Exxon Valdez Oil Spill
State/Federal Natural Resource Damage Assessment Final Report

Effects of Hydrocarbons on Bivalves Following the *Exxon Valdez* Oil Spill

Fish/Shellfish Study 13
Final Report

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Study History: Fish/Shellfish Study 13 was initiated to sample bivalve populations within Prince William Sound while Fish/Shellfish Study 21 sampled bivalve populations in Cook Inlet, the Kodiak Area and the Kenai Peninsula following the *Exxon Valdez* oil spill in 1989. The studies were combined in 1990 under Fish/Shellfish Study 13. Transect sampling was used to collect Cockles *Clinocardium nuttallii*, butter clams *Saxidomus giganteus*, and littleneck clams *Protothaca staminea* at oiled and non-oiled (control) beaches within Prince William Sound, outer Kenai Peninsula, Cook Inlet and Kodiak Island Area for growth, mortality, and recruitment studies, histopathological examination and hydrocarbon analysis. Sampling of Pacific razor clams *Siliqua patula,* was added to the study in 1990 to measure hydrocarbons because of concerns for brown bears and other upper level predators that feed on razor clams on the south side of the Alaska Peninsula. An experiment was conducted in 1990 and 1991 to evaluate the growth of littleneck clams reciprocally transplanted between oiled and control sites in Prince William Sound. This report is also being issued as an Alaska Department of Fish and Game, Division of Commercial Fisheries Regional Information Report No. 2A02-01.

Abstract: We examined the effects of hydrocarbons on bivalve populations in Prince William Sound, Kenai Peninsula, Kodiak Island, and the Alaska Peninsula following the *Exxon Valdez* oil spill. The majority of sampling sites were exposed to low levels of aromatic hydrocarbons. One designated control site (Simpson Bay) was also partially contaminated by refined petroleum hydrocarbons. Bivalve tissues at oiled sties were found to have high levels of aromatic hydrocarbons. However, clam tissues were not severely affected histopathologically either in 1989 or 1990. We were unable to determine mortality rates of bivalves during this study. Growth rates of littleneck clams decreased as the levels of aromatic hydrocarbons increased. Growth rates of littleneck clams also decreased as tide level increased. There were no significant differences in recruitment of young-of-the-year (YOY) littleneck clams between control and oiled sites in Prince William Sound. The initiation of future studies is recommended to study bivalve populations throughout Prince William Sound, Cook Inlet, Kodiak Island, and the Alaska Peninsula to provide baseline information in the event of another oil spill.

Key Words: Alaska Peninsula, bivalves, butter clam *Saxidomus giganteus*, Cook Inlet, *Exxon Valdez* oil spill (EVOS), Kenai Peninsula, Kodiak Island, littleneck clam *Protothaca staminea*, Prince William Sound (PWS), Pacific razor clam *Siliqua patula*.

Project Data: *Description of Data* – Data consisted of transect sampling for bivalves at oiled and unoiled beaches in Prince William Sound, Cook Inlet and the Alaska Peninsula for growth, recruitment, and mortality studies, histopathological examination and hydrocarbon analysis. In addition, growth data of littleneck clams reciprocally transplanted

between oiled and control sites was collected in Prince William Sound. *Format* - Numbers and size of bivalves, histopathology of bivalves, and hydrocarbon concentrations in bivalves and sediment samples collected at transect sampling locations have been entered into Lotus and Excel Worksheets. Growth data from the transplant and reciprocal transplant studies have been entered in ASCII files. In addition, SAS files contain the analyses of the growth data from transect sampling and reciprocal transplant studies. Much of the data collected has been included in the final report tables, figures and appendices. Final report is available in Adobe Acrobat PDF file format. *Custodian* – Excel and Lotus Spreadsheets, ASCII files, and SAS files mentioned above reside on the Commercial Fisheries server in Anchorage. Original data forms collected during the studies reside in the ADF&G Prince William Sound area office in Cordova. Original hydrocarbon data should be available from the NMFS Auke Bay Laboratory. *Availability* – Data is available upon written request to the ADF&G Prince William Sound area office in Cordova.

Citation:

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EXECUTIVE SUMMARY

Fish/Shellfish Study 13 was initiated in 1989 to sample bivalve populations within Prince William Sound while Fish/Shellfish Study 21 sampled bivalve populations in Cook Inlet, the Kodiak Island Area and the Kenai Peninsula. The studies were combined in 1990 under Fish/Shellfish Study 13. Transect sampling was used to collect Cockles *Clinocardium nuttallii*, butter clams *Saxidomus giganteus*, and littleneck clams *Protothaca staminea* from oiled and unoiled (control) beaches for growth, recruitment, and mortality studies, histopathological examination and hydrocarbon analysis. Only butter and littleneck clam sampling was performed in 1990 to assess possible growth differences among oiled and control locations. Sampling of Pacific razor clams *Siliqua patula*, on the south side of the Alaska Peninsula, was added to the study in 1990 to measure hydrocarbons in razor clams because of concerns for brown bears and other upper level predators that feed on razor clams. An experiment was conducted in 1990 and 1991 to evaluate the growth of littleneck clams reciprocally transplanted between oiled and control sites in Prince William Sound.

Hydrocarbon concentrations were determined for sediment and clam tissue samples collected at bivalve sampling sites. All but one of the sampling sites in Prince William Sound designated as oiled prior to sampling were exposed to aromatic hydrocarbons from the *Exxon Valdez* oil spill. Additionally, one designated control site in Prince William Sound (Simpson Bay) and one in Cook Inlet (Jakolof) were contaminated by refined petroleum hydrocarbons. Hydrocarbon contamination declined at oiled sites over the course of this study.

Histopathological analysis suggests that clam tissues were not severely affected by *Exxon Valdez* oil in either 1989 or 1990. Most (67.8%) of the molluscs examined were within normal limits histopathologically. The occurrence of parasite infestation was observed in bivalves. The parasites identified in the samples were typical of those found in clams along the Pacific Coast except that the tetraphyllidian cestode *Echeneibothrium* sp. reported from littleneck clams and gaper clams in California was absent. Previously reported only from Washington on the Pacific Coast, coccidian *Pseudoklossia* sp. was present in 17 (2.9%) of the molluscs analyzed.

The mortality rates of bivalves could not be determined in this study. Empty valves, signifying dead clams were found at all sites, but it was not possible to determine the cause of death. No evidence was found that oil from the *Exxon Valdez* increased mortality in bivalves.

Growth of littleneck clams collected in Prince William Sound in 1990 and 1991 increased as the levels of aromatic hydrocarbons decreased. Growth of littleneck clams was also found to decrease as tide level increased. In addition, littleneck clams that were tagged, notched, and reciprocally transplanted grew less than clams that were not moved but were notched and replanted. The decrease in growth was independent of direction of transplant (oil to control or reverse). The decrease in growth was probably due to tagging.

The recruitment of age 0 bivalves at control and oiled sites was estimated for sites sampled in 1989 and 1990. No significant differences in recruitment were found between oiled and control

sites.

Ages of littleneck clams using the external surface method were older than those estimated from the sectioned valve method. An initial interpretation would be that the external surface method overestimated the age due to counting a first annulus where it did not exist. Age-0 littleneck clams collected throughout the year at Simpson Bay in Prince William Sound indicated that recruitment occurred from the spring to the fall season. Because we identified the first annulus during the winter, the external surface method appeared to estimate the age of littleneck clams correctly while the sectioned valve method may have under-estimated the ages.

The objectives called for identifying alternate methods and strategies for restoration of lost use, populations, or habitat where injury was identified. One major drawback in the study of bivalves was the lack of baseline or background information prior to the oil spill. Future studies on bivalve populations are recommended throughout Prince William Sound, Cook Inlet, Kenai Peninsula area, Kodiak Island area and the Alaska Peninsula. These studies would provide baseline information that would be useful should another oil spill occur.

INTRODUCTION

The grounding of the *T/V Exxon Valdez* in Prince William Sound, on March 24, 1989 caused the largest oil spill in U.S. history. Studies were initiated in April 1989 by the Alaska Department of Fish and Game (ADF&G) to ascertain the effects of unrefined hydrocarbon contamination on selected intertidal bivalve mollusc populations throughout the affected area. The *T/V Exxon Valdez* Oil Spill (EVOS) contaminated 1,390 miles of shoreline (ADNR 1993) where populations of bivalves live (Figure 1). Bivalve populations are an important component of the food web, existing as prey for sea otters *Enhydra lutis* (Ebert 1980; Garshelis 1983), sea ducks (Patten 1991, 1992), and invertebrates (Schmidt and Warme 1969; Paul and Feder 1975; Nickerson 1977; Chew and Ma 1987; Pearson *et al.* 1981; Peterson 1982, 1983). Bivalves also support subsistence and sport fisheries in the areas affected by oil (Stratton 1990). The effects of unrefined and refined hydrocarbons on bivalves have been well documented (Dow 1975, 1978; Keck *et al.* 1978; Augenfeld *et al.* 1980; Anderson *et al.* 1982, 1983). Bivalves can be particularly susceptible to contamination from an oil spill because of their sedentary nature and their widespread abundance throughout intertidal areas (Vanderhorst and Wilkinson 1979). Mussels, oysters, and clams have been used successfully in "mussel-watch" programs to assess hydrocarbon levels in coastal areas, including Prince William Sound (Risebrough *et al.* 1983; Karinen *et al.* 1991; Rice *et al.* 1993; Short and Babcock 1996). Clams are likely to accumulate petroleum hydrocarbons because bivalves metabolize hydrocarbons at a much lower rate than finfish species (Shaw 1988) and hydrocarbon sampling of sediment or seawater alone may not indicate hydrocarbon contamination when tissue samples do (Short and Harris 1996a). It was hypothesized that hydrocarbons in intertidal areas could affect bivalve populations over a long period by affecting their ability to burrow or close their valves thereby increasing predation (Pearson *et al.* 1981) or decreasing growth (Anderson 1988; Axiak and George 1987a, 1987b; Juanes 1992). Chronic hydrocarbon contamination could also cause sublethal injuries (Carr and Reish 1978; Hartwick *et al.* 1982; Chew and Ma 1987). Perhaps the most important long-term effect on bivalves is that caused by passing on concentrated contamination to sensitive consumer species, ultimately affecting the predator's growth and survival.

In 1989, Fish/Shellfish (F/S) Study 13 was implemented to sample bivalve populations within Prince William Sound while F/S Study 21 sampled bivalve populations in Cook Inlet, the Kodiak Area and the Kenai Peninsula area. The studies were combined and expanded in 1990 under F/S Study 13. Transect sampling, targeting Pacific razor clams *Siliqua patula*, was added in 1990. An experiment was conducted in 1990 and 1991 to evaluate and compare site-specific effects on the growth of littleneck clams reciprocally transplanted between oiled and control sites in Prince William Sound.

OBJECTIVES

The major goals of this study were to document hydrocarbon contamination by the *Exxon Valdez* oil spill and determine what effects such contamination could have on selected bivalve populations. The objectives were modified during the study, primarily when F/S Studies 13 and 21 were combined (Figure 2). The objectives of this study were to:

1. Determine the level of hydrocarbons in bivalves and how levels changed over time at beach sites designated as oiled or control.

2. Determine the effects of oil contamination on vital tissues and organs of bivalves;

3. Determine and compare growth rates of bivalves at oiled and control sites;

4. Determine and compare mortality rates of bivalves at oiled and control sites;

5. Document any changes in recruitment by determining the numbers of age 0 bivalves at oiled and control sites; and

6. Identify potential alternative methods and strategies for restoration of lost use, populations, or habitat where injury was identified.

METHODS

Study Area

The sampling design incorporated 29 sites from 1989 through 1991 (Table 1). Samples were collected from 13 sites in Prince William Sound, 6 sites on the Kenai Peninsula, 4 sites in the Kodiak area, and 6 sites on the Alaska Peninsula. Weather and environmental conditions were collected at each of the sampling sites during the study (Table 2).

The site selection process for the study incorporated local knowledge of clam resources and oil stranding in addition to Department of Environmental Conservation (DEC) shoreline oiling maps and NOAA overflights. Most prospective sites were identified during helicopter overflights by concentrations of shell debris in the intertidal and subtidal zones. The locations were visited during a low tide to document the presence of clams. Sites were designated as control (unoiled) or oiled based on whether the site was in the known path of the oil spill as determined by DEC over-flights or beach walks. For sites designated as oiled, the presence of oil was verified by direct observation. "Unoiled" or control sites were located out of the known path of the oil. Selection of some sites, most notably those in the Alaska Peninsula and Kodiak area, were not

visited by project staff prior to sampling but relied solely on local knowledge and DEC oiling maps.

To our knowledge, none of the sampling sites for this study were specifically included in the beach "clean-up" activities by *Exxon* or it's contractors. Of the several thousand kilometers of shoreline in Prince William Sound, some 800 km were considered to have received sufficient oiling to require some form of shoreline "clean-up" or treatment in 1989 (Federal On-Scene Coordinator 1989). Even though our sampling sites were not included in the beach "clean-up" activities, "clean-up activities did occur in the same general areas around our sampling sites. There were studies contracted by the National Oceanic and Atmospheric Administration (NOAA) Hazardous Materials Response Branch designed to evaluate the shoreline treatments in these areas and evaluate the condition of intertidal and shallow subtidal biota in Prince William Sound following the Exxon Valdez (Houghton et. al. 1991). As part of this sampling effort, these studies looked at nine locations in Prince William Sound where bivalves or hard shell clams were found: Sheep Bay, Outside Bay, Bay of Isles, Herring Bay, Snug Harbor, Block Island, Shelter Bay, Northwest Bay-West Arm, and Ingot Island). Of these nine sampling locations, we conducted sampling in two of the same general locations: Outside Bay and Snug Harbor. Houghton *et al.* (1991) classified Outside Bay as unoiled in 1989 and Snug Harbor as oiled but untreated in 1989, while we classified both sites as oiled in our study.

Razor clam sites in Cook Inlet (control) and on the Alaska Peninsula were added to the study in 1990 in response to concerns for brown bears by other researchers. This change was to measure hydrocarbon levels in razor clams and evaluate their effect on brown bears and other upper level predators.

Transect Sampling

During 1989, littleneck clams, butter clams, and cockles were collected using transect sampling at 9 sampling sites in Prince William Sound (Table 1; Figure 3), 3 sites on the Kenai Peninsula (Figure 4), and 4 sites in the Kodiak area (Figure 4). Most sites were sampled once during the season; however, sampling was repeated twice during the season at four sites (2 control sites: Hell's Hole and Simpson Bay; and 2 oiled sites: Snug Harbor and Wilson Bay) in Prince William Sound specifically to collect hydrocarbon and necropsy samples.

Three transects were sampled at each site to insure complete coverage of the beaches as the distribution of bivalves and hydrocarbons in the substrate was unknown. Three parallel transects were laid out perpendicular to the low tide line with each transect containing seven 0.25 m^2 quadrats (Figure 5). The distance between each transect was 15 m. The top of each transect began at the +1.6 m (+5.2 ft) tide level for the Prince William Sound and Kodiak Area sampling sites. The top of transects for the Cook Inlet and outer Kenai Peninsula study sites began at the +2.4 m (+8.0 ft) tide line (Figure 5). The bottom of the transect for all sites sampled ended at the daily minimum low tide level.

Sampling was accomplished during the minimum low tide of the day to secure the greatest

number of each species of bivalve from as great a tidal range as possible. Tide height was determined using a Connex TideFinder tide and current computer. A Leitz 5X hand level and stadia rod was used to establish beach elevations. The distribution of clams or cockles was determined by removing sediment to a depth of 30 cm (12 in) from a trench next to the proposed transect. The trench was excavated beginning at the top of the transect and continuing downslope until a clam or cockle was encountered. The first sample quadrat was positioned on the transect at the height where the initial clam or cockle was located in the preliminary trench. The seventh quadrat was then established at the water's edge (the end of the transect) at the lowest tide level for the day (Figure 5). The placement of the second through sixth quadrats was based on the locations of the first and seventh quadrats. The fourth quadrat was located halfway between the first and seventh quadrats. The second quadrat was situated halfway between the first and fourth quadrats while the sixth quadrat was placed midway between the fourth and seventh quadrats. The third quadrat was positioned halfway between the second and fourth quadrats. Finally, the fifth quadrat was located midway between the fourth and sixth quadrats. Such an arrangement served to concentrate sampling effort toward the middle of the transect and maximized the number of clams collected (Paul and Feder 1973).

Once quadrat placement was established, excavation of the quadrat began. A square aluminum frame (0.5 m per side) was used as a guide to establish quadrat size. A four pronged clam rake and #2 roundnose shovel were used to remove substrate to a depth of 30 cm. Initially the upper 2 cm of substrate was removed and washed through a 1 mm mesh stainless steel screen which was supported above the ground on a frame. The balance of the substrate from the quadrat was placed on a tarp and washed through a 5 mm stainless steel screen. Seawater from a Homelite Waterbug 2-cycle pump was used to rinse the substrate and reveal the clams. Clams were placed in a 6.5 l bucket and then transferred to plastic bags that had been marked to identify the site, transect, quadrat, and date of collection. Samples were kept frozen until sampling. Clams were subsequently thawed and measured for length and weight. Total length was determined to the nearest 0.01 mm using Digi-Kanon electronic calipers measuring the paired valves at their widest point (anterior to posterior). Whole clams were weighed to the nearest 0.01 g using an Ohaus 600 g electronic scale. The clam was then shucked, all meat and muscle tissue removed, and the empty shell was weighed to the nearest 0.01 g.

In 1990, littleneck clams, butter clams, and cockles were collected using transect sampling at 10 sampling sites in Prince William Sound (Table 1; Figure 6), 6 sites on the Kenai Peninsula (Figure 7), and 2 sites in the Kodiak area (Figure 7). The number of quadrats per transect was increased from seven to eight (Figure 5). Quadrats were spaced more evenly over the entire length of the transect, by dividing the change in elevation, defined as the distance from the first clam encountered in the preliminary trench to the water's edge, by eight, which yielded the elevation increment between each quadrat. In this manner the first quadrat was located one increment below the first clam, the second quadrat two increments below, etc., with the last sampling quadrat placed at the low tide level. Sample and data collection of littleneck and butter clams in 1990 was identical to 1989.

In addition, razor clams were collected at 6 sites on the Alaska Peninsula in 1990 (Figure 8). The site configuration for razor clams consisted of a single transect running parallel to the tide line, located within the 0.0 to -0.3 m (-1 ft) tide height. Razor clams were located within the transect by

looking for a dimple in the wet sand which indicated their presence under the substrate. Razor clams were collected wherever they occurred within each transect using clam shovels, or a pump and water nozzle. Total length, whole weight, and shell weight were collected from all razor clams using the same methods described earlier for littleneck and butter clams.

Hydrocarbon Sampling

During 1989 and 1990, nine composite sediment samples were collected from each beach site (three from each transect) before bivalve sampling began. A composite sample was collected by scooping 15 cc (one tablespoon) of sediment to a depth of 2-3 cm from each sample quadrat on a transect, and placing all sediments in one pre-cleaned sample jar. The small subsamples of sediments taken from each sampling quadrat provided a representative mixture of sediment composition and contamination throughout the transect. In 1990, three composite sediment samples were taken along each razor clam beach transect, in a manner similar to that described above.

Whole clams were collected to provide tissue samples for hydrocarbon analysis. In 1989 and 1990, one composite tissue sample of each species was obtained from each transect for hydrocarbon analysis. Tissue samples from each quadrat were combined to provide a representative mixture of bivalve tissue composition and contamination throughout the transect for each species present. The desired size of each composite tissue sample was 15 g. The number of bivalves to provide this sample from each transect was estimated based on the average size of individuals of each species.

Bivalve samples were limited to a particular size range in case rates of uptake, metabolism, and depuration by clams and cockles changed with size. The first two clams with a shell length from 2-5 cm removed from a sampling quadrat were placed in the sample. Each hydrocarbon tissue sample was composed of 14-16 specimens for littleneck and butter clams. When specimens of the desired size were not found in the sampling quadrats, additional specimens were collected from other sample quadrats within the same transect.

Each hydrocarbon tissue sample of cockles was composed of six individuals. Collection was accomplished by placing the first cockle from each quadrat in the sample box then randomly selecting six to comprise the sample.

Each hydrocarbon tissue sample of razor clams in 1990 was composed of six to eight individuals with shell lengths of 2-5 cm. Razor clams were randomly collected at the beginning, middle, and end of the collection transect, for a total of three samples per site.

As part of the reciprocal transplant experiment in 1990 and 1991, four-hydrocarbon tissue samples were obtained from each sampling station; one sample from each tide height and site. Each hydrocarbon tissue sample was composed of 10-15 clams with shell lengths of 2-5 cm. These clams were collected from the donor beach trench and retained as a hydrocarbon sample at the time of transplantation. An additional 15 clams were also notched, to facilitate identification

in the fall, and buried with the tagged clams in each "A" plot. These notched clams comprised the hydrocarbon sample at the time of recovery.

Sampling procedures and quality assurance were conducted as outlined in the State/Federal Damage Assessment Plan - Analytical Chemistry - Collection and Handling of Samples document (Appendix B). After collection, samples were stored frozen until analyzed for hydrocarbons by the Geochemical and Environmental Research Group at Texas A&M University. Forty-three aromatic hydrocarbon analytes included unsubstituted and alkyl-homologs of dibenzothiophene and 2- through 5-ring polycyclic aromatic hydrocarbons (PAH). Twenty-five alkane analytes included normal alkane containing 10 through 34 carbon atoms, and two branched alkanes pristane and phytane. Hydrocarbons were extracted from sediments or tissues with dichloromethane, and PAH were separated from alkanes by silica gel chromatography after solvent exchange into hexane. Alkanes and PAH were each further separated and measured by gas chromatography equipped with a flame ionization detector or by a mass-selective detector, respectively. The detection limits of measured PAH classes was about 5 ng/g dry weight in tissues, and about 1 ng/g dry weight in mussels. The amount of PAH in a sample is summarized as total PAH (TPAH), which is the sum of all the PAH isomer detected except perylene, a common diagenic PAH in Prince William Sound marine sediments. A more detailed summary of the analytical method and its accuracy, precision, and detection limits is given in Short *et al.* 1996.

The presence of Exxon Valdez oil (EVO) in samples was evaluated following the identification procedure presented by Short and Heintz 1997. This model evaluates the alternative probabilities that the PAH found in a sample is consistent with weathered EVO, or with the regional PAH background. When EVO is identified, the weathering model also provides a quantitative index of weathering denoted as *w*. A value of zero for *w* indicates unweathered EVO, and more positive values indicate progressively more weathered oil, with values greater than 10 indicative of very weathered oil.

The regional PAH background probably derives from coal eroded from terrestrial deposits at the Bering River coal field and eastwards, and transported into Prince William Sound by the Alaska Coastal Current (Short *et al.* 1999). PAH from this source are not bioavailable. The weathering model requires the simultaneous detection of 14 selected PAH classes above the method detection limit to assign probabilities, and hence cannot be used for samples with low PAH concentrations. Patterns of relative PAH abundances that were not consistent with either of these two sources were usually either dominated by naphthalenes (which may be an artifact of sample collection, storage, or analysis) or else consisted of sporadically detected PAH at concentrations that were too low to assess likely sources. Diesel oil contamination, probably during collection, was evident in a very few tissue samples as evidenced by high abundances of 2- and 3-ring PAH but low abundances of chrysenes.

Histopathology Sampling

Collection of specimens for histopathological analysis was similar to that used for hydrocarbon sampling in 1989 and 1990. A single sample of 20 live or moribund specimens of each species was collected from each beach site. This sample size should allow detection of differences in presence of tissue damage between samples obtained from beaches with different levels of oil impact (Dr. Theodore R. Meyers, Alaska Department of Fish and Game, CFMD Division, Pathology, Juneau, AK; personal communication).

Specimens were collected as they were recovered at each quadrat and placed in a wooden box. Once the samples were removed from the beach, each clam was measured, shucked, and the meat of the animal placed in a tissue cassette or wrapped in gauze and immersed in 10% buffered formalin. Upon returning from the field or after no more than 48 hours, the formalin was poured off and each sample was preserved in a 70% alcohol solution for long-term storage. Sampling procedures and quality assurance were conducted as outlined in the SOP for histologic sample preparation for bivalve mollusks (Appendix C). Histopathological examination of bivalve tissues included all criteria listed in the histopathology guidelines. Dr. Albert K. Sparks, Seattle, Washington, conducted necropsies.

Upon completion of histopathological examinations, data forms were filled out for each sample and sent to ADF&G for data entry and summary. Results were summarized based on one of six histopathological conditions as recommended by Dr. Gary Marty of the University of California, Davis. These histopathological conditions were (1) normal, (2) inflammatory reaction, (3) degenerative change, (4) expansive and non-neoplastic change, (5) obstructive and displacement change and (6) parasites.

Reciprocal Transplant of Littleneck Clams

A reciprocal transplant experiment was initiated in 1990 to determine the effects of the EVOS on growth of littleneck clams. This experiment was to determine if growth was more affected by the donor site (where the clam was before transplanting) or by the host site (where the clam was transplanted to). The experiment involved reciprocally transplanting littleneck clams between 3 control and 3 oiled sites in Prince William Sound in May 1990 and recapturing the clams in September 1990 (Figure 9). This period was thought to encompass most of the growing season of bivalves in the area (Nickerson 1977). Sampling, clam tagging and collection methodology were identical to the transplant experiment conducted in 1990 except that tagging in 1991 occurred in April instead of May and recovery took place from late August through early September.

The criteria used for selecting paired oiled/control beaches, to the extent possible, included similarity in profile, drainage and length-frequency distribution of bivalves. Two tide heights were selected, +0.45 m (+1.5 ft) and +0.90 m (+3.0 ft); each of which had yielded an adequate number of specimens during transect sampling in 1989. Standard operating procedures (SOP) for collecting and tagging littleneck clams during the reciprocal transplant experiment were

developed in 1990 and used in 1991 (Appendix A).

Clams were transplanted to the same tidal height from which they originated. Three sampling stations were established at each tidal height for a beach and comprised a site location. Each station consisted of three adjacent 0.25 m^2 quadrats placed 2 m apart and identified as plots "A", "B", and "C" (Figure 10). All plots were marked with a small duckbill anchor that was driven to a depth of 0.6 m into the upper right hand corner of each plot. A small gill net float was secured to the free end of each anchor to facilitate locating individual plots at the end of the experiment.

The transplant was accomplished by visiting a site and collecting 210 clams from a trench at each specified tide height. This number provided 70 clams per quadrat (50 for tagging and transplanting and 20 for subsequent hydrocarbon and histopathology samples at the time of recovery). A size range of 15 mm to 35 mm clam length was employed because 15 mm was considered the smallest size that could effectively be tagged while clams less than 35 mm were selected to narrow the range of ages for which differences in growth were being determined. Maximum growth appears to occur within this size range (Glude 1978).

All clams to be transplanted had a notch (2-3 mm in length) filed out to the margin of each valve. Total length (anterior to posterior) and whole weight data were collected from each clam that was tagged. Clams were tagged sequentially in lots of 50, employing an individually numbered laminated-plastic Floy (Floy Tag &Manufacturing Inc., Seattle, WA.) tag secured with a quick-drying cyanoacrylate adhesive. After preparation, transplant clams were held in buckets for transport. Each bucket held clams for one quadrat or 50 tagged and notched individuals and 20 notched individuals.

Upon arrival at the transplant site, transect and quadrat locations were established. Each "A" plot was excavated to a depth of 30 cm and all clams of the proper size were set aside for transplant or collected to comprise a hydrocarbon sample representative of contamination at the time of transplant. If the required number of clams (210) for reciprocal transplant was not obtained during quadrat excavations, additional specimens were collected from locations adjacent to the plots.

Each "B" plot was also excavated to a depth of 30 cm and all littleneck clams and sediment were removed. Clams from these plots were marked by filing a small notch into the ventral margin of each valve to indicate size at the time of transplantation. The "B" plot clams were not individually tagged. Marking the "B" plot clams in this manner made it possible to distinguish clams excavated in the spring from any clams that may have immigrated into the plot during the summer. All "B" plot clams and sediments were then returned to the plot whence they came. Each "C" plot was located but not disturbed until recovery in the fall.

At recovery in September 1990 each plot was excavated and clams removed. Notched clams from "A" plot comprised hydrocarbon and histopathology samples. Tagged clams from each "A" plot, notched clams from each "B" plot, and all clams from each "C" plot were collected and frozen. Clams were latter thawed and sampled in the lab. Total length was determined to the nearest 0.01 mm using Digi-Kanon electronic calipers measuring the paired valves at their widest point (anterior to posterior). Whole clams were weighed to the nearest 0.01 g using an Ohaus 600

g electronic scale. The clam was then shucked, all meat and muscle tissue removed, and the empty shell weighed to the nearest 0.01 g.

As part of the reciprocal transplant study, a collection of specimens for histopathological analysis followed hydrocarbon sampling. When "A" plot clams were originally tagged, additional clams were collected from the donor site trench at each tide height. These clams were notched and included with the tagged clams in "A" plot at the receptor site. At time of recovery in the fall, five clams per each "A" plot were retained for histopathological analysis.

Aging of Bivalves

Clam age was determined by counting annuli: the series of closely spaced concentric growth rings found on the external surface of the valve that were the result of the slow winter shell growth (Paul and Feder 1976). Personnel at the University of Alaska, Institute of Marine Science (IMS) in Seward, Alaska, did all clam aging. Care was taken by the ager to ensure that each annulus was a distinct line or ridge that originated and terminated near the umbo. Size at age was determined by measuring the shell length at each annulus. An effort was made to compare both valves, when possible, since annular lines should be the same on both valves of an individual.

In instances where the first few annuli were abraded or not apparent, size at age data from younger clams (with more distinct annuli) from that site were used to establish the probable location of the first annuli. When no distinct annuli were visible, lengths at those ages were not recorded. Ages were assigned based on the number of distinct annuli observed. Zero age clams were defined as those individuals that had undergone a single full growing season (May-October) and a full winter period (November-March) that had resulted in the formation of one annulus. Using this convention, any age-0 clam could be nine to 19 months old depending on the time of spawning (March-September), larval settlement (usually 3 weeks after spawning), and collection (April-September).

Originally all clams collected were to be aged. However, in 1989 only littleneck clams were aged for use in growth estimations. This was due to the low number of other bivalve species collected. In 1990, butter, littleneck, and razor clams were collected for age and growth analysis, but razor clams were not aged because hydrocarbon sampling at the razor clam sites did not show contamination by unrefined hydrocarbons. Littleneck clams were the only species of bivalve collected in 1991. The tagged clams used in the reciprocal transplant experiment were measured at the beginning and end of the experiment to determine growth rates over time. Clams submitted to IMS for aging were assigned a unique number that was inscribed on the inside of each shell using a permanent marker.

A contract was initiated with the Washington Department of Fisheries (WDF), for microstructure analysis of littleneck clam valves in 1990. The microstructure aging method involved microscopic examination of the sectioned valve. Ages were assigned by counting the presumptive annuli, which appear as dark, narrow colorations clearly differentiated from the broad, white growth bands. This alternative method of aging was employed to confirm the external aging technique

and to ascertain if a detectable interruption in growth or "disturbance check" attributable to the EVOS could be detected. Several investigators have verified the daily deposition of individual micro-incremental patterns in quahog *Mercenaria sp.* valves due to storms and heated discharges from nuclear power plants (Kennish and Olsson 1975; Lutz and Rhoads 1981).

A random sample of 600 clams collected from six transect sampling sites (Double Bay, Gibbon Anchorage, Hell's Hole, Horseshoe Bay, and Wilson Bay) was submitted for aging to WDF after being aged at IMS. Each clam had a unique number written on the inside of each valve to facilitate a direct comparison of the two aging methods.

Specimens were prepared according to thin-sectioning methods described by Clark (1981). For clams greater than 10 mm in length, the left valve was sectioned along the maximum growth axis running from the umbo to the ventral margin of the valve. On specimens smaller than 10 mm, the entire valve interior was filled with two-part epoxy to simplify handling and then sectioned as a unit along the same axis as previously described. Sectioning was accomplished using a table saw equipped with an Accutone 2 precision diamond cut off blade. When the left valve was unavailable or destroyed in preparation, the right valve was used.

After sectioning, one section of the valve was polished on a lap wheel to obtain a flat surface. The valve section was allowed to dry and then glued to a glass slide and reinforced with Cytoseal 280. The slide was completely dried and then fixed to the diamond saw. Using the saw, cross-sections, approximately 500 microns thick, were prepared. The prepared sections were viewed under a stereomicroscope at 8x power with reflected light source. The microscope was equipped with a video camera interfaced with a computer frame grabber. The image was displayed on a monitor and then frozen so that measurements could be made (Volk *et al.* 1991).

After ages had been determined from the sectioned valve, a subsample of 90 clams was selected for examination of the hinge tooth. The hinge tooth was examined for the presence of a "disturbance check" and to provide a comparison of ages determined by two different methods. The hinge tooth was also thin sectioned, viewed at 30x magnification using a stereomicroscope and reflected light and presumptive annuli identified. The hinge tooth section was measured along a curved axis that traveled from the tip of the umbo, at the same origin as valve measurements, running along the maximum growth axis of the element to its edge. Presumptive annuli were represented as obviously dark bands observed in the tooth (Volk *et al.* 1991).

DATA ANALYSIS

Histopathological Analysis of Littleneck Clams

Three analyses were completed to analyze clam histopathology data: (1) weighted analysis of variance, where the inverse of the variance of the dependent variable was used to weight the analysis; (2) weighted analysis of variance with an arcsin square-root transformation; and (3) multinomial analysis of variance. The dependent variable in these analyses was a function of the

proportion of normal clams. The proportions were generated for each site (pooled over sampling dates), year, area (Prince William Sound, Cook Inlet, and Kodiak Island) and species. Each proportion was the number of clams classified as normal divided by the total number of clams examined. Separate analyses were performed for each species, area and year. The assumptions for the multinomial analysis of variance required that the response of the subject can be classified into one and only one category (either normal or abnormal) and the response of one subject does not affect the response of any other subject.

Growth Analysis of Littleneck Clams

One objective of this study was to compare the growth of littleneck clams at control and oiled sites within Prince William Sound to determine whether petroleum hydrocarbons from the *Exxon Valdez* affected the growth of littleneck clams. The completed growth analyses were split into two major components: (1) growth from transect sampling, and (2) growth from reciprocal transplant experiment.

Growth from Transect Sampling

Growth of littleneck clams collected during transect sampling was back-calculated for one year from 1989 to 1990 by subtracting the length at age of a clam age i in 1989 from the size at age of the same clam at age i+1 in 1990. The size at age (mm) of a clam was determined by measuring the maximum length at each annulus anterior to posterior.

Analysis of growth for littleneck clams from 1989 to 1990 was based on a fixed unbalanced nested analysis of covariance (ANCOVA) model:

$$\mathbf{Y_{ijk} = m + A_i \mid B(X_{1ij})(A_i) \mid C_j \mid D(X_{2ijk}) + e_{ijk}} \qquad \mathbf{(1)}$$

where Y_{ijk} = growth of the kth littleneck clam at the ith location and jth tide level, m = grand mean, A_i = the fixed effect of the ith location, $B(X_{1ij})$ = the hydrocarbon level at the ith location (site) and the jth tide height, C_j = the fixed effect of the jth tide level, $D(X_{2ijk})$ = the length of the kth littleneck clam at the ith location and jth tide level, and e_{ijk} = the error. The bracket (|) between factors indicates that all individual factors and covariates, and all possible interactions were included in the model. There were 10 sampling locations (sites) and 4 tide levels (-0.3, 0.0, 0.3, and 0.6 m). Three measures of hydrocarbon levels were used in separate analyses. They included the total aromatic hydrocarbons (ng/g dry weight) measured in (1) clam tissues collected during transect sampling in spring of 1989, and (2) 1990, and in (3) clam tissues in spring 1990.

Growth from Reciprocal Transplant Experiment

Analysis of growth for littleneck clams during 1990 and 1991 were based on an unbalanced ANCOVA model:

$$\mathbf{Y_{ijkl} = m + A_i + B_j + C_k + D(X_{ijkl}) + e_{ijkl}} \quad \textbf{(2)}$$

where Y_{ijkl} = growth of the lth littleneck clam in the ith year, with the jth clam type, and at the kth tide level, m = grand mean, A_i = the fixed effect the year the clams were collected (1990 or 1991), B_j = the clam type ("A" = tagged, notched and transplanted clams from "A" plots or "B" = notched clams from "B" plots), C_k = the tide level (0.45 m or 0.90 m), $D(X_{ijkl})$ = the length of the lth littleneck clam in the ith year, with the jth clam type, and at the kth tide level, and e_{ijkl} = error.

The use of ANCOVA models assumed that growth was normally distributed. Any deviation from normality may have caused problems in the interpretation of the analyses. Because of this, the growth data were tested using the Shapiro-Wilk or the Kolmogorov D statistics from the univariate procedure in SAS (SAS 1987). The SAS univariate procedure uses the Shapiro-Wilk statistic when sample sizes are less or equal to 2,000 and the Kolmogorov D statistic when sample sizes are greater than 2,000.

The ANCOVA models were run using the general linear model (GLM) procedure in SAS (SAS 1987). The significance of effects in the ANCOVA models was tested using the type III sums of squares. The models were unbalanced with missing values in the growth data sets. All effects with probabilities less than 0.05 ($P < 0.05$) were considered significant.

Recruitment Analysis of Littleneck Clams

Recruitment of littleneck clams collected by transect sampling was estimated by two methods. Pure recruitment, which is the average number of 1 year olds in 1990, and recruitment normalized by site specific recruitment in three previous year classes, which is the average ratio of 1990 1-year olds to 1990 1-, 2-, and 3-year olds pooled. The differences in recruitment for the two methods were tested using a Student-t test.

RESULTS

Transect Sampling

All four species of bivalve were never collected at the same site. We believe this was due to differences in habitat preference. Littleneck clams were encountered more often and in greater numbers than any other clams. Butter clams collected in 1989 and 1990 were found in association

with littleneck clams, but in lower numbers. Cockles were also found in association with littleneck and butter clams but because of the low numbers found in 1989, sampling for this species did not continue in 1990. Razor clams were collected only in 1990, from the Alaska Peninsula.

A total of 2,181 littleneck clams was sampled in Prince William Sound in 1989, ranging in mean length from 18.8 mm in Outside Bay to 25.7 mm in Pellew Cove (Table 3). Length frequency distributions for Ellamar and Gibbon Anchorage were unimodal, while the other sites were multimodal (Figures 11-13). Only 332 butter clams and seven cockles were collected in 1989 in Prince William Sound (Table 3).

The collection of littleneck clams from sites outside Prince William Sound during 1989 was nearly as successful as sampling in Prince William Sound. A total of 2,104 littleneck clams from the Kenai Peninsula and Kodiak Area was collected during transect sampling in 1989 (Table 4). Length frequency distributions took on a variety of shapes from a unimodal distribution in Port Dick, to a multimodal or almost flat distribution at the other sites (Figures 14 and 15). Four hundred forty-four butter clams were collected at these sites in 1989. Sample sizes were not large enough to discern any distributions from length frequencies of butter clams (Figure 16).

A total of 3,923 littleneck clams was sampled in Prince William Sound in 1990 ranging in mean length from 13.4 mm at Horseshoe Bay to 23.5 mm at Double Bay (Table 5). Length frequency distributions for each site varied from unimodal at Hell's Hole and Horseshoe Bay to multimodal at most of the other sites (Figures 17 and 18). Length at age seemed to be skewed toward the smaller sizes (< 25 mm) except at Green Island and Pellew Cove.

Sampling for both littleneck and butter clams was more successful outside Prince William Sound during 1990. The total number of littleneck clams collected from the Kenai Peninsula and Kodiak Area was 2,665 and 475, respectively (Table 6). Butter clams from these locations numbered 288 and 387, respectively. Length frequency distributions of littleneck clams collected outside of Prince William Sound were generally multimodal at all sites (Figures 19 and 20). Razor clams collected from the East Side of the Alaska Peninsula numbered 1,812. Average size ranged from 73.3 mm at Hallo Bay to 105.8 mm at Crescent River (Table 6).

Hydrocarbons

Four hundred fifty-seven sediment samples (188 in 1989, 197 in 1990, and 72 in 1991) were collected and submitted for hydrocarbon analysis (Table 7). Twelve sediment samples from 1989 and 90 sediment samples from 1990 were analyzed. Three hundred ninety-six clam tissue samples were collected and submitted for hydrocarbon analysis (Table 8). Analysis was completed on 135 tissue samples from 1989, 113 tissue samples from 1990 and 26 tissue samples from 1991. Two sediment samples were compromised following collection (Jeff Short, NOAA/NMFS, Auke Bay, Alaska, personal communication) and were not used in any analyses in the study.

Hydrocarbons in Sediments

At the 4 sites examined in 1989, *Exxon Valdez* oil (EVO) was evident only at the Fox Farm site, where TPAH concentrations ranged from 289 to 10,034 ng/g (dry weight basis) among the sample replicates (compare Figures 21 and 22). The pattern of PAH abundances was not significantly different from weathered EVO in these replicates, and was not very weathered with $1 < w < 3$. Although 1 or 2 PAH analytes were below detection limits in the Simpson Bay samples, the pattern of remaining PAH abundances was consistent with the most abundant PAH characterizing the regional coal background pattern (compare Figures 23 and 24), at TPAH concentrations ranging from 1,082 to 1,097 ng/g. TPAH concentrations ranged from 484 to 1,373 at Jakalof Bay but sources were not clear. The prevalence of un-substituted homologues suggests a contribution from combustion products, which is probably augmented by PAH from other unknown sources. At Windy Bay, TPAH concentrations ranged from 101 to 284 and were too low to determine sources.

Samples from 10 sites were analyzed in 1990 and results corroborate those from 1989. EVO was consistently identified in all 9 replicate samples collected from Snug Harbor, at TPAH concentrations ranging from 345 to 2,067 ng/g. EVO was found more sporadically among replicate samples collected from Chenega Island, Gibbon Anchorage, Green Island, and Wilson Bay, where TPAH concentrations ranged from 24 to 818 ng/g. At all these sites, detected EVO was moderately weathered, with $2 < w < 6.5$. Regional backgrounds PAH from coal were found at Double Bay (compare Figures 23 and 24) at TPAH concentrations ranging from 136 to 318 ng/g among 9 replicates. At Simpson Bay, coal background PAH consistently accounted for about 1,000 ng/g of the TPAH in sediment replicates, and these were sporadically augmented by a suite of alkyl-naphthalenes (indicative of gasoline) to TPAH concentrations as high as 5,091. Elsewhere, TPAH ranged from 12 to 169 ng/g and were too low to determine sources.

Hydrocarbons in Bivalve Tissues

Exxon Valdez oil was only evident in bivalves from oiled sites in 1989. The sites where EVO was confirmed in bivalves include Fox Farm, Gibbon Anchorage, Outside Bay, Snug Harbor, Windy Bay, and Wilson Bay. Detected EVO in these bivalves was not very weathered, with $0.8 < w < 3.6$ (compare Figures 22 and 25). Concentrations of TPAH were highest in Snug Harbor bivalves, ranging from 4,100 to 34,357 ng/g (dry weight), followed by Fox Farm and Outside Bay, where concentrations ranged from 4,190 to 10,100 ng/g. Concentrations of TPAH associated with EVO in bivalves were less than 4,000 ng/g at the other two sites. The relatively low abundance of chrysenes found for some of the samples collected from Gibbon Anchorage, Windy Bay and Wilson Bay suggest diesel oil contamination, which may have been introduced during sample collection. Concentrations of TPAH in these samples were less than 2,900 ng/g.

Concentrations of TPAH in bivalves were much lower at the other sites in 1989, and usually consisted of alkylnaphthalenes or just naphthalene alone. The highest TPAH concentration in

bivalves collected in 1989 is 681 ng/g, and 90% contained less than 300 ng/g. All 30 razor clam samples from 1989 are included in this category.

Exxon Valdez oil was tentatively detected in only three bivalve samples in 1990, two from Tonsina Bay and one from Chenega Island. The detected EVO was too weathered to permit application of the identification model, but the relative abundances of the remaining PAH were consistent with extremely weathered EVO. Concentrations of TPAH in these 3 samples ranged from 630 to 1230 ng/g.

Apart from a few samples contaminated by diesel oil or possibly combustion sources, the remaining bivalve samples collected in 1990 or 1991 contained low TPAH concentrations consisting mostly of alkylnaphthalenes or just naphthalene alone. Four samples appeared to be contaminated with diesel oil at TPAH concentrations ranging from 1,400 to 7,820 ng/g, and one sample contained an apparent combustion PAH profile at a TPAH concentration of 5,890. The highest TPAH concentration of the remaining 131 samples was 615 (all naphthalene), and 90% contained concentrations less than 300 ng/g (usually just naphthalene). These last bivalve samples included the littleneck clams involved in the 1990 reciprocal transplant experiment, and all 6 razor clam samples collected in 1990.

Histopathology of Bivalves

Histopathology examination was completed on 40 samples containing 568 bivalves from 21 sites in Prince William Sound, Kodiak Island Area, outer Kenai Peninsula and Cook Inlet (Table 9). Dr. A. Sparks examined the data and concluded "patterns of pathological changes consistent with chemical injury were revealed only in samples from Jakolof Bay (control site) in which 9 of 22 clams were diagnosed as having damage of the epidermis and gills". A copy of the report submitted by Dr. Sparks has been included in this report as Appendix F.

Dr. Sparks also identified parasites in the samples typical of those found in clams along the Pacific Coast except that the tetraphyllidian cestode *Echeneibothrium* sp. reported from littleneck clams and gaper clams in California was not observed. This is probably because the most likely final host, the bat stingray *Myliobatus californica*, was not present in the study area. The occurrence of the coccidian *Pseudoklossia sp.* in 17 (2.9%) of the molluscs is interesting because it has been reported previously only from Washington on the Pacific Coast.

Most (67.8%) of the mollusks examined were within normal limits histopathologically. Infectious agents present were often "spotty" in distribution, occurring at relatively high levels in some samples and absent in others. This is not unusual in parasitic diseases and common in highly contagious infectious diseases such as viruses and bacteria.

Three analyses of the histopathological data were performed for each species, area (Prince William Sound, Cook Inlet, and Kodiak Island) and year. We presented the p values (for H_0: No oiling effect), least square means (proportion of normal clams) and residual plot (OK-acceptable, poor-unacceptable, na) for each analysis to allow a thorough examination of the analyses.

Histopathology of Littleneck Clams

Few of the 73 littleneck clams analyzed from control sites sampled in 1989 showed histologic lesions (Table 10). Three clams had moderate to severe damage to either the kidney, ovary, or gills; causation was generally idiopathic. Six other clams were minimally infected by holotrichous ciliates or an unidentified arthropod parasite.

All three analyses of histopathological data collected in 1989 from littleneck clams in Prince William Sound were not significant: weighted anova (P=0.71); weighted anova with arcsin transformation (P=0.24); and multinomial anova (P=0.34). The least square means for the proportion of normal clams from the weighted anova were 0.147 for oiled sites and 0.173 for control sites. Examination of the residuals from the weighted anova were acceptable but were unacceptable for the arcsin transformation; indicating that the homogenous variance assumption had been violated for the arcsin transformed analysis.

There was a decrease in the proportion of normal littleneck clams collected at both control and oiled sites in Prince William Sound from 1989 to 1990 (NS). At control sites, the proportion of normal clams went from 80.8% in 1989 to 42.2% in 1990. This compared to 82.4% normal clams at oiled sites in 1989 to 63.4% in 1990. In contrast, there was an increase in the proportion of clams infested with parasites at both control and oiled sites in Prince William Sound from 1989 to 1990 (NS). Clams infested with parasites at control sites increased from 13.7% in 1989 to 39.1% in 1990. This compared to an increase from 3.7% in 1989 to 29.3% in 1990 at oiled sites.

The gills were the most affected organs, followed by the kidney, ovary, and foot; idiopathic degeneration was characterized as moderate to severe. The gills of these clams were also heavily parasitized by *Nematopsis sp.*. Gill lamellae infestation by unidentified protozoans or holotrichous ciliates was also observed. The kidneys of two clams from Gibbon Anchorage were infested with an unidentified coccidia and *Pseudoklossia sp.*. *Pseudoklossia sp.* was also found in the gill lamellae of a clam from Wilson Bay. Clams from North Chenega, Green Island, and Snug Harbor were also parasitized.

Parasitism was the most common lesion in littleneck clams from control sites in Prince William Sound sampled in 1990. *Nematopsis sp.* was the most prevalent, however, some had holotrichous ciliates, and one clam from Hell's hole had a digenetic trematode. The gill was the most commonly parasitized organ, but parasites were also in the connective tissues of the mantle and epithelium of the kidney.

Twenty-one percent (n=13) of the littleneck clams sampled in 1990 from three oiled sites in Cook Inlet and the outer Kenai Peninsula had moderate to excessive degeneration, inflammation, or parasitism. Two clams from Port Dick had tissue degeneration of the gill or mantle. Either Nematopsis sp., holotrichous ciliates, or an unidentified coccidia parasitized two clams (one from Tonsina, one from Windy Bay).

No difference in the occurrence of histopathological abnormalities was detected for data collected in 1990 from littleneck clams in Prince William Sound: weighted anova (P=0.126); weighted anova with arcsin transformation (P=0.086); and multinomial anova (P=0.85). The least square

means from the weighted anova were 0.251 for oiled sites and 0.543 for control sites. Examination of the residuals from the weighted anova and arcsin transformation indicated that the homogenous variance assumption had been violated.

Of sixty-four littleneck clams from three control sites in Cook Inlet, lesions included moderate to severe degeneration of the gill in two clams, excessive renal inflammation in one clam, and mild to moderate parasitism. Eight clams (from Seldovia and Jakolof bays) and nine clams (from Tutka Bay) were infected by parasites. *Nematopsis sp.* infestation of the gills predominated.

Histopathological abnormalities in littleneck clams were significantly greater in oiled areas in 1990 in Cook Inlet: weighted anova (P=0.001); weighted anova with arcsin transformation (P=0.002); and multinomial anova (P=0.001). The least square means of the proportion of normal clams from the weighted anova were 0.086 for oiled sites and 0.568 for control sites. Examination of the residuals from the weighted anova and arcsin transformation indicated that the homogenous variance assumption had been violated.

Littleneck clams collected in 1990 from Port Bailey, a control site in the Kodiak Area, lacked any degeneration or inflammatory lesions. Parasites were found in more than 80% of the clam tissues examined. Moderate to severe *Nematopsis sp.* infestations of the gills were the most common. Holotrichous ciliates, Pseudoklossia sp., an unidentified coccidia, and an unidentified protozoan infected other organ systems. Eleven of the thirteen littleneck clams from Kupreanof Strait (an oiled site in the Kodiak Island Area) lacked any notable histopathological conditions. However, one clam was moderately parasitized by coccidia and spent testes were noted in another.

Histopathology of Butter clams

The majority of butter clams examined in 1989 at control sites (62.5%) and oiled sites (81.3%) was normal (NS; Table 11). We did observe a drop in the proportion of normal clams in 1990 at both control sites (44.0%) and oiled sites (72.4%). Histopathologic analysis revealed that butter clam tissues had parasitic infestations similar to those observed in littleneck clams. *Nematopsis sp.*, *Mytilicola sp.*, or an unknown arthropod parasite infected either the gill or intestine.

Three butter clams from oiled Prince William Sound sites sampled in 1990 had moderate to severe tissue degeneration of the gill, stomach, and digestive gland. Nine clams from four sites were infested by parasites. *Nematopsis sp.* was found in 80% of the clam tissues infested with parasites. An unknown arthropod parasite was in the intestinal lumen of a single clam from Snug harbor. The gill was the most frequently affected organ, although parasites were also found in the kidney.

No difference in the occurrence of histopathological abnormalities was detected for data collected in 1989 from butter clams in Prince William Sound: weighted anova (P=0.34); arcsin transformation (P=0.32); and multinomial anova (P=0.20). The least square means of the proportion of normal clams from the weighted anova were 0.116 for oiled sites and 0.349 for control sites. Residuals were not examined for the weighted anova and arcsin transformation.

No difference in the occurrence of histopathological abnormalities was detected for data collected in 1990 from butter clams in Prince William Sound: weighted anova ($P=0.09$); arcsin transformation ($P=0.07$); and multinomial anova ($P=0.10$). The least square means of the proportion of normal clams from the weighted anova were 0.300 for oiled sites and 0.673 for control sites. Examination of the residuals from the weighted anova were acceptable, but indicated that the homogenous variance assumption had been violated for the arcsin transformation.

Growth of Littleneck Clams from Transect Sampling

Growth of littleneck clams by tide height fluctuated between transect sampling locations in Prince William Sound (Figure 26). Growth data collected during transect sampling were tested for normality using the Kolmogorov D Statistic from the univariate procedure in SAS (SAS 1987) and growth was found to be approximately normal ($n=3,899$; $D=0.048$; probability of a greater $D<0.01$).

Growth of littleneck clams was found to vary with the level of aromatic hydrocarbons at transect sampling sites in Prince William Sound in 1990. The effects of hydrocarbons on growth of littleneck clams were tested using three ANCOVA models (Equation 1). The three full ANCOVA models used the mean level of aromatic hydrocarbons in clam tissue samples in 1989 and 1990 and sediment samples in 1990 as covariates (Tables 12-14). All three models were highly significant ($P<0.0001$) with the level of aromatic hydrocarbon having a significant effect on growth ($P\leq0.015$ for all three models). Growth was found to decrease as aromatic hydrocarbons increased. The models accounted for 9% to 26% of the variability.

There was indication that two of the sites in the study, Simpson and Double bays, had been contaminated by refined petroleum hydrocarbons. Because of this, littleneck clams collected at these locations were removed from the transect growth data and the three ANCOVA models were used to test for differences due to just *Exxon Valdez* oil and not all petroleum hydrocarbons.

The three full ANCOVA models again used the mean level of aromatic hydrocarbons in clam tissue samples in 1989 and 1990 and sediment samples in 1990 as covariates (Tables 15-17). All three models were highly significant ($P<0.0001$) with the level of aromatic hydrocarbon attributed to the *Exxon Valdez* having a significant effect on growth ($P\leq0.06$ for all three models). Growth was found to decrease as aromatic hydrocarbons increased. The models accounted 19% up to 25% of the variability.

Of the 1,799 littleneck clams tagged and reciprocally transplanted between oiled and control sites in the spring of 1990 ("A" plot), 92% were recovered in the fall of 1990 (Table 18). Of the 707 littleneck clams notched and replanted at the same sites ("B" plot) in the spring of 1990, only 76% were recovered in the fall. The number of clams notched and recovered ranged from 66 clams notched with 85% recovered at Horseshoe Bay to 205 clams notched and 73% recovered at Double Bay. The number of days between the time tagged and recovered ranged from 106 to 121 days, averaging 111 days in 1990. We also collected clams that were dug up and then placed back in the same location ("C" plot). However, we did not analyze "C" plot clams because there was no way to identify which clams had been dug up at the time of transplantation and which were dug up for the first time.

In the spring of 1991, 1,799 littleneck clams were tagged and transplanted between oiled and control sites with 72% recovered in the fall (Table 19). One thousand six hundred ninety-four littleneck clams were notched and replanted at the same sites in the spring of 1991. Of these, 62% were recovered in the fall of 1991. The number of clams notched and recovered across sites was similar except for Wilson Bay, which had a lower recovery rate (44%). The number of days between tagging and recovery ranged from 134 to 145 days, averaging 140 days in 1991 (Table 19).

Growth of clams that were notched, individually tagged and transplanted ("A" plot) was estimated by subtracting the length of each clam at the time of tagging from the length of each clam at the time of recovery. The growth of clams that were notched and replanted ("B" plot) was calculated by subtracting the length of each clam as measured from the notch at the time of notching from the total length of each clam at the time of recovery. Different methods were used to calculate growth because "B" plot clams were only notched and not individually tagged while "A" plot clams were individually tagged. Because different methods were used to calculate the growth of "A" and "B" plot clams, growth was also calculated for 90 "A" plot clams from the notch length providing a comparison of growth using the two methods. A linear regression was developed and the growth of the "B" plot clams was adjusted using the following relationship:

(3) $\text{Growth}_i = \text{ngrowth}_i + 0.0045 - 0.012(\text{total length}_i)$

where Growth_i = adjusted growth of the ith "B" plot clam, ngrowth_i = growth of the ith "B" plot clam using notching, and total length_i = total length of the ith "B" plot clam at the time of recovery. The relationship was significant between the two methods ($R^2 = 0.90$, $n = 90$). The linear regression relationship showed that the difference in growth between the two methods increased with the length of the clam.

The reciprocal transplant growth data for 1990 and 1991 were tested for normality using the Kolmogorov D Statistic from the univariate procedure in SAS (SAS 1987). The growth data from both 1990 and 1991 were approximately normal (1990; n=2,151; D=0.07; probability of a greater D<0.01: 1991; n=2,270; D=0.08; probability of a greater D<0.01).

Growth was variable between year, clam types, and tide level (Figure 27). The effect of hydrocarbons on growth of littleneck clams was tested along side the effects of year, clam type, and tide level using two ANCOVA models (Equation 2). The two full ANCOVA models used the mean level of aromatic hydrocarbons in clam tissue samples collected in the spring of each year at tagging and recovery locations as covariates. The two models were highly significant ($P<0.0001$; Tables 20 and 21). The models accounted for approximately 25% of the variability. The level of aromatic hydrocarbons measured at the tagging sites had a significant effect on growth ($P<0.0002$; Table 20) while the level of hydrocarbons measured at the recovery locations was not significant by itself ($P=0.4307$; Table 21). However, the interactions of the level of aromatic hydrocarbons at recovery locations and year, type, and tide level were significant.

Pairwise comparisons of least squares means were used to test for differences in growth by year, clam type, and tide level. Growth for both "A" and "B" plot littleneck clams at -0.90 and -0.45 m tide levels was significantly faster in 1990 than in 1991 ($P<0.0001$; Table 22). The mean difference in least squares growth between years was 1.63 mm. The "A" plot clams grew significantly slower than "B" plot clams in both 1990 and 1991 at -0.90 and -0.45 m tide levels ($P<0.0001$). The mean difference in least squares growth between clam types was -2.02 mm. Littleneck clams at -0.90 m tide level grew significantly slower than clams at -0.45 m tide level for both "A" and "B" plot clams in 1990 and "B" plot clams in 1991 ($P<0.0001$). Even though growth was not significantly different between -0.90 and -0.45 m tide levels for "A" plot clams in 1990 ($P<0.0564$), the p-value was marginally not significant and the difference in least squares growth between tide levels was -0.50 mm.

A comparison of adjusted least squares means using the mean level of aromatic hydrocarbons at recovery locations again showed significant differences in growth of clams in 1990 and 1991 by clam type and tide level. Littleneck clams grew significantly faster in 1990 than in 1991 for "A" plot clams at -0.90 m tide level and "B" plot clams at -0.90 and -0.45 m tide levels ($P<0.0001$; Table 23). However, growth of "A" plot clams at -0.45 m tide level was not significantly different ($P=0.752$). The mean difference in least squares growth was 1.01 mm. The "A" plot clams grew significantly slower than "B" plot clams in both 1990 and 1991 at -0.90 and -0.45 tide levels ($P<0.0001$). The mean difference in least squares growth between clam types was -1.97 mm. Littleneck clams at -0.90 m tide level grew significantly slower than clams at -0.45 m tide level for both "A" and "B" plot clams in 1990 and 1991 ($P<0.0001$). The mean difference in least squares growth between tide levels was -0.81 mm.

There was an indication that two of the sites in the study, Simpson and Double bays, had been contaminated by refined petroleum hydrocarbons. These sites were removed from the reciprocal transplant data and two ANCOVA models were used to test for differences due to just the *Exxon Valdez* and not all petroleum hydrocarbons. The two full ANCOVA models were highly significant ($P<0.0001$; Tables 24 and 25). The models accounted for approximately 42% of the variability. The effect of mean level of aromatic hydrocarbons at tagging and recovery locations on growth was not significant ($P<0.3431$; Table 24) and ($P=0.2751$; Table 25); however, the interactions of aromatic hydrocarbons and year, clam type, and tide height were significant.

Pairwise comparisons of least squares means were used to test for differences in growth by year, clam type, and tide level. Growth of "A" plot clams at -0.90 and -0.45 m tide levels and "B" plot

clams at the -0.90 m tide level was significantly faster in 1990 than in 1991 (P<0.0001; Table 26). Growth of "B" plot clams at the -0.45 m level was not significantly different in 1990 (P=0.2404). The difference in least squares growth between years was 1.28 mm. The "A" plot clams grew significantly slower than "B" plot clams in 1990 at -0.90 m tide level and in 1991 at the -0.90 and -0.45 m tide levels (P<0.0001). Growth of "A" and "B" plot clams was not significant in 1990 at -0.45 m tide level (P=0.1204). The mean difference in least squares growth between clam types was -2.11 mm. Littleneck clams at -0.90 m tide level grew significantly slower than clams at -0.45 m tide level for "A" plot clams in 1990 and "B" plot clams in 1991 (P<0.0001). Even though growth was not significantly different between -0.90 and -0.45 tide levels for "B" plot clams in 1990 (P=0.0769) and "A" plot clams in 1991 (P=0.0625), the p-values were only marginally not significant and the difference in least squares growth between tide levels was -0.97 mm.

A comparison of adjusted least squares means using the mean level of aromatic hydrocarbons at recovery locations again showed significant differences in growth of clams in 1990 and 1991 by clam type and tide height. Littleneck clams grew significantly faster in 1990 than in 1991 for "A" plot clams at -0.90 and -0.45 m tide level and "B" plot clams at -0.90 m tide levels (P<0.0001; Table 27). However, growth of "B" plot clams at -0.45 m tide level was not significantly different between 1990 and 1991 (P=0.1488). The mean difference in least squares growth was 1.15 mm. The "A" plot clams grew significantly slower than "B" plot clams in both 1990 and 1991 at -0.90 and -0.45 tide levels (P<0.0001). The mean difference in least squares growth between clam types was -2.84 mm. Littleneck clams at -0.90 m tide level grew significantly slower than clams at -0.45 m tide level for "A" plot clams in 1991 (P<0.0001). Growth was not significantly different between tide levels for "A" plot clams in 1990 (P=0.6308) and 1991 (P=0.8603) and "B" plot clams in 1990 (P=0.0509). However, the p-values were only marginally not significant and the difference in least squares growth between tide levels was -0.53 mm.

Aging of Bivalves

Originally, 600 littleneck clam valves were to be examined for age, but due to low recovery at some sites, only 504 littleneck clams were sent to IMS for aging. The total number of clams eventually sectioned and aged by WDF was further reduced to 361 as some valves broke when sectioned or the confusing nature of the annuli found within the valve made interpretation impossible. In addition, the microstructure analysis was conducted on the hinge teeth of 90 of the 361 clams that were sectioned. The microstructure analysis of the sectioned valve and hinge teeth showed no evidence of a sudden or consistent interruption of micro-growth increment patterns that could be attributed to the oil spill. A complete summary of the microstructure analysis was completed by Volk *et al.* (1991). The report has been included in this report as Appendix D.

Clam ages were also estimated as part of the microstructure analysis by WDF. Ages determined at IMS by external surface were compared to ages determined by examination of the sectioned valve by WDF. Sectioned valve ages estimated by readers at WDF and external surface ages determined by the reader at IMS were in agreement for 25% of the clams. Sectioned ages were greater than the external ages for 62% of the clams and lesser than external ages for 13%. The maximum difference observed in ages from the two methods was 5 years. However, ages differed

by only ±1 year for 77% of the clams. Because age data are often used to determine growth from age-length data and to ascertain age composition, we wanted to show how the age estimates from the two methods might be affected. The mean length-at-age of clams was larger at all ages using the external surface method (Figure 28). The age composition, based on the external surface method, was significantly younger than the age composition based upon sectioned valve methods (X^2=373.73 > $15.5_{P<0.05,8}$; Figure 28). Ages were also estimated from the hinge teeth of 90 of the 361 littleneck clams that were sectioned by WDF. There was 100% agreement between ages estimated from sectioned valve and hinge teeth.

Recruitment

The average number of 1-year old littleneck clams in 1990 at oiled sites was 39.7 compared to 16.0 at control sites. The average ratio of 1990 1-year olds to 1990 1-, 2-, and 3-year olds was 0.14 at oiled sites and 0.19 at control sites. There were no significant differences in recruitment between oiled and control sites using either the pure recruitment or normalized recruitment tests (Table 28).

DISCUSSION

The two major goals of this study were to document hydrocarbon contamination at specific sites and determine what effects such contamination could have on selected bivalve populations. Contamination was determined by analyzing samples of sediment and tissue. Once the level of contamination was established, the identity of the source of contamination was sought. The effects of contamination were measured in two ways: first, by comparing clam growth between sites, and second by examination of clam tissues to determine the physical effects of contamination.

The confirmed presence of EVO in bivalves from most of the PWS sites classified as oiled in 1989 generally corroborate the *a priori* site classifications with regard to oiling from the *Exxon Valdez* oil spill. Oiling status was further confirmed at sites where corresponding sediment samples were analyzed in 1989 and 1990. The Fox Farm and Snug Harbor sites were among the most heavily oiled sites by the spill (O'Clair *et al.* 1996) whereas the other sites were considerably less impacted. The absence of PAH derived from EVO in bivalves from Ellamar suggests that this site, located near the margin of the oil trajectory through the Sound, was probably misclassified as oiled.

Outside PWS, the nearly complete failure to detect EVO in sediments or bivalves sampled from sites classified as oiled is probably because these sites are generally more exposed, were less heavily oiled than sites inside PWS, and were first sampled in late summer of 1989. Thus less oil initially present was exposed to more dispersive energy for longer periods compared with the

heavily oiled sites within PWS, so the oil usually subsided below detection limits by the sampling date. Similar results were found for caged blue mussels deployed within and outside PWS in 1989 (Short and Harris, 1996b).

The PAH are probably accumulated as whole, particulate oil by the bivalves studied here. The EVO weathering model used to identify EVO in bivalves (Short and Heintz 1997) assumes that PAH are associated with whole oil, rather than as dissolved species. Successful identification of EVO in bivalve samples thus implies particulate oil accumulation. Also, the weathering states of the EVO accumulated by bivalves are comparable with those of EVO in sediments collected concurrently, which indicates that these bivalves probably accumulated oil from nearby reservoirs of EVO stranded on beaches. The general absence of EVO in bivalves collected after 1989 indicates effective depuration by summer of 1990. These same conclusions were also found for caged blue mussels, and the magnitude of EVO accumulation was related to the proximity of heavily oiled beaches (Short and Harris, 1996b).

The regional PAH background of coal that appears in sediments from Double and Simpson Bays in this study are not bioavailable (Short *et al.* 1999), so these PAH are not a source of biological stress to exposed bivalves. In coal, PAH are sequestered by a crystalline matrix, and cannot readily migrate into biological tissues. This un-availability is corroborated by the absence of corresponding PAH in bivalves from these sites. The dominant PAH detected in these bivalves was naphthalene, which is probably an artifact of the analysis, because an environmental source of naphthalene itself is not apparent in the study area. The suite of alkyl-naphthalenes that were sporadically detected at low concentrations among bivalve samples from these and other sites may have resulted from gasoline contamination during sampling, perhaps by the 2-cycle gasoline-powered pump used to reveal the bivalves during collection. Similarly, the PAH suites characteristic of diesel oil contamination evident at a few sites may have resulted from introduced contamination during sampling, although recent small diesel spills from other boat traffic cannot be discounted.

Growth was less in littleneck clams at oiled sites than at control sites in Prince William Sound for both 1990 and 1991. Despite hydrocarbon contamination at the Simpson Bay control site, growth analyses incorporating hydrocarbon results strongly suggested that the decrease in growth of littleneck clams was attributable to oil from the *Exxon Valdez*. The question that arises is "How does the oil actually affect the growth of clams?". Some possible answers have been put forth in previous studies of mollusks. For example, *Venus verrocosa* exposed to water soluble fractions of oil opened and closed their valves more often than those not exposed (Axiak and George 1987a). Such activity results in the clam expending energy that otherwise could be going into growth. Petroleum hydrocarbon contamination also induced reduced feeding rates, increased mucous production, and increased ciliary system motions thereby causing an increased energy demand on *Venus verrocosa* and resulting in a reduction in the scope of growth (Axiak and George 1987a). Hydrocarbon pollution from anthropogenic sources caused breaks in the age-length curve representing a decrease in growth of *Mya arenaria* (Appeldorn 1983). Growth did improve in the years after the onset of pollution. Anderson (1988) found that littleneck clams exhibited low tolerance to Prudhoe Bay crude oil in sediments and reductions in growth were apparent in as little as four months.

Growth of littleneck clams also decreased as tide level increased. This finding is similar to Houghton (1973), who found that littleneck clams grew better at lower tidal heights on the north side of Kiket Island, Washington. The reason for this was probably the ability of clams to feed for longer periods of time at lower tides. In addition, littleneck clams that were notched, tagged, and reciprocally transplanted experienced decreased growth compared to clams that were only notched and replanted. Tagging probably caused the observed growth differences.

Oil can have effects other than growth on clam populations. Dungeness crab *Cancer magister* were more likely to feed on littleneck clams from oiled sites as the clams did not burrow deeply enough to avoid predation (Pearson *et al.* 1981). Siphon activity was retarded in littleneck clams exposed to Prudhoe Bay crude oil (Hartwick *et al.* 1982). While this in itself was not fatal, such behavior could render littleneck clams more susceptible to predation by the seastar *Pisaster ochraceus* and octopus *Octopus dofleini* that are found in Prince William Sound. Although river otter *Lutra canadenis* is primarily a fish eater, Bowyer *et al.* (*In Press*) found that they did feed on littleneck clams in Prince William Sound. If otters or other predators could not distinguish between oiled and unoiled clams, they could ingest oiled prey.

In-vitro studies involving artificial inducement of oil have shown that littleneck clams and other filter feeding bivalves were not severely affected by unrefined hydrocarbons (Roesijadi *et al.* 1979; Anderson *et al.* 1983). However, experimental levels of oil exposure may not duplicate actual conditions associated with the *Exxon Valdez* oil spill. In some locations beaches were saturated with oil repeatedly. Concentrations were not measured in PPB or even PPM, but in terms of 100 to 1 or 1% (Jeff Short, NMFS Auke Bay, AK personal communication). Even if clams did close as oil came ashore they would have to eventually reopen for respiration and feeding. If oiling persisted or the site was subjected to a second oiling event, then the clams could have suffocated or starved (Chew and Ma 1987). These effects would be catastrophic and short lived as the oil moved on or became diluted by wave action.

There was no evidence of lesions, specifically necrosis or atrophy of the digestive gland epithelium or gonadal lesions as observed in oysters (Sindermann 1990) exposed to crude oil from the *Amoco Cadiz*. Tissue inflammation and necrotic lesions observed on *Mya truncata* exposed to chemically dispersed and undispersed crude oil on Baffin Island (Neff *et al.* 1987) were also not observed. The most common histopathological conditions observed in littleneck and butter clams were associated with the digestive system and the occurrence of parasites. However, without baseline data regarding the histopathological conditions in either littleneck or butter clams in Prince William Sound, (Nickerson 1977; Feder *et al.* 1979), it was difficult to determine what histopathological conditions were atypical. This study identified the presence of a variety parasites heretofore undocumented in clam tissues in Alaska.

Stress due to exposure to unrefined hydrocarbons alone can render an animal more susceptible to parasitism (Anderson 1988). It becomes exceedingly difficult to determine if oil directly affected parasite abundance or if stress lowered the vulnerability of clams throughout the polluted areas making them more likely to be parasitized (Overstreet and Howse 1977).

The reasons for the low level of parasites in littleneck clams sampled in 1989 from oiled sites in Prince William Sound sites (4% in oiled versus 14% in control) are not known. There was some

evidence to suggest that hydrocarbons may affect parasite distribution. Khan and Kiceniuk (1983) found the prevalence and intensity of trematodes, *Steringophorus furciger* present in winter flounder *Pseudopleuronectes americanus* exposed to extracts of Hibernian and Venezuelan oils was lower in oil-treated fish. The absence of parasites could be due to direct toxicity from ingestion or of exposure to water soluble fractions of crude oil and/or modifications due to the changing gut environment of the host. A decline in the abundance of the nematode *Anisakis* in the gut of Pacific herring *Clupea pallasi* was observed when the fish were exposed to water soluble fractions of Prudhoe Bay crude oil (Moles *et al. In Press*). Alternatively, Khan (1990) found that fishes exposed to water soluble fractions of Prudhoe Bay crude oil had more parasites than those not exposed. Although conflicting, the above results suggested that pollutants have the potential to affect the fish's immune responses.

Similar coccidians (Morado *et al.* 1984), copepods (Chew *et al.* 1964, 1965; Katkansky *et al.* 1967), trematodes, and protozoans parasitized littleneck and butter clams, as in other parts of the world. It appears that few, if any bivalve parasites caused mortality (Sparks 1985; Fisher 1988, Sindermann 1990). Histopathology sampling began in April 1989 and ended in 1991 (no samples from 1991 were examined). It could be that sampling began too late to accurately determine direct mortality, and ended too early to ascertain fully the long term effects of hydrocarbon contamination of clam tissues.

After analyzing the histopathology data, we concluded the data were too sparse to make any reliable conclusions about oiling effects, although there was some indication that clams from oiled areas may have been injured. Examination of residuals for some of the analyses showed that the homogenous variance assumption had been violated, even though weights or transformations were used. A very significant effect was also seen for littleneck clams collected in Cook Inlet in 1990 (no data were available for 1989). The difference here is large, and is probably real in spite of the failures of assumptions.

Even though aromatic hydrocarbon levels were very high in bivalve tissues, there was no strong evidence to suggest that clam tissues were severely affected by *Exxon Valdez* oil either in 1989 or 1990. The differences in incidence of parasite occurrence between sites and years showed little statistical significance. Although histopathological conditions were documented, attributing these directly to unrefined hydrocarbon contamination was not possible. Histopathological analysis has been described as a mediocre indicator of pollution stress because it lacked the necessary sensitivity and specificity (Sindermann 1990). The lack of baseline information, so critical for determining what constitute normal histopathological conditions, make identification, assessment, and explanation of our observations that much more difficult.

The mortality rates of bivalves were not directly determined among control and oiled sites. We did observe reduced growth rates of littleneck clams that coincided with increased levels of petroleum hydrocarbons. Some studies have shown direct relationships between growth and mortality for bivalves (Dow 1975, 1978; Hartwick *et al.* 1982).

No significant differences in recruitment were found between oiled and control sites. Chew and Ma (1987) found that the extent of littleneck recruitment varies greatly between areas. Larval littleneck clams undergo metamorphosis and use byssal threads to attach to the substrate as they

settle (Chew and Ma 1987). In *Mya edulis* byssal thread formation was inhibited by the water soluble fraction of Louisiana crude oil (Carr and Reish 1978). If byssal thread formation of littleneck spat is affected in a like manner, then recruitment could be affected.

Aging of littleneck clams using the external surface method produced ages younger than ages estimated from the sectioned valve method. This was similar to previous studies. Ropes and Jerald (1987) have identified inadequacies with the external surface method for determining the ages of older bivalves. The external aging method in these previous studies always produced ages younger than the sectioned valve method. The reason for the difference between methods was thought to be identification of the first annulus. To check this, young-of-the-year (YOY) littleneck clams were collected at different times of year at Simpson Bay in Prince William Sound to verify if and when the first annuli is laid down. The external surface aging method did not pick up the first annulus during the fall but did detect an annulus in the winter and early spring. Because of this, it was felt the external valve method, for the most part; accurately ages littleneck clams and the external valve method can be used to age littleneck clams in Prince William Sound.

The objectives called for identifying alternative methods and strategies for restoration of lost use, populations, or habitat where injury is identified. A major drawback throughout this study was the lack of baseline data prior to the oil spill. Baseline data should be collected on bivalve populations throughout Prince William Sound, Cook Inlet, Kodiak Island and the Alaska Peninsula. Bivalves function as good environmental indicators because they inhabit the intertidal zone, are filter feeders, ubiquitous, and prey to many vertebrates and invertebrates. A thorough understanding of their basic life history, environmental requirements and population dynamics will be important should another oil spill occur in the future.

CONCLUSIONS

1. Bivalve tissues at oiled sites were found to have high levels of aromatic hydrocarbons. In addition, one designated control sites (Simpson Bay) was partially contaminated by refined petroleum hydrocarbons.

2. Even though bivalve tissues had high levels of aromatic hydrocarbons, no strong evidence was found to suggest that clam tissues were severely affected by *Exxon Valdez* crude oil either in 1989 or 1990 based on histopathological analyses. Only the occurrence of parasites was different between oiled and control sites; occurrence of parasites was higher at control sites.

3. The mortality rates of bivalves were not directly determined among control and oiled sites.

4. The growth rate of littleneck clams collected in Prince William Sound in 1990 and 1991 was found to decrease as the level of aromatic hydrocarbons increased. Growth of littleneck clams was also found to decrease as tide level increased. In addition, littleneck clams that were notched, tagged, and reciprocally transplanted demonstrated decreased growth compared to clams that were notched and replanted. This difference in growth was probably due to tagging.

5. No differences in recruitment were found between control and oiled sites in 1989.

6. Aging of littleneck clams using the external surface method produced ages younger than ages estimated from the sectioned valve method.

7. The objectives called for identifying alternate methods and strategies for restoration of lost use, populations, or habitat where injury is identified. One major drawback in the study of bivalves was the lack of baseline or background information before the oil spill. The initiation of future studies is recommended to study bivalve populations throughout Prince William Sound, Cook Inlet, Kodiak Island, and the Alaska Peninsula. These studies would provide important baseline information if another oil spill occurs in the future.

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Table 1. Sampling locations for Fish/Shellfish Study 13 - Effects of Hydrocarbons on Bivalves in Prince William Sound, Cook Inlet, outer Kenai Peninsula, the Kodiak Island Area, and the Alaska Peninsula, 1989-1991.

Location	Oil/ Control	1989 Transect	1990 Transect	1990 Transplant	1991 Transect	1991 Transplant	Latitude	Longitude
Prince William Sound								
Double Bay	Control		X	X		X	60°27.58'	146°28.16'
Hell's Hole	Control	X	X	X		X	60°42.13'	146°22.91'
Pellew Cove	Control	X	X				60°51.59'	147°39.54'
Simpson Bay	Control	X	X	X		X	60°38.10'	145°53.12'
Ellamar	Oil	X					60°53.02'	146°46.27'
Fox Farm	Oil	X					59°58.08'	148°08.40'
Gibbon Anchora	Oil	X	X	X		X	60°16.26'	147°26.11'
Green Island	Oil		X				60°16.59'	147°25.39'
Horseshoe Bay	Oil		X	X		X	60°00.97'	147°57.46'
North Chenega	Oil		X				60°22.87'	148°00.87'
Outside Bay	Oil	X					60°38.21'	147°26.28'
Snug Harbor	Oil	X	X				60°16.03'	147°44.86'
Wilson Bay	Oil	X	X	X		X	60°02.04'	147°55.74'
Cook Inlet and Outer Kenai Peninsula								
Jakolof Bay	Control	X	X				59°28.20'	151°32.15'
Seldovia Bay	Control	X	X				59°25.40'	151°42.46'
Tutka Bay	Control		X				59°26.25'	151°23.85'
Port Dick	Oil	X	X				59°18.67'	151°18.23'
Tonsina Bay	Oil		X				59°17.97'	150°54.87'
Windy Bay	Oil	X	X				59°13.80"	151°33.00'
Kodiak Island Area								
McDonald Lagoc	Control	X					58°09.47'	152°19.39'
Port Bailey	Control	X	X				57°25.20'	152°59.77
Kupreanof Strait	Oil	X	X				57°57.67'	153°07.77'
Ruth Bay	Oil	X					58°13.00'	152°19.60'
Razor Clam Sampling - Alaska Peninsula								
Augustine Island	Control		X				59°19.78'	153°28.69'
Chinitna Bay	Control		X				59°52.80'	152°53.80'
Crescent River	Control		X				60°12.20'	152°33.20'
Chiniak Bay	Oil		X				58°32.70'	153°53.90'
Hallo Bay	Oil		X				58°25.30'	154°02.30'
Kashvik	Oil		X				57°56.40'	155°05.35'

Table 2. Weather and environmental conditions at sampling locations for Fish/Shellfish Study 13 - Effects of Hydrocarbons on Bivalves, 1989-1991.

Location	Date	Temperature (°C) Air	Temperature (°C) Sea	Salinity (ppt)	Waves	Weather
		1989				
Prince William Sound						
Ellamar	22-Apr-89	4.4	5.5	25.2	Glassy	Overcast
Foxfarm	06-May-89	5.6	5.6	25.9	Rippled	Rain
Gibbon Anchorage	03-May-89	6.7	6.9	24.2	Glassy	Partly Cloudy
Hell's Hole	21-Apr-89	3.3	6.1	24.6	Glassy	Clear
	02-Aug-89	15.0	14.6	19.6	Wavelets	Overcast
Pellew Cove	23-Apr-89	6.6	5.2	24.8	Glassy	Squalls
Outside Bay	24-Apr-89	5.6	4.2	26.2	Wavelets	Rain
Simpson Bay	20-Apr-89	4.4	5.6	25.1	Glassy	Overcast
	01-Aug-89	16.0			Glassy	Rain
Snug Harbor	04-May-89	7.8	5.1	25.4	Glassy	Showers
	04-Aug-89	13.3	12.8	19.4	Glassy	Partly Cloudy
Wilson Bay	05-May-89	6.7	4.7	25.6	Glassy	Overcast
	03-Aug-89	13.9	13.1	20.4	Rippled	Clear
Cook Inlet and Outer Kenai Peninsula						
Port Dick	21-Aug-89	17.0	13.0	24.0	Wavelet	Clear
Windy Bay	20-Aug-89	17.0	15.0	26.0	Wavelet	Clear
Jakolof Bay	16-Aug-89	18.0	12.0	26.0	Glassy	Overcast
Seldovia Bay	17-Aug-89	18.0	14.0	26.0	Wavelet	Overcast
Kodiak Island Area						
Kupreanof Strait	14-Sep-89	19.0	10.0		Glassy	Drizzle
McDonald Lagoon	16-Sep-89				Glassy	Squalls
Port Bailey	15-Sep-89	13.0	10.0		Glassy	Clear
Ruth Bay	17-Sep-89				Glassy	Rain
		1990				
Prince William Sound						
Double Bay	12-Apr-90	8.5	6.0		Rippled	Clear
Gibbon Anchorage	23-Apr-90	11.0	8.0	32.0	Rippled	Partly Cloudy
Green Island	24-Apr-90	8.0	6.0	32.0	Rippled	Partly Cloudy
Hell's Hole	11-Apr-90				Rippled	Partly Cloudy
Horseshoe Bay	27-Apr-90	14.0	6.0	32.0	Rippled	Clear
North Chenega	28-Apr-90	15.0	9.0	32.0	Rippled	Clear
Pellew Cove	29-Apr-90	8.0	6.0	29.0	Glassy	Overcast
Simpson Bay	10-Apr-90	12.0	6.0		Rippled	Clear
Snug Harbor	25-Apr-90	9.5	7.0	31.0	Glassy	Clear
Wilson Bay	26-Apr-90				Glassy	Clear
Cook Inlet and Outer Kenai Peninsula						
Jakolof Bay	25-Jul-90				Glassy	Clear
Port Dick	22-Jun-90		11.5	20.0	Rippled	Clear
Seldovia Bay	20-Jun-90		6.0	21.0	Rippled	Overcast
Tonsina Bay	21-Jun-90		8.0	22.0	Wavelets	Partly Cloudy
Tutka Bay	24-Jun-90		9.0	17.0	Glassy	Partly Cloudy
Windy Bay	23-Jun-90				Wavelets	Partly Cloudy
Kodiak Island Area						
Kupreanof Strait	21-Jul-90				Slight 2-4'	Overcast
Port Bailey	22-Jul-90				Wavelets	Partly Cloudy
Alaska Peninsula						
Augustine Island	07-Aug-90		13.2	28.0	Slight 2-4'	Overcast
Chiniak Bay	21-Aug-90		12.0	13.0	Rippled	Clear
Chinitna Bay	08-Aug-90		12.7	26.4	Slight 2-4'	Partly Cloudy
Crescent River	06-Aug-90		13.0	18.0	Glassy	Partly Cloudy
Hallo Bay	20-Aug-90		12.0	23.0	Wavelets	Clear
Kashvik Bay	19-Aug-90		14.0	27.0	Slight 2-4'	Clear
		1991				
Prince William Sound						
Double Bay	09-Sep-91	16.0	13.0	26.0	Rippled	Overcast
Gibbon Anchorage	07-Sep-91	15.0	12.0	25.0	Glassy	Overcast
Hell's Hole	20-Sep-91	16.0	13.0	23.0	Glassy	Clear
Horseshoe Bay	07-Sep-91	15.0	12.0	26.0	Rippled	Overcast
Simpson Bay	09-Sep-91	16.0	11.0	26.0	Rippled	Drizzle
Wilson Bay	08-Sep-91	16.0	12.0	26.0	Wavelets	Rain

Table 3. Sample size, mean length (mm) and standard deviation (S.D.) of littleneck clams, and number of butter clams and cockles collected at transect sampling locations in Prince William Sound, Alaska, 1989.

			Littleneck Clams			Number Collected	
Location	Date	Transect	n	Mean Length	S.D.	Butter Clams	Cockles
Ellamar	22-Apr-89	1	329	20.8	4.0	11	1
		2	97	24.1	5.4	6	0
		3	120	16.6	6.5	13	0
		Total	546	20.5	5.5	30	1
Fox Farm	06-May-89	1	140	22.5	7.2	10	1
		2	97	21.6	7.4	8	0
		3	56	23.9	7.6	8	0
		Total	293	22.5	7.4	26	1
Gibbon Anch(	03-May-89	1	256	19.7	5.6	67	0
		2	261	19.9	5.2	14	1
		3	268	19	5.3	27	0
		Total	785	19.5	5.4	108	1
Hell's Hole	21-Apr-89	1	142	21.4	6.5	27	0
		2	164	20.2	6.2	30	0
		3	111	21.9	5.9	11	0
		Total	417	21.0	6.3	68	0
Hell's Hole	02-Aug-89	1	316			0	0
		2	268			0	0
		3	160			0	0
		Total	744			0	0
Outside Bay	24-Apr-89	1	38	16.2	6.3	1	0
		2	130	17.7	7	4	0
		3	185	20.4	7	35	2
		Total	353	18.8	7.1	39	2
Pellew Cove	24-Apr-89	1	25	26.1	8.5	0	2
		2	18	25.2	9	0	0
		3	4	26	12.3	0	0
		Total	47	25.7	8.1	0	2
Simpson Bay	20-Apr-89	1	37	17.3	7.4	9	0
		2	43	22.1	9.5	20	0
		3	38	24.5	8.9	21	0
		Total	118	21.5	9.1	50	0
Simpson Bay	01-Aug-89	1	45			0	0
		2	30			0	0
		3	60			0	0
		Total	135			0	0
Snug Harbor	04-May-89	1	20	29.4	9.1	0	0
		2	11	25.5	7.8	0	0
		3	81	24.2	7.3	11	0
		Total	112	25.3	7.9	11	0
Snug Harbor	04-Aug-89	1	9			0	0
		2	25			0	0
		3	27			0	0
		Total	61			0	0
Wilson Bay	05-May-89	1	13	12.8	3.1	0	0
		2	119	19.7	9.2	0	0
		3	258	19.8	5.8	0	0
		Total	390	19.5	6.9	0	0
Wilson Bay	03-Aug-89	1	2			0	0
		2	27			0	0
		3	240			0	0
		Total	269			0	0

Table 4. Sample size, mean length (mm), and standard deviation (S.D.) of littleneck and butter clams collected from transect sampling locations in Cook Inlet and Kodiak Island Area, Alaska, 1989.

			Littleneck Clams			Butter Clams		
Location	Date	Transect	n	Mean Length	S.D.	n	Mean Length	S.D.
Cook Inlet and Outer Kenai Peninsula								
Port Dick	21-Aug-89	1	62	18.6	3.6	0		
		2	36	17.7	3.8	0		
		3	2	12.4	2.9	0		
		Total	100	18.2	3.7	0		
Jakolof Bay	16-Aug-89	1	88	31.2	12.1	3	47.3	28.5
		2	161	30.4	12.1	8	70.9	10.6
		3	123	31.1	12.0	9	38.6	16.4
		Total	372	30.8	12.1	20	49.5	21.6
Seldovia Bay	17-Aug-89	1	29			1		
		2	18			0		
		3	19			0		
		Total	66			1		
Windy Bay	20-Aug-89	1	107	24.9	6.8	0		
		2	198	19.7	6.7	1	45.8	
		3	227	18.9	6.7	0		
		Total	532	20.4	6.7	1		
Kodiak Island Area								
Kupreanof	14-Sep-89	1	94			84		
		2	138			146		
		3	30			42		
		Total	262			272		
McDonald Lagoon	16-Sep-89	1	17	35.7	12.2	2	51.1	0.3
		2	44	35.6	5.3	1	34.9	
		3	93	30.7	7.6	18	48.9	17.5
		Total	154	32.7	8	21	48.7	16.5
Port Bailey	15-Sep-89	1	116	34.6	11.7	32	36.1	20.8
		2	92	31.2	12	31	43.7	20.9
		3	64	32	13.1	50	38.2	19.9
		Total	272	32.6	12.2	113	39.1	20.5
Ruth Bay	17-Sep-89	1	61	30.9	8.9	0		
		2	59	17.2	13	2	23.2	16
		3	226	28.4	6.3	14	28.6	7.5
		Total	346	26.9	9.4	16	27.6	9.2

Table 5. Sample size, mean length (mm), and standard deviation (S.D.) of littleneck and butter clams from transect sampling locations in Prince William Sound, Alaska, 1990.

			Littleneck Clams			Butter Clams		
				Mean			Mean	
Location	Date	Transect	n	Length	S.D.	n	Length	S.D.
North Chenega	28-Apr-90	1	93	13.8	6.7	1	7.4	
		2	87	15.5	11.2	0		
		3	114	18.5	11.3	0		
		Total	294	16.3	9.9	1	7.4	
Double Bay	12-Apr-90	1	366	25.9	5.6	0		
		2	436	22.6	7.6	4	21.8	9.8
		3	153	19.9	7.5	6	25.6	13
		Total	955	23.5	7.2	10	24.5	11
Gibbon Anchorage	23-Apr-90	1	178	18.2	6.2	0		
		2	231	22.4	6.1	2	20.1	9.6
		3	225	19.8	6.7	9	20.1	9.8
		Total	634	20.2	6.6	11	20.1	9.3
Green Island	24-Apr-90	1	196	20.5	7.9	11	29.3	12.9
		2	180	24.1	8.7	1	9.5	
		3	137	25.2	9.3	1	14.8	
		Total	513	23.1	8.8	13	26.6	13.5
Hell' Hole	11-Apr-90	1	188	16.7	4.7	27	19.6	7.5
		2	340	16.2	5.9	37	21.2	7.1
		3	110	17.3	4.7	11	21.1	8.5
		Total	638	16.5	5.3	75	20.6	7.4
Horseshoe Bay	27-Apr-90	1	279	13.1	4.6	1	19.5	
		2	168	12.8	4.1	1	23.2	
		3	40	17.5	5.9	0		
		Total	487	13.4	4.7	2	21.3	2.6
Pellew Cove	29-Apr-90	1	13	17.8	10.0	1	27.3	
		2	17	25.3	9.5	1	29.0	
		3	0			0		
		Total	30	22.1	10.2	2	28.2	1.2
Simpson Bay	10-Apr-90	1	28	12.2	3.5	0		
		2	80	20.9	8.8	28	39.7	5.8
		3	67	15.7	7.4	10	31.9	16.9
		Total	175	17.8	8.4	38	37.9	9.8
Snug Harbor	25-Apr-90	1	10	21.5	11.3	1	33.3	
		2	0			0		
		3	2	11.3	6.5	0		
		Total	12	19.2	11.0	1	33.3	
Wilson Bay	26-Apr-90	1	63	14.5	6.6	0		
		2	155	17.0	6.7	0		
		3	264	11.7	5.2	0		
		Total	482	13.8	15.9	0		

Table 6. Sample size, mean length (mm), and standard deviation (S.D.) of littleneck, butter, and razor clams collected from transect sampling locations in Cook Inlet, Kodiak Island Area, and the Alaska Peninsula, 1989.

			Littleneck Clams			Butter Clams			Razor Clams		
				Mean			Mean			Mean	
Location	Date	Transect	n	Length	S.D.	n	Length	S.D.	n	Length	S.D.
Cook Inlet and Outer Kenai Peninsula											
Port Dick	22-Jun-90	1	496	14.1	8.5	24	9.9	8.2	0		
		2	420	12.0	8.5	63	7.1	3.4	0		
		3	482	8.0	8.0	34	9.1	6.9	0		
		Total	1,398	13.4	8.4	121	8.2	5.7	0		
Jakolof Bay	25-Jun-90	1	142			0			0		
		2	174			15			0		
		3	245			22			0		
		Total	561			37			0		
Seldovia Bay	20-Jun-90	1	50	35.4	8.0	1	58.6		0		
		2	46	34.3	6.9	2	45.2	13.2	0		
		3	60	33.3	8.2	0			0		
		Total	156	34.3	7.7	3	49.7	12.1	0		
Tonsina Bay	21-Jun-90	1	82	19.9	6.8	2	37.3	10.7	0		
		2	131	19.9	7.7	6	23.4	11.4	0		
		3	29	14.1	7.0	2	18.4	14.1	0		
		Total	242	19.2	7.5	10	25.2	12.3	0		
Tutka Bay	24-Jun-90	1	134	32.7	13.1	129	39.7	16.2	0		
		2	149	35.2	11.8	27	33.8	20.1	0		
		3	86	34.7	13.8	12	22.7	18.4	0		
		Total	369	34.2	12.8	168	37.7	17.5	0		
Windy Bay	23-Jun-90	1	152	22.1	6.7	0			0		
		2	196	20.1	8.9	0			0		
		3	133	19.8	8.5	0			0		
		Total	481	21.0	8.2	0			0		
Kodiak Island Area											
Port Bailey	22-Jul-90	1	93	28.1	15.1	15	48.7	20.9	0		
		2	87	27.3	14.3	28	39.2	22.3	0		
		3	114	17.5	11.7	18	32.0	19.0	0		
		Total	294	24.7	14.6	61	40.1	21.6	0		
Kupreanof Strait	21-Jul-90	1	125	15.4	10.9	166	21.3	15.9	0		
		2	68	10.2	4.8	110	21.4	17.3	0		
		3	26	11.4	8.3	64	25.6	19.2	0		
		Total	219	13.3	9.4	340	22.1	17.0	0		
Alaska Peninsula											
Augustine Island	07-Aug-90	1	0			0			272	104.0	26.9
Chiniak Bay	21-Aug-90	1	0			0			300	91.1	42.6
Chinitna Bay	08-Aug-90	1	0			0			364	86.8	53.9
Crescent River	06-Aug-90	1	0			0			305	105.8	11.1
Hallo Bay	20-Aug-90	1	0			0			235	73.3	32.7
Kashvik	19-Aug-90	1	0			0			336	104.5	21.9

Table 7. Sediment samples collected as part of Fish-Shellfish Study 13 - Effects of Hyrdrocarbons on Bivalves and submitted for hydrocarbon analysis (Page 1 of 3).

Location	Oil/ Control	Date Collected	Samples Submitted	Samples Analyzed
		1989		
Prince William Sound				
Simpson Bay	Control	04/20/89	9	3
Hell's Hole	Control	04/21/89	9	0
Pellew Cove	Control	04/23/89	9	0
Simpson Bay	Control	08/01/89	9	0
Hell's Hole	Control	08/02/89	9	0
Total			45	3
Ellamar	Oil	04/22/89	9	0
Outside Bay	Oil	04/24/89	9	0
Gibbon Anchorage	Oil	05/03/89	9	0
Snug Harbor	Oil	05/04/89	9	0
Wilson Bay	Oil	05/05/89	9	0
Foxfarm	Oil	05/06/89	9	3
Wilson Bay	Oil	08/03/89	9	1
Snug Harbor	Oil	08/04/89	9	0
Total			72	4
Cook Inlet and Outer Kenai Peninsula				
Jakolof Bay	Control	08/16/89	9	2
Seldovia Bay	Control	08/17/89	9	0
Clam Gulch	Control	04/06/89	0	0
Ninilichik	Control	04/06/89	0	0
Total			18	2
Windy Bay	Oil	08/20/89	9	3
Port Dick	Oil	08/21/89	8	0
Total			17	3
Kodiak Island Area				
Port Bailey	Control	09/15/89	9	0
McDonald Lagoon	Control	09/16/89	9	0
Total			18	0
Kupreanof Strait	Oil	09/14/89	9	0
Ruth Bay	Oil	09/17/89	9	0
Total			18	0
Total for 1989			188	12

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Table 7. (Page 2 of 3).

Location	Oil/ Control	Date Collected	Samples Submitted	Samples Analyzed
		1990		
Prince William Sound				
Simpson Bay	Control	04/10/90	9	9
Hell's Hole	Control	04/11/90	9	9
Double Bay	Control	04/12/90	9	9
Pellew Cove	Control	04/29/90	9	9
Simpson Bay	Control	09/07/90	3	0
Hell's Hole	Control	09/07/90	3	0
Double Bay	Control	09/07/90	3	0
Total			45	36
Gibbon Anchorage	Oil	04/23/90	9	9
Green Island	Oil	04/24/90	9	9
Snug Harbor	Oil	04/25/90	9	9
Wilson Bay	Oil	04/26/90	9	9
Horseshoe Bay	Oil	04/27/90	9	9
North Chenega	Oil	04/28/90	9	9
Gibbon Anchorage	Oil	09/05/90	3	0
Horseshoe Bay	Oil	09/05/90	3	0
Wilson Bay	Oil	09/06/90	3	0
Total			63	54
Cook Inlet and Outer Kenai Peninsula				
Seldovia Bay	Control	06/20/90	9	0
Tutka Bay	Control	06/24/90	9	0
Jakolof Bay	Control	06/25/90	9	0
Total			27	0
Tonsina Bay	Oil	06/21/90	9	0
Port Dick	Oil	06/22/90	8	0
Windy Bay	Oil	06/23/90	9	0
Total			26	0
Kodiak Island Area				
Port Bailey	Control	07/22/90	9	0
Total			9	0
Kupreanof Strait	Oil	07/21/90	9	0
Total			9	0
Alaska Peninsula				
Crescent Bay	Control	08/06/90	3	0
Augustine Island	Control	08/07/90	3	0
Chinitna Bay	Control	08/08/90	3	0
Total			9	0
Kashvik	Oil	08/19/90	3	0
Hallo	Oil	08/20/90	3	0
Chiniak	Oil	08/21/90	3	0
Total			9	0
Total for 1990			197	90

- continued -

Table 7. (Page 3 of 3).

Location	Oil/ Control	Date Collected	Samples Submitted	Samples Analyzed
		1991		
Prince William Sound				
Hell's Hole	no	04/16/91	6	0
Double Bay	no	04/21/91	6	0
Simpson Bay	no	04/22/91	6	0
Hell's Hole	no	08/29/91	6	0
Double Bay	no	09/09/91	6	0
Simpson Bay	no	09/09/91	6	0
Total			36	0
Gibbon Anchorage	yes	04/14/91	6	0
Horseshoe Bay	yes	04/19/91	6	0
Wilson Bay	yes	04/20/91	6	0
Gibbon Anchorage	yes	09/06/91	6	0
Horseshoe Bay	yes	09/07/91	6	0
Wilson Bay	yes	09/08/91	6	0
Total			36	0
Total for 1991			72	0
Total for 1989-1991			457	102

Table 8. Clam tissue samples collected, as part of Fish/Shellfish Study 13 - Effects of Hydrocarbons on Bivalves, and submitted for hydrocarbon analysis (Page 1 of 3).

Location	Oil/ Control	Date Collected	Samples Submitted	Samples Analyzed
		1989		
Prince William Sound				
Simpson Bay	Control	04/20/89	6	6
Hell's Hole	Control	04/21/89	6	6
Pellew Cove	Control	04/23/89	4	4
Simpson Bay	Control	08/01/89	4	4
Hell's Hole	Control	08/02/89	4	4
Total			24	24
Ellamar	Oil	04/22/89	7	7
Outside Bay	Oil	04/24/89	5	5
Gibbon Anchorage	Oil	05/03/89	7	7
Snug Harbor	Oil	05/04/89	5	5
Wilson Bay	Oil	05/05/89	5	5
Foxfarm	Oil	05/06/89	5	5
Wilson Bay	Oil	08/03/89	5	5
Snug Harbor	Oil	08/04/89	4	4
Total			43	43
Cook Inlet and Outer Kenai Peninsula				
Clam Gulch	Control	04/06/89	2	2
Ninilchik	Control	04/06/89	1	1
Ninilchik	Control	04/07/89	3	3
Clam Gulch	Control	07/07/89	24	24
Jakolof Bay	Control	08/16/89	7	7
Seldovia Bay	Control	08/17/89	4	4
Total			41	41
Windy Bay	Oil	08/20/89	4	4
Port Dick	Oil	08/21/89	3	3
Total			7	7
Kodiak Island Area				
Port Bailey	Control	09/15/89	8	8
McDonald Lagoon	Control	09/16/89	4	4
Total			12	12
Kupreanof Strait	Oil	09/14/89	4	4
Ruth Bay	Oil	0917/89	4	4
Total			8	8
Total for 1989			135	135

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Table 8. (Page 2 of 3)

Location	Oil/ Control	Date Collected	Samples Submitted	Samples Analyzed
		1990		
Prince William Sound				
Simpson Bay	Control	04/10/90	8	8
Hell's Hole	Control	04/11/90	8	8
Double Bay	Control	04/11/90	1	1
Double Bay	Control	04/12/90	5	5
Pellew Cove	Control	04/29/90	6	6
Hell's Hole	Control	05/10/90	6	2
Simpson Bay	Control	05/23/90	6	2
Double Bay	Control	05/24/90	6	2
Double Bay	Control	09/06/90	1	1
Double Bay	Control	09/07/90	6	1
Hell's Hole	Control	09/07/90	3	1
Simpson Bay	Control	09/07/90	4	2
Hell's Hole	Control	09/08/90	3	1
Simpson Bay	Control	09/18/90	2	0
Total			65	40
Gibbon Anchorage	Oil	04/23/90	8	8
Green Island	Oil	04/24/90	7	7
Snug Harbor	Oil	04/25/90	7	7
Wilson Bay	Oil	04/26/90	6	6
Horseshoe Bay	Oil	04/27/90	8	8
North Chenega	Oil	04/28/90	6	6
Gibbon Anchorage	Oil	05/09/90	6	2
Wilson Bay	Oil	05/22/90	6	2
Horseshoe Bay	Oil	05/22/90	1	1
Gibbon Anchorage	Oil	09/05/90	6	2
Horseshoe Bay	Oil	09/05/90	6	2
Wilson Bay	Oil	09/06/90	6	2
Total			73	53
Cook Inlet and Outer Kenai Peninsula				
Seldovia	Control	06/20/90	5	1
Tutka	Control	06/24/90	8	2
Jakolof	Control	06/25/90	6	2
Total			19	5
Tonsina	Oil	06/21/90	6	2
Dick	Oil	06/22/90	5	1
Windy	Oil	06/23/90	4	2
Total			15	5
Kodiak Island Area				
Bailey	Control	07/22/90	8	2
Total			8	2
Kupreanof	Oil	07/21/90	8	2
Total			8	2
Alaska Peninsula				
Crescent Bay	Control	08/06/90	4	1
Augustine Island	Control	08/07/90	4	1
Chinitna Bay	Control	08/08/90	4	1
Total			12	3
Kashvik	Oil	08/19/90	4	1
Hallo Bay	Oil	08/20/90	4	1
Chiniak Bay	Oil	08/21/90	4	1
Total			12	3
Total for 1990			212	113

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Table 8. (Page 3 of 3).

Location	Oil/ Control	Date Collected	Samples Submitted	Samples Analyzed
		1991		
Prince William Sound				
Double Bay	Control	04/20/91	4	2
Double Bay	Control	09/09/91	4	2
Hell's Hole	Control	04/17/91	4	2
Hell's Hole	Control	08/29/91	4	2
Simpson Bay	Control	04/22/91	4	2
Simpson Bay	Control	09/08/91	1	0
Simpson Bay	Control	09/09/91	4	2
Simpson Bay	Control	09/23/91	1	0
Total			26	12
Gibbon Anchorage	Oil	04/14/91	3	3
Gibbon Anchorage	Oil	04/16/91	1	0
Gibbon Anchorage	Oil	09/06/91	2	1
Gibbon Anchorage	Oil	09/07/91	2	1
Horseshoe Bay	Oil	04/19/91	3	3
Horseshoe Bay	Oil	04/20/91	1	0
Horseshoe Bay	Oil	09/07/91	4	2
Wilson Bay	Oil	04/20/91	4	2
Wilson Bay	Oil	09/08/91	3	2
Total			23	14
Total for 1991			49	26
Total for 1989-1991			396	274

Table 9. Histopathology samples collected and submitted for analysis for Fish/Shellfish Study 13 - Effects of Hydrocarbons on Bivalves, 1990-1991 (Page 1 of 2).

Location	Oil/ Control	Date	Species	Number Collected	Number Examined
Prince William Sound					
Double Bay	Control	12-Apr-90	Littleneck Clam	21	21
Double Bay	Control	9-Sep-91	Littleneck Clam	26	0
Hell's Hole	Control	21-Apr-89	Littleneck Clam	17	17
Hell's Hole	Control	2-Aug-89	Littleneck Clam	20	18
Hell's Hole	Control	11-Apr-90	Littleneck Clam	16	16
Hell's Hole	Control	29-Aug-91	Littleneck Clam	29	0
Pellew Cove	Control	23-Apr-89	Littleneck Clam	11	11
Simpson Bay	Control	20-Apr-89	Littleneck Clam	14	13
Simpson Bay	Control	1-Aug-89	Littleneck Clam	13	13
Simpson Bay	Control	10-Apr-90	Littleneck Clam	18	18
Simpson Bay	Control	9-Sep-91	Littleneck Clam	15	0
Total - Littleneck Clams				200	127
Double Bay	Control	12-Apr-90	Butter Clam	10	6
Hell's Hole	Control	21-Apr-89	Butter Clam	13	13
Hell's Hole	Control	11-Apr-90	Butter Clam	12	12
Simpson Bay	Control	20-Apr-89	Butter Clam	14	11
Simpson Bay	Control	10-Apr-90	Butter Clam	7	7
Total - Butter Clams				56	49
Ellamar	Control	22-Apr-89	Cockle	6	0
Total - Cockles				6	0
Total - Prince William Sound Control Locations				262	
Ellamar	Oil	22-Apr-89	Littleneck Clam	17	16
FoxFarm	Oil	6-May-89	Littleneck Clam	20	20
Gibbon Anchorage	Oil	3-May-89	Littleneck Clam	21	21
Gibbon Anchorage	Oil	23-Apr-90	Littleneck Clam	22	22
Gibbon Anchorage	Oil	6-Sep-91	Littleneck Clam	23	0
Green Island	Oil	24-Apr-90	Littleneck Clam	20	20
Horseshoe Bay	Oil	27-Apr-90	Littleneck Clam	11	11
Horseshoe Bay	Oil	7-Sep-91	Littleneck Clam	25	0
North Chenaga	Oil	28-Apr-90	Littleneck Clam	17	15
Outside Bay	Oil	24-Apr-89	Littleneck Clam	14	14
Snug Harbor	Oil	4-May-89	Littleneck Clam	12	11
Snug Harbor	Oil	4-Aug-89	Littleneck Clam	16	16
Snug Harbor	Oil	25-Apr-90	Littleneck Clam	4	4
Wilson Bay	Oil	5-May-89	Littleneck Clam	12	0
Wilson Bay	Oil	3-Aug-89	Littleneck Clam	10	10
Wilson Bay	Oil	26-Apr-90	Littleneck Clam	12	10
Wilson Bay	Oil	8-Sep-91	Littleneck Clam	25	0
Total - Littleneck Clams				281	190
Ellamar	Oil	22-Apr-89	Butter Clam	9	8
FoxFarm	Oil	6-May-89	Butter Clam	10	0
Gibbon Anchorage	Oil	3-May-89	Butter Clam	10	0
Gibbon Anchorage	Oil	23-Apr-90	Butter Clam	9	9
Horseshoe Bay	Oil	27-Apr-90	Butter Clam	8	8
Outside Bay	Oil	24-Apr-89	Butter Clam	8	8
Snug Harbor	Oil	4-May-89	Butter Clam	5	0
Snug Harbor	Oil	25-Apr-90	Butter Clam	3	3
Wilson Bay	Oil	5-May-89	Butter Clam	8	0
Wilson Bay	Oil	26-Apr-90	Butter Clam	9	9
Total - Butter Clams				79	45
Total - Prince William Sound Oil Locations				360	235
Total - Prince William Sound All Locations				622	235

Table 9. (Page 2 of 2).

Location	Oil/ Control	Date	Species	Number Collected	Number Examined
Cook Inlet and Outer Kenai Peninsula					
Jakolof Bay	Control	16-Aug-89	Littleneck Clam	19	0
Jakolof Bay	Control	25-Jun-90	Littleneck Clam	22	22
Seldovia Bay	Control	17-Aug-89	Littleneck Clam	16	0
Seldovia Bay	Control	20-Jun-90	Littleneck Clam	23	23
Tutka Bay	Control	24-Jun-90	Littleneck Clam	21	18
Total - Littleneck Clams				101	63
Port Dick	Oil	21-Aug-89	Littleneck Clam	13	0
Port Dick	Oil	22-Jun-90	Littleneck Clam	21	21
Tonsina Bay	Oil	21-Jun-90	Littleneck Clam	20	17
Windy Bay	Oil	20-Aug-89	Littleneck Clam	19	0
Windy Bay	Oil	23-Jun-90	Littleneck Clam	16	15
Total - Littleneck Clams				89	53
Total - Cook Inlet and Outer Kenai Peninsula All Locations				190	116
Kodiak Island Area					
McDonald Lagoon	Control	16-Sep-89	Littleneck Clam	17	0
Port Bailey	Control	15-Sep-89	Littleneck Clam	18	0
Port Bailey	Control	22-Jul-90	Littleneck Clam	20	17
Total - Littleneck Clams				55	17
Port Bailey	Control	15-Sep-89	Butter Clam	16	0
Port Bailey	Control	22-Jul-90	Butter Clam	15	0
Total - Butter Clams				31	0
Total - Kodiak Island Area Control Locations				86	17
Kupreanof Strait	Oil	14-Sep-89	Littleneck Clam	17	0
Kupreanof Strait	Oil	21-Jul-90	Littleneck Clam	14	13
Ruth Bay	Oil	17-Sep-89	Littleneck Clam	14	0
Total - Littleneck Clams				45	13
Kupreanof Strait	Oil	21-Jul-90	Butter Clam	23	0
Total - Butter Clams				23	0
Total - Kodiak Island Area Oil Locations				68	13
Total - Kodiak Island Area All Locations				154	30
Alaska Peninsula					
Augustine	Control	7-Aug-90	Razor Clam	20	0
Chinitna	Control	8-Aug-90	Razor Clam	20	0
Crescent Bay	Control	6-Aug-90	Razor Clam	20	0
Total				60	0
Kashvik Bay	Oil	19-Aug-90	Razor Clam	20	0
Chiniak Bay	Oil	21-Aug-90	Razor Clam	20	0
Hallo Bay	Oil	20-Aug-90	Razor Clam	20	0
Total				60	0
Total - Alaska Peninsula All Locations				120	0
Total - Cook Inlet, Kodiak Island, and Alaska Peninsula				464	146
Total - All Areas				1,086	381

Table 10. Histopathological conditions of littleneck clams collected at sampling locations for Fish/Shellfish Study 13 in Prince William Sound, Cook Inlet, outer Kenai Peninsula, and the Kodiak Island Area, Alaska, 1989-1990. The number of normal clams plus those with histopathological conditions may exceed the number of clams examined because a single clam can have multiple conditions.

					Histopathological Conditions									
					Inflammatory Reaction		Degenerative Change		Expansive and Non-Neoplastic Change		Obstructive and Displacement Change		Parasites	
Location	Date Sampled	Number Examined	Normal n	Normal %	n	%	n	%	n	%	n	%	n	%
Prince William Sound														
Control Sites - 1989														
Hell's Hole	Apr-89	17	15	88.2	0	0.0	1	5.9	0	0.0	1	5.9	1	5.9
Hell's Hole	Aug-89	19	16	84.2	0	0.0	0	0.0	0	0.0	1	5.3	2	10.5
Pellow Cove	Apr-89	11	7	63.6	0	0.0	2	18.2	1	9.1	0	0.0	3	27.3
Simpson Bay	Apr-89	13	12	92.3	0	0.0	2	15.4	0	0.0	0	0.0	0	0.0
Simpson Bay	Aug-89	13	9	69.2	1	7.7	0	0.0	1	7.7	3	23.1	4	30.8
Total		**73**	**59**	**80.8**	**1**	**1.4**	**5**	**6.8**	**2**	**2.7**	**5**	**6.8**	**10**	**13.7**
Control Sites - 1990														
Double Bay	Apr-90	21	10	47.6	1	4.8	2	9.5	1	4.8	7	33.3	8	38.1
Hell's Hole	Apr-90	25	8	32.0	1	4.0	0	0.0	2	8.0	8	32.0	8	32.0
Simpson Bay	Apr-90	18	9	50.0	2	11.1	2	11.1	0	0.0	6	33.3	9	50.0
Total		**64**	**27**	**42.2**	**4**	**6.3**	**4**	**6.3**	**3**	**4.7**	**21**	**32.8**	**25**	**39.1**
Oiled Sites - 1989														
Ellamar	Apr-89	16	11	68.8	2	12.5	2	12.5	0	0.0	1	6.3	0	0.0
Fox Farm	May-89	20	16	80.0	1	5.0	3	15.0	0	0.0	0	0.0	0	0.0
Gibbon Anchorage	May-89	21	20	95.2	1	4.8	1	4.8	0	0.0	0	0.0	0	0.0
Outside Bay	Apr-89	14	10	71.4	1	7.1	3	21.4	0	0.0	0	0.0	0	0.0
Snug Harbor	May-89	11	10	90.9	0	0.0	0	0.0	1	9.1	0	0.0	0	0.0
Snug Harbor	Aug-89	16	12	75.0	0	0.0	0	0.0	0	0.0	1	6.3	4	25.0
Wilson Bay	Aug-89	10	10	100.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
Total		**108**	**89**	**82.4**	**5**	**4.6**	**9**	**8.3**	**1**	**0.9**	**2**	**1.9**	**4**	**3.7**
Oiled Sites -1990														
Gibbon Anchorage	Apr-90	22	15	68.2	0	0.0	4	18.2	1	4.5	0	0.0	4	18.2
Green Island	Apr-90	20	15	75.0	0	0.0	1	5.0	1	5.0	3	15.0	3	15.0
Horseshoe Bay	Apr-90	11	11	100.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
North Chenega	Apr-90	15	4	26.7	0	0.0	0	0.0	0	0.0	10	66.7	11	73.3
Snug Harbor	Apr-90	4	2	50.0	0	0.0	1	25.0	1	25.0	0	0.0	1	25.0
Wilson Bay	Apr-90	10	5	50.0	0	0.0	1	10.0	2	20.0	5	50.0	5	50.0
Total		**82**	**52**	**63.4**	**0**	**0.0**	**7**	**8.5**	**5**	**6.1**	**18**	**22.0**	**24**	**29.3**
Cook Inlet and Outer Kenai Peninsula														
Control Sites - 1990														
Jakolof Bay	Jun-90	22	8	36.4	0	0.0	9	40.9	1	4.5	7	31.8	8	36.4
Seldovia Bay	Jun-90	21	12	57.1	2	9.5	4	19.0	2	9.5	6	28.6	8	38.1
Tutka Bay	Jun-90	21	9	42.9	0	0.0	2	9.5	2	9.5	9	42.9	9	42.9
Total		**64**	**29**	**45.3**	**2**	**3.1**	**15**	**23.4**	**5**	**7.8**	**22**	**34.4**	**25**	**39.1**
Oiled Sites - 1990														
Port Dick	Jun-90	21	19	90.5	0	0.0	2	9.5	1	4.8	0	0.0	0	0.0
Tonsina Bay	Jun-90	17	16	94.1	0	0.0	0	0.0	0	0.0	1	5.9	1	5.9
Windy Bay	Jun-90	15	13	86.7	0	0.0	0	0.0	0	0.0	1	6.7	1	6.7
Total		**53**	**48**	**90.6**	**0**	**0.0**	**2**	**3.8**	**1**	**1.9**	**2**	**3.8**	**2**	**3.8**
Kodiak Island Area														
Control Sites - 1990														
Port Bailey	Jun-90	17	4	23.5	1	5.9	0	0.0	1	5.9	12	70.6	14	82.4
Oiled Sites - 1990														
Kupreanof Strait	Jun-90	13	11	84.6	0	0.0	1	7.7	1	7.7	0	0.0	1	7.7

Table 11. Histopathological conditions of butter clams collected at sampling locations for Fish/Shellfish Study 13 in Prince William Sound, Alaska, 1989-1990. The number of normal clams plus those with histopathological conditions may exceed the number of clams

					Histopathological Conditions									
					Inflammatory Reaction		Degenerative Change		Expansive and Non-Neoplastic Change		Obstructive and Displacement Change		Parasites	
Location	Date Sampled	Number Examined	Normal n	Normal %	n	%	n	%	n	%	n	%	n	%
Prince William Sound														
Control Sites - 1989														
Hell's Hole	Apr-89	13	10	76.9	0	0.0	0	0.0	0	0.0	2	15.4	3	23.1
Simpson Bay	Apr-89	11	5	45.5	0	0.0	0	0.0	1	9.1	5	45.5	6	54.5
Total		**24**	**15**	**62.5**	**0**	**0.0**	**0**	**0.0**	**1**	**4.2**	**7**	**29.2**	**9**	**37.5**
Control Sites - 1990														
Double Bay	Apr-90	6	0	0.0	0	0.0	1	16.7	0	0.0	4	66.7	4	66.7
Hell's Hole	Apr-90	12	7	58.3	0	0.0	0	0.0	0	0.0	5	41.7	5	41.7
Simpson Bay	Apr-90	7	4	57.1	0	0.0	0	0.0	1	14.3	3	42.9	3	42.9
Total		**25**	**11**	**44.0**	**0**	**0.0**	**1**	**4.0**	**1**	**4.0**	**12**	**48.0**	**12**	**48.0**
Oiled Sites - 1989														
Ellamar	Apr-89	8	5	62.5	0	0.0	0	0.0	2	25.0	2	25.0	3	37.5
Outside Bay	Apr-89	8	8	100.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
Total		**16**	**13**	**81.3**	**0**	**0.0**	**0**	**0.0**	**2**	**12.5**	**2**	**12.5**	**3**	**18.8**
Oiled Sites -1990														
Gibbon Anchorage	Apr-90	9	7	77.8	0	0.0	1	11.1	0	0.0	1	11.1	1	11.1
Horseshoe Bay	Apr-90	8	7	87.5	0	0.0	1	12.5	0	0.0	1	12.5	0	0.0
Snug Harbor	Apr-90	3	1	33.3	0	0.0	1	33.3	0	0.0	2	66.7	2	66.7
Wilson Bay	Apr-90	9	6	66.7	0	0.0	0	0.0	0	0.0	3	33.3	3	33.3
Total		**29**	**21**	**72.4**	**0**	**0.0**	**3**	**10.3**	**0**	**0.0**	**7**	**24.1**	**6**	**20.7**

Table 12. SAS output for analysis of covariance (ANCOVA) model to test the effects of hydrocarbons on the back-calculated growth of littleneck clams at transect sample locations in Prince William Sound, Alaska, 1990. The mean level of aromatic hydrocarbons in clam tissues in 1989 was used as a covariate in the model.

General Linear Models Procedure
Class Level Information

Class	Levels	Values
LOC	5	Gibbon Hells Simpson Snug Wilson
TIDE	4	0 1 2 -1

Number of observations in data set = 3936

NOTE: Due to missing values, only 1827 observations can be used in this analysis.

Dependent Variable: GROWTH

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	63	849.9782265	13.4917179	6.49	0.0001
Error	1763	3665.2510573	2.0789853		
Corrected Total	1826	4515.2292839			

R-Square	C.V.	Root MSE	GROWTH Mean
0.188247	39.33156	1.441869	3.66593322

Source	DF	Type III SS	Mean Square	F Value	Pr > F
LOC	3	10.06998914	3.35666305	1.61	0.1840
CLAM89(LOC)	4	25.71173117	6.42793279	3.09	0.0151
TIDE	3	15.95462610	5.31820870	2.56	0.0536
LOC*TIDE	6	25.64097668	4.27349611	2.06	0.0555
CLAM89(LOC)*TIDE	9	27.59188090	3.06576454	1.47	0.1517
LENGTH	1	0.33129690	0.33129690	0.16	0.6898
LENGTH*LOC	3	6.17290707	2.05763569	0.99	0.3967
CLAM89(LOC)*LENGTH	4	18.91323563	4.72830891	2.27	0.0592
LENGTH*TIDE	3	16.46124075	5.48708025	2.64	0.0481
LENGTH*LOC*TIDE	6	25.74934366	4.29155728	2.06	0.0545
CLAM89(LOC)*LENGTH*TIDE	9	33.76889039	3.75209893	1.80	0.0628

Definition of Classes Used in ANCOVA Model

LOC = Sampling location.
CLAM89(LOC) = Mean level of aromatic hydrocarbons in clam tissues collected in 1989 and nested within sampling location (used as a covariate).
TIDE = Tide height (-1, 0, 1, 2 ft).
LENGTH = Total length of littleneck clams (used as covariate).
"*" = Indicates interactive effect of classes (i.e. LOC*TIDE = interactive effect between location and tide).

Table 13. SAS output for analysis of covariance (ANCOVA) model to test the effects of hydrocarbons on the back-calculated growth of littleneck clams at transect sample locations in Prince William Sound, Alaska, 1990. The mean level of aromatic hydrocarbons in clam tissues in 1990 was used as a covariate in the model.

General Linear Models Procedure
Class Level Information

Class	Levels	Values
LOC	10	Chenega Double Gibbon Green Hells Horseshoe Pellew Simpson Snug Wilson
TIDE	4	0 1 2 -1

Number of observations in data set = 3936

NOTE: Due to missing values, only 3900 observations can be used in this analysis.

Dependent Variable: GROWTH

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	136	2492.246241	18.325340	9.51	0.0001
Error	3763	7253.034072	1.927461		
Corrected Total	3899	9745.280313			

R-Square	C.V.	Root MSE	GROWTH Mean
0.255739	39.84827	1.388330	3.48404103

Source	DF	Type III SS	Mean Square	F Value	Pr > F
LOC	7	64.2440829	9.1777261	4.76	0.0001
CLAM90(LOC)	9	44.3215051	4.9246117	2.55	0.0063
TIDE	3	7.1771202	2.3923734	1.24	0.2931
LOC*TIDE	17	125.6243284	7.3896664	3.83	0.0001
CLAM90(LOC)*TIDE	20	138.4534277	6.9226714	3.59	0.0001
LENGTH	1	1.0921949	1.0921949	0.57	0.4516
LENGTH*LOC	8	55.0217911	6.8777239	3.57	0.0004
CLAM90(LOC)*LENGTH	9	26.1662141	2.9073571	1.51	0.1387
LENGTH*TIDE	3	2.4071326	0.8023775	0.42	0.7413
LENGTH*LOC*TIDE	18	93.1461448	5.1747858	2.68	0.0001
CLAM90(LOC)*LENGTH*TIDE	21	124.1681230	5.9127678	3.07	0.0001

Definition of Classes Used in ANCOVA Model

LOC = Sampling location.
CLAM90(LOC) = Mean level of aromatic hydrocarbons in clam tissues collected in 1990 and nested within sampling location (used as a covariate).
TIDE = Tide height (-1, 0, 1, 2 ft).
LENGTH = Total length of littleneck clams (used as covariate).
"*" = Indicates interactive effect of classes (i.e. LOC*TIDE = interactive effect between location and tide).

Table 14. SAS output for analysis of covariance (ANCOVA) model to test the effects of hydrocarbons on the back-calculated growth of littleneck clams at transect sample locations in Prince William Sound, Alaska, 1990. The mean level of aromatic hydrocarbons in sediment samples in 1990 was used as a covariate in the model.

General Linear Models Procedure
Class Level Information

Class	Levels	Values
LOC	10	Chenega Double Gibbon Green Hells Horseshoe Pellew Simpson Snug Wilson
TIDE	4	0 1 2 -1

Number of observations in data set = 3936

NOTE: Due to missing values, only 3900 observations can be used in this analysis.

Dependent Variable: GROWTH

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	136	2561.003311	18.830907	9.86	0.0001
Error	3763	7184.277002	1.909189		
Corrected Total	3899	9745.280313			

R-Square	C.V.	Root MSE	GROWTH Mean
0.262794	39.65895	1.381734	3.48404103

Source	DF	Type III SS	Mean Square	F Value	Pr > F
LOC	8	75.9088243	9.4886030	4.97	0.0001
SED90(LOC)	9	97.8807056	10.8756340	5.70	0.0001
TIDE	3	21.0178603	7.0059534	3.67	0.0118
LOC*TIDE	17	102.2396476	6.0140969	3.15	0.0001
SED90(LOC)*TIDE	21	132.8879710	6.3279986	3.31	0.0001
LENGTH	1	0.0802348	0.0802348	0.04	0.8376
LENGTH*LOC	8	47.7152611	5.9644076	3.12	0.0016
SED90(LOC)*LENGTH	9	69.0305455	7.6700606	4.02	0.0001
LENGTH*TIDE	3	1.9499357	0.6499786	0.34	0.7961
LENGTH*LOC*TIDE	17	112.4061157	6.6121245	3.46	0.0001
SED90(LOC)*LENGTH*TIDE	21	131.4807623	6.2609887	3.28	0.0001

Definition of Classes Used in ANCOVA Model

LOC = Sampling location.
SED90(LOC) = Mean level of aromatic hydrocarbons in sediments collected in 1990 and nested within sampling location (used as a covariate).
TIDE = Tide height (-1, 0, 1, 2 ft).
LENGTH = Total length of littleneck clams (used as covariate).
"*" = Indicates interactive effect of classes (i.e. LOC*TIDE = interactive effect between location and tide).

Table 15. SAS output for analysis of covariance (ANCOVA) model to test the effects of hydrocarbons on the back-calculated growth of littleneck clams at transect sample locations in Prince William Sound, Alaska, 1990. The mean level of aromatic hydrocarbons in clam tissues in 1989 was used as a covariate in the model. Simpson and Double Bays were excluded from the model because of indication of contamination by refined hydrocarbons.

General Linear Models Procedure
Class Level Information

Class	Levels	Values
LOC	4	Gibbon Hells Snug Wilson
TIDE	4	0 1 2 -1

Number of observations in data set = 2923

Dependent Variable: GROWTH

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	49	804.2645363	16.4135620	7.89	0.0001
Error	1654	3441.0123855	2.0804186		
Corrected Total	1703	4245.2769218			

R-Square	C.V.	Root MSE	GROWTH Mean
0.189449	39.33813	1.442366	3.66658451

Source	DF	Type III SS	Mean Square	F Value	Pr > F
LOC	2	9.96008807	4.98004403	2.39	0.0916
CLAM89(LOC)	3	25.60420801	8.53473600	4.10	0.0065
TIDE	3	15.88753466	5.29584489	2.55	0.0545
LOC*TIDE	5	21.05247908	4.21049582	2.02	0.0725
CLAM89(LOC)*TIDE	8	22.15964029	2.76995504	1.33	0.2232
LENGTH	1	1.04676323	1.04676323	0.50	0.4782
LENGTH*LOC	2	3.81521824	1.90760912	0.92	0.3999
CLAM89(LOC)*LENGTH	3	18.47091961	6.15697320	2.96	0.0312
LENGTH*TIDE	3	16.36372821	5.45457607	2.62	0.0492
LENGTH*LOC*TIDE	5	18.86784641	3.77356928	1.81	0.1070
CLAM89(LOC)*LENGTH*TIDE	8	28.01183426	3.50147928	1.68	0.0978

Definition of Classes Used in ANCOVA Model

LOC = Sampling location.
CLAM89(LOC) = Mean level of aromatic hydrocarbons in clam tissues collected in 1989 and nested within sampling location (used as a covariate).
TIDE = Tide height (-1, 0, 1, 2 ft).
LENGTH = Total length of littleneck clams (used as covariate).
"*" = Indicates interactive effect of classes (i.e. LOC*TIDE = interactive effect between location and tide).

Table 16. SAS output for analysis of covariance (ANCOVA) model to test the effects of hydrocarbons on the back-calculated growth of littleneck clams at transect sample locations in Prince William Sound, Alaska, 1990. The mean level of aromatic hydrocarbons in clam tissues in 1990 was used as a covariatesin the model. Simpson and Double Bays were excluded from the model because of indication of contamination by refined hydrocarbons.

General Linear Models Procedure
Class Level Information

Class	Levels	Values
LOC	8	Chenega Gibbon Green Hells Horseshoe Pellew Snug Wilson
TIDE	4	0 1 2 -1

Number of observations in data set = 2923

NOTE: Due to missing values, only 2890 observations can be used in this analysis.

Dependent Variable: GROWTH

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	106	1889.140405	17.822079	8.66	0.0001
Error	2783	5727.182088	2.057917		
Corrected Total	2889	7616.322494			

R-Square	C.V.	Root MSE	GROWTH Mean
0.248038	40.28303	1.434544	3.56116263

Source	DF	Type III SS	Mean Square	F Value	Pr > F
LOC	5	59.41225302	11.88245060	5.77	0.0001
CLAM90(LOC)	7	40.29379982	5.75625712	2.80	0.0067
TIDE	3	13.12092283	4.37364094	2.13	0.0949
LOC*TIDE	13	99.39741301	7.64595485	3.72	0.0001
CLAM90(LOC)*TIDE	16	95.52586359	5.97036647	2.90	0.0001
LENGTH	1	0.92516997	0.92516997	0.45	0.5026
LENGTH*LOC	6	52.02983496	8.67163916	4.21	0.0003
CLAM90LOC)*LENGTH	7	25.61895027	3.65985004	1.78	0.0872
LENGTH*TIDE	3	3.46756940	1.15585647	0.56	0.6403
LENGTH*LOC*TIDE	14	67.89398208	4.84957015	2.36	0.0030
CLAM90(LOC)*LENGTH*TIDE	17	88.25682803	5.19157812	2.52	0.0005

Definition of Classes Used in ANCOVA Model

LOC = Sampling location.
CLAM90(LOC) = Mean level of aromatic hydrocarbons in clam tissues collected in 1990 and nested within sampling location (used as a covariate).
TIDE = Tide height (-1, 0, 1, 2 ft).
LENGTH = Total length of littleneck clams (used as covariate).
"*" = Indicates interactive effect of classes (i.e. LOC*TIDE = interactive effect between location and tide).

Table 17. SAS output for analysis of covariance (ANCOVA) model to test the effects of hydrocarbons on the back-calculated growth of littleneck clams at transect sample locations in Prince William Sound, Alaska, 1990. The mean level of aromatic hydrocarbons in sediment samples in 1990 was used as a covariate in the model. Simpson and Double Bays were excluded from the model because of indication of contamination by refined hydrocarbons.

General Linear Models Procedure
Class Level Information

Class	Levels	Values
LOC	8	Chenega Gibbon Green Hells Horseshoe Pellew Snug Wilson
TIDE	4	0 1 2 -1

Number of observations in data set = 2923

NOTE: Due to missing values, only 2890 observations can be used in this analysis.

Dependent Variable: GROWTH

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	106	1952.190656	18.416893	9.05	0.0001
Error	2783	5664.131838	2.035261		
Corrected Total	2889	7616.322494			

R-Square	C.V.	Root MSE	GROWTH Mean
0.256317	40.06068	1.426626	3.56116263

Source	DF	Type III SS	Mean Square	F Value	Pr > F
LOC	6	75.2967384	12.5494564	6.17	0.0001
SED90(LOC)	7	94.6612750	13.5230393	6.64	0.0001
TIDE	3	14.9485576	4.9828525	2.45	0.0619
LOC*TIDE	14	91.1792990	6.5128071	3.20	0.0001
SED90(LOC)*TIDE	17	109.2634998	6.4272647	3.16	0.0001
LENGTH	1	0.9582639	0.9582639	0.47	0.4927
LENGTH*LOC	6	46.5933825	7.7655638	3.82	0.0009
SED90(LOC)*LENGTH	7	66.6351070	9.5193010	4.68	0.0001
LENGTH*TIDE	3	1.3406950	0.4468983	0.22	0.8829
LENGTH*LOC*TIDE	14	97.6245070	6.9731791	3.43	0.0001
SED90(LOC)*LENGTH*TIDE	17	113.2941676	6.6643628	3.27	0.0001

Definition of Classes Used in ANCOVA Model

LOC = Sampling location.
SED90(LOC) = Mean level of aromatic hydrocarbons in sediments collected in 1990 and nested within sampling location (used as a covariate).
TIDE = Tide height (-1, 0, 1, 2 ft).
LENGTH = Total length of littleneck clams (used as covariate).
"*" = Indicates interactive effect of classes (i.e. LOC*TIDE = interactive effect between location and tide).

Table 18. Number of clams transplanted and recovered by site for the Prince William Sound reciprocal transplant experiment conducted from May to September 1990.

Site Name (Donor Site)	Marker/ Tide Ht. (m)		"A" Plot Tagged Clams Transplant	"A" Plot Tagged Clams Recover	"B" Plot Notched Clams Transplant	"B" Plot Notched Clams Recover	Days at Large
Gibbon Anchorage	1	0.90	50	48	35	31	119
(Hell's Hole)	2	0.90	50	49	25	20	119
	3	0.90	50	48	23	17	119
	4	0.45	50	47	20	18	119
	5	0.45	50	49	23	20	119
	6	0.45	50	47	27	24	119
Hell's Hole	7	0.45	50	50	12	9	121
(Gibbon Anchorage.)	8	0.45	50	49	27	16	121
	9	0.45	50	49	13	12	121
	10	0.90	50	50	11	9	120
	11	0.90	50	46	21	16	120
	12	0.90	50	44	20	11	120
Horseshoe Bay	13	0.45	50	47	20	17	106
(Double Bay)	14	0.45	50	47	22	20	106
	15	0.45	50	48	4	3	106
	16	0.90	49	41	12	11	106
	17	0.90	50	48	2	2	106
	18	0.90	50	39	6	3	106
Wilson Bay	19	0.45	51	48	6	4	107
(Simpson Bay)	20	0.45	49	48	29	20	107
	21	0.45	50	44	21	14	107
	22	0.90	50	45	2	1	107
	23	0.90	50	47	0	0	107
	24	0.90	50	47	14	6	107
Simpson Bay	25	0.45	50	42	13	11	107
(Wilson Bay)	26	0.45	50	44	22	15	107
	27	0.45	50	45	18	12	107
	28	0.90	50	48	5	4	118
	29	0.90	50	44	37	30	107
	30	0.90	50	45	12	11	118
Double Bay	31	0.45	50	46	49	42	106
(Horseshoe B.)	32	0.45	50	44	30	15	106
	33	0.45	50	48	30	18	106
	34	0.90	50	42	19	14	105
	35	0.90	50	48	43	33	105
	36	0.90	50	34	34	27	105
Totals			1,799	1,655	707	605	Mean=111

Table 19. Number of clams transplanted and recovered by site for the Prince William Sound reciprocal transplant experiment conducted from April to September, 1991.

Site Name (Donor Site)	Marker/ Tide Ht. (m)		"A" Plot Tagged Clams Transplant	"A" Plot Tagged Clams Recover	"B" Plot Notched Clams Transplant	"B" Plot Notched Clams Recover	Days at Large
Gibbon Anchorage	1	0.90	50	40	11	8	145
(Hell's Hole)	2	0.90	50	42	10	7	145
	3	0.90	50	42	50	44	145
	4	0.45	50	43	37	26	145
	5	0.45	50	40	50	47	145
	6	0.45	50	44	50	43	145
Hell's Hole	7	0.45	50	36	47	42	134
(Gibbon Anchorage.)	8	0.45	50	42	44	41	134
	9	0.45	50	41	45	35	134
	10	0.90	50	36	50	1	134
	11	0.90	50	36	50	17	134
	12	0.90	50	24	50	13	134
Horseshoe Bay	13	0.45	50	47	50	31	141
(Double Bay)	14	0.45	50	42	50	21	141
	15	0.45	50	41	50	40	141
	16	0.90	50	23	50	22	141
	17	0.90	50	35	50	36	141
	18	0.90	50	42	50	45	141
Wilson Bay	19	0.45	49	49	50	35	141
(Simpson Bay)	20	0.45	50	46	50	44	141
	21	0.45	50	24	50	22	141
	22	0.90	50	33	50	1	141
	23	0.90	50	42	50	9	141
	24	0.90	50	34	50	20	141
Simpson Bay	25	0.45	50	34	50	45	140
(Wilson Bay)	26	0.45	50	42	50	32	140
	27	0.45	50	27	50	33	140
	28	0.90	50	36	50	32	140
	29	0.90	50	46	50	28	140
	30	0.90	50	40	50	38	140
Double Bay	31	0.45	50	33	50	39	141
(Horseshoe B.)	32	0.45	50	29	50	10	141
	33	0.45	50	17	50	43	141
	34	0.90	50	37	50	17	141
	35	0.90	50	9	50	43	141
	36	0.90	50	13	50	45	141
Totals			1,799	1,287	1,694	1,055	Mean=140

Table 20. SAS output from analysis of covariance model of littleneck clam growth data from reciprocal transplant study in Prince William Sound, Alaska, 1990-1991. The covariates used in the model were total length and the level of aromatic hydrocarbons measured in littleneck clam tissues at tagging sites in 1990 and 1991 (Page 1 of 2).

Class	Levels	Values
YEAR	2	90 91
TYPE	2	A B
TIDE	2	-0.90 -0.45

Number of observations in data set = 4442

Dependent Variable: GROWTH

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	31	5060.624784	163.245961	51.72	0.0001
Error	4410	13918.741935	3.156177		
Corrected Total	4441	18979.366719			

R-Square	C.V.	Root MSE	GROWTH Mean
0.266638	72.62089	1.776563	2.44635299

Source	DF	Type III SS	Mean Square	F Value	Pr > F
YEAR	1	3.4025905	3.4025905	1.08	0.2992
TYPE	1	0.2988438	0.2988438	0.09	0.7583
YEAR*TYPE	1	8.1531981	8.1531981	2.58	0.1081
TIDE	1	110.1303223	110.1303223	34.89	0.0001
YEAR*TIDE	1	29.9626575	29.9626575	9.49	0.0021
TYPE*TIDE	1	9.8824373	9.8824373	3.13	0.0769
YEAR*TYPE*TIDE	1	6.6884339	6.6884339	2.12	0.1455
LENGTH	1	51.9808479	51.9808479	16.47	0.0001
LENGTH*YEAR	1	11.1545789	11.1545789	3.53	0.0602
LENGTH*TYPE	1	3.6970853	3.6970853	1.17	0.2792
LENGTH*YEAR*TYPE	1	0.9471738	0.9471738	0.30	0.5838
LENGTH*TIDE	1	160.8809025	160.8809025	50.97	0.0001
LENGTH*YEAR*TIDE	1	25.6504513	25.6504513	8.13	0.0044
LENGTH*TYPE*TIDE	1	3.7971629	3.7971629	1.20	0.2728
LENGT*YEAR*TYPE*TIDE	1	1.9580647	1.9580647	0.62	0.4309
THDC	1	42.7246634	42.7246634	13.54	0.0002
THDC*YEAR	1	0.0006661	0.0006661	0.00	0.9884
THDC*TYPE	1	7.4306099	7.4306099	2.35	0.1250
THDC*YEAR*TYPE	1	11.9652723	11.9652723	3.79	0.0516
THDC*TIDE	1	207.1736905	207.1736905	65.64	0.0001
THDC*YEAR*TIDE	1	0.7539830	0.7539830	0.24	0.6250
THDC*TYPE*TIDE	1	4.0618252	4.0618252	1.29	0.2567
THDC*YEAR*TYPE*TIDE	1	5.1877198	5.1877198	1.64	0.1999
LENGTH*THDC	1	70.0287205	70.0287205	22.19	0.0001
LENGTH*THDC*YEAR	1	0.5754000	0.5754000	0.18	0.6694
LENGTH*THDC*TYPE	1	6.8352057	6.8352057	2.17	0.1412
LENGT*THDC*YEAR*TYPE	1	1.2489694	1.2489694	0.40	0.5293
LENGTH*THDC*TIDE	1	308.8011206	308.8011206	97.84	0.0001
LENGTH*THDC*YEAR*TIDE	1	0.0286717	0.0286717	0.01	0.9241
LENGTH*THDC*TYPE*TIDE	1	1.0082597	1.0082597	0.32	0.5720
LEN*THDC*YEA*TYPE*TIDE	1	1.4926118	1.4926118	0.47	0.4917

Definitions of Classes Used in Model

YEAR = Year (1990 & 1991).
TYPE = Plot type (A plot & B plot clams).
TIDE = Tide Height (-0.90 & -0.45 meters below mean low water).
LENGTH = Total length (used as a covariate).
THDC = Level of aromatic hydrocarbons at tagging sites in the Spring of 1990 & 1991 (used as a covariate).

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Table 20. (Page 2 of 2).

Least Squares Means

YEAR	TYPE	TIDE	GROWTH LSMEAN	Std Err LSMEAN	Pr > |T| H0:LSMEAN=0	LSMEAN Number
90	A	-3	2.29637248	0.06898445	0.0001	1
90	A	-1.5	3.06956464	0.10737874	0.0001	2
90	B	-3	4.44876609	0.12180588	0.0001	3
90	B	-1.5	5.32386035	0.19631257	0.0001	4
91	A	-3	1.36300702	0.07165399	0.0001	5
91	A	-1.5	1.10929536	0.11199976	0.0001	6
91	B	-3	2.77428441	0.08385538	0.0001	7
91	B	-1.5	3.39133328	0.13296683	0.0001	8

T for H0: LSMEAN(i)=LSMEAN(j) / Pr > |T|

i/j	1	2	3	4	5	6	7	8
1	.	-6.05814	-15.376	-14.5496	9.383924	9.024449	-4.40129	-7.30965
		0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
2	6.058144	.	-8.49373	-10.0746	13.2198	12.63398	2.16732	-1.88267
	0.0001		0.0001	0.0001	0.0001	0.0001	0.0303	0.0598
3	15.37599	8.49373	.	-3.78778	21.83547	20.18162	11.32327	5.064062
	0.0001	0.0001		0.0002	0.0001	0.0001	0.0001	0.0001
4	14.5496	10.07459	3.787779	.	18.9532	18.64732	11.94337	0.15051
	0.0001	0.0001	0.0002		0.0001	0.0001	0.0001	0.0001
5	-9.38392	-13.2198	-21.8355	-18.9532	.	1.908187	-12.7949	-13.4287
	0.0001	0.0001	0.0001	0.0001		0.0564	0.0001	0.0001
6	-9.02445	-12.634	-20.1816	-18.6473	-1.90819	.	-11.9002	-13.1264
	0.0001	0.0001	0.0001	0.0001	0.0564		0.0001	0.0001
7	4.401293	-2.16732	-11.3233	-11.9434	12.79494	11.90017	.	-3.92524
	0.0001	0.0303	0.0001	0.0001	0.0001	0.0001		0.0001
8	7.309649	1.882674	-5.86406	-8.15051	13.42866	13.12641	3.925242	.
	0.0001	0.0598	0.0001	0.0001	0.0001	0.0001	0.0001	

Table 21. SAS output from analysis of covariance model of littleneck clam growth data from reciprocal transplant study in Prince William Sound, Alaska, 1990-1991. The covariates used in the model were total length and the level of aromatic hydrocarbons measured in littleneck clam tissues at recovery sites in 1990 and 1991 (Page 1 of 2).

Class	Levels	Values
YEAR	2	90 91
TYPE	2	A B
TIDE	2	-0.90 -0.45

Number of observations in data set = 4442

Dependent Variable: GROWTH

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	31	4633.721949	149.474902	45.95	0.0001
Error	4410	14345.644769	3.252981		
Corrected Total	4441	18979.366719			

R-Square	C.V.	Root MSE	GROWTH Mean
0.244145	73.72616	1.803602	2.44635299

Source	DF	Type III SS	Mean Square	F Value	Pr > F
YEAR	1	59.1740349	59.1740349	18.19	0.0001
TYPE	1	34.8423365	34.8423365	10.71	0.0011
YEAR*TYPE	1	7.9895670	7.9895670	2.46	0.1171
TIDE	1	16.6946407	16.6946407	5.13	0.0235
YEAR*TIDE	1	47.0334984	47.0334984	14.46	0.0001
TYPE*TIDE	1	100.6690054	100.6690054	30.95	0.0001
YEAR*TYPE*TIDE	1	2.2094073	2.2094073	0.68	0.4099
LENGTH	1	0.0486006	0.0486006	0.01	0.9027
LENGTH*YEAR	1	48.2433785	48.2433785	14.83	0.0001
LENGTH*TYPE	1	27.6962933	27.6962933	8.51	0.0035
LENGTH*YEAR*TYPE	1	6.1138554	6.1138554	1.88	0.1705
LENGTH*TIDE	1	6.0789531	6.0789531	1.87	0.1717
LENGTH*YEAR*TIDE	1	29.0680140	29.0680140	8.94	0.0028
LENGTH*TYPE*TIDE	1	161.5425992	161.5425992	49.66	0.0001
LENGT*YEAR*TYPE*TIDE	1	1.7221895	1.7221895	0.53	0.4669
RHDC	1	2.0198235	2.0198235	0.62	0.4307
RHDC*YEAR	1	76.7373208	76.7373208	23.59	0.0001
RHDC*TYPE	1	28.0450814	28.0450814	8.62	0.0033
RHDC*YEAR*TYPE	1	27.7622540	27.7622540	8.53	0.0035
RHDC*TIDE	1	19.0371098	19.0371098	5.85	0.0156
RHDC*YEAR*TIDE	1	22.5405593	22.5405593	6.93	0.0085
RHDC*TYPE*TIDE	1	151.8225509	151.8225509	46.67	0.0001
RHDC*YEAR*TYPE*TIDE	1	2.3988529	2.3988529	0.74	0.3905
LENGTH*RHDC	1	2.5168347	2.5168347	0.77	0.3791
LENGTH*RHDC*YEAR	1	41.2452323	41.2452323	12.68	0.0004
LENGTH*RHDC*TYPE	1	55.4660469	55.4660469	17.05	0.0001
LENGT*RHDC*YEAR*TYPE	1	36.6816057	36.6816057	11.28	0.0008
LENGTH*RHDC*TIDE	1	14.1665061	14.1665061	4.35	0.0370
LENGTH*RHDC*YEAR*TIDE	1	9.7099018	9.7099018	2.98	0.0841
LENGTH*RHDC*TYPE*TIDE	1	229.7994927	229.7994927	70.64	0.0001
LEN*RHDC*YEAR*TYPE*TIDE	1	4.1782040	4.1782040	1.28	0.2571

Definitions of Classes Used in Model

YEAR = Year (1990 & 1991).
TYPE = Plot type (A plot & B plot clams).
TIDE = Tide Height (-0.90 and -0.45 meters below mean low water).
LENGTH = Total length (used as a covariate).
RHDC = Level of aromatic hydrocarbons at recovery sites in the Fall of 1990 & 1991 (used as a covariate).

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Table 21. (Page 2 of 2).

Least Squares Means

YEAR	TYPE	TIDE	GROWTH LSMEAN	Std Err LSMEAN	Pr > |T| H0:LSMEAN=0	LSMEAN Number
90	A	-3	1.80769594	0.07208435	0.0001	1
90	A	-1.5	2.46054294	0.11620573	0.0001	2
90	B	-3	4.43972017	0.12363290	0.0001	3
90	B	-1.5	5.32892907	0.19904163	0.0001	4
91	A	-3	1.36968387	0.07222727	0.0001	5
91	A	-1.5	2.41205653	0.10531561	0.0001	6
91	B	-3	2.76650375	0.08467533	0.0001	7
91	B	-1.5	3.40991550	0.13680563	0.0001	8

T for H0: LSMEAN(i)=LSMEAN(j) / Pr > |T|

i/j	1	2	3	4	5	6	7	8
1	.	-4.7741	-18.3913	-16.6337	4.292394	-4.73553	-8.62215	-10.3613
		0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
2	4.774099	.	-11.6647	-12.4452	7.972772	0.309169	-2.12793	-5.28904
	0.0001		0.0001	0.0001	0.0001	0.7572	0.0334	0.0001
3	18.39127	11.66467	.	-3.79496	21.44108	12.48497	11.16595	5.584828
	0.0001	0.0001		0.0001	0.0001	0.0001	0.0001	0.0001
4	16.63372	12.44523	3.794959	.	18.69851	12.95314	11.8464	7.94547
	0.0001	0.0001	0.0001		0.0001	0.0001	0.0001	0.0001
5	-4.29239	-7.97277	-21.4411	-18.6985	.	-8.16245	-12.5505	-13.1882
	0.0001	0.0001	0.0001	0.0001		0.0001	0.0001	0.0001
6	4.735529	-0.30917	-12.485	-12.9531	8.16245	.	-2.62292	-5.77975
	0.0001	0.7572	0.0001	0.0001	0.0001		0.0087	0.0001
7	8.622154	2.127928	-11.1659	-11.8464	12.55055	2.622925	.	-3.99907
	0.0001	0.0334	0.0001	0.0001	0.0001	0.0087		0.0001
8	10.36131	5.289041	-5.58483	-7.94547	13.18818	5.779748	3.999071	.
	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	

Table 22. Pairwise comparisons of least square means to test for differences in growth by ye type and tide level. Least square means were generated from analysis of coviarian (ANCOVA) model of littleneck clam growth data from reciprocal transplant study Prince William Sound, Alaska, 1990-1991. The covariates used in the model were length and the level of aromatic hydrocarbons measured in littleneck clam tissues tagging sites in 1990 and 1991.

Year	Clam Type	Tide Level (m)	LSMEAN Growth (mm)	Std Err LSMEAN Growth	Pr>|T|	LSMEAN Number
1990	"A" Plot	-0.90	2.30	0.07	0.001	1
1990	"A" Plot	-0.45	3.07	0.11	0.001	2
1990	"B" Plot	-0.90	4.45	0.12	0.001	3
1990	"B" Plot	-0.45	5.32	0.20	0.001	4
1991	"A" Plot	-0.90	1.36	0.07	0.001	5
1991	"A" Plot	-0.45	1.11	0.11	0.001	6
1991	"B" Plot	-0.90	2.77	0.08	0.001	7
1991	"B" Plot	-0.45	3.39	0.13	0.001	8

T for Ho: LSMEAN(i) = LSMEAN (j)/Pr>|T|									
LSMEAN Number i/j	Year	Clam Type	Tide Level (m)	LSMEAN Growth (mm)	Year	Clam Type	Tide Level (m)	LSMEAN Growth (mm)	Growth Difference
Comparison of Growth by Year (1990 vs. 1991)									
1-5	1990	"A" Plot	-0.90	2.30	1991	"A" Plot	-0.90	1.36	0.94
2-6	1990	"A" Plot	-0.45	3.07	1991	"A" Plot	-0.45	1.11	1.96
3-7	1990	"B" Plot	-0.90	4.45	1991	"B" Plot	-0.90	2.77	1.68
4-8	1990	"B" Plot	-0.45	5.32	1991	"B" Plot	-0.45	3.39	1.93
							Mean Difference LSMEANS		1.63
Comparison of Growth by Clam Type ("A" Plot vs. "B" Plot)									
1-3	1990	"A" Plot	-0.90	2.30	1990	"B" Plot	-0.90	4.45	-2.15
2-4	1990	"A" Plot	-0.45	3.07	1990	"B" Plot	-0.45	5.32	-2.25
5-7	1991	"A" Plot	-0.90	1.36	1991	"B" Plot	-0.90	2.77	-1.41
6-8	1991	"A" Plot	-0.45	1.11	1991	"B" Plot	-0.45	3.39	-2.28
							Mean Difference LSMEANS		-2.02
Comparison of Growth by Tide Height (-0.90 m vs. -0.45 m)									
1-2	1990	"A" Plot	-0.90	2.30	1990	"A" Plot	-0.45	3.07	-0.77
3-4	1990	"B" Plot	-0.90	4.45	1990	"B" Plot	-0.45	5.32	-0.87
5-6	1991	"A" Plot	-0.90	1.36	1991	"A" Plot	-0.45	1.11	0.25
7-8	1991	"B" Plot	-0.90	2.77	1991	"B" Plot	-0.45	3.39	-0.62
							Mean Difference LSMEANS		-0.50

* Significant if Pr<0.0625.

Table 23. Pairwise comparisons of least square means to test for differences in growth by ye type and tide level. Least square means were generated from analysis of coviarian (ANCOVA) model of littleneck clam growth data from reciprocal transplant study Prince William Sound, Alaska, 1990-1991. The covariates used in the model were length and the level of aromatic hydrocarbons measured in littleneck clam tissues recovery sites in 1990 and 1991.

Year	Clam Type	Tide Level (m)	LSMEAN Growth (mm)	Std Err LSMEAN Growth	Pr>|T|	LSMEAN Number
1990	"A" Plot	-0.90	1.81	0.07	0.001	1
1990	"A" Plot	-0.45	2.46	0.12	0.001	2
1990	"B" Plot	-0.90	4.43	0.12	0.001	3
1990	"B" Plot	-0.45	5.32	0.20	0.001	4
1991	"A" Plot	-0.90	1.37	0.07	0.001	5
1991	"A" Plot	-0.45	2.41	0.11	0.001	6
1991	"B" Plot	-0.90	2.77	0.08	0.001	7
1991	"B" Plot	-0.45	3.41	0.14	0.001	8

T for Ho: LSMEAN(i) = LSMEAN (j)/Pr>|T|									
LSMEAN Number i/j	Year	Clam Type	Tide Level (m)	LSMEAN Growth (mm)	Year	Clam Type	Tide Level (m)	LSMEAN Growth (mm)	Growth Difference
Comparison of Growth by Year (1990 vs. 1991)									
1-5	1990	"A" Plot	-0.90	1.81	1991	"A" Plot	-0.90	1.37	0.44
2-6	1990	"A" Plot	-0.45	2.46	1991	"A" Plot	-0.45	2.41	0.05
3-7	1990	"B" Plot	-0.90	4.43	1991	"B" Plot	-0.90	2.77	1.66
4-8	1990	"B" Plot	-0.45	5.32	1991	"B" Plot	-0.45	3.41	1.91
						Mean Difference LSMEANS			1.02
Comparison of Growth by Clam Type ("A" Plot vs. "B" Plot)									
1-3	1990	"A" Plot	-0.90	1.81	1990	"B" Plot	-0.90	4.43	-2.62
2-4	1990	"A" Plot	-0.45	2.46	1990	"B" Plot	-0.45	5.32	-2.86
5-7	1991	"A" Plot	-0.90	1.37	1991	"B" Plot	-0.90	2.77	-1.40
6-8	1991	"A" Plot	-0.45	2.41	1991	"B" Plot	-0.45	3.41	-1.00
						Mean Difference LSMEANS			-1.97
Comparison of Growth by Tide Height (-0.90 m vs. -0.45 m)									
1-2	1990	"A" Plot	-0.90	1.81	1990	"A" Plot	-0.45	2.46	-0.65
3-4	1990	"B" Plot	-0.90	4.43	1990	"B" Plot	-0.45	5.32	-0.89
5-6	1991	"A" Plot	-0.90	1.37	1991	"A" Plot	-0.45	2.41	-1.04
7-8	1991	"B" Plot	-0.90	2.77	1991	"B" Plot	-0.45	3.41	-0.64
						Mean Difference LSMEANS			-0.81

* Significant if Pr<0.0625.

Table 24. SAS output from analysis of covariance model of littleneck clam growth data from reciprocal transplant study in Prince William Sound, Alaska, 1990-1991. The covariates used in the model were total length and the level of aromatic hydrocarbons measured in littleneck clam tissues at tagging sites in 1990 and 1991. The sampling sites Simpson and Double Bays were omitted from this analysis because of possible contamination by refined hydrocarbons (Page 1 of 2).

Class	Levels	Values
YEAR	2	90 91
TYPE	2	A B
TIDE	2	-3 -1.5

Number of observations in data set = 1524

Dependent Variable: GROWTH

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	31	3224.508878	104.016415	35.46	0.0001
Error	1492	4376.700332	2.933445		
Corrected Total	1523	7601.209210			

R-Square	C.V.	Root MSE	GROWTH Mean
0.424210	57.15915	1.712730	2.99642388

Source	DF	Type III SS	Mean Square	F Value	Pr > F
YEAR	1	38.85092336	38.85092336	13.24	0.0003
TYPE	1	8.48805803	8.48805803	2.89	0.0891
YEAR*TYPE	1	0.00070411	0.00070411	0.00	0.9876
TIDE	1	10.31421347	10.31421347	3.52	0.0610
YEAR*TIDE	1	45.65487117	45.65487117	15.56	0.0001
TYPE*TIDE	1	1.71305762	1.71305762	0.58	0.4449
YEAR*TYPE*TIDE	1	0.20145226	0.20145226	0.07	0.7933
LENGTH	1	9.04653459	9.04653459	3.08	0.0793
LENGTH*YEAR	1	42.51982528	42.51982528	14.49	0.0001
LENGTH*TYPE	1	1.45741251	1.45741251	0.50	0.4810
LENGTH*YEAR*TYPE	1	0.39500077	0.39500077	0.13	0.7137
LENGTH*TIDE	1	12.26990528	12.26990528	4.18	0.0410
LENGTH*YEAR*TIDE	1	49.11388182	49.11388182	16.74	0.0001
LENGTH*TYPE*TIDE	1	0.04491169	0.04491169	0.02	0.9015
LENGT*YEAR*TYPE*TIDE	1	0.31395788	0.31395788	0.11	0.7436
THDC	1	2.63787233	2.63787233	0.90	0.3431
THDC*YEAR	1	36.54089713	36.54089713	12.46	0.0004
THDC*TYPE	1	3.49719323	3.49719323	1.19	0.2751
THDC*YEAR*TYPE	1	0.13282261	0.13282261	0.05	0.8315
THDC*TIDE	1	5.19263276	5.19263276	1.77	0.1836
THDC*YEAR*TIDE	1	54.23592105	54.23592105	18.49	0.0001
THDC*TYPE*TIDE	1	4.86313138	4.86313138	1.66	0.1981
THDC*YEAR*TYPE*TIDE	1	1.52985230	1.52985230	0.52	0.4703
LENGTH*THDC	1	7.01512454	7.01512454	2.39	0.1222
LENGTH*THDC*YEAR	1	52.78907359	52.78907359	18.00	0.0001
LENGTH*THDC*TYPE	1	1.25847725	1.25847725	0.43	0.5126
LENGTH*THDC*YEAR*TYPE	1	0.66755203	0.66755203	0.23	0.6334
LENGTH*THDC*TIDE	1	5.35205398	5.35205398	1.82	0.1770
LENGTH*THDC*YEAR*TIDE	1	58.05052524	58.05052524	19.79	0.0001
LENGTH*THDC*TYPE*TIDE	1	0.91039003	0.91039003	0.31	0.5776
LEN*THDC*YEAR*TYPE*TIDE	1	0.68571168	0.68571168	0.23	0.6288

Definitions of Classes Used in Model

YEAR = Year (1990 & 1991).
TYPE = Plot type (A plot & B plot clams).
TIDE = Tide Height (-0.90 and -0.45 meters below mean low water).
LENGTH = Total length (used as a covariate).
THDC = Level of aromatic hydrocarbons at tagging sites in the Spring of 1990 & 1991 (used as a covariate).

-continued-

Table 24. (Page 2 of 2).

Least Squares Means

YEAR	TYPE	TIDE	GROWTH LSMEAN	Std Err LSMEAN	Pr > |T| H0:LSMEAN=0	LSMEAN Number
90	A	-3	2.83057659	0.10275241	0.0001	1
90	A	-1.5	4.29256147	0.22427742	0.0001	2
90	B	-3	5.55760658	0.22451605	0.0001	3
90	B	-1.5	4.88382894	0.30736287	0.0001	4
91	A	-3	1.58306918	0.11626539	0.0001	5
91	A	-1.5	2.08233916	0.24131782	0.0001	6
91	B	-3	3.10408071	0.14108198	0.0001	7
91	B	-1.5	5.68464429	0.60857552	0.0001	8

T for H0: LSMEAN(i)=LSMEAN(j) / Pr > |T|

i/j	1	2	3	4	5	6	7	8
1	.	-5.92629	-11.0445	-6.33557	8.039956	2.852787	-1.56705	-4.6243
		0.0001	0.0001	0.0001	0.0001	0.0044	0.1173	0.0001
2	5.926285	.	-3.98634	-1.55397	10.72547	6.708907	4.485488	-2.14633
	0.0001		0.0001	0.1204	0.0001	0.0001	0.0001	0.0320
3	11.04454	3.986341	.	1.770163	15.71994	10.54362	9.252886	-0.19584
	0.0001	0.0001		0.0769	0.0001	0.0001	0.0001	0.8448
4	6.33557	1.553965	-1.77016	.	10.04438	7.169035	5.262487	-1.17458
	0.0001	0.1204	0.0769		0.0001	0.0001	0.0001	0.2404
5	-8.03996	-10.7255	-15.7199	-10.0444	.	-1.86388	-8.31989	-6.61991
	0.0001	0.0001	0.0001	0.0001		0.0625	0.0001	0.0001
6	-2.85279	-6.70891	-10.5436	-7.16903	1.863883	.	-3.65518	-5.50244
	0.0044	0.0001	0.0001	0.0001	0.0625		0.0003	0.0001
7	1.567052	-4.48549	-9.25289	-5.26249	8.319894	3.65518	.	-4.13079
	0.1173	0.0001	0.0001	0.0001	0.0001	0.0003		0.0001
8	4.624301	2.146333	0.195844	1.17458	6.619907	5.502438	4.130788	.
	0.0001	0.0320	0.8448	0.2404	0.0001	0.0001	0.0001	

Table 25. SAS output from analysis of covariance model of littleneck clam growth data from reciprocal transplant study in Prince William Sound, Alaska, 1990-1991. The covariates used in the model were total length and the level of aromatic hydrocarbons measured in littleneck clam tissues at recovery sites in 1990 and 1991. The sampling sites Simpson and Double Bays were omitted from this analysis because of possible contamination by refined hydrocarbons (Page 1 of 2).

Class	Levels	Values
YEAR	2	90 91
TYPE	2	A B
TIDE	2	-0.90 -0.45

Number of observations in data set = 1524

Dependent Variable: GROWTH

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	31	3224.508878	104.016415	35.46	0.0001
Error	1492	4376.700332	2.933445		
Corrected Total	1523	7601.209210			

R-Square	C.V.	Root MSE	GROWTH Mean
0.424210	57.15915	1.712730	2.99642388

Source	DF	Type III SS	Mean Square	F Value	Pr > F
YEAR	1	0.25626495	0.25626495	0.09	0.7676
TYPE	1	1.78845054	1.78845054	0.61	0.4350
YEAR*TYPE	1	32.73813323	32.73813323	11.16	0.0009
TIDE	1	4.00866592	4.00866592	1.37	0.2426
YEAR*TIDE	1	0.31187378	0.31187378	0.11	0.7444
TYPE*TIDE	1	15.17325116	15.17325116	5.17	0.0231
YEAR*TYPE*TIDE	1	43.70267662	43.70267662	14.90	0.0001
LENGTH	1	2.53204804	2.53204804	0.86	0.3530
LENGTH*YEAR	1	0.14264604	0.14264604	0.05	0.8255
LENGTH*TYPE	1	11.50786840	11.50786840	3.92	0.0478
LENGTH*YEAR*TYPE	1	45.88433781	45.88433781	15.64	0.0001
LENGTH*TIDE	1	0.41324235	0.41324235	0.14	0.7075
LENGTH*YEAR*TIDE	1	0.32284001	0.32284001	0.11	0.7401
LENGTH*TYPE*TIDE	1	15.48267147	15.48267147	5.28	0.0217
LENGT*YEAR*TYPE*TIDE	1	49.03726757	49.03726757	16.72	0.0001
RHDC	1	3.49719323	3.49719323	1.19	0.2751
RHDC*YEAR	1	0.13282261	0.13282261	0.05	0.8315
RHDC*TYPE	1	2.63787234	2.63787234	0.90	0.3431
RHDC*YEAR*TYPE	1	36.54089713	36.54089713	12.46	0.0004
RHDC*TIDE	1	4.86313139	4.86313139	1.66	0.1981
RHDC*YEAR*TIDE	1	1.52985230	1.52985230	0.52	0.4703
RHDC*TYPE*TIDE	1	5.19263276	5.19263276	1.77	0.1836
RHDC*YEAR*TYPE*TIDE	1	54.23592105	54.23592105	18.49	0.0001
LENGTH*RHDC	1	1.25847725	1.25847725	0.43	0.5126
LENGTH*RHDC*YEAR	1	0.66755203	0.66755203	0.23	0.6334
LENGTH*RHDC*TYPE	1	7.01512454	7.01512454	2.39	0.1222
LENGTH*RHDC*YEAR*TYPE	1	52.78907359	52.78907359	18.00	0.0001
LENGTH*RHDC*TIDE	1	0.91039003	0.91039003	0.31	0.5776
LENGTH*RHDC*YEAR*TIDE	1	0.68571168	0.68571168	0.23	0.6288
LENGTH*RHDC*TYPE*TIDE	1	5.35205398	5.35205398	1.82	0.1770
LEN*RHDC*YEAR*TYPE*TIDE	1	58.05052524	58.05052524	19.79	0.0001

Definitions of Classes Used in Model

YEAR = Year (1990 & 1991).
TYPE = Plot type (A plot & B plot clams).
TIDE = Tide Height (-0.90 and -0.45 meters below mean low water).
LENGTH = Total length (used as a covariate).
RHDC = Level of aromatic hydrocarbons at recovery sites in the Fall of 1990 & 1991 (used as a covariate).

-continued-

Table 25. (Page 2 of 2).

Least Squares Means

YEAR	TYPE	TIDE	GROWTH LSMEAN	Std Err LSMEAN	Pr > |T| H0:LSMEAN=0	LSMEAN Number
90	A	-3	2.76349555	0.10155575	0.0001	1
90	A	-1.5	2.87542530	0.20953879	0.0001	2
90	B	-3	5.53935977	0.22333655	0.0001	3
90	B	-1.5	4.81971402	0.29285815	0.0001	4
91	A	-3	1.21409531	0.11662576	0.0001	5
91	A	-1.5	1.17212204	0.20798118	0.0001	6
91	B	-3	3.14029025	0.14007106	0.0001	7
91	B	-1.5	5.88553881	0.67728746	0.0001	8

T for H0: LSMEAN(i)=LSMEAN(j) / Pr > |T|

i/j	1	2	3	4	5	6	7	8
1	.	-0.48069	-11.3143	-6.63367	10.01906	6.875628	-2.17784	-4.55867
		0.6308	0.0001	0.0001	0.0001	0.0001	0.0296	0.0001
2	0.48069	.	-8.69872	-5.39929	6.927739	5.769348	-1.05087	-4.24581
	0.6308		0.0001	0.0001	0.0001	0.0001	0.2935	0.0001
3	11.31426	8.698717	.	1.953965	17.16689	14.31032	9.100247	-0.48542
	0.0001	0.0001		0.0509	0.0001	0.0001	0.0001	0.6275
4	6.633672	5.399294	-1.95396	.	11.4382	10.15487	5.173319	-1.44442
	0.0001	0.0001	0.0509		0.0001	0.0001	0.0001	0.1488
5	-10.0191	-6.92774	-17.1669	-11.4382	.	0.176026	-10.568	-6.79725
	0.0001	0.0001	0.0001	0.0001		0.8603	0.0001	0.0001
6	-6.87563	-5.76935	-14.3103	-10.1549	-0.17603	.	-7.84909	-6.65266
	0.0001	0.0001	0.0001	0.0001	0.8603		0.0001	0.0001
7	2.177841	1.050866	-9.10025	-5.17332	10.56795	7.849094	.	-3.9693
	0.0296	0.2935	0.0001	0.0001	0.0001	0.0001		0.0001
8	4.558666	4.245813	0.485416	1.444419	6.797246	6.652655	3.969302	.
	0.0001	0.0001	0.6275	0.1488	0.0001	0.0001	0.0001	

Table 26. Pairwise comparisons of least square means to test for differences in growth by year, clam type and tide level. Least square means were generated from analysis of coviariance (ANCOVA) model of littleneck clam growth data from reciprocal transplant study in Prince William Sound, Alaska, 1990-1991. The covariates used in the model were total length and the level of aromatic hydrocarbons measured in littleneck clam tissues at tagging sites in 1990 and 1991. The sampling sites Simpson and Double bays were omitted from this analysis because of possible contamination by refined hydrocarbons.

Year	Clam Type	Tide Level (m)	LSMEAN Growth (mm)	Std Err LSMEAN Growth	Pr>|T|	LSMEAN Number
1990	"A" Plot	-0.90	2.83	0.10	0.001	1
1990	"A" Plot	-0.45	4.29	0.22	0.001	2
1990	"B" Plot	-0.90	5.56	0.22	0.001	3
1990	"B" Plot	-0.45	4.88	0.31	0.001	4
1991	"A" Plot	-0.90	1.58	0.12	0.001	5
1991	"A" Plot	-0.45	2.08	0.24	0.001	6
1991	"B" Plot	-0.90	3.10	0.14	0.001	7
1991	"B" Plot	-0.45	5.68	0.61	0.001	8

T for Ho: LSMEAN(i) = LSMEAN (j)/Pr>|T|										
LSMEAN Number i/j	Year	Clam Type	Tide Level (m)	LSMEAN Growth (mm)	Year	Clam Type	Tide Level (m)	LSMEAN Growth (mm)	Growth Difference	Pr>|T|
Comparison of Growth by Year (1990 vs. 1991)										
1-5	1990	"A" Plot	-0.90	2.83	1991	"A" Plot	-0.90	1.58	1.25	0.0001 *
2-6	1990	"A" Plot	-0.45	4.29	1991	"A" Plot	-0.45	2.08	2.21	0.0001 *
3-7	1990	"B" Plot	-0.90	5.56	1991	"B" Plot	-0.90	3.10	2.46	0.0001 *
4-8	1990	"B" Plot	-0.45	4.88	1991	"B" Plot	-0.45	5.68	-0.80	0.2404
						Mean Difference LSMEANS			1.28	
Comparison of Growth by Clam Type ("A" Plot vs. "B" Plot)										
1-3	1990	"A" Plot	-0.90	2.83	1990	"B" Plot	-0.90	5.56	-2.73	0.0001 *
2-4	1990	"A" Plot	-0.45	4.29	1990	"B" Plot	-0.45	4.88	-0.59	0.1204
5-7	1991	"A" Plot	-0.90	1.58	1991	"B" Plot	-0.90	3.10	-1.52	0.0001 *
6-8	1991	"A" Plot	-0.45	2.08	1991	"B" Plot	-0.45	5.68	-3.60	0.0001 *
						Mean Difference LSMEANS			-2.11	
Comparison of Growth by Tide Height (-0.90 m vs. -0.45 m)										
1-2	1990	"A" Plot	-0.90	2.83	1990	"A" Plot	-0.45	4.29	-1.46	0.0001 *
3-4	1990	"B" Plot	-0.90	5.56	1990	"B" Plot	-0.45	4.88	0.68	0.0769
5-6	1991	"A" Plot	-0.90	1.58	1991	"A" Plot	-0.45	2.08	-0.50	0.0625
7-8	1991	"B" Plot	-0.90	3.10	1991	"B" Plot	-0.45	5.68	-2.58	0.0001 *
						Mean Difference LSMEANS			-0.97	

* Significant if Pr<0.0625.

Table 27. Pairwise comparisons of least square means to test for differences in growth by year, clam type and tide level. Least square means were generated from analysis of coviariance (ANCOVA) model of littleneck clam growth data from reciprocal transplant study in Prince William Sound, Alaska, 1990-1991. The covariates used in the model were total length and the level of aromatic hydrocarbons measured in littleneck clam tissues at recovery sites in 1990 and 1991. The sampling sites Simpson and Double bays were omitted from this analysis because of possible contamination by refined hydrocarbons.

Year	Clam Type	Tide Level (m)	LSMEAN Growth (mm)	Std Err LSMEAN Growth	Pr>|T|	LSMEAN Number
1990	"A" Plot	-0.90	2.76	0.10	0.001	1
1990	"A" Plot	-0.45	2.88	0.21	0.001	2
1990	"B" Plot	-0.90	5.54	0.22	0.001	3
1990	"B" Plot	-0.45	4.82	0.29	0.001	4
1991	"A" Plot	-0.90	1.21	0.12	0.001	5
1991	"A" Plot	-0.45	1.17	0.21	0.001	6
1991	"B" Plot	-0.90	3.14	0.14	0.001	7
1991	"B" Plot	-0.45	5.89	0.68	0.001	8

T for Ho: LSMEAN(i) = LSMEAN (j)/Pr>|T|										
LSMEAN Number i/j	Year	Clam Type	Tide Level (m)	LSMEAN Growth (mm)	Year	Clam Type	Tide Level (m)	LSMEAN Growth (mm)	Growth Difference	Pr>|T|
Comparison of Growth by Year (1990 vs. 1991)										
1-5	1990	"A" Plot	-0.90	2.76	1991	"A" Plot	-0.90	1.21	1.55	0.0001 *
2-6	1990	"A" Plot	-0.45	2.88	1991	"A" Plot	-0.45	1.17	1.71	0.0001 *
3-7	1990	"B" Plot	-0.90	5.54	1991	"B" Plot	-0.90	3.14	2.40	0.0001 *
4-8	1990	"B" Plot	-0.45	4.82	1991	"B" Plot	-0.45	5.89	-1.07	0.1488
						Mean Difference LSMEANS			1.15	
Comparison of Growth by Clam Type ("A" Plot vs. "B" Plot)										
1-3	1990	"A" Plot	-0.90	2.76	1990	"B" Plot	-0.90	5.54	-2.78	0.0001 *
2-4	1990	"A" Plot	-0.45	2.88	1990	"B" Plot	-0.45	4.82	-1.94	0.0001 *
5-7	1991	"A" Plot	-0.90	1.21	1991	"B" Plot	-0.90	3.14	-1.93	0.0001 *
6-8	1991	"A" Plot	-0.45	1.17	1991	"B" Plot	-0.45	5.89	-4.72	0.0001 *
						Mean Difference LSMEANS			-2.84	
Comparison of Growth by Tide Height (-0.90 m vs. -0.45 m)										
1-2	1990	"A" Plot	-0.90	2.76	1990	"A" Plot	-0.45	2.88	-0.12	0.6308
3-4	1990	"B" Plot	-0.90	5.54	1990	"B" Plot	-0.45	4.82	0.72	0.0509 *
5-6	1991	"A" Plot	-0.90	1.21	1991	"A" Plot	-0.45	1.17	0.04	0.8603
7-8	1991	"B" Plot	-0.90	3.14	1991	"B" Plot	-0.45	5.89	-2.75	0.0001 *
						Mean Difference LSMEANS			-0.53	

* Significant if Pr<0.0625.

Table 28. Recruitment estimates for littleneck clams from transect sampling locations where collections ocurred in 1989-1990.

Location (Year)	Number of Littleneck Clams Collected Age 0	1	2	3
Kodiak Island				
Control Sites				
Port Bailey (1989)	2	115	12	30
Port Bailey (1990)	12	59	38	16
Prince William Sound				
Control Sites				
Hell's Hole (1989)	0	6	26	49
Hell's Hole (1990)	0	8	111	191
Pellew Cove (1989)	0	2	1	7
Pellew Cove (1990)	1	1	2	4
Simpson Bay (1989)	0	5	11	25
Simpson Bay (1990)	0	4	24	27
Oiled Sites				
Gibbon Anchorage (198	0	13	95	224
Gibbon Anchorage (199	0	17	112	107
Snug Harbor (1989)	0	1	5	6
Snug Harbor (1990)	0	0	2	3
Wilson Bay (1989)	0	5	16	56
Wilson Bay (1990)	0	102	115	73

	Average # 1 year olds in 1990 (=1989 year class).		Average ratio of 1990 1 year olds to 1990 0,1,2,and 3 year olds pooled.	
	Oiled	Control	Oiled	Control
Mean	39.7	16.0	0.1	0.2
SD	54.7	27.5	0.2	0.2
Number	3	4	3	4
Student t-tes	not significant		not significant	

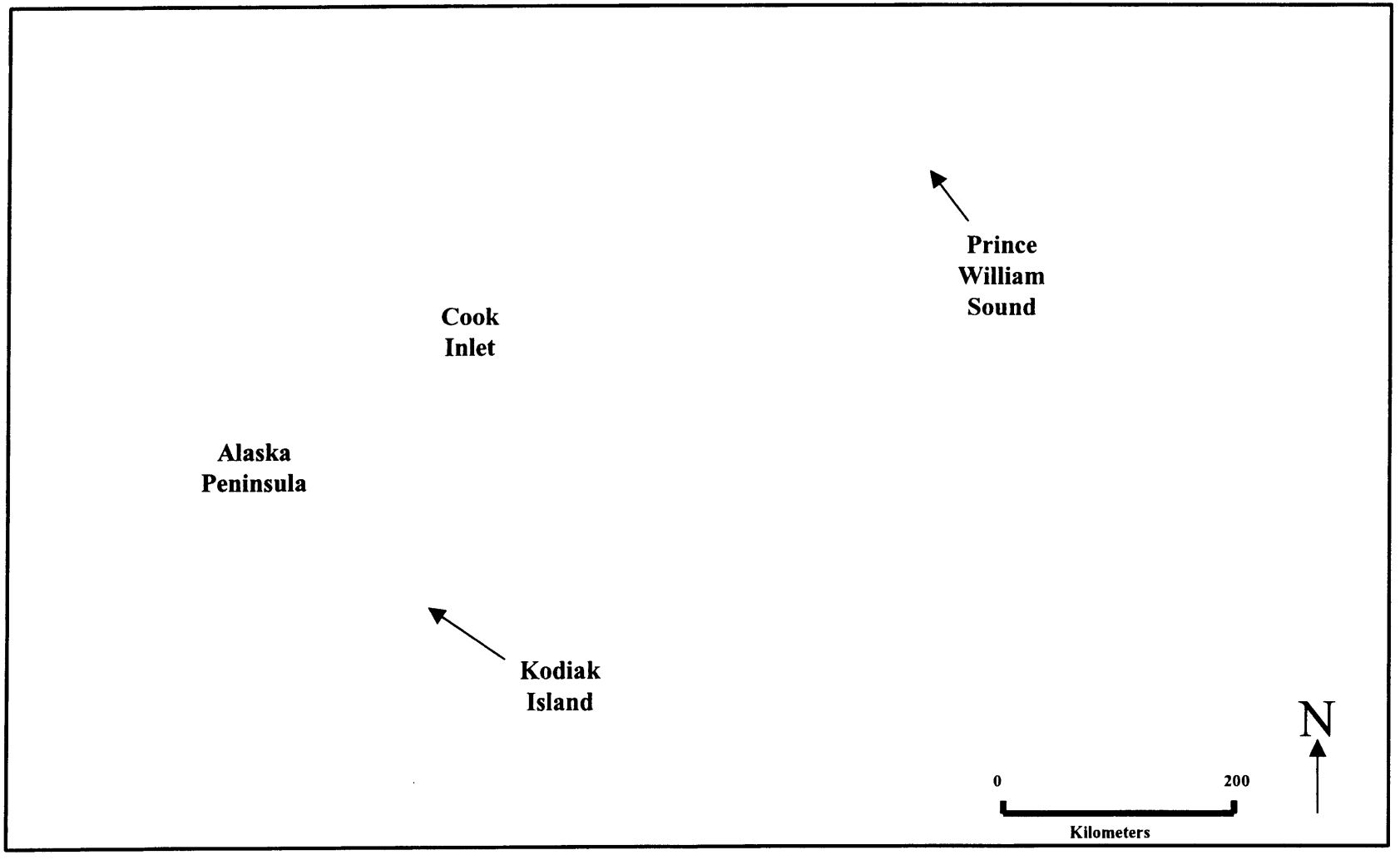

Figure 1. Map of Alaska, including Prince William Sound, Cook Inlet, Kodiak Island and the Alaska Peninsula.

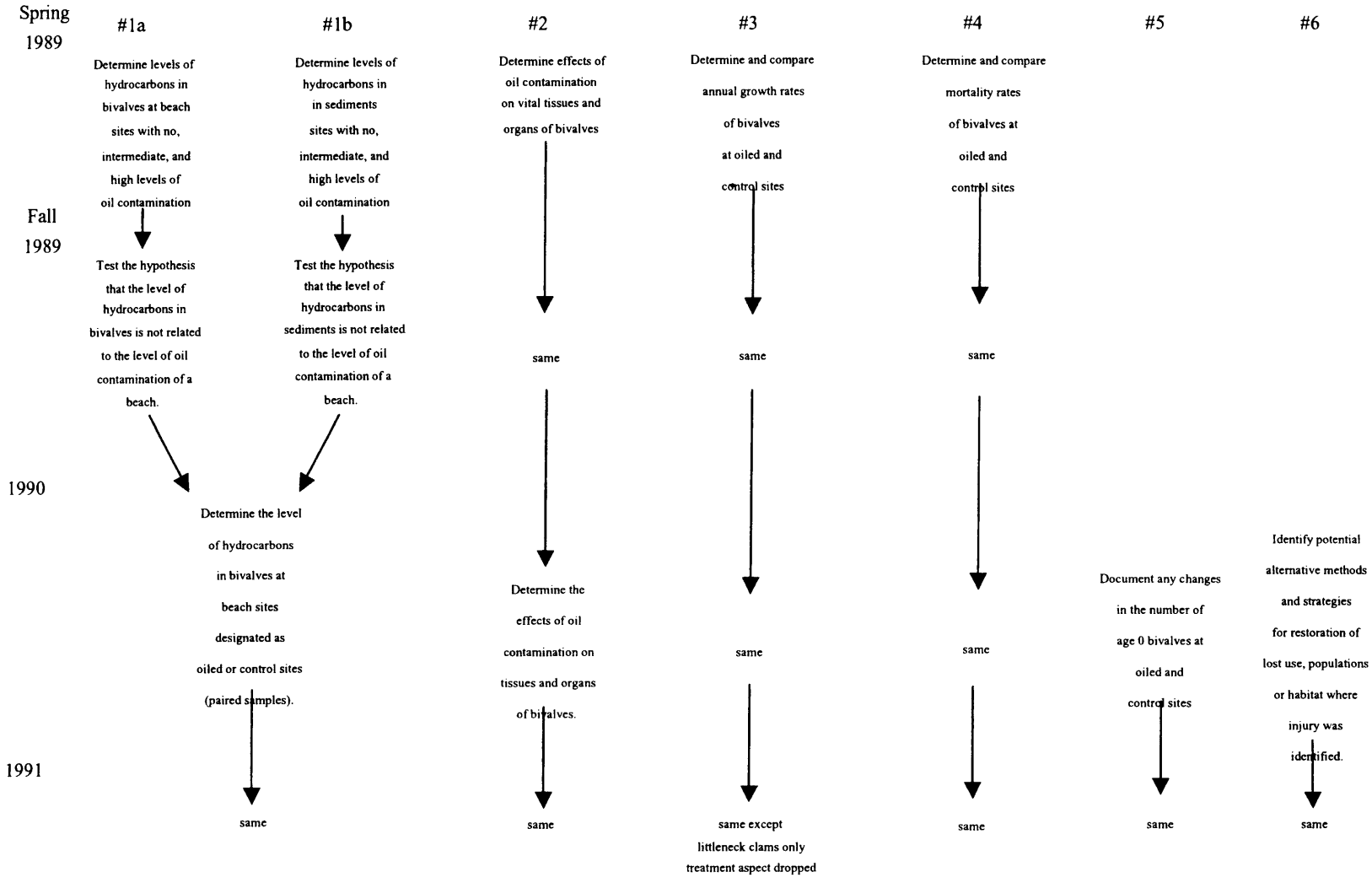

Figure 2. Summary of objectives for Fish/Shellfish Study 13 - Effects of Hydrocarbons on Bivalves, 1989-1991.

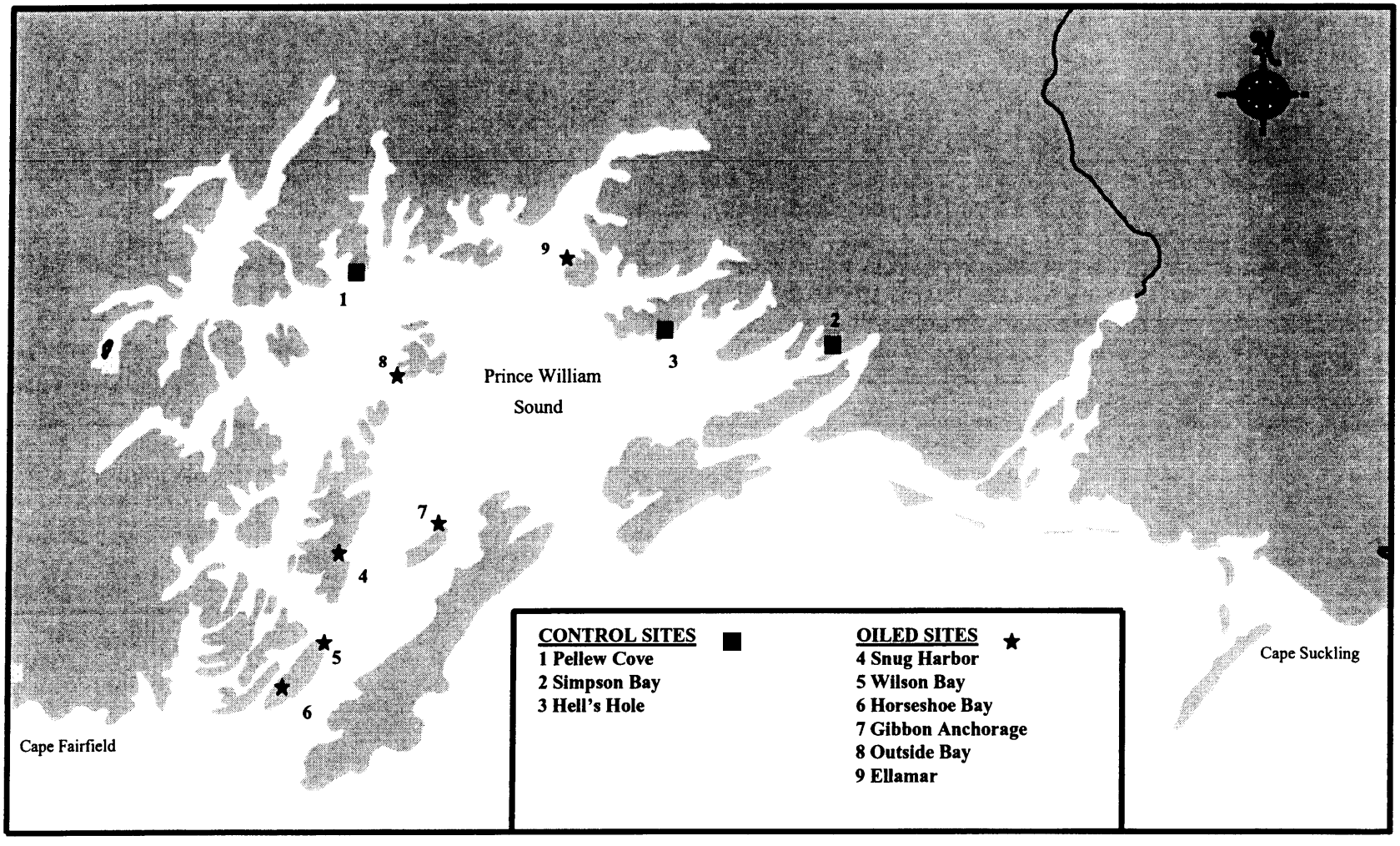

Figure 3. Transect sampling locations in Prince William Sound, Alaska, 1989.

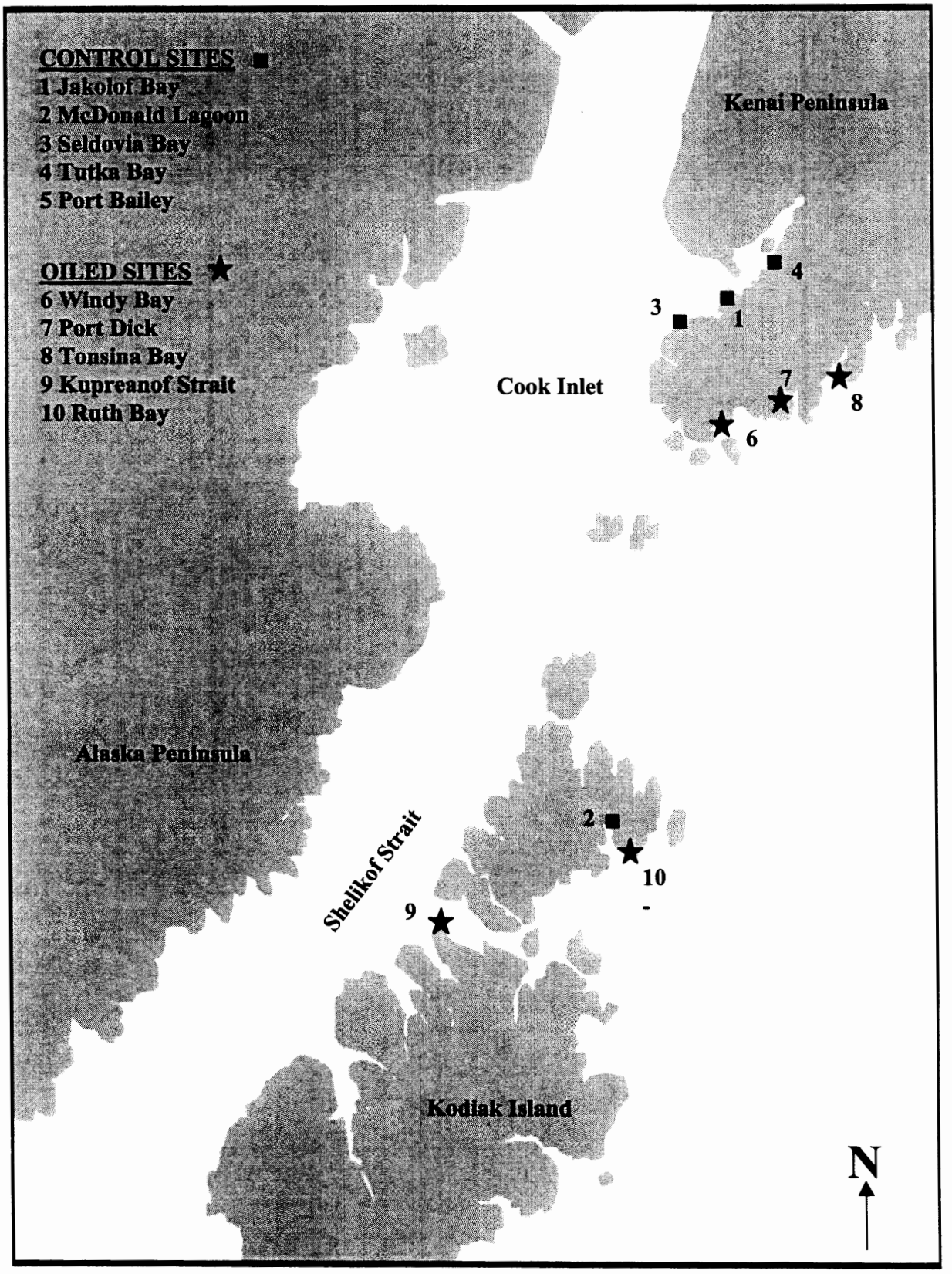

Figure 4. Transect sampling locations for butter and littleneck clams in Cook Inlet, Kodiak Island, and the Alaska Peninsula, 1990.

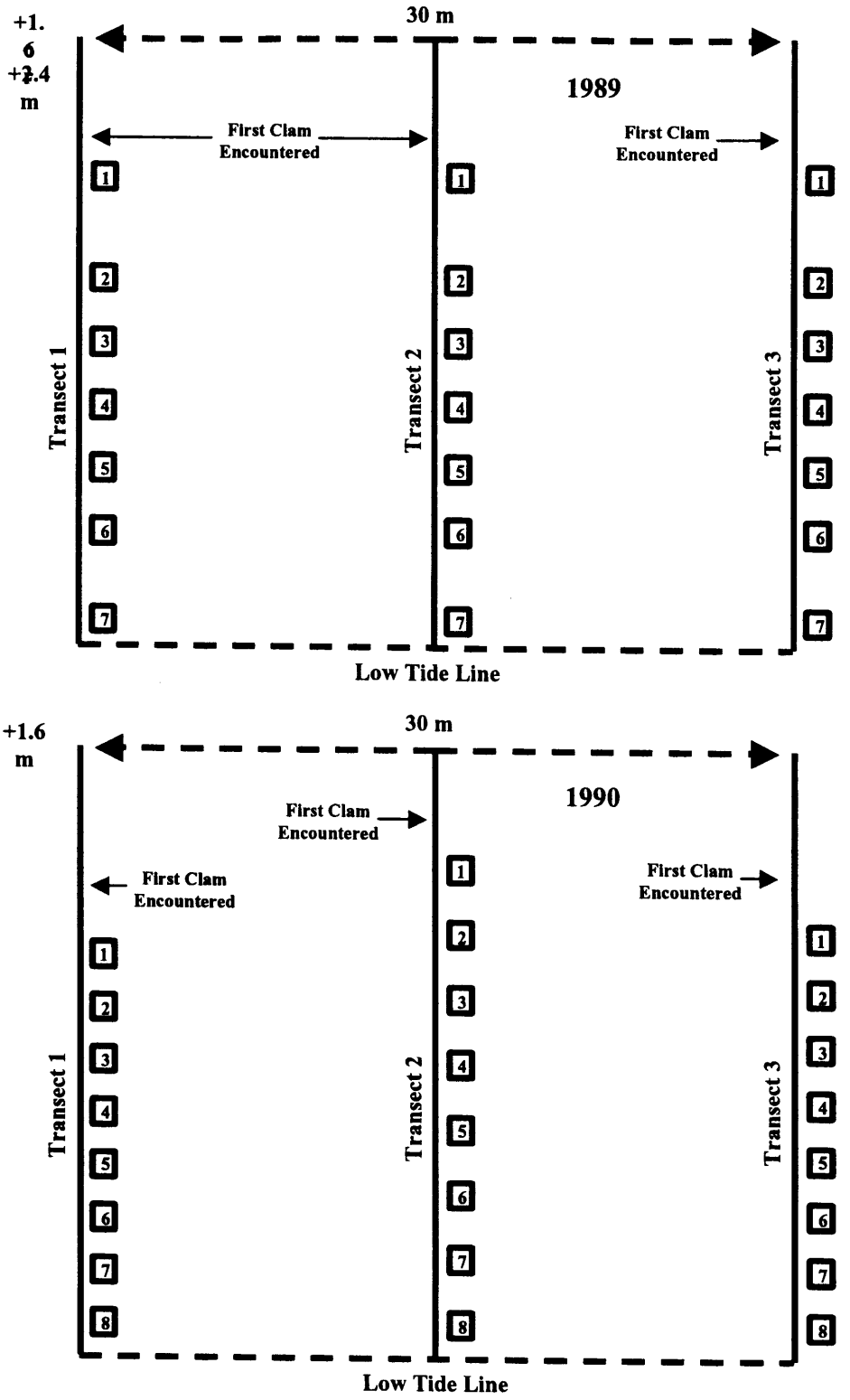

Figure 5. Placement of quadrats during transect sampling in 1989 and 1990

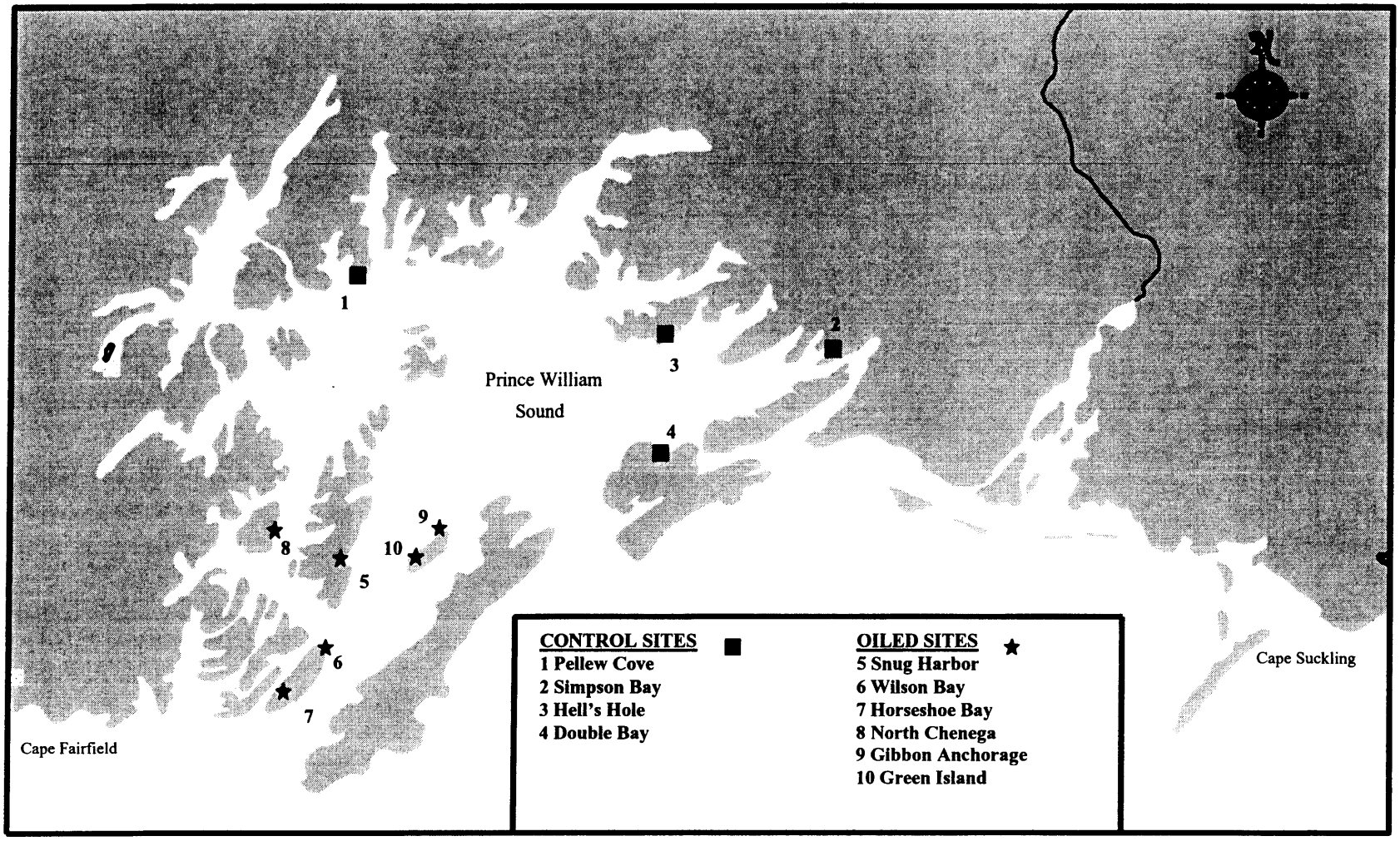

Figure 6. Transect sampling locations in Prince William Sound, Alaska, 1990.

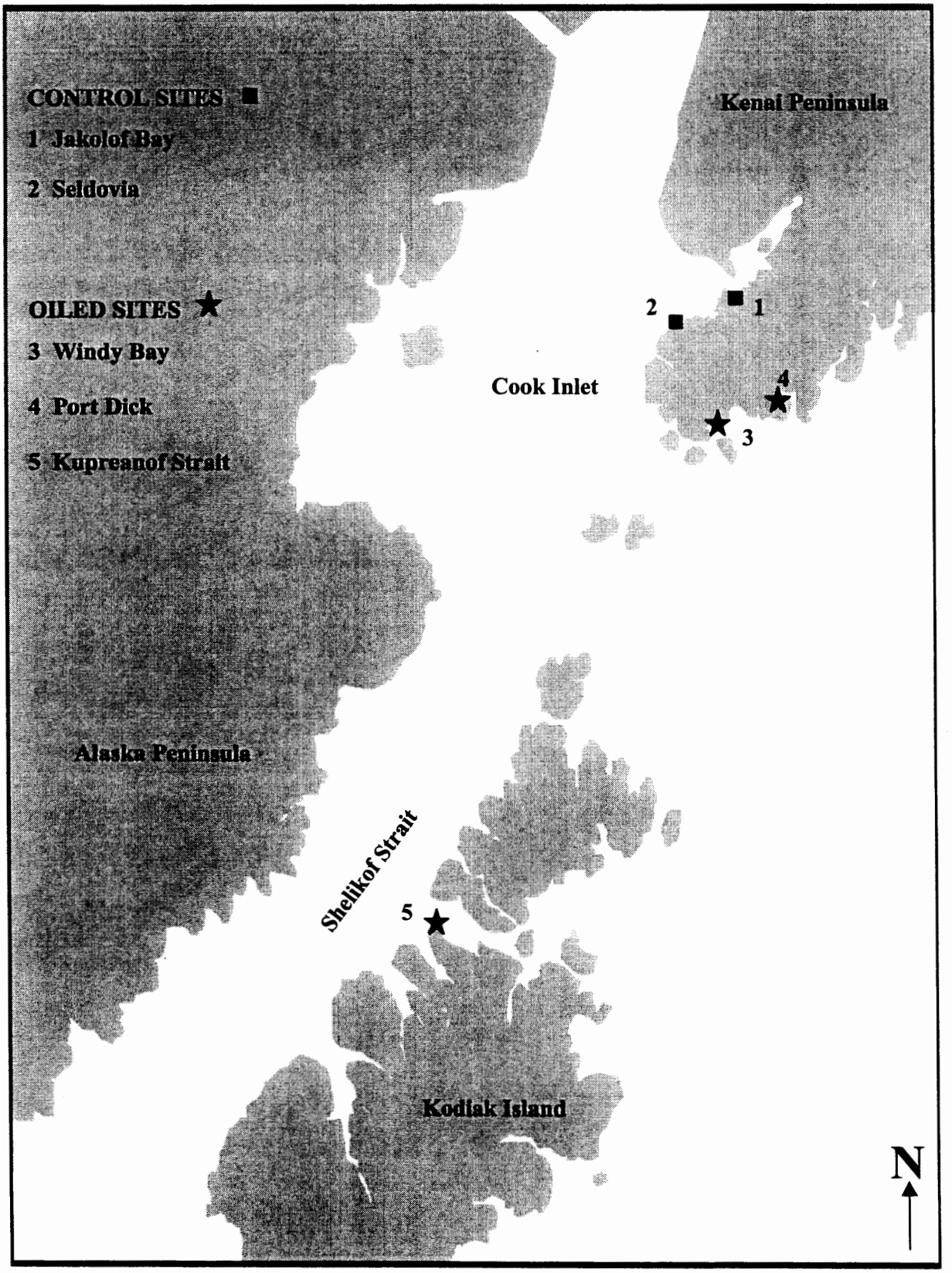

Figure 7. Transect sampling locations for butter and littleneck clams in Cook Inlet, Kodiak Island, and the Alaska Peninsula, 1990.

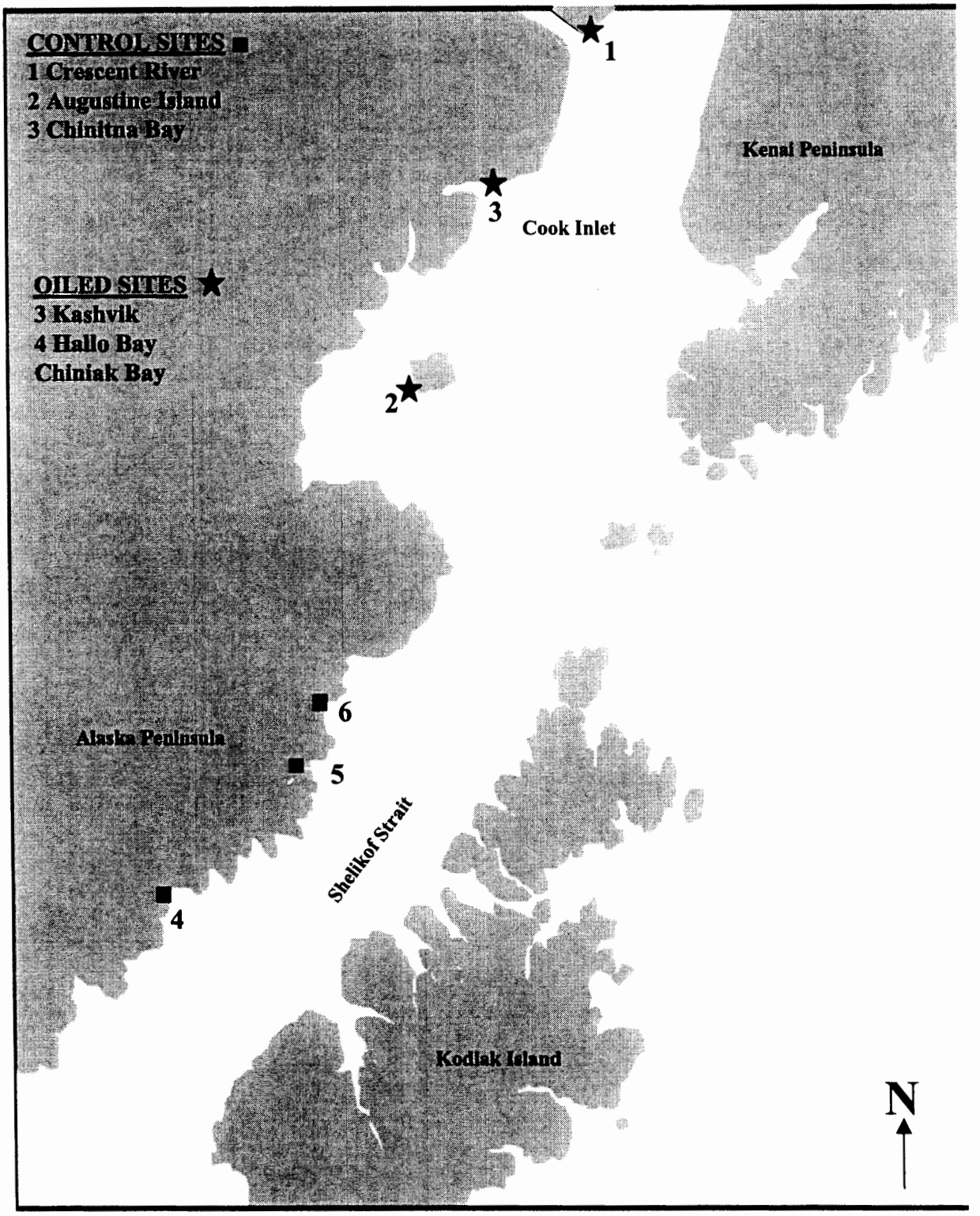

Figure 8. Transect sampling locations for razor clams in Cook Inlet, Kodiak Island, and the Alaska Peninsula, 1990.

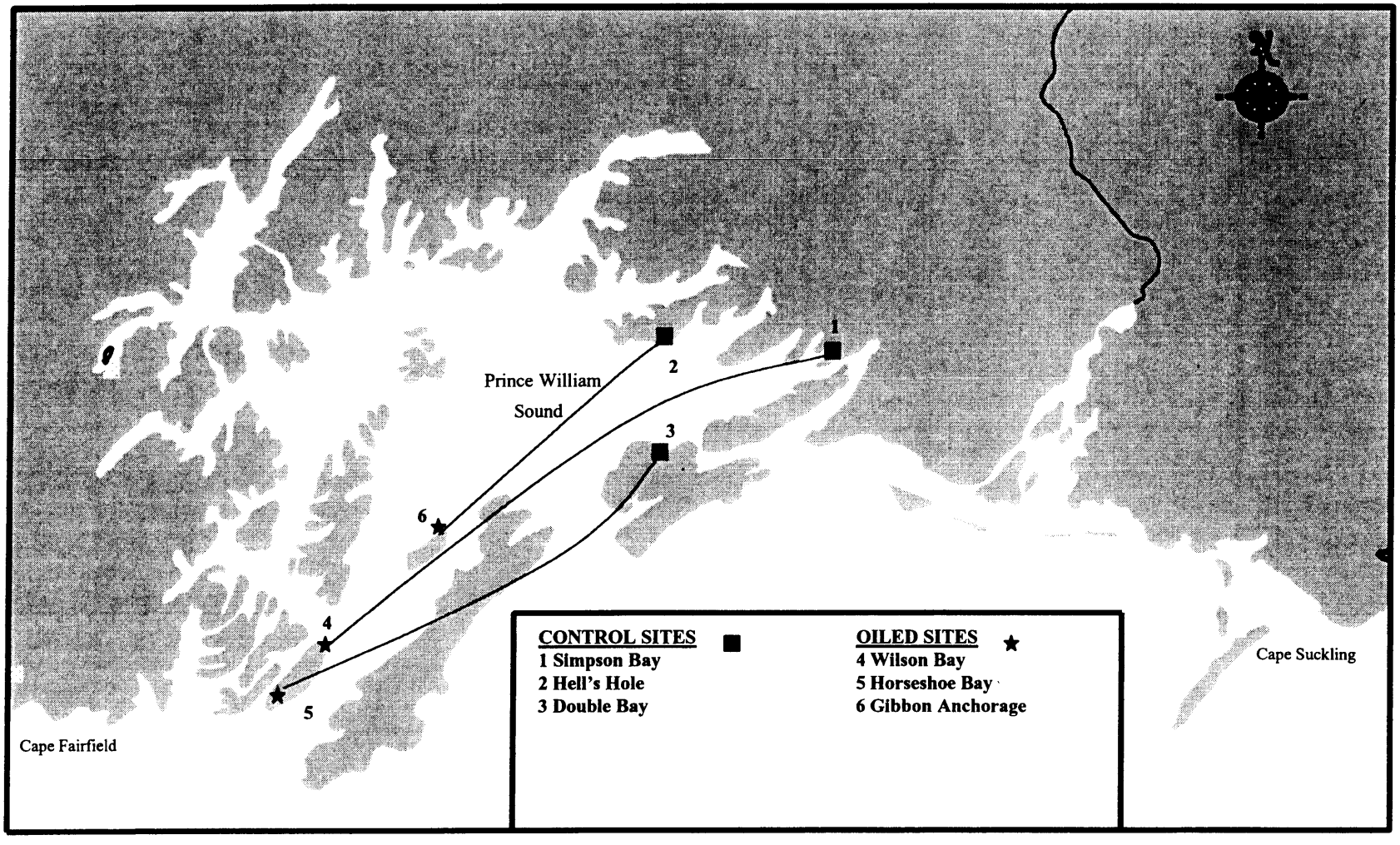

Figure 9. Sampling locations for reciprocal transplant of littleneck clams in Prince William Sound, Alaska, 1990-1991.

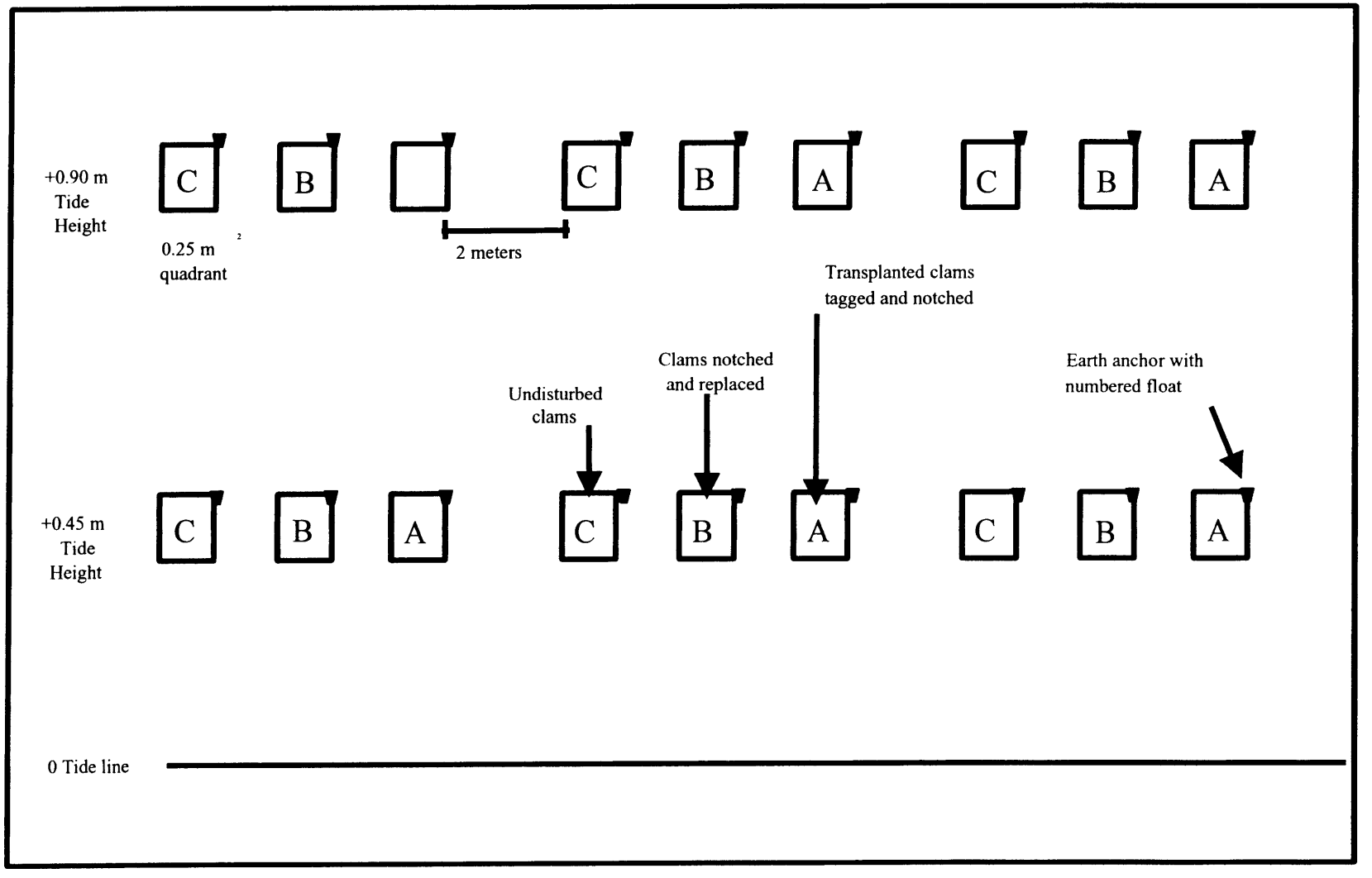

Figure 10. Beach plot configuration for reciprocal transplant of littleneck clams in Prince William Sound, Alaska, 1990-1991.

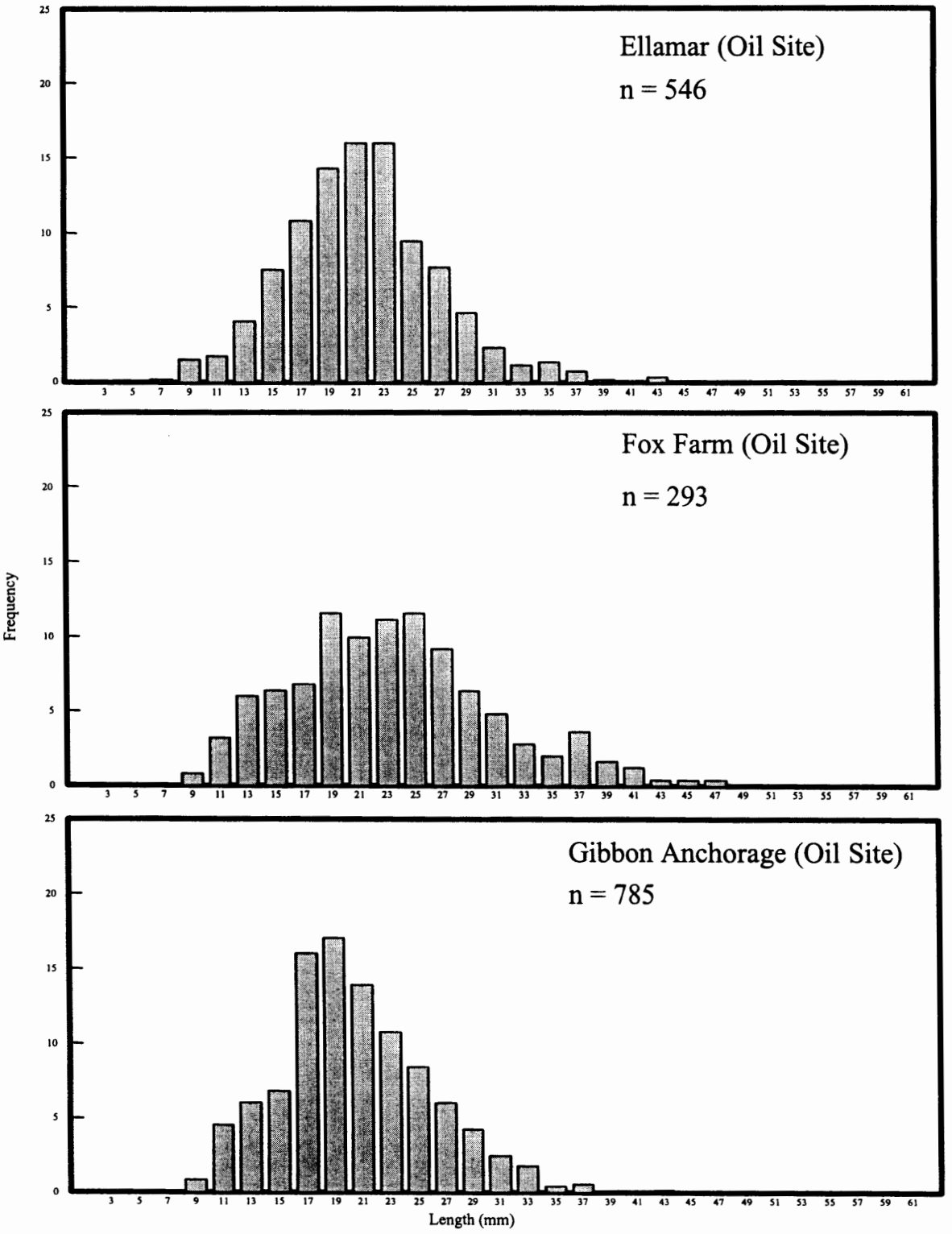

Figure 11. Length frequencies of littleneck clams collected at transect sampling locations in Prince William Sound, Alaska, 1989: Ellamar (top), Fox Farm (middle) and Gibbon Anchorage (bottom).

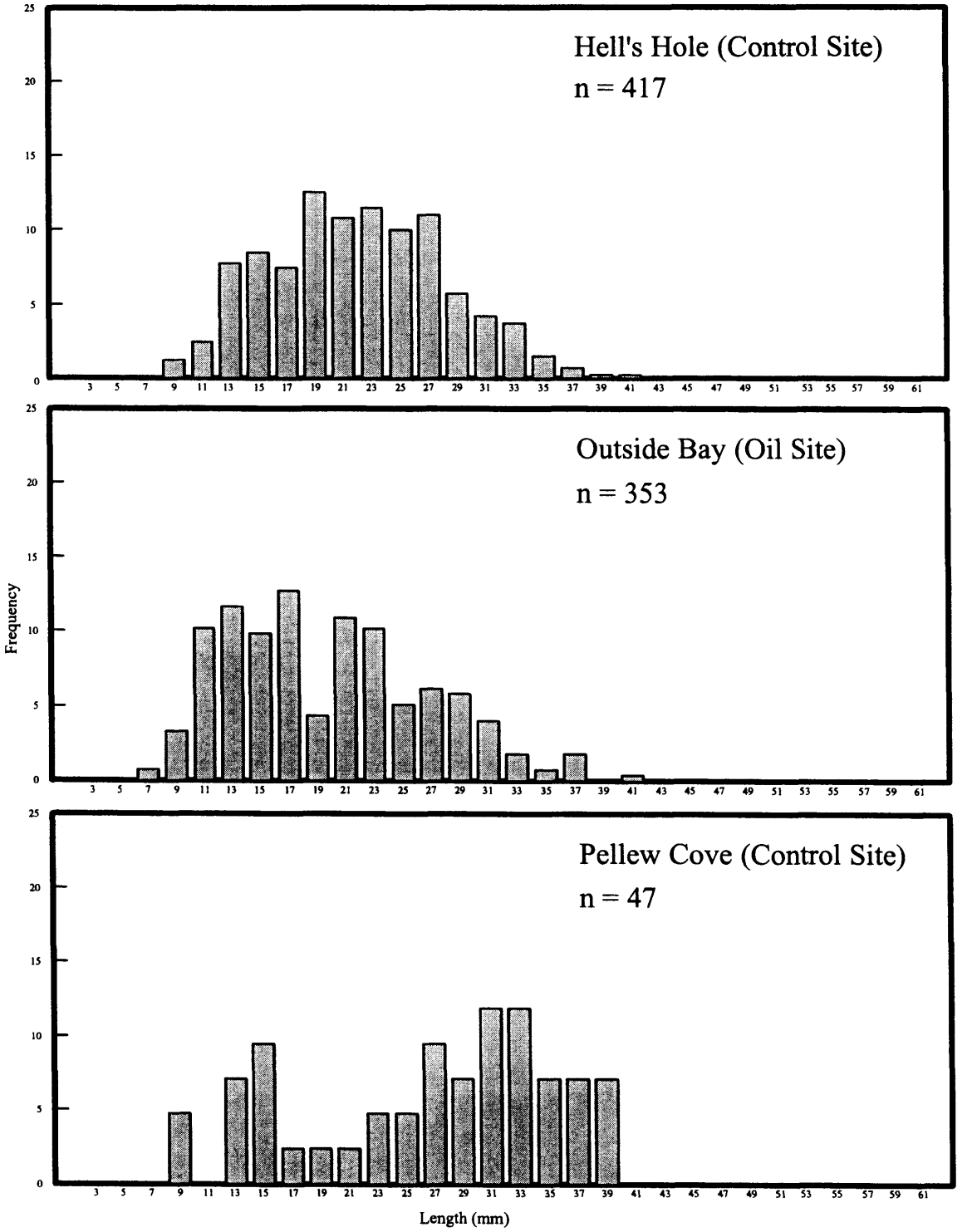

Figure 12 Length frequencies of littleneck clams collected at transect sampling locations in Prince William Sound, Alaska, 1989: Hell's Hole (top), Outside Bay (middle) and Pellew Cove (bottom).

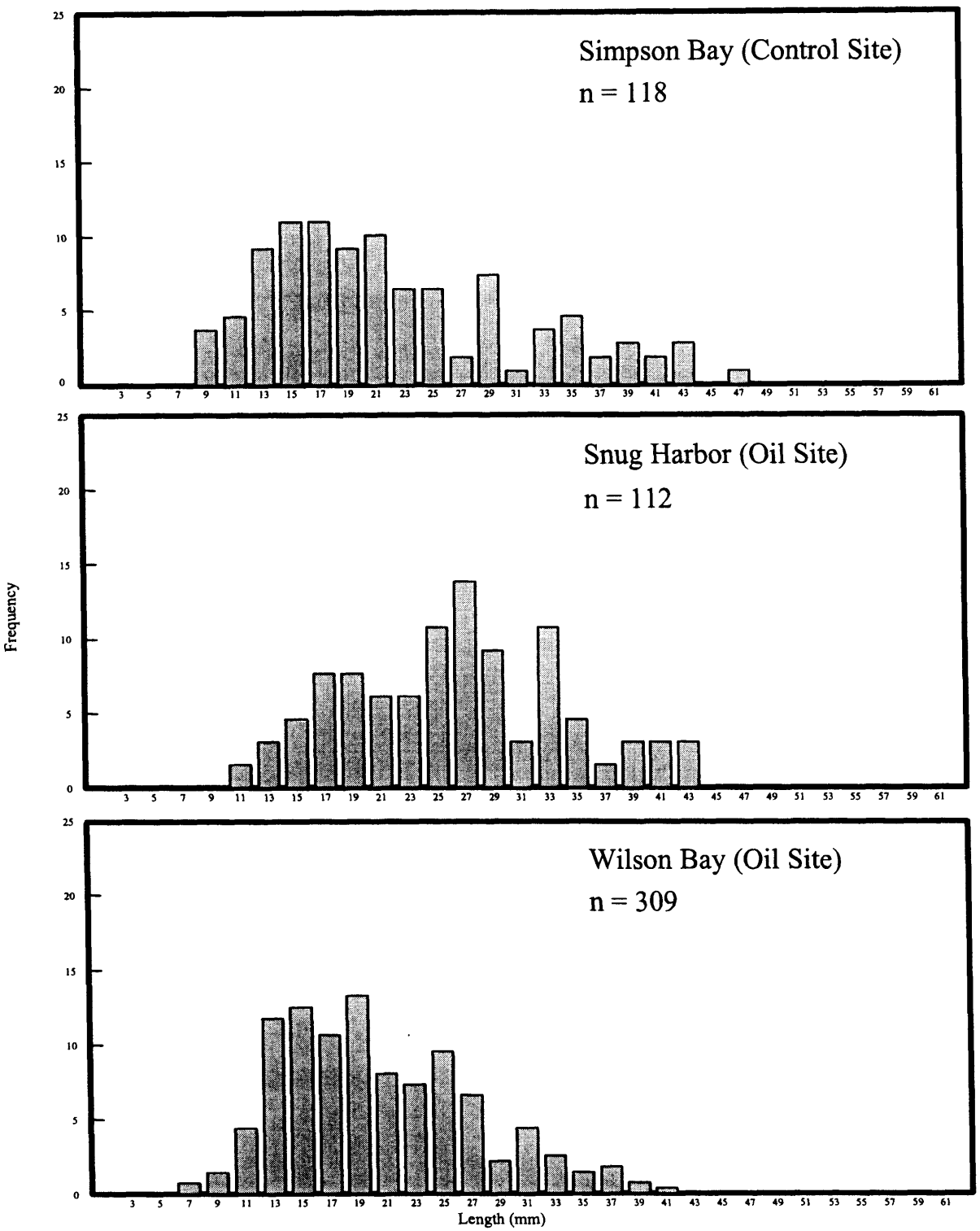

Figure 13. Length frequencies of littleneck clams collected at transect sampling locations in Prince William Sound, Alaska, 1989: Simpson Bay (top), Snug Harbor (middle) and Wilson Bay (bottom).

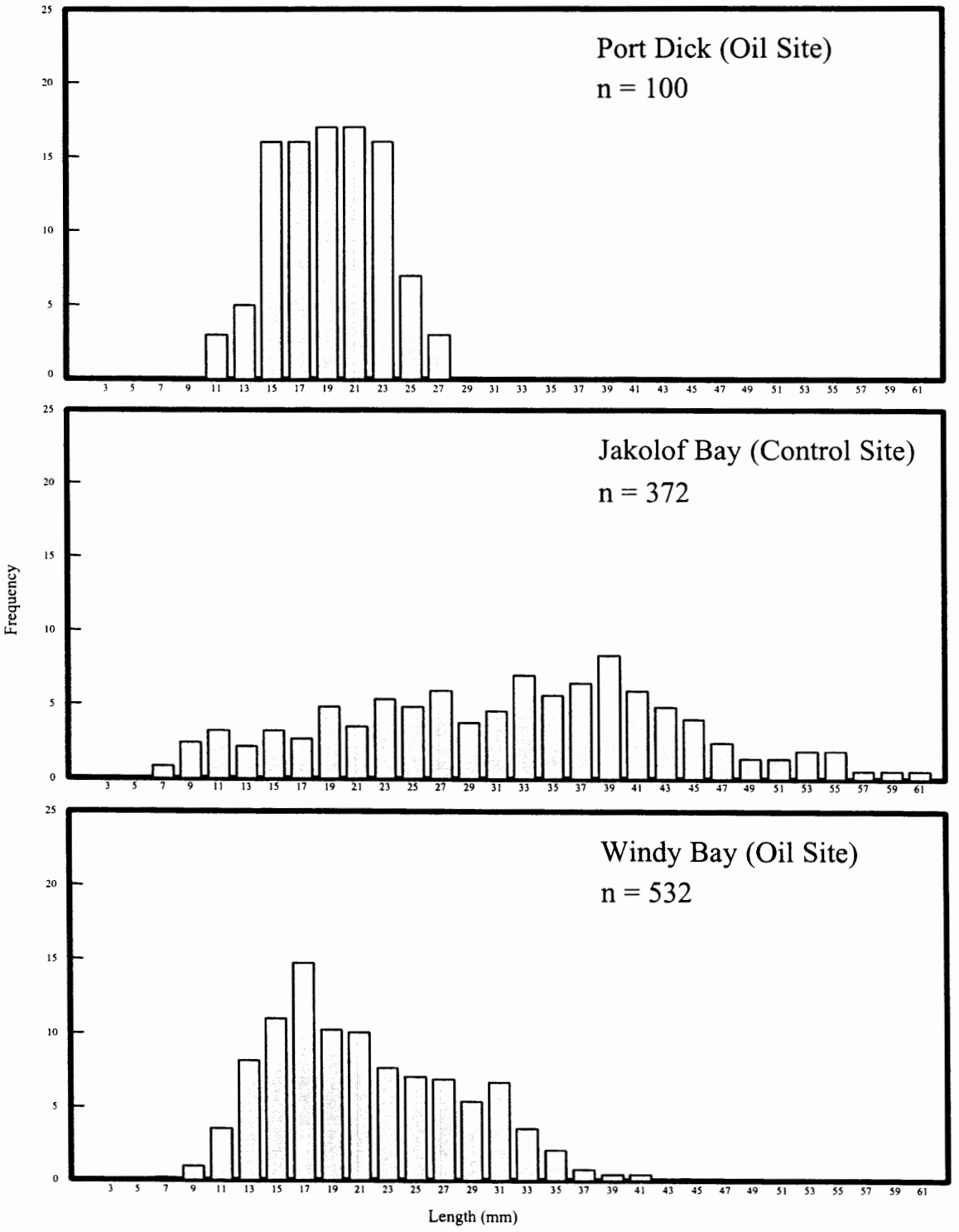

Figure 14. Length frequencies of littleneck clams collected at transect sampling locations in Cook Inlet and the outer Kenai Peninsula, Alaska, 1989: Port Dick (top), Jakolof Bay (middle) and Windy Bay (bottom).

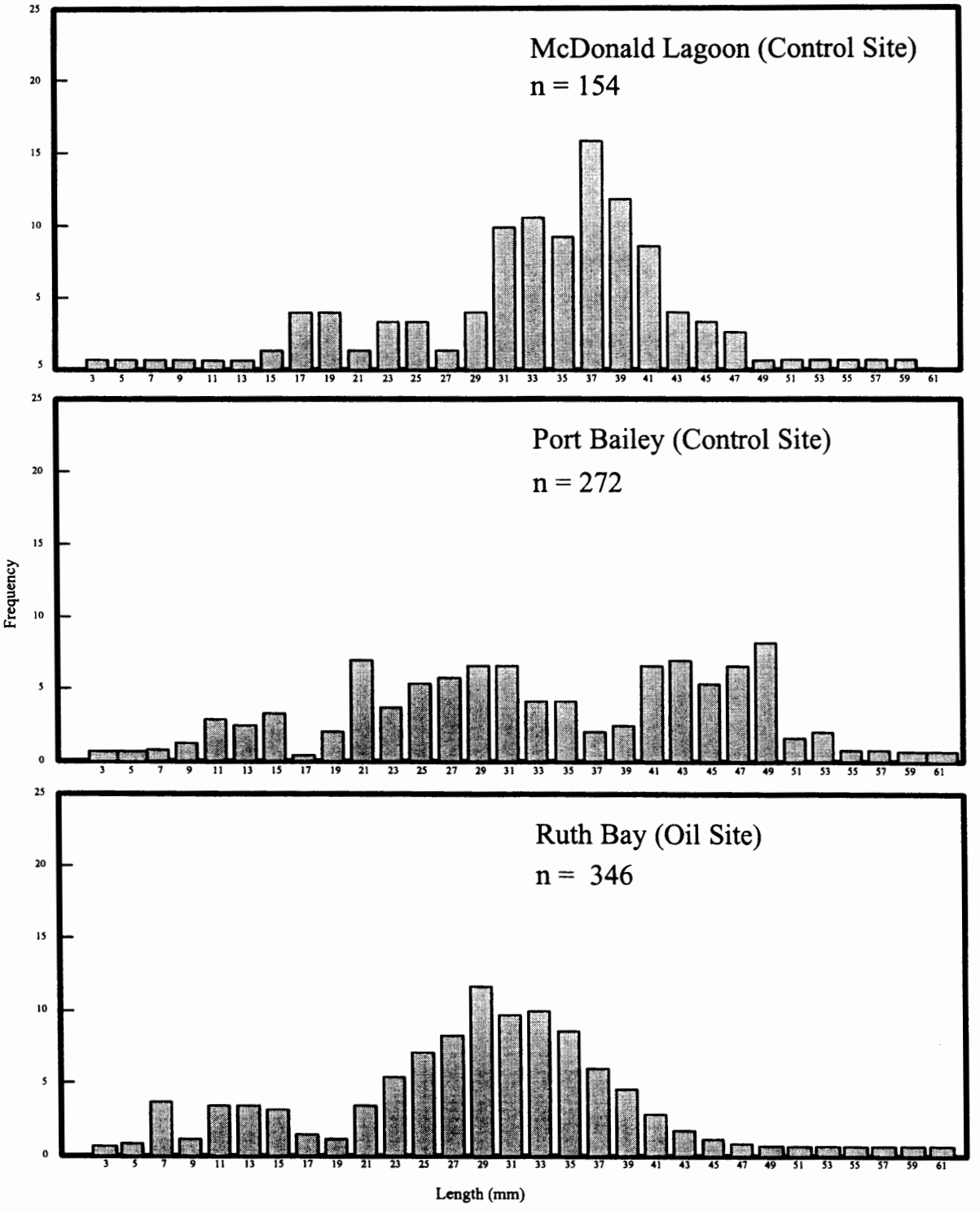

Figure 15. Length frequencies of littleneck clams collected at transect sampling locations on Kodiak Island, Alaska, 1989: McDonald Lagoon (top), Port Bailey (middle) and Ruth Bay (bottom).

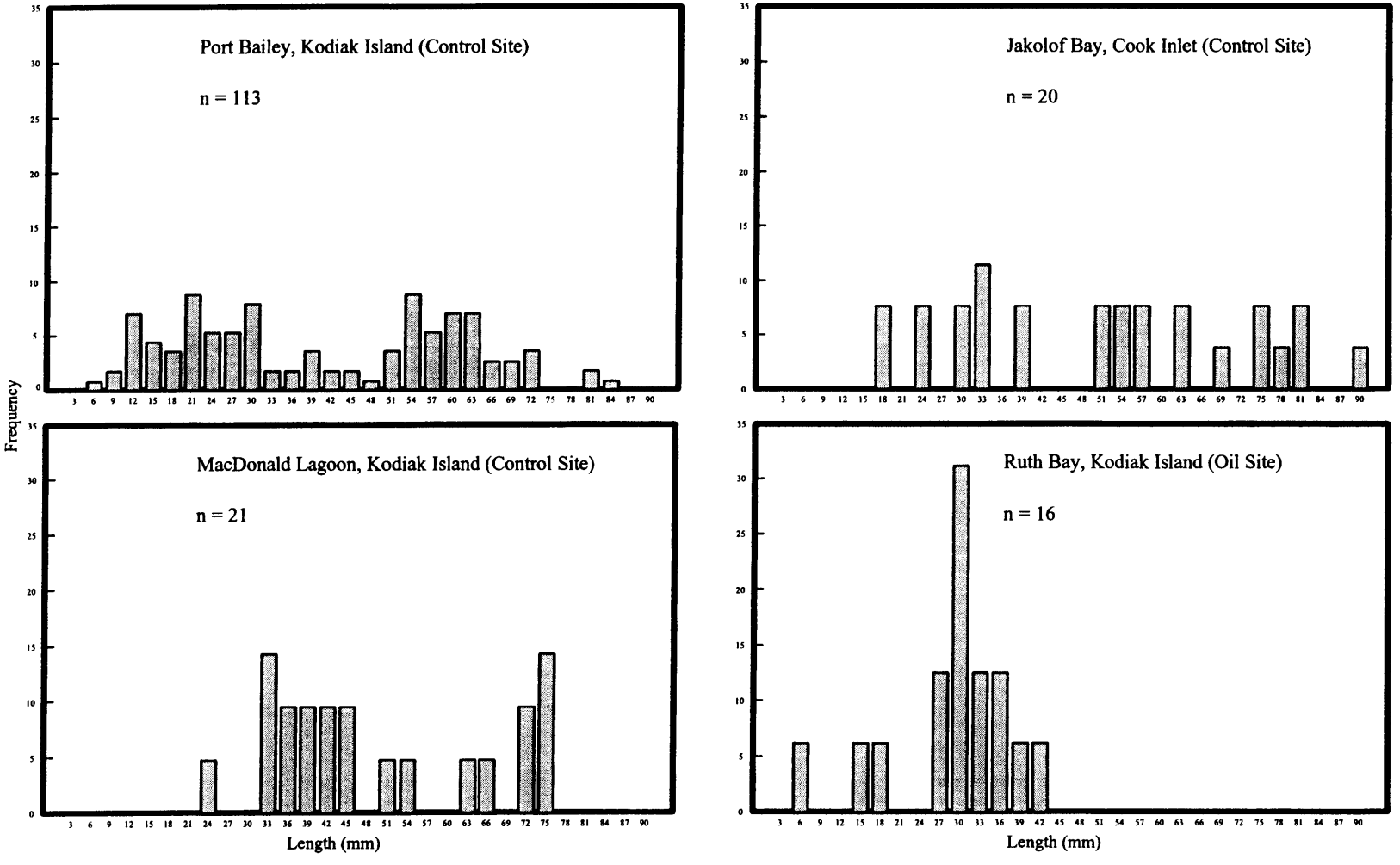

Figure 16. Length frequencies of butter clams collected at transect sampling locations in Cook Inlet and Kodiak Island, Alaska, 1989: Port Bailey (top left), Jakolof Bay (top right), McDonald Lagoon (bottom Left) and Ruth Bay (bottom right).

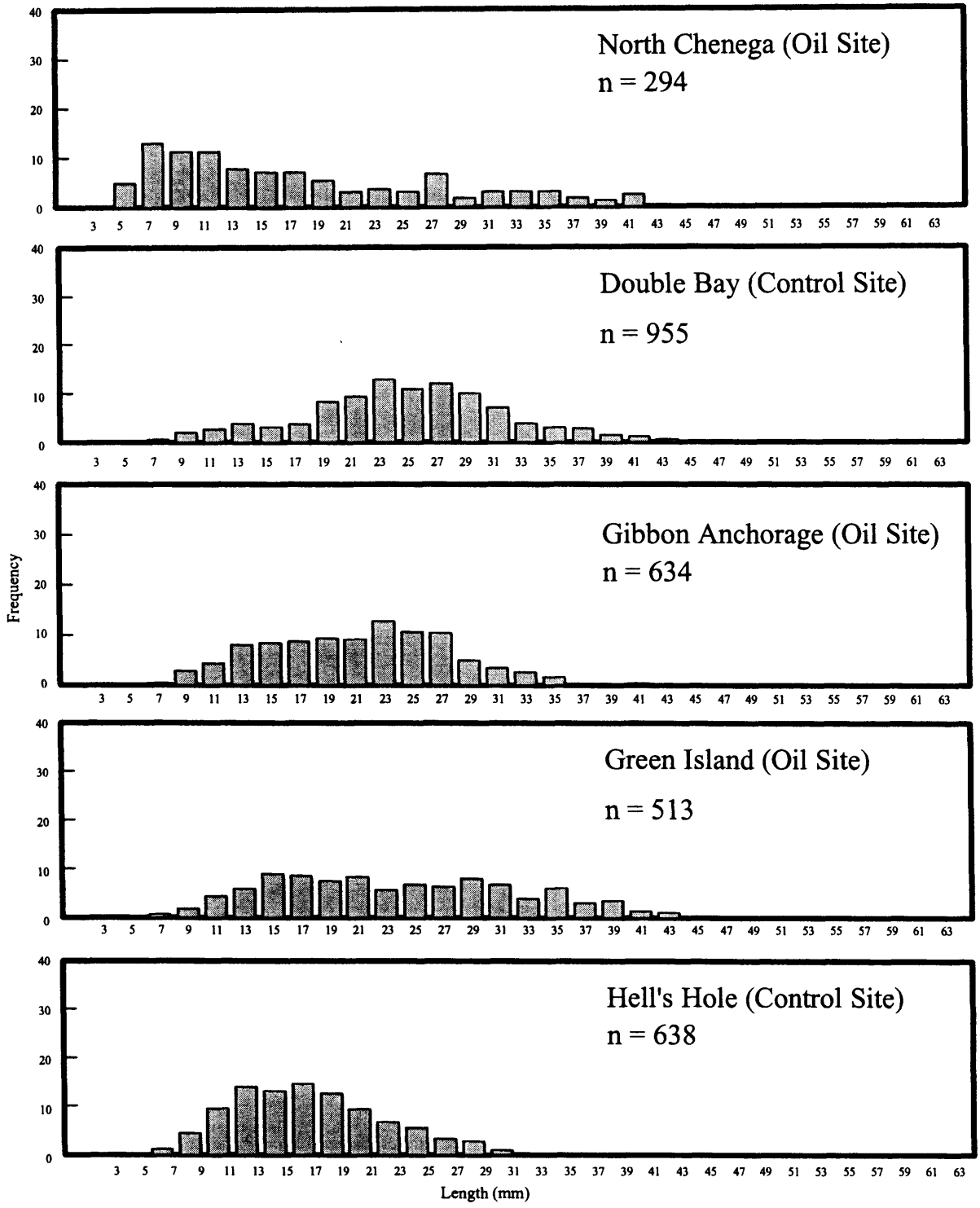

Figure 17. Length frequencies of littleneck clams collected at transect sampling locations in Prince William Sound, Alaska, 1990: North Chenega (top), Double Bay (Second), Gibbon Anchorage (middle), Green Island (fourth) and Hell's Hole (Bottom).

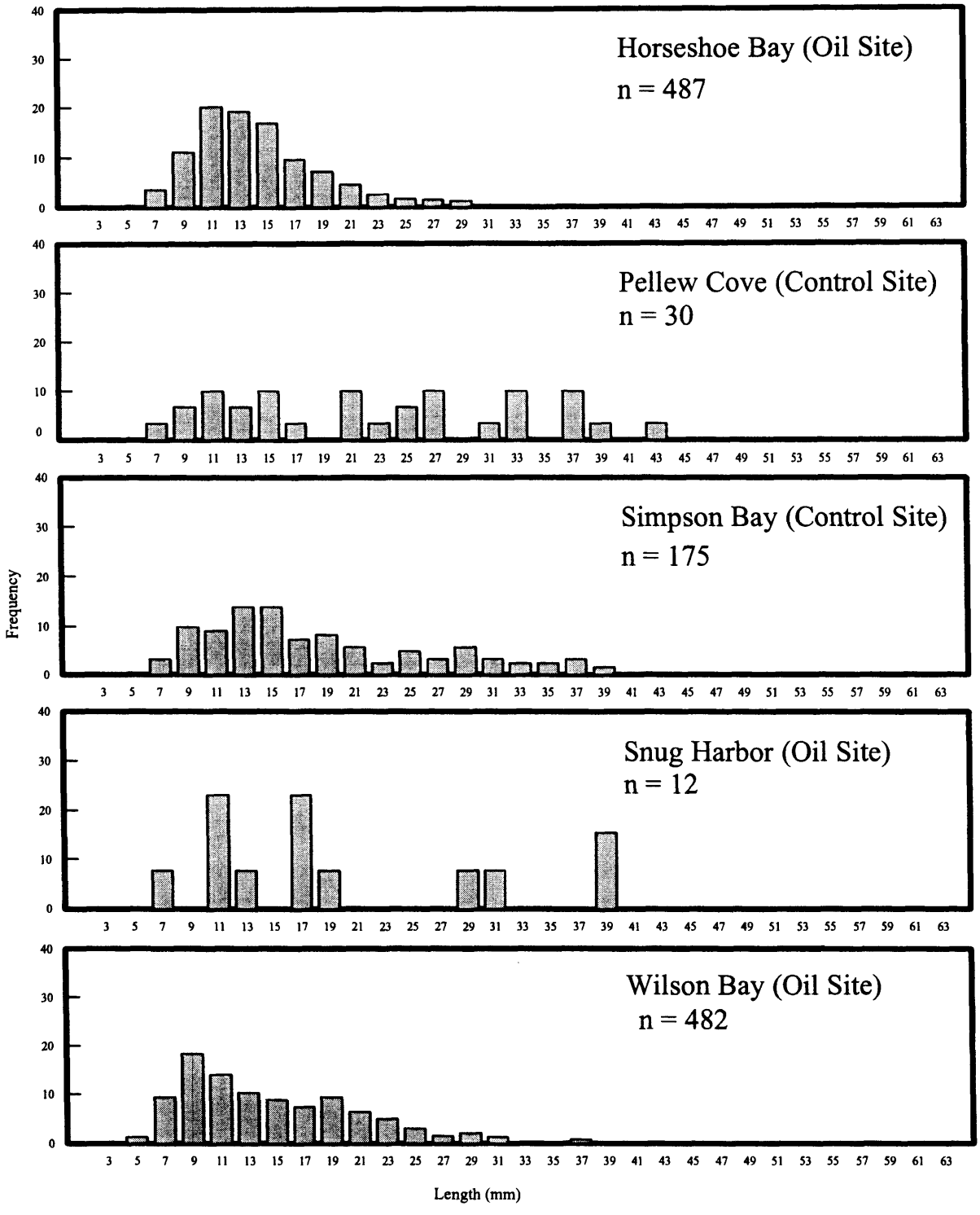

Figure 18. Length frequencies of littleneck clams collected at transect sampling locations in Prince William Sound, Alaska, 1990:

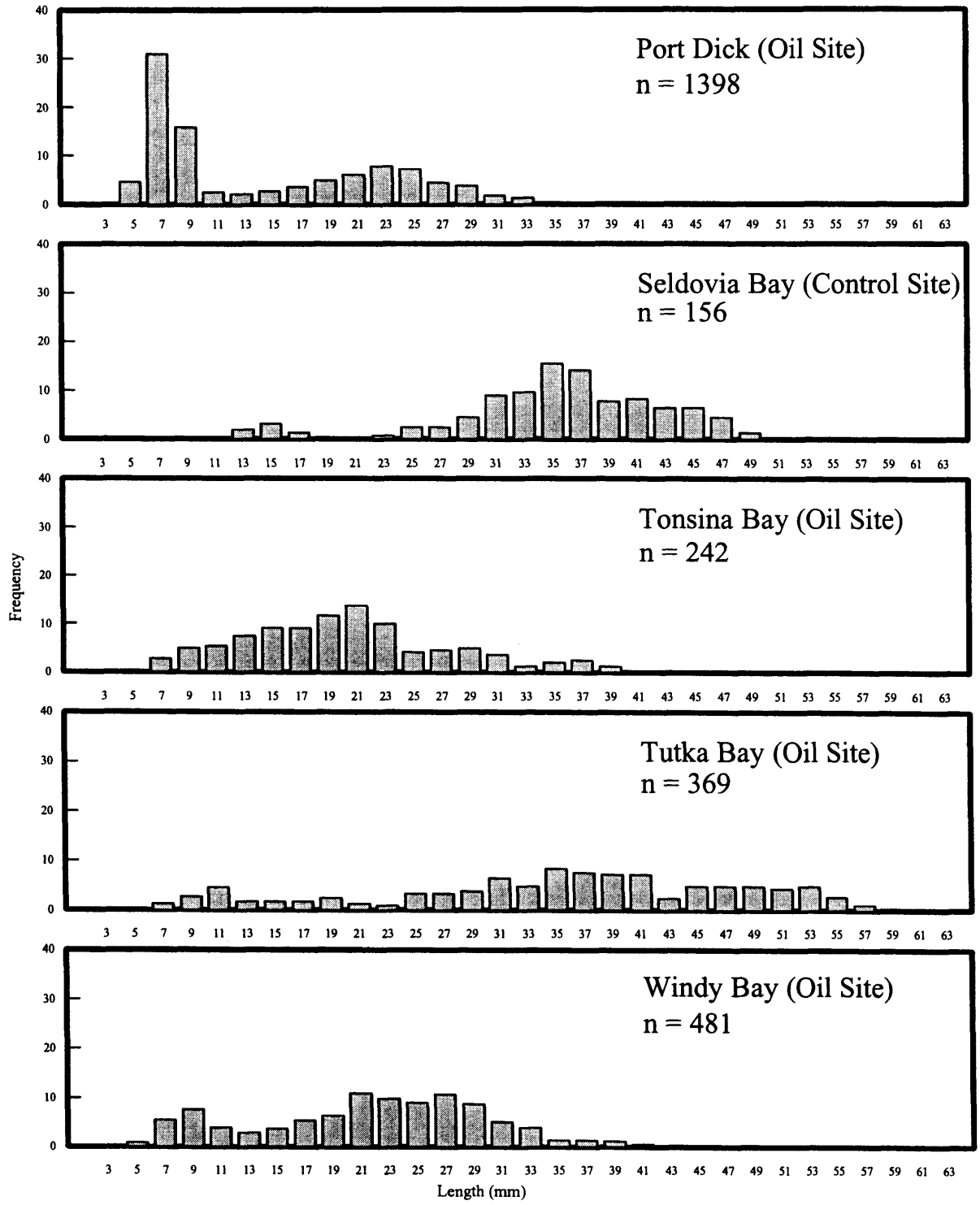

Figure 19. Length frequencies of littleneck clams collected at transect sampling locations in Cook Inlet, Alaska, 1990: Port Dick (top), Seldovia Bay (second), Tonsina Bay (middle), Tutka Bay (fourth) and Windy Bay (bottom).

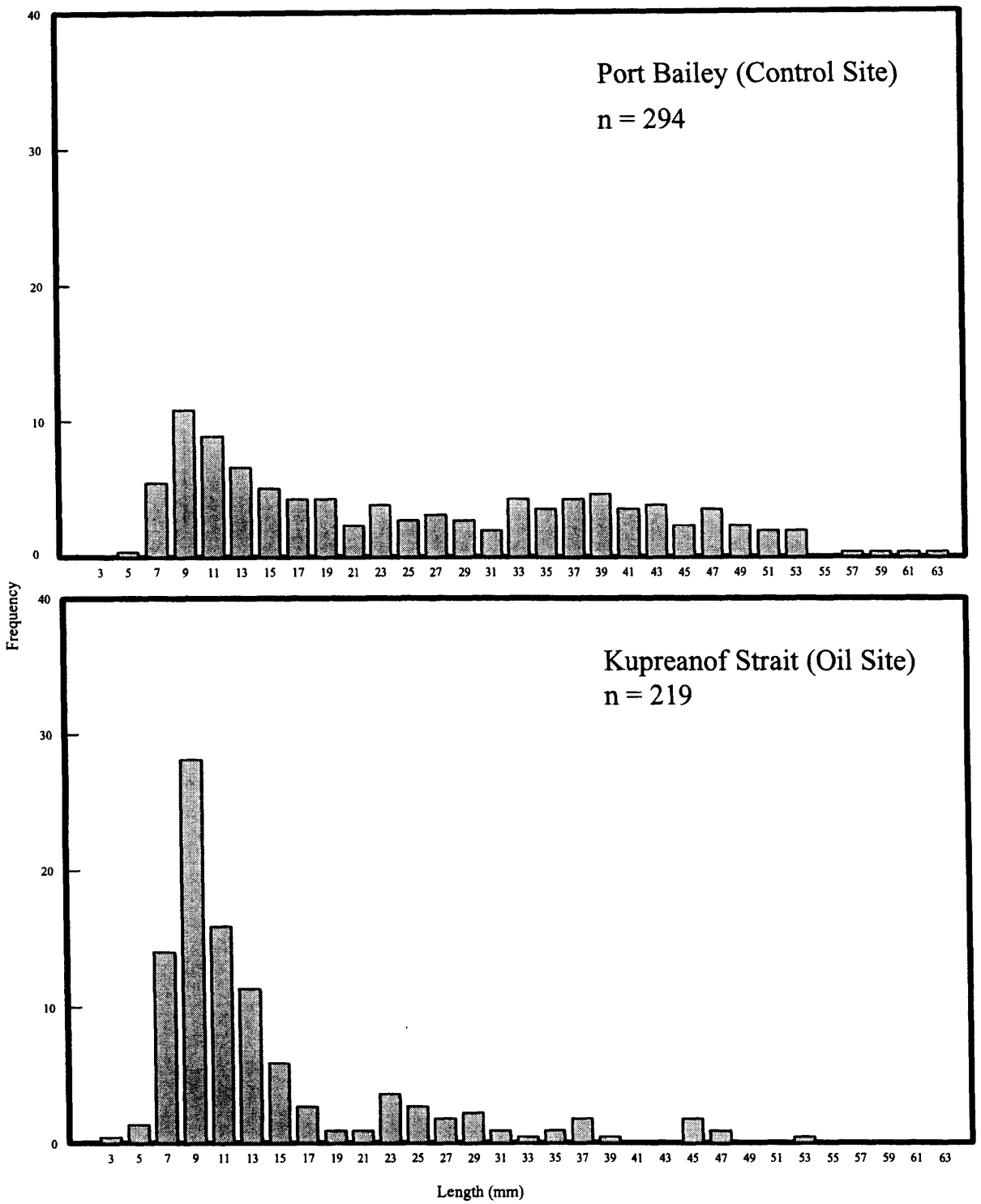

Figure 20. Length frequencies of littleneck clams collected at transect sampling locations on Kodiak Island, Alaska, 1990: Port Bailey (top) and Kupreanof Strait (bottom).

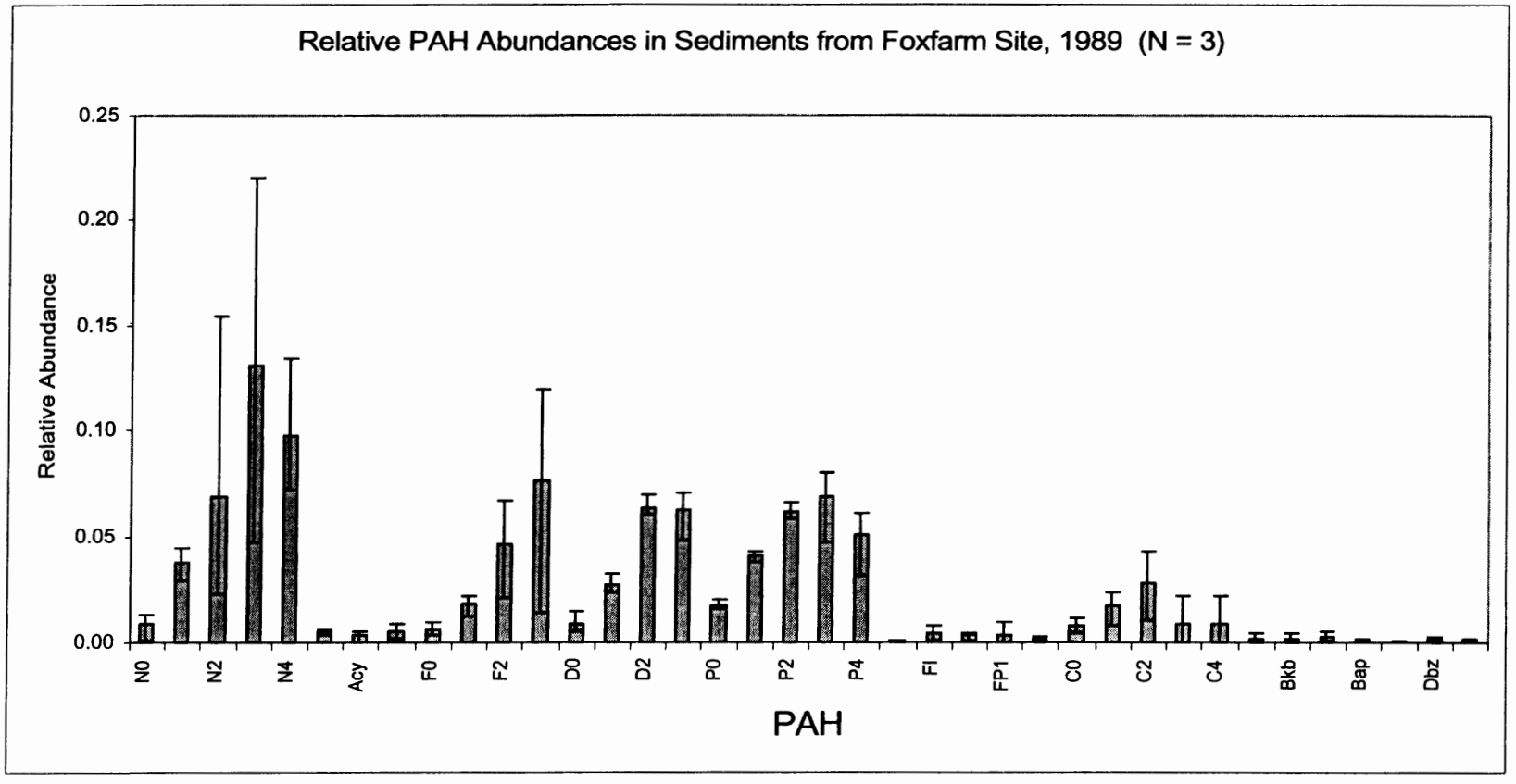

Figure 21. Relative PAH abundances in 3 heavily oiled sediments collected from the Fox Farm site in 1989. Solid bars depict the mean relative abundance, and thin vertical bars depict the range. The PAH analytes are abbreviated with numbers indicating alkyl carbon atoms as follows: N = naphthalenes; Bi = biphenyl; Acy = acenaphthylene; Ace = acenaphthene; F = fluorenes; A = anthracene; P = phenanthrenes/anthracenes; D = dibenzothiophenes; Fl = fluoranthene; Py = pyrene; FP1 = C1-fluoranthenes/pyrenes; Ben = benz[*a*]anthracene; C = chrysenes; Bfb = benzo[*b*]fluoranthene; Bkb = benzo[*k*]fluoranthene; Bep = benzo[*e*]pyrene, Bap = benzo[*a*]pyrene; Idp = indeno[1,2,3-*c,d*]pyrene; Dbz = dibenz[*a,h*]anthracene; Bzp = benzo[*g,h,i*]perylene.

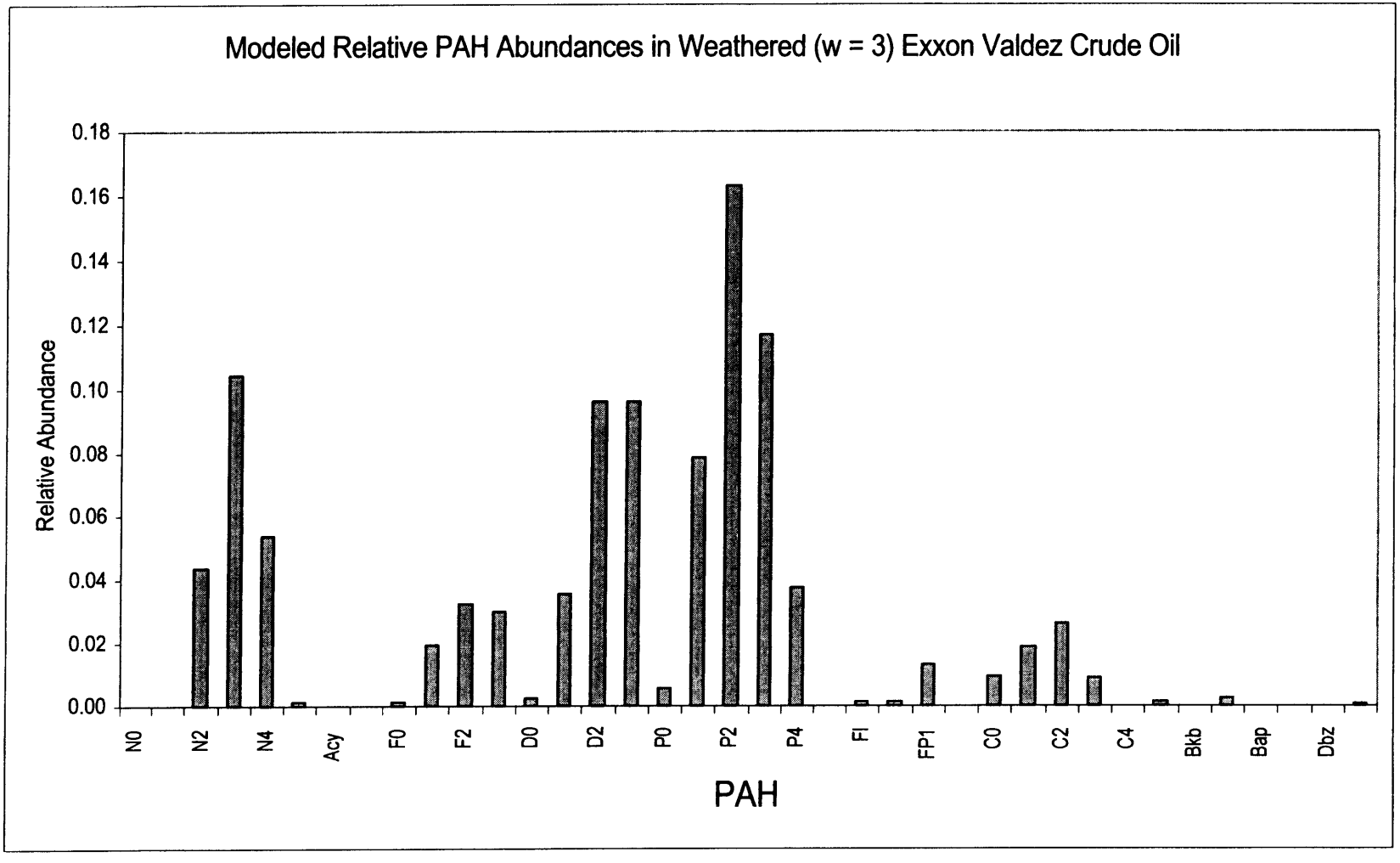

Figure 22. Relative PAH abundances calculated for weathered *Exxon Valdez* oil according to the weathering model of Short and Heintz (1997). Compare the composition depicted here with those in figures 21 and 25. See figure 21 for further details.

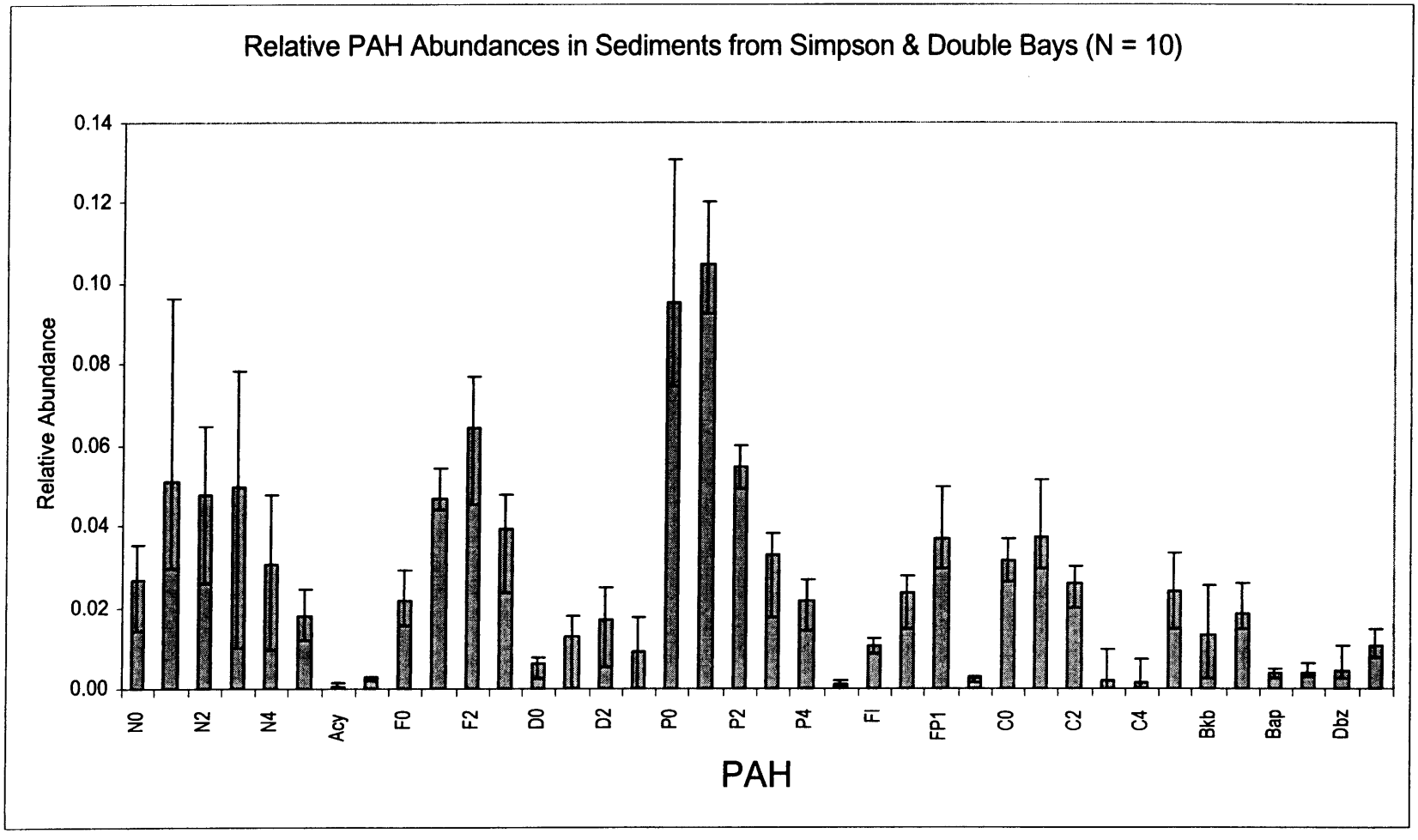

Figure 23. Relative PAH abundances in sediments from Simpson and Double Bays. Relative abundance is the ratio of a PAH to the sum of detected PAH. Samples from Simpson Bay (N = 3) were collected in 1989, and those from Double Bay (N = 7) were collected in 1990. One sample from Double Bay was omitted because several PAH were below the method detection limits (TPAH = 136 ng/g). See figure 21 for further details.

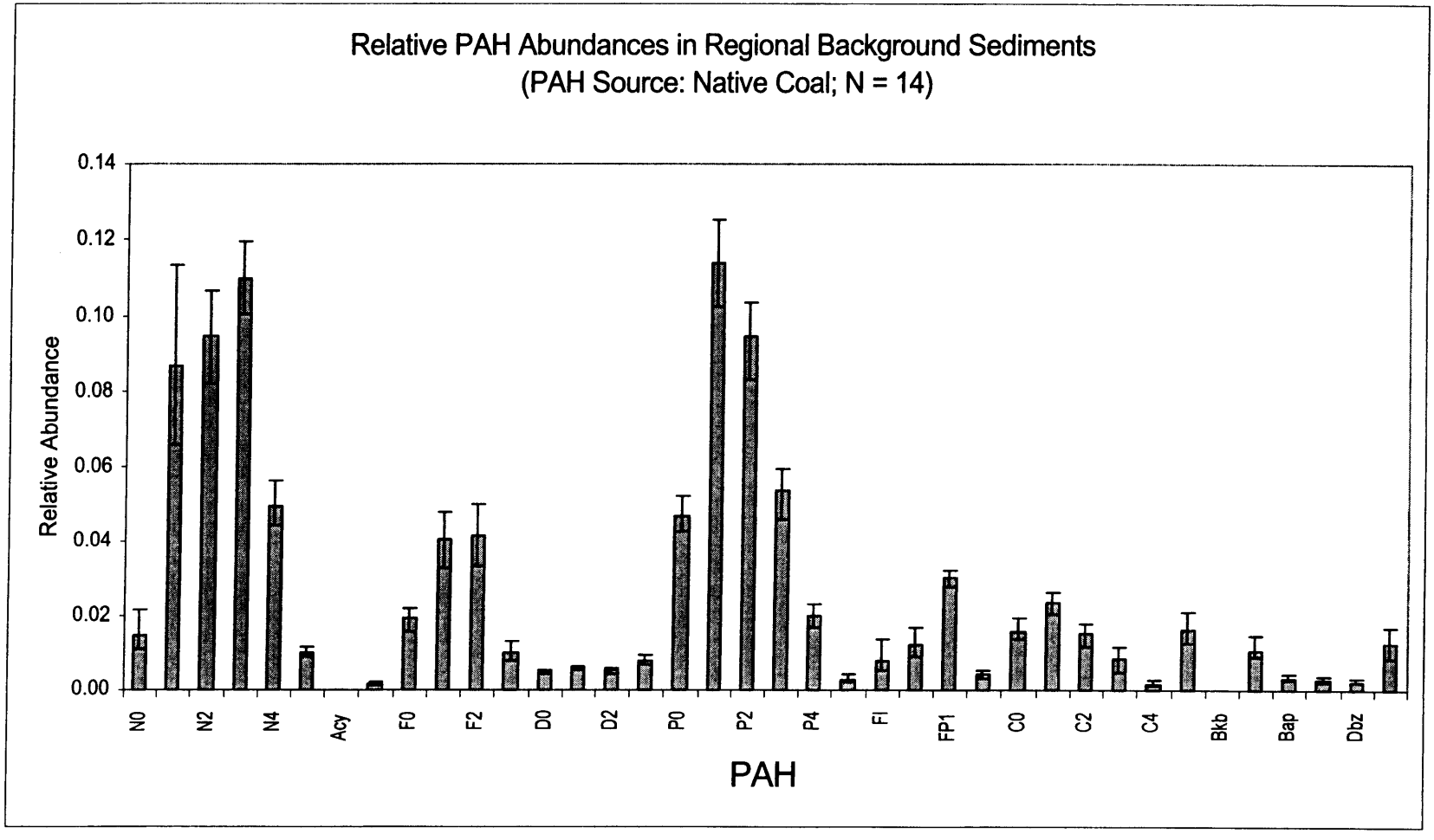

Figure 24. Relative PAH abundances in 14 benthic sediment samples from Prince William Sound and the northern Gulf of Alaska. The pattern of relative PAH abundance depicted here has been ascribed to particulate coal eroded from terrestrial deposits along the northern Gulf of Alaska margin near Katalla and eastward by Short *et al.* (1999). See figure 21 for further details.

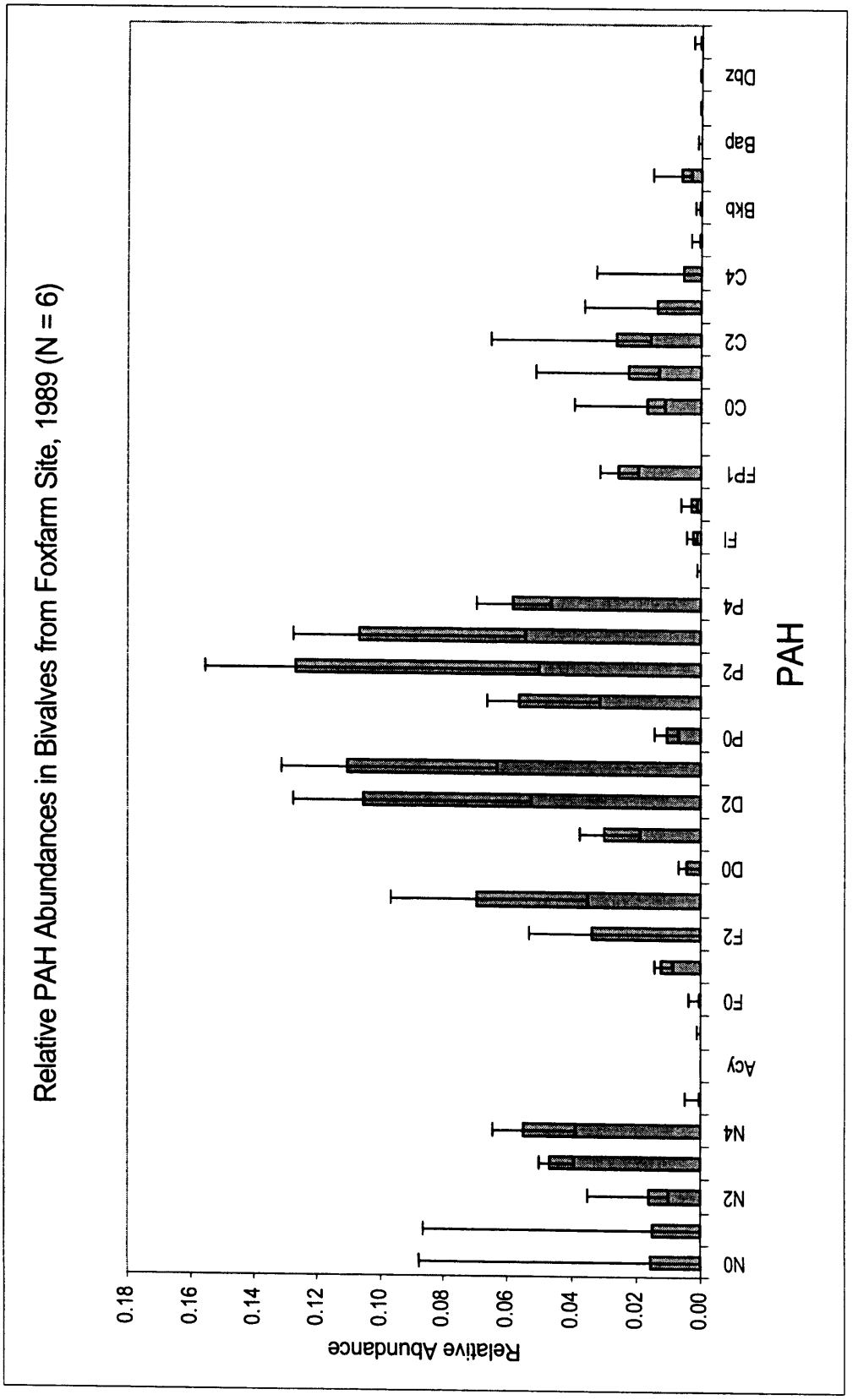

Figure 25. Relative PAH abundances in 6 oiled bivalve samples collected from the Fox Farm site in 1989. See figure 21 for further details.

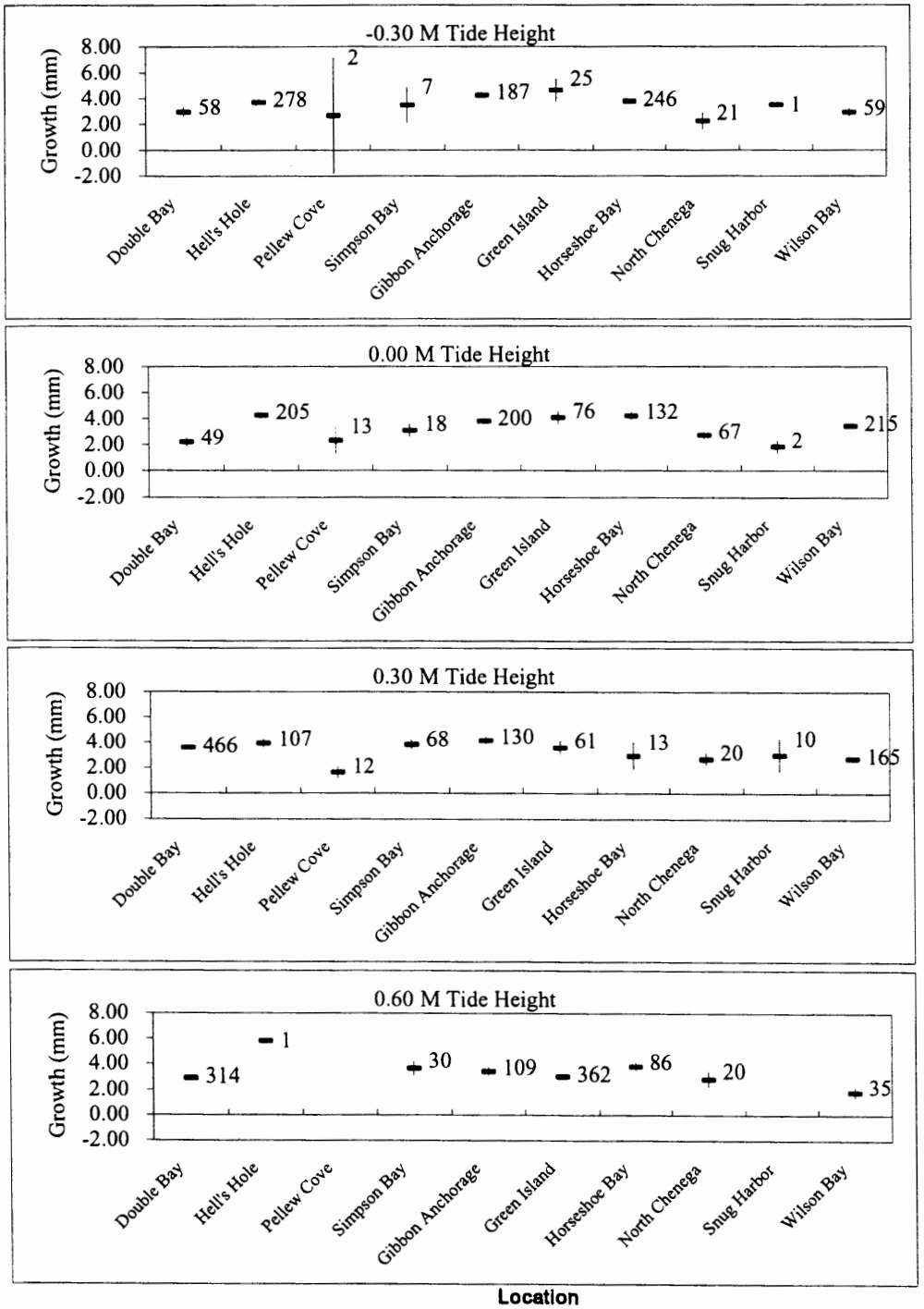

Figure 26. Mean growth, by tide height, of littleneck clams collected at transect sampling sites in Prince William Sound, 1990. Growth was estimated from 1989 to 1990 by back-calculating the length of each clam in 1989 from its' last annulus and subtracting it from the total length of each clam in 1990. Horizontal lines represent mean growth. Vertical lines represent ± 2 standard errors. Sample sizes are noted next to each line.

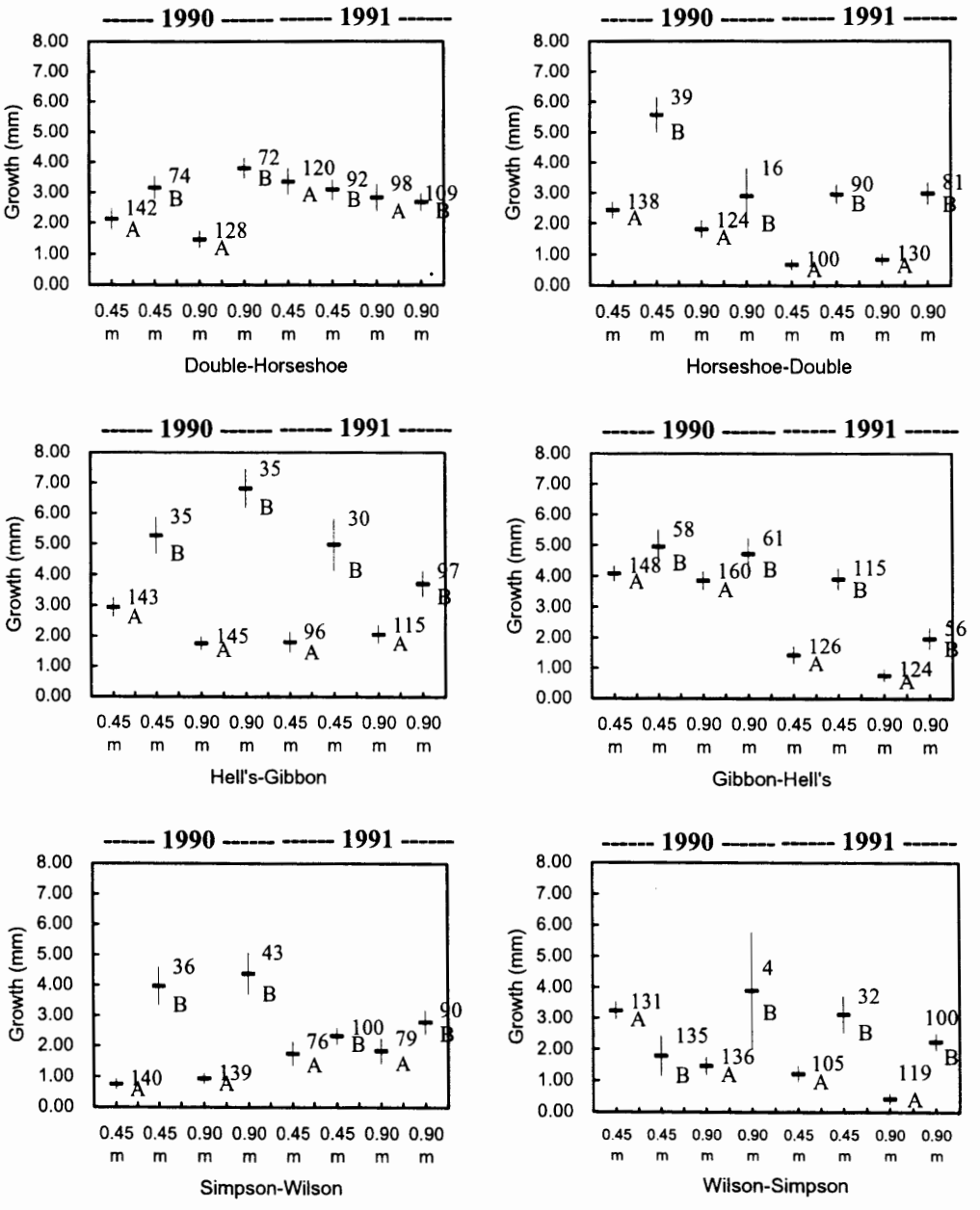

Figure 27. Mean growth of littleneck clams collected during the reciprocal transplant experiment in Prince William Sound, 1990-1991. Horizontal lines represent mean growth. Vertical lines represent ± two standard errors. Sample sizes are noted beside each bar. Clam types (A or B) are noted beside each bar. Type A clams were tagged, notched, and transplanted to a reciprocal transplant location while type B clams were notched and replanted at the same location. Tide heights (0.45 m or 0.90 m) are noted at the bottom of each graph.

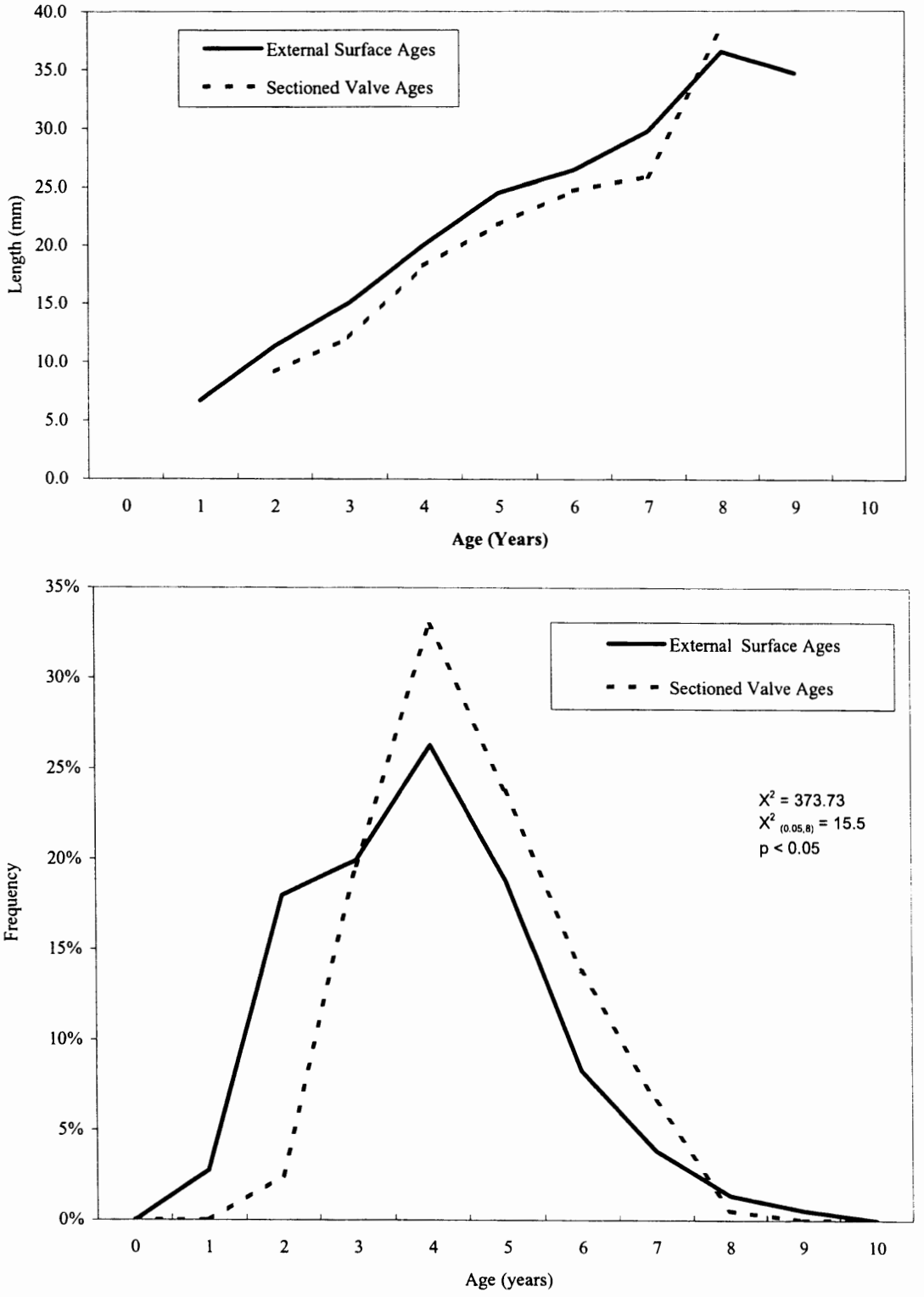

Figure 28. Mean length at age (mm) and age composition of littleneck clams in Prince William Sound based on external valve ages and sectioned valve ages. A total of 361 littleneck clams were aged using both external and sectioned valve aging techniques.

APPENDIX A

Standard Operating Procedures (SOP)

For

Collecting and Tagging Littleneck Clams

April 4, 1991

The reciprocal clam-tagging project can be broken down into three steps:

1. CLAM COLLECTION,
2. CLAM TAGGING, and
3. CLAM TRANSPLANTATION.

CLAM COLLECTION

Clams will be collected at two tide heights, +0.45 m (+1.5 ft) and +0.90 m (+3.0 ft), on each beach. Upon arrival the tide heights will be found using tide tables, a hand level and stadia rod. Clams will be collected by raking a trench along each tide height until 180 to 200 have been collected. These will be held in buckets and the water changed at intervals to reduce handling stress.

CLAM TAGGING

Tagging is to take place at the Forest Service's Green Island cabin. Clams are tagged in groups of 50 at a time. One person will select clams for correct size (15mm to 36mm) and least abrasion and using a small tapered file, notch each valve at its margin and place it on a screen/frame to dry. Another person will remove excess water, take whole weight and total length of each clam and place it on a grid consisting of five columns by ten rows. The third person functions as the data recorder. Tags are applied to clams beginning at the upper left-hand corner of the grid and moving down the page. A generous dollop of the super glue gel with the tag pushed into it's center allowing the glue to rise around the edge of the tag seems to be the best method of tag application. After the fifty clams are tagged, a slip of paper noting the time that they were completed is placed with them and they are set aside to dry for 1 to 1.5 hours. At this point they are placed in a bucket of seawater which should be changed regularly to both reduce stress and to remove glue residues. An additional 10 to 20 clams with both valves notched should be placed with each group of 50 tagged clams. These 10 to 20 additional clams will function as the hydrocarbon samples at the time of recovery in September. When complete, there should be six buckets of tagged clams for each site.

CLAM TRANSPLANTATION

Upon arriving at the transplant site the two tide heights will be located. Three plots along each tide height will be identified by driving an anchoring device with a marker buoy attached to each (see Figure 10). The tagged clams will be placed in the quadrat marked "A" in each plot. This

quadrat will be excavated to a depth of 30cm (12 in) and all clams removed. Ten to 20 of these individuals will be used in a hydrocarbon sample and should be placed in the precleaned aluminum foil sheets provided in the wooden boxes. The balance of the clams excavated may function as donor clams for the reciprocal beach or if they are not needed, may be distributed along the beach in another location. The tagged clams should be placed in the excavated quadrat with the anterior (siphon) end up. When correctly placed the umbo points downward. Once the 50 tagged clams and the 10 to 20 additional clams have been placed in the quadrat, the remainder of the earth should be laid over and some flat rocks placed over the quadrat to protect the clams from sea otter predation.

Next, another quadrat, "B" is located a distance of one quad-width (1/2 m) to the left of quadrat "A" (when looking toward the upper tide line). The quadrat is excavated, all clams removed, counted, both valves notched and replaced in the quadrat. At least 50 specimens should be in each "B" quadrat and if 50 are not found then an effort should be made to find additional clams at the correct tide height to supplement this number.

APPENDIX B

State/Federal Damage Assessment Plan,

Analytical Chemistry,

Collection and Handling of Samples

TABLE OF CONTENTS

INTRODUCTION

In response to the release of more than 10 million gallons of crude oil into Prince William Sound, the State of Alaska and four Federal Agencies, the Departments of Agriculture, Commerce and Interior and the Environmental Protection Agency are acting together to assess the damages to the natural resources. Authority for this action is provided by the Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA) and the Clean Water Act (CWA).

A damage assessment requires documentation of the exposure of the resources to oil released from the EXXON VALDEZ, identifying which resources were injured by that exposure, measuring the magnitude of the adverse affects on each resource over time and assigning economic values for that injury. Once this is done, monetary compensation can be sought from the potentially responsible parties to restore and/or replace the injured resources.

Recovery of monetary damages may involve civil court actions. It will then be necessary to prove that the samples were collected in a scientifically approved manner and that the samples were protected from outside contamination (non-incident related) and accidental mix-ups during handling and analyses. It is, therefore, extremely important that every sample be readily identified and their location and analytical status known and documented at all times.

This document and the associated training sessions, were prepared to assist field personnel in collecting samples that will provide scientifically sound and legally defensible data to support the State/Federal Natural Resource Damage Assessment for the EXXON VALDEZ oil spill.

RECORD KEEPING AND DOCUMENTATION

Standard operating procedures (SOPs) for all sampling procedures, including chain of custody procedures; sampling protocols; cleaning and preparation of sample collection and storage devices; and labeling, handling, and sample preservation and holding time must be written in detailed, clear, simple and easy to follow language.

Personnel must be knowledgeable and experienced in the described sampling techniques and must adhere to the SOPs.

Any changes in procedures must be recorded in detail in the field logbook. The log entry must include reasons that the change in procedure was unavoidable.

Field logbooks are issued by the Team Leader or their representative. The logbooks should be serially numbered, sturdy, bound books with sequentially numbered pages. Waterproof logbooks should be used if available.

Field data sheets, if used, must be consecutively numbered by project. The field data sheets must

be referred to in entries in logbooks which reference, the precise data sheet involved and the relationship to specific data in the logbook noted.

All information pertinent to field activities, including descriptive notes on each situation, must be recorded in indelible marker in the field logbook. The information must be accurate, objective, up-to-date and legible. It should be detailed enough to allow anyone reading the entries to reconstruct the sampling situation. Additional information may be provided by field data sheets, sample tags or photographs.

Entries should be made in the logbook or on field data sheets with indelible marker at the earliest possible time. Notes should never be written on scrap paper and then transferred to the logbook.

Entries into field logbooks or field data sheets are signed or initialed, and dated by the person making the entry at the time of entry.

Each day's entries are closed out with a horizontal line, date and initial.

Errors in field logbooks or other records are corrected by drawing a single line through the error, entering the correct information and signing and dating the correction. Never erase an entry or any part of an entry.

Do not remove pages from the logbook.

Completed logbooks and field data sheets are returned to the Team Leader or their representative to be archived in a central location under chain-of-custody procedures until the Trustees indicate that they may be released.

SAMPLE IDENTIFICATION AND LABELLING

A tag or label identifying the sample must be completed and attached to each sample. Waterproof (indelible) marker must be used on the tag or label. The minimum information to be included on the tag is the sample identification number, the location of the collection site, the date of collection and signature of the collector (who, what, where and when). This information and any other pertinent data such as the common and scientific names of the organism collected, the tissue collected and any remarks are recorded in the logbook. Field sample data sheets, photographs, any pertinent in-situ measurements (such as temperature, salinity, depth) and field observations are recorded in the logbook.

The location of the sampling site is determined with the aid of USGS grid maps, NOAA charts or navigational systems such as Loran C. The site locations should be plotted on a chart of appropriate scale and photocopies incorporated into the logbook. In addition, a clear, detailed descriptive location as well as the latitude and longitude, in degrees, minutes and seconds, of the collection site must be recorded in the logbook.

SAMPLING EQUIPMENT AND SAMPLE CONTAINERS

All sample containers must be either organic-free (solvent-rinsed) glass or organic-free (solvent-rinsed) aluminum foil. Lids for the glass containers must be lined with either Teflon or solvent-rinsed aluminum foil.

Certified-clean glass jars are available from various vendors and if obtainable, may be used without cleaning.

Sample collection and storage devices are cleaned by washing with soap and hot water, rinsed extensively with clean water and then rinsed with either methylene chloride or acetone followed by pentane or hexane and allowed to dry before use.

First rinse: tap water, then re-rinse in distilled water.
Second rinse: methylene chloride or acetone
Third rinse (if acetone is used): pentane or hexane

The solvents (methylene chloride, acetone, pentane and hexane) used for cleaning sample collection and storage devices must be of appropriate quality for trace organic residue analysis and be stored in glass or Teflon containers, not plastic.

New glass jars or unused aluminum foil does not need to be washed with soap and water. They must, however, be solvent-rinsed as described above before use.

Glass jars may be cleaned by heating to 440°C for a minimum of 1 hour.

Clean glassware should be stored inverted or tightly capped with either solvent-rinsed aluminum foil or Teflon-lined caps.

The dull side of the aluminum foil should be the side that is solvent-rinsed. Pre-cleaned squares may be stored with the clean sides folded together.

All equipment that comes in contact with the sample such as dredges or dissecting equipment must be solvent-rinsed before contacting each sample. Equipment should be steam-cleaned or washed with soap and hot water at the end of each day or between sampling locations.

SAMPLING PROCEDURES

The method of collection must not contaminate the samples. Do not collect any subsurface samples through surface slicks. Do not collect any samples with oil-fouled equipment, such as nets or dredges. Do not touch or collect any sample with your bare hands.

Sample container volume must be appropriate to sample size; fill the jar to just below the shoulder. Overfilled jars will break when they freeze; under filled jars will allow the sample to dry out.

At least one field blank and replicate sample should be taken for each collection site, batch of samples or 20 samples taken. (A field blank is a sample container opened in the field, closed and stored as if it contained a sample. A replicate sample is a second sample from the same site.) Rinsate blanks should be taken if appropriate.

Water Sampling

The method must be described or adequately referenced in sampling SOPs. Recommended sample size is 1-4 liters depending on the analytical methodology.

Water samples for volatiles analyses should be taken in 40 ml amber vials with no headspace or bubbles.

Sediment Sampling

Any accepted methods of collecting undisturbed surface sediment samples such as box cores, hand corers, or grabs may be used. The method must be described or adequately referenced in sampling SOPs. Recommended sample size is 10-100 grams (a 4 oz. jar).

Tissue Sampling

Organisms to be analyzed for petroleum hydrocarbons should be freshly killed or recently dead. Decomposed organisms are rarely of any value for analysis.

Whole organisms may be stored in solvent-rinsed glass jars or wrapped in solvent-rinsed aluminum foil.

Tissue sections may be taken either on site from freshly killed organisms or in the laboratory from carefully collected and preserved - cold or frozen - whole organisms. Tissue should include flesh and internal organs, especially liver. Recommended sample size is 10-15 grams.

Tissue samples need to be protected from external contamination at time of collection. Contents of the intestinal tract, external slim coating, contaminated collecting utensils, etc. are all potential sources of contamination when collecting internal tissue samples.

All instruments used in handling samples must be made of a non-contaminating material (e.g., stainless steel, glass, Teflon, aluminum) and solvent-rinsed between each sample collection.

Instruments used for exterior dissection must not be used for internal dissection.

Avoid hand contact with tissue sample.

Collect stomach and intestinal tract last.

Bird eggs are wrapped in solvent-rinsed aluminum foil and transported by any convenient means that will prevent breakage. They should be opened or refrigerated as soon as possible. Eggs are opened by cutting them with a solvent-rinsed scalpel or by piercing the air cell end and pouring/pulling the contents out. Avoid including pieces of eggshell with the contents or touching the contents with your hands. Total weight, volume (measured or calculated), length, width and contents weight must be recorded for each egg. Bile is collected by removing the gall bladder, puncturing it with a scalpel fitted with a new #11 blade, and collecting the contents in a 4 ml amber glass vial.

SAMPLE PRESERVATION AND HOLDING TIME

Samples must be kept cool, i.e., on ice.

Samples that are to be frozen, sediment and tissue, should be frozen quickly and rapidly. That is, these samples should be frozen as soon after collection as possible and the freezing process should be rapid.

Frozen samples must be kept frozen, at -20°C or less, until extracted or prepared for analysis. Repeated freezing and thawing of samples can destroy the integrity of the samples resulting in questionable data or the loss of data.

Water Samples

All water samples must be immediately extracted with methylene chloride or preserved with HCl to ph<2. If preserved, water samples are stored in the dark at 4°C and extracted within 7 days. All extracts must be stored in the dark in airtight chemically clean containers until analysis.

Sediment and Tissue Samples

Samples should not be extracted until immediately before analysis; if there is a lag between sample extraction and sample analysis, extracts must be stored in airtight containers kept in the dark at 4°C.

SAMPLE SHIPPING

All samples, except water samples, must be kept frozen throughout the shipping process.

Samples must be packaged to prevent breakage. Glass jars should be individually wrapped so that they will not contact each other if padding shifts in transit (which Styrofoam chips do). Bubble wrap or the divided boxes that new jars are shipped in work well. Pack samples in insulated containers (e.g., ice chests) with enough frozen mass to remain frozen in transit.

It is the responsibility of the sample shipper to arrange for sample receipt. Do not send samples off without arranging for pickup and storage.

To insure that samples are not compromised, shipment should not be initiated later in the week than Wednesday nor should samples be shipped in any week in which there is a holiday.

Shipments must comply with Department of transportation regulations.

CHAIN-OF-CUSTODY PROCEDURE

Samples must be kept in such a manner that they cannot be altered either deliberately or accidentally. Any indication that a sample has been subjected to tampering or physical alteration could disqualify it as evidence for possible legal action.

The field sampler is personally responsible for the care and custody of the samples collected until they are transferred under chain-of-custody procedures.

A sample is considered in "custody" if:

it is in your actual physical possession or view;
it is retained in a secured place (under lock) with restricted access, or it is placed in a container and secured with an official seal(s) such that the sample cannot be reached without breaking the seal(s)

Evidence tape or sample seals are used to detect unauthorized tampering of samples following

sample collection. The seal must be attached in such a way that it is necessary to break it in order to open the container. Seals must be affixed to the container before the samples leave the custody of sampling personnel.

All samples must be accompanied by a chain-of-custody record or field sample data record (Figure 1). When samples are transferred from one individual's custody to another's, the individuals relinquishing and receiving the samples will sign and date the chain of custody record. This record documents the transfer of custody of samples from the sampler to another person or to a specified analytical laboratory.

Shipping containers must be custody-sealed for shipment. The seal must be signed before the container is shipped. The chain-of-custody record must be dated and signed to indicate any transfer of the samples. The original chain-of-custody record accompanies the shipment; a copy is retained by the sample shipper. If samples are sent by common carrier, copies of all bills of lading or air bills must be retained as part of the permanent documentation.

Whenever samples are split, a separate chain-of-custody record is prepared for those samples and marked to indicate with whom the samples are being split.

APPENDIX C

Standard Operating Procedures (SOP)

Histological Sample Preparation for Bivalve Mollusks

Alaska Department of Fish and Game

Commercial Fisheries Management and Development

Pathology

NOTE: Only live or moribund bivalves will be suitable for processing. Histopathological changes caused by toxic chemicals are often very subtle at best. Tissues in dead bivalves autolyze very quickly and will mask these changes. Do not collect and process dead bivalve mollusks.

1. The fixative to be used in Bouin's solution (formula attached).

2. The volume of fixative should be 10 times the volume of the tissue. This is important since any less fixative may result in tissue autolysis and worthless samples.

3. The sample size per site and species will be 20 bivalves, live or moribund.

4. Bivalves less than 6 cm in length (shucked) can be fixed whole by dropping into preservative. Animals must be shucked cleanly from the shell by severing adductor muscles (diagram) prior to fixation. Discard the shell unless there is some type of shell deformity or otherwise abnormal valve. In such a case the shell should be included and attached to the donor animal by wrapping both in gauze.

5. Larger bivalves will need about 3 incisions (anterior, mid, posterior) made across the surface of the animal about midway through the tissues. Do not cut completely through the animal so that individual specimens remain intact and tissues do not become mixed.

6. Tissue and shell abnormalities must be noted on a necropsy field sheet (attached) respectively numbered for a particular animal (bag in gauze and label if necessary). If no abnormalities within the 2 specimens are observed then a single field sheet will suffice for that sample series. The field sheet(s) will also contain the label information below and must accompany the samples in a zip loc bag.

7. A label with bivalve species, size range and life stage, date of sample, location of sample and contact person's name, address and telephone number must be placed within each of the sample jars.

8. Do not mix samples of different species within the same jar of fixative. Each species requires a separate sample jar(s).

9. Place sample jars and zip loc bag containing sample data into a suitable shipping package with adequate packing material to prevent breakage. Plastic jars or containers for fixative and samples work best. Be sure lids are on tight and do not leak.

10. Mail to FRED Fish Pathology Lab: 333 Raspberry Rd., Anchorage 99502 (907-267-2244) or P.O. Box 3-2000, Juneau 99802 (907-465-3577).

11. Notify the fish pathology lab prior to sample shipment so that samples may be expected and tracked enroute.

12. Any questions regarding sample preparation should be directed to:
Dr. Ted Meyers

Principal Fish Pathologist III
ADF&G, FRED Division
Juneau Pathology Lab
P.O. Box 3-2000
Juneau, AK 99802 (907) 465-3577

FOLLOW-UP

If you are shipping samples to NMFS ABL, you will be notified of shipment arrival and condition by ABL personnel. After samples have been checked-in at ABL, you will receive a copy of the signed and dated chain of custody sheet and a printout of the data entered into the PWS database for all samples in the shipment. You will be asked to verify this information and to return a signed and dated copy of the verification to ABL.

BOUIN'S SOLUTION
FIXATIVE FOR HISTOLOGICAL SAMPLES OF FISH, BIVALVES, AND CRABS
FRED PATHOLOGY, ADF&G

1. Picric Acid, saturated aqueous solution*	750.0 ml
2. 37 - 40% Formalin	250.0 ml
3. Glacial acetic acid	50.0 ml

*Dissolve 20 g picric acid into 1000 ml distilled water with the aid of heat. Allow to cool, decant and use the supernatant fluid.

APPENDIX D

Size at Age Determination for *Protothaca staminea*

Collected from Oiled and Non-oiled Sites in

Prince William Sound, Alaska

Final Report for Contract No. Ihp-90-078.

Washington Department of Fisheries

Olympia, Washington

1991

By

C. V. Volk, J. J. Grimm, and L. A. Peterson

INTRODUCTION

A major source of environmental pollution is the accidental release of oil and its many derivatives into the natural environment. As this problem continues, there is a growing need and effort to evaluate their impacts upon near shore communities. While the effects of oil spills are undoubtedly wide ranging, a large body of work has focused upon impacts to survival and growth of intertidal bivalves. Much of this work suggests that a variety of bivalve species may be significantly impacted by these events.

The effects of oiling on bivalve growth and survival have been investigated on a variety of species with several different types of oil, in both natural and laboratory settings. Deposit feeding clams seem to be particularly susceptible to oiling and high mortalities of Macoma balthica, M.inquinata and M. nasuta have been observed on exposure to Prudhoe Bay Crude Oil and Bunker C.(Shaw et al,1976;Augenfeld et.al. 1981;Volk, unpub. data). Goesjadi and Anderson (1979) have documented a larger quantity of aromatic hydrocarbons accumulated in the tissues of the deposit feeding M. inquinata than the filter feeding P. staminea on exposure to crude oil. While mortalities of Protothaca staminea were much lower in these studies, they were still significantly greater than those in control groups (Augenfeld et.al.1981; Volk unpub. data).

Despite this increased ability to survive oiling, significant reductions in growth have been noted for the filter feeding Mytilus edulis soon after exposure to North Sea Crude Oil (Stromgren, 1987; Stromgren et al, 1986) and for Protothaca staminea exposed to experimental crude oiling (Anderson et al, 1981). The latter study also documented a greater growth reduction when the oil was mixed with the sediments to a depth of 10 cm. rather than just spread over the surface.

Axiak et.al (1988) investigated the effects of Kuwait Crude Oil on Venus verrucosa at the cellular level and found atrophy of the digestive cells in the digestive gland after 150 days exposure. Similar results have been reported for M. edulis (Lee et al 1972), Mya arenaria (Fong, 1976), and M. balthica (Clement et al 1980). Axiak and George (1987) concluded that food absorption efficiency was reduced in Venus verrucosa exposed to Kuwait crude and that this resulted in a significant reduction in energy available for somatic growth in this species. Several other authors have made similar conclusions for other bivalve species (Widdows et.al.1982; Stickle et.al.1985; Gilfillan et.al.,1977; Keck et.al.,1978). Axiak and George (1987) also documented reduced feeding rates in V. verrucosa on exposure to Kuwait crude oil due to reduced pumping activities of cilia. Once again, it was concluded that this resulted in a reduced scope for growth in this species.

Behavioral and morphological effects of oil exposure on bivalves have also been documented. Exposure of Mytilus edulis to South Louisiana crude, no. 2 fuel oil and outboard motor oil all inhibited the formation of byssal threads in this species(Carr and Reisch,1978). Macoma balthica exposed to Iranian crude oil burrowed more slowly (Linden,1977) and P. staminea burrowed more slowly and to a shallower depth on exposure to crude oil (Pearson et.al.1981). The inhibition of burrowing depth led to an increased predation rate on these clams by crabs. Siphon activities were also impaired in P. staminea on exposure to high concentrations of Alberta crude. (Hartwick et.al.,1982). This list is by no means exhaustive, however, the suggestion is clear that

bivalve mollusks exhibit higher mortalities and reductions in growth when exposed to oiling. While these studies produce compelling evidence of the lethal and sub-lethal toxic effects of oil on bivalve growth and survival, they generally do so in a laboratory or experimental field situation where animals are sequestered and presented with oil, then their ensuing growth measured following the event and compared to controls. While these experiments are not necessarily flawed, they may only produce relative growth information since a variety of other factors related to the experimental situation may also be impacting growth in experimental and control populations. Thus, it would be desirable to measure the changes in growth rate which may have occurred in a natural population following an actual oil spill. This is certainly the goal of damage assessment efforts following such an event. Unfortunately, pre-spill growth information is usually not available with which to compare the post-spill growth in marked populations from impacted areas. One possible way to circumvent this problem would be to compare the growth of clams from impacted and non-impacted areas during the same time period following a spill. However, under this scenario, it is not possible to control for the myriad affects on growth of habitat specific parameters such as food availability, population density, and a host of other factors. What is necessary is to compare the growth of these organisms before and after the spill in the same habitat which has been impacted by the oil spill. An approach to this idea is to utilize the growth histories recorded in bivalve shells as a way to document the pre and post-spill , age-specific growth of these bivalves as a means to test the hypothesis that no impact upon clam growth has occurred as a result of such an event.

The use of bivalves for documenting environmental change has been applied on a variety of levels in recent years. Appledoorne (1981) utilized the changes in length at age relationships in populations of Mya arenaria to document the effects of oil spills and mining activities on the growth of this species. However, this study determined age by length frequency analysis where age groups were recognized according to length modes. Brothers (1979) has reviewed the problems of utilizing length frequency analysis for age determination and among them is the fact that many older organisms grow very slowly resulting in compression of length modes. Furthermore, bivalve growth even in younger specimens can be quite variable and age may only explain a small percentage of the variation in size (Peterson et.al.1983).

The use of annual growth interruptions to accurately age individuals can greatly improve population demographic analyses. A host of studies have demonstrated annual periodicities of internal growth interruptions in a variety of species (Shaul and Goodwin, 1982; Peterson et al, 1983,1985; Fritz and Haven, 1983, Rhoades and Pannella, 1970; and many more). Paul and Feder (1973) have used growth interruptions on the external surface of Protothaca shells from Alaska to age these clams. These patterns are very obvious on the external surface of Alaskan clams (personal observation) and may be reliable age indicators at that latitude. Harrington (1987) has demonstrated an annual periodicity of shell growth interruptions in P. staminea on the West Coast.

Kennish (1980) has presented a good example of recruitment, growth and mortality dynamics in a Mercenaria population utilizing shell growth interruptions to accurately determine age. While this kind of population analysis is potentially very useful for documenting effects of environmental changes, a concern expressed by a number of investigators (Clark,1974; Gould,1979,Jones,1981) is that presumed annuli be validated as an accurate reflector of age. This is a general concern for

the use of any periodic patterns in calcified tissues and variability in size at age relationships between different localities (Harrington, 1987; Peterson et.al.,1983;) suggests that local validation may also be important.

The immediate impacts of environmental perturbations on individual growth have also been investigated by analyzing changes in microgrowth increments recorded between annuli in many bivalves. A variety of periodic patterns have been recognized in these micro-increment records including sub-daily, daily, fortnightly and annual, as well as aperiodic events such as storms (Lutz and Rhoads,1981). A number of investigators have verified the daily deposition of individual micro-increments in several species (Kennish and Olsson 1975;Kennish,1981, Lutz and Rhoads, 1981; Clark, 1968; Fritz and Lutz, 1986; and others). Kennish and Olsson(1975) have shown that the growth record in micro- increment patterns reflected short-term growth effects caused by thermal effluent from a nuclear power plant. The timing of heated discharges from the plant were easily recognized in the clam shells.

The goal of this study was to provide thin-section preparations of P. staminea shells collected from each of six sites in Prince William Sound Alaska, three sites oiled by the 1989 Exxon Valdez oil spill and three non-oiled sites. These preparations were viewed using video-microscopy and based upon the recognition of presumed annual interruptions in these sections, the age-specific size of each clam was determined and the data saved to a data-base file. The size at age data will be subjected to a growth model chosen by The Alaska Department of Fish and Game in order to test the hypothesis that no significant difference in growth rates of P. staminea occurred during the year of the Valdez oil spill in Prince William Sound as opposed to previous years. Although it is not the purpose of this study to actually test the hypothesis of growth changes in P. staminea as a result of the oil spill, the pitfalls and potential errors of age determination, which is the key component to this data, shall be discussed. It should be understood that until such time as ADFG deems appropriate, this data shall remain proprietary.

MATERIALS AND METHODS

Collection of Organisms

Protothaca staminea over a large size range were collected from each of six sites, three oiled and three which were not oiled by the Valdez oil spill of 1989. Specimens were collected along transects at several different tidal heights in the study areas. Specimens collected and received to The WDF calcified tissue analysis laboratory are documented in Table 1. The authors had no part in the collection of specimens and details regarding these collections should be addressed to the ADFG crew responsible for the collections.

Specimen Preparation

Specimens were prepared generally in accordance with the thin-sectioning methods outlined by Clarke (1981), and modified slightly at our laboratory. For specimens greater than about 10mm., the left valve of each specimen was sectioned along the maximum growth axis running from the umbo to the margin of the valve. The precise sectioning axis was adjusted slightly to include the smaller of the hinge tooth elements in order to include that structure in the section. Cutting of the shells was accomplished with an Accutome 2 precision diamond cut off saw equipped with a table saw top. On specimens smaller than 10mm., the entire valve was mounted in a block of polyester resin to support the small and difficult to handle elements, then sectioned as a unit along the same axis as just described. Where the left valve was unavailable or destroyed in preparation , the right valve was employed.

Following sectioning, one of the sectioned valve halves was then lapped with 600 grit fixed carborundum abrasive on a rotating lap wheel to obtain an approximately flat surface. This was subsequently polished on a lap wheel with 1 micron alumina slurry applied to a texmet pad surface, until most of the scratches were removed. After drying, this valve segment was then glued to a glass slide and reinforced with cytoseal, then allowed to dry at least overnight. This slide was then fixed to the diamond saw using a vacuum equipped chuck mount, and most of the shell lying above the glass surface of the shell removed, leaving a cross-sectional slice of the shell approximately 500 microns thick. This was then lapped on 600 grit carborundum until approximately 100-200 microns remained above the surface of the slide. The rough surface was again polished with 1 micron alumina until most scratches were removed. At this point the preparations were complete and ready for viewing.

Data Collection

For size at age determinations on valves, the sectioned specimen was placed under a stereo-microscope and viewed at 8 power with a reflected light source. The microscope was equipped with a video camera interfaced with a computer frame grabber. The image was displayed on a monitor and frozen so that measurements could be made by drawing measurement radii on the video screen with a mouse. This process was accomplished using Bioscan's Optimas image analysis software.

On the frozen video image of the sectioned valve, a reference point was established at the tip of the umbo. From there, a radius was drawn along the outside perimeter of the valve to the end of the valve, and total radius was determined. Then, at each annulus, a "flag" was placed which marked the position of that annulus and determined its distance from the reference point established at the umbo (see discussion regarding the recognition of annuli). Thus, the sizes at age represented in the data show the true incremental accrual of calcified material to the growing valve margin. For smaller specimens, this process could all be accomplished with one video image. That is, all of the shell could be accommodated in one video frame at this low magnification. However, the majority of specimens required that measurements be done in two parts since the whole clam could not be captured in one image. A small number of very large clams required the use of three images to accomplish this task. To the extent possible, this data was collected on all specimens prepared.

On a smaller number of specimens from each group, size at age data was also collected from the sectioned hinge tooth. The tooth section was viewed at 30x magnification using a stereo-microscope and reflected light, and size of the tooth was determined at each presumed annulus. The hinge tooth section was measured along a curved axis which travelled from the tip of the umbo, at the same origin as valve measurements, running along the maximum growth axis of the element to its edge (Fig. 1A). Presumed annuli were represented as obviously dark bands recorded in the tooth (see discussion).

Data collected from these measurements was manipulated within the Statgraphics statistical and graphics package in order to "paste" together the data acquired from each discrete image. Size at age tables were created using Excel spreadsheet program and the completed data set was copied to R base data base package, which is enclosed on diskette.

RESULTS AND DISCUSSION

All Protothaca specimens received from the Alaska Department of Fish and Game were prepared according to the methods outlined above, however, some clams were not analyzed for size at age data due either to the poor quality of their preparation or the confusing nature of their growth interruptions. In the first case, most specimens which were rejected for poor preparation were the smallest of clams. Although we attempted to get readable preparations from these fragile and difficult to handle specimens by mounting them whole in resin blocks before sectioning, a significant number of these small clams were cracked during preparation to the extent that no reliable data could be collected. Some larger specimens were also rejected because of cracking or breakage, however, they represented a much lower proportion of rejects than did the smaller clam sizes.

In the second case, although preparation quality was not the issue, some specimens contained a very confusing pattern of growth interruptions which we could not reliably interpret. Many of these specimens contained several very closely spaced growth interruptions early in life which were reminiscent of annuli, but whose proximity suggested it was unlikely that such slow growth would have occurred during these early years. While the author realizes that slow growth might indeed be the case, because these were generally anomalous patterns compared to the large majority of specimens, we felt that it was most prudent to reject them. The other category of unanalyzed specimens contained many closely spaced and difficult to distinguish interruptions near the distal portion of the valve in older specimens. In this case, it is very likely that growth was very slow, however, it was impossible to reliably identify individual annuli due to the dramatically interrupted growth record characteristic of these shells. A summary of specimens received, prepared and rejected is presented in Table 1.

The results of our Protothaca valve measurements and determinations of size at age are presented in Table 2. For each clam from the site specific transect quadrats, the size of the valve at each presumed annulus was determined. In a number of cases, values for the first annulus are missing

from the tables, indicating that the first annulus was not apparent and the investigator assumed that the earliest recognizable annulus corresponded to their second winter (see discussion below). Mean sizes at age and their standard deviations for all quadrats combined are also presented in these tables. Fig. 2 shows plots of these mean sizes at age for each site.

The most important aspect of this data collection concerns the accurate recognition of growth interruptions in Protothaca shells which corresponded to annular depressions or interruptions in growth. This is important in view of the fact that a variety of environmental influences may produce growth interruptions for varying lengths of time and these may all be reflected as interruptions in the regular accrual of shell material to the valve. Kennish (1980) has suggested that different events which cause interruptions in the growth of bivalve shells may produce characteristic effects in incremental growth patterns of the growing valve. For instance, an interruption caused by a sudden event such as a storm, may be characterized by normally wide micro-increments leading up to this event, and a sudden interruption of these increments caused by the storm. Growth may then be expected to resume approximately at its pre-storm pace shortly following these short-lived events, assuming the event has not produced some detrimental effects to the organism. In contrast, annular interruptions in growth are generally characterized by a gradual slowing of growth, evidenced by a gradual narrowing of micro-growth increments leading up to a dramatic interruption in the outer shell layer. Following this seasonal interruption, micro-growth increments would gradually increase in size as the new growing season begins. Fig. 3 shows the entire record of microgrowth increments recorded between two successive annuli in one specimen. One can clearly see that micro-increments following and prior to the annular interruptions are much narrower than those recorded during the rapid growth season.

In general, presumed annular interruptions appeared as deep notches in the outer shell layer, with the interruption extending through the middle shell layer of the valve (Fig.4). The interruptions in the incremental growth of the shell were typically very wide and obviously more deeply expressed than a variety of minor interruptions observed in the valve. Fig 5 demonstrates the progression of interruptions which this investigator has assumed to be annuli in two specimens from Prince William Sound. It is clear that these particular interruptions were the most dramatic recorded in the valve and these specimens are typical of presumed annulus expression in most specimens. Note that in both examples there are a number of other ancillary interruptions in the incremental deposition of calcium to the outer shell layer, however, they are obviously not as dramatic as those presumed to be annuli. Fig. 6 shows a high magnification image of an annulus, depicting the deep notching typical of these interruptions. Following this major interruption is another growth interruption, however, it is clear that this is not as wide or as dramatically notched into the outer shell layer as the annulus. Due to their close proximity, it is unlikely that one would score both as annuli.

Because these specimens were collected in spring, at the beginning of that year's growing season, it was assumed that very little growth would have occurred in most specimens for that year. As a result, even though annuli are typically difficult to observe right at the edge of a calcified element, it was generally assumed that one was present in these Protothaca.

One of the drawbacks to this study design was that periodic collections throughout a year, or at least a growing season, were not made to verify the accrual of shell material since a presumed

annulus. This would help to verify the notion that these particular growth interruptions were indeed annuli. Although this information is not yet available for Prince William Sound Protothaca, in Washington State, we have made collections through an entire year which have confirmed to us the expected appearance of annular interruptions. While this does not necessarily eliminate the possibility of confusions arising from a false annulus produced by a growth interruption similar to that occurring during the winter, it does add confidence to the notion that the investigator knows the general appearance of annuli in Protothaca valves and we are confident in the similarity between annual growth interruptions of Washington and Alaska clams.

In addition to the general validation concerns of annuli in these clam shells, of particular importance for their aging is the recognition of the first annulus. In figure 4, the first annulus is quite obvious, however, as we have mentioned, this was not always the case for all specimens. Morphological constraints and vagaries in preparation quality make the first annulus somewhat more difficult to recognize than annuli recorded later. Morphologically speaking, the outer shell layer in the region near the umbo, where the first annulus may be expected to form, is typically quite thin which makes the interruption in this layer less clear than that which is so obvious later in the growth of the valve. In addition, since P. staminea may have a protracted spawning season, recruitment of individuals to the beach will also occur over a protracted period, which will create potentially large variations in the placement of that first annulus on the shell depending upon how much of the first growing season is available to the newly recruited clam. This is certainly a major factor in Washington, however, due to a shorter growing season and perhaps a narrower window for successful recruitment in Prince William Sound, the problem may not be as dramatic for these populations. Nonetheless, I have observed significant variation in the placement of that first presumed annulus in these clams as reflected in the size at age tables, even among individuals of the same age. As a result, the reader will note that the author has frequently assumed the presence of a first annulus even when it was not visible for measurement. This was done largely on the subjective determination that the first visible, or easily determined annulus, was significantly more distal on the valve than most specimens. This is an obvious difficulty and such subjective determinations are not desirable, however, in some cases it seemed logical to do so. Examination of the largest recruits of the year at the end of the growing season would allow one to approximate the maximum size at annulus one for that population and thus, remove some of the subjectivity from this determination. Where the first annulus was thought to be unrecognizable, the decision whether or not assign the first apparent annulus as the first or second could be made on the basis of this maximum size for age 1 clams. Without this information, the author was left to make his best determination and the readers should be aware of this potential source of variability in the data and treat it accordingly.

Although only performed on a limited number of specimens, it is also interesting that age information was gleaned from the sectioned hinge tooth as well as the valve itself. In no case was there a discrepancy of ages determined from the hinge tooth versus that from the valve itself (TABLE 2) and it appears that the same record of incremental growth was preserved in the hinge tooth as that in the valve. The main difficulty in utilizing the hinge tooth as an ageing tool is similar to the valve in that recognition of the first annulus was often difficult. This may be particularly related to preparation difficulty as that portion near the umbo is sometimes lost in preparation of the tooth. The problem will be compounded for later recruits due to the very close placement of this annulus to the umbo. Fig. 6 shows hinge teeth preparations from a variety of

specimens of different ages and several ( ) show the first annulus very clearly.

It was originally thought that, in concept, due to the dramatic nature of this oil spill on some of the beaches of Prince William Sound, that the impacts of this event on clam growth might be directly observed in the incremental growth of the valve in the form of a significant interruption in this record. However, it is important to keep in mind that because the spill occurred in late March, it is unlikely that any effect in the micro-increment growth pattern would be recognized due to this event because it occurred prior to the time of rapid spring growth for these animals. We saw no evidence of a sudden and consistent interruption of micro-growth increment patterns which could be attributed to this event.

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TABLE AND FIGURE LEGEND

TABLE 1. Accounting of specimens received, prepared and analyzed by The Washington Department of Fisheries Calcified Tissue Analysis Laboratory for each site and quadrat from Prince William Sound *Protothaca staminea* collections. Categories include those from which data was collected. preparations of poor quality and of a confusing nature from which no data was collected, and those form which no data was collected for other reasons.

TABLE 2. For each site and specimen number, the size of the clam valve at each annulus, total clam length and clam age. Mean values and standard deviations for all annulus measurements combined are also given. All measurements are in millimeters. A) Double Bay B) Gibbon Anchorage C) Hells Hole D) Horseshoe Bay E) Wilson Bay.

TABLE 3. For some sites and specimens, the hinge tooth size at each annulus, total calm length and clam age. Mean values and standard deviations for all annulus measurements combined are also given. All measurements are in millimeters. A) Double Bay B) Gibbon Anchorage C) Hells Hole D) Horseshoe Bay E) Wilson Bay.

FIGURE 1. Photographs of hinge tooth sections from six specimens showing presumed annuli. All magnifications are 30x. A) Gibbon Bay, T2Q3, #3; age 6. Photograph also shows axis used for hinge tooth measurements. B) Gibbon Bay, T1Q8, #5; age 7. C) Gibbon Bay, T2Q3, #20; age 4. D) Gibbon Bay, T1Q3, #17; age 5. E) Wilson, T3Q6, #36; age 9. F) Gibbon Bay T1Q8, #3; age 8.

FIGURE 2. Plots of mean size ate age for all quadrats combined form each collection location. A) Double Bay B) Gibbon Anchorage C) Hells Hole D) Horseshoe Bay E) Simpson Bay F) Wilson Bay. Data points are mean values taken from Table 2.

FIGURE 3. Complete record of micro-growth increments between annuli for Gibbon Bay, T3Q3, #9. Magnification = 100x.

FIGURE 4. Presumed annulus in the valve of *Protothaca staminea.* Magnification = 200x.

FIGURE 5. Composite images of *P. staminea* valve showing deeply notched growth interruptions presumed to be annuli. Numbers correspond to the age represented by each annulus. Magnification = 30x. A) Gibbon Anchorage, T2Q8, #1; age 5. B)Double Bay, T1Q3, #13; age 6.

FIGURE 6. Plots of mean hinge tooth size at age for some locations and quadrats. Mean values are taken from Table 3.

APPENDIX E

Clam and Crab Histopathology Project

Alaska Department of Fish and Game

Division of Oil Spill Impact Assessment & Restoration

Contract No. IHP-91-036

Final Report

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INTRODUCTION

The material for this investigation was collected by personnel of the Alaska Department of Fish and Game and sent to Dr. Gary D. Marty, Department of Veterinary Pathology, University of California at Davis for processing. I received microscope slides containing stained sections, labeled only with the sample or collection number and the individual specimen number. This was in accordance with the protocol recommended by the HISTOPATHOLOGY TECHNICAL GROUP FOR OIL SPILL ASSESSMENT STUDIES IN PRINCE WILLIAM SOUND, ALASKA. As a member of that group, I insisted that the persons examining material be totally unaware of the location of the sampling site relative to proximity to the oil spill or subsequent movement of spilled oil.

Although the contract called for examination of clams and crabs, no crab material was received. A total of 578 molluscs were examined, 522 clams and 26 chitons. At the request of Dr. Joe Sullivan, the results of microscopic examination of the chitons were reported immediately upon completion and the last 35 clam diagnoses were reported to Mr. Jay Johnson in early April so his final report could be completed. The remainder of the microscopic findings were reported quarterly as stipulated in the contract.

RESULTS AND DISCUSSION

The summary reports consisted of: Diagnosis Sheets reporting the microscopic findings for each animal, using the NMFS RACE PATHOLOGY coding system for computer input by ADF&G; a summarization of the Diagnosis Sheets containing, in tabular form, the case no., date read, and diagnosis; and a short discussion of the possible significance of the abnormalities observed.

The results of microscopic examination of the 578 molluscs submitted to me are summarized in the accompanying table. They are grouped by collection (sample number) so that any patterns of abnormalities related to collection site (and presumably to exposure to oil) would be discernable. Even a cursory examination of the table reveals that patterns of pathological changes consistent with chemical injury are apparent only in Sample No. NO48P, in which 9 of 22 clams were diagnosed as having damage of the epidermis and gills.

The parasites identified in the samples are typical of those found in clams along the Pacific Coast (personal observations and published reports) except that the tetraphyllidian cestode *Echeneibothrium* sp. reported from littleneck clams and gaper clams in California were not observed. This is probably because the most likely final host, the bat sting ray (*Myliobatus californica*), was not present. The occurrent of the coccidian *Pseudoklossia* sp.? in 17 (2.9%) of the molluscs is interesting because it has been reported previously only from Washington on the Pacific Coast.

Most (67.8%) of the molluscs examined were within normal limits histopathologically. Infectious agents present were often "spotty" in distribution, occurring at relatively high levels in some samples and absent in others. This is not unusual in parasitic diseases and common in highly contagious infectious diseases such as viruses and bacteria.