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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Table of contents

Table of contents continued:

Page

Table of contents continued:

Page

Table of contents continued:

Page

Table of contents continued:

Table of contents continued:

List of Figures

List of Figures continued:

Page

List of Figures continued:

Page

List of Figures continued:

Page

List of Figures continued:

Page

List of Figures continued:

Page

List of Figures continued:

Page

List of Figures continued:

Page

List of Figures continued:

Page

List of Figures continued:

Page

List of Tables

List of Tables continued:

Page

List of Tables continued:

Acknowledgements

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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.

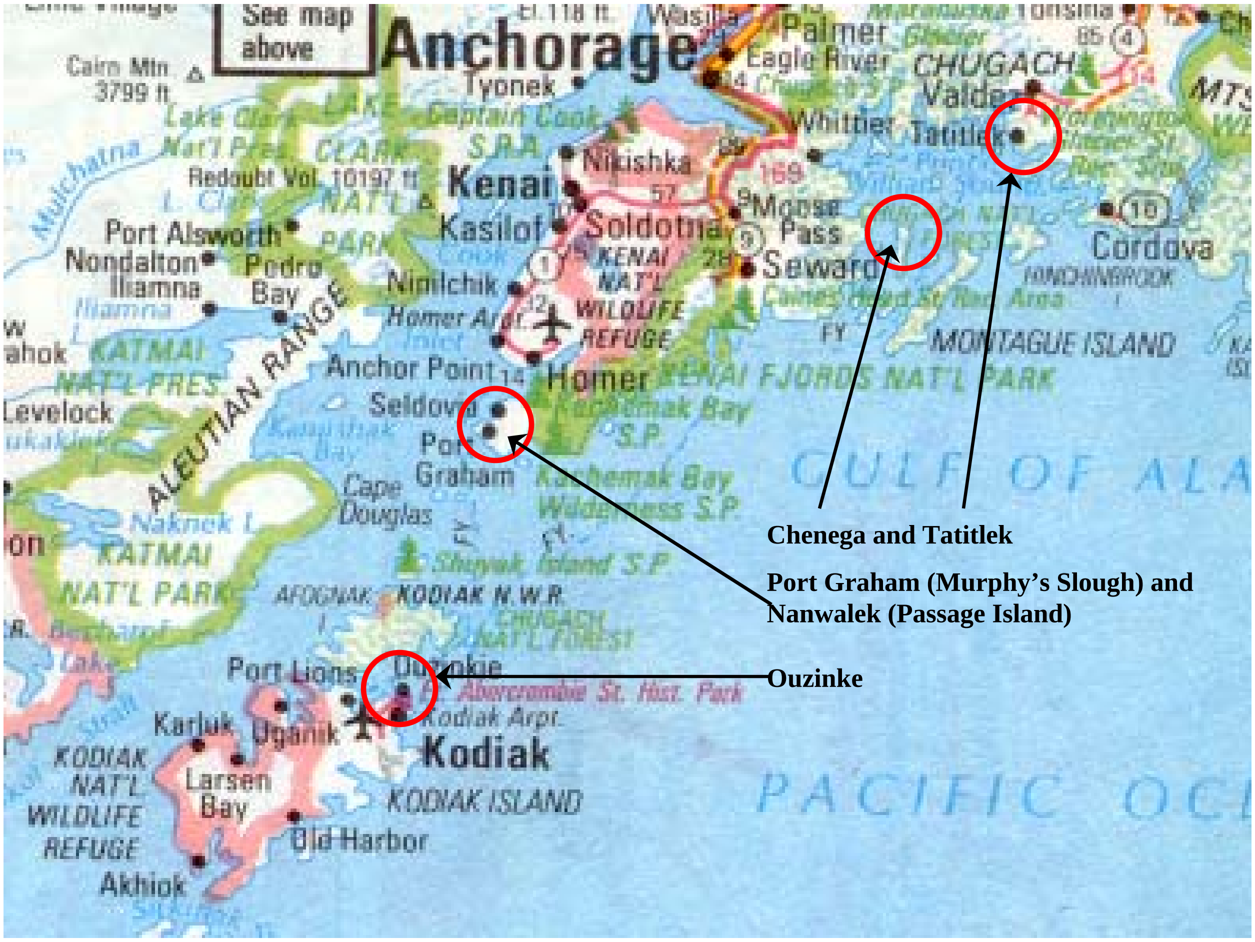

Figure 1. Location of native villages participating in the Chugach Regional Resource Council bivalve inventories and native littleneck clam enhancement studies. Bivalve inventories were completed at selected beaches near all five villages. Enhancement studies were undertaken at Murphy's Slough, Passage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

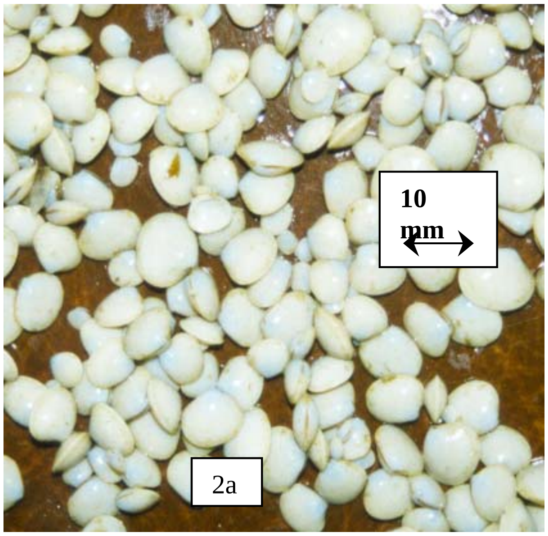

Figure 2a. Hatchery produced native littleneck clam seed ready for planting; 2b. Four-year-old native littleneck clams still showing the polished appearance of the early shell; 2c. Wild native littleneck clam from Tatitlek.

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 ($\geq$ 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 $\geq$ 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 $\geq$ 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)	$\geq$ 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.

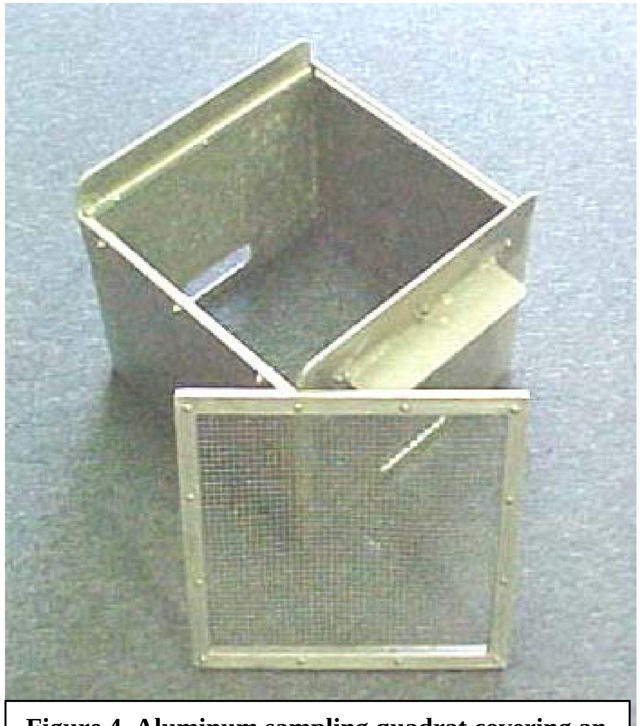

Figure 4. Aluminum sampling quadrat covering an area of 0.1 m^2 with a removable ¼" sieve

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.

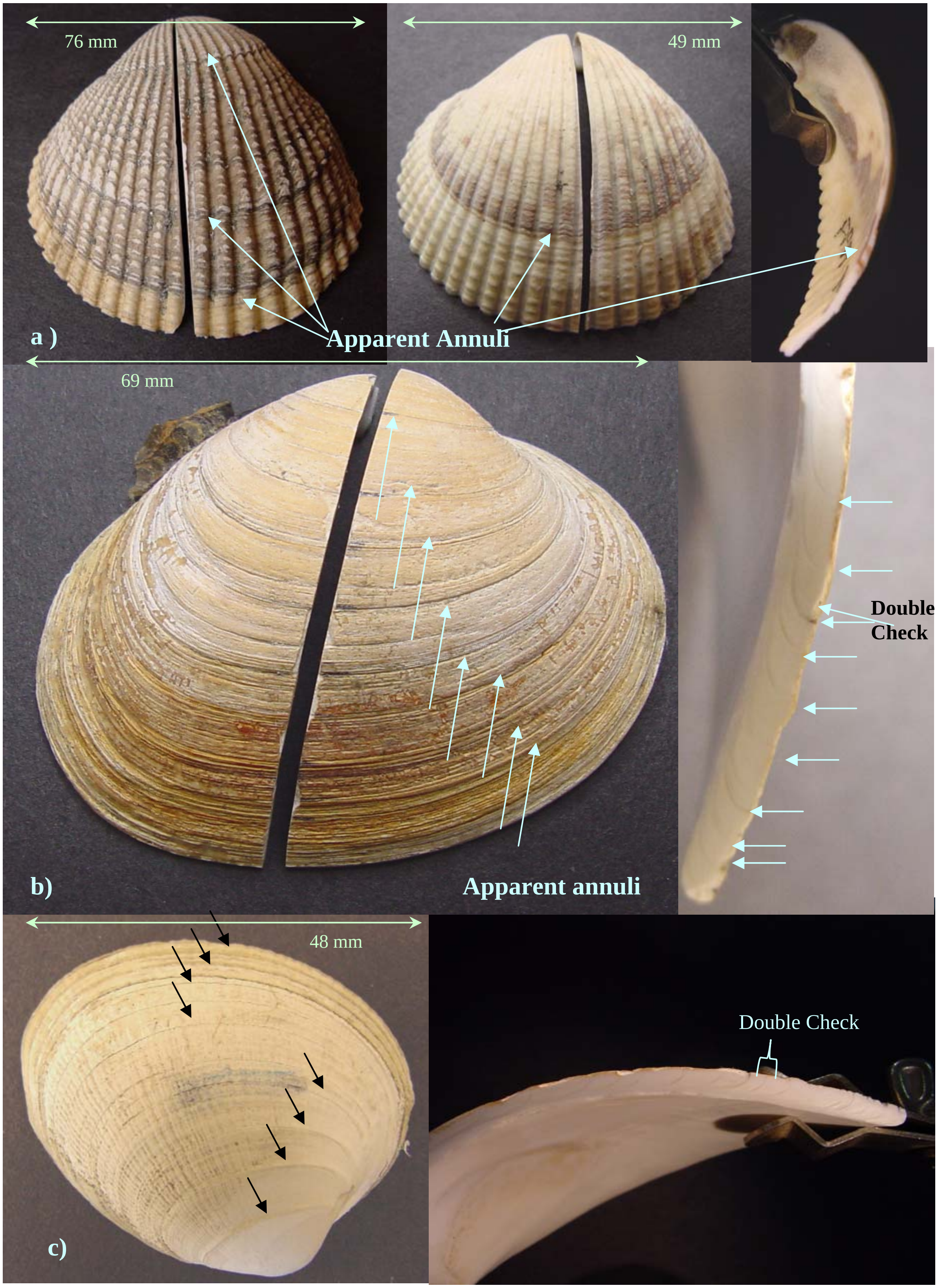

Figure 5. Typical valves of a) Nuttall's cockle (*Clinocardium nuttallii*), b) butter clams (*Saxidomus giganteus*) and c) native littleneck clams (*Protothaca staminea*).

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 ^{o}C 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^{o}C until they were analyzed. The sediments were dried in an oven at 92 ^{o}C 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 ^{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 combusted at 550 ^{o}C 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^{o}C and weighed. A 350 ml sample of thoroughly mixed water was suction filtered and the residue dried at 103 $\pm$ 2 ^{o}C to determine TSS. Total volatile solids were determined following combustion of the sample at 550 ^{o}C.

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