Reproductive biology of farmed purple-hinged rock scallop (*Crassadoma gigantea*)

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Abstract

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Native species are increasingly chosen for aquaculture expansion to avoid negative environmental impacts associated with the introduction of foreign species. However, the culture of native species is not without risks, as interbreeding with wild populations and can impact naturally occurring genetic diversity and population structure. The risk of adverse impacts depends, among other factors, on the frequency of interbreeding and genetic diversity of hatchery produced animals. Thus, cultured stocks that reach sexual maturity prior to harvest and have reduced genetic diversity because of small effective broodstock size, N_b, are particularly risky. Here, we evaluated both factors, first by establishing age and size at maturity and second, by determining the distribution of reproductive success and N_b in a voluntary mass spawn of purple hinged rock scallop, *Crassadoma gigantea.*

Age and size at first maturation were determined by repeat sampling of a known age cohort grown in Dabob Bay WA for 25 months. To establish the effects of local environment on

first maturation additional scallops were deployed in Totten Inlet, Dabob Bay, and Neah Bay for ten months and sampled once at the end of growout. Size and age at first maturation was 55.2 mm shell height and 25.1 months of age, well before the projected harvest size and age for cultured *C. gigantea*. The additive effect of age and size produced the most accurate prediction of first maturation in C. gigantea, and there was a significant effect of environment on first maturation suggesting that under optimal conditions first maturation could be even earlier than our size and age prediction.

To determine the distribution of reproductive success in a mass spawn of cultured *C. gigantea,* scallops were deployed at the three Washington State sites were sequenced using restriction site-associated DNA sequencing to identify SNPs for parentage assignment in COLONY. Three methods were then used to calculate the N_b from sibship assignments (COLONY), parentage assignment without parents (PwoP) and linkage disequilibrium (LDNe). A total of 408 loci were identified for parentage analysis and offspring were assigned to 74.5% of candidate parents at one or more sites. Survival was family specific, as N_b was significantly different across sites and N_b/N ratios were significantly lower than 1.00 at all sites (Totten Inlet=0.33, Dabob Bay=0.26, Neah Bay=0.15). Low N_b/N ratios suggest a loss of genetic diversity in the hatchery which is worsened by mortality on during growout and are an important consideration when determining the risk of genetic diversity loss in wild populations in the event of interbreeding. Unless wild population N_e/N ratios are orders of magnitude lower than their cultured conspecifics than escapes will reduce the genetic diversity of wild populations.

Our study determined that interbreeding between cultured and wild C. gigantea is possible, but the magnitude of this risk is reduced by the likely protandry and skewed sex ratio of young C. gigantea. In addition, increasing the number of broodstock used in each spawn, equalizing parental contributions prior to fertilization and using partial or full factorial spawn designs will help increase the N_b of cultured *C. gigantea*.

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Chapter 1. Age and Size at First Maturation of Farmed Purple Hinged Rock Scallops, *Crassadoma gigantea*, and its Implications for Management

1.1 ABSTRACT

Native species are increasingly chosen for aquaculture expansion to avoid negative impacts associated with the introduction of foreign species. However, the culture of native species is not without risks, as interbreeding with wild populations can impact their genetic diversity and population structure. In order to quantify the potential for interbreeding, information on the age and size at first maturation of farmed individuals is needed. Currently under consideration for commercial aquaculture production, the Purple Hinged Rock Scallop, *Crassadoma gigantea*, is endemic to the northeastern Pacific Coast. Current knowledge of *C. gigantea* reproduction consists primarily of observations on maturation seasonality in wild populations, but the relationship between age, size, and maturation across spatial scales has not been explored. Therefore, the aim of this study was to determine the age and size at maturity for farmed rock scallops in Washington State and to establish the effects of local environment on first maturation. By repeat sampling of a known age cohort, we determined the size and age at 50% first maturation to be 55.25 mm shell height and 25.1 months of age. We found that the additive effect of age and size produced the most accurate prediction of first maturation in *C. gigantea*, and that there was a significant effect of environment on first maturation. Our study determined that interbreeding between cultured and wild *C. gigantea* is possible, but the magnitude of this risk is reduced by the likely protandry, low fecundity, and skewed sex ratio of young *C. gigantea*.

1.2 INTRODUCTION

Global aquaculture production has increased 25.7% from 2000 to 2018 (FAO 2020). This increase has led to diversification in the aquaculture industry and created a demand for new species to cultivate. When choosing a new species for aquaculture, growers must select between cultivating a native or non-native species. Non-native species have the potential for negative ecological impacts on wild environments when escapes occur via invasive species introduction or new disease or parasite introductions. These introductions can lead to biodiversity loss, ecosystem degradation and native species extinction via direct competition, predation or indirectly via trophic cascades (Canonico et al. 2005, McKindsey et al. 2007, De Silva et al. 2009, Toledo-Guedes et al. 2014, Ju et al. 2019). The Food and Agriculture Organization of the United Nations (FAO) has therefore recommended caution with non-native species culture (Arthur et al. 2009). Following this recommendation and adopting precautionary approaches to aquaculture expansion, many natural resource managers now restrict permitting for new non-native species culture (Troell et al. 2014, Gentry et al. 2017). Many growers have therefore turned to farming native species (Beattie 1992, Paltzat et al. 2008, Davis 2015, Stekoll 2019) to avoid the negative environmental impacts and regulatory limitations associated with growing non-native species (Naylor et al. 2001).

The culture of native species, however, is not without its own risks. In addition to disease transmission (Lafferty et al. 2020) and impacts on environmental carrying capacity (Gallardi 2014), there are genetic risks to wild populations. If cultured individuals interbreed with wild conspecifics, there is the potential for loss of genetic diversity and fitness. This risk depends on the level of genetic differentiation between cultured and wild populations and the extent of interbreeding between them (Waples et al. 2012). The degree of interbreeding depends on the proximity of wild stocks, number of individuals escaping, spawn timing, spawn frequency, and the sex ratio of cultured groups (Waples et al. 2012, Baskett et al. 2013, Baskett and Waples 2013). Life history parameters are therefore critical to evaluate the potential for interbreeding and need to be estimated prior to cultivation. Many of these risks can be mitigated through appropriate management and husbandry but require a comprehensive knowledge of the native species' biology, ecology, and population dynamics (Lehnert et al. 2013, Gibbs and Browman 2015, Lin et al. 2015). In particular, age at first maturity in relation to harvest age is critical information when determining the potential for interbreeding.

First maturation in shellfish species, defined as the age or size at which 50% of individuals reach sexual maturity (Ropes 1968), typically corresponds to individual age and/or size and can vary among populations (Stearns and Koella 1986, Bayne 2004). First maturation timing in bivalves is quite varied, especially in the family Pectinidae, where age at maturation ranges from 2 months for the Atlantic Calico scallop, *Argopecten gibbus,* to 5 years for the Iceland scallop, *Chlamys islandica* (Barber and Blake 2016). Size at maturation for the family varies from 20mm, for *A. gibbus,* to >90mm for Ballot's saucer scallops, *Amusium balloti* (Barber and Blake 2016). In addition to age and size, environmental factors are known to influence first maturation (Giese 1959, Fabioux et al. 2005, Dieckmann and Heino 2007, Harney et al. 2013). For example, Cruz et al. (2000) deployed Catarina scallops, *Argopecten ventricosus,* in two separate bays on the Baja California Peninsula, one on the Pacific Ocean and the other on the Gulf of California. Scallops in the Pacific Ocean Bay reached first sexual maturation at age four months while none of the scallops in the Gulf of California bay matured. Thus, to characterize first maturation it is critical to determine not only size and age at first maturation but also the extent to which the local environment influences maturation.

The Purple Hinge Rock Scallop, *Crassadoma gigantea*, (J.E. Gray, 1825) is currently under consideration by the United States Pacific Coast shellfish industry for future commercial aquaculture production. Commercial production techniques as well as bottlenecks to production have been identified by Taylor Shellfish Farms, WA, Monterey Abalone Co., CA, and Annette Island Community, AK under the auspices of a Western Regional Aquaculture Center grant (2014-2017; PI: Paul Olin, CA Sea Grant, PI: Jonathan Davis, WA Puget Sound Restoration Fund). Wild populations of *C. gigantea* are distributed from Baja California to Alaska. Unlike most other pectinids, *C. gigantea* permanently cement to rocky substrate after free swimming as juveniles, making the species well-suited to a wide variety of culturing locations and techniques (Macdonald and Bourne 1989, Culver et al. 2006). Knowledge of *C. gigantea* reproduction is limited to estimates of spawning season from direct observation of wild populations. Wild populations have been observed spawning over an extended period, from October to January in southern California (Jacobsen 1977), from June to August in Puget Sound (Laurén 1982), and from June to October in British Columbia (Macdonald and Bourne 1989). However, information on the relationship between age, size, and maturation, as well as how these parameters vary among locales is lacking. To this end three growout areas were identified in Washington State, which represent potential locations for future *C. gigantea* aquaculture; Hood Canal and South Sound in Puget Sound, and the Strait of Juan de Fuca.

The Puget Sound can be divided into four main basins: Hood Canal, South Sound, Whidbey basin, and Main basin. Each basin is defined by a depression in the sea floor with a shallow sill separating it from adjacent basins (Burns 1985). Dabob Bay, in the Hood Canal was chosen due to the prevalence of shellfish aquaculture in and around the bay and its unique water chemistry, caused by the sill at the entrance to the bay (Steinberg et al. 2011). The Dabob Bay water column stratifies in summer resulting in warm surface temperatures, ideal for growing shellfish, and the water column mixes in late fall and early spring allowing increased productivity in the bay (Ebbesmeyer et al. 1980, Horner et al. 2005).

South Sound basin is home to the many aquaculture operations which could be easily converted to *C. gigantea* culture. The water column in South Sound is less stratified than in Hood Canal, due to its relatively shallow depth and greater tidal mixing (Babson et al. 2006) and this allows for high annual primary production which can support large aquaculture operations (Dumbauld et al. 2009). South Sound also lacks any known *C. gigantea* populations (Bob Sizemore, Washington Department of Fish and Wildlife, personal communications), which reduces the risk of genetic interactions in this basin.

Finally, Neah Bay, located on the western end of the Strait of Juan de Fuca is subject to coastal upwelling and therefor has cooler water temperatures and high acidification (Feely et al. 2008). Acidification has been shown to reduce calcification rates in juvenile Eastern Oysters, *Crassostrea virginica* (Waldbusser et al. 2011), juvenile Olympia Oysters, *Ostrea lurida* (Hettinger et al. 2012), and juvenile Hard Clams, *Mercenaria* (Miller and Waldbusser 2016) and could also be a stressors for *C. gigantea* juveniles. However, Neah Bay contains a large population of wild *C. gigantea* and is sheltered from the storms and strong currents found in the Strait of Juan de Fuca, making it a likely location for *C. gigantea* aquaculture. These three areas differ in annual water temperature and primary productivity but are all potential sites for future scallop aquaculture.

The aim of this study is to determine the age and size at maturity for farmed rock scallops in Washington State and to test the effects of local environment on first maturation. Our findings will help inform managers and growers to reduce the risk of interbreeding between wild and cultured *C. gigantea* populations.

1.3 METHODS

1.3.1 *Scallop Culture*

Wild adult *Crassadoma gigantea* (n=54) were opportunistically collected by divers at depths between 5 and 15 meters near Cypress Island and Burrows Channel in San Juan County, Washington State, on February 2, 2015, and transported to the Taylor Shellfish Farms Hatchery in Dabob Bay, WA (Figure 1). The scallops were held on the bottom of two 600L tanks (n=27 scallops per tank) and fed a live algae diet comprised of 10 species (Table 1) at a concentration of 100K to 150K cells per milliliter for conditioning. The water temperature was increased from 10°C to 14°C over a period of 12 days to expedite and synchronize gametogenesis.

Sex of these scallops was determined by visual inspection of the gonad. A thin broom bristle was inserted into the byssal notch of each individual and slowly moved across the mantle until the scallop gaped. A small wedge was then placed between the valves to prevent the valves from closing and therefore enable a visual examination of the gonad, deep red to orange for female and pale white for male. Individuals with no color and therefor no gamete were listed as unknown.

On April 17, 2015, a portion of the scallops volitionally spawned; 50 million fertilized eggs were collected from this spawn and reared at 16°C in flow-through 250L conical tanks. Larvae were fed the same live algae diet given to the broodstock. At 38 days post fertilization (May 25, 2015), settlement competency was observed (presence of an eye spot and foot, absence of a velum). Larvae were moved to two cylindrical tanks 60cm wide by 180cm tall and filled with artificial seaweed (Fukui North America, Ontario, Canada) to facilitate metamorphosis and byssal attachment.

In September 2015, five months after spawning, juvenile scallops (n=6000; mean shell height 5mm) were removed from artificial seaweed and transferred to 30 pearl nets with 3 mm mesh (n=200 per net). The pearl nets were suspended from floating lines in Dabob Bay adjacent to the Taylor Shellfish Farms Hatchery. The nets were hung in six stacks, containing five pearl nets spaced 30 cm apart. Each stack was suspended five meters below the surface to limit biofouling on nets. Thirteen months post-spawn (May 2016), the scallops (mean shell height 10 mm) were transferred to five hanging lantern nets with 4 mm mesh. Each lantern net was composed of eight compartments, and each compartment was stocked with 150 scallops. Eighteen months post fertilization (MPF) (October 2016), 400 scallops (mean shell height 40 mm) were transferred to two 10 mm mesh cages (n=200 per cage) and redeployed five meters below the surface in order to limit biofouling on cages. To determine age and size at first maturation, ten scallops were sampled at random from alternating cages semimonthly from January 2016 thru December 2017. Herein, we refer to this sampling group as the *temporal experiment*.

In addition to the temporal experiment, a second group of 1350 scallops were deployed to three Washington sites to investigate the influence of environment on first maturation. The sites, off long lines in Dabob Bay, mussel floats in Totten Inlet and in a marina in Neah Bay (Figure 1) represent an assortment of potential grow out conditions for cultured *C. gigantea*. From October 2016 to June 2017, nine 10mm mesh hanging cages (n=50 scallops per cage) were deployed in three stacks of three cages at each site. All stacks were suspended 3 meters below the surface in order to limit biofouling on the cages. The water temperature was measured at each site using a Hobo Pendant Temperature Logger (Onset, Bourne, MA) attached to one of the cages. The logger took a temperature reading every 30 minutes and daily maximum, minimum, and average temperature were calculated from these readings. Live scallops were collected and transported to the Taylor Shellfish Farms Hatchery on June 2, 2017 (Neah Bay) and June 9, 2017 (Dabob Bay and Totten Inlet) for gonad sampling. To account for any impacts of stocking density on maturation, survival was estimated from total counts of live scallops per cage at collection. This sampling group will henceforth be referred to as the s*patial experiment*.

1.3.2 *Tissue Sampling*

Shell height (mm), sex, and reproductive stage were recorded from each scallop. Shell height, defined as the greatest distance from umbo to the shell margin, was measured using digital calipers to the nearest 0.1mm. Tissue samples were collected from each individual for maturation analysis. For scallops with a shell height greater than 30.0 mm the gonad organ was fixed. If shell height was less than 30.0 mm the whole body was fixed, as the gonad was too small to remove without damage. The tissue samples were placed in histology cassettes (n=5 per cassette), fixed, and preserved using the PAXgene system (PreAnalytiX, Hombrechtikon, Switzerland) following the manufacturer's recommended procedure. The preserved tissues were then sent to Histology Consultation Services Inc. (Everson, WA) to be processed by routine paraffin histology, slide mounted and stained with hematoxylin–eosin (Luna 1968).

1.3.3 *Sex and Reproductive Classification*

The mounted and stained tissue sections were examined via light microscopy at 40x and 100x magnification. Sex was identified by the presence of male or female germ cells. Individuals without visible germ cells were classified as "unknown". Reproductive stage was assigned to each individual by examining three to four regions of the gonad section. Within each region, up to eight randomly chosen acini were classified until a total of 24 acini had been classified. Acini were assigned to one of six reproductive stages: inactive (0), early active (1), late active (2), ripe (3), partially spawned (4), and spent (5), modified from Ropes (1968). The criteria used to assign acini to each gonad stage are listed in Table 2 and Figure 2. The reproductive stage was assigned per individual using the mode of the 24 classified acini. Stages 0, 1, and 2 were defined as immature and stages 3, 4, and 5 as mature. Hermaphrodites, which were rarely encountered, contained limited quantities of male and female germ cells at different developmental stages precluding categorization for maturation analysis.

1.3.4 *Analysis*

Temporal Experiment: Following Ogle (2018), logistic regression models in R (version 3.4.2, (R Core Team 2017) using packages *FSA: Fisheries Stock Analysis* (version 0.8.16, (Ogle 2017)) and *car* (version 0.3.6, (Fox and Weisberg 2011)) were used to estimate age and size at first sexual maturity for all temporal samples. The general model for both age and size at first maturation was:

$$\log\left(\frac{p}{1-p}\right) = \alpha + \beta_1 X \tag{1.1}$$

where the dependent variable p was the probability of being “mature”, $1 - p$ was the probability of being “immature” and the independent variable, X, was either size or age. First sexual maturity was defined as the age or size at which 50% of sampled scallops were in stage 3, 4 or 5. Maturity was modeled as a function of age, shell height, and their potential interactions using generalized linear models (listed in Table 3). Models were evaluated using the Akaike Information Criterion adjusted for small sample size (*AICc*; (Akaike 1981). A binomial test was used to determine if sex ratios differed significantly from 1:1.

Spatial Experiment: The significance of differences in survival, reproductive stage (0-6), and maturity (immature (stage 0-2) vs mature (stage 3-5)) between sexes and among sites were tested via χ^2-tests. A χ^2-test was also used to determine if the sex ratio differed among sites and a *two*-sample *t*-test was used to determine if shell height differed significantly between males and females. A two-way ANOVA, using Type III sum of squares, was conducted to determine the effect of location and maturity (immature vs. mature) on shell height. To test the normality assumption, a Shapiro-Wilk test was run on the residuals of the two-way ANOVA. To test the homoscedasticity assumption, Levene's test was run on the ANOVA. A Tukey post-hoc test was used to identify which locations resulted in significantly different shell heights.

To determine if the percentage of mature individuals at each site was significantly different from the temporal experiment prediction for age and size, the uncertainty of each logistic regression was calculated. This was done using each site's age or average shell height at final sampling and bootstrapping each regression 1000 times. For each bootstrap a draw was simulated from a binomial probability given the proportion mature and sample size at each site to determine the uncertainty in the binomial outcome. The p-values were calculated from the frequency of draws greater than or equal to the observed percentage of mature individuals at each site.

1.4 RESULTS

1.4.1 *Scallop Culture*

Using visual inspection of the gonad we identified 47 individuals with a discernable sex and 4 individuals of unknown sex. For individuals where sex was discernable 22 individuals were female and 25 were male.

1.4.2 *Temporal Experiment*

A total of 428 individuals were sampled and classified for reproductive status during the entire temporal experiment. Of these, 217 were males, 15 females, 4 hermaphrodites and 192 were classified as unknown because germ cells were not observed (Figure 3). The overall ratio of males to females, 14.5:1, differed significantly from 1:1 (binomial test, $p < 0.0001$). During the first 15 months of sampling the majority of individuals were classified as unknown with a spike in males observed from November to January (18.9 to 20.1 months post fertilization (MPF)). Beginning in April (23.1 MPF) most individuals were male. The first female was identified in March (22.1 MPF) and the first hermaphrodites in May (24.0 MPF) (Figure 3).

Over the course of the experiment, 297 individuals were found to be immature (Stages 1, 2 and 3) and 127 individuals were mature (Stages 4, 5 and 6) (Figure 4). The first mature individuals were detected in January 2016 (20.6 MPF), but most samples were not identified as mature until July 2016 (25.4 MPF). There is evidence for two waves of maturation in a single year, from January to April (20.6 to 23.1 MPF) and again from June until sampling was completed (25.4 to 31.6 MPF) (Figures 4 and 5). Single predictor logistic regressions revealed the age at 50% maturation to be 25.10 months after fertilization (Figure 6) and the shell height at 50% maturation to be 55.25mm (Figure 7). The additive effect of age and size was the best predictor of first maturation (Table 3).

1.4.3 *Spatial Experiment*

Average daily water temperature as well as daily maximum and minimum differed among the three sites (Figure 8). Totten Inlet had the lowest daily temperature variation while Dabob Bay had the largest. Among sites, maximum temperature varied more than minimum temperature (Table 4).

Mortalities were low during the first eight months of growout with most scallop deaths observed in the last two weeks of growout. No evidence of predation was found at any site in any of the cages during the experiment. Survival differed significantly among sites ($\chi^2 = 671.15$, $df = 2$, $p < 0.001$), as did sex ratio ($\chi^2 = 18.16$, $df = 2$, $p = 0.0001$). Totten Inlet had the highest survival and highest proportion of males while Neah Bay has the lowest survival and the lowest proportion of males (Table 4). A significant difference was observed between male and female shell height ($t = 3.7$, $df = 251$, $p < 0.001$). Average shell height was 61.6mm (±6.8) for females and 59.2mm (±7.3) for males.

The proportion of mature individuals differed significantly among sites ($\chi^2 = 151.3$, $df = 2$, $p < 0.0001$) with the lowest proportion of mature individuals observed in Dabob Bay and the highest proportion in Totten Inlet, where no immature individuals were observed. Reproductive stage also differed significantly among sites ($\chi^2 = 580.03$, $df = 5$, $p < 0.000$). In Neah Bay and Totten Inlet most individuals were classified as Stage 4 and in Dabob Bay the majority of individuals were classified as Stage 5. More Stage 5 and 6 individuals were observed in Neah Bay and Dabob Bay than in Totten Inlet (NB: 40%, DB: 50%, TI: 25%) (Table 4).

The two-way ANOVA with type III sum of squares indicated a statistically significant effect of location on shell height (ANOVA, $F = 13.98$, $df = 2$, $p < 0.001$) but not of maturity on shell height (ANOVA, $F = 0.33$, $\mathrm{df} = 1$, $p = 0.57$) (Figure 9Figure 9). The Shapiro-Wilk normality test was significant ($W = 0.98$, $p < 0.001$) indicating a departure from a normal distribution however the Levene's test was not significant ($F = 1.51$, $df = 5$, $p = 0.18$), indicating compliance with the homoscedasticity assumption. A post-hoc Tukey comparison of means found that shell height in Totten Inlet was significantly greater than both Dabob Bay ($p < 0.001$) and Neah Bay ($p < 0.001$) (Figure 9Figure 9).

Bootstrap analyses indicated that there were significantly more mature scallops at all three sites than predicted by the age at maturation model ($p < 0.001$ for Totten Inlet and Neah Bay and $p = 0.023$ for Dabob Bay (Figure 6). Using the average size at each site, Totten Inlet and Neah Bay had significantly more mature individuals than the temporal experiment's size at maturation prediction ($p < 0.001$) while the proportion mature at Dabob Bay was not significantly different from the size at maturation model prediction ($p = 0.49$) (Figure 7).

1.5 DISCUSSION

The intent of this study was to determine the age and size at which 50% of farmed *C. gigantea* reach first maturation and to establish whether local environment affects the timing of first maturation. To our knowledge, this experiment is the first to examine the dynamics of first maturation in the Purple Hinge Rock Scallop. By repeat sampling of a known age cohort, we determined the size and age at first maturation to be 55.25 mm shell height and 25.1 months of age. We found that the additive effect of age and size produced the most accurate prediction of first maturation in *C. gigantea*, and that there was a significant effect of environment on first maturation.

1.5.1 *Age and size at maturity*

Age and size at first maturation for *C. gigantea* in Washington State*,* 55.25 mm shell height and 25.1 months of age, was within the range of the family Pectinidae. Our estimates for age at first maturation are similar to other scallop species that overlap in range along the Pacific Coast of North America. Both *Chlamys rubida,* the Pink Scallop and *Chlamys hastata,* the Spiny Scallop mature at 24 MPF (Parsons et al. 2016), only one month earlier than *C. gigantea*. In contrast, *C. gigantea* matures later than scallops that occur further south, *Argopecten ventricosus* the Pacific Calico Scallop (4-5 months) (Cruz et al. 2000) and *Nodipecten subnodosus* the Giant Lion-paw Scallop (10 months) (Arellano-Martínez et al. 2011) but earlier than *Patinopecten caurinus* the Weathervane Scallop (3-4 years) (Hennick 1970) which occurs further north. In contrast, this latitudinal gradient in age at maturation does not exist in abalone species that also range along the

Pacific Coast of North America (Tutschulte and Connell 1981, Breen et al. 1988, Shepherd et al. 1992).

Model comparison found that the additive effect of age and size produces the most accurate prediction of first maturation in *C. gigantea* (Table 3), demonstrating that the inclusion of both metrics increases the predictive capacity of the model. This result is not surprising given use of both predictors is common when plasticity in first maturation is evident (Grecian et al. 2003, Olsen et al. 2005, Sharpe and Hendry 2009). This is particularly true for cultured species where hatchery environments can extend spawn seasons beyond the norms of wild populations, producing juveniles months before and/or after wild populations would normally reproduce (Breese and Malouf 1975, Utting and Spencer 1991). The resulting change in spawn schedule can alter the normal correlations for age and size if temperature and food availability vary with the season. In these situations a Probabilistic Reaction Norm (PRN) is often used to determine first maturation as a function of both age and size (Heino et al. 2002, Dieckmann and Heino 2007). While outside the scope of this investigation, establishing a PRN for *C. gigantea* would allow managers to predict first maturation more accurately in cultured populations instead of relying on a single predictor.

1.5.2 *Environmental effects*

The significant difference in percentage of mature individuals found across the three spatial experiment sites could have been the result of differences in growth rate and thus size at maturation. Totten Inlet had the highest proportion of mature scallops as well as the largest average shell height. Differences in size at maturation have been observed across an environmental gradient in the abalone *Haliotis iri* (Hooker and Creese 1995) and the Catarina Scallop, *Argopecten ventricosus* (Cruz et al. 2000), where accelerated growth led to earlier maturation. The faster growth in Totten Inlet could be a result of optimal temperature conditions, as Totten Inlet had the smallest daily temperature variation and less fluctuations in temperature over the course of the experiment when compared to the other sites (Figure 8Figure 8).

In addition to maturation and growth, significant differences in survival were observed across the growout locations, with the highest survival in Totten Inlet and the lowest in Neah Bay. While survival was significantly different, it did not impact maturation as Totten Inlet and Neah Bay had the highest and lowest survival, but both sites had similar proportions of mature individuals (Table 4). While the exact cause of mortality is unknown, predation can be ruled out as no evidence for predators were found in any of the cages during growout. The majority of mortalities at Neah Bay and Dabob Bay were observed during the last two weeks of growout. It is likely that the high daily temperature fluctuations recorded in Dabob Bay during these weeks (Figure 8Figure 8) contributed to the observed mortality at that site as mass mortalities have been attributed to sudden changes in temperature in the Giant Scallop, *Placopecten magellanicu* (Dickie and Medcof 1963), and the Japanese Scallop, *Patinopecten yessoensis* (Jo et al. 2009). An alternate explanation for the observed differences in survival among sites could be that survival differed among families (see Chapter 2). While outside the scope of this experiment, future investigations could elucidate whether differential family survival contributed to the observed survival differences.

Scallops were deployed in Dabob Bay for both the spatial and temporal experiments, enabling a direct comparison of model predictions between the two experiments. As expected, the proportion of mature scallops for the spatial experiment was within the temporal experiment prediction interval for size at maturation. However, we found a marginally significant difference (p = 0.023) in the prediction for age at maturation, the temporal model predicted 63% mature but spatial sampling found 71% mature (Figure 6). This discrepancy was likely due to the difference in stocking density between experiments. The spatial experiment used a much lower initial stocking density of 50 individuals per cage, compared to the initial stocking density of 200 individuals per cage in the temporal experiment (decremented by 10 individuals every other week). The lower stocking density in the spatial cages likely reduced food competition, allowing for a marginally earlier maturation in the spatial scallops. In contrast, the observed proportion mature in both Neah Bay and Totten Inlet were significantly higher than the temporal experiment's predictions for both age and size, suggesting that first maturation could be even earlier than our model prediction if growing conditions are optimal (Figures 6 and 7).

1.5.3 *Implications for Management*

First maturation, when 50% of individuals reach sexual maturity, has been determined for many aquaculture species, including Geoduck, *Panopea generosa* (Vadopalas et al. 2015), Olympia Oyster (*Ostrea lurida)* (Hare et al. 2011), Blue Mussel, *Mytilus edulis* (Toro et al. 2002) and Manila Clam, *Ruditapes philippinarum* (Moura et al. 2018). However, perhaps a more critical measure for determining interbreeding potential is the age and size of the first mature individual in a population. For *C. gigantea,* the first mature scallop appeared after only 20 months and had a shell height of 40 mm, a full five months earlier and 15mm smaller than the first maturation calculation (Figure 5). All that is needed for interbreeding with wild individuals is a single mature captive individual. First maturation in *C. gigantea* falls well before the predicted harvest age of three to four years and size (100-110mm shell height) determined by Davis et al. (unpublished). Therefore, an interbreeding potential between cultured and wild *C. gigantea* does exist during growout.

Interbreeding can occur via two mechanisms: directly between cultured and wild animals, or indirectly if cultured individuals mate with each other and their progeny reproduce with wild individuals (Waples et al. 2012). Direct interbreeding requires two primary conditions. First, spawn times of both groups must overlap. Spawning of wild *C. gigantea* in Puget Sound has been observed from June to August (Laurén 1982). This timing corresponds to our observations, suggesting that spawn timing of cultured and wild *C. gigantea* is synchronous. Second, wild origin and cultured scallops must be close enough for gametes between the two groups to mix. Fertilization success in many marine broadcast spawners is highly dependent on gamete concentration, in particular sperm concentration (Levitan and Petersen 1995). Gamete concentration is of particular importance for aggregating populations like *C. gigantea*, which typically have small population sizes that in turn limit the total number of gametes produced in a single spawning event. Fertilization success drops dramatically at a distance of more than 1 to 4 meters in the abalone *Haliotis laevigata* (Babcock and Keesing 1999) and greater than 1 meter for the scallop *Placopecten magellanicus* (Bayer et al. 2016). If these distances are similar for *C. gigantea,* then the risk of direct interbreeding between wild and cultured scallops could be managed by avoiding farming in close proximity to wild populations. Further investigation is needed to determine a suitable distance for *C. gigantea*.

Indirect interbreeding is influenced by the number of F1 offspring from cultured individuals escaping into the wild. The number of escapees can be controlled by limiting the number of crosses between cultured individuals, for example, by a highly skewed sex ratio. Sex ratios at all sites were highly skewed towards males for all growout month, (last temporal sampling was at 32 MPF) demonstrating protandry for *C. gigantea.* For the months of May, June, and July in 2017, 97% of the scallops sampled were male and none were classified as unknown. Protandry is not unexpected in bivalves given male dominated sex ratios in other species shortly after first maturation; for example, in New Zealand geoduck (*Panopea zelandica*) (Gribben and Creese

2003), Japanese Scallops (*Patinopecten yessoensis)* (Silina 2018), Pacific Oyster (*Crassostrea gigas*) (Guo et al. 1998) and Eastern Oysters (*Crassostrea virginica)* (Mackie 1984, Wilbur et al. 1984). These species are all protandrous, starting life as males and transitioning to females later in life. Protandry limits the potential for mating between farmed individual as there are relatively few females in young protandrous cultured groups, thus a limited number of eggs which can be fertilized by cultured males and become escaped F1 offspring.

The risk of direct interbreeding in protandrous species is further diminished by the relative fecundity of wild and cultured conspecifics. Reproductive output in *C. gigantea* increases with size (MacDonald et al. 1991) so the fecundity of smaller cultured populations may well be less than that of their larger wild counterparts. Bull (1976) found that while New Zealand Scallops, *Pecten novaezelandiae,* reach sexual maturity in their first year, they did not spawn but rather resorbed gametes. Reproductive output in their second year was three times greater than the first. Therefore, it is highly likely that cultured rock scallops have far lower fecundity than their larger wild conspecifics due to their smaller size and young age. If the fecundity of cultured scallops is less than that of wild conspecifics, than the magnitude of genetic risks may be relatively lower. Quantifying the effect of protandry on individual reproductive success of wild and cultured *C. gigantea*, while outside the scope of our investigation, is an important next step to help clarify the magnitude of risk associated with interbreeding in *C. gigantea*.

Our study has determined that both direct and indirect interbreeding between cultured and wild *C. gigantea* is possible, as first maturation occurs prior to scallops reaching a commercially viable size. The magnitude of this risk is reduced by the likely protandry, low fecundity, and skewed sex ratio of young *C. gigantea*. These risks may be further reduced by distancing growout operations away from substantive wild populations and prioritizing harvest at smaller sizes whenever possible. Further investigations into relative fecundities, changes in sex ratios over time, and growout location effects are critical to understand the extent and likelihood of genetic risks to wild populations from farmed *C. gigantea*.

Chapter 2. Reproductive Success in a Mass Spawn of Purple Hinged Rock Scallop, *Crassadoma gigantea,* Reared in Different Environments and Implications for Aquaculture

2.1 ABSTRACT

Low effective population size over census population size (N_e/N) ratios are common in marine invertebrates and can be the result of high variance in reproductive success, unequal sex ratios, high genetic load or fluctuating population size. Naturally occurring low N_e/N ratios can be further diminished if cultured individuals interbreed with wild conspecifics, as large commercially produced cohorts often have small N_e. Low N_e in cultured cohorts can be due to few parents used in a spawn, spawn designs with non-random mating, unequal sex ratios and or high variance in reproductive success. Hatchery production techniques for *Crassadoma gigantea* rely on mass spawns, with unknown impacts on the N_e and genetic diversity of the resultant offspring. Here, we estimated the effective number of breeders N_b, producing a single cohort of offspring in a mass spawn and determined how environment and differential survival after outplanting impact parental contribution to the next generation. Offspring were deployed in three Washington State sites (Totten Inlet, Dabob Bay, Neah Bay) for ten months. Restriction site-associated DNA sequencing was used to identify 408 SNPs for parentage assignment**.** Although 75% of parents contributed to the spawning event, the effective number of breeders was only 17-35% (N_b=9-18) of the census number of parents (N=51) due to skewed sex ratios and high variance in reproductive success. Even though all offspring originated from the same spawning event, estimates of N_b differed between sites, suggesting family specific survival. Increasing the number of broodstock used in each spawn, equalizing parental contributions prior to fertilization, using partial or full factorial spawn designs, and maximizing survival would help increase the N_b of cultured *C. gigantea* and

reduce the risk of genetic diversity loss in wild populations in the event hatchery individuals escape, or if hatchery and wild individuals interbreed.

2.2 INTRODUCTION

Traditionally, marine populations were thought to harbor high levels of genetic diversity due to expected negligible genetic drift associated with their large size (Hauser and Carvalho 2008). However, the rate of genetic drift depends on the effective population size (N_e) and not the census size (N) (Allendorf et al. 2012). Although the N_e/N ratio is expected be less than one in most species, it is considerably lower in some marine species (Hauser and Carvalho 2008), particularly in marine invertebrates (Hedgecock and Sly 1990, Hedgecock et al. 1992, Hedgecock et al. 2006), resulting in profound implications for evolutionary processes.

One hypothesis that aims to explain low N_e/N ratios in marine species is the Sweepstakes Reproductive Success hypothesis, in which a low N_e/N ratio is attributed to high variation in relative reproductive success (Hedgecock 1994). High variation in relative reproductive success may be due to vulnerable early life history stages of marine invertebrates and stochastic fluctuations in marine environments that cause family specific survival. High genetic load in many marine organisms, especially bivalves (Bierne et al. 1998, Launey and Hedgecock 2001), can also contribute to high variation in relative reproductive success. Specifically, genetic load can result in incompatibilities between individuals, limit the success of some parent crosses and result in non-random mating (Plough 2016). Lastly, fluctuating population size, common to marine invertebrates, can decrease the N_e/N ratio (Lind et al. 2009).

Naturally occurring low N_e/N ratios in wild populations can be further diminished when captive and wild populations mix. Hatcheries produce large numbers of offspring for commercial harvest and non-random mating, unequal sex ratios and high variance in reproductive success can drastically reduce N_e/N ratios in these cohorts (Ryman and Laikre 1991). In addition, commercial cohorts are often produced with relatively few parents compared to wild reproduction and result in a further reduction in cohort N_e. Through interbreeding of cultured and wild conspecifics, N_e/N ratios in wild populations can decrease (Hedgecock and Coykendall 2007, Waples et al. 2012). Some breeding designs, such as full factorial crosses and designs with equalized parental contribution, can maximize the effective number of breeders and mitigate reductions in N_e (Fiumera et al. 2004, Busack and Knudsen 2007). However, husbandry constraints and space limitations make these option unfeasible for many commercial hatchery operations, leading to a common practice of pooled or mass spawns, with many parents placed in a single tank and allowed to spawn volitionally (Boudry et al. 2002, In et al. 2016). Reductions in allelic richness associated with genetic drift have been found when practices based on mass spawns were used in Pacific Oyster, *Crassostrea gigas* (Hedgecock and Sly 1990, Li et al. 2009), Eastern Oyster, *Crassostrea virginica* (Gaffney et al. 1992), Giant Clam, *Tridacna gigas* (Benzie and Williams 1996), Rainbow Abalone, *Haliotis iris* (Smith and Conroy 1992) and Chinook Salmon, *Oncorhynchus tshawytscha* (Withler 1988).

Such processes occurring during the production of a single cohort of species with overlapping generations are best measured as the effective number of breeders, N_b. This measure is particularly useful tool for hatcheries where cohorts are produced each year and N_b can be used to ensure that high levels of diversity are maintained (Ackerman et al. 2017, Bartron et al. 2018) and thus prevent the loss of genetic diversity in wild populations if interbreeding or escapes occur (Waples et al. 2012).

The Purple Hinge Rock Scallop, *Crassadoma gigantea*, (J.E. Gray, 1825) is currently under consideration by the United States Pacific Coast shellfish industry for future commercial aquaculture production. Wild populations of *C. gigantea* are distributed from Baja California to Alaska. Unlike many other pectinids, *C. gigantea* permanently cement to rocky substrate after free swimming as juveniles, making the species well-suited to a wide variety of culture locations and techniques (Macdonald and Bourne 1989, Culver et al. 2006). The life span of *C. gigantea* is estimated to be at least 20 years (MacDonald et al. 1991) making them a relatively long-lived species. Although *C. gigantea* fecundity has not been estimated it is likely to be high, given other scallop species produce hundreds of thousands to millions of eggs in a single spawn (Cochard and Devauchelle 1993).

Recent work by Davis et al. (personal communication) to establish production techniques for the culture of *C. gigantea* found the creation of full sib families to be extremely difficult for this species, and mass spawns were deemed the most viable option for commercial production. However, the impact of this breeding design on the N_b of cultured *C. gigantea* is yet unknown. Determining the N_b of cultured *C. gigantea* is necessary to begin quantifying the potential risk to wild populations if interbreeding occurs with hatchery-produced individuals. Growers will be dependent on wild populations as a source for broodstock due to current regulations, which discourage the use of cultured animals as broodstock to reduce the risk of domestication selectin in cultured groups (Brady Blake, personal communication). Therefore, growers and natural resource managers are interested in minimizing loss of genetic diversity and fitness in wild *C. gigantea* populations to ensure that diverse sources of broodstock are available for hatcheries and to maintain the adaptive potential of wild populations.

In this study, we aim to determine whether progeny from a mass spawning event of cultured *C. gigantea* randomly represent each potential parent. The objectives of this study were to (1) assign parentage of progeny produced in a mass spawn, (2) determine how environment and differential survival impact the contribution of each parent at different growout sites and (3) estimate N_b at each location to determine the genetic diversity at each location. We utilized restriction site-associated DNA sequencing (RADseq), a next generation sequencing method that can identify thousands of genetic markers randomly distributed across the target genome (Baird et al. 2008), to identify single nucleotide polymorphisms (SNPs) for parentage assignment. RADseq can facilitate discovery of many SNPs for parentage assignment in a short period of time (Andrews et al. 2016). Results from this study will provide critical baseline data that can be used to make recommendations for best management practices to maintain high genetic diversity in cultured *C. gigantea.*

2.3 METHODS

2.3.1 *Scallop Culture*

Wild adult *Crassadoma gigantea* (n=51) were opportunistically collected by divers at depths between 5 and 15 meters near Cypress Island and Burrows Channel in San Juan County, Washington State, USA on February 2, 2015, and transported to the Taylor Shellfish Farms Hatchery in Dabob Bay, WA (Figure 1). Scallops from these sites belong to the same genetic population that is very weakly differentiated from other populations along the coast (Lowell 2021). The scallops were held on the bottom of two 600L tanks (n=27 scallops per tank) and fed a live algae diet comprised of 10 species (Table 1) at a concentration of 100K to 150K cells per milliliter for conditioning. The water temperature was increased from 10°C to 14°C over a period of 12 days to expedite and synchronize gametogenesis.

Sex was determined by visual inspection of the gonad. A thin broom bristle was inserted into the byssal notch of each individual and slowly moved across the mantle until the scallop gaped open. A small wedge was then placed between the valves to prevent them closing and allow a visual examination of the gonad. Sex was determined by the gonad color, orange for female and white for male. Individuals with no gamete in their gonad and therefor no color to help identify sex were listed as unknown.

On April 17, 2015, the scallops were moved to a single tank and allowed to spawn volitionally; 50 million fertilized eggs were collected from this spawning event and reared at 16°C in flow-through 250 L conical tanks. Larvae were fed the same live algae diet given to the broodstock. At 38 days post spawn (May 25, 2015), settlement competency was observed (presence of an eye spot and foot, absence of a velum). Larvae were moved to two cylindrical tanks 60 cm wide by 180 cm tall and filled with artificial seaweed (Fukui North America, Ontario, Canada) to facilitate metamorphosis and byssal attachment.

In September 2015, five months after spawning, juvenile scallops (n=6000; mean shell height 5mm) were removed from artificial seaweed and transferred to 30 pearl nets with 3 mm mesh (n=200 per net). The pearl nets were suspended from floating lines in Dabob Bay WA in six stacks of five pearl nets each. The nets were spaced 30 cm apart and each stack was suspended three to five meters below the surface. Thirteen months post-spawn (May 2016), the scallops (mean shell height 10 mm) were transferred to five hanging lantern nets with 4 mm mesh suspended five meters below the surface. Each lantern net was composed of eight compartments, and each compartment was stocked with 150 scallops.

2.3.2 *Field Sites and Growout*

Eighteen months post spawn (October 2016) three random subsamples (n=450 each) were deployed to three Washington sites which represent potential locations for future *C. gigantea* aquaculture; Hood Canal, South Sound and the Strait of Juan de Fuca (Figure 1). The sites, in Dabob Bay (long lines), in Totten Inlet (mussel floats) and in Neah Bay (marina dock) were chosen to represent an assortment of potential grow out conditions for cultured *C. gigantea* (Figure 1). Dabob Bay, in the Hood Canal was chosen due to the prevalence of shellfish aquaculture in and around the bay and its unique water chemistry, caused by the sill at the entrance to the bay (Steinberg et al. 2011). The Dabob Bay water column stratifies in summer resulting in warm surface temperatures, ideal for growing shellfish, and the water column mixes in late fall and early spring allowing increased productivity in the bay (Ebbesmeyer et al. 1980, Horner et al. 2005). Dabob Bay scallops were deployed off long lines. South Sound basin is home to the many aquaculture operations which could be easily converted to *C. gigantea* culture. The water column in South Sound is less stratified than in Hood Canal, due to its relatively shallow depth and greater tidal mixing (Babson et al. 2006) and this allows for high annual primary production which can support large aquaculture operations (Dumbauld et al. 2009). South Sound also lacks any known *C. gigantea* populations (Bob Sizemore, Washington Department of Fish and Wildlife, personal communications), which reduces the risk of genetic interactions in this basin. South Sound scallops were deployed in Totten Inlet off commercial mussel rafts. Neah Bay, located on the western end of the Strait of Juan de Fuca is subject to coastal upwelling and therefor has cooler water temperatures and high acidification (Feely et al. 2008). Acidification has been shown to reduce calcification rates in juvenile Eastern Oysters, *Crassostrea virginica* (Waldbusser et al. 2011), juvenile Olympia Oysters, *Ostrea lurida* (Hettinger et al. 2012), and juvenile Hard Clams, *Mercenaria mercenaria* (Miller and Waldbusser 2016) and could also be a stressors for *C. gigantea* juveniles. However, Neah Bay contains a large population of wild *C. gigantea* and is sheltered from the storms and strong currents found in the Strait of Juan de Fuca, making it a likely location for *C. gigantea* aquaculture. Neah Bay scallops were deployed off docks in a marina.

At each site, nine 10mm mesh hanging cages (n=50 scallops per cage) were deployed in three stacks of three cages. All stacks were suspended 3 meters below the surface. The water temperature was monitored at each site using Hobo Pendant Temperature Loggers (Onset, Bourne, MA) attached to one of the cages. The loggers were programmed to record temperature every 30 minutes; daily maximum, minimum, and average temperature were calculated from these readings. All sites were visited twice monthly to download temperature readings and visually inspect the scallops and cages. Twenty-six months post spawn (June 2017), scallops were retrieved and transported to the Taylor Shellfish Farms Hatchery in Quilcene, WA on June 2, 2017 (Neah Bay) and June 9, 2017 (Dabob Bay and Totten Inlet) for sampling. Survival was calculated directly from total counts of live scallops per cage and mantle tissue samples were collected from all surviving individuals and stored in 100% ethanol. Differences in survival among sites were tested via χ^2-*tests*.

2.3.3 *Sequencing and Filtering*

For each growout site, 100 surviving offspring were randomly chosen for tissue sampling and genotyping. If less than 100 individuals survived at a given site, all samples were genotyped at that location. In addition to the offspring samples, tissue samples were collected and sequenced from all but one adult (n=50), which died after spawning but prior to sampling. DNA was extracted using the E.Z.N.A. Mollusc DNA Kit by Omega Bio-tek (Norcross, Georgia) and quantified using the Quant-iT PicoGreen dsDNA Assay Kit by Invitrogen (Waltham, Massachusetts) and assessed for quality using gel electrophoresis. Samples with poor quality DNA were discarded. DNA samples were normalized in 20uL PCR-grade water to 500ng. Five candidate parents and seven offspring were replicated after DNA extraction to allow for sequence quality checks during sequence filtering and to estimate genotyping error.

Restriction Site Associated (RAD) libraries were prepared following the protocols outlined by Etter et al. (2011). Barcodes, unique six base identifier sequence attached to an Illumina P1 adapter, were ligated to restricted DNA using T4 DNA ligase (Thermo Scientific, Waltham, MA). Individuals were then pooled into sublibraries containing a maximum of 12 individuals. Sublibraries were sheared using a Bioruptor sonicator and the resulting fragments were size selected using a MinElute Gel Extraction Kit by Omega Bio-tek (Norcross, GA). Illumina P2 adapters were annealed to the sheared DNA in each sublibrary, which was then PCR amplified. Finally, amplified sublibraries were pooled into four libraries, three with 96 individuals and one with 43 individuals. Libraries were 100bp paired end sequenced on an Illumina HiSeq 4000 at the Northwest Genomics Center (University of Washington, Seattle, WA).

RAD sequences were processed using the analysis pipeline iPyrad (Eaton 2014). Raw fastq files were demultiplexed, fastq quality was checked using FastQC (Andrews 2010) and loci identified with a *de novo* assembly. Major parameter choices included 82% clustering similarity within and between samples, minimum read depth of 10 reads per genotype per individual, a maximum of 20 SNPs per RAD locus, a maximum of 8 indels per RAD locus, and a maximum of 25% missing data per locus (Appendix 1). A catalog of loci was generated from parent samples using a *de novo* assembly. Parameters for this catalog were the same as above, except that a maximum of 50% missing data was allowed per locus. This catalog was used to genotype parents and offspring. Individual loci were filtered for a minor allele frequency of at least 0.25 to increase parentage assignment power (Vandeputte and Haffray 2014), for biallelic SNPs only, and for one random SNP per RAD locus, using a combination of custom python scripts and vcftools (Danecek et al. 2011).

Additional filtering steps were completed using GenAlEx (Peakall and Smouse 2006, Peakall and Smouse 2012). Loci and individuals with greater than 10% missing data were removed. Inbreeding coefficients (F_{IS}) were calculated for each candidate parent locus and loci were removed if F_{IS} was greater than 0.1 or less than -0.1. Replicated individuals (n=12) were compared for to estimate genotyping error. Only one replicate was retained for parentage analysis.

2.3.4 *Parentage Assignment*

The parentage assignment program COLONY (v2.0.6.6) (Jones and Wang 2010), which uses a full-pedigree likelihood method to simultaneously infer parentage and sibship among individuals, was used to determine each offspring's parents. Allelic dropout rate, when one or both allelic copies are not amplified during PCR amplification and false allele error (misscoring of an allele) were set at 3% and chosen based on percent mismatch calculated from individuals with replicated genotypes. Polygamy was allowed for males and females, and the probability of a true father or mother being included in candidate parents was set to 98% to account for the one candidate parent that was not genotyped. If the sex of a candidate parent was unknown it was included in both the mother and father candidate files.

2.3.5 *Growout Site Analysis*

To test whether the distribution of reproductive success was equal across growout sites we performed a Fishers exact test. The same test was then preformed to determine if family contributions varied across growout sites and to determine if the parent pairing assigned to each offspring was random or if specific parings were more likely at each site.

The effective number of breeders, N_b, was estimated for each site with three different methods. The first estimate was based on sibship assignments in COLONY (Wang 2009). The second method was based on parentage assignment without parents (PwoP) using equation 2.1 from Waples and Waples (2011) where k_i is the number of offspring produced by each parent and S is the sample of progeny.

$$N_e = \frac{2S-2}{\frac{\sum k_i^2}{2S}-1} \tag{2.1}$$

The PwoP method is similar to the COLONY estimator but allowed us to test the effect of unsampled parents created by COLONY on N_b estimates by calculating N_b with and without those parents. This method was also used to test whether offspring sample sizes affected N_b estimates by re-estimating N_b from 1000 resampling iterations of 54 offspring (smallest offspring sample size of all three sites at Neah Bay) with replacement at all three sites. Resampling was also conducted with a smaller sample size for Totten Inlet offspring (n=25). The significance of differences in N_b estimates between sites was estimated by determining the proportion of resampling iterations that produced more extreme N_b values than the empirical estimates.

2.4 RESULTS

2.4.1 *Growout*

Average daily water temperature as well as daily maximum and minimum differed among the three sites (Figure 15). The number of surviving scallops differed significantly across sites ($\chi^2 = 671.15$, $df = 2$, $p < 0.001$), with 98%, 66% and 14% of individuals surviving in Totten Inlet, Dabob Bay and Neah Bay respectively. No evidence of mortality due to predation was observed during growout and most mortalities at Dabob Bay and Neah Bay were observed in the two weeks prior to collection and sampling.

2.4.2 *Sequencing and Filtering*

A total of 321 individuals were included in the RAD libraries for sequencing (Table 6). The reference catalog contained 9462 loci. Filtering in iPyrad resulted in 2583 loci and the loss of seven offspring (Table 6). Additional filtering for one SNP per RAD locus and a minor allele frequency of at least 0.25 resulted in 1157 loci. Filtering for loci with missing data using GenAlEx reduced the loci count to 947. Filtering loci for an F_{IS} greater than -0.1 and less than 0.1 in GenAlEx resulted in 408 loci in 287 individuals for parentage assignment. The majority of individuals removed during filtering came from Dabob Bay (Table 6).

Twelve replicated individuals were compared for agreement across 1157 loci (Table 7) and the average percent mismatch for these individuals was 3%. Most individuals had less than 5% mismatch between replicates but two individuals had a mismatch greater than 30%. For one individual, one replicate had a heterozygosity almost twice as high as the other and was probably contaminated. Based on this observation, and the distribution of multilocus heterozygosity among all individuals (Figure 10), five individuals with a heterozygosity larger than 0.6 were considered contaminated and removed from analyses. The other pair of replicates, which differed at 50% of loci, likely stemmed from different individuals, and were both discarded (Table 7). For all other individuals, the replicate with the more complete data set was retained.

2.4.3 *Parentage Assignment*

COLONY estimates for both allelic dropout rate and false allele error were highly variable among loci, but lower than our estimates for both errors (3%, based on the percent mismatch calculated from replicated individuals) used as a prior of the analysis (Figure 11).

The candidate parent pool contained 22 females, 25 males and 4 unknown individuals. Parentage analysis in Colony resulted in offspring assignment to 84% of candidate males and 77% of candidate females (Figures 12 and 13). Mean reproductive success was comparable between males and females, but the variance in reproductive success was much higher in females than in males (Table 8).

In addition to the genotyped candidate parents, COLONY generated four parents, one male and three females, and assigned them to 29 offspring (Table 9). Only one of these offspring was assigned to two COLONY generated parents and was excluded from further analysis. The other 28 offspring were assigned to one real and one computer-generated parent and were considered single parent assignments.

Given that only one of the 51 candidate parents was missing, it was impossible for all four parents generated by COLONY to be true parents. One of the females generated by COLONY (CF02) had had much higher estimated allele probabilities when compared to the other three parents generated by COLONY. The average probability across all first estimated alleles was 0.99 with only 7% of loci generating a second allele estimate. A total of 22 offspring were assigned to this female (Table 9). The remaining three parents generated by COLONY were assigned to 9 offspring (Table 9) and can be ruled out as true individuals given the high occurrence of second and third estimated alleles. These individuals were most likely produced due to the high dropout rate and false allele error at some loci (Figure 11) and would not have been generated if error rates were lower.

2.4.4 *Growout Site Analysis*

Offspring of 32 parents were found in Totten Inlet and Dabob Bay, while offspring of only 22 parents were found in Neah Bay (Figure 12). Sex ratios of successful parents were skewed towards males at all sites, and this skew decreased with increasing survival (male : female; Neah Bay 1.29:1, Totten Inlet 1.46:1 and Dabob Bay 1.75:1) (Table 8). The distribution of reproductive success was significantly different among sites, both including (Fisher's exact test, *p*=0.046) and excluding (Fisher's exact test, *p*=0.029) the four parents generated by COLONY. The majority of parents at each site produced relatively few offspring (Figure 13), but one female (F13) and two males (M01 and M05) had high reproductive success, with 38%, 45% and 67% of offspring at Totten Inlet, Dabob Bay and Neah Bay respectively assigned to F13, and 23%, 41% and 56% of offspring at Totten Inlet, Dabob Bay and Neah Bay respectively assigned to M01 and M05 (Figure 12 and 14). The proportion of offspring produced by these three most prolific parents therefore declined with increasing survival among sites.

A total of 73 fullsib families were identified across all sites excluding fullsib families that included one of the four parents generated by COLONY. Fullsib family size ranged from one to eight across the three growout sites with most fullsib families at each site containing only one offspring (Figure 12, Table 8). Family contributions were not significantly different among sites including (Fisher's exact test, *p*=0.331) and excluding (Fisher's exact test, *p*=0. 305) the four parents generated by COLONY. A Fisher's exact test found that parent pairings given individual reproductive success of males and females deviated from random in Totten Inlet (*p*=0.000) and Dabob Bay (*p*=0.028) but not at Neah Bay (*p*=0.070), possibly because of small sample sizes.

Estimates of N_b ranged from 9 to 18 and varied depending on the calculation method (Table 10). Regardless of the method, estimates were largest for Totten Inlet and a smallest for Neah Bay (Table 10). Inclusion of the parents generated by COLONY did not have a major impact on N_b estimates at the three sites (Table 10). Sample size did not impact PwoP N_b estimates as resampling 25 and 54 offspring in Totten Inlet produced similar N_b estimates (Figure 15). Resampled N_b estimates from 54 offspring at Totten Inlet and Dabob Bay were significantly larger than the empirical N_b estimate in Neah Bay (Totten Inlet p=0.000, Dabob Bay p=0.005) (Figure 15). At all sites PwoP $N_b/N_{hatchery}$ ratios were less than one (Totten Inlet = 0.33, Dabob Bay = 0.26, Neah Bay = 0.15).

2.5 DISCUSSION

The aim of this study was to determine whether progeny from a mass spawn of cultured *C. gigantea* represent a random contribution from each parent and to identify how environment and differential survival impact parent contribution. Using RAD sequencing to identify parentage of a cultured *C. gigantea* cohort, we found that the production of a single generation using a mass spawn design did not represent a random contribution from each parent, and growout location had a significant impact on both parental contribution and estimates of N_b. Overall, N_b estimates were low, and may constitute a risk to wild populations, should hatchery and wild individuals interbreed.

2.5.1 *Individual Reproductive Success*

Skewed parental contributions are common in marine invertebrate populations as mature individuals are highly fecund and produce offspring that experience high early mortality (Giese 1959, Hedgecock and Pudovkin 2011, Plough 2016). This skew can be amplified in cultured mass spawn designs due to variability in parent candidate conditioning and fecundity, nonrandom mating, or family specific mortality (Boudry et al. 2002, Petersen et al. 2008, Straus et al. 2015). All these factors could have contributed to the skewed parental contributions we observed in our mass spawn of *C. gigantea*.

Not all parent candidates reproduced in our mass spawn, as only 74.5% of potential parent candidates were assigned to offspring. Some of these parents may have spawned but their offspring died during the larval phase (Boudry et al. 2002, Foote et al. 2019) or during growout (Degremont et al. 2005, Scanes et al. 2020). Alternatively, parents with no offspring may have lacked viable gametes and not spawned with the other successful parents, which is likely the case for adults where sex was not distinguishable. Wild populations of *C. gigantea* spawn for an extended period of time, from June to August in Puget Sound (Laurén 1982), and from June to October in British Columbia (Macdonald and Bourne 1989). Thus, variability in maturation is expected in *C. gigantea* broodstock.

In addition to variability in conditioning and survival, variance in male reproductive success was evident as two males accounted for 41% and 56% of offspring at Dabob Bay and Neah Bay respectively (Figure 14). Male reproductive variance can result from differences in condition factor between males (Fessehaye et al. 2006) or sperm competition (Beirao et al. 2019) and both could have contributed to the differences in male contributions we observed. In addition, the position of candidate parents within the spawn tank can influence the likelihood of successful fertilization, as the distance required for sperm to travel to an oocyte impacts the likelihood of fertilization (Zheng et al. 2004, Levitan 2005, Suquet et al. 2010). Condition factor, sperm competition and physical position all could have contributed to the nonrandom parent pairing observed at Totten Inlet and Dabob Bay.

2.5.2 *Effective Number of Breeders*

A key challenge for any aquaculture operation is maintaining high genetic diversity and maximizing the N_b of each hatchery produced cohort. The maximum N_b possible for a given cohort is equal to two times the size of the broodstock population ($N_{hatchery}$), thus an $N_b/N_{hatchery}$ ratio of two maintains the highest genetic diversity possible given a broodstock population size of $N_{hatchery}$ (Lande 1987). Estimated $N_b/N_{hatchery}$ ratios at all sites deviated from 2.0, with the lowest ratio in Neah Bay. These ratios were reduced at all sites by skewed parental contributions, mentioned above, and skewed sex ratio (Table 8). In Neah Bay, where survival was lowest, the $N_b/N_{hatchery}$ ratio was 50% less than the $N_b/N_{hatchery}$ ratio in Totten Inlet, where survival was highest. This is likely due to family specific mortality as similar reductions in N_e/N ratios have been observed Pacific Salmon (*Oncorhynchus spp*.) due to nonrandom mating at early life stages (Waples 2002). Low $N_b/N_{hatchery}$ ratios are a concern if cultured offspring interbreed with wild populations, as there is potential for loss of genetic diversity and fitness in wild populations (Ryman and Laikre 1991, Waples et al. 2012).

The potential effects of hatchery escapees on genetic diversity of wild populations also depends on the difference in N_e/N ratio between hatchery and wild populations (Waples et al 2016). Currently there are no N_e or N estimates for wild *C. gigantea* populations. It is likely the N_e/N ratio of wild populations will be low given low ratios have been observed in bivalves which have similar reproductive strategies to *C. gigantea,* high fecundity coupled with high early mortality (Hedgecock et al. 2006, Hedgecock and Pudovkin 2011, Hughes et al. 2019). In addition, the patchy distribution of wild populations could further reduce N_e if reproductive success varies between patches. Future research should focus on determining the N_e and N of wild *C. gigantea* populations to determine the potential loss in genetic diversity if wild and cultured individuals interbreed.

Minimizing genetic diversity loss will be imperative as genetic diversity can impact a species' ability to adapt to changing ocean conditions and disease (Gamfeldt et al. 2005, Reusch et al. 2005, Ehlers et al. 2008). Growers will be dependent on wild populations as broodstock sources, given Washington Fish and Wildlife managers currently prohibit use of cultured animals as broodstock (Brady Blake, personal communication). Thus, loss of genetic diversity in wild populations of *C. gigantea* could have significant impacts on the future of *C. gigantea* aquaculture if wild populations lose genetic diversity and adaptive ability through interbreeding with cultured conspecifics.

2.5.3 *Implications for Management*

Changes in hatchery and farm practices can be made to reduce skewed parental contributions and increase N_b/N ratios in future cultured groups. Current hatchery production techniques for *C. gigantea* rely on volitional mass spawns to produce enough viable larvae for production (Davis et al., personal communication) as space limitations in many facilities and spawn induction challenges prohibit rearing separate families during the larval and nursery phases. Isolating individuals once they begin to spawn would allow contributions from individuals to be equalized prior to fertilization, and fertilized gametes could then be pooled after fertilization (Fiumera et al. 2004). Isolation would also allow for full or partial factorial mating designs, which could reduce the impact of sperm competition and candidate parent location on fertilization (Nascimento-Schulze et al. 2021). Selecting growout sites and techniques that maximize survival would help reduce family specific mortality and thus minimize reductions in the N_b for cultured cohorts of *C. gigantea* (Nascimento-Schulze et al. 2021).

In addition to cross design and growout site selection, conditioning large numbers of broodstock for each spawn (Grant et al. 2017) will increase the potential N_b and genetic diversity of each cohort. Caution should be used when choosing broodstock, as domestication selection has been observed after a single generation in captivity (Christie et al. 2012). Collecting new wild broodstock each spawn season will help prevent segregation of wild and cultured populations and limit potential fitness consequences due to domestication selection if wild and cultured populations interbreed (Ford 2002, Baskett and Waples 2013, Waters et al. 2018).

Our study has determined that progeny from a single spawning event of cultured *C. gigantea* did not represent a random contribution from each parent and that skewed parental contributions and sex ratios lowered N_b at all sites. While these findings are true for this event, further investigations are required to determine if our observations are consistent across hatchery populations. Our data are a useful basis for such research, as they would allow design and optimization of a high throughput parentage assignment panel for mass screening (Campbell et al. 2015). Until more is known about the diversity of cultured *C. gigantea* populations, and the N_e of wild populations, increasing the number of broodstock used in each spawn, equalizing parental contributions prior to fertilization and using partial or full factorial spawn designs could help reduce the impact of low effective population size in cultured cohorts of *C. gigantea*.

TABLES AND FIGURES

Table 1: Microalgae strains grown at the Taylor Shellfish Farm Hatchery in Quilcene WA.

Microalgae Strain	**Collection Location**
Chaetoceros muelleri	Oceanic Institute, Hawaii, USA
Isochrysis galbana	Marine Biological Station, Port Erin, Isle of Man
Nannochloropsis oculata	Skate Point, Isle of Cumbrae, Scotland
Nannochloropsis oculata	Skate Point, Isle of Cumbrae, Scotland
Pavlova lutheri	Milliport, Isle of Cumbrae, Scotland
Rhodomonas salina	Milford, Connecticut USA
Skeletonema menzelii	Chase Creek, Dennis, Masachusetts USA
Tetraselmis suecica	Bembridge, Isle of Wight, England
Thalassiosira pseudonana	San Juan Island, WA USA
Tisochrysis lutea	Mataiva, Tahiti, Society Islands

Table 2: Criterial for assigning male and female acini to one of six gonad stages in *C. gigantea*.

Stage	**#**	**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 spermatogonia and < 5% spermatozoa.	Open acini with a monolayer of small oogonia attached to the acini wall.
Late Active	2	Acini contain spermatogonia 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 spermatozoa).	Empty acini (< 5% filled with free oocytes).

Table 3: Summary of results from analysis of the four generalized linear models. Regression coefficients (standard error) and *p* values are listed for each model. Akaike information criterion (AIC) results have been corrected for small sample size.

Model	Intercept	Shell Height	Age	Age : Shell Height	*AICc*
Size + Age	-15.72 (1.75) ***	0.07 (0.03) ***	0.47 (0.06) ***		212.78
Size * Age	-25.02 (12.04) **	0.25 (0.22)	0.83 (0.47) *	-0.01 (0.01)	214.14
Age	-13.72 (1.45) ***		0.55 (0.06) ***		218.50
Size	-10.57 (1.14) ***	0.19 (0.02) ***			298.18

* $p<0.1$; ** $p<0.05$; *** $p<0.01$

Table 4: Summary of environmental and individual results from the spatial experiment conducted at three Washington State locations. Immature is classified as any individual in the inactive, early active, or late active stage. Mature is classified as any individual in the ripe, partially spawned or spent stage.

	Neah Bay	**Dabob Bay**	**Totten Inlet**
Maximum Temp (C°)	13.3	19.3	16.1
Minimum Temp (C°)	4.9	6.4	4.2
Total Individuals Sampled for Histology	62	298	440
Proportion Male	66%	71%	80%
Proportion Female	24%	21%	18%
Proportion Hermaphrodite	5%	1%	0%
Proportion Unknown Sex	5%	7%	2%
Inactive (Stage 1)	0%	0%	0%
Early Active (Stage 2)	3%	7%	0%
Late Active (Stage 3)	2%	23%	0%
Ripe (Stage 4)	55%	20%	74%
Partially Spawned (Stage 5)	29%	35%	20%
Spent (Stage 6)	11%	15%	5%
Proportion Mature at Sampling	95%	70%	100%
Average Shell Height (standard deviation)	56.7mm (±6.4)	58.0mm (±6.5)	60.8mm (± 7.8)
Survival	14%	66%	98%

Table 5: The number of parent candidates and offspring retained at the beginning of each major filtering step; RAD library assembly, iPyrad filtering and GenAlEx filtering. Offspring were sampled from three growout locations, Totten Inlet, Dabob Bay and Neah Bay.

Population	**RAD libraries**	**iPyrad**	**GenAlEx**
Parent Candidates	50	50	50
Totten Inlet Offspring	104	101	100
Dabob Bay Offspring	108	105	83
Neah Bay Offspring	59	58	54
Total	**321**	**314**	**287**

Table 6: Comparison of RAD sequencing libraries replicates (n=12). Replicates included parent candidates (M = male, F = female) and offspring from each of the three growout sites (Totten Inlet, Dabob Bay and Neah Bay). Individual heterozygosity and missing data are listed for each replicate as well as percent mismatch across the 1157 loci. Individuals with heterozygosity higher than 0.6 were considered contaminated individuals and removed from further analysis.

Site / Group	**Individual ID**	**Ind. Heterozygosity**		**% mismatch**	**Missing Data**	
		Rep. 1	**Rep. 2**		**Rep. 1**	**Rep. 2**
Dabob	1	0.416	0.416	0.18%	0.1945	0.1867
Dabob	2	0.493	0.491	0.09%	0.1815	0.1824
Dabob	3	0.459	0.486	2.23%	0.2031	0.1893
Dabob	4	0.784	0.417	31.18%	0.1945	0.1850
Dabob	5	0.462	0.421	50.00%	0.1832	0.1945
Neah Bay	1	0.448	0.450	0.00%	0.1815	0.1806
Totten Inlet	1	0.438	0.434	0.26%	0.1832	0.1858
Parent Candidate	M07	0.454	0.450	0.09%	0.2334	0.1962
Parent Candidate	M25	0.423	0.421	0.18%	0.1858	0.1841
Parent Candidate	F12	0.506	0.456	4.34%	0.1997	0.1832
Parent Candidate	F13	0.551	0.552	0.09%	0.1832	0.1919
Parent Candidate	U04	0.435	0.438	0.18%	0.1850	0.1893

Table 7: The number (*n*) and proportion of candidate parents (n=51) assigned to offspring and the number (*n*) of fullsib families found at each site. Average number of offspring per candidate parent (including parents with no offspring) and variance are listed as well as the average fullsib family size and standard variance across sites.

Site	**Father**			**Mother**			**Fullsib Family**	
	n	**Prop.**	**Mean (variance)**	***n***	**Prop.**	**Mean (variance)**	***n***	**Mean (variance)**
Totten Inlet	18	72%	3.96 (14.71)	14	64%	4.23 (61.52)	53	1.26 (1.89)
Dabob Bay	19	76%	3.28 (15.21)	13	59%	3.23 (48.09)	42	0.97 (1.97)
Neah Bay	14	56%	2.16 (12.81)	8	36%	2.05 (40.33)	24	0.62 (1.91)

Table 8: Number of offspring assigned to each parent generated by COLONY at each site.

	Totten Inlet	**Dabob Bay**	**Neah Bay**	**Total**
COLONY Male 1	1	1	0	2
COLONY Female 1	2	2	1	5
COLONY Female 2	7	10	5	22
COLONY Female 3	0	1	1	2
Total	10	14	7	

Table 9: The number (*n*) and proportion of candidate parents (n=51) assigned to offspring and the number (*n*) of fullsib families found at each site. Average number of offspring per candidate parent (including parents with no offspring) and variance are listed as well as the average fullsib family size and standard variance across sites.

Site	**Father**			**Mother**			**Fullsib Family**	
	n	**Prop.**	**Mean (variance)**	***n***	**Prop.**	**Mean (variance)**	***n***	**Mean (variance)**
Totten Inlet	18	72%	3.96 (14.71)	14	64%	4.23 (61.52)	53	1.26 (1.89)
Dabob Bay	19	76%	3.28 (15.21)	13	59%	3.23 (48.09)	42	0.97 (1.97)
Neah Bay	14	56%	2.16 (12.81)	8	36%	2.05 (40.33)	24	0.62 (1.91)

Table 10: Effective number of breeders, N_b, estimates using sibship assignments in COLONY, and assignment without parents (PwoP). PwoP was calculated with and without parents generated by COLONY (CGP). Upper (UCI) and lower (LCI) 95% confidence intervals were calculated using bootstrapping in COLONY.

Method	**Totten Inlet**			**Dabob Bay**			**Neah Bay**		
	N_b	**UCI**	**LCI**	N_b	**UCI**	**LCI**	N_b	**UCI**	**LCI**
COLONY	18	43	11	15	30	8	9	24	5
PwoP + CGP	17.54	n/a	n/a	15.02	n/a	n/a	8.75	n/a	n/a
PwoP - CGP	16.30	n/a	n/a	13.43	n/a	n/a	7.60	n/a	n/a

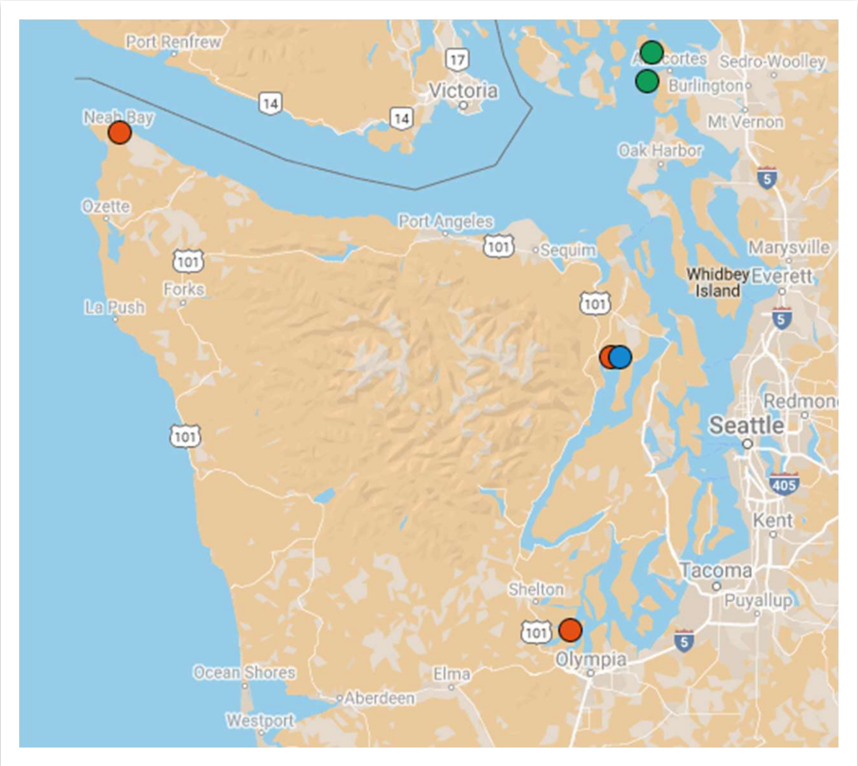

Figure 1: Broodstock collection sites (green), Taylor Shellfish Farms Hatchery (blue) and growout locations (red) in Washington State.

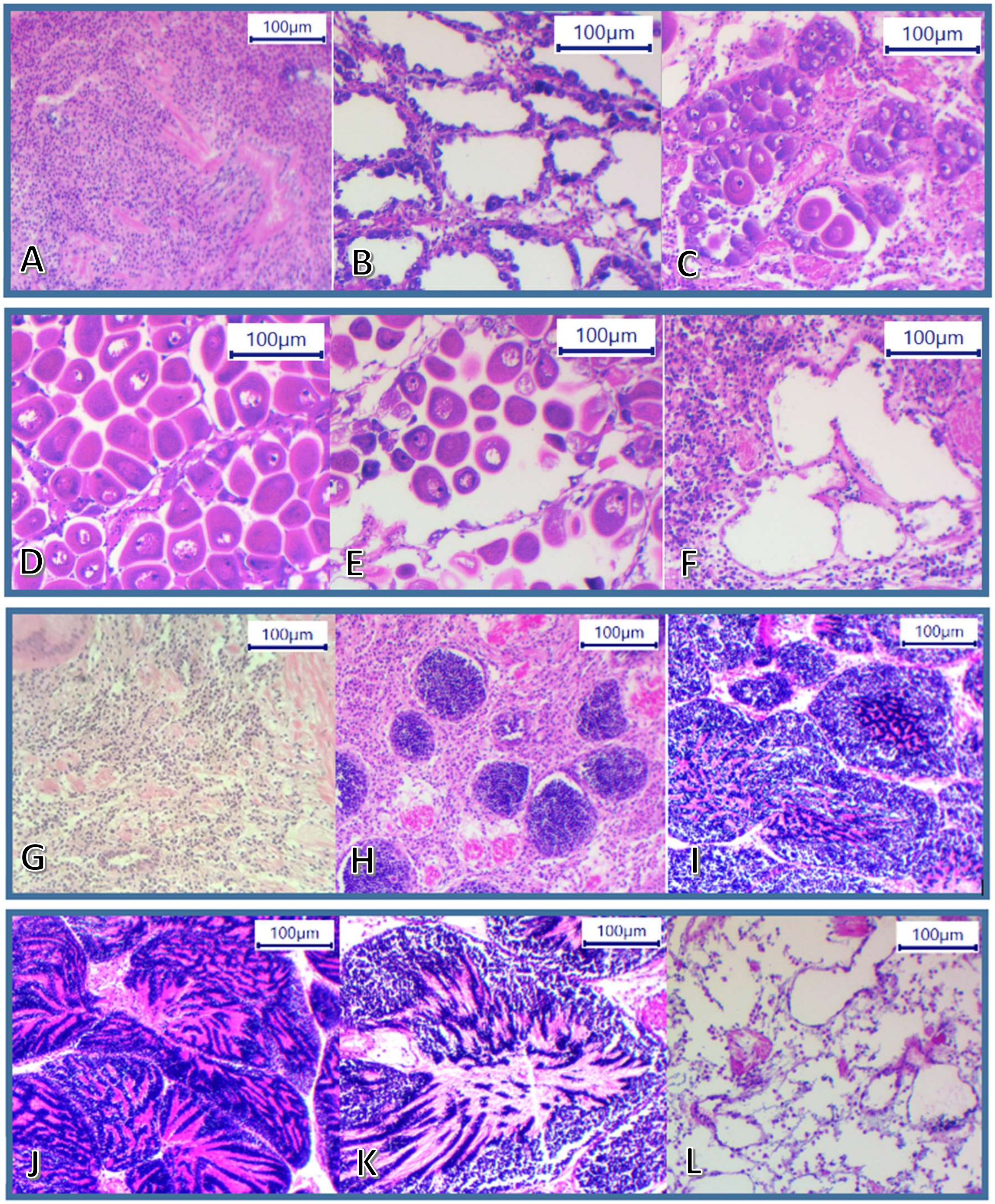

Figure 2: Histology sections of each reproductive stagefrom *C. gigantea* gonad samples with hematoxylin and eosin. Male stage: **A** inactive, **B** early active, **C** late active, **D** ripe, **E** partially spawned, **F** spent. Female stage: **G** inactive, **H** early active, **I** late active, **J** ripe, **K** partially

spawned, **L** spent.

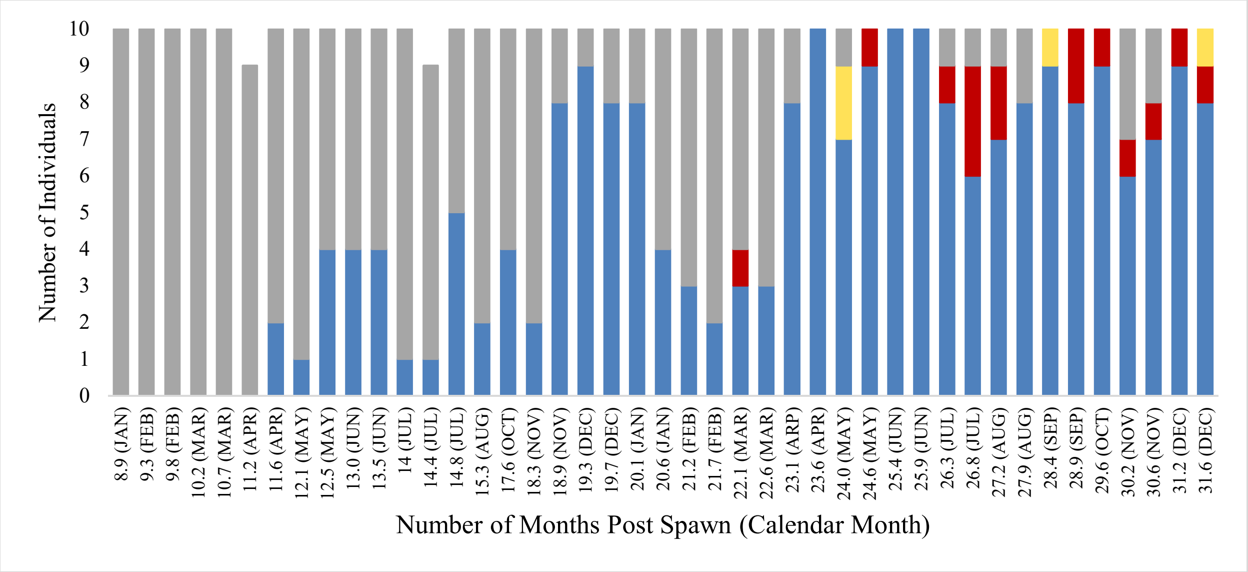

Figure 3: Number of *C. gigantea* identified as male (blue), female (red), hermaphrodite (yellow) or unknown sex (grey) on a given sample day. The x-axis shows each sample date as months post fertilization with the calendar month in parentheses. Sex was identified by the presence of spermatids, oocytes or both. Individuals without visible spermatids or oocytes were classified as unknown.

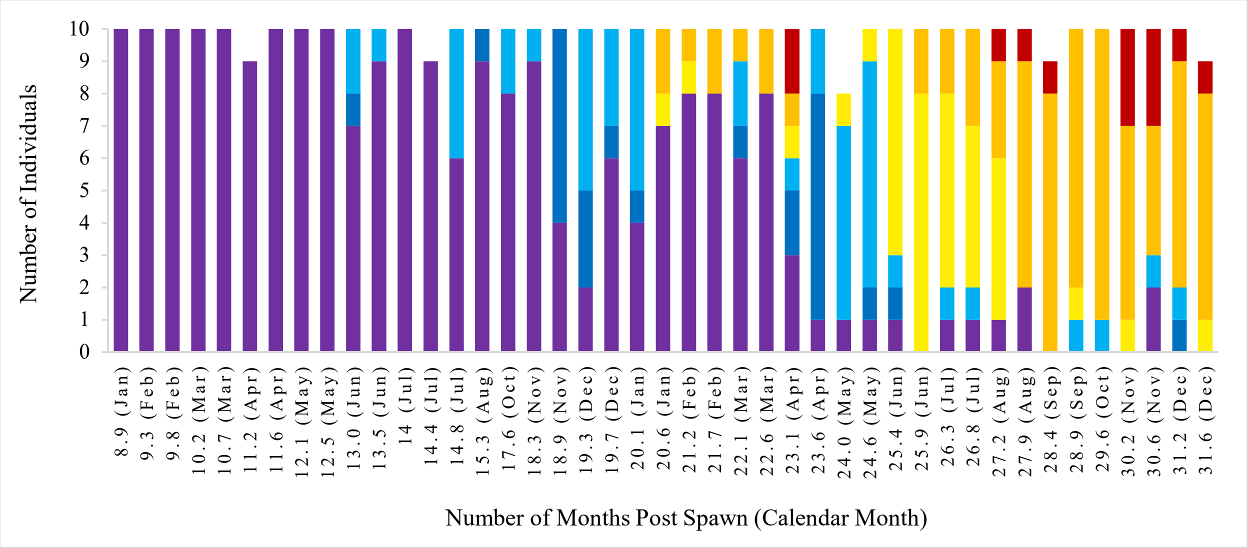

Figure 4: Number of *C. gigantea* juveniles assigned to each reproductive stage at each sample date. Stage 1 inactive (purple), stage 2 early active (dark blue), stage 3 late active (light blue), stage 4 ripe (yellow), stage 5 partially spawned (orange), and stage 6 spent (red). The x-axis shows each sample date as months post fertilization with the calendar month in parentheses. For each individual 24 acini were randomly chosen and an individual's stage was determining by taking the mode stage of sampled acini.

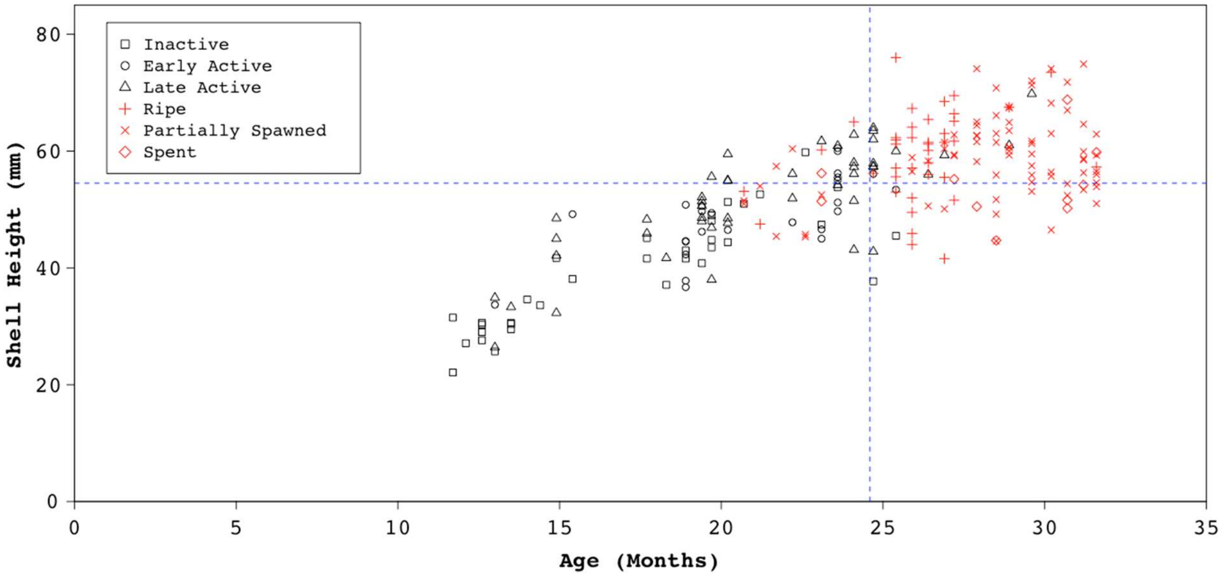

Figure 5: Size and age of *C. gigantea* assigned to six reproductive stages. Stage 1 inactive (square), stage 2 early active (circle), stage 3 late active (triangle), stage 4 ripe (plus), stage 5 partially spawned (x), and stage 6 spent (diamond). Immature individuals (inactive, early active, late active) are black and mature individuals (ripe, partially spawned, spent) are red. Blue lines indicate age (25.10 months post fertilization) and size (55.25mm) at first maturation determined by single predictor logistic regressions.

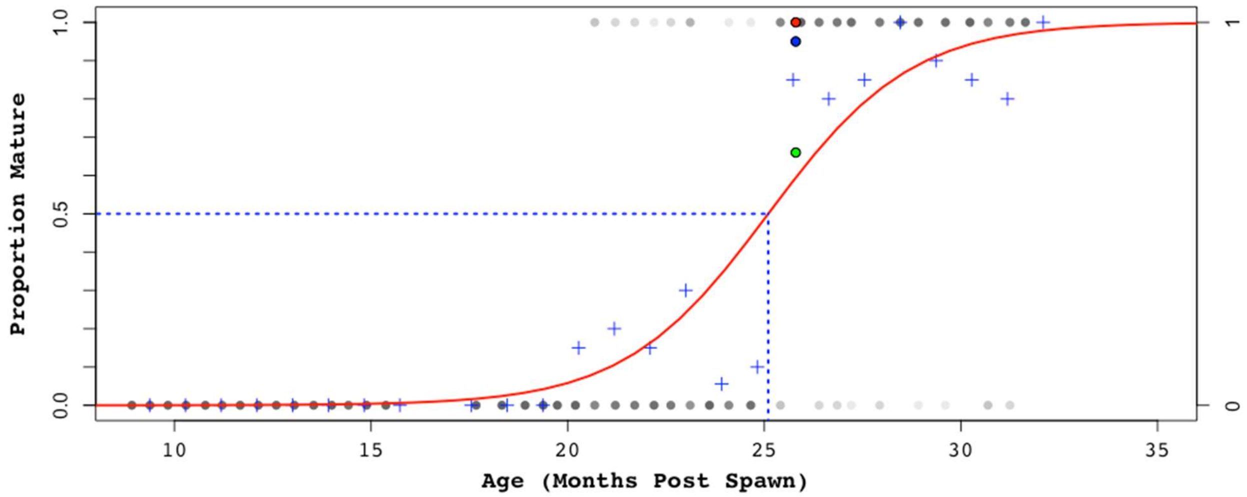

Figure 6: Fitted logistic regression for *C. gigantea* age at maturation (red line). Individual data is shown as gray-shaded dots, with darker dots indicating more individuals and the proportion of individuals that are mature at each sample timepoint are shown as blue crosses. The blue dotted line shows the age at which 50% of scallops are mature (25.10 months post fertilization). The proportion of mature individuals found at each site in the spatial experiment is also shown at 25.8 months post fertilization; Dabob Bay (green dot), Neah Bay (blue dot) and Totten Inlet (red dot).

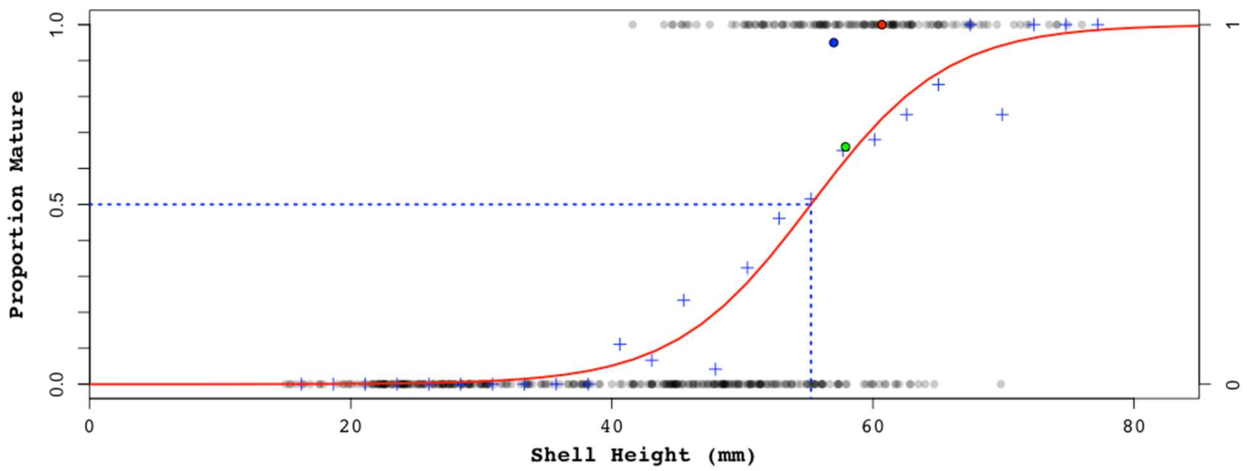

Figure 7: Logistic regression for *C. gigantea* size at maturation. Individual data is shown as gray-shaded dots, with darker dots indicating more individuals and the proportion of individuals that are mature at each sample timepoint are shown as blue crosses. The blue dotted line shows the size at which 50% of scallops are mature (55.25mm shell height). The proportion of mature individuals and average shell height found at each site in the spatial experiment is also indicated for each site in the spatial experiment; Dabob Bay (green dot), Neah Bay (blue dot) and Totten Inlet (red dot).

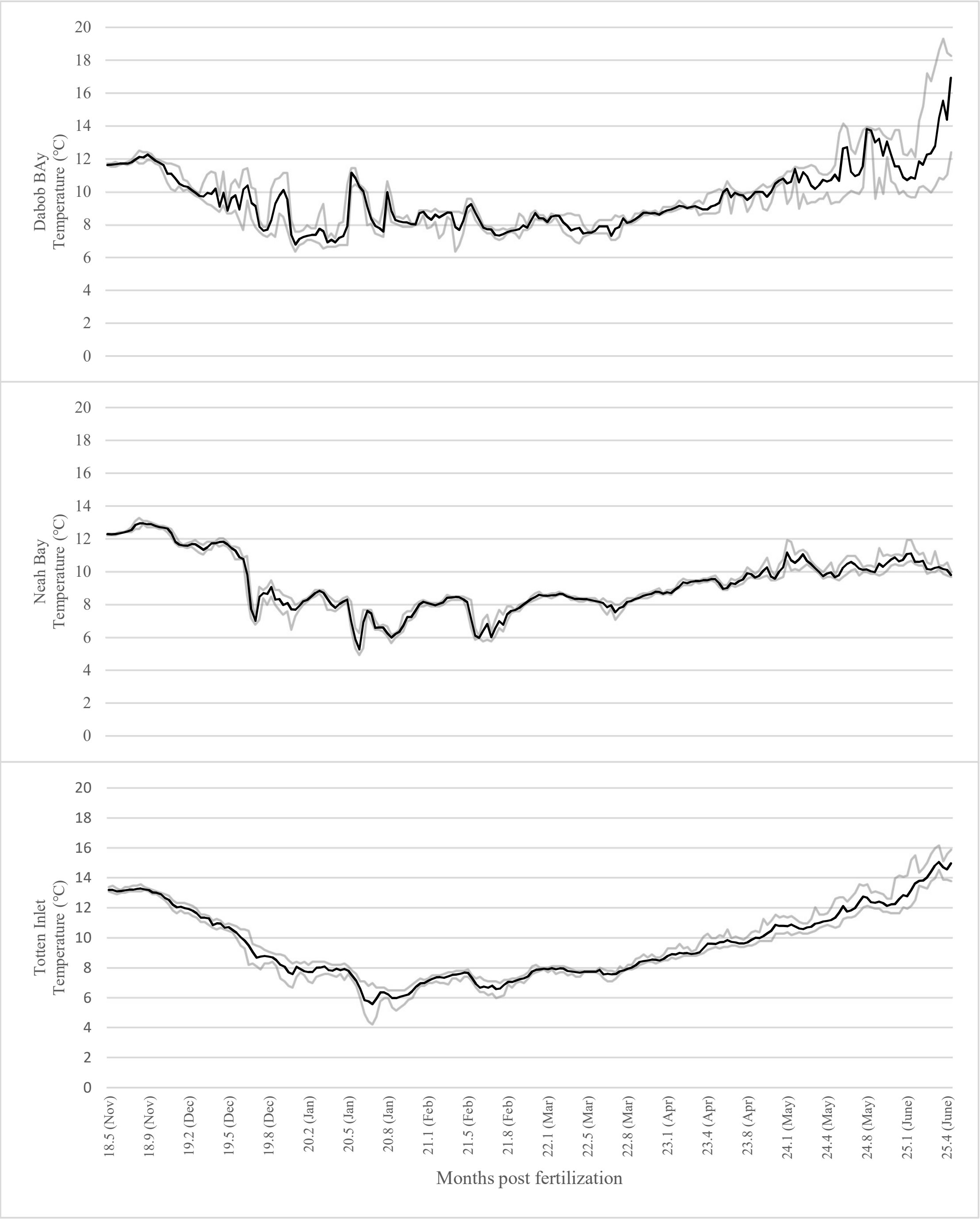

Figure 8: Average daily water temperatures (°C) recorded at each of the three growout locations from October 2016 to June 2017. Black lines show the average daily temperature and grey lines show daily minimum and maximum.

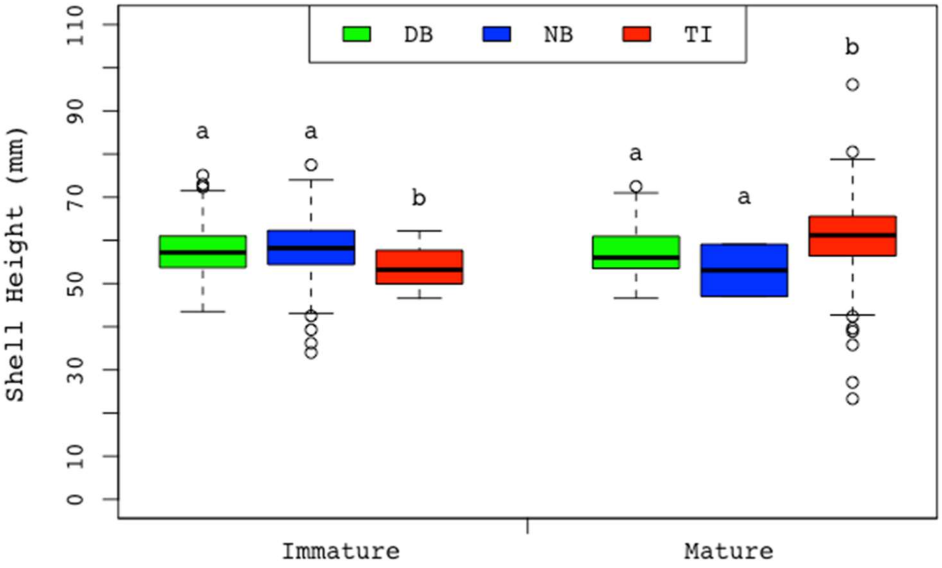

Figure 9: Shell heights of immature and mature cultured *C. gigantea* collected at three Washington State locations in the spatial experiment. Dabob Bay (DB) in green, Neah Bay (NB) in blue and Totten Inlet (TI) in red. Letters above box plots are to identify significant differences, use of the same letter indicates a non-significant difference.

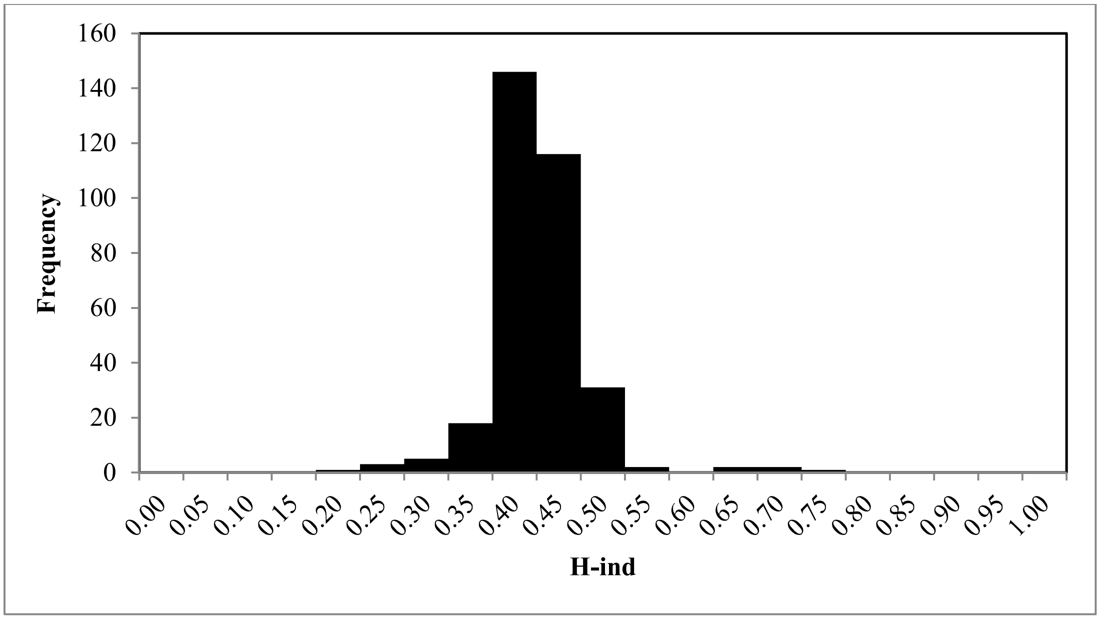

Figure 10: Distribution of individual heterozygosity across genotyped offspring and candidate parents.

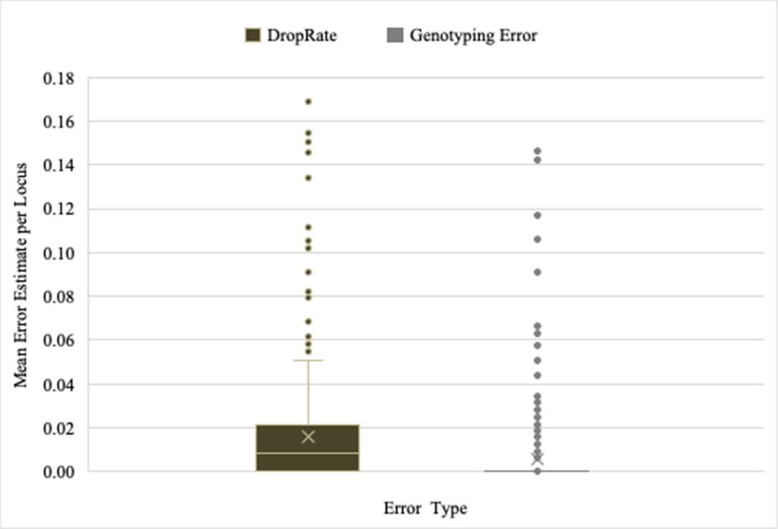

Figure 11: Box and whisker plots of COLONY estimates for mean allelic dropout rate (DropRate) and mean false allele error (Genotyping Error) per locus. Mean (x) and outlier error estimates (dots) are shown for both sequencing errors**.**

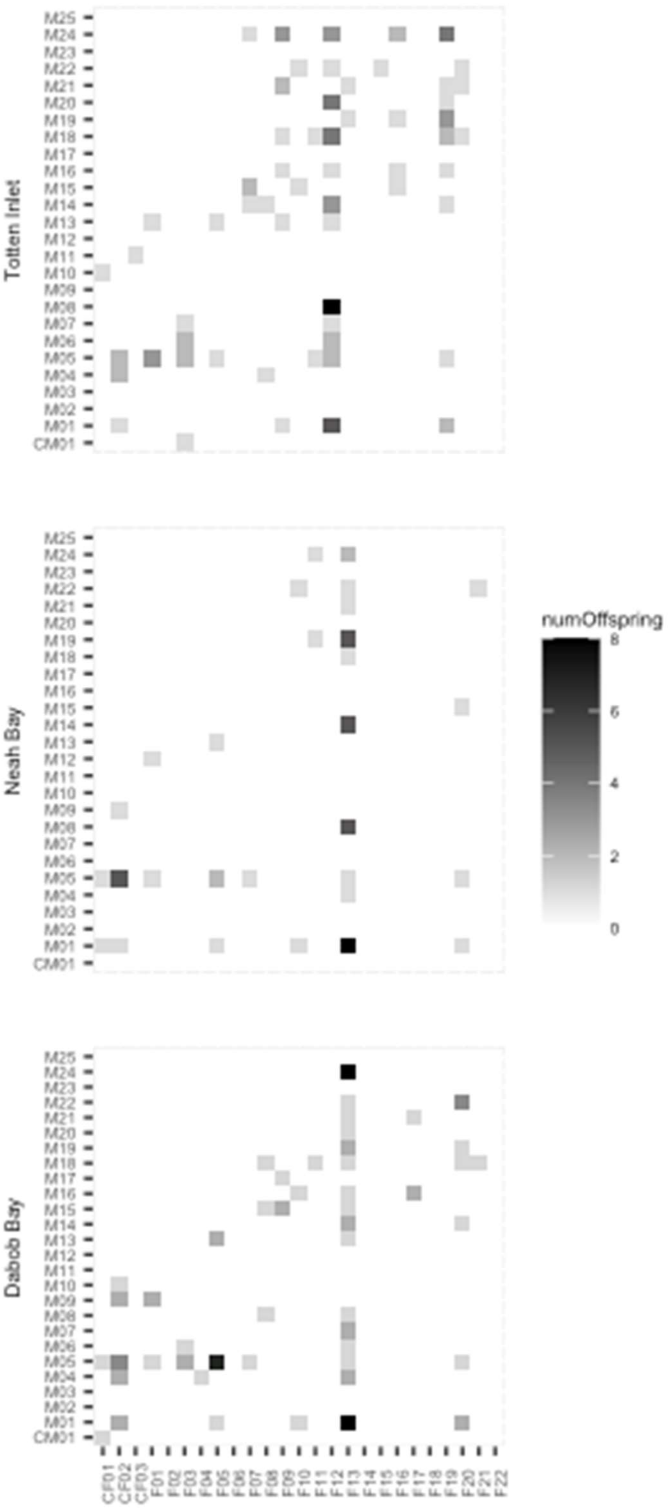

Figure 12: Parentage assignment for *C. gigantea* offspring at three growout sites; Totten Inlet, Neah Bay and Dabob Bay. Candidate Males (M) are shown on the y-axis and candidate females (F) on the x-axis. Candidates that are listed with a "C" are computer generated by COLONY.

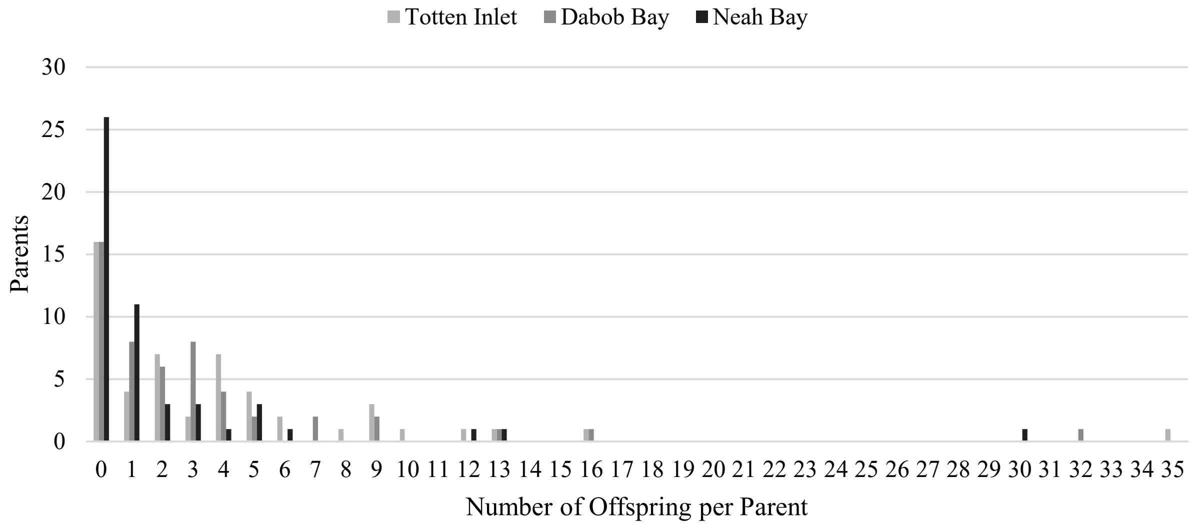

Figure 13: Distribution of reproductive success at three growout sites; Totten Inlet, Dabob Bay and Neah Bay. Number of offspring assigned to each parent are listed on the x axis and number of parents with x offspring are on the y axis. COLONY computer generated parents have not been included.

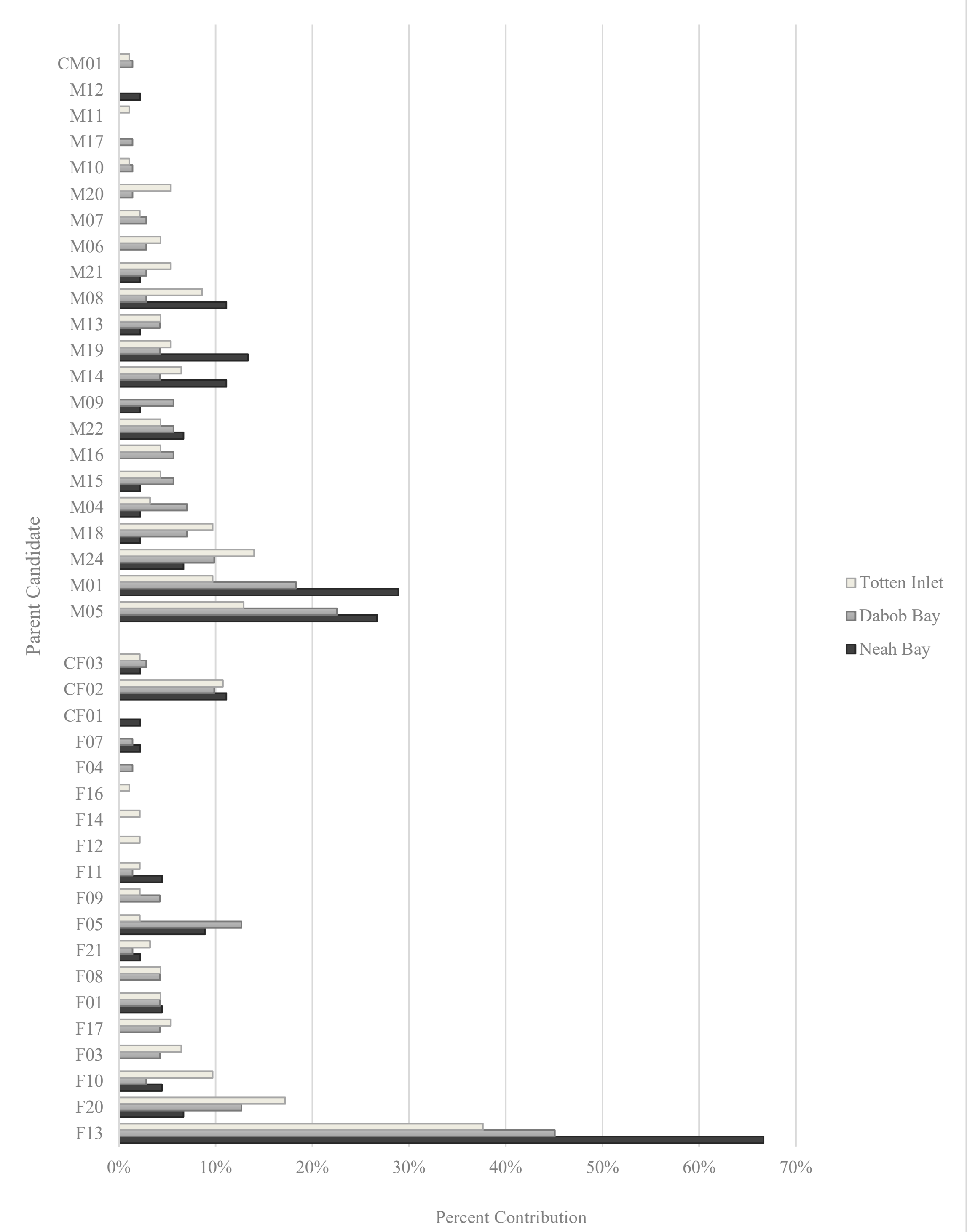

Figure 14: Percentage of offspring assigned to candidate mothers and fathers at three growout sites; Totten Inlet, Dabob Bay and Neah Bay. Candidate males are listed with an "M", females with an "F" and COLONY computer generated parents with a "C".

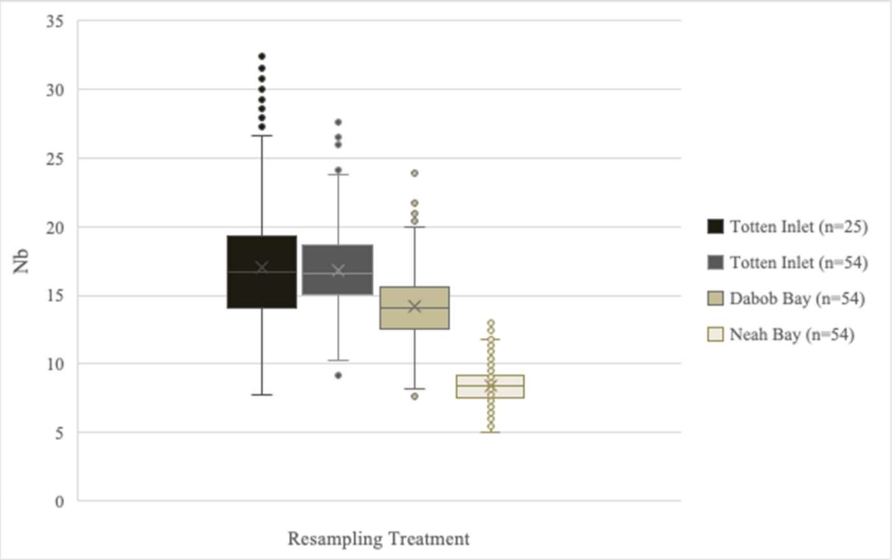

Figure 15: Box and whisker plots of N_b estimates from resampling at Totten Inlet, Dabob Bay and Neah Bay to determine if sample size impacts N_b estimates. Resampled offspring at each site (n=54) was equal the sample size at Neah Bay. A second resampling was run on Totten Inlet offspring (n=25) to determine if a large reduction in offspring sampled would impact $\boldsymbol{N_b}$ estimates. Resampling was repeated 1000 and for each resampling and individual reproductive success was calculated from the sampled offspring's parents. N_b was calculated using parentage assignment without parents (PwoP). Mean (x) and outliers (dots) are shown for each resampling treatment.

APPENDIX A

List of values used for all iPyrad assembly parameter input files.

iPyrad Parameter	**Value Used**
max_low_qual_bases	5
phred_Qscore_offset	33
mindepth_statistical	10
mindepth_majrule	10
maxdepth	2000
clust_threshold	0.82
max_barcode_mismatch	0
filter_adapters	2
filter_min_trim_len	35
max_alleles_consens	10
max_Ns_consens	5, 5
max_Hs_consens	100, 100
min_samples_locus	250
max_SNPs_locus	20, 20
max_Indels_locus]	8, 8
max_shared_Hs_locus	1.0
trim_reads	0, 0, 0, 0
trim_loci	0, 0, 0, 0

BIBLIOGRAPHY

Ackerman, M. W., B. K. Hand, R. K. Waples, G. Luikart, R. S. Waples, C. A. Steele, B. A. Garner, J. McCane, and M. R. Campbell. 2017. Effective number of breeders from sibship reconstruction: empirical evaluations using hatchery steelhead. Evolutionary Applications **10**:146-160.

Akaike, H. 1981. A new look at the statistical model identification. Engineering Technology & Applied Sciences:22-22.

Allendorf, F. W., S. N. Aitken, and G. H. Luikart. 2012. Conservation and the genetics of populations. 2nd edition. Wiley-Blackwell, Somerset, UK.

Andrews, K. R., J. M. Good, M. R. Miller, G. Luikart, and P. A. Hohenlohe. 2016. Harnessing the power of RADseq for ecological and evolutionary genomics. Nature Reviews Genetics **17**:81-92.

Andrews, S. 2010. FastQC: A quality control tool for high throughput sequence data [Online]. Available online at: http://www.bioinformatics.babraham.ac.uk/projects/fastqc/.

Arellano-Martínez, M., B. P. Ceballos-Vázquez, C. Ruíz-Verdugo, E. Pérez de León, R. Cervantes-Duarte, and P. M. Domínguez-Valdéz. 2011. Growth and reproduction of the lion's paw scallop *Nodipecten subnodosus* in a suspended culture system at Guerrero Negro lagoon, Baja California Sur, Mexico. Aquaculture Research **42**:571-582.

Arthur, J. R. B.-R., Melba G., M. L. Campbell, C. L. Hewitt, M. J. Phillips, and R. P. Subasinghe. 2009. Understanding and applying risk analysis in aquaculture: a manual for decision-makers. Food and Agriculture Organization of the United Nations, Rome.

Babcock, R., and J. Keesing. 1999. Fertilization biology of the abalone, *Haliotis laevigata*: laboratory and field studies. Canadian Journal of Fisheries and Aquatic Sciences **56**:1668-1678.

Babson, A. L., M. Kawase, and P. MacCready. 2006. Seasonal and interannual variability in the circulation of Puget Sound, Washington: A box model study. Atmosphere-ocean **44**:29-45.

Baird, N. A., P. D. Etter, T. S. Atwood, M. C. Currey, A. L. Shiver, Z. A. Lewis, E. U. Selker, W. A. Cresko, and E. A. Johnson. 2008. Rapid SNP discovery and genetic mapping using sequenced RAD markers. PLoS One **3**:e3376.

Barber, B. J., and N. J. Blake. 2016. Reproductive Physiology. Pages 253-299 Scallops - biology, ecology, aquaculture, and fisheries.

Bartron, M. L., N. M. Sard, and K. T. Scribner. 2018. Evaluation of effective number of breeders and coancestry among progeny produced using common hatchery mating strategies. Transactions of the American Fisheries Society **147**:185-194.

Baskett, M. L., S. C. Burgess, and R. S. Waples. 2013. Assessing strategies to minimize unintended fitness consequences of aquaculture on wild populations. Evolutionary Applications **6**:1090-1108.

Baskett, M. L., and R. S. Waples. 2013. Evaluating alternative strategies for minimizing unintended fitness consequences of cultured individuals on wild populations. Conservation Biology **27**:83-94.

Bayer, S. R., R. A. Wahle, P. A. Jumars, and D. C. Brady. 2016. Measuring scallop fertilization success in the field: chamber design and tests. Marine Ecology Progress Series **551**:141-154.

Bayne, B. L. 2004. Phenotypic flexibility and physiological tradeoffs in the feeding and growth of marine bivalve molluscs. Integrative and Comparative Biology **44**:425-432.

Beattie, J. H. 1992. Geoduck enhancement in Washington state. Bulletin of the Aquaculture Association of Canada **92**:18-24.

Beirao, J., T. B. Egeland, C. E. Purchase, and J. T. Nordeide. 2019. Fish sperm competition in hatcheries and between wild and hatchery origin fish in nature. Theriogenology **133**:201-209.

Benzie, J. A. H., and S. T. Williams. 1996. Limitations in the genetic variation of hatchery produced batches of the giant clam, *Tridacna gigas*. Aquaculture **139**:225-241.

Bierne, N., S. Launey, Y. Naciri-Graven, and F. Bonhomme. 1998. Early effect of inbreeding as revealed by microsatellite analyses on *Ostrea edulis* larvae. Genetics **148**:1893-1906.

Boudry, P., B. Collet, F. Cornette, V. Hervouet, and F. Bonhomme. 2002. High variance in reproductive success of the Pacific oyster (*Crassostrea gigas*, Thunberg) revealed by microsatellite-based parentage analysis of multifactorial crosses. Aquaculture **204**:283-296.

Breen, P. A., O. Canada. Department of Fisheries and, and N. A. Sloan. 1988. Northern abalone, *Haliotis kamtschatkana*, in British Columbia: fisheries and synopsis of life history information. Fisheries and Oceans, Communications Directorate, Ottawa, CAN.

Breese, W. P., and R. E. Malouf. 1975. Hatchery manual for the pacific oyster. Oregon State University, Agriculture Experiment Station.

Bull, M. 1976. Aspects of the biology of the New Zealand scallop, *Pecten novaezelandiae* Reeve 1853, in the Marlborough Sounds.

Burns, R. 1985. The shape & form of Puget Sound. Washington Sea Grant: Distributed by University of Washington Press, Seattle, WA.

Busack, C., and C. M. Knudsen. 2007. Using factorial mating designs to increase the effective number of breeders in fish hatcheries. Aquaculture **273**:24-32.

Campbell, N. R., S. A. Harmon, and S. R. Narum. 2015. Genotyping-in-Thousands by sequencing (GT-seq): A cost effective SNP genotyping method based on custom amplicon sequencing. Molecular Ecology Resources **15**:855-867.

Canonico, G. C., A. Arthington, J. K. McCrary, and M. L. Thieme. 2005. The effects of introduced tilapias on native biodiversity. Aquatic Conservation: Marine and Freshwater Ecosystems **15**:463-483.

Christie, M. R., M. L. Marine, R. A. French, and M. S. Blouin. 2012. Genetic adaptation to captivity can occur in a single generation. Proceedings of the National Academy of Sciences of the United States of America **109**:238-242.

Cochard, J. C., and N. Devauchelle. 1993. Spawning, fecundity and larval survival and growth in relation to controlled conditioning in native and transplanted populations of *Pecten maximus* (L.): evidence for the existence of separate stocks. Journal of Experimental Marine Biology and Ecology **169**:41-56.

Cruz, P., C. Rodriguez-Jaramillo, and A. M. Ibarra. 2000. Environment and population origin on first sexual maturity of Catarina scallop, *Argopecten ventricosus* (Sowerby II, 1842). Journal of shellfish research **19**:89-93.

Culver, C. S., J. B. Richards, and H. M. Page. 2006. Plasticity of attachment in the purple-hinge rock scallop, *Crassadoma gigantea*: Implications for commercial culture. Aquaculture **254**:361-369.

Davis, J. P. 2015. Recent developments in purple-hinged rock scallop culture on the US west coast. Journal of shellfish research **34**:706-706.

De Silva, S. S., T. T. T. Nguyen, G. M. Turchini, U. S. Amarasinghe, and N. W. Abery. 2009. Alien Species in Aquaculture and Biodiversity: A Paradox in Food Production. Ambio **38**:24-28.

Degremont, L., E. Bedier, P. Soletchnik, M. Ropert, A. Huvet, J. Moal, J. F. Samain, and P. Boudry. 2005. Relative importance of family, site, and field placement timing on survival, growth, and yield of hatchery-produced Pacific oyster spat (*Crassostrea gigas*). Aquaculture **249**:213-229.

Dickie, L. M., and J. C. Medcof. 1963. Causes of Mass Mortalities of Scallops (*Placopecten magellanicus*) in the Southwestern Gulf of St. Lawrence. Journal of the Fisheries Research Board of Canada **20**:451-482.

Dieckmann, U., and M. Heino. 2007. Probabilistic Maturation Reaction Norms: their history, strengths, and limitations. Marine Ecology Progress Series **335**:253-270.

Dumbauld, B. R., J. L. Ruesink, and S. S. Rumrill. 2009. The ecological role of bivalve shellfish aquaculture in the estuarine environment: A review with application to oyster and clam culture in West Coast (USA) estuaries. Aquaculture **290**:196-223.

Eaton, D. A. 2014. PyRAD: assembly of de novo RADseq loci for phylogenetic analyses. Bioinformatics **30**:1844-1849.

Ebbesmeyer, C. C., C. A. Barnes, C. C. Ebbesmeyer, and C. A. Barnes. 1980. Control of a fjord basin's dynamics by tidal mixing in embracing sill zones. Estuarine and coastal marine science **11**:311-330.

Ehlers, A., B. Worm, and T. B. H. Reusch. 2008. Importance of genetic diversity in eelgrass *Zostera marina* for its resilience to global warming. Marine Ecology Progress Series **355**:1-7.

Etter, P. D., J. L. Preston, S. Bassham, W. A. Cresko, and E. A. Johnson. 2011. Local de novo assembly of RAD paired-end contigs using short sequencing reads. PLoS One **6**:e18561.

Fabioux, C., A. Huvet, P. Le Souchu, M. Le Pennec, and S. Pouvreau. 2005. Temperature and photoperiod drive *Crassostrea gigas* reproductive internal clock. Aquaculture **250**:458-470.

FAO. 2020. The state of world fisheries and aquaculture 2020. Rome.

Feely, R. A., C. L. Sabine, J. M. Hernandez-Ayon, D. Ianson, and B. Hales. 2008. Evidence for upwelling of corrosive “acidified" water onto the continental shelf. Science **320**:1490-1492.

Fessehaye, Y., Z. El-Bialy, M. A. Rezk, R. Crooijmans, H. Bovenhuis, and H. Komen. 2006. Mating systems and male reproductive success in Nile tilapia (*Oreochromis niloticus*) in breeding hapas: A microsatellite analysis. Aquaculture **256**:148-158.

Fiumera, A. C., B. A. Porter, G. Looney, M. A. Asmussen, and J. C. Avise. 2004. Maximizing offspring production while maintaining genetic diversity in supplemental breeding programs of highly fecund managed species. Conservation Biology **18**:94-101.

Foote, A., D. Simma, M. Khatkar, H. Raadsma, J. Guppy, G. Coman, E. Giardina, D. Jerry, K. Zenger, and N. Wade. 2019. Considerations for maintaining family diversity in commercially mass-spawned penaeid shrimp: A case study on *Penaeus monodon*. Frontiers in Genetics **10**:12.

Ford, M. J. 2002. Selection in captivity during supportive breeding may reduce fitness in the wild. Conservation Biology **16**:815-825.

Fox, J., and S. Weisberg. 2011. An R companion to Applied Regression. Sage, Thousand Oaks, CA.

Gaffney, P. M., C. V. Davis, and R. O. Hawes. 1992. Assessment of drift and selection in hatchery populations of oysters (*Crassostrea virginica*). Aquaculture **105**:1-20.

Gallardi, D. 2014. Effects of bivalve aquaculture on the environment and their possible mitigation: A review. Fisheries and aquaculture journal **5**.

Gamfeldt, L., J. Wallen, P. R. Jonsson, K. M. Berntsson, and J. N. Havenhand. 2005. Increasing intraspecific diversity enhances settling success in a marine invertebrate. Ecology **86**:3219-3224.

Gentry, R. R., H. E. Froehlich, D. Grimm, P. Kareiva, M. Parke, M. Rust, S. D. Gaines, and B. S. Halpern. 2017. Mapping the global potential for marine aquaculture. Nature ecology & evolution. **1**:1317-1324.

Gibbs, M. T., and H. I. Browman. 2015. Risk assessment and risk management: a primer for marine scientists. Ices Journal of Marine Science **72**:992-996.

Giese, A. C. 1959. Comparative physiology: annual reproductive cycles of marine invertebrates. Annual Review of Physiology **21**:547-576.

Grant, W. S., J. Jasper, D. Bekkevold, and M. Adkison. 2017. Responsible genetic approach to stock restoration, sea ranching and stock enhancement of marine fishes and invertebrates. Reviews in Fish Biology and Fisheries **27**:615-649.

Grecian, L. A., G. J. Parsons, P. Dabinett, and C. Couturier. 2003. Effect of deployment date and environmental conditions on growth rate and retrieval of hatchery-reared sea scallops, *Placopecten magellanicus* (Gmelin, 1791), at a sea-based nursery. Journal of shellfish research **22**:101-109.

Gribben, P. E., and R. G. Creese. 2003. Protandry in the New Zealand geoduck, *Panopea zelandica* (Mollusca, Bivalvia). Invertebrate Reproduction & Development **44**:119-129.

Guo, X., D. Hedgecock, W. K. Hershberger, K. Cooper, and S. K. Allen, Jr. 1998. Genetic determinants of protandric sex in the pacific oyster, *Crassostrea Gigas* Thunberg. Evolution **52**:394-402.

Hare, M. P., L. Nunney, M. K. Schwartz, D. E. Ruzzante, M. Burford, R. S. Waples, K. Ruegg, and F. Palstra. 2011. Understanding and Estimating Effective Population Size for Practical Application in Marine Species Management. Conservation Biology **25**:438-449.

Harney, E., T. J. Van Dooren, S. Paterson, and S. J. Plaistow. 2013. How to measure maturation: a comparison of probabilistic methods used to test for genotypic variation and plasticity in the decision to mature. Evolution **67**:525-538.

Hauser, L., and G. R. Carvalho. 2008. Paradigm shifts in marine fisheries genetics: ugly hypotheses slain by beautiful facts. Fish and Fisheries **9**:333-362.

Hedgecock, D. 1994. Does variance in reproductive success limit effective population sizes of marine organisms. Genetics and evolution of aquatic organisms:122-134.

Hedgecock, D., V. Chow, and R. S. Waples. 1992. Effective population numbers of shellfish broodstocks estimated from temporal variance in allelic frequencies. Aquaculture **108**:215-232.

Hedgecock, D., and K. Coykendall. 2007. Genetic risks of marine hatchery enhancement: The good, the bad, and the unknown. Pages 85-101 *in* T. Bert, editor. Ecological and Genetic Implications of Aquaculture Activities. Kluwer Academic Publishers, Norwell, MA

Hedgecock, D., S. Launey, A. I. Pudovkin, Y. Naciri, S. Lapègue, and F. Bonhomme. 2006. Small effective number of parents (*Nb*) inferred for a naturally spawned cohort of juvenile European flat oysters *Ostrea edulis*. Marine Biology **150**:1173-1182.

Hedgecock, D., and A. I. Pudovkin. 2011. Sweepstakes reproductive success in highly fecund marine fish and shellfish: A review and commentary. Bulletin of Marine Science **87**:971-1002.

Hedgecock, D., and F. Sly. 1990. Genetic drift and effective population sizes of hatchery-propagated stocks of the Pacific oyster, *Crassostrea gigas*. Aquaculture **88**:21-38.

Heino, M., U. Dieckmann, and O. R. Godo. 2002. Measuring probabilistic reaction norms for age and size at maturation Evolution **56**:669-678.

Hennick, D. P. 1970. Reproductive cycle, size at maturity, and sexual composition of commercially harvested weathervane scallops (*Patinopecten caurinus*) in Alaska. Journal of the Fisheries Research Board of Canada **27**:2112-2119.

Hettinger, A., E. Sanford, T. M. Hill, A. D. Russell, K. N. S. Sato, J. Hoey, M. Forsch, H. N. Page, and B. Gaylord. 2012. Persistent carry-over effects of planktonic exposure to ocean acidification in the Olympia oyster. Ecology **93**:2758-2768.

Hooker, S. H., and R. G. Creese. 1995. Reproduction of paua, *Haliotis iris* Gmelin 1791 (Mollusca: Gastropoda), in north-eastern New Zealand. Marine and Freshwater Research **46**:617-622.

Horner, R. A., J. R. Postel, C. Halsband-Lenk, J. J. Pierson, G. Pohnert, and T. Wichard. 2005. Winter-spring phytoplankton blooms in Dabob Bay, Washington. Progress in oceanography **67**:286-313.

Hughes, A. R., T. C. Hanley, J. E. Byers, J. H. Grabowski, T. McCrudden, M. F. Piehler, and D. L. Kimbro. 2019. Genetic diversity and phenotypic variation within hatchery-produced oyster cohorts predict size and success in the field. Ecological Applications **29**:14.

In, V.-V., W. O'Connor, M. Dove, and W. Knibb. 2016. Can genetic diversity be maintained across multiple mass selection lines of Sydney rock oyster, *Saccostrea glomerata* despite loss within each? Aquaculture **454**:210-216.

Jacobsen, F. R. 1977. The reproductive cycle of the purple hinged rock scallop, *Hinnites Multirugosus* Gale 1928 (Mollusca : Bivalvia). San Diego State University.

Jo, Q., S.-k. Kim, C. Lee, M. M. Rahman, C.-S. Lee, and B. Oh. 2009. A report on the mass mortality of the farmed japanese scallop, *Patinopecten yessoensis,* on the Korean Coasts of the East Sea. The Korean Journal of Malacology **25 (2)**:93-96.

Jones, O. R., and J. Wang. 2010. COLONY: a program for parentage and sibship inference from multilocus genotype data. Molecular Ecology Resources **10**:551-555.

Ju, R. T., X. Li, J. J. Jiang, J. Wu, J. Liu, D. R. Strong, B. Li, and S. Knutie. 2019. Emerging risks of non-native species escapes from aquaculture: Call for policy improvements in China and other developing countries. Journal of Applied Ecology **57**:85-90.

Lafferty, K. D., D. C. Behringer, and B. R. Silliman. 2020. Marine disease ecology. Oxford University Press, Oxford.

Lande, R. B., G. 1987. Effective population size, genetic variation, and their use in population management. Viable populations for conservation. Cambridge University Press, Cambridge

Launey, S., and D. Hedgecock. 2001. High genetic load in the Pacific oyster *Crassostrea gigas*. Genetics **159**:255-265.

Laurén, D. J. 1982. Oogenesis and protandry in the purple-hinge rock scallop, *Hinnites giganteus*, in upper Puget Sound, Washington, U.S.A. Canadian Journal of Zoology **60**:2333-2336.

Lehnert, S. J., J. W. Heath, D. D. Heath, and E. Taylor. 2013. Ecological and genetic risks arising from reproductive interactions between wild and farmed Chinook salmon. Canadian Journal of Fisheries and Aquatic Sciences **70**:1691-1698.

Levitan, D. R. 2005. The distribution of male and female reproductive success in a broadcast spawning marine invertebrate. Integrative and Comparative Biology **45**:848-855.

Levitan, D. R., and C. Petersen. 1995. Sperm limitation in the sea. Trends in Ecology & Evolution **10**:228-231.

Li, R. H., Q. L, and R. H. Yu. 2009. Parentage determination and effective population size estimation in mass spawning Pacific Oyster, *Crassostrea gigas*, based on microsatellite analysis. Journal of the World Aquaculture Society **40**:667-677.

Lin, Y., Z. Gao, and A. Zhan. 2015. Introduction and use of non-native species for aquaculture in China: status, risks and management solutions. Reviews in Aquaculture **7**:28-58.

Lind, C. E., B. S. Evans, J. J. U. Taylor, and D. R. Jerry. 2009. The consequences of differential family survival rates and equalizing maternal contributions on the effective population size (*Ne*) of cultured silver-lipped pearl oysters, *Pinctada maxima*. Aquaculture Research.

Lowell, N. 2021. A genetic risk assessment of native shellfish aquaculture. University of Washington.

Luna, L. G. 1968. Manual of histologic staining methods of the Armed Forces Institute of Pathology. 3rd ed. edition. Blakiston Division, McGraw-Hill, New York.

Macdonald, B. A., and N. F. Bourne. 1989. Growth of the pirple-hinged rock scallop, *Crassadoma gigantea* (Grey, 1825), under natural conditions and those associated with suspended culture. Journal of shellfish research **8**:179-186.

MacDonald, B. A., R. J. Thompson, and N. F. Bourne. 1991. Growth and reproductive energetics of three scallop species from British Columbia (*Chlamys hastata, Chlamys rubida,* and *Crassadoma gigantea*). Canadian Journal of Fisheries and Aquatic Sciences **48**:215-221.

Mackie, G. L. 1984. Bivalves. Pages 351-418 The Mollusca, Volume 7: Reproduction. Academic Press, New York.

McKindsey, C. W., T. Landry, F. X. O'Beirn, and I. N. Davies. 2007. Bivalve aquaculture and exotic species: A review of ecological considerations and management issues. Journal of shellfish research **26**:281-294.

Miller, C. A., and G. G. Waldbusser. 2016. A post-larval stage-based model of hard clam *Mercenaria mercenaria* development in response to multiple stressors: temperature and acidification severity. Marine Ecology **558**:35-49.

Moura, P., P. Vasconcelos, F. Pereira, P. Chainho, J. L. Costa, and M. B. Gaspar. 2018. Reproductive cycle of the Manila clam (*Ruditapes philippinarum*): an intensively harvested invasive species in the Tagus Estuary (Portugal). Journal of the Marine Biological Association of the United Kingdom **98**:1645-1657.

Nascimento-Schulze, J. C., T. P. Bean, R. D. Houston, E. M. Santos, M. B. Sanders, C. Lewis, and R. P. Ellis. 2021. Optimizing hatchery practices for genetic improvement of marine bivalves. Reviews in Aquaculture.

Naylor, R. L., S. L. Williams, and D. R. Strong. 2001. Aquaculture - a gateway for exotic species. Science **294**:1655-1656.

Ogle, D. H. 2017. FSA: Fisheries Stock Analysis. R package version 0.8.16.

Ogle, D. H. 2018. fishR. Vignette - Maturity Schedules, http://derekogle.com/fishR/.

Olsen, E., G. R. Lilly, M. Heino, M. J. Morgan, J. Brattey, and U. Dieckmann. 2005. Assessing changes in age and size at maturation in collapsing populations of Atlantic cod (*Gadus morhua*). Canadian Journal of Fisheries and Aquatic Sciences **62**:811-823.

Paltzat, D. L., C. M. Pearce, P. A. Barnes, and R. S. McKinley. 2008. Growth and production of California sea cucumbers (*Parastichopus californicus Stimpson*) co-cultured with suspended Pacific oysters (*Crassostrea gigas,* Thunberg). Aquaculture **275**:124-137.

Parsons, G. J., R. B. Lauzier, and N. F. Bourne. 2016. Scallops of the West Coast of North America. Pages 697-717 Scallops - Biology, Ecology, Aquaculture, and Fisheries.

Peakall, R., and P. E. Smouse. 2012. GenAlEx 6.5: genetic analysis in Excel. Population genetic software for teaching and research--an update. Bioinformatics **28**:2537-2539.

Peakall, R. O. D., and P. E. Smouse. 2006. GenAlEx 6: genetic analysis in Excel. Population genetic software for teaching and research. Molecular Ecology Notes **6**:288-295.

Petersen, J. L., A. M. Ibarra, J. L. Ramirez, and B. May. 2008. An induced mass spawn of the hermaphroditic lion-paw scallop, *Nodipecten subnodosus*: genetic assignment of maternal and paternal parentage. Journal of Heredity **99**:337-348.

Plough, L. V. 2016. Genetic load in marine animals: a review. Current Zoology **62**:567-579.

R Core Team. 2017. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria.

Reusch, T. B. H., A. Ehlers, A. Hammerli, and B. Worm. 2005. Ecosystem recovery after climatic extremes enhanced by genotypic diversity. Proceedings of the National Academy of Sciences of the United States of America **102**:2826-2831.

Ropes, J. W. 1968. Reproductive cycle of the surf clam, *Spisula solidissima*, in offshore New Jersey. Biological Bulletin **135**:349-365.

Ryman, N., and L. Laikre. 1991. Effects of supportive breeding on the genetically effective population size. Conservation Biology **5**:325-329.

Scanes, E., L. M. Parker, W. A. O'Connor, M. C. Dove, and P. M. Ross. 2020. Heatwaves alter survival of the Sydney rock oyster, *Saccostrea glomerata*. Marine Pollution Bulletin **158**.

Sharpe, D. M. T., and A. P. Hendry. 2009. Life history change in commercially exploited fish stocks: an analysis of trends across studies. Evolutionary Applications **2**:260-275.

Shepherd, S. A., M. J. Tegner, S. A. Guzmán del Próo, and A. International Symposium on Abalone International Symposium on. 1992. Abalone of the world: biology, fisheries, and culture. Blackwell Scientific Publications, Cambridge, MA.

Silina, A. V. 2018. Sex change in scallop *Patinopecten yessoensis*: response to population composition? Peerj **6**:e5240.

Smith, P. J., and A. M. Conroy. 1992. Loss of genetic-variation in hatchery-produced abalone, *Haliotis iris*. New Zealand Journal of Marine and Freshwater Research **26**:81-85.

Stearns, S. C., and J. C. Koella. 1986. The evolution of phenotypic plasticity in life-history traits: Predictions of reaction norms for age and size at maturity. Evolution **40**:893-913.

Steinberg, P. D., M. T. Brett, J. S. Bechtold, J. E. Richey, L. M. Porensky, and S. N. Smith. 2011. The influence of watershed characteristics on nitrogen export to and marine fate in Hood Canal, Washington, USA. Biogeochemistry **106**:415-433.

Stekoll, M. S. 2019. The seaweed resources of Alaska. Botanica Marina **62**:227-235.

Straus, K. M., B. Vadopalas, J. P. Davis, and C. S. Friedman. 2015. Reduced genetic variation and decreased effective number of breeders in five year-classes of cultured geoducks (*Panopea generosa*). Journal of shellfish research **34**:163-169.

Suquet, M., C. Labbe, R. Brizard, A. Donval, J. R. Le Coz, C. Quere, and P. Haffray. 2010. Changes in motility, ATP content, morphology and fertilisation capacity during the movement phase of tetraploid Pacific oyster (*Crassostrea gigas*) sperm. Theriogenology **74**:111-117.

Toledo-Guedes, K., P. Sanchez-Jerez, M. E. Benjumea, and A. Brito. 2014. Farming-up coastal fish assemblages through a massive aquaculture escape event. Marine Environmental Research **98**:86-95.

Toro, J., R. Thompson, and D. Innes. 2002. Reproductive isolation and reproductive output in two sympatric mussel species (*Mytilus edulis, M. trossulus*) and their hybrids from Newfoundland. Marine Biology **141**:897-909.

Troell, M., R. L. Naylor, M. Metian, M. Beveridge, P. H. Tyedmers, C. Folke, K. J. Arrow, S. Barrett, A.-S. Crépin, P. R. Ehrlich, Å. Gren, N. Kautsky, S. A. Levin, K. Nyborg, H. Österblom, S. Polasky, M. Scheffer, and B. H. Walker. 2014. Does aquaculture add resilience to the global food system? Proceedings of the National Academy of Sciences of the United States of America. **111**:13257-13263.

Tutschulte, T., and J. H. Connell. 1981. Reproductive biology of three species of abalones (*Haliotis*) in southern California. The Veliger **23**:195-+.

Utting, S. D., and B. E. Spencer. 1991. The hatchery culture of bivalve mollusc larvae and juveniles. Ministry of Agriculture, Fisheries and Food Directorate of Fisheries Research, Lowestoft.

Vadopalas, B., J. P. Davis, and C. S. Friedman. 2015. Maturation, spawning, and fecundity of the farmed pacific geoduck *Panopea generosa* in Puget Sound, Washington. Journal of shellfish research **34**:31-37.

Vandeputte, M., and P. Haffray. 2014. Parentage assignment with genomic markers: a major advance for understanding and exploiting genetic variation of quantitative traits in farmed aquatic animals. Frontiers in Genetics **5**.

Waldbusser, G. G., E. P. Voigt, H. Bergschneider, M. A. Green, and R. I. E. Newell. 2011. Biocalcification in the eastern oyster (*Crassostrea virginica*) in relation to long-term trends in Chesapeake Bay pH. Estuaries and coasts **34**:221-231.

Wang, J. L. 2009. A new method for estimating effective population sizes from a single sample of multilocus genotypes. Molecular Ecology **18**:2148-2164.

Waples, R. S. 2002. Evaluating the effect of stage-specific survivorship on the Ne/N ratio. Molecular Ecology **11**:1029-1037.

Waples, R. S., K. Hindar, and J. J. Hard. 2012. Genetic risks associated with marine aquaculture. U.S. Dept. Commerce.

Waples, R. S., and R. K. Waples. 2011. Inbreeding effective population size and parentage analysis without parents. Molecular Ecology Resources **11 Suppl 1**:162-171.

Waters, C. D., J. J. Hard, M. S. O. Brieuc, D. E. Fast, K. I. Warheit, C. M. Knudsen, W. J. Bosch, and K. A. Naish. 2018. Genomewide association analyses of fitness traits in captive-reared Chinook salmon: Applications in evaluating conservation strategies. Evolutionary Applications **11**:853-868.

Wilbur, K. M., A. S. Tompa, N. H. Verdonk, and J. A. M. van den Biggelaar. 1984. Reproduction. Elsevier Science & Technology, Saint Louis, MO.

Withler, R. E. 1988. Genetic consequences of fertilizing chinook salmon (*Oncorhynchus tshawytscha*) eggs with pooled milt. Aquaculture **68**:15-25.

Zheng, H., G. Zhang, X. Liu, F. Zhang, and X. Guo. 2004. Different responses to selection in two stocks of the bay scallop, *Argopecten irradians irradians* Lamarck (1819). Journal of Experimental Marine Biology and Ecology **313**:213-223.