5.0. Development of hatchery, nursery and growout methods for Nuttall's cockle (*Clinocardium nuttallii*). During the 1995 shellfish surveys at the Alaskan Native villages of Tatitlek, Port Graham and Nanwalek, villagers repeatedly expressed a preference for cockles (*Clinocardium nuttallii*). Residents of Port Graham reported that cockles were common in the 1970's and early 1980's, but virtually disappeared several years before the *Exxon Valdez* oil spill. Very few cockles were observed in any of the quantitative or qualitative surveys conducted at Port Graham, Tatitlek, or Nanwalek. Excellent cockle habitat was observed in qualitative shellfish surveys at Port Graham and Tatitlek. The common cockle from the Eastern Atlantic (*Cerastoderma edule*) is prized in some areas of Europe and blood cockles of the genus *Anadara* are grown and marketed in Asia. However, Nuttall's cockle, common in sandy intertidal areas of the eastern Pacific, is not cultivated and is not commonly harvested commercially. In part, that is because this bivalve does not keep well under refrigeration (author's personal experience) and therefore has a limited commercial shelf-life. The result is that little work has been accomplished with respect to developing hatchery techniques for propagating this animal. A search of the ASFA and BIOSYS bibliographic databases revealed few citations dealing with the genus *Clinocardium.* All of those identified in the search were obtained from the University of Washington library system together with many of the references pertaining to other cockle species.

5.1. Background. In addition to being a favored food of Alaskan Natives, cockles appear to grow rapidly in Washington State. Little information regarding aging techniques appropriate to cockles (*Clinocardium nuttallii*) was obtained in the literature and no age at length data was available for either Washington or Alaska. Gallucci and Gallucci (1982) observed "the Pacific cockle's checks or growth lines are known to be unreliable for aging purposes. They opined that apparent "false checks" were a consequence of a spawning period that extends over 2/3 of the year and an existence at the sediment surface, which accentuates the impact of environmental fluctuations. The authors did not provide a reference supporting their assertion regarding the unreliability of apparent annuli in cockles and used the von Bertalanffy growth model to predict a size of 34.3 to 50.3 mm at the end of one year and 65.4 to 76.8 mm at three years of age in Oregon. Cockle valves do show very distinct checks in Washington State and Alaska. Cockle valves were collected at Chenega and Ouzinke in Alaska and at Thorndyke Bay in Washington State and the apparent annuli used to determine a length at age relationship. The results are presented in Figure (97) for Thorndyke Bay and in Figure (98) for Chenega. The results suggest that a minimum harvest size of 38 mm was reached in between 3.5 and 4.0 years. This initial interpretation, based on apparent checks, suggested that cockles reached a valve length of only 1.0 cm during their first year. That is significantly less than the size predicted by Gallucci and Gallucci (1982). In addition, the coefficients describing maximum valve length derived from the von Bertalanffy model were unrealistically high at 17.2 and 26.4 cm. Cockles are commonly found to valve lengths of 7 to 8 cm in the Pacific Northwest and a few reach 10 cm (Brooks, unpublished). The unrealistically large predicted length could be due to counting false checks as annuli or it could be associated with relatively fast growth throughout the cockle's life with death occurring before the animals exceed 10 cm. Resolution of these hypotheses requires an analysis of the length of cockles of known age.

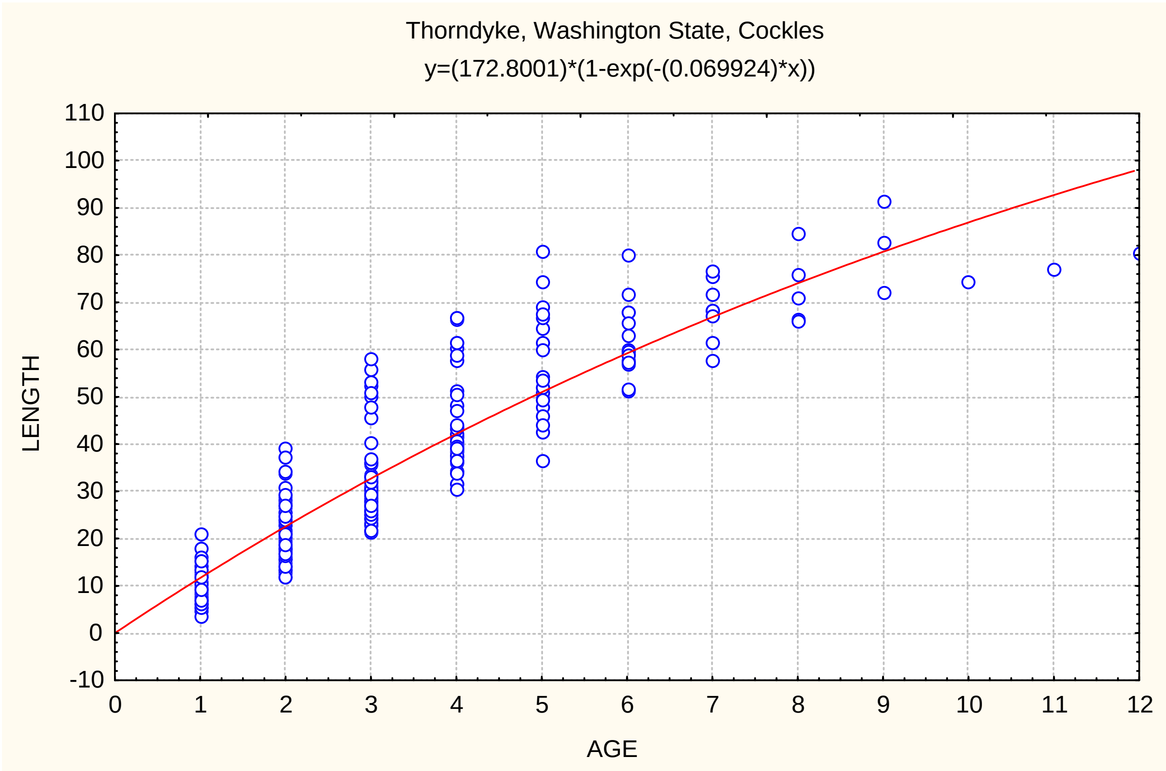

Figure 97. Length at age with von Bertalanffy model predictions for cockles collected from Thorndyke Bay in Washington State.

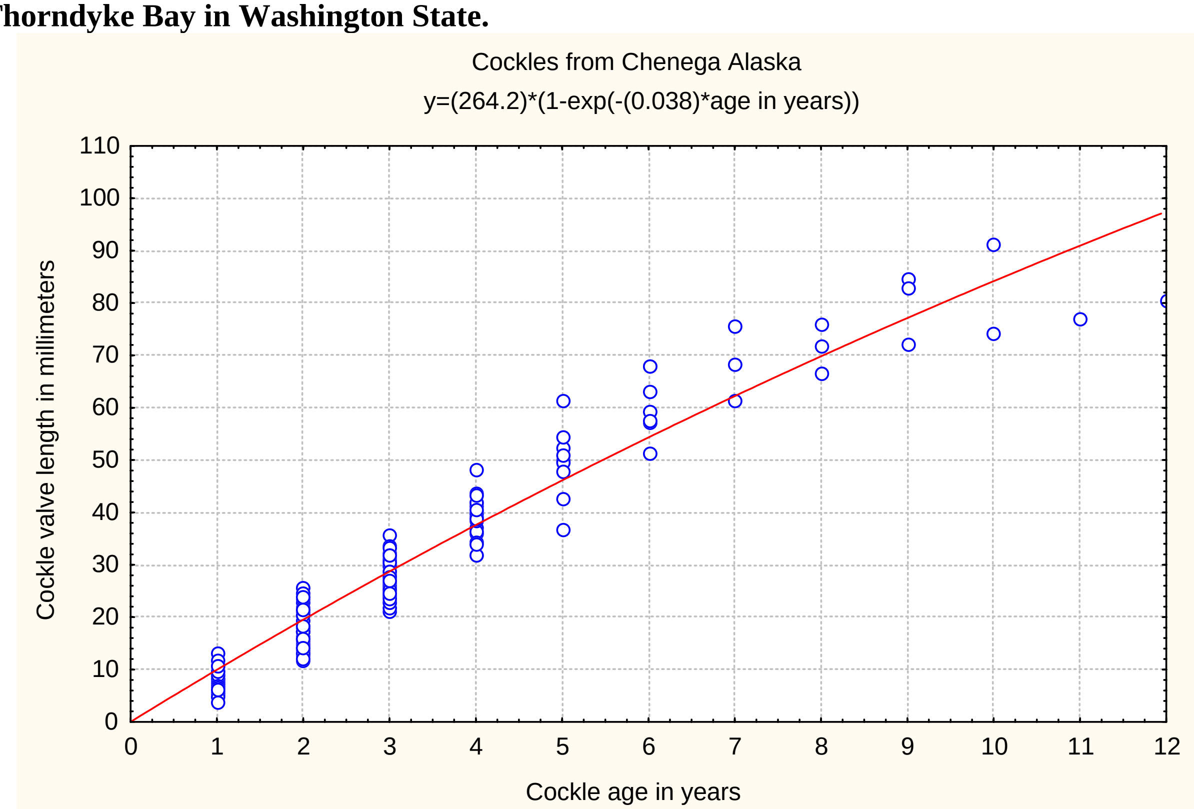

Figure 98. Length at age with von Bertalanffy model predictions for cockles (*Clinocardium nuttallii*) from Chenega, Alaska.

5.2. Reproduction of Nuttall's cockle. Robinson and Breese (1982) histologically examined gonadal tissue from cockles (*Clinocardium nuttallii*) collected from Yaquina Bay and Tillamook Bay, Oregon. They observed ripe gonads from March through September and assumed a summer spawning season. Robinson (personal communication) noted that they did spawn cockles in June but could not grow the larvae through metamorphosis. Gallucci and Gallucci (1982) confirmed that spawning could occur from April to November with a proposed peak in July and August. However, these author's discussed the possibility of a minor spawn in April and May, followed by a major spawning period from July to September.
Strathmann ((1987) confirmed a breeding season of April through November with peak reproduction between July and August in this species. The hermaphroditic nature of this species was noted by Strathmann (1987). She added that oocytes are ca. 80 μm in diameter and have jelly coats over 50 μm thick. At 15 ^{o}C, first cleavage took place within one hour and early veligers developed within 18 hours. None of the literature (including Strathman, 1987) reported spawning cockles and raising them through metamorphosis.

5.3. Materials and methods. Several activities were initiated in an effort to define hatchery, nursery and growout methods for *Clinocardium nuttallii*. This was a cooperative effort between Aquatic Environmental Sciences, Mr. Dick Poole at the Lummi native shellfish hatchery in Washington State and Mr. Ed Jones at the Taylor Resources Hatchery and nursery facility on Dabob Bay, Washington.

5.3.1. Cockle spawning. Cockles were collected from Thorndyke Bay in Washington State from April until October during 1996 and 1997. They were held in marine aquaria at 15 ^{o}C overnight. Each cohort contained 20 to 30 cockles with valve lengths greater than 50 mm. Initial spawning attempts were made with the cockles placed in 10 μm filtered and pasteurized seawater maintained at 15 ^{o}C. The temperature was raised rapidly by six degrees C through the addition of heated seawater. In the first series of attempts during April 1996, a single animal released a moderate quantity of ova. No sperm were released. Microscopic examination of tissues at the base of the foot revealed mature ova in several individuals – but no sperm.

During the second spawning effort (late August 1996), cockles were placed in clean sand in individual Pyrex dishes and maintained in aquaria at a temperature of 16 ^{o}C to mimic the ambient temperature observed in Thorndyke bay at the time of collection. The temperature of the water was rapidly raised to ca. 22 ^{o}C. On the first attempt, two males released sperm, which was used in an attempt to stimulate other cockles to spawn. Microscopic examination of the sperm indicated that they were viable. However, no additional animals spawned and no eggs were obtained. On the next day, the experiment was repeated. Sperm were obtained and a small quantity of immature ova that averaged 30 μm in diameter. A dilute sperm suspension was added to the ova in seawater (30 ppt) at 18^{o}C. No cell cleavage was observed. Removal of gonadal tissue from the spawning female revealed what appeared to be mature ova packed in oocytes. However, no mature ova were expelled (at least none were observed). Two hundred milliliters of a dense suspension (2 x 10^{6}) of phytoplankton (*Chaetoceros calcitrans* and *Thalassiosira pseudonana*) were added to the 15-liter aquaria used in each of these trials after one hour of unsuccessful spawning. The addition of food did not stimulate spawning. Attempts to spawn cockles continued in 1997 at both the Taylor Resources Hatchery on Hood Canal and at Aquatic Environmental Sciences without success. The injection of 0.9 cc of a 0.2 molar solution of seratonin into the proximal junction of the cockle's foot regularly yielded sperm – but not eggs.

Mr. Dick Poole, hatchery manager at the Lummi native hatchery received approximately 400 cockles, in plastic mesh bags, on April 12, 1998. These were placed in tanks of filtered, 30 o/oo seawater heated to 21°C in preparation for spawning Manila clams. The cockles spawned overnight without further intervention. The trochophore larvae were siphoned into other tanks at a density of ca. 2 larvae/ml for rearing at temperatures between 17 and 23°C. Parameters under which the larvae were raised through metamorphosis are provided in Table (25). The 1998 cohort metamorphosed at 200 to 300 µm on April 25, 1998. The larval stage was reported to have lasted only two weeks. The set larvae were transferred to the Suquamish tribe for planting at 500 microns valve length on April 29, 1998. They were lost (died) while being held overnight in buckets.

Table 25. Spawning and rearing conditions used by the Lummi shellfish hatchery for production of Nuttall's cockle (*Clinocardium nuttallii*) seed.

Parameter	Range	Notes
Spawning season:	Unknown – spawned only from wild stocks collected in April.	
Spawning temperature:	20 to 22°C	Spawn in mass - siphon into other tanks to dilute to 1.85 larvae/ml.
Rearing temperature:	17 to 23°C	Limits not investigated
Salinity:	20 to 30 o/oo	
Food:		
Larvae - up to 120µm.	20,000 to 50,000 cells/ml of a mixed diet containing *Isochrysis galbana, Pavlova lutheri, Chaetoceros calcitrans* and *Skeletonema costatum (3)*	
Larvae – 120 to 220 µm.	<100,000 cells/ml of a mixed diet containing *Isochrysis galbana*, Tahitian *Isochrysis, Chaetoceros calcitrans, Skeletonema costatum (3), Thalassiosira pseudonana* (clone 3H), *Chaetoceros gracilis* – fed twice daily.	
Signs of metamorphosis:	Foot shows at 200 to 220 microns. Metamorphosed larvae were caught on a 149 µm screen.	

Note: The regimen for feeding twice daily included feeding *Isochrysis* and Tahitian *Isochrysis* in the morning. The remaining species were fed in the afternoon. Phytoplankton cell densities were raised to 20,000 to 50,000 cells/ml in the culture tanks at each feeding.

5.3.2. Nursery and growout phases of cockle production. The following protocol was designed to evaluate the growth and mortality of Nuttall's cockles (*Clinocardium nuttallii*) under a variety of culture conditions. The Lummi hatchery successfully spawned and reared cockles again during the first week of April 1999 and transferred them to Mr. Paul Williams (Suquamish tribe) and Aquatic Environmental Sciences on June 2, 1999. A subsample was taken for length frequency analysis and the cockles placed in an upweller at the Taylor United hatchery.

Approximately 3,000 cockles were seeded into window screen covered trays on June 12, 1999. A subsample of this seed was randomly selected for length-frequency analysis. The remaining seed was retained in the Taylor United shellfish hatchery for outplanting on July 29, 1999. Approximately 9,000 cockle seed were transferred to Aquatic Environmental Sciences for the following trials at Dr. Joth Davis's shellfish culture site in Thorndyke Bay on Hood Canal, Washington. Substrate in this area consists of organically enriched fine and intermediate sands with small amounts of silt and clay (Brooks, unpublished). Nuttall's cockles are abundant throughout Thorndyke Bay.

A. Nine cohorts of 100 cockles each were individually measured and planted, in three replicates at the –1.0, 0.0 and +1.5' MLLW tidal levels, in half-Norplex™ bags. The –1.0' level was established at low water (1240) on July 30, 1999. The remaining tidal heights were established using a properly leveled transit and aluminum stadium.

B. Three cohorts each of 50 and 200 cockles were measured and planted in half-Norplex™ bags at the 0.0' MLLW level on July 29, 1999.

C. Six thousand cockles under planted at a density of 60/square foot under plastic netting in Thorndyke Bay at the 0.0' MLLW tide level on July 29, 1999.

Cockles were placed in half Norplex™ bays and one end sealed with a split PVC pipe and electrical ties. The bags were placed in shallow depressions dug into the substrate and filled with sieved sand such that the top one-inch of the bag protruded above the natural level of substrate. All tests, excepting the tidal height test, were conducted at the 0.0' MLLW level. The study layout is provided in Figure (99).

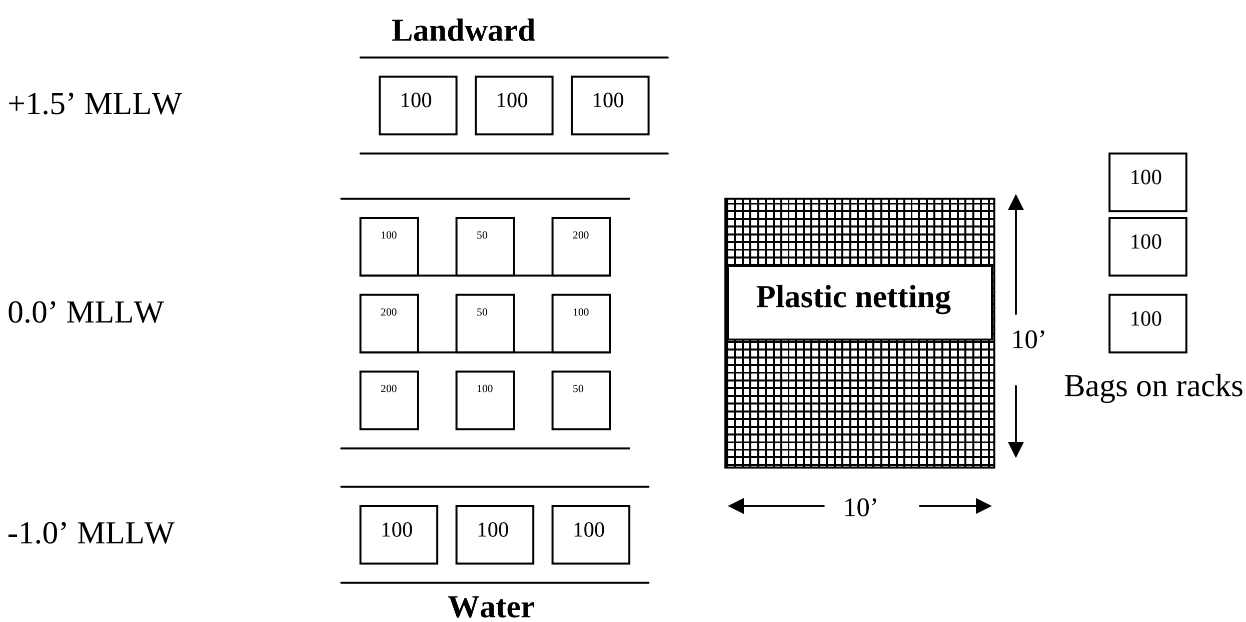

Figure 99. Layout of cockle (*Clinocardium nuttallii*) studies conducted in Thorndyke Bay, Washington State during 1999 and 2000.

These cultures were sieved and the cockles counted and measured on October 27, 1999. Unfortunately, this portion of the CRRC project was cancelled due to lack of funding in November 1999. All of the cultures were removed except for one of the replicates containing 50 cockles/bag. Cockles in that bag were sampled for a final time on June 14, 2000. The data were entered in a Statistica™ database for evaluation.

5.4. Results of cockle nursery and growout experiments. Figure (101) describes the length of all cockles planted in this study as a function of age after setting at the Lummi hatchery. Slow growth occurred at the Lummi hatchery where the cockles were held without adequate food because of commitments to produce clam and oyster seed until June 2, 1999. Initial sampling of the received stocks revealed a mixed stock containing Pacific oyster seed (*Crassostrea gigas*) and cockles (*Clinocardium nuttallii*). A random sample of the seed revealed 164 living and 170 dead cockles together with 129 living and 6 dead Pacific oysters. Cockles in this mixed culture survived at a lower rate (49%) than did the Pacific oysters (96%). This suggests that juvenile *Clinocardium nuttallii* are more fragile and perhaps difficult to maintain in culture than Pacific oysters. The differences may also be because the cockles were treated similarly to Manila clams and optimum culture conditions for this species have not been determined. To the best of the author's knowledge, the Lummi hatchery is the only facility that has successfully reared larvae of this species through metamorphosis in quantity.

5.4.1. Cockle nursery experiments. Cockles grew rapidly from 3.05 mm to 10.75 mm mean valve length during six weeks of nursery. Approximately 1000 juvenile cockles were simultaneously placed in seed bags at the 0.0' MLLW tide level in Thorndyke Bay to compare growth in this nursery method with the hatcheries downwelling system. The mean valve lengths of a subsample of 100 cockles from each culture, measured after 46 days of culture, are provided in Figure (100). A *t-test* with different variance estimates for each culture indicated that the differences were statistically significant at $\alpha = 0.05$ ($t = 3.51$, $p = 0.0005$). The reasons for this difference were not explored.

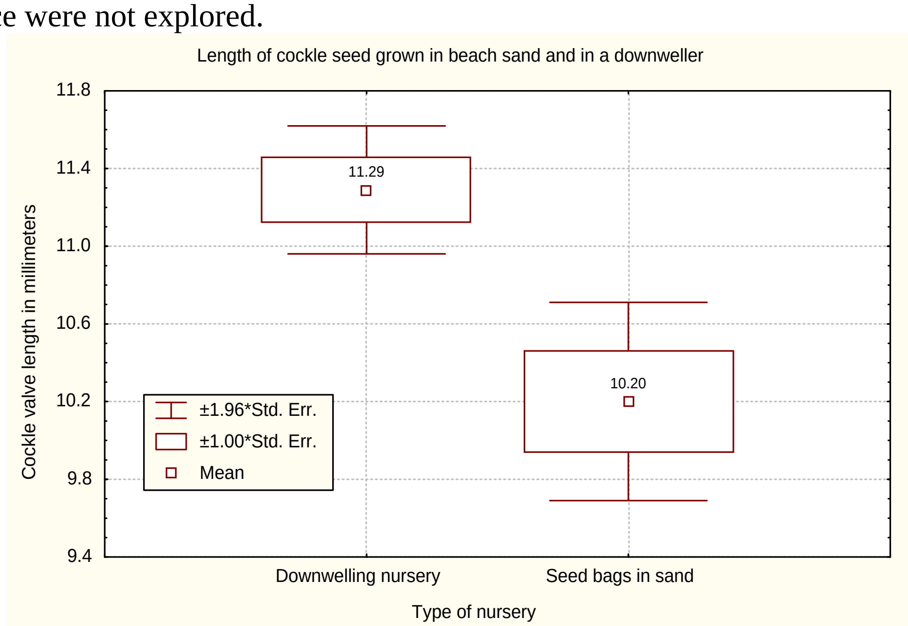

Figure 100. Comparison of the lengths of 100 cockle seed sampled from Taylor Resources hatchery downwelling nursery system and a beach culture planted in Thorndyke Bay in seed bags at the 0.0' MLLW tide level.

5.4.2. Growth of cockles in Thorndyke Bay. A history of the growth of cockles, as measured by valve lengths, is provided in Figure (101). Cockles grew rapidly following their placement either in the downwelling nursery or in seed bags. The given value for age 103 is a mean of the two nursery treatments. Cockles examined on June 14, 2000, following 319 days of growout had grown from a mean valve length of 10.75 mm to 46.10 mm. Other cockle experiments (Brooks, unpublished) suggest that little growth occurs during the winter months and that most of the growth occurs during the spring of the year following spawning.

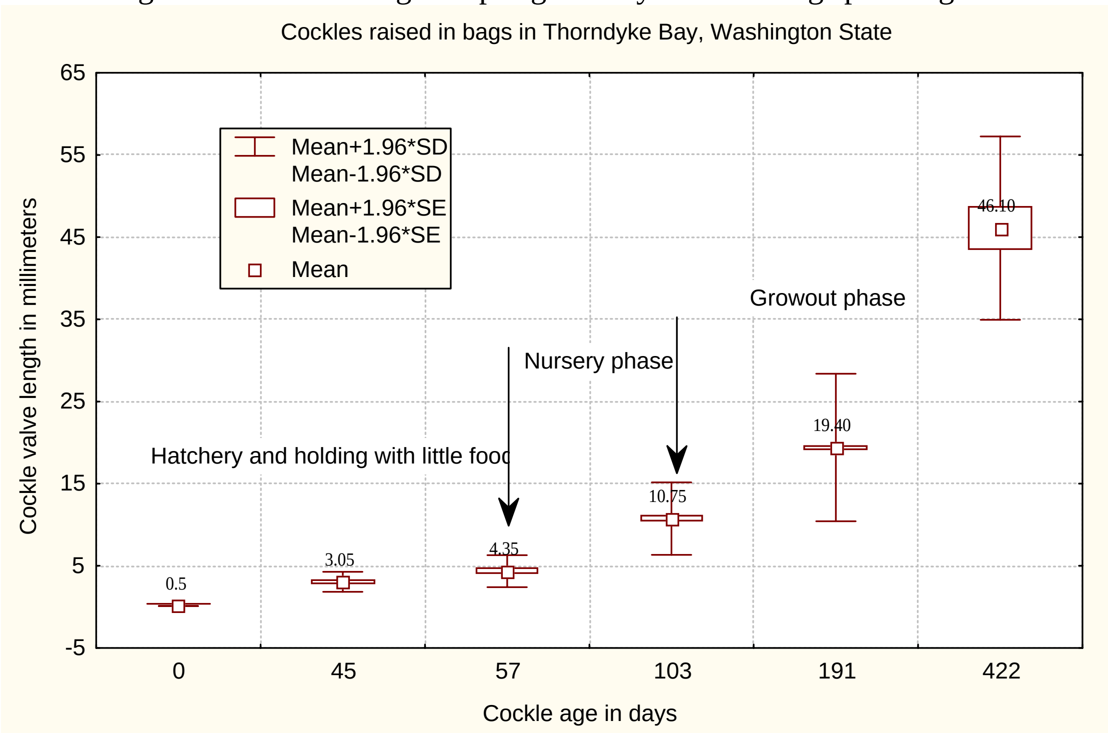

Figure 101. Nuttall's cockle (*Clinocardium nuttallii*) valve lengths as a function of age post setting. The cockles were spawned during the first week in April 1999 and held on minimum rations until June 2, 1999 when they entered Taylor Resources' nursery on Dabob Bay, Washington. The cockles were outplanted to Thorndyke Bay on July 29, 1999 and evaluated in October 1999 and June 2000.

Analysis of variance indicated significant differences in growth as a function of both planting density (F = 115.9; p = 0.00) and tidal height (F = 234; p = 0.00) during the first 88 days of growout in Thorndyke Bay. Figure (102) describes valve length statistics as a function of tidal height. Post hoc testing using Scheffe's test indicated that in this experiment, mean cockle length on October 27, 1999 at an age of 191 days was significantly shorter for cockles grown at +1.5' MLLW when compared with those grown at 0.0' MLLW (p = 0.00) or at –1.5' MLLW (p = 0.00). Significant differences were not detected in cockles grown at the two lower elevations (p = 0.38).

Analysis of variance also indicated significant differences (F = 115.8; p = 0.00) in cockle valve lengths at 191 days of age as a function of planting density. These differences are described in Figure (103). Cockles grown at the lowest density of 50 cockles per half Norplex™ bag had significantly longer (p= 0.000 in either case) mean valve lengths (24.55 mm) than those grown at densities of 100/bag (19.75 mm) or 200/bag (19.10 mm). The standard error of the mean in both cultures grown at the higher densities was low enough such that and these differences were also significant (p = 0.045).

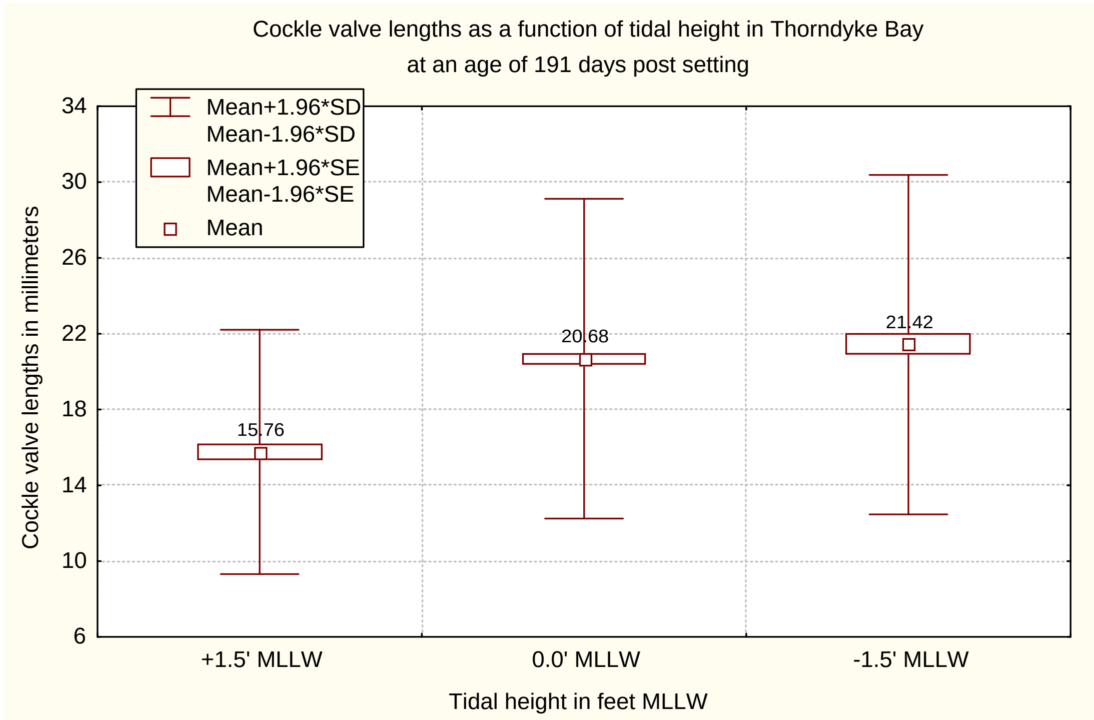

Figure 102. Mean valve lengths for cockles grown to an age of 191 days at three tidal heights in Thorndyke Bay, Washington. Cockles were seeded in three replicates each at a rate of 100 animals per half Norplex™ clam bag.

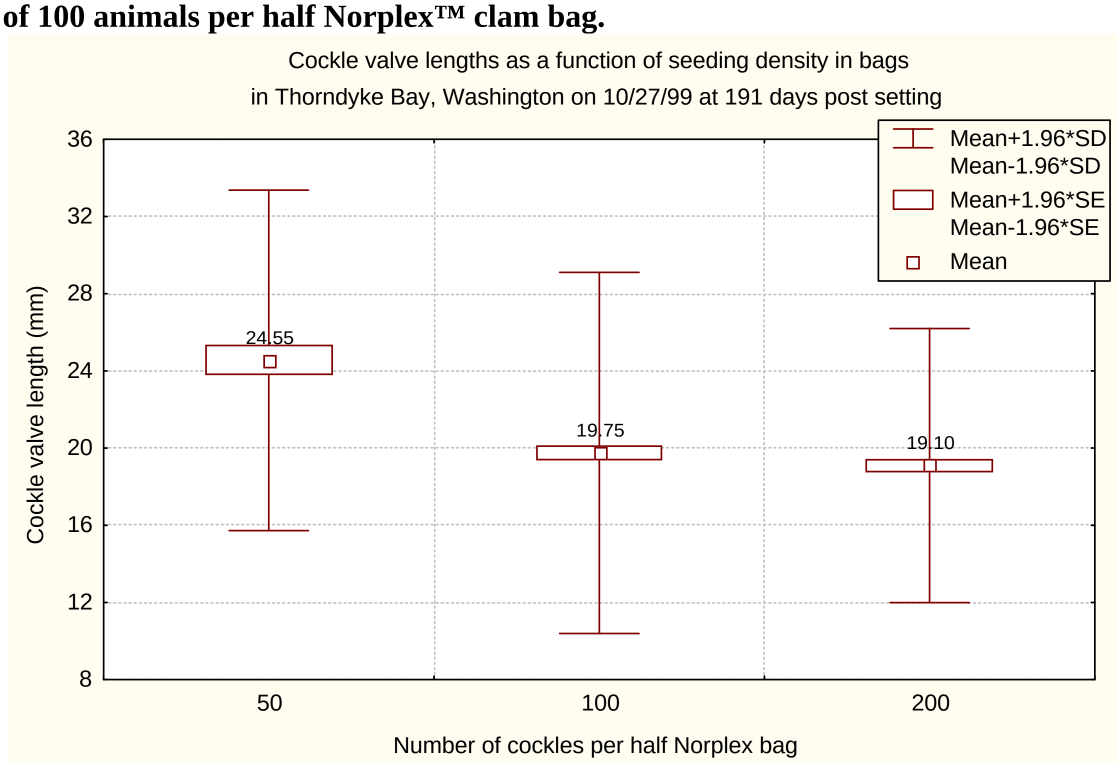

Figure 103. Cockle (*Clinocardium nuttallii*) valve lengths observed following 88 days of growout in Thorndyke Bay (Age = 191 days) as a function of planting density. The differences between each group were significant at $\alpha = 0.05$. Data included three replicates at each density. All replicates were grown at the 0.0' MLLW tide level.

5.4.3. Cockle survival during growout in Thorndyke Bay. The proportion cockles surviving in each replicate on October 27, 1999, following 88 days of growout in the field was transformed using the arcsin(sqrt(proportion)) transformation (Zar, 1984) and subjected to ANOVA. In general, more cockles survived at lower densities and at lower tidal elevations. However, none of the differences were statistically significant at $\alpha = 0.05$. The results are summarized in Figures (104) for density and (105) for tidal elevation.

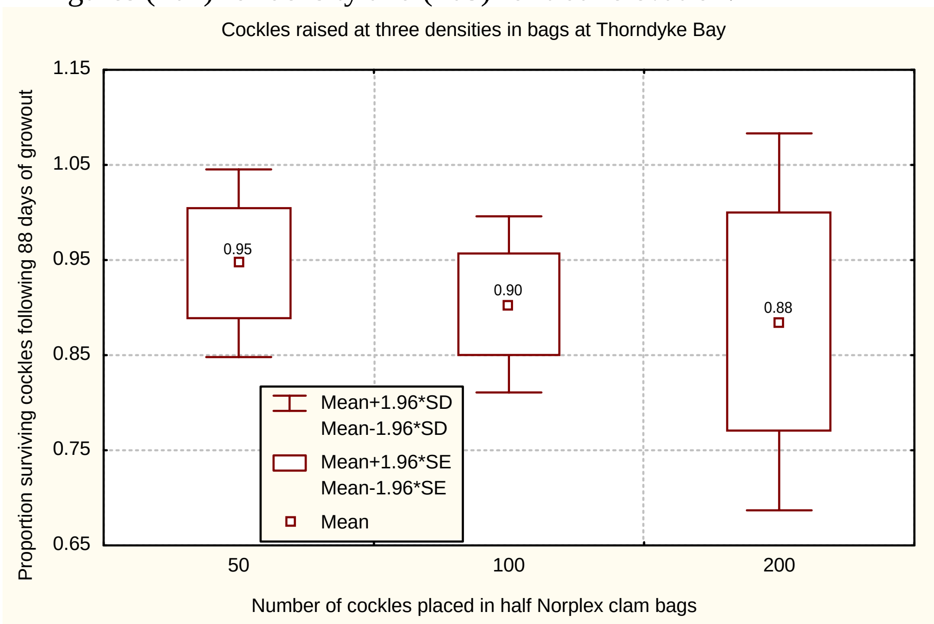

Figure 104. Survival of cockles following 88 days of growout. The bivalves were planted at three densities at the 0.0' MLLW tide level in Thorndyke Bay, Washington. None of the observed differences were statistically significant at $\alpha = 0.05$.

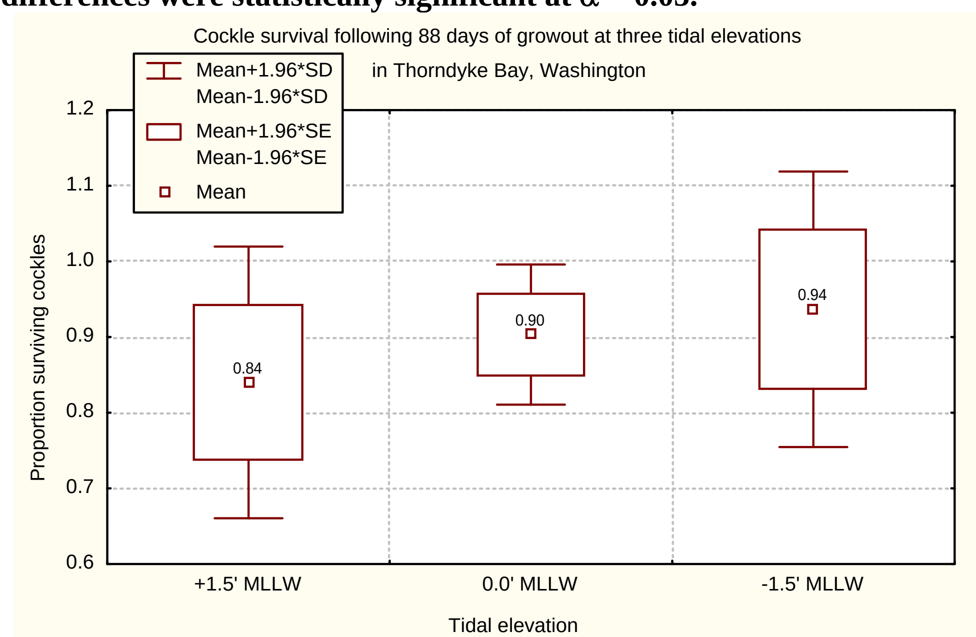

Figure 105. Survival of cockles following 88 days of growout. The bivalves were planted at a density of 100 cockles per half Norplex™ clam bag at three tidal elevations in Thorndyke Bay, Washington. The observed differences were not statistically significant at $\alpha = 0.05$.

5.4.4. Reconciliation of length at age analysis. Gallucci and Gallucci (1982) used the von Bertalanffy growth model to predict a size of 34.3 to 50.3 mm at the end of one year and 65.4 to 76.8 mm at three years of age in Oregon. The results reported here are consistent with their Oregon observations at one year and inconsistent with the predictions made in section 5.1 of this report. Gallucci and Gallucci (1982) noted, "the Pacific cockle's checks or growth lines are known to be unreliable for aging purposes". They opined that apparent "false checks" were a consequence of a spawning period that extends over 2/3 of the year and an existence at the sediment surface, which accentuates the impact of environmental fluctuations. Figure (106) is a photograph of cockles of known age from this study. Two sets of valves are shown for November 27, 1999. The smaller cockles were removed from the highest density culture and the largest cockles are representative of those observed in the 50-cockle/half-bag density. The valve on the right was representative of those evaluated in the 50-cockle/half-bag cohort examined on June 14, 2000. An apparent winter annulus is highlighted.

Figure 106. Representative cockles (*Clinocardium nuttallii*) from nursery and growout studies. Cockles were spawned by the Lummi hatchery, nurseried at Taylor Resources and grown in Thorndyke Bay, Washington.

The apparent first annulus was well defined in all cockles from this cohort. Approximately 15 cockle valves were sectioned and polished with a 600-grit whetstone. These sections revealed distinct discontinuities in the shell's structure caused by an apparent excursion of the inner lamellar layer through the outer prismatic layer to the shell's surface. These excursions were sometimes rather broad (several millimeters) and colored brown corresponding with the exterior color, which does not generally permeate the white prismatic layer. These apparent annuli, visible in section, always corresponded with significant exterior checks. However, additional exterior checks were not always associated with these discontinuities in the sectioned material. These apparently false exterior checks only occurred during and following the initial annulus. They may be associated with spawning and/or other stressful events as suggested by Gallucci and Gallucci (1982). This study did not last beyond one year and this hypothesis could not be confirmed. However, the weight of evidence strongly supports the hypothesis of Gallucci and Galluci (1982). Based on these results, it is recommended that future cockle ages be determined by sectioning the valves. In this study, that was accomplished very quickly (3 to 5 minutes per animal) by cutting with a 0.89 mm thick carborundum disk of 37.5 mm diameter attached to a Craftsman™ variable speed rotary tool operated at ca. 22,000 rpm. This was followed by light sanding of the edge on 220-grit aluminum oxide sandpaper, finishing on a 600-grit whetstone in water and examination under a stereomicroscope. A typical set of valves from Ouzinke is described in Figure (107) with the apparent true and false annuli marked.

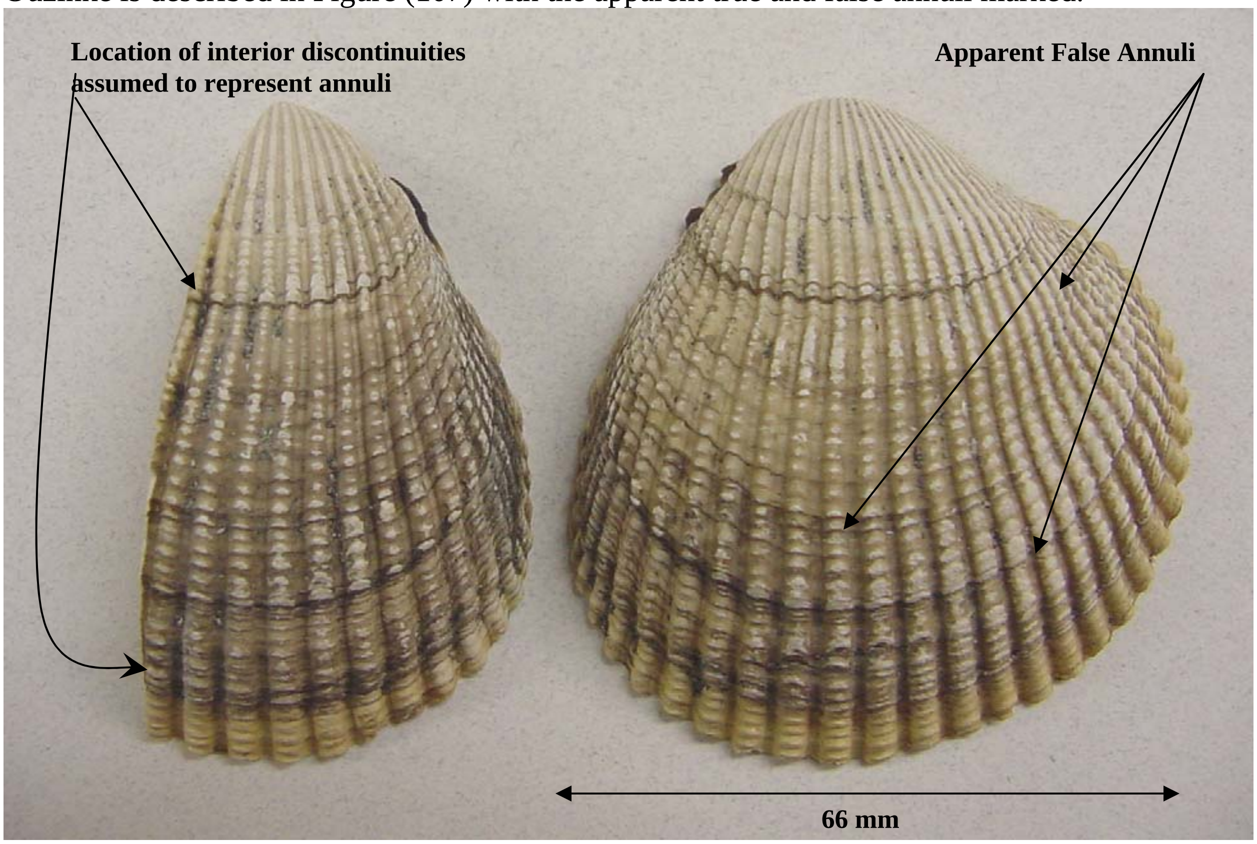

Figure 107. Cockle (*Clinocardium nuttallii*) valves from Ouzinke, Alaska with the annuli identified in sectioned material identified on the left and apparent false annuli on the valve's exterior annotated on the right. The valve length in this cockle was measured at 66 mm and was judged to have lived through two winters.

5.5. Cockle study summary. These preliminary studies with Nuttall's cockle (*Clinocardium nuttallii*) suggest the following:

- Nuttall's cockle was spawned and reared through metamorphosis in the Lummi hatchery using the parameters described in Table (25). The stated parameters worked, but additional research is required to determine optimum parameters for hatchery production. Experience suggests that newly set larvae are fragile and subject to high mortality when improperly handled. However, what constitutes "proper handling" was not determined in this study.

- Nuttall's cockle was successfully grown in a commercial nursery to a length of 11 mm in six weeks. The animal grew adequately but more slowly when held in seed bags at the 0.0' MLLW tide level in Thorndyke Bay, Washington for an identical period.

- Cockles were successfully grown to market size in 11 months of field growout. They grew more quickly during the first 88 days of field culture at tidal levels $\leq$ 0.0' and at lower densities within the tested range of 100 to 400 cockles per full Norplex™ clam bag.

- Cockles planted at ca. 600/m^2 under plastic netting dispersed during the first 88 days of culture. That statement is made because cockles were found only within the roots of scattered eelgrass in the plot. Empty cockleshells were not found in the sediments suggesting little or no mortality after burrowing in. They simply disappeared. This suggested that juvenile cockles may be mobile and a series of experiments were designed to monitor their movement using a short-term mark and recapture methodology. These experiments were not initiated because the study was terminated due to lack of funding.

- Statistically significant differences in cockle survival at varying tidal heights and densities were not observed over 88 days of field growout. However, consistent trends indicating higher survival at lower intertidal elevations and lower densities were observed. A continuation of these trends during a 10 to 12 month growout might lead to significant differences. That determination will have to wait for a longer-term study.

Most importantly, the mean valve length of cockles raised at a density 50/half bag at the 0.0' MLLW tide level reached 46 mm in 11 months. This suggests that cockles, a species preferred by Alaskan natives, could become a viable part of future shellfish enhancement programs. Obviously, the results from Washington State may not be directly applicable to Alaska due to differences in climate. However, these results suggest that further study by the Qutekcak hatchery and CRRC is warranted.

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