friedman 2008; Bouma et al. 2012). The exact size or age that wild pinto abalone begin to reproduce is unknown. However, in a separate trial experiment, captive-bred abalone from wild parents (referred to as F1s) between 3 and 4 years of age with shell lengths of approximately 40 mm were successfully spawned in a hatchery setting creating thousands of viable gametes. Though the controlled hatchery setting and potential hatchery selection of F1s may have had heavy influences on the success of this spawn, the ability of F1s to spawn at this age and size may suggest that wild abalone are reproductive around the same time.

The declining trend in the population continued after the recreational fishery closure in 1994. As habitat did not appear to limit the species, the decision was made in 2002 to initiate a captive breeding programme and attempt to re-establish spawning aggregations (Sowul et al. 2022). Wild adults are collected each year and brought back to the hatchery and become broodstock or potential parents of captive-bred juveniles. These broodstock abalone are then spawned in a hatchery with resulting single-parent family crosses allowed to mature to the juvenile stage before being released into the wild at select sites. Since the first releases in 2009 through the latest releases in 2023, almost 50,000 juveniles have been placed on 29 sites in Washington waters.

Carson et al. (2019) evaluated the pinto abalone conservation aquaculture operation in Washington for the time period of 2009–2017 (15,000 juveniles released at 12 sites) and found that the observed survival of released individuals (mean age = 20 months, mean size = 24 mm) over the first 10 months in the wild averaged 10.2%. The mark-recapture experiments in that study estimated that divers locate only 20%–40% of abalone present on any given post-release survey, so actual survival is much higher. Additionally, release site was the primary factor influencing the survival of juveniles, the effects of site greatly outweighed those of family, and there was no support for an effect of size-at-release on survival (Carson et al. 2019).

The apparent independence of size and survival suggested that perhaps juveniles could be released sooner, thereby decreasing the cost per individual and increasing the number that can be released annually for the same investment of resources. This was supported by a study on a different abalone species, *Haliotis iris*, that suggested 10 mm juveniles might be the most cost-effective size for release (Roberts et al. 2007). Here, we present the results of a pilot experiment and a 4-year study of restoration-scale releases of mixed-age juvenile groups. Our goal was to:

1. measure the survival and growth of mixed-age releases compared to releasing entire cohorts after 20 months in the hatchery as had been the strategy from 2009 to 2017, and
2. evaluate the results in conjunction with hatchery space and cost to determine if the restoration programne can be conducted more efficiently than it has been.

We note here that the experimental design balanced both the desire to evaluate the effects of age- or size-at-release on survival as well as a pressing need to prioritize restoration goals for a species in danger of local extirpation. Although Carson et al. (2019) show that site choice is highly influential, some features of robust experimental design, such as standardizing the release densities and sites for each trial year, were sacrificed to achieve desired restoration results such as establishing aggregations in new areas and releasing all available individuals. Other aspects of an ideal experimental design, such as random assignment of individuals to treatment groups, were simply not feasible due to logistical constraints (see mixed-age release methods below).

2 | Methods

2.1 | Study Area

Studies described here took place in the US portion of the Salish Sea, in the vicinity of the San Juan Islands. Specific sites on shallow rocky reefs were selected according to criteria outlined in

Carson et al. (2019), including complex cobble, boulder or bedrock seafloor with abundant macroalgae and exposure to swift tidal currents. Sites ranged from 1.5 to 12 m depth at mean lower-low water elevation. Descriptions or maps of specific study locations will not be provided here out of concern about illegal harvest.

2.2 | Pilot Experiment to Measure Survival of Abalone Released at Younger Ages

Juvenile pinto abalone from seven genetically distinct families, fertilized in the hatchery between June and September 2016, were used for this experiment. The wild parents of each cross were collected by divers from the San Juan Islands in spring of 2016 and held in the hatchery until spawning was induced in summer. Each family of juveniles was held in a separate flow-through seawater tank at the NOAA Mukilteo Research Station and fed diatoms or macroalgae *ad libitum* until the release experiments. Hatchery methodology is described in more detail in Carson et al. (2019). For this pilot experiment, subsets of the cohort were released at average ages of 9 and 14 months to compare with survival of all previous cohorts, which had been released at an average age of 20 months.

To maximize overall survival during the experiment and focus on the variable of interest (age/size at release), the experimental area was chosen for proximity to the release site 'Omaha', which had the highest juvenile survival/retention from 2009 to 2015 releases (Carson et al. 2019). Six individual release areas, each approximately 30 m apart, were selected on the −5 m depth contour stretching along the east-facing coastline of an island in the San Juan Archipelago. Each area contained bedrock, boulder and cobble habitat deemed suitable for abalone rearing, and each was numbered from 1 (northernmost) to 6 (southernmost). Each area received one young abalone module (YAM) consisting of two cylindrical 0.35 m^3 commercial crab traps fastened together. The top mesh of one trap and the bottom mesh of the other were removed to construct one cylinder of 4-cm stainless steel mesh approximately 1 m high and 1 m in diameter. Each YAM was filled with crustose coralline algae-covered cobbles 0.2–0.5 m in diameter collected on-site. Macroalgae were not added to the YAMs. Small pieces of macroalgae may have drifted through the mesh, though it is unlikely. Cobble was also collected and transported in seawater back to the hatchery, where two additional YAMs were constructed as laboratory controls, each inside its own flow-through sea water tank.

In April 2017, a subset of each of the seven hatchery families were combined and loaded into 10 cm diameter PVC tubes for transport into the field. These animals averaged 9 months since fertilization (range of 7–10 months) and had an average shell length of 8.2 mm (±3.7 mm SD). This subset was distributed to three field YAMs and one hatchery control YAM, keeping the proportions from each family equal across each. Field-bound tubes were transported in aerated seawater totes as quickly as possible to release areas for deployment via divers the next day. Field areas 1, 3 and 5 each received two PVC tubes totalling 107 animals per YAM. Tubes were carefully embedded in the top layer of cobble inside each YAM. Two additional tubes totalling 102 animals were placed inside one of the hatchery control YAMs. Hatchery control YAMs were fed macroalgae throughout the experiment. All additional members of this cohort (409 individuals) were left in their family groups and culture tanks for the next treatment.

In September 2017, the remaining animals from each family were combined and loaded into tubes for transport as before. These animals averaged 14 months since fertilization (range of 12–15 months) and had an average shell length of 13.7 mm (±6.7 mm SD). Mortality in the hatchery for this group was higher than anticipated. Therefore, field YAMs 2, 4 and 6 only received 81 animals each, with the second hatchery control YAM receiving 78 individuals.

Four dive surveys of field YAMs were conducted following the two releases (Figure 1). The first survey was conducted in September 2017, 5 months post-release. During these dives, the remaining animals from the cohort, which had spent 5 months longer in the hatchery, were released to YAMs 2, 4 and 6. In March 2018 at 20-month age, selected YAMS from both release cohorts were surveyed. The 20-month time point corresponded to the typical release age of the 2009–2017 restoration releases. The YAMs were surveyed again in October 2018 (27 months old) and a final time in March 2019 at 32 months. This final survey corresponded to the age at which survival of the 2009–2017 restoration releases was assessed. To minimize disturbance that may impact animal retention, not every module was assessed during each survey. The laboratory controls were also surveyed in the same months, with the exception of the final survey. The hatchery operation moved to a new facility in 2019, and therefore, laboratory controls had to be removed after the October 2018 survey.

During surveys, each YAM was opened in situ by divers, and each piece of cobble was carefully removed and inspected for the presence of juvenile abalone (Figure 1). Each abalone shell length was measured using callipers, and the cobble with abalone attached placed in a plastic tub. After the survey, all cobble was replaced back into the YAM, with special care taken to replace the abalone cobbles gently before closing the module. A 3-m-radius circle around each module was carefully searched for the presence of juvenile abalone that travelled out of the YAM. Percent survival and average size were calculated for each treatment and life stage.

2.3 | Mixed-Age Releases

Juvenile abalone resulting from wild parent crosses were produced at the NOAA Mukilteo Research Station or the NOAA Manchester Chew Center for Shellfish Research and Restoration each year from 2017 to 2021, following the methods described above and in Carson et al. (2019). Parental broodstock abalone for these cohorts ranged in size from 95 to 167 mm (mean 131 mm). Each family was reared in its own 35–50-gal flow-through seawater tank until just before release, when families were combined into release groups by site in equal proportions. In 4 years of releases from 2019 to 2022, over 28,000 juveniles were placed at a subset of 24 field sites in the San Juan Islands or vicinity (Table 1). Sites are chosen based on a myriad of factors that are typically associated with pinto abalone presence, including presence of complex rocky reef substrate, algae and kelp biodiversity and presence of wild abalone. Figure 2 shows an example of one site chosen for its high coverage of crustose coralline algae, a species that emits chemical cues for larval abalone

FIGURE 1 | A diver deconstructs a young abalone module (YAM), made from modified commercial crab traps, during a survey to observe juvenile survival post-release. Divers took great care to fully inspect each boulder placed inside the YAM while moving boulders in and out of the modules.

TABLE 1 | Summary of release trial cohorts between 2019 and 2022.

Cohort	Spawn year	Release year	Number of families	Individuals per site	Average shell length (mm)	Number of sites	Number of successful sites
A2	2017	2019	13	287	19.3	6	4
B1	2018	2019	14	806	9.2	6	4
B2	2018	2020	7	62	11.0	6	6
C1	2019	2020	24	541	15.3	6	6
C2	2019	2021	13	297	19.1	10	6
D1	2020	2021	24	822	9.9	10	6
D2	2020	2022	18	541	13.5	8	6
E1	2021	2022	18	370	11.2	8	6

Note: The first cohort (A) was only released as 2-year-olds. The last cohort (E) had only been released as 1-year-olds at the time of publication. Number of families is the number of unique male × female crosses providing offspring. Note that the number of families for the second release of each cohort is lower due to no additional individuals within a family retained post-Year 1 release or surviving in the hatchery to Year 2. Average shell length is as measured at the time of release. Successful sites are those with observed survival > 2% in the first, 10-month post-release survey.

settlement, as well as extensive rocky reef substrate. Each release included 9-month average age (first-year) individuals and 20-month average age (second-year) individuals. Due to the logistical constraints of safely removing and transporting the smallest individuals from each family, individuals could not be randomly assigned to release treatment. The first-year individuals included were those that were large enough to be handled (> 5 mm shell length). The second-year individuals included were those that had not been released in the prior year (due to small size and/or family representation) and that survived an additional 11 months in the hatchery to reach releasable size.

Second-year cohorts (A2, B2, C2 and D2) were marked on the shell with a dot of CorAffix cyanoacrylate adhesive (Two Little Fishies Inc., Miami Gardens, FL) dyed with Eye Candy Mica Pigment Powder (Eye Candy Pigments, Daytona Beach, FL), to distinguish them from first-year cohorts (B1, C1, D1 and E1) released simultaneously (Figure 3). Following the methods

FIGURE 2 | A juvenile pinto abalone site photographed immediately after release showing complex boulder habitat and the presence of crustose coralline algae. The photo was taken in the early spring before significant macroalgae growth. The PVC tubes used to transport the abalone are visible in the photo, and juvenile abalone are visible on the inner edge of the PVC tube in the lower right quadrant of the photo.

described in Carson et al. (2019), juveniles from both release treatments and all available families were placed approximately 50–75 at a time in 10-cm PVC tubes capped with 1 mm mesh to allow water flow. Tubes were transported from the hatchery by truck and boat to field sites in aerated, insulated seawater totes. As soon as practical, the abalone were taken underwater by divers at pre-selected sites, who nestled the tubes among cobble, boulder and bedrock reef before removing the mesh. These release sites, some of which contained abalone from previous releases, were an approximate 80 m^2 rectangle of shallow rocky reef, marked by metal pitons placed in the rock at each corner.

Approximately 10 months after release, each site was carefully surveyed by divers using methods described in Carson et al. (2019) to count and measure live abalone and collect any empty shells. Resulting survival for the older cohort was calculated by dividing the number of marked (second-year) abalone present in or around the site by the number released. Survival for first-year cohorts was calculated by dividing the number of unmarked juveniles present by the number of first-year abalone released. Tag loss was assumed to be minimal as none was observed in the hatchery but would artificially inflate first-year survival at the expense of second-year survival. In some cases, unmarked first-year abalone were distinguished from unmarked, existing abalone on a site based on size. All survival reported here is naive (observed) survival and does not include an adjustment for detection, known to be 20%–40% in this size class among the complex substrates selected for release sites (Carson et al. 2019). Calculated survival also does not include animals that have survived but moved outside the area surveyed.

3 | Results

3.1 | Pilot Experiment to Measure Survival of Abalone Released at Younger Ages

The subset of juvenile abalone released at 9-month average age were placed into field and laboratory YAMs in April of 2017. YAM 1 was surveyed 5 months later, and only 2 of the 107 abalone were found. Although the plan had been to leave YAMs 3 and 5 alone until subsequent surveys, with the low survival in YAM 1 an additional YAM (3) was surveyed. Twenty-four abalone were found in that module, for a combined survival of 12% since release. All three modules were surveyed in March of 2018, nearly 1 year after release (20 months of age), and 29 of the original 321 abalone remained (9.0% survival), all located in or around YAMs 3 and 5. Six months later (October 2018; 27 months of age), 19 abalone were located (5.9% survival). In the final survey in March 2019, at average age of 32 months, and 2 years after release, 14 were located (4.4% survival). Juveniles placed in the laboratory control YAM had 58% survival during the first 5 months of residence. At the end of the laboratory portion of the experiment in October 2018, 40% remained. Survival during each field and laboratory survey is summarized in Table 2.

During the initial 5 months that the first release was in field modules, another subset of the cohort remained in their captive rearing tanks separated by family (14-month release columns in Table 2). Seventy-six per cent of those survived to their release date in September 2017 as 14-month-old average age. Subsequent survival in the field is reported starting from the 9-month-old release (April 2017), as this hatchery mortality is a downside of waiting longer to release. Only one module (4) was surveyed in March 2018, but all three (2, 4 and 6) were surveyed in October 2018 and March 2019. Survival of the 14-month release individuals at each survey was similar to that of the 9-month-old release for both field and laboratory YAMs (Table 2).

FIGURE 3 | A close-up of a release tube in which both second-year (blue dots on the shell) and first-year (unmarked) juveniles are shown. A diver is visible in the background.

TABLE 2 | Observed survival (%) and size (average shell length in mm) of juvenile abalone released at 9 and 14 months after fertilization in the young abalone module (YAM) experiment.

Date	Average age	9-month release (field)	9-month release (lab)	14-month release (field)	14-month release (lab)
April 2017	9 months	Starting point for growth and survival, all treatments (8 mm)			
September 2017	14 months	12% (13 mm)	58% (15 mm)	76% (14 mm)[a]	76% (14 mm)[a]
March 2018	20 months	9% (21 mm)	48% (28 mm)	9% (13 mm)	47% (27 mm)
October 2018	27 months	6% (34 mm)	40% (41 mm)	6% (27 mm)	36% (38 mm)
March 2019	32 months	4% (36 mm)		2% (32 mm)	

Note: Survival is calculated as a percentage of the 9-month-old individuals.

[a] These individuals held in family groups in original rearing tanks for an additional 5 months before release into field and laboratory modules at 14 months.

Both release treatments had nearly the same average size in September 2017, regardless of where they had spent the preceding 5 months: in rearing tanks, in a hatchery control or in the wild. However, 9-month release animals were larger on average than the 14-month releases at 20 and 27 months. In a one-tailed *t*-test at the 95% confidence level, the average size of 9-month release was ~40% (8 mm) larger in March 2018 ($p = 0.002$). In October 2018, the 9-month releases were still ~20% larger (7 mm) larger ($p = 0.046$). In the final survey at 3 years old, there was no difference in mean size ($p = 0.326$), although the 9-month release average value was still greater by 4 mm (~10%).

Throughout the four field surveys, 92% of observed juvenile abalone were found inside the modules, and two-thirds of those inside were on the top layer of cobble. The percentage of all abalone encountered that were located outside the modules increased over time, with 0% found in the perimeter in September 2017, 5% in both March and October 2018 and 30% in March 2019. At each survey, the average size of those found in the perimeter was larger than those remaining in the module, consistent with the previous finding that animals are more emergent as they grow (Carson et al. 2019). Additionally, as macroalgae was not placed in the YAMs during the experiment, abalone that had emerged from the YAMs had access to a richer food source than the abalone that stayed in the YAMs. It is unknown whether the growth itself is the driver for abalone leaving the YAMs or if those that left the YAMs experience greater growth rates due to access to macroalgae outside of the YAMs. In the final survey, the animals found outside modules ($n = 6$) averaged 52.3 (±14.0 SD) mm shell length, whereas those inside ($n = 14$) averaged 27.5 (±8.3) mm. Over the course of the experiment, more animals were found outside the 9-month-old release YAMs (7) than the 14-month modules (3). The fact that 9-month releases grew faster and therefore more likely to be emergent may explain this observation.

3.2 | Mixed-Age Releases

For 4 years, 9-month and 20-month juveniles from different spawn years were released simultaneously in a mixed-age release effort. We present observed (naive) survival rates here, without any adjustment for detection. In 2019, cohorts A2 and B1 were placed on 6 new sites. In the follow-up survey in early 2020, overall survival on only four of the sites (2.8%) was deemed high enough to retain the sites for future releases. Survival on the other two (1.3%) was not high enough for retention, and those sites were removed from the comparison between cohorts. The A2 cohort, released at 20 months, averaged 4.7% survival from release to first survey. However, survival in the hatchery that occurred between 9 months (when they could have been released) to the release at 20 months was 82.2%. Therefore, overall survival from 9 to 32 months was 3.9%. In contrast, the B1 cohort, released simultaneously as 9-month-olds to the same sites had 0.8% survival between 9 and 20 months. Including data taken during surveys the following year, the overall survival from 9 to 32 months for the B1 cohort was 0.6% (Table 3 and Figure 4).

In 2020, cohorts B2 and C1 were released on 6 sites that had been established between 2009 and 2016, had received previous juvenile releases that demonstrated high survival and had existing adult populations of abalone present. Overall survival for cohorts B2 and C1 at these sites was correspondingly high (4.5%); thus, data from all 6 sites are included. Survival of the older cohort (B2) was 4.6% in the field, but when considering the hatchery survival of 54.1% after the B1 cohort was released, the survival from 9 to 32 months was 2.5%. Survival of the younger group (C1) on these sites was very similar (4.5%). We were unable to collect follow-up data on survival for this cohort at 32 months, but based on 2019 and substantial past data on survival after a second year in the wild, we estimate the 9- to 32-month survival to be 3.6%.

In 2021, cohorts C2 and D1 were released on 10 sites, 7 established and 3 new. Overall survival on 6 of the sites was higher (4.8%), and these data were included in the comparison between cohorts, but survival on 4 sites (0.7%) was not. The older cohort survived well in the field (8.3%), with an overall survival of 4.5% for ages 9–32 months after considering hatchery survival of 53.8%, once the C1s were released. The younger cohort D1 survived at 3.7% through the first year in the field for an estimated survival of 3.0% between 9 and 32 months.

TABLE 3 | Comparison of survival between two release treatments.

	Released at 9 months			Released at 20 months		
Spawn year	**Cohort**	**Field survival to 20 months**	**Field survival to 32 months**	**Cohort**	**Hatchery survival to 20 months**	**Hatchery + field survival to 32 months**
2017	[a]	—	—	A2	82.2%	3.9%
2018	B1	0.8%	0.6%	B2	54.1%	2.5%
2019	C1	4.5%	~3.6%	C2	53.8%	4.5%
2020	D1	3.7%	~3.0%	D2	19.5%	0.6%
2021	E1	7.6%	~6.1%	[b]		

Note: Survival is the number re-sighted in 20- and 32-month surveys as a percentage of all individuals alive at 9-month average age. Number 2 cohorts, A2–D2, survival at 20 months is hatchery survival prior to release. Each pair (A2 + B1, B2 + C1, C2 + D1 and D2 + E1) was released to a different set of sites. Only data from sites with overall survival > 2% are shown to reduce the impact of site choice each year. Values marked with a '~' are estimated, based on an 80% survival in the second year in the wild observed in the B1 cohort and several years of prior data (Carson et al. 2019).

[a] The first cohort (A) was only released as 2-year-olds (A2).

[b] The last cohort (E) had only been released as 1-year-olds (E1) at the time of manuscript preparation.

Lastly, in 2022, cohorts D2 and E1 were released on 8 sites: 2 existing and 6 new. Overall survival at 6 of the sites (4.6%) was high enough for inclusion, but data from 2 sites (0.6% survival) were dropped. The D2 cohort survived poorly in both the hatchery (19.5%) and field (2.9%) for a 9- to 32-month survival of 0.6%. In contrast, the E1 cohort survived well to 20 months (7.6%) for an estimated 9- to 32-month survival of 6.1%.

Shell length in mm was measured at the hatchery prior to release and in the surviving individuals found in diver surveys. Each set of families was split into release treatments on the basis of size, with larger individuals being released in their first year. In the B cohorts, animals averaging 9 mm shell length were released first (B1), growing to 23 mm on average in the field at 20 months, and an average of 58 mm at 32 months. The B2 cohort averaged 5 mm at 9 months, 11 mm at 20 months when they were released and 30 mm a year later at 32 months. The C and D cohorts followed the same pattern (Table 4), with the size of those larger individuals released earlier resulting in larger size in later field surveys.

4 | Discussion

4.1 | Pilot Experiment to Measure Survival of Abalone Released at Younger Ages

The juveniles released into YAMs at 9-month average age survived at the same rate, or better, than those released at 14-month average age (Table 2). Furthermore, the younger abalone released in the wild had faster growth rates initially through 27 months. It is curious that the difference in growth did not manifest during the period where the two treatments were in different environments (hatchery vs. field). Instead, it was detected in March of 2018 after both treatments

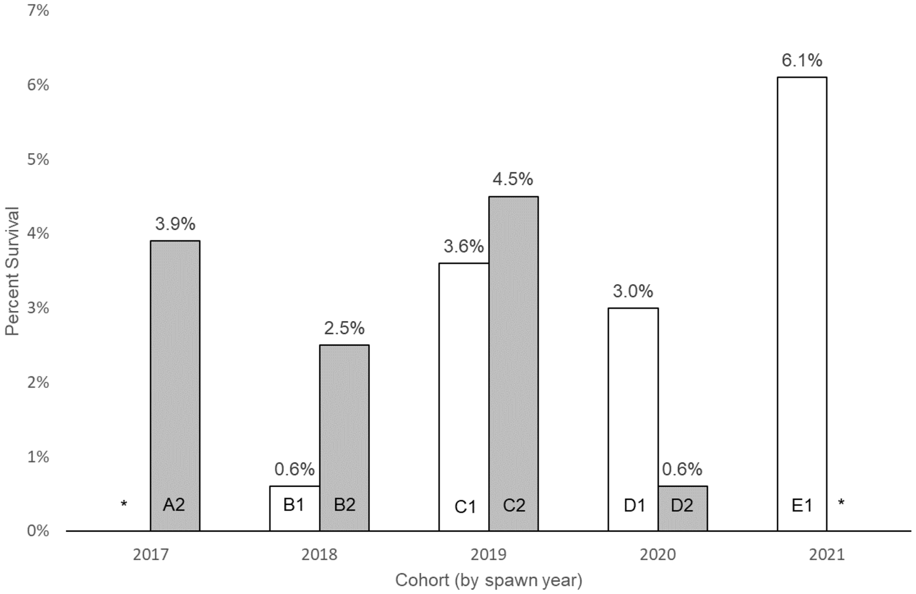

FIGURE 4 | Comparison of measured or estimated survival rates in the hatchery and field from 9- to 32-month average age between juveniles released at 9 months (white bars) and 20 months (grey bars). Details about each cohort (e.g., A2) are found in Table 1. Juveniles produced in the 2017 spawn were only released at 20 months old due to mixed-age releases beginning in 2019 (thus all abalone produced in 2017 were 20 months old at first release). Juveniles produced in 2021 were released at 9 months old in 2022, and the juveniles from this cohort held over and released at 20 months old were outside of the timeframe of this study.

TABLE 4 | Comparison of maximum shell length (SL) between two release treatments.

	Released at 9 months				Released at 20 months			
Spawn year	**Cohort**	**9-month SL (mm)**	**20-month SL (mm)**	**32-month SL (mm)**	**Cohort**	**9-month SL (mm)**	**20-month SL (mm)**	**32-month SL (mm)**
2017					A2	8	19	43
2018	B1	9	23	58	B2	5	11	30
2019	C1	15	27	[a]	C2	8	19	40
2020	D1	10	20	[b]	D2	5	14	30
2021	E1	11	26	[b]				

Note: At 9 months, each cohort (e.g., B) was split into larger individuals that could be safely transferred/released (e.g., B1) and smaller individuals left in the hatchery for release next year (e.g., B2). Second-year release growth data at 20 months are just prior to release.

[a] Not all sites received a second-year survey to measure growth in the 9-month release cohorts.

[b] Cohort E is still in progress as of 2023.

overwintered in the wild. It is possible that the acclimatization to the natural environment over the summer, when algal food is most abundant, allowed the 9-month-old releases to feed better over the winter and promote growth. If so, the timing of introduction right after the algal growing season (e.g., September) is less favourable. Growth aside, there was no evidence that holding animals the extra 5 months in the hatchery had any benefit to survival and in fact may have been detrimental to survival (Table 2). This is before considering the additional resources used to maintain those animals in the hatchery for an extra 5 months.

When comparing the survival of 9-month-old releases to that of the previous cohorts released at 20 months, it must be acknowledged that previous releases were not deployed into YAMs, but instead larger release tubes were nested into cobble on the seafloor without additional structure (Carson et al. 2019). It is possible that the mesh of the YAMs provided protection from larger predators or provided some other benefit; however, the abalone that stayed inside the YAMS had less access to macroalgae due to the mesh covering. The lack of macroalgae inside the YAMS may explain the higher growth rate of abalone that ventured outside the YAMS. Methodological differences aside, the 4% survival from release to 32 months was the same for the 9-month age treatment and previous 20-month age releases. Carson et al. (2019) report a 10% average survival for this period, but those data were derived from individually marked juveniles tracked over many years. Individuals encountered in subsequent surveys must have been alive but not detected during the first survey and were therefore included in the estimate of first-year survival. When those results are re-evaluated to only include individuals sighted in the first survey, survival adjusts to an average of 4%.

Detection rates of juvenile abalone in any given survey of an 8 × 10 m release site are low and estimated to be between 20% and 40% (Carson et al. 2019). It is possible that detection in the YAM pilot experiments here is significantly higher, given the smaller scale and module design. If so, the actual survival of 20-month releases may be significantly higher than those released at 9 months.

In the end, the pilot experiment suggested that further comparisons between 9-month and 20-month release treatments were warranted. Even if there is a survival cost to releasing earlier, the magnitude of that cost should be weighed against the additional resources used to rear longer. The idea of a “middle ground” 14-month release, which would always be in the autumn given the timing of spawning, was discarded based on the lack of a survival benefit and possible cost to growth in the pilot experiment.

4.2 | Mixed-Age Releases

When an entire year class is retained over 20 months in the hatchery, it restricts the tank space available to rear a new cohort the summer after the first-year class was spawned. On the other hand, variable growth rates of juvenile abalone at times can prevent an entire year class from being released (at 9 months) prior to the next year's spawn. The smallest individuals, those at 5 mm shell length and smaller, are vulnerable to injury during loading and transport to release sites. Therefore, when a portion of the year class, made up of average to above-average sized individuals, has been released in April, the remaining individuals can be consolidated into fewer tanks prior to setting the larvae produced from summer spawning activities. Mixed-age releases of 9-month and 20-month average ages allow for maximizing the overall hatchery output.

Additionally, during years in which hatchery production of abalone are high, juvenile density within rearing tanks can affect overall growth within each tank. It has been observed within the hatchery that tanks containing high densities of juveniles result in decreased growth rates due to competition for food and space within the tank (the exact density threshold per tank changes based on many variables, such as aquaria circulation systems, cultured biofilm availability, macroalgae availability and early larval survival rates). During these high production years when tank space is limited, it is arguably beneficial for both the abalone and the hatchery facility to release these animals rather than keeping them in a hatchery environment where their growth is likely to be stunted. The faster growth rates observed within cohorts released at 9 months of age support that these juveniles, though faced with the natural stressors found in the wild, will likely reach reproductive age quicker in the wild than they would in the hatchery, thus greater supporting the programme's goal of boosting reproductive aggregations within Washington State.

Each year, a new set of families is produced from wild parent crosses, and those parents can then be returned to the wild. Dimond et al. (2022) stress the importance of increasing the number of broodstock to maintain genetic diversity in the restored populations. Mixed-age releases allow for the easy integration of two sets of families to be placed on the same restoration site simultaneously.

Releasing juvenile abalone as early as logistically possible, in this case 9 months for the majority of each cohort, may alleviate concerns of domestication selection and hatchery acclimatization. There is some evidence that hatchery-reared pinto abalone do not respond appropriately to avoid wild predators. Hansen and Gosselin (2016) found that pinto abalone reared in the hatchery longer (4 years) had a higher deficit of anti-predator behaviour compared to those that had been in the hatchery only 1 year. However, a more recent study using a different species, *Haliotis tuberculata*, did not find evidence of hatchery acclimatization (Chauvaud, Day, and Roussel 2021).

It is difficult to truly assess the survival of mixed-age releases compared to what would have been the result had the whole year class been released at one time. When comparing the survival of each young cohort (e.g., B1) to its corresponding family members released later (e.g., B2), it is important to remember that the individuals were not assigned randomly to release treatment. The smallest individuals at 9 months comprise the older cohort the next year. The traits that led to slower hatchery growth may influence the subsequent survival in the wild. Furthermore, the two treatments were placed on different sets of sites and in different release years. We restrict our analysis to only sites where survival was favourable for all abalone (> 2% annual) to remove some of the impact of site choice on the two portions of the same year class, but it is not eliminated altogether. The effect of release year and site can be controlled by comparing the survival of a young cohort (e.g., B1) to individuals of the older cohort (e.g., A2) placed simultaneously on those same sites. Confounding that comparison is the fact that the two cohorts comprised different sets of families.

The results from our field trials do not definitively answer the question of whether it is always advantageous to wait an additional year to release individuals. The B families released in 2019 and again in 2020 would have likely had better results released only as second years, given poor survival in the first release. The C families released in 2020 and 2021 had similar survival outcomes in both years, suggesting that releasing a portion earlier was more efficient considering the cost savings. The D families had average survival for those released in 2021, but poor survival for those left in the hatchery, and poorer still when those survivors were released in 2022. First-year release was clearly the most efficient path for that cohort. The portion of the E cohort released in 2022 as 9-month-olds had excellent survival; it will be interesting to assess the survival of this cohort released as 20-month-olds in 2023 in future survey work.

In all cohorts, animals grew faster between 9 and 20 months of age in the wild than they did in the hatchery. Interpretation of this result is confounded by the fact that the fastest growing (i.e., largest) individuals in the hatchery were selected to be released first. On the other hand, when individuals in the pilot experiment were randomly assigned to treatment, growth in the wild was also faster. Also, growth per day in previous releases was slightly higher after release than in the hatchery (Carson et al. 2019). At the least, there does not appear to be a cost to growth when releasing individuals earlier. In fact, the fast growth rate of juveniles in the wild compared to juveniles kept at the hatchery better supports the restoration goals of adding aggregations to the wild population that are at or will quickly reach reproductive size.

The concept of mixed-age releases may serve as a bet hedge to the many factors that influence hatchery-reared abalone survival in the wild. Although in one of three trials it was likely less efficient to release part of a cohort the first year, we conclude that, on balance, mixed-age releases are the best way forward for this programme. The two other trials demonstrated the lack of a survival cost (and in one, even a survival benefit) to realizing the time, money and resource savings by releasing suitably sized animals as soon as practicable. Our results here concur with those of Roberts et al. (2007) who found that 10 mm shell length may be the most cost-efficient size of release for *H. iris*. The 9-month-old *H. kamtschatkana* in our study averaged 9–15 mm depending on the cohort (Table 1).

These savings of an earlier release can be evaluated by estimating cost-per-abalone released. With a set annual hatchery budget that includes the same amount of available culture tank space and staffing regardless of what age cohort is being reared, a certain production number of either a younger or older cohort can be targeted. The cost-per-abalone released within the first year, based on this hatchery budget and target production, is estimated at US$15 per abalone as of 2023. But if all juveniles are reared for a second year, approximately half the available tanks would be required for settlement and culture of a new cohort (so that there are juveniles in production for a subsequent year). This, in combination with hatchery mortality for abalone that are held longer, reduces the capacity to rear the older cohort by 60%, and the cost-per-abalone released for the older cohort is US$37.50. When combined with possible benefits to growth, reduction in hatchery acclimatization and/or selection, better rotation of hatchery resources and culture tank space and combination of 2 years' genetic crosses into one release, mixed-age releases are likely most efficient. This strategy will hopefully aid in achieving the goal of self-sustaining pinto abalone populations in Washington State. We recommend that researchers in the conservation of aquatic species consider the concept of releasing captive-bred individuals at multiple ages to balance logistics and efficiency with achieving goals of increased survival and genetic diversity of restored populations.

Author Contributions

Study design: All authors. Hatchery lead: Joshua V. Bouma. Hatchery work: Joshua V. Bouma and Caitlin S. O'Brien. Field lead: Kathleen A. Sowul. Field work: All authors. Data analysis: Kathleen A. Sowul, Joshua V. Bouma, Henry S. Carson and Caitlin S. O'Brien. Manuscript drafting: Kathleen A. Sowul and Henry S. Carson. Review of manuscript: Joshua V. Bouma, Taylor N. Frierson, Emily L. Loose, Caitlin S. O'Brien and Bethany C. Stevick.

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Ethics Statement

No permits were required for this research. The authors are employees and affiliates of the permitting agency for research with species listed as endangered in the State of Washington.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

Data from this research are available at 10.6084/m9.figshare.28069589. The file includes site-specific data for each of the hatchery releases described in the manuscript, as well as counts and shell lengths in the pilot and main experiments used to calculate the reported growth and survival. Note that geographic locations for any sites are not included given the sensitivity of the locations of this endangered species.

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