Final Report
Chugach Regional Resources Commission Bivalve Enhancement Program
Bivalve inventories and native littleneck clam (*Protothaca staminea*) culture studies
***Exxon Valdez* Oil Spill Trustee Council Project Number 95131**

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Acknowledgements

The author wishes to acknowledge the *Exxon Valdez* Trustee's for financing the five years of study leading to this report. Ms. Patty Brown-Schwalenberg and Mr. David Daisy at the Chugach Regional Resource Council provided excellent administrative support and Patty's time working in the field was viewed as a sign of her true commitment. This project would not have been successful without the leadership and guidance of Mr. Jeff Hetrick who coordinated all of the logistics and helped village residents complete their quarterly sampling. His special relationship with village elders developed over many years of service was invaluable. The aforementioned participants are all professionals who assisted natives in completing this project.

This last statement is important because these studies were designed to be conducted in large part by Native Americans – not by scientists. The significance of achieving success in culturing a new species in a new environment during the first attempt should not be underestimated. That success would not have been possible without the sincere interest and dedication of the dozens of village residents who participated in these studies.

On a personal note, the author wishes to acknowledge the friendliness and enthusiasm of the people at Ouzinke – particularly Mr. Roger Larionoff whose picture graces the cover of this report. His sincere friendship, developed over a very short period, will never be forgotten.

All of these people deserve the lion's share of credit for completing this project. It is the author's hope that bivalve enhancement activities will continue at these villages and be expanded to Ouzinke and Chenega in the near future. The cockle studies were continued without funding because of the native's obvious preference for this species. The technology for enhancing this species is clearly outlined in this report. That technology, developed in Washington State, needs to be transferred to the Quetekcak hatchery and ultimately to village shellfish culture teams. The recent development of hatchery and nursery techniques by the Washington State Department of Fish and Wildlife will enable this preferred species to also be added to the list of candidates for enhancing native shellfish resources. An adequate supply of wholesome clams will surely help Native Alaskan's sustain the heritage and culture that they obviously cherish.

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Introduction. This report is presented in five sections. Section 1.0 contains background information pertinent to the entire report. Section 2.0 describes the materials and methods used for bivalve inventories conducted in 1995 and 1996 at traditional subsistence harvest beaches near the native villages of Port Graham, Tatitlek, Nanwalek, Chenega and Ouzinke located in South Central Alaska (Figure 1). Section 3.0 describes the results of the bivalve inventories. Section 4.0 describes the methods, methods and results for native littleneck clam growout studies conducted near the villages of Port Graham, Tatitlek and Nanwalek. Section 5.0 describes preliminary investigations into the culture of Nuttall's cockle.

The purpose of this project was not to determine the causes of a perceived decline in subsistence bivalve resources, but to evaluate the potential for enhancing native littleneck clam (*Protothaca staminea*) populations using culture methods developed in Puget Sound for Manila clams (*Tapes philippinarum*). This study was designed as a *hands-on* effort that relied on Chugach Regional Resources Commission Staff and residents of each village to maintain the cultures and to collect much of the data. This hands-on approach was considered important if village residents were to develop the skills and understanding necessary to continue shellfish enhancement activities following completion of the study. The results are due, in large part, to the efforts of Mr. Jeff Hetrick from CRRC's staff and the residents of Tatitlek, Nanwalek, Port Graham, Chenega and Ouzinke.

Village residents received training in shellfish culture techniques and the specific tasks required in completing the study. In addition, each village was provided with the equipment and datasheets needed to prepare the beaches, seed the clams and to collect data during each sampling event. Appendix (1) contains the training materials used to acquaint village residents with the biology of native little clams, the study design and collection of data.

Lastly, it should be realized that these growout studies were conducted in parallel with refinement of hatchery production methods at the Qutekcak facility and with development of a nursery system. Future improvements in the hatchery and nursery phases hold the promise of producing seed spawned in late winter or early spring that can be grown in nurseries to an optimum planting size of greater than 10.0 mm in time for fall planting on the last daytime low tides of the same year. That capability was not available during this study resulting in the use of limited quantities of undersized seed for the growout studies.

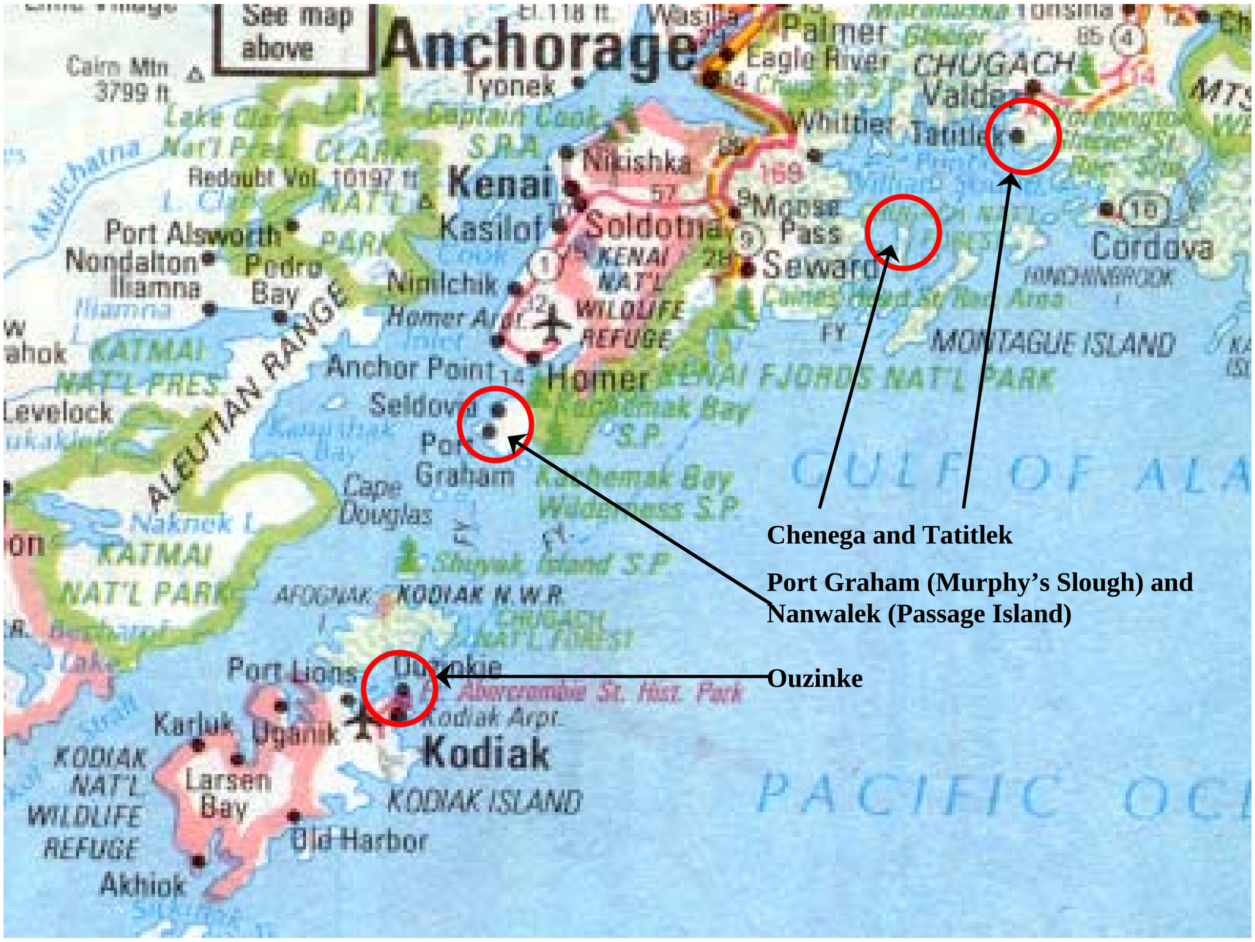


Figure 1. Location of native villages participating in the Chugach Regional Resource Council bivalve inventories and native littleneck clam enhancement studies. Bivalve inventories were completed at selected beaches near all five villages. Enhancement studies were undertaken at Murphy's Slough, Passage Island and Tatitlek.

1.0. Background information. The existence of extensive shell middens throughout the North Pacific Coast attests to the historic importance of bivalves in the diet of Native Americans. Clams have provided an important subsistence food resource in the native villages of Tatitlek, Nanwalek and Port Grahams as well as many other villages located within the area affected by the *Exxon Valdez* oil spill. However, clam populations have declined markedly at these villages in the recent past. The reasons for these declines are not well documented – but the loss of a traditional food source is significant to Native Americans. In response to concerns expressed by village elders, the Chugach Regional Resource Commission (CRRC), in cooperation with the Alaska Department of Fish and Game (ADFG), requested and received funding from the *Exxon Valdez* Oil Spill Trustee Council to re-establish populations of clams in areas readily accessible from the villages of Tatitlek, Nanwalek and Port Graham.

1.1. Littleneck clam life history. The native littleneck clam (*Protothaca staminea*) occurs in estuaries, bays, sloughs and open coastlines along the Pacific coast of North America from the Aleutian Islands to Baja California (Fitch 1953; Abbott 1974).

1.1.1. Reproduction. Sexual maturity appears to be size, rather than age dependent. It is reached at a valve length of 25 to 35 mm (Quayle, 1943). Reproductive competence is achieved between the second and eighth year of life (Paul and Feder, 1973). In Prince William Sound, Feder, *et al.* (1979) observed limited spawning in late May with a major release of gametes during June. Female *Protothaca staminea* gonads were observed in a spawning phase from early June through September. In contrast, males were in spawning condition throughout most of the year. Fraser (1929) reported limited spawning during January in Departure Bay, British Columbia and he found planktonic larvae (veligers) of this species in February.

Strathmann (1987) noted that larval culture temperatures of 10-15 ^{o}C were optimal with some survival to 20 ^{o}C. She noted that larvae survive at 32 parts per thousand (o/oo) salinity, but not at 27 o/oo. Spawning appears to be temperature related (Quayle 1943) and an examination of USFWS (1968) suggests that the sea surface temperatures are warming rapidly from less than 8 ^{o}C to >10 ^{o}C during June and July of each year in South Central Alaska.

Larval clams are planktonic for three to four weeks. Therefore, they may be dispersed over large areas by wind and tides or they may remain in localized areas (Mottet, 1980). Successful recruitment is dependent on a wide range of environmental parameters and it may vary significantly from year to year. Large year classes may be separated by either missing or subdued year classes (Rodnick and Li, 1983). Maximum life span has previously been reported at 13 years (Fitch, 1953; Paul *et al.,* 1976; Rudy and Rudy, 1970). However, ADFG (1995) reported native littleneck clams to 14 years of age.

Littleneck clams grow continuously throughout their lives. However, growth slows as clams age and is dependent on local environmental conditions; including tidal height, currents, food availability, temperature and salinity (Quayle and Bourne 1972; Trowbridge *et al.* 1996).

1.1.2. Distribution as a function of tidal elevation. The native littleneck clam inhabits the intertidal zone from approximately –2.5’ to +6.0’ MLLW in Prince William Sound, Alaska (Nickerson, 1977). Nickerson (1977) observed peak native littleneck biomass at +1.5’ MLLW with reduced biomass above +3.0’ or below –1.5’ MLLW. Feder and Paul (1973) observed maximum numbers of littleneck clams at tidal heights ranging from +1.4’ to –1.7’ MLLW with very few clams observed at tidal elevations ≤ 1.9’ MLLW. However, Goodwin (1973) reported that this species is infrequently found at subtidal depths in Puget Sound, Washington. Consistent with these reports, Quale (1960) reported that littleneck clams in British Columbia were concentrated at “about the half-tide level”. He also noted that they occured in reduced numbers at subtidal depths. This literature suggests that highest densities of native littleneck clams are typically found between –1.7’ and +3.0’ MLLW.

1.1.3. Substrate preferences. Mottet (1980) provides an excellent review of the interaction between sediment physicochemical characteristics, hydrodynamics and clam habitat preferences. Unfortunately, her treatise does not specifically include the native littleneck clam. Quayle (1941) noted that littleneck clams can be found in a variety of substrates but appeared most typically in mixed substrates of “pebbles and fine mud”. In the Pacific Northwest, littleneck clams are seldom encountered in muddy or sandy areas, they prefer loosely packed substrates consisting of a mixture of cobble, gravel, shell, sand and mud (Rutz 1994; Nickerson 1977; Feder and Paul 1973; Strathman 1987). Alexander *et al.* (1993) identified native littleneck clams as a *Substrate Sensitive* species found in sand – silt and clay substrates in San Francisco Bay and Peterson (1980) reported native littleneck clams from muddy and clean sand environments in Magu Lagoon, California. Hughes and Clausen (1980) also reported native littleneck clams from muddy substrates in Newport Bay, California. The literature suggests that while this species inhabits fine-grained sediments in the southern parts of its range, it prefers mixed substrates containing cobble, gravel, sand, silt and clay in Washington, British Columbia and Alaska.

Unfortunately, none of these reports included analyses of important physicochemical characteristics such as sediment grain size distribution, organic content measured as total organic carbon (TOC) or total volatile solids (TVS) and perhaps most importantly, sediment total sulfides ($S^{=}$). Goyette and Brooks (1999) and Brooks (2000a, 2000b) have shown that small changes in these physicochemical parameters have significant effects on infaunal communities – including large and small bivalves. Freese and O'Clair (1987) reported that survival of *Protothaca staminea* was inversely related to sediment concentrations of hydrogen sulfide and ammonia and directly related to pore water dissolved oxygen concentrations. Despite this report, the author (Brooks, unpublished) has observed large (>38 mm valve length) native littleneck clams surviving in anaerobic sediments where their shells become blackened by iron sulfides.

Native littleneck clams, like Manila clams, require stable substrates (Toba *et al.* 1992; Quayle and Newkirk 1989). They can be washed out of erosional environments or buried in depositional areas (Peterson, 1985).

1.1.4. Habitat Suitability Index (HIS) for native littleneck clams. Rodnick and Li (1983) developed a Habitat Suitability Index for native littleneck clams. They concluded that littleneck clams prefer a mixed substrate of gravel, sand and mud and that this species burrows to approximately 15 cm. Rodnick and Li (1983) considered tidal elevation an important endpoint and cited Nickerson's (1977) observation that native littleneck recruited in greatest numbers at tidal heights between –1.4' and +1.4' Mean Lower Low Water (MLLW) in Galena Bay, Prince William Sound. This observation is consistent with that of Amos (1966) and Paul *et al.* (1976) who observed maximum clam densities near the 0.0' MLLW tide level.

Rodnick and Li (1983) noted that thermal stress causes death in native littleneck clams at a few degrees below 0^{o}C and above 35^{o}C. Rutz (1994) reported the absence of clams below a freshwater runoff stream in Kosciusko Bay, Southeast Alaska. Brooks (unpublished) has also observed a paucity of native littleneck clams in Puget Sound near small streams. However, the largest commercial harvester of littleneck clams in Washington State (Mr. Reed Gunstone, personal communication) noted that littleneck clams are sometimes found in areas subjected to lowered salinities. He added that their short shelf life following commercial harvest during periods of high freshwater runoff suggests significant stress at reduced salinity. These observations are consistent with those of Quayle and Newkirk (1989) who noted that growth in native littleneck clams is optimum at salinities between 20 and 30 o/oo and that they can tolerate salinities as low as 10 to 12 o/oo for periods up to one month.

Goodwin (1973) observed higher hardshell clam (including native littleneck clams) densities in areas with high maximum current speeds (optimum between 77.1 and 154.3 cm/sec). His data are summarized in Table (1)

Table 1. Relationship between current speed and the biomass of hardshell clams observed in Puget Sound, Washington by Goodwin (1973).

Current Speed (cm/sec)	g/m^2 (butter clams)	G/m^2 (littleneck clams)
0.0 to 25.3	808	252
25.3 to 50.7	671	145
50.7 to 101.3	710	353
> 101.3	1580	646

1.2. Marking clams and other bivalves. Numerous methods are available for marking clams and other bivalves with valve lengths greater than ca. 1.5 to 2.0 cm. Marking techniques for aquatic species have been reviewed by Rounsefell (1963) and Mottet (1980).

- **Etching of valves with marks or numbers** (Brooks 1991) used a tungsten carbide tipped etching tool to inscribe numbers into the valves of mussels *Mytilus edulis galloprovincialis* and *Mytilus edulis trossulus* having valve lengths greater than 3.0 cm. This provided an individual mark that lasted for at least three years. Trowbridge *et al.* (1996) notched the margin of native littleneck clams with a valve length of between 1.5 and 3.5 cm and Peterson and Quammen (1982) marked ca. 2.5 cm native littleneck clams by etching the valves' surfaces.

- **Gluing plastic tags on the exterior of valves.** Brooks (1991) marked mussels with 3/16" diameter plastic tags, cut from microscope slide boxes with a paper punch and fixed to the valves with epoxy glue (West System™). These tags lasted for over one year in field growout experiments.

- **Vital stains and paints.** The preceding techniques are not considered appropriate for marking small bivalve seed < 15 mm valve length because of the stress involved and fragility of their valves (Trowbridge *et al.* 1996, Mottet 1980). The most common method for marking juvenile bivalves is staining with a vital stain such as neutral red (Loosanoff and Davis, 1947), alizarin red (Hidu and Hanks, 1968) or by spray painting (Glock and Chew, 1979). Vital stains may be identifiable for several weeks (Rounsefell, 1963) and fluorescent spray paints for up to 15 months. However, all of these marking techniques tend to become eroded and indistinguishable over longer periods.

- **Morphological characteristics of hatchery reared bivalves.** Mottet (1980) noted that hatchery reared seed can frequently be differentiated from natural seed by examining the "early shell". In this instance, seed produced in the Qutekcak hatchery and nursery system displayed a polished appearance prior to outplanting (Figure 2a). In general, the relatively large polished early shell remained a visible mark during much of the study (Figure 2b) – especially when compared with wild clams (Figure 2c). Because these studies started with very small seed and lasted for four years, no effort was made to mark the hatchery seed. It was considered unlikely that paints or dyes would last four years and the seed was too small to mark by etching or affixing tags. In addition, no evidence of natural native littleneck clam recruitment (newly recruited juveniles, living native littleneck clams, or native littleneck clam shells) was observed at the Port Graham study beach in Murphy's Slough and the growth data was not confounded by natural recruitment. The hatchery trait illustrated in Figure (2a) was helpful, but it did not produce an unequivocal mark for identifying hatchery seed. Naturally recruited clams in this study showed a range of early shell morphologies – likely associated with the season of spawning. Seed spawned early in the growing season possibly produced a larger early polished shell, while those spawned late in the season produced the smaller unsculptured early shell illustrated in Figure (2c).

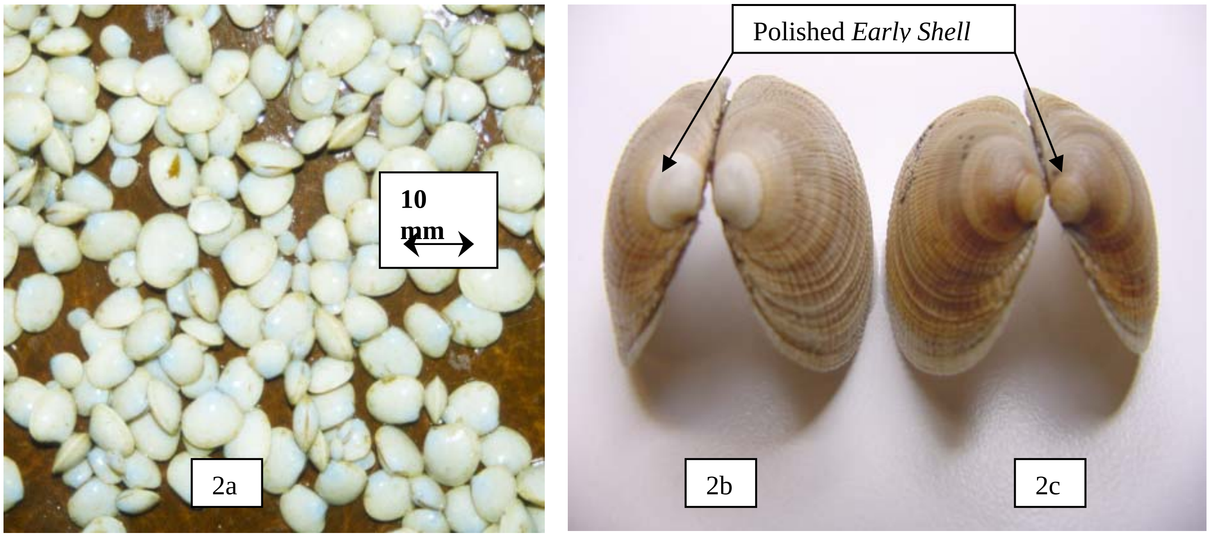


Figure 2a. Hatchery produced native littleneck clam seed ready for planting; 2b. Four-year-old native littleneck clams still showing the polished appearance of the early shell; 2c. Wild native littleneck clam from Tatitlek.

1.3. Aging of bivalves. There is a rich literature describing the aging of numerous bivalve species using incremental changes in shell growth. Shell growth in marine bivalves is greatest during the spring and summer in the presence of elevated temperatures and food supplies. Feder and Paul (1973) estimated the age of native littleneck clams by counting prominent discontinuities in the circular valve sculpture. Valve sculpturing associated with growth results from any physiological stress, including unusually low tides, reproductive activity, unsuccessful predation, disease, etc. However, Feder *et al.* (1976) consider annular shell morphology adequately reliable for aging most Prince William Sound clams because of high seasonality of growth on intertidal beaches, which are subject to freezing during low tides in January and February. The greater the seasonal variation in these primary factors, the greater the differences in shell growth will be (Quayle and Bourne 1972). Latitude has a significant effect on both temperature and the length of the growing season. For instance, Harrington (1986) demonstrated that growth rates and the lifespan of *Protothaca sp.* were strongly influenced by temperature and therefore by latitude along the Pacific coast of North America. Of particular importance, he noted that littleneck clams from southern extremes of their range (southern California to Baja) demonstrated rapid initial growth followed by significant decelerations in growth rates (as measured by the width of individual annuli). In contrast, *Protothaca sp.* from the northern portions of their range (Prince William Sound) grew more slowly and at a more constant rate.

Other stresses such as spawning, emersion during low tides, lowered salinity, handling, and storms can also influence shell growth, albeit on a microscopic scale (Crabtree *et al.*, 1980). The analysis of diurnal and seasonal patterns in bivalves shells has been explored in depth by archaeologists. Microscopic examination of daily growth lines in *Mercenaria mercenaria* has shown annual changes in increment line thickness associated with slow winter growth and 14 day cycles of thick and thin daily increments associated with tides (Pannella and MacClintock, 1968).

Era (1985) demonstrated that stressful salinities of 12 and 19.5 o/oo reduced daily incremental growth in *Protothaca staminea* to the same degree, as did emersion during semi-diurnal tidal cycles.

Ropes (1884, 1985) described procedures for aging surf clams (*Spisula solidissima*) and Feder *et al.* (1976) aged *Spisula polynyma* in Prince William Sound by identifying winter annuli recorded in the valves. Paul and Feder (1976), Paul *et al.* (1976), Trowbridge *et al.* (1996), Weymouth *et al.* (1931) and Bechtol and Gustafson (1998) described the aging of *Protothaca staminea, Mya arenaria* and *Siliqua patula* in Prince William Sound by counting winter annuli. Paul *et al.* (1976) determined the age of butter clams (*Saxidomus giganteus*) in Prince William Sound using the same techniques. For purposes of the current study, Ham and Irvine (1975) provided a detailed evaluation of various methods for determining daily, seasonal and annual growth increments in native littleneck clams, butter clams (*Saxidomus giganteus*) and Nuttall's cockles (*Clinocardium nuttallii*) from British Columbia.

Despite the well-understood theory of the relationship between bivalve shell growth and the environment, interpretation of the sometimes-complex patterns is equivocal and requires experience. This is particularly true for older individuals because of umbonal erosion and the closer spacing of annuli at ages greater than five to six years (Ropes and Jearld, 1987). Alexander *et al.* (1993) found that shell morphology in the native littleneck clam is habitat dependent – specifically that concentric lamellae are pronounced on individuals living in coarse-grained sediments and less pronounced in individuals from fine-grained sediments along the Pacific Northwest coast. Hughes and Clausen (1980) and Peterson and Ambrose (1985) noted that increments in bivalve shells result from 1) size and age differences, 2) microhabitat differences, 3) migrational behavior and 4) genetic variability. These authors advised caution in interpreting bivalve growth from an analysis of shell structure.

Trowbridge *et al.* (1996) investigated growth recorded in the valves of *Protothaca staminea* in Prince William Sound. The *Executive Summary* in Trowbridge *et al.* (1996) contains contradictory statements regarding the comparative accuracy of sectioning valves or counting external checks. At page xiv, the summary states, "Ages of littleneck clams using the external surface method were younger than those estimated from the sectioned valve method." However, the body of the report and the author's conclusions clearly state that the external method is more accurate and that <u>the sectioning method tends to underestimate the age of native littleneck clams</u>. Trowbridge *et al.* (1996) made several points worth reiterating here:

- Annular interruptions in shell growth appeared as deep notches in the outer shell layer, with the interruption extending through the middle shell layer of the valve. The interruptions in incremental growth were typically wide.

- Some individual shells present confusing patterns and should be discarded for purposes of determining age at length.

- The possibly long protracted spawning season results in significant differences in the first years growth.

- They recorded significantly faster growth in 1990 compared with 1991, suggesting that environmental factors important to shellfish growth may vary significantly from year to year.

- They concluded that the sectioned valve method under-estimated the age of littleneck clams and that the external surface aging method was more accurate.

1.4. Length at age for native littleneck clams in Alaska. Feder and Paul (1973) estimated that it required 8 to 10 years for native littleneck clams to reach a valve length of 30 mm throughout Prince William Sound. Nickerson (1977) estimated that *Protothaca staminea* recruited into a harvestable class size (≥ 38 mm valve length) at an average age of 7.5 years in Prince William Sound, while the butter clam (*Saxidomus giganteus*) required only 5.5 years to reach the same valve length. Rutz (1994) estimated the mean age of recruitment into the class having ≥ 38 mm valve length at between 10 and >12 years in Kosciusko Bay, Southeast Alaska. His data suggested that approximately 2% of the littleneck clams reached 38 mm in 7 to 9 years. Bechtol and Gustafson (1998) examined littleneck clam growth at Chugachik Island in Cook Inlet, Alaska and estimated that 0.4% of the clams attained a valve length of 38 mm at age 5. In their study of natural populations, 83.4% of the native littleneck clams reached harvest size of 38 mm at ages of 7 to 8 years. Most recently, Figure (21) in the Trowbridge *et al.* (1996) report suggested a maximum valve length of 36 to 37 mm in native littleneck clams that were ≥ 9 years old. These reports are summarized in Table (2).

Table 2. Reported age of native littleneck clams (*Protothaca staminea*) at which they recruit to a legal harvest size of 38 mm in Prince William Sound, Alaska.

Author	**Mean age to reach 38 mm valve length**
Feder and Paul (1973)	8 to 10 years
Nickerson (1977)	7.5 years
Rutz (1994)	10 to >12 years
Bechtol and Gustafson (1998)	5 to 8 years
Trowbridge *et al.* (1996)	≥ 9 years

The present study was not designed to examine the efficacy of various methods for aging clams. However, it does provide a unique opportunity to examine this issue using clams of known age. This statement is considered unequivocal for the Murphy's Slough site because native littleneck clams or remnant shells of this species were not observed within at least one kilometer of the beach during the baseline survey and no evidence of natural native littleneck clam recruitment was observed at any time during this study.

1.5. Bivalve predators. Sea otters (*Enhydra lutris*) are well-recognized predators on crab, sea urchins and bivalve mollusks, including *Saxidomus giganteus* and *Protothaca staminea* (Kvitek and Oliver 1992; Kvitek *et al.* 1993; Doroff and DeGange 1994). *Saxidomus giganteus* was reported as the most frequent otter prey item (Kvitek and Oliver 1992; Kvitek *et al.* 1993; Doroff and DeGange (1994). Recent sea otter predation is evidenced by excavations in the substrate and broken bivalve shells. No reports describing interaction between sea otters and intensive or extensive aquaculture were identified in the literature.

Other predators include crabs (Pearson *et al.* 1981; Pearson *et al.* 1981), white-winged scoters (Sanger and Jones 1992), fish (Peterson and Quammen, 1982) and gastropods – particularly in the family Naticidae (Kent 1981; Peitso *et al.* 1994; Quayle and Newkirk 1989).

Starfish, particularly *Pycnopodia helianthoides* and *Evasterias troschellii* prey on littleneck clams (Toba *et al.* 1992). All of these predators are reported to take small and large littleneck clams up to their maximum size. Pearson *et al.* (1979) determined that Dungeness crabs can locate buried native littleneck clams by detecting clam extracts in the water. Boulding and Hay (1984) observed that predation by *Cancer productus* on *Protothaca staminea* increased with increasing clam density. This may have implications for the intensive culture of native littleneck clams in areas where crab predation is a problem. Both *Cancer productus* and *Cancer magister* are capable of tearing through light plastic netting used to protect clams from large gastropods and starfish.

1.6. Bivalve culture. Native littleneck clams have not previously been used for intensive commercial culture or for subsistence enhancement in the Pacific Northwest because hatchery reared seed has not been available. However, numerous publications discuss the intensive and extensive cultivation of Manila clams in the Pacific Northwest (Quayle and Newkirk, 1989; Toba *et al.* 1992; Mottet 1980; Magoon and Vining 1981).

Successful enhancement begins with good site selection. Toba *et al.* (1992) discuss several factors important for extensive or intensive clam culture. The following parameters were discussed with village elders during the study site selection process:

- Sufficient area at an appropriate tide level (-1.5 to + 2.5’ MLLW for native littleneck clams);

- Appropriate substrate composition containing a mixture of gravel, sand, ground shell and mud with enough organic matter (> ca. 1% TVS) to bind the sediments;

- Exposure. Sediments become unstable and may move excessively when exposed to high wind and wave conditions. The fine sediment that holds gravel and sand together washes away, leaving a loose matrix of gravel and sand. As the beach shifts, small clams are either washed out of the substrate or buried under new accumulations. Clam cultivation in high-energy sites requires some form of intervention to stabilize the substrate.

- Log damage. The potential for storm damage and catastrophic loss must be assessed. This is particularly important for intensive cultures where the investment in time and money can be high. Knowledge gained from local elders was considered invaluable in choosing enhancement sites. An understanding of storm tracks, fetch, upland vegetation, the presence of logs, debris, and beach slope and composition can be used in assessing this factor. Intensive cultures should not be placed in areas subject to excessive log damage.

- Oxygen availability in sediments. Native littleneck clams survive in anaerobic sediments. However, in optimum conditions, the depth of the redox potential discontinuity (RPD) should be at least 2 cm and preferably greater than seven to ten centimeters. A deep RPD suggests adequate pore water movement, which is desired during low tides, particularly during winter to reduce the potential for freezing.

- Temperature. Beach substrates can freeze during nighttime winter low tides in the Pacific Northwest (Bower, *et al.* 1986) causing significant mortality. This suggests that Alaskan clam culture should not be attempted high intertidal elevations – particularly in the winter.

- Salinity. Areas heavily influenced by freshwater should be avoided for two reasons. First, native littleneck clams do not thrive in areas subject to prolonged periods with salinities less than 20 o/oo and second, streams tend to meander across intertidal areas. As the streams meander, they create new channels that wash away shallow infauna, including clams.

- Primary production. Native littleneck clams feed primarily on living phytoplankton and detritus that is part of the seston. The intensity and extent of enhancement projects must consider the availability of food. This may be particularly important in Alaska where primary productivity is limited by short summer growing seasons. Brooks (2000c) has brought together the literature necessary to determine carrying capacities for coastal embayments. The methodologies are not restricted to specific environments and could be applied in Alaska for estimating bivalve carrying capacity in small to medium size embayments.

- Longshore currents. Goodwin (1973) observed increased clam biomass in areas with strong currents. These currents bring food over the shellfish bed. However, as pointed out by Toba *et al.* (1992) and Nosho and Chew (1972), strong longshore currents can also redistribute clam seed, significantly reducing their density.

- Predation. Areas where predators congregate, particularly scoter ducks, should be avoided. As previously noted, the potential interaction between sea otters and intensive clam culture has not been investigated.

- Water Quality. The water quality of areas near human habitation should be carefully evaluated prior to enhancing shellfish stocks. Leaking septic systems and industrial pollution can contaminate shellfish making them unfit for human consumption. Growing area certification in accordance with the National Shellfish Sanitation Program Part I (NSSP, 1995) should be accomplished during initial culture trials and an *Approved Harvest Classification* determined prior to undertaking any significant enhancement effort.

- Paralytic shellfish poisoning (PSP). Neurotoxins synthesized by some dynoflagellates, like *Alexandrium catanella*, are concentrated in the tissues of bivalves, particularly butter clams. Intensive shellfish enhancement should not be undertaken in areas where blooms of toxic phytoplankton have been frequently observed. In addition, areas from which shellfish are harvested for human consumption should be frequently tested for PSP. Kvitek *et al.* (1993) hypothesized that high concentrations of brevetoxins in butter clams may exclude sea otters from some areas of Southeast Alaska.

- Human resources available to tend intensive shellfish cultures should be determined. Some techniques require a significant investment in time and energy. These techniques should be reserved for easily accessible beaches of optimum substrate composition. In addition, different villages may partition their time differently. In some, the intensive culture of shellfish may be a rewarding and appropriate activity. In others, village members may have outside jobs with little time to devote to caring for intensive shellfish cultures. Enhancement methods must recognize village needs and desires - they must "fit" with the village's lifestyle. Recommendation of specific enhancement techniques should only follow a careful determination of the villages needs and desires.

- Assessment of natural recruitment. Natural recruitment depends on many factors as discussed by Mottet (1980). Native littleneck clams can be absent for a number of reasons including failure to recruit new cohorts because of local hydrodynamics. Predation on new recruits and beach instability can chronically reduce or eliminate young clams from an area. The point is that the absence of clams does not mean that a beach is unsuitable for cultivating native littleneck clams. However, artificial seeding is expensive and an assessment of clam recruitment should be undertaken irrespective of the presence of adults. This can only be accomplished by sieving sediments on small (1 mm) sieves and examining the retained material under a microscope or magnifying glass. All clams retained on 1.0 mm screens should be accounted for in surveys. Alternatively, some areas may have excellent growth but they may not sustain harvests because of limited or sporadic recruitment. The frequency of successful recruitment can be assessed by evaluating age frequency histograms. However, this requires that the clams be carefully aged and valve lengths measured.

1.7. Clam culture techniques. Manila clam culture techniques used in the Pacific Northwest are reviewed in depth by Toba *et al.* (1992), Mottet (1980) and Magoon and Vining (1981). Taylor (1989) provides interesting insight into growout techniques used by commercial clam producers in the Pacific Northwest. The following increasingly intensive culture methods are commonly used for Manila clams in the Pacific Northwest.

1.7.1. Predator control. Where natural recruitment is sufficient, beaches can be enhanced by simple predator control measures such as trapping crabs and picking or trapping starfish and predatory gastropods (Quayle and Newkirk 1989).

1.7.2. Supplemental seeding. Supplemental seed can be added to beaches holding clams, but where recruitment is either too low or sporadic to sustain desired harvest levels.

1.7.3. Substrate modification. Beaches not meeting the physicochemical attributes described in Section 1.5 can still be used for shellfish culture. However, they often require modification and/or protection in order to warrant the expense of planting clams. Substrates that are too soft and muddy to support optimal clam growth can be modified by the addition of gravel and/or crushed shell (Toba *et al.* 1992).

1.7.4. Plastic netting described in Figure 3a excludes many predators and can help stabilize substrates on beaches subject to excessive sediment movement. Netting does not exclude all predators. For instance, some gastropods can burrow under the nets and numerous predators can recruit through the mesh at a young age and prey on small clams. Miller (1982) and Anderson *et al.* (1982) have reported the effectiveness of lightweight plastic netting for improving survival of Manila clams. For instance, at the end of two years, Anderson *et al.* (1982) reported 57 percent survival under ¼” x ½” netting compared with only 1% survival for unprotected Manila clams seeded at three to four mm valve length in Filucy Bay, Washington. Similar increases in survival were observed at three other test sites. Very low survival (4 to 6%) was reported at two sites regardless the protection. Toba recommended ¼” mesh for small seed averaging 3 to 4 mm valve length and ½” mesh for planting 6 to 8 mm seed. Netting typically comes in 17-foot wide rolls. The rolls are cut into 100’ lengths for ease of handling. Netting can be secured by burrying the edges approximately 6” deep around the perimeter or by sewing a leadline around the perimeter and stapling the leadline to the substrate using rebar bent in a “J” shape.

1.7.5. The use of plastic clam bags is described in (Figure 3b). Rogers (1989) and Toba *et al* (1992) discuss the culture of Manila clams in plastic cages. These cages are available in several sizes with different mesh openings designed for different stages of culture. In protected environments, the cages can simply be set into the substrate as shown in Figure (3b). In exposed environments the cages are attached to polypropylene lines running down the rows using electrical ties or to ½" steel rebar. Tying the cages together in this fashion helps to stabilize the culture reducing the potential for loss of individual cages and reducing the degree of sediment movement within the culture area. Toba *et al.* (1992) reported clam survival of 51 to 79 percent during a 17-month growout in Puget Sound. The bags measured 32" x 18" x 4" deep. Survival was not a function of density at between 300 and 1,500 clams per bag (75 to 375 clams/square foot). However, clam growth was highest at the lowest density (13.1 grams/clam) and decreased linearly as density increased to 6.8 grams/clam at 1,500 clams/bag. Toba *et al.* (1992) recommend a density of 500 – 700 Manila clams/bag, equivalent to 125 to 175 clams/sf.

3a

3b

Figure 3a) One-half inch square plastic netting being used to protect a goeduck (*Panopea abrupta*) culture and 3b) Manila clams being cultured in plastic cages. Both cultures are in Thorndyke Bay, Washington State.

1.8. Commercial clam harvest management in Alaska. The Alaska Department of Fish and Game (ADFG, 1995) conducted clam surveys for native littleneck clams (*Protothaca staminea*) in Kachemak Bay in the Southern District of the Cook Inlet Management Area. The purpose of this study was to examine the affects of commercial harvests from Department of Environmental Conservation certified beaches. This ADFG study did not examine small clams (< ≈ 15 mm) in the 1992 - 1994 surveys. Therefore, ratios of sublegal to legal size clams were skewed toward the legal clams. They observed clams from age three to age 14 and found that minimum legal size (38 mm valve length) was achieved in *Protothaca staminea* between the ages of 5 and 10 years. They concluded that growth was variable and slow.

In addition, ADFG (1995) concluded that recruitment was sporadic and that native littleneck clam populations were characterized by generally low to moderate recruitment with periodically strong year classes. The study did not examine intersite length-frequency or age-frequency distributions to determine if strong year classes occurred during the same years on all beaches in Kachemak Bay, suggesting that strong recruitment was a function of generally favorable environmental conditions - or if strong year classes were present on only a few beaches in any one year - suggesting that variable wind and current patterns, or other stochastic processes, may concentrate shellfish larvae at different beaches in different years. ADFG (1995) did find significant quantities of shellfish on all beaches in Kachemak Bay and their estimates of the number of legal and sublegal (>15 mm) size clams per square meter are provided in Table (3).

Table 3. Numbers per square meter of legal (≥38 mm valve length) and sublegal (<38 mm valve length) clams (*Protothaca staminea*) observed on five beaches in Kachemak Bay by the Alaska Department of Fish and Game in 1994.

Beach (year)	# legal size clams	# sub-legal size clams
Chugachik (1994)	36.4	42.8
Jakolof Bay East (1993)	19.0	1.3
Jakolof Bay West (1993)	17.9	10.5
Tutka (1993)	13.6	4.8
Halibut Cove (1994)	77.5	96.5
Sadie Cove (1993)	27.6	35.2

Other findings of interest in the ADFG (1995) report include the following:

- *Protothaca staminea* were generally found buried in sediment to depths of 25 to 31 cm. However, clams were found at unspecified depths greater than this.
- The biomass of clams at the most heavily harvested beaches (Chugachik and Jakolof) was slowly declining.
- Clam growth was highly variable and clams reached minimum harvest size (≥ 38 mm) at between 5 and 10 years of age.

ADFG (1995) examined several years of data at sampled beaches and compared changes in available biomass of legal size clams with department harvest records. The results are summarized in Table (4). This information suggests that, while beach response to harvest is variable, the beaches examined in their study could not sustain harvests greater than perhaps 10 to 15% per year. This seems reasonable when the median age to recruitment into the legal size population averaged 7.5 years. The ADFG (1995) data suggests that an adequate management plan will be essential to the development of a sustainable subsistence shellfish resource anywhere in Alaska.

Table 4. Changes observed in ADFG estimates of the biomass (reported in pounds) of legal size clams found on five beaches in Kachemak Bay between 1990 and 1994.

Beach	Year (biomass)	Year (biomass)	Percent Harvest	% Biomass Change
Chugachik	1992 (249,929)	1994 (131,485)	10.8% (‘92); 20.5% (‘94)	-47.4%
Jakolof	1992 (110,025)	1993 (108,227)	16.9% (’92); 12.0% (’93)	-1.6%
Sadie Cove	1993 (95,506)	1994 (135,467)	none reported	+41.8%

1.9. Environmental effects associated with bivalve culture. The intensive culture of any animal brings with it environmental changes. Brooks (1993, 1995) and Dumbauld *et al.* (2001, In press) documented a more diverse and abundant invertebrate community in cultivated Pacific oyster beds than was found in adjacent eelgrass meadows that had been displaced by oyster culture. Brooks (2000a, 2000b and 2000c) has documented the environmental response to salmon aquaculture and the raft culture of mussels. Organic loading from intensive aquaculture can exceed the assimilative capacity of local sediments causing reduced oxygen tension and increased concentrations of total sediment sulfide, causing significant changes in the infaunal and epifaunal community. However, as shown by Brooks (2000a), these effects are generally ephemeral and invertebrate communities return to normal within a period of weeks to perhaps two years during fallow periods. Newman and Cooke (1998) discussed the environmental response to the addition of gravel and/or crushed shell to fine substrates to improve the potential for littleneck clam and/or oyster cultivation in the Pacific Northwest.

Kaiser *et al.* (1996) studied the environmental response to intertidal Manila clam culture under plastic netting in England. They found that infaunal abundance was greater within the netted culture than at reference sites. A similar number of species (20-22) was observed in all areas. Harvesting of the clams by suction dredge resulted in a significant reduction of infauna. However, seven months later, no differences between the cultured plots and reference areas were found. Kaiser *et al.* (1996) did not observe statistically significant ($\alpha = 0.05$) differences in total volatile solids (TVS), percent silt/clay or photosynthetic pigments (chlorophyll α) in sediments collected under netted cultures and when compared with those from reference areas.

In follow-up studies, Spencer *et al.* (1996) compared physicochemical and biological response in netted plots with and without clams and unnetted control areas. They observed a significant, but small increase in organic content from 2.42% to 3.37% on netted plots when compared with unnetted controls. They also observed a four fold increase in the accumulation of new sediments under the netted plots when compared with the controls. The green algae *Enteromorpha sp.* settled on the nets resulting in an increase in the number of littorine snails. Deposit feeding polychaetes like *Ampharete acutifrons* and *Pygospio elegans* dominated the netted areas. In general, the authors concluded that the netting increased both the sedimentation rate and productivity of the cultivated areas. At the end of the 30-month growout cycle, Spencer *et al.* (1997) observed that increased sedimentation had elevated the beach profile by 10 cm under the netting. Clam survival was poor (500 clams/m^2 seeded and an average of only 26 clams/m^2 harvested or 5.2% survival). At the end of the culture cycle, 236 times as many herbivorous snails (*Littorina littorea*) were observed on the netted plots when compared with the controls. The number of species was significantly higher on the netted clam ground when compared with the controls (8:5) and total abundance was nearly three times higher within the clam culture than at controls (31.9:11.2/0.018 m^2 quadrat). Shannon's and Simpson's indices were also higher in the cultured plots when compared with the controls. At the end of the culture period, Spencer *et al.* (1997) concluded that the observed biological responses indicated that organic enrichment occurred within the net-covered areas. The degree of enrichment did not exceed the assimilative capacity of the sediments and the abundance of infaunal and epifaunal increased in cultured areas.

Spencer *et al.* (1998) continued their study by examining the biological and physicochemical response to suction dredge harvesting of the netted plots. They found that suction dredging significantly reduced both the abundance and diversity of infauna. However, the harvested area remediated quickly and no differences between the cultivated and control plots were observed 12 months after harvesting. Similar effects were reported for cage culture of Manila clams in the citations provided by Spencer *et al.* (1997). This review suggests that the intensive culture of bivalves under netting (or in cages) may result in the following effects:

- Increased sedimentation rates – particularly silt and clay;
- Increased organic content in sediments;
- Increases in the abundance of some infauna – particularly deposit feeding annelids;

- Increases in the number of taxa;
- Decreases in all of the metrics following removal of the nets and harvesting of the clams;
- A return to reference physicochemical and biological conditions within a relatively short period of weeks to perhaps a year.

1.10. Background summary. The review provided herein discusses only the growout phase of clam production. Hatchery and nursery production will be discussed in other sections of the CRRC report. In the Pacific Northwest, native littleneck clams prefer intertidal environments with mixed substrates containing gravel, sand and mud. They prefer salinities greater than 20 o/oo but can survive lower salinity for periods of up to a month. Their survival and growth depends on temperature, food availability, substrate stability, and predator avoidance. Crabs, gastropods, ducks, sea otters and fish all prey on native littleneck clams. Native littleneck clam abundance depends on larval recruitment and the foregoing environmental constraints. Some of these constraints, like substrate composition and stability, recruitment of juveniles and predator control, can be artificially ameliorated. Other constraints, such as hydrodynamics and food availability are beyond the control of humans and become critical aspects of site selection and management planning.

Bivalve cultivation in the Pacific Northwest is a mature industry with well-developed practices for the hatchery production, nursery, and growout of Pacific oysters, Manila clams and goeducks. These technologies, developed over the last 30 years, have enabled shellfish growers in British Columbia, Washington State and Oregon to meet the ever-increasing public demand for bivalve mollusks. Similar technologies have not been developed for native littleneck clams because they grow more slowly, do not open as reliably on steaming, and have a shorter shelflife. However, the similarities in habitat needs between Manila clams and native littleneck clams suggests that culture techniques developed for the former may also prove useful in enhancing subsistence harvests at native villages in Alaska.

1.11. Purpose of this study. The purpose of this part of the CRRC enhancement effort was to evaluate possible growout methods for native littleneck clams near native villages in South Central Alaska. It must be emphasized that the purpose of this project was not to conduct a rigorous scientific study. One week of supervised fieldwork during a single low tide series was scheduled each year between 1995 and 1999. This fieldwork was designed to establish growout studies and to train village shellfish teams to maintain the cultures and collect the necessary quarterly data. The project began in 1995 by interviewing elders at Tatitlek, Nanwalek and Port Graham to identify traditional subsistence harvest beaches appropriate for study and to gain an understanding of the village's desires. Bivalve inventories were also accomplished in 1995 at each of these villages to assess existing subsistence resources and to evaluate nominated beaches for enhancement potential.

Based on input from village elders, three general enhancement techniques were investigated using the small quantity of native littleneck clam seed available from the Qutekcak hatchery in 1996. This growout study evaluated the survival and growth of clams in bags, under plastic netting, and seeded without protection into cultivated substrates. Clams were planted at varying densities in 1998 at Murphy's Slough to evaluate density effects on growth and mortality.

The study design invoked for this project was limited by the available field resources and the small quantities of seed available from the Qutekcak hatchery during their start-up phase, which occurred in parallel with these growout studies. The protocols were designed to provide baseline information and statistically testable data relevant to the following questions and/or hypotheses.

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?

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?

Question (3) What length of time is required for native littleneck clams to reach a minimum valve length of 38 mm at Tatitlek, Nanwalek or Port Graham.

Question (4) Did observed lengths at ages one through four correspond to predictions made by the von Bertalanffy model? Regression coefficients for the von Bertalanffy model were developed from data collected during the 1995 bivalve inventories.

Question (5) Did the number of apparent annuli observed in native littleneck clams at Murphy's slough correspond with the known age of these clams? Clams in bags were of known age at Port Graham because there was no evidence of recruitment or of a pre-existing population of native littleneck clams near the study site.

Question (6) Was there excessive winter mortality in clam populations physically constrained to remain within a few centimeters of the sediment surface in bags? This question is of particular interest in Alaska where air temperature can drop to less than zero degrees centigrade for extended periods during winter and where surficial sediments may freeze.

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 no disturbance?

Hypothesis (2) Was clam survival significantly enhanced when the cultures were protected by plastic netting compared with similar seeding in unprotected areas? This question is important because the protection of seeded clams requires additional expense – both in materials and in labor to install and maintain the integrity of the plastic netting. If clams survive sufficiently well in unprotected cultures, then the need for plastic netting might be eliminated.

Hypothesis (3) Did statistically significant changes occur in the percent fines (silt and clay < 63 μm diameter) and/or in the proportion total volatile solids (TVS) observed in sediments under plastic netting when compared with areas seeded, but not protected? Significant increases in these two physicochemical parameters would require that areas with marginally high levels of either parameter, with respect to the environmental needs of *Protothaca staminea,* be given special consideration when designing future enhancement efforts. The enhancement beaches selected for this study provided a range of sediment physicochemical conditions ranging from relatively fine, high TVS sediments in Murphy's Slough to the highly exposed and rocky beach at Passage Island.

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?

Details of each year's results are provided in Brooks (1995, 1997, 1998 and 1999). This final report summarizes the findings and addresses the questions and hypotheses posed above.

2.0. Materials, methods and results for the bivalve inventories conducted in 1995 and 1996 at Port Graham, Nanwalek, Tatitlek, Chenega and Ouzinke. Upon arrival at each village, goals and desires were discussed with tribal elders and/or members familiar with shellfish harvesting. Specific questions and information included the following:

1. Reasons for choosing the sites to be sampled;
2. Traditional village use of shellfish and sources of supply;
3. Accessibility of each site for tending of intensively cultured shellfish resources;
4. Resources (Villager time, boats, etc.) available to the project;
5. Review recent shellfish harvests at the beach to be surveyed;
6. Village understanding of the current condition of local shellfish resources;
7. Village understanding of the reasons that shellfish are no longer abundant;
8. Availability of alternate beaches for survey;
9. Village preferences for mussels, cockles, native littleneck clams, butter clams, horse clams and soft-shell clams (*Mya truncata*);
10. Traditional predator control measures used by the village.

2.1. 1995-1996 bivalve inventory sampling design. The information discussed above was used to identify one or more beaches for evaluation near each village. A brief reconnaissance survey was conducted before the planned inventory to evaluate candidate beaches. A series of test digs were then undertaken to qualitatively evaluate substrate quality and existing or pre-existing shellfish resources by examining living clams and empty shells. The highest tide level at which clams were found was identified and the width of the area to be surveyed was determined and assessed for stratification by substrate type. This information formed the basis of a systematic random survey beginning at the highest elevation on the beach at which clams were found. This procedure was reversed at Passage Island because the crew arrived there at low tide. The number of transects and the number of samples per transect were determined based on the area of the beach, homogeneity of the substrate, and the time and human resources available for collecting samples during a single low tide.

The length and width of the productive area was measured using a 300' fiberglass tape. The length was divided by the number of transects plus one to obtain a transect interval. A random number between zero and the interval length was then selected and the first orthogonal transect placed at the random distance from the margin of the productive beach. Additional orthogonal transects were laid out at the specified intervals. Each transect was run at right angles (orthogonal) to the water line. The width of the beach was divided by the number of samples to be collected on each transect plus one to obtain a sample station interval. The first sample station was located at a random distance (between zero and the calculated sample interval) from the highest point on the beach at which clams were observed. Additional samples were taken at the specified interval. A single horizontal transect was also evaluated at Chenega, Ouzinke and Port Graham. These transects were evaluated at 0.0' MLLW where the orthogonal transects revealed the highest clam densities. For each sample station, red wire flags were labeled with the sample station designation and placed in the substrate at the appropriate point by the survey crew leader.

These flags followed each sample until sieving and picking of clams was completed at an upland station.

Individual samples were collected with the aid of 3/32" thick aluminum plate quadrats that covered 0.1 m^2 (Figure 4). The quadrats were pushed down into the substrate during excavation. This prevented sloughing of the sides and provided a precise sample area. Each sample was dug to a depth at which no additional clams were obtained. The ¼" screen is removable allowing the fixture to be used for either sampling or sieving the contents. In the current studies, most sediments were sieved on a 1 mm stainless steel screen to evaluate recruitment.

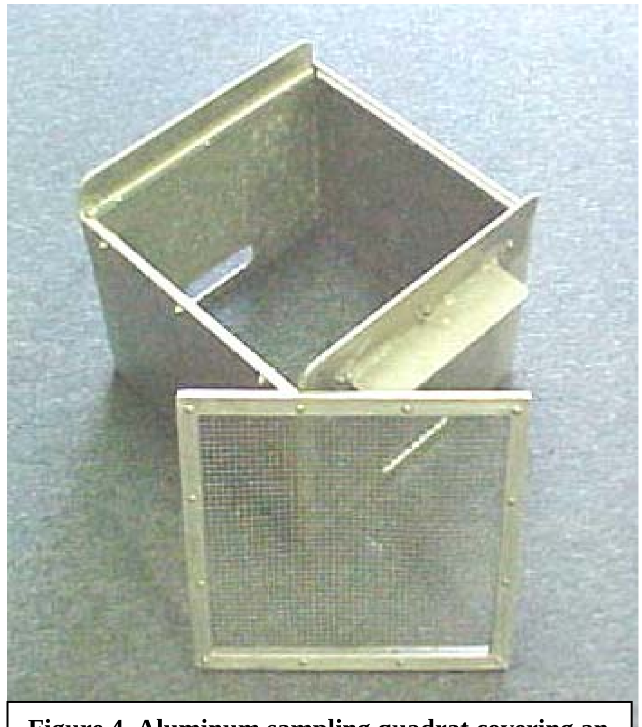


Figure 4. Aluminum sampling quadrat covering an area of 0.1 m^2 with a removable ¼" sieve

The beach slope was determined during each survey by placing a properly leveled Berger™ Model SAL-1 Automatic Level at the lowest point inundated at low tide. The elevation of each sample station was then determined relative to this reference point using an aluminum stadium. The height, above Mean Lower Low Water (MLLW), was calculated by assuming that the actual low tide equaled the predicted low tide. Small, but undetermined, errors in beach elevation might have been caused by differences between the actual and predicted low tide caused by winds and/or barometric pressure. In view of the benign weather experienced during these surveys, any errors were likely small.

2.2. Clam sample processing. A Write in the Rain™ label was placed in each sample bag with the substrate removed from the quadrat. The samples were then placed in boats for transport to a suitable upland sorting location. Sediment samples were sieved on 6.4 and 1.0 mm sieves and all clams and whole clamshells removed from each of these sieves and placed in pre-labeled, one gallon, ZIPLOCK™ bags. Where juvenile clams (< 6 mm valve length) were observed under a magnifying glass, the entire sample retained on the 1.0 mm sieve was retained for picking under a dissecting microscope. The free label placed in the bags during field sampling followed the sample into the ZIPLOCK™ bag. All samples were placed on blue ice in a cooler and shipped via overnight mail to Aquatic Environmental Sciences for processing.

2.3 Aging of bivalve shells. All clams in each sample were aged using the techniques described by Feder and Paul (1973) and Ham and Irving (1975), weighed, and their maximum valve length at each apparent annulus measured to the nearest 0.01 mm. Figure (5) provides photographs of the exterior shell surfaces and sections for a) Nuttall's cockle (*Clinocardium nuttallii*); b) butter clams (*Saxidomus giganteus*) and; c) native littleneck clams (*Protothaca staminea*). Presumptive annuli are identified in each photograph. The presumed annuli or *checks* appeared as deep notches in the prismatic layer following a general thickening of the entire shell.

Note the apparent doubled or paired dark annuli in the sectioned butter clam valve. These closely spaced checks were also apparent at many presumptive annuli in the sectioned valves of native littleneck clams of known age in this study. They appear characteristic of some annuli produced in butter clams and native littleneck clams from Alaska. The dark lines demarking annuli in sectioned valves appear to be extensions of the inner nacreous shell layer, which is continuously laid down by the mantle on the interior of bivalves, through the prismatic layer to the exterior of the valve. In some sectioned specimens, the prismatic layer was worn away, exposing only the harder nacreous layer. In these cases, the first and perhaps second annuli were not apparent in sections.

Funding was not provided for the sectioning of valves in this study and therefore only a limited number of bivalves (27) were sectioned. The results were generally consistent with the findings of Trowbridge *et al.* (1996).

- A few individuals in all three species showed evidence of double checks at one or more presumptive annuli. In some instances, these checks became very complex and consisted of a series of closely spaced dark extensions of the underlying lamellar structure through the white prismatic shell layer. These were most apparent in cockles (Figure 4a).

- Cockles were the most difficult valves to read because of what were apparently false checks on the exterior of the valves. This will be discussed in Section 5 of this report. The first four or five annuli in native littleneck and butter clams were more closely associated with discontinuities in sectioned material and few false checks were apparent.

- The valves of older native littleneck clams from Quzinke were badly eroded near the umboes. This made reading the first and second annulus very difficult because the exposed prismatic layer was nearly eroded away and it is in this layer that the annulus is observable in sectioned material. This is consistent with the findings of Trowbridge *et al.* (1996) who noted that the sectioning procedure tended to underestimate age when compared to counting presumptive annuli on the exterior of the valves.

- For purposes of this study, only data collected using the exterior valve checks was included in the database. Some specimens were discarded because their valves were either too worn for accurate interpretation or because the patterns were too difficult to interpret.

- Growth in valve length decreases with time in all of these species and the annuli laid down at older ages in butter and native littleneck clams were frequently too closely spaced to distinguish. Because of the difficulty in reading the older ages in most large butter clam valves, these were not included in the present database when computing regression coefficients for the von Bertalanffy equation.

It should be emphasized that bivalve aging techniques have not been verified in any of these species by comparing apparent annuli with clams of known ages from setting onward. In addition, the interpretation of annuli is equivocal and requires some training and skill on the part of the researcher – much as the reading of fish scales does. For those readers familiar with reading salmon scales, *crossovers* and *incomplete circuli* are characteristic of annuli in salmon scales. These same characteristics were observed at presumptive annuli in both butter and native littleneck clams from Alaska.

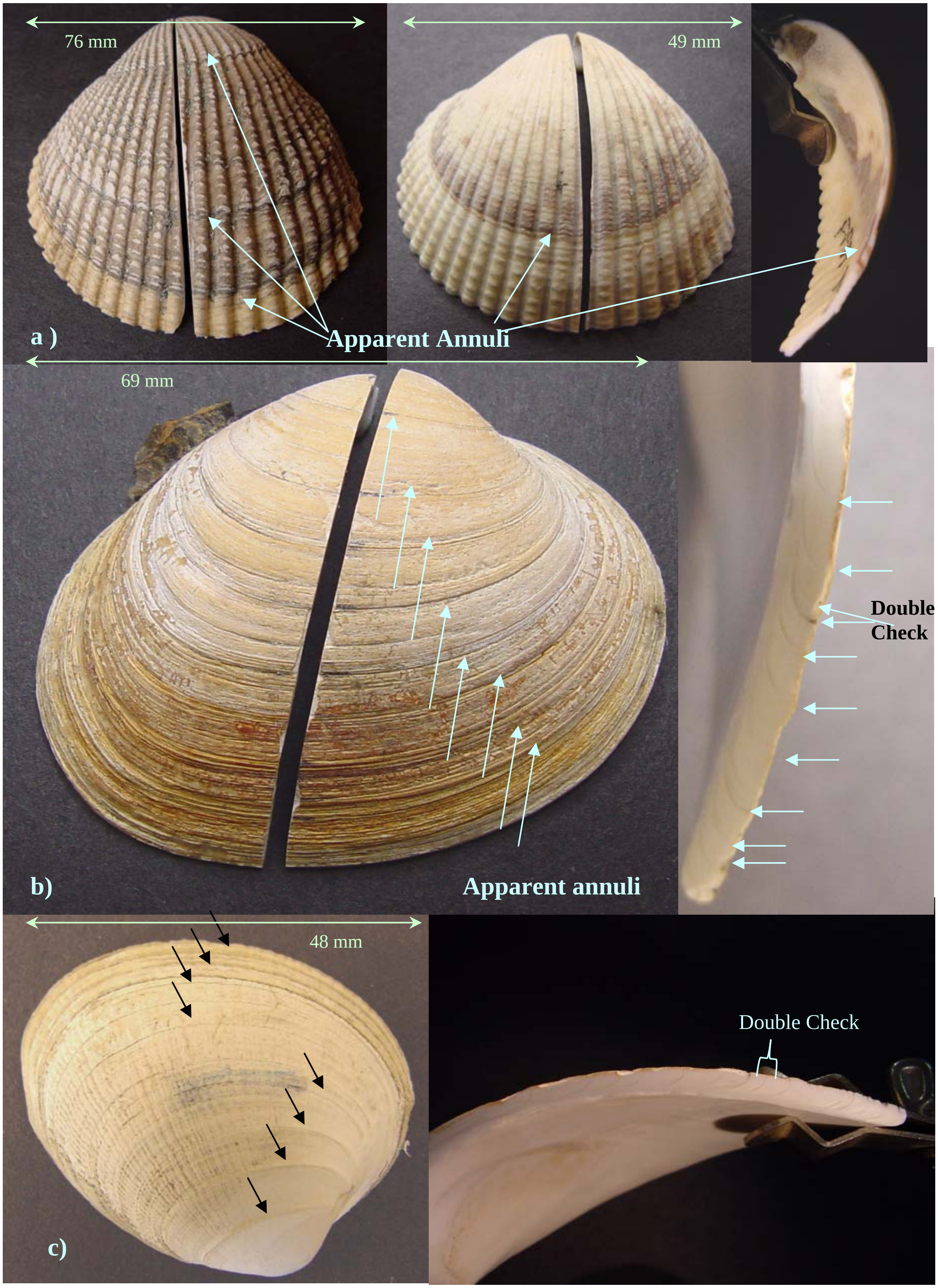


Figure 5. Typical valves of a) Nuttall's cockle (*Clinocardium nuttallii*), b) butter clams (*Saxidomus giganteus*) and c) native littleneck clams (*Protothaca staminea*).

2.4. Clam wet and dry tissue weight determinations. Wet tissues in clams with valve lengths greater than ca. 15 mm were shucked, blotted dry and weighed and then dried at 90 ^oC and reweighed. A dry tissue condition factor equal to 1000*Dry tissue weight)/Length$^{2.1}$ was then determined.

2.5. Substrate characterization. Four to twelve sediment samples were taken from randomly chosen sample stations at each beach surveyed. The depth of the Reduction Oxidation Potential Discontinuity (RPD) was determined using a clear corer and centimeter rule. Approximately 250 grams of surficial sediment (upper 2 centimeters of the sediment column) were placed in centrifuge vials and stored on ice. Large cobble and gravel greater than 2 cm diameter was excluded from the samples - but noted on the data sheets. This was accomplished because it was considered inappropriate to attempt to transport several hundred pounds of rock and cobble from remote beaches to the laboratory. In addition, bivalves are likely more influenced by the structure of sediment fractions finer than 2 cm particle size than they are by the larger components, excepting that large rock may provide a partial refuge from some predators.

2.5.1. Sediment grain size samples were stored at 4^oC until they were analyzed. The sediments were dried in an oven at 92 ^oC and processed using the dry sieve and pipette method (Tetratech, 1987). The sieves used for the sediment analysis had mesh openings of 2, 0.89, 0.25 and 0.063 mm. Particles passing the 0.063 mm sieve were analyzed by sinking rates in a column of water (pipette analysis). In addition, sediments were evaluated in the field for color, presence of attached macroalgae, presence of oil sheens and odors indicating hydrogen sulfide or petroleum.

2.5.2. Sediment total volatile solids. A separate, 50 gram surficial sediment sample, consisting only of that fraction smaller than coarse sand was taken from the top two centimeters, placed in scintillation vials and stored on ice. These samples were dried at 103 $\pm$ 2 ^oC in aluminum boats that had been pre-cleaned by ashing at 550 ^oC for 30 minutes. Drying continued until no further weight reduction was observed. The samples were then combusted at 550 ^oC until no further weight loss was recorded. Total Volatile Solids were calculated as the difference between the dried and combusted weights and expressed as a proportion of the dry weight.

2.6. Water column characterization. Three 500 ml water samples were collected at each study site. The samples were collected at mid depth from undisturbed water with a minimum depth of one meter. Samples were placed on ice and shipped via overnight express to Aquatic Environmental Sciences' laboratory for the following analyses:

2.6.1. **Total suspended solids (TSS) and total volatile solids (TVS).** A 0.45 μm glass filter was combusted at 550^oC and weighed. A 350 ml sample of thoroughly mixed water was suction filtered and the residue dried at 103 $\pm$ 2 ^oC to determine TSS. Total volatile solids were determined following combustion of the sample at 550 ^oC.

2.6.2. Dissolved oxygen was monitored *in-situ* with a YSI Model 57 Oxygen Meter. The probe had a new membrane and was calibrated with water-saturated air immediately prior to each measurement.

2.6.3. Salinity and temperature were monitored, *in-situ,* with a YSI Model 33 SCT meter that was calibrated at 0.0 and 29.6 ppt the day prior to sampling.

2.6.4. pH was determined using a dual point calibrated (pH 7 and 10) JENCO mP-Vision 6009 meter. The pH meter was calibrated in the field just prior to each set of measurements.

2.6.5. Current speeds were measured by placing a drogue in the water and timing its transit along a two-meter stick. Three replicate measurements were made in succession midway between high and low tides and again at slack tide. The surveys were conducted during spring tides and it is postulated that the observed speeds measured midway between high and low tides are representative of the near maximum surface currents at each site. These point estimates do not provide a definitive understanding of local currents, but they do provide a sense of the minimum and maximum current speeds characteristic along each beach.

2.7. Data analysis. Data was entered into an Excel™ spreadsheet and imported into a STATISTICA™ database. All discrete data was log transformed. Proportional data was transformed using the arcsine-square root transformation (Zar, 1984). An alpha (probability of making a Type I error) of 0.05 was used in all statistical testing and 95% confidence limits are reported where appropriate. Non-linear regression analysis was used to define regression coefficients for the von Bertalanffy growth model. This model was chosen because of its historical use in shellfish population studies and because it is easily interpreted. The Gompertz equation (Boltz and Burns 1996; Pennington 1979) is simply and exponential fit to natural log transformed length data. It has seen use in modeling fish growth as a function of age based on annuli interpreted from otoliths (Boltz and Burns, 1996).

The Gompertz equation might also be appropriate where heteroscedasticity or non-normally distributed residuals require a logarithmic transformation. Regression techniques are fairly robust to deviations from the underlying assumptions (including requirements for homoscedasticity and normality of residuals). However, based on comments received regarding Brooks (1995b), the residuals in each analysis were examined for homoscedasticity and tested for normality using both the Kolmogorov-Smirnov and Chi-squared goodness of fit tests (Neter *et al.*, 1985). Residuals were not significantly different from a normal distribution in every case at α = 0.05 and the von Bertalanffy model was used throughout this analysis.

3.0. Results for baseline bivalve inventories. Subsistence beach bivalve inventories were completed during a series of low tides during August 26 and 27, 1995 at Passage Island, Murphy's Slough and Tatitlek. Beaches near the villages of Chenega and Ouzinke were surveyed on June 29 and July2, 1996. The results of these inventories are presented in the following sections.

3.1. Bivalve inventory results for Tatitlek. Mr. Steve Totemoff and Mr. Gary Kompkoff were consistent in their comments that shellfish, particularly butter and native littleneck clams, have historically been an important subsistence food source. They noted that local shellfish resources had been depleted and commented that sea otter predation was a major concern. The Village of Tatitlek has an ongoing floating aquaculture industry focusing on the Pacific oyster (*Crassostrea gigas*). The Village has adequate boat and human resources. Villagers indicated that they were willing to expend significant effort to restore their shellfish resources. The beach surveyed on August 27, 1995 during a predicted –0.9' MLLW tide. It was located immediately adjacent to the village at 60^{o} 51.82' N by 146^{o} 41.15' W and is depicted in Figure (5). The surveyed area of beach measured 100 feet wide by 350 feet long. It was bounded on the north by sand and mud substrates covered with a healthy eelgrass (*Zoostera cf. japonica*) bed. The substrate was hardened by boulders and rock outcroppings to the south. The area in between contained substrate suitable for native littleneck clams.

3.1.1. Beach characterization. Figure (6) is a photograph of the sampled beach. A schematic diagram of the sampling design is provided in Figure (7). All of transect (A) and the lower portions of transect (B) were located in the sandy, eelgrass dominated strata, and six transects (C, D, E, F, G and H) were established on the gravel – cobble beach. Four sample stations were evaluated at 22 to 24' intervals on each of the seven orthogonal transects (A through F and H). Transect G was run parallel to the beach at a tidal elevation of +0.5' (MLLW) with an interval of 60'. Thirty-five shellfish samples were collected on seven transects at Tatitlek.

Figure 6. Traditional bivalve subsistence beach near the village of Tatitlek in South Central Alaska. The black garbage bags contain samples awaiting transport to an upland processing station.

The beach considered suitable for native littleneck clam production has a shallow slope (3.6%) and well-oxygenated substrates to a depth of at least 10 cm. Ten sediment samples were evaluated for sediment grain size and total volatile solids. Excluding large rock and cobble, Tatitlek clam beach sediments were 65.7% gravel, 25.87% sand and 8.33% fines (silt and clay). Tatitlek clam beach sediments contained an average of 1.31 $\pm$ 0.65 percent volatile solids. As might be expected, Total Volatile Solids were moderately well correlated (Pearson Correlation Coefficient = 0.39, P = 0.000) with the proportion fines observed in the sediment. Conditions in the sandy eelgrass meadow were quite different. The Reduction-Oxidation Potential Discontinuity was located at depths as shallow as 4 cm. This was accompanied by a slight hydrogen sulfide smell. Sediments were composed of 8.7 percent gravel, 53.6 % sand and 37.7 % fines (silt and clay). Total Volatile Solids were slightly higher in sediments under the eelgrass beds at 1.7 ± 0.11 percent. The presence of hydrogen sulfide can be attributed to reduced pore water circulation in the fine-grained sediments.

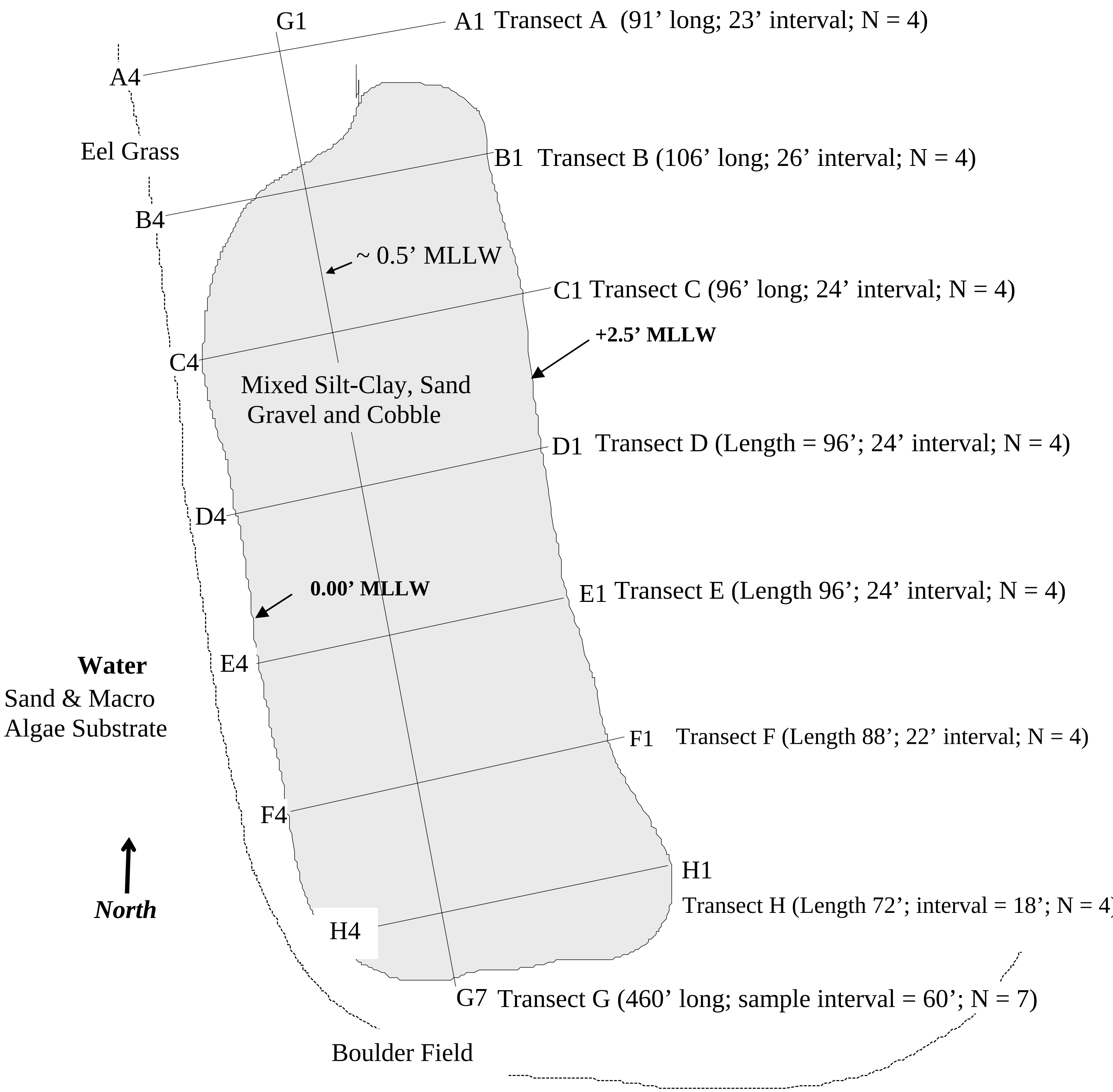


Figure 7. Schematic diagram of the Tatitlek Village shellfish beach. The beach has surveyed in August of 1995.

3.1.2. Water column characterization. Conditions at Tatitlek were acceptable for native littleneck clam culture. Water temperature was 12.0 ^{o}C, salinity equaled 26.0 ppt and dissolved oxygen was 12.5 ppm, which was slightly supersaturated. Currents at slack tide were measured parallel to the beach (085 ^{o}Magnetic) at 9.4 cm/sec. However, Village sources stated that currents are generally strong at this location and can exceed six knots (304 cm-sec^{-1}) during strong tidal exchanges. The three water samples collected at this beach averaged 3.27 mg TSS/L and 2.3 mg TVS/L. These values suggest moderate primary productivity and few suspended inorganic particulates.

3.1.3. **Bivalve population characterization.** A total of 660 living bivalves were collected in samples at Tatitlek. The distribution of these is provided in Table (5).

Table 5. Summary of bivalves collected in 35, 0.1 m^2 samples at the Tatitlek Village beach on August 27, 1995.

Species	Number
Protothaca staminea (native littleneck clam)	480
Saxidomus giganteus (butter clam)	97
Macoma inquinata (indented macoma)	72
Macoma nasuta (bentnose macoma)	4
Hiatella arctica (Arctic hiatella)	4
Mya truncata (truncate softshell)	1
Tresus cf. capax (fat gaper)	1
Clinocardium nuttallii (Nuttall's cockle)	1
Unidentified	1

Gaper, butter and native littleneck clams and cockles have potential as subsistence shellfish resources. Local villagers stated a preference for butter clams, native littleneck clams and cockles. Of these, only the butter and native littleneck clams were found in reasonable abundance.

3.1.4. Butter clams. Ninety-seven (97) living butter clams were retrieved from these samples. Their length-frequency distribution is provided in Figure (7). Most clams were small and less than two years old. Only three legal size (>38 mm valve length) butter clams were observed in all 35 samples. Descriptive statistics are provided in Table (6).

Non-linear regression was accomplished on aged living and empty butter clam valves to determine von Bertalanffy model coefficients. The resulting equation explained 92.89% of the variation and the ANOVA determined probability that the regression coefficients were all equal to zero was P = 0.000. The residuals were normally distributed. However, some caution is in order because no clam valves exceeding 79 mm were included in the database. Therefore, the maximum size of 126 mm is not well determined.

$$\text{Length of butter clams (mm)} = 126.5(1 - \exp^{-0.075 \text{ x age in years}})$$

Table 6. Summary descriptive statistics for living and dead butter clams sampled at the Tatitlek Village beach on August 27, 1995. Length and age statistics include 103 empty butter clam valves, which were measured and aged. Other statistics do not.

	Valid N	Mean	Minimum	Maximum	Std. Dev.
Length (mm)	200	34.32	2.00	79.00	23.45
Whole weight (g)	97	2.43	0.0012	47.88	6.88
Age	200	4.52	0.01	12.00	3.47
Dry Condition Factor	45	0.20	0.007	0.94	0.16

Because of their propensity to retain paralytic shellfish poisons and lack of adequate hatchery technology, butter clams are not considered appropriate for enhancement at this time. However, the Washington State Department of Fish and Wildlife shellfish laboratory at Point Whitney has spawned and raised butter clams in their hatchery (Mr. Brady Blake, personal communication). Several year classes (Ricker, 1975) are evident in the length frequency histogram provided in Figure (8), which also demonstrates a lack of legal size butter clams on this beach. Figure (9) suggests that butter clams recruit regularly to this beach, but that they typically do not survive beyond five years of age or to lengths greater than 38 mm. Predator control will be an important element in any effort to enhance shellfish resources on this beach.

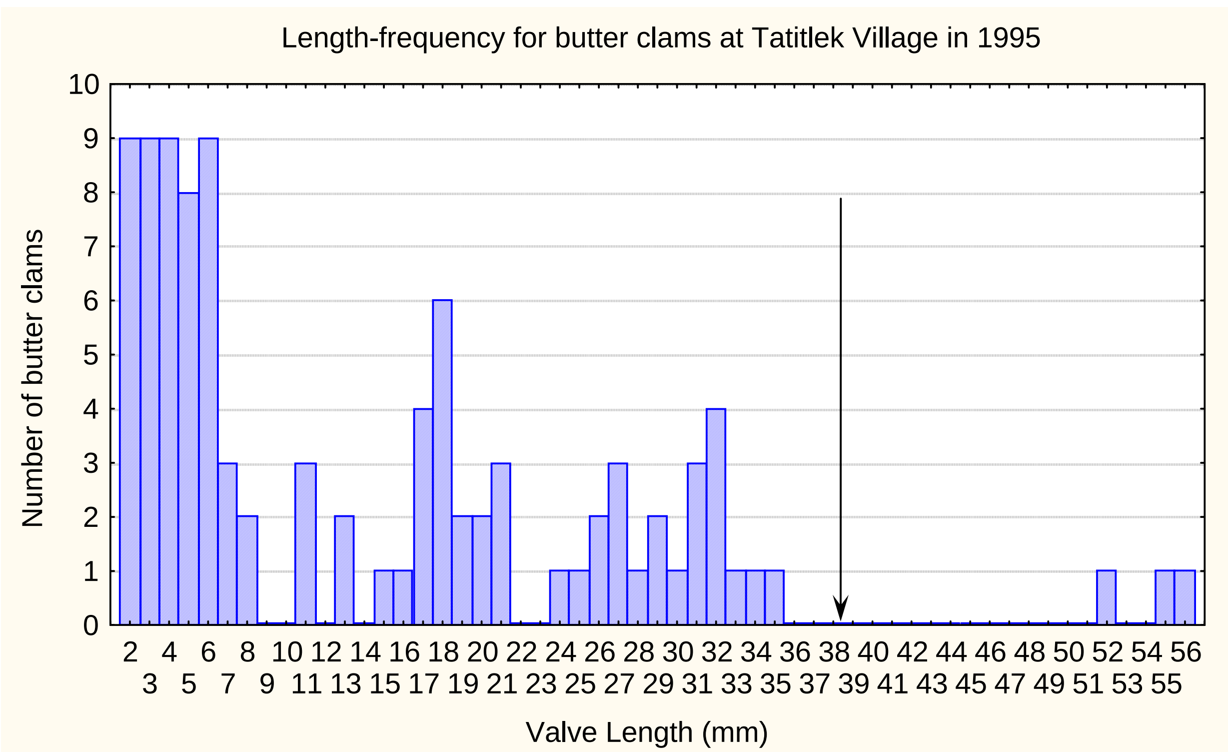


Figure 8. Length frequency histogram for butter clams (*Saxidomus giganteus*) collected in 35, 0.1 m^2 samples at the Tatitlek Village shellfish beach on August 27, 1995. The vertical line describes the minimum legal size (38 mm).

Figure (10) is a photograph taken at low tide on the Tatitlek beach. Large numbers of sunstars (*Pycnopodia helianthoides*) were observed at and below +0.5' MLLW and frilled dogwinkles (*Nucella lamellose*) were observed at tidal elevations greater than ca. +2.0' MLLW. Figure (11) is a photograph of a few of the hundreds of small clams observed on this beach that had been drilled by gastropods. In addition to these predators, numerous shore crabs were observed and sea otters were encountered offshore. Large clams were not found on this beach. However, broken butter clam shells provided equivocal evidence of historical sea otter predation.

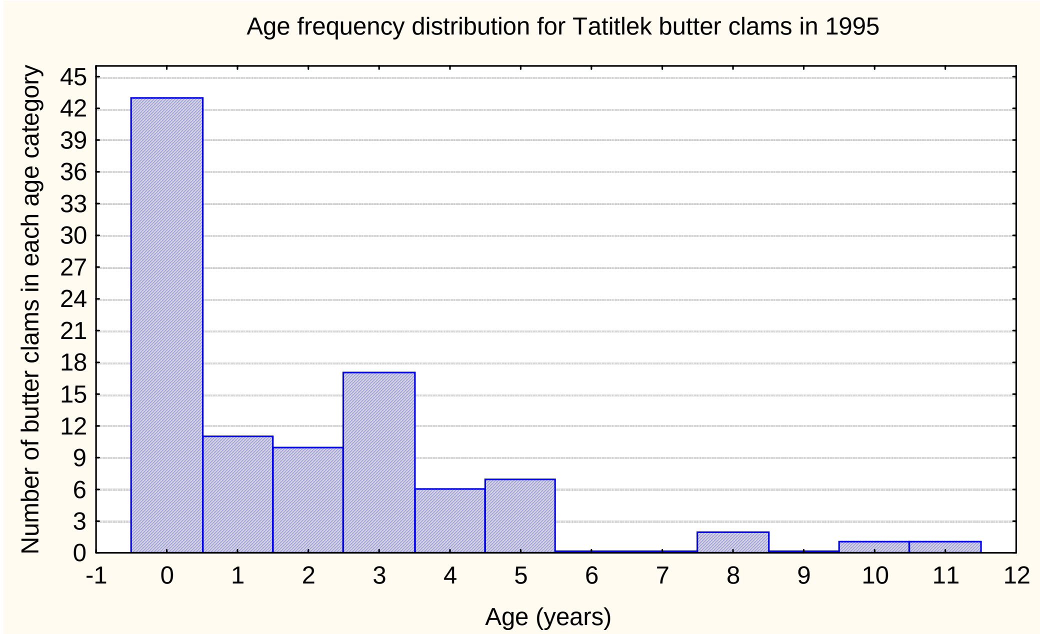


Figure 9. Age-frequency histogram for butter clams (*Saxidomus giganteus*) collected in 35, 0.1 m^2 samples at the Tatitlek Village shellfish beach on August 27, 1995.

Figure 10. Sunstars (*Pycnopodia helianthoides*) and frilled dogwinkles (*Nucella lamellose*) observed on the subsistence beach adjacent to the native village of Tatitlek in Alaska.

Figure 11. Juvenile butter clams (*Saxidomus giganteus*) collected in sediment samples from the subsistence beach adjacent to the native village of Tatitlek in Alaska.

3.1.5. Native littleneck clams. Four Hundred-eighty (480) native littleneck clams were sieved from 35 Tatitlek sediment samples. Summary statistics describing littleneck clams are presented in Table (7). The largest native littleneck clam had a valve length of 45 mm and weighed 19.34 grams. Seventeen (17) legal size clams (valve length $\geq$ 38 mm) were observed in all 35 samples. This equates to a density of approximately 73.9 g-m^{-2} or 0.016 pounds per square foot. This is approximately one tenth the minimum density considered economical for commercial clam harvests in Washington State (Paul Taylor, personal communication). The conclusion is that there is currently little opportunity for subsistence harvest of butter or native littleneck clams at this Tatitlek village beach.

Comparison of Figures (12) and (13) clearly shows the correspondence between the length and age of at least the first four year classes. Furthermore, these figures suggest that predation, from a variety of sources is taking most clams before they reach 38 mm valve length. No missing year classes are apparent in Figures (12) or (13) suggesting constant recruitment of native littleneck clams to this beach. It appears that shellfish production at this site is limited primarily by predation, disease or loss of clams associated with substrate movement. Based on the history of Manila clams in Puget Sound, a minimum juvenile density of 20 to 30 per 0.1 m^2 is desired for reasonable production. Current native littleneck clam recruitment is approximately four per 0.1 m^2 and survival is unacceptable. The previous discussion regarding predation on butter clams is appropriate for native littleneck clams as well.

Table 7. Summary descriptive statistics for living native littleneck clams sampled in 35, 0.1 m^2 quadrats at the Tatitlek Village beach on August 27, 1995.

	Valid N	Means	Minimums	Maximums	Std. Dev.
Elevation (feet above MLLW)	476	0.84	-1.10	3.12	0.83
Valve length (mm)	579	17.17	1.80	45.00	11.20
Whole weight (gm)	472	2.02	0.001	19.35	3.45
Dry tissue weight (gm)	264	0.69	0.09	3.02	0.69
Wet tissue weight (gm)	264	1.69	0.10	8.11	1.60
Age (years)	576	1.95	0.00	8.00	1.73
Dry Condition Factor	263	0.18	0.02	0.65	0.12

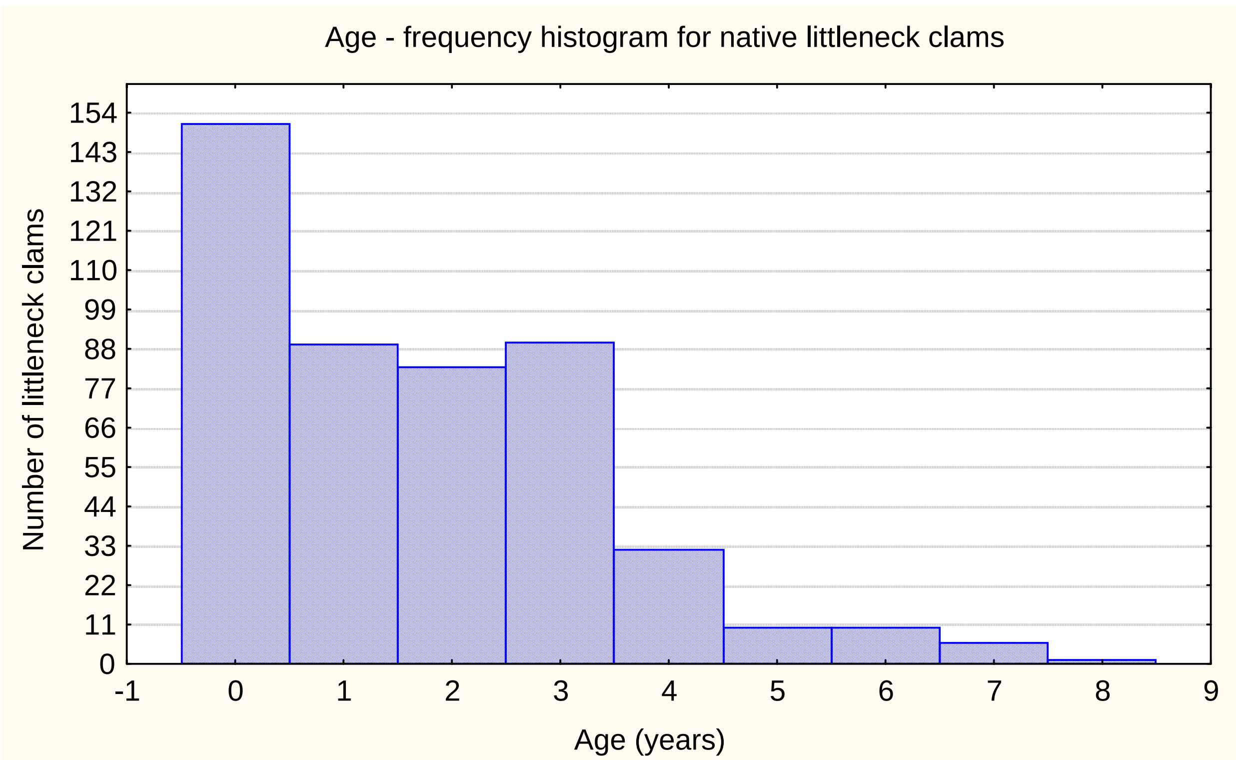


Figure 12. Age – frequency histogram for littleneck clams collected in 35, 0.1 m^2 quadrats at the Tatitlek Village on August 27, 1995.

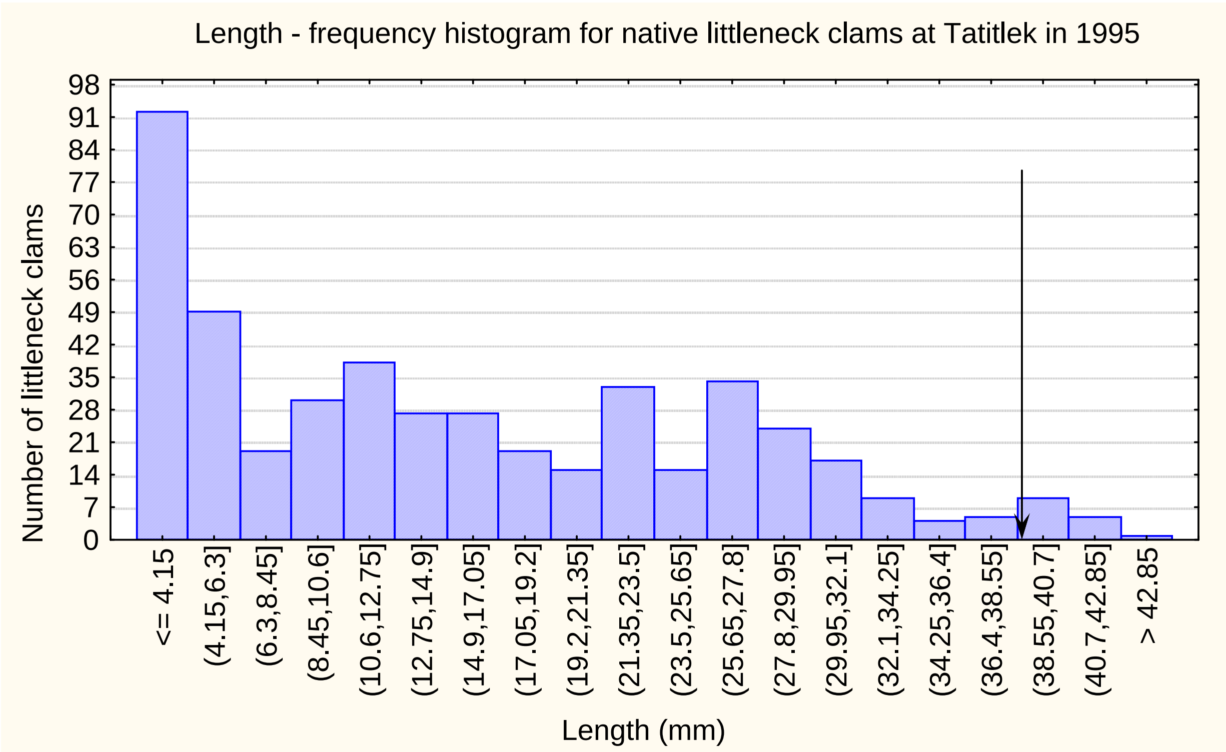


Figure 13. Length – frequency histogram for littleneck clams collected in 35, 0.1 m^2 quadrats at the Tatitlek Village on August 27, 1995. The thin vertical line represents the minimum legal size of 38 mm.

3.1.6. Distribution of clams as a function of tidal height at Tatitlek. Figures (14) and (15) compare the distribution of butter and native littleneck clams as a function of tidal height at Tatitlek. These figures are interesting in that they indicate an optimum tidal range of approximately 0.0’ to +2.0’ MLLW for native littleneck clams and an optimum of 0.0’ to 1.0’ MLLW for butter clams. It should be noted that the substrate changes to primarily sand at tidal elevations less than –1.5’ at this beach. This substrate change and the presence of large numbers of starfish at lower intertidal elevations are factors that may be responsible for limiting the clam population in deeper water. Also note that both butter clams and native littleneck clams were found at tidal elevations as high as +3.0’ MLLW. The data for native littleneck clams at Tatitlek suggests that the area between –1.0’ and +2.5’ is suitable for native littleneck clam production on this beach. This is essentially the same range described by Nickerson (1977) and Feder and Paul (1973).

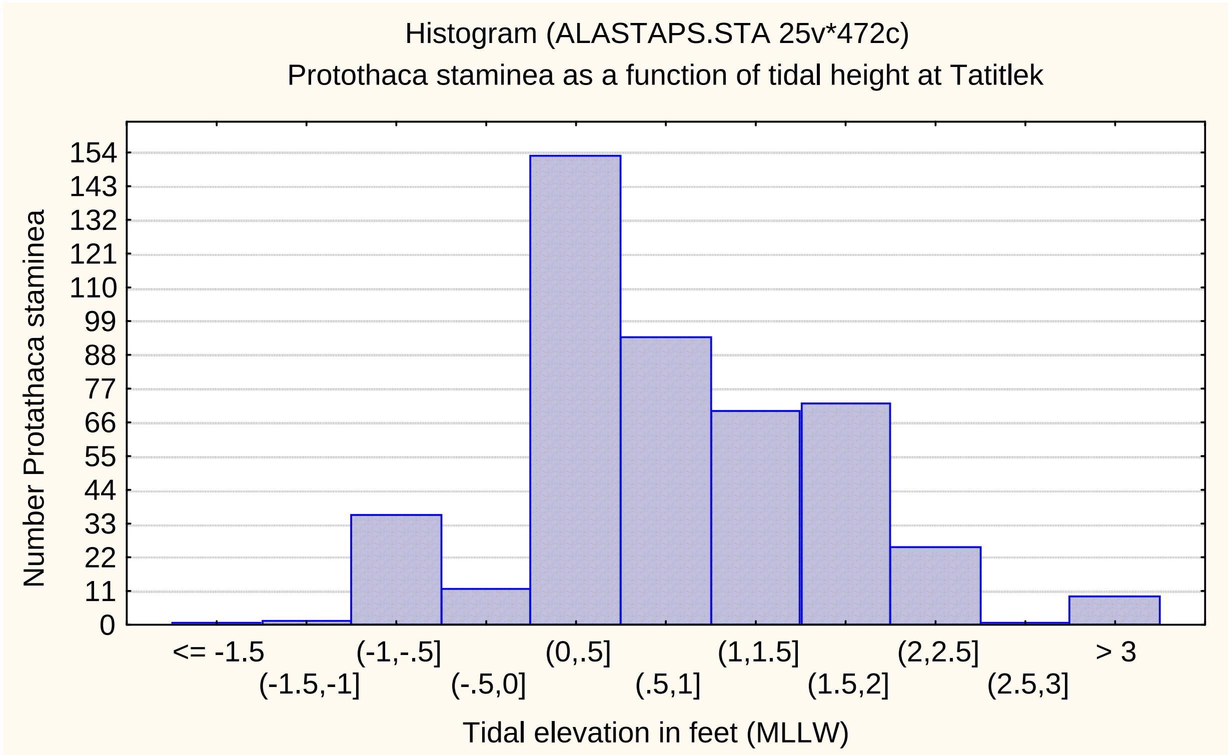


Figure 14. Tidal elevation – frequency histogram for littleneck clams collected in 35, 0.1 m^2 quadrats at the Tatitlek Village shellfish beach on August 27, 1995.

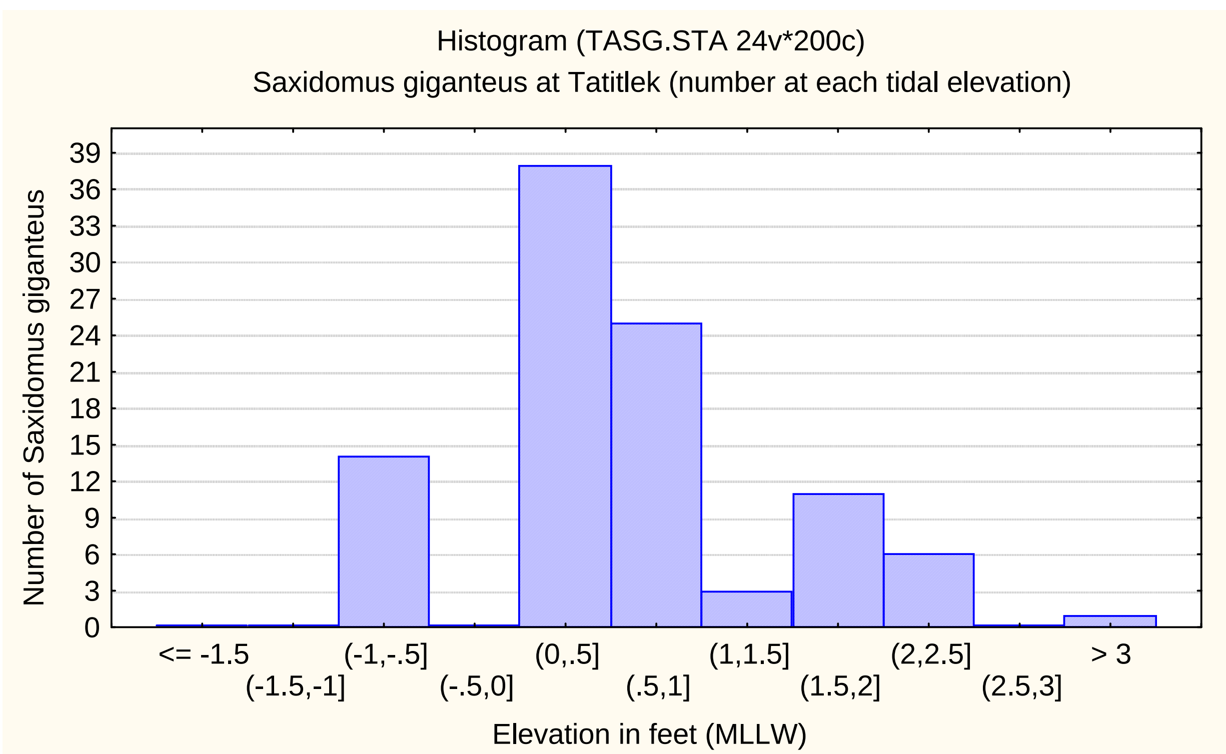


Figure 15. Tidal height – frequency histogram for butter clams (*Saxidomus giganteus*) collected in 35, 0.1 m^2 quadrats at the Tatitlek Village shellfish beach on August 27, 1995.

Average growth increments were calculated by dividing each clam's valve length by its age. This information is presented graphically in Figure (16). The coefficients determined in a linear analysis were statistically significant at $\alpha = 0.05$ but the predictive equation explained less than 3% of the variation in the database. Figure (16) suggests that within the tidal range investigated (which includes all elevations at which clams were found in this survey), mean native littleneck valve growth declined slowly with increasing tidal height. This was particularly true for the clams at the highest elevation (>3.0' MLLW), which apparently grew slower than those found at intermediate elevations. Figure (16) suggests that clam growth should be reasonably constant at beach elevations between –1.5' and +2.5' MLLW.

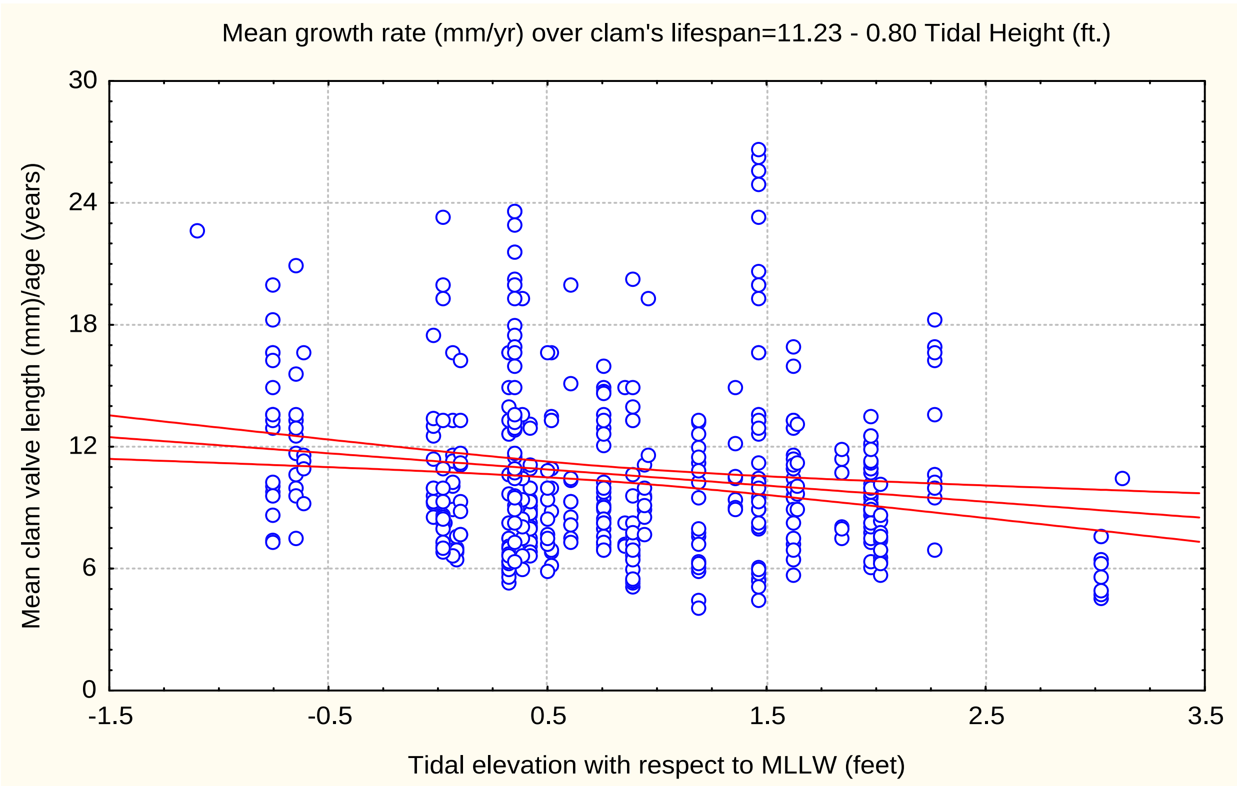


Figure 16. Growth increments (mm/year) as a function of tidal height (feet above MLLW) for native littleneck clams (*Protothaca staminea*) collected in 35, 0.1 m^2 quadrats at the Tatitlek Village shellfish beach on August 27, 1995. 95% confidence limits on the mean are provided as dashed lines in this figure.

3.1.7. Age-length analysis for native littleneck clams at Tatitlek. Regression coefficients were developed for the von Bertalanffy model using nonlinear regression. The resulting expression (Figure 17) explained 87.2% of the variation and the ANOVA determined probability that the regression coefficients were all equal to zero was P = 0.000. The residuals appear normal. However, some caution is in order because no clam valves exceeding 45 mm were included in the database. In Puget Sound, native littleneck clams grow to lengths in excess of 65 mm (Brooks, unpublished). However, clams older than 8 years were not observed at Tatitlek and the maximum predicted valve length (47.61 mm) may be inaccurate.

$$\text{Length of native littleneck clams (mm)} = 47.61(1 - \exp^{-0.2548 \text{ x age in years}})$$

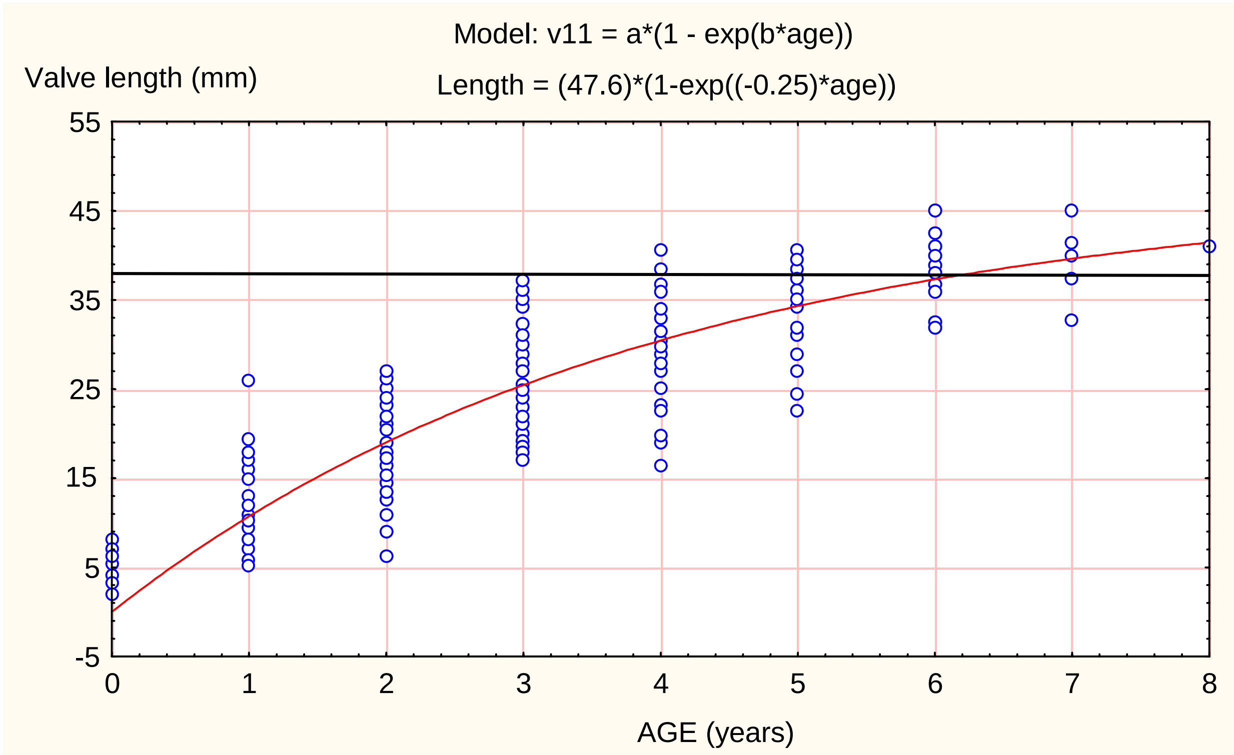


Figure 17. Length (mm) versus age (years) for native littleneck clams (*Protothaca staminea*) collected in 35, 0.1 m^2 quadrats at the Tatitlek Village on August 27, 1995. The solid horizontal line represents the minimum legal size limit ($\geq$ 38 mm).

The von Bertalanffy equation, and accompanying scatterplot, indicates that clams recruit into the legal size population ($\geq$ 38 mm), at between 4 years and >7.0 years. The average age at recruitment was six years.

3.1.8. Edible tissue versus clam length analysis. A length – wet tissue weight histogram is provided in Figure (18) and an age – wet tissue weight histogram in Figure (19). One of the possible management options involves harvesting clams at a shorter minimum valve length. However, Figures (18) and (19) suggest that this is not an appropriate alternative.

An examination of the length-frequency data in Figure (13) suggests that clams are being removed by predation at approximately 30 mm. That length is coincident with the length range where wet tissue weights are beginning to increase significantly as a function of length in Figure (18). Even at 38 mm, clams are still well within the exponential growth phase. In this database, clams that were 8 years old, with a valve length of 42 to 45 mm, had wet tissue weights of approximately 7.5 grams. This is significantly higher than the wet tissue weight of 4.5 grams associated with six-year-old clams just reaching the current minimum harvest size of 38 mm. Reducing the minimum harvest size to 30 mm (a size preceding the heaviest predation) would result in a harvest of approximately 2.5 grams wet tissue weight per clam. Nickerson (1977) observed peak increases in the rate of biomass increase (first derivative of biomass versus time) at an age of approximately 7 years (corresponding to a valve length of ca. 38 mm) with a slow decline in this important rate at older ages. Wet tissues are eaten – not the whole animal, and this discussion suggests that lowering the minimum size at harvest to avoid predation would significantly reduce the subsistence value of the resource to native Alaskans.

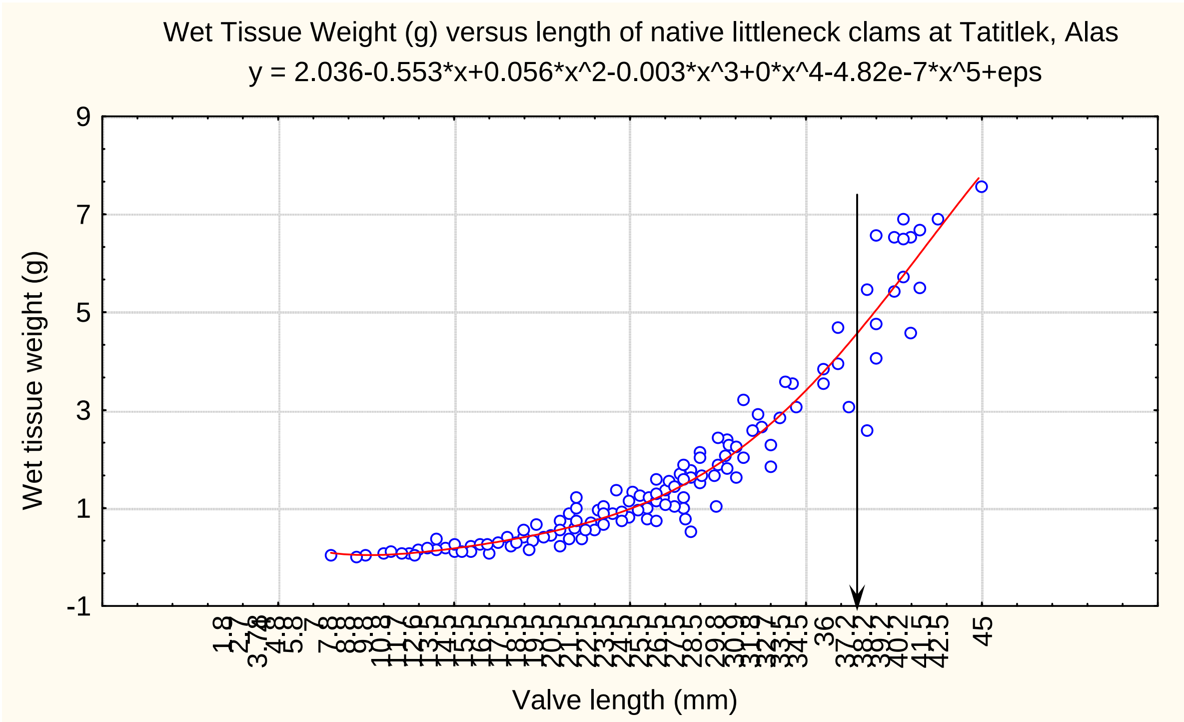


Figure 18. Length (mm) versus wet tissue weight (in grams) for native littleneck clams (*Protothaca staminea*) collected in 35, 0.1 m^2 quadrats at the Tatitlek Village shellfish beach on August 27, 1995. The vertical solid line represents the minimum legal size.

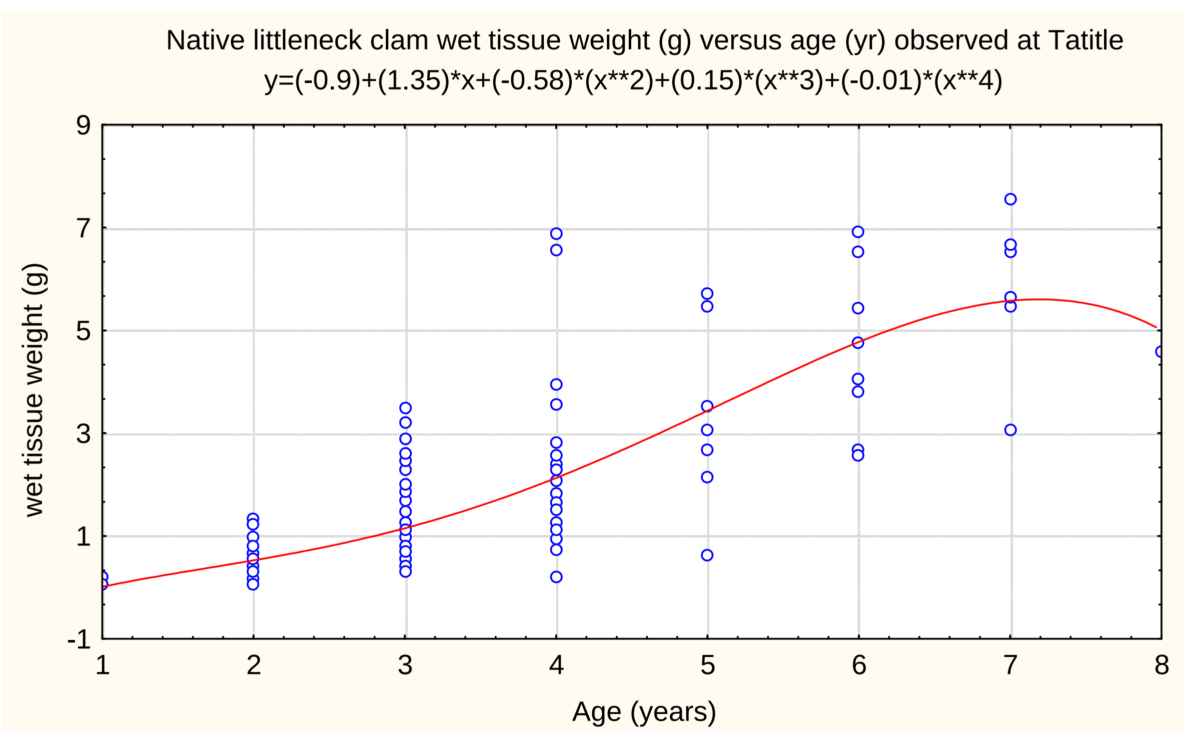


Figure 19. Age (yr) versus wet tissue weight (grams) for native littleneck clams (*Protothaca staminea*) collected in 35, 0.1 m^2 quadrats at the Tatitlek Village shellfish beach on August 27, 1995.

3.1.9. **Predator density.** Predators and/or their activities were obvious at the Tatitlek beach. Numerous small round holes (approximately 0.6 meters in diameter and 15 centimeters deep) were found on the beach. Villagers' assured us that these pits were created by sea otters and that no harvests had been conducted at this site for several years. The lack of clams larger than 55 mm on this beach suggests that otters would find little suitable prey here. It is the author's opinion that many of these holes were created by sunstars (*Pycnopodia helianthoides*), which were very abundant (Figure 8) at the 0.5' MLLW tide level and below. In an attempt to estimate the role of sunstar predation on this beach, three randomly selected stations were established on transect G. At each station, a single quadrat (3 m x 3 m) was established and the number of presumed pits and starfish were counted. The results are presented in Table (8). This examination suggests that starfish and possibly sea otters were having a significant impact on bivalve resources. Interestingly, although there was a significant amount of *Fucus sp.* on this beach, only one small urchin was observed. In addition, gastropods (Figure 10) were consuming many newly recruited clams at Tatitlek.

Table 8. Number of starfish (*Pycnopodia helianthoides, Pisaster ochraceus*) and presumed sea otter (*Enhydra lutris*) pits observed at the Tatitlek village shellfish beach on August 27, 1995. All counts are provided in numbers per square meter.

Sample Station	Pits	*Pycnopodia*	*Pisaster*
G2	0.44	1.0	0
G3	0.22	0.22	0
G6	0.0	0.56	0.11

3.1.10. Summary and recommendations for native littleneck clam enhancement at the village of Tatitlek. The following summary and conclusions are based on this survey:

- **Existing bivalve resources.** Few clams were available for harvest at this Tatitlek village beach. However, there were significant quantities of small mussels (*Mytilus edulis trossulus*), along the extreme high tide line. In many parts of the world, blue mussels are considered a delicacy. Villagers suggested that this is not a traditional food. However, their sheer volume at this site, and their acceptance in other parts of the world, suggest that this could be a valuable subsistence resource. This is particularly true because mussels are amenable to floating culture. The seed could be harvested from the high intertidal areas where the mussel grows slowly, and placed in lantern nets at the Village's oyster culture facility – or away from piling on the new ferry terminal.

- **Substrate suitability.** The Tatitlek Village Beach contains approximately one acre of ground suitable for native littleneck clam enhancement or culture. The physical and chemical parameters examined in this survey are all within acceptable limits. Clam growth, density and size suggest non-significant differences in culture potential over the area of surveyed beach. If the predation problem is solved, several physical enhancement practices could be employed here to increase natural recruitment and to make this rocky, high-energy, beach more amenable to intensive clam culture.

- **Culture depth (tidal level and depth in sediments).** Native littleneck clams were found at depths greater than 15 cm on this beach. This may be a regional adaptation for survival during cold winter, nighttime, low tides. Typically, cultured clams are protected from potential predators by placing them in sturdy mesh bags. These bags are then partially buried in the substrate. If Alaskan littleneck clams dig deeper to avoid freezing in winter, the placement of clams in bags at shallow depths could jeopardize the cultures. Therefore, consideration should be given to placing bags at lower tidal elevations or to burying the bags deeper in the substrate.

- **Predation.** Significant starfish predation was observed at Tatitlek. Several sunflower stars were observed with intact native littleneck clams in their guts. In addition, while sea otters were not observed preying on bivalves, the evidence observed during this survey suggests that they may be significant predators on larger clams. If confirmed, sea otter predation presents a new dimension in predator control. Clam and oyster cages are fairly rigid and capable of excluding starfish, large drills and all but the most aggressive crabs. However, it is unlikely that these plastic mesh cages would discourage a determined sea otter. Reasonable and effective methods to control sea otter predation (if it is eventually documented) may present a challenge.

Starfish, crabs and predatory gastropods should periodically be removed from the beach. This would likely reduce early loss of clams and allow more of the natural set to reach a minimum harvest size – absent any other enhancement effort.

- **Natural clam recruitment** to the Tatitlek Village beach occurred in low numbers in each of the last eight year-classes observed. No year classes were missing. However, natural recruitment (or at least survival until August 27, 1995) was too low in each year class to stock this beach to harvest densities greater than 0.15 pounds per square foot.

- **Age and size at harvest.** The age length analysis suggested that native littleneck clams recruit to the legal size population ($\geq$ 38 mm) at between four and >7.0 years. The wet tissue weight – length and wet tissue weight – age analysis indicates that harvesting at a valve length less than 38 mm would be an inefficient use of the resource.

- **Butter clams.** *Saxidomus giganteus* recruit naturally to this beach. However, few butter clams survived to harvest size. Due to the lack of hatchery and nursery technology, and propensity to retain brevetoxins, butter clam enhancement is not recommended at this time.

- **Cockles** are a traditional (and preferred) shellfish for Alaskan natives. The primary beach surveyed in this effort was too rocky, with too few fines, to warrant cockle enhancement. The beach lying to the northwest was sandy and suitable for cockle production. However, this beach was covered with a luxurious eelgrass (*Zoostera cf. japonica*) bed. Disruption of the ecologically valuable eelgrass meadow in an effort to enhance the cockle resource is not recommended.

- **Mussels.** The presence of large quantities of blue mussel (*Mytilus edulis trossulus*) seed should not be overlooked. These mussels are eagerly sought in other parts of the world. If the copious seed supply were removed from the high intertidal, placed in lantern nets, and submerged continually at the Villages aquaculture facility, it could quickly provide as much shellfish as the village might desire.

- **Management recommendations.** The beach at Tatitlek represents a higher energy environment that was not considered optimum because of sediment instability. Otherwise, it appeared to be of acceptable quality for growing littleneck clams. Sustained subsistence harvests will require additional seed of the largest possible size; development of effective predator control measures; and establishment of a well-designed management plan. Without effective predator control, any enhancement plan will be futile.

The easiest and quickest way to increase the supply of subsistence shellfish is to utilize the mussel resource by placing seed in lantern nets and submerging them continuously where they will quickly grow to an adequate size. Based on the Villagers' lack of interest in mussels, any mussel culture effort should be combined with efforts to increase the residents' appreciation of mussels as a valuable (and delicious) source of food.

3.2. Bivalve inventory results at Passage Island for the village of Nanwalek. Mr. Dale Bowers was very helpful and expressed a great deal of desire to re-establish a subsistence shellfishery near Nanwalek. In addition to Passage Island, which is a traditional harvest area, Mr. Bowers identified other beaches that might be candidates for enhancement. The beach closest to the village lies at a low tidal elevation and is very exposed to a long fetch across Cook Inlet. Primarily for the second reason, the beach at Passage Island was chosen for these studies.

Mr. Bowers expressed concern that traditional shellfish resources were depleted and unable to supply village needs. He felt that sea otter predation was a major problem. The village had adequate boat and human resources and indicated a willingness to expend significant effort to restore their shellfish resources. Passage Island is located about 11.5 nautical miles from the village along a very rugged and exposed coastline. Tending a shellfish culture at Passage Island from Port Graham would be problematical, especially in winter.

3.2.1. **Passage Island beach characterization.** The beach most suitable for enhancement was located at 59^{o} 22.11' N by 151^{o} 52.53' W. It measured 130 feet wide by 140 feet long and covered 0.42 acres (Figure 20). It was bounded on the west by a boulder field and by deep water on all other sides. Brown kelp (*Laminaria sp.)* was abundant in the nearshore area. The beach contained large quantities of broken horse clam (*Tresus capax*) and butter clam (*Saxidomus giganteus*) shells associated with what could have been otter pits. The area contained reasonable substrate for native littleneck clams. It was not considered suitable for cockles. Although Passage Island provided some protection, the beach was exposed to storm winds from the southeast and represented a higher energy environment than was desired for the growout studies (Figure 21). The beach was accepted in deference to village elders.

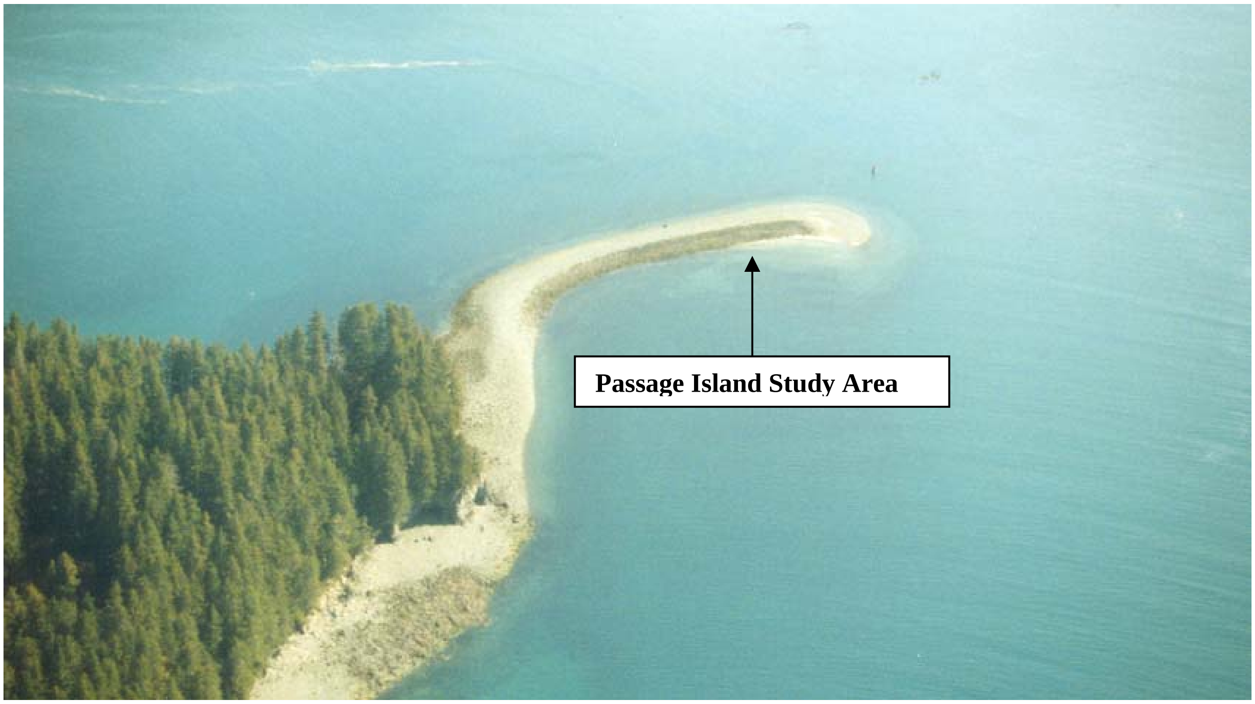


Figure 20. Aerial photograph of the eastern tip of Passage Island with the bivalve study area delineated.

Figure 21. A portion of the beach surveyed at Passage Island.

As described in Figure (22), three transects (A, B and C) were examined in that part of the beach where clams were found. Six shellfish and three sediment samples were analyzed on each of these transects giving a total of 18 shellfish and 9 sediment samples. In addition, 19 bivalves were collected in a random dig to supplement the growth data. These additional cases were not included in assessing bivalve response to environmental parameters such as tidal height.

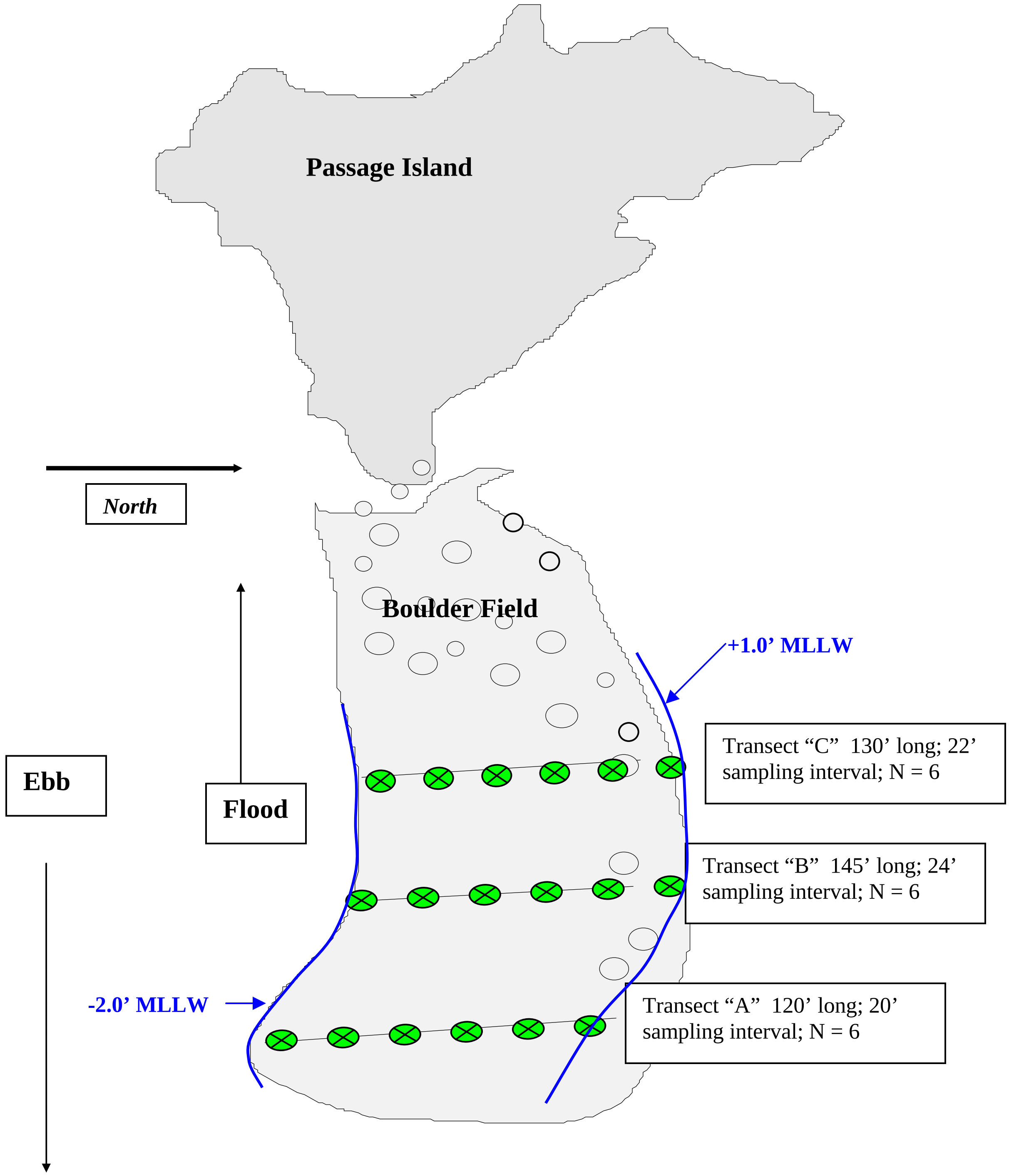


Figure 22. Schematic diagram of the Nanwalek Village shellfish beach at Passage Island. The beach has surveyed in August of 1995.

The beach considered suitable for native littleneck clam production had a shallow slope (2.3%) and well-oxygenated substrates to a depth of greater than 20 cm. Nine sediment samples were evaluated for sediment grain size and total volatile solids. Passage Island clam beach sediments contained 52.1 $\pm$ 39.3 % gravel, 38.7 $\pm$ 34.6% sand and 9.2 $\pm$ 4.83 % fines (silt and clay). Sediment composition was highly variable with the percent gravel ranging from 16 to 80.6%. Sediments contained an average of 1.30 $\pm$ 0.89 percent volatile solids. Total volatile solids at this beach are within an acceptable range for native littleneck clams. There was a very rich infauna at this site and annelids were significantly larger than usually found in Puget Sound.

3.2.2. Water column characterization. The water's temperature was 12.0 ^{o}C, salinity 30.2 ppt, and dissolved oxygen was 11.4 mg/L (which was saturated). Currents near slack flood tide were measured parallel to the beach (090 ^{o}Magnetic) at 2.8 cm/sec. However, Village sources stated that currents are generally strong at this location. The three water samples averaged 8.77 mg TSS/L and 3.23 mg TVS/L. These values suggest good primary productivity and moderate suspended inorganic particulates on the sample date.

3.2.3. Bivalve population characterization. One hundred sixty-two living bivalves were collected in the 18 systematic random samples collected at Passage Island. An additional 19 bivalves were collected in random samples and 49 empty butter and native littleneck clam shells were collected to supplement the age – length analysis. The distribution of shellfish obtained from the systematic survey is provided in Table (9).

Table 9. Summary of bivalves collected in 18, 0.1 m^2 samples at the Nanwalek Village beach at Passage Island on August 26, 1995.

Species	Number
Protothaca staminea (native littleneck clam)	105
Macoma inquinata (indented macoma)	4
Saxidomus giganteus (butter clam)	37
Macoma nasuta (bentnose macoma)	6
Macoma balthica (Baltic macoma)	2
Hiatella arctica (Arctic hiatella)	1
Mya truncata (truncate softshell)	2
Other	5
Total	162

Gaper, butter and native littleneck clams and cockles have potential as subsistence bivalve resources. Local villagers stated a preference for butter clams, native littleneck clams and cockles. Of these preferred species, only the butter and native littleneck clams were found on Passage Island.

Butter clams. Forty-one butter clams were observed in these samples. Their length-frequency distribution is provided in Figure (23). Most of the observed clams were new recruits less than two years old. Six legal size butter clams were observed in the 18 samples. Descriptive statistics for a limited number of variables are presented in Table (10).

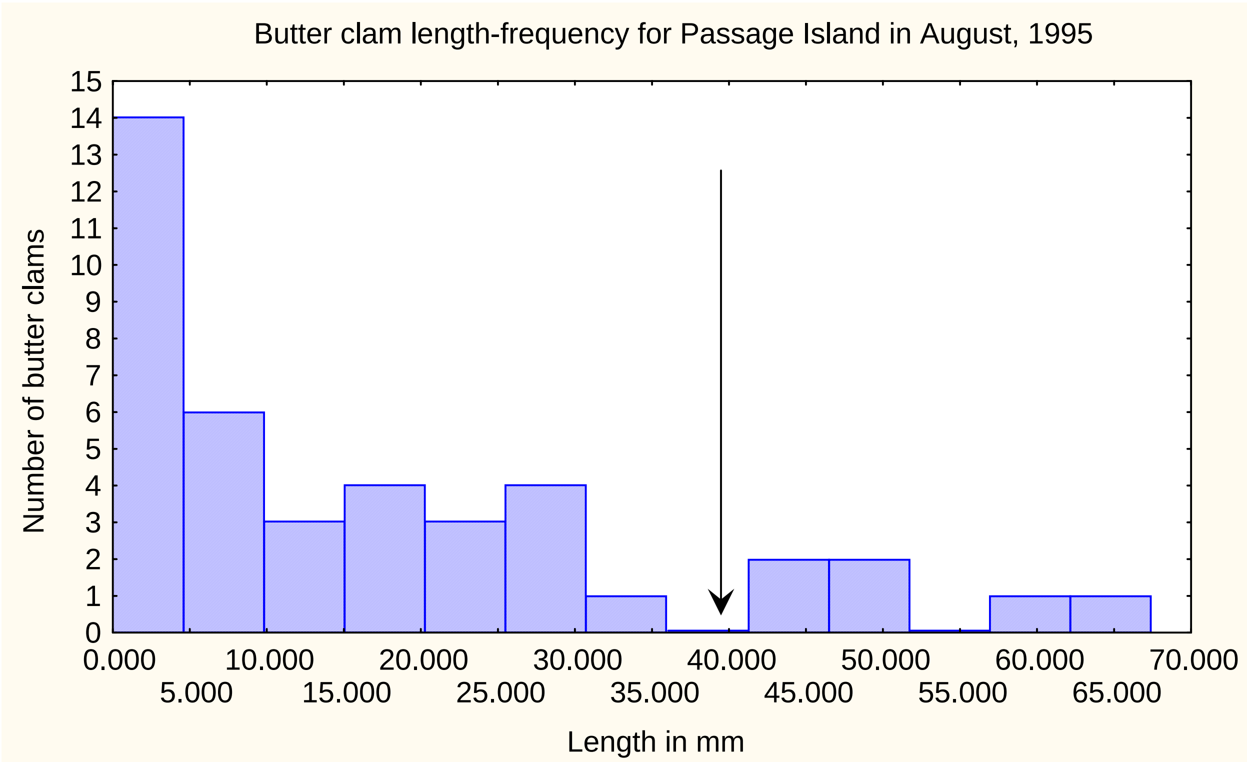


Figure 23. Length frequency histogram for butter clams (*Saxidomus giganteus*) collected in 18, 0.1 m^2 samples at Nanwalek Village's, Passage Island beach on August 26, 1995. The thin vertical line locates the legal limit ($\geq$38 mm).

Table 10. Summary descriptive statistics for living butter clams sampled at the Nanwalek Village's Passage Island beach on August 26, 1995.

	Valid N	Mean	Minimum	Maximum	Std. Dev.
Length (mm)	41	19.97	2.00	70.00	18.19
Whole weight (g)	41	7.22	0.0024	77.00	16.14
Age	41	2.65	0.00	13.00	3.08
Dry Condition Factor	20	0.25	0.06	0.58	0.15

Non-linear regression was accomplished on aged living and empty butter clam valves to determine coefficients for the von Bertalanffy model. The resulting equation explained 94.7% of the variation and the ANOVA determined probability that the regression coefficients were all equal to zero was P = 0.000. Observed and predicted values are presented in Figure (24).

The resulting Von Bertalanffy equation for Passage Island is compared with the results from Tatitlek. The results of the Passage Island data reflect the paucity of large clams in these samples. In addition, the larger coefficient on age suggests that butter clams grow more quickly at Passage Island than at Tatitlek.

$$\text{Butter clam length at Passage Island (mm)} = 84.4(1 - \exp^{-0.126 \text{ x age in years}})$$

$$\text{Butter clam length at Tatitlek (mm)} = 126.5(1 - \exp^{-0.075 \text{ x age in years}})$$

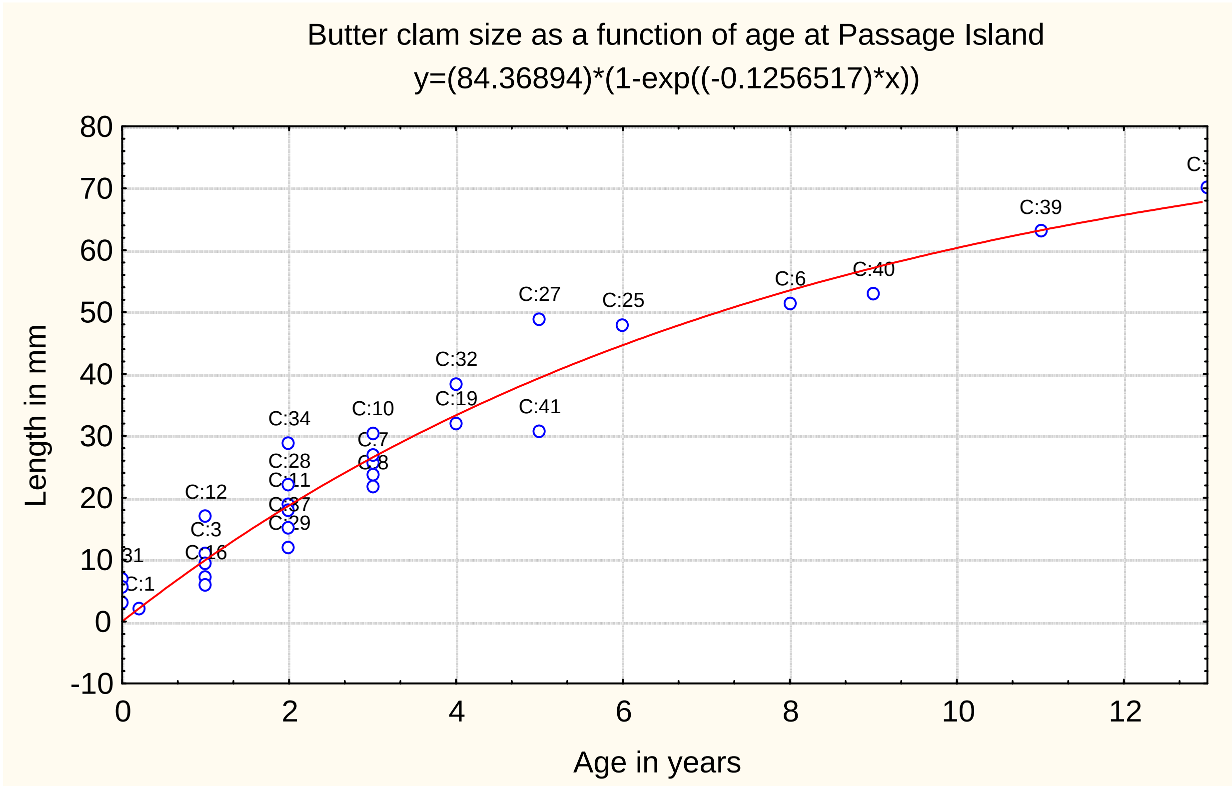


Figure 24. Solution to the von Bertalanffy model for butter clams collected in eighteen, 0.1 m² quadrats at Passage Island, Alaska, in August 1995.

An age-frequency histogram for butter clams from Passage Island is presented in Figure (25). Butter clams recruited into the legal size population at between four and five years of age (mean = 4.75 years). However, very few reached a legal size of ≥ 38 mm. Most of the mortality occurred at ages less than three years. This suggested that predators such as drills, starfish or birds were taking the small clams. From the presence of possible otter pits on the beach, the otters were exacerbating the situation by taking the few remaining large clams. The There were no apparent missing year classes for ages less than six years and recruitment of butter clams to Passage Island appears to occur regularly. However, recruitment has not been in sufficient numbers to sustain a healthy population in the presence of natural predation and mortality.

Butter clams appear to have grown well on Passage Island. However, because of their propensity to retain paralytic shellfish poisons and lack of adequate hatchery technology, this species is not considered appropriate for enhancement. Therefore, it will not receive further attention in this report. Predator control (especially starfish and drills) could have a positive affect on the number of butter clams eventually available for subsistence harvests.

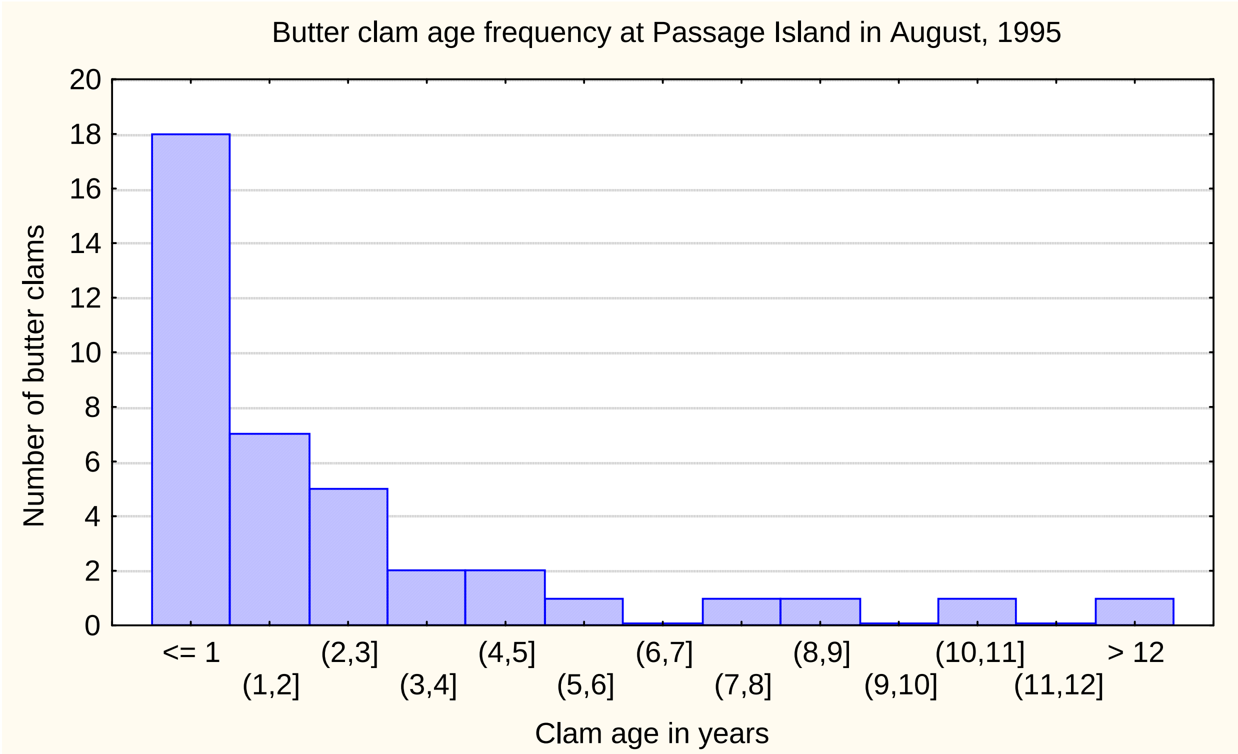


Figure 25. Age-frequency histogram for butter clams (*Saxidomus giganteus*) collected in 18, 0.1 m^2 samples at Nanwalek Village's, Passage Island shellfish beach on August 26, 1995.

Native littleneck clams. One hundred five (105) native littleneck clams were observed in the eighteen samples from Passage Island. Seven additional littleneck clams were obtained in the random digging efforts. Summary statistics for littleneck clams are provided in Table (11).

Table 11. Summary descriptive statistics for living native littleneck clams sampled in 18, 0.1 m^2 quadrats at the Nanwalek Village's beach at Passage Island on August 26, 1995.

	Valid N	Mean	Minimum	Maximum	Std. Dev.
Tidal height (ft)	18	0.099	-1.80	1.03	0.72
Length (mm)	112	26.07	2.30	52.00	9.79
Whole weight (g)	112	6.03	0.001	31.90	6.08
Age (years)	112	3.51	0.00	9.00	1.80
Dry condition factor	101	0.27	0.05	0.51	0.10
Wet tissue weight (g)	101	1.80	0.03	7.96	1.65

The largest native littleneck clam had a valve length of 52 mm and weighed 31 grams (15 per pound). Eight (8) legal size clams were obtained from the 18 quadrats included in the systematic random sample. That is less than one legal size clam per square foot and demonstrates the lack of subsistence harvest available on the beach at Passage Island.

An age frequency histogram for native littleneck clams on Passage Island is presented in Figure (26). The 1994 and 1995 year classes were very low suggesting sporadic recruitment. However, this is confounded by the presence of significant numbers of drilled clamshells in the size range three to four mm. Older clams appeared to be removed from the population shortly after reaching legal size (4 to 5 years).

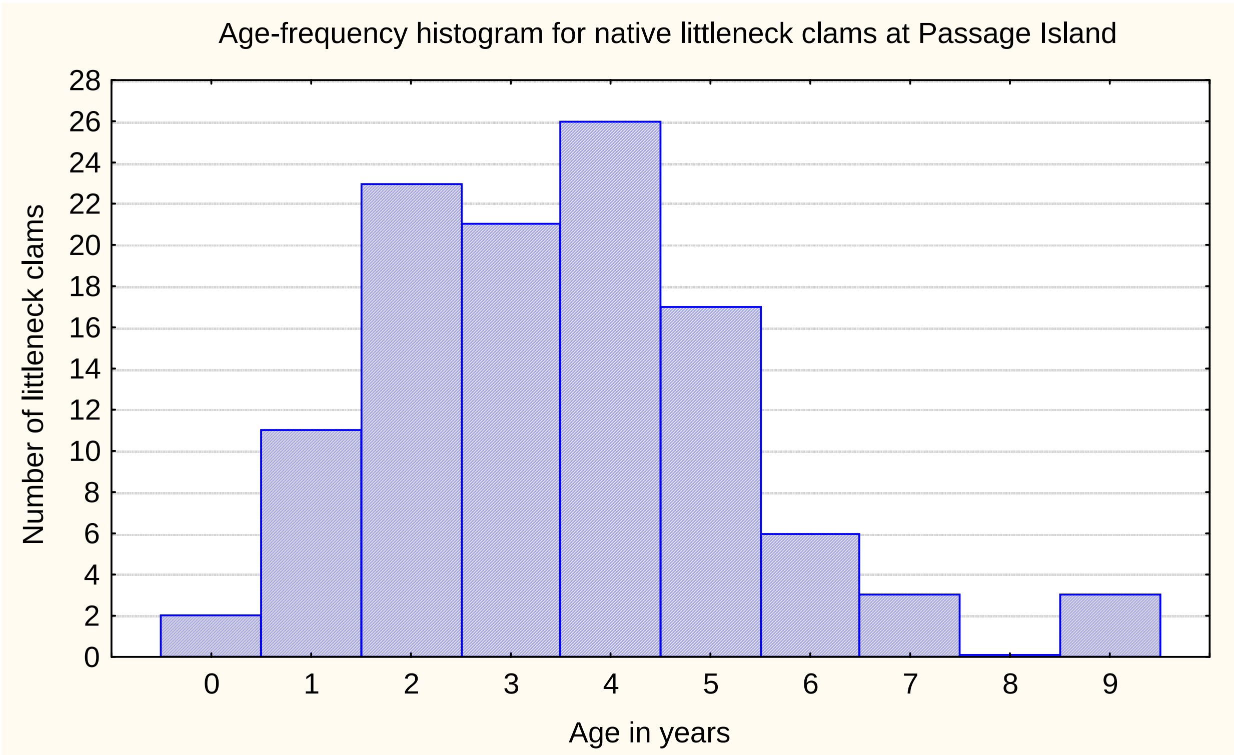


Figure 26. Age – frequency histogram for littleneck clams collected in 18, 0.1 m^2 quadrats at Passage Island on August 27, 1995.

Further examination of the population was accomplished through the length – frequency histogram provided in Figure (27). This histogram also suggests low recruitment in the recent past. The frequency observed in each of the year classes in Figure (26) should be divided by 1.8 to obtain the number of recruits per square meter since the area examined to obtain the data was 0.1 m^2/quadrat x 18 quadrats = 1.8 meters. Doing this suggests that recruitment, on average, was approximately 13 clams per square meter – far below the minimum of 400 to 700 clams per square meter typically seeded to fully utilizes beaches in Puget Sound.

This analysis indicates that current clam densities are insufficient to warrant subsistence harvests at Passage Island. Even if recruitment is enhanced, it appears that predation will still remove clams from the Passage Island population before they reach a minimum legal size. Starfish and drills are relatively easy to control. However, this beach will be difficult to protect from sea otters because of its remoteness from the village. If Passage Island is to become a valuable shellfish resource for the Village of Nanwalek, then reliable predator control measures must be developed. Seeding the beach without predator control will simply supply sub legal size clams for predators.

Figure (28) describes the distribution of native littleneck clams as a function of tidal height at Passage Island. Most of the clams were found within a narrow tidal range of –0.5’ to +1.5’ MLLW. Substrates to –1.8’ MLLW were included in this survey. However, very few clams were found at these lower elevations.

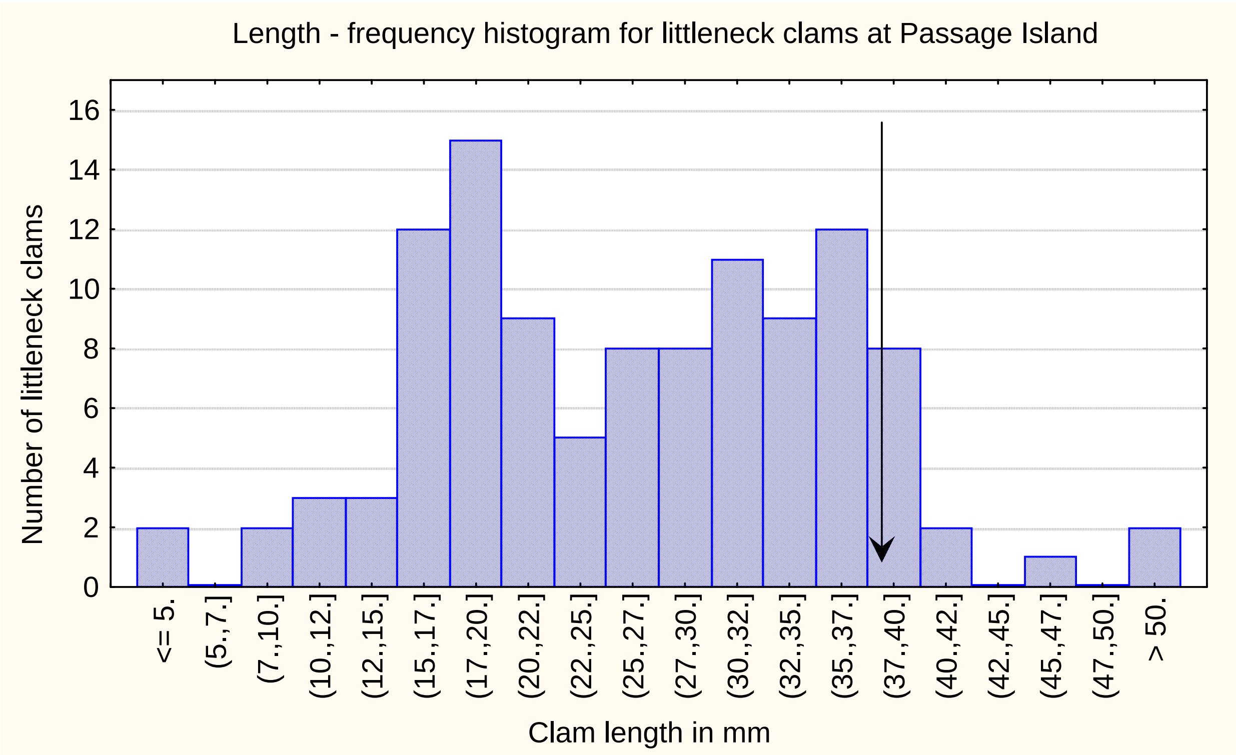


Figure 27. Length – frequency histogram for littleneck clams collected in 18, 0.1 m^2 quadrats at the Passage Island shellfish beach on August 26, 1995. The thin vertical line represents the minimum legal size of 38 mm.

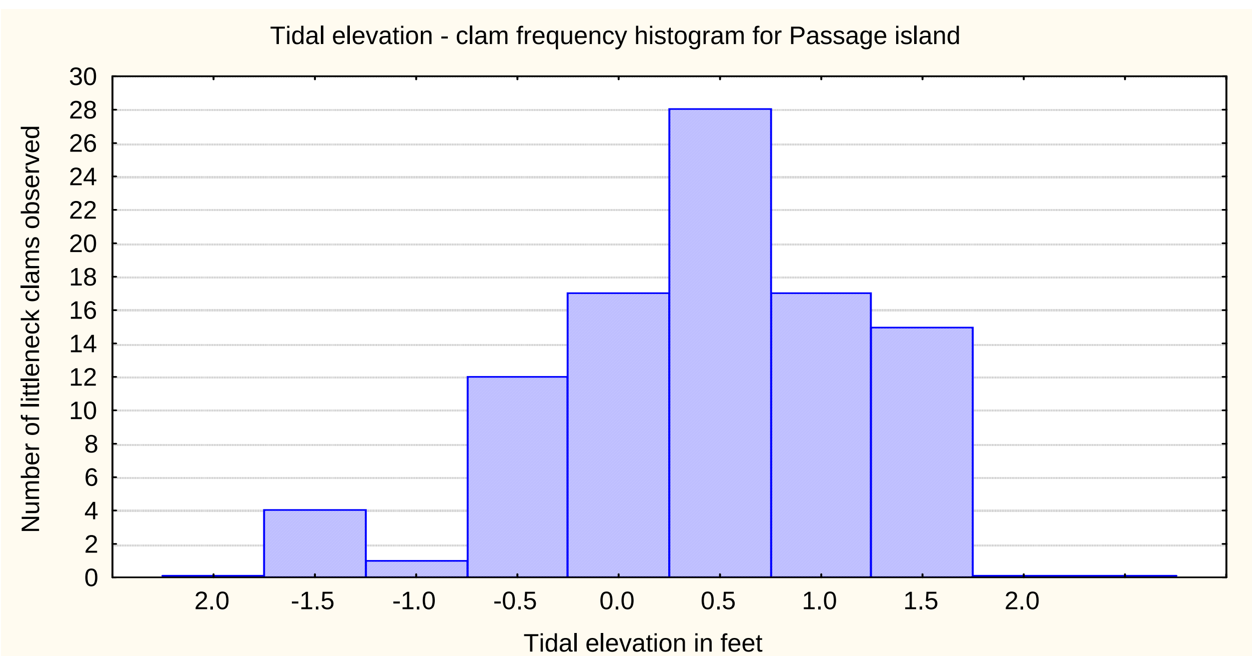


Figure 28. Tidal elevation – frequency histogram for littleneck clams collected in 18, 0.1 m^2 quadrats at Passage Island on August 26, 1995.

3.2.4. Environmental influence on clam size, age and growth. Parameters with variation were included in a square matrix of Pearson correlation coefficients. This matrix indicated that biological parameters such as total valve length, mean annual growth increments, whole-animal weight, wet tissue weight and condition factor were not significantly ($\alpha = 0.05$) dependent on environmental factors within the tested strata. This conclusion is consistent with that at Tatitlek.

3.2.5. Native littleneck clam growth as a function of age and length. Average annual growth increments were calculated by dividing the total valve length by clam age and examined as a function of age. Incremental growth of native littleneck clams at Passage Island is described in Figure (29). Some clams in the 10 to 15 mm size range appeared to have achieved that size in a single year. In other clams of the same size, an apparent annulus appears at about 1.5 mm, suggesting minimal growth during the first year. Perhaps those clams were spawned late in the year and over-wintered just after metamorphosis. The larger clams, without the check at 1.5 mm, may have spawned early in the spring or summer and enjoyed an entire growing season before winter. This could explain the large variation observed in growth increments for the one-year-old clams.

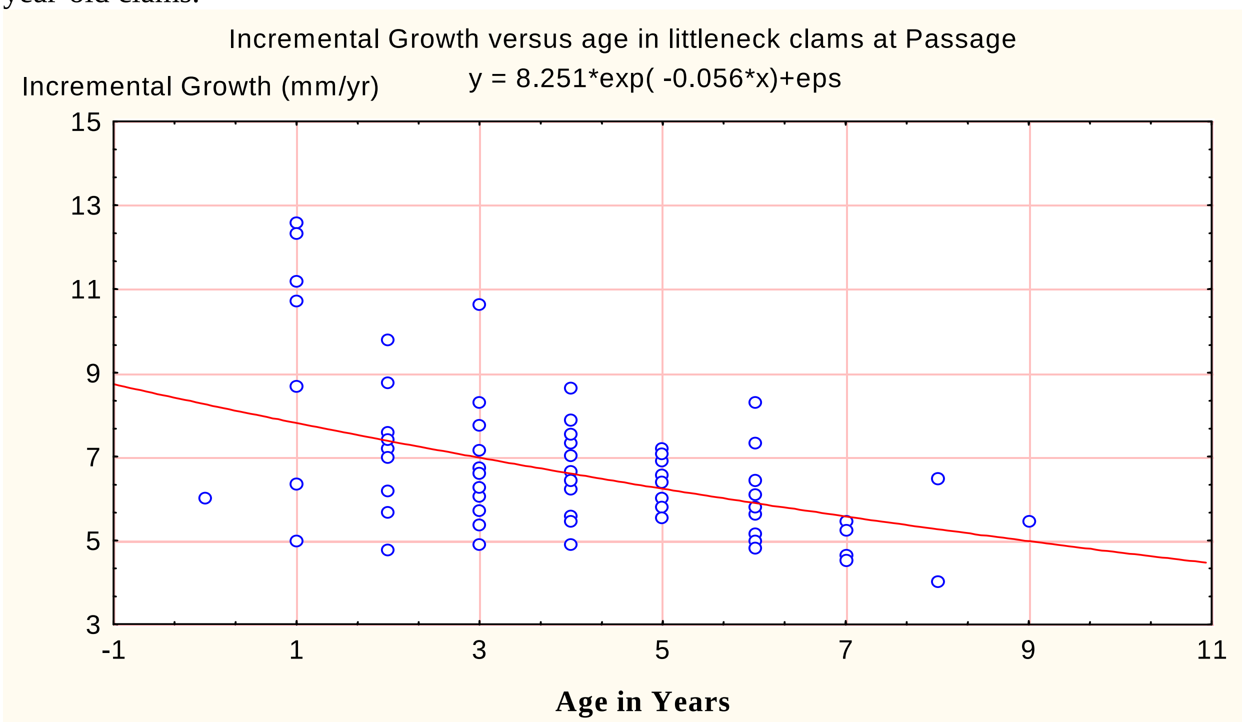


Figure 29. Average annual growth increments (mm/year) as a function of age (years) for native littleneck clams (*Protothaca staminea*) collected in 18, 0.1 m^2 quadrats at Passage Island, Alaska on August 26, 1995.

The data in Figure (29) suggests that incremental growth in valve length decreases significantly after age six. The average incremental growth methodology used in this analysis underestimates the reduction in growth as a function of age. Furthermore, it should be noted that native littleneck clam valve shape changes with age. The clams depth and width increases more that the length increases in older clams. Therefore, wet tissue weights continue to increase significantly in older clams, even though growth in valve length slows. This was nicely demonstrated by Nickerson (1977) who showed peak rates of length increase occurred at about three years of age in littleneck clams while peak increases in biomass occurred at an age of between six and seven years.

Within the area surveyed on Passage Island, clam growth does not appear to be a function of tidal height. The observed growth increments are plotted against tidal height in Figure (30). The regression coefficients were not statistically significant.

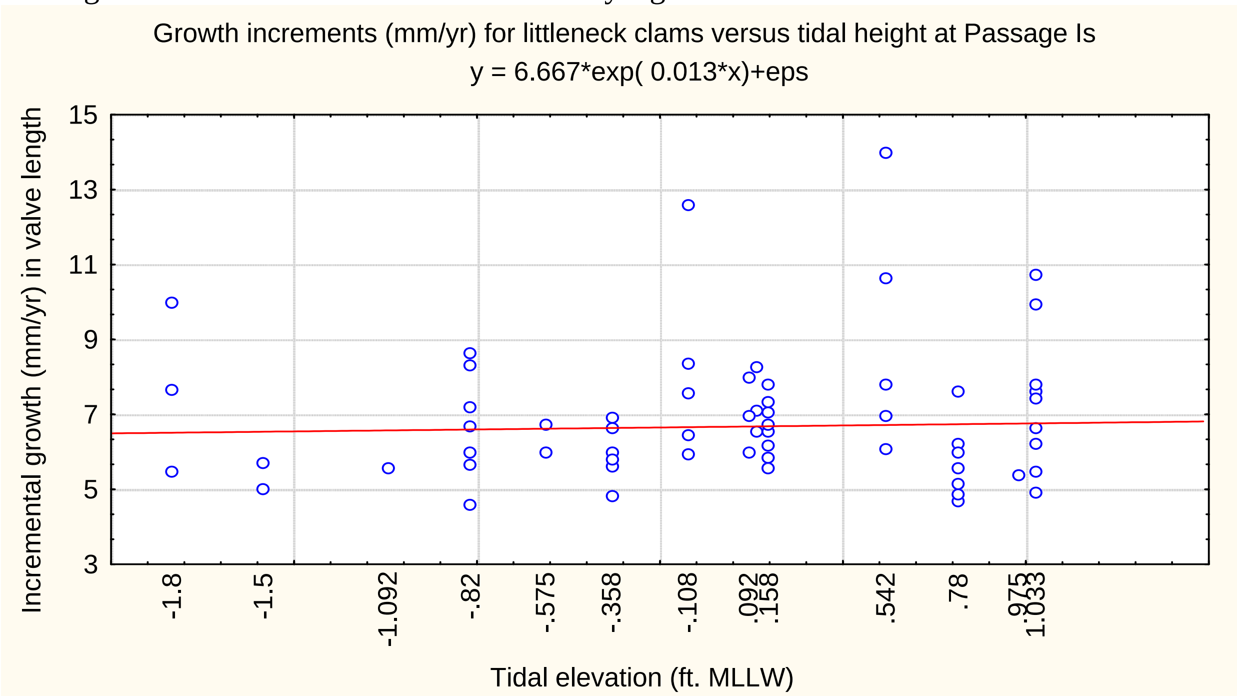


Figure 30. Growth increments (mm/year) as a function of tidal height (feet above MLLW) for native littleneck clams (*Protothaca staminea*) collected in 18, 0.1 m^2 quadrats at Passage Island, Alaska on August 26, 1995.

3.2.6. Native littleneck clam age – length analysis at Passage Island.

Regression coefficients were developed for the von Bertalanffy model using nonlinear regression. The resulting equation explained 81.2% of the variation and the ANOVA determined probability that the regression coefficients were all equal to zero was P = 0.000. The residuals were normally distributed. A full range of clam valve lengths was available for the analysis and it appears valid. Predicted and observed values of valve length, as a function of age, are presented, together with the regression line in Figure (31). This equation was solved for a length of 38 mm to obtain an average age of recruitment into the legal size population of 5.76 years. This was approximately one year longer than was required for butter clams at Passage Island.

$$\text{Length of native littleneck clams at Passage Island (mm)} = 56.45(\exp^{-0.194*\text{age in years}})$$

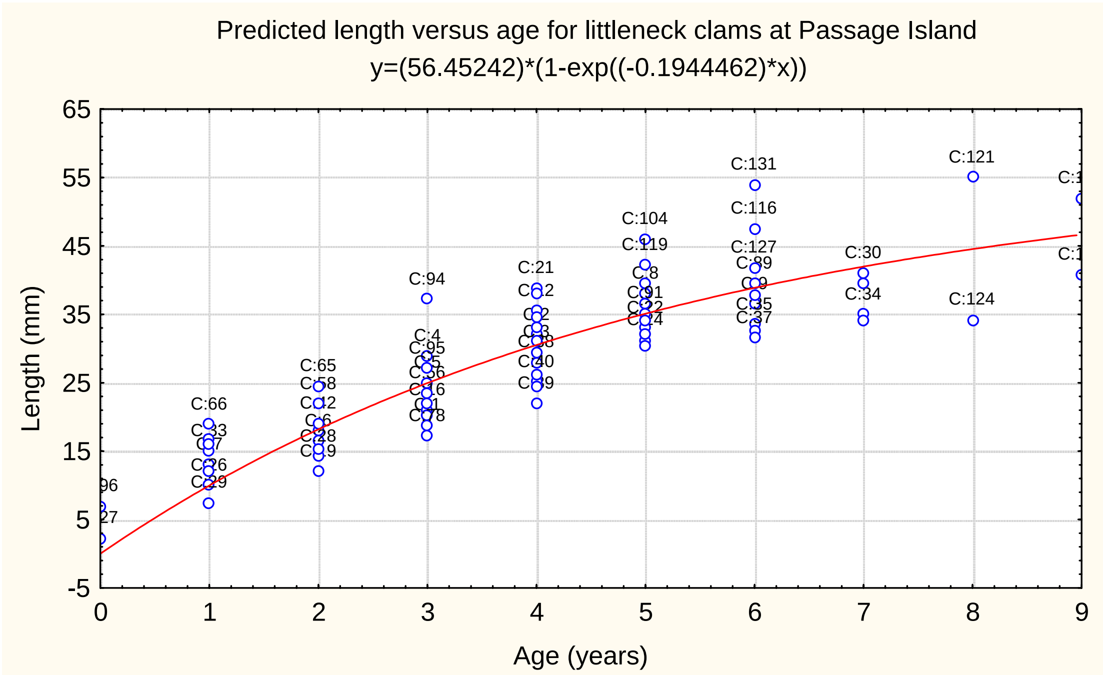


Figure 31. Valve length (mm) as a function of age (years) for native littleneck clams (*Protothaca staminea*) collected in 18, 0.1 m^2 quadrats at Passage Island, Alaska on August 26, 1995.

3.2.7. Edible native littleneck clam tissue versus clam length analysis. A length – wet tissue weight histogram is provided in Figure (32) and an age – wet tissue weight histogram in Figure (33). We tissue weights were increasing exponentially near the minimum legal size suggesting that harvest should be delayed as long as predation allows.

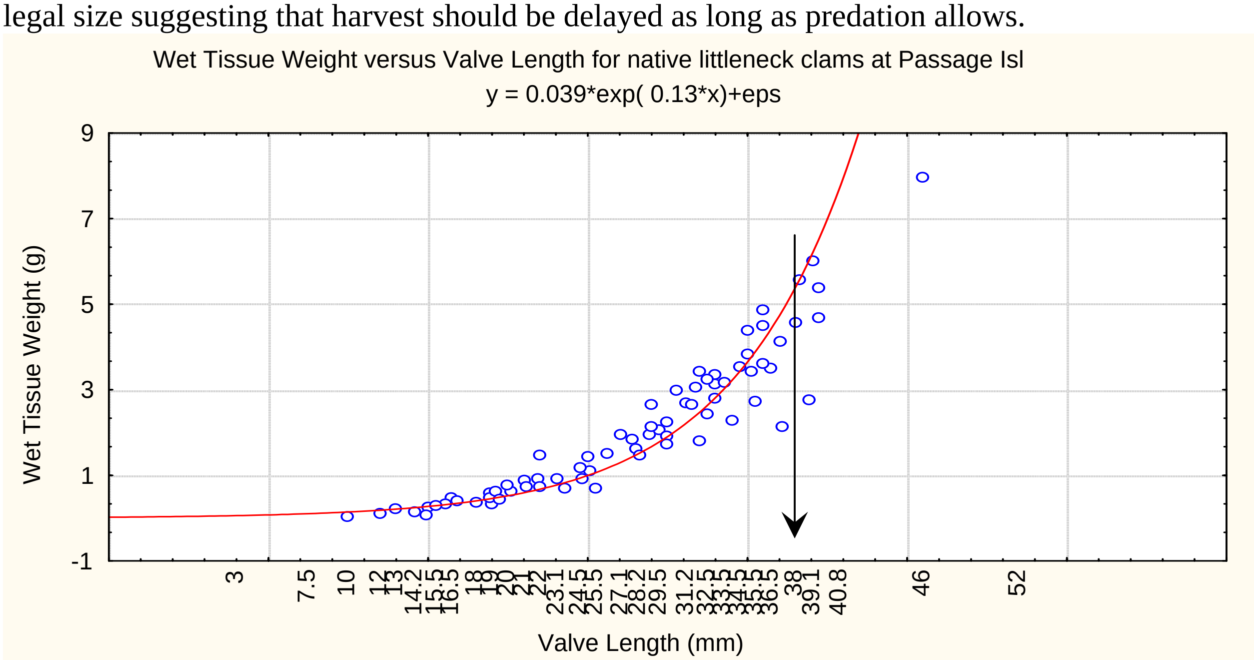


Figure 32. Length (mm) versus wet tissue weight (grams) for native littleneck clams (*Protothaca staminea*) collected in 18, 0.1 m^2 quadrats at Passage Island on August 26, 1995. The vertical solid line represents the minimum legal size.

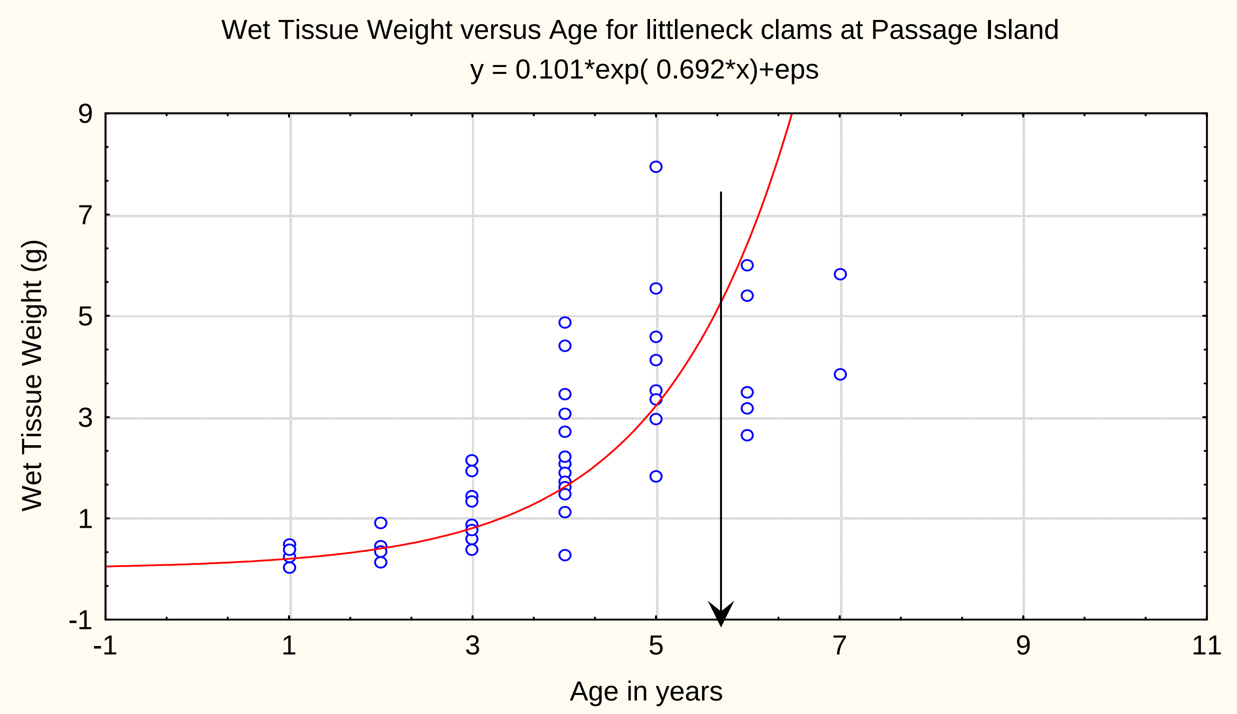


Figure 33. Age (yr) versus wet tissue weight (g) for native littleneck clams (*Protothaca staminea*) collected in 18, 0.1 m^2 quadrats at Passage Island on August 27, 1995. The vertical solid line represents the minimum legal size.

An examination of the data density in Figure (24) suggests a decrease in the rate of accumulation of wet tissues beyond an age of six years. However, there are too few data points for clams larger than 38 mm valve length to have confidence in this hypothesis and the available data suggests that growth is exponential to at least six years.

3.2.8. Predators at Passage Island. Large numbers of predators were not observed at Passage Island. Small drills (cf. *Nucella lamellosa*) were observed, as were numerous (100's) of very small (<4 mm) drilled clamshells similar to those observed at Tatitlek (see Figure 11). Numerous, small round pits (approximately 0.6 meters in diameter and 15 centimeters deep) were found on the beach. These pits were very similar to those observed at Tatitlek and may have been associated with either sunstar or sea otter predation. Villagers' assured us that these pits were from sea otters and that no harvests had been conducted at this site for several years.

3.2.9. Bivalve biomass available for subsistence harvests. The numbers of clams observed on Passage Island are insufficient to warrant subsistence harvests.

3.2.10. Summary conclusions and recommendations for native littleneck clam enhancement at Passage Island. The following conclusions and recommendations are based on this survey:

- Insufficient bivalve resources were found on this beach to warrant subsistence harvests.

- Sustained subsistence harvests will require additional seed, development of effective predator control measures, and an appropriate management plan. Optimizing solutions to these problems will require site specific studies to develop an understanding of clam growth and mortality, effective predator controls and tidal elevation versus culture depth requirements to prevent freezing during cold winter night-time low tides.

- It should be emphasized that any enhancement plan must solve the currently unacceptable predation rates on shellfish stocks. Without effective predator control, any enhancement plan will be futile. The remoteness of Passage Island from Nanwalek will make future study or enhancement activities difficult.

- The age length analysis suggests that native littleneck clams recruit into the legal size population at approximately 5.75 years. The wet tissue weight – length and wet tissue weight – age analysis indicates that harvesting at a valve length less than 38 mm would be an inefficient use of the resource.

- Growth of butter clams appeared to be somewhat faster than for native littleneck clams at Passage Island. Butter clams appeared to enter the legal size population at approximately 4.75 years. Very few had survived to harvest size on the date of this survey. Due to the lack of hatchery and nursery technology, and propensity to retain brevetoxins, butter clam enhancement is not recommended at this time.

- The high exposure of this site to wind and waves implies that an enhancement plan should include implementation of options such as bags or plastic netting that help stabilize substrates. Otherwise, seeded clams will either be washed out of the sediments or buried. However, these more intensive practices require regular maintenance if they are to be effective. The remoteness of Passage Island from the village of Nanwalek will make winter maintenance difficult.

3.3. Results for Murphy's Slough near the native village of Port Graham.

Subsistence shellfish resources near Port Graham were discussed with Mr. Pat Norman and local salmon hatchery personnel. Typical of other villages, Mr. Norman expressed a sincere interest in re-establishing a subsistence clam fishery near Port Graham. Village residents felt that the recent decline in shellfish production was caused by the 1964 earthquake and an increase in the sea otter population. A once plentiful cockle population had disappeared and Mr. Norman was particularly interested in re-established a cockle fishery. The bivalve inventory and beach characterization was accomplished on August 25 and 26, 1995 during a predicted low tide of –1.3' MLLW.

Port Graham shellfish culture team

3.3.1. **Beach characterization.** Initially, two beaches in Duncan and Tulcan Sloughs were identified for survey. Test digging on the evening of August 25, 1995 found few butter or native littleneck clams in these sloughs, which were expansive, relatively shallow, and crisscrossed by several streams. The substrate was composed of moderate quantities of fines (silt-clay) and significant quantities of small (<3 to 5 cm) broken shale. The angular nature of the shale resulted in a more tightly packed substrate that would likely inhibit the burrowing of bivalves. No butter or littleneck clams were found anywhere in Duncan or Tulcan Sloughs. The clam in greatest abundance was the truncated softshell (*Mya truncata*). These clams were 4 to 6 cm in valve length. They could provide the basis for a very limited subsistence shellfishery. However, this species does not appear to be prized and the clam density was very low – making subsistence harvests difficult.

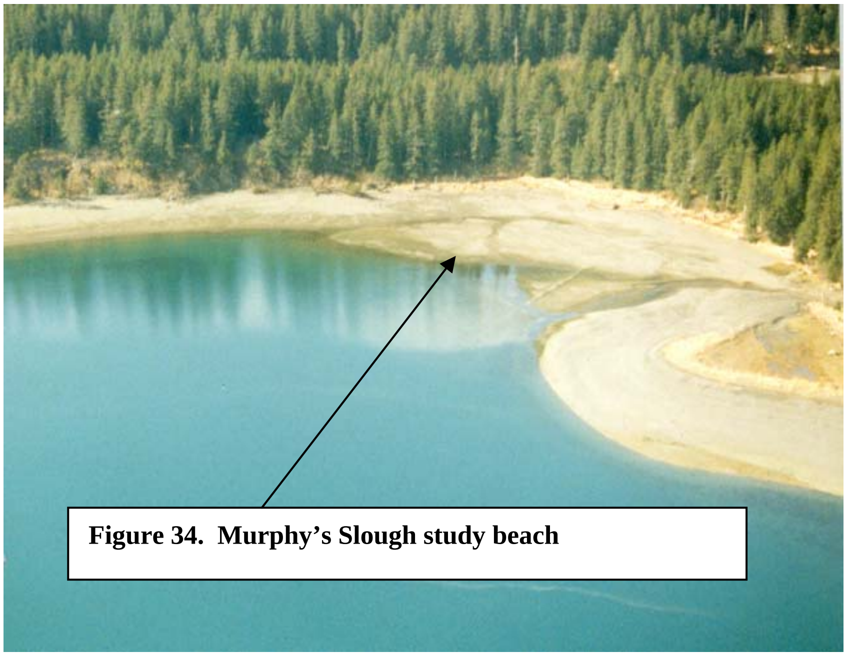


Figure 34. Murphy's Slough study beach

Because of the paucity of shellfish in Duncan Slough, a beach located around the point to the east of Tulcan Slough called Murphy's Slough (Figures 34 and 35) was surveyed. This beach measured 200' wide by 1000' long and covered approximately five acres. The beach slopes gradually into deep water and was very well protected from storm winds. The substrate was composed of moderately coarse, broken, shale. It was not compacted and a significant quantity of pore water was present. Murphy's Slough appeared to be a good beach for shellfish enhancement or intensive culture. Figure (34) is an aerial photograph of the beach; Figure (35) describes the substrate; and Figure (36) is a schematic diagram describing the systematic random sample used to evaluate this beach.

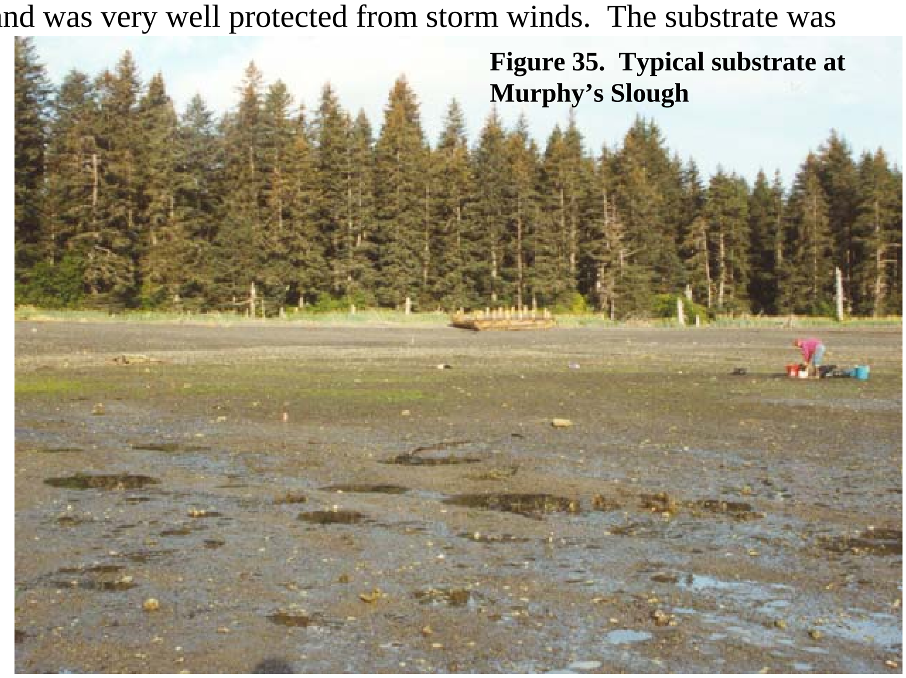


Figure 35. Typical substrate at Murphy's Slough

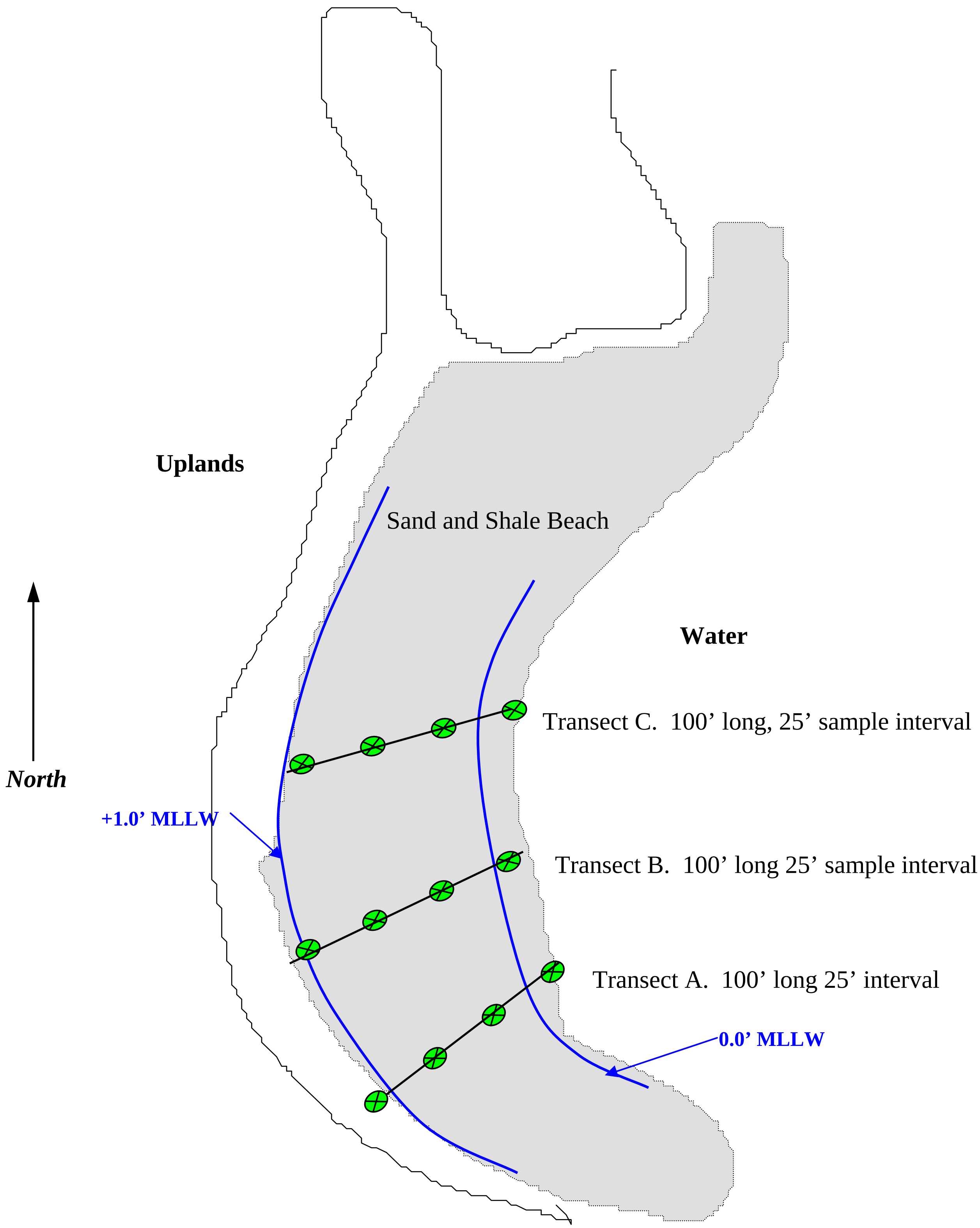


Figure 34. Schematic diagram of the Port Graham Village shellfish beach at Murphy's Slough. The beach has surveyed in August of 1995. The 12 each 0.1-m2 samples collected during the survey are identified in green.

Four sediment samples were collected on each transect (12 total) and sieved on 1.0 mm screens to identify clams. In addition, four sediment samples were collected from randomly chosen sample stations for physicochemical analysis. The beach considered suitable for native littleneck clam production had a moderately shallow slope (3.9%) and the substrate was essentially homogeneous throughout the survey area. Sediments were loosely packed and contained significant amounts of pore water. They were well oxygenated to a depth of greater than 20 cm. Murphy's Slough beach sediments were composed of 66.8 $\pm$ 27.8 % gravel, 21.3 $\pm$ 22.3% sand, 11.9 $\pm$ 5.6 % fines (silt and clay) and contained 1.21 $\pm$ 0.99 percent volatile solids. Sediment composition at this beach was considered suitable for native littleneck clam culture.

3.3.2. Water column characterization. The water's temperature was cooler than at other beaches (10.8 ^{o}C) and the salinity was 29.5 ppt. Dissolved oxygen was unexpectedly low at 7.9 ppm. Currents near slack flood tide were measured parallel to the beach at less than three cm/sec. This slough is located near the head of Port Graham. Strong currents are not anticipated and shellfish growth may be inhibited by an insufficient volume of phytoplankton rich water flowing over the bed. The three water samples collected at this beach averaged 15.2 mg-L^{-1} TSS and 4.6 mg-L^{-1} TVS suggesting a moderate quantity of inorganic and organic material in the water column.

3.3.3. Bivalve population characterization. Shellfish were not abundant at this site and only 65 living bivalves were collected in twelve systematic random samples at Murphy's Slough near Port Graham. An additional 41 empty bivalve shells were collected at random locations on the beach. The distribution of clams obtained from the systematic survey is provided in Table (12).

Table 12. Summary of living bivalves collected in 12, 0.1 m^2 samples from Murphy's Slough on August 26, 1995.

Species	Number
Macoma inquinata (indented macoma)	2
Saxidomus giganteus (butter clam)	39
Macoma nasuta (bentnose macoma)	17
Mya truncata (truncate softshell)	4
Clinocardium nuttallii (Nuttall's cockle)	2
Other	1

Gaper, butter and native littleneck clams and cockles have potential as subsistence shellfish resources. Local villagers stated a preference for butter clams, native littleneck clams and cockles. Of these, only the butter clam was found in Port Graham. However, all of the 39 butter clams collected were new recruits with valve lengths of less than 3.5 mm. The deposit feeding bentnose clam, *Macoma nasuta* prefers sandy sediments and tolerates low levels of dissolved oxygen. Most of the relatively large (to 38 mm) clams were of the genus *Macoma*. This species is not considered a valuable human food. The four softshell clams collected in these samples ranged in size from 27 to 51 mm valve length. However, their abundance was too low to warrant subsistence harvests.

Approximately 20 acres of what Port Graham residents identified as traditional shellfish beaches were examined in this survey. Clams were essentially absent from Duncan slough and Tulcan slough. Traditional subsistence species were essentially absent from Murphy's Slough. A third beach located approximately three nautical miles east of Murphy's slough was also investigated. This beach was small and was qualitatively sampled by digging a large area. Only a few butter clams were recovered together with perhaps two-dozen soft shell clams.

No beaches currently supporting a subsistence fishery were identified in this survey. Native littleneck clams were absent in nearly all samples and only two cockles were observed. A small number of butter clam recruits were observed at Murphy's Slough. Because of the paucity of clams taken in Port Graham, a meaningful analysis of the population is not possible.

The head of Port Graham is relatively shallow and contained an extensive intertidal area that appeared suitable for clam production. Because this survey was conducted on a marginal low tide, the suitable substrate lying at elevations less than –1.3' MLLW were not surveyed. It is possible that subsistence quantities of clams are available at these lower elevations. The following comments regarding the paucity of shellfish resources in Port Graham are offered in light of that caveat.

3.3.4. Summary and conclusions for Murphy's Slough near Port Graham.

Based on this survey and analysis, the following conclusions can be reached:

- No harvestable populations of clams were found at the beaches (and tidal elevations) surveyed in Port Graham.

- Several beaches near Port Graham appeared suitable for the intensive culture of clams and cockles. The bottoms were relatively flat and firm. In some areas, the broken shale was well packed making the substrate unsuitable for burrowing bivalves. In others areas, like Murphy's Slough, sediments were loose and contained significant quantities of interstitial water with a very deep RPD. Of all the beaches examined during these surveys, Murphy's Slough presented the best opportunity for cockle enhancement.

- Phytoplankton production and supply may ultimately limit clam production in Murphy's Slough. Brooks (2000c) has described methodologies for assessing the bivalve carrying capacity for small inlets and bays. These methodologies could be applied in Port Graham.

- The lack of native littleneck clams and butter clams in Murphy's Slough appears related to poor or no recruitment of these species. No littleneck clam and few butter clam juveniles were observed. Cockles, once plentiful according to village residents, were nearly absent and no juveniles were observed. The near term re-establishment of a subsistence shellfish resource will require outside sources of seed.

- Like other beaches surveyed in 1995, the intertidal at Murphy's Slough was pock marked with what appeared to be otter pits. There were numerous broken butter clam shells lying next to some of these pits. This suggests that otters may be taking the few clams that reach legal size. Juvenile (< 5 mm) butter clam shells were observed with drill holes in them. However, few gastropods were observed. Predation by starfish and drills did not appear as severe in Murphy's

Slough as it did at Tatitlek. However, adequate predator control or exclusion is recommended as part of any enhancement project.

- Murphy's Slough presents better cockle habitat than any other beach examined in these surveys. In addition to being a preferred food by Villagers, it appears that cockles grow rapidly in Alaska. The few cockleshells that were collected suggest that a minimum legal size of 38 mm could be achieved within perhaps three years. In Puget Sound, Washington, feral populations of cockles (*Clinocardium nuttallii*) appear to reach commercial size ($\geq$ 38 mm) in one to two years (Brooks, unpublished). The combination of cockle and native littleneck enhancement could provide a reasonably short-term supply of cockles and a longer-term supply (within five to seven years) of native littleneck clams. Cockles are not commonly raised in intensive culture and to the best of the author's knowledge; no commercial hatcheries were producing seed. Section 5 of this report describes successful, but preliminary, efforts to produce and nursery cockle seed in hatcheries and to raise them to market size in growout experiments.

- Mussel (*Mytilus edulis trossulus*) seed is present in the upper parts of the intertidal at numerous places near Port Graham. These mussels could be caged and hung from buoys in the bay to provide an almost immediate (one year) supply of shellfish. However, like the other native village residents, mussels do not appear to be a traditional subsistence food source and some experimentation and outreach would be required. The high potential productivity associated with blue mussels should be explored, at least on an experimental basis, by the Port Graham village.

3.4. Recommendations for native littleneck clam enhancement at Tatitlek, Nanwalek (Passage Island) and Port Graham (Murphy's Slough)

3.4.1. Nanwalek and Tatitlek. Passage Island and the Tatitlek beach represent high-energy environments with significant quantities of large rock. Otherwise, they are representative of typical native littleneck clam habitat. Both beaches enjoy high current speeds. These physical conditions offer the promise of relatively fast native littleneck growth. Intensive culture requires areas of relatively uniform substrate from which cobble larger than 7.5 cm has been removed. This preparation required significant hand labor at both beaches. The rock was placed seaward from the test cultures to help retain water during low tides and to encourage recruitment of wild larvae (Toba *et al.* 1992). Tatitlek is recommended for native littleneck clam enhancement – but not for cockle enhancement. Native littleneck clam suitability studies are recommended for Passage Island. However, the remoteness of Passage Island will make sampling difficult, particularly in winter, and maintenance of intensive cultures problematic. Both beaches will require bag culture and/or plastic netting to stabilize sediments.

3.4.2. Port Graham. There are several extensive beaches in Port Graham that could be used for bivalve culture. The beach at Murphy's Slough has an ideal substrate. Future bivalve production will likely be more limited by food (detritus and living phytoplankton) than by the availability of suitable substrates. Preliminary growth and mortality studies to substantiate this areas suitability for bivalve culture should be followed by an analysis of the systems bivalve carrying capacity before planning significant enhancement efforts. Based on this authors experience, small to moderate scale subsistence enhancement efforts (10 to 100 acres) are unlikely to approach the carrying capacity of Murphy's Slough.

3.4.3. Predator Control. Control of starfish and drills is easily accomplished by hand picking and removal to an upland area. No direct or unequivocal evidence of sea otter control predation in intertidal areas was observed during these surveys. However, if it occurs, it may present a significant problem. The literature did not reveal any reference to this subject because intensive bivalve culture is uncommonly practiced in areas with large otter populations.

3.4.5. Harvest management plan. Harvest management of shellfish resources in Alaska is of special importance because of the anticipated slower growth, particularly of native littleneck clams. Individual management plans should be developed by each village to insure that shellfish produced in enhancement projects are harvested in a sustainable way.

Figure (37) presents a scatterplot of all native littleneck clams measured and aged in the 1995 surveys. The scatterplot is fitted with a nonlinear solution to the von Bertalanffy model. The results suggest that native littleneck clams enter the legal population at an age greater than four years and that only half of the clams appeared to reach a valve length of 38 mm by age seven.

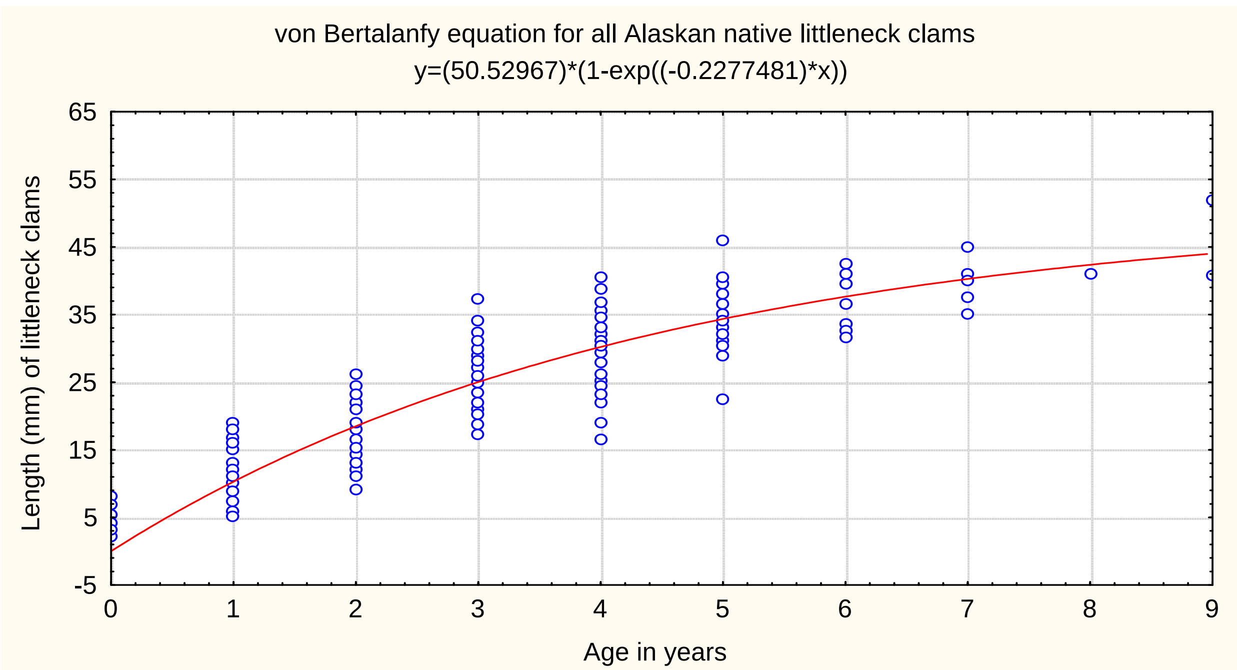


Figure 37. Scatterplot describing length of native littleneck clam valves as a function of age in 1995 samples collected at shellfish beaches near Tatitlek and Nanwalek. A nonlinear solution to the von Bertalanffy model is provided and the resulting regression plotted on the graph.

Feder and Paul (1973) found minor variations in the incremental growth of valves in littleneck clams from Prince William Sound. They found an average age of recruitment into the legal size population of 8 to 10 years. That is on the high end of the 5 to 10 year age at recruitment estimated by ADFG (1995). Figure (37) suggests that native littleneck clams reach a minimum size of 38 mm at an age between five years and >9 years. Solving the von Bertalanffy equation given in Figure (37) for age at a length of 38 mm suggests that the average clam reaches a minimum legal size at 6.12 years of age. These estimates are all similar on the top end but this report and ADFG (1995) suggests that recruitment into the minimal legal size class occurs at an earlier age than suggested by Feder and Paul (1973) or Nickerson (1977).

3.5. Bivalve inventory results for the village of Chenega. Mr. Jeff Hetrick (CRRC) conducted interviews with tribal elders prior to undertaking the 1996 surveys. Based on those interviews, an intertidal area in Crab Bay located at 60^{o} 04.24' N by 147^{o} 59.80' W was selected for inventory (Figure 38). Steve Ward, Gail Evanoff, Vern Totemoff, Meadow Christensen, Kean and Donia from the village of Chenega participated in the bivalve survey. Similar to other villages, residents stated a preference for cockles, butter and native littleneck clams. They noted that traditional shellfish resources had been depleted for several years, for unknown reasons. Chenega had adequate boat and human resources and there was some interest in participating in the study. Village residents expressed more interest in having shellfish to eat. The presence of a CRRC Flupsy in Chenega may stimulate additional interest.

Figure 38. Intertidal area in Crab Bay near the village of Chenega surveyed for bivalves on June 29, 1996.

3.5.1. Beach characterization. The beach surveyed in 1996 is accessible from the village by either boat or four-wheel drive vehicle via an overland route. The survey area is outlined in Figure (38). The total area of the bay is approximately 40 acres. However, an un-named stream enters from the north (Figure 38). Numerous, abandoned stream channels were observed running across a broad expanse of the intertidal. These channels suggested that much of the area was unsuitable for clam culture because of the periodic scouring effect of the stream. The bay contains a patchy distribution of eelgrass (*Zoostera marina*) at tidal levels below ca. – 2.0' MLLW. Numerous excavations, attributed to sea otters by village residents, were observed. Starfish (*Pycnopodia helianthoides*) and drills (*Nucella lamellosa*) were present, but in low numbers. The surveyed area measured approximately 115' wide by 236' deep (Figures 38 and 39). It laid in front of a substantial berm, which was currently carrying the stream well to the east. It appeared to be relatively stable and there was no evidence of recent stream erosion in the surveyed area. Much of the bay's substrate was composed of broken shale that was too hard for burrowing species. The surveyed area contained a suitable mix of fines and gravel for hardshell clams. Beach substrates were biologically active with large numbers of *Nereis sp.* and sipunculids. A preliminary reconnaissance survey supported the author's visual assessment that the chosen area contained the highest abundance of bivalves in this bay.

As described in Figure (39), three transects (A, B and C) were laid out normal to the beach and a fourth transect was examined parallel to the beach at the 0.0' MLLW tide level. Four samples were collected on transects A and D and six samples on Transects B and C for a total of 20, 0.1 m^2 quadrats.

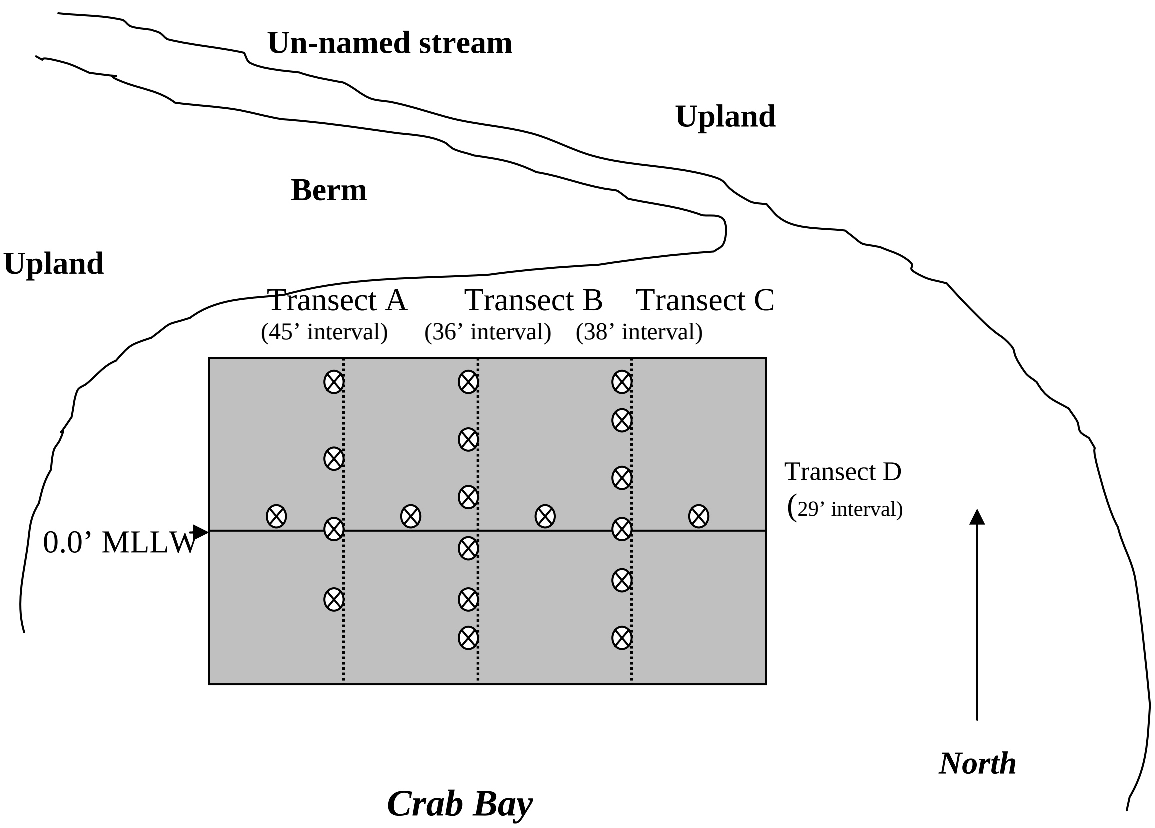


Figure 39. Schematic diagram of the Village of Chenega shellfish beach on Crab Bay. The beach has surveyed on June 29, 1996.

The beach considered suitable for native littleneck clam production had a very shallow slope ranging from 2% along Transect A to 1% along Transect C. The reduction oxidation potential discontinuity was deeper than 10 cm at all stations. Eight sediment samples were evaluated for sediment grain size and total volatile solids. These samples contained 57.5 $\pm$ 8.3% gravel, 33.6 $\pm$ 8.5% sand, 8.5 $\pm$ 2.6% silt and clay, and 2.8 $\pm$ 0.8 percent total volatile solids. Macroalgae (*Fucus* and *Enteromorpha*) contributed to the TVS content.

3.5.2. Water column characterization. Water temperature was 13.8 ^{o}C and salinity varied from 28.0 ppt at Transect (A), located furthest from the stream to 25.0 ppt at Transect C, which was closest to the stream. Currents at slack tide were measured parallel to the beach at 2.5 cm/sec. The pH varied between 7.75 and 7.76.

The three water samples collected at this beach averaged 6.7 mg TSS/L and 3.8 mg TVS/L Turbidity (nephelometric units) varied between 0.69 and 1.00 NTU. These values suggest moderate quantities of organic seston and suspended inorganic particulates. These results provide no basis for eliminating this beach from consideration for enhancement.

3.5.3. Bivalves observed in sediment samples from Crab Bay. One hundred and nine (109) living bivalves were retrieved from samples at Crab Bay. These bivalves are enumerated in Table (13). Clams were not found in sufficient abundance to support subsistence harvests.

Table 13. Summary of bivalves collected in 20, 0.1 m^2 samples at Crab bay near the Village of Chenega on June 29, 1996.

Species	Number
Protothaca staminea (native littleneck clam)	97
Saxidomus giganteus (butter clam)	6
Clinocardium nuttallii (Nuttall's cockle)	6
Total living bivalves	**109**

Butter Clams. Six butter clams were observed in these samples. Their length-frequency distribution is provided in Figure (40). Most of the observed butter clams were new recruits less than two years old. Only one legal size butter clam was observed in the 20 samples. Descriptive statistics for a limited number of variables are presented in Table (14).

Table 14. Summary descriptive statistics for living butter clams retrieved from Crab Bay sediment samples near the Village of Chenega on June 29, 1996.

	Valid N	Mean	Minimum	Maximum	Std. Dev.
Length (mm)	6	12.80	3.82	46.5	16.6
Whole weight (g)	5	4.33	0.14	21.4	9.6
Age	6	2.17	0.00	8.0	2.9
Dry Condition Factor	2	0.38	0.01	0.69	0.44

Non-linear regression was accomplished on aged living and empty butter clam valves to determine von Bertalanffy model coefficients. Residuals were normally distributed (Kolmogorov-Smirnov; d = 0.054; P is n.s. @ $\alpha = 0.05$) and there was no evidence of heteroscedasticity. The resulting equation explained 96.13% of the variation and the ANOVA determined probability that the regression coefficients were all equal to zero was P = 0.000. A broad range of clam lengths and ages were included in the analysis (many of which were measured from empty valves) and the longest clam (123.4 mm maximum length) exceeded the maximum predicted by the von Bertalanffy equation. This expression is likely a good predictor of butter clam length as a function of age.

Length of butter clams in Crab Bay, Chenega (mm) $= 113.5(1 - \exp^{(-0.0672 \text{ x age in years})})$

The paucity of living butter clams with valve lengths $\geq$ 38 mm attests to the lack of a subsistence resource on this beach. It should be noted that despite the fact that most of the observed butter clams were new recruits, recent recruitment was very low at this beach (2.0/m^2 in 1995). Therefore, predator control (especially starfish and sea otters) may have a minor, but positive affect on the number of butter clams eventually available for subsistence harvest.

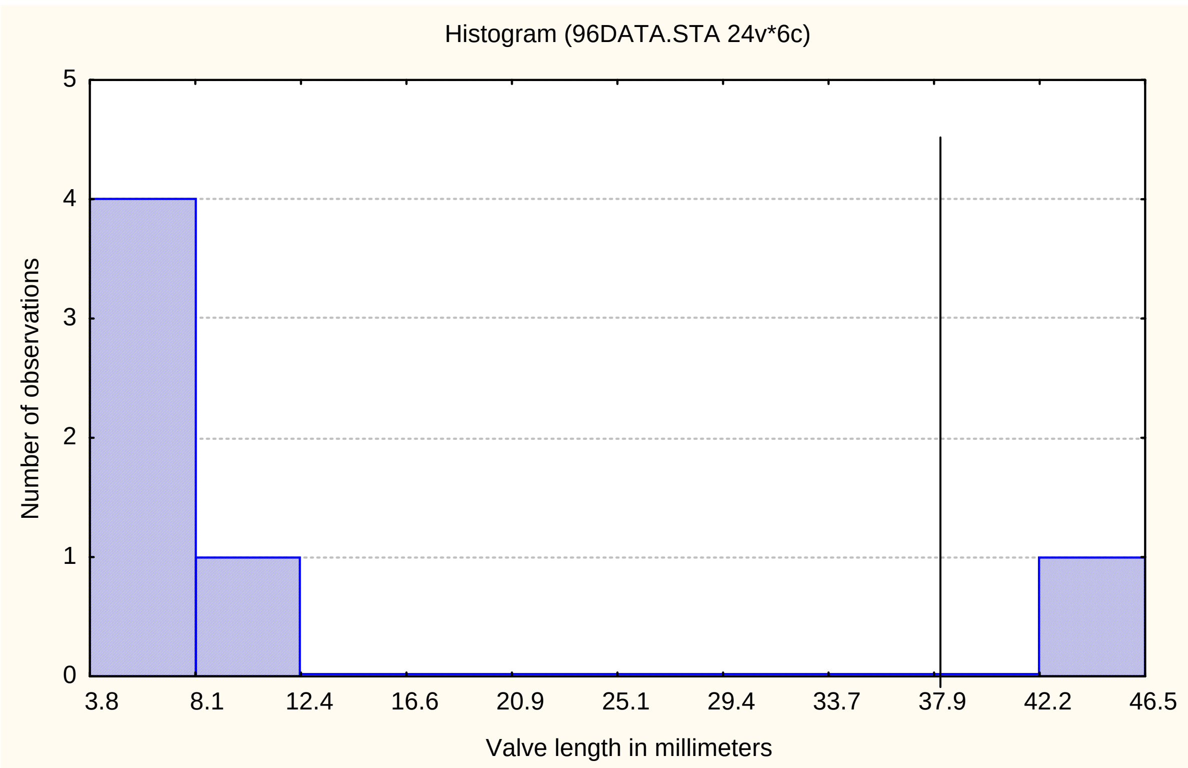


Figure 40. Length frequency histogram for butter clams (*Saxidomus giganteus*) collected in 20, 0.1 m^2 samples at the Chenega Village shellfish beach on June 29, 1996. The thin vertical line locates the legal limit (>38 mm).

Cockles. Six cockles (*Clinocardium nuttallii*) were observed in these samples. Summary statistics are presented in Table (15) and a length-frequency histogram is provided in Figure (41).

Table 15. Summary descriptive statistics for living cockles sampled in 20, 0.1 m^2 quadrats at the Chenega Village shellfish beach in Crab Bay on June 29, 1996.

	Valid N	Mean	Minimum	Maximum	Std. Dev.
Valve length (mm)	6	27.90	11.56	49.09	13.36
Whole weight (gm)	6	7.20	0.26	23.92	8.65
Age (years)	5	2.40	2.00	4.00	0.89
Dry Condition Factor	4	0.34	0.232	0.41	0.08

The largest cockle had a valve length of 49.1 mm and weighed 23.9 grams. Only one legal size cockle (valve length $\geq$ 38 mm) was observed in the 20 samples. There is currently no opportunity for subsistence harvest of cockles at this Chenega Village beach.

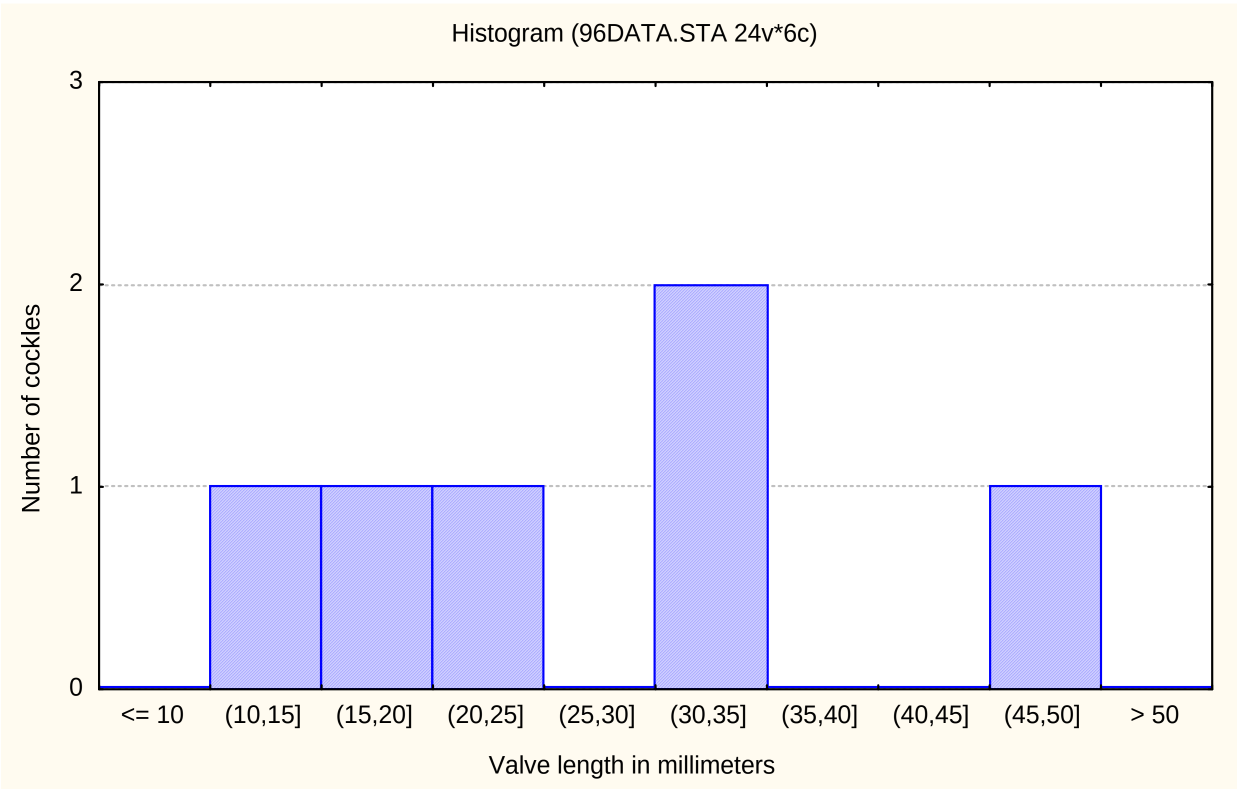


Figure 41. Length-frequency histogram for living cockles (*Clinocardium*) collected in 20, 0.1 m^2 samples during the bivalve survey in Crab Bay near the village of Chenega on June 29, 1996.

Native littleneck clams. Ninety-seven (97) native littleneck clams were observed in Crab Bay sediment samples. Very pronounced circular sculpture, apparently not associated with growth checks was observed in eight of these clams. Summary statistics describing native littleneck clams are presented in Table (16).

Table 16. Summary descriptive statistics for living native littleneck clams sampled in 20, 0.1 m^2 quadrats at the Chenega Village shellfish beach in Crab Bay on June 29, 1996.

	Valid N	Mean	Minimum	Maximum	Std. Dev.
Valve length (mm)	97	21.89	2.63	47.90	7.68
Whole weight (gm)	97	5.64	0.036	25.84	3.77
Age (years)	95	4.00	0.00	7.00	4.41
Dry Condition Factor	82	0.28	0.19	0.40	0.08

Figure (42) presents an age - frequency histogram for Crab Bay native littleneck clams. The native littleneck population is dominated by three and four year old clams that likely settled in 1992 and 1993. Figure (42) suggests that recruitment is sporadic at this site (or juvenile survival is poor). It appears that relatively strong year classes set in 1992 and 1993 but that recruitment since then has been minor. Juvenile clams should be found at a minimum density of 20 to 30 per 0.1 m^2 for optimum production. Current recruitment is estimated at 3.5 per 0.1 m^2 - or about 15% of optimum. This is close to the value of four recruits per m^2 observed at Tatitlek in the 1995 survey (Brooks, 1995).

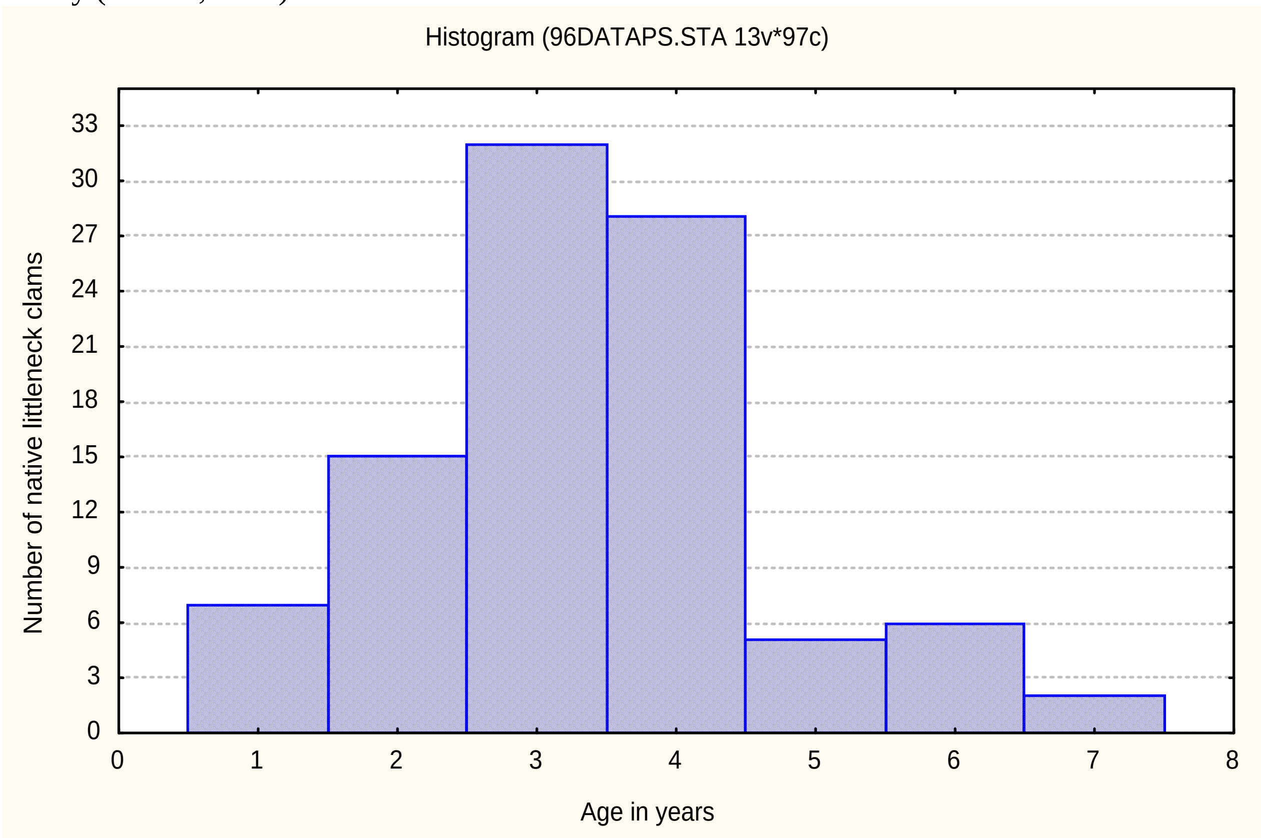


Figure 42. Age – frequency histogram for littleneck clams collected in 20, 0.1 m^{-2} quadrats at Crab Bay on June 29, 1996.

Further examination of the population was accomplished using the length - frequency histogram provided in Figure (43), which indicates that larger clams are being eliminated from the population, either by predation or because of local harvest. Fewer than five legal size littleneck clams (valve length >38 mm) were obtained in the entire survey. Insufficient edible shellfish (butter, native littleneck clam and cockles) are available at this site for subsistence harvests. This suggests that under natural conditions, shellfish production at this site is limited primarily by inadequate recruitment, and perhaps by overharvest or predation.

3.5.4. Bivalve distribution as a function of tidal height. Figure (44) describes the distribution of native littleneck clams as a function of tidal height in Crab Bay. This figure supports previous surveys indicating that the optimum tidal elevation for native littleneck clams is ca. 0.0’ MLLW. It should be noted that the substrate changes to primarily sand at tidal elevations less than -1.5’ at this beach. Therefore, it was expected that native littleneck and butter clams would be absent below this elevation. It is also interesting to note that both butter clams and native littleneck clams were found at tidal elevations near +3.0’ MLLW. The data for native littleneck clams suggests that the area between -1.5’ and +0.5’ is suitable for native littleneck clam production on this beach.

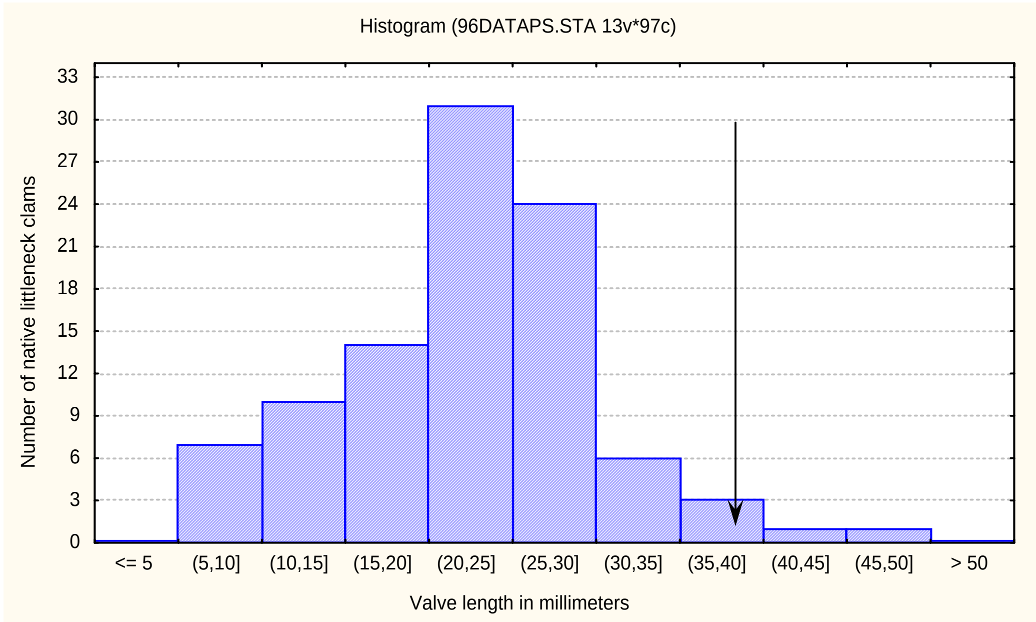


Figure 43. Length - frequency histogram for littleneck clams collected in 20, 0.1 m^2 quadrats at Crab Bay on June 29, 1996. The thin vertical line represents the minimum legal size of 38 mm.

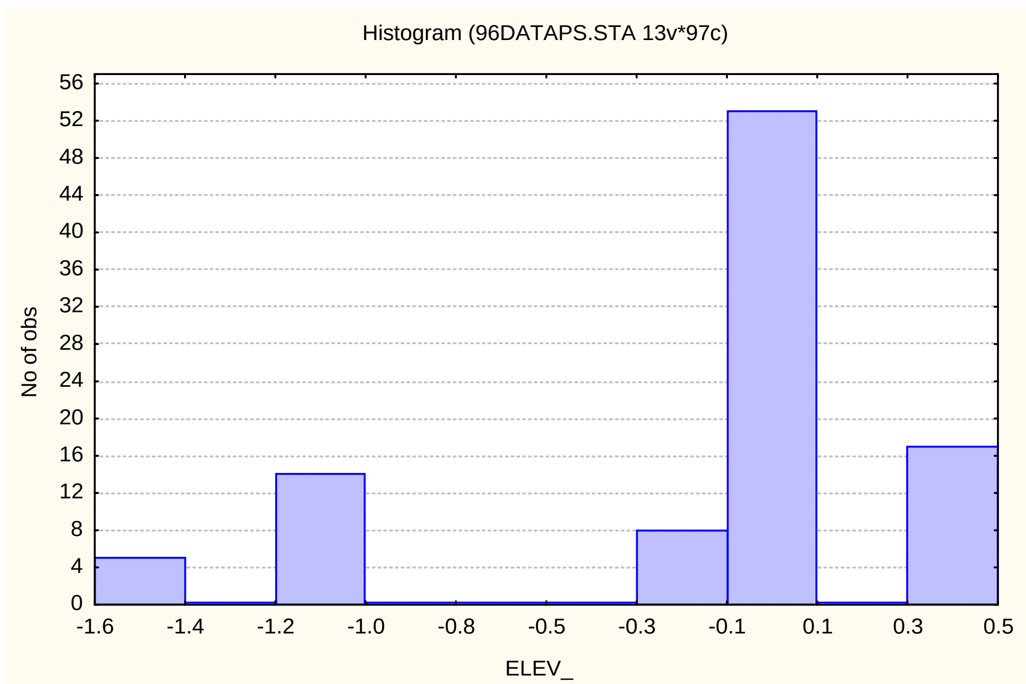


Figure 44. Tidal elevation – clam frequency histogram for littleneck clams collected in 20, 0.1 m^2 quadrats in Crab Bay near the village of Chenega on June 29, 1996.

3.5.5. Influence of environmental factors on growth of native littleneck clams. The physicochemical and biological variables evaluated in this study were included in a square matrix providing Pearson correlation coefficients. This matrix suggested that biological parameters such as length, average annual incremental shell growth, whole-animal weight, wet tissue weight, and condition factor were not strongly dependent on environmental factors within the tested strata. Even though some of the correlation coefficients are significant, the corresponding Coefficients of Determination indicate that they explained a very small part of the variation in dependent physiological variables. This conclusion was supported by cluster analysis, principle components analysis, regression analysis and Analysis of Variance. Only AGE was a truly significant factor effecting clam size, growth and condition. A summary of the most pertinent correlation's is provided in Table (17).

Table 17. Summary of most relevant Pearson correlation coefficients. The probability (p) that the coefficient equals zero is also provided. Significant coefficients (at $\alpha = 0.05$) are bolded. For all variables, the valid number of cases was 88.

	Tidal elevation	Sediment TVS	Salinity
Length	0.013 P = 0.290	**0.088** **p = 0.005**	**0.352** **p = 0.000**
Growth increment	0.009 P = 0.370	0.000 P = 0.990	0.001 P = 0.730
Whole animal weight	0.008 P = 0.410	**0.220** P = 0.000	**0.550** P = 0.000
Age	0.017 P = 0.230	**0.090** **P = 0.004**	**0.310** **P = 0.000**
Dry Condition Factor	0.013 P = 0.290	0.016 P = 0.230	**0.120** **p = 0.001**

Clam length was positively correlated with sediment total volatile solids (TVS) and salinity. There was a moderate size stream flowing into Crab Bay behind a berm lying between the upland and the intertidal. This stream entered the bay to the east where it was having a small effect on salinity during this summer sampling period. It likely has a much larger effect during the winter and spring. In addition, it possibly breaches the berm periodically resulting in a disruption of intertidal sediments, which either buries or exposes clams. There was evidence of several old stream channels meandering across the eastern part of this beach. The presence of this stream likely reduces the number of older clams in its meander plain. This is suggested by the positive correlation between length, whole-animal weight, and age, with salinity in Table (18). The positive correlation between dry condition factor and salinity is likely because higher condition has been observed in older clams and older clams were more prevalent in the western part of the survey area where salinities are highest and the stream has least influence. If the budget had allowed a determination of actual internal valve volume, rather than relying on length, then this correlation would likely not have been as significant. However, it can also be postulated that periodically reduced salinities may reduce feeding times, resulting in the positive correlation between salinity and condition factor.

Physiological parameters (length, wet tissue weight, condition index, whole animal weight) were not significantly correlated with tidal elevation. That is likely the result of the rather narrow intertidal band within which *Protothaca sp.* was found on this beach (-1.6' to + 0.5' MLLW) with the large majority of the littleneck clams being found at 0.0' MLLW.

Average growth increments were calculated by dividing the valve length by the clam's age. This procedure should be viewed as a crude approximation of growth because it does not recognize that incremental growth is negatively correlated with age (r^2_a = -0.16; P = 0.000). However, for purposes of determining the average growth increment as a function of tidal height, it gives a reasonable assessment of the optimum tidal height at which to cultivate clams on this beach. This information is presented graphically in Figure (45). The line represents a best polynomial fit to the data with 95% confidence limits on the mean displayed. Figure (45) suggests that within the tidal range investigated (which includes all elevations at which clams were found in this survey), native littleneck valve growth is acceptable for culture purposes. A decline in incremental growth was observed at tidal elevations below ca. –1.0' MLLW. These observations are consistent with those reported by Brooks (1995b) for beaches near Tatitlek, Port Graham and Nanwalek.

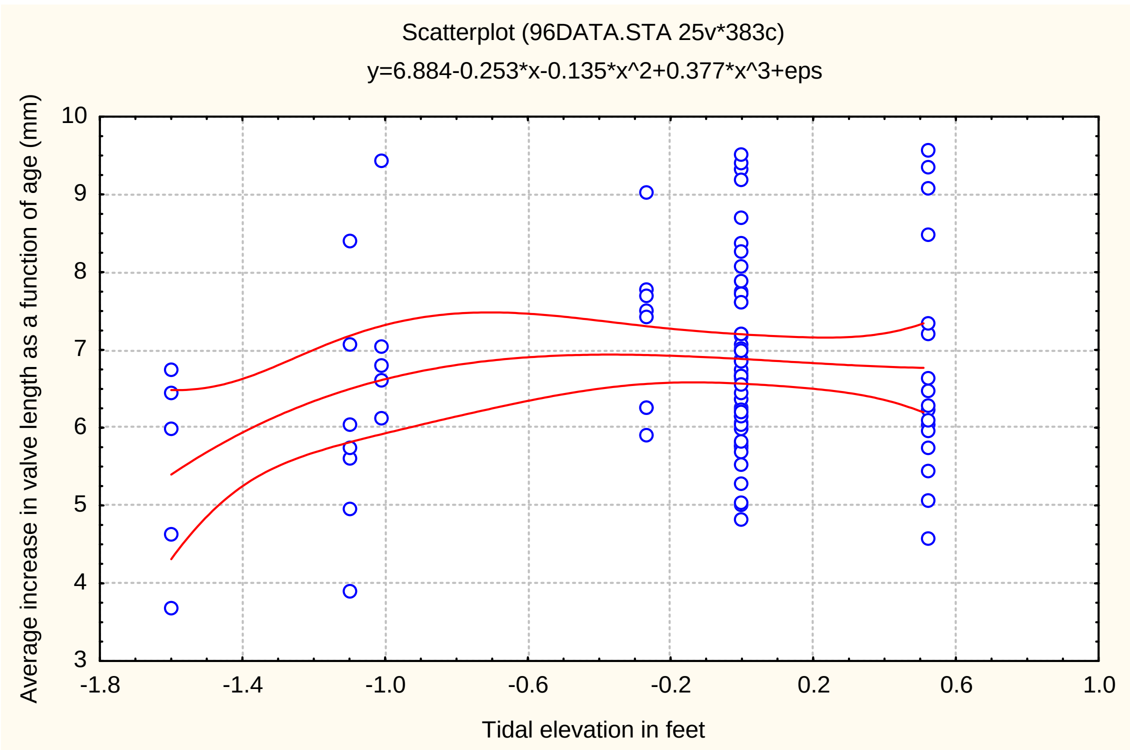


Figure 45. Growth increments (mm/year) as a function of tidal height (feet above MLLW) for native littleneck clams (*Protothaca staminea*) collected in 20, 0.1 m^2 quadrats at the Chenega Village shellfish beach on June 29, 1996. Ninety-five percent confidence limits on regression predictions are provided.

3.5.6. Age at length determination for native littleneck clams at Chenega. Regression coefficients were developed for the von Bertalanffy model using non-linear regression. The resulting regression explained 87.2% of the variation and the ANOVA determined probability that the regression coefficients were all equal to zero was P = 0.000. The regression residuals were not significantly different from a normal distribution (Kolmogorov-Smirnov, d = 0.0508), P is n.s. at α = 0.05). However, some caution is in order because no clam valves exceeding 47.9 mm were included in the database. In Puget Sound, native littleneck clams grow to lengths in excess of 65 mm (Brooks, unpublished). However, native littleneck clams older than seven years were not observed at Crab Bay for unknown reasons. A scattergram, including the regression line is provided in Figure (46). The von Bertalanffy equation, and accompanying scatterplot, suggests that native littleneck clams begin recruiting into the legal size population at six years of age and the average age of recruitment is seven years.

Native littleneck von Bertalanffy model for Crab Bay   Length = 55.9(1 - exp$^{-0.155 \text{ x age in years}}$)

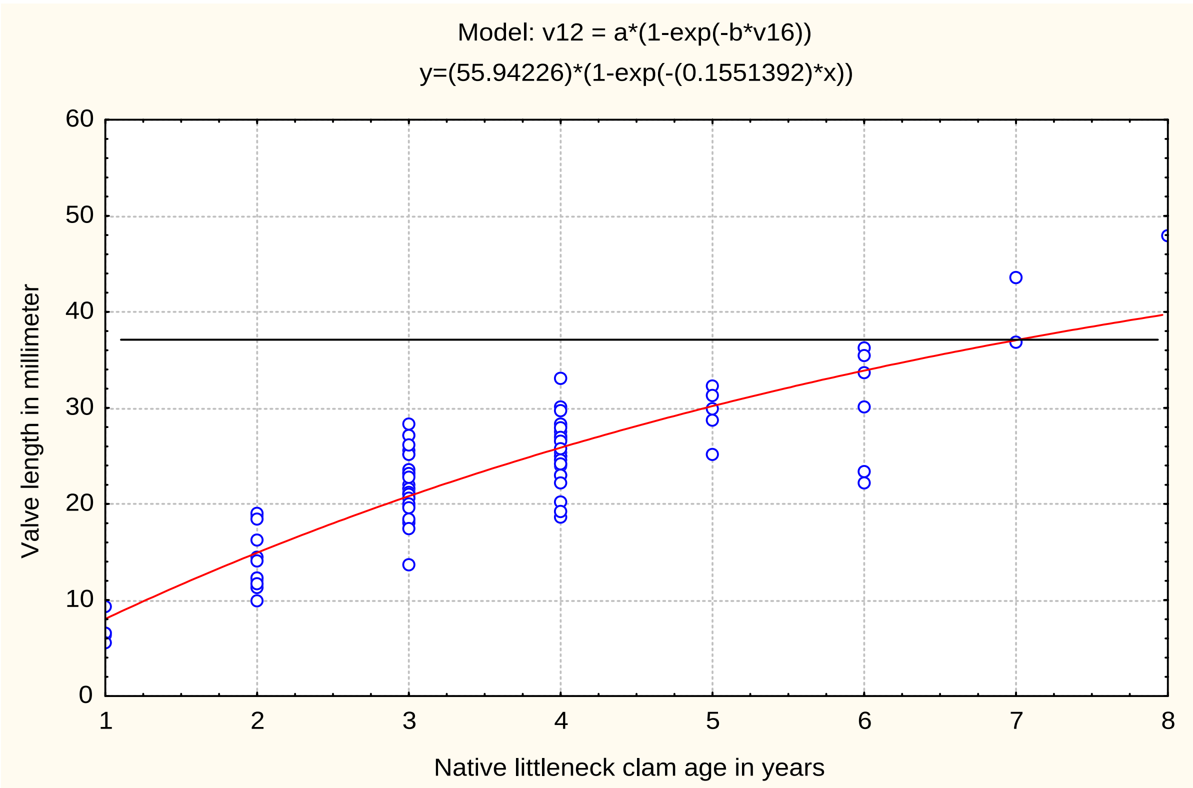


Figure 46. Length (mm) versus age (years) for native littleneck clams (*Protothaca staminea*) collected in 20, 0.1 m^2 quadrats at Chenega Village on June 29, 1996. The solid horizontal line represents the minimum legal size limit ($\geq$ 38 mm).

3.5.7. Wet tissue analysis. A length - wet tissue weight histogram is provided in Figure (47) and an age - wet tissue weight histogram in Figure (48). These results are consistent with those presented earlier and demonstrate that wet tissue weights are increasing exponentially near 38 mm valve length. This suggests that if predation and/or disease can be controlled, then the clams should be allowed to grow to at least 45 mm prior to human harvest.

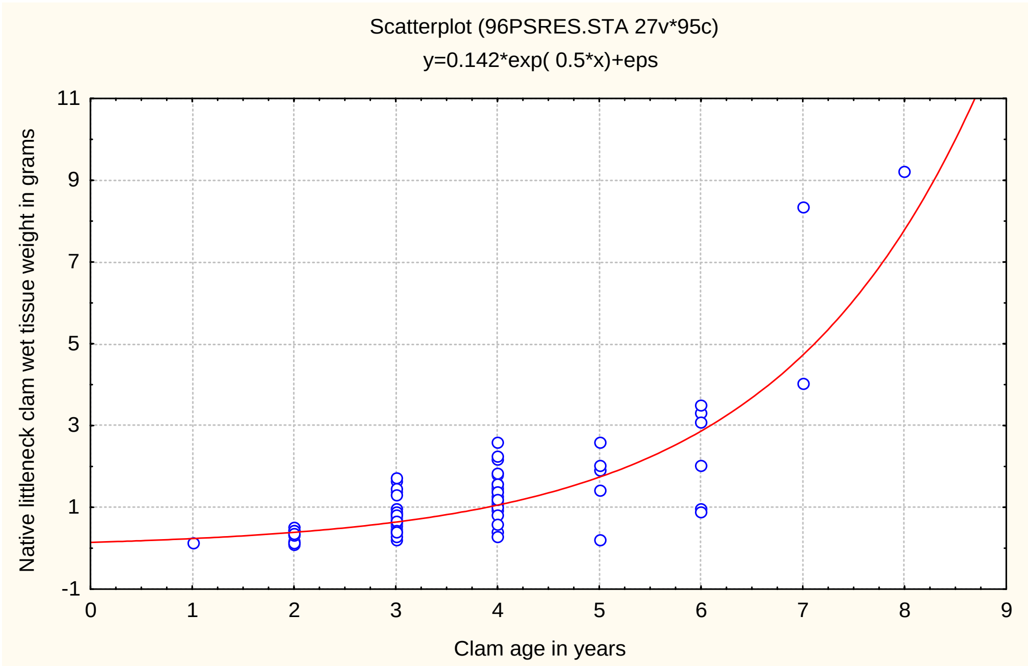


Figure 47. Wet tissue weight (grams) versus age (years) for native littleneck clams (*Protothaca staminea*) collected in 20, 0.1 m^2 quadrats at the Chenega Village shellfish beach on Crab Bay surveyed on June 29, 1996.

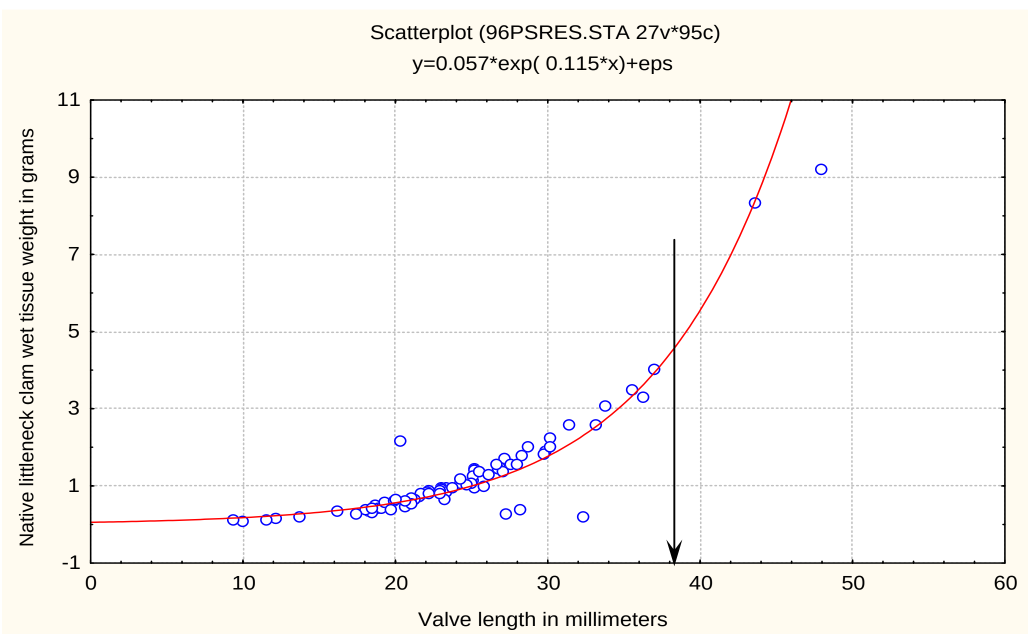


Figure 48. Wet tissue weight (grams) versus length (mm) for native littleneck clams (*Protothaca staminea*) collected in 20, 0.1 m^2 quadrats at the Chenega Village shellfish beach on June 29, 1996. The vertical solid line represents the minimum legal harvest size.

An examination of the length-frequency data provided in Figure (43) suggests that clams are being removed from the population at between four and five years of age and at a size of ca. 30 to 32 mm. Figure (48) indicates that wet tissues are accumulating rapidly between ca. 25 mm and at least 43 mm. A clam that is 8 years old with a valve length of approximately 42 to 45 mm will have wet tissue weights of approximately 7.5 grams. This is significantly higher than the wet tissue weight of 4.5 grams associated with a six-year-old clam just reaching the current minimum harvest size of 38 mm. Reducing the minimum harvest size to 30 to 32 mm (a size preceding the heaviest predation) would result in a harvest of approximately 2.5 grams wet tissue weight per clam. This discussion suggests that reducing the minimum harvest size is not an appropriate management tool to increase the subsistence food value of the existing clam population at Crab Bay. These conclusions are identical to those resulting from an analysis of the Tatitlek, Port Graham and Nanwalek data reported in Brooks (1995b).

3.5.8. Predator density at Chenega. Very few starfish were observed on this beach at the time of the survey. A small number of drills (*Nucella lamellosa*) were present in a patchy distribution throughout the bay. The intertidal associated with Crab Bay was covered with holes approximately 0.5 m in diameter and 15 to 20 cm deep. Village residents noted that some harvesting has occurred there. However, they associated most of the holes with sea otter predation. It was not possible to partition larger clam losses between human harvest and predation based on observation and the information received. However, several areas appeared to have been heavily disrupted.

3.5.9. Shellfish sanitation. Three water samples were collected at Chenega and shipped, on ice to Aquatic Environmental Sciences where they were examined for fecal coliform bacteria using the five tube MPN system. Observed fecal coliform levels were <2 in all three samples indicating no evidence of contamination during the period of this survey. Shellfish enhancement should coincide with the collection of sufficient water samples to certify this beach in accordance with procedures established in the National Shellfish Sanitation Program, Part I.

3.5.10. Summary, conclusions and recommendations for native littleneck clam enhancement at Crab Bay near the village of Chenega. The following conclusions and recommendations are based on this survey and analysis:

- **Shellfish biomass available for harvest.** There is currently no bivalve biomass available for harvest at this Chenega Village beach. The small number of cockles collected during the survey suggests that this species is adapted to this environment and could be cultured, pending development of hatchery, nursery, and grow-out methods.

- **Beach suitability.** The Crab Bay beach contains greater than ten acres of ground suitable for native littleneck clam and cockle enhancement or culture. The physical and chemical parameters examined in this survey are all within acceptable limits. Clam growth, density and size suggest non-significant differences in culture potential over the area of surveyed beach. The small number of legal size clams observed in this survey suggest that both a predator control program and a harvest management plan will be essential to optimizing future harvests. Enhancement of the eastern third of this beach is not recommended because of the potential for disruption associated with a change in the existing stream channel.

- **Predation.** Significant starfish predation was not observed in this survey. Sea otters were not observed preying on bivalves at any beach. The nature of the intertidal disturbances suggests that they were associated either with human harvest or with sea otters. Drills were observed, albeit in low numbers. Any effort at beach enhancement should include a predator watch and removal of starfish, drills, drill egg cases, and crabs. Predation by sea otters should be documented, when and if it occurs. Clam and oyster cages are fairly rigid and capable of excluding starfish, large drills and all but the most aggressive crabs. However, it is unlikely that these plastic mesh cages would discourage a determined sea otter. Caged bivalves should be examined periodically and predators removed before they can consume large quantities of shellfish.

- **Recruitment** of native littleneck clams to the beach on Crab Bay occurred in low numbers in each of the last eight-year classes. No year classes were missing. However, recruitment, or at least survival of juvenile clams until June 29, 1996, was too low and inadequate in each year class to provide for sustained, subsistence shellfish harvests.

- **Age at harvest.** The age length analysis suggests that native littleneck clams recruit to the legal size population at an average of seven years. The wet tissue weight – length, and wet tissue weight – age, analysis indicates that harvesting at a valve length less than 38 mm would be an inefficient use of the resource. This beach would likely benefit from development of a harvest management plan by elders in the Village of Chenega.

- **Butter clams.** *Saxidomus giganteus* recruited in small numbers to this beach. However, few butter clams survived past the juvenile stage. The reasons for this were not determined. Due to the lack of hatchery and nursery technology, and propensity to retain brevetoxins, butter clam enhancement is not recommended at this time.

- **Cockles** are a traditional (and preferred) shellfish for native Alaskans'. The intertidal area of Crab Bay provides suitable substrates for cockle enhancement once culture methods are developed.

3.6. Bivalve inventory results for the village of Ouzinke. The Village of Ouzinke provided a very warm welcome to the CRRC study team. The people of Ouzinke were enthusiastic and eager to participate in this study and expressed a desire for enhanced subsistence shellfish resources. This exuberance carried through to the work at hand, which was undertaken in a professional and dedicated manner. The author whishes to express his sincere appreciation to the following participants who made this survey extremely enjoyable. A special thank-you to my guide, Mr. Roger Larionoff whose knowledge of the local area was invaluable.

Roger Larionoff	Paul Panamarioff	Maria Skonberg	Lylia Pestrikoff
Melody Anderson	David Pestrikoff	Bill Boskofsky	Sandra Muller

The people of Ouzinke expressed a great deal of interest in the intensive or semi-intensive culture of clams and cockles for subsistence purposes. The surveyed beach lies across *Narrow Strait* at a distance of approximately 2.7 kilometers from the village near Precoda Island (locally referred to as Cat Island). It was relatively small, but suitable for culture purposes. The strait and beach are reasonably well protected and should be accessible during most of the year. Numerous other small beaches, suitable for enhancement, were observed near Ouzinke. Brief reconnaissance surveys indicated that several of these beaches currently held harvestable numbers of butter clams (*Saxidomus giganteus*). There was a suitable beach situated in front of the Village. However, the number of people and heavy use suggested that it might not meet National Shellfish Sanitation Program requirements for an *Approved Harvest Classification*. Sea otter predation was not evident on any of the several beaches examined near Ouzinke.

3.6.1. Beach characterization. The surveyed beach is located at 57^{o} 48.12' N and 152^{o} 30.05'W. The area judged suitable as native littleneck clam habitat measured 50 to 70' feet wide by 120 feet long (0.17 acres). It was bounded on the west by a cobble field and on the east by a small stream flowing through fine sediments. Brown kelp (*Fucus cf. distichus* and *Laminaria cf. saccharina.)* was abundant in the nearshore area. The beach contained large quantities of broken butter clam (*Saxidomus giganteus*) shells. No "otter pits" were observed on this beach. Beach substrates consisted of mixed gravel (28 to 51%), sand (44 to 67%), and lesser amounts of silt and clay (5 to 6%). This mix is suitable for native littleneck clams. This beach is not suitable for cockles. However, a discontinuous eelgrass meadow within Camel Bay contained numerous cockleshells and appeared prime habitat for *Clinocardium* enhancement.

Figure (49) is a photograph of the beach. The inset is a silver fox that remained within 10 to 20 feet of the author during sample collection – enticed by an occasional shore crab thrown to him.

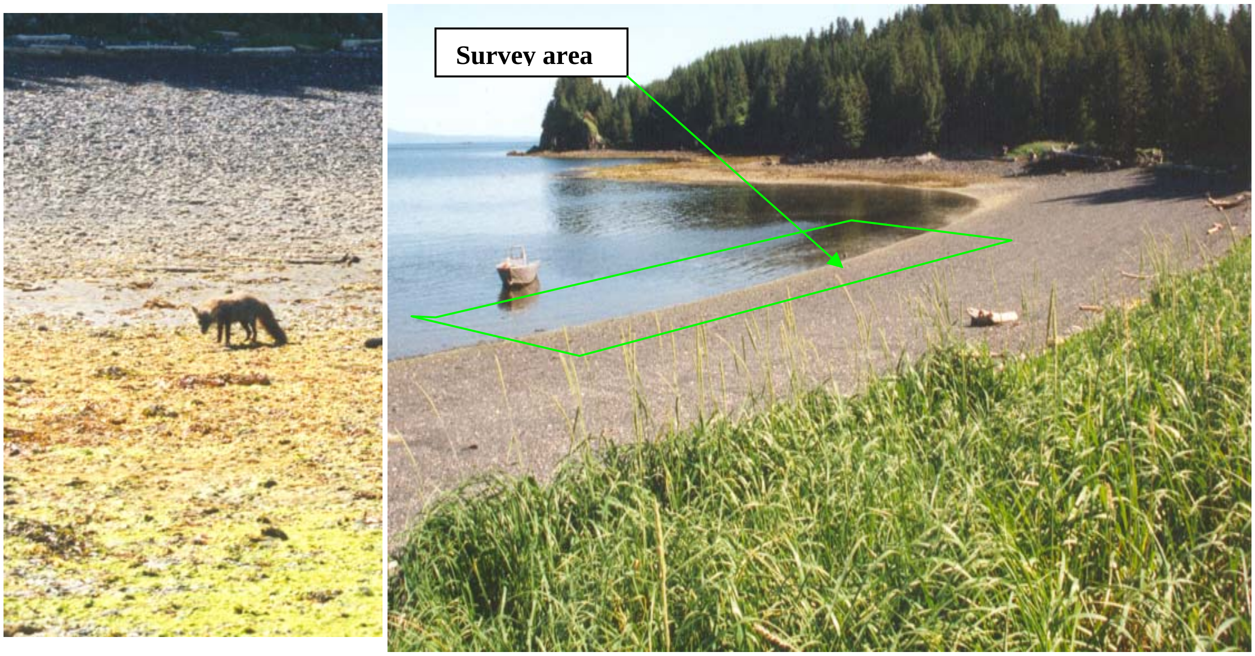


Figure 49. Subsistence harvest beach located on the northern side of Narrow Strait near the native village of Ouzinke. The inset depicts the algae covered substrate typical of this beach.

Figure (50) describes the four transects (A, B, C and D) that were examined in the most suitable clam habitat observed at this beach. Six 0.1 m^2 shellfish samples were collected at 10' intervals (with a random start) along Transects A, B and C. Four 0.1 m^2 shellfish samples were collected along Transect D, surveyed at the 0.0' MLLW tidal elevation. A single sediment sample was analyzed, at a randomly chosen sample station, on each of transects A, C and D. This design resulted in a total of 22 shellfish and 3 sediment samples. In addition, the valves from 22 empty butter, softshell and littleneck clams were collected to supplement the age-length database. Data resulting from the analysis of empty valves was used only to determine coefficients for the von Bertalanffy model.

The beach considered suitable for native littleneck clam production had a shallow slope (2%) and aerobic sediments to a depth of greater than 20 cm. The foreshore consisted of a sand and gravel dunefield that had been stabilized by vegetation. This foreshore separates two embayments. A significant amount of seawater was observed percolating through intertidal sediments in the survey area.

Three sediment samples were evaluated for sediment grain size and total volatile solids. Sediments averaged 41.2 $\pm$ 29.6 % gravel, 53.2 $\pm$ 29.3% sand, 5.6 $\pm$ 1.7% fines (silt and clay) and 1.92 $\pm$ 0.85% TVS. Sediment composition on the surveyed portion of this beach is suitable for native littleneck culture. However, sediments on either side of the surveyed area are either too coarse or too fine to provide optimum culture conditions.

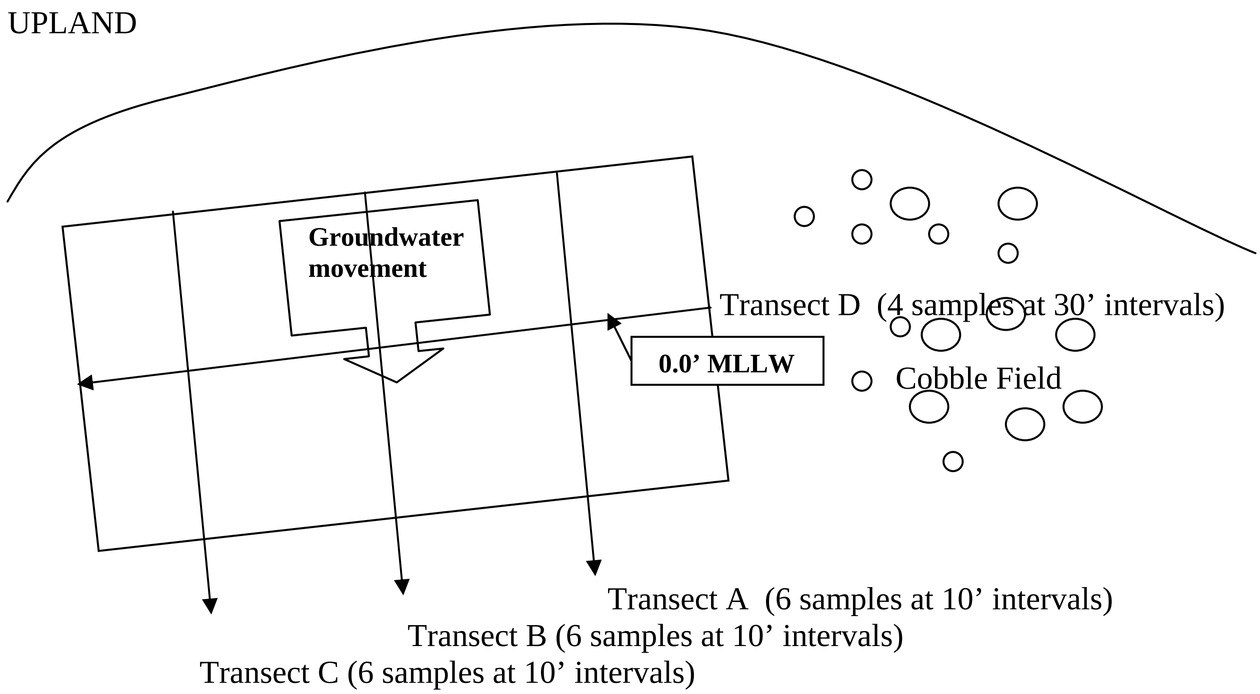


Figure 50. Schematic diagram of the Ouzinke Village shellfish beach located on the southern shore of Narrow Strait. The beach has surveyed on July 2, 1996.

3.6.2. Water characterization. The water temperature was 13.2 ^{o}C and salinity 31.2 ppt. Currents measured on the early ebb tide averaged 3.9 cm/sec and flowed east. The three water samples collected at this beach averaged 6.43 mg TSS/L and 2.33 mg TVS/L. These values suggested moderate to low levels of both primary productivity and suspended inorganic particulates. They do not suggest any reason why this beach would not be suitable for clam enhancement.

3.6.3. **Bivalve population characterization.** Eighty-three living bivalves were collected in the 22 systematic random samples from this beach (Table 18). An additional 19 bivalves were collected in random samples.

Table 18. Summary of bivalves collected in 22, 0.1 m^2 samples at the Ouzinke Village beach at Narrow Strait on July 2, 1996.

Species	Number
Protothaca staminea (native littleneck clams)	19
Saxidomus giganteus (butter clams)	61
Mya truncata (truncate softshell clams)	3

Softshell, butter and native littleneck clams have potential as subsistence shellfish resources. Local villagers stated a preference for butter clams, native littleneck clams and cockles. Of these, only the butter and native littleneck clams were found on the surveyed beach. Large, empty valves of *Clinocardium nuttallii* were observed in an eelgrass meadow and intertidal area at Camel Bay (local name) located three kilometers west of the surveyed beach.

Butter Clams. Sixty-one (61) butter clams were observed in these samples. Over half of the observed butter clams were new recruits less than two years old. Twenty-two legal size butter clams were observed in the 22 samples. Descriptive statistics for a limited number of variables are presented in Table (19). Figure (51) provides a length-frequency summary for butter clams collected during this survey. A vertical line is displayed at the minimum legal size of 38 mm valve length.

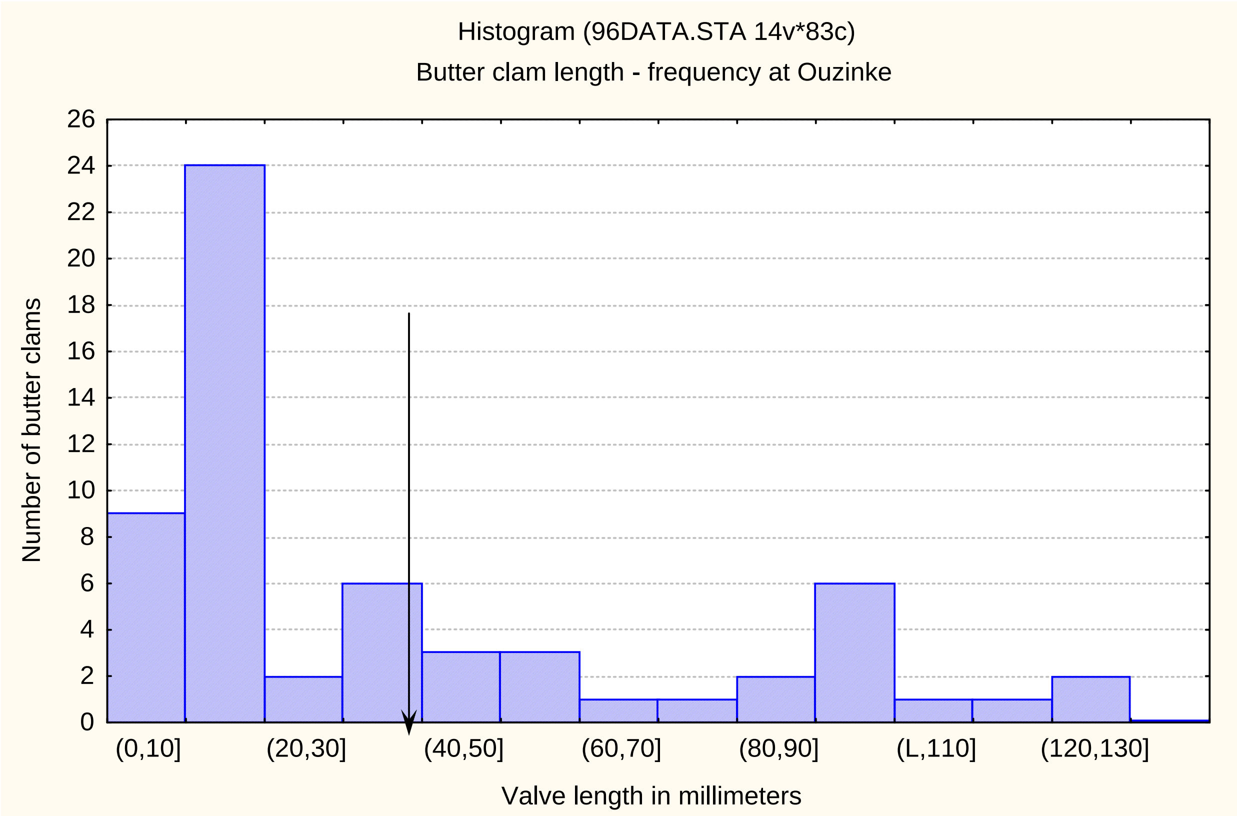


Figure 51. Length frequency histogram for butter clams (*Saxidomus giganteus*) collected in 22, 0.1 m^2 samples at the Ouzinke Village shellfish beach on July 2, 1996. The vertical line locates the legal limit ($\geq$38 mm).

Non-linear regression was accomplished on aged living and empty butter clam valves to determine coefficients for the von Bertalanffy model. The resulting equation explained 94.1% of the variation and the ANOVA determined probability that the regression coefficients were all equal to zero was P = 0.000. Residuals in the analysis were not significantly different from a normal distribution (Kolmogorov-Smirnov; d = 0.087; p = n.s. @ α = 0.05). Observed and predicted values are presented in Figure (52).

The resulting Von Bertalanffy growth equation for Ouzinke is compared with the results from Tatitlek and Nanwalek below. Large clams were not observed at either Passage Island or Tatitlek, but were observed in this survey. The larger asymptotic size predicted for Ouzinke may be due to the inclusion of larger clams in the database or it may reflect reduced predation (or other hypotheses). Living butter clams as large as 123 mm valve length were collected at Ouzinke. However, the valves on several of these were too worn for aging. The smaller coefficient on age suggests that butter clams grow more quickly at Ouzinke than at either Passage Island or Tatitlek or it may result from the inclusion of older and larger clams in this database.

Length of butter clams at Ouzinke (mm) $= 171.3(1 – \exp^{-0.050 \text{ x age in years}})$

Length of butter clams at Passage Island (mm) $= 84.4(1 - \exp^{-0.126 \text{ x age in years}})$

Length of butter clams at Tatitlek (mm) $= 126.5(1 - \exp^{-0.075 \text{ x age in years}})$

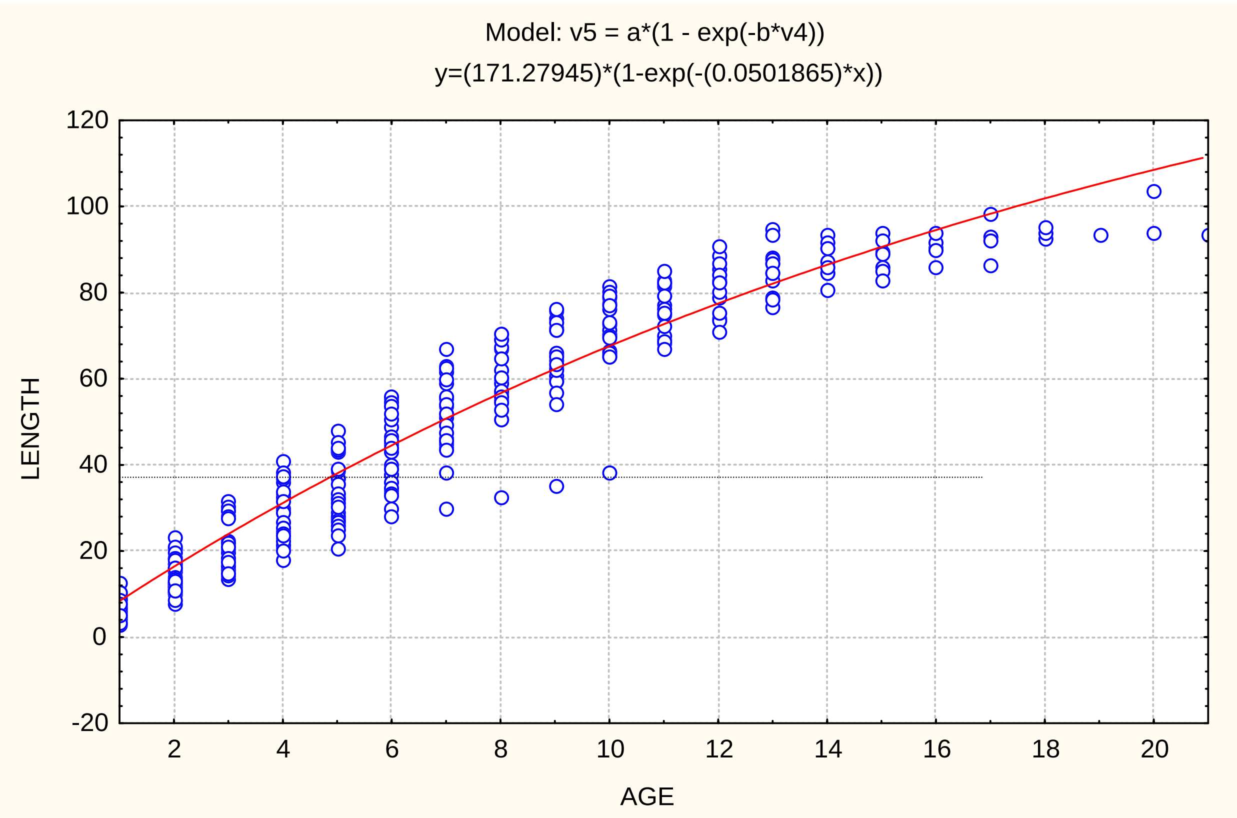


Figure 52. Solution to the von Bertalanffy model for butter clams collected in 22, 0.1 m^2 samples at the Ouzinke Village shellfish beach on July 2, 1996. The horizontal line represents the minimum legal size (38 mm).

Table 19. Summary descriptive statistics for living butter clams sampled at the Ouzinke Village's shellfish beach on July 2, 1996.

	Valid N	Mean	Minimum	Maximum	Std. Dev.
Length (mm)	61	37.9	4.22	123.4	35.3
Whole weight (g)	61	53.3	0.16	444.1	104.3
Age	60	6.1	0.00	21.0	5.7
Dry Condition Factor	34	0.9	0.13	2.2	6.2

An age-frequency histogram for butter clams is presented in Figure (53). Butter clams appeared to recruit into the legal size population at between age four and seven years (mean = 5.0 years). Recruitment of butter clams to this Ouzinke beach appears to occur regularly, but not in sufficient numbers to sustain subsistence harvests. If recruitment in 1994 and 1995 was indicative of other years, a significant proportion of the new recruits appear to have survived and entered the harvestable population. A number of hypotheses could be invoked to explain the higher survival in this location. It is remote from the Exxon Valdez oil spill and may represent undisturbed conditions. However, presumptive otter pits were not found on this beach and very few drills or starfish were observed. Therefore, it is also possible that reduced predation is responsible for the increased number of large clams. Numerous other hypotheses could be invoked. None of these was investigated as part of this study.

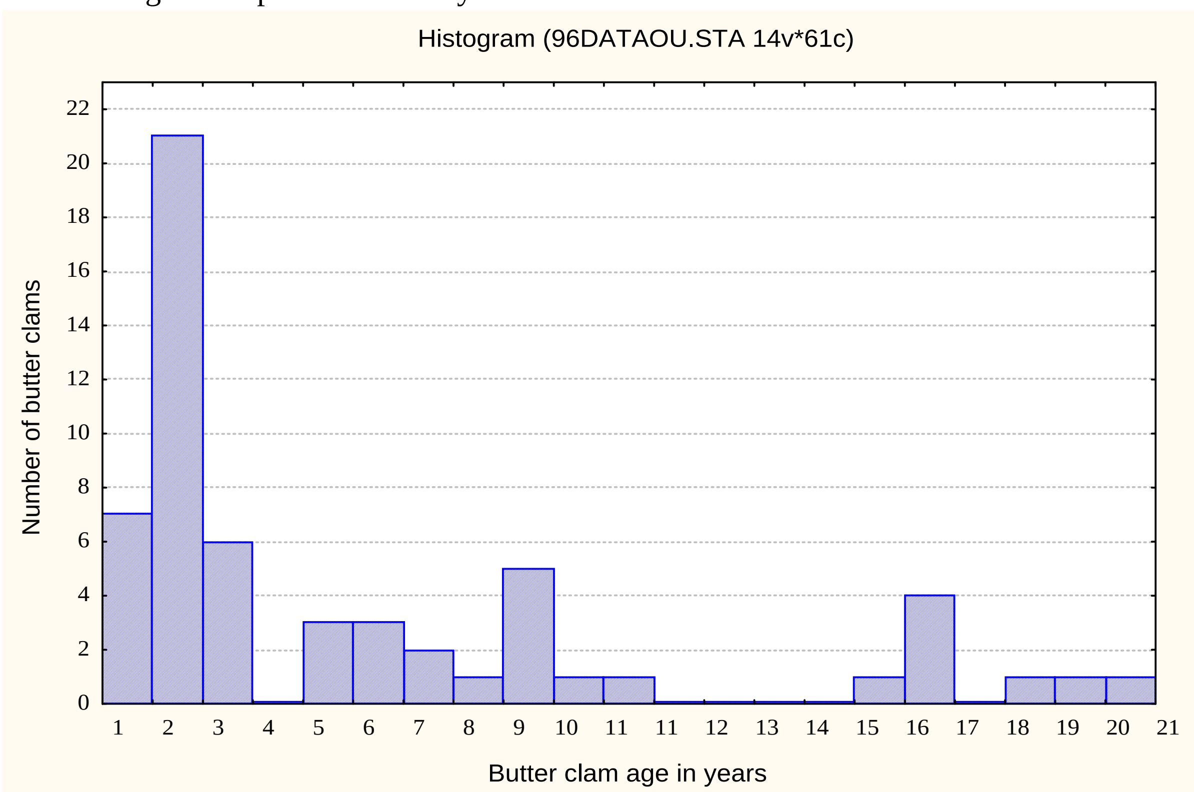


Figure 53. Age-frequency histogram for butter clams (*Saxidomus giganteus*) collected in 22, 0.1 m^2 samples at the Ouzinke Village, Narrow Strait, shellfish beach on July 2, 1996.

Butter clams were growing and apparently surviving well on this Ouzinke beach. However, because of their propensity to retain paralytic shellfish poisons and lack of adequate hatchery technology, this species is not considered appropriate for enhancement. It should be noted that recruitment of butter clams is low but occurs regularly on this beach. This suggests that significant harvests of any kind would quickly deplete the standing biomass. A sound harvest management plan, developed and implemented by the elders of the Village of Ouzinke could help sustain these stocks.

3.6.4. Harvestable biomass of butter clams at Ouzinke. This is the first beach surveyed by the CRRC study team that contained subsistence quantities of shellfish. The average sample weight of butter clams in each sample was 93.1 grams. The harvestable biomass (including 95% confidence limits on the mean), within the 60' x 120' survey area was 670.3 ± 297.3 kilograms. Most of these clams were collected near 0.0' MLLW.

3.6.5. Native littleneck clams. Nineteen (19) native littleneck clams were observed in the 22 samples collected at the Ouzinke shellfish beach on Narrow Straits. Summary statistics describing littleneck clams are presented in Table (20).

Table 2o. Summary descriptive statistics for living native littleneck clams sampled in 22, 0.1 m^2 quadrats at the Ouzinke Village's beach on Narrow Strait on July 2, 1996.

	Valid N	Mean	Minimum	Maximum	Std. Dev.
Length (mm)	19	29.6	6.97	55.01	16.61
Whole wt. (g)	19	12.1	0.07	43.03	13.91
Age (years)	19	4.9	1.00	11.00	3.36
Dry Condition	14	0.48	0.23	0.79	0.18
Wet Tissue Wt (g)	14	6.96	0.55	18.53	5.83

The largest native littleneck clam had a valve length of 55 mm and weighed 43 grams (10.5 per pound). Eight (8) legal size native littleneck clams were obtained from the 22 quadrats included in the systematic random sample. That is less than one legal size clam per square foot and demonstrates the lack of subsistence littleneck harvest available on this beach. Figure (54) suggests steady, but low recruitment (or survival of recruits past settlement) at this beach.

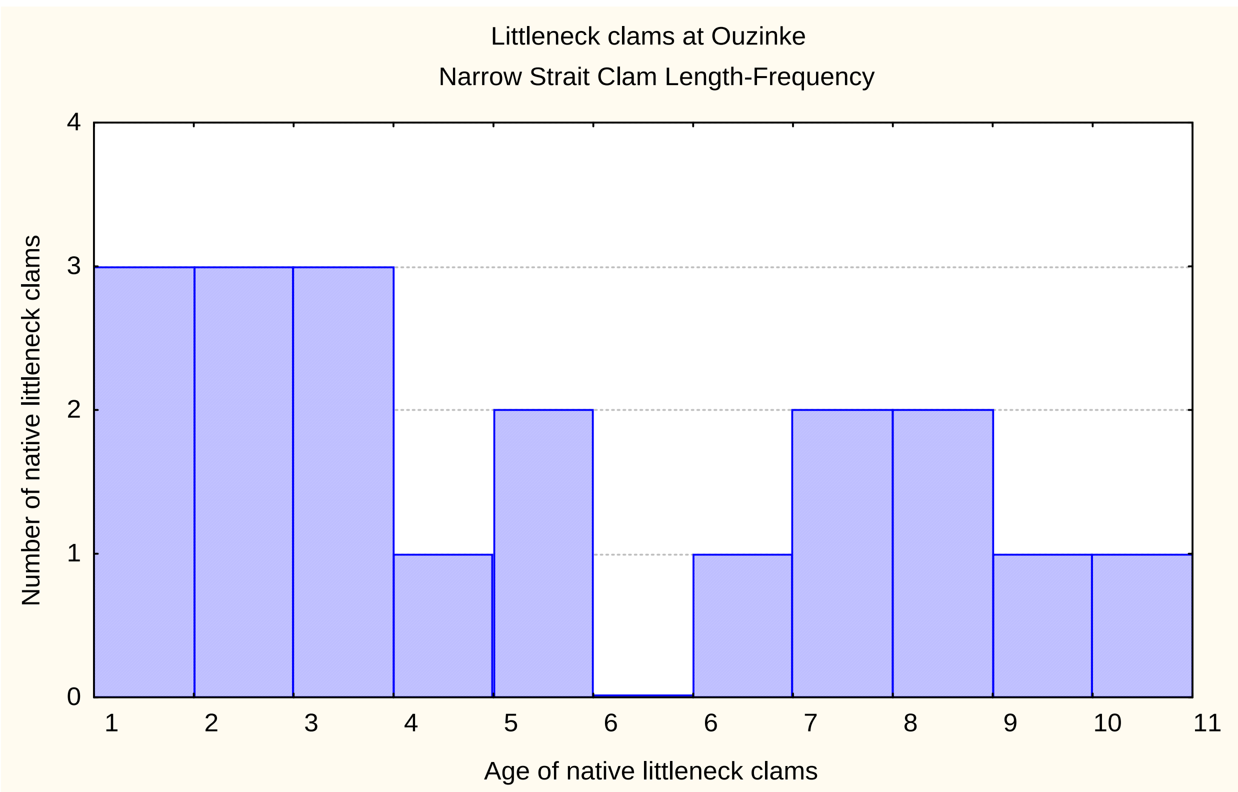


Figure 54. Age – frequency histogram for littleneck clams collected in 22, 0.1 m^2 quadrats at the Ouzinke shellfish beach on July 2, 1996.

Further examination of the population was accomplished using the length - frequency histogram provided in Figure (55). These two histograms suggest that recruitment is generally reliable but low at this site. It also appears reasonable to conclude that (assuming current recruitment reflects past recruitment) survival is good. The frequency observed in each of the year classes in Figure (51) should be divided by 2.2 to obtain the number of recruits per square meter. Doing this suggests that recruitment in 1993, 1994 and 1995 resulted in between one and two littleneck clams surviving per square meter until 1996. This is far below the minimum of 200 to 300 clams per square meter needed to fully utilize a quality habitat such as this. It appears that supplemental seed would benefit future bivalve harvests at this beach.

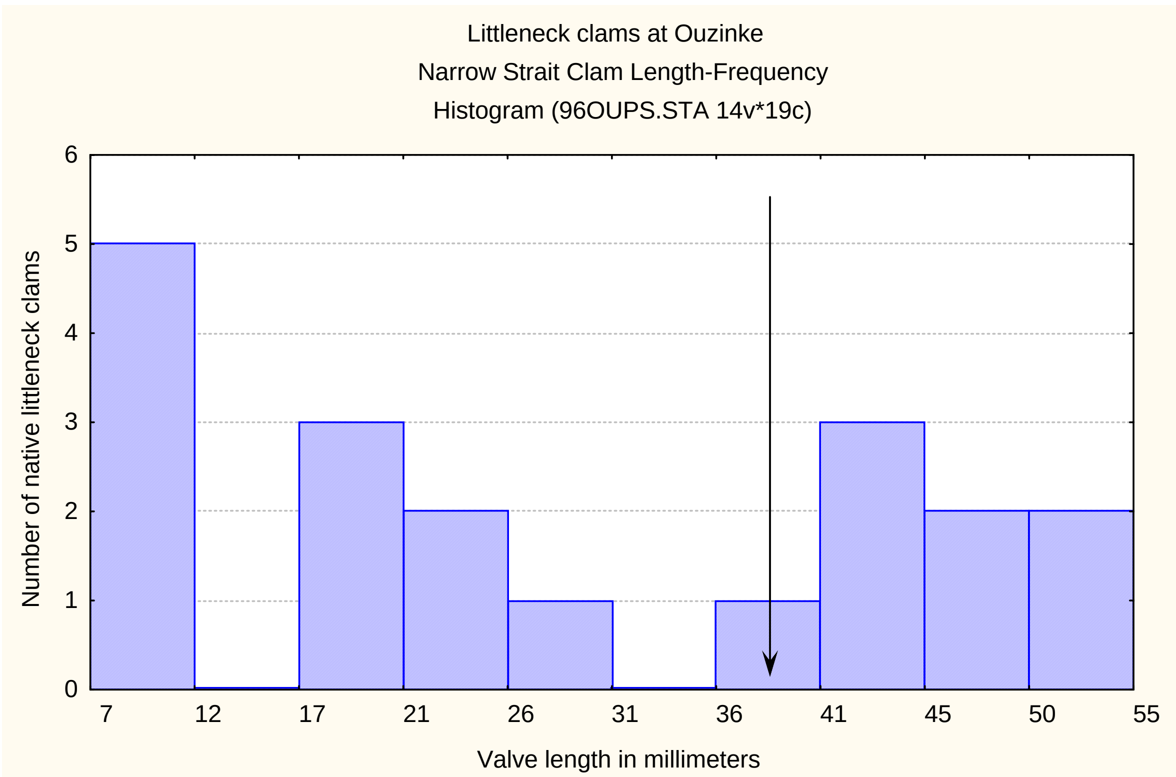


Figure 55. Length - frequency histogram for littleneck clams collected in 22, 0.1 m^2 samples collected at this Ouzinke beach on July 2, 1996. The vertical line represents the minimum legal size of 38 mm.

Current clam densities are insufficient to warrant subsistence harvests of littleneck clams at this Ouzinke beach. However, a few littleneck clams will be retrieved during a butter clam harvest. Older native littleneck clams are present as a significant proportion of recent recruitment. However, too few native littleneck clams were obtained in this survey to warrant any conclusion regarding survival. The relative absence of predators suggests that extensive cultivation without a need for predator exclusion netting may be appropriate on this beach.

Figure (56) describes the distribution of native littleneck and butter clams as a function of tidal height at this Ouzinke beach. Unlike other beaches surveyed in this study, most of the littleneck clams were found at relatively low intertidal elevations. This may reflect reduced starfish predation. However, the few native littleneck clams retrieved did not provide a basis for drawing significant conclusions.

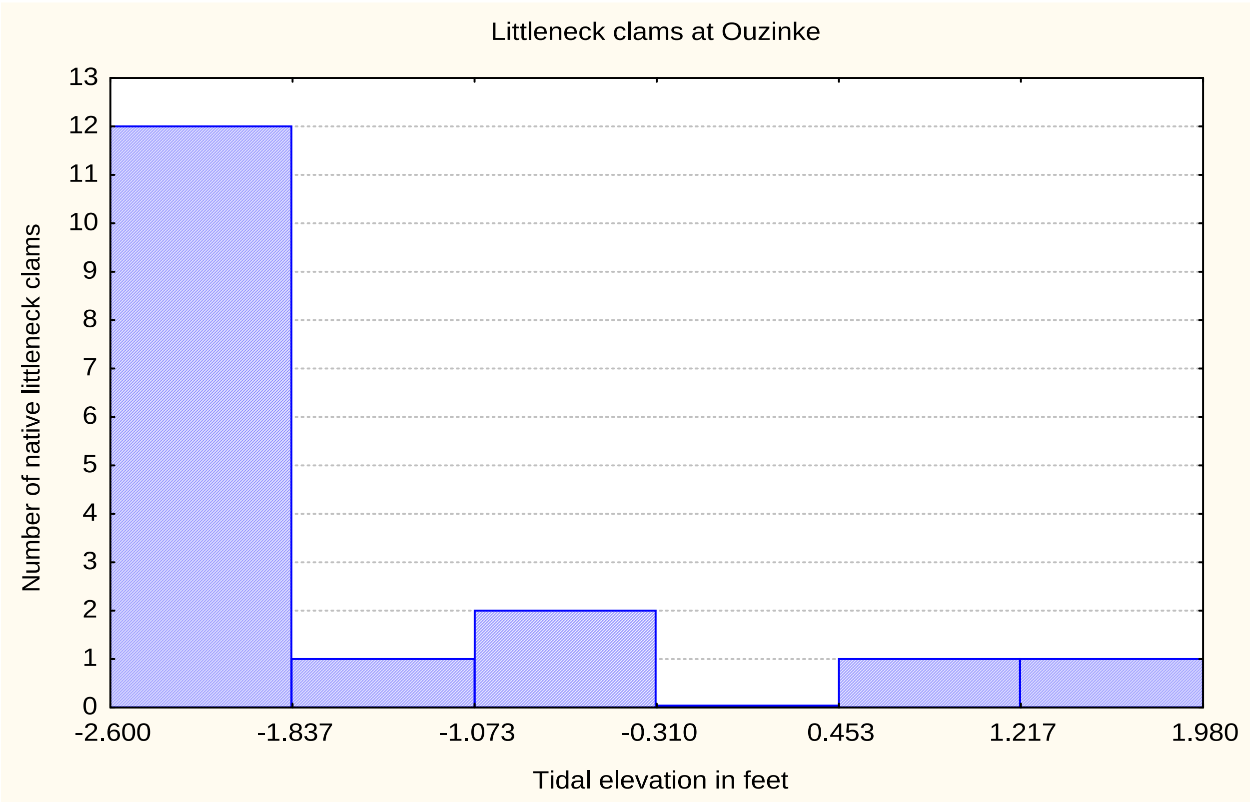


Figure 56. Tidal elevation - frequency histogram for littleneck clams collected in 22, 0.1 m^2 quadrats at the Village of Ouzinke shellfish beach on Narrow Strait on July 2, 1996.

3.6.6. Age-length analysis for native littleneck clams at Ouzinke. Regression coefficients were developed for the von Bertalanffy model using nonlinear regression. The resulting equation explained 93.7% of the variation and the ANOVA determined probability that the regression coefficients were all equal to zero was P = 0.000. The residuals were not significantly different from a normal distribution (Kolmogorov-Smirnov; d = 0.11; p = n.s. @ α = 0.05). Clam lengths to 55 mm were available for the analysis. Predicted and observed values of valve length, as a function of age, are presented, together with the regression line in Figure (57). This equation was solved for a length of 38 mm to obtain the average age of recruitment into the legal size population. Based on the von Bertalanffy model, the average age of recruitment to a size $\geq$ 38 mm was 6.13 years. The unexpectedly high maximum length of 73.8 mm may be associated with higher growth rates throughout the lifespan of this species in this part of Alaska. Under any circumstances, clams with valve lengths longer than 55 mm were not included in the database and extrapolation to lengths greater than that is inappropriate.

$$\text{Native littleneck clam length at Ouzinke (mm)} = 73.8(1 - \exp^{-0.118*\text{age in years}})$$

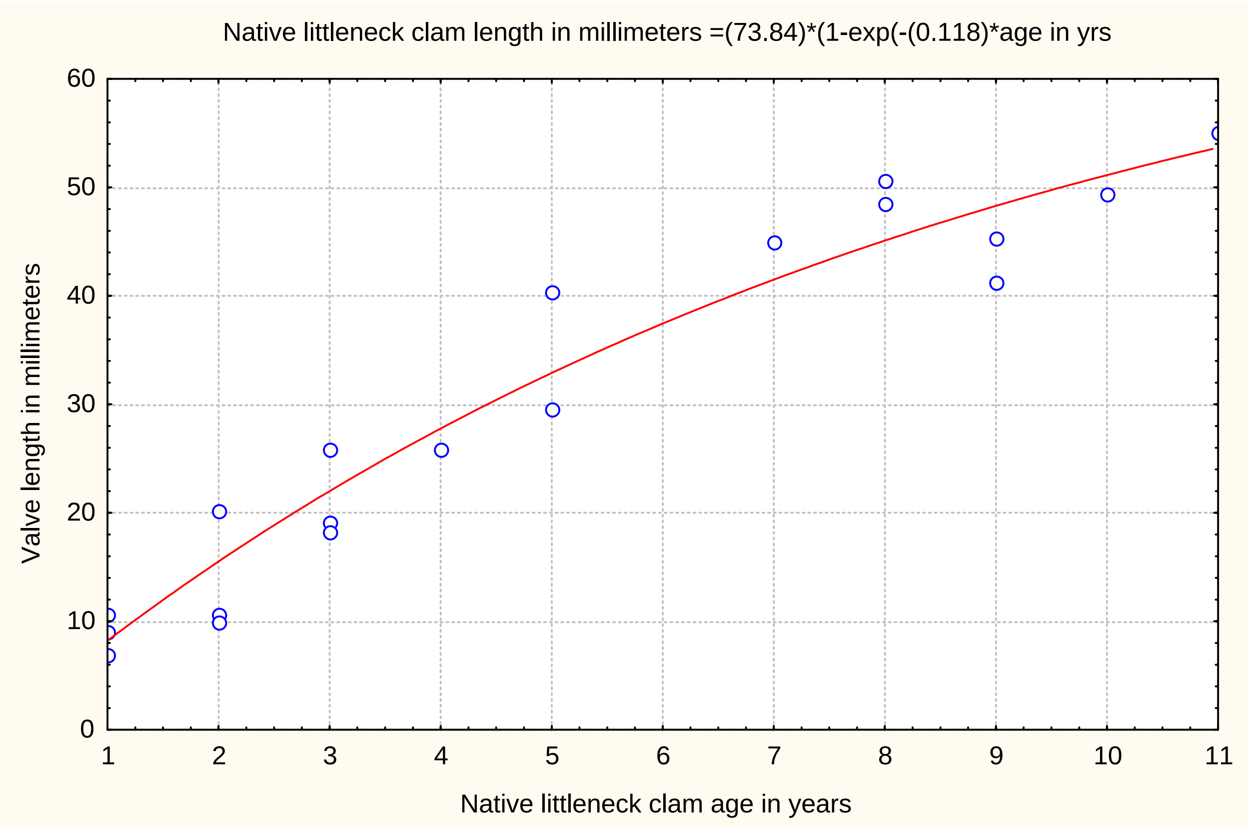


Figure 57. Valve length (mm) as a function of age (years) for native littleneck clams (*Protothaca staminea*) collected in 22, 0.1 m^2 quadrats at the Ouzinke shellfish beach on Narrow Strait on July 2, 1996.

3.6.7. Bacteriological water quality at the Ouzinke shellfish beach on Narrow Strait. Three water samples were collected at the survey beach and returned to Aquatic Environmental Sciences at 4^{o}C where they were examined for fecal coliform bacteria using the five tube MPN method. Fecal coliform bacteria were < 2/100 ml in all samples. This analysis does not satisfy the needs of the National Shellfish Sanitation Program. However, it suggests that there is no continuing source of fecal coliform bacteria at this beach. Certification should be obtained for the receiving water from responsible agencies prior to any major enhancement effort.

3.6.8. Summary, conclusions and recommendations for native littleneck clam enhancement at the village of Ouzinke's, Narrow Strait shellfish beach. Based on this survey and analysis, the following conclusions can be reached:

➢ **Shellfish biomass available for harvest.** There is currently a significant shellfish biomass available for harvest on this beach and on several other beaches in the local area. Butter clams comprise the majority of the harvestable biomass. The total biomass on this single beach has been estimated at 670.3 $\pm$ 297.3 kilograms. The majority of these are large (older) butter clams. The apparent low clam recruitment level suggests that subsistence harvests would quickly deplete the standing stock. This could be avoided by invoking a locally supported management plan.

- **Beach suitability for bivalve enhancement.** The surveyed Ouzinke beach contains approximately one- fifth acre of ground suitable for native littleneck clam enhancement or culture. The physical and chemical parameters examined in this survey are all within acceptable limits. The beach is readily accessible from the village. The apparent absence of large numbers of predators makes this area unique among the five village beaches surveyed in 1995 and 1996. There is an opportunity here to implement a more extensive enhancement trial.

  The observation of a significant flow of saltwater from the interdunal area above the beach is a positive aspect of this beach that will reduce the potential desiccation and overheating in the summer and freezing during winter low tides. Nearly all aspects of native littleneck growth are enhanced by significant amounts of interstitial water movement.

- **Bivalve predation.** Evidence of significant predation on bivalves was not observed in this survey.

- **Bivalve recruitment.** Recently past bivalve recruitment to this Ouzinke beach has been too low to sustain long-term subsistence or recreational harvests.

- **Native littleneck clams (*Protothaca staminea*).** Few native littleneck clams were observed in samples from this Ouzinke beach. The reason is thought to be poor juvenile recruitment. The age length analysis suggests that native littleneck clams recruit to the legal size population at approximately 6.5 years of age.

- **Butter clams (**_Saxidomus giganteus_) also recruit in low numbers to this beach. Growth appeared somewhat faster than for native littleneck clams and butter clams entered the legal size population at approximately 5.0 years of age. There was a harvestable standing biomass of butter clams on this, and several other beaches in the area. However, the large biomass consists of older clams that will not be quickly replaced following harvest.

- **Cockles (*Clinocardium nuttallii*)** are a traditional (and preferred) shellfish for Alaskan natives. The primary beach surveyed in this effort was too rocky, with too few fines, to warrant cockle enhancement. However, excellent cockle habitat was observed in Camel Lagoon approximately 3 kilometers west of the surveyed beach.

- **Mussels (*Mytilus edulis trossulus*)** were not observed in abundance on any of the surveyed beaches in the Ouzinke area. Unless local sources of seed are identified, mussel culture would require the importation of hatchery produced seed or seed collected from other locations.

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

Native littleneck clam growth and mortality study

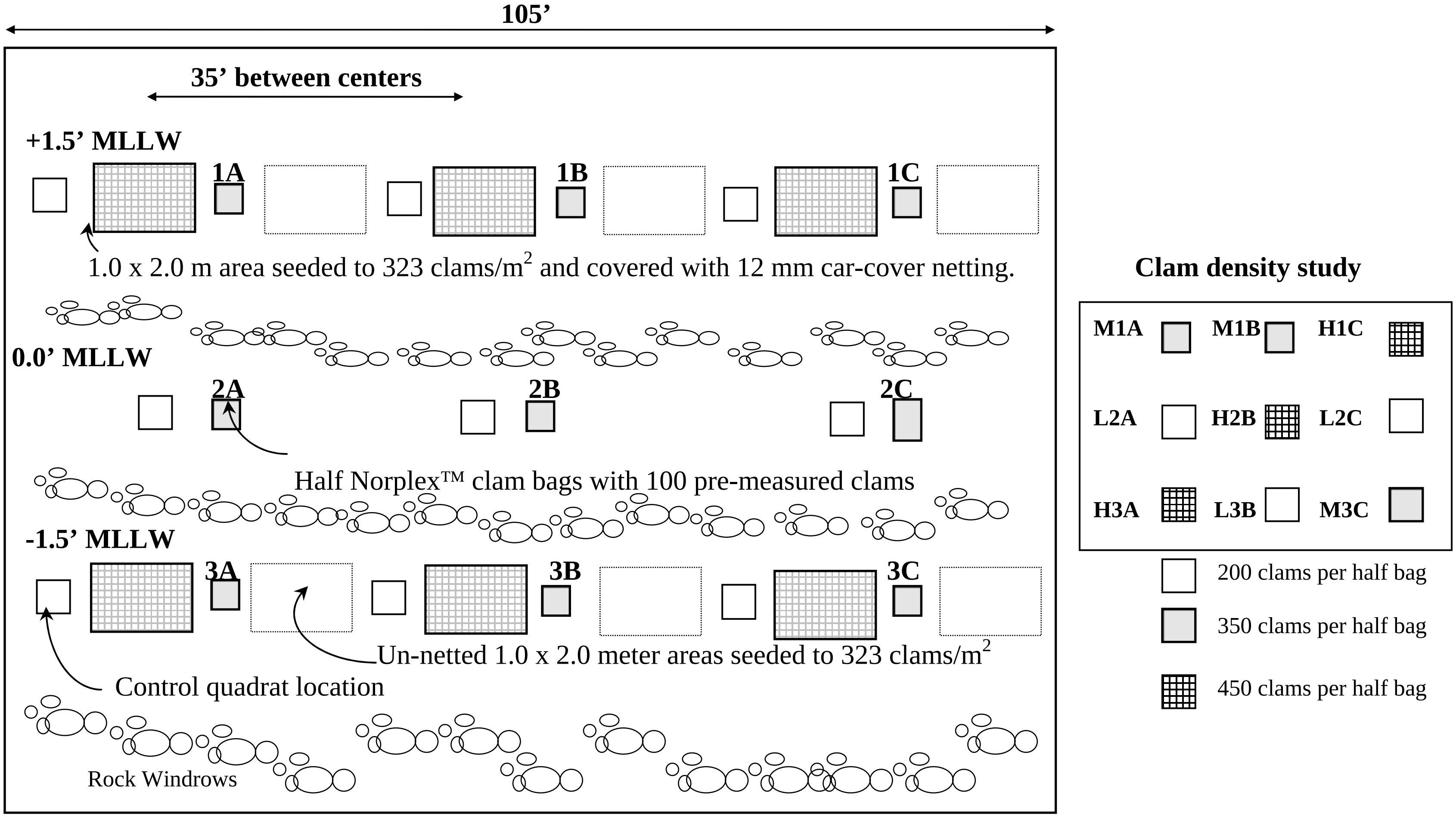


Figure 58. Study design for clam enhancement studies at previously surveyed beaches at the villages of Tatitlek, Nanwalek and Port Graham. The density study was added in 1998. It is shown to the right of the existing study site in this figure. Individual treatments were separated by four feet and ten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

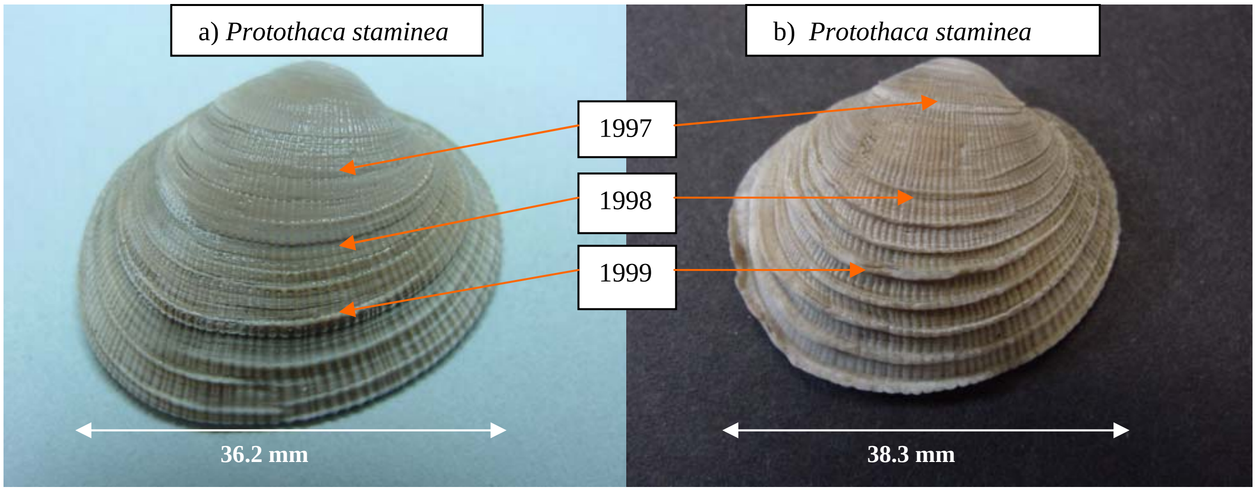


Figure 62) Native littleneck clam planted in 1996 in Murphy's Slough at a tidal height of a) +1.5' MLLW and b) –1.5' MLLW and collected on September 9, 1999 following 1162 days (3.2 years) of growout. Winter annuli formed in January of 1997, 1998 and 1999 are marked. Annuli are assigned to the month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

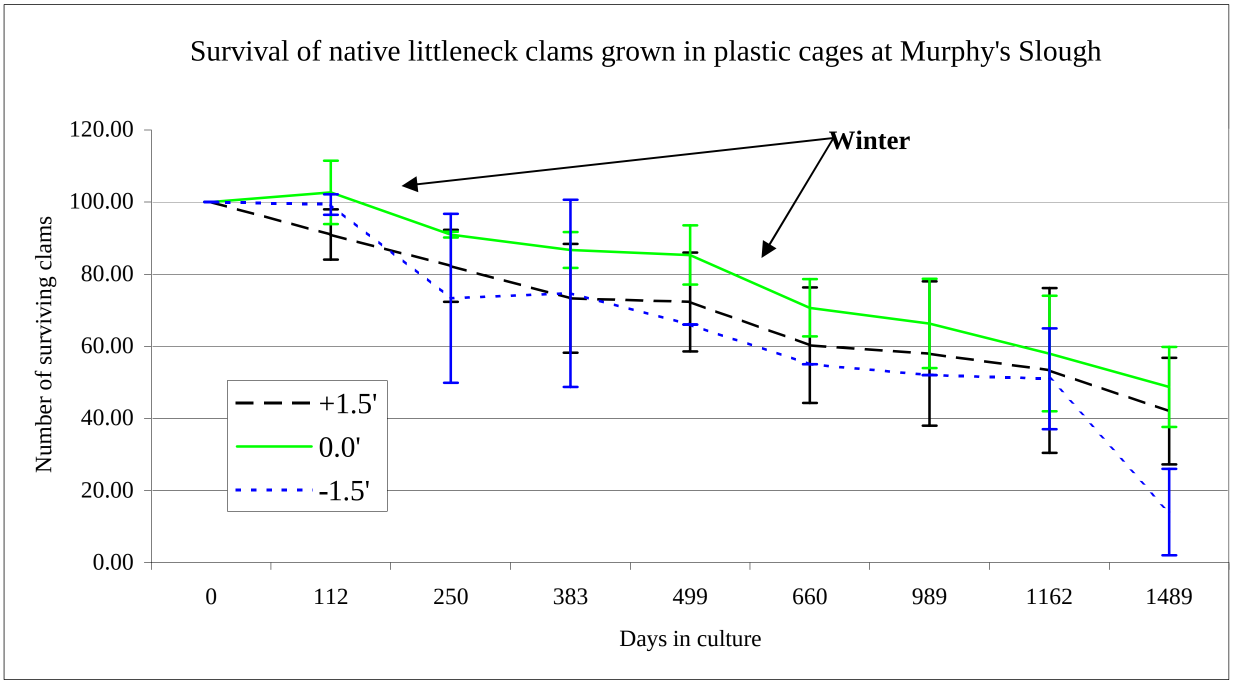


Figure 63. Mean number of surviving clams in replicate bags at three tidal heights in Murphy's Slough, Port Graham, Alaska as a function of date.

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.

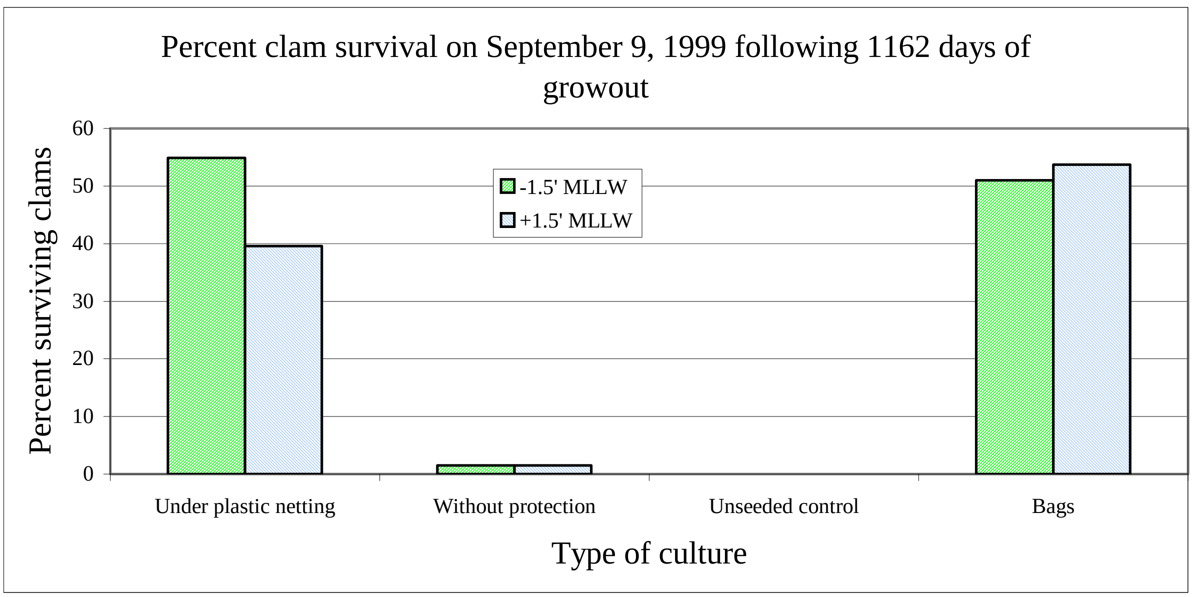


Figure 65. Percent surviving native littleneck clams cultivated in Murphy's Slough. Data compare survival on September 9, 1999 with planting density on July 4, 1996.

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).

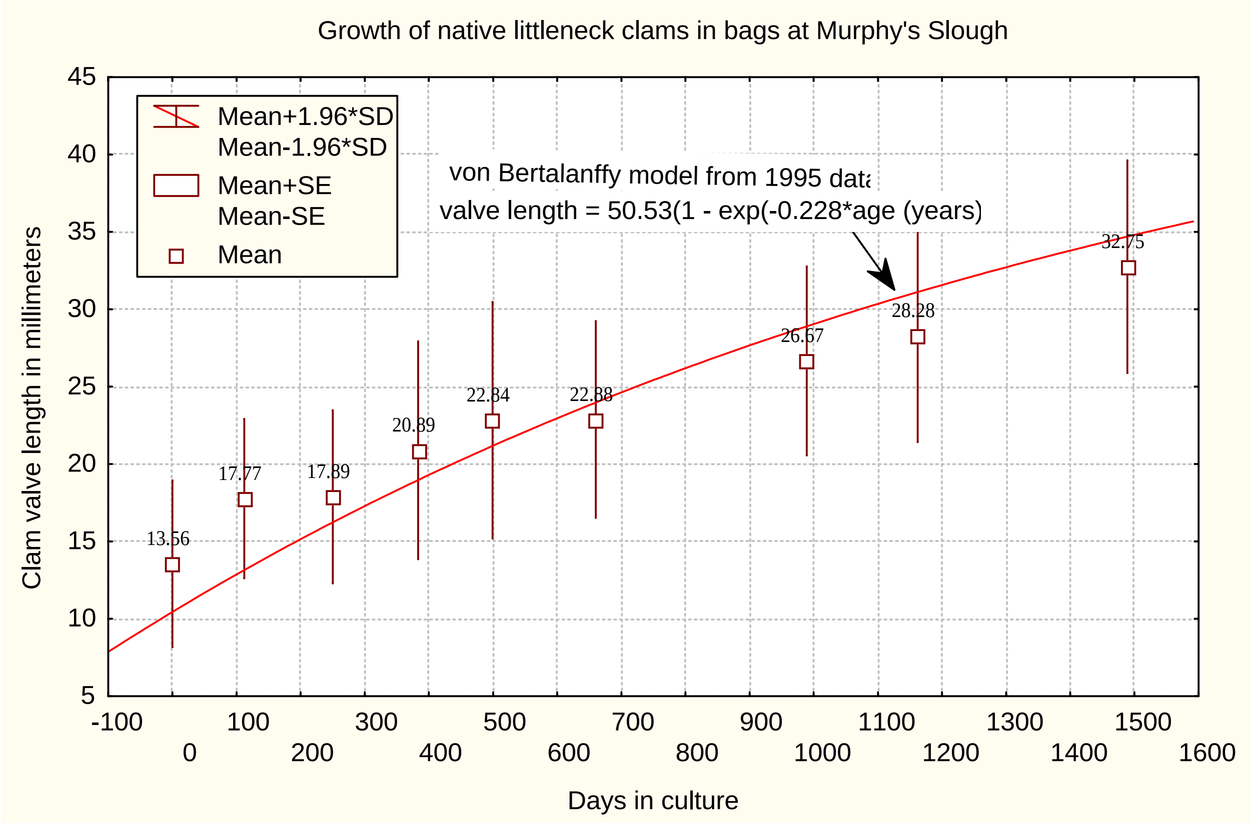


Figure 66. Mean lengths of native littleneck clams cultured at all tidal elevations in bags at Murphy's Slough between July 5, 1996 and August 1, 2000. The von Bertalanffy growth model developed for native littleneck clams from the baseline bivalve inventories conducted in 1995 as part of this effort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

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.

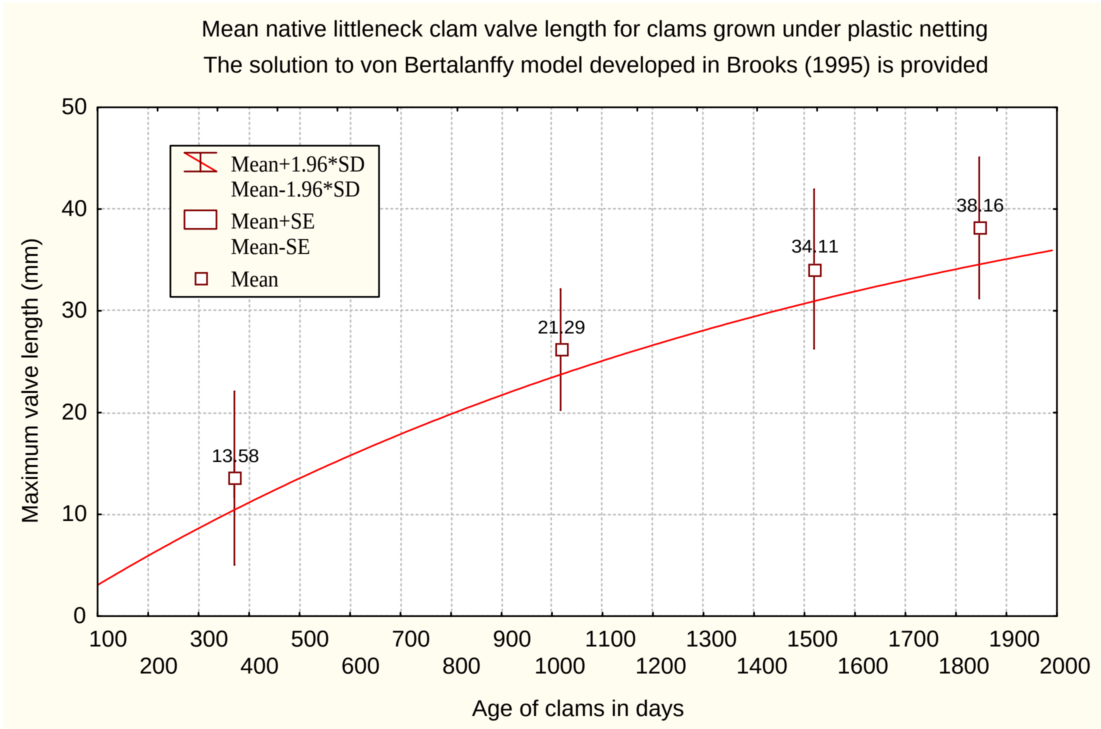


Figure 67. Comparison of the observed growth of native littleneck clams under plastic netting in Murphy's Slough with the von Bertalanffy model predictions based on the 1995 baseline surveys at Tatitlek and Passage Island.

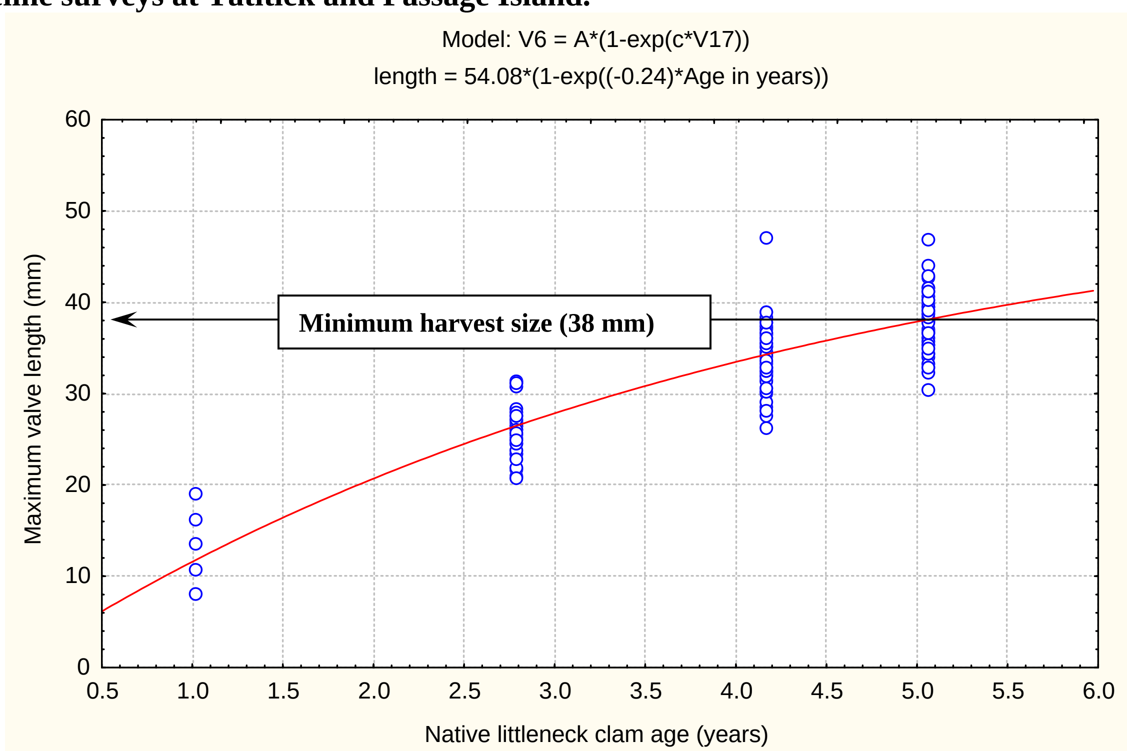


Figure 68. Solution to the von Bertalanffy model for native littleneck clams grown in Murphy's Slough under plastic netting. The clams were spawned in 1995, seeded on the beach in 1996 and monitored in 1998, 1999 and 2000.

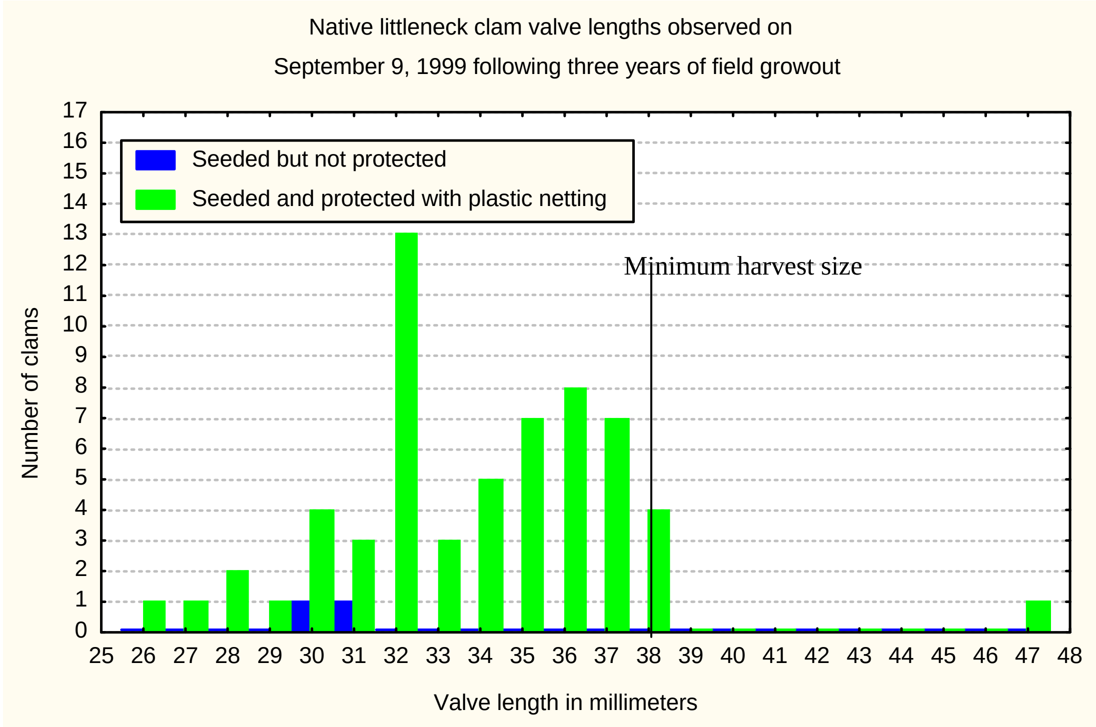


Figure 69. Length-frequency histogram describing artificially propagated native littleneck clams sampled from areas protected by plastic netting (green) and without protection (blue). The culture was initiated in 1996 and sampled in 1999.

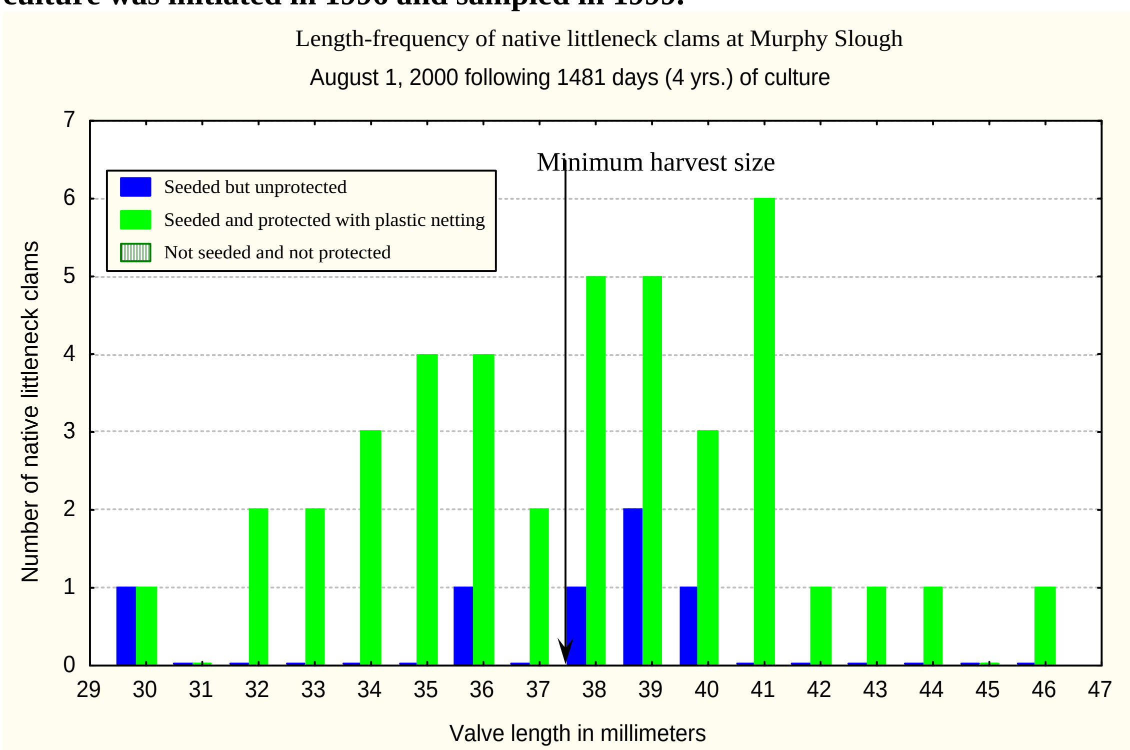


Figure 70. Length-frequency histogram describing artificially propagated native littleneck clams sampled from areas protected by plastic netting (green) and without protection (blue). The culture was initiated in 1996 and sampled in 2000.

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.

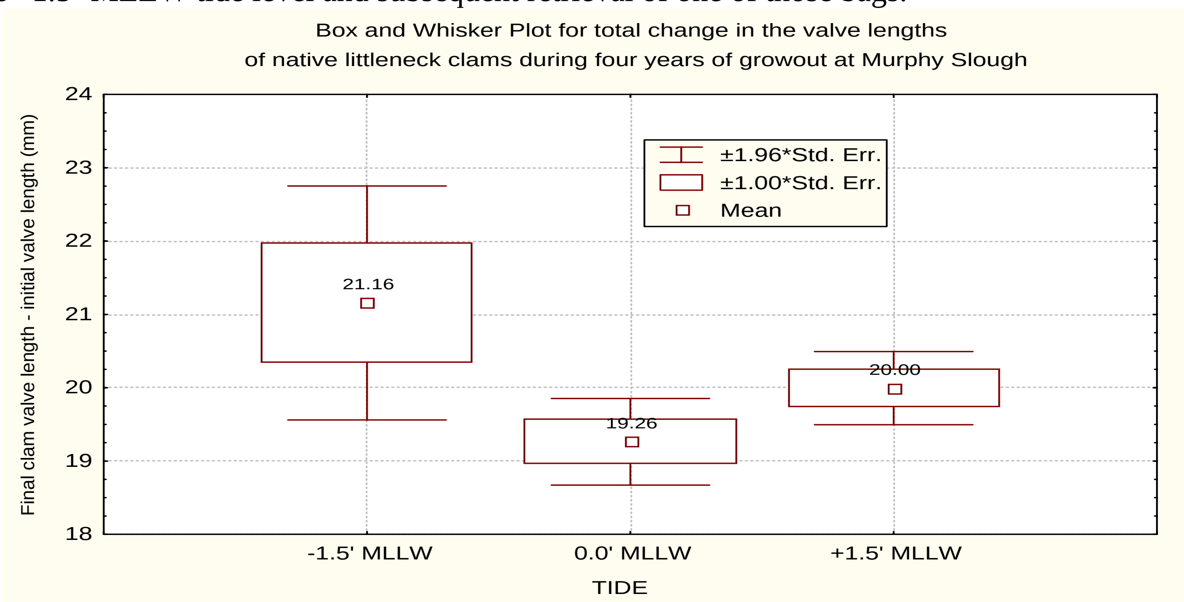


Figure 71. Box and whisker plots describing the difference in initial and final mean valve lengths of native littleneck clams grown in bags at Murphy's Slough from 1996 until 2000 as a function of tidal height.

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 ($\chi^2 = 0.12$, $\chi^2_{\text{critical} = 26.3}$, $\nu = 16$) from the distribution described by Feder and Paul (1973) for total native littleneck clam weight versus length. In fact, they are essentially identical.

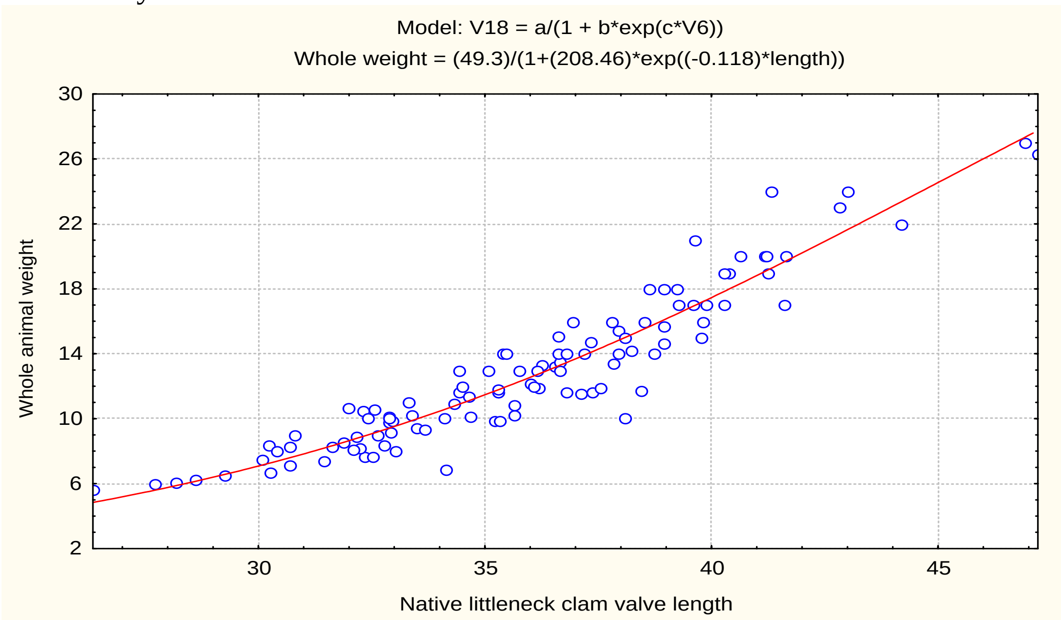


Figure 72. Logistic growth curve model fit to whole-animal weights (grams) and valve lengths (mm) observed in clams collected grown under plastic netting at Murphy's Slough, Alaska.

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.

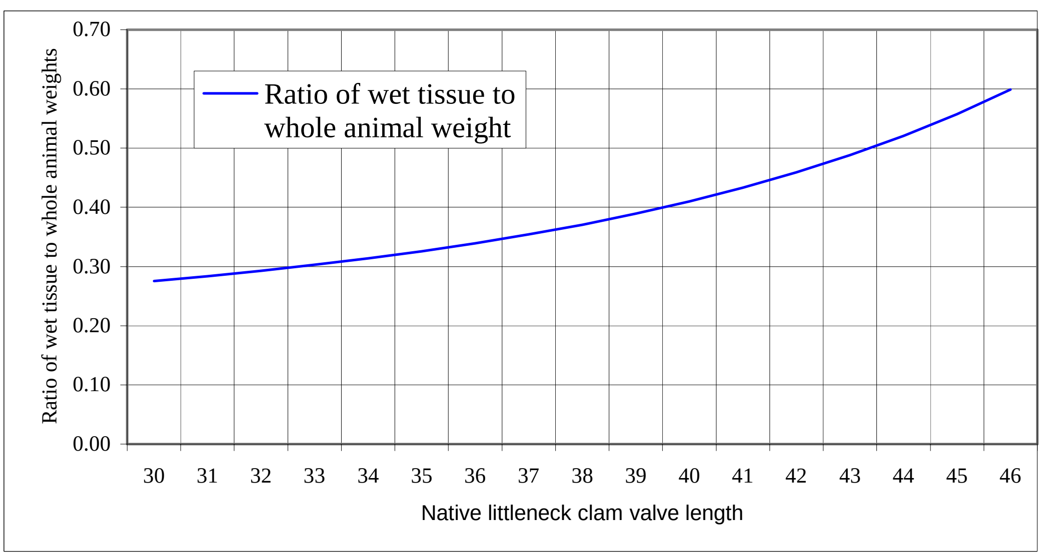


Figure 73. Ratio of wet tissue to whole-animal weights for native littleneck clams as a function of a function of valve length (mm).

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.

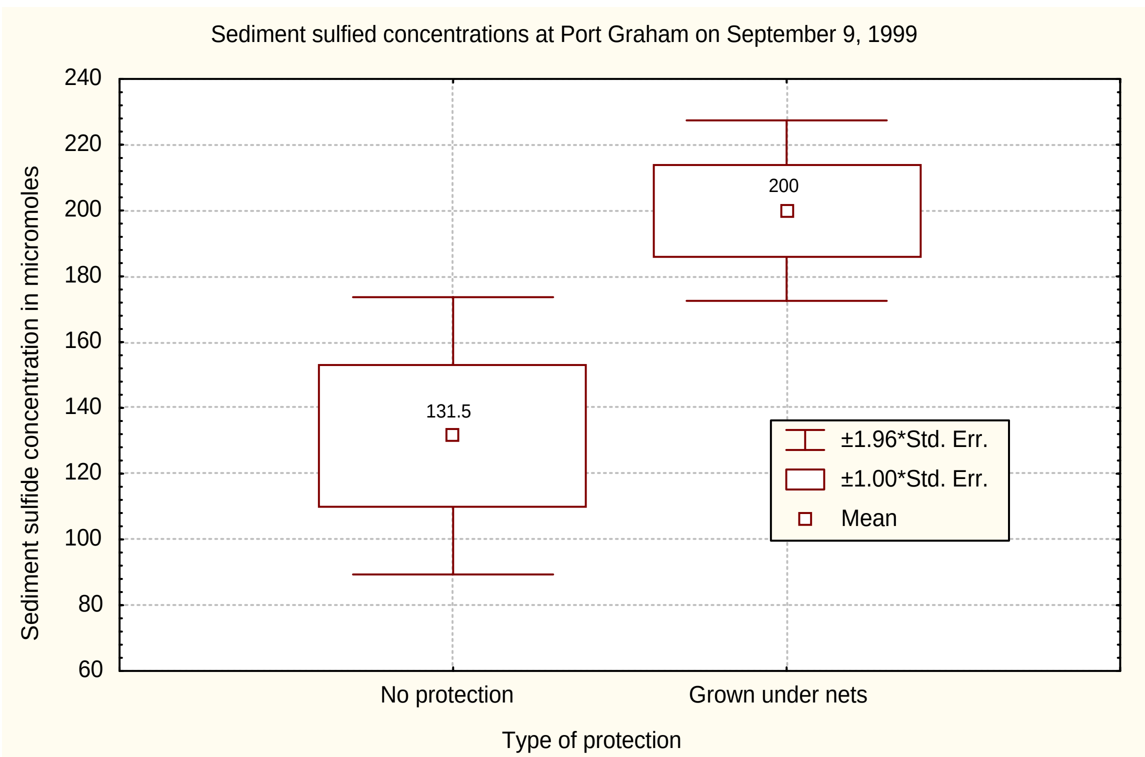


Figure 74. Box and whisker plot comparing the concentration of total sediment sulfides in Murphy's Slough sediments under plastic netting with sediments from unprotected treatment plots.

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 $\pm$ 0.6 mg/L (mean $\pm$ 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 $\pm$ 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

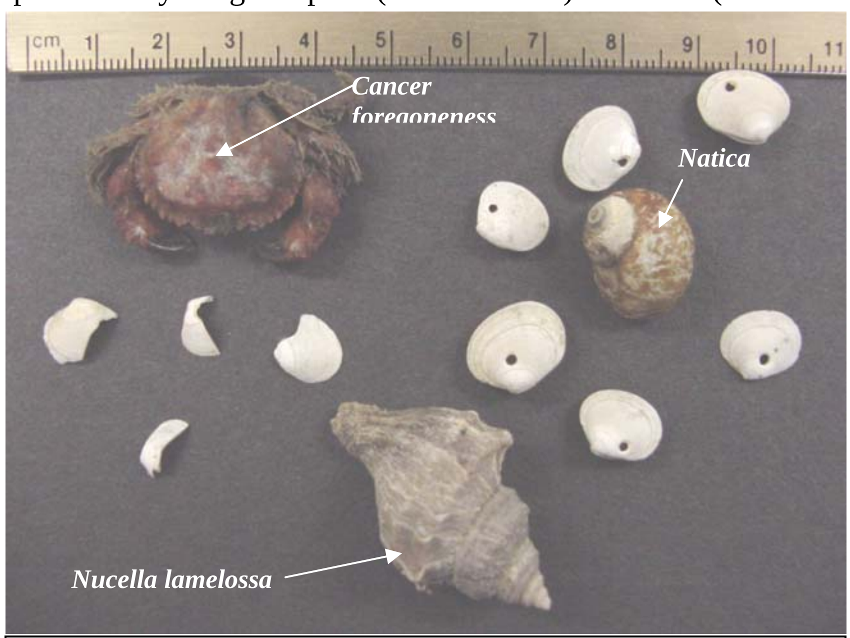


Figure 75. Gastropods (with drilled native littleneck clams) and *Cancer oregonensis* (with characteristic broken clam shells) found in bags at Murphy's Slough and Tatitlek during 1999.

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.

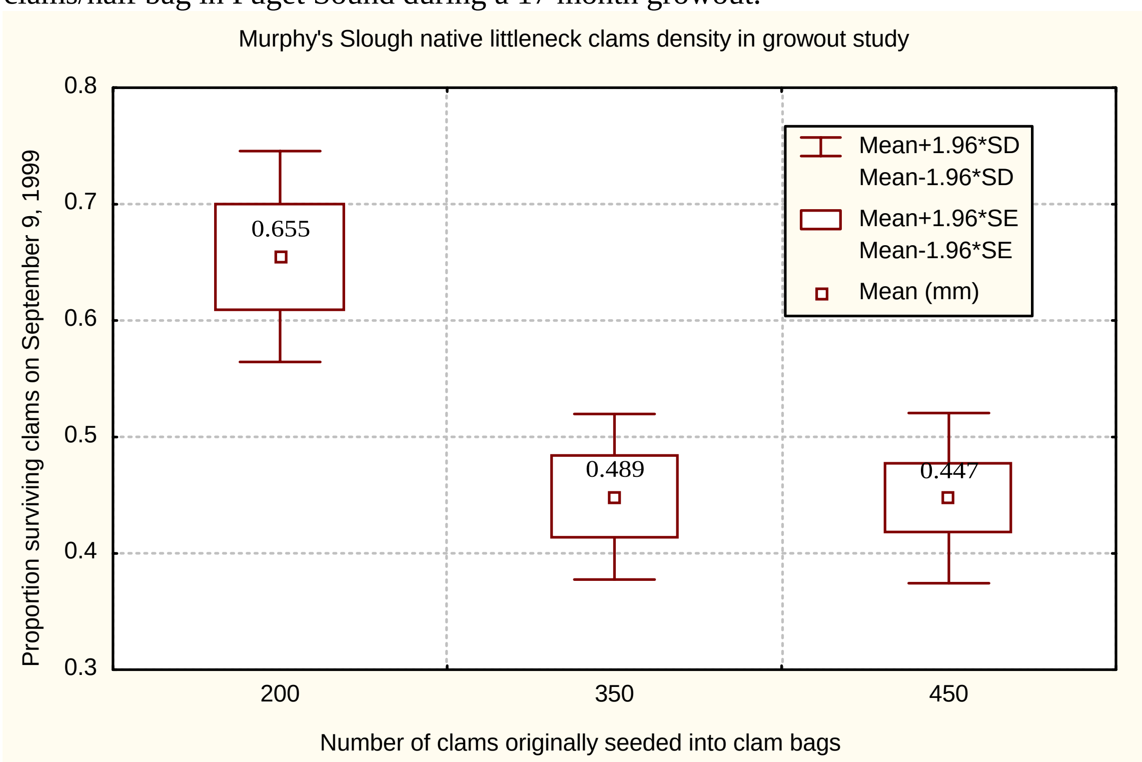


Figure 76. Proportion surviving native littleneck clams planted at densities of 200, 350 and 450 clams per half clam growout cage in Murphy’s Slough on April 25, 1998 at the 0.0’ MLLW tide level and evaluated on September 9, 2000.

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).

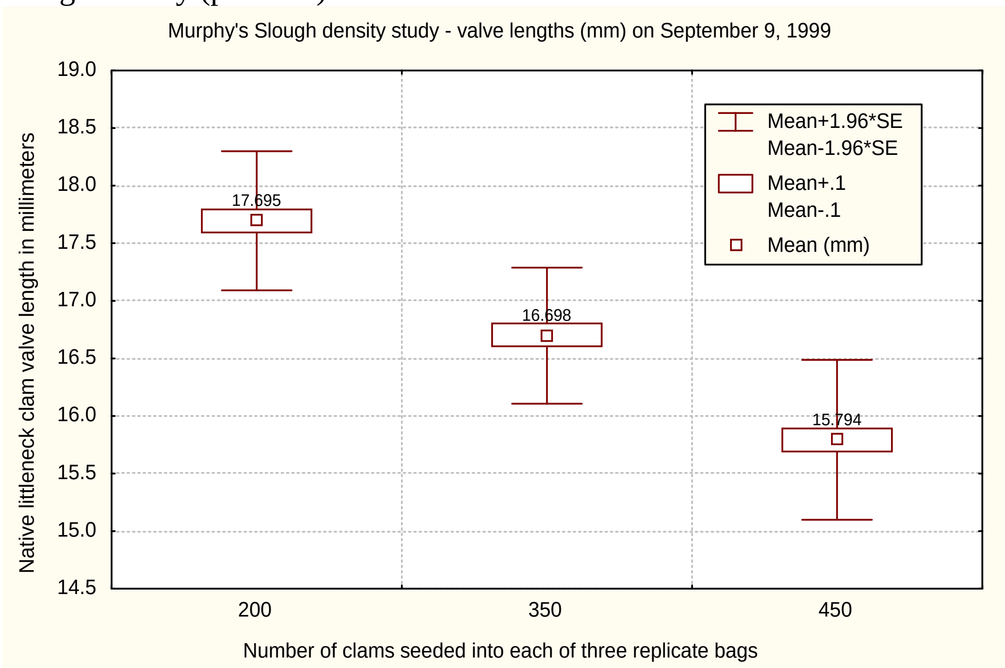


Figure 77. Mean valve lengths in three replicates of native littleneck clams planted at densities of 200, 350 and 450 clams per half clam growout cage in Murphy's Slough on April 25, 1998 at the 0.0' MLLW tide level and evaluated on September 9, 2000.

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.

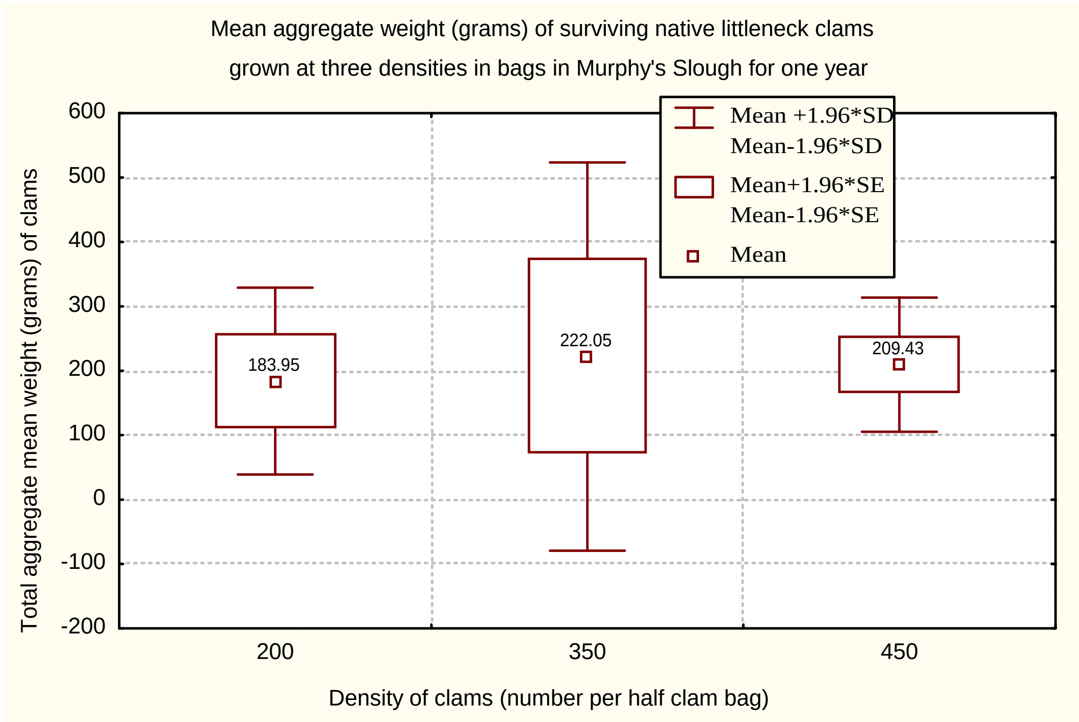


Figure 78. Mean aggregate weight (grams) of native littleneck clams grown in bags for one year at three densities at 0.0' MLLW in Murphy's Slough, Alaska.

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.

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.

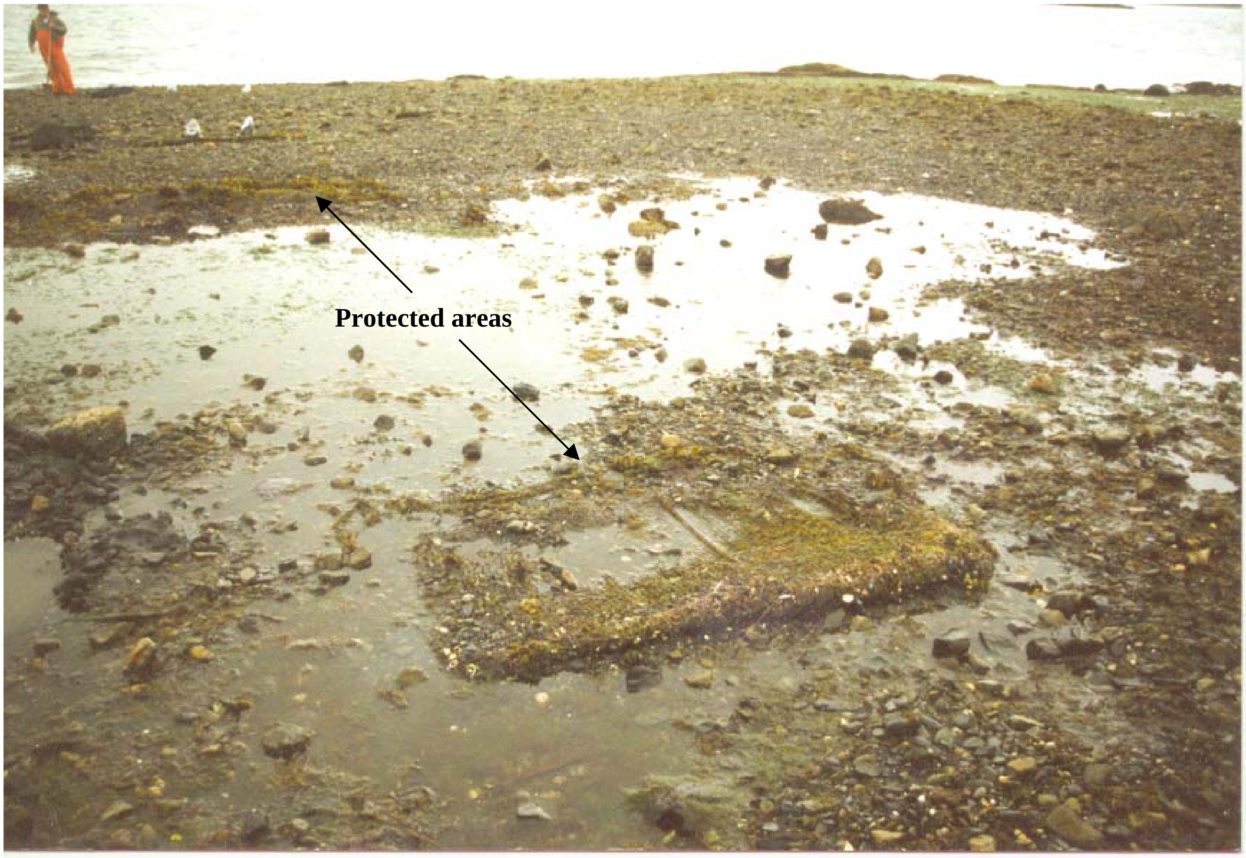


Figure 81. Enhancement plots (1A) and (1B) on the Tatitlek shellfish beach. Beach substrates were stabilized under the seeded area that was protected with plastic netting. The unprotected area, located to the right in this photograph, was badly eroded and no clams were retrieved in two replicate samples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

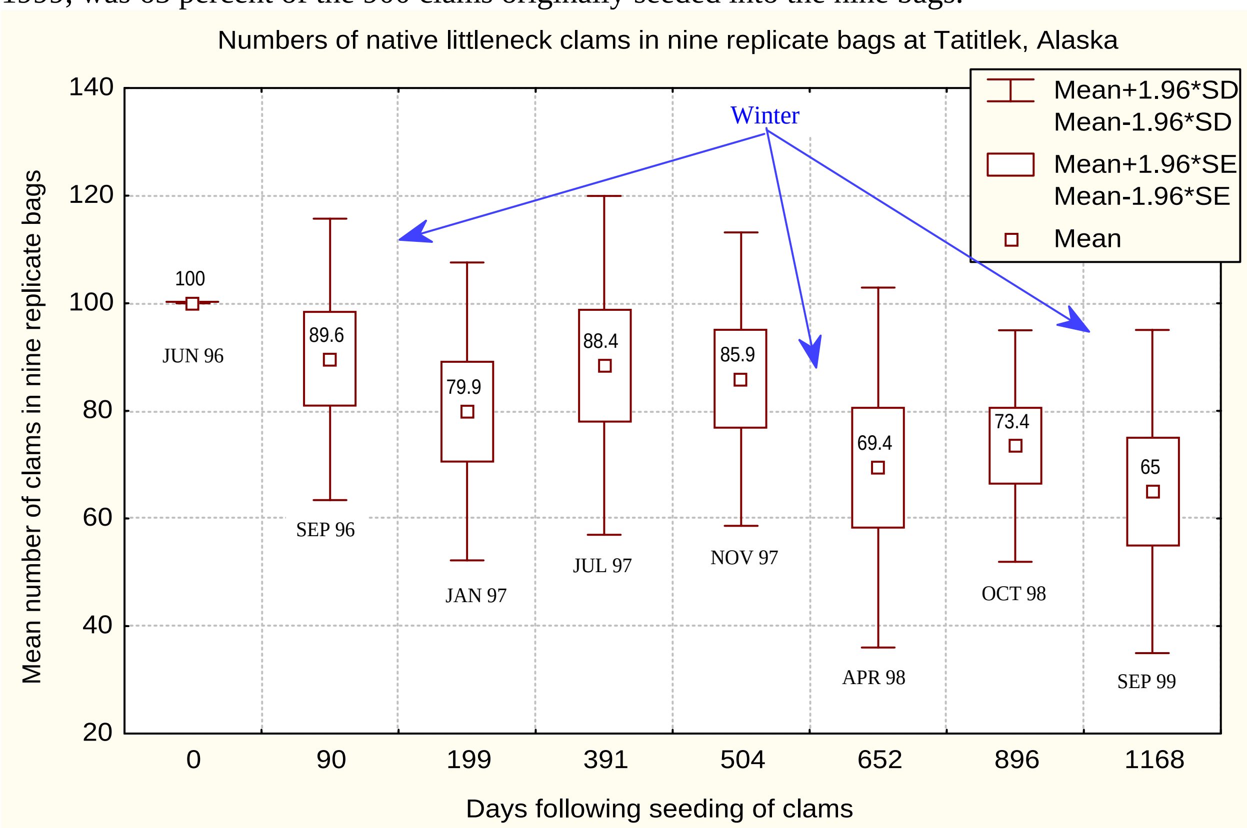


Figure 82. Mean number of surviving native littleneck clams in bags as a function of time (days) following planting on June 29, 1996 at the beach adjacent to the village of Tatitlek, Alaska. Significant differences in survival as a function of tidal height were not observed and the data was pooled.

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 ($p = 0.05$).

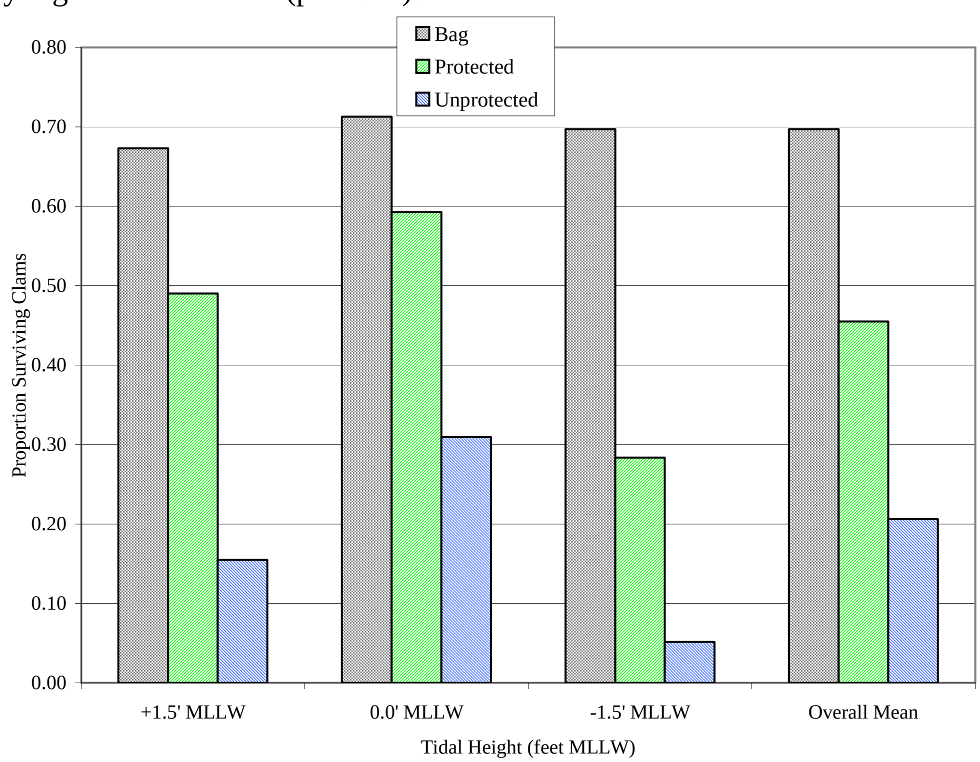


Figure 83. Proportion surviving native littleneck clams at Tatitlek as a function of tidal height and treatment (Bags, Protected with Plastic netting, or seeded but left Unprotected).

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.

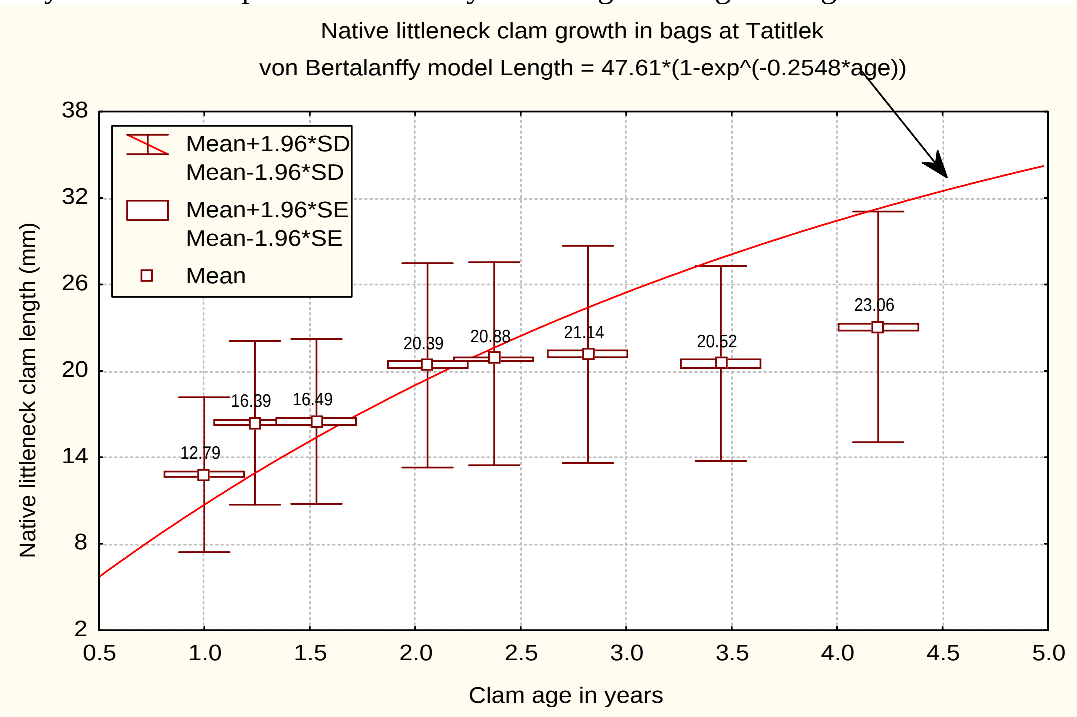


Figure 84. Mean lengths of native littleneck clam cohorts cultured at all tide heights in bags at Tatitlek between June 27, 1996 and September 9, 1999. Clams in bags were measured quarterly for the first two years during this study.

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

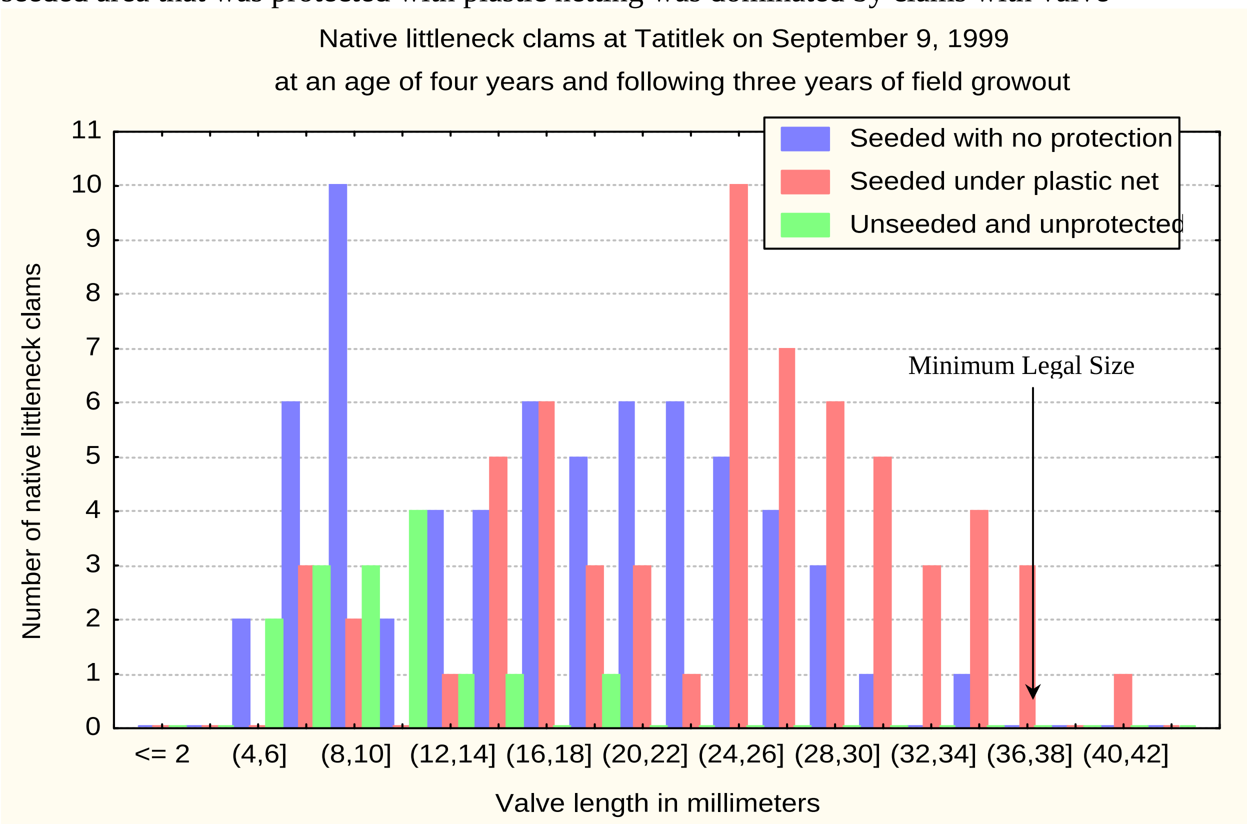


Figure 85. Length-frequency histogram describing the distribution of native littleneck clams retrieved on September 9, 1999. Significant differences in valve length as a function of tidal height were not observed and the results pooled.

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.

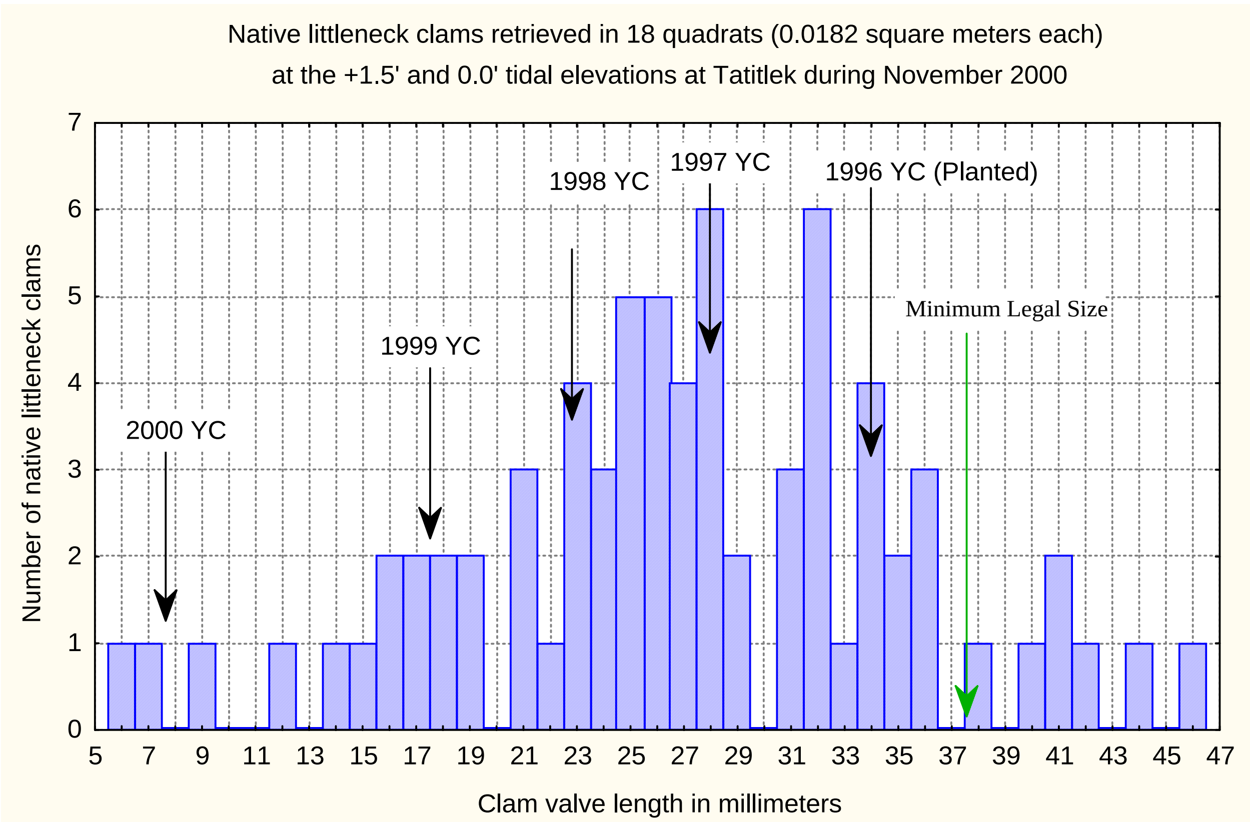


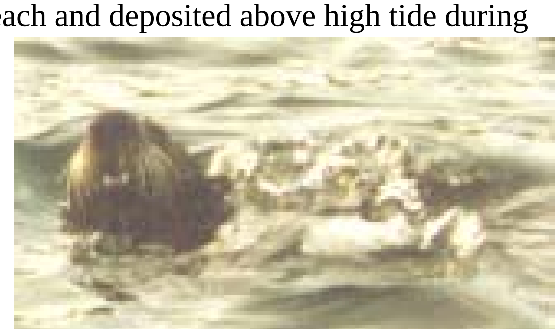
Figure 86. Mean lengths of native littleneck clams cultured under plastic netting at Tatitlek between June 27, 1996 and September 9, 1999. These clams were sampled once each year in 1998, 1999 and 2000.

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.

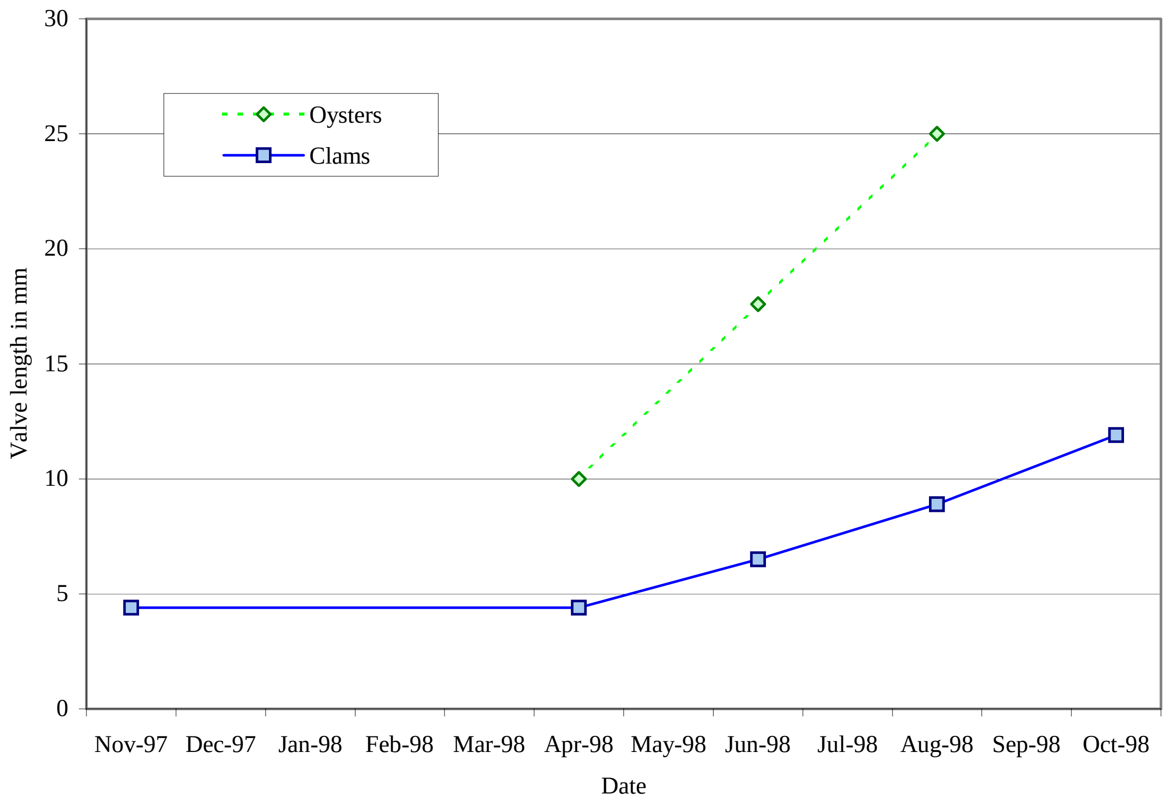


Figure 88. Growth of oysters (*Crassostrea gigas*) and clams (*Protothaca staminea*) in the tidally driven Flupsy at Tatitlek during 1998. Oysters were planted following the July 1998 measurements.

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.

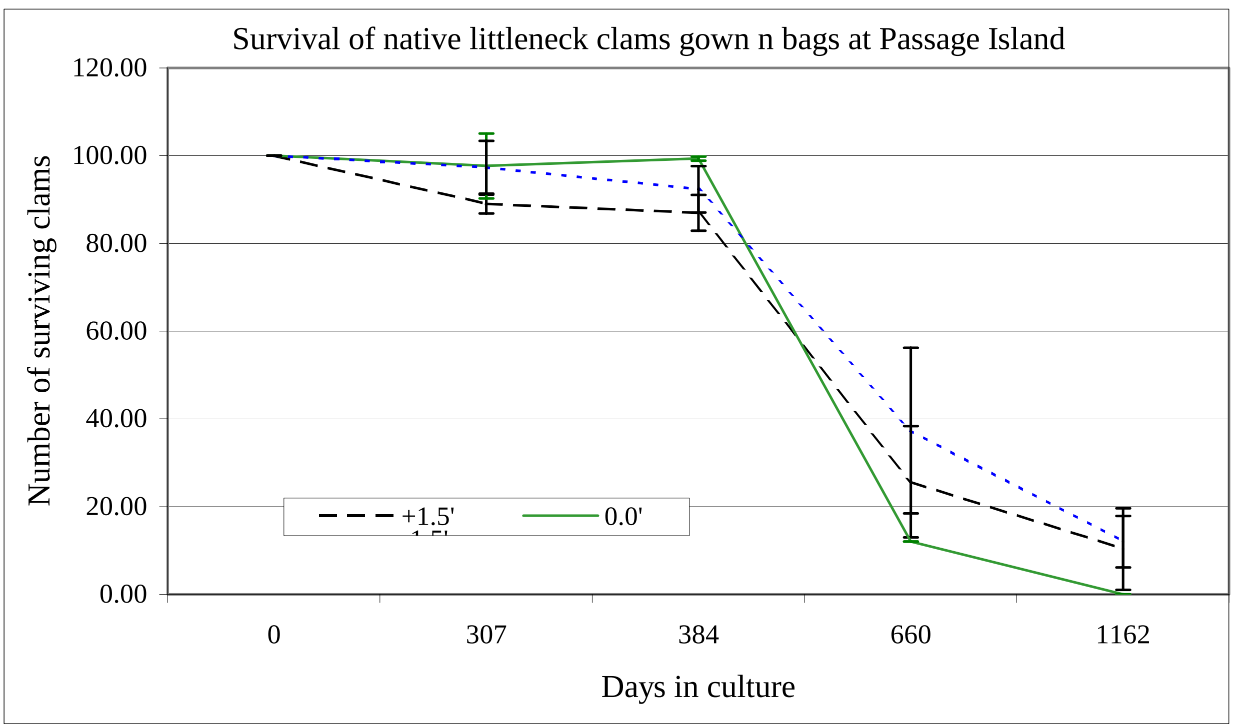


Figure 90. Number of surviving clams grown in bags at Passage Island, Alaska through September 8, 1999.

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.

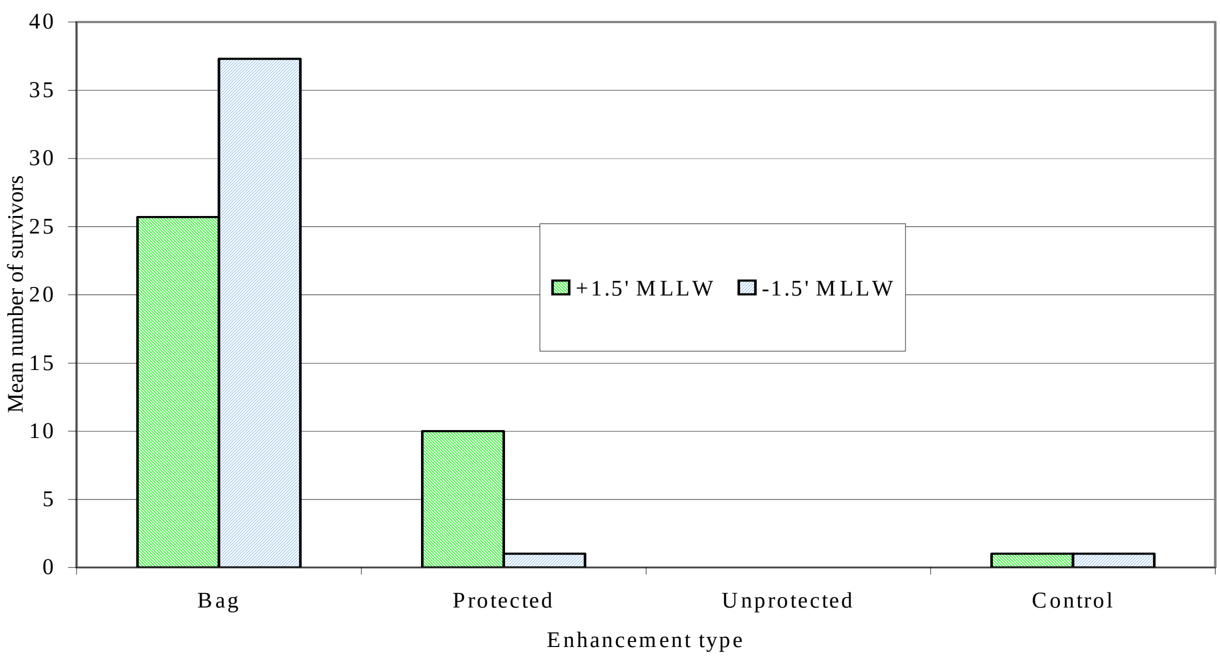


Figure 91. Survival of native littleneck clam (*Protothaca staminea*) seed planted in the intertidal area of Passage Island during 1996 and evaluated on April 24, 1998.

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.

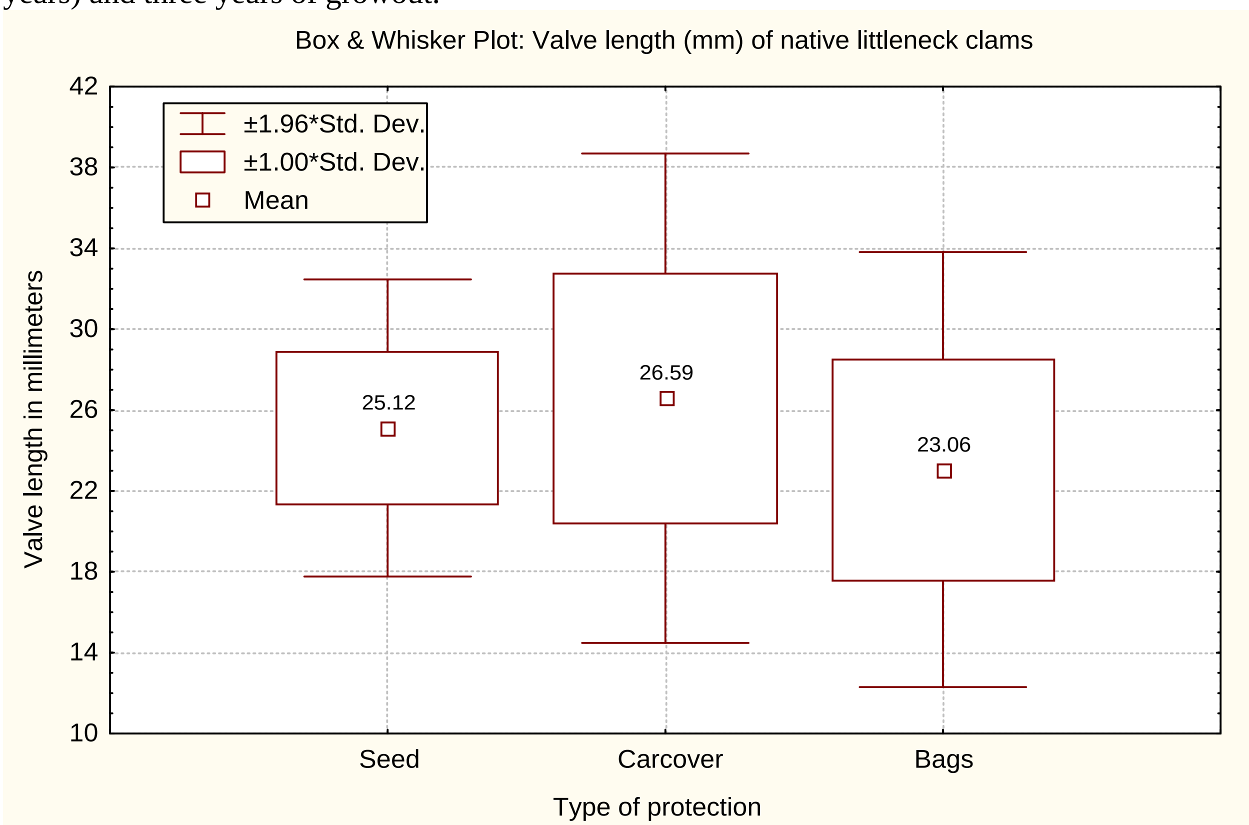


Figure 92. Final valve lengths of native littleneck clams grown at Passage Island for three years. Clams were seeded into cultivated sediments and either protected with plastic netting (Carcover™) or unprotected (Seed). Nine additional cohorts of 100 clams each were grown in plastic clam cages. Differences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

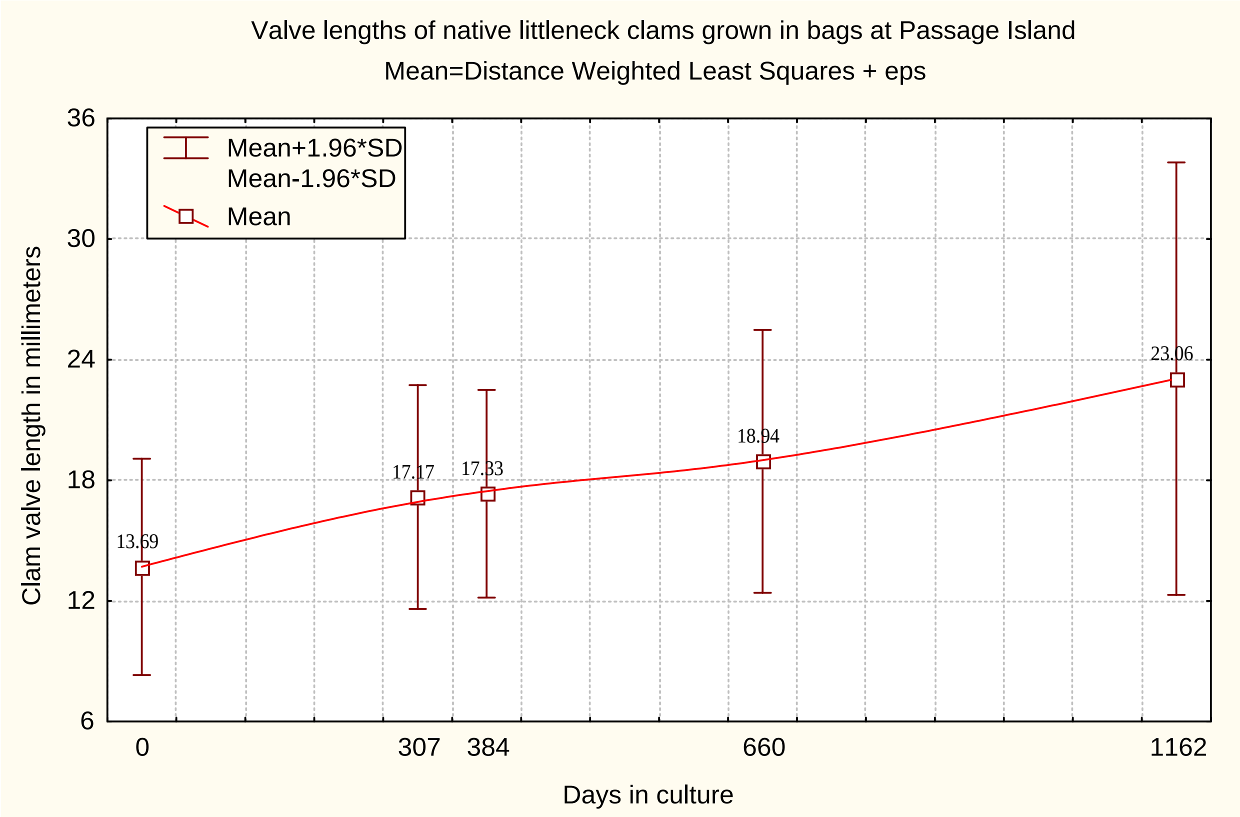


Figure 93. Mean length (mm) of clams grown in bags at all tidal elevations on Passage Island, Alaska as a function of seed age.

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.

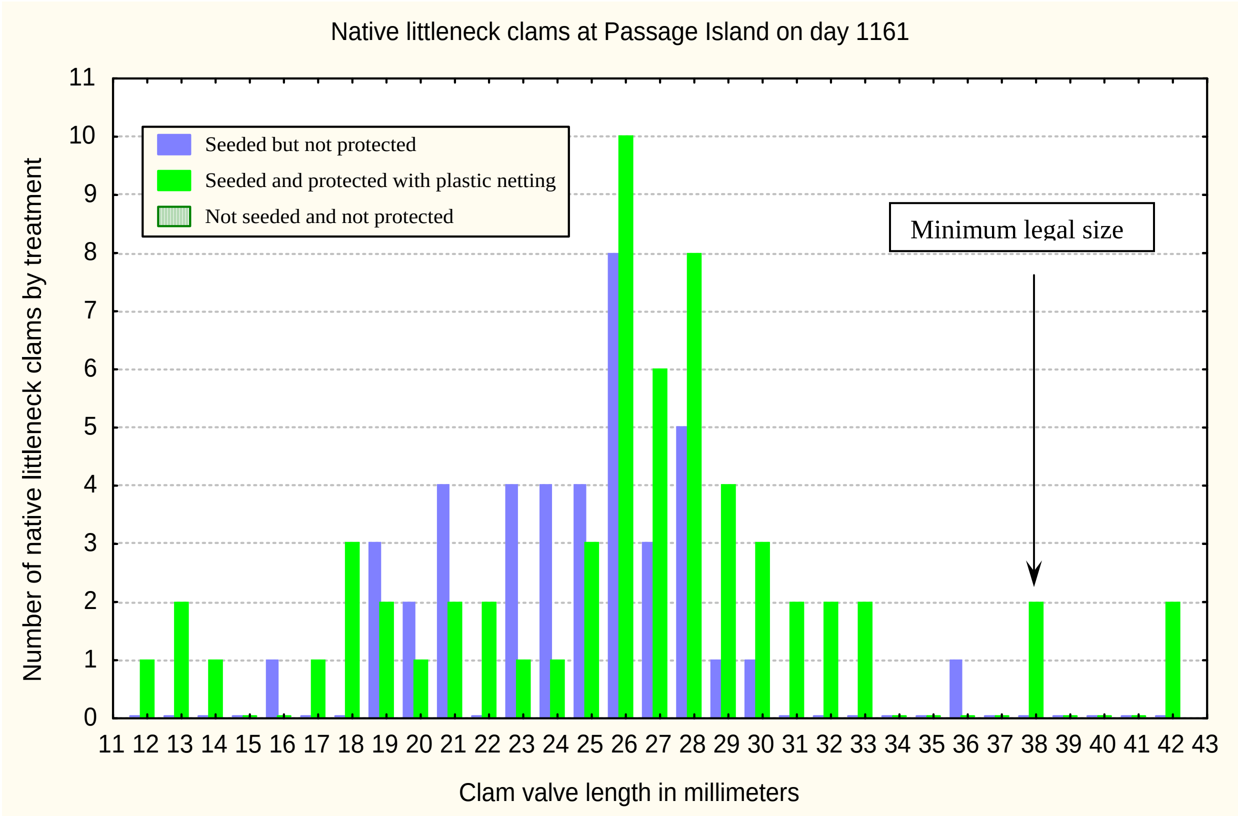


Figure 94. Length frequency histogram describing the population of native littleneck clams observed on September 8, 1999 at Passage Island, Alaska. Clams depicted in green were retrieved from plots protected with plastic netting. Clams in blue were seeded but not protected. No native littleneck clams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

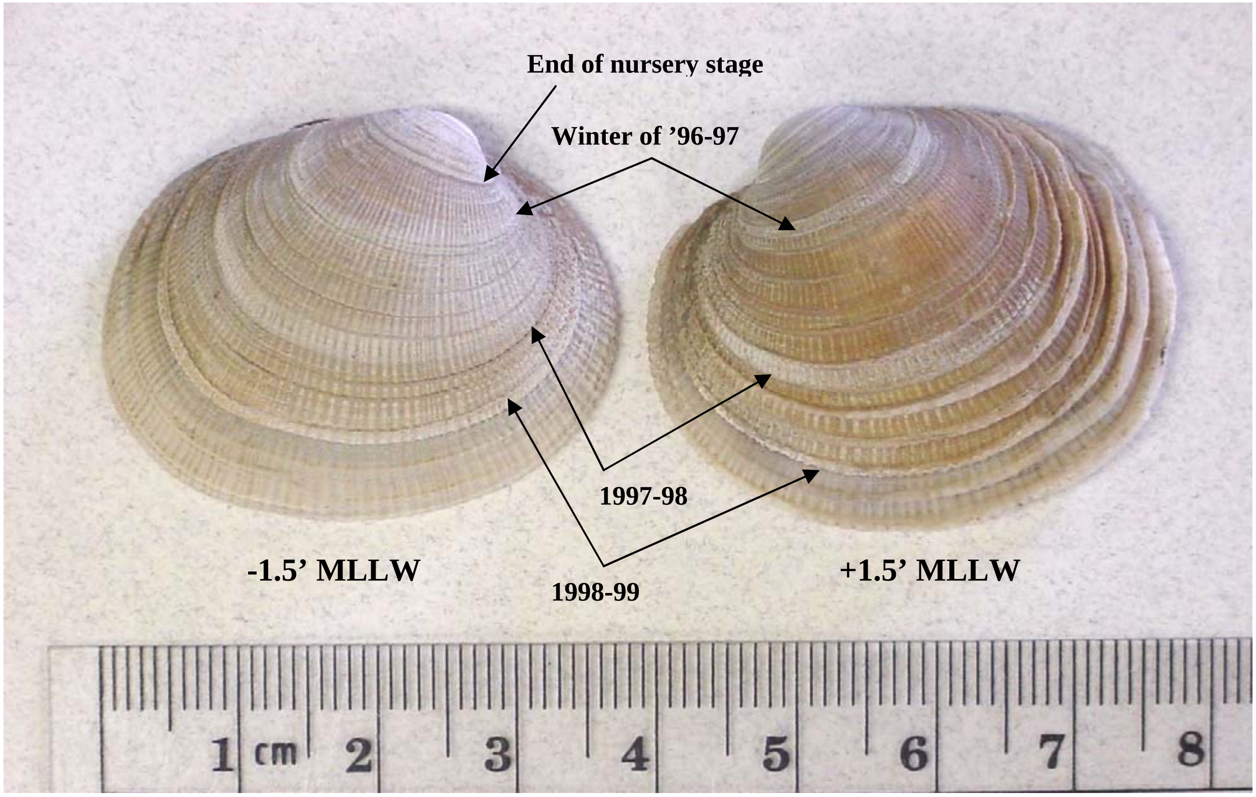


Figure 95. Differential valve sculpturing observed in native littleneck clams cultured at two different tidal elevations under plastic netting in Murphy's Slough, Alaska. Apparent annuli are identified.

- **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.

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

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

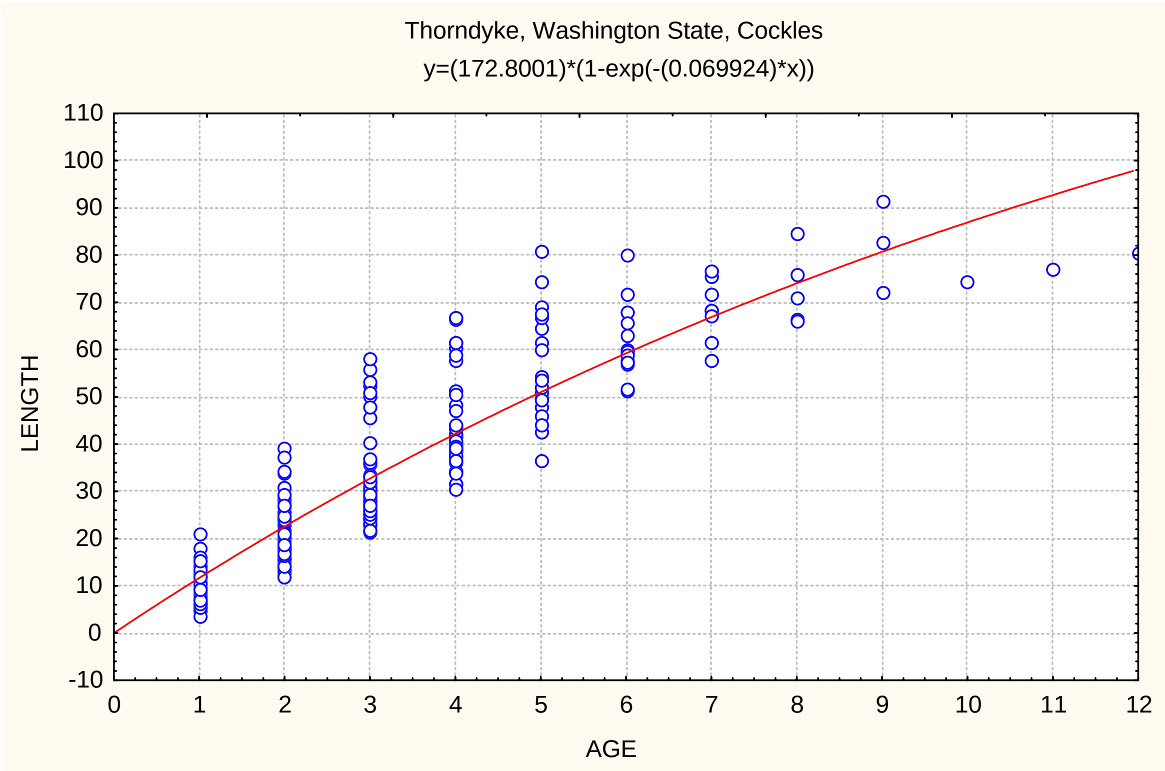


Figure 97. Length at age with von Bertalanffy model predictions for cockles collected from Thorndyke Bay in Washington State.

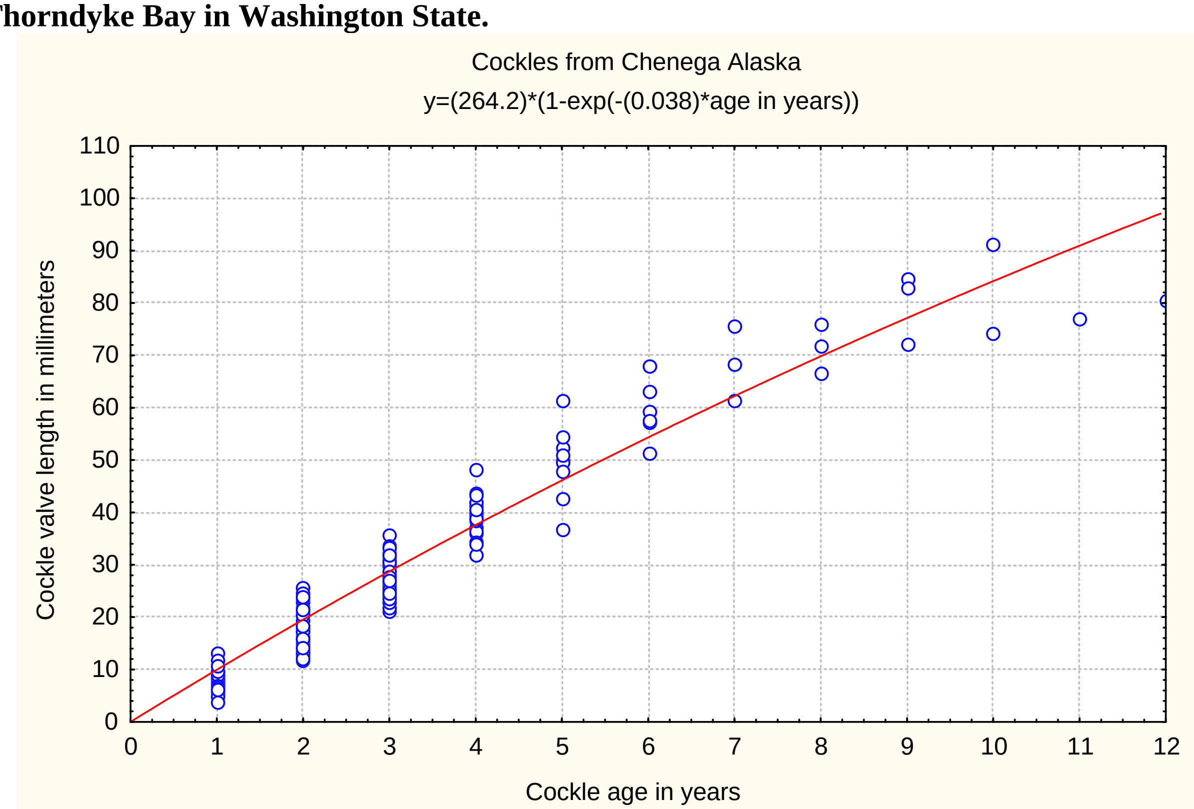


Figure 98. Length at age with von Bertalanffy model predictions for cockles (*Clinocardium nuttallii*) from Chenega, Alaska.

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

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

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

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

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

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

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

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

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

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

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

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

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

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

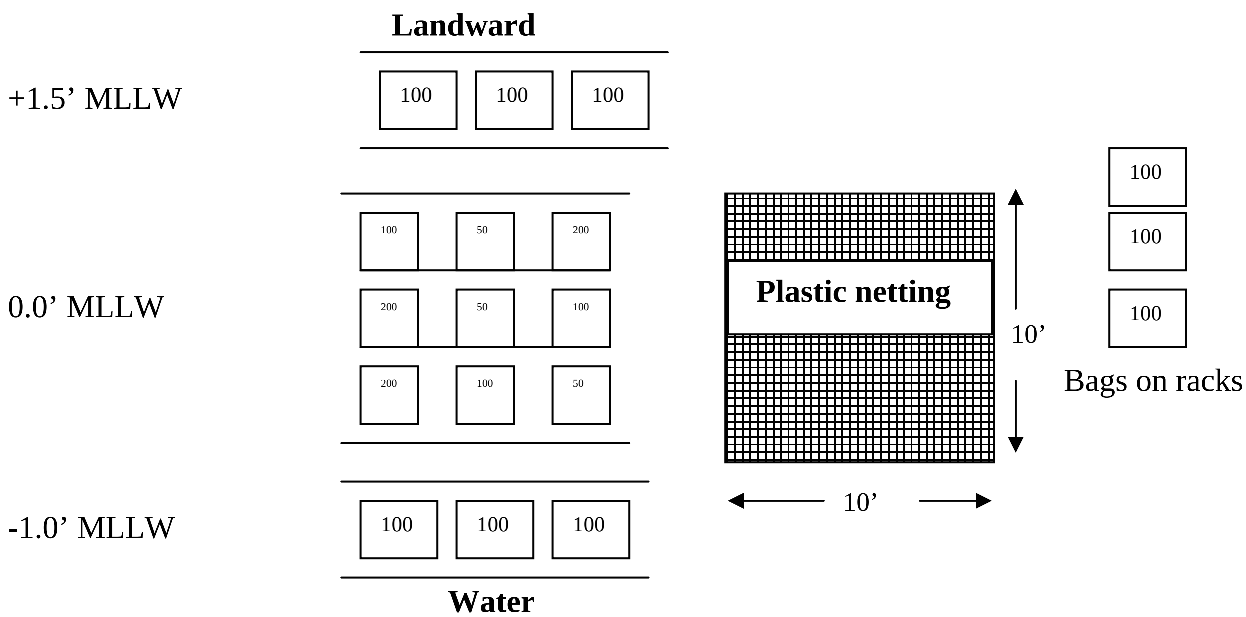


Figure 99. Layout of cockle (*Clinocardium nuttallii*) studies conducted in Thorndyke Bay, Washington State during 1999 and 2000.

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

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

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

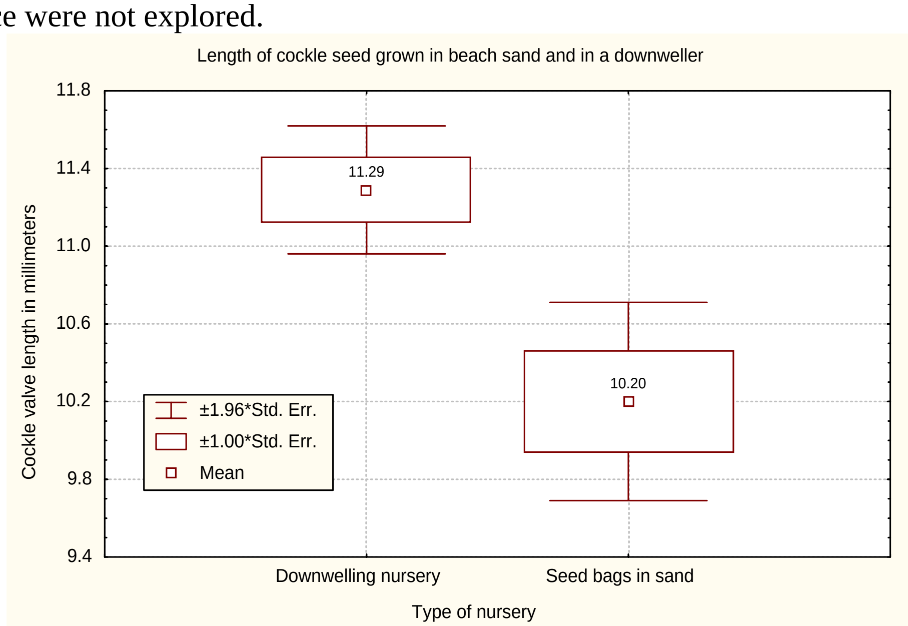


Figure 100. Comparison of the lengths of 100 cockle seed sampled from Taylor Resources hatchery downwelling nursery system and a beach culture planted in Thorndyke Bay in seed bags at the 0.0' MLLW tide level.

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

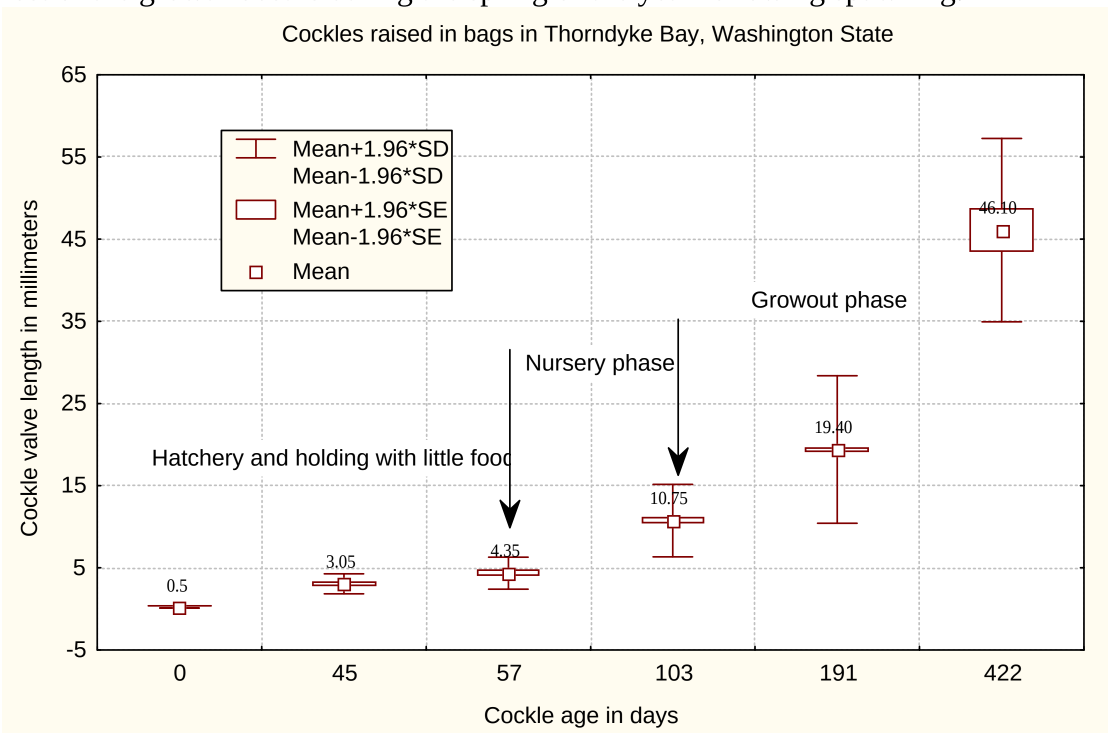


Figure 101. Nuttall's cockle (*Clinocardium nuttallii*) valve lengths as a function of age post setting. The cockles were spawned during the first week in April 1999 and held on minimum rations until June 2, 1999 when they entered Taylor Resources' nursery on Dabob Bay, Washington. The cockles were outplanted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

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

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

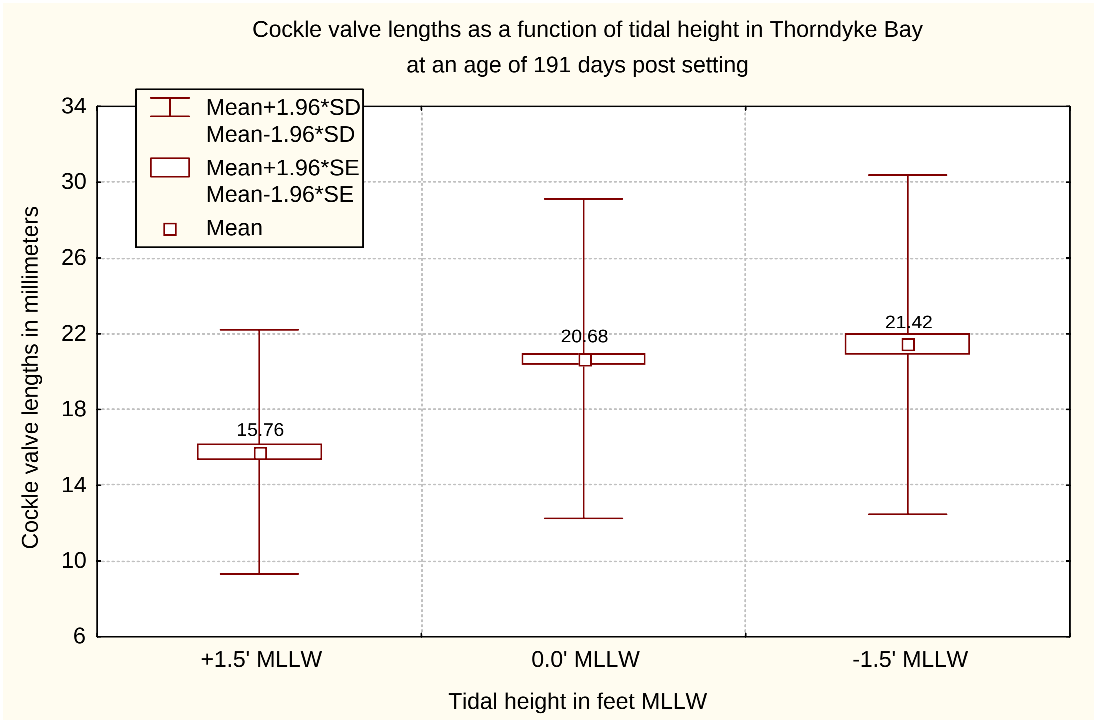


Figure 102. Mean valve lengths for cockles grown to an age of 191 days at three tidal heights in Thorndyke Bay, Washington. Cockles were seeded in three replicates each at a rate of 100 animals per half Norplex™ clam bag.

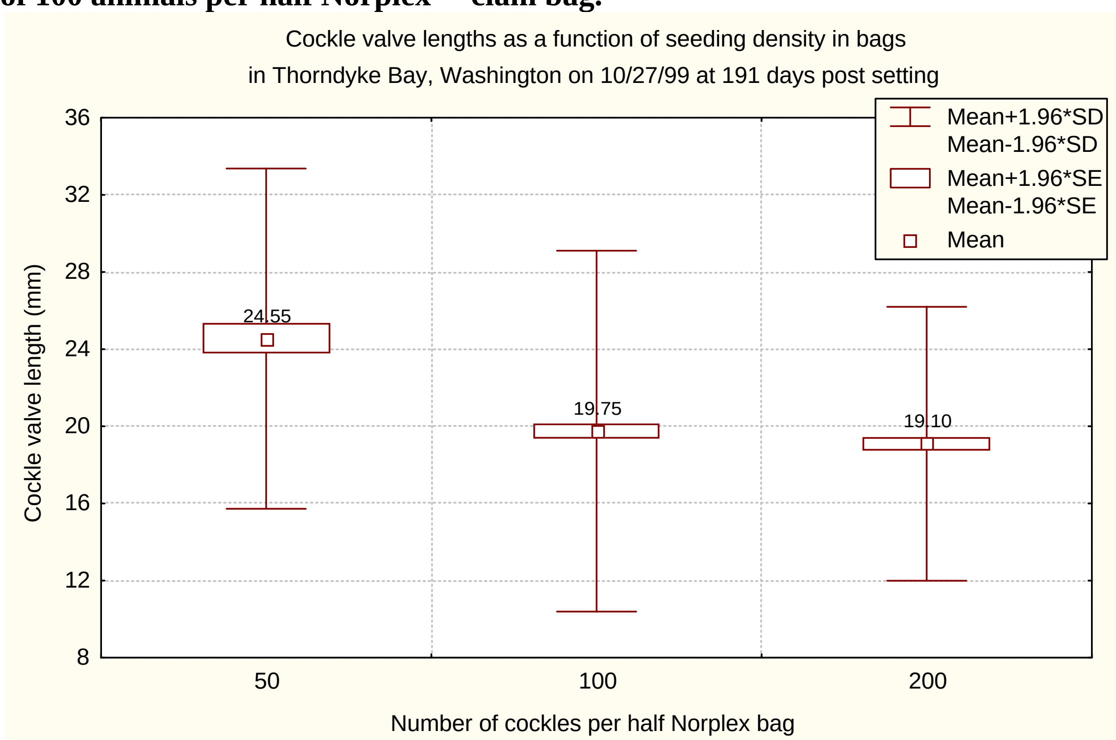


Figure 103. Cockle (*Clinocardium nuttallii*) valve lengths observed following 88 days of growout in Thorndyke Bay (Age = 191 days) as a function of planting density. The differences between each group were significant at $\alpha = 0.05$. Data included three replicates at each density. All replicates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

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

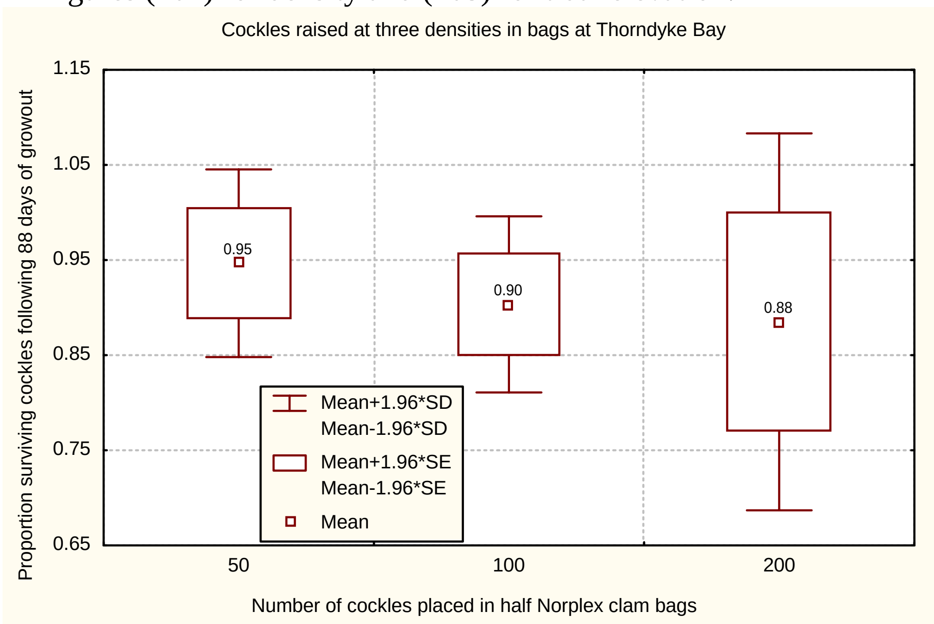


Figure 104. Survival of cockles following 88 days of growout. The bivalves were planted at three densities at the 0.0' MLLW tide level in Thorndyke Bay, Washington. None of the observed differences were statistically significant at α = 0.05.

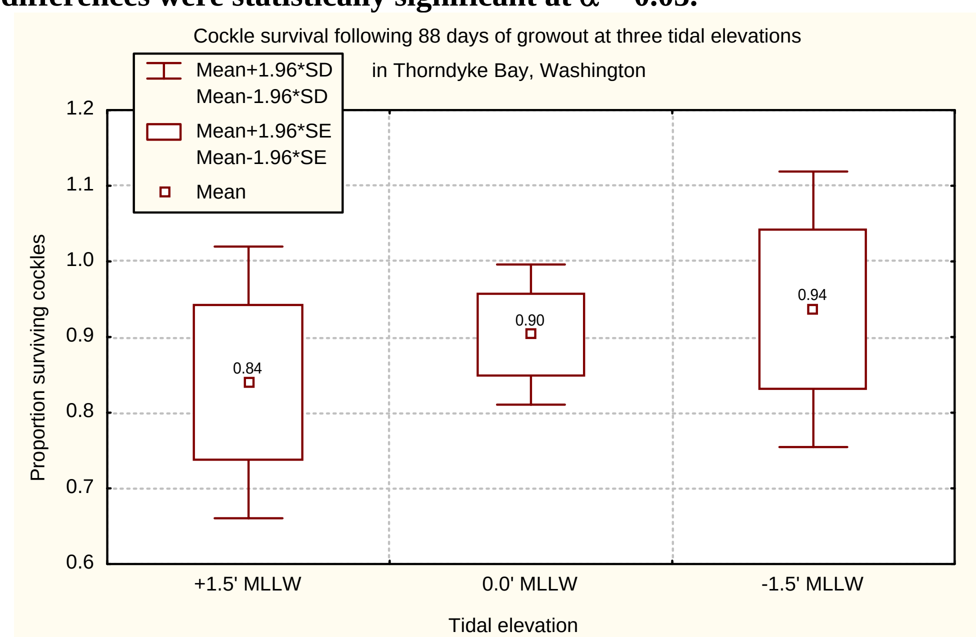


Figure 105. Survival of cockles following 88 days of growout. The bivalves were planted at a density of 100 cockles per half Norplex™ clam bag at three tidal elevations in Thorndyke Bay, Washington. The observed differences were not statistically significant at α = 0.05.

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

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

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

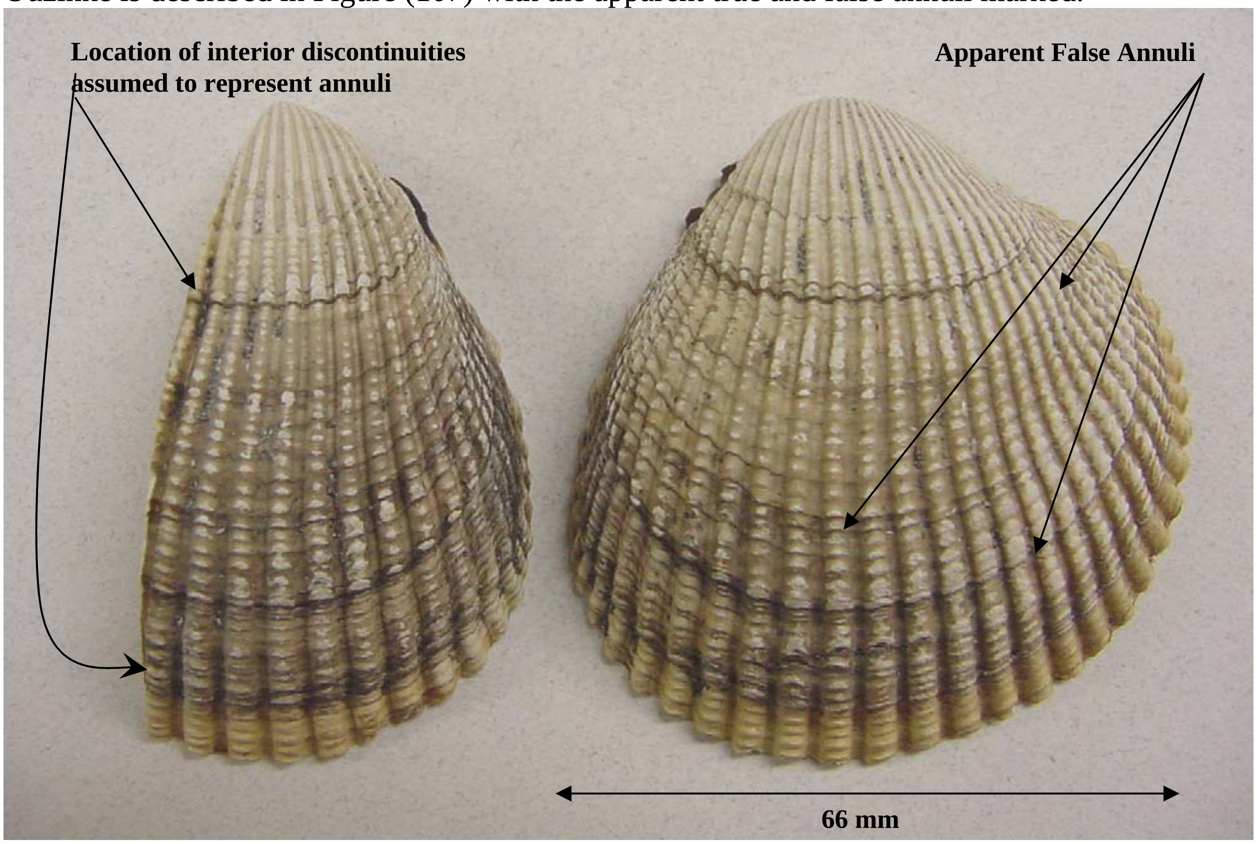


Figure 107. Cockle (*Clinocardium nuttallii*) valves from Ouzinke, Alaska with the annuli identified in sectioned material identified on the left and apparent false annuli on the valve's exterior annotated on the right. The valve length in this cockle was measured at 66 mm and was judged to have lived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

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

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

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

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

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

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

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

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