Part I: Summary

PROJECT TITLE: Profitable and biosecure rock scallop culture for the West Coast

REPORT GIVEN IN YEAR: 2015

REPORTING PERIOD: 8/1/2014 to 7/30/2015

AUTHORS: Paul Olin, Brent Vadopalas, Joth Davis, Karl Menard

FUNDING LEVEL: Total funds allocated to date:

First Year Request: 2013-2014;	$113,238
Second Year Request: 2014-2015;	$115,906
Total funds allocated to date:	$229,144

PARTICIPANTS:
Washington:
Joth Davis, Taylor Shellfish, Baywater Inc., and Puget Sound Restoration Fund
Molly Jackson, Taylor Shellfish
Sara Wykoff, Taylor Shellfish
Ed Jones, Taylor Shellfish
Dave Pederson, Taylor Shellfish
Brent Vadopalas, University of Washington
Earl Steele, Bellingham Technical College, Fisheries & Aquaculture Sciences (host at Drayton Harbor)
Vivian Berry, Suquamish Tribe (host at Agate Pass)
Ryan Crim, Puget Sound Restoration Fund (host at Clam Bay)
Betsy Peabody, Puget Sound Restoration Fund
Hans Daubenberger, Port Gamble S'Klallam Tribe (host at Port Gamble)
Bethany Stevick, Washington Department of Fish & Wildlife (shell annuli exam)

California:
Paul Olin, California Sea Grant
Carrie Culver, California Sea Grant
Karl Menard, Bodega Marine Laboratory, hatchery and growout site
Arty Seavey, Monterey Abalone Company, growout site
Andrew Kim, Monterey Abalone Company

Alaska
Jeff Hetrick, hatchery and growout site

PROJECT OBJECTIVES: The overall goal of this project is to advance marine aquaculture along the west coast of the U.S. by demonstrating production techniques for a new, competitive and biosecure product; the purple-hinge rock scallop, *Crassadoma gigantea*. The primary

emphasis of our proposed research is the development of methods to increase the biosecurity associated with farming a native species in close contact with wild conspecifics, while at the same time comparing the relative performance of triploid vs. diploid stocks. In essence, we aim to greatly expand the geographic range of scallop culture, and reduce genetic contact between farmed and wild populations by using 3N seed generated by 4Nx2N matings. We also seek to compare culture techniques used in the different areas to determine the most efficient and profitable way to culture rock scallops.

Specific Research Objectives:
1. Production of quantities of diploid and triploid rock scallop seed for growout trials, comparing growth, reproductive status, and survival of 3N and 2N progeny at multiple growout sites, and culture techniques in Alaska, Washington and California, including four collaborations with First Nation Tribes.

2. Triploidy investigations:
- Determining the optimal timing for production of 3N scallops by inhibition of second polar body extrusion.
- Determining the optimal dosage of 6-DMAP for production of 3N scallops by inhibition of second polar body extrusion
- Determining 3N rock scallop gamete and 3N x 2N embryo ploidy and viability

3. Creation of tetraploid stocks for mating to diploid stock insuring offspring are 100% triploid. Studies will include:
- 3N female x 2N male, inhibition of first polar body extrusion
- 3N female x 2N male, inhibition of second polar body extrusion
- 2N female x irradiated sperm from 2N male, inhibition of extrusion of both polar bodies.
- 2N female x 4N male

Specific Outreach Objectives:
1. Training shellfish growers through pilot demonstration growout at 14 commercial shellfish farms including four with Tribal affiliations, and transfer of hatchery technology for the production of diploid and triploid seed to a hatchery in California and Alaska through focused technology transfer workshops. Disseminating information through a) presentations at shellfish conferences including the PCSGA annual conference and the annual Washington Sea Grant Shellfish Growers Workshop as west coast shellfish growers are well represented at these venues; b) submission of articles and manuscripts to newsletters, trade publications, and peer-reviewed scientific journals; and c) creation of a production manual for the purple-hinge rock scallop describing hatchery and growout protocols.

ANTICIPATED BENEFITS: The goal of this proposed research and outreach is to expand the West Coast shellfish industry through creation of triploid seed and demonstration of efficient culture methods for the native purple-hinge rock scallop. A viable and growing shellfish industry in the United States is critical to maintain rural economies that are dependent on marine resource development and working waterfronts. Shellfish aquaculture is a low trophic level means of seafood production that provides many benefits to coastal communities and the environment, while at the same time increasing the supply of locally produced safe and nutritious seafood for

American consumers. Benefits include employment, economic diversification and growth, attractions for local ecotourism programs and shellfish festivals. There is also a strong desire to develop native species for aquaculture development to diversify the shellfish industry, help alleviate production declines associated with oyster seed shortages, and avoid concerns often voiced today about the use of non-native species.

PROGRESS AND PRINCIPAL ACCOMPLISHMENTS: Summarize the objectives and progress toward them during the year as a regional project unit. The work and findings should be presented as group activities for each distinct line of investigation, with credit for specific contributions as needed. Measurement data are to be given in metric units; however, a dual system of measurement may be used to express results. Where the project has not progressed to the stage of accomplishments, a brief description should be given of the activities of investigators/participants, detailing the status and expectations for the following year.

PROJECT OBJECTIVES: List each objective.

Specific Research objectives include:

1. Production of quantities of diploid and triploid rock scallop seed for growout trials, comparing growth, reproductive status, and survival of 3N and 2N progeny at multiple growout sites, and culture techniques in Alaska, Washington and California.

Pursuant to feedback on Year 1 progress received from the WRAC board, diploid production was emphasized in Year 2, including removing and mitigating production bottlenecks and increasing knowledge and development of husbandry techniques. Production of triploids was thus de-emphasized during Year 2.

YEAR 2 Production

Washington State Trials

The number of rock scallop broodstock in the Taylor hatchery was increased significantly in Year 2 because of the complementary work on rock scallop adaptive genetics currently underway under the auspices of a NOAA Aquaculture grant (A new native species for shellfish aquaculture and precautionary guidelines to protect wild populations: local adaptation, population structure and broodstock development in rock scallops (*Crassadoma gigantea*), Lorenz Hauser, PI). A total of four broodstock populations were thus available for production (Port Gamble, n=15, Dabob Bay , n=55, Cypress Island, n=75, and Sekiu, n=60). A series of six spawns occurred at the Taylor hatchery in 2015. Three volitional spawns occurred, apparently related to elevated temperatures and lunar phase.

Scallop production increased slightly between 2014 and 2015 rising from 5,000 in 2014 to a final count of around 7,500 seed in 2015. Almost all of these resulted from a single larval rearing cycle using a Kalwall tube with air diffusion. This is a promising indication that water motion and turbulence may seriously impact larval survival and that survival can be increased significantly by manipulations of aeration and water circulation. In 2015, the Taylor Hatchery had some serious production problems related to supersaturation and elevated temperatures.

Production bottlenecks identified to date include acquisition of high quality gametes, the need for improvement in hatching and larval husbandry, and a better understanding of how to promote setting. We are adaptively managing our larval rearing protocols to overcome these hurdles and increase seed production.

Gametes: Based only on qualitative criteria such as rounding, buoyancy, and fertilization rates, it appears that volitionally spawned oocytes are generally of higher quality than those obtained using serotonin injections. Obtaining clean gametes from rock scallops is extremely difficult because of the many associated facultative symbionts, some of which prey on rock scallop larvae. Efforts to reduce contaminants have been implemented via multiple rinses, UV sterilization, and broodstock preparation.

Hatching: Two types of hatching systems have been investigated at the Taylor Hatchery. Static 40,000 liter tanks performed better than smaller higher density systems. This is likely because at lower densities, unhealthy zygotes and contaminants cannot as easily infect healthy zygotes.

Larval Husbandry: Growth of larvae was highly variable among batches and populations. Despite broodstock maintained in common conditions and similar larval dietary ration, different batches of larvae progressed through the screen sizes (48, 60, 70, 80, 100, 118, 135, 160, 180 um) at different rates. This may be due to carryover maternal effects that varied the provisioning of certain oocyte populations, differential presence of probiotic organisms, or undetected bacterial infections that slowed growth. In a few cases, ciliate and copepod infestations were persistent problems despite careful screening and rinsing. The current strategy for larval care is to improve filtration and conduct thorough rinsing at the first two screenings.

Setting: Improving setting performance was discussed at length at the annual WRAC rock scallop workgroup meeting in March 2015 in Monterey, CA. Settlement substrates and flow characteristics were identified as important parameters to address. At the Taylor hatchery, three experimental settlement systems were set up to yield information on these parameters. In one of these a 9" d disc diffuser was placed in the bottom of the tube to ensure water movement and minimize dead zones. Almost all of the 7,500 seed produced came from this single Kalwall tube with air diffusion.

California Larval Rearing Trials
WRAC Scallop Culture Protocol (Draft) 2014-2015
Aquatic Resources Group
UC Davis Bodega Marine Laboratories

Broodstock Acquisition/Cleaning- Broodstock were hand collected locally in Bodega Harbor from concrete floating dock structures. Additional broodstock were acquired from Monterey Abalone Company and the Santa Barbara Channel.

Broodstock Maturation and Handling- Broodstock held in the lab awaiting spawning or being conditioned for spawning are kept on flow through seawater with some access to natural phytoplankton. Additional feeding typically occurs every other day with a slurry of suspended

cultured phytoplankton and Reed Mariculture shellfish diet. Animals are visually assessed periodically to assess gonad maturation

Broodstock Spawning- Broodstock is induced to spawn using serotonin injections, typically .25 – 1cc directly into the adductor muscle. Challenges related to serotonin induction include random gamete release with males typically releasing sperm quickly and females releasing eggs in a delayed time frame, i.e. males in 30 to 60 minutes vs females releasing 45 minutes to 6 hours.

Fertilization- Standard bivalve fertilization techniques were utilized. Eggs were collected, gently rinsed on a 20 micron wet screen, re-suspended in a known volume of clean seawater and counted. Sperm was collected, poured through a 20 micron screen to remove tissue and contaminants, and also counted in a known water volume. Sperm was added to a fertilization bucket containing eggs until desired concentration was achieved and confirmed by observation on the microscope. Average sperm to egg ratio was 5 to 7 sperm per egg with a range of 3 to 20 sperm per egg.

Post Fertilization Zygote Care and Stocking Larvae- Zygotes were stored in large round black static incubation bins, typically for 48 to 56 hours at 16 to 17°C. Swimming trochophore larvae, were harvested by decanting, consolidated on a 20 micron wet screen, rinsed to reduce contamination, re-suspended in a known volume, and stocked to larval culture cones at desired density, 1-2 larvae/ml, for grow out. Our last and most successful spawn had 650,000 larvae stocked to a 500 liter silo.

Larval Culture Pre-Settlement- Standard bivalve larval rearing techniques were used, wet screen down every third day, segregation of poor performing animals, good animals suspended in a known volume and counted, 500 liter silo cleaned and rinsed with heated fresh water and rinsed with heated filtered seawater, silo refilled with heated clean filtered seawater, algae fed to the desired concentrations, and animals returned to silo.

Larval Culture Post-Settlement- The larval silo was fitted with an internal standpipe that was topped with a banjo screen. This allowed a continuous flow of filtered makeup to be added to the culture. Different types of settling substrates were suspended in the culture. Algae was added to the cone daily, based on residual algae levels (80K-100K).

Juvenile Scallop Culture- The current crop of settled scallops are being held in a recirculating sea water system at 16 -17C. The animals are batched on 10" wide 175 micron screens with water supplied from above. They were moved out of the flow through settling silo on day 134. They are fed sterile algae daily, (C-Iso., Pav., Thal.) with constant densities ranging from 80k – 160K, with an average density of 100K.

Out planting Scallop Seed- Seed are currently being on grown at Bodega marine Laboratory but we have secured permission to out plant juveniles in Bodega Harbor on an oyster aquaculture flupsy privately owned by Chris Starbird.

Nutrition- Initial efforts to culture scallop larvae relied mainly on *C-Isochrysis sp.* These efforts produced weak larvae with poor survivorship and nutrition was identified as a limiting factor early on. Subsequent efforts relied on a mixed diet of *C-Isochrysis sp.*, *Nannochloropsis sp.*, *Pavlova sp.*, *Chaetoceros sp.*, and *Thallasiosira sp.*

Triploid Production

The following specific objectives have been met:

- Determining the optimal timing for production of 3N scallops by inhibition of second polar body extrusion. *This has been determined to be a 20 min treatment, 55-60 min post fertilization at 17 C.*
- Determining the optimal dosage of 6-DMAP for production of 3N scallops by inhibition of second polar body extrusion. *This has been determined to be 425 uM 6-DMAP.*

The following specific objectives have not yet been accomplished as we need to have mature triploid males and females:

- Determining 3N rock scallop gamete and 3N x 2N embryo ploidy and viability

2. Creation of tetraploid stocks for mating to diploid stock insuring offspring are 100% triploid.

The following specific objectives have not yet been accomplished as we need to have mature triploid females:

- 3N female x 2N male, inhibition of first polar body extrusion
- 3N female x 2N male, inhibition of second polar body extrusion

The following objective will be pursued in Year 3:

- 2N female x irradiated sperm from 2N male, inhibition of extrusion of both polar bodies.

The following objective will be pursued once we have mature tetraploids:

- 2N female x 4N male

3. Compare growth, reproductive status, and survival of 3N and 2N progeny at 14 growout sites in Alaska, Washington and California, including four collaborations with First Nation Tribes.

Growout trials have been initiated at three locations in Washington including the Suquamish Tribal site at Agate Pass, and two sites in California. Initial partners remain interested but insufficient production of seed has precluded initiating field trials at additional locations. Seed production remains the biggest challenge. Rock scallops from a previous project were collected to enable estimation of the size at cementation based on the imprint of the pearl nets, and the mean size at cementation for these Washington State rock scallops was 31 mm.

Specific Outreach objectives include:

1. Training shellfish growers through pilot demonstration growout at 14 commercial shellfish farms including four with Tribal affiliations, and transfer of hatchery technology for the

production of diploid and triploid seed to a hatchery in California and Alaska through focused technology transfer workshops. Disseminating information through a) presentations at shellfish conferences including the PCSGA annual conference and the annual Washington Sea Grant Shellfish Growers Workshop as west coast shellfish growers are well represented at these venues; b) submission of articles and manuscripts to newsletters, trade publications, and peer-reviewed scientific journals; and c) creation of a production manual for the purple-hinge rock scallop describing hatchery and growout protocols.

1. Joth Davis hosted Carrie Culver in Washington on June 8-9, 2015 for a technology transfer visit. They designed and implemented two commercial growout trials that are currently underway. Carrie subsequently established similar growout trials using naturally recruited seed at the Monterey Abalone Company. Broodstock from Monterey Abalone Company were shipped to Bodega Marine Laboratory for spawning in early September.

2. Joth Davis visited the Alutiig Pride Shellfish Hatchery in Seward, AK at the invitation of Jeff Hetrick on June 10-12, 2015. While there he consulted with hatchery staff on the details of spawning and rearing rock scallops. Time was spent reviewing all larval and seed rearing techniques used in Washington State including the use of serotonin injections to induce spawning in scallops. It was a useful and informative visit for all involved.

USEFULNESS OF FINDINGS: State how the findings may be or have been used for public benefit. Include specific examples, where possible. Estimates of acceptance and application of results, and of any economic values inherent in or accruing from them will be helpful in enlisting support for future research and outreach education. (Statements from this section may be used in future budget hearings and news releases).

To date we have identified mixed algal diets that support larval development, survival, and settlement of purple-hinge rock scallops. We have also evaluated a number of different larval rearing tank designs and water circulation techniques and identified some that seem promising. Determination of optimal protocols for consistent and reliable production of seed with higher survival will require further evaluation and replication.

Protocols for producing triploid seed have been established and future work on production of tetraploids and haploid sperm is planned.

These are all requisite steps in pursuit of our ultimate goal to develop protocols for production of scallop seed in sufficient quantities to involve all our commercial partners and establish a scallop sector in the shellfish industry.

SUPPORT: Use the format shown below to indicate all sources of funding and additional other support, federal and non-federal, for this project. Specify the name of the "other" sources as a footnote to the table.

INSTITUTIONAL NAME: Taylor Shellfish Co. approximate contributions

Salaries	
Oversight—Dave, Benoit	$1000
Prep and systems setup	$1700
Broodstock care	$965
Spawns	$2938
Seed	$865
Additional staffing	$1617
Total salaries	**$9085**
Benefits	**$2271**
Algae	2500
Supplies	$450
Equipment	$450
Hatchery space, utilities	$4,000

BUDGET: Annual reports for projects requesting a further year of funding must contain itemized budget breakdowns for each budget item for each PI. For each PI, use the budget spreadsheet sample and template found on G5 and G6. Because of increased federal scrutiny, failure to follow requirements on submission of budget information may jeopardize the request for next year's funds.

INSTITUTIONAL NAME: UC San Diego, Scripps Institute of Oceanography

	2014
Salaries:	$7,943
Student Title Graduate Student Researcher (GSR)	$7,943 9 months @ 25%
Hourly Title Student Assistant II undergraduate	
Benefits:	**238**
GSR	1.725%
Student Asst.	
Travel	$7,213
Equipment	
Supplies	$9,000
Other	$12,600
Total	**36,994**

INSTITUTIONAL NAME: University of Washington

	2014
Salary—0.25 FTE	$21933
Benefits 30.5%	$7088

Travel mileage and ferry to Taylor Shellfish Hatchery	$1341
Supplies	0
Equipment	0
Other Contractual Services Data storage Motor pool reimbursement Conference registration—NSA/PCSGA 2014 Conference registration—NSA annual meeting 2015 Total other contractual services	 $157 $54 $215 $450 $876

INSTITUTION NAME: Puget Sound Restoration Fund

	2014
Salaries:	**$13,332**
Technician Title Hatchery Technician	4 months @ 33%
Benefits:	**$2,400**
Technician Title Hatchery Technician	18%
Travel	$2,000
Equipment (used flow cytometer)	$0
Supplies	$2,000
Other (LD phone)	$2,010
Total	**$21,742**

WORK PLANNED FOR NEXT YEAR: Define specific work planned for the following year. State any proposed changes in direction or emphasis, or in the responsibilities or assignments of the participants.

Work will continue in year three as outlined below. The only significant change is that a concentrated effort will be sustained in California, Washington, and Alaska to identify optimal larval husbandry protocols to consistently produce hundreds of thousands of seed

Production of diploids

- **Year 1** production was >5,000 viable 30 mm seed.
- **Year 2** production was 7,500 in Washington and 200 in California
- **Year 3** production will continue at an expanded level of effort, which will be enhanced by knowledge gained from husbandry trials in Year 2.

Production scale triploid induction—

This was not achieved in Year 2, but is an important objective to achieve in Year 3 because there are a series of follow-on objectives that depend on having mature 3Ns:

- Determining 3N rock scallop gamete and 3N x 2N embryo ploidy and viability

- 3N female x 2N male, inhibition of first polar body extrusion
- 3N female x 2N male, inhibition of second polar body extrusion

Creation of tetraploid stocks--

The following objective will be pursued in Year 3:

- 2N female x irradiated sperm from 2N male, inhibition of extrusion of both polar bodies.

Additional Objectives:

Evaluate Kalwall Tube setting system

- In September/October, 2015, scallop larval rearing and settlement trials will be quantified between two aeration systems and among four substrate types.

Conduct Cementation Experiments:

- 100L tank substrate options—
  - color: dark, light
  - material: PVC, fiberglass, etc.
- glue to plates—
  - this experiment initiated in August 2015 is underway

Evaluate Maturation:

- size class preserving: histology
  - In 2015, we found very small scallops from CA (wild YOY) were already mature in the first year. Assessment of Washington scallops will be undertaken to document seasonality, size, and age at maturation.

Aging :

- Known age shells will be sent to WDFW to examine for presence of clear annuli.

PUBLICATIONS, MANUSCRIPTS, OR PAPERS PRESENTED: Attach a separate list of all publications to date. Under the subheadings of Publications in print and Manuscripts, list journal articles, popular articles, outreach materials, videos, technical reports, theses, dissertations, etc. (reference the "Transactions of the American Fisheries Society" for the preferred format). Under Papers presented, include the author(s), title, conference/ workshop, and date(s).

Jackson, M., Wykoff, S., Davis J., and B. Vadopalas. 2014. Advances in Rock Scallop *Crassadoma gigantea* Culture: Seed Production and Induction of Triploidy. 68th Joint annual meeting of the National Shellfisheries Association Pacific Coast Section and the Pacific Coast Shellfish Growers Association, Vancouver, Washington, USA, September 22-25.

Vadopalas, B, Jackson M., and J. P. Davis. 2015. Induction of triploidy in the purple-hinged rock scallop *Crassadoma gigantea* (Gray, 1825). 107th Annual Meeting of the National Shellfisheries Association, Monterey, California, USA, March 22-26.

Davis, J. P. 2015. Recent developments in purple-hinge rock scallop culture on the US west coast. Northeast Aquaculture Conference and Exposition and the Milford Aquaculture Seminar, Portland, ME, January 14-16, 2015.

SUBMITTED BY:

September 13, 2015

Project PI: Paul Olin

APPROVED: September 14, 2015

Project Monitor: Fred Conte