

Exxon Valdez Oil Spill
Restoration Project Annual Report

Clam Restoration Project

Restoration Project 97131
Annual Report

This annual report has been prepared for peer review as part of the *Exxon Valdez* Oil Spill Trustee Council restoration program for the purpose of assessing project progress. Peer review comments have not been addressed in this annual report.

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Study History: The project effort was initiated under Restoration Project 97131, the subject of this annual report. This is the third year of a scheduled five-year project.

Abstract: Cost effective procedures for establishing safe, easily accessible subsistence clam populations near Native villages in the oil spill region will be established. The Qutekcak hatchery in Seward will annually provide about 800,000 juvenile littleneck clams and cockles. Historical information, local and agency expertise, and research will be used to identify areas to seed and method. Total seeded area during the project will not exceed 5 hectares. Follow-up research on success of seeding will be conducted. Development work will be confined to areas near the Native villages of Eyak, Tatitlek, Nanwalek and Port Graham.

Key Words: Beach survey, clam growout areas, clam restoration, cockles (*Clinocardium nuttalli*) Exxon Valdez oil spill, Eyak, littleneck clam (*Prothothaca staminea*), Nanwalek, Port Graham, shellfish hatchery, shellfish nursery, Tatitlek.

Project Data: (will be addressed in the final report)

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Executive Summary

Clams were once a major subsistence resource in the Native communities of Nanwalek and Port Graham in lower Cook Inlet and Tatitlek in Prince William Sound. Local clam populations have been decreasing in recent years and their contribution to the subsistence harvest has been greatly reduced. There are probably several reasons for this including changes in currents and beach patterns, increasingly heavy sea otter predation and the *Exxon Valdez* oil spill. The oil spill impacted the wild clam populations and their importance as a subsistence food in two ways. First, some clam beds suffered from direct oiling. Second, even though the oil did not directly impact many clams, they have a tendency to accumulate, concentrate and store the toxic contaminants from non-lethal amounts of oil. This has badly eroded the confidence of the villagers in the healthfulness of the remaining wild clam populations as a subsistence food.

The project goal is to provide the project villages with safe, reliable, easily accessible sources of clams for subsistence use. Project objectives for 1997, the third year of the project, were to continue to improve hatchery production techniques for littleneck clams (*Protothaca staminea*), develop hatchery culture procedures for cockles (*Clinocardium nuttalli*), continue work with the nursery pond near the hatchery as well as experiment with a tidally driven fluidized upwelling nursery system (FLUPSY), continue littleneck clam growout studies on beaches near Tatitlek, Port Graham and Nanwalek, test predator control coverings on razor clam beaches near Eyak, and initiate PSP testing of the designated subsistence beaches. The following is a rundown of the activities under each objective.

Hatchery: The Qutekcak Shellfish Hatchery located on the Institute of Marine Science grounds in Seward has been in operation since October 1993. This is a small, temporary facility will be used until the State of Alaska's Mariculture Technical Center/Hatchery Complex is built and ready for occupancy. The Qutekcak Hatchery will be leasing the hatchery portion of the State facility. Anticipated occupancy of the new hatchery is January 1998.

The hatchery experienced a good rate of success in producing littleneck clam beginning in February 1997. The biggest change from previous methodology involved reducing the broodstock conditioning temperature from 13°C to 9.5°C. This change resulted in the brood clams completing rapid gametogenesis and the resulting zygotes having high rates of normal development to D-veligers. Extensive abnormal development of early larvae has not recurred since this change. However, irregular two to four day periods when new algae cultures fail to grow combined with sudden larval mortality, despite carefully standardized procedures, demonstrate ongoing sudden water quality changes. This phenomenon is receiving continued investigation.

The small size of the pilot hatchery is an impediment to production and survival rates. Each clam spawn easily produced more larvae than capacity allowed. Consequently, the spawning was quenched after about 5 million eggs were released. Littleneck clam larvae have proven very sensitive to larval rearing densities typically found at other hatcheries. Older larvae must be reared at a density of less than one larva per 2 milliliters to obtain even slow growth.

Survival through metamorphosis was highly variable ranging from 10 to 80 percent. Insufficient space in the pilot hatchery necessitates placing the setting pediveligers into the same downwelling system containing spat from prior settings. This cohabitation reduced food availability and water quality for the setting pediveligers leading to increased mortality during the

stressful metamorphic process. Approximately 200,000 spat from the first four spawns and an additional 150,000 spat from the last three spawns survived metamorphosis.

In an effort to gain an understanding of why the hatchery experienced several episodes of poor algae production and large losses of larval clams the hatchery enlisted the aid of EVS Environment Consultants and Dr. Ralph Elston of AquaTechnics, Inc. These consultants performed both histo-pathological examinations of clam larvae and water quality analyses to try to isolate the cause(s) of this problem. Nothing definitive has been discovered to date and further tests are scheduled if or when the problem occurs again.

Work under a contract with Aquatic Environmental Sciences, Port Townsend, WA, to develop spawning techniques for the cockle is ongoing. To date the contractor has been unable to produce any fertilized eggs using an array of standard spawning inducing techniques. Unfortunately, there is nothing in the literature describing successful methods of inducing spawning in this animal. It should be noted that similar difficulties have been experienced in the initial attempts to spawn a variety of shellfish. Work on cockles under this project has been curtailed for FY 98, but will continue under a different project.

The cockle is a popular subsistence species. Its high value warrants additional research to produce a successful method to spawn them.

Pre-Nursery Pond: The hatchery utilizes a 1 million liter pond to culture algae for its pre-nursery. The 30m by 37m pond is 5 meters at its deepest point. Raw seawater from a 60-meter deep intake is pumped into the pond to bring in nutrient rich water. The flow can be controlled to allow for adequate flushing yet maintain the ambient air temperature. An air compressor is used to aerate and circulate water in the pond to eliminate stratification and increase phytoplankton production. Fertilizer solutions are added daily to increase the intensity and duration of phytoplankton blooms. Physical parameters of the seawater including temperature, salinity, pH, and redox potential are monitored and water samples are collected at various intervals for nutrient level analysis. Identification of the most abundant phytoplankters as well as secchi disk readings are also made. The food laden pond water is pumped through dense trays of small (1+mm) littleneck clam spat. Spat reaching 3+ mm are removed and sent to both the tidal FLUPSY at Tatitlek and the pump driven FLUPSY at Chenega Bay. Outdoor rearing of clam spat in pond upwellers continued through October at which time they were returned into the hatchery.

The pond received a much needed draining and cleaning in FY 97, which greatly reduced the quantities of suspended particulate inhibiting diatom growth. Many yards of mud were washed off the sides and then vacuumed off the bottom liner with a "super sucker" vacuum tank truck. After this cleaning a dense diatom bloom was easily sustained all summer and even through November when seawater temperatures fell to 4° C or 5° C. The pond was enriched with the same fertilizer at F/2 ratios as described in the last report. Trace mineral and CO₂ enrichment were not used because of the high costs of these compounds and the over-abundance of pond algae for existing numbers of clam spat.

Outdoor microalgae culture in large 10,000-liter tanks proved very successful and reliable in FY 97. Culture densities typically grew to an impressive 300,000 cells per milliliter of *Skeletonema costatum*, *Thalassiosira gravida*, and *Chaetoceros spp.* Unfiltered seawater from 60 m deep

intake was pumped into the pond-side tanks, fertilized and aerated with only natural illumination for about five days until harvest. A bloom of a lipid rich, green *Tetraselmis striata* was also maintained for three months in one of these outdoor tanks; harvesting half the culture every few days. This microalgae can be pumped directly into the pre-nursery upwellers to feed the larger spat or drained into the pond as a large-scale inoculate.

Tidal FLUPSY: A tidal fluidized upwelling system (tidal FLUPSY) was designed and constructed to test its potential as a remote nursery system for the EVOS clam project. Remote nursery systems offer several advantages over nursery culture at the hatchery. One is that it frees up hatchery space and personnel that can be better used in hatchery production. Another is that several remote nursery systems offer a redundancy of supply in case one of the systems fails. A third is that remote nursery systems can be located near the growout areas thus reducing transport costs. The big disadvantage to remote nursery systems is that the cost of pumping water at a remote location in Alaska makes them impractical.

A tidal FLUPSY is designed as a low maintenance non-mechanical method to nursery shellfish. The unit, when anchored, directs tidal and current flow into a flume that forces large quantities of water (and plankton) to flow through upwelling chambers containing juvenile bivalves.

An aluminum tidal FLUPSY identical in size and dimension to the system described in Baldwin, et al. "Construction and Operation of a Tidal-Powered Upweller Nursery System" 1995, South Carolina Sea Grant Consortium, was built and set up in late August in the Tatitlek Narrows near the village of Tatitlek. Since there was no clam seed available, the unit was seeded with 50,000 oyster seed averaging 15mm in length. The seed were removed from the FLUPSY in late November and had increased 20% in size to an average length of 18 mm. The FLUPSY was badly damaged in a winter storm and was rebuilt.

In lieu of clam seed in the spring of 1997 the FLUPSY was seeded with about 50,000 oyster seed on May 25, 1997. Average length was 7.0 mm. The oyster seed was removed from the FLUPSY on September 10 at which time they had doubled in size to an average length of 14.5 mm. Approximately 83% of the oyster seed survived.

Around 10,000 littleneck clam seed were placed in the FLUPSY on October 31. They remained in the FLUPSY overwinter and did not fare well. Only 15% of the seed survived the winter and growth was negligible.

This was the second year of problems with overwintering seed in a tidal FLUPSY. This is obviously not a good strategy and will not be continued. Seed placed in a tidal FLUPSY in the spring should be of sufficient size for planting in growout areas by late summer. Further confirmation of this will be obtained in FY 98 with clam seed that was placed in the FLUPSY in late March 1998.

Growout Studies: Growout study work in FY 97 concentrated on determining growth and survival of littleneck clam seed planted on beaches near the Tatitlek, Port Graham and Nanwalek villages in FY 96. These beaches were identified during the FY 95 beach surveys. About 8,100 clam seed per village were used in three separate tests with same tests conducted at each village. One test involved placing 100 measured clams in each of nine "Norplex" clam bags. Three bags each were nestled into the substrate to a minimum depth of 4 inches at the -1.5 MLLW tide level, the "zero" tide level (mean lower low water) and the +1.5 MLLW tide level. These clams are

being used for detailed growth and mortality studies. The remaining clams were divided into 12 subsamples of about 600 clams each. Six of the subsamples were seeded at the +1.5 MLLW tide level, three under netted "car cover" and three uncovered. The remaining six subsamples were seeded at the -1.5 MLLW tide level in a similar arrangement.

The littleneck clam seed were placed in the test plots between June 27 and July 4, 1996. After that the clams in the Norplex bags were checked for survival and growth at quarterly intervals through November 1997 or a period of 17 months. The Nanwalek site was not sampled during the last two quarters so survival and growth data are only available for a 12-month period ending July 22, 1997.

The survival rate during this period was very good. Survival was greater than 80% at all three village sites. This is as good or better than the survival rate for manila clams grown under culture in Puget Sound. Growth rates for the Tatitlek and Port Graham sites fell on the high side of what was expected. Growth (increase in valve length) at these sites for the 12 month period ending in July 1997 was just under 8 mm. Growth for the full 17 month period was around 9 mm. Growth at the Nanwalek site was a lot less, averaging less than 4 mm for the 12 month period ending in July.

The Nanwalek site appears to be a much poorer site from the standpoint of growth. It is unclear why at this point. The major difference between the Nanwalek site and the other two sites is that it is a more exposed and higher energy beach. Although this doesn't seem to effect survival, it may be retarding clam growth.

For the Nanwalek and Port Graham sites there was no significant difference in growth rates among the clams planted at the -1.5 MLLW, MLLW, and +1.5 MLLW tide levels. There was a significant difference in growth among the three tide levels at the Tatitlek site with clams at the +1.5 MLLW tide level growing 1 mm and 2.5 mm larger than clams at the MLLW tide level and the -1.5 MLLW tide level respectively. It is uncertain at this point what may have caused this growth differential. There is wild littleneck clam seed in the area and it is possible that some of this seed got introduced into the Norplex bags when new gravel was put in the bags during one or more of the quarterly samplings. Sampling protocol calls for the gravel to be sifted with a ¼ inch sieve before being put in the bags. However, if this procedure wasn't followed, wild clam seed of the same year class could have gotten into the bags. If this is what happened it will also skew the survival rate. This isn't a problem at the Port Graham and Nanwalek sites because there are no wild littleneck clams near either area. Additional sampling in FY 98 and FY 99 should provide at least a partial answer to what caused the different growth rates among the three tide levels at Tatitlek.

During FY 96 a total of six two square meter plots were cultivated and seeded at the -1.5 MLLW tide level with another six at the +1.5 MLLW tide level. Half these plots were protected with "car cover" beach netting and half were left exposed. The purpose of the covered plots was to determine whether clams in this environment had similar growth and survival rates as those in the Norplex bags. The purpose of the uncovered plots was to evaluate the potential for enhancing shellfish beds without protection.

In FY 97 both the covered and uncovered plots were sampled for growth and survival at the Port Graham site during the November sampling. Otherwise, these plots have been left undisturbed. Growth in both the covered and uncovered plots at Port Graham was not significantly different

than in the Norplex bags. Survival in the covered plots was around 94%, which is significantly higher than the 80% survival rate in the Norplex bags. Survival in the uncovered plots was about 8%. Additional sampling will need to be done on these plots, but it appears that protecting the clams with netting (or a bag) greatly enhances their chances of survival. Further testing will also be needed to determine if whether a cultivated plot protected with netting offers a better environment for clam survival or if the poorer survival in the Norplex bags is an artifact of the higher sampling rate.

Clam seed that was intended for seeding in the growout areas in FY 97 did not reach sufficient size until late summer. The next available tide cycle for planting this seed occurred in mid October. Unfortunately foul weather during this tide cycle prevented the crew from getting out on the grounds and planting the seed. About half of this seed was later shipped to Tatitlek and Port Graham for holding until spring. About half of this seed was lost overwinter. Seed not sent to the villages was returned to the hatchery to overwinter in the hatchery pond. In the future all seed that cannot be planted out during a given year will be returned to the hatchery.

Eyak Razor Clam Studies: This is the second year of a project designed to provide baseline information for future efforts to restore and enhance razor clam populations *Siliqua patula* for subsistence use by the Village of Eyak near Cordova..

Initially, it was believed that there was some number of sub-legal (too small for legal harvest) clams on nearby beaches that would grow to harvestable size if predation could be reduced. However, this turned out not to be the case as very few clams of any size were found during the FY 96 beach surveys. The clams that were found were placed in a 4 meter by 3-meter predator control study area and covered with 12-millimeter mesh netting. The netting was torn off during a severe winter storm so no information from this study was collected.

In FY 97 the objective was changed from locating a population of sub-legal razor clams and applying predator control measures to capturing as many razor clams as possible, with an emphasis on sub-legals, and transporting them to a selected growout area to conduct growth and mortality studies and evaluate predator control measures. A total of 82 clams were collected in June and July of 1997. They were mostly three and four year olds (legal harvest size is achieved at around age seven). These clams were marked and buried at the study site at 6-inch intervals. Car cover netting was than placed over all the clams and anchored down.

The clams were last sampled in March 1998. Fourteen clams were recovered at that time, four of which had lost their mark. Average growth on the clams was around 10%. This was less than what was expected from the literature. The stress of moving the clams, being under car cover netting, and/or being in an area with poor growing conditions may be factors that caused the less-than-expected growth.

Further funding under this project for the razor clam work has been curtailed. An attempt will be made to locate an alternative funding source to finish out the work that was started. One interesting point that became apparent during the course of this study is that there are virtually no razor clams, adult or sub-legal, in the Cordova area. This is an area that once had the largest razor clam population in the state.

PSP Testing: The presence of the commercial mariculture operation at Tatitlek eliminates the

need for PSP testing of the local subsistence clam beds. It has proven difficult to collect sufficient samples from the Port Graham and Nanwalek beaches for PSP analysis. In addition the State of Alaska is charging \$125 for PSP tests not required by regulation. Because of this no sampling was done under this project in FY 97.

PSP testing will need to be a major part of any effort aimed at restoring clams for subsistence use. However, this effort no longer fits well within the scope of this project. The Chugach Regional Resources Commission is now in the process of applying for funding to both expand the work started here to other villages in the oil spill region and to develop a comprehensive PSP testing program that can be used to ensure that clams harvested from these beaches are safe to eat.

Recommendations and Conclusions: The separate facets of the study are going well at this point. However, there needs to be better coordination between each facet. For instance, communication and coordination in shipping seed from the hatchery to the remote nurseries and from the nurseries to the growout areas need improvement. A definitive analysis of the clam growth in the tidal FLUPSY still hasn't been done. Emphasis will be placed on completing this analysis in FY 98 and FY 99.

More work needs to be conducted, but at this point it appears that a subsistence clam restoration effort is quite feasible for littleneck clams. The most likely scenario would be for the hatchery to produce 3 mm to 5 mm seed ready for placement in a FLUPSY by mid April. The FLUPSY would produce 8 mm to 12 mm seed ready for planting on the beaches in late summer. These clams would reach harvestable size in about three years.

Although a four year time frame between spawning the clams and harvesting them is long, it would not be an impediment as long as survival rates remained high. Additional work on this project will determine if that is the case.

Introduction

The purpose of this project is to develop cost effective procedures for establishing managed populations of clams in areas that are readily accessible from Native villages in the oil spill region. These clams will be used as a source for subsistence food to replace the natural clam resource that has been lost, damaged or depleted. The villages of Port Graham, Nanwalek, Tatitlek and Eyak will take part in the development process.

Clams were once an important subsistence food in the Native villages. Clam populations in areas that are reasonably accessible to the villages have decreased to very low levels in recent years. Consequently, the role of clams in the subsistence diet in these villages has been greatly reduced. And, with a few exceptions, the role of clams in the subsistence diet of most Native villages in the oil spill area is a lot less than it was historically.

There are probably a number of reasons why local clam populations are currently at low levels. Since clams are basically an unmanaged resource in the oil spill area, there are no quantifiable data available that could point to the actual circumstances that lead to the sharp reduction in these clam populations. However, there are events that likely played a major role. These include changes in beach configurations resulting from the 1964 earthquake, increasingly heavy sea otter predation, human over-harvest and the *Exxon Valdez* oil spill.

The oil spill impacted the wild clam populations and their importance as a subsistence food in two ways. First, many clam beds suffered from direct oiling. The impact of the oil on the clam beds in Windy Bay, for instance, destroyed one of the more important clam beds in the lower Kenai Peninsula. With the current timber harvesting operations soon to provide road access from Port Graham and Nanwalek to the Windy Bay area, the loss of the clam resource there had a major impact on these villages. Second, even though many clams weren't killed from the oil, they have a tendency to accumulate and concentrate the toxic contaminants from non-lethal amounts of oil. This has badly eroded the confidence of the villagers in the healthfulness of the remaining wild clam populations as a subsistence food.

In order to re-establish local clam populations as a subsistence resource for the Native villages a program needs to be developed to enhance the depleted stocks and the replace damaged ones. Over the past ten years the nursery systems and field growout technologies have sufficiently evolved to make clam enhancement and reseeding efforts feasible. This technology can be readily applied to increasing the clam resource near the villages to determine which applications would be best suited for the task at hand.

This program was initiated in FY 95 as a demonstration project. The first year objectives were to decide what species of clams will be used for the project, determine the potential of the Qutekcak Shellfish Hatchery to produce seed for the project and develop the system for identifying the growout areas near the villages of Port Graham/Nanwalek and Tatitlek.

After consultation with the Native villagers, experts in clam production techniques and a literature search, littleneck clams (*Protothaca staminea*) and cockles (*Clinocardium nuttalli*) were selected as the species that will be used in the restoration effort. The butter clam (*Saxidomus giganteus*), a popular species with the Native villagers, was rejected because of its slow growth characteristics and propensity to retain the Paralytic Shellfish Poison toxin for extended periods.

Littleneck clam broodsource for both Port Graham/Nanwalek and Tatitlek have been cleared for use in the Qutekcak Shellfish Hatchery in Seward. A Nanwalek/Port Graham source of cockle broodstock has also been cleared for hatchery use, but the state fish pathologist is withholding clearance for a Tatitlek cockle broodstock pending further analysis.

As part of the study to identify growout areas near the villages a literature search was conducted through the University of Alaska to identify all previous research on littleneck clam life histories and population surveys. Time was spent with Alaska Department of Fish & Game (ADF&G) shellfish biologists from lower Cook Inlet and Prince William Sound to review and discuss clam surveys and management plans, and residents of the villages of Port Graham, Nanwalek and Tatitlek were interviewed to identify nearby areas that either now or once had significant populations of littleneck clams. Beach surveys were then conducted near Port Graham, Nanwalek and Tatitlek. Several sites were identified as suitable for use in this project.

The hatchery produced several small batches of littleneck clam seed. However, survival through metamorphosis was poor. An experienced shellfish hatchery manager was brought into the hatchery to ensure that the proper culture procedures were in place and to improve larval health and survival. Changes made to the broodstock conditioning, spawning, setting and rearing procedures have begun to pay off. Production and survival rates have been at acceptable levels

since February 1997. There still appears to be a periodic problem with seawater quality. Research into this problem is continuing

Dr. Ken Brooks of Aquatic Environmental Sciences in Washington State has been contracted to develop the protocols for the hatchery/nursery production of cockles. A tidally driven fluidized upwelling nursery system (tidal FLUPSY) was set up near Tatitlek to test its potential for nursery production. Test plots on beaches near Tatitlek, Nanwalek and Port Graham have been seeded with littleneck clams for growth, mortality and predator control studies, and predator control coverings are being tested on razor clam beaches near Eyak.

The new State owned hatchery was ready for occupancy in January 1998. The facility is being leased and operated by the Qutekcak Native Tribe who will contract with the project to conduct the hatchery and pre-nursery work. This new facility will greatly enhance operations and allow the project to increase production as well as expand into cockles. The facility will have increased algae production capabilities which, in addition to permitting increased seed production, will allow the project to expand investigations on pre-nursery production at the hatchery. The shellfish hatchery manager hired by the project in FY 96 will remain on staff for at least the duration of this project.

Because very little culture or enhancement work has been done previously with littleneck clams or cockles, this project is breaking a lot of new ground. This is perhaps good news from the standpoint of contributing to the knowledge pool, but it is slowing the project down. The hatchery, nursery and growout procedures that are being developed for this project must be adapted from previous work on other species. The growout work will first require the development of a database on growth and mortality for both species to help determine the best enhancement approach.

Objectives

1. Hatchery Processes- Develop reliable, cost effective hatchery techniques for the littleneck clam (*Protothaca staminea*) and the cockle (*Clinocardium nutalli*). Produce a 3mm-5mm seed in the hatchery within 19 weeks after spawning.
2. Nursery- Develop cost effective, reliable techniques to grow 5mm hatchery seed to an out-planting size of 10mm - 15mm within 12 weeks.
3. Growout - Describe current local clam populations through interviews and resource assessments. Locate sites, develop reliable, cost effective growout techniques, and evaluate the efficacy of proposed methods.
4. Safety Testing - Set up a program for testing clams from the subsistence beaches for the presence of paralytic shellfish poisoning (PSP).

Methods

Hatchery: The Qutekcak Shellfish Hatchery located on the Institute of Marine Science grounds in Seward has been in operation since October 1993. During this time the hatchery was designed

and assembled and has evolved into a small pilot-scale operation. The staff has successfully set larvae of the Pacific oyster (*Crossostrea gigas*) and raised them to 15 mm for the aquatic farm industry. In addition, the hatchery has successfully conditioned, spawned, set and raised the native littleneck clam (*Protothaca staminea*) to 10mm. As part of this project the hatchery will also attempt to produce cockle (*Clinocardium nutalli*) seed.

The Qutekcak shellfish hatchery experienced a good rate of success in producing Littleneck clam spat in FY 97. Hatchery operations were conducted in the existing pilot facility for all of FY 97. The new hatchery facility that Qutekcak is leasing from the State was ready for occupancy in January 1998. As of this writing (May 1998) the move into the new facility is nearly complete. Spawning the clam broodstock has been very successful both in terms of ease of inducing spawning on demand and in high percentages of gamete viability. Almost all brood clams have completed rapid gametogenesis when conditioned below 10° C and zygotes have demonstrated high rates of normal development to D-veligers unlike spawns prior to February of 1997 and described in the last annual report. Reducing the broodstock conditioning temperature from 13° C (summertime high) to 9.5° C (spring water temperature) partially accounts for why extensive abnormal development of early larvae has not recurred since February 1997. However, irregular two to four day periods when new algae cultures fail to grow combined with sudden larval mortality despite carefully standardized procedures demonstrate ongoing sudden water quality changes.

Attached to this report are three consultant's reports. The first summarizes the results of histopathological examinations of clam larvae sampled from three different spawns. The second and third reports describe the results of a bioassay of hatchery seawater using a sensitive algal spore production test and the results of an oyster larvae bioassay of hatchery seawater.

The first report by Dr. Ralph Elston reveals significant bacterial infection of the one of three clam larvae samples during a period when the larvae of that particular group were dying rapidly. Thirty-six percent had "terminal infections" in various stages but he noted a small number of bacterial cells visible on the external shell surface suggesting that hygienic conditions in the culture are generally good but that the causative bacteria may be releasing an exotoxin. The second sample was of surviving larvae near terminal size and only 6 % had infections that were identical to the first sample. He commented on how poorly developed they were for 28 days of age. The third sample had no infections or lesions although a few bacterial cells were observed on the external shell surface. They were sampled from a typical slow growing group in the pilot hatchery. They served as our control for the first two samples. The exotoxin suggested by Dr. Elston might also originate from other sources than bacteria growing in the culture or even from bacteria at all. Histology cannot identify the source. I believe this suggestion supports our observations and experience that we suffer periods of bad seawater quality. Recent research has shown that the addition of organic compounds to seawater can cause larval mortality either by stimulating low background levels of bacteria to increase and/or increasing their virulence.

Larvae samples are still being collected on a regular basis for more histology by Dr. Elston, however, the real source of the problem appears to lie in the water, which is where more attention should be directed. Daily samples of ambient seawater are being collected for total dissolved organic carbon (DOC) analysis by the University of Washington Seawater Chemistry lab. It is possible that the seafood processor may be changing DOC levels and related microbial dynamics

to the detriment of our larvae cultures. Having pre-, during and post- incident DOC sample might help us to verify this suspicion.

The second report, a toxicity identification evaluation (TIE) by EVS Environment Consultants, describes a sensitive *Champia sp.* algal bioassay of our pilot hatchery seawater shows that significantly fewer (2/3 less) spores were produced relative to local seawater from British Columbia. The hatchery seawater was collected during a period of algal and larval mortality. We will collect more seawater during future problem periods for further assay experiments designed to begin identifying possible toxins in the water.

The third consultants report, also by EVS Environment Consultants, found no difference in survival but a significant decrease in size of oyster larvae grown for six days in seawater we supplied them during a problem period in the pilot hatchery compared to larval growth in their own seawater. The larvae also appeared paler in the hatchery seawater, many with tissues contracting from the shell. Because the effect on the larvae was at a relatively low level and involved subjective observations they recommend using the apparently more sensitive *Champia sp.* algal bioassay for future investigations into toxicity.

The frequency or perhaps severity of seawater quality problems may prove less of a problem in the new hatchery. Although not strictly pertinent to the 97 FY reporting period our first trial clam spawn in the new facility this April was a great success. The larvae grew twice as fast as in the pilot hatchery and 13 times as many mature larvae were placed into setting systems as ever before. Several reasons may account for our early success in this facility. The new, independent seawater system extends into deeper waters (250 feet). The one-micron filtered seawater receives about 4 to 6 times the UV dosage (60,000 to 90,000 microwatt/sec.cm²) as before. We also added probiotic bacteria ("PBD-31", Enviro-Reps. Intl.) to the larval tanks. The mix of beneficial bacterial strains purportedly inhibits pathogenic bacteria in shrimp hatcheries and grow-out facilities. And finally, the new larvae tanks themselves are 150 times the size (30,000 liters) of the tanks in the pilot hatchery. Different chemical and bacterial dynamics may occur in these much larger volumes of seawater than in 200-liter tanks.

Work under a contract with Aquatic Environmental Sciences, Port Townsend, WA, to develop spawning techniques for the cockle is ongoing. To date the contractor has been unable to produce any fertilized eggs using an array of standard spawning inducing techniques. Unfortunately, there is nothing in the literature describing successful methods of inducing spawning in this animal. It should be noted that similar difficulties have been experienced in the initial attempts to spawn a variety of shellfish. Work on cockles under this project has been curtailed for FY 98, but will continue under a different project.

Nursery: Pre-Nursery Hatchery Pond: The hatchery utilizes a 1 million liter pond to culture algae for its pre-nursery. The 30m by 37m pond is 5 meters at its deepest point. Raw seawater from a 60-meter deep intake is pumped into the pond to bring in nutrient rich water. The flow can be controlled to allow for adequate flushing yet maintain the ambient air temperature. An air compressor is used to aerate and circulate water in the pond to eliminate stratification and increase phytoplankton production. Fertilizer solutions are added daily to increase the intensity and duration of phytoplankton blooms. Physical parameters of the seawater including temperature, salinity, pH, and redox potential are monitored and water samples are collected at

various intervals for nutrient level analysis. Identification of the most abundant phytoplankters as well as secchi disk readings are also made. The food laden pond water is pumped through dense trays of small (1+mm) littleneck clam spat. Spat reaching 3+ mm are removed and sent to both the tidal FLUPSY at Tatitlek and the pump driven FLUPSY at Chenega Bay. Outdoor rearing of clam spat in pond upwellers continued through October at which time they were returned into the hatchery.

The pond received a much needed draining and cleaning in FY 97, which greatly reduced the quantities of suspended particulate inhibiting diatom growth. Many yards of mud were washed off the sides and then vacuumed off the bottom liner with a "super sucker" vacuum tank truck. After this cleaning a dense diatom bloom was easily sustained all summer and even through November when seawater temperatures fell to 4° C or 5° C. The pond was enriched with the same fertilizer at F/2 ratios as described in the last report. Trace mineral and CO₂ enrichment were not used because of the high costs of these compounds and the over-abundance of pond algae for existing numbers of clam spat.

Remote Nursery Systems: Remote nursery systems offer several advantages over nursery culture at the hatchery. One is that it frees up hatchery space and personnel that can be better used in hatchery production. Another is that several remote nursery systems offer a redundancy of supply in case one of the systems fails. A third is that remote nursery systems can be located near the growout areas thus reducing transport costs. The big disadvantage to remote nursery systems is that the cost of pumping water at a remote location in Alaska made them impractical.

Recently, work conducted under the South Carolina Sea Grant program lead to the development of a tidally driven remote nursery system. This system, called a Tidally Driven Floating Upwelling System (tidal FLUPSY), uses the strength of tidal currents to force sea water, with its accompanying load of phytoplankton, through cages containing small clams. The system appears to work quite well and is easy to maintain. Because the system is driven by a natural energy source readily available in Alaska, it appears to have great promise here.

About 50,000 oyster seed were seeded equally into 4 of the 12, 18"L x 18"W x 24"D bins on May 25 1997. The mean length of the oyster seed was 7mm with a range from 4.5mm to 10mm. The oysters are measured at their longest point using monostat vernier calipers. The oysters are photocopied for ease of measuring and record keeping. The oysters were checked at approximately three-week intervals. They were stirred but were not sorted.

Approximately 10,000 native littleneck clams were shipped from the Qutekcak Shellfish Hatchery on October 31, 1997 and placed in the tidal FLUPSY. The clams averaged 4.8mm with a maximum size of 6.8mm and a minimum size of 2.0mm. The clams were measured at their longest point using monostat vernier calipers. Because the seed were smaller than the 1/4" mesh in the FLUPSY bin they were placed in a 6" PVC pipe with mosquito mesh on the bottom.

Growout:

Growout Techniques: Seeding Intertidal Areas: In 1995 a series of baseline surveys were conducted in the vicinity of Tatitlek, Port Graham and Nanwalek to select a cross-section of beaches that might be suitable for growout. One beach per village was selected. The Nanwalek beach is representative of moderate energy beaches, the Tatitlek beach is representative of open gravel beaches with good tidal exchange and the Port Graham beach is representative of

protected areas. The Port Graham and Nanwalek beaches are located within two miles of one another and were tended by the same crew.

The intent of the beach growout work is to establish similar growth and mortality, and predator control studies on each of the three beaches and compare the results. This information will be used to determine the kind of clam production, for each of the two species, that can be expected from each beach type, and what predator control measures seem to work best on each beach.

The seeding study involved the placement of littleneck clam seed clams (10 mm to 15 mm valve length) in a replicate, blocked design which will examine growth and mortality as a function of tidal height and in the presence or absence of protective predator exclusion devices. A uniform seeding density of 30 seed clams per square foot was utilized.

Growth and mortality of Caged Clams: One hundred seed clams were placed in "Norplex™" clam bags for a detailed growth and mortality study. The valve lengths of all clams placed in these bags were measured to the nearest 0.01 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 can be used as the mean lengths of the clams seeded into other parts of the study.

Clam bag ends were secured with electrical ties on one end and a 1" 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 6". The top surfaces of each bag extended a minimum of 1" above the substrate. Each bag was secured to a piece of 1/2" rebar driven into the substrate to a minimum depth of 18" or when hitting bedrock. Identical study lay-outs were used at all three Villages.

Bags are being retrieved at three month intervals and all contents removed from the bags. The number of surviving clams, and the number of empty clam shells, are determined. The valve length of each clam is measured and recorded. Fouling organisms are removed from the bags and clams replaced in the bags with a shovelfull of sieved (1/2") gravel. Clam bags are then carefully nestled back in the sediment.

Clam enhancement using Car-cover netting: A minimum of 4' was required between each treatment and block. This provided access to the treatment for sampling without disturbing adjacent plots. All large (>10.0 cm diameter) rock and cobble were 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. Car-cover netting was precut to a dimension of 2m x 3m. It was secured by burying in a ca. 20cm deep 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.). After all plots were prepared, the tidal elevation of the center of each plot or bag was measured against a known tidal elevation. Sediment samples were taken adjacent to each set of the treatments for baseline analysis of total volatile solids and sediment grain size. In addition to treatment samples, control stations were established for annual sampling and processing in a similar manner. During annual monitoring, sediment samples will be taken from each of the car-covered, uncovered seeded area and control to determine the biophysical effects associated with the various treatments.

Clam enhancement without protective netting: Additional 1.0 x 2.0 meter sites were prepared as described above except that car-cover netting was not installed.

Seeding: 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. The valve length of clams removed was measured and recorded. Three random samples of seed for each beach were weighed and counted to obtain an average weight per clam. A total clam weight equivalent to 600 clams was seeded into each 1.0 x 2.0 meter area as the tide floods. Clams were seeded through the car cover netting.

Data recording: Clams in the enhancement evaluation will be examined annually during the 1997, 1998 and 1999 field work. Clam plots will be evaluated by noting the presence of predators, and covering the netted plots and collecting three randomly selected 0.1 M² samples from each plot. The clams in the samples will be counted, measured in-situ and immediately replaced at a shallow depth with the substrate taken from the quadrat. New netting will then be installed.

Razor Clam Predator Control: The razor clam project was started at the request of Eyak tribal members who during a meeting with the Chugach Regional Resource Commission (CRRC) requested assistance in restoring their razor clam populations. At that time Eyak tribal members expressed concern that the only razor clams available were sub-legal.

The field crew was unable to locate significant numbers of clams in any one area during the FY 96 field season. Because of this it was decided to collect a number of clams and replant them in a single area on "Bud's Bar" (see Diagram # 1) under car cover netting. The netting was washed or blown off during a winter storm and very little information was obtained from this study.

Bud's Bar was again used in FY 97. A growout area (4 ft x 10 ft.) was prepared at -1.5' tide. A higher tide location, by .5 ft, was selected for the second test plot to allow for more frequent access. The plot selected in 1996 was at -2.0' and was not accessible during most of the tide sequences. The area was prepared by removing debris off of the surface and was dug to 6" to remove any miscellaneous material and loosen the substrate. The area appears to be suitable since the razor clams collected in 1996 and cultured nearby overwintered and had survived to this point.

After the area was cleared 1/4" hard plastic netting (Vexar) was placed over the area and anchored at both ends with rebar. Hard plastic cover was used in place of car cover. During the 1996 season the car cover used was hard to work with because it tore easily and was difficult to uncover. And although there was no evidence of predation hard plastic would probably offer more protection from predators.

Areas within 5 miles of Bud's Bar were dug at low tides (-2 or greater) through July (see Diagram #1). Nickerson had previously identified these areas as having substantial razor clam populations. During low tide sequences two to four diggers would walk the beach looking for razor clams to show.

Any clams captured during the digs were removed, measured, aged and placed under the hard plastic cover at plot #2. The razor clams were measured using Manostat vernier calipers. The razor clam valves were measured between the longest points. The age of the razor clams were estimated by counting rings on the exterior of the valves. This is not a very good method to use

however the clams would have to be sacrificed to accurately estimate their age. After the clams were sampled, the shells were dried and numbered using white and red fingernail polish.

While digging for clams special attention was made to sift through the sand and try to find small razor clams. Random areas were dug with the shovel and the overturned sand was examined for small clams.

The two plots, 1996 #1 and 1997 #2, were checked on a regular basis. The test plots were checked for a final time for this project in March 1998.

PSP Testing: A PSP testing program is needed for the subsistence beaches at Nanwalek and Port Graham. A collection effort for PSP testing for the Port Graham/Nanwalek area was attempted on two occasions in FY 97.

Results

Hatchery: Each clam spawn easily produced more larvae than capacity allowed at the pilot hatchery. Consequently, the spawning was quenched after about 5 million eggs were released. Littleneck clam larvae have proven very sensitive to larval rearing densities typically found at other hatcheries. Older larvae must be reared at a density of less than one larva per 2 milliliters to obtain even slow growth. This results in a theoretical maximum of 500,000 larvae per group with the limited larval tank volumes available in the pilot facility.

Eight groups of Littleneck clam larvae were reared in the pilot hatchery during 1997. Larvae grew slowly requiring from 25 to 38 days to reach the mature pediveliger stage and survival was somewhat low (Figure 1). All but one group of larvae produced competent pediveligers that were placed into downwelling setting systems to complete their metamorphosis (Table 1).

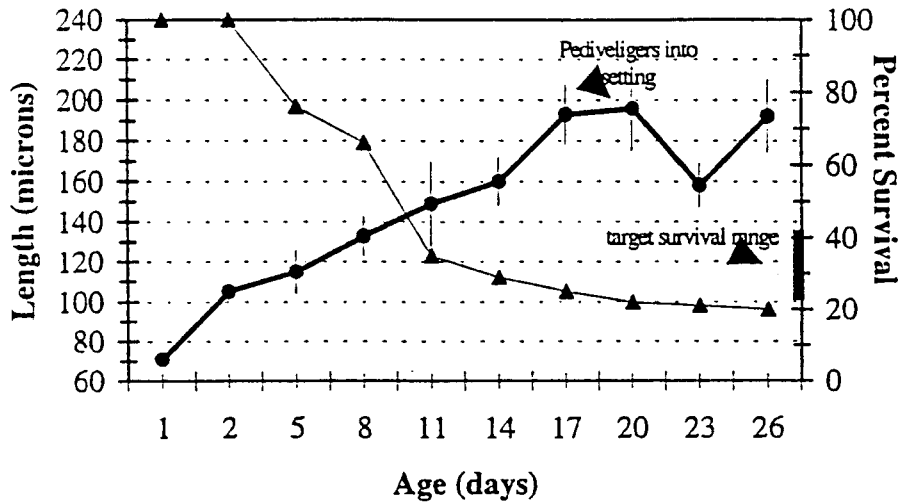
Survival through metamorphosis was highly variable ranging from 10 to 80 percent. Insufficient space in the pilot hatchery necessitates placing the setting pediveligers into the same downwelling system containing spat from prior settings. This cohabitation reduced food availability and water quality for the setting pediveligers leading to increased mortality during the stressful metamorphic process. Approximately 200,000 spat from the first four spawns and an additional 150,000 spat from the last three spawns survived metamorphosis.

Table 1. Larval Clam Production For FY 97

Date	Spawn Group	No. pediveligers into setting
3/17 - 4/24	1	63,000
5/5 - 6/9	2	104,000
6/11 - 7/18	3	50,000
7/9 - 8/4	4	454,000
8/12 - 9/9	5	330,000
9/5 - 9/27	6	*poisoned by fumes
9/20 - 10/18	7	202,000

* fumes from IMS maintenance on a freeze drier in our building poisoned both clam and scallop larvae under culture in the hatchery at that time.

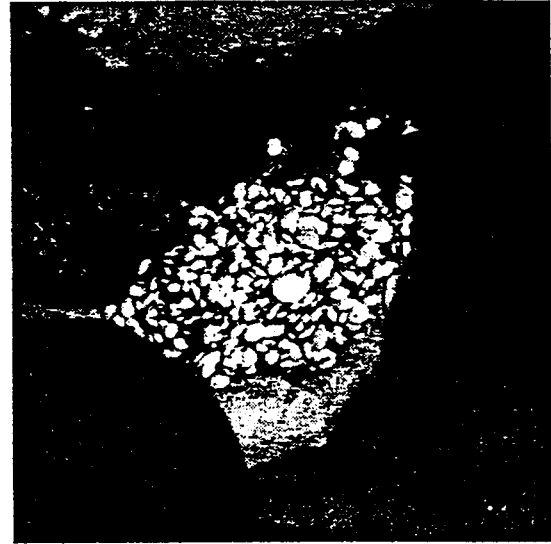
Figure 1. Clam Larval Growth and Survival of Group Four



Nursery: Hatchery Pre-Nursery: Spat reaching 1 mm in size were transferred outside during the summer and fall into upwellers circulating seawater from the algae pond. In September, these spat were graded into 3-5 mm and 5 - 10 mm groups. These were transferred to the PWS nursery upweller for nursery stage rearing (Table 2). The sub 3-5 mm spat remained for further growth. Outdoor rearing of clam spat in pond upwellers (Figures 2 &3) continued through October at which time they were returned into the hatchery.

Table 2. 1997 Hatchery Clam Spat Production

Date	Size	No. Spat to PWS
9/6/97	3-5 mm	17,000
	5-10 mm	10,000
10/13, 11/1	3 mm +	22,500



Figures 2 & 3. Littleneck clam spat reared in hatchery pond upwellers

Outdoor microalgae culture in large 10,000-liter tanks proved very successful and reliable in FY 97. Culture densities typically grew to an impressive 300,000 cells per milliliter of *Skeletonema costatum*, *Thalassiosira gravida*, and *Chaetoceros spp.* Unfiltered seawater from 70 m depth was pumped into the pond-side tanks, fertilized and aerated with only natural illumination for about five days until harvest. We also maintained a bloom of a lipid rich, green *Tetraselmis striata* for three months in one of these outdoor tanks; harvesting half the culture every few days. This microalgae can be pumped directly into the pre-nursery upwellers to feed the larger spat or drained into the pond as a large-scale inoculant.

The pond received a much needed draining and cleaning this summer, which greatly reduced the quantities of suspended particulate inhibiting diatom growth. Many yards of mud were washed off the sides and then vacuumed off the bottom liner with a "super sucker" vacuum tank truck. After this cleaning a dense diatom bloom was easily sustained all summer and even through November when seawater temperatures fell to 4° C or 5° C. The pond was enriched with the same fertilizer at F/2 ratios as described in the last report. Trace mineral and CO₂ enrichment were not used because of the high costs of these compounds and the over-abundance of pond algae for existing numbers of clam spat.

Remote Nursery Systems: The 50,000 oyster seed that was placed in the tidal FLUPSY on May 25 were removed on September 10, sorted, measured and placed in lantern nets on the Tatitlek aquatic farm site. In the intervening 3½ months about 83% of the seed survived to grow to an average length of 14.5 mm with a range from 6 mm to 34 mm.

The 10,000 littleneck clams placed in the FLUPSY on October 31 remained overwinter. Many were lost during a storm which tipped the rearing containers and allowed for the small clams to float out. The remainder of the clams, approximately 1,500, was placed in culture bags at the growout area in March 1998. No growth data was collected.

Growout: Results of the littleneck clam enhancement studies at Tatitlek, Port Graham and Nanwalek, and the attempt to develop hatchery culture techniques for the cockle can be found in the report by Dr. Kenneth M. Brooks, "Part I: Native littleneck clam (*Protothaca staminea*) enhancement studies at the villages of Nanwalek, Port Graham and Tatitlek; Part II: Development of Spawning Techniques for the Basket Cockle (*Clinocardium nutalli*)", April 16, 1998, located in Appendix I.

Razor Clam Studies: Mr. Bud Janson, who is enrolled in the Native Village of Eyak, was responsible for capturing the razor clams for this study. Mr. Janson and his crew dug six low tides attempting to locate as many razor clams as possible. Ten local sand bars (Diagram #1) were dug in an attempt to find razor clams. Many of these areas yielded no clams which was surprising since they once supported large populations (Nickerson). The paucity of clams appears to be worse than expected. An inherent problem with capturing razor clams is the unpredictability of when they will "show", however, an experienced digger will manage to find some amount of clams if they are in the area.

Area beaches were dug during several tides and captured razor clams were transferred to the test site. All captured clams were measured, their age estimated and then numbered with fingernail polish and placed in the growout study area. Samples that were difficult or confusing to age were not estimated.

A total of 82 clams were captured near the study area during the 1997 field season (Diagram #2). These clams were placed in rows at 6" intervals under the cover after they were captured.

No clams smaller than 45mm were found. Three empty shells, which were approximately 15mm in length, were found in July near the surface. This was the only appearance of juvenile razor clams in the area. There appears to have been no significant recruitment to the beach for several years. It also appears that most of the razor clams captured may be from the same year class since the estimated ages and relative uniformity of the clams lengths suggests that they all may be cohorts.

The 43 clams captured in 1996 were checked at plot #1 throughout the 1997 field season. The northern side of the car cover had been buried under 6" inches of sand and had to be dug out and replaced. There were clams still under the cover but they were not sampled. They were scheduled to be sampled and numbered in 1998 prior to the removal of funding

The test plots were checked for a final time in 1997 on September 17th and 18th. No damage was noticed and razor clams were showing under the cover.

The final sampling of the test plots occurred on March 31, 1998. Sampling razor clams is extremely difficult because it is hard to locate the clams and mortality is likely to occur from the digging and handling. To completely sample an area would take an extensive effort. 15 clams were observed at Plot #1 (1996) but they were not sampled.

Fourteen clams were retrieved and measured from plot #2 and placed back in the test area. Of the 14 clams recovered 4 had lost their numbers or were illegible. It is likely that many of the clams will lose their markings by the next sampling period. A different method of numbering should be devised.

Table 1 shows the results of the clam sample in March 1998. All of the clams sampled had grown. The lowest measured growth was 1.2% and the largest was 20.2 %. The average was approximately 10%. This is lower than would be expected based on information from Nickerson. The slower growth could be attributed to stress from handling or possible poorer growing conditions under the predator cover.

Table 3. Length, estimated age and percent of growth of recaptured clams at Bud's Bar

Sample #	Length (mm)	Est. Age	% Growth	Sample #	Length (mm)	Est. Age	% Growth
26	92	4	8.2%	NR	102	5	
58	81	4	8.0%	68	100	5	20.5 %
7	112	5	4.7%	28	91	4	7.1%
53	82	4	15.5 %	NR	78	4	
24	65	3	12.1 %	15	91	4	9.6%
19	86	4	1.2%	NR	89	5	
NR	102	5		78	86	4	10.3 %

PSP Testing: The presence of the commercial mariculture operation at Tatitlek eliminates the need for PSP testing of the local subsistence clam beds. It has proven difficult to collect sufficient samples from the Port Graham and Nanwalek beaches for PSP analysis. In addition the State of Alaska is charging \$125 for PSP tests not required by regulation. Because of this no sampling was done under this project in FY 97.

PSP testing will need to be a major part of any effort aimed at restoring clams as a major part of the subsistence diet. However, this effort no longer fits well within the scope of this project. The Chugach Regional Resources Commission is now in the process of applying for funding to both expand the work started here to other villages in the oil spill region and to develop a comprehensive PSP testing program that can be used to ensure that clams harvested from these beaches are safe to eat.

Discussion

Hatchery: The hatchery is finally achieving survival rates for the littleneck clam that are in line with general hatchery survival rates. There are still periodic problems in the hatchery that are thought to be caused by water quality. Investigations into this problem are continuing.

The new State hatchery facility that will be operated by the Qutekcak Native Tribe beginning in January 1998 will greatly expand production capabilities. If a subsistence clam enhancement

program in the oil spill region proves feasible the new hatchery will be able to supply the needed seedstock.

Developing the techniques for spawning cockles is proving more elusive than was originally thought it would be. Funding for cockle work under this project beyond FY 97 has been curtailed. However, cockles are a very popular subsistence shellfish and they are worth the additional effort it will apparently take to develop the procedures for producing hatchery seed. Funding for this work beyond FY 97 is being obtained from another source.

Nursery: The hatchery pond was cleaned out in FY 97 and this greatly improved algae production. The pond is now being used solely for plankton production that is fed to seed in upwellers surrounding the pond. Microalgae culture in outdoor 10,000 liter outdoor tanks is also being used with good success.

The tidal FLUPSY looks promising. However a more definitive analysis of its potential needs to be done before it can be determined just how effective this apparatus will be. Emphasis will be placed on obtaining this analysis in FY 98 and FY 99.

Growout: The growth and mortality and the predator control studies for littleneck clams are going well. Seed planted in FY 96 had good survival 80+% after 17 months except for seed that was planted without protection. Seed not placed in net bags or under car cover had about an 8% survival rate. Growth was about as expected. It appears that a harvestable size will be achieved within three to four years after being planted. No seed was planted in FY 97 due to none being available from the hatchery until mid September and getting weathered out on the next favorable tide cycle. This seed will be planted in the spring of FY 98.

The razor clam studies near Eyak are more problematical. There are not numbers of undersize razor clams on the beaches near the village as was originally thought. In addition to implementing predator control measures to give the clams a chance to grow to harvest size it will be necessary to relocate clams from more distant, less accessible areas to these beaches. Growth on clams that were moved for other beaches and placed under car cover on the study beach had a lower than expected growth rate. This tends to indicate that such an approach may not be practical.

Funding under this project for razor clam work was curtailed after FY 97. An attempt is being made to secure other funding to finish out the work that was started here.

PSP Sampling: The presence of the commercial mariculture operation at Tatitlek eliminates the need for PSP testing of the local subsistence clam beds. It has proven difficult to collect sufficient samples from the Port Graham and Nanwalek beaches for PSP analysis. In addition the State of Alaska is charging \$125 for PSP tests not required by regulation. Because of this no sampling was done under this project in FY 97.

PSP testing will need to be a major part of any effort aimed at restoring clams for subsistence use. However, this effort no longer fits well within the scope of this project. The Chugach Regional Resources Commission is now in the process of applying for new funding. If obtained these funds will be used to both expand the work started here to other villages in the oil spill

region and to develop a comprehensive PSP testing program that can be used to ensure that clams harvested from these beaches are safe to eat.

Conclusions

The Clam Restoration Project remains on track in spite of some significant problems that have cropped up along the way. There needs to be better communication and coordination among the different facets of this project especially in transferring clam seed. Procedures and protocols will be developed in FY 98 and FY 99.

The work completed to date indicates that a littleneck clam restoration project may be feasible. The most likely scenario at this point would be for the hatchery to produce 3 mm to 5 mm seed ready for placement in a FLUPSY by mid April. The FLUPSY would produce 8 mm to 12 mm seed ready for planting on the beaches in late summer. These clams would reach harvestable size in about three years. The Qutekcak Shellfish Hatchery will relocate to the new State facility in January 1998. Hatchery culture techniques for the cockle will hopefully be developed. The tidal FLUPSY will get a thorough testing in FY 98 and FY 99. Enough information will be collected from the littleneck clam growout studies to indicate whether or not a subsistence clam enhancement program is feasible.

The Eyak razor clam study found that there were virtually no razor clams in an area that once had the largest populations of these clams in the state. Transferring clams from other areas to accessible areas near Eyak does not appear feasible at this point.

References

References can be found in the separate reports located in Appendix I.

Appendix I Project Report: Part I: Native littleneck clam (*Protothaca staminea*) enhancement studies at the villages of Nanwalek, Port Graham and Tatitlek; Part II: Development of Spawning Techniques for the Basket Cockle (*Clinocardium nutalli*).

Project Title

*Part I: Native littleneck clam (Protothaca staminea) enhancement studies
at the Villages of Nanwalek, Port Graham and Tatitlek*

*Part II: Development of
Spawning Techniques for the Basket Cockle (Clinocardium Nutallii)*

*Chugach Regional Resources Commission Shellfish Enhancement Program
Exxon Valdez Oil Spill Trustee Council
Project Number 95131*

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Report Date:

April 16, 1998

*Part I: Native littleneck clam (Protothaca staminea) enhancement studies
at the villages of Nanwalek, Port Graham and Tatitlek*

*Shellfish Restoration Program
EVOS DPD Project #95131*

Introduction. The purpose of this project is to establish populations of clams in areas that are readily accessible from the villages of Tatitlek, Nanwalek and Port Graham. When appropriate methods for shellfish enhancement have been developed at these villages, the enhancement program will likely be expanded to include other Alaskan Indian Villages such as Ouzinkie and Chenega Bay. These clams will be used as a source of subsistence food to replace the natural clam resource that has been lost or depleted.

Shellfish enhancement studies. Beaches at Tatitlek, Nanwalek and Port Graham were surveyed in 1995 (Brooks, 1995). The results of those surveys have been used to develop site specific littleneck clam and mussel enhancement study projects at these same villages.

There are numerous techniques that can be used to enhance shellfish populations, particularly clam populations. The purpose of the present study is to assess growth and mortality of native littleneck clams under controlled conditions, which minimize the potential for predation. This information is important in verifying growth rates predicted by ADFG (1995), Feder and Paul (1973) and Brooks (1995) using apparent winter valve checks. Specific enhancement recommendations will be made pending outcome of these studies.

The 1996 shellfish enhancement studies began with the placement of seed clams (*Protothaca staminea*, 5 mm to 15 mm valve length) in a replicated, blocked design to examine growth and mortality as a function of tidal height and in the presence or absence of "car-cover" predator exclusion netting. Clams were seeded at ca. 33/ square foot in all treatments.

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 with minimal feed 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. For purposes of aging, these clams were considered to be 12 months old because of the year of starvation. A smaller cohort of 1,200 clams was available from the 1995 spawn. These juveniles were grown indoors in upwellers until May of 1996, when they were transferred to pearl nets hanging in the pond. At one year of age they averaged 17.9 ± 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 1995 and 1996 Qutekcak Hatchery annual reports for this project. These clams were mixed at the hatchery and randomly subsampled to provide three stocks of ca. 8,067 clams for each village. Subsamples were shipped to each village within two days of placement in the study plots during 1996.

Seeding of netted and un-netted substrates. Littleneck clams provided by the Qutekcak hatchery were divided into 12 subsamples of approximately 600 clams each. Clams were sprinkled onto the netted and un-netted sites as the flood tide covered them. This required a total of 600 clams/station x 2 treatments (netted and uncovered) x 2 tidal heights (+1.5 feet and -1.5' MLLW) x 3 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 each village (24,300 seed clams total).

Maintenance. Village culturists were encouraged to monitor these studies on a weekly basis, or as tidal conditions permit. They were cautioned that all rips in the netting must be repaired and all predators removed. Badly damaged nets should be replaced with as little disturbance to the culture as possible. Water temperature, air temperature and salinity should be measured and recorded at least bi-weekly.

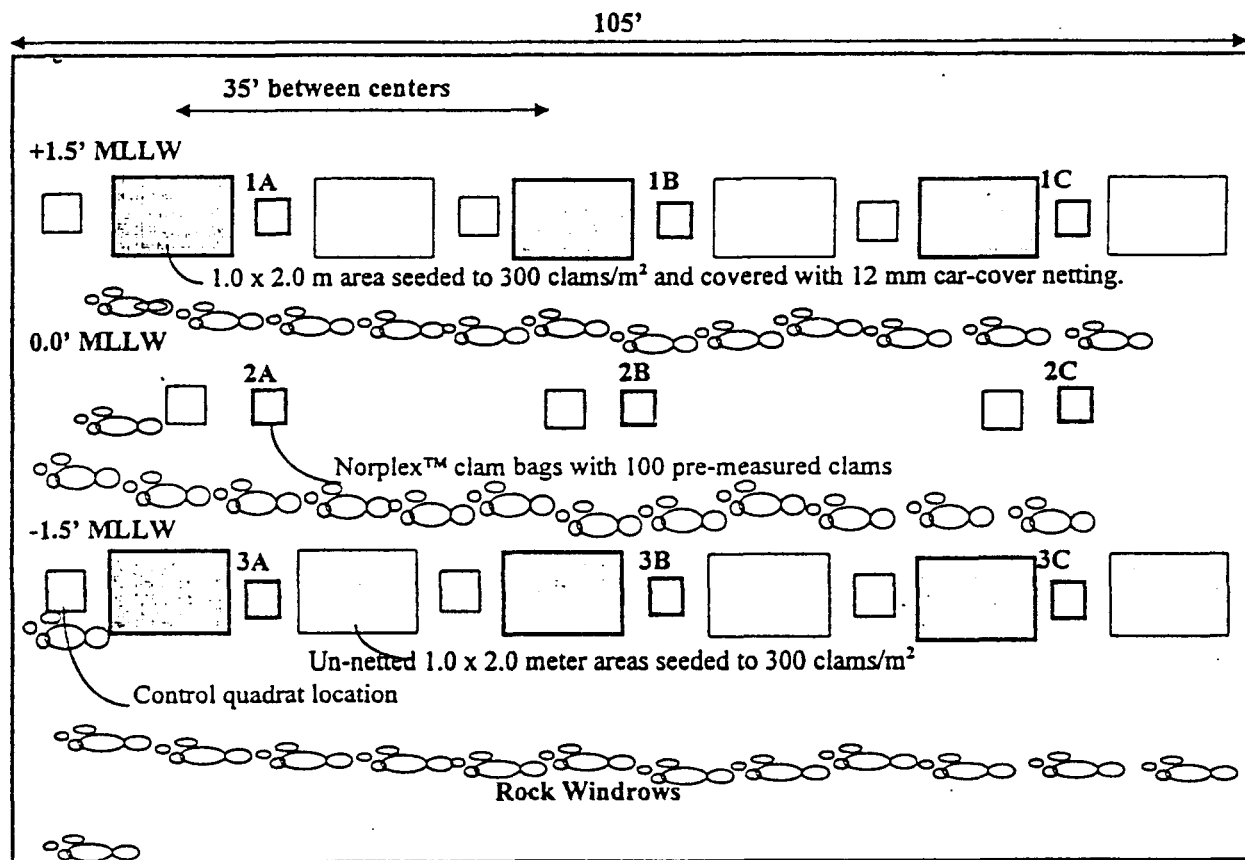


Figure 1. Study design for clam enhancement studies at previously surveyed beaches at the villages of Tatitlek, Nanwalek and Port Graham.

Data collection for netted and un-netted treatments. Clams in netted and un-netted plots will be examined annually during 1998 and subsequent field seasons. Clam plots will be evaluated by noting the presence of predators, uncovering the netted plots and collecting one or

Growth and mortality of caged clams. One hundred seed clams were placed in "Norplex™" clam bags for a detailed growth and mortality study. The valve lengths of all clams placed in these bags will be 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 on the data sheets provided by Aquatic Environmental Sciences.

Clam bag ends were secured with four electrical ties on one end and a piece of split PVC pipe (1.25" diameter) 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 a minimum of 1" above the substrate. Each bag was secured, with extra large electrical ties, to a piece of 1/2" rebar driven into the substrate to a minimum depth of 18" or when hitting bedrock. Identical study lay-outs, described in Figure (1), were used at all three Villages. 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 clam shells 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 renested in the sediment and the 100 premeasured clams sprinkled on top of the sediment in the bag prior to securing the 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 opening the next bag.

Clam enhancement using Car-cover netting. A minimum of 4' was required between each treatment and block. This provided access to the treatment for sampling without disturbing adjacent plots. All large (>10.0 cm diameter) rock and cobble were 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. Car-cover netting was precut to a dimension of 3m x 2m. It was secured by burying in a ca. 20 cm deep trench on all four sides of each 3.0 meter by 1.0 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.). After all plots were prepared, the tidal elevation of the center of each plot or bag was measured against a known tidal elevation. Sediment samples were taken adjacent to each set of the treatments for baseline analysis of total volatile solids and sediment grain size. In addition to treatment samples, control stations were established for annual sampling and processing in a similar manner. During annual monitoring, sediment samples will be taken from each of the car-covered, uncovered seeded area and control to determine the biophysical effects associated with the various treatments.

Clam enhancement without protective netting. Additional 1.0 x 2.0 meter sites were prepared as described above except that car-cover netting was not installed.

two randomly selected 0.018 m² samples, collected to a depth of 15 to 20 cm from each plot. The clams in the samples will be counted, measured and replaced at a shallow depth with the substrate taken from the quadrat. The netting will then be replaced.

A sediment sample will be collected from the top two cm of the quadrat in each sample plot. The RPD will be measured at each of these points and the sediment sample retained for total volatile solids and sediment grain size analysis. The substrate will be characterized to include the following:

- A. Substrate color
- B. Presence of attached macroalgae
- C. Presence of predators
- D. Evidence of excessive littoral drift or log damage
- E. Oily sheen
- F. Odor (hydrogen sulfide, ammonia or petroleum)
- G. A photographic record of the site will be made to describe the general area, seeding treatments, shoreline, fetch, and substrate type.
- H. Water temperature and salinity will be measured.
- I. At a minimum, each annual beach survey will include:
 - 1. 18 sediment samples (50 gm each) for sediment grains size analysis
 - 2. 18 sediment samples for Total Volatile Solids analysis.

Sediment grain size will be determined using the sieve and pipette method (Plumb, 1981). Sediments greater than 1 cm will be pooled. Additional sieves sizes will include 2 mm, 1 mm, 500 μ m, 125 μ m, 63 μ m. Silt (>3.9 μ m) and clay (<3.9 μ m) will be differentiated using the pipette method.

Sediment Total Volatile Solids will be determined by drying a sediment sample at 103 \pm 2 $^{\circ}$ C until no further weight reduction is observed and then ashing the sample at 550 $^{\circ}$ C until no further weight loss is recorded (PSEP, 1986).

Results – The results of baseline surveys completed at Tatitlek, Nanwalek and Port Graham were provided in Brooks (1995). The results of the 1996 field season, during which these experiments were laid out, are provided in Brooks (1997) and will not be repeated in this annual report. The 1997 field season was delayed until October, 1997, waiting for the release of clam seed from the Qutekcak hatchery. Unfortunately, weather prevented travel from Homer to Port Graham, Nanwalek or Tatitlek. Dr. Brooks and Mr. Hetrick remained in Homer for two days and then decided to abort because the weather was not predicted to break until too late in the low tide series. The gear and half the clam seed was sent to Port Graham and unfortunately lost when the hatchery and fish processing plant burned down. The remainder of the 1997 seed was set in a flupsy in Chenega and will be planted in 1998. Sampling planned for 1997 will be accomplished at the end of April in 1998. Caged clams were examined by Villagers on the dates shown in Table (1). Results observed at these three villages are discussed individually.

Table 1. Sampling dates on which caged native littleneck clams were planted and subsequently examined at the Alaskan Native Villages of Nanwalek, Port Graham and Tatitlek. The approximate age of the clams is given in parentheses.

Nanwalek	Port Graham	Tatitlek
July 3, 1996	July 5, 1996	June 27, 1996
May 6, 1997	October 26, 1996	September 27, 1996
July 22, 1997	March 11, 1997	January 14, 1997
No-data	July 22, 1997	July 22, 1997
No-data	November 15, 1997	November 15, 1997

Results for Passage Island near the Village of Nanwalek. The Village of Nanwalek was not able to examine clams at the Passage Island study site during the winter of 1996-97. The site is likely too remote to safely sample during winter night-time low tides. The number of survivors observed in each replicate at this site are described in Table (2).

Table 2. Number of surviving littleneck clams maintained in Norplex™ cages at the village of Nanwalek in South-central Alaska as a function of tidal height and clam age. The approximate tidal height (feet above MLLW) is provided. Average numbers and standard deviations are provided.

Number of surviving clams at -1.5' MLLW

Age (days)	Replicate (1)	Replicate (2)	Replicate (3)	Average (STDS)
365 (July 3, 1996)	100	100	100	100 (0.0)
671 (May 6, 1997)	88	99	106	97.7 (7.4)
748 (July 22, 1997)	100	99	99	99.3 (0.5)

Number of surviving clams at 0.0' MLLW

Age (days)	Replicate (1)	Replicate (2)	Replicate (3)	Average (STDS)
365 (July 3, 1996)	100	100	100	100 (0.0)
671 (May 6, 1997)	92	87	88	89 (2.2)
748 (July 22, 1997)	92	88	82	87.3 (4.1)

Number of surviving clams at +1.5' MLLW

Age (days)	Replicate (1)	Replicate (2)	Replicate (3)	Average (STDS)
365 (July 3, 1996)	100	100	100	100 (0)
671 (May 6, 1997)	89	100	103	97.3 (6.0)
748 (July 22, 1997)	86	92	101	93.0 (6.2)

Increases in the numbers of clams are noted in several of these replicates. Samples sent to AES from the November 11, 1997 sampling at Port Graham included *Macoma nasuta*, *Cylocardia cf. crebricostatai* (with 22 radial ribs) and *Saxidomus giganteus*. The mesh on the clam bags has ¼" openings and it is likely that clams are recruiting into the bags. The use of a mesh size small enough to exclude new recruits would significantly reduce water flow through the bags. This author has found that paint wears off clam shells too quickly to be of real use and seed clams are too small for etching or notching. Species other than *Protothaca staminea* will be removed from the bags by Dr. Brooks during the April, 1998 field season. Survival, as a function of tidal elevation, is summarized in Figure (2). The null hypothesis that survival was equal at all intertidal elevations can be rejected in an analysis of variance with $\alpha = 0.056$. Post Hoc testing using Duncan's Test (with critical ranges) indicated that the number of survivors was significantly higher at -1.5' MLLW when compared with the 0.0' tidal height. Other comparisons were not significant. The mean number of survivors for all groups was 96. This should be considered a preliminary number because it is highly likely that other species have recruited into the bags and included by Villagers in their evaluations. These will be removed during the 1998 field season and revised data presented in 1999.

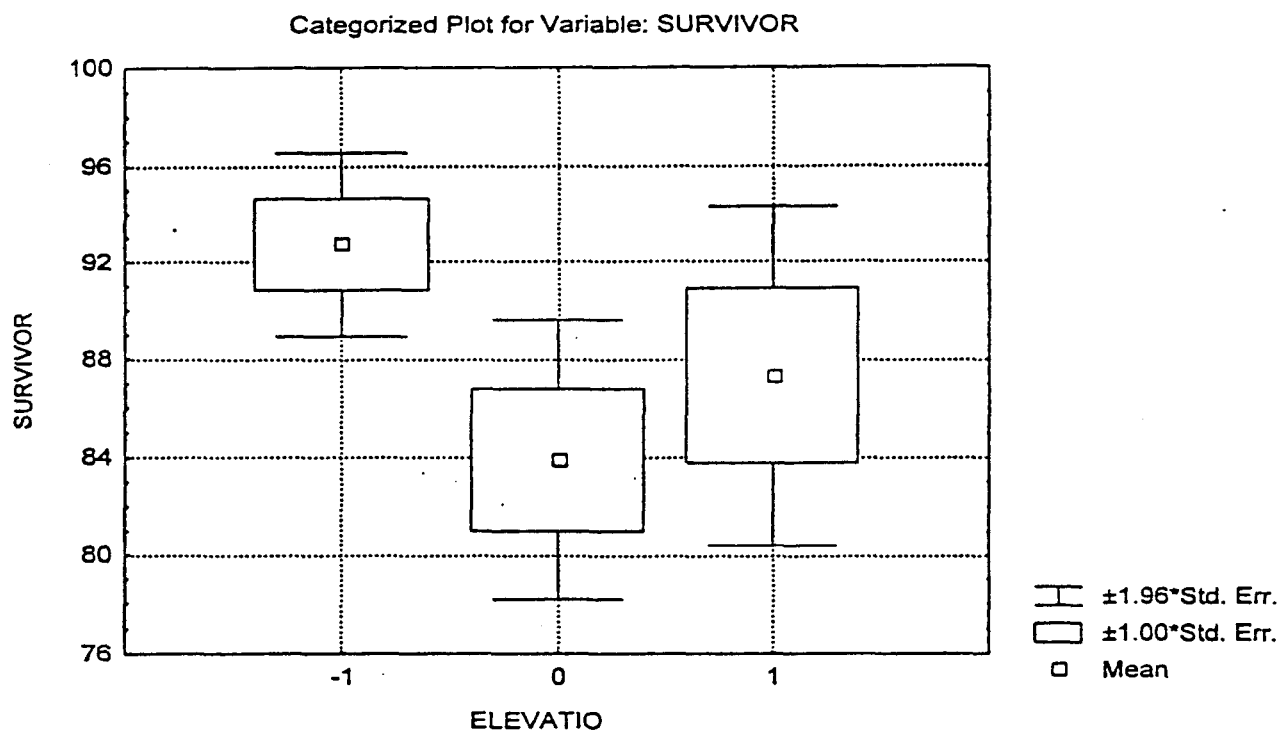


Figure 2. Survival of caged native littleneck clams at Passage Island between July 3, 1996 and July 22, 1997 at tidal elevations of -1.5', 0.0' and +1.5' MLLW. The box is ± 1.00 standard errors of the mean and the whisker is ± 1.96 standard errors of the mean.

Growth of native littleneck clams at Passage Island near the native village of Nanwalek in South Central Alaska. The average length of caged littleneck clams measured between July 1996 and July 1997 is summarized in Table (3) and Figure (3).

Table 3. Valve lengths (in millimeters \pm one standard deviation) of caged, native littleneck clams at Passage Island from planting on June 27, 1996 until July 22, 1997, as a function of intertidal elevation in feet above Mean Lower Low Water (MLLW). Each value is the mean of three replicates.

Clam Age (days)	-1.5'	0.0'	+1.5'
365	13.3 \pm 0.3	13.3 \pm 0.7	14.5 \pm 1.5
671	17.0 \pm 0.2	16.7 \pm 0.7	17.8 \pm 1.3
748	17.1 \pm 0.3	17.0 \pm 0.2	17.9 \pm 1.2

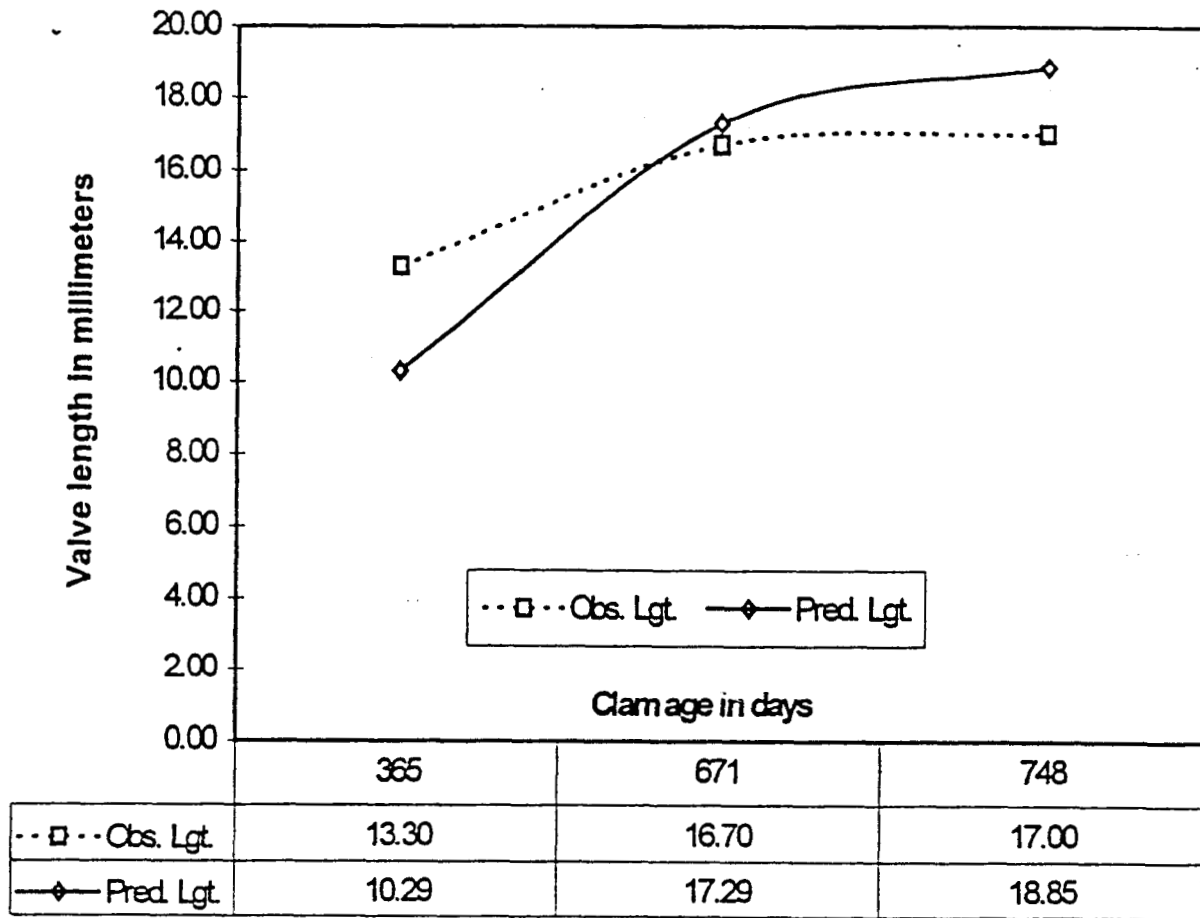


Figure 3. Length of caged native littleneck clams, as a function of age, at Passage Island near the native village of Nanwalek. The von Bertalanffy growth curve developed by measuring apparent annuli on the valves of native littleneck clams collected at Passage Island during 1995 is included for reference.

Several hypotheses could be invoked to explore the apparent lack of growth observed between Day 671 (May 6, 1997) and Day 748 (July 22, 1997):

- It is possible that the clams were significantly stressed during the May 6, 1997 sampling. The interval between samples was only two months and significant stress may have reduced growth during a large portion of that time. If sampling stress is responsible, it was not sufficient to cause high mortality during the same period.
- It is possible that spring blooms exhausted one or more nutrients in the water by May and that phytoplankton production was reduced during the May to July period. This hypothesis will be explored and rejected when examining the complete data set available for nearby Port Graham.
- It is also possible that clams are simply not growing as fast at Passage Island as predicted. Figure (4) supports this hypothesis. The increase in valve length from seeding in July 1996 to the July, 1997 sampling is described in Figure (3) as the *Growth Increment*.

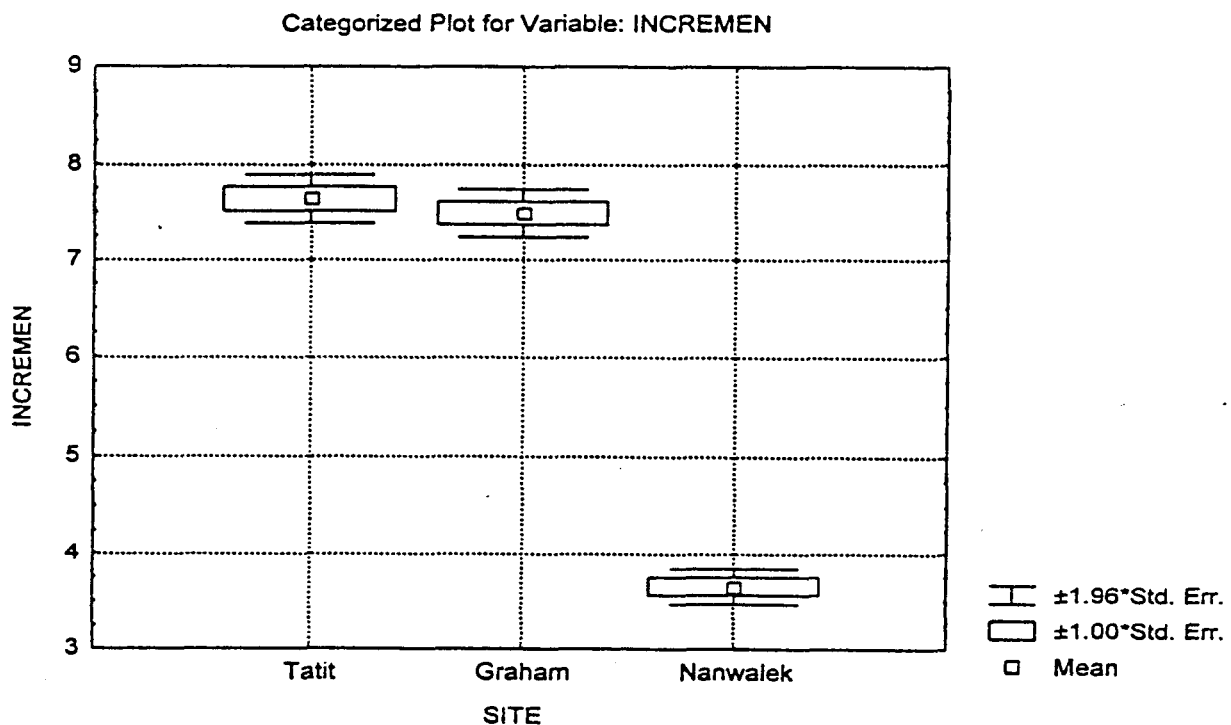


Figure 3. Mean incremental growth (in millimeters ± 1.0 and 1.96 standard errors) of native littleneck clams grown in cages near the native villages of Tatitlek, Port Graham and Nanwalek. The period of growth varied between 378 days at Port Graham and 386 days at Tatitlek.

The null hypothesis that incremental growth was the same at all sites was rejected by Analysis of Variance ($N = 2,561$; $F = 355.8$; $P < 0.00$). Post Hoc testing using Duncan's test indicates that incremental growth at Tatitlek and Port Graham was not different at $\alpha = 0.05$ ($p = 0.38$) but that growth at both of these sites was significantly faster than at Nanwalek ($p = 0.000011$ and 0.000009 respectively). Therefore, it is concluded that during this period of time, valve length increased more slowly at Nanwalek (Passage Island) than at the other two sites. Caged clams at Nanwalek were not measured in the spring of 1997, when caged clams were examined at both Tatitlek and Port Graham. Assuming that sampling procedures were the same at all villages, this suggests that sampling stress was not the cause of the reduced growth.

Brooks (1995) used age-length data to estimate incremental growth as a function of age at Passage Island. The results for native littleneck clams indicated that incremental increases in valve length varied between 5 mm/y and 12.5 mm/y with a mean of 7.6 mm/y at 1.5 years of age. Observed increases in the valve length of caged clams at Passage Island between July 1996 and July 1997 was about half this amount. In 1998, this study will assess growth in bags and in the netted and un-netted plots that have not been disturbed for two years at Passage Island. The 1998 results will shed further light on growth at this site.

Summary for Nanwalek (Passage Island). Toba *et al.* (1992) reported 17 month survival rates of 51 to 79 percent for Manila clam (*Venerupis japonica*) seed planted in bags at densities of 300 to 1,500 clams per full bag. Survival at Passage Island averaged 94 percent over a 13 month period. While the two studies involve different species cultured over slightly different periods of time, survival results at Passage Island are encouraging. The first year's growth data at this site is disappointing. Incremental increases in valve length approximately half of that predicted by the Bertalanffy growth equation for this site and half of that observed at either Port Graham or Tatitlek.

Passage Island was the site chosen by the Village of Nanwalek for this study. In retrospect, Passage Island has proven a poor study site because it is too remote from the village – especially in winter and possibly because of poor growth. No data was collected during the winter of 1996 – 1997 and the cultures were not tended to remove fouling fauna and flora from the bags or predators from the beach. Based on this lack of success, 1998 enhancement efforts will examine several beaches in closer proximity to Nanwalek in an effort to find a suitable location. However, the culture studies initiated in 1996 will be continued at Passage Island.

Results for Port Graham (Murphy Slough). The Village of Port Graham was able to measure and count clams in all nine replicates during each scheduled period. Native littleneck clams were not observed at any beach near the Village of Port Graham during the 1995 baseline survey. A five acre area of intertidal in Murphy Slough was chosen for these enhancement studies because it consisted of 67% unconsolidated and broken shale, 21% sand and 12% fines (silt and clay). The beach contained significant amounts of free flowing pore water and appeared well protected with stable substrates. The 1995 baseline survey did not reveal native littleneck clams of any size. However, Dr. Brooks' experience and the substrate's composition suggested that the chosen beach in Murphy Slough would be suitable for the growth of native littleneck clams. In part, the shellfish studies being conducted on this beach will provide a test of the hypothesis that suitable native littleneck growing areas can be

identified, based on the physicochemical characteristics of the water column and sediments—regardless of supporting evidence for the existence of previous littleneck clam populations.

Survival of native littleneck clams in Murphy Slough. As previously described, a total of nine caged replicates of 100 native littleneck clams were placed in a three by three blocked experiment on July 4, 1996. These bags were retrieved and all bivalves counted and their valve lengths measured at quarterly intervals. The number of survivors is provided in Table (3).

Table 3. Number of surviving littleneck clams maintained in Norplex™ cages in Murphy Slough near the Village of Port Graham in South-central Alaska as a function of tidal height and clam age. The approximate tidal height (feet above MLLW) is provided. Average numbers and standard deviations are provided.

Number of surviving clams at -1.5' MLLW

Age (days)	Replicate (1)	Replicate (2)	Replicate (3)	Average (STDS)
365 (July 4, 1996)	100	100	100	100 (0.0)
479 (October 26, 1996)	100	90	95	95 (8.8)
610 (March 11, 1997)	90	80	99	90 (0.8)
743 (July 22, 1997)	83	98	102	94 (5.0)
856 (November 15, 1997)	57	87	102	82 (8.2)

Number of surviving clams at 0.0' MLLW

Age (days)	Replicate (1)	Replicate (2)	Replicate (3)	Average (STDS)
365 (July 4, 1996)	100	100	100	100 (0.0)
479 (October 26, 1996)	83	90	100	91 (7.0)
610 (March 11, 1997)	69	85	93	82 (10.0)
743 (July 22, 1997)	53	78	89	73.3 (15.1)
856 (November 15, 1997)	54	76	87	72.3 (13.7)

Number of surviving clams at +1.5' MLLW

Age (days)	Replicate (1)	Replicate (2)	Replicate (3)	Average (STDS)
365 (July 4, 1996)	100	100	100	100 (0)
479 (October 26, 1996)	96	103	99	99 (2.9)
610 (March 11, 1997)	93	41	88	74 (23.4)
743 (July 22, 1997)	95	39	93	75.7 (25.9)
856 (November 15, 1997)	Unable to locate	Unable to locate	86	86.0 (0.0)

Surviving clams in each bag were photocopied. Examination of those photocopies indicates that in addition to the planted native littleneck clams, the bags contained very small numbers (none to four) of clams of the species *Macoma cf. nasuta*, *Cyclocardia cf. crebricostatai* (with 22 radial ribs), *Saxidomus giganteus* and *Hiatella sp.*. Therefore, these survival data are slightly inflated and should be considered preliminary. However, based on the photocopies, the number of new recruits is likely small and survival rates are not expected to significantly decrease. These other species will be removed during the 1998 field season. The field crew was unable to locate Replicates (1) and (2) at the highest elevation (+1.5 MLLW) during the November 15, 1997 evaluation. A thorough search for these bags will be made during the 1998 field season. No significant difference in survival has been observed as a function of tidal height ($F = 0.78$; $p = 0.518$) at Murphy Slough.

Toba *et al.* (1992) observed 51 percent survival of Manila clams grown in bags in Puget Sound at the end of 17 months culture. Seeding density was similar to that used in Murphy Slough. Survival at Port Graham averaged 80% over the nine replicates during the sixteen months of evaluation. This is significantly greater than commercial survival documented by Toba *et al.* (1992).

During 1996, a total of six two square meter areas were cultivated and seeded at a tidal elevation of -1.5' MLLW with another six at +1.5' MLLW. Half of these areas were protected with beach netting and half were left exposed. The uncovered areas were included to evaluate the potential for enhancing shellfish resources by seeding without protection, as suggested by the EVOS reviewer in 1995 comments.

The initial seeding density was approximately 33 clams per square foot. On November 15, 1997, twelve 0.018 m² random samples were collected from these areas in Murphy Slough. These samples revealed 94% survival of clams protected with beach netting and 8% survival in unprotected areas. These results are consistent with survival rates of 33 to 66 percent under beach netting and 0.0 to 14 percent for unprotected clams in Puget Sound reported by Toba *et al.* (1992).

Higher survival rates are reported herein for clams seeded under beach netting and left undisturbed for 16 months (<94%>) when compared with caged clams that were removed from the substrate, counted and measured at quarterly intervals (80%). The significance of these differences will be tested in 1998 following similar evaluation at Tatitlek and Nanwalek.

Growth of native littleneck clams at Murphy Slough near the Alaskan Village of Port Graham. The average valve length of native littleneck clams grown in bags at Port Graham is described in Figure (4). Growth stanzas are evident in 1996 and 1997. Increased growth was observed during the period between July 4, 1996 (Day 365) and October 26, 1996 (Day 479). This was followed by reduced growth during winter months from October 16, 1996 (Day 479) through March 11, 1997 (Day 610). Increased growth was again noted from March, 1997 (Day 610) and July 22, 1997 (Day 743) followed by minimal growth between July, 1997 and November, 15, 1997 (Day 856). The sampling intervals are too coarse to identify periods of maximum growth with any certainty. In addition, these data represent only one full growing season and equations predicting growth will not be attempted until more data is available. The Bertalanffy growth equation developed for nearby Passage Island is included in Figure (4) for

reference. A Chi-Squared test for goodness of fit between the observed lengths and those predicted by this equation will be completed in 1998 following two growing seasons.

The mean valve lengths of caged clams increased by an average of 7.48 ± 0.5 mm (mean \pm 95% confidence intervals) from July 4, 1996 to July 22, 1997. This increase is not significantly different from the increase of 7.58 mm predicted by Brooks (1995) based on age-length data from nearby Passage Island. It should be noted that the mean length of littleneck clams grown under beach netting and undisturbed for 16 months was 23.9 mm. However, this length was not significantly greater than the average of 22.6 for clams grown in cages at the same site (ANOVA; $F = 1.16$, $p = 0.319$).

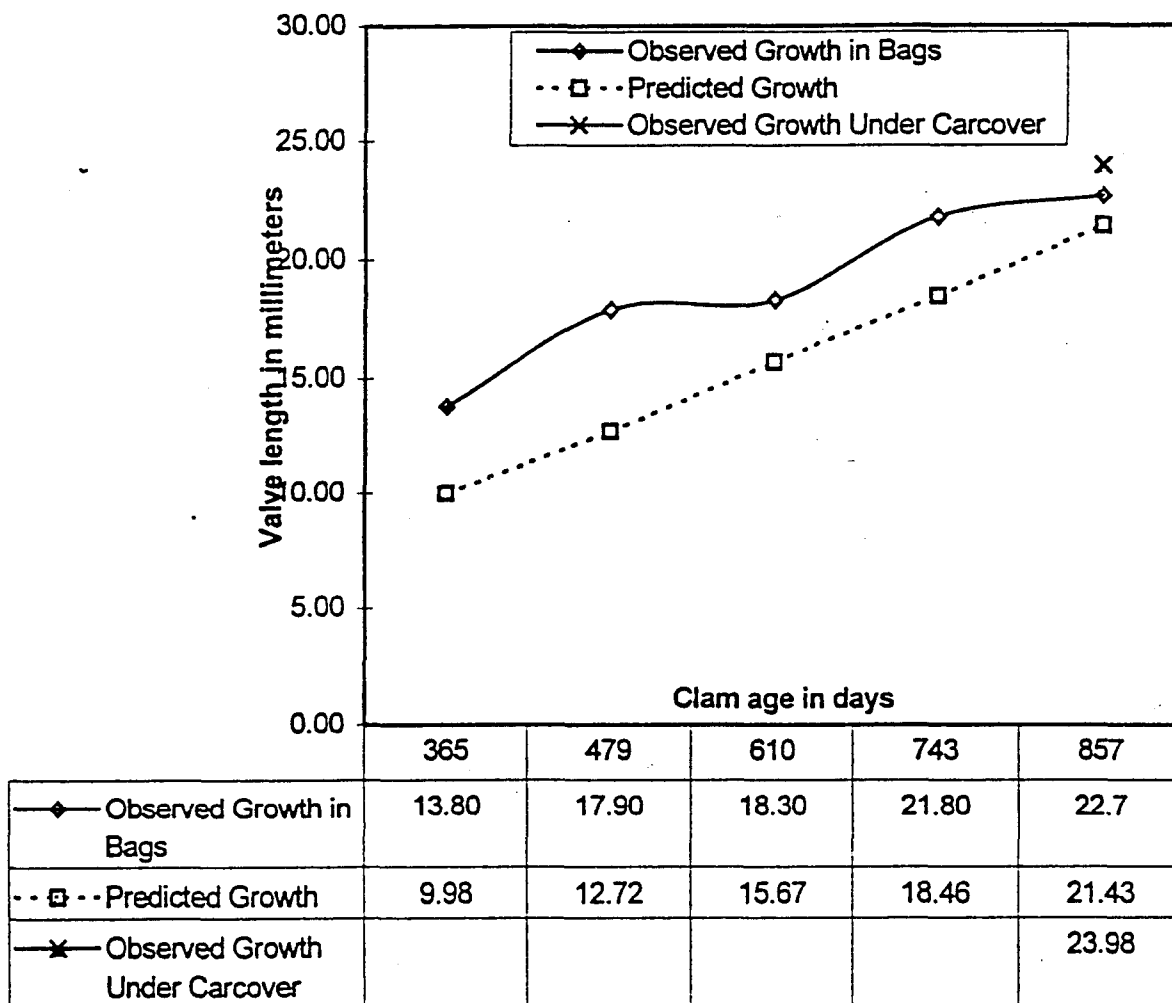


Figure 4. Mean length of native littleneck clams grown in cages at Murphy Slough near Port Graham, Alaska as a function of time. A single datapoint is provided describing the mean valve length of native littleneck clams grown undisturbed under beach netting during the same period of time. All clams were planted on July 4, 1996 at an age of approximately 365 days.

Summary for Murphy Slough. It should be emphasized that these data are only for the first year of what is expected to be a four or five year growout period. The following statements are based on this preliminary data:

- During the first 16 months of these field trials, native littleneck clams grown in bags or under beach netting have survived as well or better than commercially grown Manila clams in Puget Sound.
- Native littleneck clams did not survive well absent the beach netting. The reasons for that mortality have not been investigated and are beyond the scope of the current investigation.
- Growth data is consistent with predictions made using the Bertalanffy growth equation for nearby Passage Island.
- Stress, associated with the quarterly disturbance of caged clams may be evident in the increased mean valve length observed in undisturbed clams grown under beach netting. However, the increased mean valve length is not statistically significant.
- Despite the fact that littleneck clams were not found during the 1995 baseline survey in Murphy Slough, this area has (to date) shown promise as an acceptable area for enhancing clam resources to the Village of Port Graham.
- The equation for incremental growth given in Brooks (1995) for Passage Island can be used to estimate the time required for the cultured clams in Murphy Slough to reach a minimum harvest size of 38 mm. The total time estimate for field culture is 4.4 years. This suggests that the average valve length of clams planted in July of 1996 will reach 38 mm in the Fall of 2000 or Spring of 2001.

In summary, native littleneck clams seeded at Murphy Slough in July of 1996 are surviving well and their growth, to date, has been well predicted using the Bertalanffy Growth Curve constructed using age length data from nearby Passage Island. To date the experiments in Murphy Slough are very encouraging and suggest that intensive culture techniques can be used to produce legal size native littleneck clams with four to five years of field culture.

Results for the Village of Tatitlek. The beach chosen for enhancement is located immediately adjacent to the Village of Tatitlek. The Village culture team was able to collect data during each quarter since these clams were planted on June 27, 1996. Weather prevented the planned October, 1997 field work. That detailed examination of the cultures will be accomplished in April, 1998. The results of the Villages' data collection is presented in the following paragraphs.

Survival of native littleneck clams at the native Village of Tatitlek. As previously described, a total of nine caged replicates of 100 native littleneck clams were placed in a three by three blocked experiment on June 27, 1996. These bags were retrieved and all bivalves counted and their valve lengths measured at quarterly intervals. The number of survivors is provided in Table (4).

Table 4. Number of surviving littleneck clams maintained in Norplex™ cages adjacent to the Village of Tatitlek in South-central Alaska as a function of tidal height and clam age. The approximate tidal height (feet above MLLW) is provided. Average numbers and standard deviations are provided.

Number of surviving clams at -1.5' MLLW

Age (days)	Replicate (1)	Replicate (2)	Replicate (3)	Average (STDS)
365 (June 27, 1996)	100	100	100	0.0
454 (September 27, 1996)	100	90	95	95 (4.1)
559 (January 14, 1997)	99	80	90	89.7 (7.8)
751 (July 25, 1997)	102	83	98	94.3 (8.2)
867 (November 15, 1997)	102	57	87	82 (18.7)

Number of surviving clams at 0.0' MLLW

Age (days)	Replicate (1)	Replicate (2)	Replicate (3)	Average (STDS)
365 (June 27, 1996)	100	100	100	100 (0.0)
454 (September 27, 1996)	70	81	86	79 (6.7)
559 (January 14, 1997)	54	66	86	68.7 (13.2)
751 (July 25, 1997)	97	70	104	90.3 (14.7)
867 (November 15, 1997)	81	80	99	86.7 (8.7)

Number of surviving clams at +1.5' MLLW

Age (days)	Replicate (1)	Replicate (2)	Replicate (3)	Average (STDS)
365 (June 27, 1996)	100	100	100	100 (0.0)
454 (September 27, 1996)	88	77	116	93.7 (16.4)
559 (January 14, 1997)	81	71	92	81.3 (8.6)
751 (July 25, 1997)	59	81	102	80.7 (17.6)
867 (November 15, 1997)	78	80	93	83.7 (6.6)

Increases in the numbers of clams in the bags were observed in most replicates between January 14, 1997 and July 15, 1997. This includes the Spring and early Summer season during which natural recruitment of *Protothaca staminea* and *Saxidomus giganteus* is expected (Strathmann, 1987). Length-frequency data in Brooks (1995) indicated consistent recruitment of both species. Based on the 0+ age group (1995 cohort) in that analysis, the number expected

to recruit to an area equal to the area covered by these half bags is approximately 9/bag which is consistent with the increases observed in 1997. A length-frequency histogram for the clams measured on Day 751 at Tatitlek did not reveal clams with valve lengths less than 12.2 mm. Therefore, if the additional clams were new recruits to the area, it is more likely that they represented the 1996 year class and that they were introduced when new substrate was added while replanting the clams following their measurement on January 14, 1997. All of this is conjecture that will hopefully be at least partially resolved when the clams are examined in 1998.

Growth of native littleneck clams adjacent to the Village of Tatitlek in South-Central Alaska. The average valve length of clams grown in cages at Tatitlek is described in Figure (5).

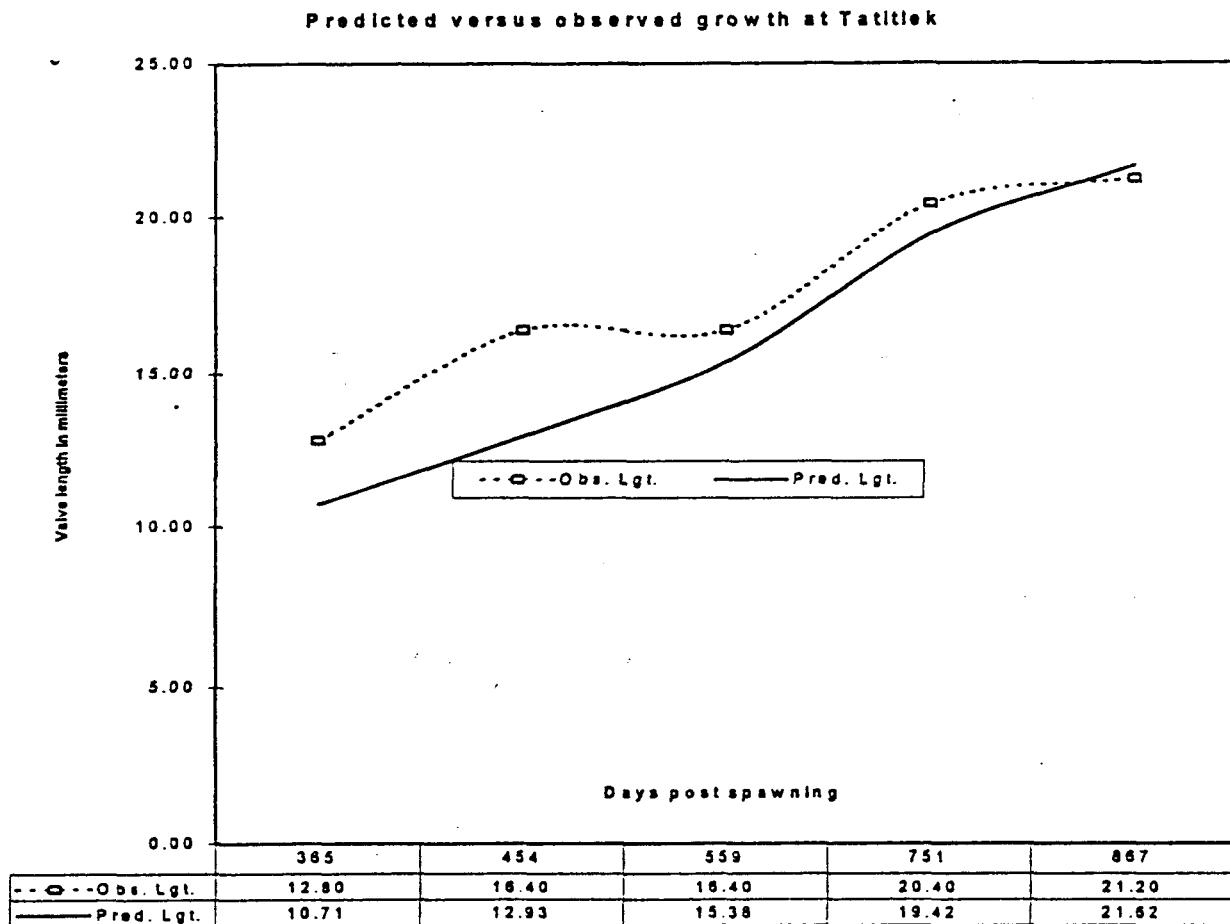


Figure 5. Mean length of native littleneck clams grown in cages adjacent to the native village of Tatitlek, Alaska, as a function of time. Clams were planted on June 27, 1996 at an age of approximately 365 days.

Two annual growth stanzas are apparent in Figure (5). Rapid growth is evident during the periods of July to September, 1996 and again between January and July, 1997. Reduced growth was recorded between September 1996 and January 1997 and again between July, 1997 and November 1997.

Sediments added to the clam bags during replanting of the cultures should have been sieved on ¼" screens. Therefore, any new clams introduced with that sediment would have had valve lengths less than perhaps 8 mm. The addition of these smaller clams would decrease the overall average length recorded in each cage during the next sampling period.

From planting on June 27, 1996 until the November 15, 1997 examination, the average valve length of clams in the Tatitlek cages increased by 6.11 mm/y. Significant differences ($\alpha = 0.05$) were observed in incremental increases in valve length as a function of elevation on the beach. This is graphically illustrated in Figure (6). Tidal elevations corresponding to the values on the x-axis are (1) = -1.5' MLLW, (2) = 0.0' MLLW and (3) = +1.5' MLLW. Differences in incremental growth, as a function of tidal elevation within this range, were not observed by Brooks (1995) during the baseline survey.

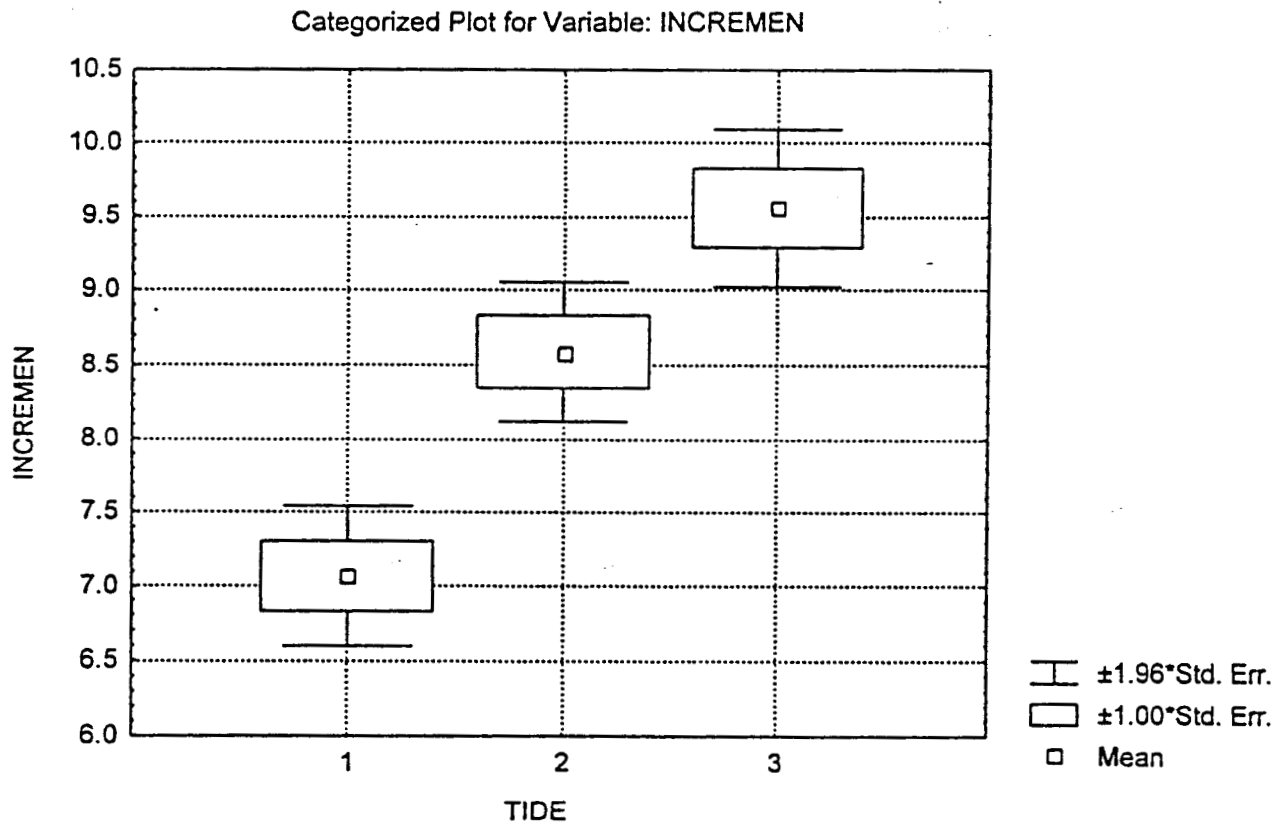


Figure 6. Box and whisker plots (Mean \pm 95% confidence intervals) of the mean incremental growth in the valve lengths of native littleneck clams cultured in cages at intertidal levels of (1) = -1.5' MLLW, (2) = 0.0' MLLW and (3) = +1.5' MLLW. Incremental growth is the increase in valve length between planting on June 27, 1996 and November 15, 1997.

The true significance of these differences associated with tidal elevation are questionable because additional clams were inadvertently introduced into the cultures as previously discussed. A thorough examination of the clams in each cage will be made in 1998 and all clams of a different species removed. It is considered inappropriate to remove native littleneck clams – no matter how small because there is a possibility that some clams did not grow. This was not a problem in Port Graham because there was no evidence of native littleneck clam recruitment in the baseline survey and no evidence of adult clams of the same species in the area. However, the baseline survey at Tatitlek did reveal consistent native littleneck clam recruitment and this may be confounding the results – particularly in the first year. Recruitment in 1998 and beyond should show up as a distinct year class of small clams in either the cages or under the protective beach netting.

Summary for Tatitlek. The caged cultures appear to be surviving satisfactorily. Taken on face value, observed growth of the caged native littleneck clams appears somewhat slower than predicted by the Bertalanffy growth equation based on age-length analysis of wild clams. An attempt to clarify these issues will be made by Dr. Brooks during the 1998 field season. Irrespective of these technical issues, the clams are surviving and growing reasonably well at this site and further enhancement is certainly warranted.

Summary and conclusions from the 1997 field season. This study completed one full year of field trials in 1997. The following statements and conclusions are made in light of the experience gained during that year:

- The Passage Island site is too remote from the village of Nanwalek for intensive culture. We will continue the existing studies at this site but will shift 1998 and subsequent enhancement efforts to a location nearer the village.
- Villagers at Tatitlek and Port Graham should be commended for completing the winter sampling during 1996-97.
- Significant mortality was not observed at any site during the winter of 1996-97. This does not mean that it will not occur in the future. However, based on these results, the study will examine clams in the bags only twice per year. Once on the first daylight low tides in the spring and again on the last daylight low tides in the fall. This will reduce stress on both the clams and Villagers.
- Port Graham, which showed no evidence of a previous population of native littleneck clams, has demonstrated an ability to support high clam survival and the best growth of any of the beaches tested. These results suggest that site selection, based on the physicochemical characteristics of the water and sediments, is an effective means of identifying future areas for enhancement.

- Clams at all of these sites are growing and surviving at rates which, if they continue, indicate that enhancement of Village littleneck clam resources can be accomplished using available technologies.
- At Port Graham, it appears that native littleneck clams can be grown from a mean valve length of 12 mm to the minimum legal harvest size of 38 mm in just over four years. What has not been determined is the growth of littleneck clams in the flupsy. This growth needs to be quantified in a reasonably rigorous manner. That can be accomplished by measuring six randomly selected sub-samples of 50 juvenile clams at monthly intervals. The measurement should begin on introduction to the flupsy and continue until September. If the clams have reached a minimum valve length of six to ten millimeters by September, then it is recommend that they be seeded into appropriate intertidal areas. In this scenario, clams could be spawned in late winter and grown to ca. 3.0 mm in hatchery upwellers by March or early April. They would then be grown in the upwellers to a minimum size of six to ten millimeters and planted in the same fall. It appears possible that following four more years of field growth, the clams would reach minimum harvest size in a total of 4.5 to 5.0 years.

The planned 1997 field season included a full range of measurements designed to test the following hypotheses at each Village:

1. Growth of caged clams can be predicted by the Bertalanffy growth equations for Passage Island and Tatitlek developed in Brooks (1995).
2. Stress induced by repeated disturbance of caged clams did not result in significantly reduced growth or survival.
3. Growth and survival of planted native littleneck clams were equal at all intertidal elevations between -1.5' MLLW and + 1.5 MLLW during the first year of growth.
4. Survival and growth of clams is equal in protected and unprotected cultures.
5. The depth of the reduction-oxidation potential discontinuity is not significantly less in sediments under beach netting when compared with unprotected areas of the beach.
6. The percent fines (silt and clay < 0.63 μ m diameter) is not significantly increased in sediments under beach netting when compared with unprotected areas of the beach.
7. The percent Total Volatile Solids is not significantly different in sediments under beach netting when compared with sediments collected from seeded but unprotected areas or control areas that received no disturbance.

In addition, an experiment designed to assess clam density effects was developed and planned for installation at Port Graham and Tatitlek in 1997. Unfortunately, that portion of the field season designed to provide data for these tests, and to set up the density experiment, was cancelled due to weather. This work has been rescheduled for April, 1998. The results of the 1998 field work will be documented in a report and presented as a contributed paper to the 1998 Lowell Wakefield Fisheries Symposium – American Fisheries Society Meeting to be held

in Anchorage, Alaska on September 30 – October 3, 1998. A copy of the accepted abstract is included as Appendix (2) to this report. In addition, a response to comments made by the EVOS reviewer is provided as Appendix (3). That response speaks for itself.

Part II: Development of Spawning Techniques for the Basket Cockle (Clinocardium Nutallii)

Background. Strathmann (1987) described *Clinocardium nuttallii* as a simultaneous hermaphrodite with concurrent male and female follicles in the gonad. Robinson and Breese (1982) noted annual spawning in June through October in bays on the Oregon coast. Gallucci and Gallucci (1982) observed gametogenesis in cockles from the San Juan Islands during the period between October and June with spawning in April to November (mostly in July to August). Based on these published reports, cockles were collected during low tides in June through September, 1997 and attempts made to spawn them. Based on apparent annuli in cockles collected in Thorndyke Bay, Washington, Brooks (1997) used non-linear regression to obtain coefficients for the Bertalanffy growth equation. This analysis suggested that cockles grow at a rate of approximately 10 mm per year during the first five years of life. Those results were inconsistent with the observations of shellfish growers in Washington State and with the work of Gallucci and Gallucci (1982) who noted the presence of false checks on cockle valves and predicted valve lengths of 34.3 to 50.3 mm at the end of one year and 65.4 to 76.8 mm at the end of three years.

Methods. In July of 1997, four hundred juvenile cockles were collected from plastic tubes used to protect seed goeuds (*Panope abrupta*). These cockles were assumed to have set from a spring spawning. The seed was placed in Norplex™ clam bags and set in Thorndyke Bay, Washington State at a tidal level of +1.5' MLLW. The cockles were measured on September 15, 1997 and again on April 17, 1998.

Attempts to spawn cockles were made on June 18, July 18, August 16 and September 15, 1997. In each case, 15 to 20 cockles were collected from the sandy beach. Cockles were held for two days in three five-gallon aquaria filled with 10 µm filtered seawater (28 parts per thousand salinity). The initial temperature was set at 14 °C and slowly increased to 16 °C. The adult cockles were fed twice each day by introducing one liter of mixed, cultured algae at a density of ca. 2×10^6 cells/ml to give an initial cell density of 10^5 /ml. The aquaria were continually aerated. An attempt to initiate spawning was made by rapidly increasing the temperature to 10 °C and adding 500 ml of mixed algae.

Further attempts to induced spawning were made by injecting 0.7 cc of Serotonin (5-hydroxytryptamine) into the proximal areas of the foot. The Serotonin was prepared by adding 1.9 mg of Serotonin (Sigma, H-7752) to 10 mg of one micron filtered seawater (Strathman, 1987). The valves of filtering cockles were held open by inserting a ¼" wooden (*Alnus rubra*) wedge in the gape. This allowed injection with minimal stress.

Following each spawning effort, cockles were shucked and sectioned at one centimeter intervals dorsally and ventrally from the juncture of the foot with the body. Wet squashes were made from tissues contained in each section and examined under a compound microscope at 400x for the presence of ova and/or sperm.

Results. The mean length of the 400 cockle seed planted in Thorndyke Bay on July 31, 1997 was 13.1 cm. Two of the replicates were missing when the cockles were re-examined on September 15, 1998. Seventy-seven percent of the cockles survived in Replicate one and 53 percent survived in Replicate Two. The valve length of these cockles had increased from 13.1 \pm 2.6 mm on July 31, 1997 to 39.5 \pm 7.2 mm on September 15, 1997. The mean increase in valve length was 0.56 mm/day. These cockles will be examined in May of 1998 and the experiment repeated with new seed. If confirmed, these data strongly support the thesis that basket cockles can be grown from seed to market size in one season in Puget Sound. Confirmation of this rapid growth should be made in Prince William Sound.

Sperm was regularly obtained during each spawning attempt – eggs were not. As soon as an animal was observed spawning, it was removed to an individual finger bowl. The injection of 0.7 cc of Serotonin into the proximal portion of the foot, produced sperm within 15 minutes on each trial. On one occasion, immature ova (ca. 35 μ m diameter) were obtained. These ova did not have well defined nuclei and no polar bodies were observed. No cleavage was achieved following fertilization with 0.2 ml of a dense sperm suspension containing to achieve a sperm concentration of ca. 10⁸ sperm/ml ova. The ova were gently washed on a 20 μ m Nytex™ screen after 20 minutes and incubated in clean seawater filtered to 10 μ m.

Sectioning of these animals revealed follicles (spermatogonia) filled with sperm. Only immature ova and or empty follicles were observed, suggesting that the animals had previously spawned. Based on these observations, our attempts to spawn cockles will begin in April, 1998 and end in July.

Conclusions. Cockle seed collected in Dabob Bay, Washington and replanted in Thorndyke Bay grew from 13.1 mm to 39.5 mm in six weeks. This appears to be the first documented quantitative growth data *Clinocardium nuttallii* in the Pacific Northwest. No success has been achieved in spawning this animal and the evidence from squashes prepared in July through September suggests spawning earlier in the year (April to June?). Additional efforts will be made in the spring of 1998 to spawn cockles collected in Washington State. Assuming that appropriate hatchery procedures can be developed and assuming that growth of juveniles is also rapid in Alaska, this bivalve holds promise as an additional species for enhancement. This is particularly true since it is a species preferred by natives.

References

- Amos, M.H. 1966. Commercial clams of the North American Pacific Coast. Fish and Wildl. Serv. Bureau of Commercial Fisheries. Circular 237.
- Alaska Department of Fish and Game. 1995. Kachemak Bay Littleneck Clam Assessments, 1990-1994. ADFG, Regional Information Report No. 2A95-19.
- Bolz, G.R. and B.R. Burns. 1996. Age & Growth of Larval Atlantic herring, *Clupea harengus*; a comparative study. Fish. Bull. VOL 94, pp. 387 - 397.
- Brooks, K.M. 1995. Baseline shellfish survey of tidelands near the Tatitlek, Nanwalek and Port Graham Villages in support of the Nanwalk/Port Graham/Tatitlek Clam Restoration Project; Exxon Valdez Oil Spill Trustee Council Project Number 95131. 51 pp.
- Brooks, K.M. 1997. Part I: Baseline shellfish survey of tidelands near the Alaskan Villages of Ouzinke and Chenega; Part II: Native littleneck clam (*Protothaca staminea*) enhancement studies at the villages of Nanwalek, Port Graham and Tatitlek; Part III; Literature Search and Development of Spawning Techniques for the Basket Cockle (*Clinocardium nuttallii*). Chugach Regional Resources Commission Shellfish Enhancement Program; Exxon Valdez Oil Spill Trustee Council, Project Number 95131.91 pp.
- Feder, H.M. and A.M. Paul. 1973. Abundance Estimations and Growth-Rate Comparisons for the Clam *Potothaca staminea* from Three Beaches in Prince William Sound, Alaska, with Additional Comments on Size-Weight Relationships, Harvesting and Marketing. Alaska Sea Grant Program Report No. 73-2. Insitute of Marine Science, University of Alaska, Fairbanks, Alaska 99701
- Feder, H.M., J.C. Hendee, P. Holmes, G. J. Mueller and A.J. Paul. 1979. Examination of a Reproductive Cycle of *Protothaca staminea* Using Histology, Wet Weight-Dry Weight Ratios, and Condition Indices. Veliger, Vol. 22; No. 2. pp 182 - 187.
- Fitch, J.E. 1953. Common marine bivalves of California. Calif. Dep. Fish Game Fish Bull. 90. 102 pp.
- Goodwin, C.L. 1973. Distribution and Abundance of Subtidal Hard-shell Clams in Puget Sound, Washington. Washington State Department of Fisheries, Technical Report No. 14. 81 pp.
- Paul, A.J., and H.M. Feder. 1973. Growth, recruitment and distribution of the littleneck clam, *Protothaca staminea*, in Galena Bay, Prince William Sound, Alaska. U.S. Natl. Mar. Fish. Serv. Fish. bull. 71:665-667.

- Pennington, M.R. 1979. Fitting a growth curve to field data. *In*: J.K. Ord, g.P. Patil, and C. Taille (eds.), *Statistical distributions in ecological work*, p. 419-428. Inat. Coop. Publ. House, Fairland, MD.
- Plumb, R.H. 1981. Procedures for handling and chemical analysis of sediment and water samples. Technical Report EPA/CE-81-1. U.S. Army Corps of Engineers, Vicksburg, MS.
- Quale, D.B. 1943. Sex, gonad development and seasonal gonad changes in *Paphia staminea* Conrad. *J. Fish. Res. Board Can.* 6:140-151.
- Quale, D.B. 1960. The intertidal bivalves of British Columbia. B.C. Prov. Museum, Dept. of Education. Handbook No. 17.
- Quale, D.B., and N. Bourne. 1972. The clam fisheries of British Columbia. *Fish. Res. Board Can. Bull.* 179:1-70.
- Robinson, A.M. and W.P. Breese. 1982. The spawning season of four species of clams in Oregon. *J. Shellfish Res.* 2: 55-57.
- Rodnick, K. and H.W. Li. 1983. Habitat Suitability Index Models: Littleneck Clam. U.S. Fish and Wildlife Service Report Number FWS/OBS-82/10.59, September 1983.
- Rudy, P., and L.H. Rudy. 1979. Oregon estuarine invertebrates: an illustrated guide to the common and important invertebrate animals. U.S. Fish Wildl. Serv. Biol. Serv. Program. FWS/OBS-79/111:166-168.
- Rutz, T. 1994. Clam Survey, Kosciusko Bay Clam Culture Plots, Southeast Alaska - 1994. Alaska Department of Fish and Game commercial fisheries Management and Development Division, Juneau, Alaska. Regional Information Rpt. No. 5J94-21.20 pp.
- Strathman, M. F. *Reproduction and Development of Marine Invertebrates of the Northern Pacific Coast, Data and Methods for the Study of Eggs, Embryos, and Larvae.* University of Washington Press. 670 pp.
- Toba, E.R., D.S. Thompson, K.K. Chew, G.J. Anderson and M.B. Miller. 1992. Guide to Manila Clam Culture in Washington. Washington Sea Grant Program, University of Washington, Seattle, Washington. 41 pp.
- Trowbridge, C., T. Baker and J.D. Johnson. 1996. *Exxon Valdez Oil Spill, State/Federal Natural Resource Damage Assessment Final Report. Effects of Hydrocarbons on Bivalves.* Fish/Shellfish Study 13. Final Report. Alaska Department of Fish and Game, Division of Commercial Fisheries Management and Development, 333 Raspberry road, Anchorage, Alaska 99518-1565.

**Appendix II Project Report: Results of Razor Clam Study for the
Village of Eyak**

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**Results of Razor Clam
Studies for the Village of Eyak**

**EVOS Project 97131
Chugach Region Clam Restoration**

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**Results of Razor Clam
Studies for the Village of Eyak**

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1. Introduction and Background

This is the second year of a project designed to provide baseline information for future efforts to restore and enhance razor clam populations Siliqua patula for subsistence use and harvest for the Village of Eyak near Cordova. This effort is part of Exxon Valdez Oil Spill (EVOS) Restoration Project 97131 **Chugach Region Clam Restoration**.

Razor clams were once the basis for an important commercial, subsistence and recreational fishery near Cordova known as the "razor clam capital of the world" with annual harvests of several million pounds. Presently, populations are so low that no commercial fishery has been prosecuted since 1988 and recreational harvests are minimal. The decline is attributed to environmental changes in flow from the Copper River, land shifts from the 1964 earthquake and sea otter predation.

Members of the Eyak tribe located near the City of Cordova expressed a desire to reestablish razor clam populations within the area to restore a traditional subsistence food source.

Review of 1996

The objectives and results for the first year of the project are summarized below:

1) Conduct Survey and Interviews

- determined traditional areas of use and harvest of shellfish especially razor clams.
- identified traditional harvest areas on maps and determined "local names".
- identified access to beaches and anchorages and described landmarks.
- developed an understanding of local perspectives of recent harvests and reasons for declining populations.

2) Physical and chemical characterization of selected beach substrates

- substrate samples were collected at a test beach site and analyzed for particle size and organic content.

3) Physical and chemical characterization of beach area water column

- water chemistry such as temperature, salinity and dissolved oxygen were

collected.

- water samples were collected for Total Suspended Solids (TSS) and Volatile Suspended Solids (VSS). The results suggest good primary productivity and a few suspended particulate.

4) Shellfish population characterization

- evaluated shellfish populations on a selected beach, very few shellfish were found.
- sampled beach areas for existing populations of razor clams, no clams were found.
- selected "Bud's Beach" for enhancement.

5) Predator Control

- transferred local razor clams to the study area.
- began study of a predator control method utilizing "car cover".

The project was successful in accomplishing all of the tasks outlined in the 1996 Detailed Project Description (DPD). A year end report was submitted to the Exxon Valdez Oil Spill Trustee Council in March 1997.

The 1996 field work was successful in providing a basis for further work. The test area being void of significant numbers of razor clams offered an opportunity to work in an area that appears to be excellent razor clam habitat but does not have any "noise" from resident populations. The results of the 1996 field season provided the basis for the goals outlined in the 1997 DPD.

1997 Objectives

The DPD outlined for 1997 focused on predator control. Work done in Puget Sound and Canada suggests that it may be possible to enhance clam populations by applying predator control screening.

Because of the inability to locate clams on randomly selected areas during the 1996 field season the 1997 DPD was modified. Random sampling was eliminated as a means of capturing razor clams for the study. A concerted effort was made to dig adjacent areas to try to capture as many clams as possible and transfer them to the test plots for testing the predator netting.

Because of the migratory nature of cockles, they were eliminated from

the 1997 DPD.

The main objectives of the 1997 work plan were to capture as many razor clams as possible, preferably juveniles, and transport them to a growout area and conduct a growth and mortality study while continuing to evaluate predator control methods.

2. Materials and Methods

A growout area (4 ft x 10 ft) was prepared at "Bud's beach" at -1.5' tide. A higher tide location, by .5 ft, was selected for the second test plot to allow for more frequent access. The plot selected in 1996 was at -2.0' and was not accessible during most of the tide sequences. The area was prepared by removing debris off of the surface and was dug to 6" to remove any miscellaneous material and loosen the substrate. The area appears to be suitable since the razor clams collected in 1996 and cultured nearby overwintered and had survived to this point.

After the area was cleared 1/4" hard plastic netting (Vexar) was placed over the area and anchored at both ends with rebar. Hard plastic cover was used in place of car cover. During the 1996 season the car cover used was hard to work with because it tore easily and was difficult to uncover. And although there was no evidence of predation hard plastic would probably offer more protection from predators.

Areas within 5 miles of Bud's Beach were dug at low tides (-2 or greater) through July. (Diaram #1). Nickerson had previously identified these areas as having substantial razor clam populations. During low tide sequences two to four diggers would walk the beach looking for razor clams to show.

Any clams captured during the digs were removed, measured, aged and placed under the hard plastic cover at plot #2. The razor clams were measured using Manostat vernier calipers. The razor clam valves were measured between the longest points. The age of the razor clams were estimated by counting rings on the exterior of the valves. This is not a very good method to use however the clams would have to be sacrificed to accurately estimate their age. After the clams were sampled, the shells were dried and numbered using white and red fingernail polish.

While digging for clams special attention was made to sift through the sand and try to find small razor clams. Random areas were dug with the shovel and the overturned sand was examined for small clams.

The two plots, 1996 #1 and 1997 #2, were checked on a regular basis. The test plots were checked for a final time for this project in March 1998.

3. Results

Mr. Bud Janson, who is enrolled in the Native Village of Eyak, was responsible for capturing the razor clams for this study. Mr. Janson and his crew dug six low tides attempting to locate as many razor clams as possible. Ten local sand bars (Diagram #1) were dug in an attempt to find razor clams. Many of these areas yielded no clams which was surprising since they once supported large populations (Nickerson). The paucity of clams appears to be worse than expected. An inherent problem with capturing razor clams is the unpredictability of when they will "show", however, an experienced digger will manage to find some amount of clams if they are in the area.

Area beaches were dug during several tides and captured razor clams were transferred to the test site. All captured clams were measured, their age estimated and then numbered with fingernail polish and placed in the growout study area. Samples that were difficult or confusing to age were not estimated.

A total of 82 clams were captured near the study area during the 1997 field season (Diagram #2). The 82 clams were placed in rows at 6" intervals under the cover after they were captured (Table 1).

No clams smaller than 45mm were found. Three empty shells, which were approximately 15mm in length, were found in July near the surface. This was the only appearance of juvenile razor clams in the area. There appears to have been no significant recruitment to the beach for several years. It also appears that most of the razor clams captured may be from the same year class since the estimated ages and relative uniformity of the clams lengths suggests that they all may be cohorts.

The 43 clams captured in 1996 were checked at plot #1 throughout the 1997 field season. The northern side of the car cover had been buried under 6" inches of sand and had to be dug out and replaced. There were clams still under the cover but they were not sampled. They were scheduled to be sampled and numbered in 1998 prior to the removal of funding

The test plots were checked for a final time in 1997 on September 17th and 18th. No damage was noticed and razor clams were showing under the cover.

Table 1. Age and lengths of razor clams captured at Bud's Beach, 1997

Sample #	Length (mm)	Est. Age	Sample #	Length(mm)	Est. Age
1	90	4	42	65	3
2	95	4	43	70	3
3	100	4	43	70	3
4	100	4	45	83	4
5	100	4	46	78	4
5	100	4	47	80	4
7	100	4	48	82	4
8	108	4	49	85	4
9	105	4	50	81	4
10	108	?	51	77	4
11	75	4	52	80	4
12	80	4	53	71	4
13	80	4	54	73	4
14	80	4	55	82	4
15	83	4	56	45	?
16	89	4	57	73	3
17	85	4	58	75	4
18	85	4	59	93	4
19	85	4	60	78	4
20	90	4	61	86	4
21	90	4	62	87	4
22	60	3	63	76	3
23	65	3	64	86	4
24	58	3	65	64	3
25	90	4	66	81	4
26	85	4	67	50	3
27	74	?	68	83	4
28	85	4	69	45	3
29	103	5	70	65	3
30	78	4	71	65	3
31	60	4	72	90	4
32	85	4	73	65	3
33	84	4	74	61	3
34	88	4	75	52	3
35	82	4	76	78	4
36	88	4	77	85	4
37	80	4	78	78	4
38	85	4	79	85	4
39	85	4	80	77	4
40	82	4	81	75	4
41	65	3	82	60	3

The final sampling of the test plots occurred on March 31, 1998. Sampling razor clams is extremely difficult because it is hard to locate the clams and mortality is likely to occur from the digging and handling. To completely sample an area would take an extensive effort. 15 clams were observed at Plot #1 (1996) but they were not sampled.

Fourteen clams were retrieved and measured from plot #2 and placed

back in the test area. Of the 14 clams recovered 4 had lost their numbers or were illegible. It is likely that many of the clams will lose their markings by the next sampling period. A different method of numbering should be devised.

Table 2 shows the results of the clam sample in March 1998. All of the clams sampled had grown. The lowest measured growth was 1.2% and the largest was 20.2%. The average was approximately 10%. This is lower than would be expected based on information from Nickerson. The slower growth could be attributed to stress from handling or possible poorer growing conditions under the predator cover.

Sample #	Length (mm)	Est. Age	% Growth	Sample #	Length (mm)	Est. Age	% Growth
26	92	4	8.2%	NR	102	5	
58	81	4	8.0%	68	100	5	20.5%
7	112	5	4.7%	28	91	4	7.1%
53	82	4	15.5%	NR	78	4	
24	65	3	12.1%	15	91	4	9.6%
19	86	4	1.2%	NR	89	5	
NR	102	5		78	86	4	10.3%

Unfortunately, the funding for this portion of the Exxon Valdez Oil Spill (EVOS) Restoration Project 97131 **Chugach Region Clam Restoration** was not made available for FY98. The plan was to dig the total area of the test plots in July 1999 which would have given a minimum of one full year of growth and survival data.

4. Recommendations

1. Seek additional funding to finalize the current growout and predator protection studies.
2. Complete studies of the specific life histories of Razor Clams in the Cordova area.
3. Look at additional enhancement techniques such as transplanting with juvenile clams from other areas.
4. Investigate hatchery techniques for producing juvenile razor clams.

5. References

Nickerson, R. B. 1975. A critical analysis of some razor clam (Siliqua patula Dixon) populations in Alaska. Alaska Dep. Fish and Game, Juneau. 194 pp.

6. Appendices

Trip Reports

April 5 1997

Preparation work was done to rebuild the clam project site. The covering was twisted and did not cover all of the study area. One inch rebar was cut to 6' lengths to be wire tied to the ends of the covering.

April 6 Tide 7:46 A.M. -2.5

I went to the project site to redo the covering. Upon arrival at the site, the covering was balled up. I dug the covering out of the sand and tore large holes in it as I dug it out. The solution to this problem is to use heavier covering because if I can tear holes in it easily then so can predators.

There are 4 clams observed under the old covering and 2 were recovered and planted under the new covering. I placed the new covering next to the old one and attached 1" rebar with wire ties. I then surveyed the area around the site and dug seven legal size clams and planted them under the cover for a total of nine clams.

April 7 8:01 A.M. -2.1'

Left town around 7:00 A.M. arrived 15 minutes later. Went to grassy island bar where the razor clam project is located. I found the plastic covering balled up. I don't know if it was from sea otter or tidal activity. The solution would be to stake the covering down better. After I straightened out the covering I went and surveyed the beach for razor clams and found a total of five legal size clams (4"+), no undersized.

April 8 No work due to weather.

April 9 9:29A.M. -2.5'

The first bar I went to was Big Point Bar. 1 undersized clam was found. The second bar I went to was the north end of concrete Bar, no clams were found. The third bar was Rock Quarry Bar and 0 clams were found here also. The one thing I did notice was a lot fresh dead clams. That is I found a lot of shells on all three bars. I also went back to Grassy Island Bar and found another 3 legal sized clams and 0 undersized.

April 10 10:14 A.M. -2.0'

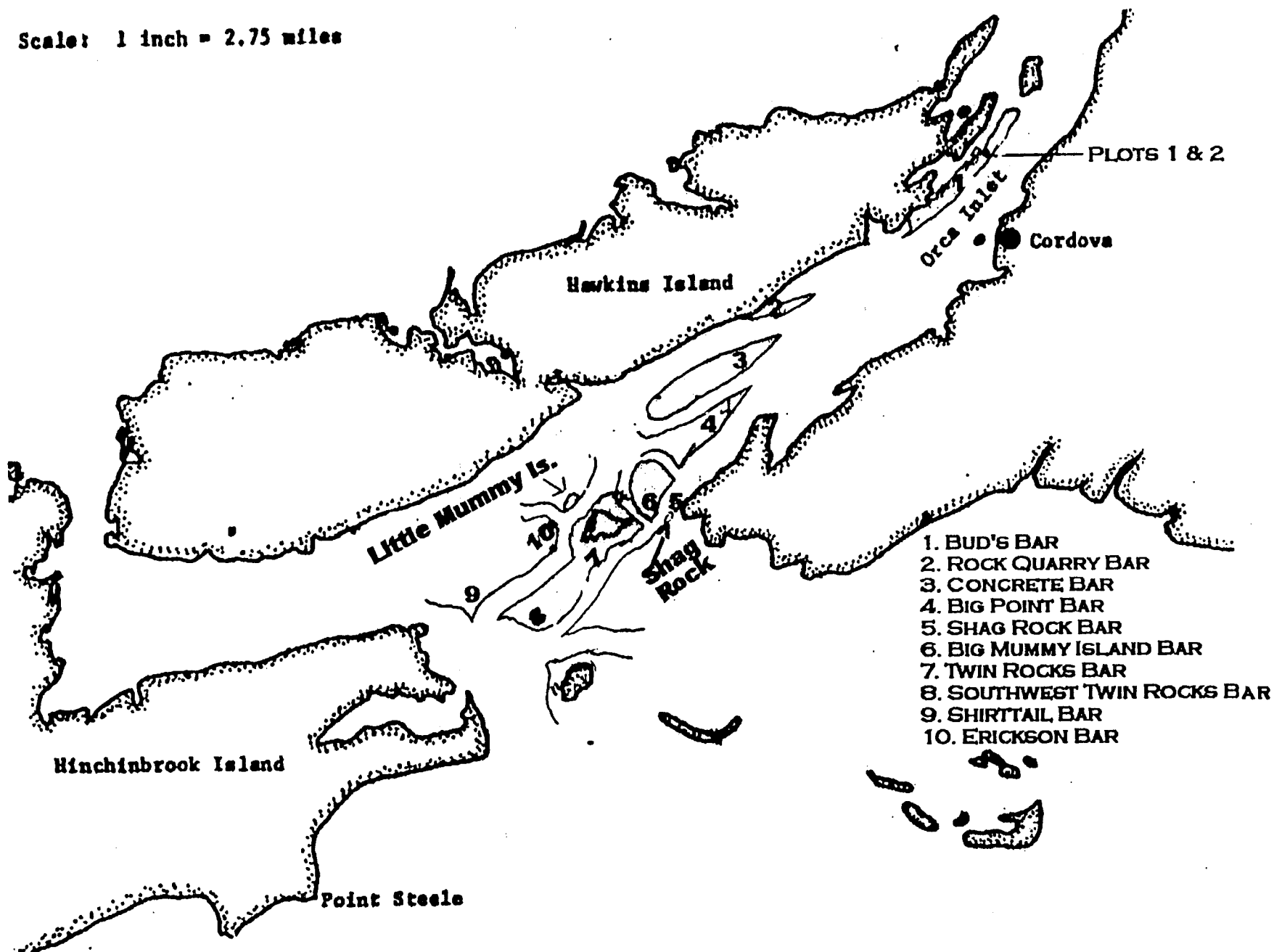
The first bar I surveyed was Shag Rock and 2 undersized clams were found. The second was Big Mummy Island Bar. No clams were found on this bar. Also noted was about a dozen sea otters hauled out on these bars and more feeding in the channels by the bars.

April 11 1997 10:59 A.M. -1.1'

The bar and area surveyed was the Hartney Bay region. No clams were found. Noted a couple of depressions in the sand that appeared to be the remains of sea otters digging clams.

The next run of tides are 4/23 to 4/26 and 5/5 to 5/10.

Scale: 1 inch = 2.75 miles

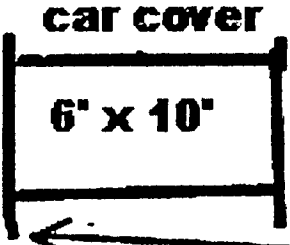


II-II

1. BUD'S BAR
2. ROCK QUARRY BAR
3. CONCRETE BAR
4. BIG POINT BAR
5. SHAG ROCK BAR
6. BIG MUMMY ISLAND BAR
7. TWIN ROCKS BAR
8. SOUTHWEST TWIN ROCKS BAR
9. SHIRTTAIL BAR
10. ERICKSON BAR

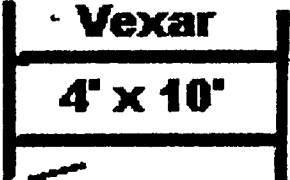
Grassy Is.

Plot# 1
At -2.0 ft.
43 legal size clams
planted in 1996.

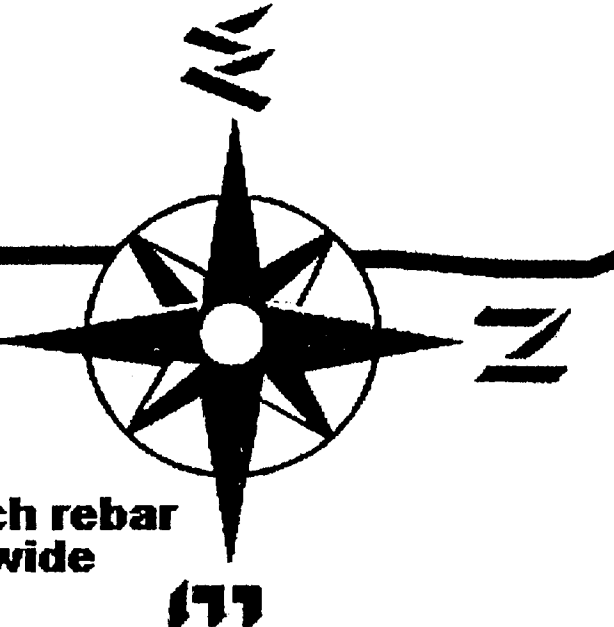


1 inch rebar
6ft. wide

Plot# 2
at - 1.5 ft.
82 undersized clams
planted in 1997.



1 inch rebar
6 ft. wide



Bud's Bar

Cordova

II-12

**Appendix III Qutekcak Shellfish Hatchery FY 97 Report with attached
Histo-pathology and Water Quality Reports**

**Qutekcak Shellfish Hatchery
FY 97 Progress Report
to the
Chugach Regional Resources Commission
on Littleneck Clam Production**

The Qutekcak shellfish hatchery experienced a good rate of success in producing Littleneck clam spat in FY 97. Hatchery operations were conducted in the existing pilot facility for all of FY 97. The new hatchery facility that Qutekcak is leasing from the State was ready for occupancy in January 1998. As of this writing (May 1998) the move into the new facility is nearly complete. Spawning the clam broodstock has been very successful both in terms of ease of inducing spawning on demand and in high percentages of gamete viability. Almost all brood clams have completed rapid gametogenesis when conditioned below 10° C and zygotes have demonstrated high rates of normal development to D-veligers unlike spawns prior to February of 1997 and described in the last annual report. Reducing the broodstock conditioning temperature from 13° C (summertime high) to 9.5° C (spring water temperature) partially accounts for why extensive abnormal development of early larvae has not recurred since February 1997. However, irregular two to four day periods when new algae cultures fail to grow combined with sudden larval mortality despite carefully standardized procedures demonstrate ongoing sudden water quality changes. Research into this problem is addressed below in **Hatchery Health Management**.

Clam Larvae Culture

Each clam spawn easily produced more larvae than capacity allowed at the pilot hatchery. Consequently, the spawning was quenched after about 5 million eggs were released. Littleneck clam larvae have proven very sensitive to larval rearing densities typically found at other hatcheries. Older larvae must be reared at a density of less than one larva per 2 milliliters to obtain even slow growth. This results in a theoretical maximum of 500,000 larvae per group with the limited larval tank volumes available in the pilot facility.

Eight groups of Littleneck clam larvae were reared in the pilot hatchery during 1997. Larvae grew slowly requiring from 25 to 38 days to reach the mature pediveliger stage and survival was somewhat low (Figure 1). All but one group of larvae produced competent pediveligers that were placed into downwelling setting systems to complete their metamorphosis (Table 1).

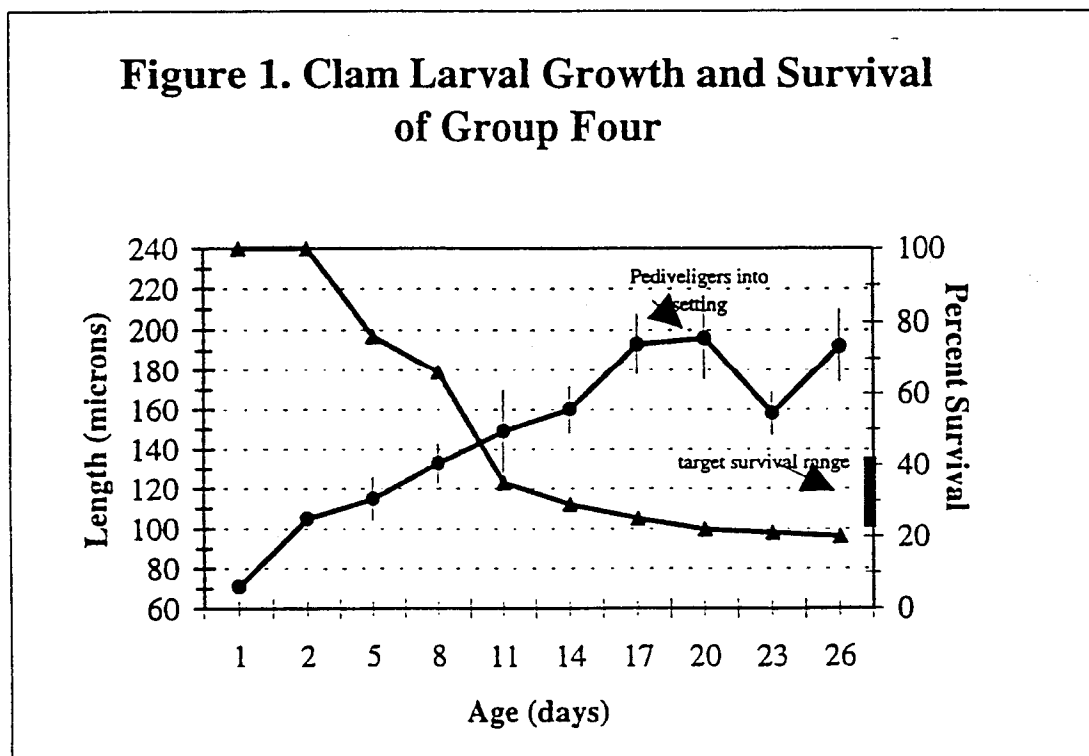
Survival through metamorphosis was highly variable ranging from 10 to 80 percent. Insufficient space in the pilot hatchery necessitates placing the setting pediveligers into the same downwelling system containing spat from prior settings. This cohabitation reduced food availability and water quality for the setting pediveligers leading to increased mortality during the stressful metamorphic process. Approximately

200,000 spat from the first four spawns and an additional 150,000 spat from the last three spawns survived metamorphosis.

Table 1. Larval Clam Production For FY 97

Date	Spawn Group	No. pediveligers into setting
3/17 - 4/24	1	63,000
5/5 - 6/9	2	104,000
6/11 - 7/18	3	50,000
7/9 - 8/4	4	454,000
8/12 - 9/9	5	330,000
9/5 - 9/27	6	*poisoned by fumes
9/20 - 10/18	7	202,000

- fumes from IMS maintenance on a freeze drier in our building poisoned both clam and scallop larvae under culture in the hatchery at that time

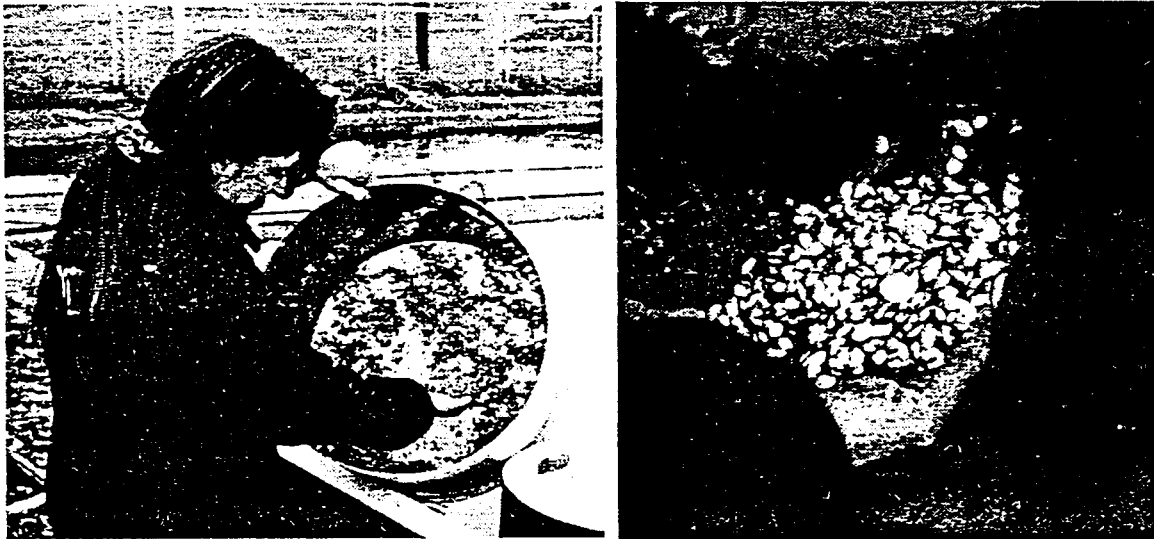


Pre-nursery Spat Rearing

Spat reaching 1 mm in size were transferred outside during the summer and fall into upwellers circulating seawater from the algae pond. In September, these spat were graded into 3-5 mm and 5 - 10 mm groups. These were transferred to the PWS nursery upweller for nursery stage rearing (Table 2). The sub 3-5 mm spat remained for further growth. Outdoor rearing of clam spat in pond upwellers (Figures 2 &3) continued through October at which time they were returned into the hatchery.

Table 2. 1997 Hatchery Clam Spat Production

Date	Size	No. Spat to PWS
9/6/97	3-5 mm	17,000
	5-10 mm	10,000
10/13, 11/1	3 mm +	22,500



Figures 2 & 3. Littleneck clam spat reared in hatchery pond upwellers

Outdoor microalgae culture in large 10,000-liter tanks proved very successful and reliable in FY 97. Culture densities typically grew to an impressive 300,000 cells per milliliter of *Skeletonema costatum*, *Thalassiosira gravida*, and *Chaetoceros spp.* Unfiltered seawater from 70 m depth was pumped into the pond-side tanks, fertilized and aerated with only natural illumination for about five days until harvest. We also maintained a bloom of a lipid rich, green *Tetraselmis striata* for three months in one of these outdoor tanks; harvesting half the culture every few days. This microalgae can be

pumped directly into the pre-nursery upwellers to feed the larger spat or drained into the pond as a large-scale inoculant. The pond received a much needed draining and cleaning this summer, which greatly reduced the quantities of suspended particulates inhibiting diatom growth. Many yards of mud were washed off the sides and then vacuumed off the bottom liner with a "super sucker" vacuum tank truck. After this cleaning a dense diatom bloom was easily sustained all summer and even through November when seawater temperatures fell to 4° C or 5° C. The pond was enriched with the same fertilizer at F/2 ratios as described in the last report. Trace mineral and CO₂ enrichment were not used because of the high costs of these compounds and the over-abundance of pond algae for existing numbers of clam spat.

Hatchery Health Management

Attached to this report are three consultant's reports. The first summarizes the results of histo-pathological examinations of clam larvae sampled from three different spawns. The second and third reports describe the results of a bioassay of hatchery seawater using a sensitive algal spore production test and the results of an oyster larvae bioassay of hatchery seawater.

The first report by Dr. Ralph Elston reveals significant bacterial infection of the one of three clam larvae samples during a period when the larvae of that particular group were dying rapidly. Thirty-six percent had "terminal infections" in various stages but he noted a small number of bacterial cells visible on the external shell surface suggesting that hygienic conditions in the culture are generally good but that the causative bacteria may be releasing an exotoxin. The second sample was of surviving larvae near terminal size and only 6 % had infections that were identical to the first sample. He commented on how poorly developed they were for 28 days of age. The third sample had no infections or lesions although a few bacterial cells were observed on the external shell surface. They were sampled from a typical slow growing group in the pilot hatchery. They served as our control for the first two samples. The exotoxin suggested by Dr. Elston might also originate from other sources than bacteria growing in the culture or even from bacteria at all. Histology cannot identify the source. I believe this suggestion supports our observations and experience that we suffer periods of bad seawater quality. Recent research has shown that the addition of organic compounds to seawater can cause larval mortality either by stimulating low background levels of bacteria to increase and/or increasing their virulence.

We are continuing to regularly collect larvae samples for more histology by Dr. Elston, however, I feel the real source of the problem lies in the water which is where we should direct more attention. We are, therefore, collecting daily samples of ambient seawater for total dissolved organic carbon (DOC) analysis by the University of Washington Seawater Chemistry lab. Our theory is that the seafood processor may be changing DOC levels and related microbial dynamics to the detriment of our larvae cultures. Having pre-, during and post- incident DOC sample might help us to verify this suspicion.

The second report, a toxicity identification evaluation (TIE) by EVS Environment Consultants, describes a sensitive *Champia sp.* algal bioassay of our pilot hatchery

seawater shows that significantly fewer (2/3 less) spores were produced relative to local seawater from British Columbia. The hatchery seawater was collected during a period of algal and larval mortality. We will collect more seawater during future problem periods for further assay experiments designed to begin identifying possible toxins in the water.

The third consultants report, also by EVS Environment Consultants, found no difference in survival but a significant decrease in size of oyster larvae grown for six days in seawater we supplied them during a problem period in the pilot hatchery compared to larval growth in their own seawater. The larvae also appeared paler in the hatchery seawater, many with tissues contracting from the shell. Because the effect on the larvae was at a relatively low level and involved subjective observations they recommend using the apparently more sensitive *Champia sp.* algal bioassay for future investigations into toxicity.

The frequency or perhaps severity of seawater quality problems may prove less of a problem in the new hatchery. Although not strictly pertinent to the 97 FY reporting period our first trial clam spawn in the new facility this April was a great success. The larvae grew twice as fast as in the pilot hatchery and 13 times as many mature larvae were placed into setting systems as ever before. Several reasons may account for our early success in this facility. The new, independent seawater system extends into deeper waters (250 feet). The one-micron filtered seawater receives about 4 to 6 times the UV dosage (60,000 to 90,000 microwatt/sec.cm²) as before. We also added probiotic bacteria ("PBD-31", Enviro-Reps. Intl.) to the larval tanks. The mix of beneficial bacterial strains purportedly inhibits pathogenic bacteria in shrimp hatcheries and grow-out facilities. And finally, the new larvae tanks themselves are 150 times the size (30,000 liters) of the tanks in the pilot hatchery. Different chemical and bacterial dynamics may occur in these much larger volumes of seawater than in 200-liter tanks.

SHELLFISH DIAGNOSTIC EXAMINATION REPORT

Q97-60

Page 1 of 3

prepared for:

December 24, 1997

Mr. John Agosti
Qutekcak Shellfish Hatchery
PO Box 369
Edward, AK 99664

Reference No.: AQ97-60

History: Native littleneck clams (*Prototheca staminea*) are being cultured in the Qutekcak Shellfish Hatchery and experience periodic morbidity and mortality. The following samples were collected, fixed at the hatchery and submitted for histological examination:

- 1) 9-19-97, 14 day old clam larvae.
- 2) 10-22-97, 28 day old clam larvae.
- 3) 11-6-97, 16 day old clam larvae.

Histological findings:

- 1) *Histological(14 day clam larvae)*: 200+ examined. These veliger larvae are in an early stage of the larval development cycle relative to setting. A significant proportion (36%) had terminal bacterial infections. These infections were in various stages of development. The infections are initiated by bacteria attaching to the external shell surface or periostracum. Subsequently, the bacteria invade along the shell surface to the internal shell surface where they progressively infect mantle tissue. After subsequent deep invasion of the mantle on the internal shell surface, the bacteria are able to infect the visceral cavity. There is little capacity for repair in these larvae and the infection is considered terminal once the peripheral mantle, near the shell margin, is infected. The high proportion of infected larvae in this sample indicates that the infection would lead to the failure of this culture. The relatively small number of bacterial cells suggests that the causative bacteria may produce an exotoxin. There were few bacterial cells visible on the external shell surface suggesting that hygienic conditions in the culture are generally good but that the causative bacteria is aggressive

AquaTechnics Inc. PO Box 687 Carlsborg, WA 98324
Tel: 360-683-2376 Fax: 360-683-2550 email: relston@olympus.net

and specific with respect to these infections.

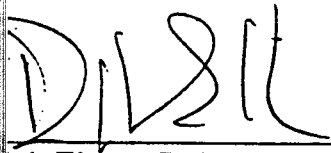
- (2) *Histological (28 day clam larvae):* 200+ examined. These were veliger larvae but appear poorly developed for 28 days post-fertilization, although the normal length of the larval period under the growth conditions used at the hatchery was not indicated. There were a few bacterial infections in this group (6%). These infections were identical to those noted above in sample (1). The proportion may indicate that the infection is only recently introduced to the tank or that it progresses more slowly due to the age of the larvae. However, these 28 day larvae are morphologically very similar to the 14 day larvae in sample (1). There were no other significant lesions noted in this group.
- (3) *Histological (16 day clam larvae):* 200+ examined. These were very early veliger larvae. There were no bacterial infections or any other significant lesions noted in this group. There were a few bacterial cells observed on the external shell surface.

Comments:

The results show the presence of moderate to severely invasive bacteria in the 14 day clam larvae, occurring at a high prevalence. A similar infection at a lower prevalence was noted in the 28 day larvae but not in the 16 day larvae. These samples were collected over about a six week time period so the differences in infection rate may be the result of changing conditions in the hatchery. The infection observed in the two samples is similar to the infection observed in oyster larvae from the Qutekcak hatchery earlier this year (Case number AQ97-23). Although the identity of the bacteria cannot be determined from histological examination, the similarity of the infection pattern suggests that the same species of bacteria could be involved in both the clam and oyster infections.

These are very aggressive infections and larvae have little capacity for defense or repair of damage once the infection is initiated. Additionally, it is important to note that the single histological examinations are a point in time snapshot that do not indicate the rate of progress of the infection. In past cases where a sequence of infection has been observed, these usually are rapidly progressing infections that can result in morbidity and mortality of greater than 50% of a culture in as little as one to two days.

As noted in the previous report, these histological observations suggest that bacterial management needs to be further addressed in this system. The usual sources of bacterial contamination and amplification, including water source and system, brood stock and stock and expanded algal cultures, should be investigated. Since there is relatively little experience in the bivalve hatchery industry with native littleneck clam larval culture, it is possible that this species may be associated with particularly aggressive bacterial infections that may affect other species, once they are established in the hatchery. A more detailed examination of hatchery bacteriology may be necessary to identify the source and key management strategies for preventing these infections.



Ralph Elston, PhD

December 24, 1997
Date

EVS CONSULTANTS

Champia parvula ALGAL REPRODUCTION TOXICITY TEST DATA SUMMARY

Client Outokumpu Shellfish Hatchery EVS Analysts AFM, EJT, JES^{AM}, LJS
 EVS Project No. 31808-01
 EVS Work Order No. 9700911 Test Initiation Date Dec 3, 1997

SAMPLE

Identification #1
 Amount Received 1X20L
 Date Collected Not given
 Date Received Dec. 1, 1997

TEST SPECIES INFORMATION

Culture Source/Data Received in-house culture
 Culture Batch -
 Reference Toxicant Sodium Dodecyl Sulphate (SDS)
 Current Reference Toxicant Result (IC50 and 95% CL) 0.41 and 0.19-0.66 (mg/L SDS)
 Reference Toxicant Warning Limits (mean ± 2SD) * 0.92 ± 1.43 0.89 ± 1.53 (mg/L SDS)

TEST CONDITIONS

Exposure Period
 Temperature Range (°C) 23.0-24.0
 pH Range 7.6-8.4
 Dissolved Oxygen Range (mg/L) 6.5-10.1
 Salinity Range (ppt) 27-32
 Photoperiod (L:D h) 16:8
 No. Organisms/Rep Five females and one male/Rep
 Other -

Recovery Period

Temperature Range (°C) 23.5-25.0
 Other -

Toxicity Test Results Mean number of cystocoups produced in sample = 8.4
Mean number of cystocoups produced in control = 25.2

Data Verified By E. McPherson Date Verified Jan 20/98

EVS CONSULTANTS

Champia parvula TOXICITY TEST - WATER QUALITY DATA

Client Dukekak Shellfish Hatchery
 EVS Project No. 3/808-01
 EVS Work Order No. 970911

Sample ID sample #1
 Test Initiation Date/Time Dec 3, 1997 / 1330h
 Recovery Initiation Date Dec 5, 1997 / 1300h
 Test Termination Date Dec 12, 1997

INITIAL WATER QUALITY	SAMPLE	TEST MEDIUM (EVS SW/ENW. SW)	RECOVERY MEDIUM
Temperature (°C)	23.0	24.0 / 24.0	22.5
Dissolved Oxygen (mg/L)	8.0 → 6.9 aerated 10min	6.6 / 6.5	7.3
pH	7.6 → 7.6	8.1 / 8.2	7.7
Conductivity (µmhos/cm)	—	—	—
Salinity (ppt)	32	29 / 28	28
Hardness (mg/L as CaCO ₃)	6000	Not Applicable	Not Applicable
Alkalinity (mg/L as CaCO ₃)	96	Not Applicable	Not Applicable

TEST PERIOD - WATER QUALITY MONITORING

Concentration (µg)	Temperature (°C)			pH			Salinity (ppt)			DO (mg/L)		
	0	24	48	0	24	48	0	24	48	0	24	48
DO	23.0	24.0	23.5	7.6	7.7	8.2	32	30	30	6.9	7.7	9.1
EVS SW CONTROL	24.0	24.0	23.5	8.1	7.6	8.4	29	28	28	6.6	8.0	10.1
ENW. SW CONT.	24.0	24.0	23.5	8.3	7.6	8.0	28	27	27	6.5	7.6	8.3
Techn. Initials	AFM	AFM	EM	AFM	AFM	EJT	AFM	AFM	EM	AFM	AFM	EM

RECOVERY PERIOD - TEMPERATURE (°C) MONITORING (initial daily entries)

Day	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
24°C	25.0	24.5	24.0	23.5	23.5	23.5	23.5

Sample Description clear, colourless solution
 WQ Instruments Used: Temp. cal/hy therm. pH II-A-29 DO II-A-20 Cond/Sal. II-C-22
 Comments SW = seawater fan added to testing chamber

Test Set Up By AFM/ETT Data Verified By C. M. Plusa Date Verified Jan 20/98

EVS CONSULTANTS
Champia parvula TOXICITY TEST - CYSTOCARP COUNTS

Client Quikcak Shellfish Hatchery
 EVS Project No. 3/808-01
 EVS Work Order No. 9700911

Sample ID Sample #1
 Test Initiation Date Dec 3 1997 / 1330h
 Recovery Initiation Date Dec 5 1997 / 1300h
 Test Termination Date Dec 12 1997
 Date Counted Dec 12 1997

(% C'n)	(V) c'n	Rep	Individual Plant Cystocarp Counts					Comments or Observations	Tech. Init.
			1	2	3	4	5		
10		A	17	18	0	14	9	red tips, pale stems	et
		B	8	11	7	10	10	red tips, pale stems	et
		C	11	6	3	7	16	red tips, pale stems	et
		D	2	2	11	4	2	red tips, pale stems	et
ENV. SW LOW	101	A	11	0	16	17	0 ^⓪	} Plant is dead (48h). } Do not appear healthy.	AFY
		B	34	29	18	10	0		AFY
		C	0	9	11	2	3		AFY
		D	0	8	4	6	3		AFY
EV SW cont.	can	A	4 ^⓪	17	32	60	22	⓪ Plants are not healthy - green + fragmented	AFY
		B	3 ^⓪	35	13	26	18		AFY
		C	14	23	31	60	10		AFY
		D	45	30	15 ^⓪	15	32		AFY
		A							
		B							
		C							
		D							
		A							
		B							
		C							
		D							

Data Verified By C. McPherson

Date Verified Jan 20/98

Test ID: EVS6268 Protocol: EPAM 87 Sample Type: SEDIMENT1-Marine Lab ID: EVS-Environment Consultants					Test: AL-Algal Reproduction Test Species: CP-Champia parvula Sample ID: #1 Start Date: 3/12/97 End Date: 12/12/97				
ID	Rep	Group	C1	C2	C3	C4	C5	Notes	
1	1	EVS Control	4	17	32	60	22		
2	2	EVS Control	3	35	13	28	18		
3	3	EVS Control	14	23	31	60	10		
4	4	EVS Control	45	30	15	15	32		
5	1	Env.Can.Cont.	11	0	16	17	0		
6	2	Env.Can.Cont.	34	29	18	10	0		
7	3	Env.Can.Cont.	0	9	11	2	3		
8	4	Env.Can.Cont.	0	8	4	6	3		
9	1	100.00	17	18	0	14	9		
10	2	100.00	8	11	7	10	10		
11	3	100.00	11	8	3	7	16		
12	4	100.00	2	2	11	4	2		

Comments: Qutekcek Shellfish Hatchery; 3/808-01; WO#9700911

Algal Reproduction Test-Reproduction

Start Date: 3/12/97 Test ID: EVS6266 Sample ID: #1
 End Date: 12/12/97 Lab ID: EVS-Environment Consultant Sample Type: SEDIMENT1-Marine
 Sample Date: Not given Protocol: EPAM 87 Test Species: CP-Champia parvula
 Comments: Qutakcak Shellfish Hatchery; 3/808-01; WO#9700911

Conc %	1	2	3	4
EVS Control	27.000	19.000	27.600	27.400
Env. Cont.	8.800	18.200	5.000	4.200
100	11.600	9.200	8.600	4.200

Conc %	Mean	SD	Transform: Untransformed				N	1-Tailed		
			Mean	Min	Max	CV%		t-Stat	Critical	MSD
EVS Control	25.250	4.174	25.250	19.000	27.600	16.531	4			
Env. Cont.	9.050	6.422	9.050	4.200	18.200	70.957	4			
*100	8.400	3.085	8.400	4.200	11.600	36.732	4	6.492	1.943	13.089

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.82878	0.749	-1.2459	0.25189
F-Test indicates equal variances (p = 0.63)	1.83018	47.4883		
The control means are significantly different (p = 5.50E-03)	4.23033	2.44691		
Hypothesis Test (1-tail, 0.05)				
Homoscedastic t Test indicates significant differences				

Statistical comparisons were made against the EVS control water

EVS CONSULTANTS

Champia parvula ALGAL REPRODUCTION TOXICITY TEST DATA SUMMARY

Client DukeKirk Shellfish Hatchery
 EVS Product No. 31808-01
 EVS Work Order No. 9700911

EVS Analysts AFY, EJT, LJS
 Test Initiation Date Dec 3, 1997

SAMPLE

Identification # 2
 Amount Received 1 x 20L
 Date Collected Not given
 Date Received Dec. 1, 1997

TEST SPECIES INFORMATION

Culture Source/Data Received in-house culture
 Culture Batch -
 Reference Toxicant Sodium Dodecyl Sulphate (SDS)
 Current Reference Toxicant Result (IC50 and 95% CL) 0.41 and 0.19-0.66 (mg/L SDS)
 Reference Toxicant Warning Limits (mean ± 2SD) 0.92 ± 1.43 0.89 ± 1.53 (mg/L SDS)

TEST CONDITIONS

Exposure Period

Temperature Range (°C) 23.0-24.0
 pH Range 7.6-8.4
 Dissolved Oxygen Range (mg/L) 6.5-10.1
 Salinity Range (ppt) 27-32
 Photoperiod (L:D h) 16:8
 No. Organisms/Rep Five females and one male/Rep
 Other -

Recovery Period

Temperature Range (°C) 23.5-25.0
 Other -

Toxicity Test Results Mean number of cystocarpus produced in sample = 8.0
Mean number of cystocarpus produced in control = 25.2

Data Verified By E. Metherson Date Verified Jan 20/98

EVS CONSULTANTS
Champia parvula TOXICITY TEST - WATER QUALITY DATA

Client Quekcoak Shellfish Hatchery
 EVS Project No. 3/808-01
 EVS Work Order No. 9700911

Sample ID Sample #2
 Test Initiation Date/Time Dec. 3, 1997 / 1330 hr
 Recovery Initiation Date Dec 5, 1997 / 1300 h
 Test Termination Date Dec. 12, 1997

INITIAL WATER QUALITY	SAMPLE	TEST MEDIUM (EVS SW / ENV. CAN. SW)	RECOVERY MEDIUM
Temperature (°C)	<u>23.0</u>	<u>24.0 / 24.0</u>	<u>22.5</u>
Dissolved Oxygen (mg/L)	<u>8.2 → 7.0 aerated 10 min</u>	<u>6.6 / 6.5</u>	<u>7.3</u>
pH	<u>7.6 → 7.5</u>	<u>8.1 / 8.2</u>	<u>7.7</u>
Conductivity (µmhos/cm)	<u>---</u>	<u>---</u>	<u>---</u>
Salinity (ppt)	<u>32</u>	<u>29 / 28</u>	<u>28</u>
Hardness (mg/L as CaCO ₃)	<u>5700</u>	<u>Not Applicable</u>	<u>Not Applicable</u>
Alkalinity (mg/L as CaCO ₃)	<u>100</u>	<u>Not Applicable</u>	<u>Not Applicable</u>

TEST PERIOD - WATER QUALITY MONITORING

Concentration (µg/L)	Temperature (°C)			pH			Salinity (ppt)			DO (mg/L)		
	0	24	48	0	24	48	0	24	48	0	24	48
DO	<u>23.0</u>	<u>24.0</u>	<u>23.5</u>	<u>7.9</u>	<u>7.7</u>	<u>8.3</u>	<u>32</u>	<u>30</u>	<u>30</u>	<u>7.0</u>	<u>7.9</u>	<u>8.5</u>
EVS SW CONTROL	<u>24.0</u>	<u>24.0</u>	<u>23.5</u>	<u>8.1</u>	<u>7.6</u>	<u>8.4</u>	<u>29</u>	<u>28</u>	<u>29</u>	<u>6.6</u>	<u>8.0</u>	<u>10.1</u>
ENV. CAN. SW CONT.	<u>24.0</u>	<u>24.0</u>	<u>23.5</u>	<u>8.3</u>	<u>7.6</u>	<u>8.0</u>	<u>28</u>	<u>27</u>	<u>27</u>	<u>6.5</u>	<u>7.6</u>	<u>8.3</u>
Tech. Initials	<u>AFM</u>	<u>AFM</u>	<u>EL</u>	<u>AFM</u>	<u>AFM</u>	<u>EL</u>	<u>AFM</u>	<u>AFM</u>	<u>EL</u>	<u>AFM</u>	<u>AFM</u>	<u>EL</u>

RECOVERY PERIOD - TEMPERATURE (°C) MONITORING (initial daily entries)

Day 0 <u>AFM</u>	Day 1 <u>EL</u>	Day 2 <u>AFM</u>	Day 3 <u>AFM</u>	Day 4 <u>AFM</u>	Day 5 <u>AFM</u>	Day 6 <u>AFM</u>	Day 7 <u>AFM</u>
<u>24.0</u>	<u>25.0</u>	<u>24.5</u>	<u>24.0</u>	<u>23.5</u>	<u>23.5</u>	<u>23.5</u>	<u>23.5</u>

Sample Description clear, colourless solution
 WQ Instruments Used: Temp. WT-100, pH II-A-29, DO II-A-20, Cond./Sal. II-C-22
 Comments 1 Fan added to testing chamber

Test Set Up By AFM/EST Data Verified By C. N. P. USA Date Verified Jan 20 1998

EVS CONSULTANTS Champia parvula TOXICITY TEST - CYSTOCARP COUNTS

Client Rutekcah Shellfish Hatchery
 EVS Project No. 3/808-01
 EVS Work Order No. 9700911

Sample ID Sample # 2
 Test Initiation Date Dec 3 1997
 Recovery Initiation Date Dec 5 1997 / 1330 h
 Test Termination Date Dec 12 1997 / 1300 h.
 Date Counted Dec 12 1997

% (v) Concn	Rep	Individual Plant Cystocarp Counts					Comments or Observations	Tech. Init.
		1	2	3	4	5		
10	A	17	7	4	18	12	red tips, pale stems	ett
	B	13	12	7	3	3	healthy and red.	ett
	C	2	1	1	8	1	red tips, pale stems	ett
	D	4	6	34	7	0	red tips green stems	ett
ENV AN EVS SW CONC of	A	11	0	16	17	0 ^⓪	⓪ Plant dead at 48h } All plants do not } appear healthy.	AFM
	B	34	29	18	10	0		
	C	0	9	11	2	3		
	D	0	8	4	6	13		
ENV SW CONC T.	A	4 ^⓪	17	32	60	22	⓪ Plant is not healthy - green + fragmented	AFM
	B	3 ^⓪	35	13	26	18		
	C	14	23	31	60	10		
	D	45	30	15 ^⓪	15	32		
	A							
	B							
	C							
	D							
	A							
	B							
	C							
	D							

Data Verified By C. McPherson

Date Verified Jan 20/98

Test: AL-Algal Reproduction Test				Test ID: EVS8267					
Species: CP-Champia parvula				Protocol: EPAM 87					
Sample ID: #2				Sample Type: SEDIMENT1-Marine					
Start Date: 3/12/97		End Date: 12/12/97			Lab ID: EVS-Environment Consultants				
P	ID	Rep	Group	C1	C2	C3	C4	C5	Notes
	1	1	EVS Control	4	17	32	60	22	
	2	2	EVS Control	3	35	13	28	18	
	3	3	EVS Control	14	23	31	60	10	
	4	4	EVS Control	45	30	15	15	32	
	5	1	Env.Can.Cont.	11	0	16	17	0	
	6	2	Env.Can.Cont.	34	29	18	10	0	
	7	3	Env.Can.Cont.	0	9	11	2	3	
	8	4	Env.Can.Cont.	0	8	4	6	3	
	9	1	100.0	17	7	4	18	12	
	10	2	100.0	13	12	7	3	3	
	11	3	100.0	2	1	1	8	1	
	12	4	100.0	4	6	34	7	0	

Comments: Qutekcek Shellfish Hatchery; 3/808-01; WO#9700911.

Algal Reproduction Test-Reproduction

Start Date: 3/12/97 Test ID: EVS6287 Sample ID: #2
 End Date: 12/12/97 Lab ID: EVS-Environment Consultant Sample Type: SEDIMENT1-Marine
 Sample Date: Not given Protocol: EPAM 87 Test Species: CP-Champia parvula
 Comments: Qutekcak Shellfish Hatchery; 3/808-01; WO#9700911

Conc %	1	2	3	4
EVS Control	27.000	19.000	27.600	27.400
Env. Cont.	8.800	18.200	5.000	4.200
100	11.800	7.600	2.600	10.200

Conc %	Mean	SD	Transform: Untransformed				N	t-Stat	1-Tailed	
			Mean	Min	Max	CV%			Critical	MSD
EVS Control	25.250	4.174	25.250	19.000	27.600	16.531	4			
Env. Cont.	9.050	6.422	9.050	4.200	18.200	70.957	4			
*100	8.000	3.963	8.000	2.600	11.600	49.540	4	5.994	1.943	16.094

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution ($p > 0.01$)	0.79498	0.749	-1.1253	-0.4292
F-Test indicates equal variances ($p = 0.93$)	1.1093	47.4883		
The control means are significantly different ($p = 5.50E-03$)	4.23033	2.44691		
Hypothesis Test (1-tail, 0.05)				
Homoscedastic t Test indicates significant differences				

Statistical comparisons were made against the EVS control.

EVS CONSULTANTS

Alkalinity/Hardness Measurements

Client: Quekkek Shellfish Hatchery
 Project #: 1808-01
 Work Order: 9700911

Test Type: 7-d algal reproduction toxicity test
 Test Species: Champia parvula
 Start Date: Dec. 3, 1997 (exposure period); Dec. 5, 1997 (recovery period)
 End Date: Dec. 5, 1997 (exposure period); Dec. 12, 1997 (recovery period)

Sample ID	Subsample Date	Alkalinity Measurement					Hardness Measurement			
		Subsample Volume (mL)	Initial H ₂ SO ₄ Volume (mL)	Volume to pH 4.5 (mL)	Total Volume to pH 4.2 (mL)	Alkalinity (mg/L as CaCO ₃)	Subsample Volume (mL)	Initial EDTA Volume (mL)	Final EDTA Volume (mL)	Hardness (mg/L as CaCO ₃)
#1 ¹	Jan 2, 1998	50	0.0	4.9	5.0	96	1.0	0.0	6.0	6000
#2 ¹	Jan 2, 1998	50	0.0	5.1	5.2	100	1.0	0.0	5.7	5700

¹ 1 mL of sample diluted with 49 mL of distilled water for hardness measurement; no dilution used for alkalinity measurement.

II-19

Copy
Jan 20, 1998

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 0604862 9519
 EVS CONSULTANTS
 012/012



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FAX TRANSMITTAL SHEET

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TO: John Agosti

DATE: March 3, 1998 OUR FILE #: 3/808-01

FAX #: 1-907-224-5282 TEL #:

SENT BY: Howard C. Bailey, Ph.D., R.P. Bio. # OF PAGES: 3

RE: TIE testing of hatchery water and algae

Dear John:

Attached are the results from the 6-day larval oyster development test performed with the hatchery water samples collected in November 1997 and in February 1998. The larvae reared in your water from November did not appear to be as healthy as those in control treatments; however, these results were based on subjective measurements. A longer term test may be more effective at measuring a sub-lethal endpoint, as variations in larval size may have masked an effect on growth in this 6-day test.

Please free to call if you have any further questions. I will be out of the country for the next two months, but can be reached through e-mail (hbailey@evs.bc.ca) or through the office, as I will be collecting messages daily. James Elphick will be in the office for the next two weeks and will be able to assist you directly with any questions you might have in that period of time.

Regards,

A handwritten signature in cursive script, appearing to read 'Howard', is written over a horizontal line.

Howard

ORIGINAL: Sent by Mail []
Filed [X]

Sent by Courier []
Held Pending Your Response []



ENVIRONMENT CONSULTANTS

Dyster larval development test

Hatchery water (two November samples and one February sample) and hatchery algae (one November sample and one February sample) were tested using 7-d old diploid veliger larvae (Coast Seafoods). Water treatments were compared to control water (EVS laboratory seawater and Environment Canada seawater) and algal treatments in November hatchery water to November hatchery water fed with an algal paste (*Tahitian isochrysis*, *Isochrysis galbana*, *Pavlova lutheri*, and *Nannochloropsis oculata*). Larvae were exposed in 1-L volumes with a larval density of approximately 2 per mL and were fed daily with approximately 60,000 algal cells per mL. Beakers were aerated gently throughout the test, and were maintained in the dark at 22°C.

Water treatments were renewed at 48-hr intervals by removing and replacing 500-mL of solution in each beaker. Water was removed by syphon from a standpipe with an 80 micron Nitex screen to prevent removal of larvae.

Larvae were exposed for 6 days, at which point, the beakers were mixed gently and 10-mL aliquots subsampled and formalized. Larvae were analyzed for survival, growth and appearance. A minimum of 15 larvae were measured for each treatment. Determinations of survival and incidence of broken shells were based on counts of 50 - 70 larvae.

There was no reduction in survival in any of the treatments. Larval size was significantly smaller than in the EVS control water in one of the hatchery samples from November ($p < 0.05$). No other samples showed a significant effect of exposure on larval size. Small differences in size may have been masked by relatively large variance in size of larvae in all treatments.

Larvae appeared to be less dense (paler) in November hatchery water samples fed with algal-paste than the EVS and Environment Canada control treatment larvae. This effect was amplified by feeding larvae in the November hatchery water sample with November hatchery algae - many of the larvae in this treatment had tissues which were contracted from the shell. However, this effect was not present in the November hatchery water sample fed with February hatchery algae. The larvae appeared marginally less dense than control larvae in the February hatchery water sample fed with algal paste. These effects may be significant with respect to larval survival; however, they are qualitative measures which cannot be easily compared. Small numbers of empty shells were also noted in some of the treatments, and were associated with both the February and November water samples. None were found in either of the control treatments, or in the hatchery water sample fed with February hatchery algae.


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Treatment Water (Algae)	Size (μm)	% Shells Empty	Larval appearance
EVS Control (algal paste)	127.8 \pm 13.3	0	Dense, dark, shell full
Env. Canada Control (algal paste)	123.8 \pm 13.3	0	Dense, dark, shell full
November water 1 (algal paste)	125.4 \pm 17.4	4	Paler, shell full
November water 2 (algal paste)	119 \pm 13.6	2	Paler, shell full
February water (algal paste)	130.9 \pm 17.4	5	Marginally paler than control
November water (November algae)	123.8 \pm 16.7	6	Paler, contracted from shell
November water (February algae)	131.6 \pm 15.9	0	Dense, dark, shell full

Conclusions

Subjectively, the larvae appeared least healthy in waters from November 1997. Empty shells were present at a low rate in your waters but not in the control waters. Clearly the larvae fed the February algae did the best, suggesting some interaction or compensatory mechanisms were occurring.

Based on the relatively low level of effect, it would be difficult to pursue a TIE on the oyster larvae. A longer exposure time would probably make the endpoint more definitive, but would also require greater effort. An alternative choice might be to test the February water with *Champia* and, if effects are apparent, look for the cause of toxicity with this species.

Toxicity Test Report (Draft)

Introduction

Toxicity tests with the red alga *Champia parvula* were conducted on two water samples received from Qutekcak Shellfish Hatchery on 1 Dec 1997. The purpose of these tests was to determine if water from the Hatchery might produce adverse sub-lethal effects on marine organisms, a point of concern given that this water is used in the culture of algae and various life history stages of bivalves. The tests compared survival and cystocarp production in the two test waters to water obtained from Burrard Inlet, British Columbia. Another water sample, obtained from the Environment Canada Laboratories in North Vancouver, BC, was also tested. This sample appeared murky which was likely a result of contamination from recent stormwater runoff into the vicinity of the intake pipe. Consequently, it was not included in the comparison.

Methods

The test methods followed USEPA (1988) guidelines for conducting toxicity tests with estuarine and marine organisms. *Champia* were obtained from in-house cultures. Male and female branch tips were exposed together in the test waters for 48 hr in 250 mL flasks containing 100 mL of solution. At this time, the female tips were transferred to culture medium. After seven days in culture medium, survival and cystocarp development evaluated under a dissecting microscope. There were four replicates per treatment. The nominal test temperature was 23C and the exposures were conducted under a 16hrL:8hrD photoperiod at a light intensity of approximately 500ft-c.

Results

Water quality parameters were within the range of tolerance for this test organism. Test temperature ranged between 23 and 25C during the study. Dissolved oxygen concentrations were between 6.5 and 10.1 mg/L and pH was between 7.6 and 8.4. The salinity range was 27-32 ppt.

There was no effect on survival in any of the test treatments. However, cystocarp production was appreciably reduced in both of the test samples compared with tips exposed to water from Burrard Inlet. More specifically, cystocarp production averaged 8.0 and 8.4 cystocarps per plant (standard deviation of 4.0 and 3.1) in the two Hatchery samples compared with 25.2 cystocarps per plant (standard deviation of 4.2) in plants reared in water from Burrard Inlet.

The data were evaluated for normal distribution and equality of variances. Since these assumptions were confirmed, the differences between the test waters and control were evaluated with homoscedastic t-tests and were significant at $p < 0.05$.

Conclusions

The results indicate that cystocarp production in the two water samples received from Qutekcak

Shellfish Hatchery was significantly less than in a water sample obtained from Burrard Inlet, BC. Further testing would be required to determine the cause of reduced reproduction and whether it would affect bivalves or algae under production at the Hatchery.

Reference

USEPA. 1988. Short-term methods for estimating the chronic toxicity of effluents and receiving waters to marine and estuarine organisms. EPA/600/4-87/028. Environmental Monitoring and Support Laboratory, Office of Research and Development, U.S. Environmental Protection Agency, Cincinnati, OH.

Environmental Services for
Industry and Government:

Vancouver, Canada
Seattle, U.S.A.



**ENVIRONMENT
CONSULTANTS**

Our File #: 3/808-01

May 14, 1998

John Agosti
Qutekcak Shellfish Hatchery
Box 309
101 Railway Avenue
Seward, AK
USA 99664

Dear Mr. Agosti:

Re: *Champia parvula* toxicity testing conducted on Sample #1 and #2

The following is a draft toxicity test report of the *Champia parvula* algal reproduction tests on Samples #1 and #2, received December 1, 1997. The test method used, results, and conclusions are provided.

INTRODUCTION

Toxicity tests with the red alga *Champia parvula* were conducted on two water samples received from Qutekcak Shellfish Hatchery on 1 Dec 1997. The purpose of these tests was to determine if water from the Hatchery might produce adverse sub-lethal effects on marine organisms, a point of concern given that this water is used in the culture of algae and various life history stages of bivalves. The tests compared survival and cystocarp production in the two test waters to water obtained from Burrard Inlet, British Columbia. Another water sample, obtained from the Environment Canada Laboratories in North Vancouver, BC, was also tested. This sample appeared murky which was likely a result of contamination from recent stormwater runoff into the vicinity of the intake pipe. Consequently, it was not included in the comparison.

METHODS

The test methods followed USEPA (1988) guidelines for conducting toxicity tests with estuarine and marine organisms. *Champia* were obtained from in-house cultures. Male and female branch tips were exposed together in the test waters for 48 hr in 250 mL flasks containing 100 mL of solution. At this time, the female tips were transferred to culture

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**ENVIRONMENT
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John Agosti

Page 2

May 14, 1998

medium. After seven days in culture medium, survival and cystocarp development was evaluated under a dissecting microscope. There were four replicates per treatment. The nominal test temperature was 23°C and the exposures were conducted under a 16hrL:8hrD photoperiod at a light intensity of approximately 500ft-c.

RESULTS

Water quality parameters were within the range of tolerance for this test organism. Test temperature ranged between 23 and 25°C during the study. Dissolved oxygen concentrations were between 6.5 and 10.1 mg/L and pH was between 7.6 and 8.4. The salinity range was 27-32 ppt.

There was no effect on survival in any of the test treatments. However, cystocarp production was appreciably reduced in both of the test samples compared with tips exposed to water from Burrard Inlet. More specifically, cystocarp production averaged 8.0 and 8.4 cystocarps per plant (standard deviation of 4.0 and 3.1) in the two Hatchery samples compared with 25.2 cystocarps per plant (standard deviation of 4.2) in plants reared in water from Burrard Inlet.

The data were evaluated for normal distribution and equality of variances. Since these assumptions were confirmed, the differences between the test waters and control were evaluated with homoscedastic t-tests and were significant at $p < 0.05$.

CONCLUSIONS

The results indicate that cystocarp production in the two water samples received from Qutekcak Shellfish Hatchery was significantly less than in a water sample obtained from Burrard Inlet, BC. Further testing would be required to determine the cause of reduced reproduction and whether it would affect bivalves or algae under production at the Hatchery.



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John Agosti
Page 3
May 14, 1998

REFERENCE

USEPA. 1988. Short-term methods for estimating the chronic toxicity of effluents and receiving waters to marine and estuarine organisms. EPA/600/4-87/028. Environmental Monitoring and Support Laboratory, Office of Research and Development, U.S. Environmental Protection Agency, Cincinnati, OH.

Yours truly,

EVS ENVIRONMENT CONSULTANTS

for Howard C. Bailey, Ph.D., R.P. Bio.
Senior Ecotoxicologist

Appendix IV Response to Reviewer Comments on FY 96 Report

*Aquatic Environmental Sciences
644 Old Eaglemount Road
Port Townsend, WA 98368
Phone and FAX (360) 732-4464
Email brooks@olympus.net*

November 3, 1997

Ms. Patricia Brown-Schwalenberg
Executive Director, Chugach Regional Resources Commission
4201 Tudor Centre Drive, Suite 211
Anchorage, Alaska 99518

Dear Patty,

I have received Dr. Spies' comments, and those of his reviewer, dated September 9, 1997. I understand that no response to those comments is required. However, their nature is such that I feel a response is necessary. There appears to be several areas of misunderstanding with respect to the objectives of Section 3. GROWOUT (EVOS DPD Project #95131). The following comments are framed in the context of the grant and of Dr. Spies' letter dated September 9, 1997.

GENERAL COMMENTS.

Purpose of the Study. It has been my view that the purpose of this project, as described in the grant proposal, is to help Alaskan Native Villages improve their subsistence levels of bivalve shellfish. We have approached this project with the specific goal of involving Villager's in every possible aspect of the study and of transferring responsibility for the care of their shellfish to them at the earliest possible date. During 1995, we conducted lengthy interviews with Village elders and Village shellfish growers. They expressed an interest in intensive shellfish culture techniques and expressed little interest in extensive enhancement, even though we explained that extensive enhancement would require far less effort. These native desires are in large part responsible for our emphasis on intensive techniques.

Based on these interviews and our understanding of the constraints associated with native littleneck growth and mortality in Alaska, our plan has been to enhance subsistence shellfish resources through implementation of intensive shellfish culture techniques used to produce clams, primarily the Manila clam, in the Pacific Northwest. It must be emphasized that the Manila clam (*Tapes philippinarum*) is widely grown in the Pacific Northwest, and we know a lot about its culture. The native littleneck clam (*Protothaca staminea*) is not intensively cultured and techniques for hatchery production of seed, nursery of juveniles and growout to market size have not been developed. The reason is simple. The Manila clam is used worldwide, it has proven easier to work with in the hatchery, and it brings a higher price in the marketplace (currently ca. \$1.80/pound for native littlenecks and \$2.70/pound for Manila clams). Originally, we anticipated some failure in the field studies associated with the transfer of this technology. However,

to date, those failures have not occurred and all clams appear to be surviving and growing well.

In response to our 1995 report, the EVOS reviewer suggested that we consider extensive enhancement as described for *Mercenaria mercenaria* by Peterson *et al.* (1995). I obtained a copy of this paper and shared its contents with several shellfish growers in Washington State. The concept was not endorsed by a single person contacted for the following reasons:

- At harvest sizes of 38 to 50 mm valve length, there are 14 to 20 native littleneck clams per pound – ultimately yielding a few ounces of edible wet tissues. Native littleneck clams are not readily visible on the beach and are typically recovered by digging an area of intertidal to a depth of ca. 8" and recovering the clams in the overturned substrate. In general, commercial and substantial recreational harvests require minimum clam densities of at least 0.2 pounds of clams per square foot (three to four clams per square foot). This estimate is based on personal communications with major shellfish growers in Washington State and Oregon during my seven years of experience evaluating shellfish beds for a variety of clients, including the U.S. Government. Our Statement of Qualifications lists some of these clients. Seeding clams at one per square meter would require digging up as much as 14 square meters of substrate to obtain dinner for one. That simply is not reasonable.

- In the Pacific Northwest, the area under rocks provides habitat for a host of clam predators including shore crabs (*Hemigrapsus nudus*) and juvenile red rock crabs (*Cancer productus*). The placement of clam seed under rocks would simply result in their immediate destruction by these predators. Intensively cultured shellfish beds are typically cultivated to loosen the substrate and to remove as many predators as possible. Large rock is frequently removed and placed seaward in berms to increase the natural catch of seed and to increase the deposition of organic material behind the berms in the high energy environments characterized by cobble and rock. Mussels, oysters and clams utilize finely divided detritus as a major food source. That is the reason it is important to measure organic carbon or volatile solids in evaluating a beach.

The point I would like to make is that we did consider the EVOS reviewer's suggestion to consider extensive enhancement. However, there was little support for extensive enhancement from Villagers and no support for extensive enhancement as described in Peterson *et al.* (1995) from those most familiar with clam culture in the Pacific Northwest. This suggestion was not ignored. It was rejected for the reasons given above.

We did cultivate and seed three areas at each of three tidal elevations without providing additional protection from predators and without an attempt to increase substrate stability using any kind of netting. This treatment was described in our 1996 report. **The first sampling will occur in 1998** and we will likely be able to test the null hypothesis that clam recruitment, survival and growth is equal in all four of the treatments established in 1996. Those treatments included clams in bags, clams under Carcover, cultivated beach seeded but unprotected, and a control area cultivated but not seeded or protected.

The reviewer expressed concern that, "a complete array of density experiments is not proposed that will assess density dependence in a scientifically sound design." An evaluation of density dependent growth and mortality is a goal in the grant – a component of the grant that was scheduled to begin in 1997. The experimental design was not provided earlier because I felt that it should include the preliminary growth and mortality results from the first six months evaluation. In addition, the growout studies are dependent on the production of clam seed at the Qutekcak hatchery. We received the 1996 – 97 crop of clam seed in October 1997 and based on the number of clams provided, we are now able to design the 1997 field experiments. The point is that it would be helpful to avoid criticizing the team for not including study elements in annual reports that are not scheduled to begin until after the report is submitted.

Aging of shellfish. We have used traditional methods for assessing shellfish age. The use of checks and annuli in various structures is a widely used technique for assessing age. Scales, otoliths, fin rays and opercula have been used for many years as indications of age in fish. Likewise, the apparent annuli in bivalve shells have been used for the same purpose. These annuli can be difficult to discern in southern latitudes. Their interpretation in northern latitudes remains somewhat uncertain and requires experience. However, age information, obtained in this manner, has formed the basis for many studies (Feder and Paul, 1973, Paul and Feder, 1973, ADFG, 1995, Trowbridge *et al.*, 1996, Rutz, 1994, Bayne, 1976). No study was found in the literature in which caged or marked clams of known age were followed for several years to validate the efficacy of apparent annuli in estimating age. The results of our caged growth and mortality study will provide the first study of this kind. The point I would like to make is that this is a traditional technique, used by many researchers for decades to assess bivalve age. The purpose of this grant was not to verify these techniques and no funding was provided to accomplish that task. However, our experimental design will provide additional information helping future researchers understand these techniques.

In 1996, the EVOS reviewer referenced the ADFG Fish/Shellfish Study 13 and cautioned that, "the use of external lines can readily miss the first winter's growth check and thereby systematically **underestimate** age by one year." He discussed the implications of **underestimating** age in our analysis. We received a copy of the ADFG (1996) report in April, 1996, several months after submitting our 1995 annual report. What ADFG (1996) actually stated is:

Page 15, *Comparison of Aging Techniques of Littleneck Clams*

" . . . The age composition, based on the external surface method, was **significantly older** than the age composition based upon sectioned valve methods . . ."

Contrary to the reviewer's caution, ADFG (1996) found that the external check system (used in our study) likely results in assigning older ages to clams – not younger ages. Thus, if there is an error, we have **overestimated** the age of clams, **not underestimated** them as cautioned by the reviewer. Conclusion 7 in ADFG (1996) notes that, "Based upon these findings, we put forth that the external surface method is correct and the section valve method is actually under-estimating the age of littleneck clams."

We have used the external surface method in our estimation of clam ages and contrary to the reviewer's assertion, ADFG (1996) supports this methodology. Under any circumstances, the external surface method is relatively less time consuming and expensive than acetate peels or valve sectioning. The funding for this study did not support the use of the more expensive and less accurate methods proposed by the reviewer. Aging valves using the more time consuming and expensive methods would have required a significantly higher level of funding for this project.

We have used the same methods to age cockles and butter clams. A student at the University of Washington is currently undertaking a Masters thesis in which he will investigate the aging of butter clams using the external valve surface technique. Those results should be available prior to submission of our final report. We will consider his conclusions and make any necessary corrections to our butter clam database at that time. The point I wish to make is that I did investigate the reviewer's comment and found it based on a misinterpretation of ADFG (1996). While I agree that there is some subjectivity in assessing clam age using apparent annuli, it is a relatively inexpensive and useful technique.

Use of the von Bertalanffy model. I am not sure why the reviewer is averse to the use of the von Bertalanffy equation to predict length at age. Again, this is a long used model that has worked well for many researchers. Bayne (1976) provides a good review of the model's use. In any case, the von Bertalanffy equation is the model I have chosen to use in this study because of its historic use in shellfish research and because its interpretation is intuitive. The purpose of this study was not to evaluate alternate models for predicting length at age. We are simply using a well-known tool to make predictions. The bottom line is that our results meet the underlying assumptions for regression analysis and the von Bertalanffy model has generally explained better than 95% of the variation in each database. Therefore, it appears that this relationship will meet our need to predict length at age in developing management plans for cultured and wild shellfish found on specific beaches used by the various villages.

At the end of four or five years, our caged growth and mortality study will provide a test of both the external check method and our ability to predict length at age using a number of models. When that data is available, we will assess predictions made by the von Bertalanffy and Gompertz models. Granted, our data will be somewhat confounded by the various protective systems, however, because we are evaluating four treatments, we will be able to examine differences associated with the treatments.

At page 10 of the 1996 report, the reviewer asks why I used a Log transformation for discrete data. The underlying assumptions for regression analysis (and most parametric statistics) include a condition that the data be continuously distributed. Discrete data are not continuously distributed and it is common to use a $\text{Log}(X + 1)$ transformation to meet this condition. One is added to the discrete number (X) to provide for inclusion of zero counts (which otherwise would equal $-\infty$). I have assumed that the reader is familiar with these considerations. Based on common practice in the literature, I believe that remains a reasonable assumption.

Measurement of physical and chemical data. Too many studies fail to measure basic physical and chemical environmental parameters important to shellfish. This historic failure reduces our current ability to predict the productivity of intertidal areas for culture purposes. Optimum environmental conditions vary significantly, particularly between genera. Typically, cockles (*Clinocardium nuttallii*) are found in sandy substrates, while *Mya arenaria* prefers sandy substrates with higher silt-clay content. *Protothaca staminea* is primarily found in mixed cobble, gravel, sand, silt and clay substrates with sufficient silt and clay to indicate a reasonable degree of substrate stability. For Washington State species, Magoon and Vining (1981) summarize these requirements.

Section 2.1.b. of the grant requires that we evaluate the composition of substrates using the graduated sieve method. Based on my experience, we have expanded the parameters being evaluated to include Total Volatile Solids, and a host of organoleptic clues (depth of the RPD), presence of H₂S, NH₃, petroleum, etc. The additional cost is minimal and no additional funds have been sought from EVOS for this added work. We have analyzed the water column for a suite of parameters, not in an attempt to characterize the water column over a period of time, but rather to look for physical or chemical "red flags" indicating that a particular beach is inappropriate for clam culture.

There are numerous reasons why bivalve culture may fail on any particular beach. The Murphy Slough beach at Port Graham will provide a good test of the use of physicochemical parameters in determining the suitability of a beach to support shellfish culture. In this instance, very few hardshell clams were observed at Murphy Slough – even though the substrate appeared excellent for the intensive culture of clams. There are numerous possible reasons why clams were not found in this area, the most likely reason being that currents inhibit recruitment. However, it is also possible that fresh water intrusion or any one of a number of other factors might be responsible. Based on our examination of the physicochemical parameters included in this study and the nearshore watershed, I believe that recruitment failure is the most likely factor. Our growth and mortality studies are designed to evaluate that hypothesis. If the clams grow and survive normally (compared with our studies at other sites and with wild clams), that will support the hypothesis that the absence of clams is associated with poor recruitment rather than with other environmental factors. If they die, or do not grow well, then it is likely that other factors are more important in determining the lack of clams on this beach.

In any case, our measurement of these parameters has been accomplished, without additional cost to the grant, in an attempt to increase our probability of successfully identifying appropriate beaches for enhancement purposes. Developing a suite of parameters for quantitative analysis to determine an area's suitability for clam culture was also not a stated goal of this grant. If EVOS desires, that could be done. I have included a brief report summarizing the literature with respect to oyster culture in Oregon completed several years ago. With additional funding, I could produce a similar document for Manila and native littleneck clams.

The same comments apply to our analysis of water for fecal coliform bacteria. It is not our intention to certify any of these beaches in accordance with the provisions of the National Shellfish Sanitation Program. Rather we are collecting and analyzing water samples, when we can, looking for indications that an area would likely not meet the requirements of NSSP. It is much easier to decertify an area than it is to certify one. I

would remind the reviewer that State Shellfish Sanitation Programs generally require the collection of water samples for fecal coliform analysis over an extended period of time – they do not rely on many samples collected over a short or intermediate period. Once again, this part of our study was not required in the grant, and no additional EVOS funds have been requested.

Information gathering and literature search. The grant required that we develop baseline data on local beaches prior to planting clams for growout. In addition, we were required to conduct a literature search to, “see what information is available on **species composition and local abundance of shellfish**. This will include work conducted by EVOS funded project Fish/Shellfish 13. (emphasis added).”

We have accomplished this task through workshops conducted at each village and through discussion with village elders. We have conducted a literature search and rely on literature describing natural populations of native littleneck clams in the Pacific Northwest (Washington State to Alaska). Environmental conditions, particularly photoperiod, are so different in southern California, that specific observation there has little value in this project. However, for the final report, I will expand the literature search to include all areas of the world. Work with other clam species, such as *Mercenaria mercenaria*, is of little value in this project and it does not appear efficient to expand the search to include other species.

It is the goal of this project team to turn this enhancement program over to individual villages, with coordination and support from CRRC, as soon as possible. Therefore, we have listened very carefully to the concerns and desires expressed by participants in each village. Villagers have expressed a preference for intensive culture techniques where they work intimately with the shellfish. They have universally expressed an interest in producing the basket cockle (*Clinocardium nuttallii*) because this is a preferred food. This desire has been incorporated in our growout study by attempting to develop hatchery and growout techniques for *C. nuttallii*. This species has not been used in commercial aquaculture in the Pacific Northwest and virtually nothing is known regarding either hatchery production or growout.

There are large quantities of very small mussels (*Mytilus edulis trossulus*) at some Villages. Predation restricts these populations to the highest tide levels where they grow very slowly. I originally thought that placing these small mussels in suspended culture would increase their growth and provide a ready source of shellfish to Villagers. However, mussels were not identified as a traditional source of food at the Villages where we are working. In the end, the mussel cultures held in lantern nets were neglected and lost by the Villages. Thus, while this species might provide a source of tasty and nutritious seafood, the Villagers do not recognize it as such. This is their subsistence program and we have discontinued work with this species.

SPECIFIC COMMENTS. Some of the reviewer’s comments have been valuable and we have incorporated them in our study design. Others have not been incorporated because they would have required significant changes after the field work had been initiated. These concerns should have been expressed (and discussed) during the design phase of the grant – not after the grant was approved and the studies had begun. Other comments have been considered, but not incorporated, because I disagree with the

conclusions reached by the reviewer. It should be noted that we are devoted to improving subsistence levels of shellfish at these Villages. To that end, no reasonable or valuable suggestion is discarded out of hand. The following specific comments discuss only a few of these issues.

In this year's review he (she) expresses concern over sedimentation inside mesh bags. Mesh bags have been successfully used for years in the Pacific Northwest for the intensive culture of both oysters and clams. The use of bags and Carcover has expanded with time, not contracted. It has expanded because it works. Like all intensive techniques, their use must be managed and the grower must respond to observed conditions. In no case, in this current study, has sedimentation in the bags been a negative factor. However, I am interested in changes in particle size under Carcover netting, which has been used for many years as a tool to help stabilize substrates and exclude predators. That is the reason we are using the Sieve and Pipette method of Plumb (1981) to evaluate changes in Sediment Grain Size under Carcover.

The 1996 report was written four months into a study that will take several years before results could properly be assessed. The reviewer's repeated request for statistical tests of significance are premature. What would Dr. Spies response be to a demand for statistical testing of an experimental design after completing only the baseline part of the study? The design used for this study will result in our ability to test hypotheses regarding the growth of clams in intensive culture, substrate changes associated with the culture, and survival of clams with varying degrees of protection. Statistical testing of hypotheses must wait until we have the data upon which to base appropriate tests.

Most of the statistical analysis conducted to date has been descriptive in nature. Those descriptive statistics are valuable in guiding future work and in forming hypotheses regarding future results. We are using these preliminary results in real time to modify each succeeding year's workplan.

There are several reasons for removing large rock and cobble from intensively cultured areas. First, large material holds the Carcover off the substrate making it more vulnerable to damage. Second, large rock often becomes covered with barnacles that tear carcover. Third, and perhaps most importantly, large rock provides cover for numerous intertidal bivalve predators, such as shore crabs (*Hemigrapsus nudus*, etc.). Placing clam seed under rocks in the Pacific Northwest would simply result in a feast for the many predators found there. Native littleneck clams are infrequently found under large rock – likely because of this predation on seed. They are found in mixed substrates between large rocks. The efficacy of using this rock (or sand bags) to create berms is discussed by Toba *et al.* (1992) with reference to Tsutsumi *et al.* (1981).

Lastly, cultivating the ground to be planted loosens the substrate making it easier for clam seed to "dig-in" during seeding on an incoming tide. In asking that we determine how many of the seed dig-in and at what speed, your reviewer ignores Pacific Northwest tides which typically range from -4.5' MLLW to + 11 or 12' MLLW. We necessarily work tides that are as low as possible to maximize the time available for sampling. Our culture areas are covered with several feet of water within an hour of planting the seed.

Summary. Many of the reviewer's comments are well founded and will be incorporated in submission of the final report. However, as stated above, many of them are either premature, or suggest a lack of familiarity with the existing grant. These annual reports are not intended as completed pieces of work. My intent has been to summarize each year's effort and to glean as much information from the data as possible in an effort to optimize each following year's workplan and our probability of eventual success.

The modifiers "may, or suggests that, etc" precede the preliminary conclusions and hypotheses generated in these annual reports. These modifiers are intended to identify the statement as preliminary and based on the evidence to date – not as conclusions supported by detailed analysis of the studies' results. At the end of this study we will have the information necessary to test numerous hypotheses put forward in the annual reports. Our ability to reduce the total grow-out time by one or two years will be a matter of record – not one for debate. However, as in all lengthy studies, these analyses must await collection of an appropriate database. These preliminary conclusions are included as guidance for the team's members from year to year. Perhaps Dr. Spies needs would be better served by extracting only that portion of the annual report meeting his reviewer's criteria and using the rest of the report internally within the study team. That is an option that should be considered – it would result in far less detail in the annual report.

Constructive criticism is always welcomed in the conduct of any study. In this case, I did not participate in writing the grant. I believe the growout phase of this project is far exceeding the grant's requirements on a very limited budget. I would very much appreciate constructive criticism from Dr. Spies, or his reviewer. However, I believe that appropriate review and comment must be made in the context of the grant, as approved. The time to change the goals, objectives and methods of a grant is prior to funding. Not after the study has begun. I would be pleased to discuss any specific comment with you, Dr. Spies, or his reviewer.

Sincerely,

Dr. Kenneth M. Brooks
President, Aquatic Environmental Sciences

References.

- Bayne, B.L. 1976. Marine mussels: their ecology and physiology. Cambridge University Press, Cambridge, England. 506 pp.
- Feder, H.M. and A.J. Paul. 1973. Abundance Estimations and Growth-Rate Comparisons for the Clam *Protothaca staminea* from Three Beaches in Prince William Sound, Alsaka, with Additional Comments on Size-Weight Relationships, Harvesting and Marketing. Institute of Marine Science Technical Report No. R73-3, Alaska Sea Grant Program Report No. 73-2.
- Toba, D.R., D.S. Thompson, K.K. Chew, G.J. Anderson and M.B. Miller. 1992. Guide to Manila Clam Culture in Washington. Washington Sea Grant Program, University of Washington, Seattle, Washington.
- Trowbridge, C., T.T. Baker and J.D. Johnson. 1996. Effects of Hydrocarbons on Bivalves. EVOS Natural Resource Damage Assessment Final Report, Fish/Shellfish Study 13, Final Report.
- Tsutsumi, Y., H. Kawabe, and K. Tominaga. 1981. Investigations into what contributes to the development of nursery areas of the asari clam (*Venerupis japonica*). (In Japanese.) Kumamoto Prefectural Nori Laboratory, 1980 Report of the cooperative Aid Project for Designated Research, 23 pp.