4.0. Native littleneck clam enhancement studies. The enhancement study design illustrated in Figure (56) was used at Passage Island, Murphy's Slough and Tatitlek. The design included three replicates of each of the following treatments laid out using a properly leveled transit, aluminum stadium and a 300-foot fiberglass tape.

- One hundred native littleneck seed clams were individually measured and placed in each of nine half Norplex™ bags. These were raised at three tidal heights (-1.5', 0.0' and +1.5' MLLW). Nine hundred clams were grown in bags at each beach.

- Clams were seeded at a density of 300/m^2 into three replicated plots that had been cultivated to a depth of 15 cm to remove existing clams, large rock and to loosen the substrate. Three replicates were placed at each of two tide levels (-1.5' and +1.5' MLLW) – except at Tatitlek where three replicates were placed at each of three tide levels (-1.5', 0.0' and +1.5' MLLW). These test cultures were protected with lightweight plastic netting. The limited supply of seed from the hatchery in 1996 prevented seeding at the 0.0' MLLW height at Murphy's Slough and Passage Island.

- Clams were also seeded at a density of 300/m^2 into replicated plots identical to those described above – but without protective plastic netting. This treatment was established to examine the efficacy of extensive enhancement with minimum labor and ongoing management.

- An unmanipulated control station was established at each of the nine blocks to provide a natural reference.

- Additional seed became available from the Qutekcak hatchery in 1999. This seed was used to evaluate the effects of planting native littleneck clams at varying densities in bags placed at a tidal elevation of 0.0' MLLW in Murphy's Slough. Three replicate bags were planted at densities of 200, 350 and 450 clams per half Norplex™ bag in the randomized block experiment described in Figure (56).

A copy of the protocols and datasheets employed during the 1999 field season is provided in Appendix (2) and a copy of the database in both Statistica™ and Microsoft Excel™ formats is provided on the accompanying CDROM disk (Appendix 3). The cultures were evaluated on the dates given in Table (22). Dates highlighted in blue represent annual evaluations by the CRRC field team

4.1. Village workshops. Educational workshops were held for the villages of Tatitlek, Nanwalek and Port Graham prior to establishing the culture studies at each village. These workshops consisted of two parts. The first session began with a discussion of the 1995 surveys at each Village and a description of what was learned, including management recommendations specific to each village. This was followed with a detailed description of native littleneck clam biology, culture techniques (largely borrowed from the culture of manila clams (*Tapes philippinarum*)) and enhancement recommendations for each Village. The importance of shellfish sanitation and the requirements of the National Shellfish Sanitation Program were reviewed, as was the need for monitoring for paralytic shellfish poisoning (PSP). Three copies of the books *Introduction to Shellfish Aquaculture in the Puget Sound Region* (Magoon, Washington Department of Natural Resources, undated) and *Guide to Manila Clam Culture* (Toba, *et al.*, 1995) were distributed in each village along with copies of Brooks (1997).

The second part of each workshop was devoted to introducing village participants to the shellfish enhancement studies being undertaken at each village. The reason for each protocol element was discussed and precision and fidelity in completing the quarterly sampling emphasized. Each village was provided with a set of tools, protocols and data sheets necessary for conducting the quarterly sampling. The following equipment was provided to each village:

- Two sets of stainless steel Vernier calipers and two cafeteria trays for sorting shellfish
- One hand trowel and two clam harvest rakes
- One hard bristle brush for cleaning clam cages
- All bags, nets, electrical ties, rebar, tags, data sheets and data transmittal sheets necessary to complete the first years' sampling.

Villagers were instructed in the use of the Vernier calipers. Hands-on practice was obtained as the participants measured each of the 900 clams used in the caged growth and mortality studies. This activity was closely monitored by the CRRC study team. Nine village residents attended the combined Nanwalek (4) – Port Graham (5) session and six people were present at Tatitlek. These same people participated in preparing the study sites and planting seed. A great deal of interest (questions and discussion) was expressed by participants with regard to the biology of clams, the time required to reach legal size, and the potential for increasing subsistence harvests through enhancement.

4.2. Clam (*Protothaca staminea*) seed supply. Juvenile clams were provided by the Qutekcak Shellfish Hatchery from stocks spawned in 1994 and 1995 by Mr. Jeff Hetrick and Carmen Young. Twenty-three thousand juvenile clams from the 1994 cohort were grown indoors for one year and then transferred into gravel filled trays placed in a pond managed for optimum phytoplankton growth. Valve lengths in these two-year-old clams varied between 3.3 and 12.5 mm. A smaller cohort of 1,200 clams was available from the 1995 spawn. These juveniles were grown indoors in upwellers until May 1996, when they were transferred to pearl nets hanging in the hatcheries pond. At one year of age, they averaged 17.9 $\pm$ 0.6 mm. This rapid growth attests to the improved growth possible with even moderately enhanced nursery techniques. A description of the pond, its management, and phytoplankton productivity should be available in the Qutekcak Hatchery annual reports for this project. These clams were mixed at the hatchery and randomly subsampled to provide three stocks of ca. 8,000 clams for each village. These subsamples were shipped to each village within two days of placement in the study plots.

4.3. Study design and materials and methods.

4.3.1. Growth and mortality of caged clams. One hundred seed clams were placed in half "Norplex™" clam bags for a detailed growth and mortality study. The valve lengths of all clams placed in these bags was measured to the nearest 0.1 mm using vernier calipers. Clams placed in bags were a random sample from the seed used in other parts of the study. Therefore, the mean lengths of clams in the bags were used as the mean lengths of the clams seeded into other parts of the study. Measurement of these clams provided a chance for village culturists to use the vernier calipers and to record data. Clam bag ends were secured with four electrical ties on one end and a 1-1/4" piece of split PVC pipe on the other end. Each bag received a shovelfull of sieved (1/2" sieve) gravel. Bags were then nestled into the substrate to a minimum depth of 4". The top surfaces of each bag extended one inch above the substrate. Each bag was secured with extra

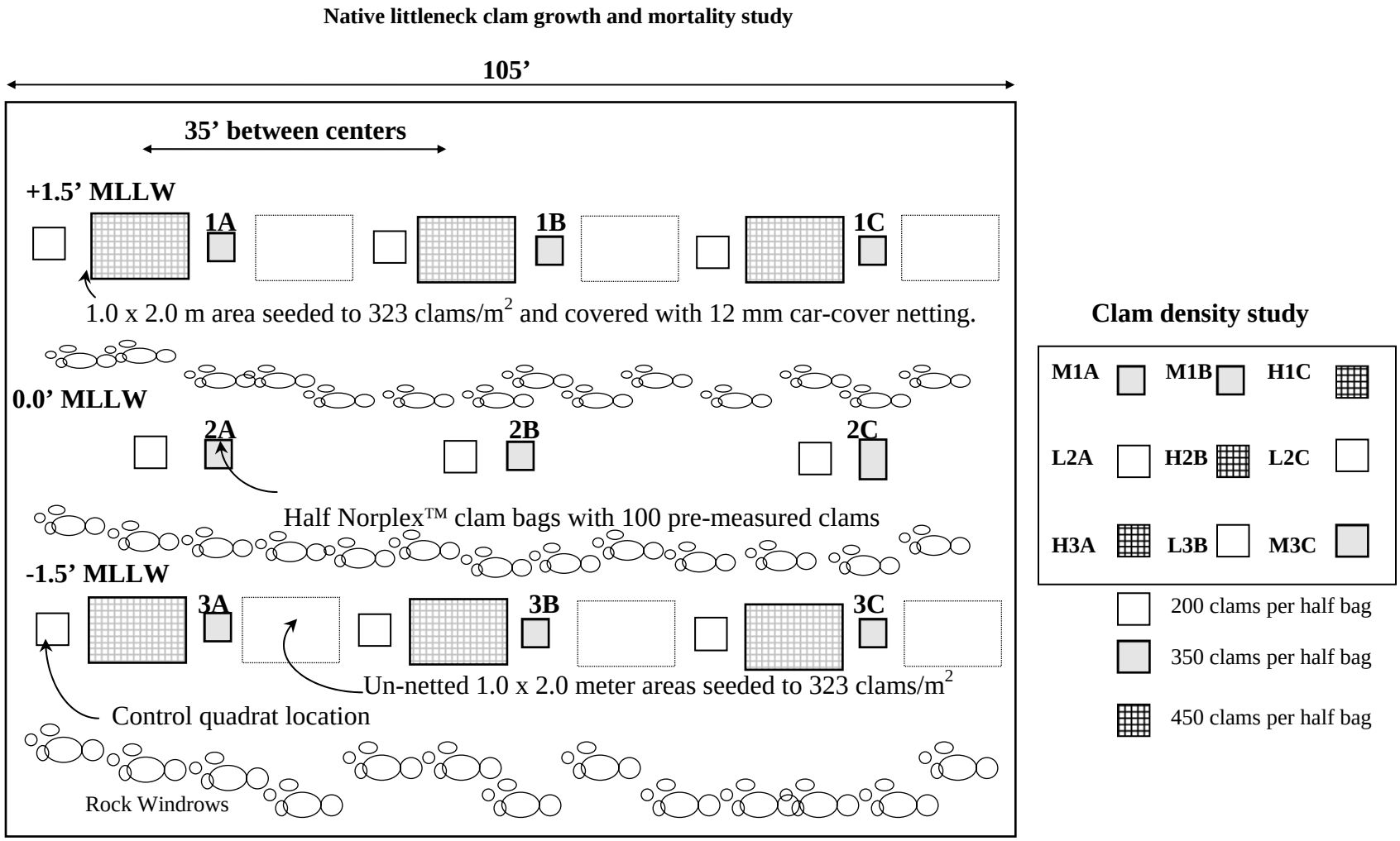

Figure 58. Study design for clam enhancement studies at previously surveyed beaches at the villages of Tatitlek, Nanwalek and Port Graham. The density study was added in 1998. It is shown to the right of the existing study site in this figure. Individual treatments were separated by four feet and ten feet of spacing was provided between the blocks.

large electrical ties, to a piece of ½" rebar driven into the substrate to a minimum depth of 18" or when hitting bedrock Identical study lay-outs, described in Figure (58), were used at all three villages – except that the protected and unprotected treatments (plus controls) were replicated at the 0.0' MLLW tide level at Tatitlek. This part of the study required measurement of 900 clam seed per village (2,700 total).

The study plan required that bags be retrieved at three-month intervals and the valve length of each surviving clam measured and recorded to the nearest 0.1 mm. All empty clamshells were to be retrieved, measured and archived. Fouling organisms were removed from the bags and a shovelfull of sieved (1/2") gravel added. Clam bags were then carefully renestled in the sediment and the 100 premeasured clams sprinkled on top of the sediment in the bag prior to securing the open end with split PVC pipe and electrical ties. Villagers were cautioned to retrieve clam bags individually and to measure and replace the clams in one bag before removing the next bag.

4.3.2. Clam enhancement using plastic netting. A minimum of 4' was left between each treatment and 10' between each block. This provided access to the various treatments for sampling without disturbing adjacent plots. All large (>10.0 cm diameter) rock and cobble was removed from the area to be seeded. The area was dug to remove all clams larger than 1.0 cm and raked to provide a smooth surface. Plastic netting was precut to a dimension of 9' x 6'. It was secured in a trench on all four sides of each 1.0-meter by 2.0-meter plot. Each plot was marked with PVC pipe. Each piece of PVC pipe had the plot number written on it (i.e. A +1.5, etc.). Sediment samples were taken adjacent to each set of treatments for baseline analysis of total volatile solids and sediment grain size. In addition to treatment samples, control stations were sampled annually and processed in a similar manner.

4.3.3. Extensive native littleneck clam enhancement (without protective netting). Six additional 1.0 x 2.0 meter sites were prepared and seeded as described above except that protective plastic netting was not installed.

4.3.4. Seeding of netted and unnetted substrates. Littleneck clams provided by the Qutekcak hatchery were divided into 12 subsamples of 600 clams each by determining the number of clams held in a four ounce beaker, followed by volumetric division. Clams were sprinkled onto the netted and un-netted sites as the flood tide covered them. This required 600 clams/station x two treatments (netted and uncovered) x two tidal heights (+1.5 feet and -1.5' MLLW) x three replicates = 7,200 clams per village. When combined with the 900 clams required in the bagged growth and mortality study, a total of 8,100 seed clams were seeded at Passage Island and Murphy Slough and 11,700 clams were seeded at Tatitlek (27,900 seed clams total).

4.3.5. Evaluation of the effects of culture density in bags on native littleneck clam survival and growth. Optimum native littleneck clam density was estimated from data for Manila clams presented in Toba *et al.* 1992. This study will examine three replicates of each of the following densities.

200 clams per half Norplex™ bag (80 clams/ square foot)
350 clams per half Norplex™ bag (140 clams/ square foot)
450 clams per half Norplex™ bag (160 clams/square foot)

All density experiment replicates were planted in an area 20' wide centered along the 0.0' MLLW station adjacent to the existing growout study site as depicted in Figure (58). The valve lengths of four randomly selected sub-samples of 50 clams each were measured. These measured clams were then mixed back into the available stock of 7,000 clams and triplicate random samples of 200, 350, and 450 clams counted out and placed in Ziploc™ bags for transport to the beach. This required 3,000 of the 7,000 available clams. One-half Norplex™ clam bags were filled with approximately 0.5 cubic feet of clean, screened sediment and nestled into depressions dug into the substrate at Murphy's Slough. The clams were sprinkled on top of the substrate during the flood tide and the ends of the bags folded and secured with electrical ties. Each bag contained one inside and one outside label. The bags were secured to 9' long pieces of ½" rebar with UV resistant, heavy-duty electrical ties. The nearly completed seeding is described in Figure (59). The substrate was backfilled against the Norplex™ bags when seeding was complete. This left approximately 2.5 cm of the bag above the substrate's surface.

Figure 59. Native littleneck clam seed density experiment initiated in April 1998 at Murphy's Slough near Port Graham, Alaska. The substrate was replaced around the perimeter of each bag when planting was complete.

4.3.6. Study site maintenance. Village culturists were encouraged to monitor these studies on a weekly basis, or as tidal conditions permitted. They were cautioned that all rips in the netting should be repaired and all predators removed. Badly damaged nets should be replaced with as little disturbance to the culture as possible.

4.3.7. Evaluation of treatments (other than bags) seeded with *Protothaca staminea* in 1996. A coffee can quadrat with a diameter of 6" (0.0182 m^2) was used to remove all substrate and clams to a depth of approximately 15 cm. This material was carefully sieved on 1.0 mm screens and the length of all clams measured using an electronic caliper. The clams were returned with the sieved sediment to the location from which they were taken. A systematic random sampling plan was used in this evaluation. The distance above and to the right of the lower left-hand corner of a PVC pipe quadrat was randomly determined for each site. The intersection of these two coordinates described the location of the sample. Samples were taken from the upper right hand quadrant of the intersection. This arrangement is described in Figure (60). Only two samples were collected from each plot to minimize disturbance of the small culture areas. The length and identity of each bivalve was recorded. Thirty-six samples were collected at Murphy's Slough and at Passage Island. Fifty-four samples were collected at Tatitlek.

Figure 60. Fixture used to define the sample location in unseeded Control and seeded areas protected with plastic netting or seeded and left unprotected.

4.3.8. Determination of sediment grain size distribution (SGS). The top two centimeters in each of the sediment samples was removed, examined for clams, homogenized in a stainless steel bowl, and then placed in a precleaned 250 ml HDPE bottle for TVS and SGS determination. Approximately 35 grams of the sample were dried in an oven at 92 ^{o}C and processed using the sieve and pipette method of Plumb (1981). The sieves used for the SGS analysis had mesh openings of 2.0, 0.89, 0.25 and 0.063 mm. Particles passing the 0.063 mm

sieve during initial wet sieving were analyzed by sinking rates in a column of water (pipette analysis). Data were arcsin(sqrt(proportion)) transformed prior to analysis.

4.3.9. Determination of sediment total volatile solids content (TVS). A 50-gram surficial sediment sample, excluding material $\geq$ 2.0 cm was taken from the top two centimeters of the substrate. These samples were dried at 103 $\pm$2 ^{o}C in aluminum boats that had been pre-cleaned by ashing at 550 ^{o}C for 30 minutes. Drying continued until no further weight reduction was observed. The samples were then ignited at 550 ^{o}C until no further weight loss was recorded. Total Volatile Solids were calculated as the difference between the dried and ashed weights as a proportion of the sample dry weight. Data were arcsin(sqrt(proportion)) transformed prior to analysis.

4.3.10. Water concentrations of fecal coliform bacteria. Three water samples were collected at each shellfish beach in autoclaved, 500 ml HDPE sample bottles by immersing the covered sample bottle to a depth of 0.5 meters in undisturbed water. The bottle cap was then removed and the bottle filled to the top with no headspace. Clean, shoulder length gloves were used during this sampling. Care was taken to not disturb sediments by wading or poling of the skiff during water sampling. Samples were held on ice at 4 ^{o}C until examined within 96 hours (holding time exceeded the recommendations of APHA, 1975). The number of fecal coliform bacteria was determined in each sample using the five tube MPN method (APHA, 1975, Method 908A). The recorded values were compared with the requirements of the National Shellfish Sanitation Program Manual of Operations, Part I (NSSP, 1995).

4.3.11. Water total volatile solids (TVS) and total suspended solids (TSS) analyses. Separate 500 ml samples of water were collected for the determination of TVS and TSS. Samples were collected in the same manner described in paragraph 4.11 and held at 4^{o}C until analyzed. TSS was determined by filtering a homogeneous sample through a Whatman glass fiber filter (0.45 μm particle retention) that had been ashed at 550 ^{o}C for 20 minutes and pre-weighed. The filter, with the residue from a 350 ml water sample, was repeatedly dried at 103 ^{o}C and weighed until no further weight loss was observed (generally one hour). The filter, with dried and weighed residue, was then ignited in a muffle furnace at 550 ^{o}C for twenty minutes. TVS and TSS were recorded as mg/L.

4.3.12. Sediment total sulfide analysis. Sediment samples for sulfide analysis were fixed in the field by adding 0.5 ml of two normal zinc acetate. Sulfide analysis was accomplished using an Orion™ ISE/pH/mV/ORP/temperature meter model 290A with a Model 9616 BNC *ionplus* Silver/Sulfide electrode. The meter has a concentration range of 0.0000 to 19900 μmoles and a relative accuracy of $\pm$ 0.5% of the reading. Detailed procedures for standards and buffer preparation, and analysis are contained in Brooks (2000b).

4.3.13. Evaluation of native littleneck clam (*Protothaca staminea*) and Pacific oyster (*Crassostrea gigas*) seed growth in the tidal Flupsy at Tatitlek. Seed oysters (*Crassostrea gigas*) and native littleneck clams (*Protothaca staminea*) were placed in the Tatitlek tidal Flupsy on April 5, 1998. The valve lengths of three random subsamples of thirty bivalves each were measured from each culture at two-month intervals until October 23, 1998. These measurements provided an estimate of the growth achieved in the post hatchery nursery phase of

enhancement. Based on results from Washington State with Manila clams, it has been hypothesized that up to one year can be eliminated from the total time to harvest size using these nursery techniques.

4.3.14. Periodic evaluation of test cultures. The one by two meter protected, unprotected and control plots were evaluated annually in 1998 and 1999 by the CRRC field team. The protocols developed for this study required quarterly sampling of the clams held in bags during 1996 and 1997. The bags were evaluated semiannually in 1998 and 1999. The actual sampling dates are provided in Table (21).

Table 21. Sampling dates for growout trials. Blue entries represent annual fieldwork supervised by the CRRC field team. Data collection on other dates was accomplished by Port Graham village residents. Days in growout are provided in parentheses.

Port Graham	Tatitlek	Passage Island
July 4, 1996 (0)	June 29, 1996 (0)	July 5, 1996 (0)
October 24, 1996 (112)	September 27, 1996 (90)	
March 11, 1997 (250)	January 14, 1997 (199)	May 6, 1997 (307)
July 22, 1997 (383)	July 25, 1997 (391)	July 22, 1997 (384)
November 15, 1997 (499)	November 26, 1997 (504)	
April 25, 1998 (660)	April 24, 1998 (652)	April 24, 1998 (660)
March 20, 1999 (989)	December 12, 1998 (896)	
September 9, 1999 (1162)	September 10, 1999 (1168)	September 8, 1999 (1162)

August 1, 2000 (1489) – Data collected by the Alaska Department of Fish and Game.

4.4. Results for Murphy's Slough (Village of Port Graham). The site is located approximately 1.0 nm from the Village of Port Graham. However, access is across sheltered water. The beach at Murphy's Slough was considered ideal for several types of intensive and extensive bivalve enhancement efforts. The intertidal area suitable for clam culture documented in the 1995 bivalve inventory had a gentle slope and covered several acres. The substrate consisted of a mixture of 59% small gravel less than 2 cm diameter, 30% sand and 11 percent silt and clay. Sediment TVS averaged 2.05 $\pm$ 0.4 percent. In 1995, this beach had a high volume of subsurface porewater observed during low tide. The RPD was consistently >10 cm and predators were restricted to a few starfish and possibly otters – as evidenced by the large number of pits, the absence of large butter clams, and the number of broken butter clam valves. Figure (61) is an aerial photograph of the study area. Two of the bags (3B and 3C) holding clams used in the growth and mortality study disappeared from this site in 1997. All other study components remained in good condition and all required samples were collected during the course of this study. The clams growing in bags were evaluated on August 1, 2000 by Ms. Nicky Szarzi with ADFW. That data is included in this study for growth studies. Samples collected outside the bags by ADFG cannot be used to determine survival because additional native littleneck clams were removed from all of the seeded plots following quantitative sampling in 1999.

Figure 61. Native littleneck clam enhancement site in Murphy's Slough near the Village of Port Graham, Alaska.

Native littleneck clams were not observed on this beach during the 1995 baseline study and none were observed outside the seeded plots during the study. The lack of an existing native littleneck clam population was of concern during the site selection process. However, the decision to use this site was based on the observed sediment physicochemistry, which typically supports littleneck clams in Washington State, British Columbia and Alaska. It was hypothesized that the lack of native littleneck clams in the area was due to lack of recruitment – perhaps associated with unfavorable surface currents during the spring and summer months. From a study perspective, the lack of native littleneck clam recruitment provided an opportunity to examine growth and survival of this species in Alaska without interference from the constant recruitment of new native littleneck clams observed at Tatitlek and Passage Island.

4.4.1. Aging native littleneck clams. The native littleneck clams in Murphy's Slough were all of known age. The presence of apparent annuli on the exterior of the valves was supported by an extension of the inner lamellar matrix secreted by the mantles inner surface through the outer prismatic layer (Morton, 1979). These dark lines of lamellar $CaCO_3$ were frequently present as doublets separated by several hundred microns. As previously noted, sectioned valves required very careful preparation or the first annulus was not recognizable because of the thinness of the prismatic layer – even in these clams that were grown in substrate for only three years. Figure (62) depicts the differing sculpturing observed in clams from the same cohort grown at two intertidal levels.

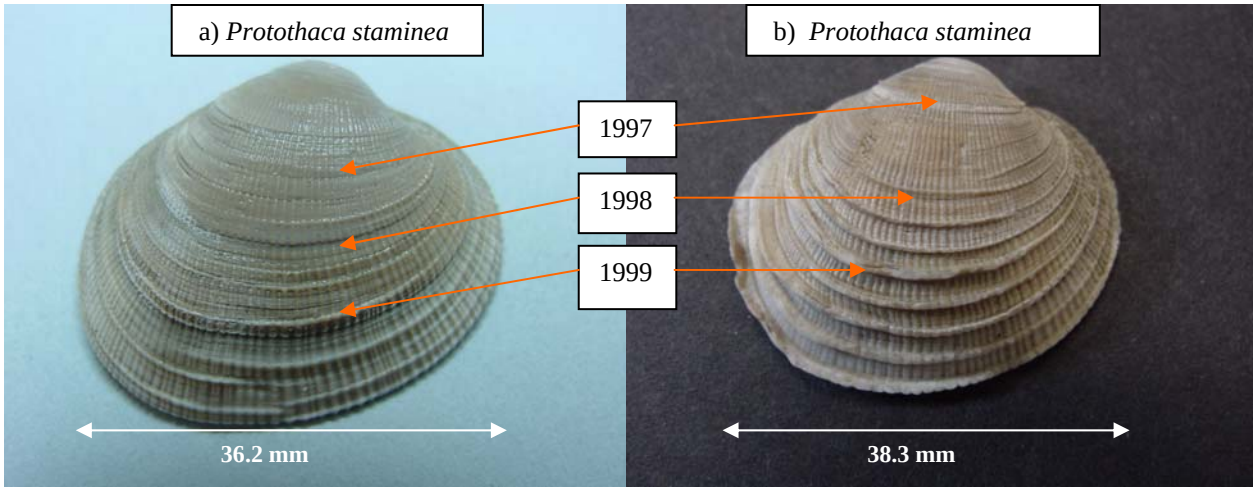

Figure 62) Native littleneck clam planted in 1996 in Murphy's Slough at a tidal height of a) +1.5' MLLW and b) –1.5' MLLW and collected on September 9, 1999 following 1162 days (3.2 years) of growout. Winter annuli formed in January of 1997, 1998 and 1999 are marked. Annuli are assigned to the month of February in the year indicated.

4.4.2. Survival of native littleneck clams in bags at Murphy's Slough. Table (22) summarizes survival of native littleneck clams between July 4, 1996 and August 1, 2000 at Murphy's Slough. Two of the three bag replicates at –1.5' MLLW were missing in 1997. One of these was recovered from deep water in 1999. However, all but two of the clams in that recovered bag had died by 2000. This compromised data from the –1.5' MLLW block. After four years of field growout, average survival was 42% at +1.5' MLLW and 48.7% at 0.0' MLLW. Figure (63) graphically describes the survival of native littleneck clams grown in bags at Murphy's Slough. It should be noted that significant winter mortality was not observed in bag cultures at the +1.5' MLLW tide level. This is important because the winter of 1998-99 was unusually cold and the clams survived well – suggesting that this factor should not inhibit bag culture at this site. Survival under plastic netting was significantly higher than survival of clams seeded and afforded no protection ($p = 0.000$). Differences in survival between clams grown in bags and those grown under plastic netting were not significantly different.

Table 22. Survival of clams grown in Murphy's Slough at three tidal elevations. Mean numbers of surviving clams in three replicate bags and the standard deviation is provided for each tidal elevation on each day. Only one bag was found on days 499, 660 and 989 in the –1.5' MLLW block. One of the two missing bags was retrieved from deep water on day 1162.

DAY	+1.5'	+1.5' STDS	0.0'	0.0' STDS	-1.5'	-1.5' STDS
0	100.00	0.00	100.00	0.00	100.00	0.00
112	91.00	6.98	102.70	8.81	99.33	2.87
250	82.30	9.98	91.00	0.82	73.30	23.42
383	73.30	15.06	86.70	4.99	74.70	25.94
499	72.30	13.72	85.33	8.18	66.00	0.00
660	60.30	16.01	70.67	7.93	55.00	0.00
989	58.00	20.02	66.33	12.39	52.00	0.00
1162	53.30	22.88	58.00	16.05	51.00	14.00
1489	42.00	14.76	48.70	11.09	14.00	12.00

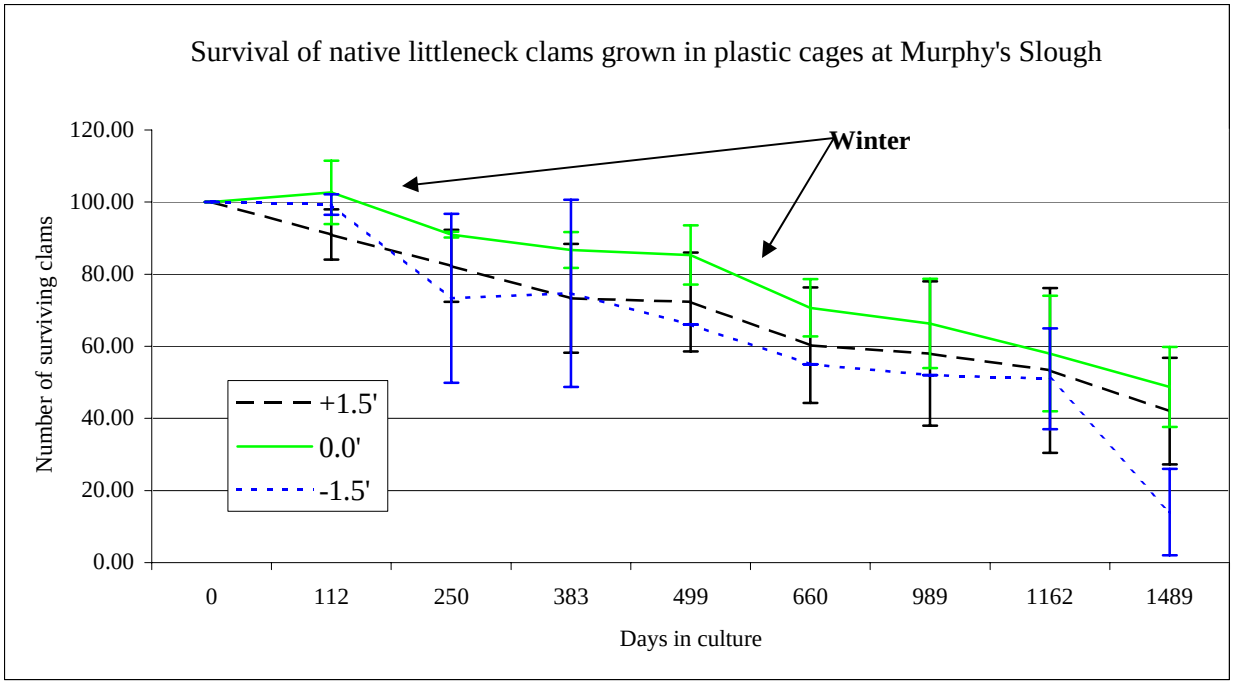

Figure 63. Mean number of surviving clams in replicate bags at three tidal heights in Murphy's Slough, Port Graham, Alaska as a function of date.

4.4.3. Survival of unprotected native littleneck clams seeded at Murphy's Slough compared with identical plots seeded and protected with plastic netting.

The intertidal area being evaluated in Murphy's Slough was stable throughout this study with no observable substrate movement. The primary purpose of the plastic netting at this site was to discourage predation by gastropods, starfish, crabs and birds. The lightweight plastic could not withstand the determined efforts of marine mammals like sea otters. However, it was thought that light to moderate algal fouling on the nets might camouflage the clams and ameliorating predation by otters. This fouling is described in Figure (64).

Clams were originally seeded in the protected and unprotected plots at a density of 300 clams per square meter. Two samples, covering an area of 0.0182 m^2 each, were collected from each of the three replicates at +1.5' MLLW and –1.5' MLLW giving six samples from each treatment at each tidal height (36 samples total). All count data were Log(N+1) transformed prior to analysis.

Figure 64. Fouled Carcover™ netting protecting native littleneck clam seed planted in 1996 at Murphy's Slough, Port Graham, Alaska.

Figure (65) describes the percent of the original 300 clams/m^2 surviving in six 0.0182 m^2 samples collected from each of the replicates at two different tidal heights (+1.5' and –1.5' MLLW) on September 9, 1999 following 1162 days of field growout. No littleneck clams were retrieved from unseeded control plots. That was consistent with the lack of native littleneck clams found in the 1995 baseline survey. Two native littleneck clams were found in the six samples collected from areas seeded but not protected with plastic netting and 31 clams were found in sediments collected from under the protected plots.

Analysis of variance indicated that tidal level within the tested range (-1.5' to + 1.5' MLLW) was not a significant factor affecting survival (p = 0.38). The type of protection afforded (bags, plastic netting, or unprotected) did significantly affect survival (p = 0.000). Post Hoc testing using Scheffe's test indicated that there was not a significant difference in survival when comparing bags and plastic netting. However, both of these forms of protection afforded statistically significantly higher survival than those seeded into cultivated ground but not protected (p = 0.000). The survival rates of 40 to 55 percent observed at Murphy's Slough following 3 years of growout under plastic netting were similar to those reported by *Toba et al* (1992) for Manila clams grown for two years in Puget Sound.

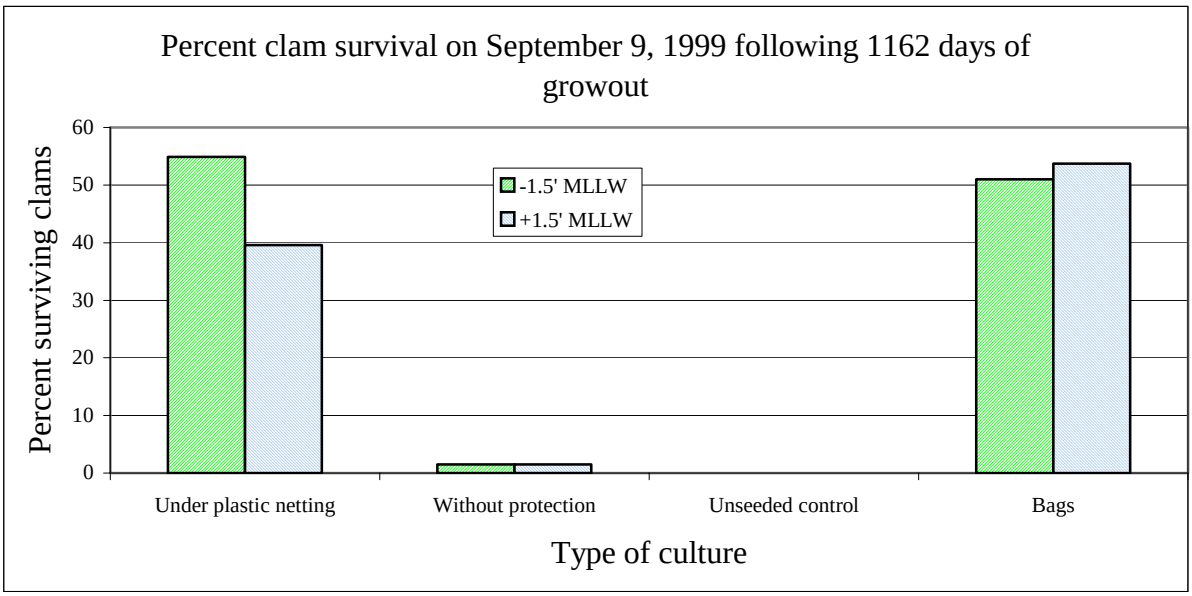

Figure 65. Percent surviving native littleneck clams cultivated in Murphy's Slough. Data compare survival on September 9, 1999 with planting density on July 4, 1996.

4.4.4. Growth of native littleneck clams in field trials at Murphy's Slough. Figure (66) describes the growth of all native littleneck clams in bags at Murphy's Slough during this study. The clams were originally planted on July 5, 1996 at an age of one year. They were last sampled on August 1, 2000 following 1489 days (4.1 years) of field growout (a total age of 5.1 years). The von Bertalanffy model developed using data from all living native littleneck clams collected at Tatitlek and Passage Island (Brooks, 1995) is included for reference.

4.4.5. Growth as a function of treatment. Statistically significant differences in growth as a function of treatment were observed (ANCOVA, F = 65.7; p = 0.000) in the September 9, 1999 data. Post hoc testing using Scheffe's test indicated that that native littleneck clams grown in bags were significantly smaller (27.03 $\pm$ 3.14 mm) and slower growing than those grown under plastic netting (34.74 $\pm$ 4.17 mm).

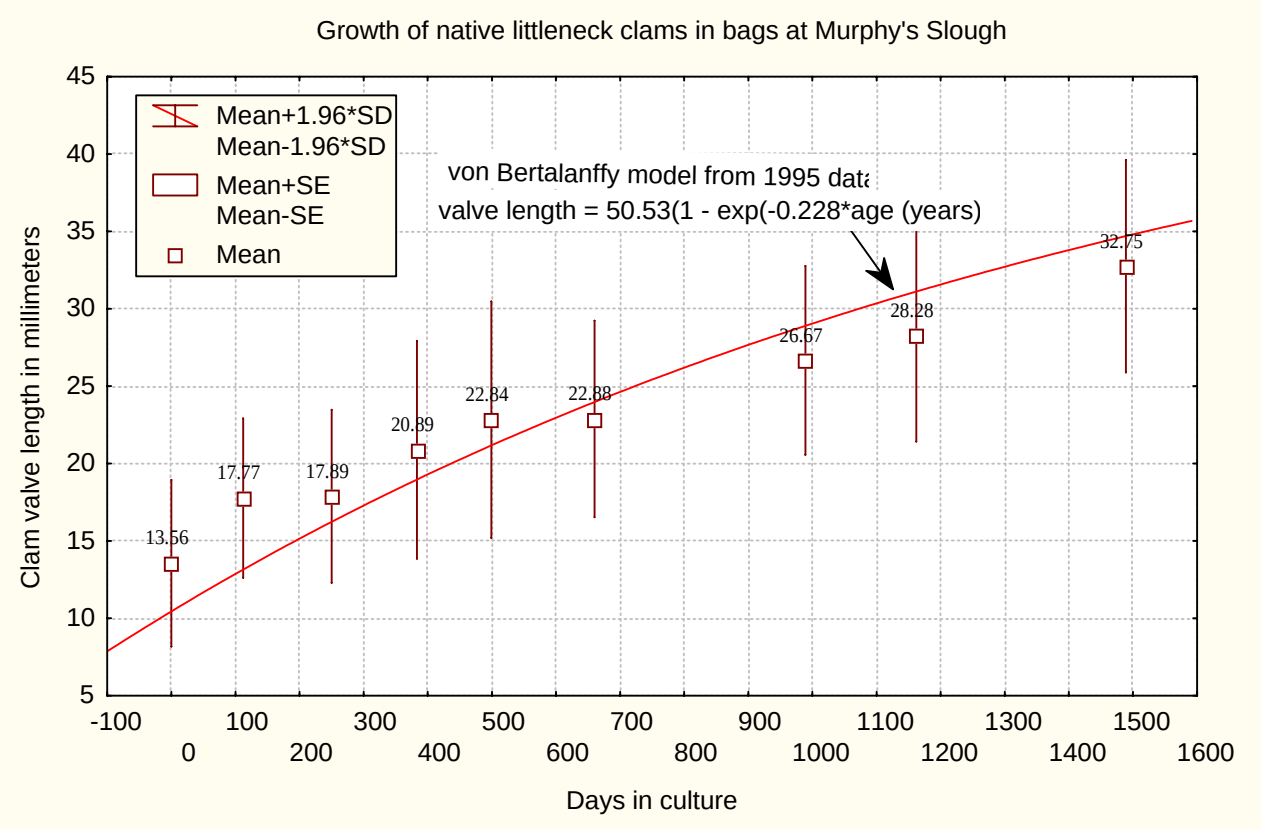

Figure 66. Mean lengths of native littleneck clams cultured at all tidal elevations in bags at Murphy's Slough between July 5, 1996 and August 1, 2000. The von Bertalanffy growth model developed for native littleneck clams from the baseline bivalve inventories conducted in 1995 as part of this effort is included for reference (Brooks 1995).

Figure (67) compares the valve lengths of native littleneck clams sampled under plastic netting with the von Bertalanffy model developed in Brooks (1995b). Clams grown under plastic netting had somewhat longer maximum valve lengths at all ages than predicted. However, the fit is remarkably similar and not significantly different. A solution to the von Bertalanffy model was defined for the clams grown under plastic netting in Murphy's Slough. The resulting model explained 74% of the variance. The residuals were normally distributed and there was no evidence of heteroscedasticity. The resulting model, presented graphically in Figure (68), is:

$$\text{Native littleneck clam valve length (mm)} = 54.1*(1 - \exp^{(-0.24*\text{age in years})})$$

The mean length of the 47 native littleneck clams recovered from beneath plastic netting in Murphy's Slough on August 1, 2000 by ADFG, following four years of growout, was 38.09 mm – slightly exceeding the minimum legal harvest size. Figure (69) is a length-frequency histogram describing the valve lengths of clams sampled under plastic netting in 1999 and Figure (70) provides similar data for 2000. Native littleneck clams began recruiting into the minimum legal harvest size in 1999, following three years of growout and more than half (57.4%) of these clams exceeded the minimum harvest size of 38 mm when last surveyed in 2000.

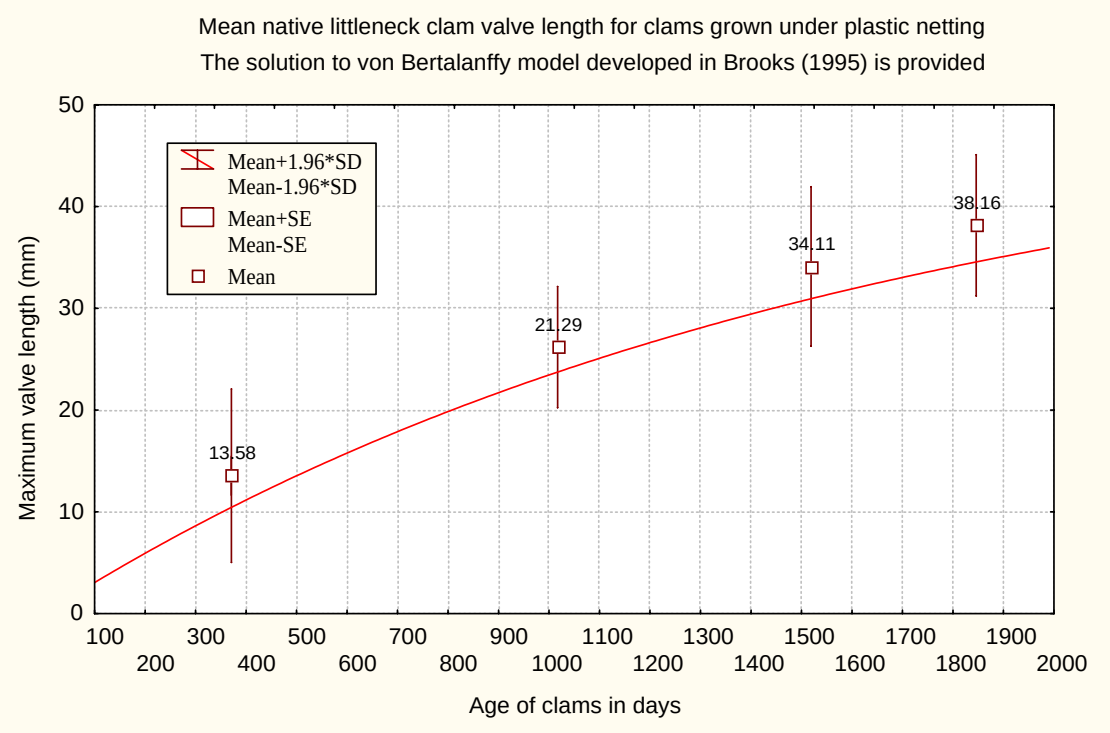

Figure 67. Comparison of the observed growth of native littleneck clams under plastic netting in Murphy's Slough with the von Bertalanffy model predictions based on the 1995 baseline surveys at Tatitlek and Passage Island.

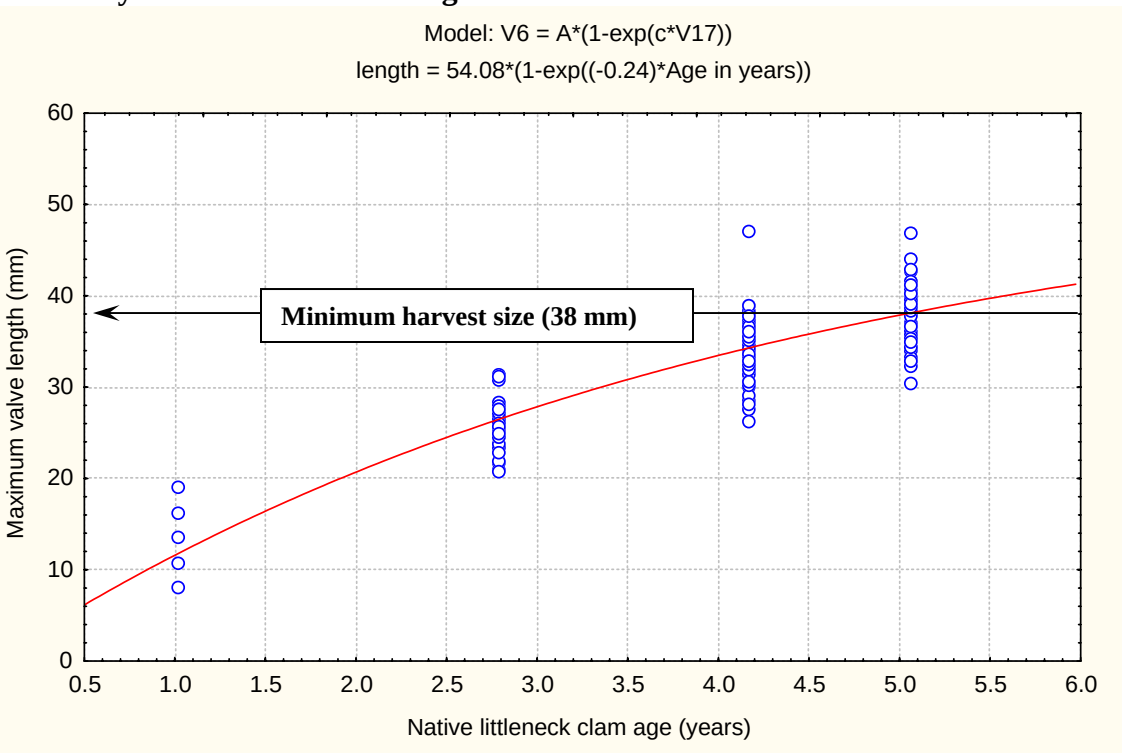

Figure 68. Solution to the von Bertalanffy model for native littleneck clams grown in Murphy's Slough under plastic netting. The clams were spawned in 1995, seeded on the beach in 1996 and monitored in 1998, 1999 and 2000.

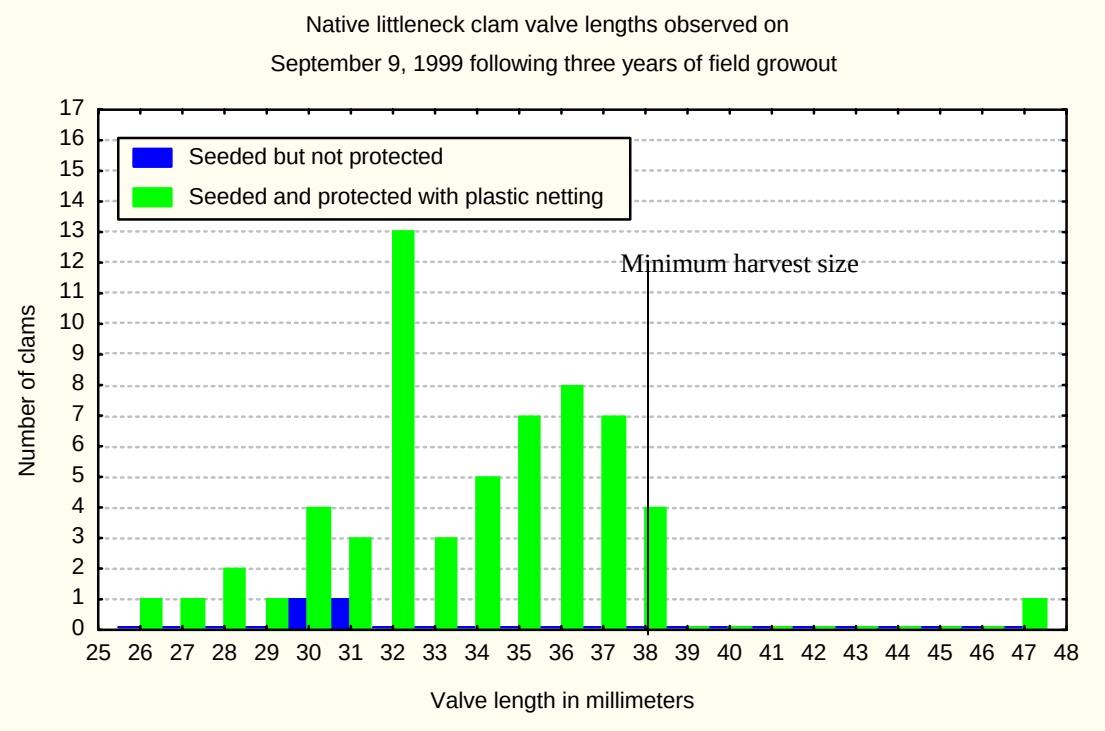

Figure 69. Length-frequency histogram describing artificially propagated native littleneck clams sampled from areas protected by plastic netting (green) and without protection (blue). The culture was initiated in 1996 and sampled in 1999.

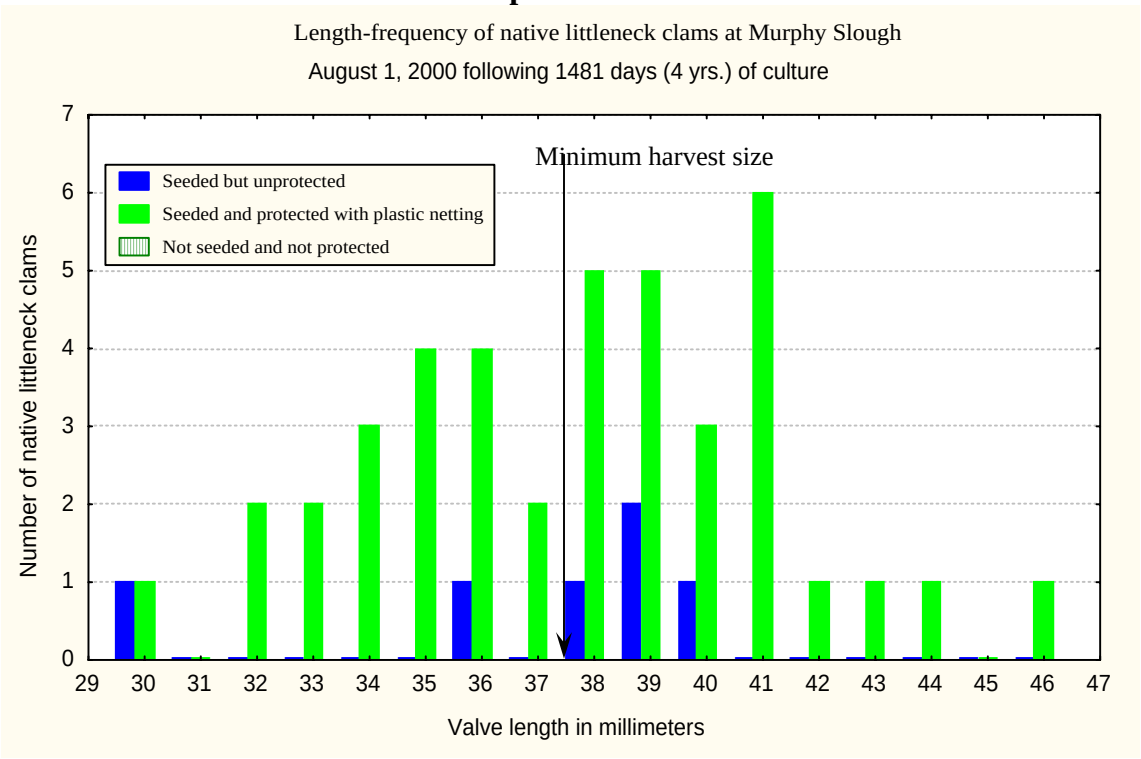

Figure 70. Length-frequency histogram describing artificially propagated native littleneck clams sampled from areas protected by plastic netting (green) and without protection (blue). The culture was initiated in 1996 and sampled in 2000.

4.4.6. Tide level effects on growth at Murphy's Slough. Analysis of covariance with initial clam length as a covariable indicated that the tidal level at which clams were grown had a significant effect on their size on each date ($F = 32.7$; $p = 0.000$). To simplify presentation of these effects, a new variable (Incremental Length) equal to the clams' length on each date minus the mean initial length of clams placed into that bag was invoked. This variable was submitted to analysis of variance and throughout most of the study, tidal effects were a significant factor affecting the incremental growth of clams. By the end of the study (August 1, 2000), differences in incremental growth of clams in bags were not as significant (ANOVA; $F = 4.2$; $p = 0.016$). Post hoc analysis using Scheffe's test (Zar, 1984) indicated that the incremental change in valve lengths for clams grown at the 0.0' MLLW tide level was significantly lower than for those grown at –1.5' MLLW ($p = 0.03$). These results are presented graphically in Figure (71). It should be noted that these results were confounded by the loss of two of the three replicate bags at the –1.5' MLLW tide level and subsequent retrieval of one of those bags.

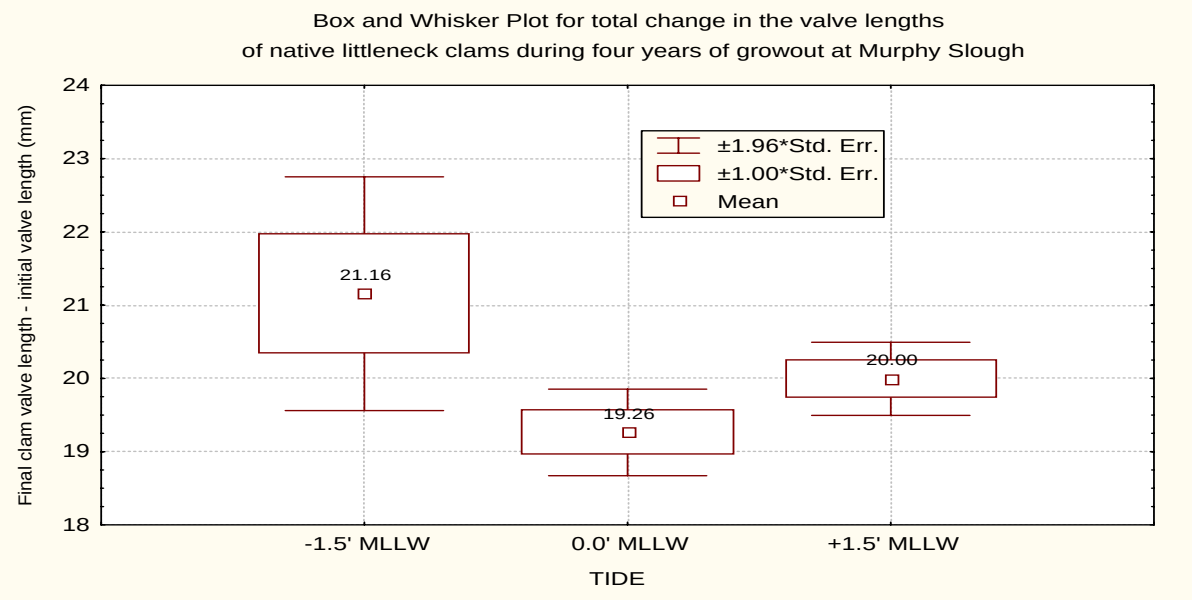

Figure 71. Box and whisker plots describing the difference in initial and final mean valve lengths of native littleneck clams grown in bags at Murphy's Slough from 1996 until 2000 as a function of tidal height.

4.4.7. Length-weight relationship for native littleneck clams grown in Murphy's Slough. All clams were returned to the various treatments following measurement until 1999. Native littleneck clams collected from under plastic netting during the 1999 field season were frozen until 2001 when their lengths and whole-animal weights were determined. All of the frozen clams lost their pallial water – but there was no evidence of freezer burn. Clams retrieved from under the plastic netting in Murphy's Slough during 2000 by ADFG were similarly weighed. That data was used to construct the length-weight scattergram provided in Figure (72). The data was fitted to a logistic regression model using the general nonlinear algorithm provided in Statistica™. The resulting regression explained 89.7% of the variation in the database and the residuals were normally distributed. The model predicts that whole-animal weights double between 30 mm and 38 mm and that they redouble between 38 and 47 mm valve length. These values are not significantly different ($\chi^2 = 0.12$, $\chi^2_{critical = 26.3}$, $\nu = 16$) from the distribution

described by Feder and Paul (1973) for total native littleneck clam weight versus length. In fact, they are essentially identical.

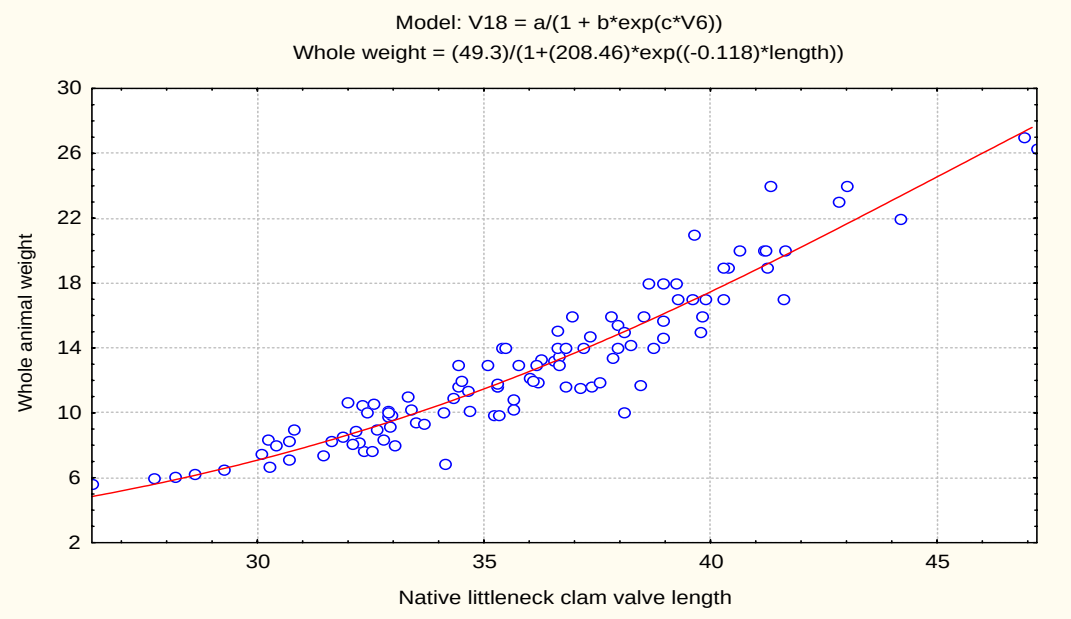

Figure 72. Logistic growth curve model fit to whole-animal weights (grams) and valve lengths (mm) observed in clams collected grown under plastic netting at Murphy's Slough, Alaska.

Brooks (1995b) analyzed wet tissue weights as a function of valve length for native littleneck clams from Passage Island and recommended that cultured clams not be harvested before the minimum legal size of 38 mm because of the rapid increase in wet tissue weights above ca. 25 mm. Wet tissue weights as a proportion of whole-animal weights for native littleneck clams determined in this study are provided in Figure (73). These data indicate that the proportion of total clam weight that is edible (wet tissues) increased from 28% at a valve length of 30 mm to 60% at a valve length of 47 mm.

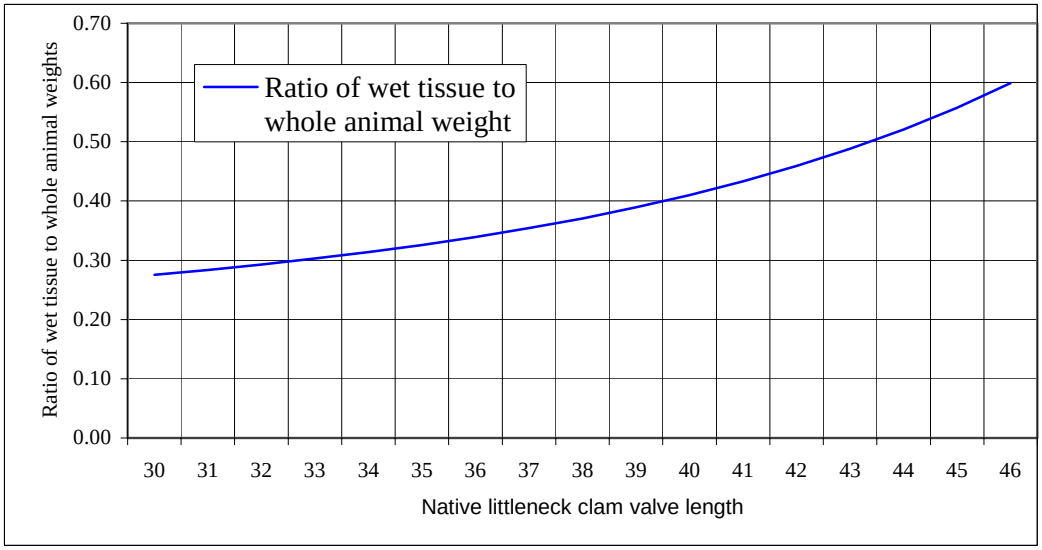

Figure 73. Ratio of wet tissue to whole-animal weights for native littleneck clams as a function of a function of valve length (mm).

4.4.8. Changes in the physicochemical properties of sediments at Murphy's Slough. Murphy's slough represents a low energy environment compared with Passage Island and Tatitlek. Some increase in the proportion fines was expected at sites protected with plastic netting when compared with unprotected plots. *T-tests* were used to assess differences in these physicochemical data. Significant differences were not observed in either the percent fines (silt and clay with particle size $\leq$63 microns) or in the proportion sedimented total volatile solids during either 1998 or 1999.

Brooks (2000b) and Brooks (2001, work in progress) found total sediment sulfide concentrations to be a valuable endpoint for assessing the infaunal and epifaunal response to organic loading from salmon farms. Sediment sulfides were evaluated in three replicate samples from unprotected cultures and under plastic netting at Murphy's Slough during the 1999 CRRC field season. The results are depicted graphically Figure (74). While not statistically significant at $\alpha = 0.05$ ($p = 0.066$), higher concentrations of sulfides were observed under the netting, suggesting that this parameter may be useful in further assessing the effects of this culture practice. It is also possible that the analysis of additional samples would reveal a significant relationship. However, the two square meter areas covered with netting to protect native littleneck clam cultures in Murphy's Slough did not significantly effect the concentrations of total volatile solids, sediment grain size distribution, or sediment total sulfides.

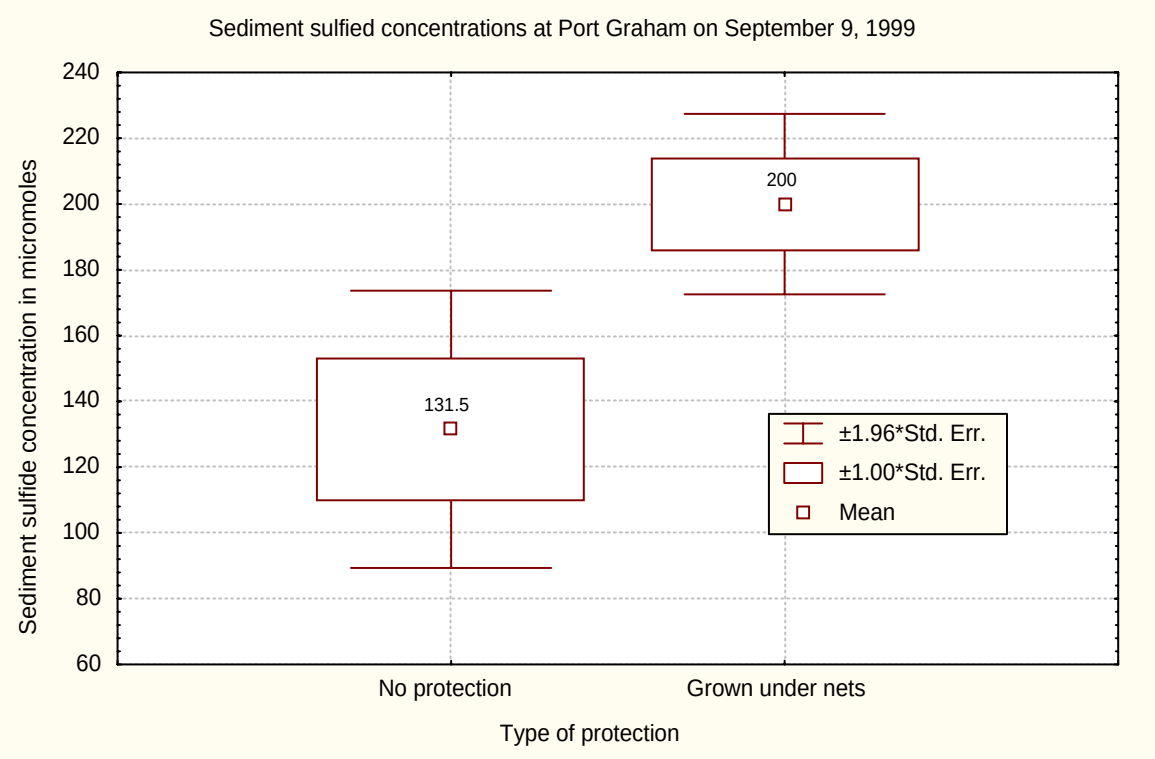

Figure 74. Box and whisker plot comparing the concentration of total sediment sulfides in Murphy's Slough sediments under plastic netting with sediments from unprotected treatment plots.

4.4.9 Fecal coliform and Total Volatile Solids in the water column at Murphy's Slough. The water temperature at Murphy's Slough on April 25, 1998 was 6.5 ^{o}C. Total Suspended Solids were measured at 2.9 $\pm$ 0.8 mg/L and the mean Total Volatile Solids was

1.3 ± 0.6 mg/L (mean ± one standard deviation). These data suggest that about half of the suspended particles retained on a 0.47 μm filter were organic and half were inorganic. The TSS and TVS concentrations observed at Murphy's Slough were approximately twice those observed at Passage Island during the same time frame.

Fecal coliform bacteria were not detected (< 2.0 fecal coliform bacteria/100 ml water) in any of the water samples collected during this study. This suggests that Murphy's Slough would likely meet the requirements for an Approved Classification as defined in Part I of the NSSP Manual of Operations. However, the 15 samples collected do not constitute an adequate survey in compliance with Part I of the NSSP Manual of Operations.

4.4.10. Native littleneck clam growth versus planting density in bags.

Three thousand native littleneck clams were planted in three replicates at each of three densities (80, 140 and 160 clams/square foot) during April of 1998. The lengths of four random samples of 50 seed clams each were used to determine the mean planting size and length distribution of the seed. The mean and 95% confidence interval for clam length at planting was (8.12 ± 0.21 mm). Clams for the density experiment were then counted from random samples into each bag.

Clams in each of these bags were retrieved on September 9, 1999. The clams were sieved and frozen at –20 ^{o}C. Their maximum valve lengths were measured and the aggregate weight of living clams remaining in each bag weighed to the nearest 0.1 grams in December 2000. Clams in two of the bags suffered severe predation by the gastropods (*Natica clausa*) and crabs (*Cancer oregonensis*) shown in Figure (75). The third predatory gastropod (*Nucella lamelossa*) shown in Figure (75) was not present in Murphy's Slough, but was abundant in the rocky intertidal environments at Passage Island and Tatitlek. The drilled valves of native littleneck clams are characteristic of predation by mollusks in the family Naticidae and the broken valves are typical of crab predation in bags. Sediments were sieved on ¼" sieves prior to seeding in 1998. The clams were drilled at valve lengths of 9.6 to 17.2 mm suggesting that this predation occurred following a period of growth. Whether the predators were introduced as very small juveniles passing the ¼" sieve or as new recruits is unknown. The point is that the five naticid gastropods and two cancer crabs reduced survival in the low density replicate PL2A to 12% and to 25% in the medium density replicate PM1B. The reduced density in these bags could contribute to increased growth if there was a density dependent growth factor

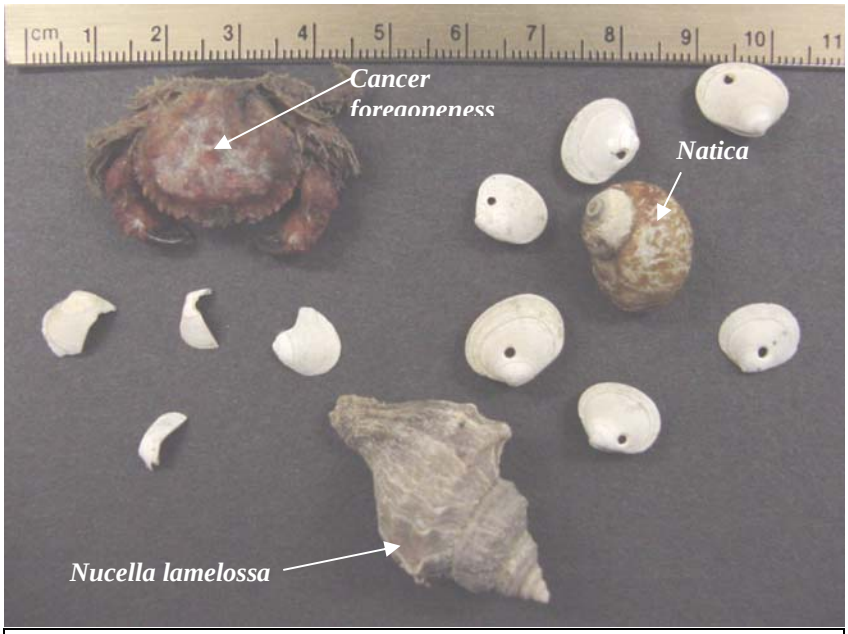

Figure 75. Gastropods (with drilled native littleneck clams) and *Cancer oregonensis* (with characteristic broken clam shells) found in bags at Murphy's Slough and Tatitlek during 1999.

and the loss due to predation certainly biased the results in terms of identifying a density dependent relationship between clam density and survival not associated with predation. Analysis of variance carried out on the entire database, including these two replicates, indicated that there were significant differences in valve lengths at the end of the study with clams in the low-density treatment growing faster than those in the medium and high-density treatments. Similarly, the proportion surviving clams was analyzed following an arcsine(square root) transformation (Zar, 1984) and a higher proportion of surviving clams was found in the low density experiment when compared with either of the other two treatments, which were similar.

The question being asked in this study was, "Does the number (density) of native littleneck clams placed in bags affect their growth and mortality during the first year of culture?" To better answer this question, the database was reanalyzed, excluding the two replicates in which predation by crabs and gastropods was known to have had a significant effect on survival and possibly on growth. The results of that analysis are summarized in Figure (76) for survival and in Figure (77) for final valve length.

Analysis of variance indicated that the proportion surviving clams was significantly different between treatments at $\alpha = 0.05$ ($p = 0.000$). Post hoc testing using Scheffe's test indicated that a higher proportion of clams grown at a density of 200 clams/bag survived than treatments with 350 or 450 clams/bag ($p = 0.000$ in each case). The minor difference in survival between the higher density treatments was not significant ($p = 0.999$). These results suggest that native littleneck clams survive better at the lower density and are different from those of Toba *et al.* (1992) who found that Manila clams survived equally well (65 to 79%) at densities of 250 to 750 clams/half bag in Puget Sound during a 17 month growout.

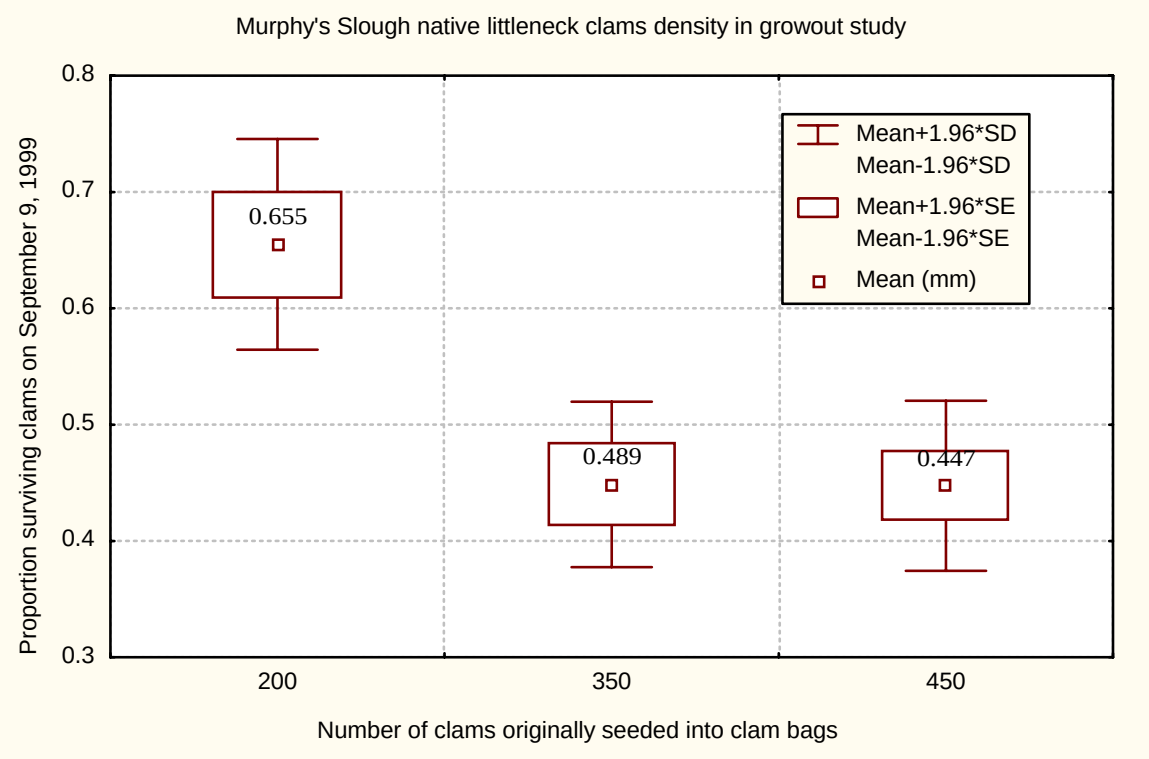

Figure 76. Proportion surviving native littleneck clams planted at densities of 200, 350 and 450 clams per half clam growout cage in Murphy's Slough on April 25, 1998 at the 0.0' MLLW tide level and evaluated on September 9, 2000.

Toba *et al.* (1992) found that the weight of Manila clams steadily decreased with increasing culture density. As shown in Figure (77) mean clam lengths decreased linearly with increasing seeding density in this experiment. Those differences were significant (ANOVA, F = 6.44, p = 0.002). Post hoc analysis using Scheffe's test indicated that the lowest density (200 clams/bag) grew significantly faster than the highest density (450 clams/bag) with the probability that the two means were equal being p = 0.002. The final valve lengths of clams grown at the intermediate density of 350 clams/bag were not significantly different from the low density (p = 0.26) or the high density (p = 0.20).

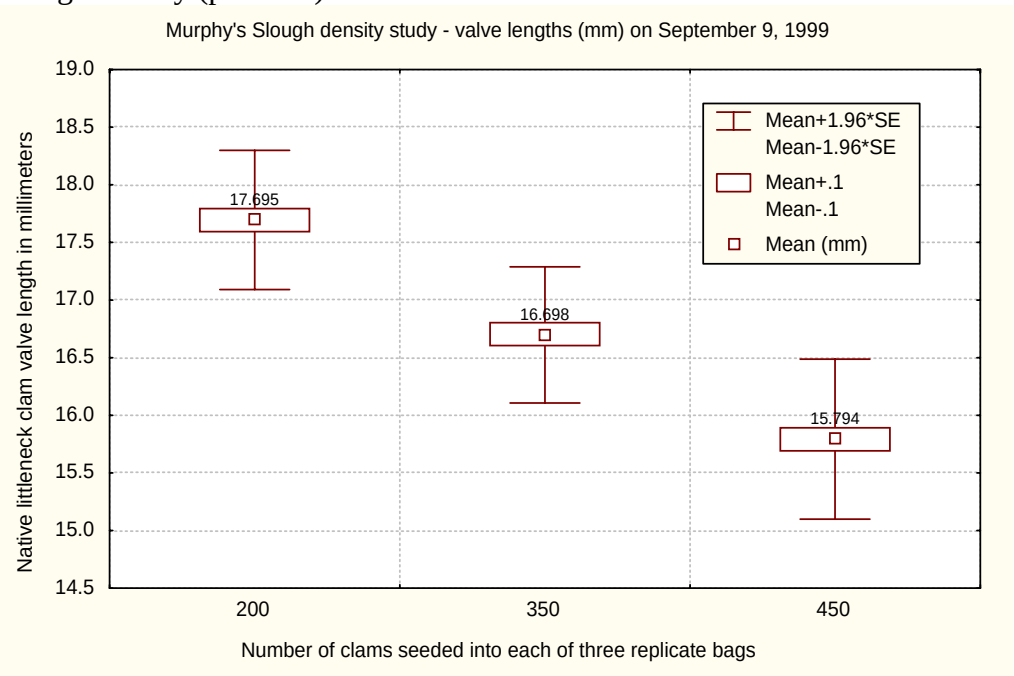

Figure 77. Mean valve lengths in three replicates of native littleneck clams planted at densities of 200, 350 and 450 clams per half clam growout cage in Murphy's Slough on April 25, 1998 at the 0.0' MLLW tide level and evaluated on September 9, 2000.

The preceding analysis suggests that native littleneck clams grown at the lowest tested density (200 clams/half bag) will survive better and achieve longer mean valve lengths after one year of growout than those seeded at higher densities. However, to grow the same number of clams, that requires the use of more bags, more space and most importantly, more labor to maintain the additional bags. Another way of looking at this issue is to examine the biomass grown under each of these conditions. The aggregate weights of all clams in each bag are described graphically in Figure (78). Analysis of variance (F = 0.09; p = 0.92) indicates that at the end of one year there was no significant difference in the aggregate weight of surviving clams at the three densities.

This information describes one management tool for future enhancement efforts. If the availability of seed of an appropriate size (6 to 10 mm) continues to be a limiting factor, then production can be improved by planting at lower density. This will improve survival and the weight of individual clams – at least at the end of the first year of culture. If seed becomes readily available and intertidal space and/or labor become limiting factors, then clams should be planted at higher density during the first year and then possibly thinned to lower densities for final growout. The last part of this statement is uncertain at this point, because growth as a function of density was not investigated beyond the first year in this study.

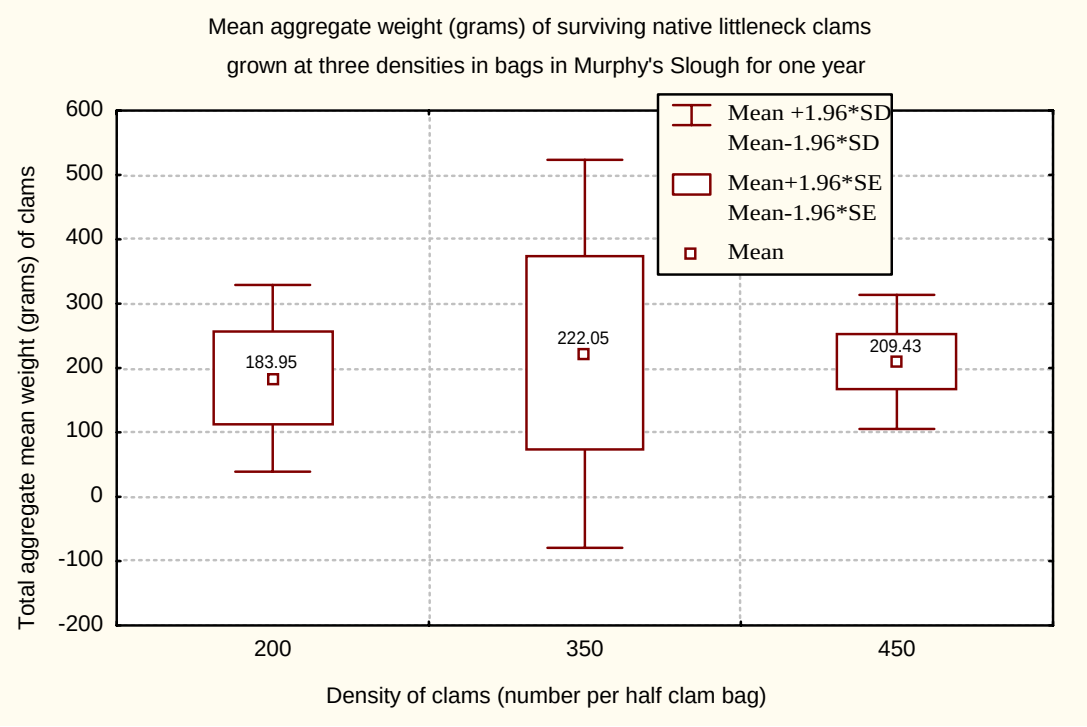

Figure 78. Mean aggregate weight (grams) of native littleneck clams grown in bags for one year at three densities at 0.0' MLLW in Murphy's Slough, Alaska.

4.4.11. Bivalve predation at Murphy's Slough. Sea Otters were observed in groups of three to four animals throughout Port Graham and near Murphy's Slough. However, no evidence of sea otter predation on the study cultures was observed. Numerous (>50) starfish (*Pycnopodia helianthoides*) were counted at the –4.0' tide level in front of the enhancement area. They were not observed at tidal elevations greater than –1.5' MLLW where the studies cultures were placed. As noted in Section 4.2.10, significant predation was related to small naticid gastropods and shore crabs. These small animals are more difficult to locate and remove than larger predators.

4.4.12. Summary for Murphy's Slough. Native littleneck clams grew and survived well enough at this site to warrant continued enhancement efforts. The following conclusions are based solely on the results at Murphy's Slough. As noted in the 1995 baseline report, the geography of this site and physicochemical composition of the sediments would be considered ideal for native littleneck clam production anywhere in the Pacific Northwest. Expectations for clam growth and survival should not be extended from Murphy's Slough to different intertidal environments. The lack of evidence for historical populations of native littleneck clams in the immediate area was of some concern at the start of these studies. However, based on the results, it appears that the absence of native littleneck clams was caused by lack of recruitment rather than environmental conditions inimical to survival and growth of the species. The following conclusions and recommendations follow from this analysis:

- Clams grown in bags and examined quarterly during the first two years of the study grew more slowly than those grown undisturbed under plastic netting did. The coefficients developed in 1995 for the von Bertalanffy model based on data from Passage Island appeared adequate to predict the growth of native littleneck clams grown in bags and frequently disturbed.

The equation given below better describes anticipated growth of undisturbed native littleneck clams under plastic netting in Murphy's Slough.

$$\text{Native littleneck clam valve length (mm)} = 54.1*(1 - \exp^{(-0.24*\text{age in years})})$$

- Previous reports have estimated that 7 to 10 years would be required in South Central Alaska for native littleneck clams to reach a minimum legal harvest size of 38 mm. Native littleneck clams cultured under plastic netting in Murphy's Slough began recruiting into the legal size range at the end of three years of growout (four years of age) and 57.4% of the clams retrieved in August 2000 had valve lengths $\geq$ 38 millimeters. These five-year-old clams had been grown in the field for four years.

- The short-term study of density effects reported herein suggested that native littleneck clams will survive better and grow more quickly when seeded into bags at lower densities of ca. 300 to 400 clams per full bag rather than at higher densities of 700 to 900 clams/bag. The mean biomass of clams produced at the three seeding densities was not significantly different. These results can be used to estimate the best seeding density as a function of the cost and availability of seed, suitable culture area and available labor resources to maintain the cultures.

- Silt and clay did not increase significantly as a proportion of the total sediment matrix under plastic netting in Murphy's Slough. Concentrations of total sulfides increased in sediments under plastic netting but the difference was not significant at $\alpha = 0.05$ ($p = 0.06$). However, sediment sulfide concentrations are increasingly recognized as a valuable tool in understanding the biological response to organic loading and it is recommended that this parameter be added to future studies examining the environmental response to intensive bivalve culture.

- Fecal coliform bacteria were not observed above the quantitation limit of two FC/100 ml in any water sample from Murphy's Slough. The 15 samples collected do not satisfy the requirements for a sanitary survey by the National Shellfish Sanitation Program. An appropriate survey should be completed to verify that Murphy's Slough warrants an *Approved* harvest classification prior to significant further enhancement.

- Native littleneck clams did not survive adequately in Murphy's Slough without some form of protection by bags or plastic netting;

- Primary predators were shore crabs and gastropods. No evidence of sea otter predation on these cultures was observed. That may be due to the small size of the clams during this study;

- High mortality associated with winter freezing temperatures was not observed in native littleneck clams grown in bags at Murphy's Slough. The volume of moving porewater at this site likely ameliorates the potential for freezing. However, the winter of 1998-99 was reported to be unusually cold by residents in Port Graham. These results should not be extended to cultures placed at higher intertidal elevations or in sediments with lower porewater volumes.

The beach at Murphy's Slough is relatively expansive with a shallow slope. The site enjoys an excellent substrate of small gravel mixed with sand, silt and clay held together with moderate amounts of organic carbon. Copious amounts of pore water were observed on all

sample days and the RPD was >15 cm in all samples. Murphy's Slough appears capable of sustaining native littleneck clams in an expanded enhancement effort.

In Puget Sound, native littleneck clams grow fastest and are most abundant where moderately fast currents deliver significant amounts of living phytoplankton and detritus. Murphy's Slough does not appear to be a well-flushed site. The availability of appropriate seston (bivalve food) and its delivery by local currents may become limiting with a significantly expanded bivalve population. In other words, it is possible that native littleneck clam enhancement in Murphy's Slough could be constrained by the available food supply before the suitable intertidal substrate is fully utilized. Dame (1996) and Brooks (2000c) have assessed and expanded various methodologies for determining the bivalve carrying capacity of coastal bays and inlets. Murphy's Slough can likely support further enhancement without undue concern for carrying capacity. However, the author recommends a carrying capacity evaluation before significant commercial culture of native littleneck clams is undertaken in Murphy's Slough.

4.4.13. Additional enhancement activities at Murphy's Slough in 1999.

Approximately 80,000 native littleneck clams were available for additional enhancement during the 1999 field season. The available seed varied in size from 2.3 to 5.1 mm with an average of 4.0 $\pm$ 0.20 mm. This is significantly smaller than the desired valves lengths of 6 to 10 mm. Obvious predators (gastropods and starfish) were removed from an area measuring 160 feet long paralleling the 0.0' MLLW tide level by 17' wide. The substrate was cultivated with rakes to a depth of approximately 5 cm. Plastic netting with leadline previously sewn into the perimeter was rolled out over the surface and the leadline staked with rebar "J" stakes. The number of seed clams per unit volume was determined. Ten random subsamples, each containing 8,000 clams (determined volumetrically), were seeded through the plastic netting on the incoming tide at a density of 30-seed/square foot. Figure (79) depicts the culture being seeded by Port Graham residents. The inset in Figure (79) shows the seed through the plastic netting.

Figure 79. Port Graham residents planting 80,000 native littleneck clams in Murphy's Slough during 1999. These clams should begin reaching a minimum legal harvest size in 2004 or 2005.