4.5. Results for the village of Tatitlek. The study site at Tatitlek lies within easy walking distance of the Village. The intertidal consists of shale outcroppings that have been broken into angular rock, cobble, gravel and finer material. Substrates tended to be somewhat compacted and coarse, and they were considered suitable for enhancement only with substantial cultivation effort. This is particularly true with intensive culture techniques that require use of plastic bags or netting. This beach was not as amenable to intensive culture techniques as was Murphy's Slough. In addition, a moderate amount of substrate movement was experienced during the winters of 1997-98 and 1998-99. However, the integrity of the study site was maintained through regular maintenance by the residents of Tatitlek. In fact, participation by Tatitlek Villagers' was excellent during all phases of this study and data was regularly collected during scheduled sampling times. Figure (80) depicts the enhancement beach and its relationship with the village.

Figure 80. Traditional subsistence beach and the site of the 1995 – 1999 native littleneck clam enhancement studies at the Village of Tatitlek.

Figure (81) is a photograph of one of the netted replicates, taken in 1998, after the first of these storms. The upper 5 to 7 cm of sediments around the plastic netting had been eroded and moved to other areas of the beach. The storms causing this erosion would have also washed small clams out of the sediments and deposited them elsewhere. Native littleneck clams were not found in the adjacent area that had been seeded but not protected. In this instance, the light plastic netting was effecting in stabilizing the area seeded with clams and an average of 65% of the seeded clams survived until last surveyed on October 27, 2000. No native littleneck clams were found in seeded but unprotected plots at the +1.5' MLLW level in 2000 and only five native littleneck clams were retrieved in nine samples collected from similarly seeded but unprotected areas at the 0.0' MLLW tide level. The storms that caused this erosion also damaged several of the netted plots. The nets were replaced during the 1998 field season.

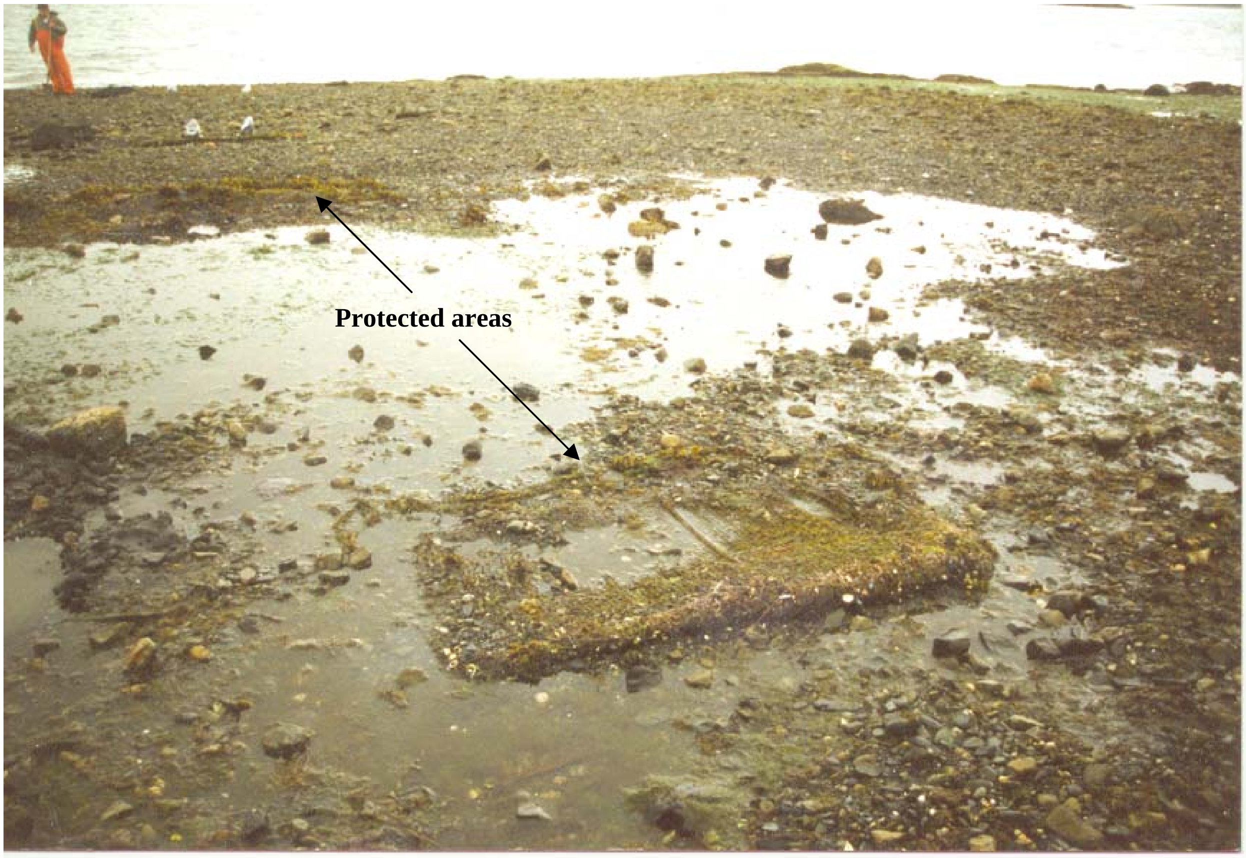

Figure 81. Enhancement plots (1A) and (1B) on the Tatitlek shellfish beach. Beach substrates were stabilized under the seeded area that was protected with plastic netting. The unprotected area, located to the right in this photograph, was badly eroded and no clams were retrieved in two replicate samples from the unprotected plot in 1999 or 3 samples in 2000.

4.5.1. Physicochemical properties of sediments at Tatitlek. Protected and Unprotected trials were installed at three tidal elevations at Tatitlek (-1.5' MLLW, 0.0' MLLW and +1.5' MLLW) in 1996. Sediment grains size and sediment TVS were evaluated in 18 samples from Protected, Unprotected, and Control areas on April 26, 1998. Total volatile solids and total sediment sulfides were evaluated in twelve samples on September 9,1999. Proportional data (TVS and fines) were arcsine(square root) transformed (Zar, 1984) and analyzed using ANOVA and *t-tests*. Statistically significant differences ($\alpha = 0.05$) were not observed for the proportion fines (silt and clay) or TVS as a function of treatment (protected or unprotected), beach elevation (tidal height), or replicate (horizontal position on the beach) during either year. Sediment total sulfides were the most sensitive indicator of organic loading. While not statistically significant ($p = 0.27$), mean sulfide concentrations were nearly three times higher under plastic netting (76.3 µmoles) compared with the seeded, but unprotected, area (27.9 µmoles). The major effect of protecting clams with lightweight plastic netting at Tatitlek was to stabilize the substrate preventing its movement during storm events. These data suggest that fines and TVS do not accumulate under small plots protected with plastic netting on moderate to high-energy beaches.

4.5.2. Survival of native littleneck clams in bags at Tatitlek. Figure (82) describes the survival of native littleneck clams in bags at Tatitlek between 1996 and 1999. Significant differences in survival as a function of tidal elevation between –1.5' and + 1.5' MLLW were not observed (ANOVA, F = 1.05, p = 0.35) at the end of the study. The increases in mean number of clams observed on July 1997 and December 1998 were due to recruitment into the bags where metamorphosed clams were protected from starfish, gastropod and possibly other predators. The decreases observed during winter months are pointed out in blue. The author did not examine these cultures in 1997 due to weather. Therefore, new recruits and species other than native littleneck clams were not removed from the bags in 1997. Butter clams (*Saxidomus giganteus*) and native littleneck clams less than 10 mm valve length were removed from the bags by the CRRC field team during the summer of 1998 and 1999. This problem is pointed out because it is likely that clams recruiting into the cages in 1997 may have grown beyond a size where they could be distinguished from the original 1996 seeding. This would cause an overestimation of clam survival and an underestimation of the samples' mean size.

The mean number of surviving clams was relatively constant during the summer months and declined most during winter. Either this may have been due to cold air temperatures during low tides or to stress associated with sediment movement around the protected cultures described in section 4.2.1. No cause and effect relationship was determined for these small winter losses during this study. The number of clams counted in bags at the end of the study on September 9, 1999, was 65 percent of the 900 clams originally seeded into the nine bags.

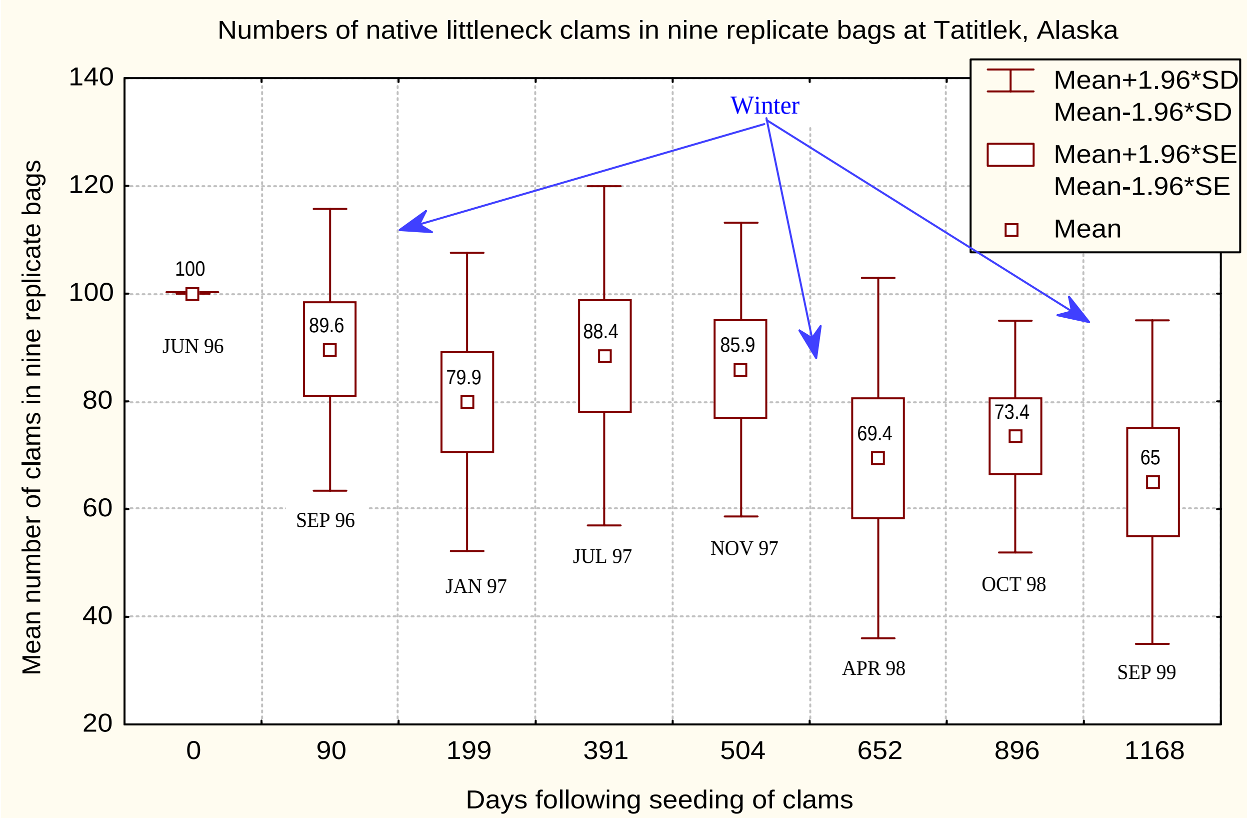

Figure 82. Mean number of surviving native littleneck clams in bags as a function of time (days) following planting on June 29, 1996 at the beach adjacent to the village of Tatitlek, Alaska. Significant differences in survival as a function of tidal height were not observed and the data was pooled.

4.5.3. Survival of native littleneck clams in various treatments. Native littleneck clam seed was planted in Protected and Unprotected two square meter plots on July 5, 1996 at Tatitlek. Planting density was 300 clams/m^2. These clams were not sampled until April 26, 1998 when two 0.0186 m^2 samples were collected from each of three replicates at each of three tidal heights. This effort resulted in 6 samples per tidal height and 18 samples for each treatment (54 samples total). The mean proportion of surviving clams in each seeded treatment on April 26, 1998 is summarized in Figure (83). Five native littleneck clams were retrieved from Control Plot (A) and six from Control Plot (B) at the highest tide level (+1.5'). No native littleneck clams were retrieved from other Control plots. Figure (79) suggests that unprotected native littleneck clam seed survived adequately (mean for all elevations of 21% through the first 18 months of growout) on this beach. However, unprotected native littleneck clams did not survive as well at the lowest tidal height tested (-1.5' MLLW). This may be due to the large number of *Pycnopodia helianthoides* observed at the lower intertidal elevations. It would be interesting to monitor this area during high tides to determine how high this echinoderm ranges. The author has frequently observed sunflower stars subtidally in Puget Sound and less frequently intertidally where *Pisaster, Mediaster and Evasterias* species are more frequently observed. The survival of native littleneck clams in bags and under Carcover™ at Tatitlek is excellent and these techniques appeared valuable for enhancing subsistence harvests of native littleneck clams. Paired sample *t-tests* indicated that the number of clams surviving with protection was significantly higher than without ($p = 0.05$).

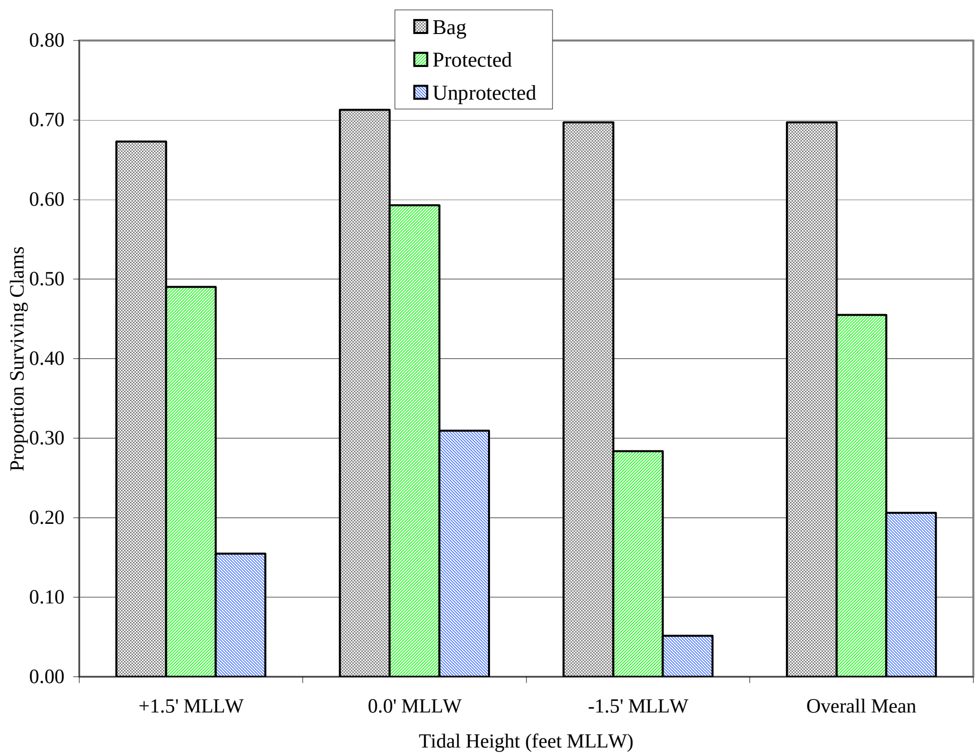

Figure 83. Proportion surviving native littleneck clams at Tatitlek as a function of tidal height and treatment (Bags, Protected with Plastic netting, or seeded but left Unprotected).

Table (23) provides summary statistics for survival and valve length observed in 54 sediment samples collected on September 9, 1999. The ratio of the number of clams observed in each of six replicate 0.0182 m^2 samples randomly collected in each treatment at each tidal height to the number seeded in 1996 is provided. This data must be interpreted with caution because as described in Brooks (1995b), recruitment of wild clams to the Tatitlek beach occurred on a regular basis from ca. 1991 to 1995. In addition, the storm during the winter of 1998 redistributed sediments and likely the clams in them over much of the beach that was not protected with plastic netting.

The discrete survival count data was transformed to continuous data using a Log(n + 1) transformation. The mean number of clams retrieved in 1999 samples differed significantly as a function of treatment (ANOVA, $F = 3.83$, $p = 0.036$). Post hoc testing using Scheffe's test indicated that significantly more clams were retrieved from under plastic netting when compared with the unseeded control areas ($p = 0.04$). The density of clams retrieved from protected and unprotected areas that had been seeded in 1996 were not significantly different ($p = 0.67$); nor were differences between seeded and unprotected areas and the control ($p = 0.21$).

The 1998 and 1999 results suggest that seeded areas contained significantly more clams at the end of three years than unseeded areas. However, while more clams were retrieved from seeded and protected areas when compared with seeded areas left unprotected, the differences were not significant at $\alpha = 0.05$. These data also support the 1995 report of consistent native littleneck clam recruitment at this beach. Together, these reports suggest that factors other than recruitment are responsible for the paucity of clams >38 mm observed on this beach.

Table 23. Proportion surviving native littleneck clams determined in six replicate 0.0182 m^2 samples collected at each of three tidal levels on September 9, 1999 following three years of field growout. The clams were originally seeded at a density of 300 clams per square meter in three replicate plots located at each of three tidal elevations. The seeded areas were cultivated and either protected with plastic netting or left unprotected.

Tidal Elevation	Type protection	Mean length (mm)	Number of clams	Proportion of seed
+1.5'	Unprotected	18.7	22	0.58
+1.5'	Protected	27.7	17	0.45
+1.5'	Unseeded control	12.8	4	NA
+1.5'	Bags	24.0	159	0.53
0.0'	Unprotected	17.6	16	0.42
0.0'	Protected	22.8	31	0.81
0.0'	Unseeded control	8.3	6	NA
0.0'	Bags	23.9	195	0.65
-1.5'	Unprotected	12.2	10	0.26
-1.5'	Protected	25.1	21	0.55
-1.5'	Unseeded control	9.6	5	NA
-1.5'	Bags	22.8	231	0.77

4.5.4. Growth of native littleneck clams in field trials at Tatitlek. Figure (84) describes the growth of native littleneck clams in bags at Tatitlek with predictions from the von Bertalanffy model developed from the analysis of length and age during the 1995 baseline survey.

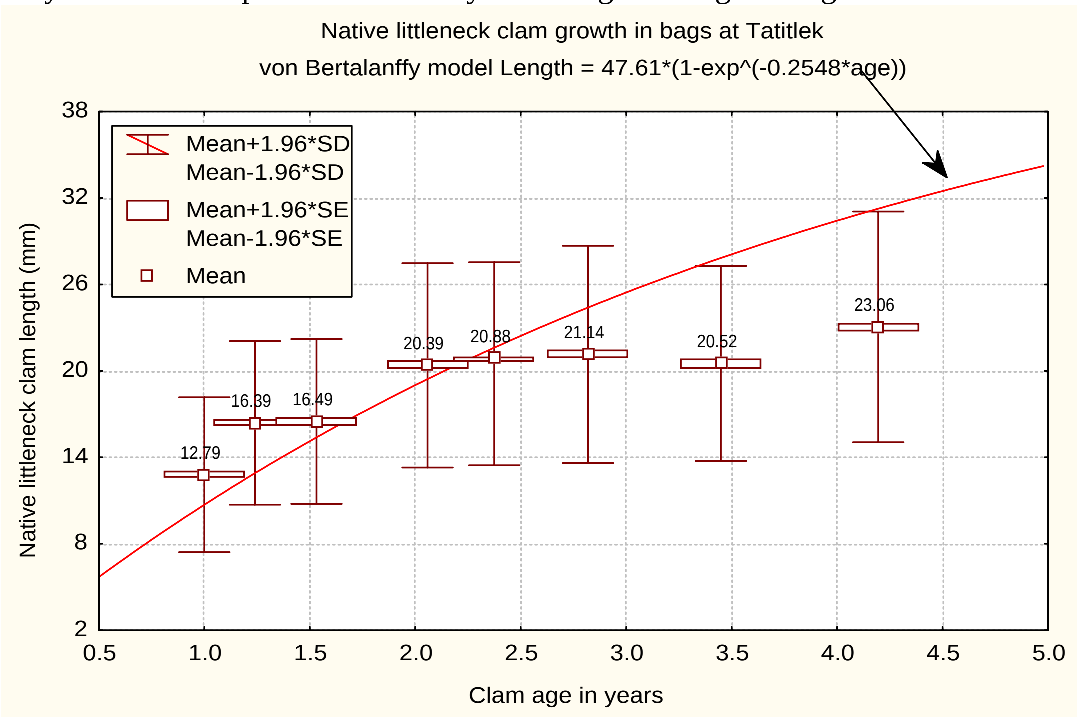

Figure 84. Mean lengths of native littleneck clam cohorts cultured at all tide heights in bags at Tatitlek between June 27, 1996 and September 9, 1999. Clams in bags were measured quarterly for the first two years during this study.

Von Bertalanffy predictions are greater than the mean for all ages greater than 2.2 years and little increase in the mean valve length of clams retrieved from bags was observed until the last year of the study. However, clams in the upper five percent of the observed sizes for clams grown in bags, as evidenced by the 1.96*standard deviation whisker in Figure (80), were growing in a manner similar to the von Bertalanffy predictions from 1998 until the end of the study.

Analysis of covariance with initial length as the covariate indicated that valve lengths on September 9, 1999 were significantly different as a function of treatment ($F = 44.20$; $p = 0.000$). Similar to the results from Port Graham, clams grown under netting had the longest mean length (27.2 mm) followed by clams grown in bags (23.49 mm). Native littleneck clams retrieved in samples from seeded, but unprotected, plots had the shortest mean valve length (17.26 mm). Post hoc testing using Scheffe’s test indicated that the differences between mean valve lengths of native littleneck clams grown in bags or under plastic netting were not significant at $\alpha = 0.05$ ($p = 0.41$). The mean length of native littleneck clams from unprotected areas was significantly shorter than the mean length from bags ($p = 0.000$) or from under netting ($p = 0.000$).

These results are likely the result of recruitment of new clams into these cultures during the study. As previously discussed, recruitment of native littleneck clams at Tatitlek appears to occur in most years. The addition of these small clams into the cultures would cause an increase in the estimated survival and a decrease in estimated growth. Native littleneck clams less than the minimum size in the previous quarterly sample were removed from the bags by the author during each annual CRRC field season. However, the 1997 fieldwork was cancelled due to weather and new recruits were not removed from the bags until April 24, 1998. It is likely that some native littleneck clams recruiting after June 29, 1996 would have grown to a size that would be indistinguishable from the original seed. It is also likely that the significant disturbance in sediments caused by storms (see Figure 77) during 1997-98 and again in 1998-99 created stress in all hardshell clams on this beach. The significantly reduced clam size in the seeded but unprotected areas was likely caused by the loss of the planted seed during storm-associated redistribution of sediments (and the clams in them). As previously noted, sediments (and the clams seeded into them) were effectively stabilized under the plots seeded and protected with netting. Each of these factors likely contributed to these results.

The purpose of this effort was to evaluate the potential for enhancing native littleneck clam subsistence resources at native Alaskan villages. Figure (85) describes the length-frequency of native littleneck clams observed at Tatitlek on September 9, 1999 as a function of the type enhancement. Native littleneck clams retrieved from reference sediments were all less than 20 mm valve length and likely represent clams less than two years old. Clams retrieved from areas that were seeded in 1996 and not protected with plastic netting show one mode at 8 mm valve length. These likely represent 1999 recruits. There is an apparent second cohort with a mode at 16 mm and a third at 20 to 22 mm. The largest clam in the seeded, but unprotected, area had a valve length of 34 mm. In contrast, the population of native littleneck clams retrieved from the seeded area that was protected with plastic netting was dominated by clams with valve

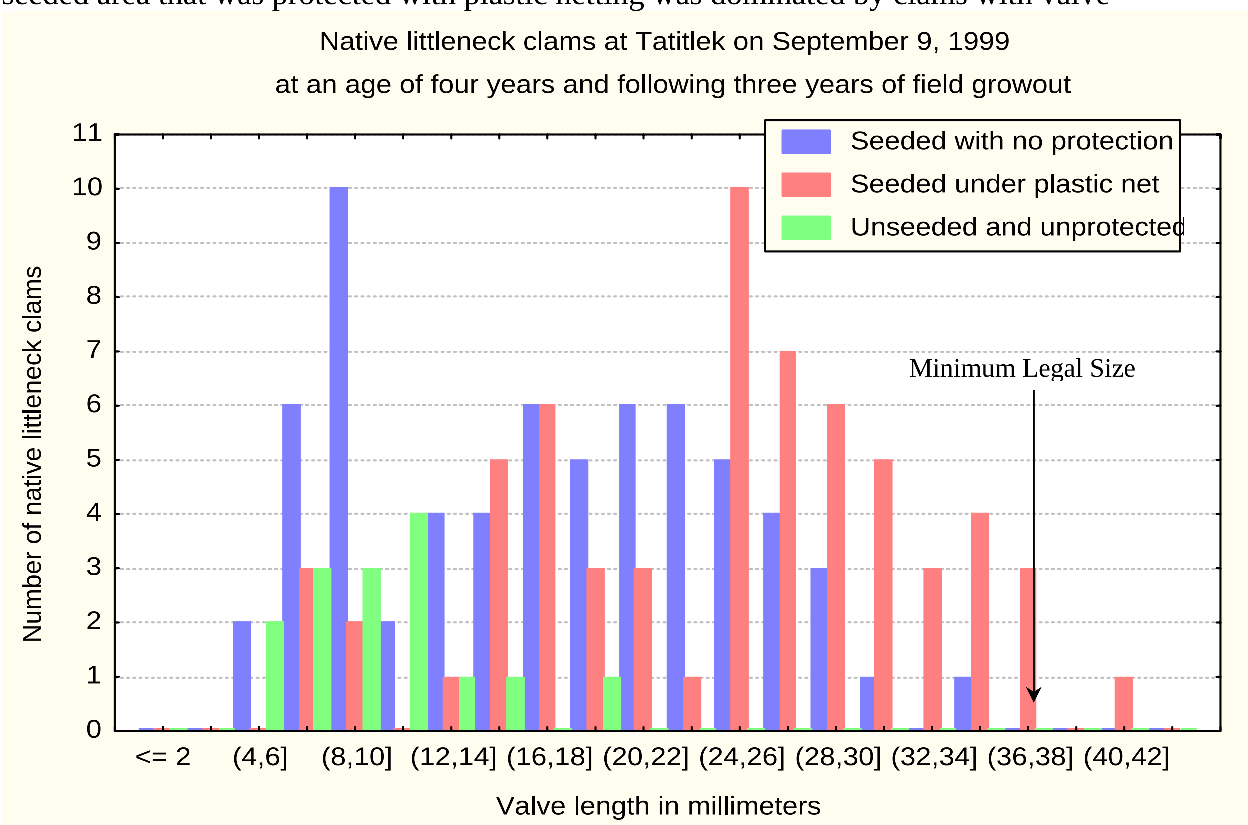

Figure 85. Length-frequency histogram describing the distribution of native littleneck clams retrieved on September 9, 1999. Significant differences in valve length as a function of tidal height were not observed and the results pooled.

lengths in the 24 to 26 mm range. One native littleneck clam retrieved from protected sediment samples recruited into the minimum legal harvest size of 38 mm during 1999 following 3 years of growout at an age of four years.

Mr. Jeff Hetrick from the CRRC field team evaluated native littleneck clams in three replicate 0.0182 m^2 sediment samples from under plastic netting at each treatment plot located at the 0.0’ and +1.5’ MLLW tidal heights during November 2000 (18 samples total). The marginal low tide prevented sampling the three replicates located at –1.5’ MLLW. The results are presented in the length-frequency histogram provided in Figure (86). The location of the apparent year classes is based on a qualitative evaluation of the distribution and location of apparent modes. The median lengths associated with each year class are consistent with the growth observed at Murphy’s Slough where the data was not confounded by natural recruitment. All clams were removed from the substrate during cultivation prior to seeding in 1996. Note that seven native littleneck clams were found with valve lengths exceeding the minimum harvest size. Despite the significant sediment instability observed on this beach at the end of four years of growout, 7.1 percent of the clams originally seeded under plastic netting had survived to harvest size.

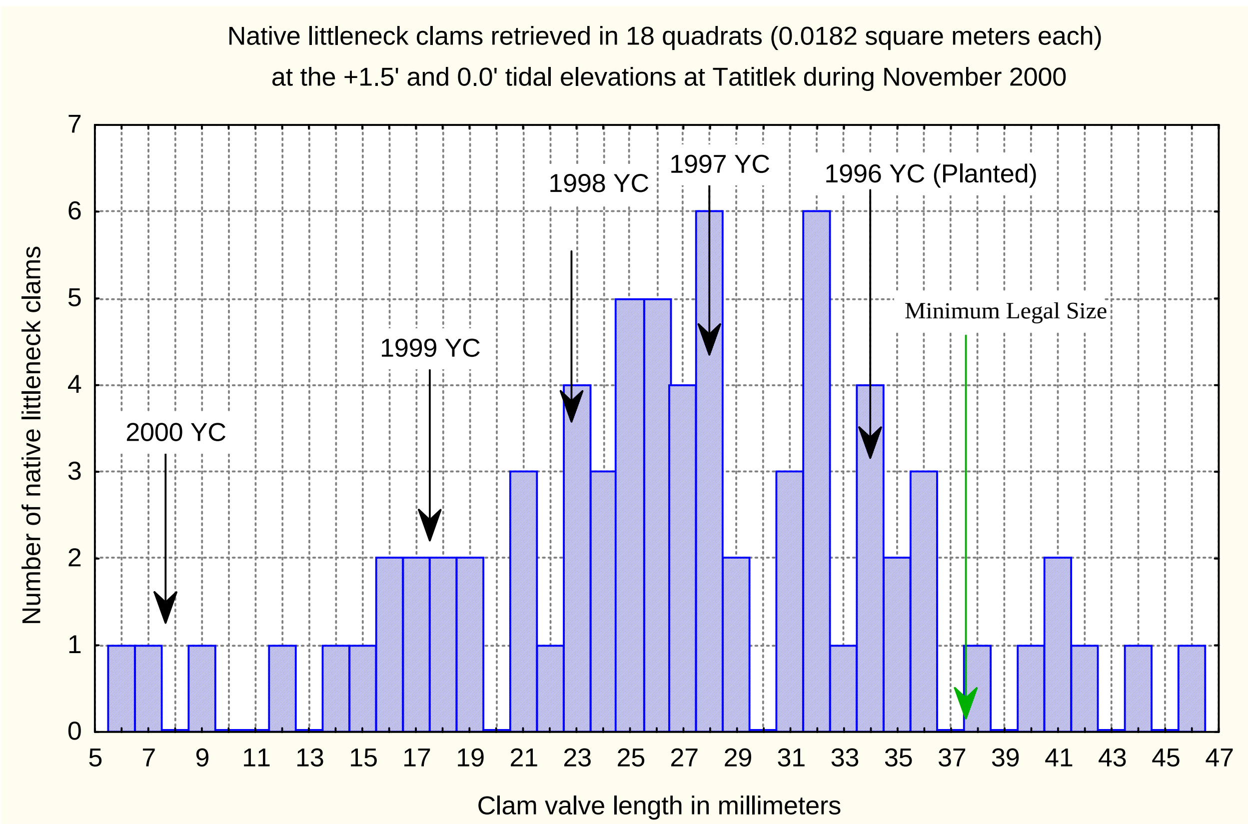

Figure 86. Mean lengths of native littleneck clams cultured under plastic netting at Tatitlek between June 27, 1996 and September 9, 1999. These clams were sampled once each year in 1998, 1999 and 2000.

Native littleneck clam survival and growth data was confounded by the annual recruitment of clams into these cultures. However, this analysis indicates that in high-energy intertidal environments, plastic netting was effective in stabilizing the substrate and in retaining clams. In 2000, following four years of field growout, 7.1 percent of the number of clams originally seeded under plastic netting had valve lengths exceeding the minimum harvest size. The number of clams recovered from bags at the end of three years of growout averaged 65% of those seeded. However, an unknown number of those clams were likely new recruits added during the late summer of 1996 or in the spring and summer of 1997 when the bags were not screened by the principal investigator. The point is that survival in bags in this stressful environment was likely less than 65%. Very few clams recruited to and survived beyond the first two years in control areas and the population of clams resident in the seeded and unprotected treatments were smaller and less numerous than those in the seeded and protected area. Statistically significant ($\alpha = 0.05$) differences in either growth or number of clams were not observed as a function of tidal height between –1.5' MLLW and + 1.5' MLLW.

4.5.5. Fecal coliform in the water column at Tatitlek on April 26, 1998. Fecal coliform (FC) bacteria were detected in all three replicate water samples from Tatitlek taken on April 26, 1998. The Most Probable Number (MPN) was 55.4 FC/100 ml, which exceeded the NSSP standard MPN of 14.0 FC/100 ml for an Approved Harvest Classification. The second part of the NSSP standard states than no more than 10% of the samples can exceed 43 FC/100 ml. Two of the three samples exceeded this value (50 and 170). The source of this fecal contamination was not determined. Birds and marine mammals are possible sources of fecal coliform bacteria in marine environments. The proximity of this site to the village of Tatitlek suggests that further work to determine the proper harvest classification of this site is warranted.

4.5.6. Total Suspended and Total Volatile Solids in the water column at Tatitlek on April 26, 1998. The water temperature at Tatitlek on April 26, 1998 was 6.5 ^{o}C. Summer temperature measured on June 27, 1996 was 12.0 ^{o}C. Total Suspended Solids were measured at 193.8 $\pm$ 95.7 mg/L and the mean TVS content was 14.1 $\pm$ 10.9 mg/L (mean $\pm$ one standard deviation). The source of the particulate inorganic matter is unknown. The high TVS suggested that there was a rich food resource in the water on this early spring day. Summer values recorded on June 27, 1996 were significantly lower at 3.27 mg TSS/L and 2.3 mg/L TVS/L.

4.5.7. Bivalve predators at Tatitlek. Gastropod egg cases, likely from *Nucella cf. lamellosa*, were abundant and numerous adult gastropods were observed at Tatitlek. An army of *Pycnopodia helianthoides* was present below the +0.5' MLLW tide level during every field trip to this beach. *Pycnopodia helianthoides* was observed at a mean density of 0.6/m^2 at the 0.5' MLLW tide level during 1995 and four to six *P. helianthoides* were counted per square meter in front of the enhancement area on April 26, 1998. Figure (87a) describes this assemblage, as it existed on the morning of April 26, 1998. Figure (87b) is a photograph of one of four-bushel baskets of starfish removed from the enhancement beach and deposited above high tide during 1996. Numerous shallow circular pits, possibly made by either sea otters or *P. helianthoides*, have been observed on this beach. It should be noted that no direct evidence of sea otter predation on clam cultures was observed during this study. *Pycnopodia helianthoides* has been observed excavating shallow depressions on this beach and

Sea otter near Port Graham

several sunflower stars have been observed with intact clams (i.e. including the valves) in their guts.

(a) **(b)**

Figure 87. a) *Pycnopodia helianthoides* below the Tatitlek enhancement beach on April 26, 1998. b) Seastars removed from the Tatitlek enhancement beach prior to initial seeding in 1996. This is one of four-bushel baskets of starfish that were removed to an upland area during one morning of predator control.

Control of predatory gastropods and starfish is easily accomplished and should be part of any shellfish enhancement program. It is possible that removal of the large numbers of starfish on the beach would allow a larger portion of the naturally set native littleneck clams to reach harvest size.

4.5.8. Growth of seed clams and oysters in the tidally driven Flupsy at Tatitlek. Figure (88) describes mean oyster (*Crassostrea gigas*) and clam (*Protothaca staminea*) lengths as a function of time in the Tatitlek tidal Flupsy. Clams did not grow during the winter between November 9, 1997 and April 5, 1998. Their valve lengths increased from a mean of 4.4 mm on April 5, 1998 to 11.9 mm on October 23, 1998. Manila clams (*Tapes japonica*) are generally planted at six to ten millimeter valve length. Figure (88) suggests that native littleneck clams, spawned in February or March, and placed in a Flupsy by early April, could achieve a valve length >10 mm and be ready to outplant by September of the same year. This is encouraging because it appears that juvenile clams can be reared to a suitable planting size in time to be planted on the last daylight tides in September or early October in Alaska. Additional Flupsy evaluation should be accomplished. This was planned for the 1999 field season. However, lack of funding prevented accomplishment of the preliminary work to accomplish this task. A copy of the 1999 field season protocols is provided in Appendix (2). These protocols provide details for an appropriate study to more thoroughly evaluate clam growth and survival in Flupsys.

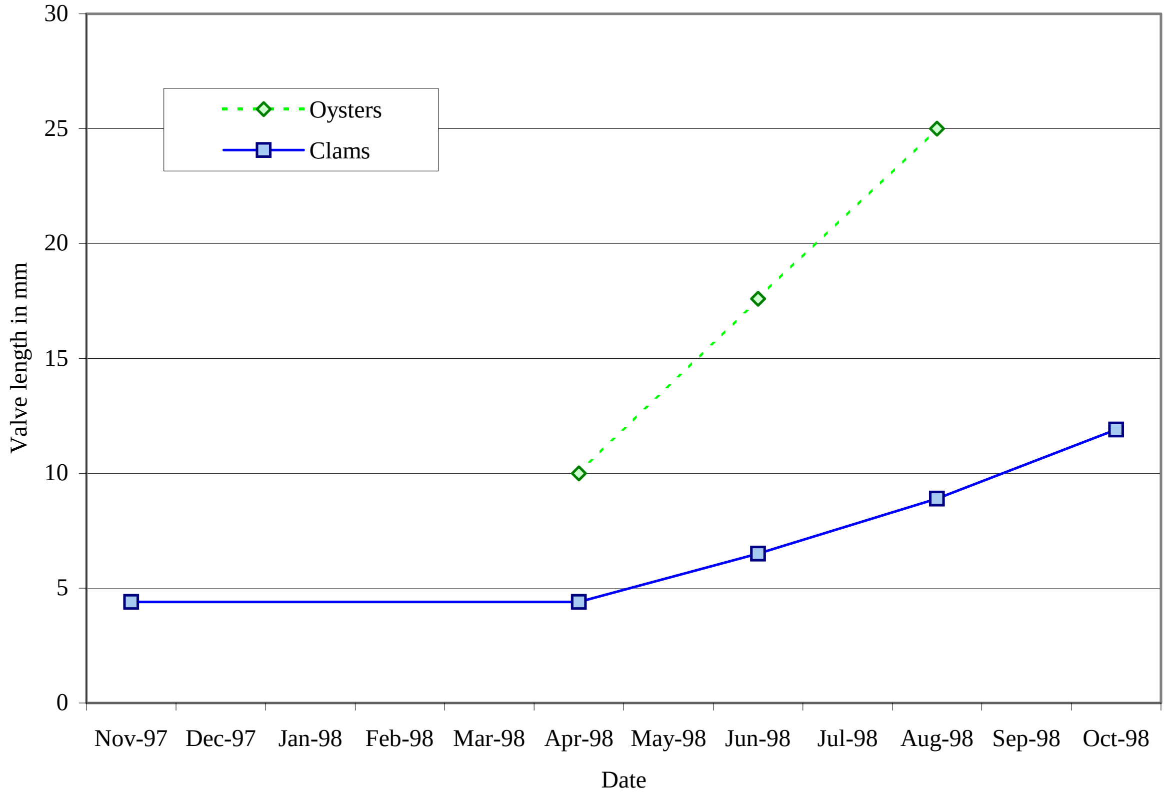

Figure 88. Growth of oysters (*Crassostrea gigas*) and clams (*Protothaca staminea*) in the tidally driven Flupsy at Tatitlek during 1998. Oysters were planted following the July 1998 measurements.

4.5.9. Summary for Tatitlek. Natural recruitment of native littleneck clams appeared to occur regularly on this beach throughout the study. This recruitment confounded the analysis of clam growth and survival. These analyses were further confounded by the substantial sediment movement caused by winter storms in 1997-98 and again in 1998-99. Despite this stress, native littleneck clams survived and grew adequately. Lightweight plastic netting appeared sufficient to stabilize the substrate and to retain the planted clams in most cases. Seven percent of the number of clams planted in 1996 had grown to greater than minimum harvest size in four years.

Of the three sites participating in these studies, Tatitlek is the only beach at which unprotected enhancement with native littleneck clams could be recommended. It may be possible to enhance the clam population by frequently removing predators and cultivating and seeding areas located above ca. 0.0’ MLLW. Some caution must be exercised in this respect, because juvenile native littleneck and butter clams were found in reasonable abundance on this beach during the 1995 baseline survey. In contrast, a total of only 3 butter and 20 native littleneck clams, with valve lengths > 38 mm, were observed in the thirty-five 0.1 m^2 samples collected during the baseline survey. This attests to the severity of predation on this beach. Any attempt to raise clams without predator netting should include a program to remove starfish and predatory gastropods from the beach at regular intervals. Initially this should be accomplished weekly or monthly.

The 1998 fecal coliform tests from Tatitlek are of concern. A sanitation survey in compliance with NSSP should be undertaken at this site before significant resources are invested in shellfish enhancement.

Approximately 60,000 clams were seeded under plastic netting at this beach during 1999. The clams were small with mean valve lengths of only four millimeters and this will likely reduce their survival. Figure (89) is a photograph of Tatitlek residents seeding clams through the plastic netting covering an area of 1700 square feet.

Figure 89. Tatitlek residents seeding 60,000 native littleneck clams through light-weight plastic netting covering 1700 square feet of the village beach. The beach had been leveled and large rock removed to form a shallow berm behind each net. The small seed, averaging 4.0 mm valve length, was seeded at a density of 380 clams/m^2 or 35 clams/square foot.

4.6. Results for Passage Island near the village of Nanwalek. The beach at Passage Island is located approximately 11.5 nautical miles (nm) from the Village of Nanwalek (English Bay). Access is along an unprotected coastline of Cook Inlet. This discouraged access to the beach during winter low tides that occur at night. Consequently, the cultures were not adequately tended and three scheduled sampling events were missed during this study. The lack of maintenance was exacerbated by the exposure of this beach to strong wave action. The consequences were that significant substrate movement occurred during the winter of 1997 – 1998. Three of the bags (1A, 2A, and 3A) were buried under 10 to 15 cm of coarse gravel and cobble as were several of the sites protected with plastic netting. Bags 2B and 2C were buried to a depth where they could not be located (>30 cm). No additional enhancement efforts are recommended, or planned, for this site. Time permitting, more protected enhancement sites, that are closer to Nanwalek, should be investigated in the future. Experience gained at this site reinforces the site selection parameters defined at the beginning of this study. Sites that are difficult to access and sites that are subject to significant substrate instability should simply be rejected for enhancement purposes.

4.6.1. Survival of native littleneck clams in bags at Passage Island. Figure (90) describes the survival of native littleneck clams in bags at Passage Island. Survival was excellent at this site until the storm event(s) of the winters of 1997-98 and 1998-99 buried some bags and left others completely uncovered. If this enhancement site were more accessible, the Villagers' might have been able to recover the buried bags and rebury the exposed bags before the clams died. However, that is conjecture. The lesson to be learned from this experience is that inaccessible and weather exposed sites are not suitable for intensive enhancement purposes.

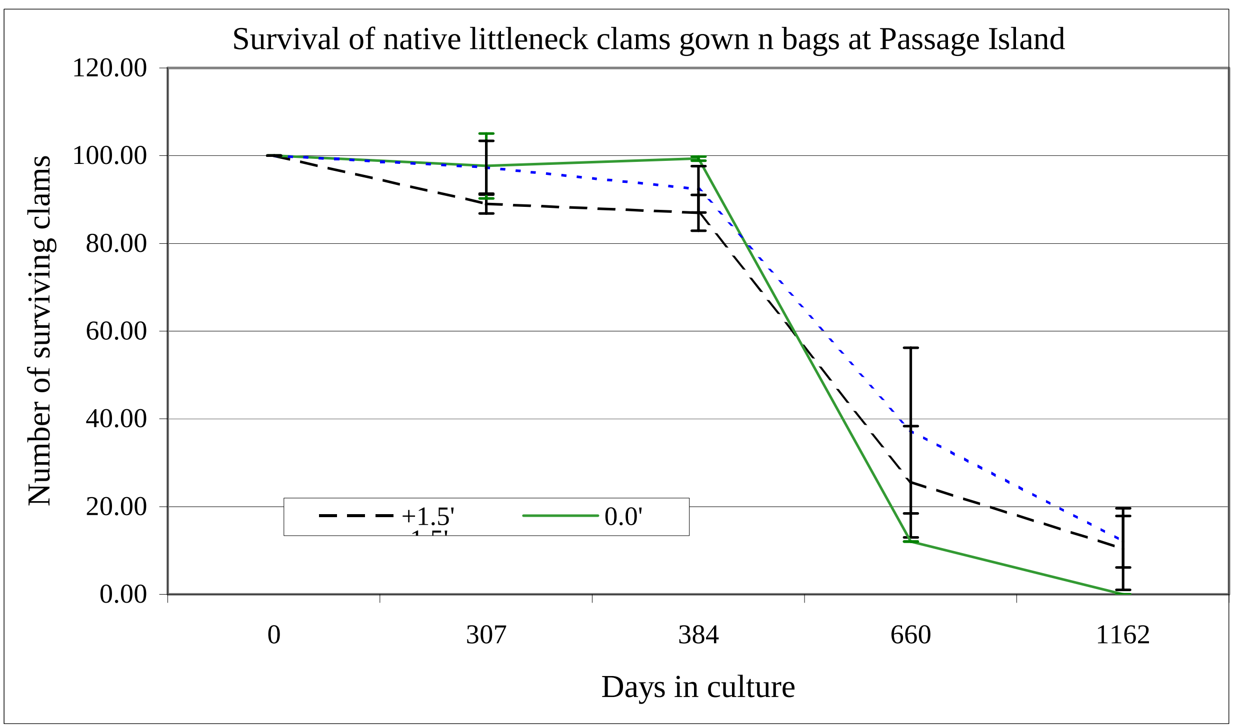

Figure 90. Number of surviving clams grown in bags at Passage Island, Alaska through September 8, 1999.

4.6.2. Survival of unprotected native littleneck clams seeded at Passage Island compared with identical plots seeded and protected with Carcover™. Plastic netting (Carcover™) has the potential to protect bivalves from many predators. As discussed in the results for Tatitlek, plastic netting also functions to stabilize substrates subject to movement. Clams were seeded at a density of 300 clams/m^2 into replicated, cultivated, plots covering two square meters each in 1996. Two samples covering an area of 0.018 m^2 were collected from each of the three replicates at +1.5' MLLW and -1.5' MLLW on April 24, 1998, providing six samples from each treatment at each tidal height. All count data were Log(N + 1) transformed prior to analysis.

Figure (91) describes the results of sampling each of these plots during April 1998. Two of the bags were lost and three were buried. However, more clams survived in bags than in the other types of culture. Plastic netting increased survival at Protected sites. Forty-five native littleneck clams were retrieved in all Passage Island samples (not including bags). Thirty-seven (37) of these were from seeded areas protected with Carcover™, one was from the seeded, but unprotected area and seven were retrieved from control plots. The netting did help stabilize the substrate and it is likely that native littleneck clam seed was washed out of the unprotected treatments or was buried too deeply to survive. The nearly total loss of clams from the seeded and unprotected treatments suggests that simply broadcasting seed onto a cultivated, but unprotected, intertidal area is not a practical enhancement technique at this high-energy site. Approximately 66 native littleneck clams were seeded in 1996 into the twelve 0.0182 quadrats sampled in April of 1998. Thirty-seven (37) of these survived, suggesting a gross survival rate of 56% in the Protected treatment. This was surprising considering the visual evidence of significant sediment movement during the winter of 1997-98 at this beach.

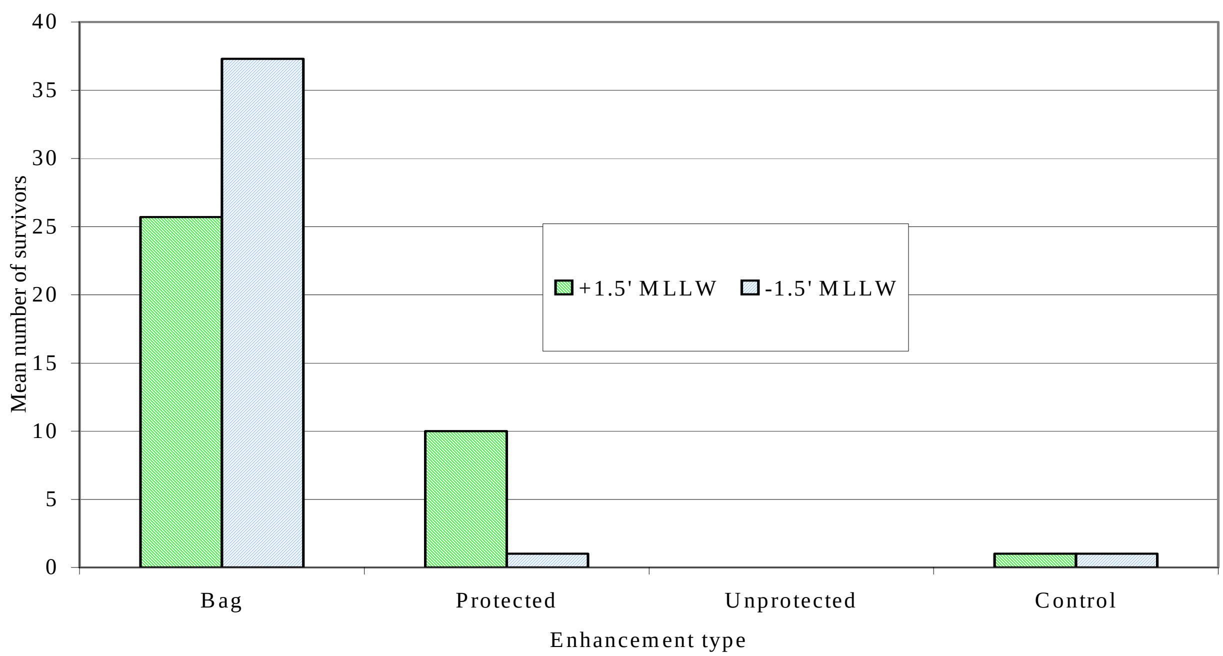

Figure 91. Survival of native littleneck clam (*Protothaca staminea*) seed planted in the intertidal area of Passage Island during 1996 and evaluated on April 24, 1998.

Paired sample *t-tests* comparing the types of enhancement indicated that significantly more clams were found under Carcover when compare with either the control (p = 0.028) or the unprotected enhancement trial (p = 0.001). Significant differences between the seeded, but unprotected trial and the control were not significant (p = 0.720). These results suggest that unstable substrates may have caused a significant loss of unprotected native littleneck clams at Passage Island and that Carcover™ netting was effective in reducing these losses.

4.6.3. Growth of native littleneck clams in field trials at Passage Island. At the end of the study, analysis of covariance with initial clam length as the covariate indicated that there were significant differences as a function of treatment (F = 17.51, p = 0.000) but not as a function of tidal height (F = 1.15, p = 0.29). The mean length of native littleneck clams grown in bags (23.05 mm) was significantly less (P = 0.000) than that of clams grown under plastic netting (26.6 mm). The valve length of clams at the end of the study that were seeded without benefit of protection was intermediate and not significantly different from those grown in bags or under netting. These results are summarized in Figure (92). Figure (93) describes the growth of native littleneck clams in bags at Passage Island. The clams were originally planted on July 3, 1996 at an age of one year. They were last sampled on September 8, 1999 at an age of 1532 days (4.2 years) and three years of growout.

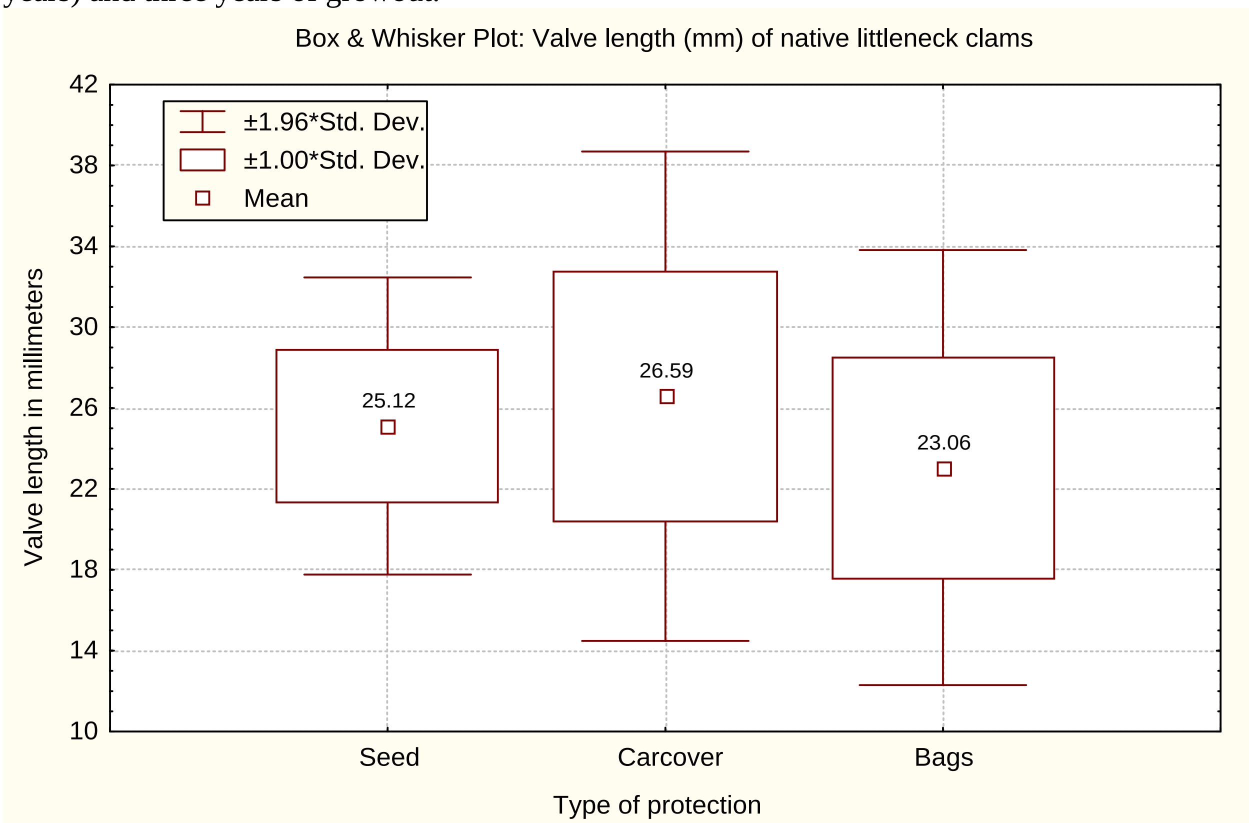

Figure 92. Final valve lengths of native littleneck clams grown at Passage Island for three years. Clams were seeded into cultivated sediments and either protected with plastic netting (Carcover™) or unprotected (Seed). Nine additional cohorts of 100 clams each were grown in plastic clam cages. Differences in growth as a function of tidal height (-1.5' to +1.5' MLLW) were not observed.

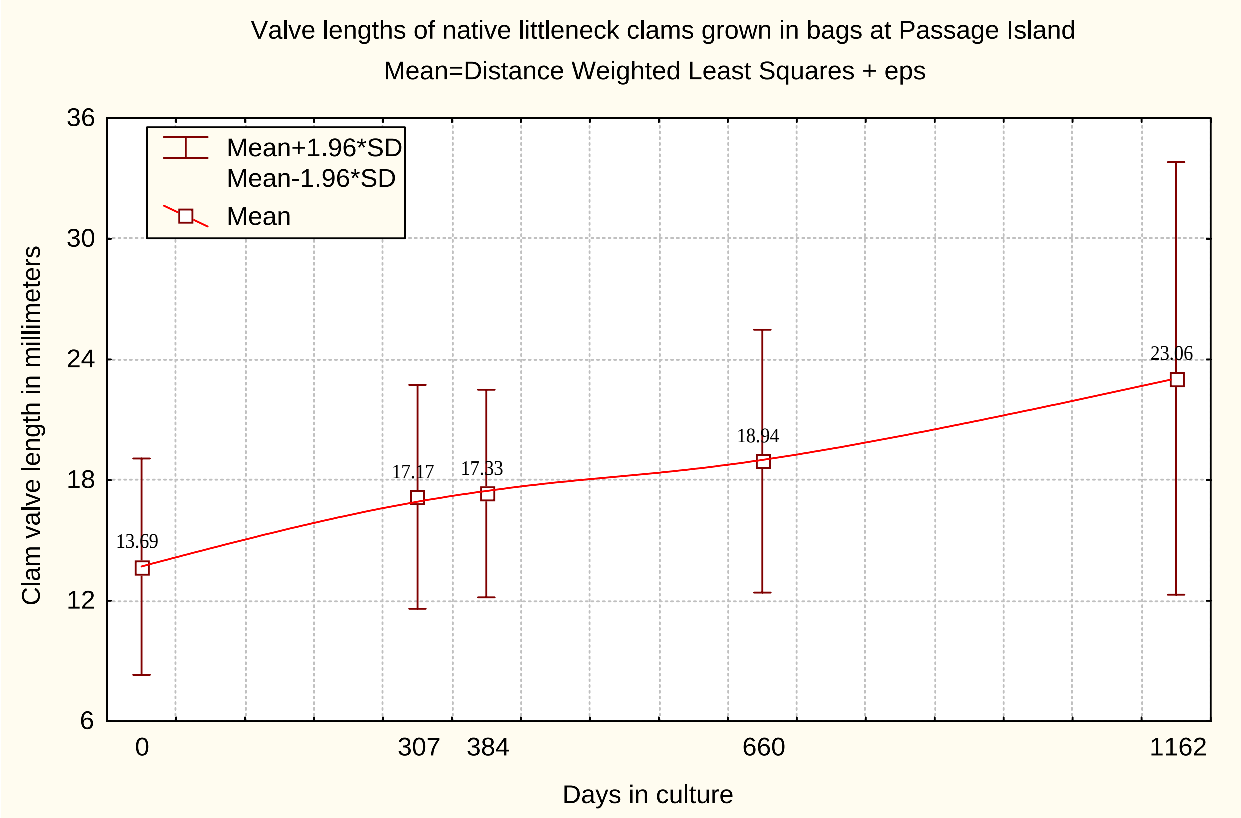

Figure 93. Mean length (mm) of clams grown in bags at all tidal elevations on Passage Island, Alaska as a function of seed age.

Figure (94) provides a length-frequency histogram for clams collected on September 8, 1999. Four clams $\geq$ the minimum legal length of 38 mm were observed. Small and recently recruited native littleneck clams were observed during the 1995 baseline survey at this site and new recruits are apparent in Figure (94). Newly recruited bivalves of a number of species were observed in bags at Passage Island during annual CRRC evaluations. Bivalve species other than native littleneck clams were removed from the bags during each annual field survey by the CRRC team. Native littleneck clams with valve lengths less than 8 mm were also removed, as this was the smallest size clam originally planted. However, it is likely that some new recruits became members of the cohort of clams counted in the bags. Because these recruits were younger (and smaller) than those planted in 1996, their inclusion would decrease the mean valve lengths observed. It has been suggested that the clams planted in 1996 should have been marked. However, the experience gained in this study supports the author's original opinion that marking techniques (tags, etching, paint, vital stains) appropriate for seed clams (12 mm valve length) will not remain visible for the duration of studies designed to last four years or more.

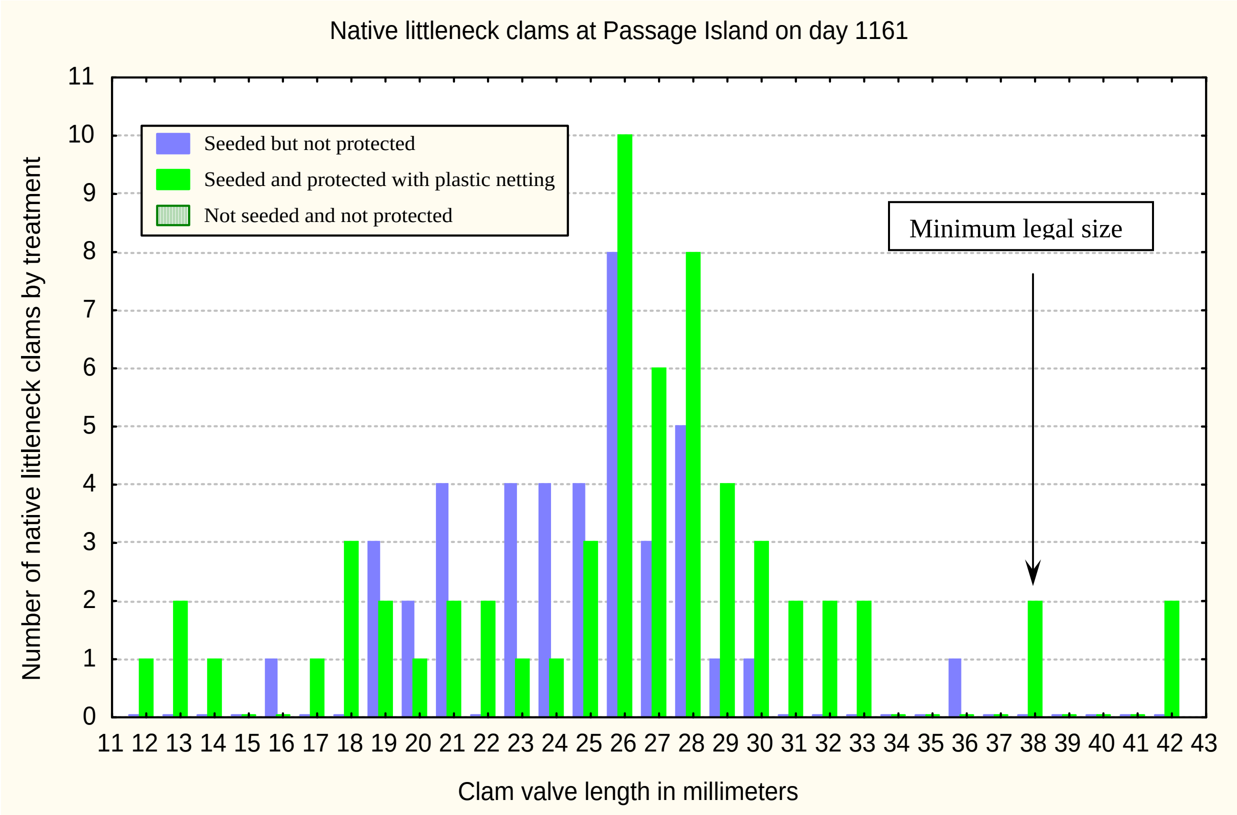

Figure 94. Length frequency histogram describing the population of native littleneck clams observed on September 8, 1999 at Passage Island, Alaska. Clams depicted in green were retrieved from plots protected with plastic netting. Clams in blue were seeded but not protected. No native littleneck clams were found in control areas during the 1999 survey.

4.6.4. Changes in the physicochemical properties of sediments at Passage Island. Sediment physicochemical characteristics are summarized for the various treatments in Table (2). The proportion fines observed under Carcover™ was significantly higher ($p = 0.013$) from the proportion observed in the seeded, but unprotected, site. No other significant differences were observed with the probability of rejecting the null hypotheses varying between 0.42 and 0.72.

Table 24. Summary of the proportion fines (silt and clay < 64 µm particle size), total volatile solids (TVS) as a proportion of sediment dry weight, and depth (cm) of the reduction oxidation potential discontinuity (RPD) observed in control areas, in seeded areas under plastic netting and in unprotected but seeded areas. All values are means of three replicates ± one standard deviation.

Type of treatment	Proportion fines	Proportion TVS	Depth of RPD (cm)
Control	0.076 ± 0.028	0.024 ± 0.007	>15
Seeded and unprotected	0.066 ± 0.005	0.022 ± 0.006	>15
Seeded and protected	0.082 ± 0.011	0.023 ± 0.007	>15

4.6.5. Fecal coliform bacteria. Fecal coliform bacteria were not detected in any of the water samples (all samples were < 2.0 FC/100 ml). This was consistent from year to year suggesting that this area would likely meet the requirements for an Approved Classification as defined in Part I of the NSSP Manual of Operations.

4.6.6. Total volatile solids and total suspended solids in the water column at Passage Island on April 24, 1998. The water at Passage Island was very clear on April 24, 1998. Total Suspended Solids were measured at 1.5 ± 0.9 mg/L and the mean Total Volatile Solids was 0.70 ± 0.03 mg/L (mean ± one standard deviation). These data suggest that about half of the suspended particles retained on a 0.47 µm glass filter were organic and half were inorganic. The TVS value of 0.70 mg/L was unexpectedly low during this spring sampling period when higher phytoplankton production was expected.

4.6.7. Summary for Passage Island. This site has proven too remote and exposed to allow for proper maintenance of intensive native littleneck clam culture either in bags or under Carcover™. The untended cultures were disrupted during winter storms in 1997-98 and again in 1998-99. Native littleneck clams survived best under protective netting, but survival of seeded clams was also adequate when no protection was provided. Very few native littleneck clams reached a minimum harvest size of 38 mm during their three-year growout at Passage Island. This is significant when compared with the results for Murphy's Slough where native littleneck clams began recruiting into the ≥ 38 mm size class at three years of age and where more than half of the clams reached this minimum harvest size at the end of four years of growout. The length of native littleneck clams was significantly less in bags when compared with those grown under plastic netting where they were left undisturbed during the first 659 days of field growout. This reduction is likely associated with the stress imposed during periodic sampling of bagged clams. This finding, coupled with the growth and survival of clams simply seeded into cultivated portions of the beach without protection, suggests that intensive cultivation should not be practiced at this site. Future enhancement is not recommended at Passage Island.

4.7. Native littleneck clam enhancement study summary. The purpose of this study was to evaluate the potential for enhancing native littleneck clam resources at member villages of the Chugach Regional Resources Commission. The study took guidance from village elders regarding their preference for study areas and enhancement methods. The findings presented in this report are the result of a team effort with contributions from CRRC, particularly Mr. Jeff Hetrick, and the residents of Tatitlek, Port Graham and Nanwalek who participated in annual field evaluations and who conducted independent sampling of clams growing in bags during the rest of the year. The study would not have been possible without their interest and participation.

Annual recruitment of native littleneck clams at Tatitlek and Passage Island confounded the growth and survival assessment at those beaches. No evidence of natural recruitment of native littleneck clams in Murphy's Slough was observed at any time during this study and those results provide unequivocal data describing the growth and survival of native littleneck clams in that and likely in similar Alaskan environments. The data from Passage Island and Tatitlek is useful in describing native littleneck clam enhancement in tidal environments exposed to higher energy. Three general questions were asked in Section 1.11 of this report and four testable hypotheses identified. Each of these is discussed in the following summary statements:

➢ **Question (1).** What was the biomass and species composition of bivalve populations on traditional subsistence beaches at the villages of Tatitlek, Nanwalek and Port Graham in 1995 and at Ouzinke and Chenega in 1996?

Eleven species of large bivalves were observed during these studies:

Nuttall's cockle	*Clinocardium nuttallii*
Native littleneck clam	*Protothaca staminea*
Butter clam	*Saxidomus giganteus*
Horse clam	*Tresus cf. capax*
Surf clam	*Spisula polynyma* (Ouzinke only)
Truncate softshell clam	*Mya truncata*
Baltic mussel	*Mytilus edulis trossulus*
Arctic hiatella	*Hiatella arctica*
Bent-nose macoma	*Macoma nasuta*
Stained macoma	*Macoma inquinata*
Baltic macoma	*Macoma balthica.*

The first seven (cockles, native littleneck clams, butter clams, horse clams, surf clams, softshell clams and mussels are prized in various parts of the world for human consumption. The remaining four species are not typically consumed. Butter clams and native littleneck clams dominated the bivalve community in mixed sediments. Macoma clams were more common in sandy sediments. The other species were infrequently found except that cockles were abundant in Camel bay near Ouzinke. Surf clams were only observed at the Ouzinke beach.

Several beaches near the village of Ouzinke held harvestable quantities of butter clams. The quantitative survey predicted 670.3 $\pm$ 297.3 kg of primarily butter clams within the 7,200 square feet of surveyed beach. None of the beaches surveyed at other villages in these inventories contained harvestable quantities of legal size clams of any species. Recruitment was low but regular at most beaches. However, nearly all of the butter and native littleneck clams were lost before they reached a minimum legal harvest size of 38 mm.

Question (2). What is the potential for enhancing native village shellfish resources using 1) unprotected supplemental seeding of cultivated beach areas; 2) supplemental seeding under protective plastic netting; or 3) intensive cultivation of clams in bags.

This study implemented proven techniques for raising Manila clams in Washington to the culture of native littleneck clams in Alaska. Growth and mortality studies were confounded at Tatitlek and Passage Island by the constant recruitment of native littleneck clams into the cultures. However, the results from these two high-energy environments did provide valuable insight into the benefits of various enhancement techniques. The study at Murphy's Slough did not suffer from this problem and those results provide unequivocal evidence of the potential for native littleneck clam enhancement in Alaska. The following statements are provided in response to this question:

➢ **Predation.** No evidence of sea otter predation on cultured clams was observed during these studies. This is likely because the clams were small. Major predators included sunstars

(*Pycnopodia helianthoides*) gastropods (*Natica clausa* and *Nucella lamellosa*) and shore crabs (*Cancer oregonensis*). These predators must be controlled before any form of enhancement will be successful. Survival was improved when protection was provided by cages or lightweight plastic netting. Bags must be inspected regularly to remove predators. In general, survival in bags and under plastic netting was greater than 40% at the end of four years of field growout. That would be considered acceptable for commercial shellfish culture in Puget Sound.

- **High-energy environments.** Plastic netting efficiently stabilized sediments and retained planted clams during significant storm events. However, these interventions must be maintained. Un-maintained cultures at Passage Island were lost because the netting was either buried or breached and bags were either washed out of the sediment or buried under as much as a foot of accumulated gravel. Maintained clam cultures on the high energy Tatitlek beach grew more slowly than those in protected Murphy's Slough did. However, 7 percent of the seeded clams recruited into the legal harvest size range at Tatitlek in four years of field growout.

- **Murphy's Slough.** The results from this quiet embayment with excellent sediment physicochemical characteristics demonstrate the potential for clam enhancement in Alaska. Forty to 55 percent of the clams planted in bags or protected with plastic netting survived until the end of the study. Twenty-seven (27) percent of the clams planted under plastic netting in 1996 exceeded the minimum legal harvest size of 38 mm when last sampled in 2000 following four years of field growout (total age = 5.1 years). Clams grown in bags were retrieved and counted eight times during this study. That disturbance resulted in slower growth in bags and the mean clam valve length was only 32.75 mm at the end of four years of field growout.

- **Effects of protection.** In general, few clams survived in unprotected cultures. The populations were supplemented by new recruits at Tatitlek and Passage Island, but losses, likely associated with gastropod and starfish predation, removed clams as they grew and the mean valve length of these populations remained significantly shorter than for the protected treatments. The benefits of protection were very apparent at Murphy's Slough were only two native littleneck clams were retrieved from seeded but unprotected areas in comparison with 31 clams from under similarly treated areas that had been covered with plastic nets.

- **Tide level effects.** Consistent differences in survival or growth were not observed as a function of tide height within the tested range of –1.5' MLLW and +1.5' MLLW. Mortality increased during winter but was not catastrophic except at Passage Island where the untended cultures were disrupted by storms.

- **Clam density effects.** Native littleneck clams survived significantly better at 200 seed per half cage when compared with densities of 350 or 450 clams per cage. The final mean length of clams increased linearly as the seeding density decreased. Native littleneck clams grown at 200 clams/half bag were two millimeters longer on average at then end of one year when compared with those planted at 450/half bag. Significant differences in the biomass of clams (total weight of all clams in a bag) were not observed at the end of the density study.

Question (3). What length of time was required for native littleneck clams to reach a minimum valve length of 38 mm at Tatitlek, Nanwalek or Prot Graham? The von Bertalanffy growth model, based on actual mean valve lengths recorded in clams grown under plastic netting in Murphy's Slough predicts that clams will grow to minimum legal size in 5.05 years. In this study, 57.4% of the native littleneck clams seeded in June 1996 and remaining alive on August 1, 2000 exceeded 38 mm valve length. In the higher energy environment of Tatitlek, only 7% of the native littleneck clams planted under plastic netting reached a minimum harvest size by November 2000.

Question (4). Do observed lengths at ages one through four correspond to predictions made by the von Bertalanffy model and **Question (5)** do the number of apparent annuli observed in native littleneck clams at Murphy's Slough correspond with the known age of these clams? The ages of native littleneck clams appear to be reasonably well recorded in winter annuli recognizable on the exterior surface of the valves. These annuli can be more or less difficult to read depending on the degree of sculpturing. This is illustrated in Figure (91) depicting two native littleneck clams, each of which was collected on September 9, 1999 at an age of four years and following three years of field culture under plastic netting at Murphy's Slough. Each winter annulus corresponded to a discontinuity observed in the sectioned valve. These discontinuities appear to be caused by an extension of the inner lamellar shell layer through the outer prismatic layer. These dark, hyaline, lines were sometimes observed as doublets separated by a few hundred microns. The first annulus was frequently not observed in sectioned valves. The apparent reason is that the prismatic layer near the umboes erodes quickly and becomes thin. An annulus is apparent as a contrast in the prismatic layer and therefore the first annulus becomes difficult or impossible to distinguish in sectioned material. Originally, it was thought that the polished exterior shell surface observed in hatchery and nursery produced clams would provide a distinguishing mark. However, the erosion discussed above obliterated that mark, as it would likely have obliterated dyes or paints used to mark the seed.

The mean valve lengths of native littleneck clams grown under plastic netting at Murphy's Slough increased in a manner consistent with von Bertalanffy model predictions derived from baseline age-length data obtained at Passage Island (Brooks, 1995). That model predicted that native littleneck clams would require an average of 5.76 years from setting to reach a minimum harvest size of 38 mm. Native littleneck clams grown in Murphy's Slough began reaching 38 mm valve lengths following three years of growout or four years of age. Fifty-seven percent (57.4%) of the native littleneck clams retrieved by the Alaska Department of Fish and Game from beneath plastic netting on August 1, 2000 had reached a minimum harvest size of 38 mm at five years of age. This is on the lower end of the 5 to 8 year prediction made by Bechtol and Gustafson (1998) and as little as half the time predicted by the other authors listed in Table (2). Murphy's Slough was considered ideal habitat for native littleneck clams by the author during the 1995 baseline survey – even though native littleneck clams were not found anywhere on this beach. These results indicate that native littleneck clams can be raised to legal size in as little as four to five years of field growout in Alaska.

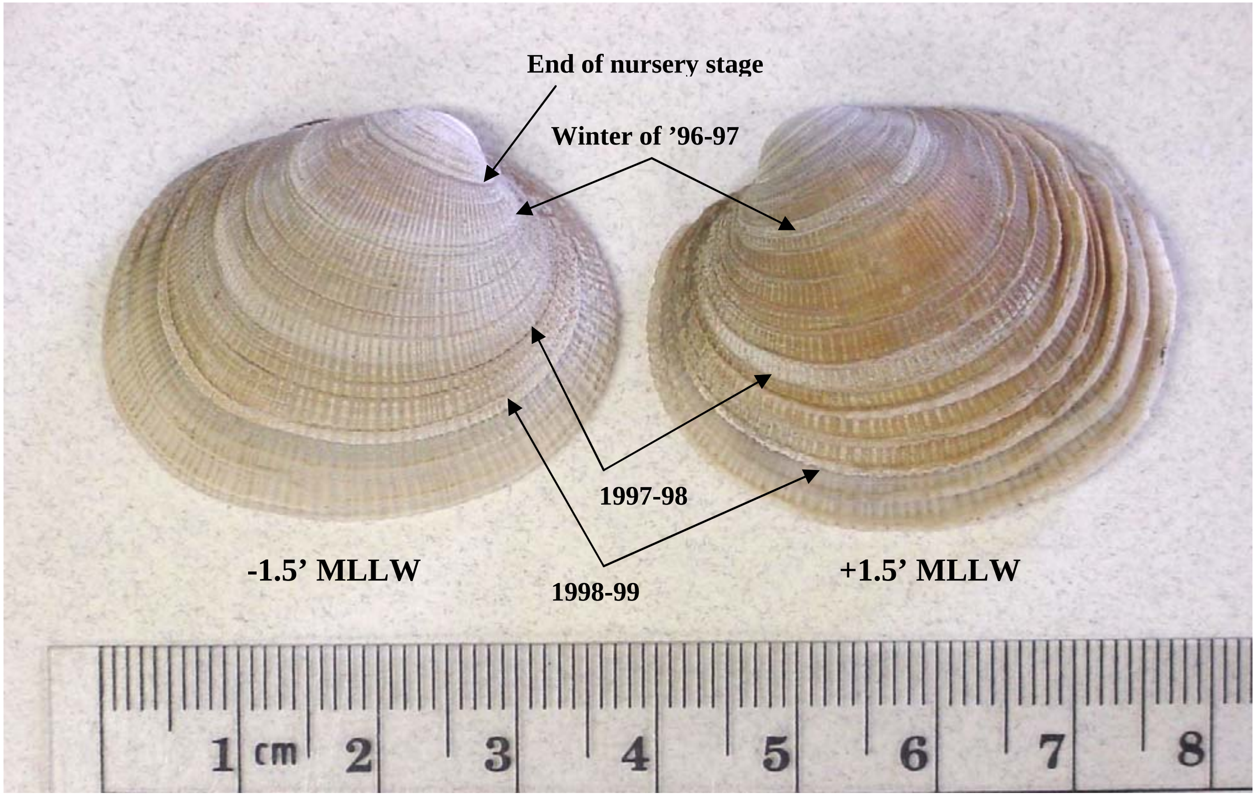

Figure 95. Differential valve sculpturing observed in native littleneck clams cultured at two different tidal elevations under plastic netting in Murphy's Slough, Alaska. Apparent annuli are identified.

- **Question (6).** Was there excessive winter mortality in clam populations physically constrained to remain within a few centimeters of the sediment surface in bags? Mortality rates increased during winter months at all three study sites. However, winter mortality was not considered catastrophic at any site during the first winter when small clams were likely most susceptible to freezing. Catastrophic mortality did occur during the winter of 1997-98 and again in 1998-99 at Passage Island when bags were washed out of the substrate in erosional areas and buried in depositional areas. No maintenance of the Passage Island study site was conducted during winter months. Tatitlek represented a similar high-energy beach. However, the bags were reset following significant storms by village residents and the plastic netting repaired or replaced. The result was that winter losses averaged only 8 to 15 percent during each of the three years of this study at Tatitlek. Approximately ten percent of the clams in bags were lost each winter at Murphy's Slough and excepting the lowest tidal elevation, where two of the bags disappeared for two years; survival at the end of four years in growout was 40 to 50%. This survival rate is similar to that reported by Toba *et al.* (1992) for Manila clams grown for two years under plastic netting in Puget Sound. The weight of evidence presented in this report suggests that mortality increases in winter, but that if the cultures are maintained, winterkill should not inhibit enhancement. This study also points out the need for proper maintenance of intensive bivalve cultures.

- **Hypothesis (1).** Were statistically significant ($\alpha = 0.05$) differences in growth and/or survival of native littleneck clams grown in bags and removed for quarterly examination observed when compared with similar seed raised under plastic netting with free vertical movement in the substrate, and examined only biannually? This hypothesis was tested at Murphy's Slough. Significant differences were observed in survival between clams provided protection in bags or under plastic when compared with the similarly seeded cohort that was not protected. However, survival differences between clams in bags or under plastic netting were not significant on September 9, 1999 following 1162 days of growout.

The null hypothesis that clams grown in the various treatments (bags, netting, unprotected) was tested using analysis of covariance with initial length as the covariate. Data from the last day of the formal study (September 9, 1999) were used in this analysis. The null hypothesis was rejected and post hoc testing revealed that the mean length of clams grown in bags (27.03 $\pm$ 3.14 mm) was significantly less than for those grown under plastic netting (34.74 $\pm$ 4.17 mm). Too few clams were retrieved from seeded but unprotected areas to allow for a meaningful analysis. The reason for the different growth rates is most likely that clams in bags were dug up, sieved and measured eight times during the study while those under plastic netting were undisturbed. This is simply another fine example of the Heisenberg *uncertainty principle.*

- **Hypothesis (2).** Was clam survival significantly enhanced when cultures were protected by plastic netting compared with similar seeding in unprotected areas? In other words, what is the potential for extensive as opposed to intensive clam enhancement. This hypothesis was tested at Murphy's Slough in 1999. Native littleneck clams were not found in unseeded control areas adjacent to each replicate in Murphy's Slough. Only two native littleneck clams were retrieved from 12 cores covering 0.0182 m^2 taken in seeded, but unprotected, areas. In contrast, 31 clams were found in the same number of samples collected from under plastic netting. The calculated survival rate varied between 40 and 55 percent in the 3 replicates. This was similar to survival in bags and consistent with Manila clam survival in Puget Sound reported by Toba *et al.* (1992). Analysis of variance on survival data indicated that the null hypothesis of equal survival should be rejected ($p = 0.000$). Post hoc testing indicated that survival in bags or under plastic netting was not significantly different but that either means of protection resulted in significantly higher survival than was observed in unprotected cultures at Murphy's Slough.

No direct evidence of sea otter predation on cultured clams was obtained at any of the test sites during this study. Significant predation was associated with starfish, particularly *Pycnopodia helianthoides*, crabs (*Cancer oregonensis*) and gastropods (*Natica clausa* and *Nucella lamelossa*) which made their way into bags at a size allowing entry through the ¼" mesh. It is likely that an improvement in native littleneck clam survival to harvest could be achieved at Tatitlek by periodic removal of starfish and predatory gastropods – regardless any other enhancement efforts.

Plastic netting was very effective at stabilizing sediments and retaining seed clams at Tatitlek. The analysis was confounded by steady recruitment of juvenile clams into all of the treatments during this study. However, the length frequency histogram provided in Figure (85) showed that clams retrieved from the unprotected areas were smaller than from the protected areas and that none of the unprotected clams had recruited into the legal size class by September 9, 1999. In contrast, protected native littleneck clams began recruiting into the legal size class at Tatitlek by September 1999 (one clam!) and 7.1% of the number of clams originally seeded under plastic netting were of legal size during a survey conducted during November 2000 by Mr. Jeff Hetrick of CRRC. Even though the analysis was confounded by recruitment at Tatitlek, the results illustrate the stabilizing effects of bags and netting in high-energy intertidal environments.

➢ **Hypothesis (3).** Did statistically significant changes occur in the percent fines (silt and clay < 63 µm diameter) and/or the proportion total volatile solids (TVS) observed in sediments under plastic netting when compared with areas seeded, but not protected? Small increases in TVS and the percent silt and clay were observed under plastic netting when compared with the unprotected treatments in Murphy's Slough. However, none of those differences were statistically significant at $\alpha = 0.05$. An increase was also observed in sediment total sulfides measured under plastic netting at Murphy's Slough and at Tatitlek. Those differences were nearly significant ($p = 0.066$) at Murphy's Slough in 1999. Neither TVS nor the proportion silt and clay were elevated at Tatitlek or Passage Island due to the higher currents and increased exposure of these beaches to storms. Consistent with the work of Brooks (2000b and 2000c) at salmon farms, these results suggest that sediment sulfides are a sensitive indicator of organic loading.

➢ **Hypothesis (4).** Were significant differences in growth and/or mortality of clams raised at different tidal heights or at different densities in plastic cages observed? This hypothesis was best tested at Murphy's Slough where the clam density experiment was initiated in 1998 and monitored in 1999. Analysis of variance resulted in a rejection of the null hypothesis that survival was equal at all three densities. Post hoc testing using Scheffe's test indicated that the 65.5% mean survival at the lowest density (200 clams/half-bag) was significantly higher when compared with either of the two higher densities (350 and 450 clams/half bag). The difference in survival between the two higher densities was not significant.

The null hypothesis that mean clam valve lengths at the end of 16 months of growth was equal for clams grown at three densities was rejected. The mean valve length of native littleneck clams decreased linearly with increasing density. The mean length of clams (17.7 mm) in the lowest density bags was nearly two millimeters longer than the mean in the highest density bags (15.8 mm). Post hoc testing using Scheffe's test indicated that the difference between the lowest and highest density was significant ($p = 0.002$). The differences between the intermediate density and either extreme were not significant.

The aggregate weight of native littleneck clams in the three density treatments was not significantly different as a function of density. The aggregate weight varied between 183.95 grams at the lowest density and 222.05 grams at the intermediate density. It decreased to 209.4 grams at the highest density. None of these differences was statistically significant.

The results of this study have unequivocally demonstrated that native littleneck clams can be grown from a mean valve length of 13.6 mm to 38.2 mm in four years of field growout. Fifty seven percent of the clams grown under plastic netting had reached a minimum harvest size of 38 mm in four years growout in Murphy's Slough. This study has also demonstrated the problems encountered with enhancement projects in high-energy intertidal areas and the effectiveness of properly maintained bags or plastic netting in ameliorating those problems. Figure (92) describes native littleneck clams seeded at an age of one year in Murphy's Slough and at the end of two and three years of field growout.

Figure 96. Representative native littleneck clams grown under plastic netting from June 1996 until September 1999.