

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

Injury to Prince William Sound Spot Shrimp

Subtidal Study Number 5  
Final Report

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Injury of Prince William Sound Spot Shrimp

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**Study History:** Four surveys have been conducted by Alaska Department of Fish and Game to assess possible damage to spot shrimp *Pandalus platyceros*. The surveys were conducted during November 1989, March 1990, and November 1990 as Fish/Shellfish Study 15 (Injury of Prince William Sound Spot Shrimp), and continued as Subtidal Study Number 5 in November 1991.

**Abstract:** Differences in pre- and post-spill spot shrimp fishing within Prince William Sound were based on the catch per unit effort (CPUE), which was significantly lower in oiled areas in 1989 and 1990, and significantly higher in the oiled area in 1991. In the unoiled area, the percentage of the female population has steadily increased from 7.3% in 1989, 11.3% in 1990, to 16.8% in 1991, while in the oiled area females increased from 2.0% in 1989, 2.5% in 1990, to 2.6% in 1991. The total number of eggs per female was less in the oiled area in 1989. No difference between oiled and unoiled areas was found in 1990 or 1991. Hydrocarbon analyses did not detect oil contamination within sampled spot shrimp, but this analysis is limited as these organisms may metabolize oil. Histopathology analyses were conducted on shrimp collected in 1989. Those in the unoiled area had more inflammatory gill lesions than those within the oiled area. Histopathology analyses showed no difference between oiled and unoiled areas, indicating little or no oil contamination to the adult population. Catch data suggested a strong relation between population structure and commercial fishing, which selects for large males and females. Any damage to the adults by EVOS would be difficult to assess due to high pre-spill fishing mortality.

**Key Words:** Spot shrimp, *Pandalus platyceros*, commercial fishing, catch per unit effort, eggs per female, histopathology, Prince William Sound.

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## EXECUTIVE SUMMARY

Since the grounding of the T/V *Exxon Valdez* caused an oil spill on March 24, 1989, four surveys have been conducted by the Alaska Department of Fish and Game (ADF&G) to assess possible damage done by the oil spill to spot shrimp *Pandalus platyceros*. The surveys were conducted November 1989, March 1990, and November 1990 as Fish/Shellfish Study 15, then November 1991 as Subtidal Study 5. This report incorporates results of all surveys, but emphasizes November surveys and their analyses.

Spot shrimp previously supported an important commercial fishery and continue to support recreation and subsistence fisheries in Prince William Sound (PWS). Adult spot shrimp are an important food for various commercially important fishes (i.e. rockfish, lingcod and pollock), while young spot shrimp are prey for various nearshore animals (i.e. juvenile rockfish). Adult spot shrimp are a representative species of the deepwater nearshore benthic ecosystem, sharing aspects of their distribution, life history and food habits with other economically important crustaceans residing in PWS (Table 1). A significant portion of spot shrimp habitat was in the direct path of the oil spill.

Since the T/V *Exxon Valdez* oil spill (EVOS), the southwest area of PWS has experienced little commercial spot shrimp fishing, while the northern area of PWS has continued to support a fishery. Despite this difference in fishing, catch per unit effort (CPUE) as measured in the number of spot shrimp per pot, was significantly lower in the oiled area in 1989 and 1990 when compared to the unoiled area, for the same years. In 1991, the CPUE was significantly higher in the oiled area. Most of the differences in CPUE have been attributed to pre- and post-spill fishing within PWS.

An interesting attribute of spot shrimp population structure is the percentage which is female. In the unoiled area, the percentage of the population that is female has steadily increased from 7.3% in 1989, 11.3% in 1990, and further rose to 16.8% in 1991, while in the oiled area the percent females has only modestly changed from 2.0% in 1989, 2.5% in 1990, to 2.6% in 1991.

The total number of eggs per female at a given size was less in the oiled area in 1989. No difference between oiled and unoiled areas was found in 1990 or 1991. The presence of dead eggs did not vary between the oiled and unoiled areas, but this would have been difficult to detect if females slough dead eggs.

Hydrocarbon analyses did not detect oil contamination within sampled spot shrimp. However, usefulness of hydrocarbon analysis on shrimp, specifically of the tissues sampled, is limited since these organisms may metabolize oil.

In March 1992, histopathology analyses, (D.V. Lightner, University of Arizona, Appendix C) were completed on shrimp collected in November 1989. Results indicated that shrimp collected in the unoiled area of PWS had more inflammatory gill lesions, a condition expected of shrimp

exposed to toxins, than those sampled within the oiled area. Other histopathology analyses did not show any difference between oiled and unoiled areas. This indicates little or no oil contamination had affected the adult portion of the spot shrimp population at that time.

Part of the analysis conducted for the 1991 survey, was a review of the commercial harvest of spot shrimp both before and after the EVOS. Catch data, obtained from annual fish tickets, suggest a strong relation between population structure (number and size distribution) and commercial fishing. The commercial fishery selects for large males and females. Therefore, any damage to the adult spot shrimp population by the EVOS would be difficult to assess due to high pre-spill fishing mortality.

## INTRODUCTION

Spot shrimp *Pandalus platyceros* previously supported an important commercial fishery in Prince William Sound (PWS), and continue to support recreation and subsistence fisheries. Important spot shrimp habitat is generally contained within what is known as the Traditional Harvest Area. This area is defined as those waters west of a line from Montague Point to Bidarka Point in PWS (Figure 1) and is the principal harvest area for the commercial spot shrimp fishery within PWS. The area is characterized by numerous, steeply cut glacial fjords and passages. A significant portion of this area was in the direct path of the oil spill from the grounding of the T/V *Exxon Valdez* on March 24, 1989. Minor isolated populations of spot shrimp near PWS include Lituya Bay to the east and the outer coast of the Kenai Peninsula to the west. The outer coast of the Kenai Peninsula was also oiled from the *Exxon Valdez* oil spill (EVOS).

Spot shrimp, like most pandalid shrimp, have five distinct life stages: larval, juvenile, male, transitional and female. Larvae are primarily released into the water column in late March and throughout April (Strathmann 1987). The larvae enter the zooplankton community, phasing through four larval molts (Price and Chew 1972), until the end of summer, late August or September (Butler 1980). Spot shrimp then settle into the intertidal and shallow subtidal zones as juveniles (Barr 1974; Bousfield and McAllister 1962). The juveniles migrate to deeper waters at about 2.5 to 3 years of age (Kimker and Donaldson 1987), where after a short time they become mature males. The spot shrimp remain as males for approximately 3 to 5 years (Kruse and Murphy 1989), but being protandrous hermaphrodites they change to females (Butler 1964, 1980; Sunada 1986). It should be noted that all shrimp are males before changing to females and it seems that all males (if they live) become females. As females they will live another 3 to 5 years and reproduce annually, if conditions permit. Females carry between 500 and 5000 eggs (depending on size) for approximately 6 months, starting in late September and early October. In March and April, with the release of the larvae, the life cycle begins anew.

Spot shrimp are a representative species of the deepwater (30 m - 250 m) nearshore benthic ecosystem. They tend to be found on rocky seafloors versus sand or silt substrates (Barr 1970, 1971 and 1974; Barr and Barr 1983; Kessler 1985), though there are records of spot shrimp being found on the softer bottoms (Barr 1970, 1971). It is uncertain what feeds upon the larval spot shrimp while they are within the zooplankton community. The juvenile spot shrimp are fed upon by various intertidal and shallow subtidal inhabitants, such as young rockfish *Sebastodes* (A. Hoffman, Alaska Department of Fish and Game, Anchorage, personal communication). Adult spot shrimp serve as a food source for a variety of fish, including rockfish (Rosenthal et al. 1988), Pacific cod *Gadus macrocephalus*, sculpin *Cottidae* and pollock *Theragra chalcogramma*, and invertebrates like octopus *Octopus dofleini* and Tanner crab *Chionoecetes* spp. (Table 1). Spot shrimp eat bottom dwelling invertebrates, mostly annelid and polychaete worms and detrital material (Barr and Barr 1983; Butler 1980). Spot shrimp share aspects of their distribution, life history or food habits with other economically important shellfish species (Table 1).

A commercial pot fishery which targets spot shrimp has been in operation since 1979. Large males and females make up the saleable catch of this fishery. Due to the lack of selectivity of the pots employed in the fishery, prior to 1990, a large portion of non-saleable smaller males and a few juveniles were caught, and discarded (thrown overboard), with assumed low survival. This is known as the deadloss of the catch. In 1990, the Alaska Board of Fisheries passed a regulation requiring the use of large rigid meshed panels to reduce the catch of non-saleable sized shrimp.

The level of the commercial spot shrimp harvest has varied over the years, with the different regions within PWS having been fished at varying effort (Figure 2). In the early 1980s, the yearly spot shrimp harvest began to increase, mostly within southwest PWS, as a function of increased effort, i.e. vessels, and by the mid-1980s, the fishery had grown considerably, taking several thousand kilograms of shrimp each year (Figure 2). This extensive fishing effort seems to have lowered the stocks considerably, thus any adverse effect by the EVOS could further hinder the recovery of the PWS spot shrimp stocks.

Spot shrimp are known to be sensitive to oil contamination in all phases of their life history. The effects of oil on spot shrimp in particular and shrimp in general are well documented (Anderson et al. 1974, 1981; Brodersen 1987; Brodersen et al. 1977; Mecklenburg et al. 1977; Rice et al. 1979, 1984; Sanborn and Malins 1980; Stickle et al. 1987; Vanderhorst et al. 1976). In many laboratory studies, susceptibility was measured as the length of time necessary for different concentrations of oil to kill half the sample (Anderson et al. 1981; Brodersen 1987; Brodersen et al. 1977; Mecklenburg et al. 1977; Rice et al. 1979, 1984; Stickle et al. 1987; Vanderhorst et al. 1976). The literature indicates that adult shrimp are susceptible to injury from oil (Anderson et al. 1974, 1981; Rice et al. 1979, 1984; Stickle et al. 1987; Vanderhorst et al. 1976), but do not accumulate hydrocarbons in their systems (Sanborn and Malins 1980). Larvae are even more susceptible to injury from oil than adults (Brodersen 1987; Brodersen et al. 1977; Carls and Rice 1980). Furthermore, small concentrations of oil can hinder successful molting of shrimp larvae (Mecklenburg et al. 1977), and slow their movement (Brodersen 1987; Carls and Rice 1980, Rice et al. 1979, 1984), both conditions would likely make them more susceptible to predation (Rice et al. 1984). In 1989, the eggs of the spot shrimp and other shellfish species (Table 1) hatched immediately before the oil spill, so zoea larvae at or near the water surface were very vulnerable to aromatic hydrocarbons. Juvenile spot shrimp from the 1988 and 1987 year classes were present at nearshore locations and also were vulnerable to direct oil contamination. Though there is little evidence of direct oil contamination at the depths inhabited by adult spot shrimp during the year of the oil spill, they may have eaten oil contaminated food. The adult population may also have been affected more directly in later years, when residual oil sank to the seafloor (Boehm et al. 1985).

## OBJECTIVES

The goal of this project was to assess the damage done to a representative species of the nearshore benthic ecosystem by the oil spill. Spot shrimp were chosen because they are economically important, they are more sedentary than other shellfish species (crustaceans specifically), and some pre-spill information existed on spot shrimp. The objectives set forth to meet this goal were:

1. Determine the catch per unit effort (CPUE) by weight, number and number per weight of spot shrimp *Pandalus platyceros* in sites within both oiled and unoiled areas, and test for significant differences among years and areas (oiled versus unoiled).
2. Compare size and age frequencies of spot shrimp among sites and by sex, using various methods of length frequency analysis and graphical representation.
3. Analyze fecundity (both eggs per female and number of females with eggs), and egg mortality between oiled and unoiled areas over time, and determine whether those effects result in adverse changes in reproductive viability.
4. Analyze tissue and egg samples for presence of hydrocarbons and compare differences between oiled and unoiled sites to test the null hypothesis that the level of hydrocarbons is not related to the level of oil contamination present at a site.
5. Compare various histopathological results between oiled and unoiled areas, to determine possible sublethal effects of oil contamination.
6. Use historic catch data from the commercial fishery to estimate and model the effects of fishing on the population structure (length-age frequency and CPUE) over time, and compare these results between oiled and unoiled areas, to separate oil induced effects from fishing effects.
7. Compile all the above information to determine the level of damage caused by the EVOS on the spot shrimp population, specifically noting level of oiling effect when compared to fishing effect.

## METHODS

### *Survey Design*

The spot shrimp habitat within PWS was divided into oiled and unoiled strata. Localized spot shrimp distribution in these areas was established by interviewing commercial fishermen. The unoiled strata included the northwestern portion of PWS, where samples were taken from Unakwik Inlet, Port Wells (Golden) and Culross Passage (Figure 1). The oiled strata included central and southwestern PWS, where samples were taken from Herring Bay, northeast Chenega Island, and north Green Island (Figure 1). The reason for comparing oiled versus unoiled sites was due to the lack of pre-spill information on the population or the stock sizes of spot shrimp within PWS. Each site is located within a commercial statistical reporting area as defined by ADF&G, for the shellfish fishery (Figure 1, Table 2).

Unakwik Inlet and Green Island were also chosen because of previous spot shrimp studies near these sites. Unakwik Inlet was the site of research on abundance and growth of spot shrimp a few years prior to the EVOS (Kimker 1984, 1985; Kimker and Donaldson 1986, 1987). Similar research occurred at Green Island but in an earlier year, 1982 (Kimker 1983). These studies represent most of the research performed within PWS on spot shrimp prior to the EVOS.

All surveys were conducted from the R/V *Montague* during November 1989, March 1990, November 1990 and November 1991. These months were chosen based upon the need to sample during egg bearing periods. By November, egg extrusion should be complete. The March survey was to provide information on the timing of larval release.

Each site was stratified into shallow, 35 to 130 m (approximately 20 to 70 fathoms), and deep, 130 to 220 m (approximately 70 to 120 fathoms) strata. A string of eleven pots constituted a station. In 1989, eleven pots spaced 9 m (5 fathoms) apart made up a station. This configuration was changed after 1989 (1990 and 1991) to provide more coverage of the depth range within a stratum; thus after 1989, eleven pots spaced 18.5 m (approximately 10 fathoms) apart on a longline constituted a station (Figure 3). In 1989, exactly two stations were set for each depth stratum, while in 1990 and 1991, at least two stations were set for each depth stratum, except at Green Island in November 1990 when three stations were set in the shallow stratum only, due to poor catches from previous surveys in the deep stratum. The number of stations set, in 1990 and 1991, varied from site to site and year to year as a function of collection success in previous years and time remaining for the completion of that year's survey. The goal was to catch at least 500 shrimp from each depth stratum at each site for length frequency analysis. If necessary, pots were redeployed additional days, targeting the areas of highest catches from the previous samples for that cruise, until the required sample size per site was achieved.

Spot shrimp were sampled using standardized commercial shrimp pot gear, which measured 40.6 x 40.6 x 91.4 cm (16 x 16 x 36 in) with a 6.4 cm (2.5 in) tunnel located 17.8 cm (7 in) into each end (Figure 4). Each pot was baited with a 2 liter (2 quart) jar of chopped bait herring. Longlines of pots were set in late afternoon and retrieved the following morning. Average soak time for each longline was about 18 hours.

#### *Relative Abundance*

Upon retrieving the pots, spot shrimp specimens for hydrocarbon and histopathology analysis were removed. The remaining pandalid shrimp were sorted by species, weighed and counted. Weights were obtained using an electronic digital scale and recorded to the nearest 2 g. If a station's catch was estimated to have an excess of 500 spot shrimp, then the station was subsampled and an estimated number of spot shrimp calculated for that station. The subsamples were obtained by taking a constant proportion of shrimp from each pot in a station. At stations where the estimated number of shrimp was less than 500, a total count was performed.

In comparing CPUE between oiled and unoiled areas, the CPUE was calculated from only those pots set the first day at a depth stratum and site combination. Redeployed pots were set to target specific areas, sampled the previous day, of known high concentrations of spot shrimp. A redeployment was specifically set to attain the 500 specimen sample size for length frequency analysis. CPUE from these pots would not represent an unbiased abundance estimate or be comparable with sites where additional fishing was not needed. Further, CPUE from these pots would represent time related dependent samples, increasing bias within the results.

There was a concern that the second day sets might introduce a bias in the length frequency analysis. However, this bias was assumed to be minor since we set on two depth strata to incorporate differences in size with depth.

To reduce handling contamination and ensure fresh spot shrimp for hydrocarbon and histopathology analyses, spot shrimp were removed from the pots immediately upon retrieval of the pot string. The total weight of spot shrimp for a pot ( $W_T$ ) was calculated as follows:

$$W_T = W_p + W_c + W_h \quad (1)$$

where  $W_p$  is the weight of spot shrimp measured for each pot shortly after recovery,  $W_c$  is the estimated weight of shrimp taken for hydrocarbon analysis and  $W_h$  is the estimated weight of shrimp taken for histopathology analysis. The weight of spot shrimp taken for hydrocarbon

analysis was estimated as follows:

$$W_c = N_c(\bar{w}_e) \quad (2)$$

where  $N_c$  is the number of spot shrimp taken from a pot used for hydrocarbon analysis and  $\bar{w}_e$  is the average weight of an egg bearing female, as calculated from a subsample of egg bearing females. The weight of spot shrimp taken for histopathology analysis was estimated in a similar way:

$$W_h = N_h(\bar{w}_s) \quad (3)$$

where  $N_h$  is the number of spot shrimp taken from a pot used for histopathology analysis and  $\bar{w}_s$  is the average weight of a spot shrimp for that year.

A general linear model was fit to the spot shrimp data, using the statistical software package *SAS* (*SAS Institute Inc. 1988*). The hypothesis of no difference in number, weight or number per weight between spot shrimp caught within the oiled area versus those caught within the unoiled area was tested at the 0.05 level (i.e.  $\alpha = 0.05$ ) for each year. CPUE<sub>ijklm</sub> is defined as the catch per unit of effort, measured in number per pot, weight per pot or number per weight per pot, of spot shrimp at oiling strata  $i$ , depth strata  $j$ , site  $k$  and sample (pot)  $m$ . Because of the potential of significant interaction terms, the full model was fit:

$$CPUE_{ijklm} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + \gamma_{k(i)} + (\beta\gamma)_{jk(i)} + \epsilon_{ijklm} \quad (4)$$

with  $\mu$  as the grand mean,  $\alpha$  as an oiled effect,  $\beta$  as a depth stratum effect,  $\gamma$  as a site effect nested within the oiling strata, all interaction terms and  $\epsilon$  as the error. When interaction terms were significant, an hypothesis test for the differences between oiled and unoiled strata was performed using the least square means (*Milliken and Johnson 1984*). When interaction terms were insignificant ( $p > 0.05$ ) then the ANOVA was run again, omitting insignificant interaction terms.

A year term was added to the model, and difference between years was tested, again at the 0.05 level (i.e.  $\alpha = 0.05$ ), separately for the oiled and unoiled areas using the following

model:

$$CPUE_{hjkm} = \mu + \delta_h + \beta_j + \gamma_k + (\delta\beta)_{hj} + (\delta\gamma)_{hk} + (\beta\gamma)_{jk} + (\delta\beta\gamma)_{hjk} + \epsilon_{hjkm} \quad (5)$$

where  $\delta$  is the year effect. The Bonferroni inequality was used to control type I error for planned a-posteriori comparisons of least square means, between 1989 and 1990, and between 1990 and 1991. Only the November samples were used in the year effect. The March 1990 sample was not used due to possible seasonal variability of spot shrimp in stock size or feeding habits.

#### *Length, Growth, Sex and Fecundity*

Sex, carapace length, and fecundity data were recorded only for spot shrimp. Carapace length was measured from the rear of the right eye socket to the posterior midpoint of the carapace (Appendix A, Figure 1) and recorded to the nearest 0.1 mm using a digital electronic caliper. Sex was identified as juvenile, male, transitional or female according to Appendix A. Egg condition (no eggs, eyed or uneyed), egg color (dark reddish brown, brown, amber, orange or blue), egg fouling, number of dead eggs, and the presence of breeding dress (if no eggs were present) was recorded for all females. A maximum of 25 ovigerous females at each station for each site was collected to estimate fecundity and egg mortality, measured as the number of dead eggs per female. Egg samples were processed and the total number of eggs per female estimated according to Kinzer (1991), using the following formula:

$$X = \left( \frac{x'}{y'} \right) Y \quad (6)$$

where  $X$  is the estimated total number of eggs,  $x'$  is the number of eggs in the subsample,  $y'$  is the dry weight of the subsample and  $Y$  is the total dry weight of the sample.

#### Length Composition

The length frequency histograms were expressed in CPUE. For each site, the percentage of each carapace size category, in 1 mm intervals from 13 mm to 58 mm, was calculated from the entire sample (both initial and redeployed samples). This percentage was then multiplied by the average CPUE for spot shrimp (size independent) at that site, from the first day samples only.

This gave the number of spot shrimp per pot at a specific size category, year and site.

The length-frequency histograms were expressed in CPUE (number of shrimp per pot) to ensure clear interpretation of the data, as compared between years and sites. The percentage of spot shrimp at a specific cohort, when compared between sites or years, may under or over emphasize the relative change in the number of spot shrimp of that cohort. For example, if a site were to have a strong recruitment one year, all other year classes might seem very low, even if there had been little change in the rest of the population from the previous year.

### Growth

In the 1990 status report (Donaldson et al. 1990), a von Bertalanffy growth curve (Frechette and Parsons 1983) was estimated for spot shrimp populations at four of six sites, using modal mixture analysis (Otter Research LTD. 1992). The von Bertalanffy growth curve is a commonly used growth model represented as follows:

$$L_t = L_\infty (1 - e^{(-k(t-t_0))}) \quad (7)$$

where  $L_t$  is the length at time  $t$  (which is usually in years but can also represent other time measures such as months, weeks or molts),  $L_\infty$  is the maximum length the shrimp's carapace is expected to reach,  $k$  is the growth parameter and  $t_0$  is the starting time for the growth curve.

Mixture modal analysis is based on the assumption that a length frequency histogram is composed of several overlapping normal curves, each curve representing a separate age group. The statistical software package *MULTIFAN* (Otter Research Ltd 1992) was used for these calculations. To perform the mixture modal analysis, the shrimp from each site were pooled across stations and depth strata, as was done in the length frequency graphs. A separate analysis was performed for each site to determine whether growth was site specific. Golden and Green Island were excluded from the analysis. Golden was excluded because all year classes, except one, were too low for analysis and Green Island was excluded due to the small sample obtained in November 1989 and March 1990.

### Sex Composition

Reproductive potential is based on both fecundity and the number of females. The number of females per pot in oiled versus unoiled areas was tested using analysis of variance. As with the ANOVA on the entire population CPUE data, only the stations set the first day were used. A square root transformation was used for analyses as outlined in Zar (1980), since data were counts and the number of females per pot was low.

A general linear model using SAS was fit to the square root of the female spot shrimp count ( $\sqrt{FEM}$ ) and the hypothesis of no difference in number was tested, at the 0.05 level (i.e.  $\alpha = 0.05$ ), between females caught within the oiled area versus those caught within the unoiled area, for each year. Because of the potential for significant interaction terms, the full model was fit:

$$\sqrt{FEM}_{ijkm} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + \gamma_{k(i)} + (\beta\gamma)_{jk(i)} + \epsilon_{ijkm} \quad (8)$$

with  $\mu$  as the grand mean,  $\alpha$  as an oiled effect,  $\beta$  as a depth stratum effect,  $\gamma$  as a site effect nested within the oiling strata, all interaction terms and  $\epsilon$  as the error. Where interaction terms were significant, the differences between oiled and unoiled strata were tested using the least square means (Milliken and Johnson 1984). When interaction terms were insignificant ( $p > 0.05$ ) then the ANOVA was run again, omitting insignificant interaction terms.

A year term was added to the model, and the difference between years was tested separately for the oiled and unoiled areas using the following model:

$$\sqrt{(FEM)_{hjkm}} = \mu + \delta_h + \beta_j + \gamma_k + (\delta\beta)_{hj} + (\delta\gamma)_{hk} + (\beta\gamma)_{jk} + (\delta\beta\gamma)_{hjk} + \epsilon_{hjkm} \quad (9)$$

where  $\delta$  is the year effect. The Bonferroni inequality was used to control type I error for planned a-posteriori comparisons of least square means, between consecutive years. Only November samples were used in the year effect. The March 1990 sample was not used due to seasonal variability in the distribution of spot shrimp.

#### Fecundity and Related Parameters

Differences among sites for spot shrimp fecundity and relative clutch size were examined using analysis of covariance. SAS was used to perform a general linear model fit to the clutch size data. The hypothesis of no difference in number of eggs per female was tested, at the 0.05 level (i.e.  $\alpha = 0.05$ ), between spot shrimp catches within the oiled area versus those within the unoiled area, for each year. Because of the potential of significant interaction terms, the full

model was fit:

$$NUMEGGS_{ijkm} = \mu + x_{ijkm} + \alpha_i + \beta_j + (\alpha\beta)_{ij} + \gamma_{k(i)} + (\beta\gamma)_{jk(i)} + \epsilon_{ijkm} \quad (10)$$

with NUMEGGS as the number of eggs per female,  $\mu$  as the grand mean,  $x$  as a covariate for carapace length,  $\alpha$  as an oiled effect,  $\beta$  as a depth stratum effect,  $\gamma$  as a site effect nested within the oiling strata, all interaction terms and  $\epsilon$  as the error. Where interaction terms were significant, hypothesis testing for the differences between oiled and unoiled strata was performed using the least square means (Milliken and Johnson 1984). When interaction terms were insignificant ( $p > 0.05$ ) then the ANOVA was run again, omitting insignificant interaction terms.

A year term was added to the model, and difference between years was tested separately for the oiled and unoiled areas using the following model:

$$NUMEGGS_{hjkm} = \mu + x_{hjkm} + \delta_h + \beta_j + \gamma_k + (\delta\beta)_{hj} + (\delta\gamma)_{hk} + (\beta\gamma)_{jk} + (\delta\beta\gamma)_{hjk} + \epsilon_{hjkm} \quad (11)$$

where  $\delta$  is the year effect. The Bonferoni inequality was used to control type I error for planned a-posteriori comparisons of least square means. Only the November samples were used in the year effect. The March 1990 sample was not used due to seasonal variability in the number of spot shrimp with eggs.

Females were divided into three major categories, females with eggs, females without eggs but in breeding dress and females without eggs and not in breeding dress. Breeding dress occurs after the molt immediately preceding extrusion of eggs, characterized by the presence of long, simple, and plumose setae on the protopodites of pleopods (Butler 1980). To test for significance between females with eggs versus those without eggs, in oiled versus unoiled areas, a log-linear model was fit using the statistical package *GLIM* (Payne 1987). We tested at the 0.05 significance level (i.e.  $\alpha = 0.05$ ) for association between females with eggs, and oiling strata, with sites nested within oiling strata. A chi-square statistic was used to test for differences among sites in the number of spot shrimp in breeding dress. Analyses to determine possible effects of the oil spill on the number of dead eggs per female were conducted using a Mann-Whitney test on ranked data (Conover 1980).

### *Hydrocarbon Analysis*

To prevent contamination, specimens for hydrocarbon testing were taken from the pot immediately after its removal from the water, before being weighed and processed. Three female spot shrimp formed one composite sample of muscle and one composite sample of eggs. Each composite was taken from a different pot. Two replicates of the composite were taken randomly from one station in the stratum and the third replicate came from the other station.

The number of specimens needed for each hydrocarbon analysis depended on the size of specimens collected. The experiment was designed to detect a difference of 1.2 standard deviations in hydrocarbon content with the probability of making a type I error equal to 0.05 (i.e.  $\alpha = 0.05$ ) and making a type II error equal to 0.10 (i.e.  $\beta = 0.10$ ). At least 15 g of tissue were needed for each analysis. Based on average size of adults, three spot shrimp were needed to provide this amount of tissue. Three hydrocarbon samples from each treatment level were needed to detect contamination among the levels (B. Clark, personal communication).

### *Histopathology Analysis*

Specimens for histopathological analysis were taken from each catch before it was weighed and sorted (by sex). Twenty spot shrimp from a single station in each stratum were selected, preserved and handled following recommendations of the Histopathology Technical Group (Appendix B). After 1989, the fixation agent was changed from 10% neutral buffered formalin to Davidson's fixative, since formalin fixation in shrimp causes marked shrinkage, hardening and destruction of tissues (Bell and Lightner 1988)

Histopathology specimens were sent to D.V. Lightner, University of Arizona, for examination of the gills and associated appendages; the digestive tract (hepatopancreas, foregut, and midgut); the ventral nerve cord and thoracic ganglia; the heart; the antennal gland; the hematopoietic tissues; the gonads and developing embryos; and the cuticle (sites with shell disease lesions or presumed wounds). Three items, inflammatory gill lesions, concentrations of the gill parasite *Lagenophrys*, and melanized cuticular lesions, were of most interest in determining exposure to toxins. Inflammatory gill lesions and *Lagenophrys* infestation severity were both rated on a scale from 0 to 3, with 0 being least severe. Melanized cuticular lesions were recorded as either present or absent (Appendix C).

To test for significance of gill lesion severity between in oiled versus unoiled areas, a log-linear model was fit, using the interactive statistical package *GLIM*. We tested at the 0.05 significance level (i.e.  $\alpha = 0.05$ ) for interaction between gill lesion severity, and oiling strata, with sites nested within oiling strata. A similar analysis was performed for *Lagenophrys* severity. If interaction was significant, the first two severity levels were combined (0 and 1), the last two

severity levels were combined (2 and 3), and the log-linear analysis was re-computed. A log-linear analysis was also performed on the count of shrimp both with and without melanized cuticular lesions. Testing again examined the interaction of lesions (with or without) and oiling strata, with sites nested within oiling strata.

#### *Environmental Observations*

Environmental data were recorded at each site. Water temperature, salinity and dissolved oxygen content were recorded at one meter depth intervals using a Seabird Electronics CTD (model SBE19, serial # 192488-297) at a location near the deepest portion of the second stratum of each site.

#### *Population Model Using Catch Data*

The nature of a pot fishery led to variable levels of exploitation across PWS. Fishermen tend to "prospect" for productive spot shrimp habitat, returning to the same spot until their CPUE drops to a level thought to be less economic than a new "prospective" area. Fishermen would then move on to fish a new area allowing the original, in theory, to recover. Fishing effort, even with a maximum of 86 boats in 1987, may never have been enough to impact the entire population of spot shrimp uniformly in any given year. Instead, a moving pattern of depletion and recovery, often slow, across years, bays and most importantly, statistical reporting areas was created.

To assess the effects of the commercial fishery on the spot shrimp population structure, yearly catch data were obtained from the ADF&G fish ticket database. A fish ticket is a sales receipt required by law to be submitted to ADF&G within seven days of landing. Fish tickets give information on the amount caught, where the catch was made, i.e. what statistical reporting area, how many landings were made, how many pots were used, when the catch was made and when it was sold. The most reliable information on a fish ticket is the amount caught (Hilsinger 1987). Due to the low level of spot shrimp fishing prior to 1979, only catch data after 1979 (1980 - 1991) were used. Data were divided into specific statistical reporting areas, as defined by the ADF&G commercial fisheries, and by year. Only statistical reporting areas which include our sample sites were used (Figure 1 and Table 2).

To evaluate the effect of past fishing effort on the population structure and determine whether differences in abundance between oiled and unoiled areas were influenced more by fishing rather than the oil spill, age (length-frequency) models were made for each statistical reporting area having an oil spill sample site within it. These models were compared to the observed length-frequency graphs from our surveys.

A preliminary model was specified to provide a meaningful structure to commercial catch information, which was the only information available on pre-spill stock conditions. The model would also aid in deciding whether more time and effort should be spent on developing a more complex and realistic model. This model was not designed to estimate stock abundance or overall size/sex composition but rather to indicate whether current population structure could have resulted from fishing alone. Units of measurement were not produced in the output, since our purpose was to model relative frequency of length categories and not actual population size, therefore each statistical reporting area's model is independent of the other areas.

The assumptions for the model were as follows:

1. A specific carapace length relates to a specific age. For example, instead of referring to 3 year old shrimp, we referred to 19 mm shrimp (Table 3). Values were approximated from the *MULTIFAN* fit of the von Bertalanffy growth curve.
2. Spot shrimp begin to recruit to commercial fishing gear (pots) at 19 mm (3 years old). Since not all shrimp reached our sampling depths at the same age, we assumed 50% of all 19 mm individuals were susceptible to be caught in our samples, 75% of 24 mm (age 4) individuals and 100% of all larger individuals.
3. A constant 2% natural cohort mortality occurred yearly from 19 mm to 49 mm.
4. No spot shrimp lived longer or grew larger than 49 mm, or 12 years. This length was picked because few shrimp were caught with a carapace length greater than 49 mm, specifically none in the oiled area.
5. Recruitment was constant within each statistical reporting area for the virgin population.
6. Fishing mortality affected all spot shrimp between 28 mm and 49 mm evenly. For example, if 100 kg are taken from a stock of 1,000 kg (between 28 mm and 49 mm), then each age (length) class loses 10%. Although spot shrimp recruit to the gear at lengths less than 28 mm, no information on the survival rate of these discarded spot shrimp was available and therefore deadloss was ignored in the model.
7. All rates are instantaneous. Natural and fishing mortality occurred at the end of the year, while recruitment occurred at the beginning of the year. No seasonal variability was considered.
8. Deadloss was ignored, since no quantitative measure was available.
9. Females were defined as shrimp between 41 mm and 49 mm.

10. Shrimp were exploited uniformly within a given year and statistical reporting area.
11. Fishermen accurately reported on the fish ticket both weight and location of catch.

Catch data, recorded on fish tickets in pounds of spot shrimp, were expressed in kilograms for use in the simulation model. Estimates for a cohort were in biomass and not number of shrimp. Changes in number of shrimp per kilogram for each age class were not considered in order to reduce model complexity. However, since each cohort was considered separately and the catch each year was removed evenly by percentage from each cohort, the biomass per cohort was considered to be adequate for this model. For a more complex model the change in size (by weight) at age should not be ignored.

For each statistical reporting area, an initial stock size was estimated. This initial abundance was defined as the biomass of each age group before fishing started in 1980. The initial abundance was set with the smallest values possible to ensure that fishing and natural mortality never removed all shrimp in a specific age group before they attained 49 mm (i.e. no negative numbers of older shrimp). In other words, we used values that provided low numbers of catchable shrimp (since all stocks were depressed by 1991) that resulted in reasonable values ( $> 0$ ) in 1991.

The procedure to find the initial stock size was done in an iterative manner. A suspected minimum population biomass was entered into the program. This minimum biomass usually resulted in the premature termination of the program, because the program encountered a cohort biomass which was less than zero. The program would be re-run with a biomass greater than the first estimate. If this resulted in a premature termination of the program, then the initial biomass would be raised again and continued until a value giving all positive cohort biomass estimates was reached. When all cohort biomass estimates were positive the program was re-run with an initial biomass estimate between the largest biomass which caused premature termination and the smallest biomass which gave positive cohort estimates. This last step was repeated until, the virgin population biomass estimate, which provided positive cohort estimates, was only ten thousand kilograms greater than the largest estimate which caused premature termination.

For a given year,  $j$ , (starting with 1980), the percentage of a cohort to survive to that year was calculated, along with recruits joining the population. The total saleable shrimp (28 mm to 49 mm) in kilograms,  $sumyr_j$ , was estimated as:

$$sumyr_j = \sum_{i=5}^{12} cohort_{ij} \quad (12)$$

where  $j = 1980, 1981, \dots, 1991$  and  $cohort_{ij}$  are the cohorts age 5 through 12 in year  $j$ . The

percentage of shrimp remaining after fishing, *survive*, for year *j*, was calculated as:

$$survive_j = \frac{sumyr_j - catch_j}{sumyr_j} \quad (13)$$

where *catch* is kilograms harvested in year *j*. For the next year, *j+1*, and thus the next age, *i+1*, each cohort was estimated as:

$$cohort_{i+1,j+1} = 0.98 cohort_{ij} survive_j \quad (14)$$

where 0.98 is the proportion of the cohort surviving in the absence of fishing. No fishing mortality was considered for cohorts younger than age five in year *j+1*, and their biomass was calculated as:

$$cohort_{i+1,j+1} = 0.98 cohort_{ij} \quad (15)$$

We assumed that no cohort survived more than 12 years or 49 mm. The first cohort (*i* = 1), for year *j+1*, was calculated from females surviving from the previous year's (*i,j*), fishing and natural mortality. All cohorts present in 1980, the first year of the simulation, were given the same initial biomass value within each specific statistical reporting area. Recruitment remained constant, each year, unless the female population dropped below a specific biomass level. Below this value the females were assumed to reproduce at a density related rate, namely as the female stock decreased more larvae were assumed to be reproduced, and the *i* = 1 cohort in year *j+1* was calculated as:

$$cohort_{1,j+1} = \frac{\sum_{i=9}^{12} 0.98 survive_j cohort_{ij}}{3} \quad (16)$$

The above steps were repeated, until the year to be estimated was reached. The report for a specific year was actually the amount of shrimp available for the coming year (i.e. an estimate for 1989 was the amount catchable in 1990 before fishing). This time lag was used to make results coincide with actual surveys, which occurred in November near the end of the commercial shrimp fishing season.

## RESULTS

Damage assessment surveys were conducted during, November 1989, March 1990, and November 1990, under Natural Resource Damage Assessment Fish/Shellfish Study 15. An additional survey was done November 1991 under, Natural Resource Damage Assessment Subtidal Study 5. The first two surveys (November 1989 and March 1990) sampled spot shrimp during the same egg bearing period (Donaldson et al. 1990). The 1991 status report (Trowbridge et al. 1991) documented results of the November 1990 survey, and compared them to those obtained from the November 1989 survey. No March survey was done in 1991. While the present report discusses all surveys, only November surveys (1989, 1990 and 1991) were used for annual comparisons.

Data for this project are archived in the Anchorage office of the ADF&G. Where possible, raw data sets are documented and kept in electronic form to facilitate use in future assessment and restoration activities. Electronic copies of relevant working files that were created and used during analysis are logged and archived, along with electronic copies of reports and other printed matter associated with this project.

Surveys sampled spot shrimp at the same six sites each year: Unakwik Inlet, Golden, Culross Passage, Herring Bay, Chenega Island and Green Island (Figure 1). In November 1989 24 stations were set: two stations, each represented a string of pots (Figure 3), at each of two depth strata, at each of the six sites (Table 4). In March 1990, 35 stations were set (Table 5). Additional pot strings were set at Culross Passage, Herring Bay, Chenega Island and Green Island to catch enough shrimp for length frequency distributions. In November 1990, 40 stations were set (Table 6). Additional pot strings were set at Golden, Culross Passage, Herring Bay, Chenega Island and Green Island. Due to time constraints and the poor catch in previous years, only the shallow depth stratum was fished at the Green Island site. In November 1991, 51 stations were set (Table 7). More than the original 4 pot strings per site were set at all sites. Also, a new site, Snug Harbor, was included during the 1991 survey.

### *Relative Abundance*

The average number and weight of pandalid shrimp caught per pot varied from year to year (Table 8). In general, spot shrimp was the most common species of shrimp captured during surveys. However, pink shrimp *Pandalus borealis* were the most abundant shrimp species at Herring Bay in 1990, and at Green Island in both 1989 and 1990, while coonstripe shrimp *Pandalus hypsinotus* were most abundant at Golden and Culross Passage in 1991. Spot shrimp was the most abundant species by weight for all years and sites, except Green Island in 1990 when pink shrimp was the most abundant by weight. Coonstripe shrimp was second most abundant species by weight at Unakwik, Golden and Culross Passage (all unoiled sites) each year. Pink shrimp was the second most abundant species by weight at Herring Bay and Chenega

(both oiled sites) in 1989 and 1990, while coonstripe shrimp was the second most abundant species by weight in 1991 for these two sites. Humpy shrimp *Pandalus goniurus* and rough patch shrimp *Pandalus stenolepis* were caught in very low numbers at only a few stations. This basically agrees with our understanding of shrimp distribution in PWS. No further analysis was made on the other species, since catches tended to be inconsistent and often too low to perform meaningful analysis.

The ANOVA model used on the CPUE for each year was:

$$CPUE_{ijkm} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + \gamma_{k(i)} + \epsilon_{ijkm} . \quad (17)$$

The depth stratum-site interaction term was not used due to the empty cell for Green Island's deep strata in the 1990 survey (i.e. the deep strata was not fished at all in 1990 at Green Island). The depth stratum effect was not significant for the number of shrimp per kilogram per pot in 1989 or 1990; however, it was kept in the model to maintain consistency with the other linear models. The sum of squares,  $R^2$ , and F value of each ANOVA, as tested by year, were different between years for the number of shrimp per pot, weight per pot and number of shrimp per kilogram per pot, (Table 9). The  $R^2$  values tended to be 0.35 and 0.50 indicating similar fit in most years and variables, however the number of shrimp per kilogram per pot had lower  $R^2$  values in 1990 and 1991, implying a poorer fit in those years.

The average weight of spot shrimp per pot was significantly lower in the oiled area than the unoiled area in 1989 and 1990; however there was no significant difference in 1991 (Table 10). The oiled area had significantly fewer shrimp in number per pot in 1989 and 1990 than the unoiled area; however, in 1991 the oiled area had significantly more shrimp per pot (Table 10). Finally, the number of shrimp per kilogram per pot was significantly less in the unoiled area every year (Table 10), in other words, the average size of spot shrimp was significantly higher in the unoiled areas each year.

Due to the significance of interaction terms a least square mean contrast was used to compare strata CPUE within oiled and unoiled areas. The average weight of spot shrimp per pot within the shallow stratum was significantly lower in the oiled area than the unoiled area in 1989 and 1990, with no significant difference in 1991 (Table 11). The average weight of spot shrimp per pot within the deep stratum was significantly lower in the oiled area than the unoiled area in 1990 and 1991, with no significant difference observed in 1989. The shallow stratum of the oiled area had significantly fewer shrimp in number per pot in 1989 and 1990 than the unoiled area; however, in 1991 the oiled area had significantly more shrimp per pot (Table 11). For the deep stratum, the oiled area had significantly fewer shrimp in 1990 only. The number of shrimp per kilogram per pot was significantly less in the unoiled area for the shallow stratum every year (Table 11). In other words, the average size of spot shrimp was significantly larger in the unoiled areas each year. The number of shrimp per kilogram per pot was significantly lower in the unoiled deep stratum in 1989, but no significant difference was observed in 1990 or 1991.

The ANOVA model used to test between year effects for the two oil strata was:

$$CPUE_{hjkm} = \mu + \delta_h + \beta_j + \gamma_k + (\delta\gamma)_{hk} + \epsilon_{hjkm}. \quad (18)$$

As with the previous model, interaction terms involving depth strata were not incorporated due to the missing data for Green Island in 1990. The sum of squares and F value were different between the oiled and unoiled areas in all cases (Table 12), as might be expected since significant difference was found for each year. The R<sup>2</sup> value was similar for the oiled and unoiled area models, for weight per pot and number per pot, however with the number per kilogram per pot, the R<sup>2</sup> value in the unoiled area was nearly twice that found in the oiled area.

Average weight of shrimp per pot did not vary significantly between 1989 and 1990, in either the oiled or unoiled area (Table 13). However, between 1990 and 1991, there was a significant decrease in the weight per pot in the unoiled area, and a significant increase in the oiled area. The unoiled area has had a decrease in the number of shrimp per pot from year to year, with 1989 to 1990 being insignificant at the  $\alpha = 0.05$  but significant at the  $\alpha = 0.10$ , and 1990 to 1991 being highly significant. The average number of shrimp per pot, in the oiled area was significantly lower in 1990 than in 1989. However, there was a significant increase in the number of shrimp per pot in the oiled area from 1990 to 1991. Furthermore, there were significantly more shrimp per pot in 1991 than in 1989 ( $p < 0.0001$ ) in the oiled area. The number of shrimp per kilogram per pot has been significantly lower each year in both the oiled and unoiled areas (Table 13).

Average weight of shrimp per pot did not vary significantly between 1989 and 1990, at any sites except Unakwik Inlet which had a significant increase and Herring Bay which had a significant decrease (Table 14). Between 1990 and 1991, within the unoiled area, there was a significant decrease in the weight per pot at Golden, but there was no significant difference at Culross Passage or Unakwik. However, there was a significant increase in the average weight per pot at all sites within the oiled area between 1990 and 1991. All sites within the unoiled area have had a decrease in the number of shrimp per pot from year to year, except between 1990 and 1991 at Culross Passage in which there was no significant difference. The average number of shrimp per pot, in the oiled area was significantly lower in 1990 than in 1989 at Herring Bay only. However, there was a significant increase in the number of shrimp per pot at all sites within the oiled area from 1990 to 1991 (Table 14). The number of shrimp per kilogram per pot has been significantly lower each year at Golden and Culross within the unoiled area, but no significant difference at Unakwik Inlet between years (Table 14). The number of shrimp per kilogram per pot was not significantly different between years at Herring Bay, nor at Green Island between 1989 and 1990. However there was a significant decrease in the number of shrimp per kilogram per pot at Chenega Island between consecutive years and at Green Island between 1990 and 1991 (Table 14).

## *Length, Growth, Sex and Fecundity*

### Length Composition

Length histograms (Figures 5 and 6) are very different at each site. Scales on the graphs for the various sites are different because certain sites (i.e. Golden) had a much higher CPUE than others. All sites, except Culross Passage, seem to have a strong mode for a specific year class, and there seems to be little or no recruitment in 1991 (17 mm to 22 mm), except at Herring Bay and possibly Culross Passage.

The graphs seem to support the results of the previous CPUE analyses. The unoiled sites stocks are decreasing at all lengths, while the oiled sites decreased between 1989 and 1990 but increased between 1990 and 1991. Further, there are few large shrimp in the oiled areas.

### Growth

The mixture modal analysis performed on November 1989 and March 1990 survey catch data, showed little difference in shrimp growth rates among sites or between the oiled and unoiled areas (Table 15). The growth parameter,  $k$ , has two values, one approximately half that of the other (Table 15). The smaller  $k$  value represented biannual molt (two molts per year), while the larger value represented annual growth. Use of the smaller growth parameter and half-year time intervals provided a better graphical fit to the normal curve and a greater maximum likelihood value. Both fits, however, represent the same basic growth rate (Table 15).

### Sex Composition

The adult spot shrimp population was dominated by males at all sites (Table 16). There seemed to be a higher percentage of males within the oiled area than within the unoiled area for all years, although there was site to site variability in both oiling strata. Trends in male CPUE were similar to total population CPUE because of the high percentage (> 75%) of males at each site, therefore no rigorous statistical test was performed.

The number of females per pot did not always follow population CPUE (Figures 7 and 8). The final ANOVA model used each year on the square root of the number of females per pot was:

$$\sqrt{FEM_{ijkm}} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + \gamma_{k(i)} + \epsilon_{ijkm} . \quad (19)$$

The depth stratum-site interaction term was not used due to the empty cell for Green Island's deep strata in the 1990 survey. The oiling effect and site effect, nested within the oiling effect, were consistently the most significant effects in the ANOVA model (Table 17). This illustrates the importance of oiling strata and site variability on the number of females per pot. The  $R^2$

values seemed to have a greater year to year variability than did the ANOVA model fit to the entire population (Table 9).

Each year of the study and within both depth strata, the unoiled area had significantly more females than the oiled area, given in Table 18. This was in contrast to the patterns seen in overall spot shrimp numbers between oiled and unoiled areas, since in 1991 the oiled area had significantly more spot shrimp than did the unoiled area.

The ANOVA model to test between year effects for the two oil strata was:

$$\sqrt{FEM_{hjkm}} = \mu + \delta_h + \beta_j + \gamma_k + (\delta\gamma)_{hk} + \epsilon_{hjkm}. \quad (20)$$

As with all previous models, interaction terms involving depth strata were not incorporated. The sum of squares,  $R^2$ , and F values were extremely different between the oiled and unoiled areas. The sum of squares were as much as 40 times greater in the unoiled area,  $R^2$  values were sometimes 4 times greater, and F values were nearly 10 times as much (Table 17). This probably occurred because very few females per pot were found in the oiled area (Table 16).

The unoiled and oiled areas did not change in the same manner from year to year. In the unoiled area, there was a significant increase in females between 1989 and 1990, and then a significant decrease between 1990 and 1991. There was a significant increase in females between 1989 and 1990 at two of the sites within the unoiled area, Unakwik Inlet and Golden, but there was no difference at Culross Passage. However, there was only one site, Golden, which indicated a significant decrease between 1990 and 1991, with both Unakwik Inlet and Culross Passage having no significant difference between these years. In the oiled area and all sites within the oiled area, there was no difference in the number of females between 1989 and 1990. There was a significant increase in females between 1990 and 1991 in the oiled area, and specifically observed at Herring Bay and Chenega Island (Table 18). This increase was not observed to be significant at the Green Island site. Again variation in female abundance did not follow total population trends.

#### Fecundity and Related Parameters

The number of eggs per female and sample sizes varied among sites (Table 19). The analysis of covariance model fit to the number of eggs per female, with carapace length as the covariate, was:

$$NUMEGGS_{ijkm} = \mu + x_{ijkm} + \alpha_i + \gamma_{k(i)} + \epsilon_{ijkm}. \quad (21)$$

The stratum and all stratum interaction terms were removed due to insignificance ( $p > 0.15$ ) in all cases. Though the sum of squares,  $R^2$  and F values tended to be different each year (Table

20), carapace length was the most significant term (accounted for the greatest amount of variability) in every case. For 1990 and 1991, the second most important component in the analysis of covariance model was the site effect, while the least important component was the oiling effect. In 1989, oiling and site effects were similar and marginally significant.

The analysis of covariance indicated that a significant difference in clutch size existed between the oiled and unoiled areas only for 1989 (Table 21). A linear comparison of the fecundity relationship, number of eggs versus carapace length between oiled and unoiled areas produced similar results: a significant difference between oiled and unoiled areas only in 1989. Furthermore, more complex regression models (i.e. nonlinear regression) provided no better information than did the linear regression fits.

The analysis of covariance model for each oiling stratum and between years was:

$$NUMEGGS_{hjkm} = \mu + x_{hjkm} + \delta_h + \gamma_k + (\delta\gamma)_{hk} + \epsilon_{hjkm}. \quad (22)$$

As with the above analysis of covariance, the stratum term and its associated interaction terms were found to be insignificant ( $p > 0.20$ ) in all cases. The different statistics for the analysis of covariance (sum of squares,  $R^2$ , and F values) varied between the oiled and unoiled area (Table 20), but  $R^2$  values were generally similar. The most significant term, again, was the carapace length covariate.

Analysis of covariance and linear comparisons for the unoiled area showed no significant difference in clutch size between 1989 and 1990, but significantly fewer eggs per clutch in 1991 (Table 22). However, further analysis of site variability, indicates no significant difference between years for Golden and Culross Passage, and Unakwik Inlet has had a significant decrease each year (Table 23). In the oiled area, analysis of covariance and linear comparison between years indicated there was a difference in clutch size between 1989 and 1990, although it was marginal ( $p=0.0553$ ) for analysis of covariance. Analysis of covariance showed a significant difference in clutch size between 1990 and 1991, while linear comparison showed no difference among slopes. The two sites used for this analysis varied in opposite manners. There was a significant increase in the number of eggs per female between 1989 and 1990 at Herring Bay, but no difference was detected at Chenega Island; then there was a significant decrease between 1990 and 1991 at Chenega Island but no difference at Herring Bay (Table 23).

The number of females with or without eggs varied most in the unoiled area (Table 24). In 1989, there was no significant association ( $p = 0.7313$ ) between oiling, with sites nested within the oiling strata, and the number of females with or without eggs. In 1990, there was no significant association between oiling, with sites nested within the oiling strata and the number of females with or without eggs, although it was marginally insignificant ( $p = .0939$ ). However, in 1991, there was significant association ( $p = 0.00006$ ) between oiling, with sites nested within oiling strata, and the number of females with or without eggs.

Females were pooled across sites, within the two oiling strata. This was done first for the analysis of percent females without eggs in breeding dress versus females without eggs not in breeding dress, and second for the analysis of egg mortality (number of dead eggs per female). The analysis of females in breeding dress or not in breeding dress, was pooled because there were few or no females without eggs at most sites. For the analysis of egg mortality, pooling across oiling strata was done because there were few females with dead eggs, making analysis with nesting very difficult.

The analysis of percentage of females in breeding dress should be viewed with caution, since the number of females without eggs in 1989 and 1990 was low (Table 24). In 1989 and 1991 there was a significantly higher percentage of females not in breeding dress in the oiled area, while in 1990 there was no difference between the oiled and unoiled area. There was no significant difference between oiled and unoiled areas for the number of dead eggs per female for any year.

#### *Hydrocarbon Analysis*

A total of 262 samples for hydrocarbon contamination analysis were taken during the three years of study (Appendix D). To date only 17 samples collected from spot shrimp in 1989 (7 from unoiled sites and 10 from oiled sites) have been analyzed. No oil contamination was detected in any of these samples. All other 245 samples have yet to be analyzed, and are unlikely to be analyzed.

#### *Histopathological Analysis*

A total of 48 samples were collected for histopathology analysis (Appendix D). Only 12 samples from the 1989 survey have been analyzed (Lightner and Redman 1992; Appendix C). A total of 120 shrimp were examined for severity of gill lesions, and presence of the gill parasite *Lagenophrys* (Table 25). A count of shrimp with melanized cuticular lesions, with theorized cause being of toxic nature, was also performed (Table 25).

Inflammatory gill lesion occurrence was considered to be the best indicator of a toxic affect (Sindermann 1990; D.V. Lightner personnal communication). The log-linear fit of all 4 severities showed a significant ( $p = 0.0001$ ) association with the oiling affect, with a site affect nested within the oiling affect (Table 25). When severities were combined, a significant ( $p = 0.0003$ ) association was again observed. However, it was the unoiled area that had more severe gill lesions than did the oiled area.

Results of the analysis of *Lagenophrys* on gills were different. When all 4 severities were considered in the log-linear fit, no significant ( $p = 0.065$ ) association was observed between severity and oiling, again with site affect nested within the oiling affect (Table 25). Since the statistical significance was marginal, severities were combined and re-analyzed. Results again

indicated no significant ( $p = 0.1887$ ) association between severity and oiling.

A total of only 10 shrimp out of 120 examined had the melanized cuticular lesions (Table 25). The log-linear fit found no significant ( $p=0.3642$ ) association between the number of shrimp with lesions and the oiling effect, with the site effect nested within the oiling effect.

### *Environmental Observations*

Temperatures (Figures 9 - 11) were most variable within the shallow depth range of the spot shrimp's habitat (35 - 130 m). For the unoiled area Unakwik Inlet and Golden had similar temperature profiles, while the Culross Passage temperature was lower in the first 100 m, in 1989. Within the oiled area, Herring Bay and Chenega Island tended to be similar all years. However the Green Island temperature was lower than the others between 1 - 81 m during 1989 and 1990, and higher between 81 - 181 m during 1991. Salinity (Figures 12 - 14) and dissolved oxygen concentration (Figures 15 - 17) gradients were similar for all years at all sites within spot shrimp habitat ( $> 35$  m).

### *Population Model Using Catch Data*

Commercial catch has varied among statistical reporting areas within both unoiled (Figure 18) and oiled (Figure 19) study areas. In general catches were greatest in the oiled area (Southwest PWS) during the early 1980's, peaking in 1982 and then declining (Figure 2). Catches were greatest in the unoiled area (Northwest PWS) during the mid to late 1980's. Our population model used this pattern of fishing to simulate the population structure for 1989-1991 in the absence of an oil spill.

Results from the model consisted of predicted length frequency distributions for shrimp within the five statistical areas for 1989, 1990 and 1991 (Figures 20 and 21). Within the unoiled area, abundance declined most dramatically in statistical reporting area 20304, but all areas showed declining recruitment and had very few females. Lack of recruitment and few females also occurred in population simulations of the oiled statistical reporting areas.

The model was also used to project the length frequency distributions for 1992 and 1993 (Figures 22 and 23). The unoiled area is expected to have little recruitment and few females, although females are projected to become more abundant in 1993. The oiled area is also expected to have little recruitment. However the projected increase in females in 1992 and 1993 suggest that recruitment should increase in 1995 and 1996, especially in statistical reporting area 20101 (Figure 23).

## DISCUSSION

### *Relative Abundance, Length, Growth and Sex Composition*

CPUE (Tables 9 - 11), length frequency distributions (Figures 5 - 8) and sex composition (Table 16) were highly variable among sites, even within the same oiling stratum. After reviewing the history of the commercial fishing, we believe that much of the site variability was due to different patterns of fishing within PWS. Sites were spread throughout the spot shrimp's habitat and, as a result, were located within different statistical reporting areas (except for Herring Bay and Chenega Island), and were probably fished at different intensities (Figures 18 and 19). A strong fishing effect on site specific population structure was further suggested by noting that the two statistical reporting areas with the highest catch in 1990 and 1991, 20300 and 20304 (Figures 18 and 19), contained both sites which had the greatest drop in CPUE between 1990 and 1991, Golden and Culross Passage (Table 14 and Figure 5). In addition, continuous fishing allowed between 1985 and 1988 within statistical reporting area 20101 (Donaldson 1989), may be the explanation for the extremely low CPUE observed at Green Island, which is within the 20101 statistical reporting area, in all three survey years (Table 8). Differences in environmental conditions were also considered as a cause for the site variability, however the environmental conditions observed (Figures 9 - 17) were not at a level thought to cause harm to shrimp (Jamieson and Pikitch 1988 and Rice et al. 1984).

In general, trends in the oiling strata may also be explained by fishing. In 1989 and 1990, the oiled area had fewer shrimp which were smaller than those caught in the unoiled area. In 1991, however, there were more spot shrimp in the oiled area than the unoiled area, although average size of spot shrimp was still smaller within the oiled area (Tables 8, 10, 11 and 13; Figures 5 and 6). Since the fishery targets on large males and females, it concentrates its efforts on the breeding population, and therefore impacts larval stocks for 2 to 4 years from one year of fishing, since spot shrimp are multi-year spawners. The oiled area was highly exploited in the early to mid-1980's (Figures 2 and 19). Low abundance (< 1 shrimp/pot) of spot shrimp greater than 34 mm may be the result (Figures 6 and 8). The unoiled area was exploited more heavily in the mid- to late-1980's (Figures 2 and 19). Low recruitment observed in the unoiled area in the last two years may be the result (Figure 5). The lower number of shrimp per pot in 1991 in the unoiled area was probably due to continued fishing of spot shrimp within the unoiled area in 1990 and 1991 (Figures 18 and 19). No fishing was allowed in the oiled area in 1990 and very little fishing occurred in 1991.

Strong evidence that most stock structure differences between oiling strata and among study sites could have been caused largely by fishing, makes it difficult to demonstrate effects due to the EVOS. However, we have observed anomalies in our data which suggest effects did occur. First, when *MULTIFAN* was used to fit a von Bertalanffy curve to the length frequency data of November 1989 and March 1990, shrimp in oiled and unoiled areas were shown to have similar growth rates (Table 15). However, in November 1990 and November 1991, growth of spot

shrimp in oiled areas was actually slightly less than for those caught in the unoiled areas (observational comparison, not statistical). This was unexpected since spot shrimp caught in the oiled area were smaller and most growth studies on pandalid shrimp have indicated faster growth for younger individuals (Anderson 1991; Butler 1964, 1980) . Unfortunately, due to time, funding and staffing limitations *MULTIFAN* was not run with 1990 or 1991 survey length frequency distributions. While, a slower growth rate may be attributed to environmental considerations, it would not be surprising to see such an effect from oil contamination.

Another anomaly was the decrease in CPUE from 1989 to 1990 in the oiled sites followed by a dramatic increase in 1991 (Tables 8 and 12, and Figures 6 and 8). The increase in CPUE was to a level greater than that observed in 1989, occurred within all size cohorts, and therefore could not be attributed to juvenile recruitment. A change in sampling efficiency was considered as a possible cause of this anomaly. However this abundance change occurred only in the oiled area and was cyclic in nature, rather than monotonic. Since spot shrimp in PWS are relatively sedentary (Kimker and Donaldson 1987), any migration could be considered unusual behavior. We were unable to identify a specific mechanism for this, and, with the lack of knowledge on the behavior of spot shrimp in central and south PWS, this anomaly could not be conclusively attributed to the EVOS.

#### *Fecundity and Related Parameters*

The results from our analysis of reproduction parameters was also inconclusive. All comparisons between oiled and unoiled areas with regards to spot shrimp reproductive parameters were either statistically insignificant, as in the case of the number of eggs per female (except in 1989)(Table 21 and 22); inconsistent as with the number of females with or without eggs (Table 24); or suspect when the sample size is low, as with females in breeding dress (Table 24).

#### *Hydrocarbon and Histopathology*

Our inability to detect hydrocarbon contamination in spot shrimp may have been due to the small number analyzed, the ability of spot shrimp to process hydrocarbons in muscles and eggs (Sanborn and Malins 1980), or an absence of contamination. Other Natural Resource Damage Assessment (NRDA) studies found hydrocarbon contamination at depths which spot shrimp inhabit. The conclusions of NRDA Subtidal Study 1A (Feder 1991) regarding depth of contamination were based on the greater abundance of opportunists and sediment-water interface feeders at oiled sites than at unoiled sites. This suggested that there was a major disturbance from oil contamination for stations at 40 m, and a significant disturbance at 100 m and greater depths. Two sites in NRDA Subtidal Study 1A, Chenega Bay and Herring Bay, were near two of our sites, Chenega Island and Herring Bay. NRDA Subtidal Study 1B (Braddock et al.

1991), showed most-probable-number measurements (Brown and Braddock 1990) of oil-degrading microorganisms (cells per g dry sediment) in sediments and water samples to be greater at oiled sites than control sites at depths of about 100 m, in 1990. NRDA Subtidal Study 1B included some sites near the Chenega Island and Green Island sites. NRDA Air/Water Study number 2 (Rice and O'Clair 1990) used total hydrocarbon concentration (in ppm) analysis, and found significant hydrocarbons to depths of 100 m. In NRDA Subtidal Study 4 (Wolfe 1991), percent oyster larval mortality was greater in 1990 at oiled sites than reference sites at depths of both 20 m and 100 m.

Histopathology analysis gave no indication of contamination. Inflammatory gill lesions on spot shrimp should have been the best indicator of toxic contamination (Dr. Lightner personal communication). However, the unoiled area had more severe cases of these lesions than did the oiled area. The oiled area seemed to have more severe cases of *Lagenophrys* on the gills and more melanized cuticular lesions, but differences were found to be statistically insignificant. Furthermore, these gill parasites and cuticular lesions were more indicative of slower molt and not necessarily due to direct toxic contamination (Dr. Lightner, personal communication). As noted earlier, these results were based on samples from the 1989 survey; no other histopathology samples were analyzed, although samples were taken. Furthermore, hydrocarbon analysis results of the above projects had stronger indications of oil contamination within spot shrimp habitat in 1990 than in 1989. Therefore, histopathological analysis of samples taken in 1990 may have been helpful in making year to year comparisons.

#### *Population Model Using Catch Data*

An important objective for the last year of this project was to separate potential effects of commercial fishing on spot shrimp populations from that of the oil spill. We attempted to model spot shrimp population dynamics by simulating CPUE and size structure from observed commercial catches, theorized natural mortality, and estimated recruits per female. The model constructed from the fish ticket data over simplified the system and had many untested assumptions.

Due in part to simplifications and assumptions, the model did not fit all the sites equally well. For statistical reporting area 20301 (Figure 20), the model poorly predicted the overall change in abundance seen at the Unakwik Inlet study site from 1989 through 1991 (Figure 5). At this site, the model overestimated the number of recruits (shrimp < 24 mm), and underestimated the number of females (shrimp > 38 mm). Within statistical reporting area 20300, on the other hand, the fit was much better and the model mimicked the strong year class at 28 mm in 1989 at Golden, along with its decline from 1989 to 1991 (Figure 5). However, the model underestimated the number of females at this site as well. The model predicted a steady decline in the population of statistical reporting area 20304 (Figure 20), but did not capture the observed increase in smaller shrimp (< 24 mm) in 1990 for the Culross Passage study site (Figure 5). The population model predicted fairly well our survey results seen in 2 of the 3 sites in the

unoiled area.

Within the oiled area an immediate problem for the model was evident: both Herring Bay and Chenega Island were within statistical reporting area 20100 but had different population structures. The population model did not predict the trend in abundance seen from 1989 through 1991 at the Herring Bay or Chenega study sites (Figure 6). The model could not predict the increased abundance observed in 1991, since the model assumed spot shrimp were sedentary. Furthermore, the predicted decline in abundance by the model between 1990 and 1991, was the result of minor fishing within statistical reporting area 20100 in 1991 (Figure 21). The fishing within statistical reporting area 20100 was conducted near Chenega Island according to fishermen log books, which might explain why Herring Bay and Chenega Island did not increase to the same extent from 1990 to 1991. The model for statistical reporting area 20100, did not predict the recruitment as seen at Herring Bay in 1991 and overestimates females (Figure 6). The model also overestimated the number of females at the Chenega Island study site. The model seemed to predict the basic shape of the length frequency distribution of the Chenega Island study site, but the predicted distribution was about 3 to 5 mm ahead of the actual mode.

The population model for statistical reporting area 20101 (Figure 21) successfully predicted the shape of the Green Island length frequency distributions, showing few small shrimp (< 25 mm) in 1991 and one dominant mode. However, the predicted length frequency distribution was 4 to 7 mm ahead of the actual mode, as described in the length frequency distribution from the survey data (Figure 6). Also, the model did not predict the increase in overall abundance observed in 1991, since our model was based on a closed population with no immigration or emigration. Explanations for the various changes in abundance from 1989 through 1991 observed in the survey data include (1) immigration and emigration, and (2) a change in attraction to baited pots. If either or a combination of these two phenomena occurred, this occurrence was unique to the oiled area, and therefore, may be related to a disturbance such as the oil spill.

In the absence of commercial fishing, the population model predicted little or no recruitment in any of statistical reporting areas (Figure 22 and 23). Within statistical reporting areas 20301, 20300 and 20304, the number of females was underestimated in most years by the model and within statistical reporting areas 20100 and 20101, the number of females was overestimated by the model. From these results, further discussion on recruitment would be inappropriate.

The model did not fit all site length frequency distributions equally well; errors in shape and mode location were observed. This indicated that a more complex model will be needed, which better simulates annual growth, before it will be possible to separate fishing and oiling effects. Further, the model has not been tested on non-stressed populations. Finally, a better understanding of migration and larval drift of spot shrimp is needed since movement within PWS seemed to occur.

## CONCLUSIONS

Other studies (Braddock et al. 1991) have shown that oil was present at the depth adult spot shrimp inhabit. We know shrimp are affected by oil, causing death at relatively low levels of contamination, and that predators of adult spot shrimp were affected by the EVOS (i.e. rockfish, Hoffmann et al. 1991). We could not find conclusive evidence that spot shrimp within PWS were themselves affected by the EVOS. Our results suggest that observed stock abundance and structure could mostly be explained by the extensive fishing effort for this species prior to the EVOS. Unfortunately, pre-spill biological information on spot shrimp in PWS was limited to results of studies at Unakwik Inlet and Green Island. Finally, investigators could not pursue the spot shrimp study as intensely as perhaps was necessary, due to time, funding and staffing considerations.

While we were unable to conclude that spot shrimp within south and central PWS areas were adversely affected by the EVOS, it seems very unlikely that spot shrimp were not affected, especially the larvae which were in the water column at the time of the oil spill. The design of the present study and the support provided were insufficient for a thorough investigation of the EVOS effects on spot shrimp. This is especially true given that we have not yet fully sampled the 1989 brood year.

The study was designed and conducted with deadlines and in competition with other projects. Given adequate time and funding, the study could have possibly intensified as follows: More sites could have been used, including more sites from different statistical reporting areas, as well as more replicates within each statistical reporting area. Greater attention could have been placed on fishing effects, both prior to and after the EVOS. As requested by the investigators, more emphasis and attention could have been focused on larval and juvenile spot shrimp, which are more susceptible to oil. Perhaps sites outside the oiled area could have been set aside (i.e. closed) from commercial fishing to maintain a more natural unfished population outside the spill area for comparative purposes. More histopathology samples could have been analyzed (a situation beyond the control of the principle investigator), along with more hydrocarbon analysis.

A more accurate model could have been attempted to better determine fishing effects. Lastly, better interaction with other studies should have been pursued actively throughout the study.

There has been some good biological information gained from this study. It is now known that spot shrimp populations within PWS mature and grow more slowly than those in the southern part of their range (Butler 1964, 1980). We have documented new predators, namely octopus, Pacific cod and pollock which were observed preying upon spot shrimp. Finally, spot shrimp seem to have been harvested more extensively than was originally thought prior to the oil spill.

Whether caused by fishing pressure or oil spill contamination, PWS spot shrimp are now at a low level of abundance and the pot fishery has been closed, for an indeterminate period. As

stated earlier, the study has shown that the spot shrimp of PWS grow more slowly than those found to the south, which means recovery of this species will likely take many years. If the larvae of 1989 were adversely affected (as we hypothesize), recovery will take even longer in the central and southern areas of PWS. Furthermore, effects of reduced spot shrimp abundance on the PWS ecosystem is unknown but likely to be detrimental, to at least some species. It is our hope that the information gained on spot shrimp from these studies, due to the EVOS, will help the spot shrimp resource managers better manage this resource. We also hope that these studies will provide insight into the best approach for assessing damage to spot shrimp, if another oil spill occurs.

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Table 1. Life history comparisons for economically important shellfish in the Exxon Valdez oil spill affected area.<sup>a</sup>

Shellfish Species	Egg Bearing Period	Principle Hatching Occurs	Planktonic Larval Period	Settlement Period	Juvenile Habitat	Adult Food <sup>b</sup> Preference
Spot Shrimp <i>(Pandalus platyceros)</i>	Oct-Mar	March-April	March-August	Late Summer	Inshore and shallower than adults, rock crevices and kelp patches	detritus, worms (annelids and polychaetes)
Pink Shrimp <i>(Pandalus borealis)</i>	Nov-April	March-April	March-August	Late Summer	Inshore and shallower than adults.	polychaetes, mysids and other crustaceans (In general, pandalid shrimp feed on detritus, amphipods, euphausiids, annelids, and other shrimps).
Tanner Crab <i>(Chionoecetes bairdi)</i>	April-May (11 mo)	March-May	March-August	Late Summer	Inshore and shallower than adults	polychaetes, ophiuroids, fishes, <i>Nuculatenuis</i> , <sup>c</sup> bivalves, shrimp, amphipods, crab
Red King Crab <i>(Paralithodes camtschatica)</i>	April-May (11 mo)	March-May	March-August	Mid-June to Late Summer	Inshore and shallower than adults	molluscs, brittle stars, polychaetes, snails, sand dollars, pelecypods, basketstars, sea urchins
Blue King Crab <i>(Paralithodes platypus)</i>	April-May (11 mo)	March-May	March-August	Late Summer	Rock shellhash substrates	molluscs, brittle stars, polychaetes
Brown King Crab <i>(Lithodes aequispina)</i>	Variable year round	Variable year round	Unknown	Unknown	Shallower depths than adults	echinoderms, polychaetes, hydroids, molluscs, amphipoda, decapoda

<sup>a</sup>Most information is from the literature and applies to the species throughout its range.

<sup>b</sup>Food habits are from the general literature and represent prey items utilized throughout their range.

<sup>c</sup>From study of Tanner crab in Prince William Sound (Feder and Hobart 1981).

Table 2. Survey sites for the spot shrimp oil spill assessment survey and the respective statistical reporting areas within Prince William Sound.

Survey Site	Statistical Reporting Areas
Unakwik Inlet	20301
Golden (Port Wells)	20300
Culross Passage	20304
Herring Bay	20100
Chenega Island	20100
Green Island	20101
Snug Harbor <sup>a</sup>	20101

<sup>a</sup> Snug Harbor was only sampled in the 1991 survey.

Table 3. Assumed sizes at age used to model the Prince William Sound spot shrimp population and the effect of the commercial fishery.

Age in Years	Approximate Length of Carapace in mm
3 <sup>a</sup>	19
4	24
5	28
6	32
7	35
8	38
9	41
10	44
11	47
12	49

<sup>a</sup> An age 3 and 19 mm are the youngest shrimp consistently caught.

Table 4. Sampling locations for the November, 1989 oil spill assessment survey of spot shrimp in Prince William Sound.

Site	Depth Stratum <sup>a</sup>	Station <sup>b</sup>	Latitude <sup>c</sup>	Longitude <sup>c</sup>	Minimum Depth <sup>d</sup>	Maximum Depth <sup>d</sup>	Number of Pots	Soak Time <sup>e</sup>
Unakwik	1	A	60.59.80	147.32.68	32	43	11	16
Unakwik	1	B	61.00.00	147.32.34	45	55	11	18
Unakwik	2	A	60.59.86	147.32.79	70	82	11	17
Unakwik	2	B	61.00.15	147.32.39	90	100	11	18
Golden	1	A	60.57.67	148.01.85	35	50	11	17
Golden	1	B	60.57.92	148.01.41	52	65	11	17
Golden	2	A	60.57.72	148.01.95	70	94	11	17
Golden	2	B	60.57.92	148.01.74	95	100	11	17
Culross	1	A	60.39.36	148.11.72	55	70	11	17
Culross	1	B	60.39.18	148.11.51	40	60	11	17
Culross	2	A	60.36.11	148.10.41	70	100	11	17
Culross	2	B	60.36.00	148.10.92	70	90	11	16
Herring	1	A	60.27.61	147.44.28	35	65	11	17
Herring	1	B	60.28.06	147.45.49	50	62	11	17
Herring	2	A	60.28.55	147.45.42	75	85	11	17
Herring	2	B	60.28.32	147.45.51	70	85	11	16
Chenega	1	A	60.24.67	147.58.04	50	70	11	17
Chenega	1	B	60.23.12	147.58.96	45	55	11	17
Chenega	2	A	60.24.55	147.58.19	70	80	11	18
Chenega	2	B	60.23.28	147.58.36	85	100	11	17
Green	1	A	60.19.09	147.29.11	35	46	11	18
Green	1	B	60.18.37	147.28.90	57	64	11	17
Green	2	A-	60.18.55	147.29.98	70	80	11	17
Green	2	B	60.18.31	147.30.36	80	88	11	17

<sup>a</sup> 1 = shallow (35 - 130 m); 2 = deep (130 - 220 m).

<sup>b</sup> Station letter represents order in which the stations were set, by depth strata.

<sup>c</sup> Latitude and longitude are listed to the one-hundredth of a minute.

<sup>d</sup> Depth is in meters.

<sup>e</sup> Rounded to the nearest hour.

Table 5. Sampling locations for the March, 1990 oil spill assessment survey of spot shrimp in Prince William Sound

Site	Depth Stratum <sup>a</sup>	Station <sup>b</sup>	Latitude <sup>c</sup>	Longitude <sup>c</sup>	Minimum Depth <sup>d</sup>	Maximum Depth <sup>d</sup>	Number of Pots	Soak Time <sup>e</sup>
Unakwik	1	A	60.59.80	147.30.68	35	60	11	16
Unakwik	1	B	60.59.96	147.32.12	45	70	11	16
Unakwik	2	A	60.59.91	147.32.79	70	95	11	16
Unakwik	2	B	61.00.10	147.32.41	82	95	11	16
Golden	1	A	60.57.64	148.01.88	30	70	11	16
Golden	1	B	60.57.89	148.01.44	25	60	11	16
Golden	2	A	60.57.76	148.01.80	70	100	11	16
Golden	2	B	60.57.90	148.01.65	70	95	11	16
Culross	1	A	60.38.95	148.11.58	32	45	11	18
Culross	1	B	60.38.73	148.11.36	43	63	11	18
Culross	2	A	60.35.77	148.11.33	70	96	11	18
Culross	2	B	60.35.73	148.11.68	70	85	11	18
Culross	2	C	60.35.66	148.12.14	70	85	11	18
Herring	1	A	60.27.69	147.44.35	35	70	11	16
Herring	1	B	60.28.01	147.45.77	37	58	11	18
Herring	1	C	60.28.21	147.45.68	60	62	8	18
Herring	1	D	60.28.09	147.45.54	55	60	11	17
Herring	1	F	60.27.84	147.45.65	63	48	11	16
Herring	2	A	60.28.57	147.45.43	70	95	11	18
Herring	2	B	60.28.31	147.45.56	70	85	11	18
Chenega	1	A	60.24.65	147.58.05	52	70	11	17
Chenega	1	B	60.23.13	147.58.95	45	63	11	17
Chenega	2	A	60.24.52	147.58.26	72	83	11	17
Chenega	2	B	60.23.21	147.58.55	72	80	11	17
Chenega	2	C	60.24.66	147.58.14	70	85	6	17
Green	1	A	60.19.02	147.29.08	40	60	11	17
Green	1	B	60.18.90	147.29.28	31	65	11	17
Green	1	C	60.17.80	147.29.94	60	68	6	17
Green	1	D	60.19.12	147.29.15	35	60	11	17
Green	1	E	60.19.03	147.29.25	40	50	11	17
Green	1	F	60.18.95	147.29.54	28	50	11	17
Green	2	A	60.17.31	147.31.57	72	80	11	17
Green	2	B	60.17.13	147.32.01	70	80	11	17
Green	2	C	60.17.03	147.32.25	70	80	11	17
Green	2	D	60.16.77	147.32.27	70	75	6	17

<sup>a</sup> 1 = shallow (35 - 130 m); 2 = deep (130 - 220 m).

<sup>b</sup> Station letter represents order in which the stations were set, by depth strata.

<sup>c</sup> Latitude and longitude are listed to the one-hundredth of a minute.

<sup>d</sup> Depth is in meters.

<sup>e</sup> Rounded to the nearest hour.

Table 6. Sampling locations for the November, 1990 oil spill assessment survey of spot shrimp in Prince William Sound.

Site	Depth Stratum <sup>a</sup>	Station <sup>b</sup>	Latitude <sup>c</sup>	Longitude <sup>c</sup>	Minimum Depth <sup>d</sup>	Maximum Depth <sup>d</sup>	Number of Pots	Soak Time <sup>e</sup>
Unakwik	1	A	60.59.91	147.32.88	40	65	11	17
Unakwik	1	B	61.00.03	147.32.65	33	64	11	17
Unakwik	2	A	60.59.87	147.33.06	73	102	11	17
Unakwik	2	B	61.00.09	147.32.53	70	94	11	17
Golden	1	A	60.57.73	148.01.86	30	65	11	42
Golden	1	B	60.57.98	148.01.33	25	57	11	42
Golden	2	A	60.57.89	148.01.76	70	95	11	42
Golden	2	B	60.58.05	148.01.53	70	75	11	42
Golden	2	C	60.58.16	148.01.43	70	94	11	42
Culross	1	A	60.39.09	148.11.41	30	65	11	16
Culross	1	B	60.38.87	148.10.87	31	61	11	16
Culross	1	C	60.36.02	148.12.18	35	70	11	19
Culross	1	D	60.36.10	148.11.51	47	47	11	19
Culross	1	E	60.36.07	148.11.88	45	45	11	18
Culross	2	A	60.36.10	148.11.50	70	90	11	16
Culross	2	B	60.36.08	148.11.52	75	100	11	16
Culross	2	C	60.36.00	148.11.77	70	110	11	16
Culross	2	D	60.35.99	148.12.04	70	85	11	18
Culross	2	E	60.36.04	148.11.65	73	100	11	19
Herring	1	A	60.28.13	147.45.82	40	58	11	19
Herring	1	B	60.28.30	147.45.73	50	65	11	19
Herring	1	C	60.28.52	147.45.59	59	70	11	19
Herring	1	D	60.28.36	147.45.72	50	63	11	18
Herring	1	E	60.28.79	147.45.63	50	55	11	19
Herring	1	F	60.27.34	147.44.34	50	58	11	16
Herring	2	A	60.28.49	147.45.52	75	80	11	19
Herring	2	B	60.28.33	147.45.62	60	75	11	19
Herring	2	C	60.28.37	147.45.37	75	93	11	19
Herring	2	D	60.28.47	147.45.59	70	75	11	18
Herring	2	E	60.28.59	147.45.51	70	90	11	18
Herring	2	F	60.28.77	147.45.52	73	85	11	18
Chenega	1	A	60.24.79	147.58.04	50	70	11	18
Chenega	1	B	60.23.24	147.58.91	41	67	11	18
Chenega	1	C	60.24.71	147.58.45	36	64	11	18
Chenega	2	A	60.24.66	147.58.18	70	86	11	18
Chenega	2	B	60.23.32	147.58.61	70	93	11	18
Chenega	2	C	60.23.49	147.58.41	70	90	11	18
Green	1	A	60.19.15	147.29.19	47	68	11	18
Green	1	B	60.19.18	147.25.20	41	60	11	18
Green	1	C	60.19.01	147.29.57	35	50	11	18

<sup>a</sup> 1 = shallow (35 - 130 m); 2 = deep (130 - 220 m).

<sup>b</sup> Station letter represents order in which the stations were set, by depth strata.

<sup>c</sup> Latitude and longitude are listed to the one-hundredth of a minute.

<sup>d</sup> Depth is in meters.

<sup>e</sup> Rounded to the nearest hour.

Table 7. Sampling locations for the November, 1991 oil spill assessment survey of spot shrimp in Prince William Sound.

Site	Depth Stratum <sup>a</sup>	Station <sup>b</sup>	Latitude <sup>c</sup>	Longitude <sup>c</sup>	Minimum Depth <sup>d</sup>	Maximum Depth <sup>d</sup>	Number of Pots	Soak Time <sup>e</sup>
Unakwik	1	A	60.59.54	147.32.90	27	70	11	18
Unakwik	1	B	60.59.58	147.32.84	35	70	11	18
Unakwik	1	C	60.59.77	147.32.60	40	65	11	19
Unakwik	2	A	60.59.59	147.32.97	70	121	11	18
Unakwik	2	B	60.59.70	147.32.81	75	95	11	19
Unakwik	2	C	60.59.89	147.32.54	75	100	11	19
Golden	1	A	60.57.62	148.01.94	31	67	11	17
Golden	1	B	60.57.77	148.01.65	28	62	11	17
Golden	1	C	60.57.70	148.01.78	30	63	11	18
Golden	1	D	60.57.53	148.02.09	35	59	11	18
Golden	2	A	60.57.74	148.01.93	73	90	11	17
Golden	2	B	60.57.94	148.01.66	70	90	11	17
Golden	2	C	60.57.62	148.02.16	75	98	11	19
Golden	2	D	60.57.65	148.02.09	73	103	11	18
Golden	2	E	60.57.52	148.02.36	72	107	11	18
Golden	2	F	60.57.40	148.02.56	72	94	11	18
Culross	1	A	60.39.20	148.12.36	35	55	11	18
Culross	1	B	60.38.96	148.12.13	28	68	11	18
Culross	1	C	60.38.83	148.12.10	34	64	11	18
Culross	1	D	60.36.04	148.11.85	32	65	11	17
Culross	1	E	60.36.08	148.11.35	30	68	11	17
Culross	1	F	60.36.11	148.11.23	35	70	11	17
Culross	2	A	60.36.03	148.11.28	72	100	11	19
Culross	2	B	60.36.01	148.11.47	75	85	11	19
Culross	2	C	60.35.97	148.11.89	73	93	10	19
Culross	2	D	60.35.96	148.11.66	75	90	11	17
Culross	2	E	60.36.02	148.11.31	70	100	11	17
Herring	1	A	60.28.69	147.46.02	36	70	11	18
Herring	1	B	60.28.61	147.45.98	30	61	11	18
Herring	1	C	60.28.31	147.46.07	28	43	9	19
Herring	2	A	60.28.75	147.45.88	71	93	11	18
Herring	2	B	60.28.61	147.45.80	72	85	11	18
Herring	2	C	60.28.36	147.45.88	73	83	11	19
Herring	2	D	60.28.45	147.45.80	76	80	7	24
Chenega	1	A	60.24.67	147.58.26	32	64	11	17
Chenega	1	B	60.24.61	147.58.16	38	55	11	18
Chenega	1	C	60.22.97	147.59.43	39	54	11	18
Chenega	2	A	60.24.66	147.58.43	73	85	11	18
Chenega	2	B	60.23.20	147.58.84	70	100	11	18
Chenega	2	C	60.23.29	147.58.69	73	94	9	18
Green	1	A	60.17.25	147.31.00	44	68	11	17
Green	1	B	60.16.49	147.32.82	41	55	11	17
Green	1	C	60.16.47	147.32.89	36	49	11	18
Green	1	D	60.16.17	147.33.35	41	50	11	18
Green	2	A	60.18.90	147.32.04	70	80	11	17
Green	2	B	60.16.45	147.33.16	70	96	11	18
Green	2	C	60.16.69	147.33.24	75	80	11	18
Green	2	D	-0- <sup>f</sup>	-0- <sup>f</sup>	72	85	11	18
Snug	1	A	60.14.61	147.40.85	35	52	11	17
Snug	2	A	60.14.51	147.40.33	70	90	11	17
Snug	2	B	60.14.33	147.40.54	71	92	7	17

<sup>a</sup> 1 = shallow (35 - 130 m); 2 = deep (130 - 220 m).

<sup>b</sup> Station letter represents order in which the stations were set, by depth strata.

<sup>c</sup> Latitude and longitude are listed to the one-hundredth of a minute.

<sup>d</sup> Depth is in meters.

<sup>e</sup> Rounded to the nearest hour.

<sup>f</sup> Specific location not recorded.

Table 8. Average weight (kg) and number per pot of Pandalid shrimp captured during the November oil spill impact assessment surveys. Only catches from the first day's set at each site were used for spot shrimp.

Site	Year	Average number of shrimp per pot			Average weight (kg) of shrimp per pot		
		Spot	Pink	Coonstripe	Spot	Pink	Coonstripe
Unakwik	1989	25.2	9.5	15.7	0.638	0.028	0.118
	1990	45.2	8.5	14.1	1.051	0.025	0.111
	1991	27.8	4.6	26.9	0.832	0.016	0.193
Golden	1989	57.9	8.7	10.1	0.832	0.034	0.068
	1990	31.9	11.9	10.8	0.721	0.048	0.079
	1991	9.5	11.8	20.8	0.291	0.050	0.128
Culross	1989	19.7	1.7	6.5	0.262	0.006	0.044
	1990	7.6	4.0	3.6	0.111	0.015	0.021
	1991	4.5	4.2	5.6	0.091	0.017	0.033
Herring	1989	18.6	31.2	2.9	0.280	0.098	0.022
	1990	8.6	43.3	8.1	0.138	0.126	0.044
	1991	33.0	15.9	23.9	0.571	0.055	0.147
Chenega	1989	24.9	17.0	6.1	0.264	0.063	0.045
	1990	28.5	18.2	8.2	0.400	0.062	0.046
	1991	38.0	5.3	14.5	0.701	0.021	0.104
Green*	1989	3.8	7.9	0.1	0.038	0.025	0.001
	1990	1.3	13.3	1.6	0.024	0.062	0.008
	1991	7.1	6.0	1.7	0.130	0.025	0.011
Unoiled	1989	34.3	6.6	10.8	0.578	0.022	0.077
	1990	26.5	7.0	7.6	0.585	0.026	0.054
	1991	14.0	7.1	15.9	0.405	0.029	0.103
Oiled	1989	15.8	18.7	3.0	0.194	0.062	0.023
	1990	15.1	31.7	7.2	0.220	0.098	0.040
	1991	21.3	9.1	10.8	0.387	0.035	0.071

\* Only 33 pots set in the shallow stratum at this site in November 1990.

Table 9. Statistics from unbalanced ANOVAs fit to the CPUE from spot shrimp survey data, collected in November 1989, 1990 and 1991 in Prince William Sound.

Parameter	Year	Sum of Squares	R <sup>2</sup>	F Value	p-value
(Weight (kg) of shrimp)/pot	<u>1989</u>	32.76	0.414	25.82	0.0001
	Oiling	9.72		53.64	0.0001
	Stratum	10.26		56.58	0.0001
	Stratum*Oiling	3.77		20.81	0.0001
	Site(Oiling)	9.01		12.43	0.0001
	<u>1990</u>	44.29	0.510	45.53	0.0001
	Oiling	14.90		107.82	0.0001
	Stratum	3.67		26.53	0.0001
	Stratum*Oiling	3.77		27.25	0.0001
	Site(Oiling)	23.06		41.72	0.0001
	<u>1991</u>	46.29	0.351	27.04	0.0001
	Oiling	0.06		0.28	0.5963
	Stratum	10.39		48.57	0.0001
	Stratum*Oiling	0.62		2.88	0.0906
	Site(Oiling)	34.31		32.07	0.0001
(Number of shrimp)/pot	<u>1989</u>	128,099	0.407	24.18	0.0001
	Oiling	22,283		29.44	0.0001
	Stratum	52,475		69.33	0.0001
	Stratum*Oiling	8,755		11.57	0.0008
	Site(Oiling)	45,653		15.08	0.0001
	<u>1990</u>	80,737	0.398	28.89	0.0001
	Oiling	19,884		49.80	0.0001
	Stratum	8,693		21.77	0.0001
	Stratum*Oiling	4,969		12.45	0.0005
	Site(Oiling)	51,750		32.40	0.0001
	<u>1991</u>	117,224	0.349	26.79	0.0001
	Oiling	4,776		8.73	0.0033
	Stratum	30,165		55.15	0.0001
	Stratum*Oiling	7,674		14.03	0.0002
	Site(Oiling)	70,102		25.63	0.0001

-Continued-

Table 9 (continued) page 2 of 2

Parameter	Year	Sum of Squares	R <sup>2</sup>	F Value	p-value
((Number of shrimp)/(kg)/pot	1989	106,539	0.508	32.47	0.0001
	Oiling	17,243		36.79	0.0001
	Stratum	0.01		0.00	0.9967
	Stratum*Oiling	3,859		8.23	0.0045
	Site(Oiling)	82,206		43.85	0.0001
	1990	82,670	0.215	10.71	0.0001
	Oiling	25,248		22.90	0.0001
	Stratum	62		0.06	0.8123
	Stratum*Oiling	8,716		7.91	0.0053
	Site(Oiling)	47,111		10.68	0.0001
	1991	37,597	0.276	16.21	0.0001
	Oiling	10,638		36.70	0.0001
	Stratum	2,576		8.89	0.0031
	Stratum*Oiling	5,129		17.69	0.0001
	Site(Oiling)	1,711		5.91	0.0001

Table 10. Statistical comparison of CPUE, from spot shrimp survey, between oiled and unoiled areas for each year, using the least square means (lsm) from an ANOVA analysis.

Parameter	Year	Unoiled lsm	Oiled lsm	p-value
(Weight (kg) of shrimp)/pot	1989	0.578	0.194	0.0001
	1990	0.653	0.188	0.0001
	1991	0.412	0.387	0.5963
(Number of shrimp)/pot	1989	34.63	15.91	0.0001
	1990	29.31	12.33	0.0001
	1991	14.19	21.35	0.0033
((Number of shrimp)/(Weight(kg))/pot	1989	68.44	87.51	0.0001
	1990	54.76	75.74	0.0001
	1991	38.45	50.77	0.0001

Table 11. Statistical comparison of CPUE, from spot shrimp survey, between oiled and unoiled depth stratum for each year, using the least square means (lsm) from an ANOVA analysis.

Parameter	Year	Depth Stratum	Unoiled lsm	Oiled lsm	p-value
(Weight (kg) of shrimp)/pot	1989	Shallow	0.894	0.271	0.0001
	1989	Deep	0.261	0.116	0.0519
	1990	Shallow	0.885	0.186	0.0001
	1990	Deep	0.420	0.189	0.0001
	1991	Shallow	0.535	0.588	0.4410
	1991	Deep	0.290	0.186	0.0001
(Number of shrimp)/pot	1989	Shallow	54.86	24.40	0.0001
	1989	Deep	14.40	7.41	0.1561
	1990	Shallow	39.18	13.70	0.0001
	1990	Deep	19.44	10.95	0.0193
	1991	Shallow	18.50	34.44	0.0001
	1991	Deep	9.88	8.27	0.6200
((Number of shrimp)/(Weight(kg))/pot)	1989	Shallow	64.09	91.88	0.0001
	1989	Deep	72.80	83.14	0.0322
	1990	Shallow	48.42	81.10	0.0001
	1990	Deep	61.11	70.38	0.1444
	1991	Shallow	37.31	57.47	0.0001
	1991	Deep	39.60	44.08	0.0919

Table 12. Statistics from unbalanced ANOVAs fit to the CPUE from spot shrimp survey data for different oiling conditions, collected in November 1989, 1990 and 1991 in Prince William Sound.

Parameter	Oiling	Sum of Squares	R <sup>2</sup>	F value	p-value
(Weight (kg) of shrimp)/pot	<u>Unoiled</u>	71.92	0.392	32.33	0.0001
	Year	4.63		9.37	0.0001
	Stratum	20.72		83.84	0.0001
	Site	35.69		72.21	0.0001
	Year*Site	7.55		7.64	0.0001
	<u>Oiled</u>	32.01	0.316	26.48	0.0001
	Year	10.63		39.56	0.0001
	Stratum	6.27		46.72	0.0001
	Site	14.83		55.19	0.0001
	Year*Site	2.72		5.07	0.0005
(Number of shrimp)/pot	<u>Unoiled</u>	175,712	0.382	30.86	0.0001
	Year	34,307		27.12	0.0001
	Stratum	51,307		81.10	0.0001
	Site	49,984		39.51	0.0001
	Year*Site	35,718		14.12	0.0001
	<u>Oiled</u>	121,481	0.307	25.03	0.0001
	Year	23,545		21.83	0.0001
	Stratum	36,610		67.90	0.0001
	Site	66,039		61.24	0.0001
	Year*Site	7,116		3.30	0.0110
((Number of shrimp)/(Weight(kg))/pot	<u>Unoiled</u>	183,415	0.493	44.64	0.0001
	Year	63,771		69.84	0.0001
	Stratum	6,122		13.41	0.0003
	Site	89,334		97.83	0.0001
	Year*Site	26,712		14.63	0.0001
	<u>Oiled</u>	121,888	0.277	18.08	0.0001
	Year	76,836		51.29	0.0001
	Stratum	14,409		19.24	0.0001
	Site	13,946		9.31	0.0001
	Year*Site	17,731		5.92	0.0001

Table 13. Statistical comparison of CPUE, from the spot shrimp survey in Prince William Sound, between years for the two oiling strata, using the least square means (lsm) from an ANOVA analysis.

Parameter	Oiling	1989	1990	1991	p-value
(Weight (kg) of shrimp)/pot	Unoiled	0.578	0.651		0.2217
	Unoiled		0.651	0.419	0.0001
	Oiled	0.194	0.150		0.3171
	Oiled		0.150	0.470	0.0001
(Number of shrimp)/pot	Unoiled	34.63	29.40		0.0854
	Unoiled		29.40	14.61	0.0001
	Oiled	15.90	9.88		0.0345
	Oiled		9.88	26.19	0.0001
((Number of shrimp)/(Weight(kg)))/pot	Unoiled	68.44	55.01		0.0001
	Unoiled		55.01	38.20	0.0001
	Oiled	86.93	75.56		0.0047
	Oiled		75.56	53.02	0.0001

Table 14. Statistical comparison of CPUE, from the spot shrimp survey in Prince William Sound, between years for site of the two oiling strata, using the least square means (lsm) from an ANOVA analysis.

Parameter	Oiling	Sites	1989	1990	1991	p-value
(Weight (kg) of shrimp)/pot	Unoiled	Unakwik	0.638	1.030		0.0003
	Unoiled	Unakwik		1.030	0.832	0.0502
	Unoiled	Golden	0.832	0.768		0.5259
	Unoiled	Golden		0.768	0.333	0.0001
	Unoiled	Culross	0.262	0.154		0.2834
	Unoiled	Culross		0.154	0.091	0.4878
	Oiled	Herring	0.279	0.138		0.0478
	Oiled	Herring		0.138	0.590	0.0001
	Oiled	Chenega	0.264	0.400		0.0584
	Oiled	Chenega		0.400	0.697	0.0001
	Oiled	Green	0.038	0.000		0.1418
	Oiled	Green		0.000	0.121	0.0050
(Number of shrimp)/pot	Unoiled	Unakwik	25.57	44.19		0.0008
	Unoiled	Unakwik		44.19	27.79	0.0014
	Unoiled	Golden	57.87	34.27		0.0001
	Unoiled	Golden		34.27	11.58	0.0001
	Unoiled	Culross	20.46	9.73		0.0369
	Unoiled	Culross		9.73	4.47	0.2565
	Oiled	Herring	19.28	8.58		0.0190
	Oiled	Herring		8.58	34.50	0.0001
	Oiled	Chenega	24.91	28.50		0.4279
	Oiled	Chenega		28.50	37.74	0.0237
	Oiled	Green	3.51	0.00		0.0515
	Oiled	Green		0.00	6.32	0.0038
((Number of shrimp)/(Weight(kg))/pot)	Unoiled	Unakwik	40.59	41.22		0.8936
	Unoiled	Unakwik		41.22	35.16	0.1626
	Unoiled	Golden	68.58	44.72		0.0001
	Unoiled	Golden		44.72	32.30	0.0036
	Unoiled	Culross	96.13	79.08		0.0002
	Unoiled	Culross		79.08	47.14	0.0001
	Oiled	Herring	68.37	66.16		0.6930
	Oiled	Herring		66.16	55.78	0.0505
	Oiled	Chenega	91.02	71.64		0.0004
	Oiled	Chenega		71.64	51.29	0.0001
	Oiled	Green	101.4	88.87		0.1705
	Oiled	Green		88.87	51.99	0.0001

Table 15. Parameters for a von Bertalanffy growth curve, for Prince William Sound spot shrimp with estimated carapace lengths for specific ages.

Parameter	Unoiled Area				Oiled Area			
	Unakwik Inlet		Culross Passage		Herring Bay		Chenega Island	
Growth Parameter ( <i>k</i> )*	0.080 (0.16)		0.067 (0.13)		0.080 (0.16)		0.080 (0.16)	
Maximum Carapace Length ( <i>L</i> <sub>∞</sub> , in mm)	57.0		57.8		55.2		55.8	
	<u>Age</u>	<u>Length</u>	<u>Age</u>	<u>Length</u>	<u>Age</u>	<u>Length</u>	<u>Age</u>	<u>Length</u>
Age (in years) and Estimated Length (in mm)	2.8	20.3	3.2	20.3	2.7	19.4	2.4	17.7
	3.3	23.1	3.7	22.7	3.2	22.3	2.9	20.7
	3.8	25.7	4.2	25.0	3.7	24.8	3.4	23.4
	4.3	28.1	4.7	27.1	4.2	27.2	3.9	25.9
	4.8	30.4	5.2	29.1	4.7	29.3	4.4	28.2
	5.3	32.4	5.7	30.9	5.2	31.3	4.9	30.3
	5.8	34.3	6.2	32.6	5.7	33.2	5.4	32.2
	6.3	36.0	6.7	34.3	6.2	34.8	5.9	34.0
	6.8	37.6	7.2	35.8	6.7	36.4	6.4	35.7
	7.3	39.1	7.7	37.2	7.2	37.9	6.9	37.3
	7.8	40.5	8.2	38.5	7.7	39.2	7.4	38.7
	8.3	41.8	8.7	39.8	8.2	40.4	7.9	40.0
	8.8	42.9	9.2	40.9	8.7	41.6	8.4	41.2
	9.3	44.0	9.7	42.0	9.2	42.6	8.9	42.4
	9.8	45.0	10.2	43.0	9.7	43.6	9.4	43.4
<b>SITES COMBINED</b>								
Growth Parameter ( <i>k</i> )*	0.080 (0.16)				0.080 (0.16)			
Maximum Carapace Length ( <i>L</i> <sub>∞</sub> , in mm)	57.4				54.9			
	<u>Age</u>	<u>Length</u>	<u>Age</u>	<u>Length</u>	<u>Age</u>	<u>Length</u>	<u>Age</u>	<u>Length</u>
Age (in years) and Estimated Length (in mm)	2.8	20.7	2.6	18.6	3.3	23.6	3.1	21.4
	3.8	26.2	3.6	24.0	4.3	28.6	4.1	26.3
	4.8	30.8	4.6	28.5	5.3	32.8	5.1	30.6
	5.8	34.7	5.6	32.4	6.3	36.5	6.1	34.2
	6.8	38.1	6.6	35.8	7.3	39.6	7.1	37.2
	7.8	40.9	7.6	38.6	8.3	42.2	8.1	39.9
	8.8	43.4	8.6	41.0	9.3	44.4	9.1	42.0
	9.8	45.4	9.6	43.1				

\* The first value provided for the growth parameter is in half-year increments and the value in parenthesis is for the parameter in yearly increments.

Table 16. Spot shrimp sex ratio and average catch by sex within Prince William Sound, from the November spot shrimp surveys in 1989, 1990 and 1991.

Site	Year	Percent of Population		Average Number/pot	
		Male	Female	Male	Female
Unakwik	1989	83.9	16.1	21.27	4.08
	1990	85.4	14.5	37.82	6.44
	1991	78.1	21.9	21.71	6.08
Golden	1989	96.4	3.6	55.78	2.09
	1990	89.2	10.8	30.41	3.69
	1991	77.6	22.4	8.01	2.30
Culross	1989	96.0	4.0	19.86	0.82
	1990	93.8	6.2	8.98	0.59
	1991	98.1	1.9	4.38	0.08
Herring	1989	96.5	3.5	18.61	0.67
	1990	96.6	3.4	8.28	0.29
	1991	96.9	3.1	34.15	1.10
Chenega	1989	98.9	1.1	24.64	0.28
	1990	98.5	1.5	28.06	0.44
	1991	97.7	2.3	36.76	0.85
Green	1989	100.0	0.0	3.53	0.00
	1990	96.9	3.1	1.23	0.04
	1991	97.8	2.2	6.44	0.15
Unoiled	1989	92.7	7.3	31.78	2.50
	1990	88.7	11.3	23.50	3.00
	1990	83.6	16.4	11.71	2.29
Oiled	1989	98.0	2.0	15.49	0.31
	1990	97.5	2.5	14.72	0.38
	1991	97.4	2.6	20.75	0.55

**Table 17.** Statistics from unbalanced ANOVAs fit for each year and oiling conditions, to the square root of the number of females per pot from spot shrimp survey data, collected in November 1989, 1990 and 1991 in Prince William Sound.

Parameter	Year	Sum of Squares	R <sup>2</sup>	F Value	p-value
(Number of Females) <sup>1/2</sup> /pot	<u>1989</u>	35.02	0.327	17.78	0.0001
	Oiling	14.41		51.22	0.0001
	Stratum	1.97		7.01	0.0086
	Stratum*Oiling	1.94		6.91	0.0091
	Site(Oiling)	16.69		14.83	0.0001
	<u>1990</u>	117.64	0.556	54.83	0.0001
	Oiling	49.54		161.62	0.0001
	Stratum	5.80		18.93	0.0001
	Stratum*Oiling	7.25		23.67	0.0001
	Site(Oiling)	56.70		46.24	0.0001
	<u>1991</u>	104.03	0.422	38.84	0.0001
	Oiling	22.02		57.56	0.0001
	Stratum	10.96		28.56	0.0001
	Stratum*Oiling	2.09		5.48	0.0198
	Site(Oiling)	67.69		44.24	0.0001
Parameter	Oiling	Sum of Squares	R <sup>2</sup>	F Value	p-value
(Number of Females) <sup>1/2</sup> /pot	<u>Unoiled</u>	174.98	0.410	34.90	0.0001
	Year	10.79		9.69	0.0001
	Stratum	27.01		48.49	0.0001
	Site	116.49		104.54	0.0001
	Year*Site	10.71		4.81	0.0008
	<u>Oiled</u>	7.38	0.110	6.75	0.0001
	Year	1.81		7.42	0.0007
	Stratum	0.62		5.06	0.0249
	Site	4.81		19.77	0.0001
	Year*Site	0.57		1.17	0.3236

Table 18. Statistical comparison for the square root of the number of females per pot, between oiled and unoiled areas for each year, followed by the comparison between years for the two oiling strata, using the least square means (lsm) in both cases, from the ANOVA analysis. The data is from the spot shrimp survey of Prince William Sound.

Parameter	Year	Stratum	Unoiled lsm	Oiled lsm	p-value
(Number of Females) <sup>1/2</sup> /pot	1989		1.292	0.825	0.0001
	1989	Shallow	1.465	0.826	0.0002
	1989	Deep	1.120	0.824	0.0015
	1990		1.687	0.840	0.0001
	1990	Shallow	1.995	0.822	0.0001
	1990	Deep	1.380	0.857	0.0001
	1991		1.438	0.952	0.0001
	1991	Shallow	1.683	1.048	0.0001
	1991	Deep	1.193	0.855	0.0002
Parameter	Oiling	1989	1990	1991	
		lsm	lsm	lsm	p-value
		Unoiled	1.292	1.681	0.0001
		<u>Unoiled</u>	1.681	1.438	0.0037
		Unakwik	1.766	2.399	0.0001
		Unakwik	2.399	2.199	0.1864
		Golden	1.137	1.787	0.0001
		Golden	1.787	1.328	0.0014
		Culross	0.975	0.855	0.4287
		Culross	0.855	0.787	0.6220
		Oiled	0.825	0.822	0.9351
		<u>Oiled</u>	0.822	0.950	0.0010
		Herring	0.953	0.880	0.2830
		Herring	0.880	1.062	0.0049
		Chenega	0.815	0.899	0.2193
		Chenega	0.899	1.027	0.0370
		Green	0.707	0.686	0.7996
		Green	0.686	0.760	0.3164

Table 19. Prince William Sound spot shrimp egg count information for each site, from the November surveys only.

Site	Year	Number of Samples	Minimum Number of Eggs Per Female	Maximum Number of Eggs Per Female	Average Number of Eggs per Female
Unakwik	1989	91	826	3,297	2,165
	1990	98	417	3,326	1,979
	1991	112	228	4,696	1,784
Golden	1989	43	910	3,362	2,369
	1990	85	1,417	4,273	2,527
	1991	124	833	5,076	2,502
Culross	1989	29	1,441	3,965	2,308
	1990	51	1,176	3,734	2,293
	1991	12	1,216	2,811	2,117
Herring	1989	19	343	2,759	1,691
	1990	54	818	3,009	2,036
	1991	49	144	2,876	1,862
Chenega	1989	10	1,381	2,298	1,963
	1990	26	1,044	3,120	2,034
	1991	36	897	3,627	1,729
Green*	1989	-	-	-	-
	1990	1	2,581	2,581	2,581
	1991	10	995	3,191	2,140
Unoiled	1989	163	826	3,965	2,244
	1990	234	417	4,273	2,246
	1991	248	228	5,076	2,159
Oiled	1989	29	343	2,759	1,785
	1990	81	818	3,120	2,042
	1991	95	144	3,627	1,841

\* All females caught at the Green Island site were used for hydrocarbon analysis in 1989, and only one was left for fecundity analysis in 1990.

Table 20. Statistics from unbalanced ANOVAs fit for each year and oiling conditions, to the number of eggs per female from spot shrimp survey data, collected in November 1989, 1990 and 1991 in Prince William Sound.

Parameter	Year	Sum of Squares	R <sup>2</sup>	F Value	p-value
(# of Eggs)/Female	<u>1989</u>	26,103,328	0.490	35.76	0.0001
Oiling		621,877		4.26	0.0404
Site(Oiling)		1,197,661		2.73	0.0450
Carapace Length		19,076,037		130.66	0.0001
	<u>1990</u>	48,510,858	0.434	39.44	0.0001
Oiling		140,803		0.69	0.4079
Site(Oiling)		4,053,261		4.94	0.0007
Carapace Length		31,901,276		155.60	0.0001
	<u>1991</u>	109,428,680	0.608	74.36	0.0001
Oiling		12,663		0.06	0.8063
Site(Oiling)		6,595,936		6.28	0.0001
Carapace Length		70,708,620		336.36	0.0001
Parameter	Oiling	Sum of Squares	R <sup>2</sup>	F Value	p-value
(# of Eggs)/Females	<u>Unoiled</u>	144,039,080	0.522	77.20	0.0001
	Year	1,872,261		4.52	0.0113
	Site	3,381,483		8.16	0.0003
	Year*Site	3,887,926		4.69	0.0010
	Carapace Length	97,353,122		469.58	0.0001
	<u>Oiled*</u>	27,906,669	0.482	26.33	0.0001
	Year	861,908		8.72	0.0002
	Site	376,703		0.70	0.4037
	Year*Site	2,637,719		8.32	0.0003
	Carapace Length	23,440,598		135.21	0.0001

- \* The Green Island site was not used in the analysis because no females were available for fecundity analysis in 1989 and only one female was available for analysis in 1990.

Table 21. Statistical comparison between oiled and unoiled areas for each year using the least square means (lsm) from an analysis of covariance and slope from linear regressions.

Parameter	Year	Unoiled	Oiled	p-value
<b>Analysis of Covariance</b>				
(Number of Eggs)/Female (lsm)	1989	2208.0	2031.9	0.0404
	1990	2215.0	2346.7	0.4079
	1991	2094.9	2118.7	0.8063
<b>Linear Regression (<math>E = a + bL</math>)*</b>				
Intercept (a)	1989	-4475.6	-358.5	
Slope (b)		161.64	53.41	0.0010
Significance of Regression (p-value)		0.0001	0.1546	
Intercept (a)	1990	-4142.4	-4078.1	
Slope (b)		152.51	152.13	0.9883
Significance of Regression (p-value)		0.0001	0.0001	
Intercept (a)	1991	-5295.1	-4230.4	
Slope (b)		176.53	150.55	0.8380
Significance of Regression (p-value)		0.0001	0.0001	

\* E is the number of eggs on a female and L is the carapace length of the female.

Table 22. Statistical comparison between years for the two oiling strata, using the least square means (lsm) from an analysis of covariance and slope from linear regressions.

	Oiling	1989	1990	1991	P-value
<b>Analysis of Covariance</b>					
(Number of Eggs)/Female (lsm)	Unoiled	2297.5	2288.3		0.8552
	Unoiled		2288.3	2130.0	0.0058
	Oiled <sup>a</sup>	1868.1	2040.1		0.0553
	Oiled <sup>a</sup>		2040.1	1777.2	0.0001
<b>Linear Regression (<math>E = a + bL</math>)<sup>b</sup></b>					
Slopes (b) <sup>c</sup>	Unoiled	161.64	152.51		0.6450
	Unoiled		152.51	176.53	0.0358
	Oiled	53.41	152.13		0.0067
	Oiled		152.13	150.55	0.2426

<sup>a</sup> The Green Island site was not used in this analysis because no females were available for fecundity analysis in 1989 and only one female was available in 1990.

<sup>b</sup> E is the number of eggs per female and L is the female carapace length.

<sup>c</sup> From regression analysis (Table 15).

Table 23. Statistical comparison of the number of eggs per female, from the spot shrimp survey in Prince William Sound, between years for sites within the two oiling strata, using the least square means (lsm) from an analysis of covariance.

Parameter	Oiling	Sites	1989	1990	1991	p-value
(Number of Eggs)/Female (lsm)	Unoiled	Unakwik	2324.0	2122.5		0.0025
	Unoiled	Unakwik		2122.5	1934.2	0.0029
	Unoiled	Golden	2277.2	2302.2		0.7705
	Unoiled	Golden		2302.2	2259.4	0.5047
	Unoiled	Culross	2291.3	2440.3		0.1609
	Unoiled	Culross		2440.3	2196.4	0.0955
	Oiled	Herring	1652.6	2037.1		0.0003
	Oiled	Herring		2037.1	1913.0	0.1084
	Oiled	Chenega	2083.6	2043.1		0.7808
	Oiled	Chenega		2043.1	1641.5	0.0001

Table 24. Sample size and descriptive statistics used in the statistical comparison of fecundity related parameters between the oiled and unoiled areas.

Parameter	Year	Unoiled	Oiled	p-value
Number of Females with Eggs	1989	222	30	
	1990	458	81	
	1991	407	95	
Number of Females without Eggs	1989	12	7	
	1990	40	16	
	1991	200	20	
(Average Number of Dead Eggs)/Female <sup>a</sup>	1989	6.0	3.5	0.335 <sup>b</sup>
	1990	2.1	0.4	0.152 <sup>b</sup>
	1991	3.0	4.5	0.634 <sup>b</sup>
Number of Females without Eggs not in Breeding Dress	1989	0	3	
	1990	38	16	
	1991	87	19	
Percent Females without Eggs not in Breeding Dress	1989	0.0%	42.8%	0.036
	1990	95.0%	100.0%	0.999
	1991	43.5%	95.0%	0.001

<sup>a</sup> Only females with eggs were used for these averages. The Mann-Whitney test was used to test for significance, averages were provided for reference only.

<sup>b</sup> The p-value is from a Mann-Whitney test using ranked values.

Table 25. Results of the histopathology classifications for inflammatory gill lesions, *Lagenophrys* on gills and melanized cuticular lesions, from the 1989 survey of spot shrimp (Appendix C).

Site	Depth Strata	Inflammatory Gill Lesions, Severity Grade*				<i>Lagenophrys</i> on Gills, Severity Grade*				Melanized Cuticular Lesion Count
		G0	G1	G2	G3	G0	G1	G2	G3	
Unakwik Inlet	Shallow (52A/1-10) <sup>b</sup>	3	0	3	4	4	3	3	0	0
	Deep (52B/1-10) <sup>b</sup>	3	0	4	3	6	4	0	0	0
	Total	6	0	7	7	10	7	3	0	0
Golden	Shallow (52C/1-10) <sup>b</sup>	2	8	1	0	4	5	1	0	0
	Deep (52D/1-10) <sup>b</sup>	0	2	5	3	3	3	4	0	1
	Total	2	10	6	3	7	8	5	0	1
Culross Passage	Shallow (52E/1-10) <sup>b</sup>	0	8	1	0	8	1	1	0	1
	Deep (52F/1-10) <sup>b</sup>	1	2	7	0	2	4	4	0	0
	Total	1	10	8	0	10	5	5	0	1
Herring Bay	Shallow (52G/1-10) <sup>b</sup>	8	2	0	0	6	2	2	0	0
	Deep (52H/1-10) <sup>b</sup>	10	0	0	0	2	3	4	1	0
	Total	18	2	0	0	8	5	6	1	0
Chenega Island	Shallow (52I/1-10) <sup>b</sup>	9	1	0	0	1	6	3	0	2
	Deep (52J/1-10) <sup>b</sup>	1	3	3	3	1	3	6	0	2
	Total	10	4	3	3	2	9	9	0	4
Green Island <sup>c</sup>	Shallow (52K/1-10) <sup>b</sup>	3	4	2	1	0	4	6	0	2
	Deep (52L/1-10) <sup>b</sup>	3	0	2	5	0	2	6	0	2
	Total	6	4	4	6	0	6	12	2	4
Unoiled	Shallow	5	16	5	5	16	9	5	0	1
	Deep	4	4	16	6	11	11	8	0	1
	Total (52A-F/1-10) <sup>b</sup>	9	20	21	11	27	20	13	0	2
Oiled	Shallow	20	7	2	1	7	12	11	0	4
	Deep	14	3	5	8	3	8	16	3	4
	Total (52G-L/1-10) <sup>b</sup>	34	10	7	9	10	20	27	3	8

\* The severity grade G0 is the least severe and G3 is most severe.

<sup>b</sup> Labels as found in the report of Dr. Lightner, Appendix C.

<sup>c</sup> The sample sent from Green Island was comprised of pink shrimp, due to low numbers of spot shrimp.

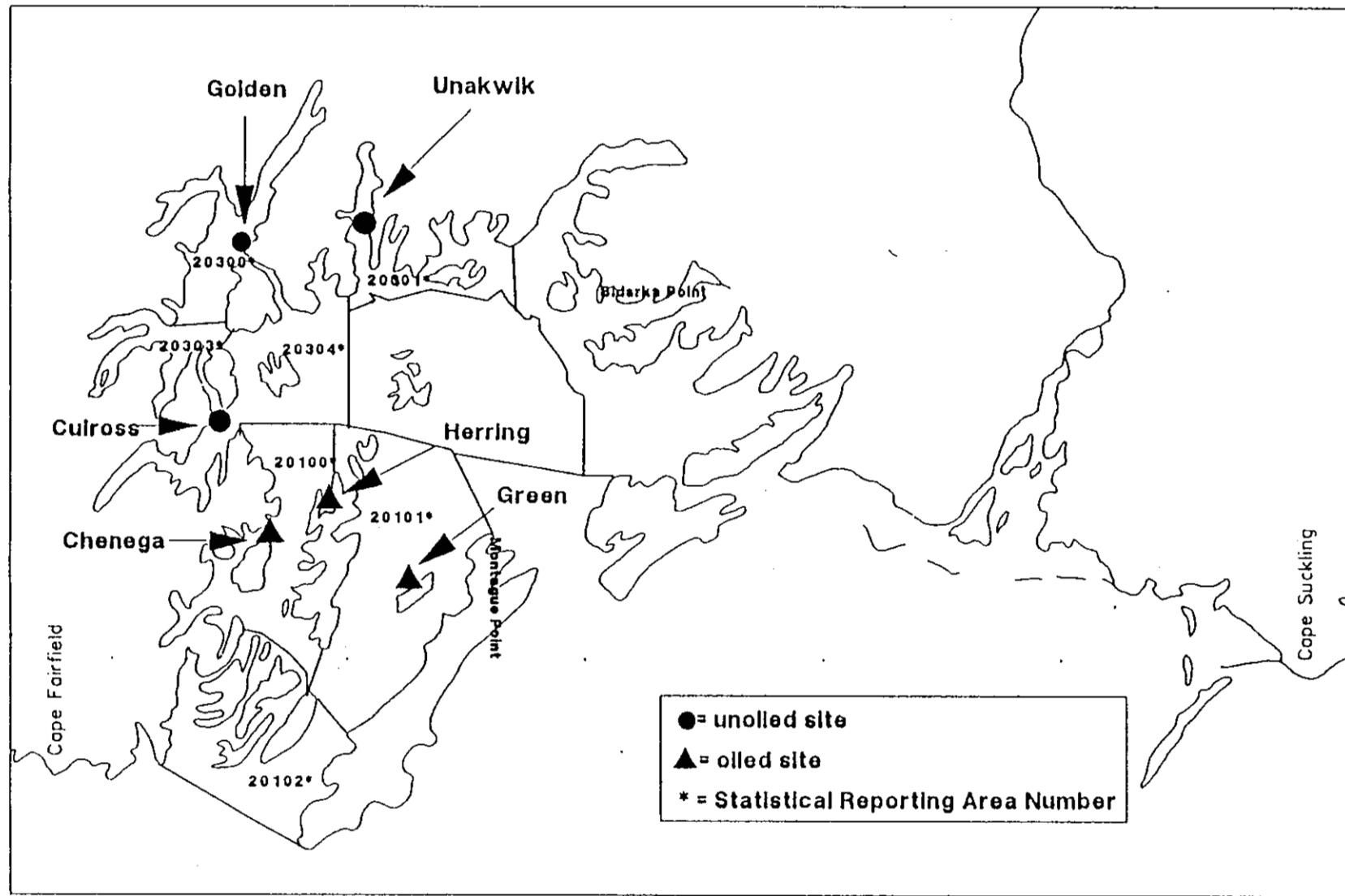


Figure 1. Sampling locations for spot shrimp in Prince William Sound for Subtidal Study 5 and the major statistical areas for reporting commercial shellfish catch.

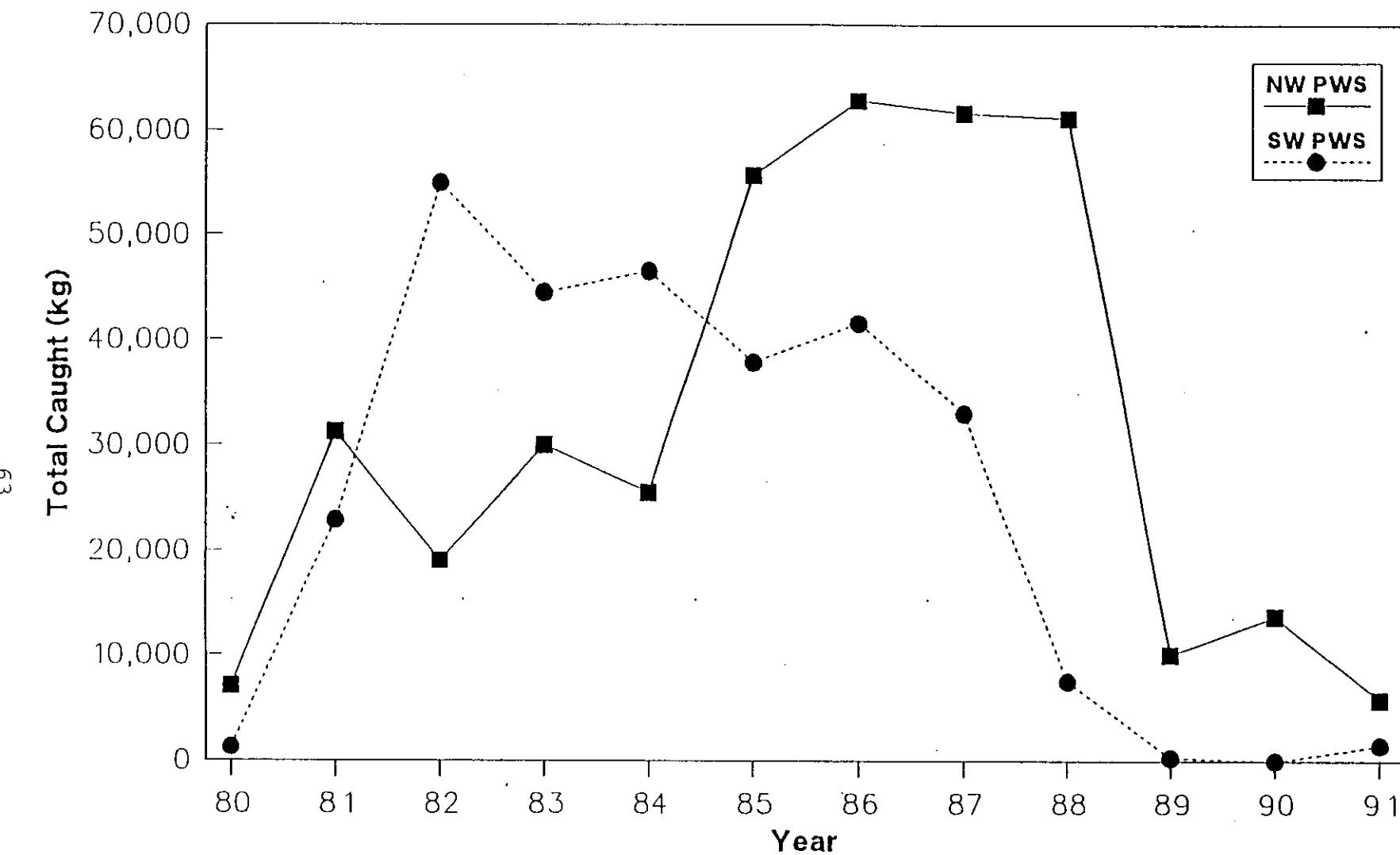


Figure 2. Spot shrimp catch from 1980 to 1991, in the northwest area (statistical reporting areas 20300, 20301, 20303 and 20304) and in the southwest area (statistical reporting areas 20100, 20101 and 20102) of Prince William Sound.

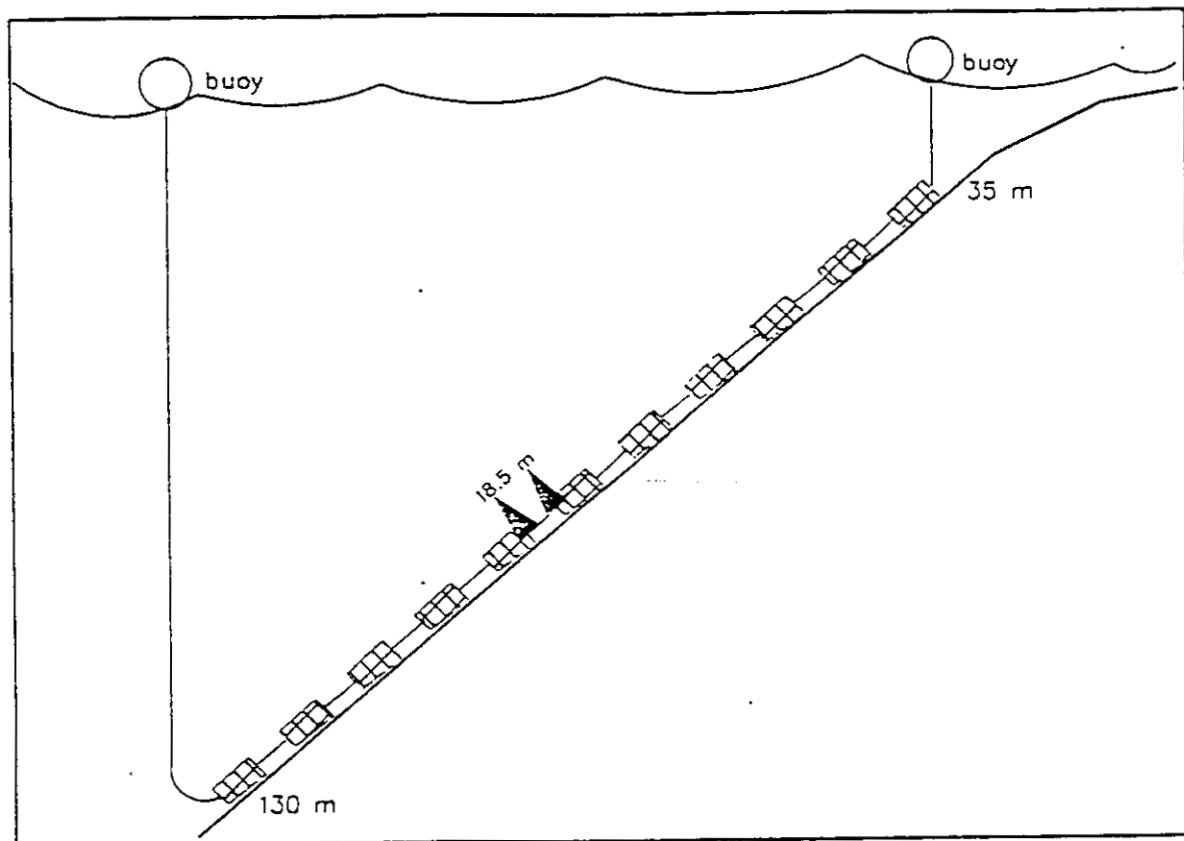


Figure 3. An eleven pot string (station), as used in the spot shrimp survey.

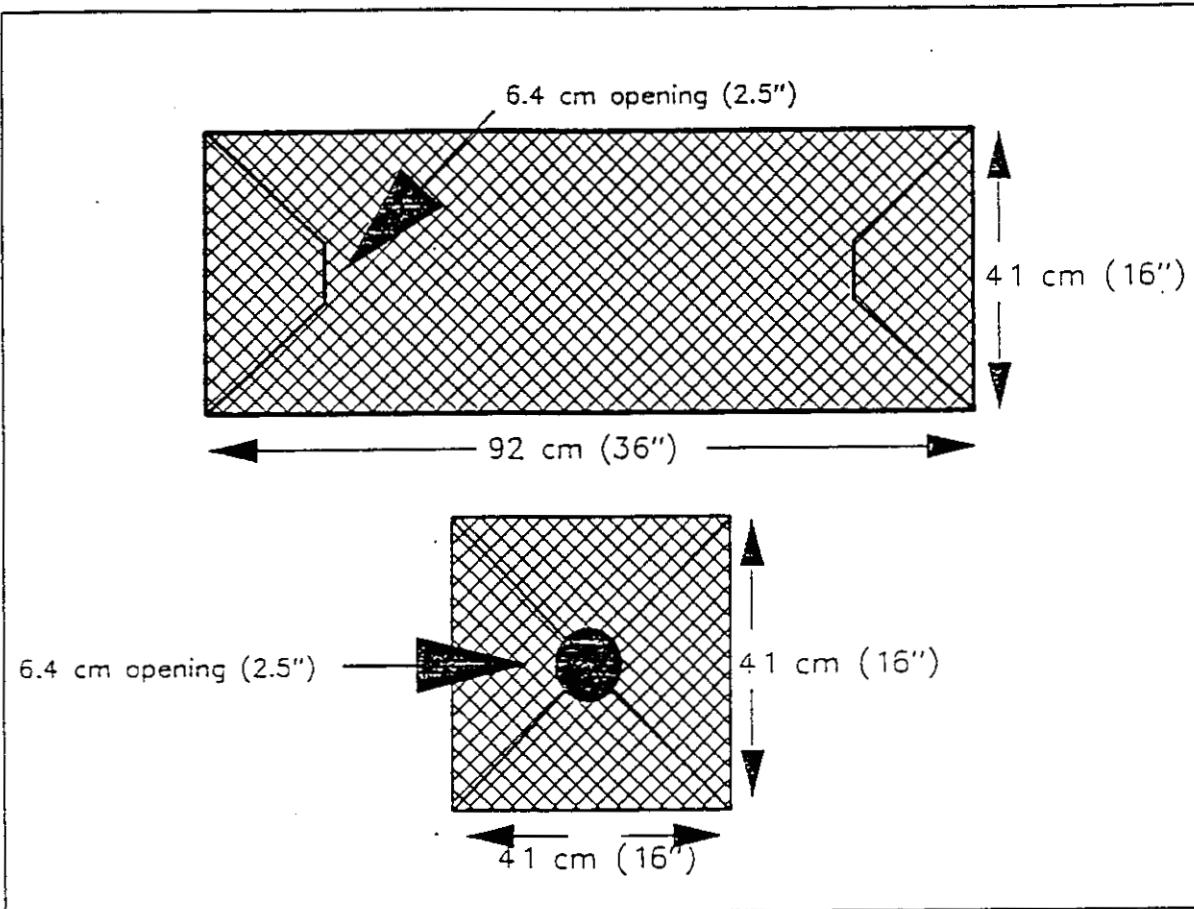


Figure 4. Specifications of the pots used to capture spot shrimp for the spot shrimp surveys.

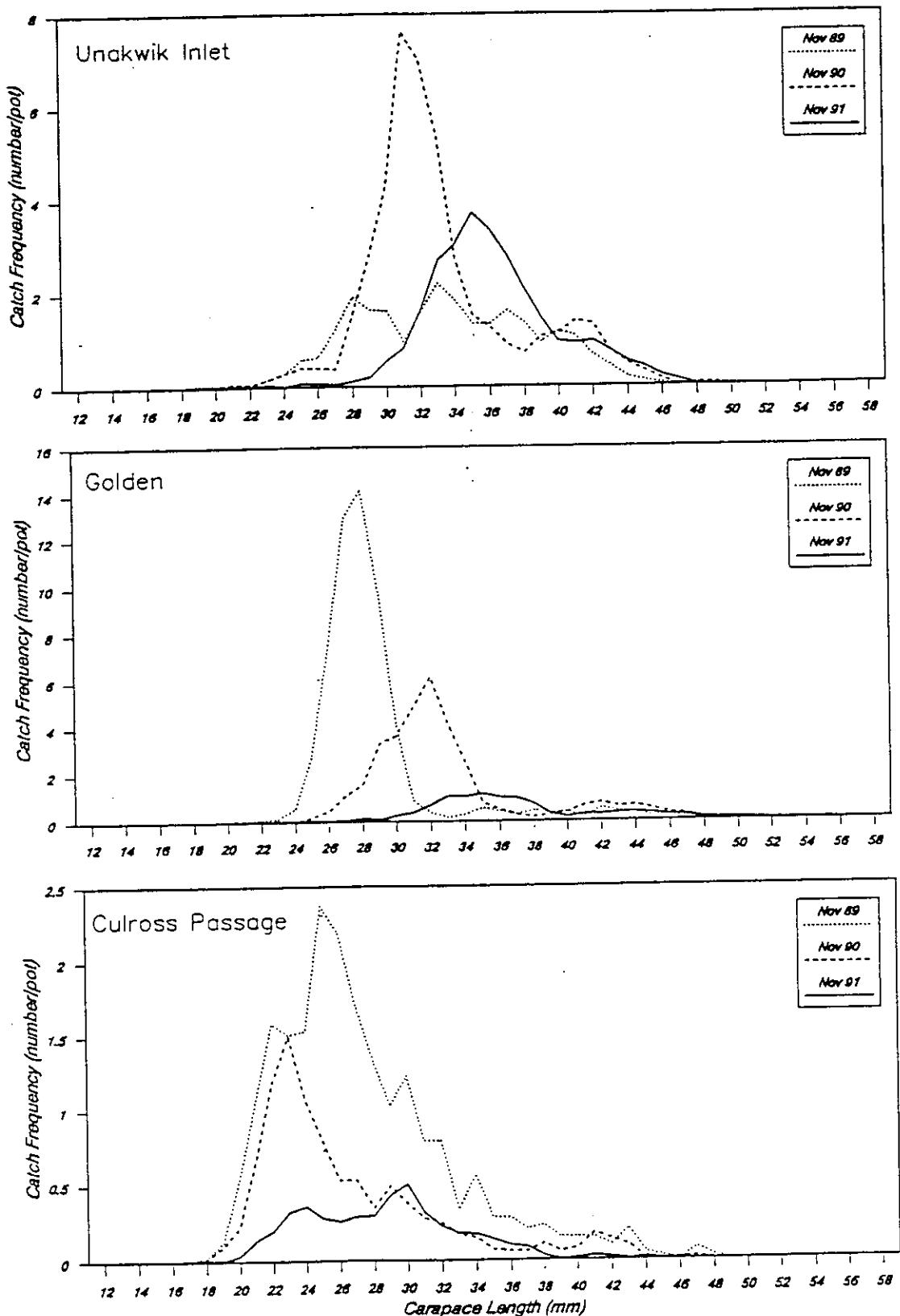


Figure 5. Carapace length frequency of spot shrimp within Prince William Sound from the unoiled sites: Unakwik Inlet (top), Golden (middle) and Culross Passage (bottom), 1989–1991.

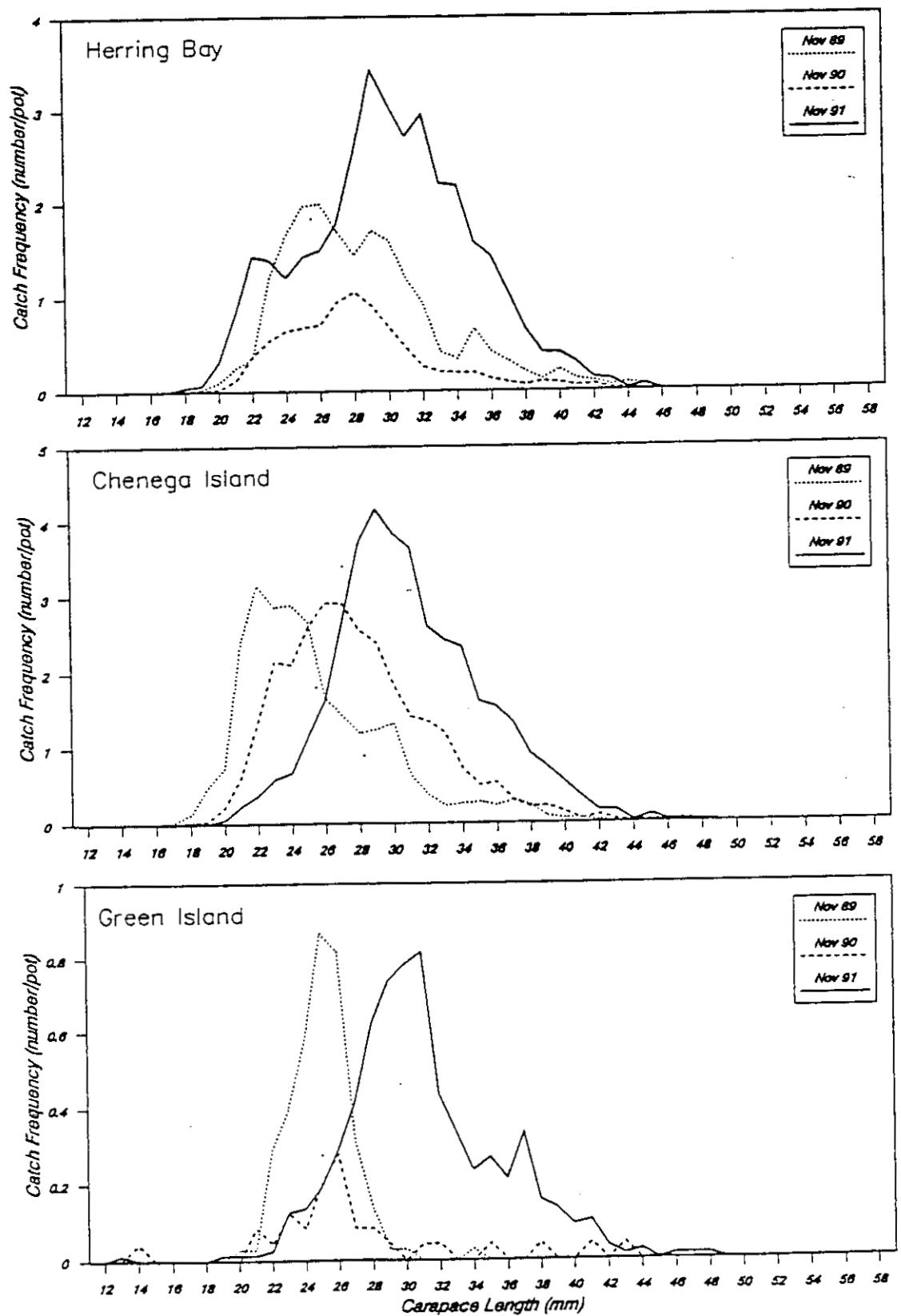


Figure 6. Carapace length frequency of spot shrimp within Prince William Sound from the oiled sites: Herring Bay (top), Chenega Island (middle) and Green Island (bottom), 1989–1991.

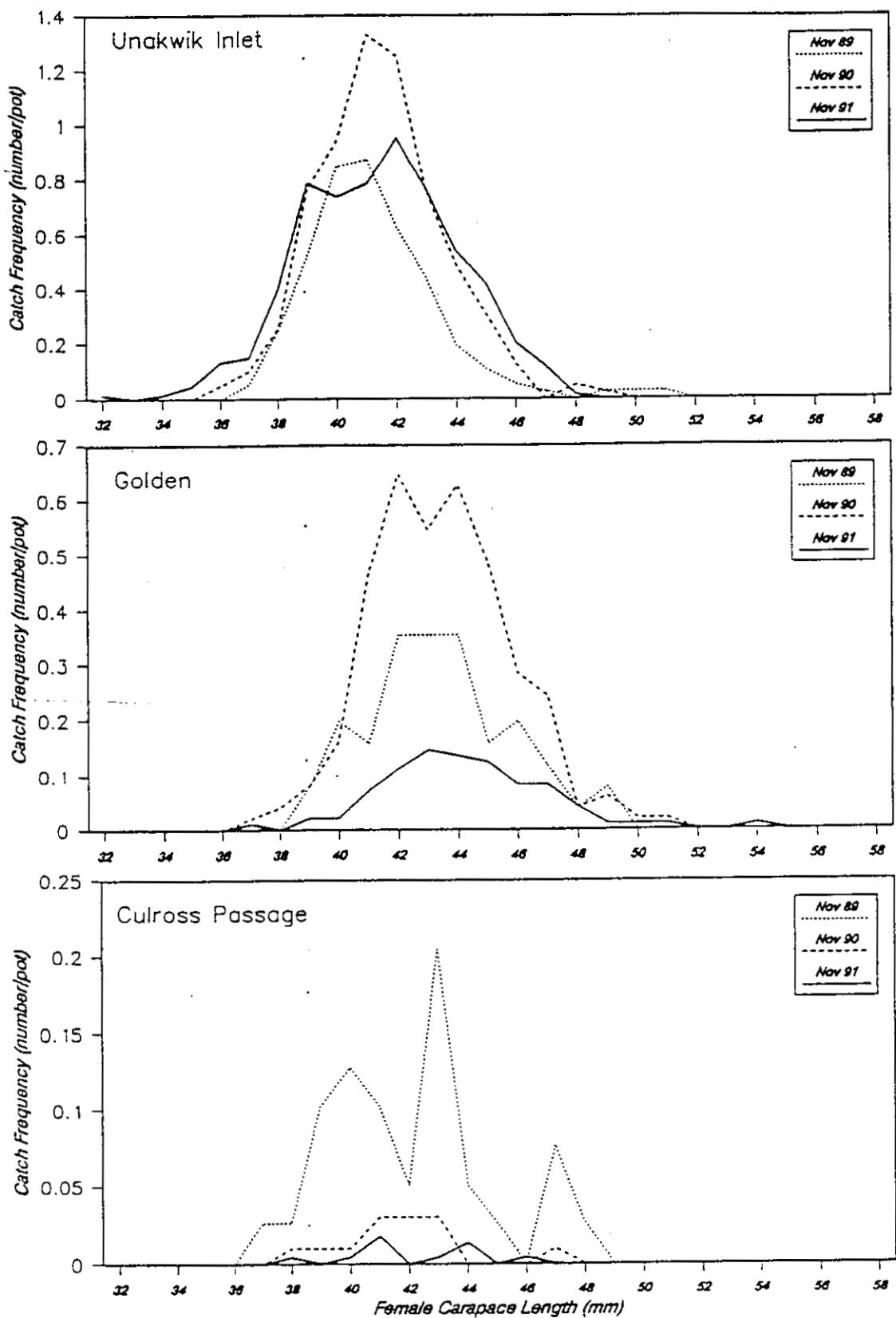


Figure 7. Female spot shrimp length frequency in Prince William Sound, from the unoiled sites: Unakwik Inlet (top), Golden (middle) and Culross Passage (bottom), 1989–1991.

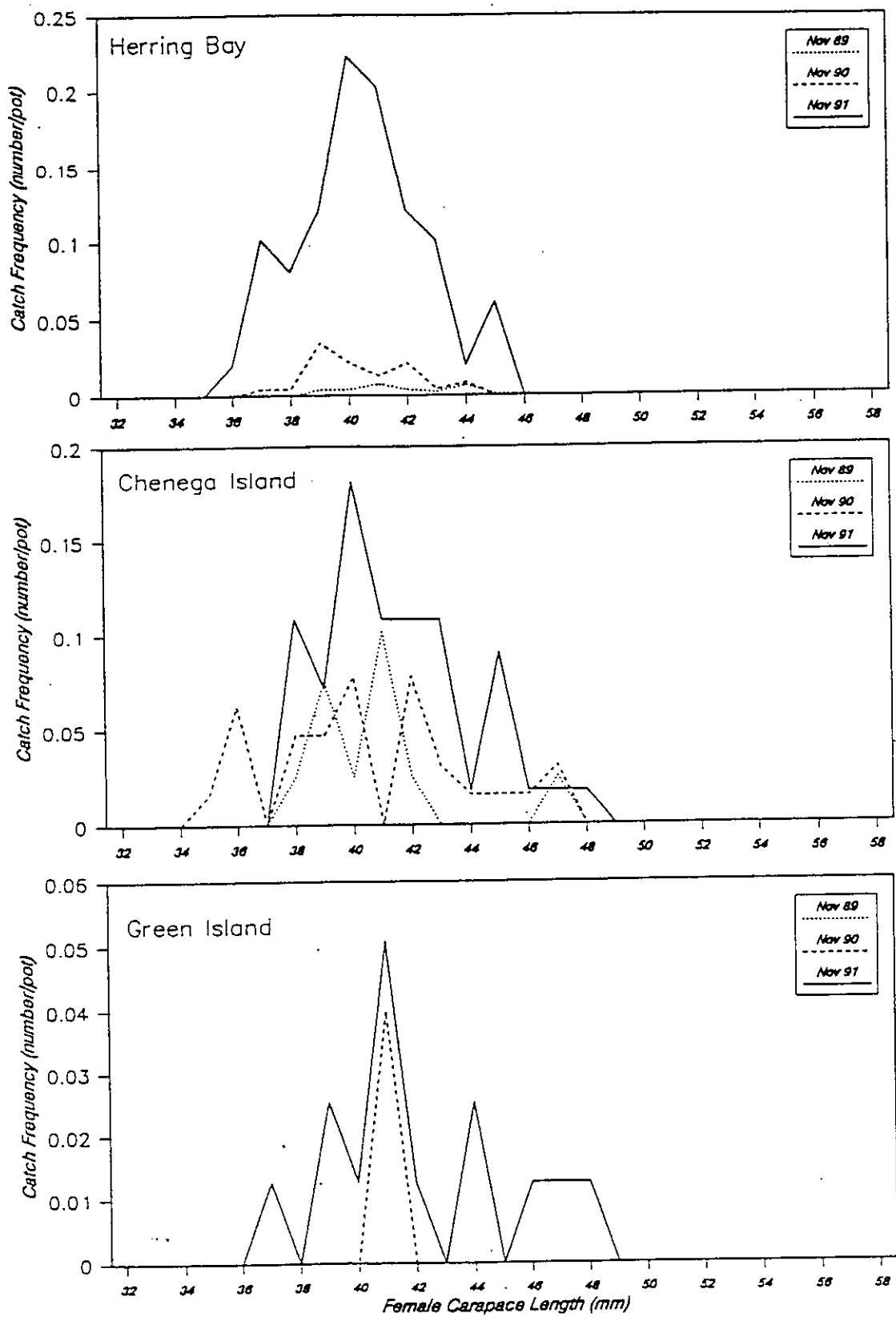


Figure 8. Female spot shrimp length frequency in Prince William Sound, from the oiled sites: Herring Bay (top), Chenega Island (middle) and Green Island (bottom), 1989–1991.

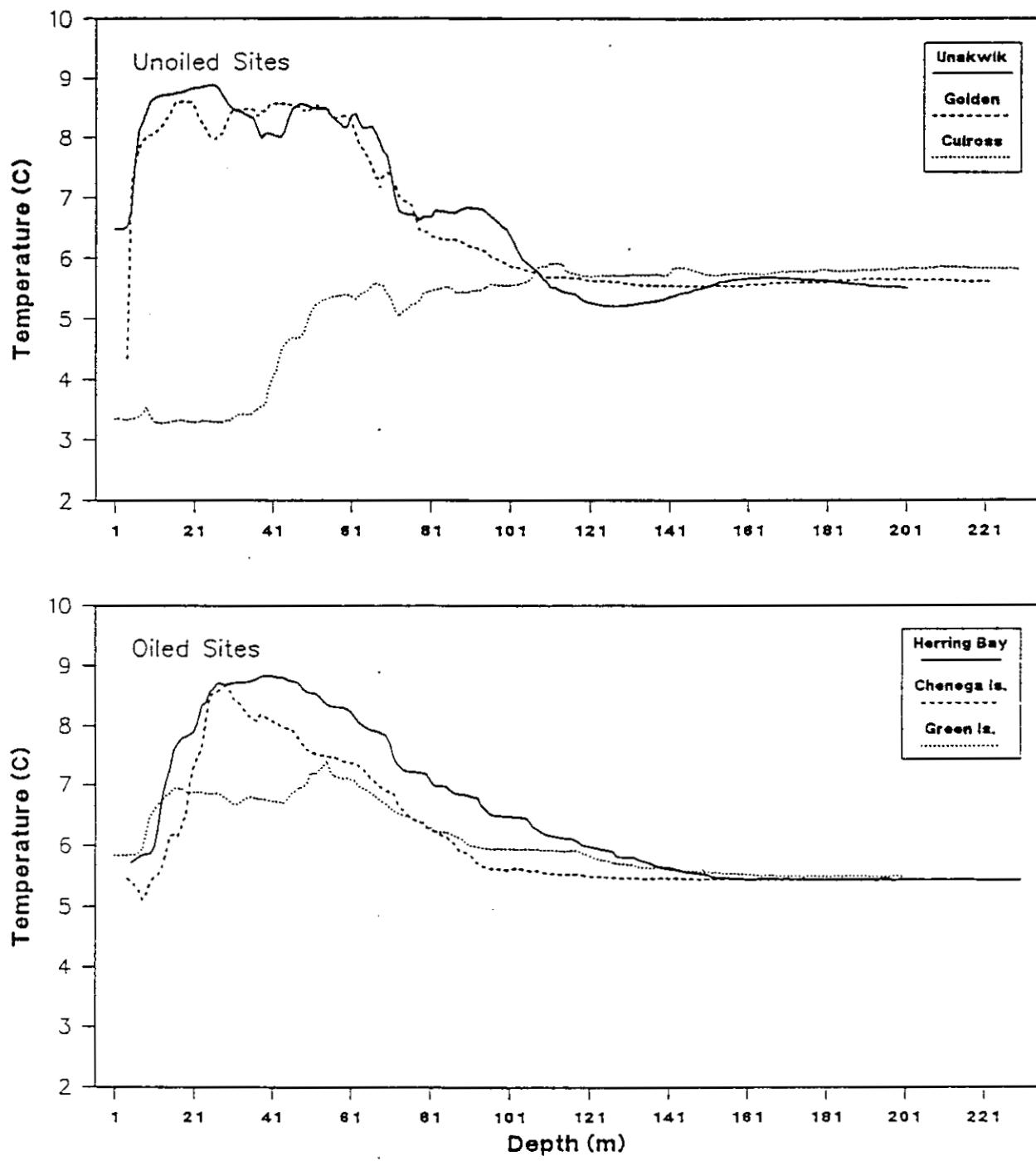


Figure 9. Temperature gradient by depth in November 1989, for unoiled (top) and oiled (bottom) sites, from the Prince William Sound spot shrimp survey.

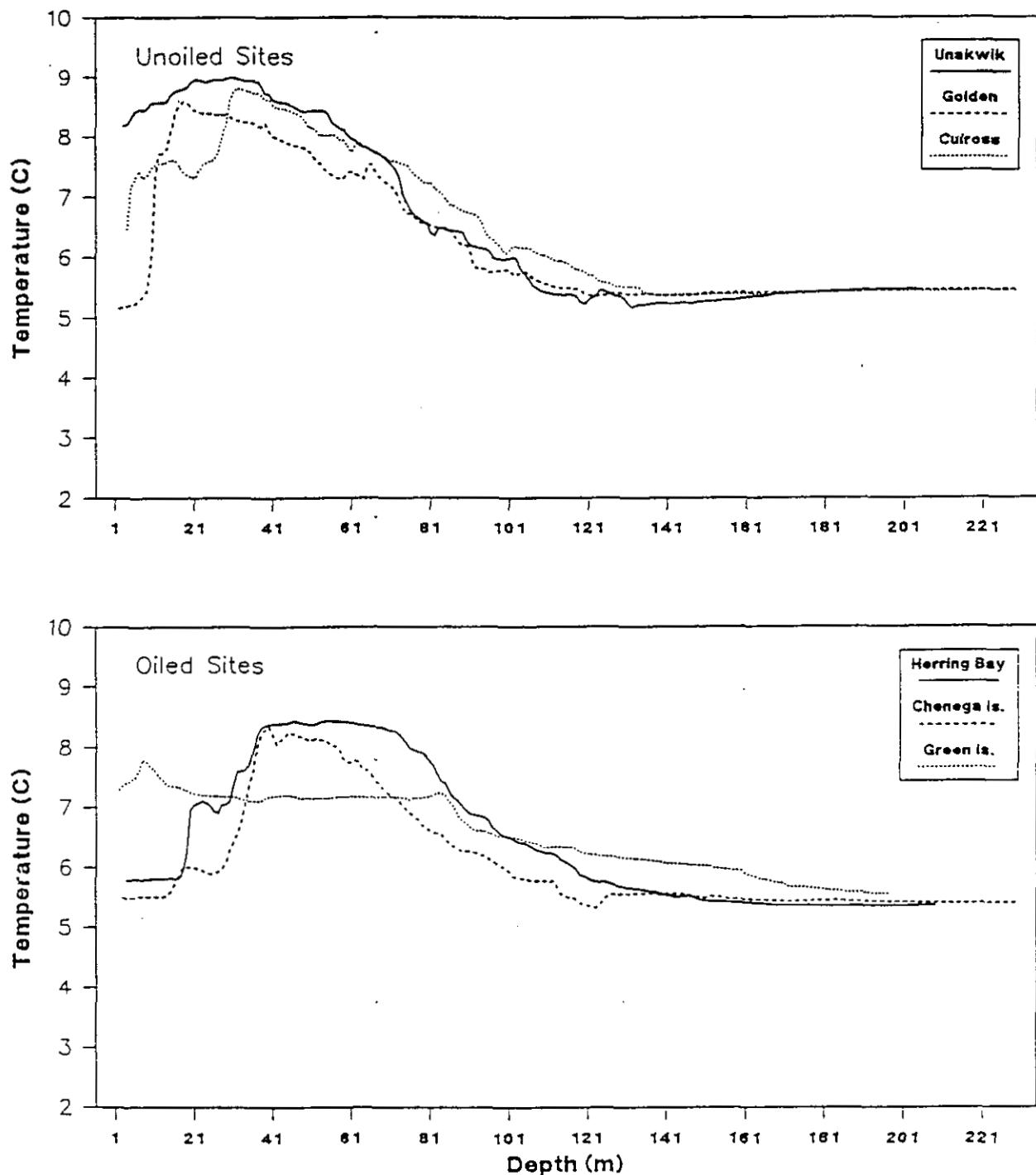


Figure 10. Temperature gradient by depth in November 1990, for unoiled (top) and oiled (bottom) sites, from the Prince William Sound spot shrimp survey.

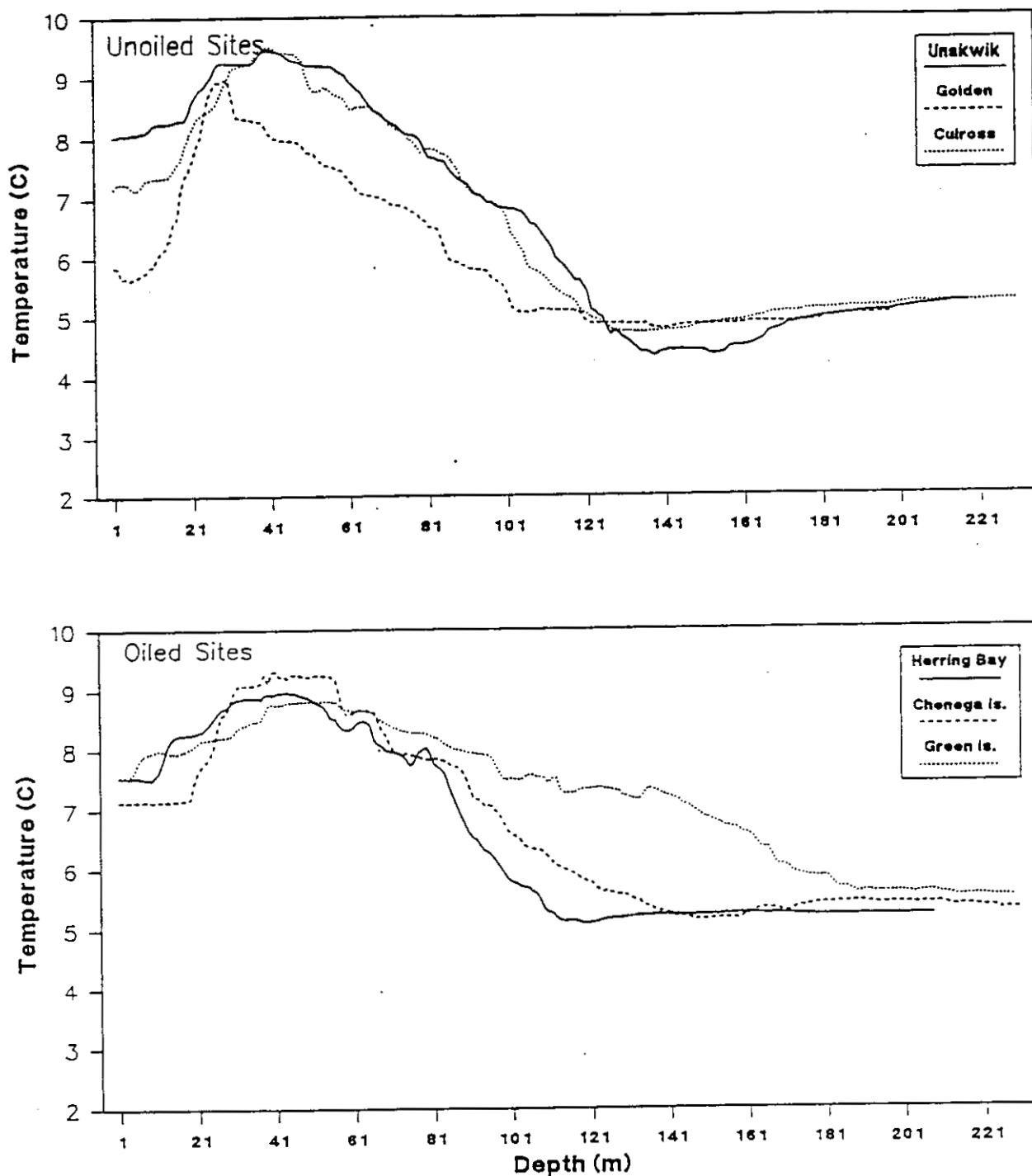


Figure 11. Temperature gradient by depth in November 1991, for unoiled (top) and oiled (bottom) sites, from the Prince William Sound spot shrimp survey.

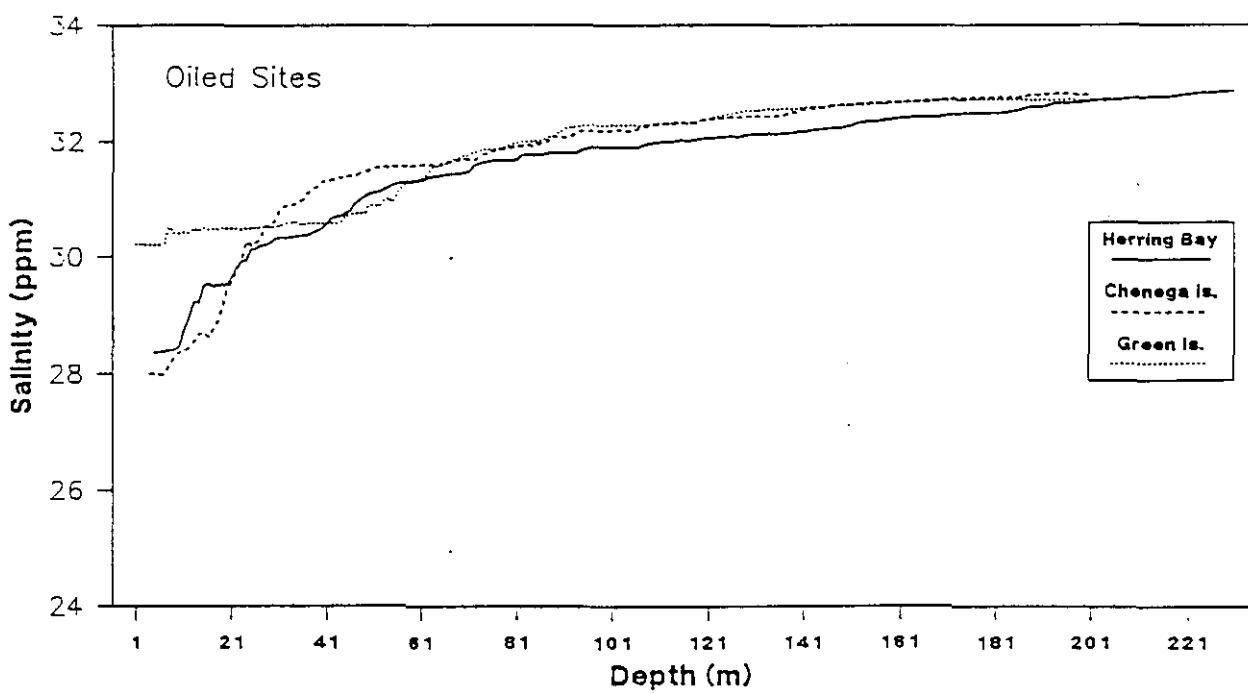
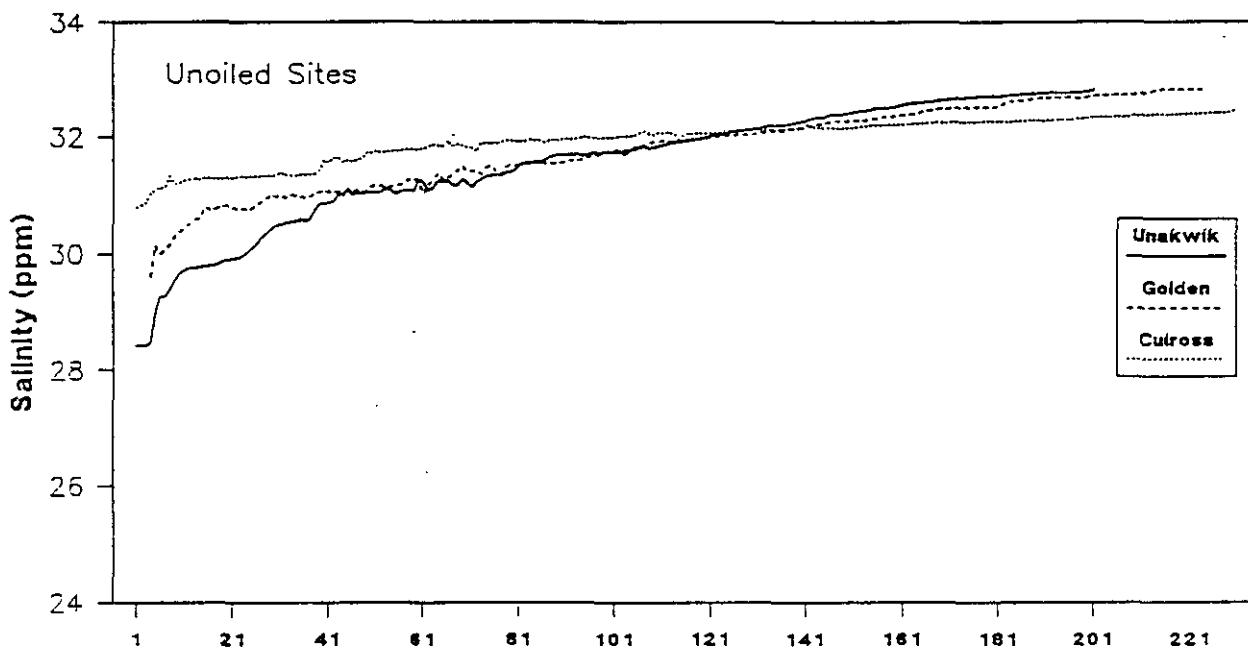


Figure 12. Salinity gradient by depth in November 1989, for unoiled (top) and oiled (bottom) sites, from the Prince William Sound spot shrimp survey.

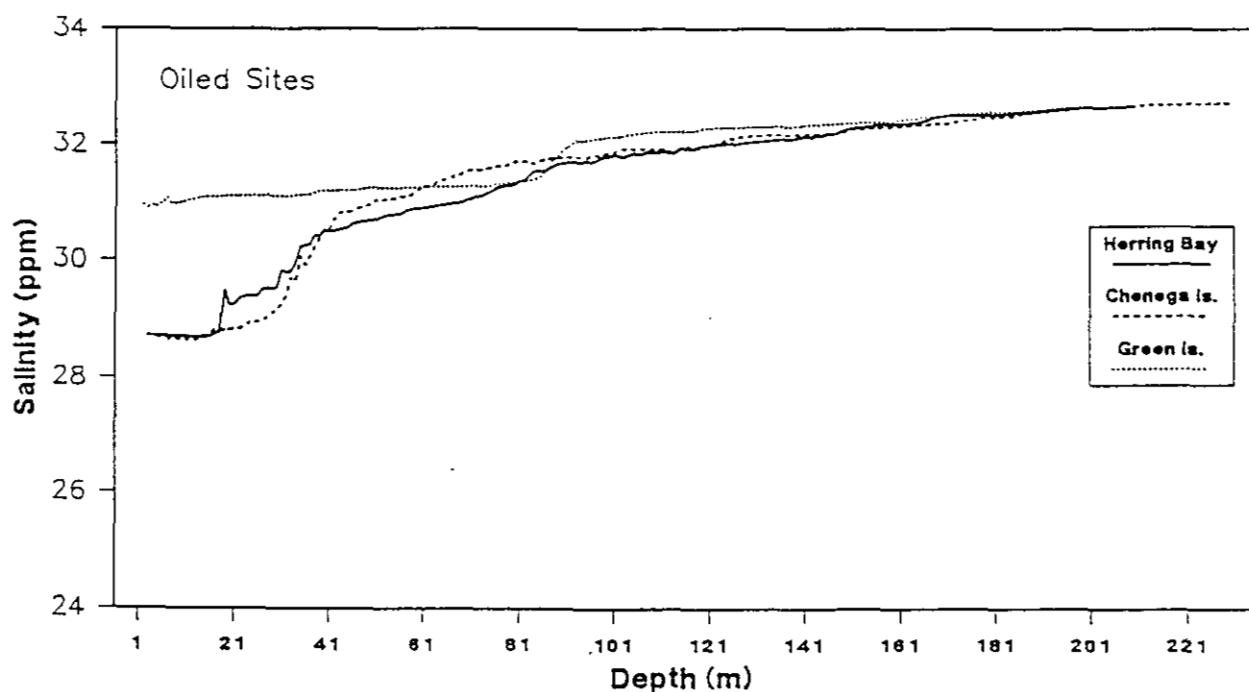
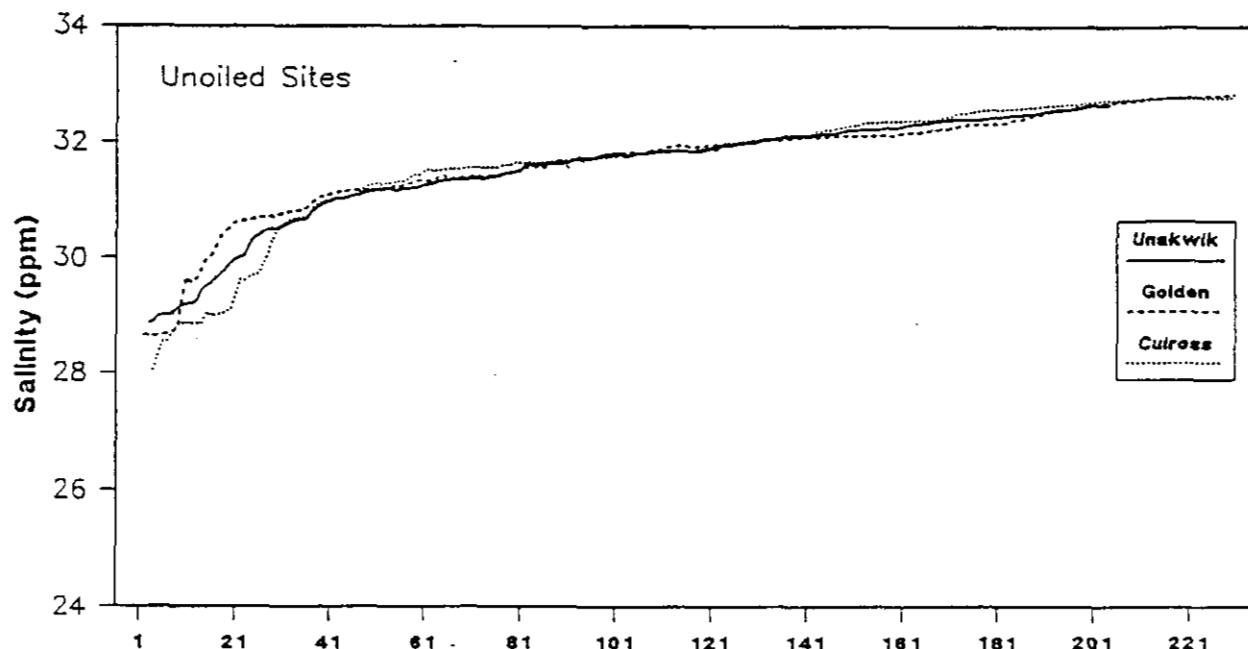


Figure 13. Salinity gradient by depth in November 1990, for unoiled (top) and oiled (bottom) sites, from the Prince William Sound spot shrimp survey.

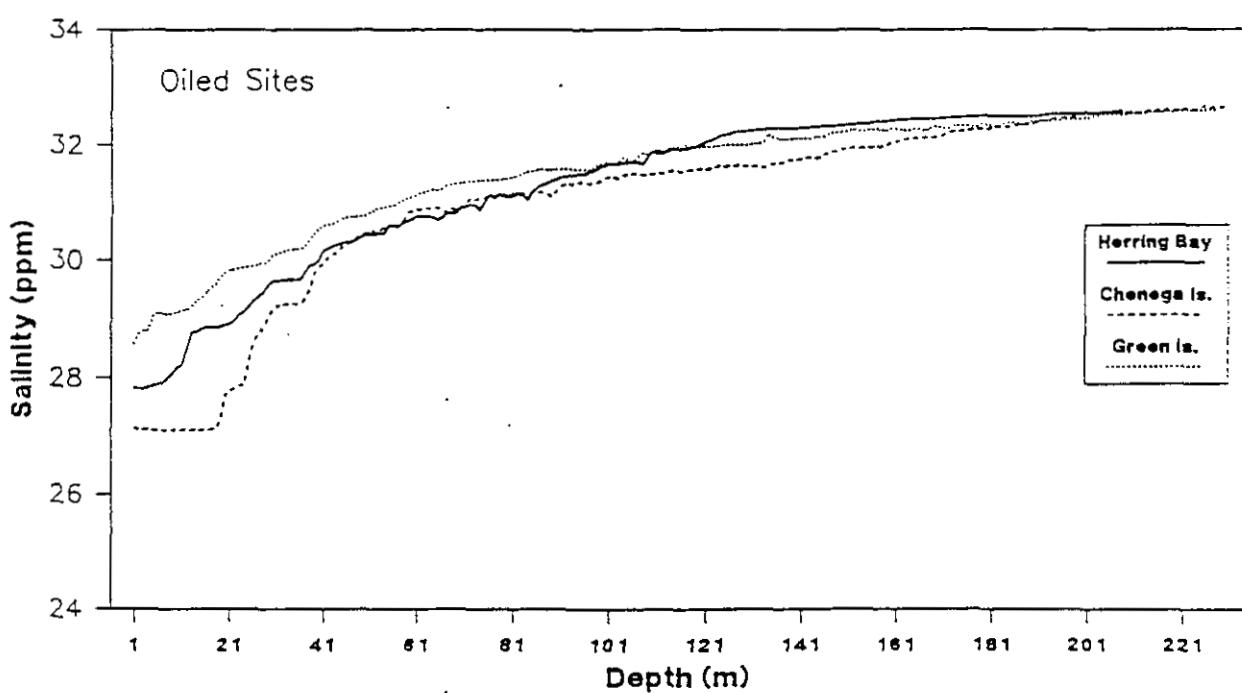
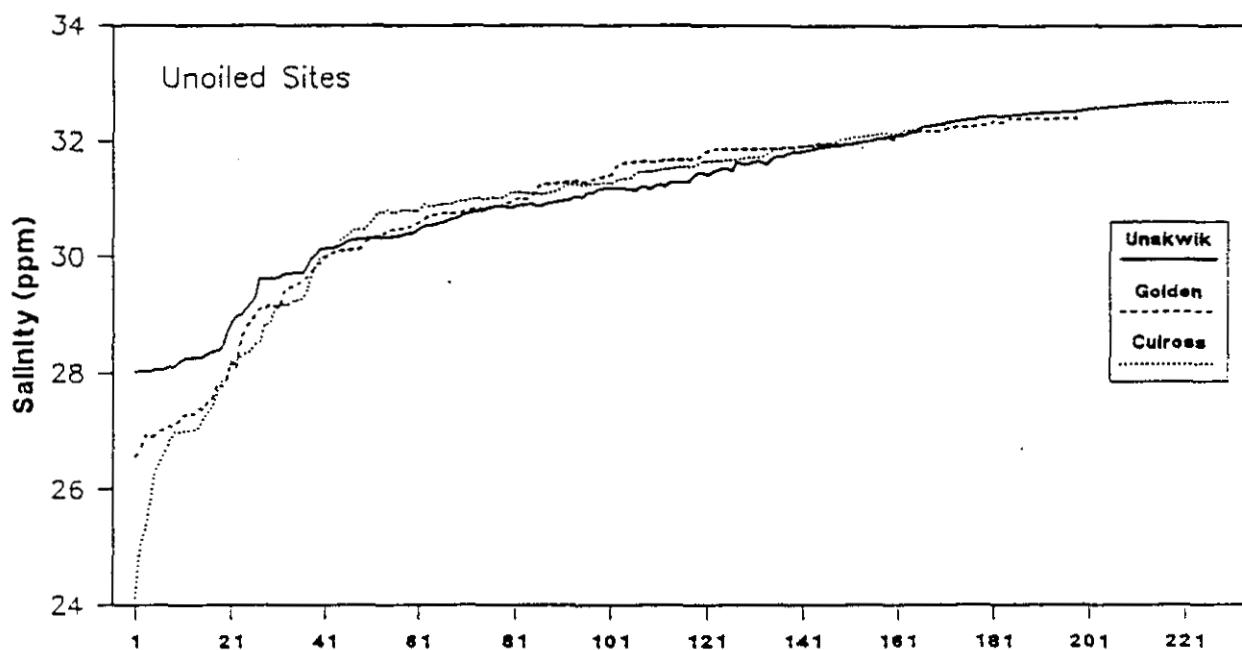


Figure 14. Salinity gradient by depth in November 1991, for unoiled (top) and oiled (bottom) sites, from the Prince William Sound spot shrimp survey.

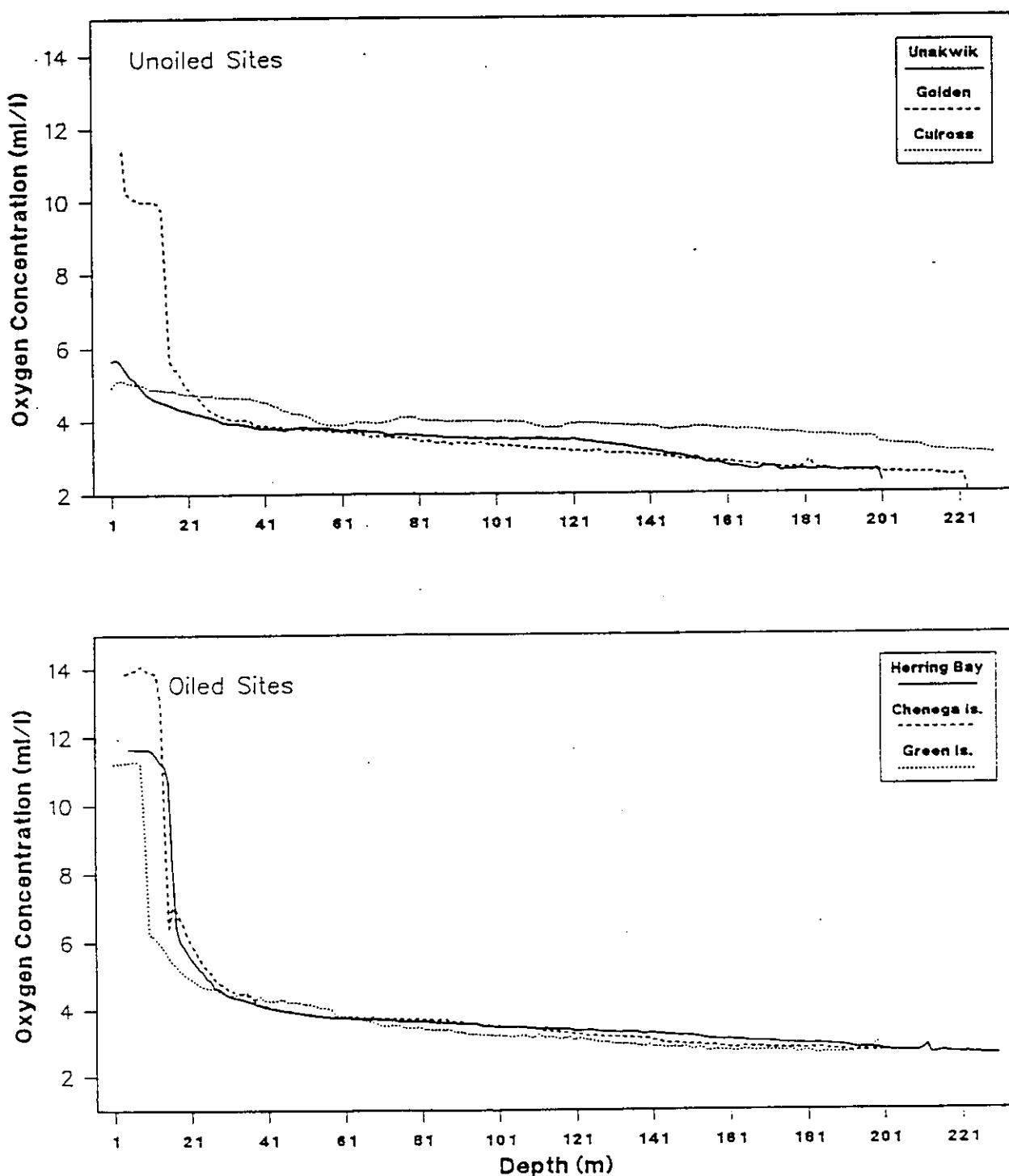


Figure 15. Oxygen concentration gradient by depth in November 1989, for unoiled (top) and oiled (bottom) sites, from the Prince William Sound spot shrimp survey.

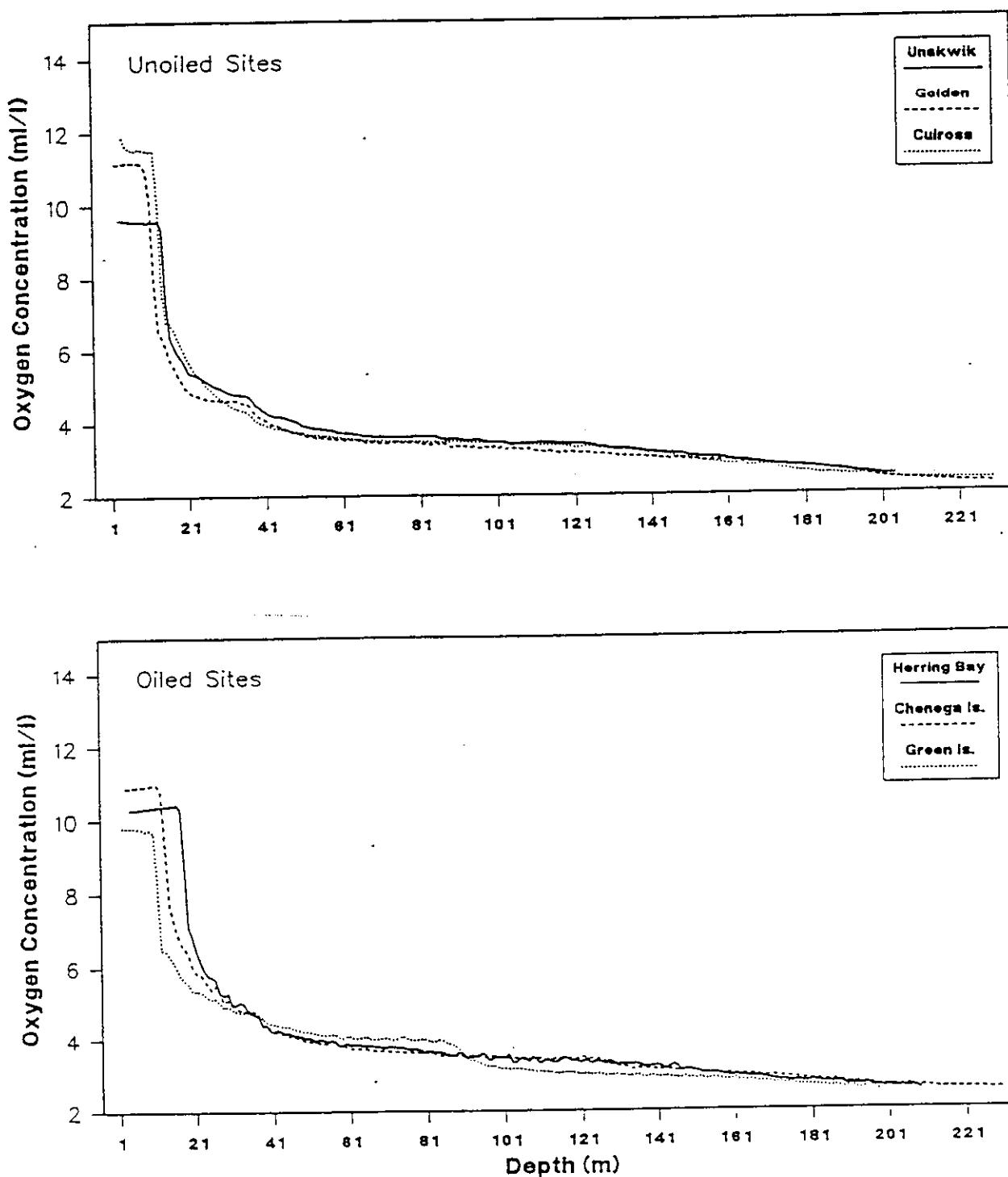


Figure 16. Oxygen concentration gradient by depth in November 1990, for unoiled (top) and oiled (bottom) sites, from the Prince William Sound spot shrimp survey.

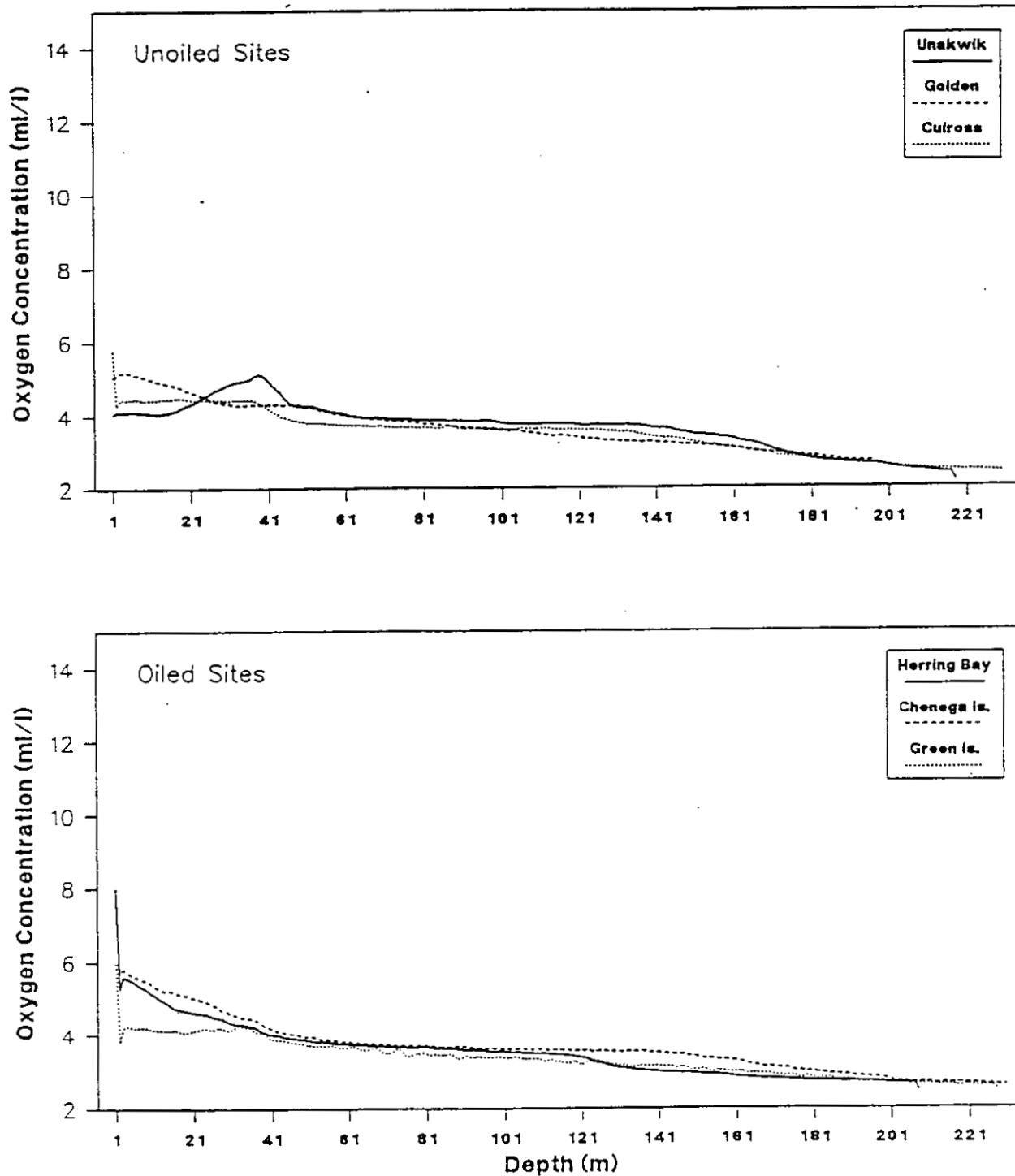


Figure 17. Oxygen concentration gradient by depth in November 1991, for unoiled (top) and oiled (bottom) sites, from the Prince William Sound spot shrimp survey.

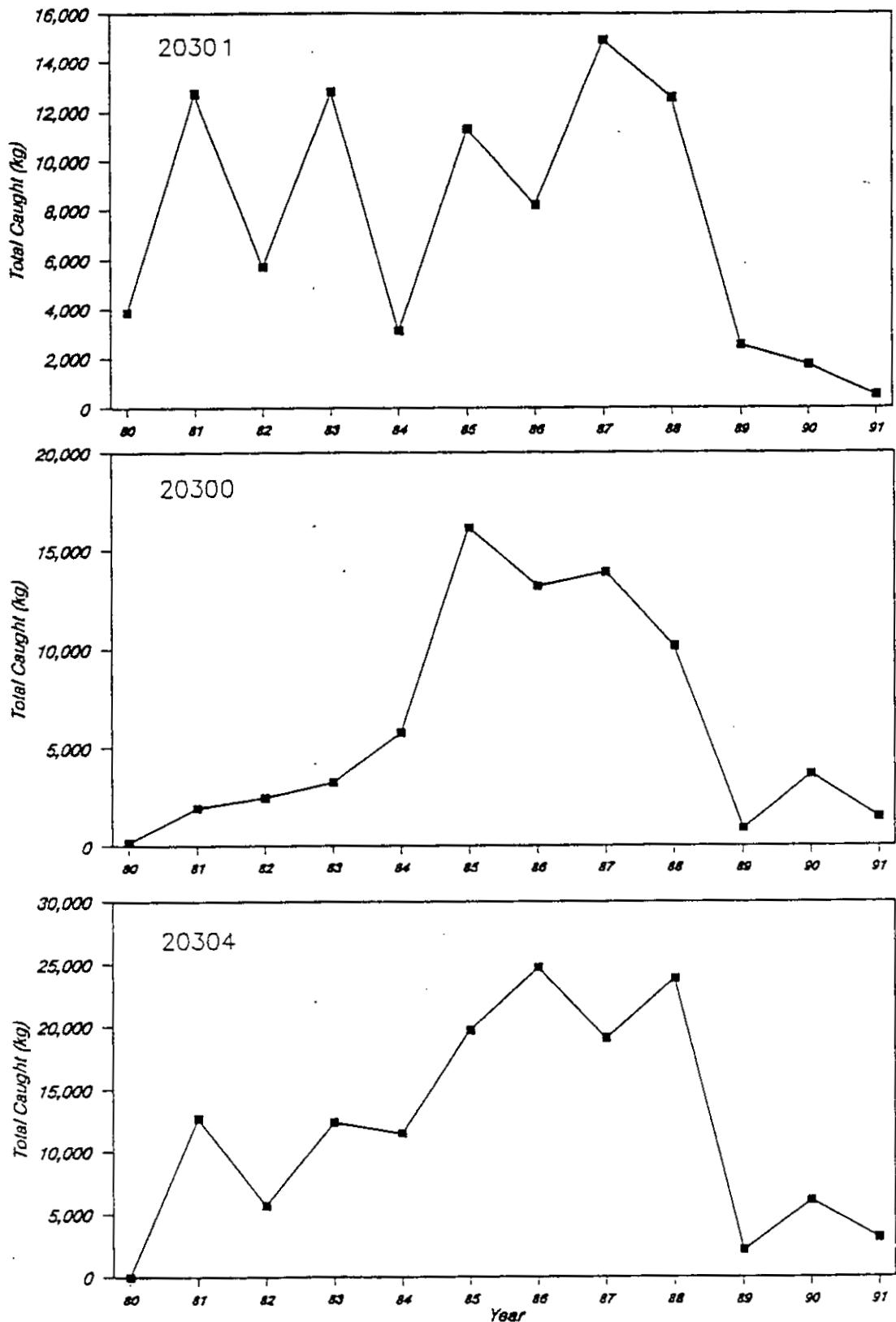


Figure 18. Total annual catch of spot shrimp caught in statistical reporting area 20301 (top) which includes the Unakwik study site, 20300 (middle) which includes the Golden study site and 20304 (bottom) which includes the Culross Passage study site, these areas are within the unoiled area.

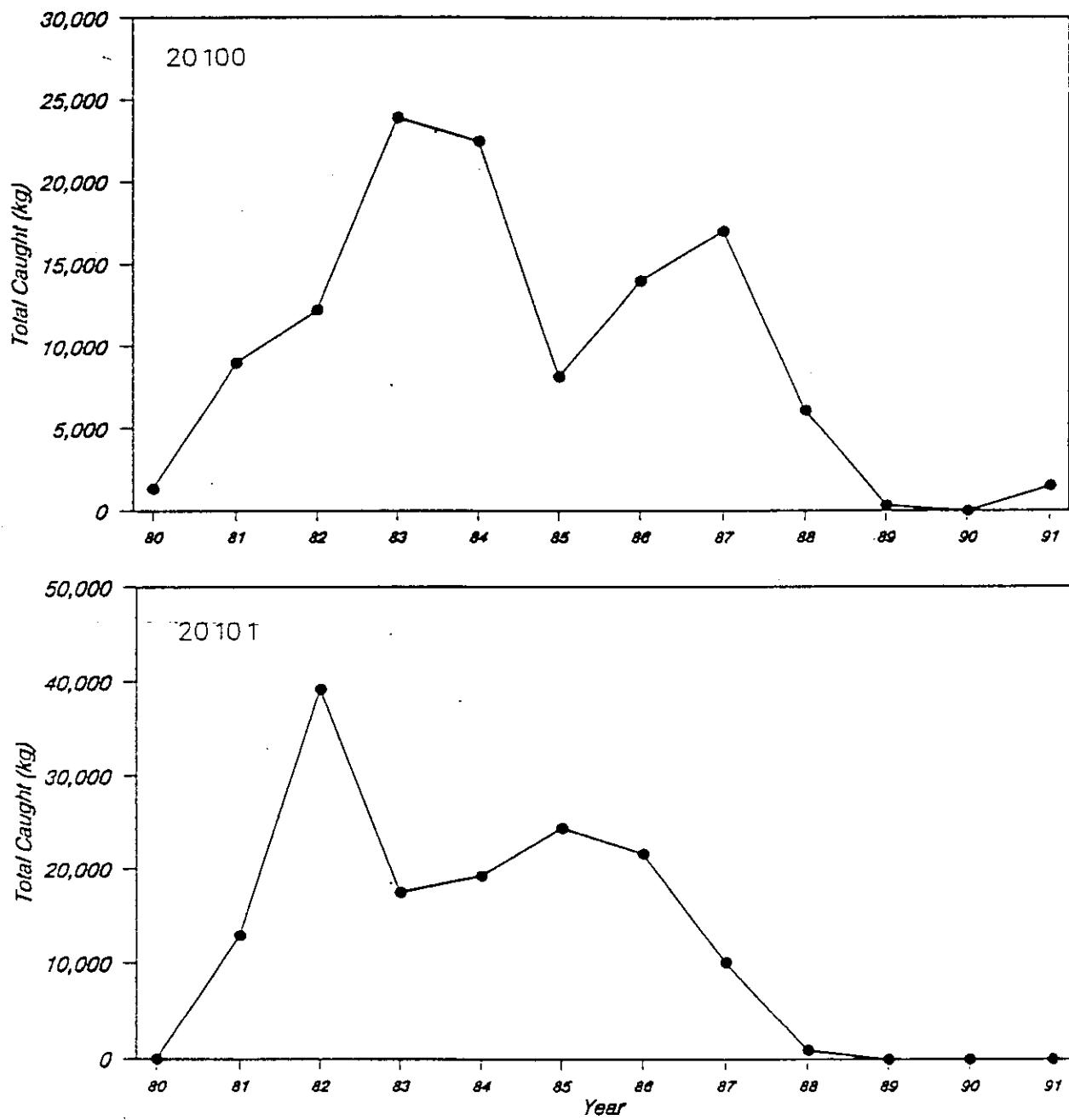


Figure 19. Total annual catch of spot shrimp caught in statistical reporting area 20100 (top) which includes the Herring Bay and Chenega Island study sites and 20101 (bottom) which includes the Green Island study site, these areas are within the oiled area.

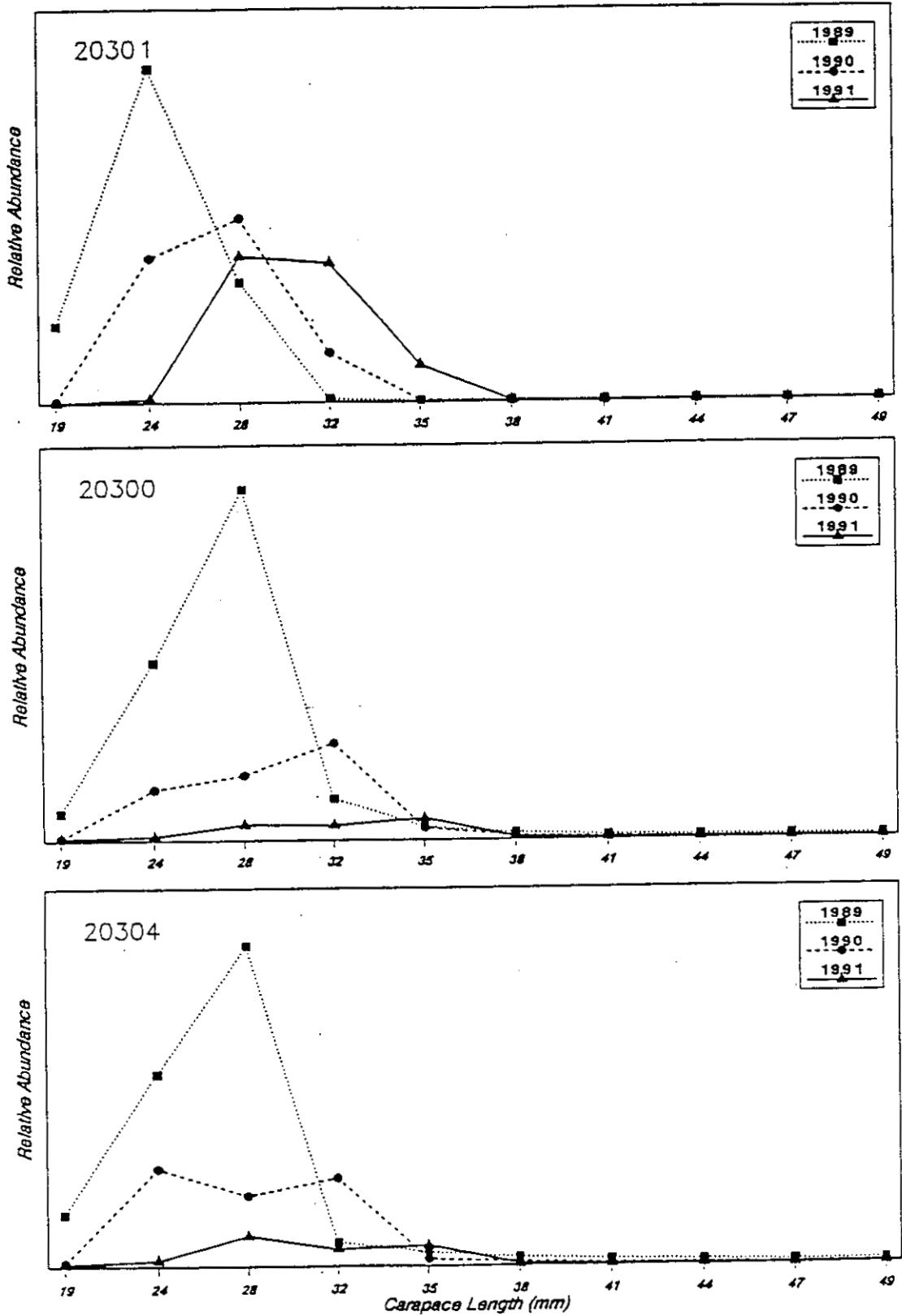


Figure 20. Carapace length frequency model output for statistical reporting area 20301 (top) which includes the Unakwik study site, 20300 (middle) which includes the Golden study site, and 20304 (bottom) which includes the Cuiross Passage study site.

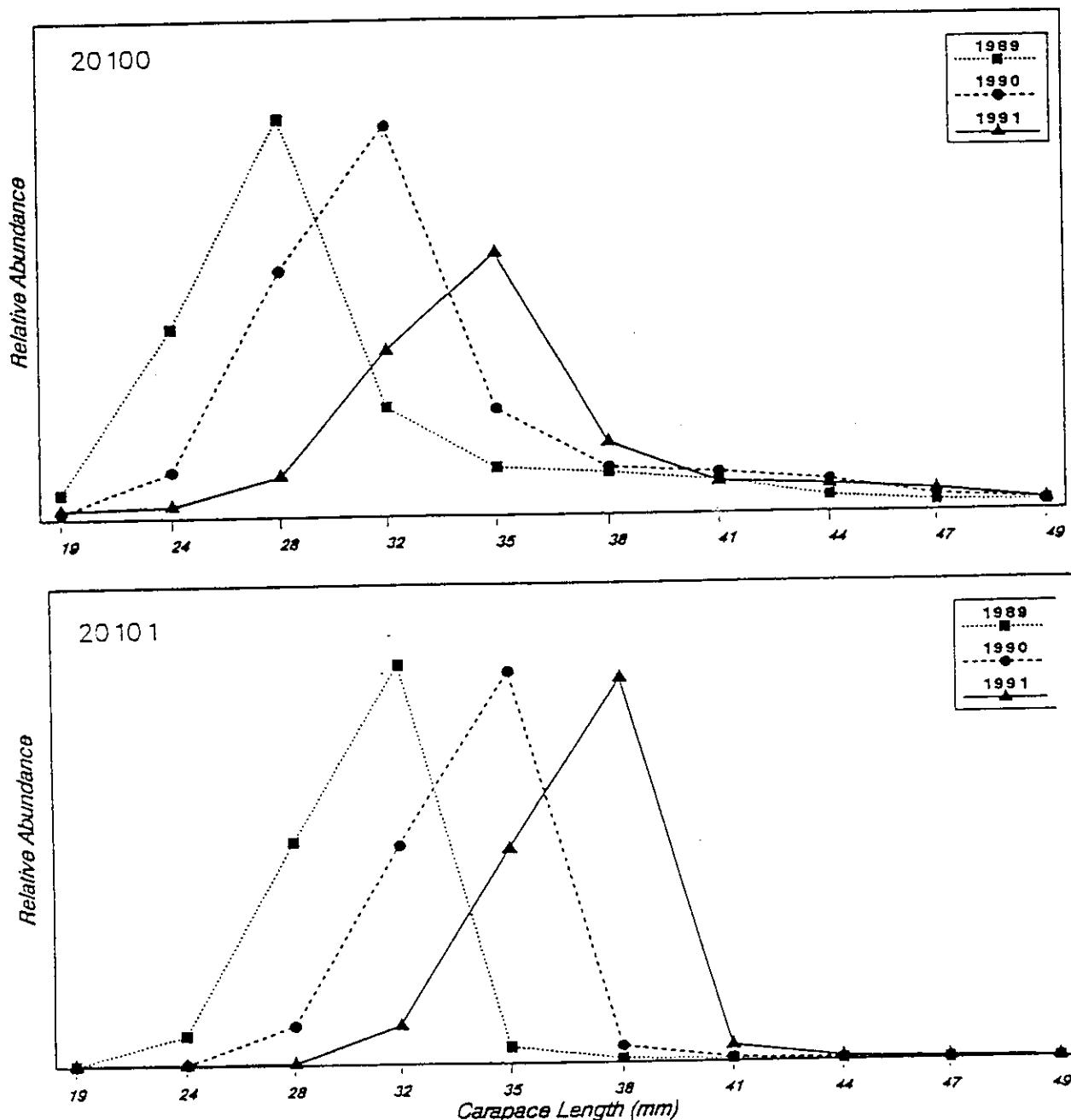


Figure 21. Carapace length frequency model output for statistical reporting area 20100 (top) which includes the Herring Bay and Chenega Islands study sites, and 20101 (bottom) which includes the Green Island study site.

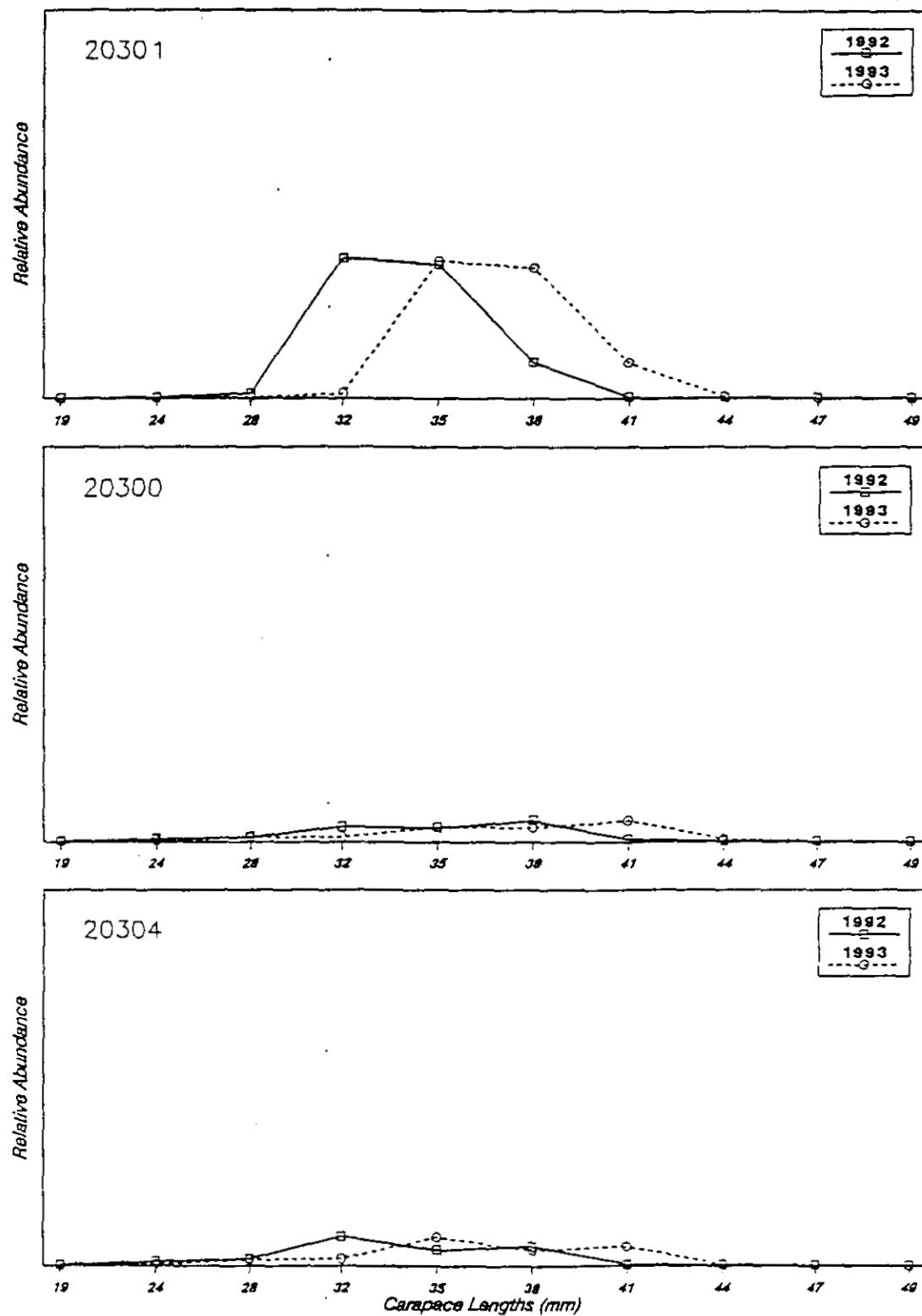


Figure 22. Carapace length frequency model output projecting for 1992 and 1993 for statistical reporting area 20301 (top) which includes the Unckwik study site, 20300 (middle) which includes the Golden study site and 20304 (bottom) which includes the Culross Passage study site.

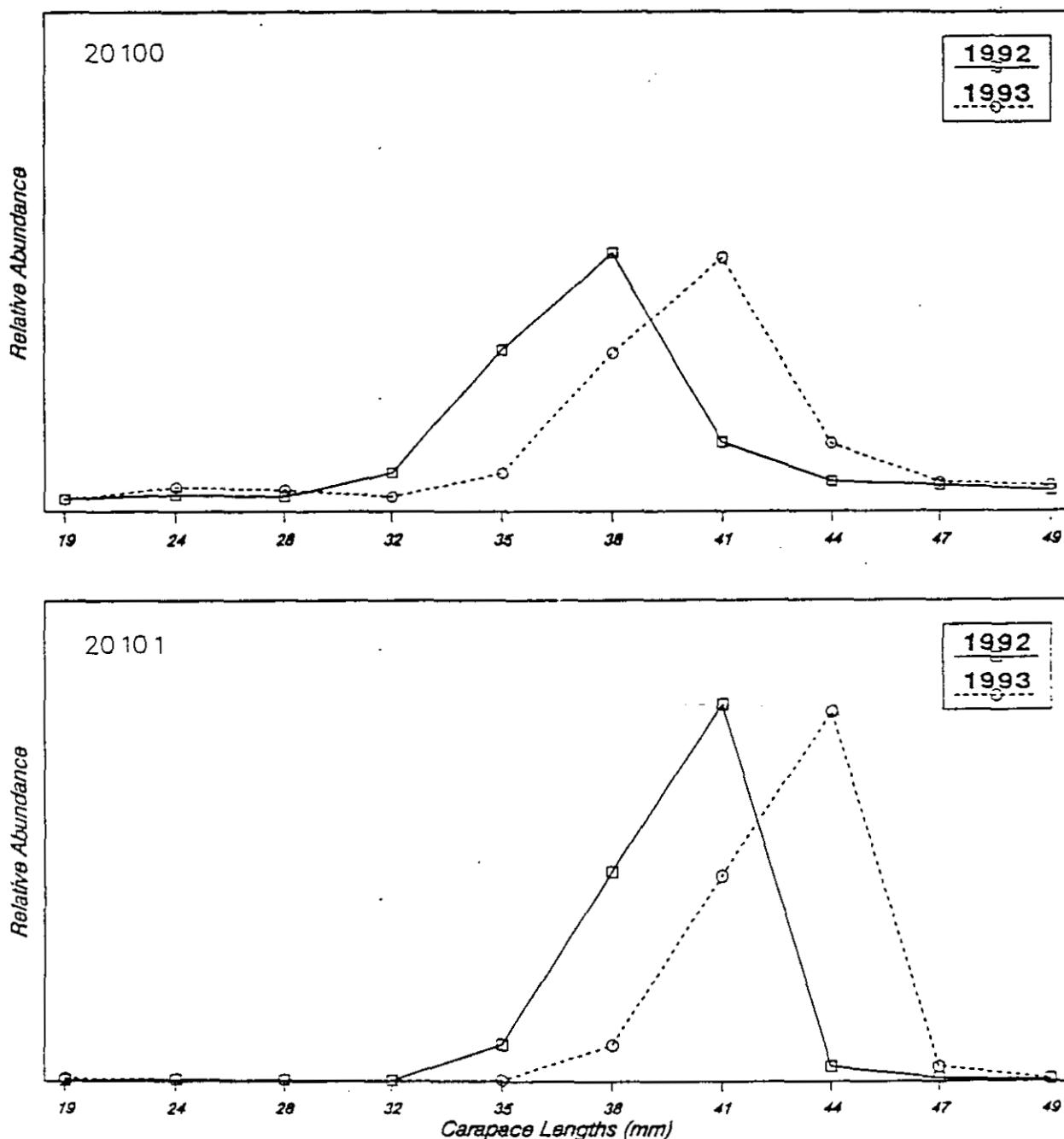


Figure 23. Carapace length frequency model output projecting for 1992 and 1993 for statistical reporting area 20100 (top) which includes the Herring Bay and Chenega Island study sites, and 20101 (bottom) which includes the Green Island study site.

**APPENDIX A.**

**Standard Operating Procedures for  
Sexing Pandalid Shrimp in Prince William Sound**

STANDARD OPERATING PROCEDURE  
FOR SEXING PANDALID SHRIMP IN THE PRINCE WILLIAM SOUND

by: Charlie Trowbridge  
Dan Coyer

November 3, 1989

INTRODUCTION

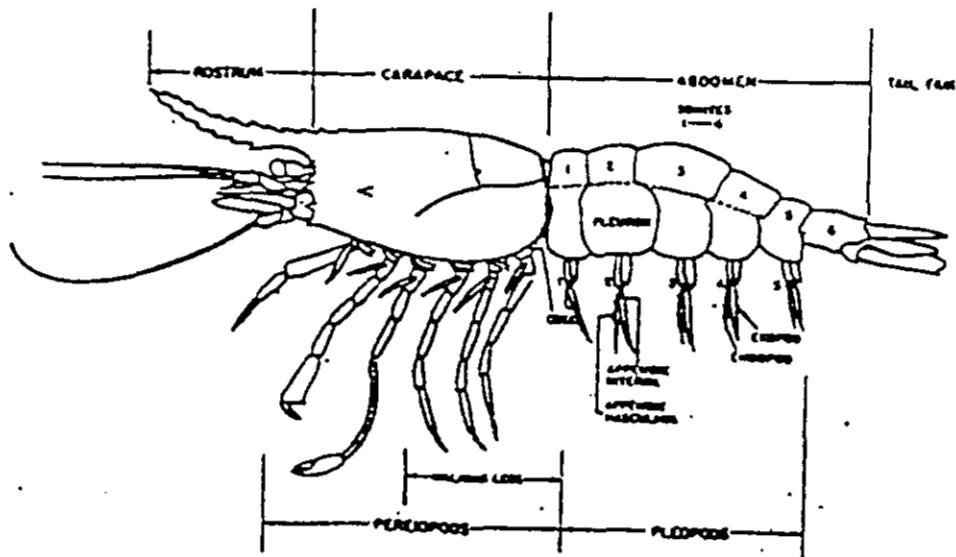
Pandalid shrimp in Alaska are typically protandric hermaphrodites, therefore, three sexual phases can be identified: male, transitional, and female. Determining the sex of a Pandalid shrimp by examining sex organs is difficult and time consuming, but using the secondary sexual characteristic of endopod development, which closely tracks gonad development, allows sex to be determined with relative ease. This is, therefore, the preferred procedure and is performed according to Butler's description in his work Shrimps of the Pacific Coast of Canada (1980).

EQUIPMENT

1. Needle probe.
2. Forceps
3. Bright light and black background.
4. Source of magnification: 3X.

SEXING THE SPECIMEN

Using the needle probe, isolate and examine the endopod of the first pleopod (see figure 1). Removing the exopod with the forceps may be helpful: If the distal margin is bifid, equally lobed with a median cleft, then the sex is male (see figure 2). If, on the medial edge, near the tip, there exists a small rigid protuberance, then the sex is transitional. If the tip is nib-shaped like the working end of a quill pen and sharply pointed, then the sex is female.



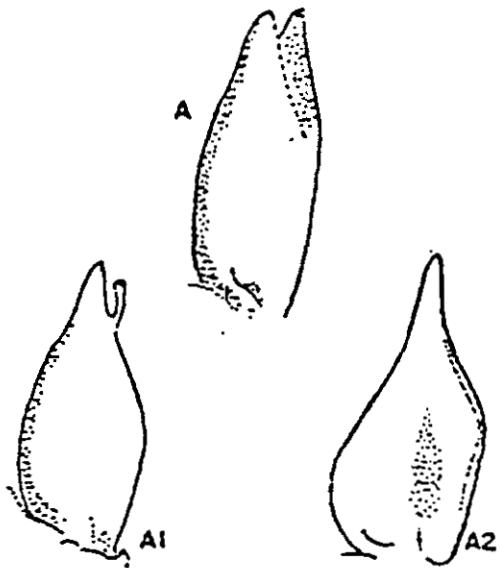


FIG. 2 Endopod of first pleopod: (A) male phase, (Al) transitional phase, (A2) female phase.

The second pleopod may also be used to sex the specimen and is especially helpful in verifying the transitional phase. A male is identified as having two small processes nearly the same length branching from the basal inner margin of the endopod (see figure 3). The medial process, the appendix masculina, is distally spined. The lateral process is the appendix interna and is tipped with "hook-like setae". A transitional is identified as having both processes with the appendix masculina clearly atrophied to approximately one-half (or less) the length of the appendix interna. The female has only the appendix interna.

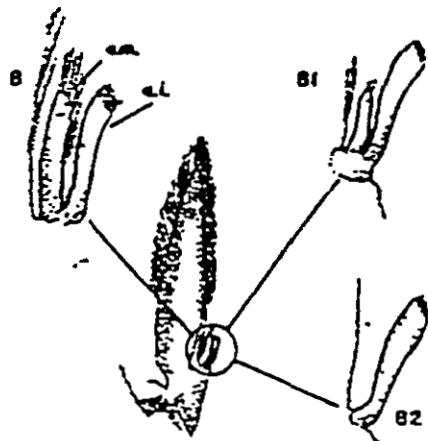


FIG. 3 Endopod of second pleopod: (B) male phase, (B1) transitional phase, (B2) female phase.

Allen (1959) also gives a detailed account of these morphological changes in *Pandalus borealis* (see figure 4). Allen's drawings are more extensive than Butler's, but both authors agree on the use of endopods in sexing Pandalid shrimp.

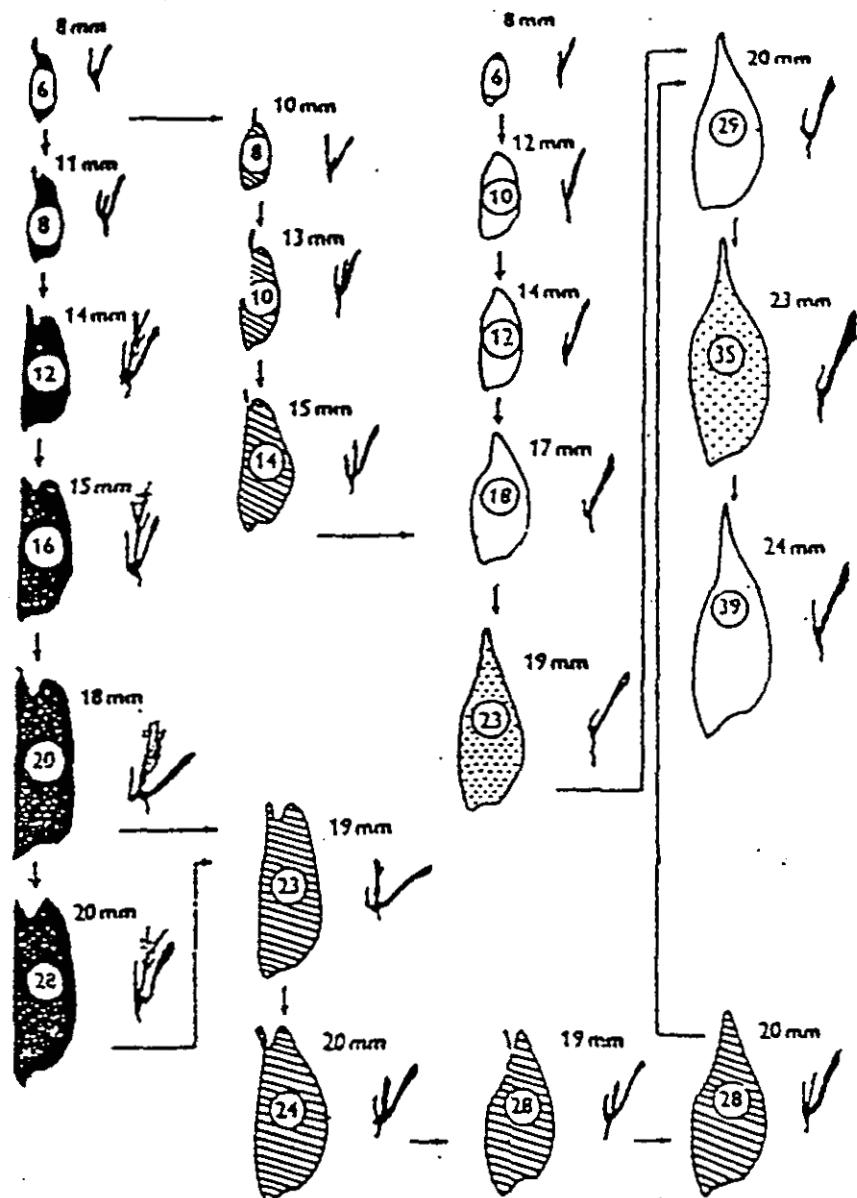


FIG. 4 Endopodite development with corresponding appendix interna and appendix masculina. Male endopodite, black; transitional, cross-hatched; female, outlined. Age in months encircled, carapace length above each figure (Allen).

#### REFERENCES

- Allen, J. A. 1959. On the Biology of *Pandalus borealis* (Kroyer), with reference to a population off the Northumberland Coast. Journal of Marine Biology Association U.K. 38:189-220 Great Britain.
- Butler, T. H. 1980. Shrimps of the Pacific Coast of Canada. Canadian Bulletin of Fisheries and Aquatic Sciences 202, Department of Fisheries and Oceans, Ottawa.

**APPENDIX B.**

**Procedures for Taking and Handling  
Hydrocarbon and Histological Samples**

STATE/FEDERAL DAMAGE ASSESSMENT PLAN

ANALYTICAL CHEMISTRY

COLLECTION AND HANDLING OF SAMPLES

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1. INTRODUCTION
2. RECORD KEEPING AND DOCUMENTATION
3. SAMPLE IDENTIFICATION AND LABELLING
4. SAMPLING EQUIPMENT AND SAMPLE CONTAINERS
5. SAMPLING PROCEDURES
  - 5.1 General
  - 5.2 Water
  - 5.3 Sediment
  - 5.4 Tissue
6. SAMPLE PRESERVATION AND HOLDING TIME
  - 6.1 Water
  - 6.2 Sediment and Tissue
7. SAMPLE SHIPPING
8. CHAIN-OF-CUSTODY PROCEDURE

## 1. 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.

## 2. 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.

### 3. 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 are 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.

#### 4. 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 do 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.

#### 5. 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; underfilled 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.

5.1 Water - 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 head space or bubbles.

5.2 Sediment - 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).

5.3 Tissue - 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 egg shell 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.

#### 6. 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.

6.1 Water - 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 air tight chemically clean containers until analysis.

6.2 Sediment and Tissue - 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 air tight containers kept in the dark at 4°C.

#### 7. 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.

## 8. 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.

## PROCEDURE FOR SHIPPING SAMPLES TAKEN FOR ANALYTICAL CHEMISTRY

### SHIPPING METHOD

- The best way to ship samples within Alaska is with ALASKA AIRLINES AIR FREIGHT. Shipments sent this way are tracked in the airline's system as frozen and are placed in frozen storage in Anchorage and/or Juneau before flights, between flights or when awaiting pick-up. Alaska Airlines Gold Streak, Delta, DHL, Federal Express, Airbourne have no freezer storage as a back up in case of delays; shipping frozen samples with any of these is much riskier.

### ADVANCE NOTIFICATION

- If samples are being sent to National Marine Fisheries Service Auke Bay Laboratory, Sid Korn (789-6021) or Nancy Barr (789-6605) MUST be notified in advance of the shipment and informed of the carrier, date and time.

### SHIPPING CONTAINERS

- All samples must be shipped in well-insulated, sturdy containers. DO NOT ship in cardboard boxes, tool chests, etc. Coolers are the best containers.

### PACKING

- KEEP SAMPLES FROZEN until they are to be packed for shipment; while they are being packed, and while they are awaiting shipment!!
- Wrap sample jars individually for shipment. Jars MUST be carefully wrapped. If possible place them in original boxes that fit inside the shipping coolers. In every case jars must not touch each other and must be padded from sides of cooler. Place padding between layers of jars as well. Cardboard dividers, bubble wrap, or strips of absorbent padding may be used in packing.
- If shipment occurs during hot weather, chilled blue ice must be placed among samples (during packing) to add extra cold mass to the shipment.
- If possible, the packed shipping container with its lid open should be in a freezer overnight so container, packing materials and samples will be well chilled.
- Remove materials to be sent from freezer as shortly before shipping time as is practical. PLACE BLUE ICE ON TOP OF SAMPLES IN CONTAINER, fill empty space with packing material.
- PLACE ORIGINAL CHAIN OF CUSTODY SHEET IN THE CONTAINER.
- Seal container with strapping tape and with signed and dated custody tape.
- MARK EACH SHIPPING CONTAINER - "KEEP FROZEN"

**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 print-out 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.

Davidson's Fixative for Shrimp  
Don Lightner  
Department of Veterinary Science  
University of Arizona  
Tucson, Arizona 85721

Shrimp for microscopy to be fixed in Davidson's fixative should be fixed live by the injection/immersion method.

1. Larvae and early postlarvae - fix by immersion in fixative with fixative volume to shrimp volume exceeding 10 to 1. Fix for 12 to 24 hours; transfer to 50% alcohol for storage or shipment in glass or plastic vials.
2. Larger postlarvae, juveniles and adults: Inject fixative into hepatopancreas, stomach, and midgut region in 4th abdominal segment; then on small shrimp open shell longitudinally for the length of the animal; or bisect or trisect larger shrimp as well as opening the shell.

Fix for 12 - 48 hours (use the longer fixation time for larger shrimp) in Davidson's, then transfer to 50 to 70% alcohol for storage and shipment.

The fixative should be made up as follows:

Davidson's Fixative (for 1 liter):

95% ethyl alcohol	330
Formalin (37% technical grade)	220
Glacial acetic acid	115
Tap water	335

Detailed fixative procedure for Davidson's fixative (injection and immersion method):

1. Select (if possible) moribund or otherwise compromised shrimp (dead shrimp are useless) and kill shrimp by injection of 0.1 to 5 ml (amount depending on size of specimen; immerse live larvae and early postlarvae without the injection step) of fixative into the hepatopancreas;
2. Then open cuticle over cephalothorax and abdomen just lateral to the dorsal midline using dissecting scissors; bisect or trisect larger shrimp (i.e. 12g or larger);
3. Then immerse shrimp in fixative with volume of fixative to tissue of at least 10 to 1.
4. Fix for 24 to 72 hrs (depending on size, longer for larger shrimp to insure adequate decalcification of exoskeleton).
5. Transfer samples to 50% ethyl alcohol.
6. Specimens (juveniles to adults) may be shipped by wrapping in cloth or paper towels saturated with 50% alcohol and packed in double plastic bags. Pack and ship larvae and postlarvae in small glass or plastic vials, that are in turn packed in double plastic bags.
7. Label each specimen container carefully in soft pencil on water resistant white paper. Please include separately any appropriate notes on gross observations, species and age of specimens, original source of shrimp or source of the parent brood stock, and source and species of other shrimp at your facility, especially if held in same tanks or ponds, etc.

## TECHNIQUES

The micrographs in this handbook were produced primarily from specimens of cultured *Penaeus stylostris*. Specimens were almost exclusively obtained from the University of Arizona's marine culture research facilities in Sonora, Mexico and Oahu, Hawaii. Additional specimens of *P. stylostris*, and other species, were obtained from various private and government facilities, and in particular from Marine Culture Enterprises' commercial facility on Oahu, Hawaii.

The techniques of specimen fixation, though simple in nature, are of the utmost importance in the preparation of meaningful microscopic slides. Inadequate or improper fixation, if not recognized as such, can often lead to misinterpretation of the sectioned material. The relatively impervious chitinous exoskeleton of shrimp does not allow for adequate fixative penetration by simple immersion. Hence, it is imperative that immersion within a fixative be immediately preceded by injection of the fixative into vital areas.

The timing of fixation is of equal importance. Specimens should be fixed immediately following removal from the water, i.e. they should not be removed from the water and carried in an empty bucket to the place where they are to be fixed. They should instead be placed in a bucket, or similar utensil, with an adequate amount of water and then carried to the site of fixation or fixed on site. Additional care should be exercised to limit the amount of handling stress that each specimen is subjected to prior to fixation. Stress mediated histopathology, due to excessive handling, could be misinterpreted as being the state of the animal in its normal environment.

Various fixatives have been used for the preservation of shrimp and other crustaceans with varying success. Among those used are Helly's (Luna, 1968), Bouin's (Luna, 1968), 10% neutral buffered formalin (Luna, 1968) and Davidson's AFA (Illumason, 1972). Our experience has shown Davidson's AFA to be the best general purpose fixative for penaeid shrimp when intended for light microscopic observations.

More precisely, the methods for specimen preparation are as follows:

### Collection

1) Collect shrimp by whatever means are available with a minimum of handling stress. For the study of presumably diseased shrimp, select those which are moribund, discolored, displaying abnormal behavior, or otherwise abnormal, except in the case of intentional random sampling for estimation of disease prevalence. Shrimp sampled for normal histology should not be abnormal in appearance nor behavior. Do not collect shrimp that are dead for any

sample, unless it can be positively determined that they have died within the last few minutes. If recently dead shrimp must be sampled, be sure to make note of this condition and estimate the time since their death.

- 2) Transport the shrimp to the laboratory via a water filled utensil. Supply adequate aeration to the container if they are to be left for a short period of time before actual fixation.

### Fixation or Preservation

- 1) Have ready an adequate supply of fixative; a rule of thumb is that a minimum of approximately 10 X their volume of fixative should be used for each specimen (e.g. a shrimp of 10 ml volume would require 100 ml of fixative).
- 2) Davidson's fixative should be made as such:
  - a) 330 ml 95% ethyl alcohol
  - b) 220 ml 100% formalin (saturated aqueous solution of formaldehyde gas, 37-39% solution).
  - c) 115 ml glacial acetic acid
  - d) 335 ml tap water (preferably distilled if available)
  - e) store at room temperature
- 3) Inject fixative (0.1 to 10 ml depending on size of shrimp), via needle and syringe (needle gauge dependant upon shrimp size; small shrimp, small needle) into the living shrimp. The site of injection should be laterally in the hepatopancreas proper (Figure 1a), in the region anterior to the hepatopancreas (Figure 1b), in the posterior abdominal region (Figure 1c) and in the anterior abdominal region (Figure 1d). Precautions should be taken to avoid skin and eye contact with the fixative. The fixative should be divided between the different regions, with the cephalothoracic region, specifically the hepatopancreas, receiving a larger share than the abdominal region. A good rule of thumb: "Inject an equivalent of 5-10% of the shrimp's body weight; all signs of life should cease".





- 4) Immediately following injection, slit the cuticle, with dissecting scissors, from the sixth abdominal segment to the base of the rostrum, paying particular attention not to cut deeply into the underlying tissue. The incision in the cephalothoracic region should be just lateral to the dorsal midline, while that in the abdominal region should be approximately mid-lateral (Figure 2).

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- 5) Shrimp larger than 12 grams, should then be transversely slit once at the abdomen/cephalothorax junction (Figure 3a) or again mid-abdominally (Figure 3b).  
6) Following injection, incisions and bisection/trisection, immerse the specimen in the remainder of the fixative.



- 7) Allow the shrimp to remain in the fixative at room temperature for 24 to 72 hr depending on the size of shrimp (larger shrimp for longer).  
8) Following proper fixation, the specimens should be transferred to 50% ethyl alcohol, where it can be stored for an indefinite period.  
9) Record a complete history of the specimen at the time of collection; gross observations on the condition of the shrimp, species, age, weight, source (pond, tank or raceway identifying number), source of parent stock, and any other pertinent historical information that may at a later time provide clues to the source and cause of the problem. Use soft-lead pencil on paper (plastic paper if possible).

#### Transportation or Shipment for Processing

- 1) remove the specimens from the 50% ethyl alcohol.
- 2) wrap with paper towels to completely cover (Figure 4a).
- 3) place towel-wrapped specimen in a sealable plastic bag and saturate with 50% ethyl alcohol (Figure 4b).
- 4) include the history, as recorded above, with the shipment (Figure 4c).
- 5) place bag within a second sealable bag.
- 6) multiple small sealable bags can again be placed within a large sealable bag (Figure 4d).



APPENDIX C.

**Final Report of Histopathology Results  
for the 1989 Sample Set**

HISTOPATHOLOGY OF PANDALID SHRIMP;  
OIL ASSESSMENT STUDIES IN PRINCE WILLIAM SOUND, ALASKA

A FINAL REPORT FOR THE 1989 SAMPLE SET

Contract Number IHP-91-037

State of Alaska, Department of Fish and Game

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Date of this report

March 6, 1992

## INTRODUCTION

The toxicity and ecological effects of crude oil and petroleum on aquatic organisms have been of major concern and the topic of research for many years. However, because crude oil is a mixture of many compounds that vary greatly from one oil field to another, its pathological effects on aquatic animals can vary as well. Because synergistic, antagonistic, and additive effects of the various toxic components of crude oil undoubtedly can occur, pathological effects may depend on the short and long term toxicity of the various compounds present (Sparks, 1985).

Sindermann (1990) reviewed the effects of the crude oil spill from the tanker Amoco Cadiz near the Brittany coast of France on marine mollusks. Short term effects included a massive mortality of 20 to 50% in the most heavily oiled sites within the first 3 months after the spill. Subsequent studies showed a variety of lesions in the mollusks studied including necrotic and inflammatory lesions in the gonads and digestive gland of Ostrea edulis; and elevated hydrocarbon levels of 2 to 5 times the levels found in mollusks in unpolluted sites 7 years after the spill. A high prevalence of hemocytic neoplasms was found in O. edulis and Cerastoderma edule (a cockle), that although suggestive, showed no direct relationship to the spill (Sindermann, 1990).

Shell disease of crustacea is frequently encountered in badly degraded estuarine and coastal waters (Sindermann, 1990). The

disease, also known as "brown spot disease", "burned spot disease", "rust disease", "appendage rot" or "appendage necrosis", and "black gill disease", is characterized primarily by the presence of melanized erosions of the crustacean cuticle (Sparks, 1985; Bell and Lightner, 1988), which in these animals covers the gills, general body surface (the shell or exoskeleton), and lines the foregut and hindgut (Bell and Lightner, 1988). However, black silt and detritus present among the gill lamellae, and sometimes associated with significant populations of epicommensal fouling organisms, has also been noted to give decapod gills a brown to black color. Sindermann (1990) has reviewed the literature that has linked elevated prevalence of shell disease and black gill disease lesions in marine decapod crustaceans to anthropogenic pollutants.

Hence, in our histological examination of the sample sets of Pandalus platyceros and P. borealis provided by the Alaska Department of Fish and Game, we sectioned and examined the specimens so that the following organ and tissues were examined in each specimen: the gills and associated appendages; the digestive tract (hepatopancreas, foregut, and midgut); the ventral nerve cord and thorasic ganglia; the heart; the antennal gland; the hematopoietic tissues; the gonads and developing embryos; and the cuticle (sites with shell disease lesions or presumed wounds). The following report summarizes our observations.

## MATERIALS AND METHODS

### Sample Receipt

On May 5th, 1991, we received via Alaska Airlines airfreight 12 sample sets of preserved shrimp from C. Trowbridge of the Cordova office of the Alaska Game and Fish Department (AKGFD). Eleven of the sample sets contained only samples of the spot shrimp, Pandalus platyceros, while one of the 12 sample sets consisted of a sample set of pink shrimp, P. borealis. All shrimp in all samples had been preserved in the field by AKGFD personnel using 10% neutral buffered formalin, but otherwise following the fixation procedures for penaeid shrimp as outlined in Bell and Lightner (1988).

Formalin fixation in decapods like shrimp causes marked shrinkage and hardening of tissues, it penetrates slowly, and, therefore, in larger shrimp autolysis may result even when special care is taken to insure proper fixation. Furthermore, because the specimens had been preserved with formalin, decalcification in Davidson's fixative (a procedure we have found to result in less tissue damage and subsequent processing difficulties than when formic acid is used) was necessary before histological processing could be initiated.

## Gross Appearance and Lesions

As the specimens in each sample set were unpacked for histological processing, they were individually weighed, sexed when possible, and examined for the presence of any grossly visible lesions or other anomalies (Table 1). From each sample set a total of five representative specimens (which included any specimens with visible abnormalities or lesions) were photographed. A set of color transparencies of the representative specimens from each sample set was provided to Dr. J. Sullivan with copies of previous status reports submitted on October 16, 1991, and January 27, 1992.

## Histology

Ten previously preserved shrimp specimens from each sample set were selected in such a manner that animals with grossly visible lesions, berried females, and animals representing each size group were sectioned. Whole shrimp were decalcified for 48 to 96 hr in Davidson's AFA prior to being "cut in" for histological processing. Procedures followed for "cutting in" specimens prior to paraffin embedding were according to Bell and Lightner (1988; p. 4) to provide a "gut-gill panorama", which provides a standardized method for histological evaluation of virtually all major tissues and organ systems of shrimp in a minimum number of tissue blocks and histological slides. Tissue infiltration, embedding, sectioning and staining were carried out as described in Bell and Lightner (1988). Duplicate histological

slides were prepared from sequential sections from each tissue block for each specimen and stained using a modified Mayer's hematoxylin and phloxine/eosin (H&E) stain as also described in Bell and Lightner (1988). No other histological stains or procedures were carried out. A duplicate set of histological slides from this study will accompany a copy of this report.

Histological sections were examined using routine bright field light microscopy. Histological sections were scanned for lesions with 4 and 10 X objectives, and examined with higher magnification objectives of 20, 40 and 100x when necessary to ascertain the nature of lesions, histological artifacts (i.e. postmortem autolysis from formalin fixation), determination of cell types, intracellular inclusions, and other histological structures. Severity of histological lesions was assigned semi-quantitative numerical ratings or grades according to the scheme given in Table 2.

Color and black and white photomicrographs were taken in parallel of representative lesions, parasites, and other abnormal tissue structures as they were encountered. Likewise, photomicrographs of presumed normal regions of tissues were also taken for comparative purposes. Duplicate sets of a portion of the color transparencies resulting from this study have been provided to AKFGD with previous reports.

### **Oil Content of Stomach Contents**

During the cutting in process it was noted that some shrimp contained masses of brown to black detrital material as the main component of the stomach contents. To determine if this material was crude oil, or if it contained a high oil content, the stomach contents of approximately 10 shrimp were pooled. These were dried on a Whatman No. 1 filter paper in a vacuum oven at 45 °C to a constant weight, then extracted with 60 ml of hexane six times, and then dried again in the same manner to a constant weight. The difference in dry weight before and after hexane extraction was considered to be the lipid and/or oil fraction.

### **RESULTS AND DISCUSSION**

#### **Gross Appearance and Lesions**

Grossly visible deviations from "normal" included: 1) the presence of melanized cuticular lesions which were considered to be either due to shell disease or to mechanical trauma acquired in the collection traps; 2) broken or missing appendages, especially if melanized; most were considered to be due to trauma during capture, fixation, and shipment; 3) the presence of regurgitated stomach contents in masses around the mouth, mouth appendages and in the gill cavity; and for 4) the presence of grossly visible epicommensal fouling organisms. Other gross observations which were noted included the stage (according to color) of development of embryonating eggs (i.e., before or after

pigmentation of the eyes becomes apparent) on berried females. All were pale yellowish, suggesting that they were recently spawned and set eggs, at an early stage in embryo development and well before development of pigmented eye spots (Table 1).

### Histological Findings

Our histological observations are summarized in Table 3. Histological examination of the sections prepared from each shrimp processed showed uniform problems with fixation that are typical with decapod crustacean tissues that are fixed with formalin. In general, the tissues of these shrimp were hard, brittle and difficult to section. Patchy to generalized autolysis of some organs and tissues (especially the ventral nerve cord and ganglia, the central region of the hepatopancreas, and the anterior midgut) was uniformly present in the majority of the specimens. However, such autolytic changes due to the method of fixation did not preclude histological detection of several distinctive types of lesions, gill and cuticular epicomensal organisms, and internal parasitic microorganisms. In tissues or organs in which we found at least some histopathology (as indicated by the presence of a parasite or by necrosis and inflammation), the frequent presence of inflammatory cells allowed a distinction to be made of the tissue changes observed in such lesions from autolytic changes due to fixation artifact.

Lesions in the Gills and Gill Cavity:

Gill lesions of variable severity comprise the most significant histological alteration found in the sample sets that might be related to exposure to oil or to a degraded environment (Table 3). The least severe lesions present in the gills consisted of focal to multifocal areas in the gill lamellae and gill rachi that showed necrosis; hemocyte infiltration, congestion, and inflammation; hemocytic nodule formation; and melanization of hemocyte inflamed foci or of hemocytic nodules (Figures 1-3).

Some shrimp showed no lesions in the histological sections examined (Table 3; Figures 1a-1d). In shrimp with low grade lesions in the gills, focal and multifocal areas of necrosis and inflammation were more common. Often, the more end or distal tips of each of the gill processes (or rachi) was affected, while more proximal portions of the same gill process were less affected or not affected (Figures 2a-2b). In the most severely affected specimens the above described lesions were present, but also present was an edematous swelling of the hemolymph channels in the central rachus of the affected gill processes (best illustrated in specimens from 52J, 52K, and 52L) (Figures 2c-2f). Hemocyte congestion and a spongiform fibrosis were also observed in the edematous areas of such gills in the more severely affected specimens (Figures 2a-2d, 2f).

Debris consisting of amorphous brown to black detrital-like material was present on gills of some specimens. Although not commonly present, such deposits were typically found adjacent to areas of the gill lamellae with the most advanced inflammatory lesions (Figures 3a-3b).

Cuticular Lesions:

Melanized cuticular lesions were noted grossly (Table 1) and representative examples were also sectioned and examined histologically. Representative examples of these lesions were found by histology to be either resolving wounds or classical examples of bacterial shell disease. Many of the cuticular lesions, while possibly due to environmental toxicants like oil, might have also been due to wounds acquired from the traps used to collect them. Melanized cuticular lesions, which were located on surfaces likely to be traumatized by the shrimps' behavior in the traps (i.e. abrasions, lacerations, puncture wounds, etc. on the dorsal surface of the second, third, or fourth abdominal segments and on the tips of the uropods, telson, rostrum, and the pereiopods are typically the result of collisions with the cage or other shrimp which occur as a result of the "tail-flip" escape movement), were considered to have resulted directly from physical trauma.

In contrast, melanized cuticular lesions present on the cuticle of other more protected areas such as the gills (discussed separately in the following section), gill accessory

structures, gill chamber, and feeding appendages, or on areas of the cuticle are more likely to be the result of toxic or bacterial etiology and not mechanical trauma. While many shrimp possessed melanized cuticular lesions that were likely to be the result of physical trauma, several shrimp also possessed this latter type of lesion, which were more likely to have resulted from toxic or bacteriological factors related to a degraded environment. Table 1 lists the prevalence of melanized cuticular lesions (no distinction is made as to whether due to trauma or to toxic or bacterial shell disease) in the sample sets. Table 3 provides data based on the histological appearance of such lesions and indicates the number of shrimp in the sample of 10 sectioned which possessed a histologically significant severity of shell disease-type lesions that were likely to have been due to factors other than trauma (Figure 3c). Sample groups 52I-52L (NSS008 to NSS012) had the highest prevalence of such lesions (Table 3).

Inclusions in Fixed Phagocytes/Reserve Cells:

A number of specimens in many of the samples showed the presence of fixed phagocytes (or reserve cells) with dense eosinophilic cytoplasmic inclusions that contained presumed pyknotic and/or karyorrhectic nuclei (Figure 3d). Such cells were most often present in the subcuticular connective tissues and among the heart muscle fibers in the heart of these shrimp. Because no inflammatory response accompanied their presence, even when abundant, I doubt that they are pathologic.

Parasites and epicommonsals:

Present on the gill lamellae of nearly every specimen of all sample groups processed were low to moderate numbers of a loricate protozoan. These were commonly present on the cuticle in recessed or highly folded areas (such as on the gill lamellae where they originate from the primary gill rachus), and less often on the cuticle of various appendages (Figures 4a-4b). While we have not attempted to classify this protozoan, we presume (based on its morphology) that it may be a species of Lagenophrys. When abundant in foci on the gills, these protozoans evoked a slight to moderate inflammatory response as indicated by the presence of hemocyte congestion of the parasitized lamellae (Figure 4a).

A metazoan epicommonsal organism was detected only on the gills of the P. borealis specimens. Because of its histological structure, we presume that the metazoan organism may be a member of the nemertean worm group (Figures 4c-4d). As no significant host response accompanied this organism, it appeared to have little direct adverse effects on the affected shrimp. None of these worms were detected among the brooding eggs of P. borealis or P. platyceros.

One specimen of P. borealis showed a remarkably heavy systemic infection by an amoeboid protozoan. This organism occurred singly and as multinucleated syncytia throughout the

hemocoel and loose connective tissues of the affected individual (Figures 4e-4f). Equally remarkable was the absence of a host inflammatory response to the parasite and the presence of ingested material in the stomach of the affected shrimp, indicating that this severely parasitized shrimp was still feeding at the time of its capture.

Gregarine trophozoites were present in low numbers in the anterior portion of the midgut of a few of the P. platyceros. In all examples observed, the number of parasites present was low and considered to be insignificant.

Miscellaneous Observations:

Of interest was the low number of functional males in the samples of P. platyceros. We noted no definite males during our unpacking and gross examination of these shrimp, and subsequent histological study of the representative specimens selected showed only a few males with testis and sperm, and some of these also had developing ovaries. Generally, the smaller individuals in these samples displayed gonadal tissues that were interpreted as immature testis, or were sufficiently developed to contain recognizable spermatozoa (Table 3). Likewise, those hermaphroditic individuals that clearly possessed testis and ovary (Figure 10), were among the smaller animals in the sample sets (Tables 1 and 3).

Likewise, the single sample set of P. borealis contained a

number of hermaphrodites which possessed both testis containing mature sperm and ovaries with developing ova (Tables 1 and 3) (Figures 5a-5b). One individual had both mature testis and ovaries and recently set brooding eggs on its pleopods. Hermaphroditic shrimp were also present in the samples of *P. platyceros*, but at far lower prevalence rates (Table 3; Figures 5c-5d).

Three specimens with possible low-grade infections of hepatopancreas tubule epithelial cells by an intracellular bacteria were observed. One shrimp in 91-52H and two in 91-52L displayed large intracytoplasmic basophilic inclusion bodies composed of presumed rickettsia or chlamydia.

#### **Oil Content of Stomach Contents**

The dry weight of pooled brown-black stomach contents taken from approximately 10 prawns was 0.80 g. Following hexane extraction and drying, the weight of the sample was 0.76 g. Subtraction of the post extraction weight from initial sample weight shows that the stomach content sample contained 0.04 g of lipid and/or oil. Hence, 5% of the original sample was lipid and/or oil. This value is well with the normal range of total lipid expected for invertebrate animal foods which constitute the diet of decapod crustaceans. It is unlikely, therefore, that the black/brown coloration of the stomach contents of these shrimp was due to their feeding on detrital material associated with benthic deposits of crude oil.

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- Sparks, A.K. 1985. Synopsis of Invertebrate Pathology Exclusive of Insects. Elsevier Science Publishers, Amsterdam. 423 p.
- Sindermann, C.J. 1990. Principal Diseases of Marine Fish and Shellfish. Volume 2, Second Edition. Academic Press. 516 p.

Table 1. Summary of gross examination observations made upon unpacking and processing of shrimp samples from 91-52.

UAZ ID	Alaska ID	Species	Number in Sample	Females w/embryos	Grossly Normal	Shell Disease /Melt Wounds	Weight (grams)		
							Smallest	Average	Largest
52A	NSS 001	platceros	20	2	11	9	5.22	19.32	37.55
52B	NSS 002	platceros	20	4	5	10	9.48	27.22	44.60
52C	NSS 003	platceros	20	1	10	10	7.36	14.33	43.12
52D	NSS 004	platceros	20	0	14	6	9.44	13.72	40.28
52E	NSS 005	platceros	20	0	12	3	6.35	14.75	45.26
52F	NSS 006	platceros	20	0	17	3	5.32	8.88	19.97
52G	NSS 007	platceros	20	1	12	8	5.94	13.10	25.15
52H	NSS 008	platceros	20	0	14	6	5.05	12.96	23.55
52I	NSS 009	platceros	20	0	13	7	5.88	9.38	15.95
52J	NSS 010	platceros	20	0	13	7	4.92	8.13	12.91
52K	NSS 011	platceros	20	0	15	5	6.63	8.98	11.48
52L	NSS 012	borealis	19	6	19	0	1.59	2.75	7.85

Table 2. The generalized scheme for assigning a numerical qualitative value to severity grade of infections, surface infestations, and disease syndrome severity as used in Table 3.

Severity Grade	Clinical or Histological Findings
0	No signs of infection by pathogen, parasite, or epicommensal present. No lesions characteristic of syndrome present.
1	Pathogen, parasite, or epicommensal present but in numbers or amounts just above diagnostic procedure minimum detection limits. Lesions characteristic of syndrome present, but "disease" not significant. Prognosis is for insignificant effect, except in developing infections by highly virulent pathogens.
2	Low to moderate numbers of pathogen, parasite, or epicommensal present. Light to moderate lesions characteristic of syndrome present. Prognosis is for possible production losses and or slight increases in mortality if no treatment (if treatable) is applied.
3	Moderate numbers of pathogen, parasite, or epicommensal present. Moderate to severe lesions characteristic of syndrome present. Potentially lethal prognosis if no treatment (if treatable) is applied.
4	High numbers of pathogen, parasite, or epicommensal present. Severe lesions characteristic of syndrome present. Lethal prognosis.

Table 3. Summary of histological observations on samples of Pandalus platyceros and P. borealis from the Alaska Game and Fish Department's OSIAR Shellfish Project \*.

UAZ ID No.	Alaska ID No.	Sex				F w/ <sup>1</sup> embryos	Inflammatory Lesions <sup>2</sup> Gills						Debris <sup>3</sup> on Gills				Lagenophrys				Melz. Cutic.: # w/signific. histopath <sup>4</sup>	
		F	M	M/F	N/D		G0	G1	G1-2	G2	G2-3	G3	G0	G1	G2	G3	G0	G1	G2	G3		
52A/1-10	NSS001	9	0	1	0	0	3	-	3	-	4	-	7	1	1	1	4	3	3	0	0	0
52B/1-10	NSS002	0	0	1	1	2	3	-	4	-	3	-	10	0	0	0	6	4	0	0	0	0
52C/1-10	NSS003	9	0	0	1	1	2	8	-	1	0	0	9	0	1	0	4	5	1	0	0	0
52D/1-10	NSS004	7	2	0	1	0	0	2	-	5	3	-	8	0	2	0	3	3	4	0	1	1
52E/1-10	NSS005	10	0	0	0	1	0	8	-	1	1	-	10	0	0	0	8	1	1	0	1	1
52F/1-10	NSS006	10	0	0	0	0	1	2	-	7	0	0	9	1	0	0	2	4	4	0	0	0
52G/1-10	NSS007	10	0	0	0	1	8	2	-	0	-	0	10	0	0	0	6	2	2	0	0	0
52H/1-10	NSS008	10	0	0	0	0	10	0	-	0	-	0	10	0	0	0	2	3	4	1	0	0
52I/1-10	NSS009	10	0	0	0	0	9	1	-	0	-	0	10	0	0	0	1	6	3	0	2	2
52J/1-10	NSS010	10	0	0	0	0	1	3	-	3	-	3	10	0	0	0	1	3	6	0	2	2
52K/1-10	NSS011	10	0	0	0	0	3	4	-	2	-	1	10	0	0	0	0	4	6	0	2	2
52L/1-10	NSS012	5	0	4	0	4	3	-	2	-	5	-	-	-	-	-	0	2	6	2	2	2

\* Abbreviations used in Table 3:

G = severity grade; see Table 2 for detailed definition.

F = female.

M = male.

M/F = functional hermaphrodite.

N/D = not determined.

Fem w/ = females with developing embryos on their pleopods.

<sup>1</sup> Embryos appear mostly normal and in some embryos organization of body segments, appendage buds, and distinct tissue types is apparent.

<sup>2</sup> Lesions range from multifocal necrosis, inflammation, hemocytic nodule formation, and melanization of areas in gill lamellae to marked hemocytic congestion and fibrosis of the hemocoel within the primary gill rachis of one or more gill processes.

<sup>3</sup> Debris: consisting of amorphous brown to black detrital-like material present on gills, but especially adjacent to areas of gills with the most advanced inflammatory lesions.

<sup>4</sup> Melanized cuticular lesions indicated here are located in areas likely to be the result of toxic or bacterial etiology and not mechanical trauma.

## FIGURE LEGENDS

Figure 1. Histological sections of normal or near normal gills from Pandalus platyceros and P. borealis. All H&E staining.

- 1a. 91-52B/3 (NSS002); plat.; normal gills. X36.
- 1b. 91-52B/3 (NSS002); plat.; normal gills. X100.
- 1c. 91-52K/4 (NSS011); plat.; normal gills. X100.
- 1d. 91-52K/4 (NSS011); plat.; normal gills. X200.
- 1e. 91-52G/6 (NSS007); plat.; G1 edema and hemocytic congestion of hemocoel in central rachus. X40.
- 1f. 91-52L/4 (NSS012); borea.; G2 hemocytic congestion of hemocoel in central rachus. X200.

Figure 2. Histological sections of gills from Pandalus platyceros and P. borealis with varying grade of necrotic and inflammatory lesions. All H&E staining.

- 2a. 91-52L/4 (NSS012); borea.; G2 hemocytic congestion of central rachus and lamellae of a gill process tip. X180.
- 2b. 91-52J/10 (NSS010); plat.; G2-3 hemocytic congestion, edema, and fibrosis of central rachus and G2 multifocal hemocytic lesions in lamellae. G2 Lagenophrys sp. X90.
- 2c. 91-52D/8 (NSS004); plat.; multifocal G3 lesions in lamellae and central rachus. X100.
- 2d. 91-52B/6 (NSS002); plat.; G2-3 hemocytic congestion, edema and fibrosis of central rachus and lamellae. X180.
- 2e. 91-52L/4 (NSS012); borea.; G3 multifocal hemocytic lesion in lamellae. G2 Lagenophrys sp. X190.
- 2f. 91-52D/8 (NSS004); plat.; large G3 focal melanized hemocytic lesion and a G3 generalized hemocytosis affecting most of a single gill process. X33.

Figure 3. Histological sections of gills, cuticle and heart from Pandalus platyceros. All H&E staining.

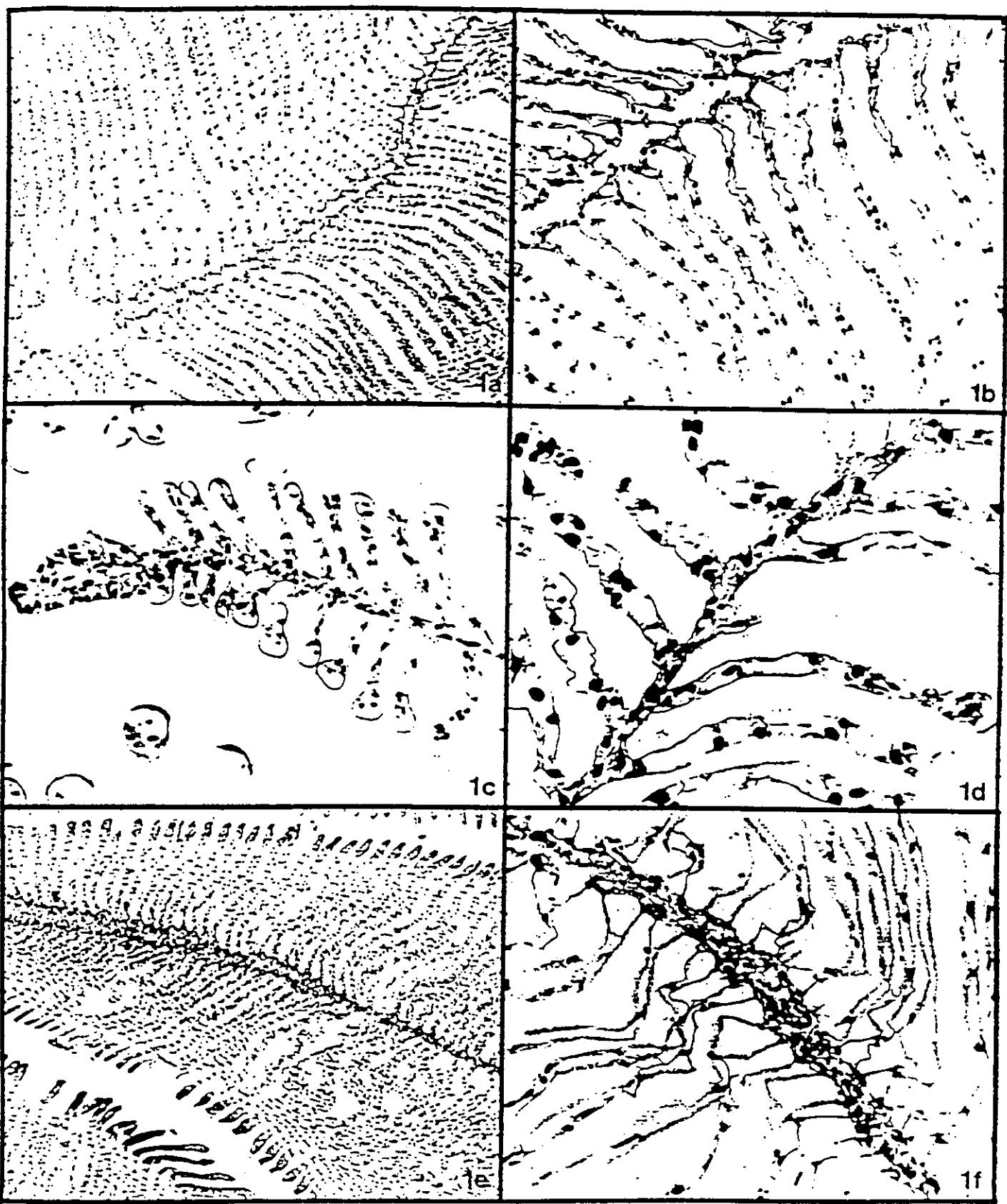
- 3a. 91-52B/3 (NSS002); plat.; G2 generalized to multifocal hemocytic congestion of gill lamellae; G2 edema, congestion and fibrosis of gill rachus; and G3 debris between adjacent lamellae. X100.
- 3b. 91-52B/3 (NSS002); plat.; G2 generalized to multifocal hemocytic congestion of gill lamellae; G2 edema, congestion and fibrosis of gill rachus; and G3 debris between adjacent lamellae. X200.
- 3c. 91-52D/8 (NSS004); plat.; focal melanized cuticular "shell disease" lesion on a maxilliped. X140.
- 3d. 91-52H/9 (NSS008); plat.; cytoplasmic inclusions in fixed phagocytes (or possibly reserve cells as these were very common in subcutis) in heart, which contain basophilic granules within an eosinophilic mass. X410.

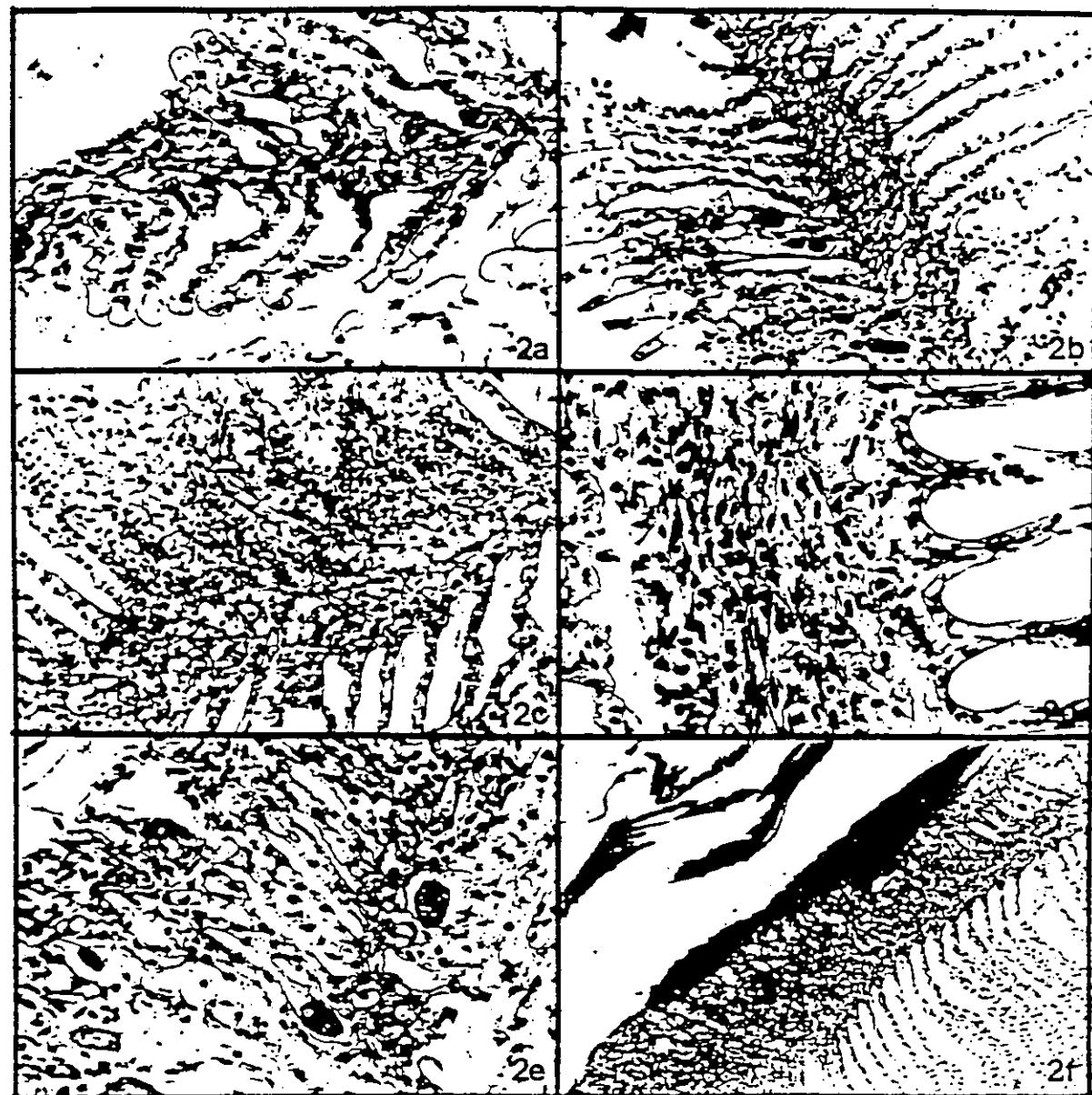
Figure 4. Histological sections of significant epicommensal and parasitic organisms from Pandalus platyceros and P. borealis. All H&E staining.

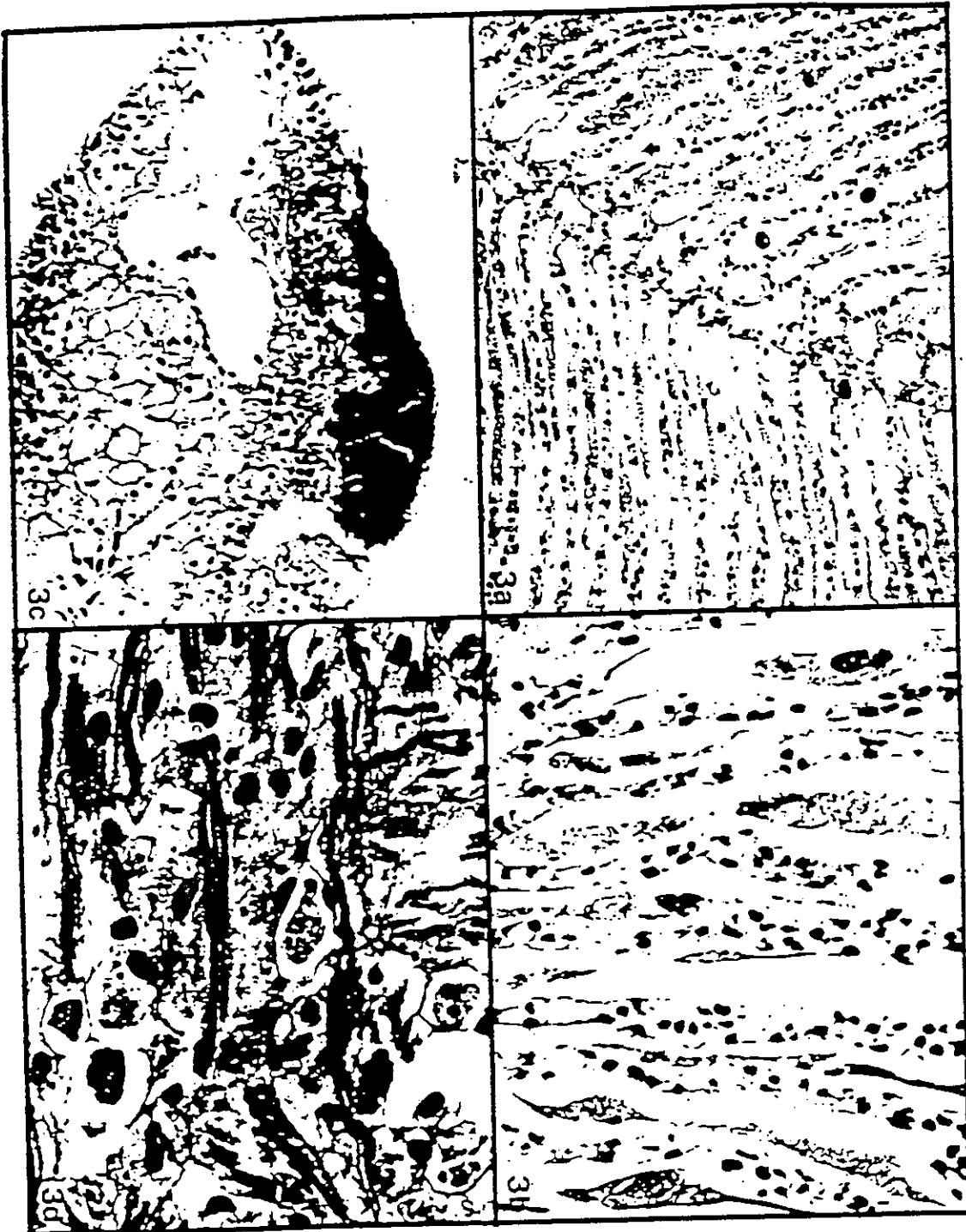
- 4a. 91-52I/3 (NSS009); plat.; G2 Lagenophrys sp. on gill lamellae. X2 20.
- 4b. 91-52I/3 (NSS009); plat.; G2 Lagenophrys sp. on gill lamellae. X410.
- 4c. 91-52L/8 (NSS012); borea.; presumed nemertean worms on gills. X400.
- 4d. 91-52L/8 (NSS012); borea.; presumed nemertean worms on gills. X215.
- 4e. 91-52L/2 (NSS012); borea.; clusters of an unidentified amoeboid protozoan parasite in loose connective tissue of the subcutis. X410.
- 4f. 91-52L/2 (NSS012); borea.; clusters of an unidentified amoeboid protozoan parasite in the lumen of the heart. X650.

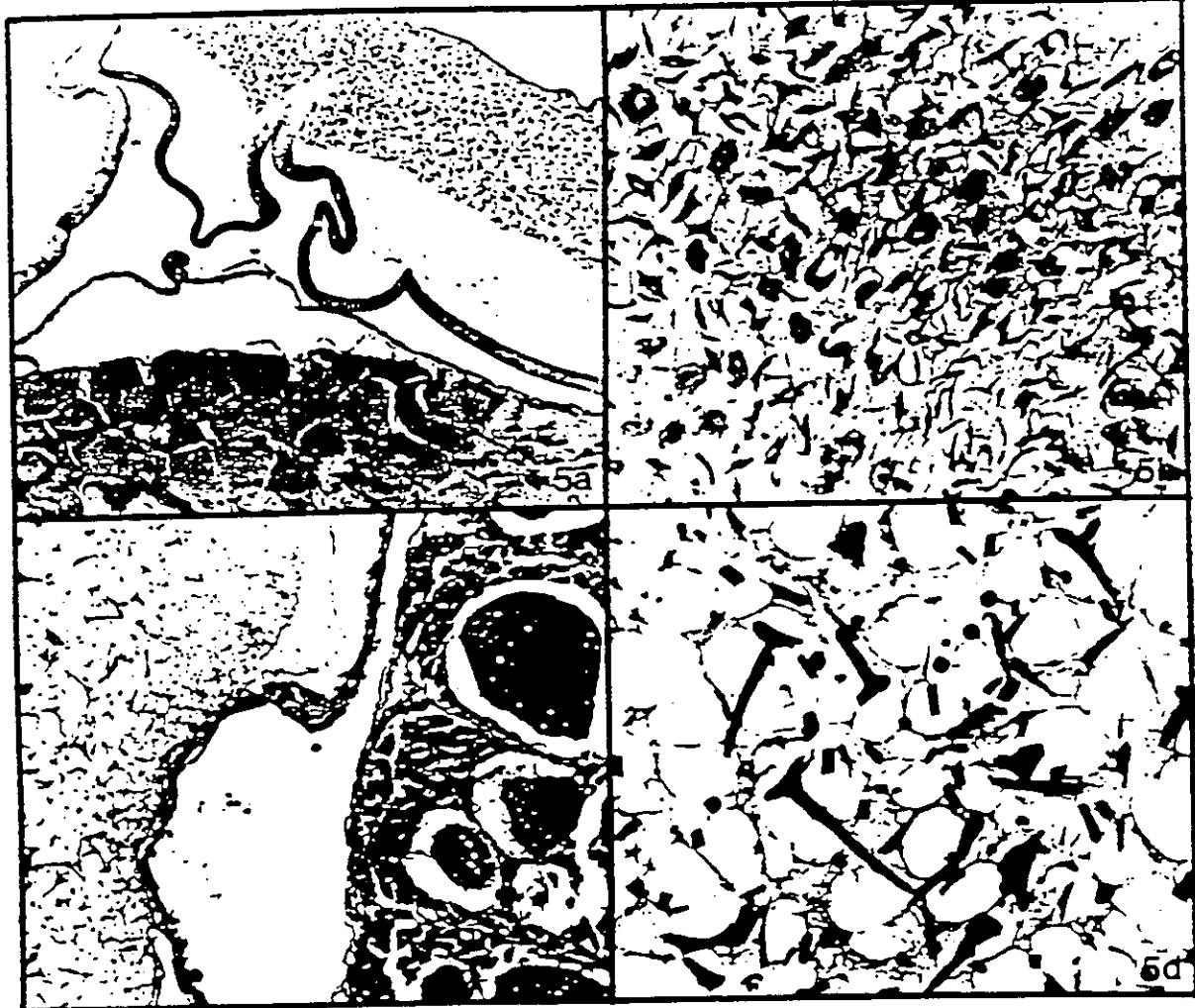
Figure 5. Histological sections of gonadal tissues from Pandalus platyceros and P. borealis. All H&E staining.

- 5a. 91-52L/10 (NSS012); borea.; gonad lobes with ovary (bottom) and testis (top). X90.
- 5b. 91-52L/10 (NSS012); borea.; mature sperm in testis lobe. X410.
- 5c. 91-52A/6 (NSS001); plat.; gonad lobes with ovary (right) and testis (left). X100.
- 5d. 91-52A6 (NSS001); plat.; mature sperm in testis lobe. X410.











**APPENDIX D.**

**Tables of Hydrocarbon and Histopathology Samples**

Site	Stratum	Station	Hydrocarbon		Analysis	Results
			Sample Number	Sample Type		
1	1	A	HSS001E	Egg	Analyzed	no oil
1	1	A	HSS001M	Muscle	None	
1	1	A	HSS002E	Egg	None	
1	1	A	HSS002M	Muscle	None	
1	1	B	HSS003E	Egg	Analyzed	no oil
1	1	B	HSS003M	Muscle	None	
1	2	A	HSS004E	Egg	None	
1	2	A	HSS004M	Muscle	None	
1	2	A	HSS005E	Egg	None	
1	2	A	HSS005M	Muscle	None	
1	2	B	HSS006E	Egg	None	
1	2	B	HSS006M	Muscle	None	
1			HSS007FB	Field Blank	None	
2	1	B	HSS008E	Egg	Analyzed	no oil
2	1	B	HSS008M	Muscle	None	
2	1	A	HSS009E	Egg	Analyzed	no oil
2	1	A	HSS009M	Muscle	None	
2	1	B	HSS010E	Egg	Analyzed	no oil
2	1	B	HSS010M	Muscle	None	
2	2	B	HSS011E	Egg	None	
2	2	B	HSS011M	Muscle	None	
2	2	B	HSS012M	Muscle	None	
2	2	A	HSS013E	Egg	None	
2	2	A	HSS013M	Muscle	None	
2			HSS014FB	Field Blank	None	
3	1	B	HSS015E	Egg	Analyzed	no oil
3	1	B	HSS015M	Muscle	None	
3	1	B	HSS016M	Muscle	None	
3	2	B	HSS017E	Egg	Analyzed	no oil
3	2	B	HSS017M	Muscle	None	
3	2	A	HSS018E	Egg	None	
3	2	A	HSS018M	Muscle	None	
3	2	A	HSS019E	Egg	None	
3	2	A	HSS019M	Muscle	None	
3	1	A	HSS020M	Muscle	None	
3			HSS021FB	Field Blank	None	

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Site	Stratum	Station	Hydrocarbon Sample Number	Sample Type	Analysis	Results
4	2	A	HSS022E	Egg	Analyzed	no oil
4	2	A	HSS022M	Muscle	None	
4	2	A	HSS023E	Egg	Analyzed	no oil
4	2	A	HSS023M	Muscle	None	
4	1	B	HSS024E	Egg	Analyzed	no oil
4	1	B	HSS024M	Muscle	None	
4	1	B	HSS025E	Egg	Analyzed	no oil
4	1	B	HSS025M	Muscle	None	
4	2	B	HSS026E	Egg	Analyzed	no oil
4	2	B	HSS026M	Muscle	None	
4	1	A	HSS027E	Egg	Analyzed	no oil
4	1	A	HSS027M	Muscle	None	
4			HSS028FB	Field Blank	None	
5	2	B	HSS029E	Egg	Analyzed	no oil
5	2	B	HSS029M	Muscle	None	
5	2	B	HSS030M	Muscle	None	
5	1	B	HSS031E	Egg	Analyzed	no oil
5	1	B	HSS031M	Muscle	None	
5	1	A	HSS032E	Egg	Analyzed	no oil
5	1	A	HSS032M	Muscle	None	
5	1	B	HSS033E	Egg	Analyzed	no oil
5	1	B	HSS033M	Muscle	None	
5	2	A	HSS034M	Muscle	None	
5			HSS035FB	Field Blank	None	
6	1	A	HSS036M	Muscle	None	
6	1	A	HSS037M	Muscle	None	
6	1	B	HSS038M	Muscle	None	
6	2	B	HSS039M	Muscle	None	
6	2	A	HSS040M	Muscle	None	
6			HSS042FB	Field Blank	None	

Site	Stratum	Station	Hydrocarbon		Analysis	Results
			Sample Number	Sample Type		
1	1	A	HSS043E	Egg	None	
1	1	A	HSS043M	Muscle	None	
1	1	A	HSS044E	Egg	None	
1	1	A	HSS044M	Muscle	None	
1	2	A	HSS045E	Egg	None	
1	2	A	HSS045M	Muscle	None	
1	2	A	HSS046E	Egg	None	
1	2	A	HSS046M	Muscle	None	
1	2	B	HSS047E	Egg	None	
1	2	B	HSS047M	Muscle	None	
1	1	B	HSS048E	Egg	None	
1	1	B	HSS048M	Muscle	None	
1			HSS049FB	Field Blank	None	
2	1	A	HSS050E	Egg	None	
2	1	A	HSS050M	Muscle	None	
2	1	A	HSS051E	Egg	None	
2	1	A	HSS051M	Muscle	None	
2	1	B	HSS052E	Egg	None	
2	1	B	HSS052M	Muscle	None	
2	2	B	HSS053E	Egg	None	
2	2	B	HSS053M	Muscle	None	
2	2	A	HSS054E	Egg	None	
2	2	A	HSS054M	Muscle	None	
2	2	B	HSS055E	Egg	None	
2	2	B	HSS055M	Muscle	None	
2			HSS056FB	Field Blank	None	
3	1	B	HSS057M	Muscle	None	
3	1	B	HSS058M	Muscle	None	
3	1	A	HSS059M	Muscle	None	
3	2	C	HSS060E	Egg	None	
3	2	C	HSS060M	Muscle	None	
3	2	A	HSS061M	Muscle	None	
3	2	B	HSS062E	Egg	None	
3	2	B	HSS062M	Muscle	None	
3			HSS063FB	Field Blank	None	

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Site	Stratum	Station	Hydrocarbon Sample Number	Sample Type	Analysis	Results
4	1	A	HSS064M	Muscle	None	
4	1	C	HSS065E	Egg	None	
4	1	C	HSS065M	Muscle	None	
4	1	B	HSS066M	Muscle	None	
4	2	B	HSS067E	Egg	None	
4	2	B	HSS067M	Muscle	None	
4	2	A	HSS068E	Egg	None	
4	2	A	HSS068M	Muscle	None	
4	2	B	HSS069E	Egg	None	
4	2	B	HSS069M	Muscle	None	
4			HSS070FB	Field Blank	None	
5	1	A	HSS071E	Egg	None	
5	1	A	HSS071M	Muscle	None	
5	1	A	HSS072E	Egg	None	
5	1	A	HSS072M	Muscle	None	
5	1	B	HSS073E	Egg	None	
5	1	B	HSS073M	Muscle	None	
5	2	B	HSS074E	Egg	None	
5	2	B	HSS074M	Muscle	None	
5	2	C	HSS075E	Egg	None	
5	2	C	HSS075M	Muscle	None	
5	2	A	HSS076E	Egg	None	
5	2	A	HSS076M	Muscle	None	
6	2	B	HSS077M	Muscle	None	
6	2	B	HSS078E	Egg	None	
6	2	B	HSS078M	Muscle	None	
6	2	B	HSS079M	Muscle	None	
6			HSS080FB	Field Blank	None	
6	1	A	HSS081E	Egg	None	
6	1	A	HSS081M	Muscle	None	
6	1	B	HSS082M	Muscle	None	
6	1	C	HSS083M	Muscle	None	
6	2	C	HSS084E	Egg	None	

Site	Stratum	Station	Hydrocarbon		
			Sample Number	Sample Type	Analysis
1	1	A	128601	Egg	None
1	1	A	128602	Muscle	None
1	1	A	128603	Egg	None
1	1	A	128604	Muscle	None
1	1	B	128605	Egg	None
1	1	B	128606	Muscle	None
1	2	A	128607	Egg	None
1	2	A	128608	Muscle	None
1	2	A	128609	Egg	None
1	2	A	128610	Muscle	None
1	2	B	128611	Egg	None
1	2	B	128612	Muscle	None
1			128613	Field Blank	None
2	1	B	128614	Egg	None
2	1	B	128615	Muscle	None
2	1	B	128616	Egg	None
2	1	B	128617	Muscle	None
2	2	A	128618	Egg	None
2	2	A	128619	Muscle	None
2	2	A	128620	Egg	None
2	2	A	128621	Muscle	None
2	1	A	128622	Muscle	None
2	1	A	128623	Egg	None
2	2	B	128624	Egg	None
2	2	B	128625	Muscle	None
2	2	B	128626	Field Blank	None
2			128627	Field Blank	None
3	1	A	128628	Egg	None
3	1	A	128629	Muscle	None
3	2	A	128630	Egg	None
3	2	A	128631	Muscle	None
3	2	B	128632	Muscle	None
3	2	C	128633	Egg	None
3	2	C	128634	Muscle	None
3	1	B	128635	Egg	None
3	1	B	128636	Muscle	None
3	1	B	128637	Muscle	None
3	1	B	128638	Egg	None
3			128639	Field Blank	None

-Continued-

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Site	Stratum	Station	Hydrocarbon Sample Number	Sample Type	Analysis	Results
4	1	B	128640	Egg	None	
4	1	B	128641	Muscle	None	
4	1	C	128642	Muscle	None	
4	2	B	128643	Muscle	None	
4	2	A	128644	Egg	None	
4	2	A	128645	Muscle	None	
4	2	A	128646	Egg	None	
4	2	A	128647	Muscle	None	
4	1	B	128648	Muscle	None	
4			128649	Field Blank	None	
5	1	B	128650	Egg	None	
5	1	B	128801	Muscle	None	
5	1	B	128802	Egg	None	
5	1	B	128803	Muscle	None	
5	1	A	128804	Egg	None	
5	1	A	128805	Muscle	None	
5	2	B	128806	Egg	None	
5	2	B	128807	Muscle	None	
5	2	C	128808	Egg	None	
5	2	C	128809	Muscle	None	
5	2	C	128810	Egg	None	
5	2	C	128811	Muscle	None	
5			128812	Field Blank	None	
6	1	A	128813	Muscle	None	
6	1	A	128814	Muscle	None	
6	1	B	128815	Muscle	None	
6			128816	Field Blank	None	

Samples from the November, 1991 oil spill assessment survey  
of spot shrimp in Prince William Sound, collected for hydro-  
carbon contamination assessment.

Site	Stratum	Station	Hydrocarbon			Analysis	Results
			Sample Number	Sample Type			
1	1	A	200401	Muscle		None	
1	1	A	200402	Egg		None	
1	1	A	200403	Muscle		None	
1	1	A	200404	Egg		None	
1	1	B	200405	Muscle		None	
1	1	B	200406	Egg		None	
1	2	A	200407	Muscle		None	
1	2	A	200408	Egg		None	
1	2	A	200409	Muscle		None	
1	2	A	200410	Egg		None	
1	2	B	200411	Muscle		None	
1	2	B	200412	Egg		None	
1			200413	Field Blank		None	
2	1	A	200414	Muscle		None	
2	1	A	200415	Egg		None	
2	2	A	200416	Muscle		None	
2	2	A	200417	Egg		None	
2	2	A	200418	Muscle		None	
2	2	A	200419	Egg		None	
2	1	B	200420	Muscle		None	
2	1	B	200421	Egg		None	
2	1	B	200422	Muscle		None	
2	1	B	200423	Egg		None	
2	2	C	200424	Muscle		None	
2	2	C	200425	Egg		None	
2			200426	Field Blank		None	
3	1	A	200427	Muscle		None	
3	1	B	200428	Muscle		None	
3	1	B	200429	Muscle		None	
3	2	C	200430	Muscle		None	
3	2	C	200431	Egg		None	
3	2	B	200432	Muscle		None	
3	2	B	200433	Egg		None	
3	2	A	200434	Muscle		None	
3	2	A	200435	Egg		None	
3			200436	Field Blank		None	

-Continued-

Appendix D. 4. (page 2 of 2)

Site	Stratum	Station	Hydrocarbon		Analysis	Results
			Sample Number	Sample Type		
4	1	A	200450	Muscle	None	
4	1	A	200501	Egg	None	
4	1	A	200502	Muscle	None	
4	1	A	200503	Egg	None	
4	1	B	200504	Muscle	None	
4	1	B	200505	Egg	None	
4	2	B	200506	Muscle	None	
4	2	B	200507	Egg	None	
4	2	C	200508	Muscle	None	
4	2	C	200509	Egg	None	
4	2	C	200510	Muscle	None	
4	2	C	200511	Egg	None	
4			200512	Field Blank	None	
5	1	A	200437	Muscle	None	
5	1	A	200438	Egg	None	
5	1	A	200439	Muscle	None	
5	1	A	200440	Egg	None	
5	2	A	200441	Muscle	None	
5	2	A	200442	Egg	None	
5	2	A	200443	Muscle	None	
5	2	A	200444	Egg	None	
5	2	B	200445	Muscle	None	
5	2	B	200446	Egg	None	
5	1	B	200447	Muscle	None	
5	1	B	200448	Egg	None	
5			200449	Field Blank	None	
6	1	A	200513	Muscle	None	
6	1	B	200514	Muscle	None	
6	1	B	200515	Muscle	None	
6	2	A	200516	Muscle	None	
6	2	D	200517	Muscle	None	
6	2	D	200518	Muscle	None	
6			200519	Field Blank	None	

## Appendix D. 5.

Samples taken from the November, 1989 oil spill assessment survey of spot shrimp of Prince William Sound, for histopathology analysis.

Site	Stratum	Station	Histopathology	
			Sample Number	Analysis
1	1	A	NSS001	Analyzed
1	2	B	NSS002	Analyzed
2	1	A	NSS003	Analyzed
2	1	B	NSS004	Analyzed
3	1	A	NSS005	Analyzed
3	2	B	NSS006	Analyzed
4	1	A	NSS007	Analyzed
4	2	B	NSS008	Analyzed
5	1	A	NSS009	Analyzed
5	2	A	NSS010	Analyzed
6	1	A	NSS011	Analyzed
6	2	B	NSS012P	Analyzed

Appendix D. 6.

Samples taken from the March, 1990 oil spill assessment survey of spot shrimp of Prince William Sound, for histopathology analysis.

Site	Stratum	Station	Histopathology Sample Number	Analysis
1	1	A	NSS013	None
1	2	A	NSS014	None
4	1	B	NSS015	None
4	2	B	NSS016	None
5	1	A	NSS017	None
5	2	B	NSS018	None

## Appendix D. 7.

Samples taken from the November, 1990 oil spill assessment survey of spot shrimp of Prince William Sound, for histopathology analysis.

Site	Stratum	Station	Histopathology Sample Number	Analysis
1	2	B	128701	None
1	1	B	128702	None
2	2	A	128703	None
3	1	B	128704	None
3	2	D	128705	None
4	1	A	128706	None
4	2	C	128707	None
5	2	A	128708	None
5	1	B	128709	None
6	1	A	128710	None

## Appendix D. 8.

Samples taken from the November, 1991 oil spill assessment survey of spot shrimp of Prince William Sound, for histopathology analysis.

Site	Stratum	Station	Histopathology	
			Sample Number	Analysis
1	1	A	128711	None
1	2	A	128712	None
2	1	A	128713	None
2	2	A	128714	None
3	1	A	128715	None
3	2	A	128716	None
5	1	A	128717	None
5	2	A	128718	None
4	1	A	128719	None
4	2	A	128720	None
6	1	A	128721	None
6	2	A	128722	None
7	1	A	128723	None