Interim Report

A. Grant # NA3NM4270112
B. Federal: $274,418 Match: $80,776 Total: $355,194
C. Project Title: **Restoration and Aquaculture of the Northern Abalone, *Haliotis kamtschatkana*, in Washington State**
D. Grantee: Drs. Carolyn S. Friedman and Kerry A. Naish (UW), Don Rothaus (WDFW), Jonathan Davis (Taylor Shellfish) and Betsy Peabody (Puget Sound Restoration Fund).
E. Award Period: September 1, 2003 through February 29, 2005.
F. Period covered by this report: September 1, 2005 – February 28, 2006
G. Summary of Progress and Expenditures to Date:

Work Accomplishments:

Objectives **-** In a threatened species, initial efforts to rehabilitate a species should be focused on obtaining the necessary information for a species recovery plan. This plan should examine the need and potential for rehabilitation first. If intervention is deemed necessary, supplementation should only proceed following research into the prospective impact of such a program on the wild target populations (Waples 1991). Our intention in this study is to obtain the baseline information necessary to make informed decisions on whether a supplementation program in pinto abalone should proceed. This information includes derivation of the population demographics around the SJI (diver surveys and genetic analyses), examining and testing culture methods that maximize both the genetic variation and the survivability of outplanted individuals, and soliciting the opinions of stakeholders through a workshop. In addition, as interest in marine species as food items grows, the development of new aquaculture species is needed. Given recent experiences with exotic species, culture of local species is desired. Hence, we will begin evaluation of the pinto abalone for commercial aquaculture. Specifically, our objectives for this proposed investigation were to:

1. Quantify adult and juvenile northern abalone population densities and characterize abalone habitat at 8-12 sites in the San Juan Island archipelago (SJIA, within and outside marine protected areas) and five other sites where abalone were once abundant. Sites for future experimentation will also be selected.
2. Initiate genetic analysis of abalone stock structure for use in species management and enhancement efforts.
3. Develop captive breeding and rearing protocols for pinto abalone.
4. Develop pilot scale pinto abalone culture methods for future commercial use.
5. Initiate quantification of behavioral differences of juvenile abalone reared in 'natural habitats' (with higher flows, live rock, red sea urchins and small fish) versus those reared using conventional methods.
6. Convene a workshop to engage public involvement in abalone restoration.

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Longer-term goals are to assess enhancement methods (adult aggregation versus outplants of various life stages)

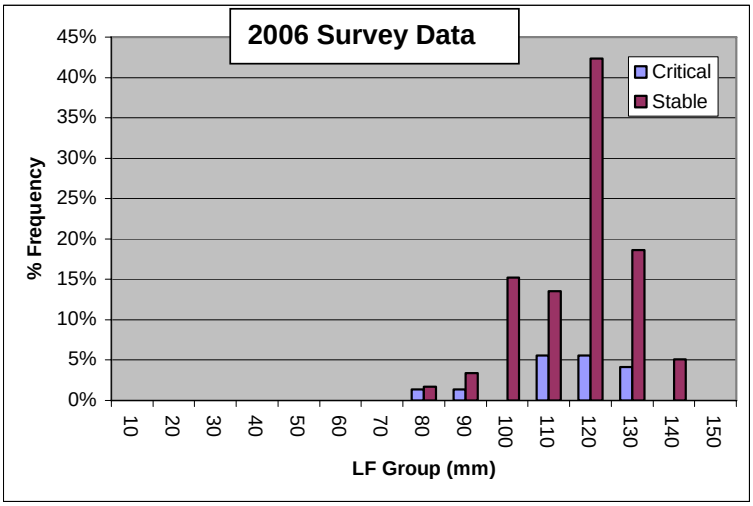
within and outside marine protected areas for abalone restoration, if deemed appropriate based on these studies.

Tasks accomplished

Objective 1: Quantify adult and juvenile northern abalone population densities and characterize abalone habitat at 8-12 sites in the San Juan Island archipelago (SJIA, within and outside marine protected areas) and five other sites where abalone were once abundant. Sites for future experimentation will also be selected.

Declining populations of pinto abalone (*Haliotis kamtschatkana*) have been monitored by the Washington Department of Fish and Wildlife (WDFW) in the San Juan Archipelago since 1992. WDFW divers had periodically tested for differences in density and size at ten index stations (See figure 1 to the left; In 1992, 1994, and1996) that range in size from 135 m^2 to 378 m^2 (averaging 230 m^2). Based on initial population trends (1992-1994), WDFW closed all pinto abalone take in the state. In order to effectively manage this species, understanding population trends is needed. All 10 sites were revisited in February 2006.

An examination of length frequency of resident abalone was quantified over time, we observed a significant increase in mean abalone size between 1996 and 2003 ($p<0.0001$) indicating that new recruits were not entering our measured populations. The solid arrows signify the mean size in 1992 and the hatched arrow illustrates a right shift in mean size in 2003. These trends have continued into 2006 (See figure below).



Objective 2: To initiate genetic analysis of abalone stock structure for use in species management and enhancement efforts.

The status of a stock should be determined a priory – and this information can be gained by combining diver surveys with genetic data. The latter will provide demographic information on the likely stock structure and effects of fragmentation on that stock. This information will be shared with the decision-making bodies in order to determine whether an enhancement program should be implemented. Genetic markers have been successfully used in the past to detect population structure in abalone (reviewed by Withler 2000); generally, differentiation between abalone populations is typical for marine organisms and is low, but population differences are significant (e.g. Brown 1991, Brown & Murray 1992, Hamm & Burton 2000, Huang et al. 2000). Structure in pinto abalone is probably characteristic of the genus. Spawning season may affect dispersal and, hence, genetic relationships and sizes of meta-populations (Tegner 1993). Red abalone spawn throughout the year and little genetic differences have been documented between geographically distant populations (Burton and Tegner 2000), while black abalone which have a limited spawning season show significant differences in allelic frequency between populations in close proximity to one another (Hamm and Burton 2000). Although pinto abalone are reported to have mature gonads and spawn during the spring, like red abalone ripe gonads are seen throughout the year (Quayle 1971). Thus, *we predict that pinto abalone within the SJIA represent one large meta-population.* However, it is necessary to derive baseline data on stock structure to assist decisions on supplementation approaches.

One of the objectives of our investigation was to initiate genetic analysis of abalone stock structure for use in species management and enhancement efforts. Before we began conducting a baseline genetic survey on the wild populations before enhancement and on the hatchery broodstock used in our rearing experiments, we optimized the methods for utilizing the twelve loci described for northern abalone (Miller *et al.* 2001). Many of the loci had null alleles, and thus we chose 6 microsatellite loci for use in examination of genetic structure of pinto abalone in eight populations throughout their range (Washington State; Alaska, Sitka; Alaska, Ketchikan; British Columbia and California).

Methods

DNA Extraction. DNA was extracted from all collected (see table below) abalone tentacles using the Qiagen DNeasy 96 Tissue Kit according to the manufacturer's protocol.

DNA Analysis. The seven loci were amplified individually by polymerase chain reaction (PCR) that employed fluorescent labeled primers. For primer sets *Hka*40, *Hka*43, *Hka*48, *Hka*56, and *Hka*65 PCR was conducted on a MJ thermal cycler (Waltham, MA) in 10 µl reactions containing 1 µl of undiluted DNA, 0.2 M of each primer. 0.4 mM dNTP's, 0.1 units of Promega *taq* DNA polymerase (Madison, WI), 2mM $MgCl_2$, and 1X PCR buffer containing 500mM KCl, 100mM Tris-HCl (pH 9.0 at 25°C) and 1% Triton® X-100. The reaction was similar for sets Hka80 and Hka85 except that we used 1.5 mM $MgCl_2$. PCR consisted of an initial denaturing step at 95°C for 5 min followed by 29 cycles of 95°C for 45 s, 54°C for 45 s, and 72°C for 45 s. For Hka56, the annealing step was carried out at 60°C for 45 s.

Multiplexed genotypes (pooled after PCR) were analyzed using a MegaBace capillary-based sequencer (Molecular Dynamics, Pharmacia). Sizes of the alleles at each of the loci were compared to an internal lane standard (Molecular Dynamics) using the MegaBace software, and genotypes for each individual at each locus were determined.

Results and discussion

We extended the range of a previous genetic survey in British Columbia, Canada, to populations found in Washington state (Table 1) where abundances are much lower than more northern pinto abalone populations. Washington state populations are likely the most vulnerable, because of decreased connectivity with the more abundant northern populations, and may require separate conservation measures. Six microsatellite loci were used to survey southern populations from California, Washington and northern populations in Alaska. Pairwise F_{ST} values were small (0.000-0.007), and populations from the interior coasts (Washington and Ketchikan) were significantly different from those on the outer coast (British Columba and Sitka in Alaska, Table 2). Population assignment tests were conducted on all data in order to test for structure in smaller samples from Washington and California. While the test did not differentiate Ketchikan or a second species *H. wallalensis* (flat abalone), a group of divergent individuals, aggregated around South Long Island, located at the confluence of the Straits of Georgia and Juan de Fuca (northern Puget Sound), were detected (See Figure below). These individuals were morphologically indistinguishable from pinto abalone, and also occurred at lower frequency in samples throughout the range. Removal of these "outliers" reduced any genetic differentiation between the Washington and the outer coast samples. The likely identity for the second form is unknown, but it may be a sympatric reproductively isolated population or a known or undescribed species. Further clarification of the taxonomy of this newly identified form is required in order to derive a full census of pinto abalone for conservation purposes, particularly in Washington state where population recruitment is currently very low.

Table 1: Description of locations, abbreviations and sample sizes for *Haliotis kamtschatkana kamtschatkana* (Pinto abalone) and *H.wallalensis* (Flat abalone) used in the population genetic analyses

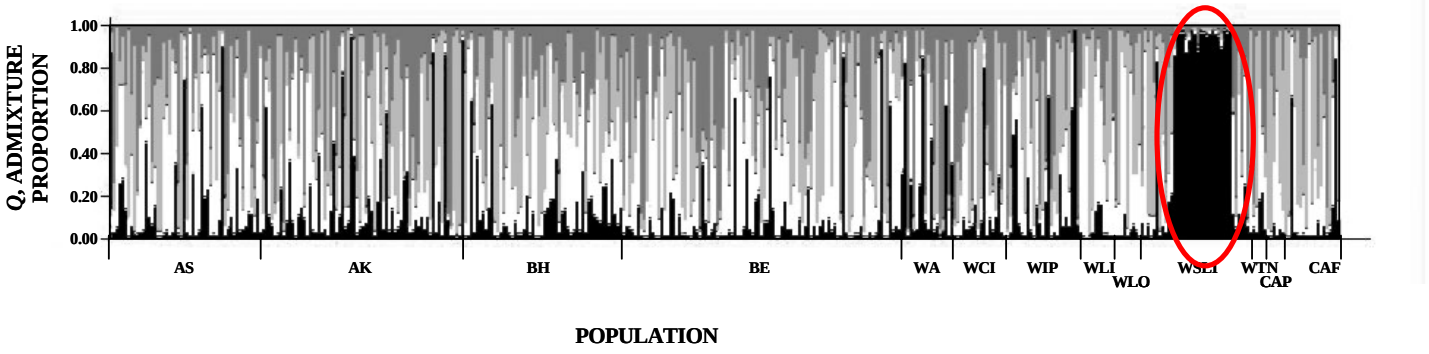
Region	Population designator	*N*	Location	Coordinates
Alaska	AS	51	Sitka sound	57 02.849 N 135 22.663 W 57 05.233 N 135 27.130 W
	AK	70	Ketchikan	55 21.840 N 131 52.296 W
British Columbia	BH	54	Hankin Point	53 42.400 N 130 24.610 W
	BE	95	Elbow Island	48 54.060 N 125 16.556 W
Washington	WA	17	Hatchery Broodstock	Exact location unknown
	WCI	18	South Cactus Island	48 38.903 N 123 07.895 W
	WIP	25	Iceberg Point	48 25.132 N 122 53.421 W
	WLI	12	North Long Island	48 26.489 N 122 54.982 W
	WLO	9	Lawerance Point	48 32.034 N 122 58.153 W
	WSLI	39	South East Long Island	48 26.344 N 122 55.108 W
	WTN	5	Turn Island	48 32.034 N 122 58.153 W
California	CAP	5	California Pinto	Exact location unknown
	CAF	20	California Flat	Exact location unknown

Table 2. F_{ST} value (below diagonal) and probability values for pairwise genotypic tests (above diagonal) for five populations of *H.k.kamtschatkana* along the West Coast of North America. Samples are named in Table 1; WA_{all} = all samples collected from the San Juan Islands, WA_{adj} = adjusted population, outlier individuals removed. Significant values determined by tests with 1000 permutations are in bold, significant results removed by the sequential Bonferroni procedure are in bold italics.

	AS	AK	BH	BE	WA_{all}	WA_{adj}
AS		0.0689	0.4193	***0.0471***	**0.0011**	**0.0431**
AK	***0.0026***		**0.0002**	**0.0000**	**0.0000**	**0.0000**
BH	0.0021	**0.0069**		0.6912	**0.0106**	0.2877
BE	0.0034	**0.0072**	0.0000		**0.0000**	**0.0044**
WA_{all}	**0.0044**	**0.0052**	***0.0064***	**0.0058**		1.0000
WA_{adj}	***0.0022***	**0.0042**	0.0031	0.0019	0.0000	

Analyses have been prepared for publication and will include estimates of genotyping error, fine scale analysis of the populations around the San Juan Islands and examination of the relationship between population differentiation and geographic structure. See attached pdf of submitted manuscript to the Journal of Experimental Marine Biology and Ecology.

Figure. Population assignment of individual Pinto abalone *H.k.kamtschatkana* using STRUCTURE (Pritchard *et al*. 2001). Four possible contributing populations were detected, and are denoted by a greyscale ranging from white to black. Sample sites for individuals are denoted along the X axis, and defined in Table 1. Divergent individuals from south Long Island are circled in red..



Objective 3: Develop captive breeding and rearing protocols for northern abalone.

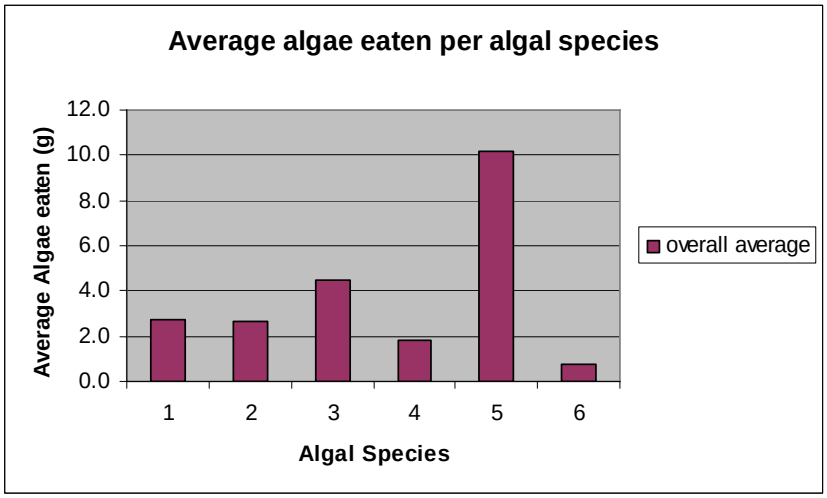
Brood stock abalone and progeny from the summer 2004 spawn were maintained during this period as during the previous 6 month period.

Objective 4: Develop pilot scale pinto abalone culture methods for future commercial use..

Captive abalone were maintained as in the previous 6 month report. No specific experiments were conducted as all efforts were directed towards producing ripe adult individuals – an effort that failed to yield any significant results. In this case, adult abalone were fed live macroalgae (mainly Nereocystis) and the temperature maintained at between 14-15 degrees C

Diet Studies

We conducted additional diet studies October 2005 using additional algal species and again identified *Nereocystis* as the favored species. Algae 5 (Nereo) is significantly different ($p = 0.0000$) from all other algal species (See below).



Algal species key
1. *Costaria costata*
2. *Cyamathere triplicata*
3. *Laminaria saccharina*
4. *Agarum fimbriatum*
5. *Nereocystis*
6. *Palmaria* mollis (Dulce)

Macroalgal culture. Dulse and/or other macroalgae WAS collected locally in the San Juan Islands, returned to the hatchery facility and vegitatively propagated in well lit (1000 watt metal halide growlamps), aerated tanks using methods similar to techniques utilized to rear *P. mollis* in co-culture with *H. rufescens* (Evans and Langdon 2000, Rosen et al. 2000, Buchal et al. 1996). Cultured *P. mollis* and Turkish towel (*Chondracanthus*) has been used to feed adult and juvenile abalone to supplement kelp collections during the maturation phase and juveniles in grow-out.

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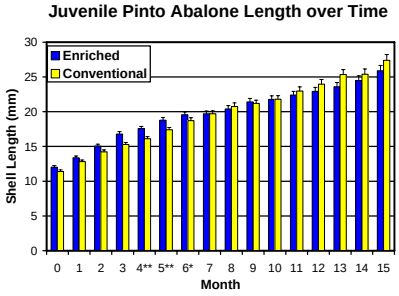
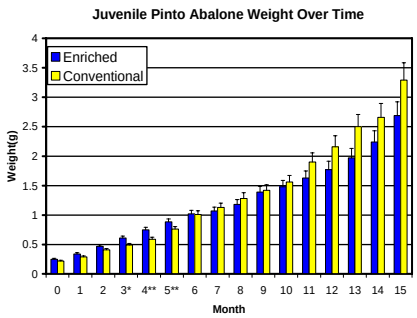
Objective 5: Initiate quantification of behavioral differences of juvenile abalone reared in 'natural habitats' (with higher flows, live rock, red sea urchins and small fish) versus those reared using conventional methods.

Juvenile Culture in Enriched and Conventional Habitats





Experiments to determine the best rearing methods for Pinto abalone destined for supplementation have been completed. Three habitat enriched tanks and three conventional tanks were set up in Spring of 2004. The enriched tanks contain coralline

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algae encrusted rocks, red and green sea urchins, and native macroalgaes, while the conventional tanks use corrugated fiberglass as a surface for benthic diatom growth. This experiment compared the survivorship, growth, and shell coloration of abalone cultured using conventional aquaculture methods to those raised in the enriched habitats. In June of 2004, 30 juvenile pinto abalone (average shell length 11.7mm) were introduced to each tank. Animals were examined, weighed, and measured every four weeks for the duration of the experiment. Abalone shell color changed dramatically during this time. When the animals were introduced to the tanks in June they had thin shells that were very silver in color. Shells were noticeably stronger within the first month, and coloration changed rapidly to a more normal color range of reds and greens. No differences in shell coloration or survivorship were seen between the two treatments. During the first five months of the study (June-October), animals in the enriched tanks grew significantly faster than animals in the conventional tanks ($P<0.05$). However, after month five, these differences disappear ($P>0.05$). Reasons for these differences are unknown, however, it is noteworthy that algal growth in the enriched tanks was much better than in the conventional tanks during the first five months of the study. After October, algal growth was better overall in the conventional tanks. These differences may be due to the presence of sea urchins that graze on benthic algae. Under summer light and temperature conditions, the added nutrients provided by the sea urchins may encourage algal growth while the urchins may keep the algal growth cropped during the winter months. This experiment was continued beyond the planned six month duration due to the unexpected nature of early results. However after fifteen months no further statistically significant differences in length or weight were observed between the treatments. Abalone growth is following a normal pattern as evidenced by the weight to length relationship graphed below.

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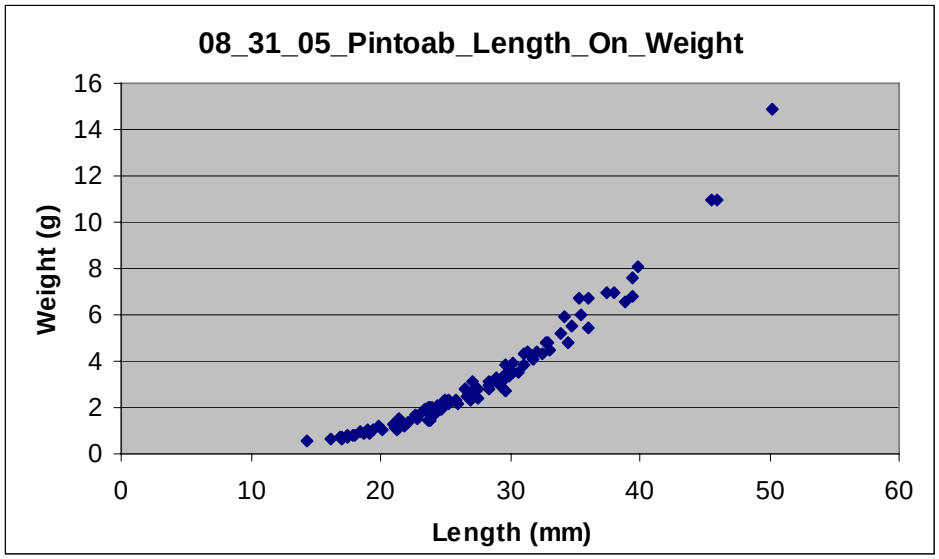

Experiments to assess habitat selection and predator avoidance in animals raised under the two treatment regimes have been completed. In these experiments, a single abalone was placed in an aquarium and monitored with a video camera for eight hours. The abalone was placed in an exposed area on the floor of the aquarium, however, there are coralline algae encrusted rocks available in one corner of the tank. Experiments were done with 96 animals, 48 from each rearing method. Additionally, half of all animals in each experiment When watching video footage, the researcher records the amount of time taken for the abalone to reach the cryptic habitat. Additionally, each time the abalone leaves or enters the cryptic habitat is recorded, enabling researchers to determine number of minutes spent in exposed versus cryptic habitat. Video data is currently being analyzed using compositional data analysis techniques. Our first hypothesis is that juvenile abalone raised in habitat enriched tanks will both find the cryptic habitat more quickly and spend more time there than do animals raised in conventional tanks. Our second hypothesis is that juvenile abalone in the presence of predators will again find the cryptic habitat more quickly and spend a greater proportion of their time there. These experiments comparing juvenile culture in enriched and conventional habitats took place at the Mukilteo Field Station, a NOAA facility run by Paul Plesha.

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Objective 6: Convene a workshop to engage public involvement in abalone restoration.

No new effort was expended during this period.

C. Expenditures: These figures have been provided directly by UW Grants and Contracts department.

D. Deviations from our original design: We have added an examination of pinto abalone phylogeography (Genetics section above) to our genetic assessments to provide a more thorough examination of pinto abalone population structure. In addition, due to the changing pattern of growth in the natural versus conventional culture system study, we are continuing beyond the initial 6 mo duration originally planned. We also added examination of juvenile recruitment modules at selected Index stations (below). Finally, we examined postlarval survival under varying environmental conditions (below).

Pinto Abalone Juvenile Recruitment Field Study

During this collaborative and interdisciplinary project, innovative abalone recruitment modules (ARMs) are being surveyed *in situ* by research divers to gather novel data on juvenile recruitment and abundance at several geographical locations in the San Juan Islands. ARMs have been shown to successfully attract juvenile abalone less than 50mm shell length in the wild and have been experimented with in pinto abalone studies in Canada (Defreitas 2003) and a variety of abalone species in California (Rogers-Bennett et al. 2004, Davis 1995). Their use is being perfected during this ongoing project. The study is assessing correlations between adult and juvenile densities, juvenile depth and habitat preferences, and patterns in recruitment success.

The ARMs are constructed from modified commercial crab pots: internal structures are

removed and the crab pots are then filled with six quartered cinder blocks. This concrete material is colonized by benthic flora and fauna, most importantly crustose coralline algae, and provides a known and consistent amount of artificial habitat. The modules are designed to act as surrogate habitat that will allow collection of juvenile recruitment data without disturbing natural habitat.

In total, 60 ARMs were deployed in August & September of 2004: 20 modules at each of the three survey sites: Long Island; located south of Lopez Island, Parker Reef; located north of Orcas Island, and Big Cactus Island; located north of San Juan Island. These sites are adjacent to three of the WDFW adult abalone index sites, have all historically shown significant abalone populations and were chosen to represent three different geographic regions within the San Juan Islands. Two different depth strata are being surveyed at each site as half of the ARMs at each site are located at a depth of 3-4 m and half are located at a depth of 6-7 m (corrected to Mean Lower Low Water). A team of UW certified research divers is surveying all ARMs at each site *in situ* three times annually (February, June, and October) from 2005-2006. The ARMs are opened and each concrete block removed by hand and closely inspected for the presence of juvenile abalone. Calipers are used to measure abalone shell lengths for those animals found within the modules. Size, density, and habitat preference is recorded. All concrete pieces are then carefully resituated in the ARM. We added 6 ARMs to the long Island site as one juvenile abalone was found east of our current ARMs. All ARMs were surveyed in October 2005 and a single juvenile abalone at Long Island was observed further illustrating the decline of Washington state pinto abalone. ***These data highlight the need for outplanting of captive bred animals for successful restoration of this species***.

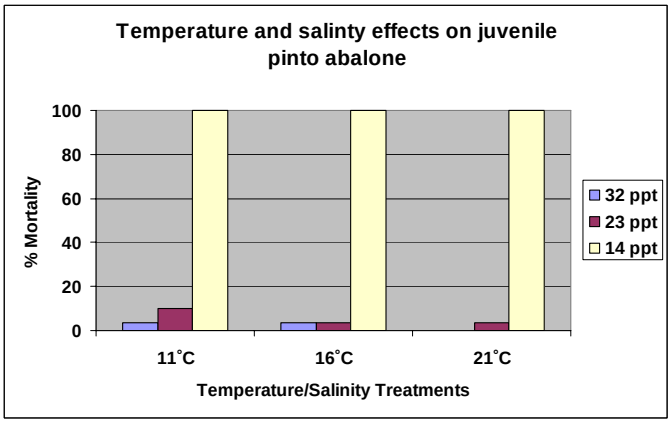
Effects of salinity and temperature on the survival of post-larval pinto abalone

Because it is possible that newly settled abalone are subject to temperature and salinity variations on seasonal and short-term scales in parts of the San Juans Islands that may influence larval settlement and post-larval survival, experiments will be conducted in the laboratory to test the tolerance and survival of juvenile pinto abalone in altered temperatures and salinities. Post-larval pinto abalone that were spawned in the Mukilteo lab in late June, 2005 will be utilized in these trials. The abalone range in size from 3-7mm and have been held in flow-through seawater for the past four months at a temperature of 14ºC and a salinity of 32 ppt.

This experiment used a 3x3 factorial design to challenge the juvenile abalone at a series of different temperatures and salinities. Temperatures were maintained at 13ºC, 17ºC, or 21ºC and salinities were maintained at 32ppt, 23ppt, or 14ppt, representing 9 treatments in total. These measurements were chosen to represent the range of conditions juvenile abalone will experience during the short-term and seasonal fluctuations in the San Juan Islands. Each treatment included five replicate groups of six animals each using a total of 270 juvenile abalone.

These trials were conducted at the Mukilteo lab facility where there is access to ambient and heated seawater. Three large but shallow glass tanks were set up as water baths, one containing a heater programmed to 21ºC, a second containing a heater programmed to 16ºC, and a third utilizing flow-through 11ºC seawater from the ambient

manifold available. The treatments were carried out in 1 liter plastic beakers so that each water bath held 15 beakers divided into three salinity categories with five replicates each. The 1 liter plastic beakers did not have any flow of seawater but each held a small airstone. The experiment lasted 14 days with each beaker being observed every two days for number of mortalities and percent survival. The percentage of survival in each of the nine treatments over the course of the study was analyzed using a two-factor ANOVA and compared to determine the tolerance levels of juvenile abalone to raised temperatures and lowered salinities. While temperature did not influence survival, depressed salinity had a significant effect ($p<0.05$; See figure below).



As results from the first experiment showed no significant temperature effects on juvenile abalone survival, a second experiment was conducted to address salinity effects only. Similar methods were followed with the exception that in this experiment all salinity treatment groups were maintained in one water bath of a constant ambient summer-time temperature of 14ºC. Juvenile abalone were challenged with a finer range of salinities with the goal of identifying a low salinity threshold or tolerance level. Salinities tested included 14, 17, 20, 23, 26, 29 and 32 ppt. Each treatment group included five replicates of six animals each, with a total of 210 animals being used in this experiment. The experiment will last 14 days. Results from this experiment were analyzed using a single-factor ANOVA. Significant losses were observed in abalone exposed to salinities less than 26 ppt ($p<0.05$) with the highest losses at salinities of 20-14 ppt (see figure below).