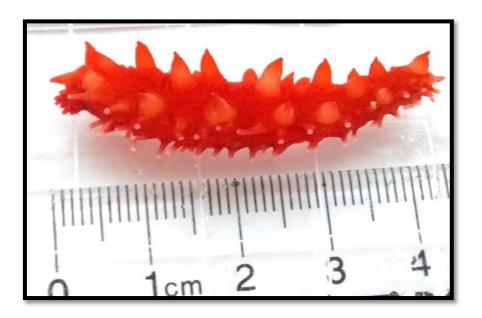
### FINAL REPORT - Saltonstall-Kennedy Program #NA15NMF4270322, 06/01/2015 - 05/31/2019

#### Development of Red Sea Cucumber (*Parastichopus californicus*) Poly-Aquaculture for Nutrient Uptake and Seafood Export

#### August 2019



#### Principal and Co-Investigators and Affiliations

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Photo: Juvenile red sea cucumber reared in the PSRF Manchester hatchery

#### **Goals and Objectives**

The overall goal of this project is to develop sea cucumber aquaculture in the United States through a collaborative effort addressing the following project objectives:

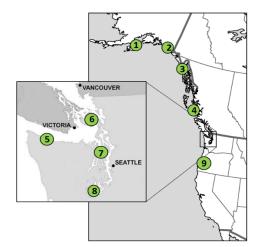
- 1. Examine the genetic population structure of spatially distinct aggregations of the sea cucumber *Parastichopus californicus*.
- 2. Develop hatchery and nursery technology in Manchester, Washington and Seward, Alaska.
- 3. Estimate mortality, initial growth, and target densities of sea cucumbers in co-culture with farmed mussels, salmonids or sablefish in Alaska (Ketchikan) and Washington (Quilcene Bay and Rich Passage).
- 4. Quantify sedimentation and water chemistry characteristics prior and during sea cucumber introduction (Dissolved oxygen, pH, carbon chemistry, nutrients, sedimentation rates and size) in Washington (Quilcene Bay and Rich Passage).
- 5. Advance federal, state and local permitting processes for sea cucumber aquaculture in Washington and Alaska.
- 6. Document and describe required steps for further development of commercial/farm-scale applications.

#### Accomplishments

### 1. Examine the genetic population structure of spatially distinct aggregations of the sea cucumber *Parastichopus californicus*.

Genetic samples were taken from collection sites in Alaska, British Columbia, Washington, and Oregon (**Figure 1**). Not shown was an additional collection site near Santa Barbara, California.

At least 50 individual sea cucumbers were sampled from each geographic region by removing a pea size portion of their muscle band via sterile technique and which was held in 100% ethanol for analysis by the University of Washington (**Figure 2**).



#### Populations

- 1. Chiniak Bay, AK
- 2. Yakutat, AK
- 3. Auke Bay, AK
- Bella Bella, BC
  Sekiu, WA
- 6. James Island, WA
- 7. Keyport, WA
- 8. Eld Inlet, WA
- 9. Charleston, OR

Figure 1: Map of collection sites.

Stewart Grant of Alaska Department of Fish and Wildlife provided samples from Alaska and British Columbia as part of a concurrent study looking at local population genetics in these areas. Sea cucumbers from California were collected by a trawl harvester out of Santa Barbara and sampled by Pacific Shellfish Institute (PSI) employee. Sea cucumbers were collected by Washington Department of Fish and Wildlife dive staff in Eld Inlet, by dive harvester Vassili Kalashnikov in the San Juan's, Strait of Juan de Fuca and Southern Oregon, and by Suquamish Tribal biologist dive staff in Keyport and processed by PSI staff.



**Figure 2**. Longitudinal mussel bands of a sea cucumber *Parastichopus californicus* with a sample taken out in the center for later genetic analysis.

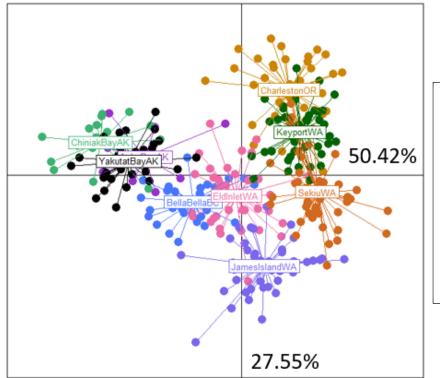
Graduate student Natalie Lowell and co-workers at the Hauser lab University of Washington described the progress made in conducting the genetic analyses. They faced a bioinformatic or data analysis challenge, in which fewer loci were observed prior to filtering with increasing sample sizes run through the dDocent pipeline (Puritz, Hollenbeck, & Gold, 2014). They circumvented this challenge by running individuals through the dDocent pipeline in batches, and merging results on shared single nucleotide polymorphisms (SNPs). SNPs were then filtered for maximum missing data of 30% per locus and 30% per individual, minimum minor allele frequency of 5%, minimum minor allele count of 5 reads, minimum genotype quality of 20, minimum genotype depth of 10 reads, and only one SNP per RAD locus. The final filtered data set produced 1680 SNPs. They ran an additional batch of repeated individuals to estimate genotype calling error in this batch method. This confirmed that genotype calling error was small at 1.02%, and entirely due to missing data as opposed to differently called genotypes.

Genepop was used to estimate global Fst and pairwise Fst (**Table 1**), and to test for global and pairwise genic differentiation (or genetic variation). They estimated global Fst to be 0.0083. A value of Fst=0 indicates complete similarity, whereas a value of Fst=1 suggests there is no overlap. The largest pairwise Fst estimates, denoting the most different population comparisons, were between Chiniak Bay, AK and Sekiu, WA, James Island, WA, and Keyport, WA (Fst  $\approx$  0.015). The global genic differentiation test was highly significant (chi2 < 7339.2, degrees of freedom = 3360), providing evidence for population structure. Pairwise genic differentiation test results demonstrated that 21 of 36 population pairs are significantly differentiated, including one pair of populations less than 100 km apart: James Island, WA and Keyport, WA (**Table 1**).

**Table 1**: Pairwise Fst. Values are shaded darker green for greater Fst values, and bolded if genic differentiation tests were significant, p < 0.05. Collection site code key: CB\_AK = Chiniak Bay, AK, YB\_AK = Yakutat Bay, AK, AB\_AK = Auke Bay, AK, BB\_BC = Bella Bella, BC, SK\_WA = Sekiu, WA, JI\_WA = James Island, WA, KP\_WA = Keyport, WA, EI\_WA = Eld Inlet, WA, and CH\_OR = Charleston, OR.

	CB_AK	YB_AK	AB_AK	BB_BC	SK_WA	JI_WA	KP_WA	EI_WA
YB_AK	0.0035							
AB_AK	0.0028	0.0021						
BB_BC	0.0084	0.0065	0.003					
SK_WA	0.0157	0.0132	0.0091	0.0086				
JI_WA	0.0156	0.0123	0.0098	0.0067	0.0048			
KP_WA	0.0141	0.0115	0.0083	0.0083	0.0036	0.009		
EI_WA	0.0098	0.0083	0.0051	0.0044	0.0065	0.004	0.003	
CH OR	0.0137	0.013	0.0085	0.0116	0.0084	0.0132	0.0037	0.0073

They conducted a discriminant analysis of principal components (DAPC) to explore patterns in variation due to population differentiation (**Figure 2**). Consistent with patterns in the DAPC, they found evidence for isolation by distance using a Mantel test (Mantel statistic r = 0.9069; p = 0.0014).



**Figure 2**: Discriminant analysis of principal components, using 298 individuals total and 1680 filtered SNPs. The first principal component explained 50.42% of the retained variance, and the second principal component explained 27.55%.

Using analyses of molecular variance (AMOVAs), significant population structure was observed at the following grouping levels: collection site, state, and within/outside Puget Sound (**Table 2**). Population differentiation and ecological association methods (Bayescan and OutFLANK) were used to identify loci putatively linked to selection. 32 loci were detected with at least one program and the sequences of these loci were aligned to the UniProt protein database. Of those with matches, gene ontologies were related to endosome membrane, smooth signaling pathways, and lysosome related functions.

In conclusion, our investigators found evidence of small but significant population structure at both small and large geographic scales, including significant differentiation among populations within Puget Sound. Population structure was significant at all groupings: across collection sites, across states/regions, and inside/outside of Puget Sound. This structure was driven at least in part by limited dispersal, as seen in the pattern of isolation by distance. Evidence for adaptive differentiation was much more limited, but this could be an artifact of the limited number of SNPs retained after filtering. It was concluded that further research will be needed to better quantify adaptive differentiation and the patterns that drive it.

**Table 2**: AMOVA results. For each of three different hierarchical grouping levels tested, the proportion of variation explained by this grouping (phi) and the p-value are reported.

AMOVA Results

Grouping	Phi	р
Collection site	0.0143615	0.001
State / region	0.01386325	0.001
Within / outside Puget Sound	0.004389083	0.001

#### 2. Develop hatchery and nursery technology in Manchester, Washington and Seward, Alaska.

A major accomplishment for this project has been the development and maintenance of an ongoing collaboration with the Alutiig Pride Shellfish Hatchery (APSH) and the Puget Sound Restoration Fund (PSRF) Ken Chew Center hatchery and the installation of the infrastructure and tools needed for sea cucumber rearing. PSRF staff worked collaboratively to develop protocols for spawning broodstock, rearing larvae and juveniles for feeding studies and multi-trophic aquaculture studies.

In 2016, PSRF staff completed design and installation of the necessary infrastructure and tools for rearing sea cucumbers. They built a 1500 square foot nursery system designed for rearing juvenile sea cucumbers and pinto abalone. They installed 40 tanks dedicated for setting and rearing juvenile sea cucumbers and fiberglass broodstock tanks (roughly 48" diameter and 26" deep) capable of holding about 25 individuals each. In 2018, they identified alternative broodstock holding locations and thus removed the broodstock tanks within the nursery to make room for additional juvenile rearing tanks (for use with sea cucumber or abalone). Existing hatchery-systems were used for spawning and larviculture work.

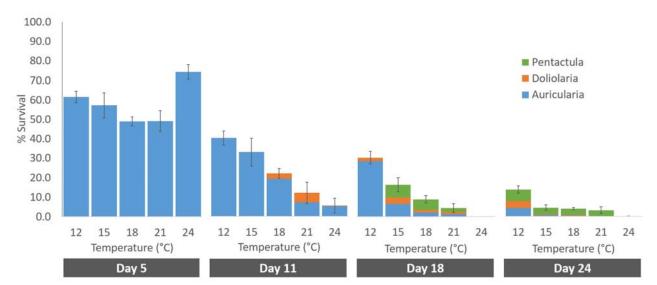
Adult sea cucumbers were collected via SCUBA from Clam Bay, directly adjacent to the Manchester hatchery, and immediately transferred to holding tanks. Animals were fed a variety of detrital material from various hatchery tanks. Multiple induced spawns were conducted in 2016, utilizing a variety of stimuli. The most successful stimuli was a +5C heat shock in a combined batch of roughly 20 animals. Males were fairly reliable typically initiating sperm release within 1 hour. Females were less reliable and only released eggs once sufficient sperm had been released in culture tanks. All spawning sea cucumbers typically displayed unique behaviors such as raising the anterior portion of their body into the water column and waving back and forth.

Initial spawning in 2016 resulted in successful fertilizations and gamete development; however, we were unsuccessful in rearing them through metamorphosis. It is believed the primary reason was the high temperatures experienced in the hatchery that year. In 2017 several successful spawns were completed producing more than 18 million eggs. A total of 11 females released eggs with quantities ranging from approximately 100,000 to 4,000,000 per female. At least 19 males spawned sufficient sperm quantities to use for fertilization crosses. In all spawning events, staff were able to isolate spawning individuals once they began releasing gametes to control parental crosses of offspring. Larvae from each parental cross were reared separately in most cases. In some instances, eggs were fertilized from multiple males separately and then pooled after fertilization. Larval survival to metamorphosis was good for 2 different spawning events, producing approximately 319,000 pentactula larvae that were set in our sea cucumber nursery system. The larvae from the other 2 spawning events failed to survive to metamorphosis, which was suspected to be temperature related. Both of those groups were being reared in mid-Summer when temperatures were high, ranging from 15-18C in the culture tanks. Each of the successful groups were reared at temperatures between 14-15C.

Juvenile survival was quite high in the first several months (roughly 25%) but growth was extremely slow. Juvenile's from both groups became visible without a microscope in early spring 2018 when they were roughly 2-10mm in length. At this point, the larger ones were beginning to develop their characteristic red pigment. Over the course of the next year, survival gradually declined (though there were no significant mortality events) and growth increased; however SK Final Report – September 2019 Page 6

notably asynchronously. By summer of 2019, we had over 1000 individuals remaining which varied dramatically in size from 1-2 cm to greater than 20cm in length. Significant variation in growth rates has been observed in other sea cucumber species, so this wasn't entirely unexpected; however, PSRF and PSI are currently exploring ways to reduce this variation through feeding trials funded through the Pacific States Marine Fisheries Commission.

In order to optimize our rearing strategies, we conducted multiple experiments aimed at identifying ideal temperatures and feeds for larvae and post-set juveniles. In 2016, we worked with Kendra Baird a Masters student at Evergreen State College to investigate the effects of temperature on larval development. Kendra designed a rearing experiment monitoring growth, survival and development at 5 seawater temperatures, 12, 15, 18, 21, and 24C. Her results showed that growth increased but survival decreased with higher temperatures (**Figure 3**). Sea cucumbers were capable of surviving in temps as high as 21C; however, survival was very low. No larvae survived at 24C. While survival was highest at 12C, growth was extremely reduced so that very few individuals made it to late larval stages. It was concluded that 15C was optimal when taking into account both survival and development. Kendra wrote her M.S. thesis on this research and received her degree in 2017.



**Figure 3**. Effects of seawater temperature on survival and development of *Parastichopus californicus* larvae at 5, 11, 18 and 24 days old (blue bars = auricularia stage larvae, orange bars = doliolaria stage larvae and green bars = pentactula stage larvae) (n = 6). Error bars are SE

Feeding experiments were conducted in 2016 and 2017 in an effort to improve both survival and growth. Sea cucumber larvae were fed a variety of microalgal diets commonly used and widely available in most shellfish hatcheries and monitored for survival and development to pentactula (metamorphosis) (**Figure 4**). Several algal strains appeared particularly good for development (*Isochrysis galbana*, *Rhodomonas salina*, and *Chaetoceros calcitrans*). Each of these strains resulted in the highest number of individuals reaching pentactula stage. Also 2 strains were identified to avoid (*Tetraselmis* sp. and *Thallasiosira pseudonana*) where both growth and survival was lower than the unfed treatment.

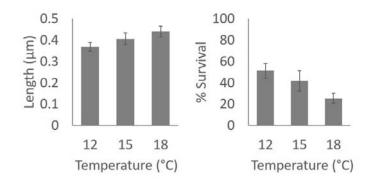


**Figure 4**. Sea cucumber in the pentactula stage (PSRF).

A later phase of this research was applied to juveniles (**Figure 5**) fed a variety of benthic diatoms. Our results showed overall very low growth rates over the course of 7 weeks, suggesting that either none of the strains were good food sources or they need something other than benthic diatoms during this stage. A subsequent experiment exposed post-set juveniles to 3 temperatures: 12, 15, and 18C. As in the feeding experiment, growth was limited in this experiment while survival was high. The juveniles were fed a mixed benthic diatom assemblage (same ones used for feeding trials) which we think limited their growth. However, we did see a trend of slightly increased growth and reduced survival with increasing temperatures (**Figure 6**).



Figure 5. Juvenile sea cucumber



**Figure 6**. Mean body length (left) and survivorship (right) of post-set *Parastichopus californicus* juveniles reared at 12, 15, and 18°C after 42 days.

In conclusion, the project identified that larval culture is best at 15C and the best larval feeds are the microalgal strains, *I. galbana, R. salina*, and *C. calcitrans*. While patterns were identified with the post-settlement culture experiments, the slow growth limited our ability to infer anything particularly significant from the results, other than the possibility that benthic diatoms may not be an important food source for post-settlement sea cucumbers of this species. For other species, benthic diatoms are reportedly good and important food sources for post-settlement juveniles. In addition, juveniles are set in the nursery system under a variety of haphazardly conditioned tanks. Typically, the highest growth and survival occurs in tanks with the most fouling.

The Puget Sound Restoration Fund will continue to maintain a sea cucumber nursery of animals produced by this grant. Feeding studies are now underway on a portion of these animals under a new project awarded by the Pacific Marine State Fisheries Commission in June 2018. Based on these findings the whole population is being fed a more robust diet. Outreach efforts continued at the Manchester facility with many school groups, professionals, government scientists and representatives visiting the hatchery in this time period. All were given tours where the sea cucumber project was discussed.

# 3. Estimate mortality, initial growth, and target densities of sea cucumbers in co-culture with farmed mussels, salmonids or sablefish in Alaska (Ketchikan) and Washington (Quilcene Bay and Rich Passage).

Alaska: Led by Dr. Charlotte Regula-Whitefield, Alaska outplant trials proved successful in determining how outplanted juveniles grow when placed into natural environments. Cages were deployed intertidally around Ketchikan along the Tongass Highway road system in regions with

different wave exposure and habitat characteristics. A total of 5 cage setups, each with three cages, were deployed in April 2016. Cages were deployed either horizontally in the subtidal zone or vertically hung off docks around Ketchikan. Overall, the caged experiments showed promising results in both their physical design as well as in juvenile *P. californicus* growth and survival. While biofouling was an initial concern, cages only needed to be maintained once during the 6 months of deployment. Maintenance included a light cleaning of the 1mm screen lining as well as removing any crab predators that had settled within the cages. In future experiments, the cage design (two halves of the Mexican trays) will be improved for maintenance purposes so that cages close more securely between inspections. In all cages, crabs grew to 2 - 4 cm in carapace length, and were large enough to begin preying on the juvenile sea cucumbers.

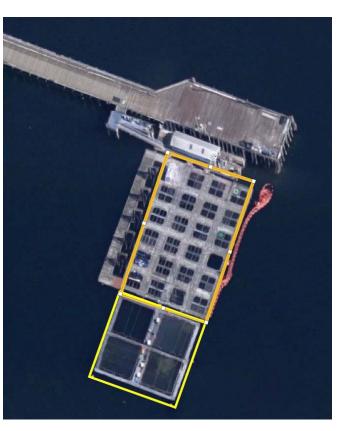
Horizontal cages had 0% juvenile *P. californicus* growth or survival. There are multiple reasons this could have occurred; most likely due to no microalgae biofilm formation. In contrast, vertical cages that were hung off of docks had high growth (roughly 30 mm) and survival (roughly 20 %). All cages also had high levels of natural sea urchin settlement, and vertical cages with higher sea urchin settlement rates appeared to have higher juvenile *P. californicus* growth. Juvenile *P. californicus* tended to be found under the bio saddles which had been placed in the cages at the start of the experiment. In subsequent cage experiments we believe it may be beneficial to have the cages packed fully with bio saddles or other plastic spacers; this would increase surface area for biofilms, provide more area for sea cucumbers, and could limit growth of crab predators.

**Washington:** Outplant growth trials started in March, 2017 in Washington State at the NOAA facility at Manchester WA (**Figure 7**). The outplant site was adjacent to the Ken Chew Center hatchery at Manchester and contained black cod (*Anoplopoma fimbria*) broodstock in a set of shallow net pens and a Viking pen full of market size black cod. A second growout site was developed at a mussel farm in Quilcene Bay Washington. Staff at a mussel farm collected wild set juveniles off mussel lines for the project. Over 50 juveniles were photo identified, weighed and measured and deployed on the mussel rafts in custom-built cages in May 2017.

During the project a small number of adult sea cucumbers held in upland tanks at the Manchester facility showed signs of skin ulcers/lesions in December 2016 and January 2017 after a significant cold spell. Around 10% of all animals died after forming these lesions. At the onset, sample organisms were collected and transferred to Dr. Ralph Elston at Aquatechnics for histological analysis. Brady Blake of the Washington Department of Fish and Wildlife (WDFW) was also consulted. A diagnostic report was received in March of 2017 without finding a cause for the lesions or mortalities. By that time the remaining sea cucumbers did not show signs of lesions. Ulcer disease (skin ulceration syndrome) is commonly reported in the sea cucumber literature and multiple bacterial species are thought to be potentially causal including the bacterial genera *Vibrio* and *Shewanella*. The latter genus is a long gram negative bacteria similar to those observed in the respiratory tissue in one of these specimens. This is clearly only suggestive but indicates that the skin ulceration syndrome seen in Puget Sound is similar to some of the cases reported in the literature.



**Figure 7.** Left overview of pier and research net-pens at NOAA's Manchester laboratory. Right, detail of pens. Pens in orange border do not touch the bottom at low tide. Each pen is: 16'x 16' x 12' WxLxD. Pens in yellow border touch the bottom at low tide.



Growth measurements were collected on a quarterly basis at the Quilcene and Manchester growout sites (Figure 8) with a total of 8 sampling events for 2 years of growth. Growth at the Quilcene site (Figure 9) was reduced in the fall and winter months but trended higher in the spring. Mussels were harvested in the fall and replaced with new mussel seed. We believe growth was reduced during this time due to a lack of detritus from overlying mature mussels. Under a new grant, a final measurement was taken place at the Quilcene site in July 2019. All sea cucumbers were harvested at this time and a split weight will be measured to provide an accurate market weight. Individuals will be frozen and provided to a direct to China exporter to assess the quality of the sea cucumbers. Overall,

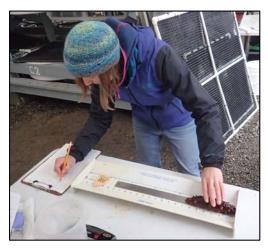


Figure 8. Quilcene sea cucumber measurements in November

sea cucumber growth was best at the mussel farm, after a boost in growth in the spring, sea cucumber growth was poor at black cod farm. This is likely due to the low numbers of black cod being reared that the farm in the second year of the project.

Settlement inside growout cages was tracked as well. Significant settlement of crab, shrimp, urchin and sea stars were noted at the Rich Passage (Manchester) site. A few red rock crab (*Cancer productus*) grew fast enough, likely feeding of other settled organisms, to start feeding on deployed sea cucumbers. Staff identified and enumerate settled species, removing predators as soon as they were spotted.

SK Final Report - September 2019

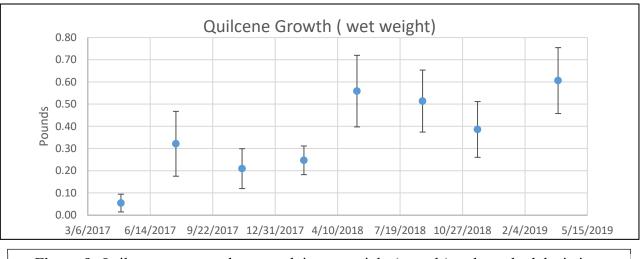


Figure 9. Quilcene sea cucumber growth in wet weight (pounds) and standard deviation.

**Local Utilization:** To assess the utilization of the local sea cucumber population near mussel farms, site surveys were conducted at the Quilcene growout site and at a Totten Inlet mussel farm. On July 31<sup>st</sup>, commercial sea cucumber harvester Vassili Kalashnikov dove inside Penn Cove's mussel farm in Quilcene bay. He recorded his observations on the presence/absence of sea cucumbers under and adjacent to the rafts and also at a control site a quarter mile North of the farm. No sea cucumbers were encountered at the control site while dense patches were found directly adjacent to the rafts with a few directly under the rafts when there was structure (rocks, rope, etc.) present. Additional surveys were conducted in the Fall and Winter of 2018 in Quilcene where significant numbers sea cucumbers were observed on structured habitats in corridors surrounding the mussel rafts. No sea cucumbers were observed at locations North or South of the mussel farm.

On September 18<sup>th</sup>, a dive survey was conducted on two Taylor Shellfish mussel farms, "Deepwater point" and "Gallagher cove", in Totten inlet, again by Vassili Kalashnikov. No sea cucumbers were encountered at multiple locations underneath, adjacent and at control sites near either farm. Additional towed video surveys were conducted at the farm and controls sites in the Fall and Winter of 2018. No sea cucumbers were encountered during these surveys.

## 4. Quantify sedimentation and water chemistry characteristics prior and during sea cucumber introduction (Dissolved oxygen, pH, carbon chemistry, nutrients, sedimentation rates and size) in Washington (Quilcene Bay and Rich Passage).

We proposed to expand the site knowledge for selected culture areas by detailing depth profiles for salinity, temperature, pH, and dissolved oxygen while collecting sedimentation/deposition rates. Water quality meters, YSI 6600 sondes, were continuously maintained at the Manchester and Quilcene sites. Quilcene data for the 2017-19 period are shown in **Figure 10**, monitoring continued for the duration of the project. Monitoring at Manchester was discontinued as of November 2018 following decommissioning of the NOAA fish culture system. Data from both sites were processed and analyzed, and provided to project partners and other interested parties including University of Washington researchers.

Carbon chemistry samples were taken during deployment and recovery of the water quality instruments for post calibration of pH and to determine other carbon chemistry values such as pCO2, TCO2 and omega aragonite saturation.

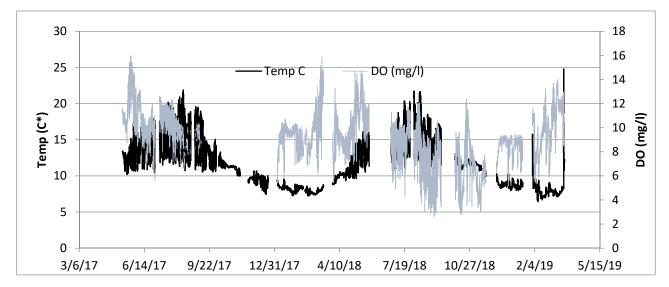
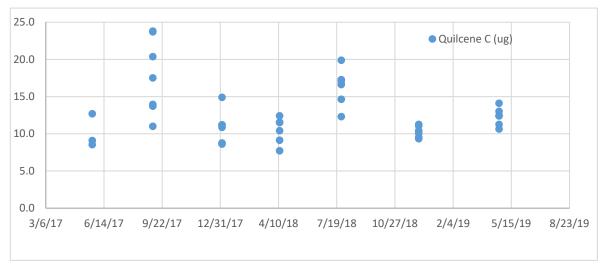
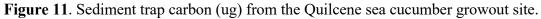


Figure 10. Water quality: temperature and dissolved oxygen (DO) at 20' depth on Quilcene mussel raft.

While no abnormal observations were reported at the Rich Passage site at Manchester, coccolithophore blooms were observed throughout much of Hood Canal during some of the reporting period. These dense phytoplankton populations affected water quality at the Quilcene Bay site. When the bloom crashed, mussel farm staff reported a mussel die off possibly linked to low dissolved oxygen. Low DO events were recorded during these times on a YSI 6600 unit.

Sediment trap samples were collected every 3 months at both sites with additional deployments needed to track changes based on lower fish densities at the Manchester site. All samples were analyzed by the University of Washington Marine Chemistry lab. The results of the carbon analyses for the project period to April 2019 are shown in **Figure 11**. These observations provided data on the availability, quantity and quality of organic biodeposits adjacent to and under mussel rafts and fish pens. The utilization and assimilation of these biodeposits by sea cucumbers was considered a key aspect of this project as it can further reduce nitrogen pollution.





## 5. Advance federal, state and local permitting processes for sea cucumber aquaculture in Washington and Alaska.

The principal output for this objective was to confer and coordinate with WDFW and NOAA staff for selection and utilization of outplant sites at a Quilcene mussel farm and a NOAA Manchester fish raft culture site. This has allowed and will continue to allow outplanting of hatchery reared sea cucumbers at Manchester and Rich Passage and natural set cucumbers at Quilcene and Point Whitney for cage culture of sea cucumber juveniles in open water and upland settings.

Efforts in Alaska were also continued via the Southeast Alaska Regional Dive Fisheries Association (SARDFA) for outplanting sea cucumbers for enhancement and co-culture with finfish and shellfish.

## 6. Document and describe required steps for further development of commercial/farm-scale applications.

Discussions continued with Alaska researchers as they conducted a feasibility study on sea cucumber enhancement and aquaculture for The Southeast Alaska Regional Dive Fisheries Association. A conference call reporting on the feasibility study was attended by PSI staff. Possible next steps include growout trials at an oyster farm and discussing the feasibility of developing a sea cucumber hatchery in Ketchikan.

In Washington additional steps toward commercialization are being pursued via new grants sources. These are addressing the feasibility of sea cucumber growout utilizing existing infrastructure and waste from other species. Shorebased trials are now being conducted at Manchester and Point Whitney Washington using readily available oyster and abalone waste, macroalgae co-culture systems and formulated feeds. All of these food sources and co-culture systems are being compared with that of growing sea cucumber via mussel waste. This SK project has shown the great potential of growing sea cucumbers with mussel waste but the addition of complimentary foods such as the brown macroalgae Sargassum may boost growth even further, reducing growout times. Discussions with WDNR staff also continued as the project team has SK Final Report – September 2019 Page 13

helped design laboratory and field based growth and cage trials.

#### **Presentations and Reporting**

PSI and PSRF staff provided many presentations and tours to the public during the project duration. The titles and venues for these presentations are listed below.

- Suhrbier, A. Cheney, D., Crim, R., Ryan, S., Baird, K., Lowell, N., Hauser, L. 2018, Giant Red Sea Cucumber (*Parastichopus californicus*) Puget Sound Hatchery Production, Growout Trials & Other Interesting Facts. Northwest Fish Culture Concepts Conference. December 2018. Portland, OR
- Suhrbier, A. Cheney, D., Crim, R., Lowell, N., Hauser, L., Elston, R. 2018, Sea Cucumber (*Parastichopus californicus*) Polyculture Trials and Hurdles in Washington State. World Aquaculture Society Meeting. March 2019. New Orleans, LA.
- Lowell, N., Vadopalas, B., Hauser, L., Jackson, M., Suhrbier, A. 2018. Population Genetics of Two Emergent Shellfish Aquaculture Species and Implications for Genetic Risk Assessments. World Aquaculture Society Meeting. March 2019. New Orleans, LA.
- Crim, R., Ryan, S., Baird, K., Suhrbier, A., Williams, P. 2018. Developing Aquaculture Techniquies for Giant Red Sea Cucumbers in Puget Sound: Broodstock, Larvae and Juvenile Culture. National Shellfisheries Association Meeting. March 2018, Seattle, WA
- Suhrbier, A. Cheney, D., Crim, R., Lowell, N., Hauser, L., Elston, R. 2017, Giant Red Sea Cucumber (*Parastichopus californicus*) Puget Sound Growout Trials & Other Interesting Facts. Pacific Coast Shellfish Growers Association – National Shellfisheries Association Pacific Coast Section Meeting. October 2017. Welches OR.
- Hetrick, J., Regula Whitefield, C. Sea Cucumber Production: The Alaska Experience. Pacific Coast Shellfish Growers Association National Shellfisheries Association Pacific Coast Section Meeting. October 2017. Welches OR.
- Suhrbier, A. Cheney, D., Crim, R., Lowell, N., Hauser, L., Elston, R. 2016. Sea Cucumber Aquaculture in Washington State, An Update on a Pilot Scale Study. Pacific Coast Shellfish Growers Association – National Shellfisheries Association Pacific Coast Section Meeting. October 2016. Chelan WA.
- Baird, K., Crim, R., Ryan, S., Suhrbier, A., 2016. The Effect of Temperature on Early Developmnet of the Giant California Sea cucumber in a Hatchery Setting. Pacific Coast Shellfish Growers Association – National Shellfisheries Association Pacific Coast Section Meeting. October 2016. Chelan WA.

- Ryan, S., Baird, K., Crim, R. 2016. The Effects of Microalgal Diet on Growth and Survival on California Sea Cucumber Larvae. Pacific Coast Shellfish Growers Association – National Shellfisheries Association Pacific Coast Section Meeting. October 2016. Chelan WA.
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