2018 WRAC FINAL REPORT

PART 1: SUMMARY

PROJECT TITLE: Triploids, tetraploids, and successful metamorphosis in purple hinge rock scallops (*Crassadoma gigantea*)
REPORT GIVEN IN YEAR 2018
PROJECT WORK PERIOD: 9/01/2015–8/31/2018

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REASON FOR TERMINATION:

4 -year project and funds exhausted.

PROJECT OBJECTIVES:

1. (low risk) Produce sufficient chemical 3Ns to enable a) growout trials at 4 sites, and b) future tetraploid induction trials that require oocytes from mature triploid females

2. (high risk) Conduct 4N induction trials following the French patent method and the gynogenetic method, both of which require only mature diploid broodstock. Develop 4N containment.
3. (low risk) Run a series of settlement experiments to describe factors that influence successful settlement and metamorphosis to the juvenile feeding stage.
4. (low risk). Continue research on survival, growth and cementation behavior of rock scallops currently deployed at 8 sites in Puget Sound and one in California.
5. The primary outreach objective is the production of a WRAC publication describing production of triploids, once a reliable protocol has been established.

PRINCIPAL ACCOMPLISHMENTS:

Objective 1: Produce sufficient chemical 3Ns to enable a) growout trials at 4 sites, and b) future tetraploid induction trials that require oocytes from mature triploid females.

In 2016-2018 the working hypothesis was that the general failures to spawn were caused by poor conditioning in the hatchery, either due to insufficient time/degree-days to condition, or to a nutritional deficit. To address these potential issues, two conditioning protocols were used. One group (group 1) of 30 broodstock was brought from Dabob Bay into the hatchery in April for a 4-5-week conditioning period of continual feeding and gradual increase in temperature from ambient (~10 C) to ~14 C. Spawn attempts and/or visual inspection of gonadogenesis for Group 1 commenced in May and were ongoing through August 2018. Generally poor reproductive condition was noted throughout this time period.

The second group (Group 2) of 15 broodstock remained in Dabob Bay to 'naturally' condition on wild phytoplankton until August. Group 2 was brought into the hatchery 10-15 days prior to spawn attempts. None of the spawn attempts in 2017-2018 of either Group 1 or Group 2 were successful; visual inspections of broodstock gonads revealed gametogenesis generally failed to progress well in both groups. Progress on this objective was thus unfortunately impeded because the broodstock did not come into condition despite sustained efforts.

Despite considerable effort by hatchery staff at both the Taylor Hatchery and at Pacific Hybreed, Inc. to uncover the cause or causes of failed production, no clarity emerged. As of this writing, it remains unknown whether failures during the larval stage were due to inadequate conditioning protocols, poor gamete quality, disease, or water chemistry. Whether the poor conditioning in rock scallops was rooted in the same causes is unknown.

Putative triploid seed

One group of 2N and 3N rock scallops produced in 2015-16 was husbanded through to 2017, albeit in extremely low numbers. To conserve the very low numbers of putative 3Ns (n=19), tissue sampling for ploidy analysis was delayed. First, a trial

tissue sampling of 2016's 2Ns (n=10) revealed no mortality after 1 month. Second, shell shape was compared between the two groups under the working hypothesis that shell morphology might change as a result of triploidy. No difference between groups was detected in any of the shell metrics, ratios, or volume. Third, when putative 3Ns attained SL between 8 and 26 mm, ctenidia from putative 3Ns and three normal 2Ns were stained with DAPI and run on a Cyflow ploidy analyzer (Sysmex Partec) flow cytometer. Mean peak values of putative 3Ns were compared to known 2Ns. No differences in mean peak values were detected. Based on these results, all of the survivors in the 3N group were diploid, not triploid.

Maturation

Substantial progress was made in understanding the variation and temporal progression of maturation in rock scallops. Cultured rock scallops were sampled semi-monthly over 32 mo. to determine age and size at first maturity, and to elucidate the variation in maturation stage at monthly intervals. In addition, spatial variation in maturation was investigated. At 50% maturation in the group, shell height is 54.5 mm and age is 24.6 mo.

Objective 2: Conduct 4N induction trials following the French patent method and the gynogenetic method, both of which require only mature diploid broodstock. Develop 4N containment.

Because rock scallop broodstock failed to condition and spawn, all 4N induction trials were conducted using both chemicals and pressure using C. gigas as proxy.

Despite repeated trials, we were unable to reproduce the results reported for the French method. Similar failures to reproduce the results were reported by others (Stan Allen, pers. Comm.). Briefly, after chemical treatment, ongoing size selection for the smaller fraction did not result in any detectable 4Ns at any time point. The conclusion was that some critical aspect of the method was not sufficiently described in the French patent.

Crassostrea gigas were also proxy for attempts to alter ploidy by applying pressure (10,000 psi) to fertilized eggs to block one or both polar bodies. The pressure method successfully induced triploidy in *C. gigas* with low mortality to the D hinge stage.

All trials, however, were curtailed in May 2017 due to ongoing serious problems with larval mortality at the Taylor hatchery. The pressure technique is a promising method to try for rock scallop 4Ns once gametes are available.

Objective 3: Run a series of settlement experiments to describe factors that influence successful settlement and metamorphosis to the juvenile feeding stage.

Progress on Objective 3 was impeded due to the issues of broodstock conditioning (described above under Objective 1); no gametes were available for larval production hence no larvae were available for settlement trials.

Objective 4: Continue research on survival, growth and cementation behavior of rock scallops currently deployed at 8 sites in Puget Sound and one in California.

Studies of growth and survivorship and cementation behavior were initiated in July 2015 and continue to the present with the latest measurements on growth (size at age), survivorship and cementation behavior taken in August, 2017. The seven sites in Washington State were selected based on disparate geography and availability of commercial partners to assist in maintaining scallops on docks and floats. Replicate arrays of shellfish cages (4 bags, 55 scallops each) were suspended at a depth of 2-3 M (except at Dabob Bay where cages were suspended below the summer thermocline (5M). At approximately 100 day intervals, survival, growth, and cementation behavior data were taken. Growth and survivorship has been satisfactory to excellent at all sites and by late August 2017 (25 months following planting as seed), the mean size (SL) of scallops for all sites was 78.9mm. Survivorship was high at most sites as well at 73% after 25 months.
Most interestingly, at all sites under investigation the percentage of scallops cementing into the meshes of the shellfish cages remains low overall with cementation rates well below 1% for scallops measured between May and August, 2017. Scallops exhibited cementation behavior beginning at about 30mm SL and the majority of animals cease to actively cement after they attained a 50-55 mm SL.

Objective 5: The primary outreach objective is the production of a WRAC publication describing production of triploids, once a reliable protocol has been established.

This objective was changed to a focus on growout techniques due to continued problems with broodstock ripening in the final year of the research. The Project team met in June 2018 and discussed this objective with the decision to move forward with a dedicated report on growout techniques in Washington and California to follow.

IMPACTS:
Title: Triploids, tetraploids, and successful metamorphosis in purple hinge rock scallops (*Crassadoma gigantea*)
Relevance: In the last decade, it has become increasingly important to look to native species to expand and diversify shellfish aquaculture in U.S. Pacific coast estuaries. High value seafood products, such as purple hinge rock scallops, are strong contenders for adding to the suite of species reared by the shellfish aquaculture industry on the west coast. Among the critical information needs when developing a new species for aquaculture are evaluating performance (growth and survival characteristics at different life stages in a commercial setting), optimal husbandry techniques and the size and age at which sexual maturation occurs. The

last parameter relates mainly to the use of breeding technology (e.g. triploidy) that can minimize potential risks associated with wild stocks of scallops interbreeding with farmed stocks. A number of commercial shellfish growers in California, Washington, and Alaska expressed interest in filling these information gaps and in pursuing general culture techniques for the purple hinge rock scallop.

Response: Efforts were organized among commercial growers, the University of Washington, the University of California, and others to develop a research program focused on developing commercial aquaculture of the rock scallop. The Western Regional Aquaculture Center funded a two-year grant to pursue growout trials in different environments in Washington State, to shed light on when farmed stocks mature, and to further develop ways to produce sterile rock scallops to help safeguard wild stocks from interbreeding with farmed stocks.

Results: Purple hinge rock scallops exhibited differences in growth at different sites in Washington, indicating that production of a marketable rock scallop can occur in as little as 3 years in some locales. The project has yielded important data on rock scallop growout performance. First, survival was good across all sites. Second, growth was excellent at three sites. Third, contrary to earlier trials, the shift to different growout gear and regular, albeit infrequent, handling effectively prevented cementation of the vast majority of juvenile rock scallops; it thus appears possible to minimize the cementation behavior by a combination of gear and handling during a critical developmental stage/size. The resulting product is attractive and can be marketed as live product or shucked adductor muscles. The relative ease of production coupled with yield (growth + survivorship) of rock scallops in seven disparate locales within Washington illustrates the high potential profitability of rock scallop aquaculture.
In the field, maturation occurs in the second year, and the vast majority are male at that point. The unforeseen difficulties with conditioning rock scallops to reach maturity in the hatchery points to important avenues for future research, and resulted in hindering further work on producing sterile stocks and optimizing seed culture in the hatchery.
Impact: The net result of the research was that significantly more is known about the performance characteristics of rock scallops grown under commercial grow out conditions. Information on growth rate, survivorship and especially cementation behavior for seven Puget Sound locations is available to the shellfish industry for their consideration. Shellfish companies are now in a better position to make informed decisions about whether to initiate further commercial development of this scallop species. State and tribal wild resource management agencies have critical information on gametogenesis and sex ratios of rock scallops maintained in the field that may help inform the potential for interbreeding of farmed and wild stocks. Results of growout study demonstrate the potential for rock scallop aquaculture, once remaining challenges can be overcome. The potential for rock scallop aquaculture has been recognized by a number of Washington Tribes and commercial shellfish aquaculture companies.

Collaborators: Significant roles were played, initially by the Taylor Shellfish Co., and continued by Baywater Inc., the University of Washington, the University of California, and Pacific Hybreed, Inc.
Contact: Jonathan P. Davis, Pacific Hybreed, Inc. jothpdavis@gmail.com

RECOMMENDED FOLLOW-UP ACTIVITIES:

It was abundantly clear that the availability of ripe broodstock scallops significantly impeded meeting the objectives of this research. The most critical need is a dedicated effort to examine specific dietary, environmental or other endogenous conditions associated with triggering gametogenesis and gamete production under hatchery conditions for this species. The research group has recently received encouragement from NOAA to develop a proposal for the 2019 Saltonstall-Kennedy Program focused on this critical problem.

Attaining sterility in rock scallops should also be vigorously pursued utilizing approaches based on traditional approaches utilizing polyploids (e.g. mated triploids from tetraploid brood stocks) or longer term via "knockout" gene technologies.

Ongoing research is focusing on the uptake and retention kinetics of biotoxin in rock scallops associated with the dinoflagellate, *Alexandrium catenella*, to help clarify risk and harvest parameters associated with harmful algal blooms in Washington State. Toxin load in different tissues and especially detoxification rates are important to assess in order to determine whether a market for whole rock scallops can be developed. Otherwise, markets for adductor muscle only will need to be pursued though this will hamper efforts to market rock scallops as whole live animals differently than other commercially available products (e.g. wild Alaskan caught weathervane scallops and Atlantic sea scallops). Other follow-up research include objectives described above that were thwarted in the current study due to lack of availability of scallop gametes including efforts to optimize rock scallop post larval settlement and nursery culture.

Additional research is needed, however on optimizing cage density of scallops based on the work reported here. It is recommended that individual density be reduced to 10 individuals per square foot after the second year of grow out in order to ensure that marketable scallops (>100mm) can be acquired within a three-year time frame.

It is abundantly clear that purple hinge rock scallop aquaculture in Puget Sound is technically feasible and that the performance of rock scallops cultured under commercial conditions appears very promising, based on live weight, adductor muscle size and quality after nearly three years of growout in a variety of locations.

SUPPORT:

YEAR	WRAC	OTHER SUPPORT					Total Support
		University	Industry	Other Federal	Other	Total	
2016-17	$62492	$0	$10000	$0	$0	$10000	$72492
2017-18	$64316	$0	$1500	$0	$0	$1500	$65816

PUBLICATIONS, MANUSCRIPTS, OR PAPERS PRESENTED

Jackson, M., Wyckoff, S., Davis, J., Vadopalas, B., 2014, How to grow rock scallops, 67th PCSGA Annual Conference, Vancouver, WA, Sept 23-25.

Jackson, M., Wyckoff, S., Davis, J., Vadopalas, B., 2015, Development of commercial hatchery production techniques for rock scallop, 68th PCSGA Annual Conference, Hood River, OR, Sept 22-25.

Jackson, M., Davis J., Vadopalas Band L. Hauser. 2015, Investigating local adaptation in Washington State purple hinge rock scallops.69th Joint Annual Meeting of the National Shellfisheries Association Pacific Coast Section and the Pacific Coast Shellfish Growers Association, Hood River, Oregon, USA, September 22-24.

Lowell, N., Davis J. Vadopalas B.,and L. Hauser. 2015. Assessing population structure and local adaptation of rock scallops to inform aquaculture practice. 69th Joint Annual Meeting of the National Shellfisheries Association Pacific Coast Section and the Pacific Coast Shellfish Growers Association, Hood River, Oregon, USA, September 22-24.

Jackson, M. Davis J., and B. Vadopalas. 2015. Update on development of commercial hatchery production techniques for purple hinged rock scallops. 69th Joint Annual Meeting of the National Shellfisheries Association Pacific Coast Section and the Pacific Coast Shellfish Growers Association, Hood River, Oregon, USA, September 22-24.

Davis, J. 2015. Prospects for purple hinge rock scallop cultivation on the west coast—studies on aquaculture potential. 69th Joint Annual Meeting of the National Shellfisheries Association Pacific Coast Section and the Pacific Coast Shellfish Growers Association, Hood River, Oregon, USA, September 22-24.

Jonathan Davis 2016. Developments in Aquaculture for Purple Hinge Rock Scallop. 70th Annual Joint Meeting of the National Shellfisheries Association-Pacific Coast Section and the Pacific Coast Shellfish Growers Association. Chelan, WA October 12-14.

Jonathan Davis 2016. Interview for article discussing the potential for purple hinge rock scallop aquaculture. “Rock Scallop: Viable Candidate for Aquaculture”. *Aquaculture North America*, June 2016.

Jackson, M., Lowell, N., Vadopalas, B., Hauser, L., 2017, The reproductive cycle of cultured purple hinged rock scallop, *Crassadoma gigantea*, in Dabob Bay Washington, 69th PCSGA Annual Conference, Welches, OR, Sept 18-21

Jackson, M et al. mms in prep. Age and size at maturation in the purple hinge rock scallop.

PART II: DETAIL
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PROJECT OBJECTIVES:

1. (low risk) Produce sufficient chemical 3Ns to enable a) growout trials at 4 sites, and b) future tetraploid induction trials that require oocytes from mature triploid females
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TECHNICAL SUMMARY AND ANALYSES:

Objective 1: Produce sufficient chemical 3Ns to enable a) growout trials at 4 sites, and b) future tetraploid induction trials that require oocytes from mature triploid females.

In 2016-2017 the working hypothesis was that the general failures to spawn in 2015-2016 were caused by poor conditioning in the hatchery, either due to insufficient time/degree-days to condition, or to a nutritional deficit. To address these potential issues, two conditioning protocols were used. One group (Group 1) of 30 broodstock was brought from Dabob Bay into the hatchery in April for a 4-5-week conditioning period of continual feeding and gradual increase in temperature from ambient (~10 C) to ~14 C. Spawn attempts and/or visual inspection of gonadogenesis for Group 1 commenced in May and were ongoing through September 2017. Generally poor reproductive condition was noted throughout this time period.

The second group (Group 2) of 15 broodstock remained in Dabob Bay to 'naturally' condition on wild phytoplankton until August. Group 2 was brought into the hatchery 10-15 days prior to spawn attempts. None of the spawn attempts in 2017 of either Group 1 or Group 2 were successful; visual inspections of broodstock gonads revealed gametogenesis generally failed to progress well in both groups. Progress on this objective was thus unfortunately impeded because the broodstock did not come into condition despite sustained efforts.

With few exceptions, production was low among species reared at the Taylor hatchery during 2016-17. Despite considerable effort by hatchery staff to uncover the cause or causes of failed production, no clarity emerged. As of this writing, it remains unknown whether failures during the larval stage were due to poor gamete quality, disease, or water chemistry. Whether the poor conditioning in rock scallops was rooted in the same causes is unknown.

2017-18 Report on Broodstock Conditioning in Purple Hinge Rock Scallops

During the final year of WRAC (2017-2018) research on purple hinge rock scallops another concerted effort was made to condition scallops under laboratory/hatchery conditions to induce the onset of gametogenesis and maturation of gametes for spawning and subsequent earing of embryos.

Rock scallops (N=17) were brought into the Baywater SeaLab (now Pacific Hybreed SeaLab) on December 12, 2017 from Taylor Shellfish Farms. Scallops were introduced to a polyethylene fish tote with a dedicated air supply at ambient temperature (12 °C) and ambient salinity (29ppt). The flow into the tote was maintained at 60L per hour for the duration of the conditioning trial.

Beginning on December 13, scallops were fed a 50/50 mix of two live diatoms cultured in the PSRF Algae greenhouse. Seawater temperature was increased and maintained at approximately 14 °C for the duration of the conditioning period. A combination diet consisting the prymnesiophyte flagellate, CISO (*Tisochrysis lutea*, CCMP463) and a diatom (*Chaetoceros gracilis*) (e.g. CHAGRA) mixed at a ration of 50/50 by cell number were fed rock scallop broodstock at a total concentration of 50,000 cells per ml. Microalgae was added to a head tank and pumped into the scallop broodstock tote via peristaltic pump (Masterflex Corp.). In addition, the rock scallops were fed REED Mariculture Shellfish Diet, with daily additions to the head tank to complement the live algae component. Total cell density fed out to scallops continuously over a 24-hour period was approximately 100,000 cells per ml). This feeding regime was maintained over the ensuing 6 weeks with CISO and CHAGRA substituted at times with other algal strains reared in the PSRF algal facility.

Prior to initiating the conditioning trial scallops were evaluated initially for gametogenic activity. Briefly, scallops were induced to open their valves by inserting a flexible filament (brush or broom straw) through the byssal notch (if present) and "tickle" the adductor muscle. This action usually induces the animal to valve "clap" in an effort to remove the irritant. The clapping, however also enables an observer to directly evaluate the sex and relative condition of the gonad. Prior to initiating conditioning in this group of scallops, a number (N=6) were evaluated for gametogenic development with no significant activity noted. It was assumed then that scallops were not ripe and potentially available for spawning.

After approximately six weeks in the Sealab with scallops maintained daily as described above, scallops were again evaluated for gametogenic activity. On examination, no significant increase in gonadal mass for either male or female scallops was noted. Following this examination this group of scallops was placed into the wild in suspension culture for further holding on the Manchester dock.

A second group of rock scallops (see photo above) was brought into the facility in mid-March for a second conditioning trial. Following the same procedures

(temperature, salinity and seawater supplied to the tanks) as described above, thirty rock scallops were brought into the Pacific Hybreed Sealab. Prior to conditioning trials, a number of scallops were examined for gonadal presence. Scallops were easily sexed at this time but significant gonadal development was not observed. In hopes of developing more substantive gametes, scallops were set up as described above and fed a combination of live algae and REED Mariculture Spat Diet. Scallops were maintained for 10 weeks, with the daily ration fed out to the scallops as described above. Gonadal development was examined in mid May, late June and mid July as described above with little or no increase in gonadal mass noted. With the exception of 2 individuals (females) that were moderately ripe of 22 individuals examined in mid August (2018), the other scallops (20 examined) exhibited reduced gonadal mass. At that time, remaining scallops were returned to the wild to be maintained on the Manchester dock.

Conclusions: After two dedicated conditioning trials utilizing 1. older scallops coming from a wild population and 2. younger scallops (3 year+ age that had been reared in the hatchery), using similar approaches, our research group was unable to induce significant gonadal development in either group of scallops. Scallops appear to be feeding well with copious amounts of biodeposits produced daily in both groups. Mortality of scallops was low with less than 5% loss over the conditioning period for either group. Coincidently, the Baywater SeaLab was also conditioning a similar biomass of Pacific oysters during the same time frame with oysters responding significantly (with significant gonadal development occurring) to the added food supply and warmer waters supplied conditioning tanks. It is largely unknown why rock scallops fail to undergo initial or renewed gametogenesis in the hatchery environment. In past years and prior to WRAC funding, routine scallop spawns were attained by bringing in wild scallops that were either in or close to full reproductive condition. Unfortunately, access to wild scallops has been largely curtailed due to regulatory concerns over relative abundance and other considerations, making it imperative to develop protocols for conditioning hatchery maintained populations.

Putative triploid seed

One group of 2N and 3N rock scallops produced in 2015-16 was husbanded through to 2017, albeit in extremely low numbers.

To conserve the very low numbers of putative 3Ns (n=19), tissue sampling for ploidy analysis was delayed. First, a trial tissue sampling of 2016's 2Ns (n-10) was conducted in June 2017 to characterize post-sampling mortality. Briefly, juveniles X-Y mm SL were immersed in a 7% $MgSO_4$ bath for ~15 min to relax adductors. When gape remained despite stimulation, a 1x3 mm section of ctenidia was resected using a 2 mm ring curette. After sampling, individuals were rinsed and returned to flow-through FSW. The group was monitored for mortality for approximately 1 mo. No mortality was detected.

Second, shell shape was compared between the two groups. Shell length (SL), width (SW) and height (SH) were measured to the nearest 0.1 mm. The SL:SH relationship revealed close concordance (X^2= 0.133, df=1, P=0.71; Fig. 1). Shell volume was estimated by assuming a circular profile with the SL (conservative) as the diameter, and doubling the calculated spherical cap volume (Fig. 2). No difference between groups was detected in any of the shell metrics, ratios, or volume.

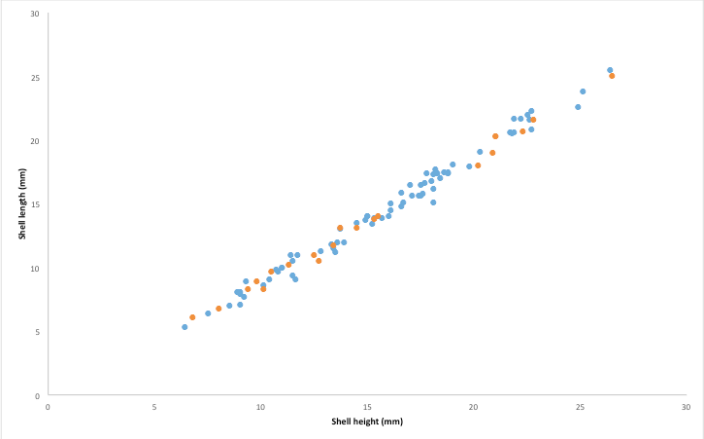


Figure 1. Relationship of shell length and shell height in juvenile rock scallop (Crassadoma gigantea) between normal diploids (blue dots) and putative triploids (orange dots).

Third, when putative 3Ns attained SL between 8 and 26 mm, tissues were sampled following the protocol outlined above on 7/14/2017. Tissues from putative 3Ns and three normal 2Ns were placed immediately on ice. Tissues were macerated in DAPI and run on a Cyflow ploidy analyzer (Sysmex Partec) flow cytometer. Mean peak values of putative 3Ns were compared to known 2Ns. No differences in mean peak values were detected. Based on these results, all of the survivors in the 3N group were diploid, not triploid.

Maturation

The current knowledge of rock scallop reproductive cycles is based on wild adult scallops of unknown age (Lauren 1982; Macdonald & Bourne 1989) collected at relatively infrequent intervals. To better characterize the maturation cycle in Dabob Bay, as well as gain data on size and age at first maturation and sex ratios, rock scallops produced at the Taylor hatchery in 2015 and deployed in Dabob Bay in 2016 were lethally sampled at semi-monthly intervals for gonad histology.

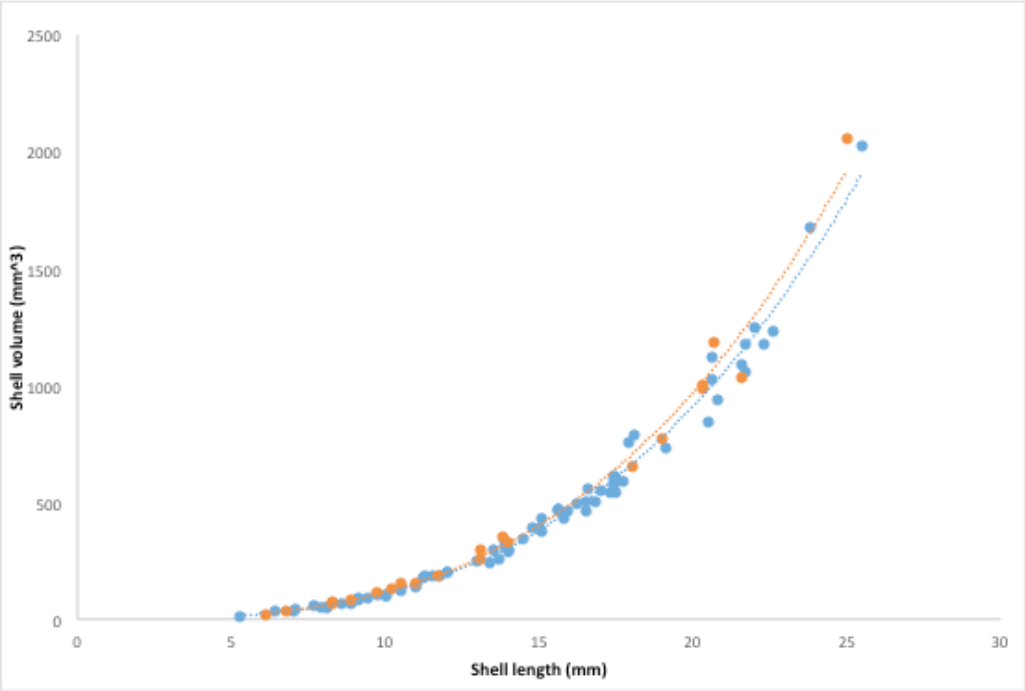


Figure 2. Relationship of shell length and estimated shell volume in juvenile rock scallop (Crassadoma gigantea) between normal diploids (blue dots) and putative triploids (orange dots).

Scallop production

Wild adult *Crassadoma gigantea* (n=54) were collected near Cypress Island (48.545749, -122.681953) and Burrows Channel (48.490116, -122.695972) in San Juan County, Washington State, USA on February 2, 2015. All collections were carried out by divers at depths ranging between 5 and 15 meters. Scallops were transported to the Taylor Shellfish Farms Hatchery on Dabob Bay WA for conditioning; broodstock were held in a single tank, fed a live algae diet, and the water temperature was gradually increased from 10°C to 14°C to facilitate gonad conditioning. A volitional spawn occurred on April 17, 2015 and broadcast spawning was allowed to continue. From this, approximately 50 million fertilized eggs were collected and reared in flow-through 250L tanks at 16°C. Larvae were fed a mixture of 16 live algae species and moved to a settlement system 28 days post spawn for metamorphosis where they were allowed to attach to artificial seaweed. In September 2015, five months post-spawn, juveniles (mean = 5 mm SH) were transferred to pearl nets (200 juveniles per net) and suspended from a floating long line in Dabob Bay, WA. Nets were arranged in seven stacks of five pearl nets. In May 2016, scallops were an average of 10 mm SH and were transferred to 8 stacks of hanging lantern nets. Each lantern net stack comprised 10 compartments, and each compartment was stocked with 150 scallops. In October 2016, a subset of scallops (n=400), were transferred to two hanging cages, each stocked at 200 scallops per cage for histology sampling.

Also in October 2016, additional subsets of scallops were deployed across three Washington State sites; Dabob Bay, Totten Inlet and Neah Bay for assessment of survival, growth, and maturation in different environments. Sites selected represented a variety of potential growing conditions for cultured *C. gigantea*. At each site, 450 juvenile scallops were deployed in nine hanging cages and each cage was stocked with 50 scallops. Cages were arranged in three stacks of three cages with the first cage 1.5 m below the surface with 0.3 m between cages. Scallops at each site were allowed to grow for 25 mo.

Tissue Sampling

Sampling for histology began from pearl nets in January 2016 and continued after transfer to lantern nets (October 2016) through December 2017 when the scallops were 32 months old. Ten scallops were randomly collected approximately every two weeks. For each individual, shell height (SH), from umbo to the shell margin, was recorded to the nearest 0.1mm, and tissue samples were preserved for histology analysis. Samples included the whole body for SH < 30.0 mm and only gonad for animals exceeding this height. Samples were then placed in histology cassettes, (n=5 per per cassette) and fixed and preserved using the PAXgene system (Qiagen) following the manufacturer's protocol. Preserved tissues were processed for routine histology, slide mounted, and stained with hematoxylin and eosin.

Sex and Reproductive Classification

Histology slides were examined via light microscopy at 40x and 100x to determine sex and the gonad development stage of each individual. Sex was identified by the presence of spermatids, oocytes or both. Individuals without visible spermatids or oocytes were classified as "unknown". A reproductive stage was assigned to each individual by examining three to four regions of the gonad. Within each gonad region, 8 random acini were classified until a total of 24 acini per specimen had been classified. Acini were assigned to one of six developmental stages; inactive (0), early active (1), late active (2), ripe (3), partially spawned (4), and spent (5), based on a modified protocol from Ropes (1968). The requirements for assigning acini to each gonad stage are listed in Table 1 and Figure 3. Stage was assigned to each individual using the mode of the 24 classified acini. Individuals at stage zero to two were classified as immature, and those at stage three to five were classified as mature.

Table 1. Criteria for assigning male and female acini to one of six gonad stages in Crassadoma gigantea.

Phase	#	Male Criteria	Female Criteria
Inactive	0	No acini visible. Abundant connective tissue.	No acini present or empty acini with no oogonia present.
Early Active	1	Acini contain spermatagonia and < 5% sperm.	Open acini with a monolayer of small oogonia attached to the acini wall.

Late Active	2	Acini contain spermatagonia and spermatozoa. The spermatozoa occupy > 5% but < 50% of the acini.	Acini contain small oogonia and larger oocytes attached to the acini wall. Free oocytes occupy < 20% of the acini.
Ripe	3	Acini contain > 50% spermatozoa.	Acini are full and contain > 20% free oocytes.
Partially Spawned	4	Acini contain > 50% spermatozoa and have partially emptied.	Acini predominantly contain free oocytes and show gaps where mature oocytes have emptied.
Spent	5	Empty acini (< 5% filled with sperm).	Empty acini (< 5% filled with free oocytes).

First Sexual Maturity

Logistic regression models (R version 3.4.2, R Core Team 2017; package FSA version 0.8.16, Derek H. Ogle 2018) were used to identify age and size at 50% sexual maturity for the population. The general model for both age and size at first maturation was:

$$\log\left(\frac{p}{p-1}\right) = \alpha + \beta_1 X \qquad \text{(eqn 1)}$$

where p was the probability of being "mature", $1 - p$ was the probability of being "immature" and X was size or age. Maturity was modeled as a function of age, shell height, and their potential interactions. The model fits were compared using Akaike Information Criterion adjusted for small sample size (AICc) (Akaike 1981), and a reaction norm for first sexual maturity was then calculated using the model with the smallest AICc (i.e. the best fit model). First sexual maturity was defined as the age and size at which 50% of sampled scallops were in phase 3, 4 or 5.

At the three growout sites to compare growth, survival, and maturation, cages were removed on June 6, 2017 and survival was recorded for each cage. For each surviving individual, shell height, shell length, shell width, whole weight, meat weight, and adductor diameter was recorded. Gonad tissue samples were preserved for histology analysis using the methods described above.

RESULTS

Sex and Reproductive Classification

A total of 428 individuals were sampled semi-monthly for histology and classified for reproductive status. Of these, 217 were determined to be male, 15 female, 4 hermaphrodites and 192 classified as unknown because they did not contain germ cells (Figure 3). For each maturation phase, the following number of individuals were assigned; inactive (224), early active (27), late active (40), ripe (44), partially spawned (77) and spent (14). A total of 291 individuals were found to be immature and 135 individuals were mature with the first mature individual found in January 2016 (Figure 4). Stage variation within sexed individuals ranged from 0 to 1.29 and

a Kruskal-Wallis rank sum test found no significant difference between males and females (Figure 6).

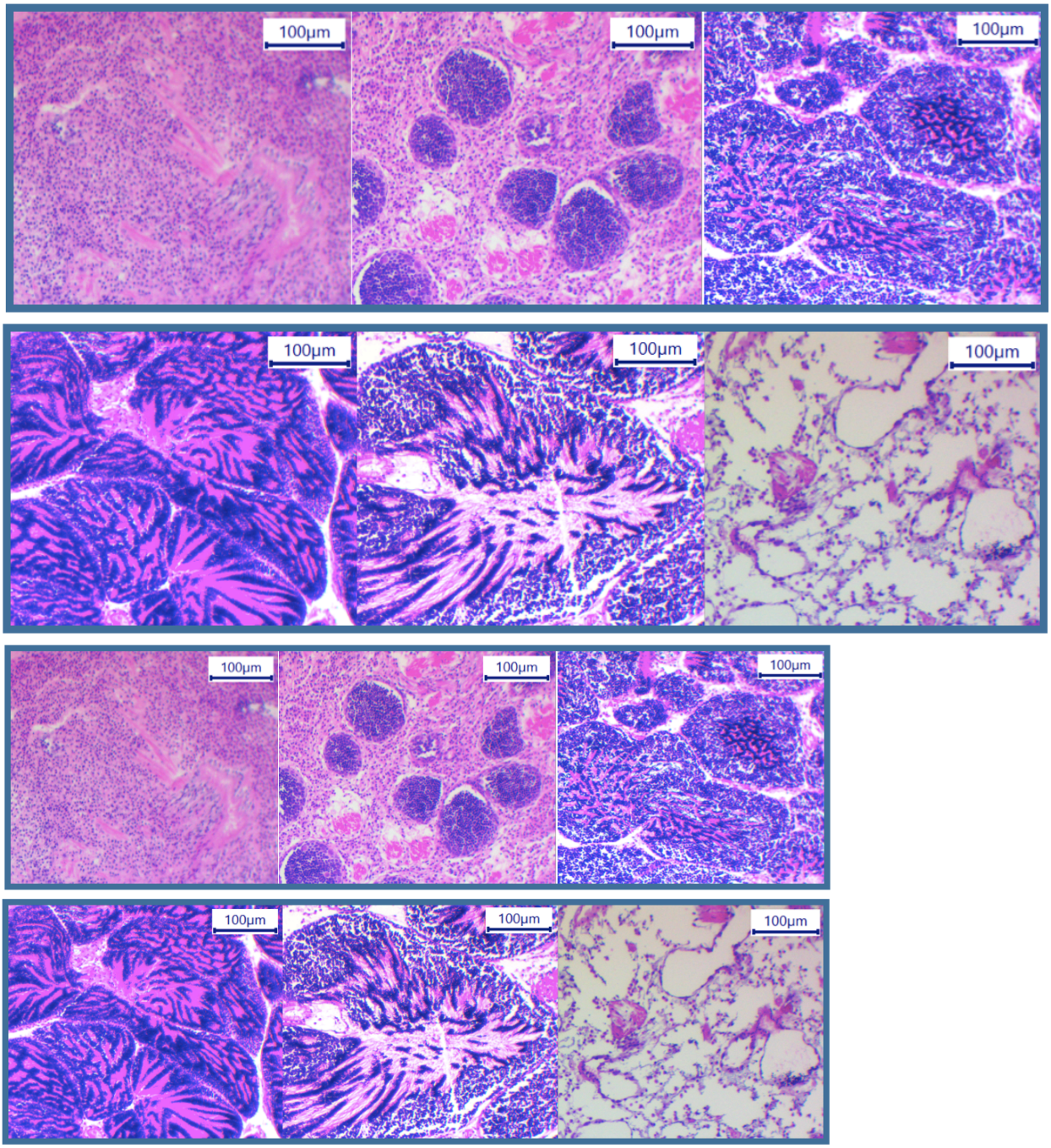


Figure 3. Photomicrographs of male and female rock scallop gonads.

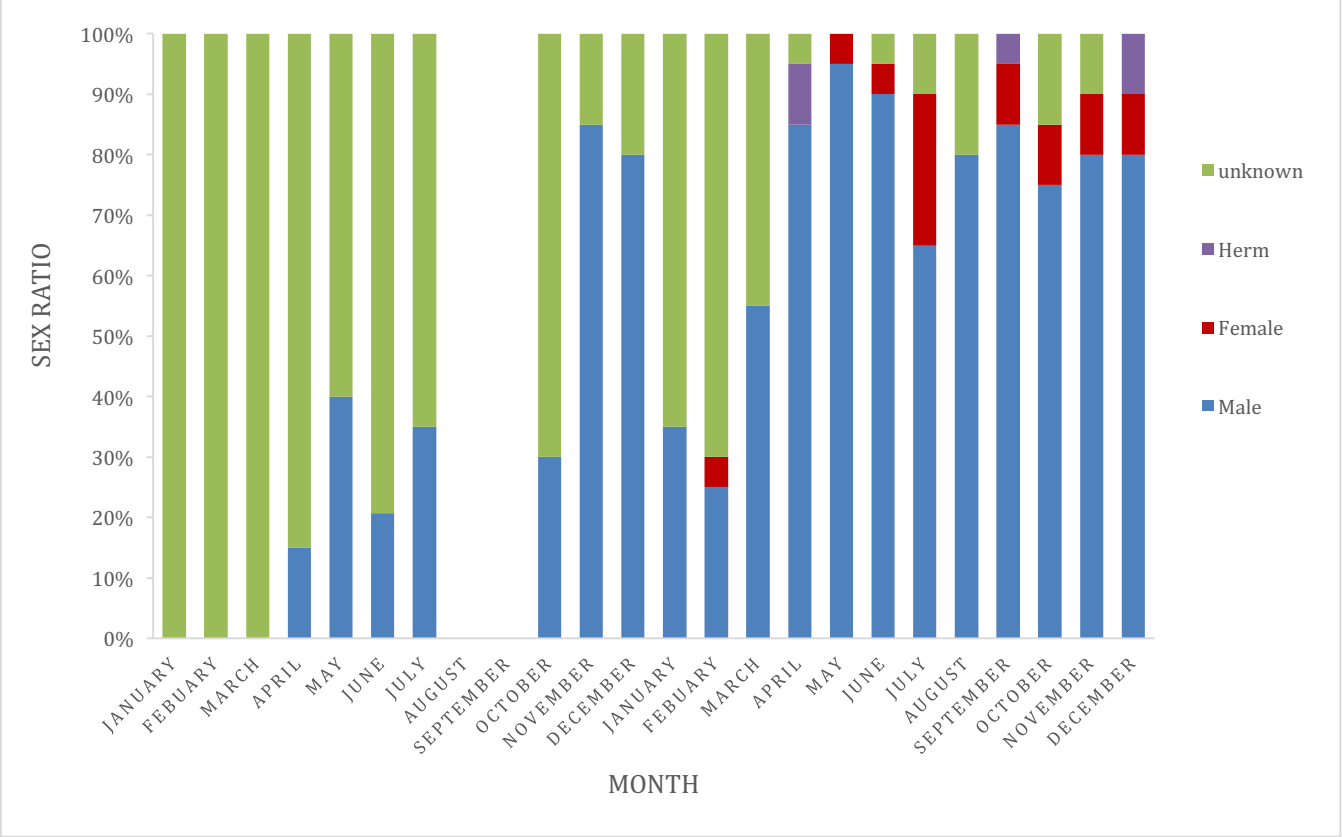


Figure 4: Proportion of Crassadoma gigantea identified as male (blue), female (red), hermaphrodite (yellow) or unidentified (grey) in a given sample month. Sex was identified by the presence of spermatids, oocytes or both. Individuals without visible spermatids or oocytes were classified as unknown.

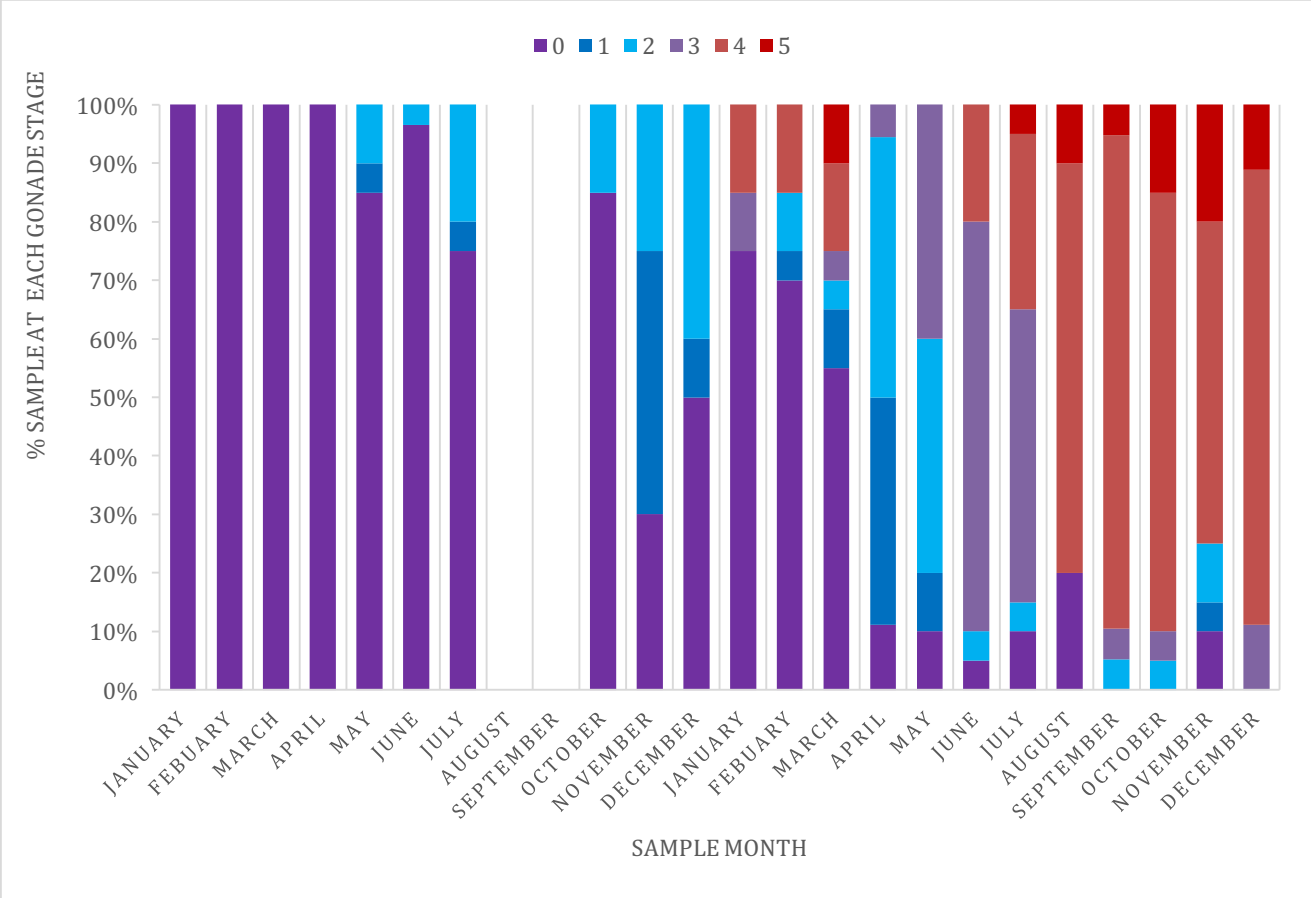


Figure 5: Proportion of Crassadoma gigantea juveniles assigned to maturation phases at each sample date. Inactive (purple), early active (dark blue), late active (light blue), ripe (yellow), partially spawned (orange), and spent (red). For each individual 24 acini were randomly chosen and an individuals phase was determining by taking the mode phase of sampled acini.

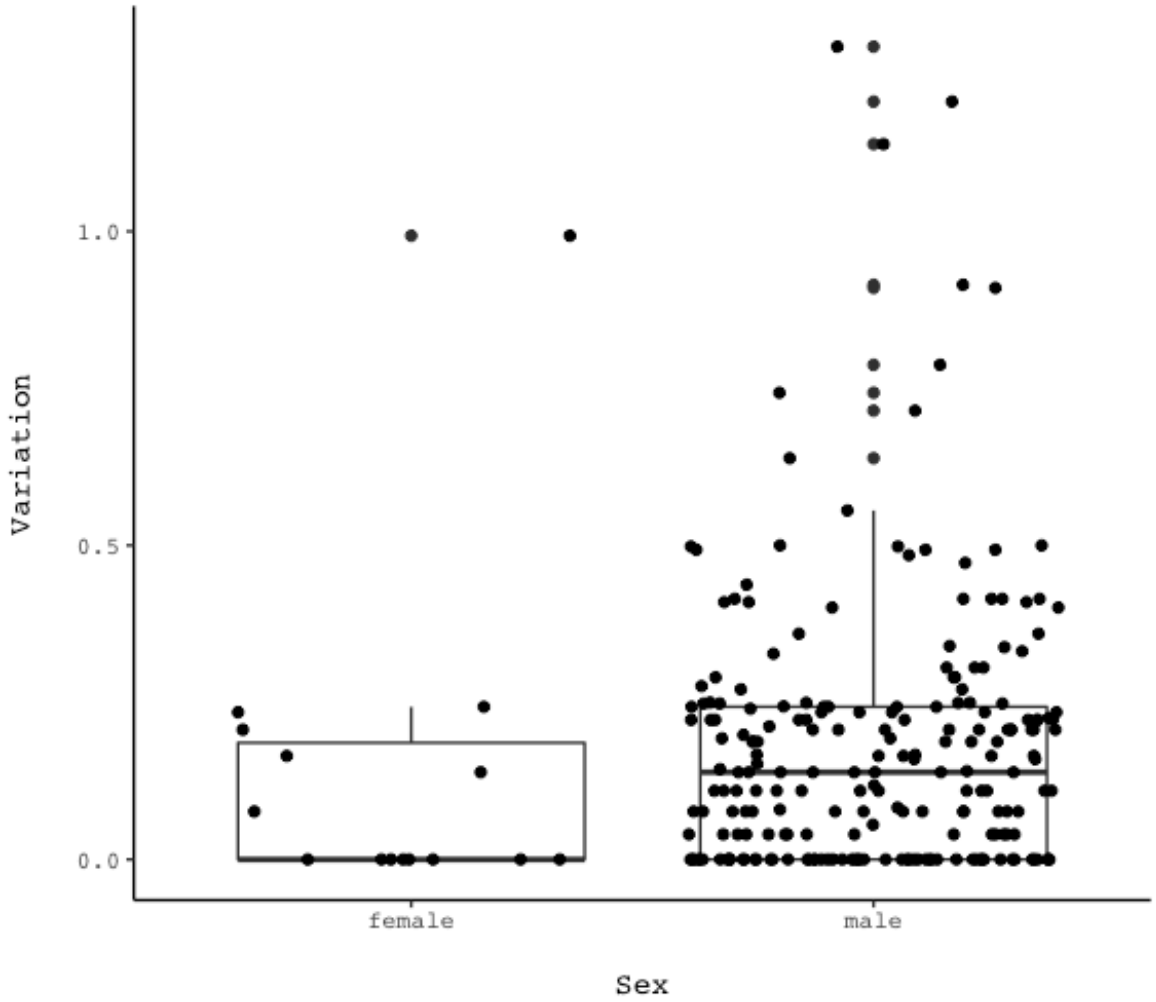


Figure: 6: Within individual acini phase variation by sex for Crassadoma gigantea. For each individual 24 acini were classified as inactive, early active, late active, ripe, partially spawned or spent. No significant difference in variation between females and males was observed (p=0.998).

First Sexual Maturity

Analysis of the models with a single predictor found age at maturation to be 24.6 months post spawn (Figure 7) and size at maturation to be 54.5 mm in shell height (Figure 8). AICc analysis found that the models with size and age, both singly and as additive predictors, best fit the data (Table 2). No interaction of size and age was detected (Table 2). The progression of maturation at size and age is depicted in Figure 9.

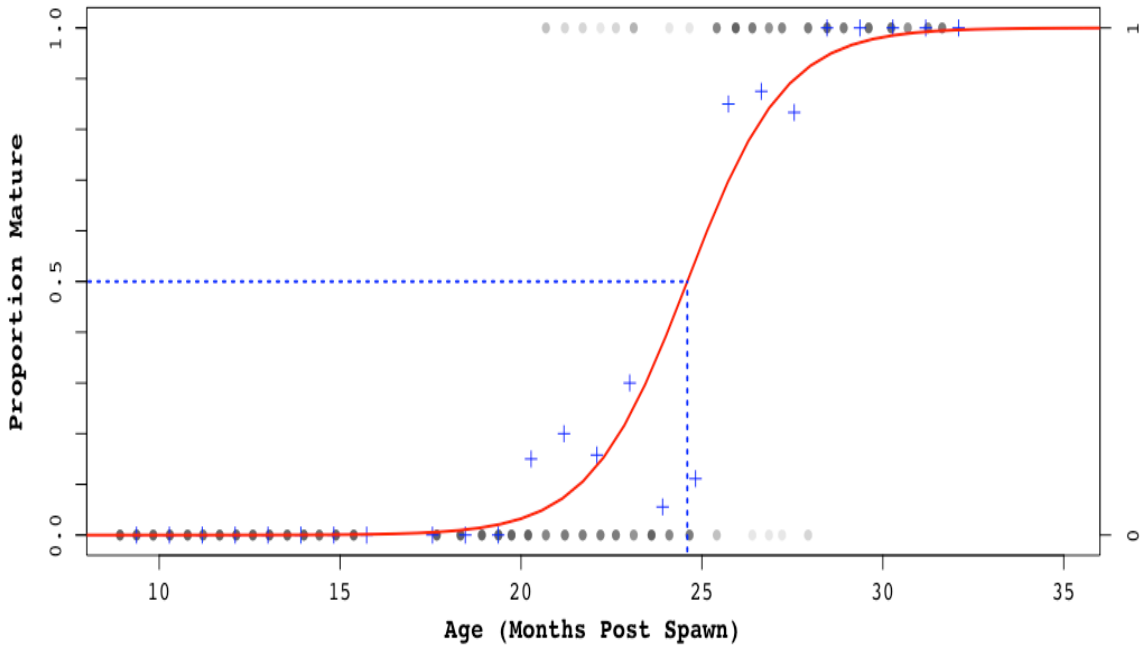


*Figure 7: Logistic regression for Crassadoma gigantea age at maturation. Proportion mature for each age is indicated by blue pluses and individual maturation is shown as grey-shaded dots, with darker dots corresponding to a greater number of individuals. The blue dotted line shows the age at which 50% of scallops are mature (***24.6 mo.)**.

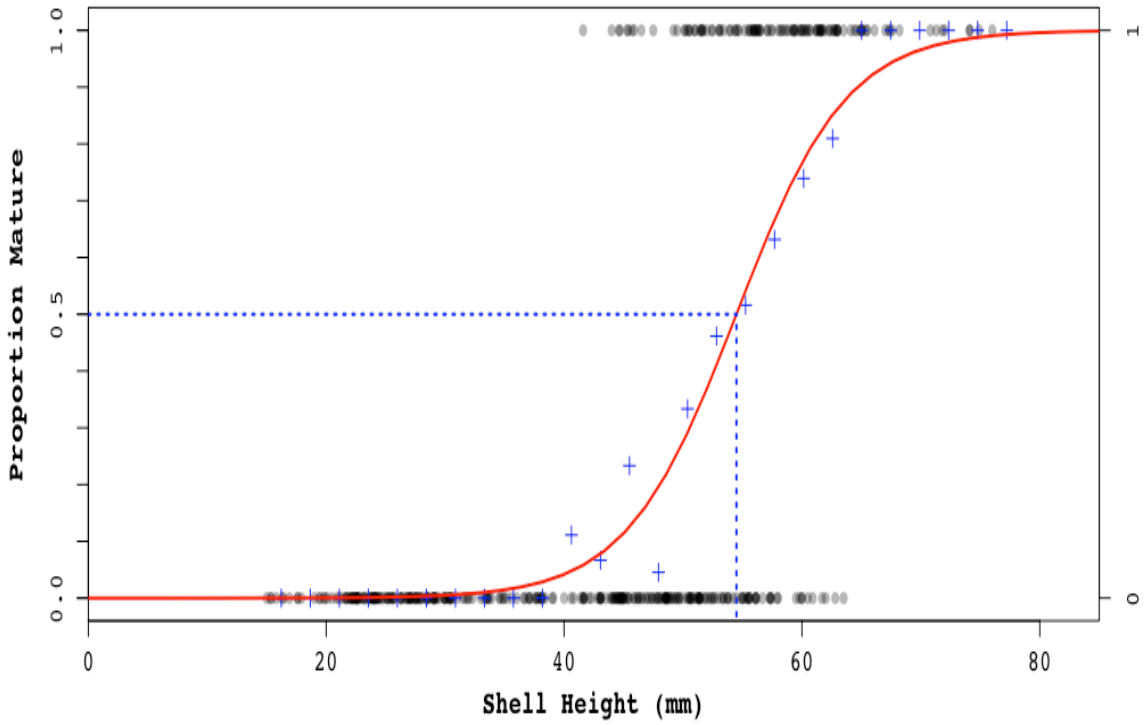


Figure 8: Logistic regression for Crassadoma gigantea size at maturation. Proportion mature for each size is indicated by blue pluses and individual maturation is shown as grey-shaded dots, with darker dots corresponding to a greater number of individuals. The blue dotted line shows the size at which 50% of scallops are mature **(54.5mm)**.

Table 2. Summary of results from analysis of the four generalized linear models. Regression coefficients (standard error), test statistics and significance values are listed for each model. AIC values are corrected for sample size.

	Dependent variable:			
	Size*Age	Size+Age	Age	Size
	(1)	(2)	(3)	(4)
Intercept	-5.93 (8.90)	-20.84 (2.56)***	-18.20 (2.13)***	-11.78 (1.33)***
Shell_Height	-0.21 (0.18)	0.08 (0.03)***		0.22 (0.02)***
Age	0.06 (0.37)	0.66 (0.09)***	0.74 (0.09)***	
Shell_Height:Age	0.01 (0.01)			
AICc	264.65	159.45	154.46	154.91
Observations	404	404	404	404
Note:				*p<0.1; **p<0.05; ***p<0.01

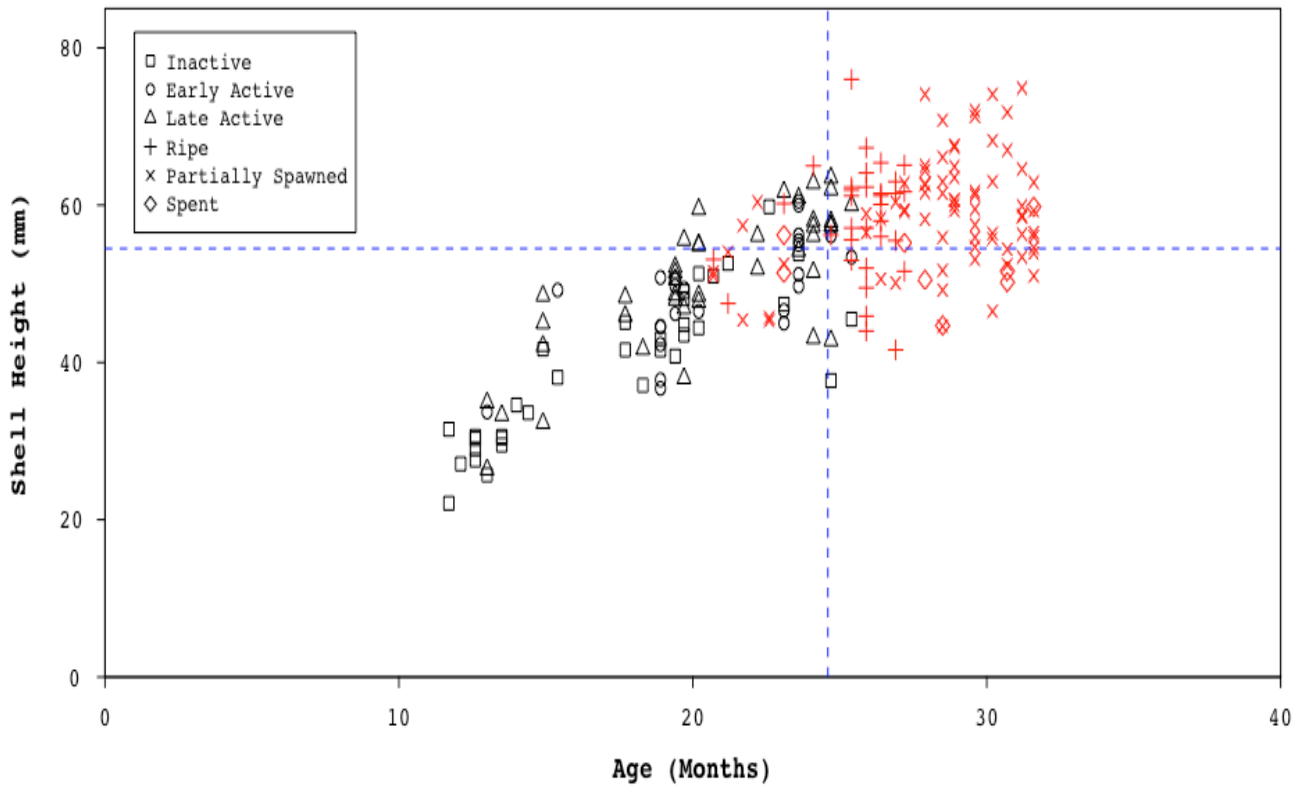


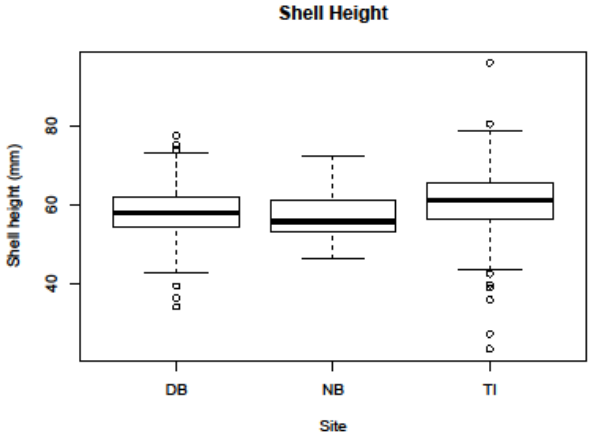
Figure 9: Size and age of Crassadoma gigantea assigned to six maturation stages. Inactive (square), early active (circle), late active (triangle), ripe (plus), partially spawned (x), and spent (diamond). Immature individuals (inactive, early active, late active) are black and mature individuals (ripe, partially spawned, spent) are red.

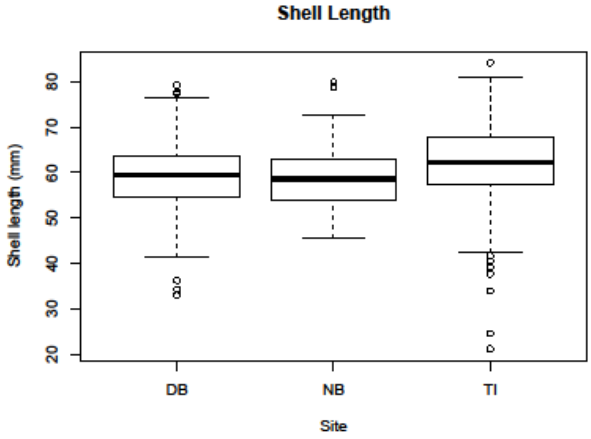
Spatial variation

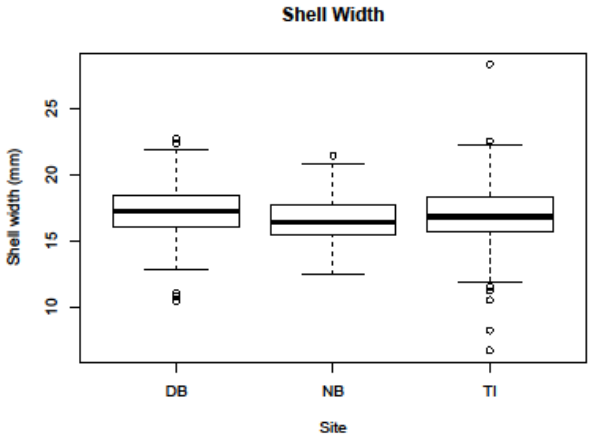
Contrary to what was expected based on where wild populations are found, scallops at Neah Bay exhibited very poor survival, while scallops in Totten Inlet (no substantive wild population in the south Puget Sound sub basin) exhibited excellent survival (Table 3). In general, greater growth was observed at Totten Inlet than at Dabob Bay or Neah Bay. Analyses of maturation at the three sites has not yet been completed. Shell and adductor morphometrics are summarized in Figure 10.

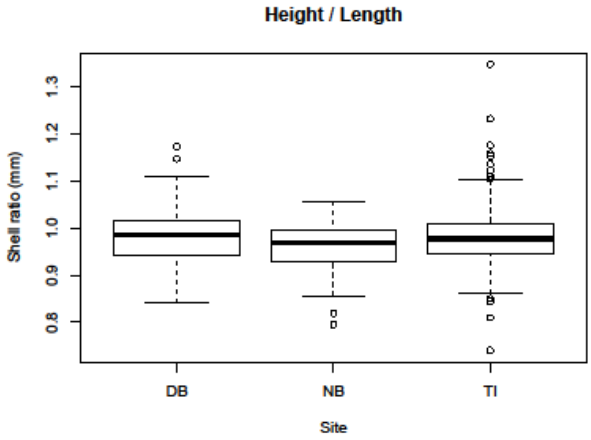
Table 3. Temperature and survival of *Crassadoma gigantea* at the 3 outplant sites.

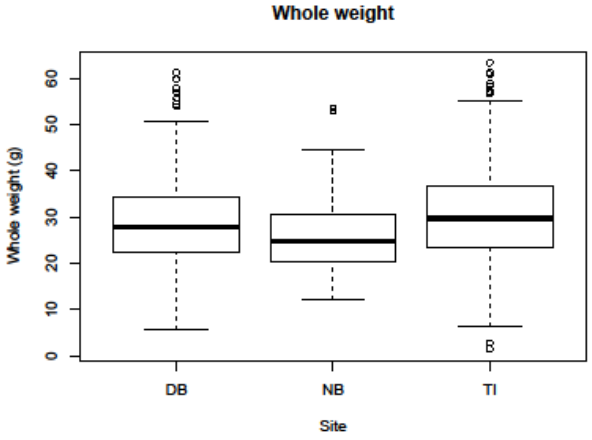
Site	*Average Temp (C)*	*Min Temp (C)*	*Max Temp (C)*	*Survival (%)*
Dabob Bay	10.34	8.03	16.63	66
Totten Inlet	10.26	6.78	17.25	96
Neah Bay	10.72	7.40	19.17	14

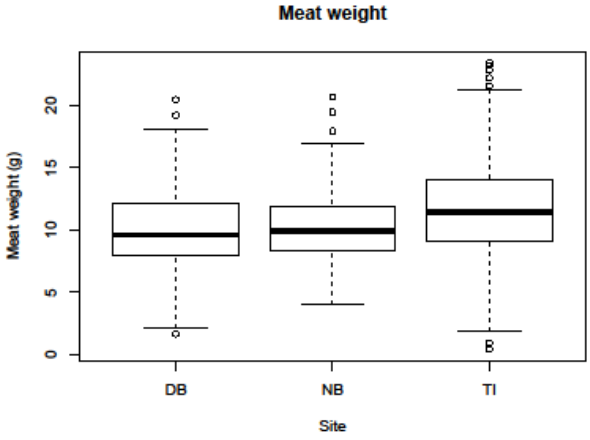

Shell Height
Shell height (mm)
40
60
80
DB
NB
TI
Site

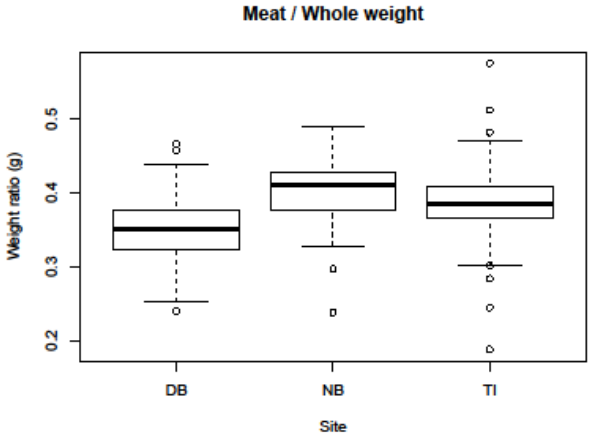

Shell Length
Shell length (mm)
20
30
40
50
60
70
80
DB
NB
TI
Site


Shell Width
Shell width (mm)
10
15
20
25
DB
NB
TI
Site


Height / Length
Shell ratio (mm)
0.8
0.9
1.0
1.1
1.2
1.3
DB
NB
TI
Site


Whole weight
Whole weight (g)
0
10
20
30
40
50
60
DB
NB
TI
Site


Meat weight
Meat weight (g)
0
5
10
15
20
DB
NB
TI
Site


Meat / Whole weight
Weight ratio (g)
0.2
0.3
0.4
0.5
DB
NB
TI
Site

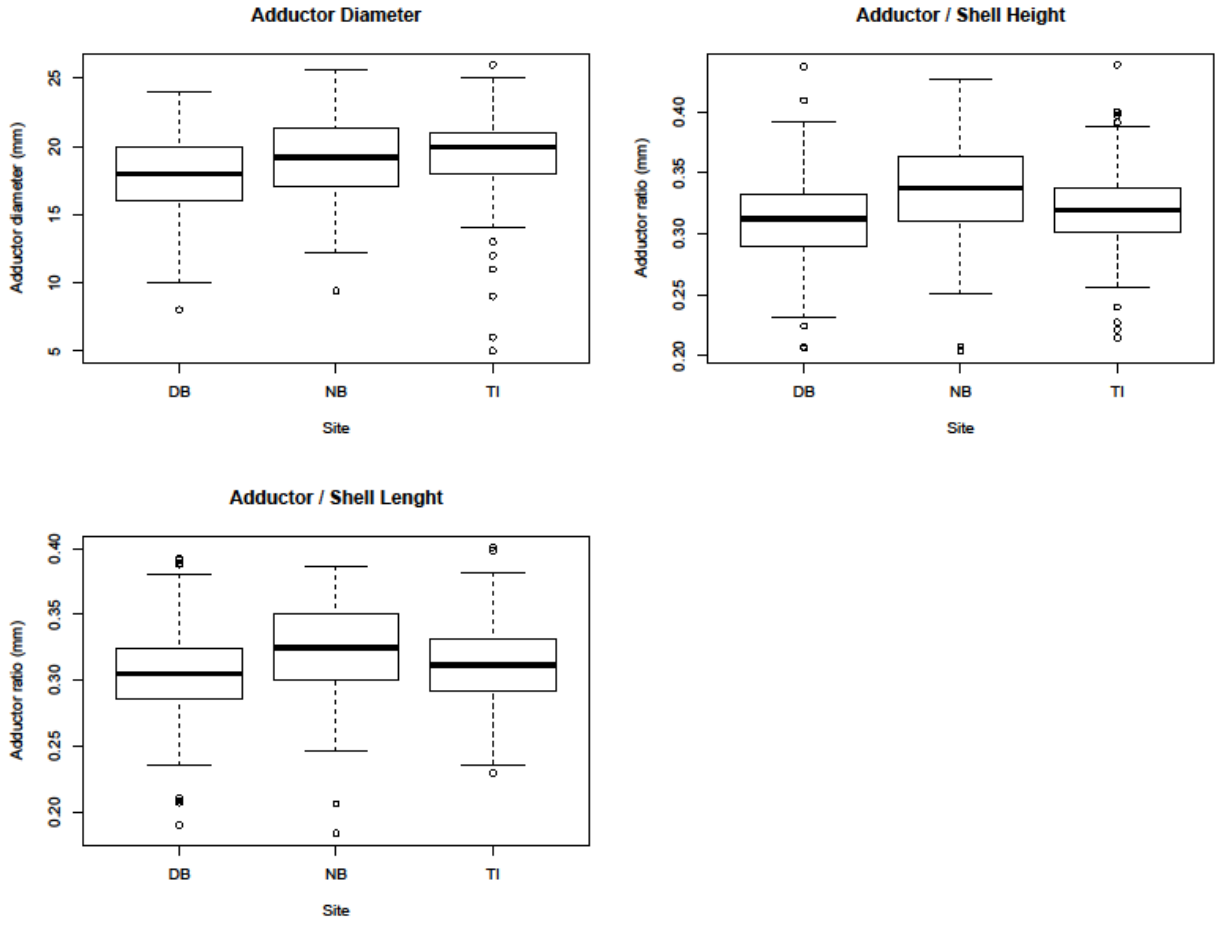


Figure 10. Box-whisker plots of morphometrics of 25 mo. old Crassadoma gigantea grown in Dabob Bay (DB), Neah Bay (NB) and Totten Inlet (TI).

Objective 2: Conduct 4N induction trials following the French patent method and the gynogenetic method, both of which require only mature diploid broodstock. Develop 4N containment.

Because rock scallop broodstock failed to condition and spawn, all 4N induction trials were conducted using both chemicals and pressure using *Crassostrea gigas* as proxy.

Despite repeated trials, we were unable to reproduce the results reported for the French method. Similar failures to reproduce the results were reported by others (Stan Allen, pers. Comm.). Briefly, after chemical treatment, ongoing size selection for the smaller fraction did not result in any detectable 4Ns at any time point. The conclusion was that some critical aspect of the method was not sufficiently described in the French patent.

Crassostrea gigas were also proxy for attempts to alter ploidy using pressure. A high pressure pump (brand) with the hydraulic oil replaced by glycerol was attached to stainless steel cylindrical pressure tube, 30 cm long x 3.5 cm diameter. Fertilized oocytes were held at 23 C, and placed in 25 cm long x 2 cm diameter PVC treatment capsules that were capped at the bottom. The open ends were closed using a wax paper membrane held in place with a PVC ring to exclude any air. The purpose of the membrane was to allow pressure equalization in the treatment capsule. The treatment capsule was placed in the water-filled pressure tube, and the tube cap was screwed in place. Water was added as necessary to exclude air from the system. Once sealed, 10,000 PSI was applied for 5-25 min post-fertilization to cause retention of PB1, PB2, or both. Resultant embryos were queried for ploidy via flow cytometry. The pressure method successfully induced triploidy in *C. gigas* with low mortality to the D hinge stage.

All trials, however, were curtailed in May 2017 due to ongoing serious problems with larval mortality at the Taylor hatchery. Lack of broodstock maturation (described under Objective 1) led to no further trials in 2018. The pressure technique appears to be a promising method to try for rock scallop 4Ns once gametes are available.

Objective 3: Run a series of settlement experiments to describe factors that influence successful settlement and metamorphosis to the juvenile feeding stage.

Progress on Objective 3 was impeded due to the ongoing issues with broodstock conditioning (described above under Objective 1). No gametes were available for larval production hence no larvae were available for settlement trials.

Objective 4: Continue research on survival, growth and cementation behavior of rock scallops currently deployed at 7 sites in Puget Sound and one in California.

Studies of growth and survivorship and cementation behavior were initiated in July 2015 with measurement taken at approximately 3-5 months. These continued through June, 2018 when a final date set was taken on a suite of morphometric parameters on three of the seven field populations. The seven sites in Washington State were originally selected for performance studies based on disparate geography and availability of commercial partners to assist in maintaining scallops on docks and floats as available (Figure 11). Sites include Hood Canal (Dabob Bay and Port Gamble), North Puget Sound (Drayton Harbor and Port Hadlock), Central Puget Sound (Agate Passage and Manchester/Clam Bay) and South Puget Sound (Totten Inlet). For each site,

Figure 11. Map showing seven Puget Sound basin sites for ongoing studies on growth and survivorship of rock scallops.

replicate arrays of shellfish cages suspended at a depth of 2-3 M (except at Dabob Bay where cages were suspended below the summer thermocline (5M). Each replicate array consisted of four ADPI Oyster growout cages containing 55 scallops at the start of the work. Each set of cages are maintained in an enclosure constructed of PVC pipe. A continuous recording temperature sensor (Onset Computer Company) was placed onto one of the two arrays at each site.

The original intent was to collect data on cementation, size at age and survivorship at 100 day intervals. All measurements were collected either by caliper, though on two occasions in 2016, photographs of individual scallops were taken for processing using image analysis software (Image J). The image analysis approach, while time efficient in the field, proved unsatisfactory due to poor image quality and was subsequently abandoned for measurements taken in July 2016. Growth and survivorship has been satisfactory to excellent at all sites and by late August 2017 (25 months following planting as seed), the mean size (shell length) of scallops for all sites was 78.9mm (Fig 12).

There was appreciable variability in growth at different sites with main basin (Manchester and Agate Passage) and Hood Canal sites demonstrating the most rapid growth.

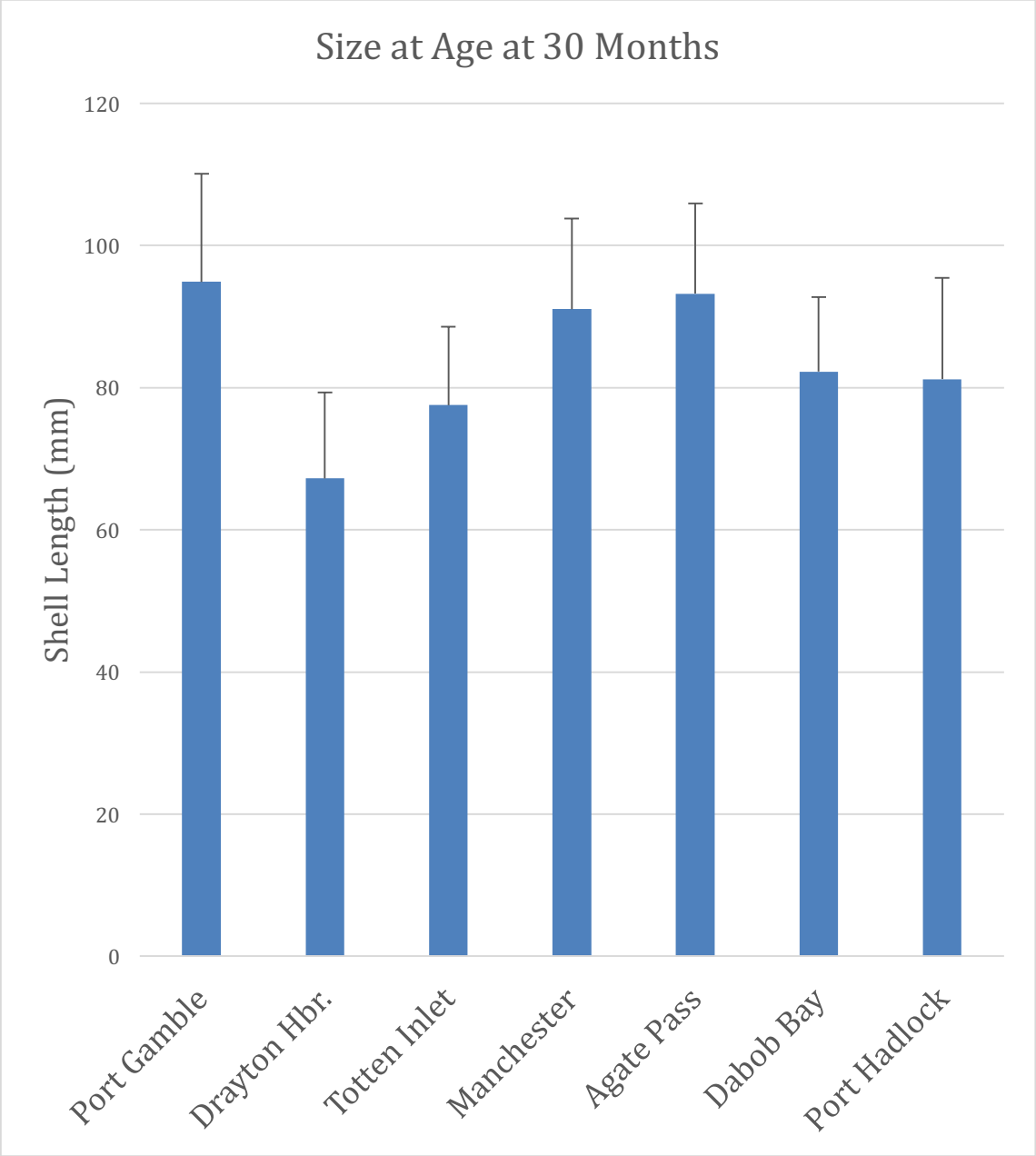


Figure 12. Mean size in mm (± 95% CI) of farmed purple hinge rock scallop, Crassadoma gigantea, after 30 months following plant out of seed at seven grow out sites in Washington State.

Survivorship was high at most sites as well at 70% after 30 months. Scallops from Drayton Harbor and Manchester experiencing the lowest survivorship (63% from plant out). Other sites experienced higher overall survivorship and was over 80% from plant out for both Totten Inlet and Agate Passage (Fig. 13).

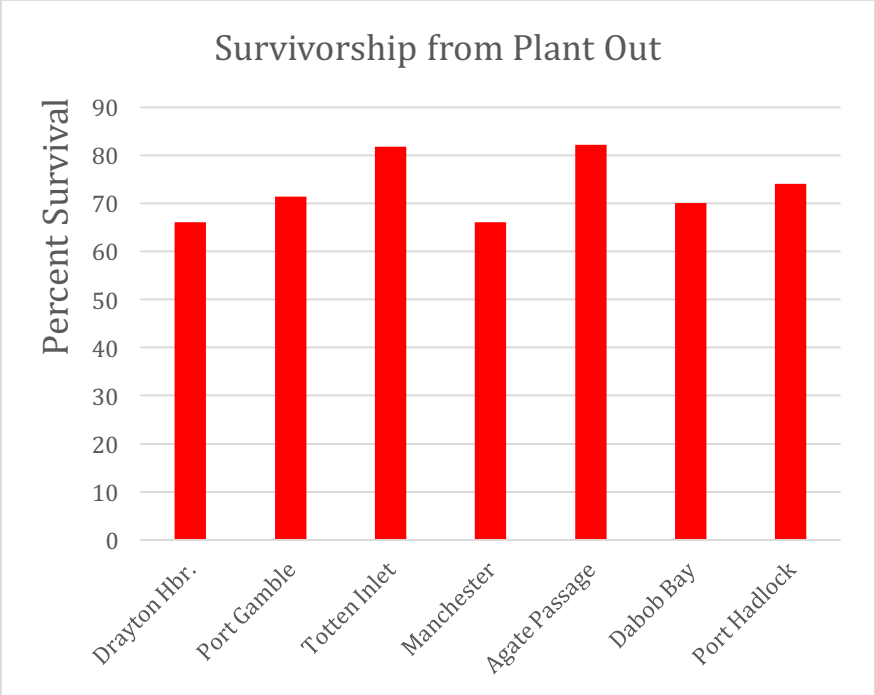


Figure 13. Cumulative survivorship from plant out 30 months earlier for seven Puget Sound sites.

Most interestingly, at all sites under investigation the percentage of scallops cementing into the meshes of the shellfish cages remains extremely low overall (Table 4) with cementation rates well below 1% for scallops measured between May, 2017 and June 2018.

In late June 2018, the Project Team of Davis, Vadopalas and Culver met to discuss findings over the course of the project in Washington State. There, detailed discussions ensued relating to next steps necessary in developing rock scallop aquaculture. The principal outreach product consisting of a new manual for potential scallop growers was discussed. Also, final sampling of scallops from three primary locations was completed. In this work, scallops were shucked and tissues assessed for biometric parameters, including whole live tissue mass, adductor muscle mass and presence of gonadal material (to assess sex ratio). A total of 40 animals were assessed for three experimental populations, including Manchester, Agate Pass and Port Gamble. Data for these populations is summarized below (Figure 14).

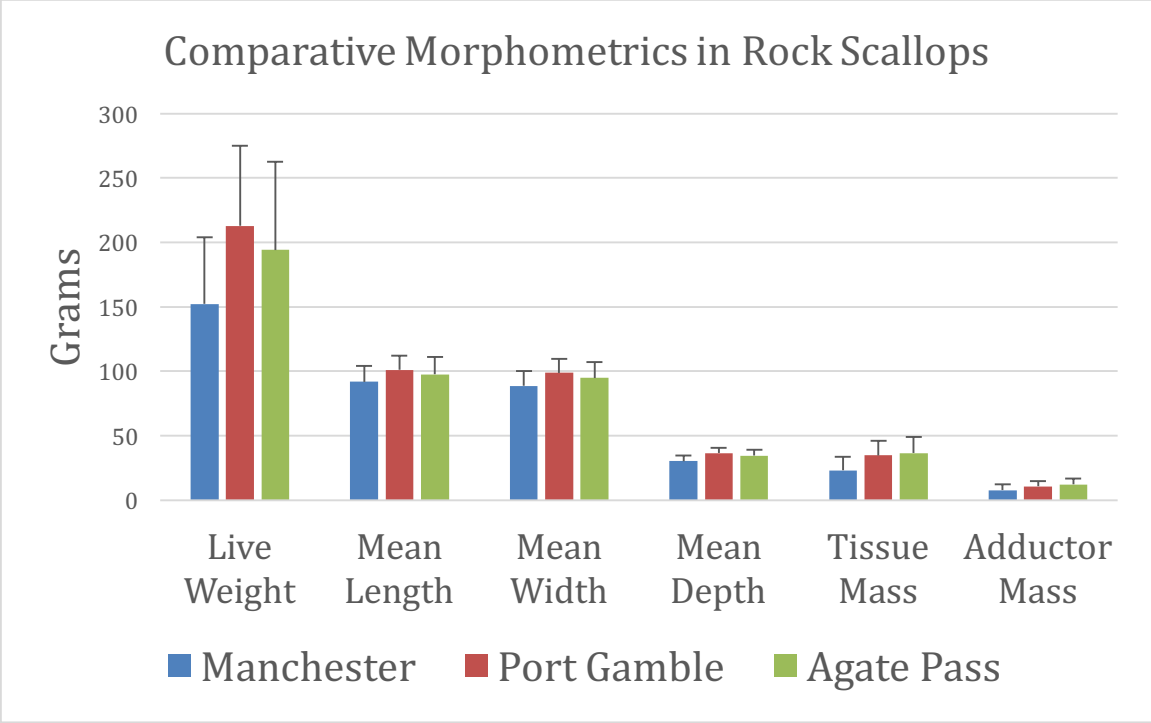
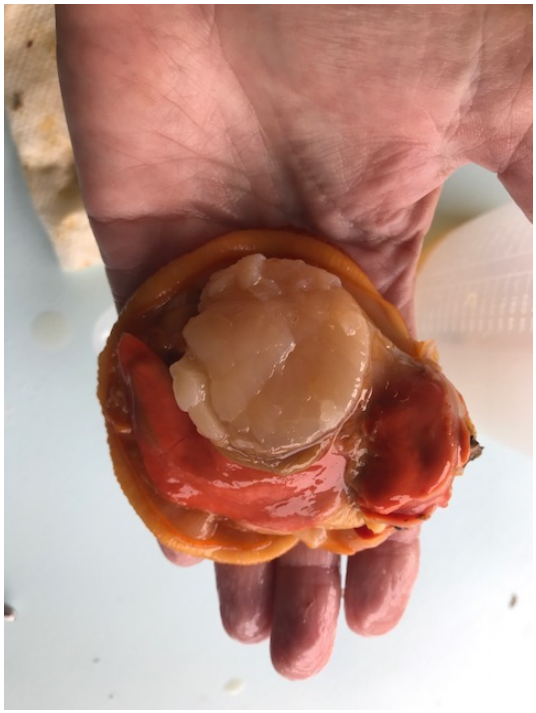


Figure 14. Comparative morphometric information on three populations of rock scallops grown from seed to harvest size in Puget Sound.

The finding that scallops exhibit cementation behavior beginning at about 30mm shell length and then largely ceasing this behavior at 50-55 mm is of significant value to the development of a rock scallop aquaculture industry sector as tray culture appears possible in this species. Frequent handling of scallops while in the juvenile, cementation phase will remain necessary, however the majority of grow out time from this size to harvest (100-110mm) does not appear to require frequent handling, at least in Washington State. Also, as the photo to the left shows, the adductor muscle in this species is large relative to the tissue mass, a significant advantage in scallop aquaculture.



Site	Cementation Rate (%)
Drayton Harbor	0
Port Gamble	0
Totten Inlet	0.55
Manchester	1.4
Agate Passage	0
Dabob Bay	0
Port Hadlock	0

Table 4. *Mean cementation rate between April and August 2017 for scallops maintained in polyethylene grow bags at seven Puget Sound sites.*

Objective 5: The primary outreach objective is the production of a WRAC publication describing production of triploids, once a reliable protocol has been established.

This objective was changed to a focus on growout techniques due to continued problems with broodstock ripening in the final year of the research. The Project team met in June 2018 and discussed this objective with the decision to move forward with a dedicated report on growout techniques in Washington and California to follow.

IMPACTS:

Title: Triploids, tetraploids, and successful metamorphosis in purple hinge rock scallops (*Crassadoma gigantea*)
Relevance: In the last decade, it has become increasingly important to look to native species to expand and diversify shellfish aquaculture in U.S. Pacific coast estuaries. High value products, such as scallops, are strong contenders for the shellfish industry. Among the critical information needs when developing a new species for aquaculture are growth and survival characteristics at different life stages, optimal husbandry techniques, the size and age at which maturation occurs, and methods to potentially mitigate any risk to wild stocks from interbreeding with farmed stocks. A number of commercial shellfish growers in California, Washington, and Alaska expressed interest in filling these information gaps and in pursuing general culture techniques for the purple hinge rock scallop.
Response: Efforts were organized among commercial growers, the University of Washington, the University of California, and others to develop a research program focused on developing commercial aquaculture of the rock scallop. The Western Regional Aquaculture Center funded a two-year grant to pursue growout trials in different environments in Washington State, to shed light on when farmed stocks mature, and to further develop ways to produce sterile rock scallops to safeguard wild stocks from interbreeding with farmed stocks.

Results: Purple hinge rock scallops exhibited stark differences in growth at different sites in Washington, indicating that production of a marketable rock scallop (100mm) can occur in as little as 3 years in some locales. The project has yielded important data on rock scallop growout performance. First, survival was good to excellent across all sites. Second, growth was excellent at three sites. Third, contrary to earlier trials, the shift to different growout gear and regular, albeit infrequent, handling effectively prevented cementation of the vast majority of juvenile rock scallops; it thus appears possible to minimize the cementation behavior by a combination of gear and handling during a critical developmental stage/size. The resulting product is attractive and may be marketed as either a whole live product or shucked for the highly valued adductor muscle (see photo below of cultured rock scallop with large adductor muscle. The relative ease of production coupled with yield (growth + survivorship) of rock scallops in seven disparate locales within Washington illustrates the high potential profitability of rock scallop aquaculture.

In the field, maturation occurs in the second year, and the vast majority are male at that point. The unforeseen difficulties with conditioning rock scallops to reach maturity in the hatchery points to important avenues for future research, and resulted in hindering further work on producing sterile stocks and optimizing seed culture in the hatchery.

Impact: The net result of the research was that significantly more is known about what it takes to farm rock scallops. Shellfish hatcheries can make informed decisions about how, and whether, to pursue development of this species for commercial culture. State and tribal wild resource management agencies have also benefited from critical information gained on gametogenesis and sex ratio in this species as it may impact the potential for interbreeding of farmed and wild stocks. Results of the growout study demonstrate the high potential for rock scallop aquaculture to blossom, once culture challenges associated with gamete acquisition can be overcome. The potential has been recognized by at least two Washington tribes and a number of shellfish aquaculture companies in Washington State and California.

Collaborators: Significant roles were played by the Taylor Shellfish Co., Baywater Inc., the University of Washington, the University of California, and Pacific Hybreed, Inc.

Contacts: Jonathan P. Davis, Baywater & Pacific Hybreed, Inc., jothpdavis@gmail.com
Brent Vadopalas, University of Washington, Seattle, WA., brentv@uw.edu

PUBLICATIONS, MANUSCRIPTS, OR PAPERS PRESENTED:

Jackson, M., Wyckoff, S., Davis, J., Vadopalas, B., 2014, How to grow rock scallops, 67th PCSGA Annual Conference, Vancouver, WA, Sept 23-25.

Jackson, M., Wyckoff, S., Davis, J., Vadopalas, B., 2015, Development of commercial hatchery production techniques for rock scallop, 68th PCSGA Annual Conference, Hood River, OR, Sept 22-25.

Jackson, M., Davis J., Vadopalas B, and L. Hauser. 2015, Investigating local adaptation in Washington State purple hinge rock scallops.69th Joint Annual Meeting of the National Shellfisheries Association Pacific Coast Section and the Pacific Coast Shellfish Growers Association, Hood River, Oregon, USA, September 22-24.

Lowell, N., Davis J. Vadopalas B.,and L. Hauser. 2015. Assessing population structure and local adaptation of rock scallops to inform aquaculture practice. 69th Joint Annual Meeting of the National Shellfisheries Association Pacific Coast Section and the Pacific Coast Shellfish Growers Association, Hood River, Oregon, USA, September 22-24.

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Davis, J. 2015. Prospects for purple hinge rock scallop cultivation on the west coast—studies on aquaculture potential. 69th Joint Annual Meeting of the National Shellfisheries Association Pacific Coast Section and the Pacific Coast Shellfish Growers Association, Hood River, Oregon, USA, September 22-24.

Jonathan Davis 2016. Developments in Aquaculture for Purple Hinge Rock Scallop. 70th Annual Joint Meeting of the National Shellfisheries Association-Pacific Coast Section and the Pacific Coast Shellfish Growers Association. Chelan, WA October 12-14.

Jonathan Davis 2016. Interview for article discussing the potential for purple hinge rock scallop aquaculture. "Rock Scallop: Viable Candidate for Aquaculture". *Aquaculture North America*, June 2016.

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Jackson, M et al. ms in prep. Age and size at maturation in the purple hinge rock scallop.

SUBMITTED BY:
Title: (Gary Freitag) Date 9/13/2018

APPROVED:
Project Monitor Date

Gary Freitag

Record of Work Group Meeting (2017-2018)

Prior to the start of the final year of the project and during summer (August 1, 2017), a project work meeting was held on Bainbridge Island. Attending were Joth Davis, Brent Vadopalas and Benoit Eudeline and on the phone were Gary Freitag and Sue Cudd. Discussed were plans for the final year of the proposed research, including the critical need to bring in younger rock scallops for conditioning trials and to make spawning attempts when possible. Also discussed was the intent to complete the both the growout study over the ensuing 12 months and remaining work associated with Molly Jackson's research on gametogenesis in this species. The proposed work progressed in late 2017 and 2018 as planned at this meeting. These are detailed in the Rock Scallop Termination Report.

During a few days in late June, 2018 a sub-work group meeting with held among Joth Davis, Brent Vadopalas and Carrie Culver in Washington State. Here, final components of the project were discussed including the intent to complete an outreach document hatchery and growout methods for this species. Also discussed were next steps in planning for closing gaps in needed research on this species, especially related to attaining a better understanding of triggers associated with gametogenesis. Also discussed were data needs for Carrie Culver prior to her leaving for the European Aquaculture meeting in France where she presented a paper on progress in rock scallop aquaculture.