F/S 13

STATE/FEDERAL NATURAL RESOURCE DAMAGE ASSESSMENT
DRAFT PRELIMINARY STATUS REPORT

Project Title:	EFFECTS OF HYDROCARBONS ON BIVALVES
Study ID Number:	Fish/Shellfish Study Number 13
Lead Agency:	State of Alaska, ADF&G; Division of Commercial Fisheries
Cooperating Agency(ies):	Federal: USFS State: DNR
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Date Submitted:	November 27, 1991

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EXECUTIVE SUMMARY

The field component of the bivalve project in 1991 was a reciprocal clam transplant between oiled and non-oiled sites in Prince William Sound (PWS). The transplant allows the comparison of growth rates between oiled and non-oiled areas and offers a means of measuring the damage caused to bivalves by the Exxon Valdez Oil Spill (EVOS). The Pacific littleneck clam, *Protothaca staminea*, was used for this study as it is the most widely distributed bivalve in the area of interest. During the transplant, hydrocarbon and histopathology samples were collected to document continuing levels of hydrocarbon contamination and to assess tissue damage to littleneck clams. The 1991 reciprocal transplant was a repeat of work done in 1990.

Growth of clams transplanted from non-oiled to oiled sites was significantly less than clams transplanted from oiled to non-oiled sites in both 1990 and 1991. The reduced growth was seen in both length and weight. The difference in growth appears to be larger in 1991 than in 1990. The decreased growth may be due to oiling, but geographic or site effects (ie. temperature, food supply, etc.) can not be discounted. Methods are being examined that will allow the separation of differences caused by oiling and site effects.

Initial results of hydrocarbon samples indicate widespread oiling in sediments and tissues from both oiled and non-oiled sites in PWS and oiled sites in Cook Inlet. To date, 823 hydrocarbon and sediment samples (294 from 1989, 409 in 1990, and 120 from 1991) have been collected and submitted for analysis. Preliminary results from 290 clams indicate that 60.7% and 79.0% of the clam and sediment samples analyzed from oiled sites showed contamination and 14.1% and 60.5% of the clam and sediment samples from non-oiled sites showed contamination. Further analysis of the samples is needed to determine the source of the hydrocarbon contamination.

The microstructure of bivalve shells provides a detailed record of past growth increments. Work has been completed on 504 clams collected from PWS in 1990 to examining their microstructure for a growth "check" corresponding to the EVOS. Researchers with the Washington Department of Fisheries observed no significant corresponding interruption in micro-growth increments. It should be noted the EVOS occurred in the early spring, which is a slow period of growth for clams.

Collection of length at age data from littleneck clams is continuing at the University of Alaska, Institute of Marine Science in Seward (IMS). Aging of clams collected in 1989 and 1990 has been completed. A total of 3,540 littleneck clams collected in PWS have been submitted for aging.

OBJECTIVES

1. Test the hypothesis that the level of hydrocarbons in bivalves and in sediments is not related to the level of oil contamination of a beach. Within Prince William Sound, the experimental levels include no oil contamination, moderate or heavy oil contamination, and oil contamination which has been mechanically treated. The experiment is designed to detect a difference of 1.9 standard deviations in hydrocarbon content with the probability of making a type I and type II error of 0.05 and 0.1, respectively. Outside of Prince William Sound, the experimental levels include no oil contamination and moderate or heavy contamination. This portion of the experiment is designed to detect a difference of 1.4 standard deviations in hydrocarbon content with the probability of making a type I and type II error of 0.05 and 0.1, respectively.

2. Document the presence and type of damage to tissues and vital organs of bivalves sampled from beaches such that differences of ±5% can be determined between impact levels 95% of the time. Impact levels within Prince William Sound are no oil contamination, intermediate or high oil contamination, and intermediate or high oil contamination in a treated condition. Outside of PWS, the levels include no oil contamination and intermediate or high oil contamination.

3. Test the hypothesis that the growth rate of littleneck, butter and razor clams is the same at beaches of no oil impact, intermediate or high levels of oil impact and intermediate or high levels of oil impact in areas which had been treated. This experiment is designed to detect a difference in mean shell height equal to the difference between the mean shell height at age i and age i+1 clams with the probability of making a type I error equal to 0.01 and probability of making a type II error equal to 0.05.

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

INTRODUCTION

Fish/Shellfish (F/S) Study 13, was initiated in 1989, and continued through 1991, to examine the affects of the oil spill (EVOS), as a result of the grounding of the *M/V Exxon Valdez* on March 24, 1991, on bivalve mollusk populations in PWS, outer Kenai Peninsula, Cook Inlet, and Kodiak Island. The EVOS contaminated near shore areas where populations of bivalves exist in all these locations. Bivalve populations are an important component of the food chain, existing as prey for sea otters and bears, and supporting subsistence and sport fisheries in these areas. The effects of oil on the growth and survival of bivalves have been well documented (Anderson *et al.* 1982, 1983; Augenfeld *et al.* 1980; Dow 1975, 1978; Keck *et al.* 1978). Bivalves may be particularly susceptible to contamination by oil because they are relatively sedentary and inhabit intertidal areas. In addition bivalve mollusks are more likely to accumulate petroleum hydrocarbons because they metabolize hydrocarbons at a much lower rate than finfish species. It is hypothesized that increased hydrocarbons in near shore areas could affect bivalves for a long period of time by increasing mortality, decreasing growth, and causing sublethal injuries.

Transect sampling for cockles *Clinocardium nuttali*, butter clams *Saxidomus giganteus*, and littleneck clams was conducted at oiled and non-oiled beaches within PWS, outer Kenai Peninsula, Cook Inlet, and Kodiak Island during 1989 as part of F/S Study 13 and F/S Study 21. Fish/Shellfish Study 13 was concerned with bivalve populations within PWS and F/S Study 21 was concerned with bivalve populations outside PWS. These two studies were combined in 1990 under F/S Study 13. Transect sampling for butter and littleneck clams was continued in 1990. The primary focus of the 1989-90 transect sampling was to obtain length at age data for bivalves by known tidal height. The data collected was to be used to determine possible growth effects between oiled and non-oiled sites. Cockles were not included in 1990 due to the low number available during transect sampling in 1989. Pacific razor clams *Siliqua patula* were added to the study and sampled on the West side of Cook Inlet and South Alaska Peninsula in 1990.

During 1991, the field sampling was limited to a reciprocal transplant of littleneck clams in PWS and the collection of hydrocarbon and histopathology samples. The reciprocal transplant experiment was conducted to further evaluate and compare site specific effects on growth of clams transplanted between oiled and non-oiled sites. Hydrocarbon and histopathological samples were collected at each site to document the presence and level of hydrocarbon contamination. The reciprocal transplant experiment was conducted in PWS in 1990 and repeated in 1991. The experiment consisted of tagging littleneck clams and transplanting them between oiled and non-oiled sites during the spring. The clams were then recovered during the fall thereby bracketing the period of maximum growth. To separate out differences in growth due to site, growth of clams that were transplanted will also be compared with the growth of resident clams (clams that were not transplanted) from each site.

A contract with the Washington Department of Fisheries was established for microstructure analysis of littleneck clams in 1990 to ascertain if a stress check was caused by the EVOS. A number of investigators have verified the daily deposition of individual microincremental patterns in quahog *Mercenaria mercenaria* due to storms and heated discharges from nuclear power plants (Lutz and Rhoads, 1981; Kennish and Olsson, 1975; and Fritz and Lutz, 1986). The microstructure examination of the clam's hingeplate and valve cross sections has been completed. Clam ages, determined by these methods, have been compared to visual aging using presumptive annuli of the external surface of the valve which was conducted by the University of Alaska, Institute of Marine Science (IMS) in Seward.

STUDY METHODOLOGY

Study Sites

Littleneck clams were sampled at six study sites in PWS that represented two levels of oil contamination (no contamination or non-oil and intermediate or high contamination or oil) (Figure 1, Table 1). Beaches with no oil contamination were Hell's Hole, Double Bay, and Simpson Bay. Beaches with moderate or heavy oil contamination were Gibbon Anchorage, Wilson Bay, and Horseshoe Bay. Transect sampling and/or reciprocal transplant experiment was conducted at each site during 1989-1991 (Table 1).

For each sample site, the following site description information was recorded: site orientation (N-NW etc.), latitude, longitude, low tide height, temperature and salinity of the water, weather and wave action (Table 2). Temperature and salinity of the water were measured at a distance of approximately 5 meters offshore from the sampled beach at the daily low slack tide.

Aging

All clams collected from quadrates A, B, and C at each tide height at each site were weighed and total length recorded. After the shells were cleaned and numbered, all shells were sent to the IMS for aging. The length at age of each clam age was recorded. IMS has been contracted to age the 5,400 shells to be collected in 1991 (450 per tide height x 2 tide heights x 6 beaches). In addition, littleneck clams collected during transect sampling in 1990 and sent to IMS for aging have been aged. The data has been entered into an RBASE database.

Microstructure Aging

Microstructure analysis was initiated to determine if a "check" attributable to

EVOS could be seen. A random sample of 600 clams comprised of 50 clams from the six 1990 transect sampling sites and representing the two beach types (no contamination, and intermediate or heavy contamination) was to be analyzed, but low recovery in some areas affected the size of the sample sent for analysis. Only 504 littleneck clams were submitted to the Washington Department of Fisheries for microstructure analysis (Volk 1991). Of these, 135 clams were not analyzed due either to the poor quality of the preparations (clams were to small to be sectioned) or the confusing nature of the growth interruptions. Therefore data was collected from 369 clams.

Reciprocal Transplant Experiment

Littleneck clams were transplanted from oiled to non-oiled beaches and from non-oiled to oiled beaches in PWS (Figure 2). Criteria for selecting paired oiled/non-oiled beaches, to the extent possible, included similarity in profile, drainage, and length-frequency distribution of bivalves.

PAIRED BEACHES

OILED		NON-OILED
GIBBON ANCHORAGE	------	HELL'S HOLE
WILSON BAY	------------	SIMPSON BAY
HORSESHOE BAY	---------	DOUBLE BAY

Two tidal heights were utilized (+1.5 ft and +3.0 ft) at paired beaches. Clams were transplanted to the same tidal height from which they originated. At each tidal height, three stations were established creating triplicate sampling stations at each height (Figure 3). Each location consisted of three adjacent clearly marked 0.25 m^2 quadrates. One quadrate (C) was marked, but was not disturbed. Another quadrate (B) was dug to a depth of 0.3 m and all of the removed clams and sediment were replaced in the quadrate. Clams from quadrate (B) had a small notch filed into the ventral edge of the valves to mark the time of disturbance. When fewer than 50 clams were available from this quadrate, additional clams from the same tide height were notched and included with the clams originating in the quadrate. The third quadrate (A) was dug to a depth of 0.3 m and all original clams were removed. The transplanted clams were placed in this quadrate along with the original sediment. The clams removed from quadrate A were collected either for use as donor clams at the reciprocal site or to augment any deficit in clams found in quadrate B.

Clams to be transplanted were obtained by digging a trench along the prescribed tidal height of the donor beach until 150 clams between 15mm and 35mm in length had been collected. Additional clams were collected from this trench for hydrocarbon and necropsy analysis. Fifteen millimeters is considered to be the smallest size which can effectively be tagged. Clams less than 35 mm were selected to narrow the range of ages for which differences in growth were determined and because the maximum growth rate appears to occur within this size range. A sample of 50 specimens transplanted into each of three plots provided 150 samples from each tidal height at each beach and 450 clams for each tidal height and level of beach impact. Sample size for growth is based on the difference between mean shell length for age i and age i+1 clams, variance in shell height for age i+1 clams, probability of making a type I error equal to 0.01 and probability of making a type II error equal to 0.05 (Netter and Wasserman 1985). The sample size was determined after comparing data for mean shell length and variance in shell length taken from Paul and Feder (1973) and

Nickerson (1977). The sample size for detecting between impact level differences in growth at age of clams in the size range of 15 mm to 35 mm was estimated at 133 clams from the Paul and Feder (1973) data and at 85 clams from the Nickerson (1977) data for each impact level. The higher estimate was rounded up to 150 clams by including the next smaller size group (age 5-6). The purpose of 3 sites for each impact level was to provide replicates at each impact level.

Transplanted clams were identified by marking each clam with a numbered Floy tag secured with a quick-drying adhesive. All marked clams had a small notch filed into the ventral edge of the valves to mark the time of transplantation. Individual clams were measured and wet and dry weights of clams were recorded so that clam condition could be compared in terms of a weight to length ratio. After tagging, clams were placed in buckets containing seawater for transport to each sampling station. In most cases, clams were held less than 24 hours, the clams destined for Hell's Hole, were not transplanted for 72 hours. The delay was due to inclement weather.

Each reciprocal sampling site was marked with a small anchor driven to a depth of 0.6 m into the upper right hand corner of quadrate A (Figure 3). A small inconspicuous buoy was attached to the anchor line to identify each plot. Stations were placed 2 m apart. The location of the stations was roughly triangulated with major beach features to aid in future location. A detailed record of project activities was maintained for each sample quadrate at each site and tide height.

Transplanted clams (quadrate A and B) and control clams (quadrate C) were recovered by digging an 0.25 m^2 hole at the appropriate tide height and station. A concerted effort was made to recovery all clams that had been transplanted. This sometimes necessitated expanding the hole along the tide height. Clams in C quadrate were occasionally very difficult to locate. When 50 control clams could not be found in the C quadrate, the control clams were supplemented by adding non-marked clams from A and B quadrate. Clams for aging were placed in plastic bags for transport and freezing. Tagged clams were bagged individually to guard against tag lose due to freezing. The clams recovered were measured and wet and dry weights were recorded. The clams were sent to the Institute of Marine Science for aging.

Collection of Sediment Hydrocarbon Samples

A total of twelve sediment samples were collected from each beach site (triplicates from each tide height, spring and fall). All sediment samples were collected before bivalve sampling was performed. The triplicate hydrocarbon samples from each tide height were composite sediment samples which were collected by scooping one tablespoon (15 cc) of sediment to a depth of 2 to 3 cm from each of the nine sample quadrates at a tide height. The small subsamples of sediment taken from each sampling quadrate provided a representative mixture of sediment composition and contamination along the tide height.

All samples were placed in precleaned 4 oz glass jars. Each jar was labelled with the site name, latitude, longitude, date, "SEDIMENT", transect number, sample number, names of the sampling team members, "BIVALVE", and "ADF&G". Data was recorded on the appropriate form.

A total of twelve composite sediment samples (three per tide height) were obtained from each beach sampled. This provided a total of 36 samples for each impact level (3 hydrocarbon sample/tide ht. * 2 tide hts./site = 6 hydrocarbon samples/site; 6 hydrocarbon samples/site * 3 sites/impact level * 2 sampling periods = 36 hydrocarbon samples/impact level). The industry standard is 8 samples for each treatment level. A sample size of 9 composite samples is considered an adequate number of samples to detect a difference in sediment contamination between impact levels at the desired α and β levels. This coverage level was doubled.

Collection of Bivalve Hydrocarbon Samples

Four hydrocarbon samples were obtained from each sampling station (tide height and site). Each hydrocarbon sample was composed of 10 to 20 clams. Ten to twenty specimens with a shell length of 2-5 cm were collected from the donor beach trench and retained for hydrocarbon analysis, to form a hydrocarbon sample at the time of transplantation. In addition, during transplantation 10 to 20 additional clams were collected from the donor beach trench for placement with tagged clams in quadrate "A" at each sample station. These clams comprised the hydrocarbon sample at the time of recovery in the fall. Each clam was placed directly in a sample container before another bivalve was obtained.

Bivalve samples were limited to a particular size range because rates of uptake, metabolism, and depuration by clams probably change with size. If specimens of the desired size were not found in each of the sampling quadrates, then the desired number of additional specimens were collected from adjacent areas at the same tidal height.

Specimens were placed together on a piece of aluminum foil cleaned with methylene chloride. The samples were held in an untreated wooden box which was opened as little as possible. Prior to freezing each sample was double wrapped in aluminum foil, logged onto a chain of custody form, and the number of specimens and sample size range tabulated. Each sample was labelled with the site name, latitude, longitude, date, species, transect number, names of the sampling team members, "BIVALVE", and "ADF&G". Data was recorded on the appropriate form.

Triplicate tissue samples from each tide height at each site (sampling station) were collected to provide a representative mixture of bivalve tissue composition and contamination across the site. The desired size of each composite tissue sample was 15 gm. The number of bivalves to provide this sample from each transect was estimated based on the average size of individuals of each species. An estimate of 3 hydrocarbon samples from each site was needed to detect contamination between impact levels.

Histopathology Samples

Collection of specimens for necropsy began only after all hydrocarbon samples had been taken. Fifteen additional clams were collected from the donor site trench at each tide height. These clams were notched and included with the tagged clams in quadrate A at the receptor site. At time of recovery in the fall, five clams per quadrate were retained for necropsy analysis. Five clams per quadrate allows 15 necropsy samples per tide height and 30 samples per site and two sampling periods provides for a total of 360 necropsy samples from all 6 sites. This sample size will facilitate detection of tissue damage of ±5% with 95% confidence between samples obtained from beaches with different levels of oil impact and detection of gross differences between beaches with no and medium or high oil impact.

Specimens were collected as they were dug. Each was measured, shucked, and the tissue placed in a tissue cassette and immersed in formalin. Sampling procedures and quality assurance were conducted as outlined in the histopathology guidelines set forth in the study plan. Histopathological analysis of bivalve tissues will include all criteria listed in the histopathology guidelines. Necropsies are being conducted by Dr. Albert K. Sparks.

STUDY RESULTS

1990 Transect Sampling

The mean shell length of littleneck clams collected during transect sampling in 1990 was highly variable (Table 3). The largest mean shell length in the oiled areas was Green Island at 23.1 mm; followed by Gibbon Anchorage, 20.3 mm; Snug Harbor, 19.2 mm; North Chenega, 16.3 mm; Wilson Bay, 13.8 mm; and Horseshoe Bay, 13.4 mm. Of the non-oiled sites, Double Bay had the largest clams on average with a mean of 23.5 mm; followed by So. Pellow Cove, 22.1 mm; Simpson Bay, 17.8 mm; and Hell's Hole, 16.5 mm. The length frequencies of littleneck clams collected was also highly variable (Figures 4 and 5). Green Island and Gibbon Anchorage had the largest and most variable lengths of littleneck clams in the oiled areas (Figure 4) and Double Bay had the largest clams in the non-oiled areas (Figure 5).

Aging Analysis

The aging of littleneck clams from seventeen sites sampled in 1989 and eighteen sites in 1990 has been completed by the University of Alaska. Aging of littleneck clams collected during 1991 has been initiated. At present only age data from 1990 transect sampling has been completed and entered into the database. Total length, whole weight, shell weight and length, and mean length at age for clams collected during the 1990 transect sampling are available (Table 4). Clams in the non-oiled areas were longer and weighed more on average than clams in the oiled areas (Table 4). Mean length at age between oiled and non-oiled sites was variable (Table 4, Figure 6). The clams in the non-oiled sites, especially Double Bay were mostly comprised of age-5 clams compared to more age-3 clams in oiled areas (Figure 6).

Growth data by quadrate will also be available to establish differences in growth by tidal height. Relative cohort strength will be predicted using all the quadrates from each site.

Documentation of young-of-the-year (YOY) clams will continue to be a part of the growth and age determinations conducted by UA-IMS. The age of clams by site is available for the 1990 transect sampling data, and 1990 reciprocal transplant experiment. As analysis of the 1989 transect data and determination of ages for clams recovered during 1991 reciprocal transplant sampling is completed, the age and size of clams by site will be compared between years.

Microstructure Aging

The microstructure analysis of littleneck clam valves has been completed. A total of 369 clam valves from six sites were examined. The complete summary of the microstructure analysis was completed by Volk *et al.* (1991). The report is available upon request from the Principal Investigator.

For each clam the size of the valve at each presumed annuli was determined. In addition, the sectioned hinge teeth of 90 clams from five sites were examined for the presence of the "check". The ages from the teeth were also compared to ages determined from the sectioned shells and ages determined by aging whole valves.

The microstructure analysis of the sectioned valve and tooth indicated no evidence of a sudden and consistent interruption of micro-growth increment patterns which could be attributed to the oil spill. But, it is important to bear in mind that the oil spill occurred prior to the observed rapid spring growth period when the clam usually experiences a greater degree of shell growth. The comparison of the sectioned tooth and valve ages indicated no disagreement between the two methods. Agreement between sectioned and whole valves was not as good. Generally there was no more than one years difference between the methods, but differences as great as four years were observed. Both agers experienced difficulty in identifying the first annuli which could explain most of the disparity.

Reciprocal Transplant Experiment

The growth of littleneck clams transplanted from oiled to non-oiled sites was greater than the growth of clams transplanted from non-oiled to oiled sites both 1990 and 1991 (Figures 7-10). The mean growth of clams transplanted to non-oiled sites was almost double that of clams transplanted to oiled sites in 1990 (Table 5). The differences in growth may be due to oiling. However, the difference may be a site specific effect such as higher water temperatures, or more food availability. Methods are presently being looked at to separate out differences due to oiling and those attributable to site differences.

Sediment and Bivalve Hydrocarbon Samples

To date, 823 hydrocarbon and sediment samples (294 from 1989, 409 from 1990, and 120 from 1991) have been collected and submitted for analysis. Results have been received from 290 of these samples. A total of 533 samples in the analytical queue at the Auke Bay Repository. Preliminary results indicate that 60.7 % and 79.0 % of the clam and sediment samples analyzed from oiled sites showed contamination and 14.1 % and 60.5 % of the clam and sediment samples from non-oiled sites show contamination.

Histopathology Samples

Histopathological analysis has been received for seven littleneck samples collected from Prince William Sound during 1989. A total of 111 clams were examined. A few individuals appeared to be in the process of repairing small surface wounds of the epidermis of the foot and mantle; several had degenerated kidney or digestive gland epithelium; and others appeared to exhibit gonadal suppression of unknown causation. Also noted was a lack of typical holotrichous ciliates in the gill chambers and other commensals and parasites. Their absence may be related to the distribution of their alternate host.

RESTORATION PLANNING

One proposal for bivalves was submitted to RPWG on November 13, 1991 (Appendix C). The restoration plan for bivalves is to conduct a bivalve restoration and enhancement project. The goal of this restoration project is to assess the restoration and enhancement needs of those bivalves populations injured by EVOS, and to determine if mariculture techniques are a valid tool to use in restoration or enhancement of the identified species. Initiation of a demonstration project would take place if cogent evidence of the applicability of mariculture techniques in restoration and enhancement is concluded. The details of the restoration proposal can be found in Appendix C.

RECOMMENDATIONS FOR 1992 SEASON

No further field sampling is recommended for the 1992 season for Natural Resource Damage Assessment (NRDA). It is recommended the 1992 season be used to complete data entry, data analyses, and a final report. Specific activities to complete would include: (1) aging of littleneck clams collected in 1991 and enter data into database; (2) hydrocarbon analysis of tissue and sediment samples; (3) histopathology analysis of necropsy samples; (4) analyses of littleneck clam data (both transect sampling and reciprocal transect data) for differences in growth and sizes at age; and (5) writing a final report for Fish\Shellfish Study 13.

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TABLES

Table 1. Sites for transect sampling and reciprocal transplant experiment in Prince William Sound, 1989–1991.

	Oil/	1989		1990		1991			
Site name	Non–Oil	Transect	Transplant	Transect	Transplant	Transect	Transplant	Latitude	Longitude
Double Bay	non–oil			X	X		X	60°27.55'	146°28.36'
Hell's Hole	non–oil	X		X	X		X	60°42.44'	146°23.00'
Pellew Cove	non–oil	X		X				60°51.10'	147°39.46'
Simpson Bay	non–oil	X		X	X		X	60°37.73'	145°53.11'
Snug Harbor	oil	X		X				60°15.21'	147°44.62'
North Chenega	oil			X				60°22.69'	147°57.34'
Gibbon Anchorage	oil	X		X	X		X	60°16.07'	147°26.12'
Green Island	oil			X				60°16.59'	147°25.39'
Horseshoe Bay	oil			X	X		X	60°00.97'	147°57.46'
Wilson Bay	oil	X		X	X		X	60°02.04'	147°55.74'

Table 2. Environmental conditions for sampling sites used in reciprocal transplant experiment of littleneck clams in Prince William Sound, 1991.

Sitename	Date	Air Temp.(°C)	Sea Temp.(°C)	Salinity (ppt)	Waves	Weather
Non–Oiled Sites						
Double Bay	09–Sept.	16	13	26	rippled	overcast
Hell's Hole	29–Aug.	16	13	23	glassy	clear
Simpson Bay	09–Sept.	16	11	26	rippled	drizzle
Oiled Sites						
Gibbon Anchorage	07–Sept.	15	12	25	glassy	overcast
Horseshoe Bay	07–Sept.	15	12	26	rippled	overcast
Wilson Bay	08–Sept.	16	12	26	wavelets	rain

Table 3. Number and length of littleneck clams recovered from oiled and non–oiled transect sampling sites in Prince William Sound, 1990.

Site	Size of Clam	Number of Clams	Minimum Length (mm)	Maximum Length (mm)	Mean Length (mm)	S.D.
Non–oiled sites						
Double Bay	Less than or equal to 15 mm	110	3.2	15.0	11.0	2.5
	Greater than 15 mm	781	15.2	44.2	25.3	5.7
	Total	891	3.2	44.2	23.5	7.2
Hell's Hole	Less than or equal to 15 mm	253	5.3	15.0	11.7	2.2
	Greater than 15 mm	338	15.0	33.7	20.2	3.9
	Total	591	5.3	33.7	16.5	5.4
Pellew Cove	Less than or equal to 15 mm	11	5.6	15.0	11.2	3.1
	Greater than 15 mm	19	16.9	41.2	28.4	7.1
	Total	30	5.6	41.2	22.1	10.3
Simpson Bay	Less than or equal to 15 mm	61	6.0	15.0	11.1	2.5
	Greater than 15 mm	62	15.0	38.5	24.3	6.8
	Total	123	6.0	38.5	17.8	8.4
Oiled sites						
North Chenega	Less than or equal to 15 mm	93	4.1	15.0	8.9	3.0
	Greater than 15 mm	75	15.4	40.9	25.5	7.4
	Total	168	4.1	40.9	16.3	9.9
Gibbon Anchorage	Less than or equal to 15 mm	153	4.8	14.9	11.8	2.2
	Greater than 15 mm	474	15.0	40.4	23.0	5.0
	Total	627	4.8	40.4	20.3	6.6
Green Island	Less than or equal to 15 mm	113	6.2	15.0	12.0	2.2
	Greater than 15 mm	413	15.0	50.5	26.1	7.4
	Total	526	6.2	50.5	23.1	8.9
Horseshoe Bay	Less than or equal to 15 mm	345	4.7	15.0	11.0	2.4
	Greater than 15 mm	139	15.0	29.4	19.3	3.7
	Total	484	4.7	29.4	13.4	4.7
Snug Harbor	Less than or equal to 15 mm	5	6.8	11.1	9.5	1.8
	Greater than 15 mm	8	15.5	38.9	25.3	9.8
	Total	13	6.8	38.9	19.2	11.0
Wilson Bay	Less than or equal to 15 mm	302	3.8	15.0	9.7	2.7
	Greater than 15 mm	180	15.1	36.2	20.7	4.6
	Total	482	3.8	36.2	13.8	6.4

Table 4. Mean whole weight, shell weight, shell length, and mean length at age of littleneck clams collected at transect sampling sites in Prince William Sound, 1990.

Site	Sample Size	Whole Weight (g)	Shell Weight (g)	Shell Length (mm)	Age 0	Age 1	Age 2	Age 3	Age 4	Age 5	Age 6	Age 7	Age 8	Age 9	Age 10	Age 11	Age 12	Age 13
Non–Oiled Sites																		
Double Bay	891	3.58	2.24	23.50	3.2	5.3	9.5	14.6	19.8	24.3	27.5	30.7	33.8	35.8	37.8	39.3	40.0	35.6
Hell's Hole	591	1.31	0.82	16.49	2.8	5.7	9.8	14.4	18.8	22.9	25.2	26.3						
Pellew Cove	30	4.21	2.42	22.08	4.6	7.9	11.5	15.1	19.3	24.5	28.3	32.1	34.4	31.5				
Simpson Bay	123	2.36	1.38	17.76	3.0	5.6	10.1	13.1	17.4	21.8	26.8	30.8	33.5	35.2	35.1			
Combined	1,635	2.68	1.67	20.51	3.0	5.5	9.7	14.4	19.2	23.6	26.6	29.2	33.7	35.6	37.8	39.3	40.0	35.6
Oiled Sites																		
Snug Harbor	13		2.14	19.24	2.7	4.9	7.5	10.7	13.6	18.6	22.4	26.2	29.3	31.9	35.9	35.8	38.4	
Green Island	526		2.44	23.03	3.2	5.3	8.9	13.3	18.1	22.6								
Gibbon	627	2.53	1.59	20.23	3.7	7.0	11.4	16.1	20.7	24.2	27.0	29.4	31.6	34.1	37.0	37.3		
Horseshoe Bay	484	0.74	0.44	13.31	2.8	5.6	9.2	13.1										
Chenega	168	1.99	1.48	16.29	3.9	6.3	9.4	13.0	16.4	20.4	23.9	27.2	30.5	32.7	35.0	36.2	36.4	40.5
Wilson Bay	482	1.04	0.62	13.75	3.7	6.3	9.5	12.9	16.5	19.1	22.3	25.7	30.1	32.9				
Combined	2,300	1.21	1.34	17.76	3.4	6.1	9.8	13.9	18.4	22.0	24.8	27.7	30.9	33.5	36.6	37.0	36.6	40.5

Table 5. Mean initial length, mean growth (mm), mean initial weight, and mean growth (g) of littleneck clams that were part of the reciprocal transplant experiment in Prince William Sound in 1990. There were six sites (three oiled and three non–oiled), and two tide heights (1.5 and 3.0 ft) at each site. Clams were reciprocally transplanted between oiled and non–oiled sites.

Donor Site	Tide Height (ft)	Mean Length (mm)	S.D.	Mean Growth (mm)	S.D.	Mean Weight (g)	S.D.	Mean Growth (g)	S.D.
Non–Oiled Sites – Transplanted to Oiled Sites									
Double Bay	1.5	27.2	3.7	0.7	0.8	5.6	2.1	−0.2	0.7
Double Bay	3.0	27.9	4.0	0.8	1.1	6.1	2.4	0.1	0.8
(transplanted to Horseshoe Bay)									
Hell's Hole	1.5	27.9	5.0	1.5	2.1	6.2	3.4	0.1	1.4
Hell's Hole	3.0	28.6	4.2	0.8	1.2	6.3	2.6	−0.3	0.8
(transplanted to Gibbon Anchorage)									
Simpson Bay	1.5	22.8	2.8	1.2	1.3	3.4	1.4	0.1	0.6
Simpson Bay	3.0	27.1	3.8	0.4	0.9	5.6	2.2	−0.3	0.7
(transplanted to Wilson Bay)									
Oiled Sites – Transplanted to Non–Oiled Sites									
Gibbon Anchorage	1.5	25.4	4.2	1.8	1.7	5.0	2.5	0.2	1.2
Gibbon Anchorage	3.0	27.7	4.1	2.0	1.6	5.9	2.6	0.5	0.9
(transplanted to Hell's Hole)									
Horseshoe Bay	1.5	24.0	4.4	3.3	2.4	4.2	2.5	1.2	0.8
Horseshoe Bay	3.0	21.6	3.2	2.9	2.4	3.1	1.6	0.8	0.7
(transplanted to Double Bay)									
Wilson Bay	1.5	21.4	3.2	1.7	1.7	2.7	1.3	0.4	0.6
Wilson Bay	3.0	22.0	4.4	1.8	1.9	3.0	1.9	0.5	0.7
(transplanted to Simpson Bay)									

FIGURES

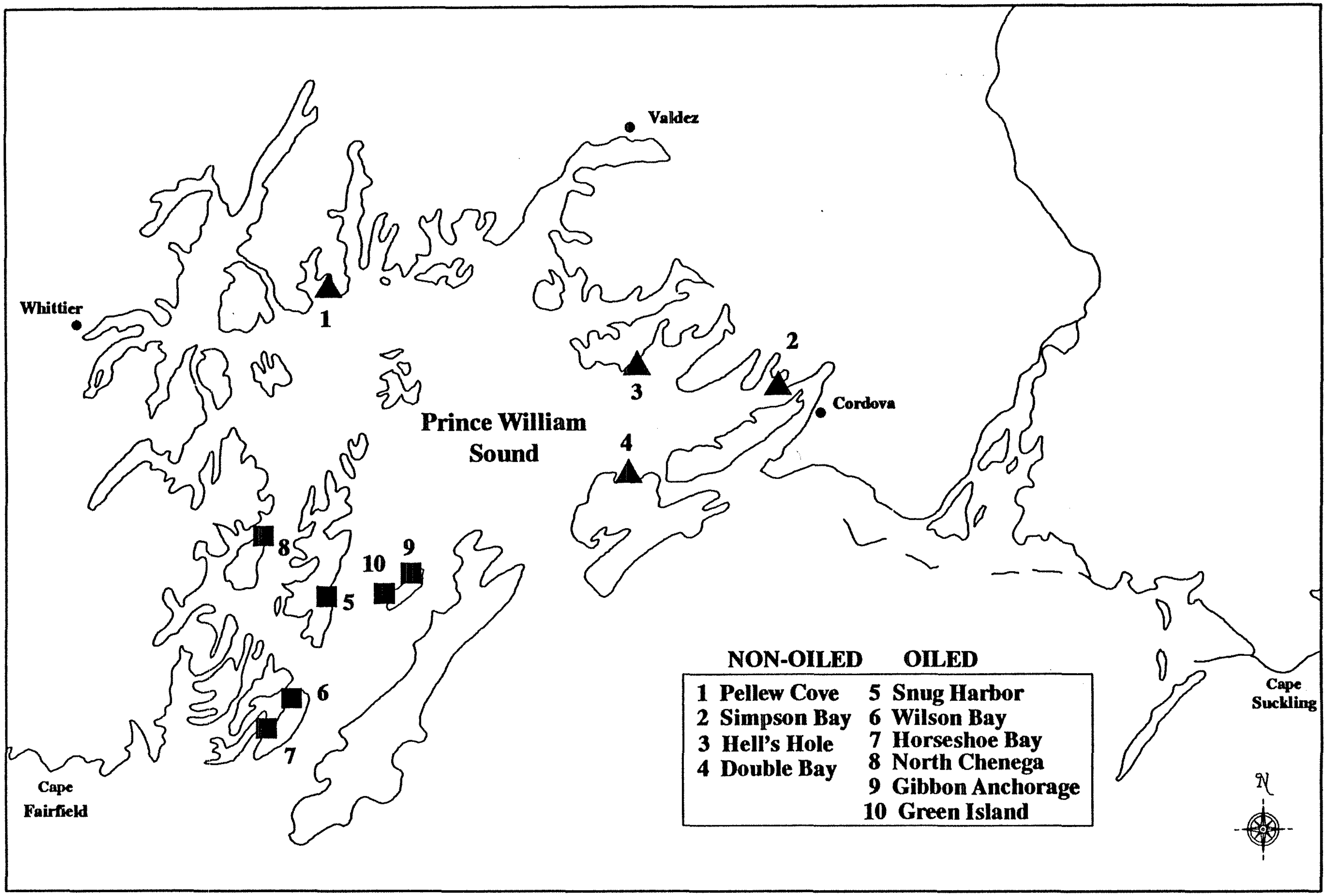

Figure 1. Sites for transect and reciprocal transplant sampling in Prince William Sound, 1989-1991.

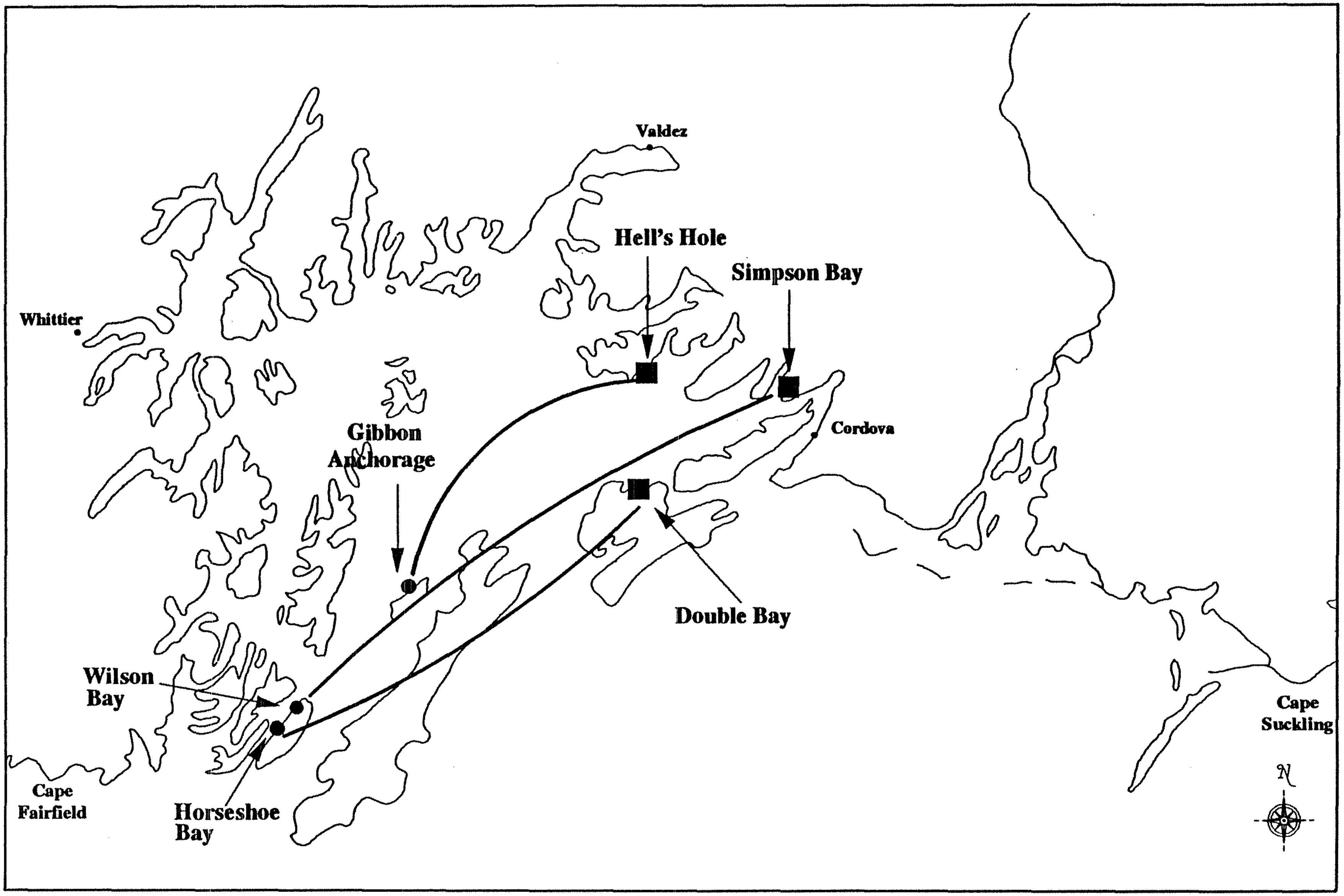

Figure 2. Paired locations for the reciprocal transplant study of littleneck clams in Prince William Sound, 1990-1991.

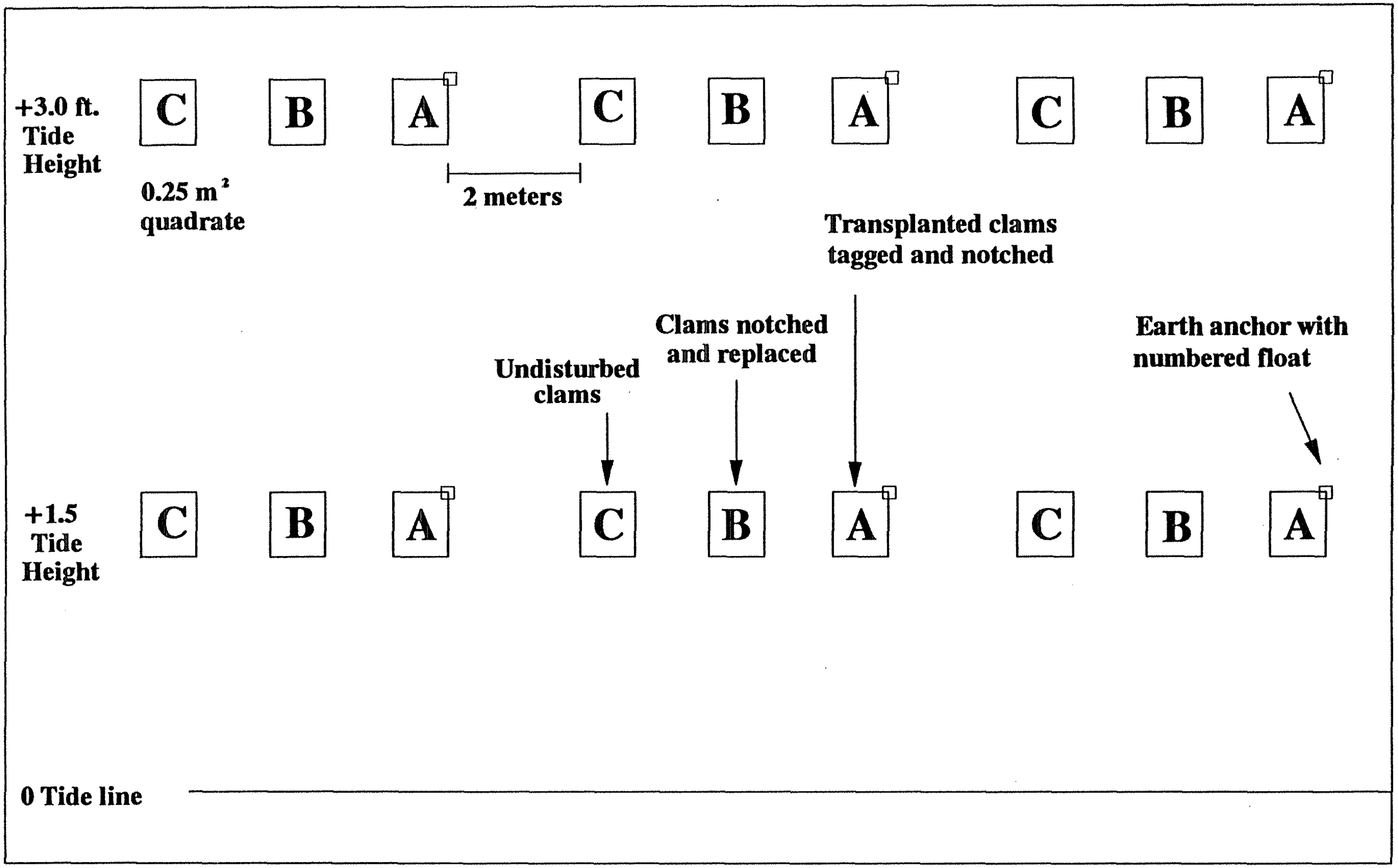

Figure 3. Beach plot configuration for reciprocal transplant study of littleneck clams in Prince William Sound, 1990-1991.

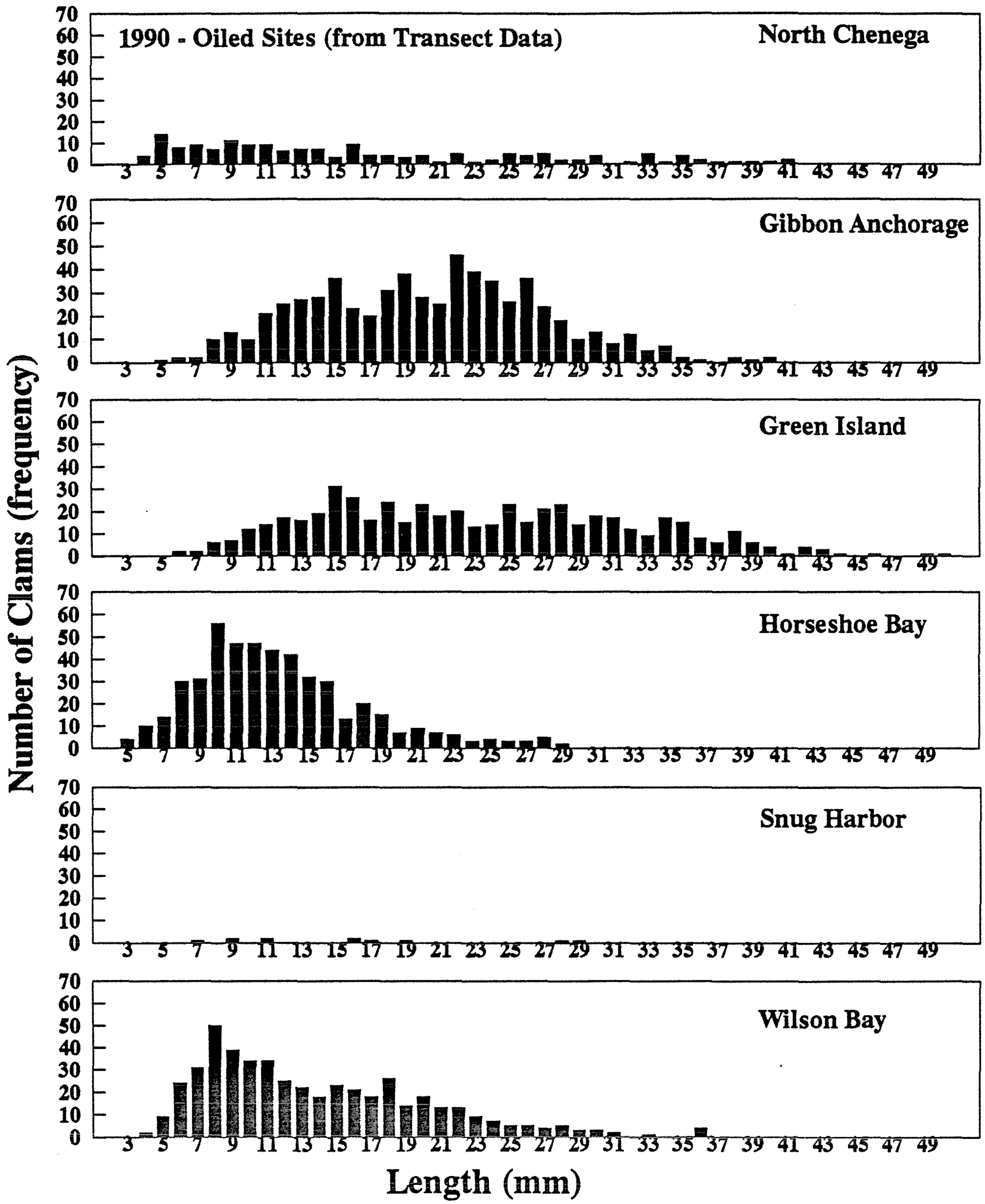

Figure 4. Comparison of length frequencies of littleneck clams collected at oiled transect sampling sites in Prince William Sound, 1990.

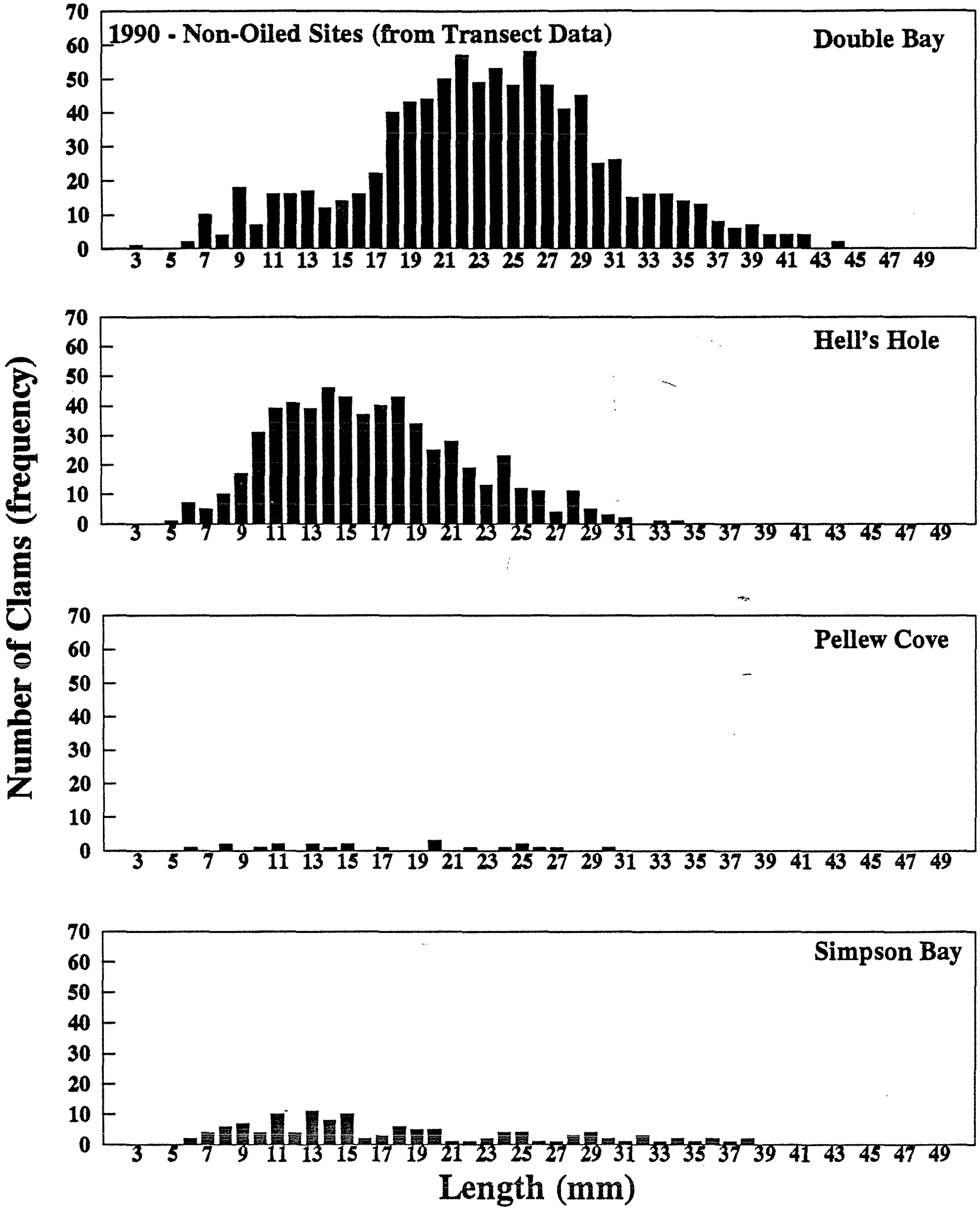

Figure 5. Comparison of length frequencies of littleneck clams collected at non-oiled transect sampling sites in Prince William Sound, 1990.

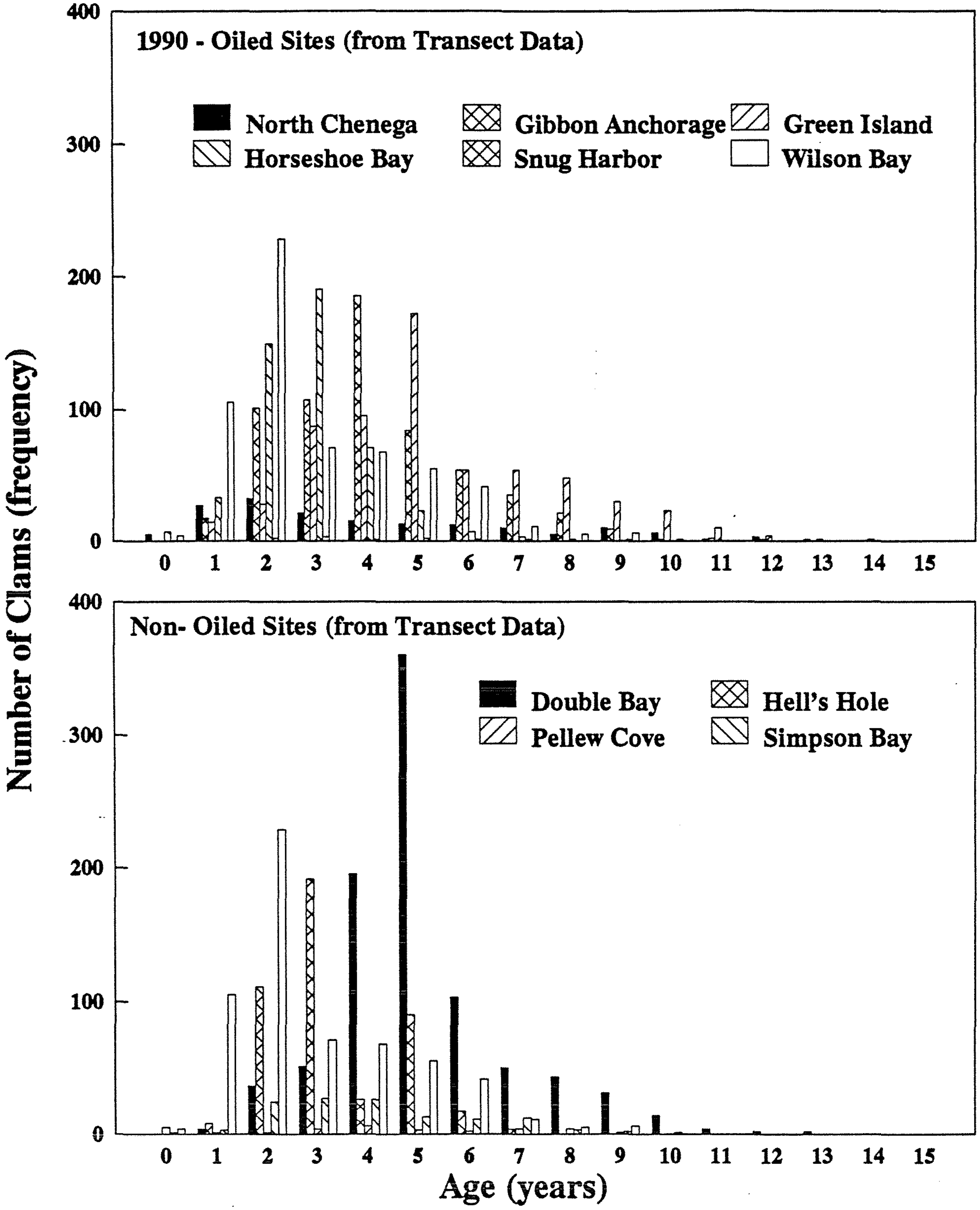

Figure 6. Comparison of age frequencies of littleneck clams collected at oiled and non-oiled transect sampling sites in Prince William Sound, 1990.

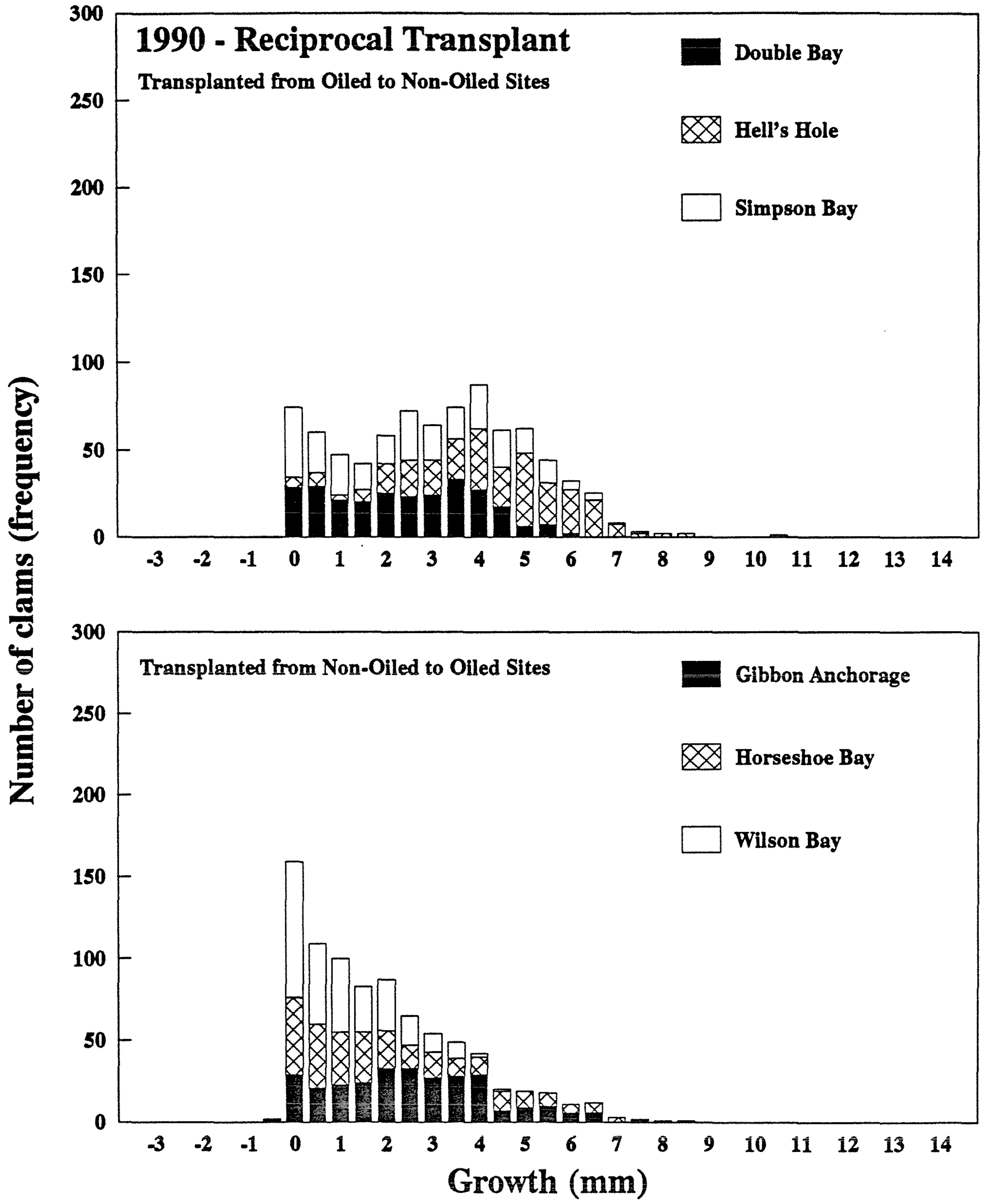

Figure 7. Comparison of growth (mm) of littleneck clams that were reciprocally transplanted and recovered between oiled and non-oiled sites in Prince William Sound, 1990.

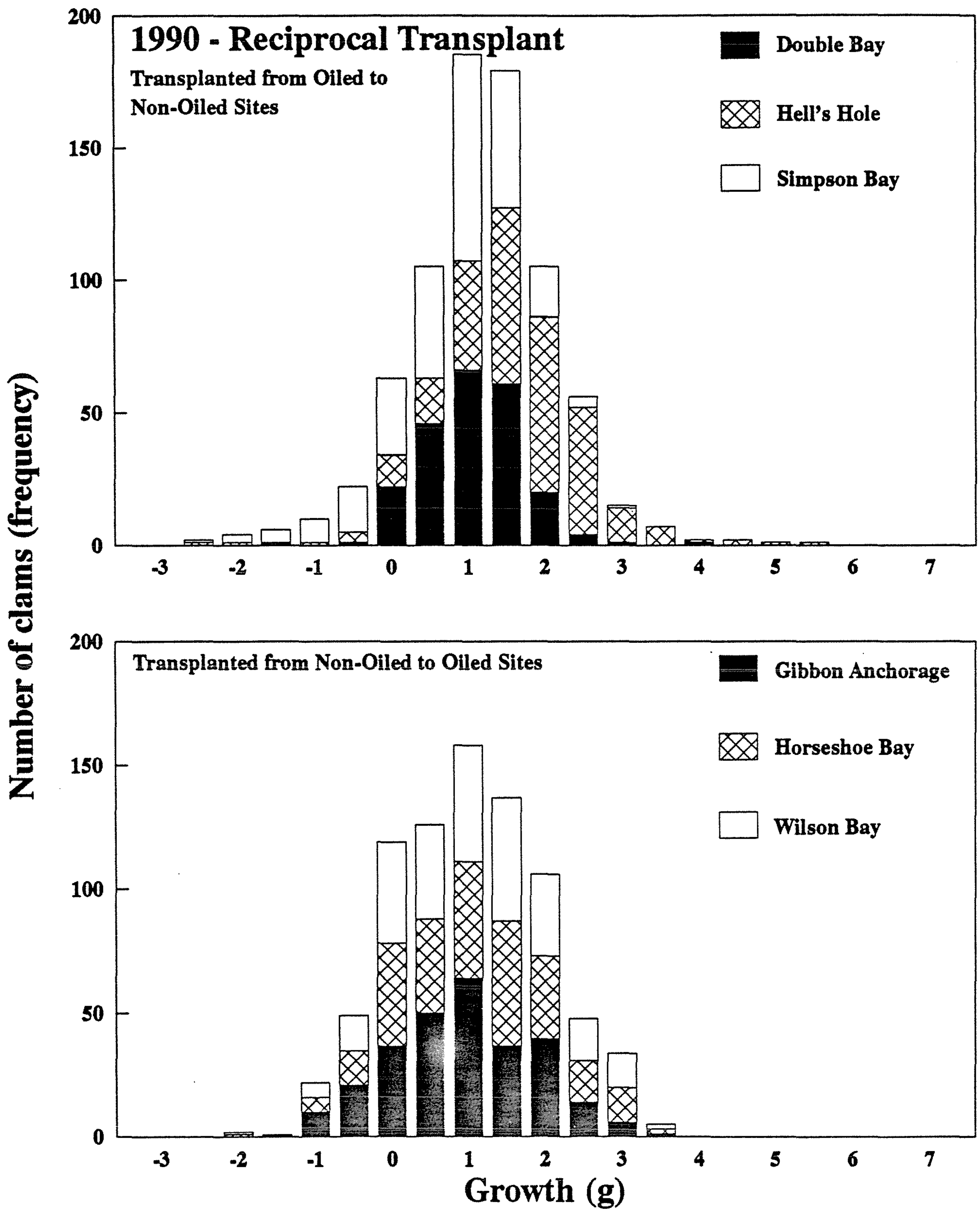

Figure 8. Comparison of growth (g) of littleneck clams that were reciprocally transplanted and recovered between oiled and non-oiled sites in Prince William Sound, 1990.

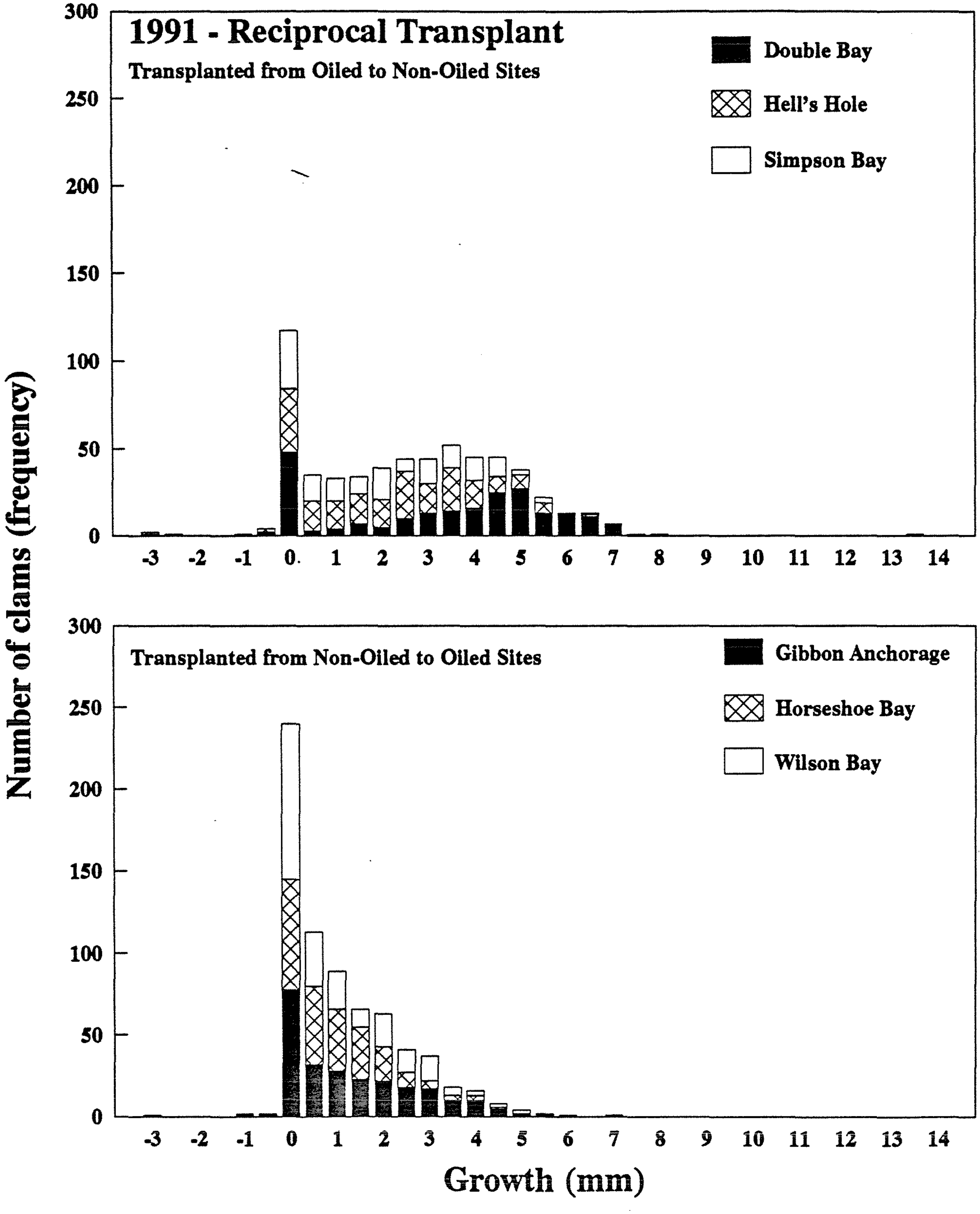

Figure 9. Comparison of growth (mm) of littleneck clams that were reciprocally transplanted and recovered between oiled and non-oiled sites in Prince William Sound, 1991.

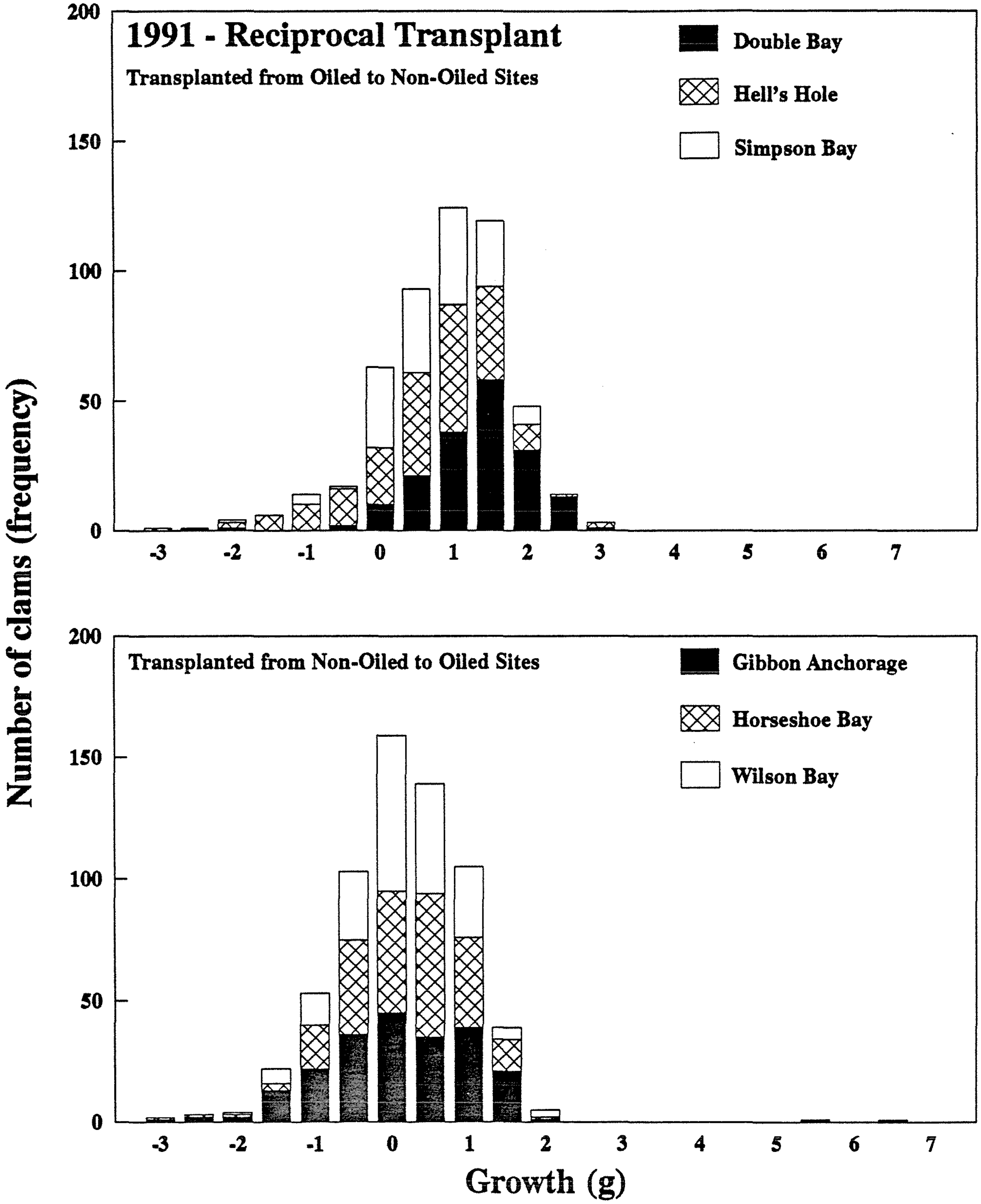

Figure 10. Comparison of growth (g) of littleneck clams that were reciprocally transplanted and recovered between oiled and non-oiled sites in Prince William Sound, 1991.

APPENDIX A. RESTORATION PROPOSALS

RESTORATION SCIENCE STUDY PROPOSAL

Draft November 20, 1991

A. Study Name: Bivalve Shellfish Restoration and Enhancement

B. Injured Species to be Addressed:

1) Littleneck clams *Protothaca staminea*
2) Butter clam *Saxidomus giganteus*
3) Blue mussel *Mytilus edulis*
4) Weathervane scallop *Patinopecten caurinus*
5) Other bivalves identified by the needs assessment

C. Principal Investigator(s)

James O. Cochran, Mariculture Coordinator, F.R.E.D. Division
Charles Trowbridge, Shellfish Biologist, Comm. Fisheries Div.
J. Johnson, Shellfish Biologist, Commercial Fisheries Division
Agency: Alaska Department of Fish and Game

D. Project Objectives:

The goal of this project is to assess the restoration and enhancement needs of those bivalves populations injured by EVOS, and to determine if mariculture techniques are a valid tool to use in restoration or enhancement of the identified species. Initiation of a demonstration project would take place if cogent evidence of the applicability of mariculture techniques in restoration and enhancement is concluded.

E. Project Methods:

1) Review and analyze relevant NRDA studies. Establish liaisons with project Principal Investigators.
2) Conduct literature searches to ascertain available mariculture techniques.
3) Establish technical feasibility which would include cost benefit analysis, outline of the permitting process, and a technology assessment.
4) Identify stocks to be enhanced or restored.
5) Describe population dynamics of stocks to be restored or enhanced.
6) Identify one project site for a demonstration project utilizing a shellfish hatchery or other technology.
7) Initiate a demonstration project.

F. Duration of the Project: (number of seasons needed to fulfill project objectives).

Year one=> Conduct NRDA needs assessment and project status. Perform literature search and establish expert contacts. Identify and catalog demonstration project site. Collect samples of bivalve species to be enhanced. Data collected would be used in conjunction with bivalve samples collected after EVOS (Fish/Shellfish Study 13), to develop population dynamics.

Year two=> Initiate demonstration project using identified restoration techniques (hatchery seed, indigenous seed, etc.).

Year three=> Assess demonstration project. Proceed with project development as appropriate.

G. Estimated cost (per year if more than one year)

Year one=> $95,000 per year

Year two=> $100,000 per year

Year three=> $105,000 per year

H. Restoration Activity or Endpoint:

A report on the feasibility of rehabilitating injured stocks using appropriate artificial enhancement techniques, and the accelerated restoration of damaged intertidal and subtidal bivalve populations if mariculture feasibility is established.

I. This study relates directly to the first general science need identified by RPWG. It will identify and evaluate artificial enhancement as a restoration option, and provide basic population information on stock structure.

J. This project needs to begin before reductions in population abundance and distribution make identification of established populations and donor stocks even more difficult. Also, by initiating the project in 1992 much of the background work can be accomplished at the time the F.R.E.D. Division's Mariculture Technology Center will be coming on line.

K. This project links to the Coastal Habitat and Prince William Sound Effects of Hydrocarbons on Bivalves Damage Assessment Projects. It will provide information to these projects and provide a potential tool for restoration and enhancement.