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

Injury to Demersal Rockfish and Shallow Reef Habitats in Prince William Sound, 1989-1991

Subtidal Study Number 6
(Fish/Shellfish Study Number 17)
Final Report

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Study History: Subtidal Study Number 6 was initiated as Fish/Shellfish Study Number 17 (Injury to Prince William Sound Rockfish) as part of the State/Federal Natural Resource Damage Assessment (NRDA) Plan in 1989 and 1990, as necropsies of dead demersal rockfish collected immediately after the spill indicated mortality due to exposure to hydrocarbons. In 1991, the project was included in the Damage Asssessment Plan as Subtidal Study Number 6 (Injury to Demersal Rockfish and Shallow Reef Habitats in Prince William Sound and along the Lower Kenai Peninsula).

Abstract: Demersal rockfish tissues were collected at four sites (two oiled and two unoiled) in Prince William Sound in both 1990 and 1991 and at four sites (two oiled and two unoiled) along the outer Kenai Peninsula in 1990. Analysis of hydrocarbon data showed that there was a significantly higher incidence of hydrocarbons in the bile of rockfish from oiled areas than unoiled areas in 1989 (P=0.005), however there were no significant differences in 1990 (P=0.933) or 1991 (P=0.844). In 1990, nine histopathologic lesions were scored by pathologists, and in 1991, 26 different lesions were scored, indicating there were significant differences between unoiled and oiled sites in two of the nine lesion scores, liver lipidosis (P=0.0086) and liver glycogen depletion (P=0.0005) in 1990; and two of the 26 lesion scores, liver lipidosis (P=0.0006) and kidney lymphocytes (P=0.0005) in 1991. No differences in lesion scores were seen between sites on the outer Kenai Peninsula in 1990. After histopathologic examination, the pathologists accurately "predicted" which sites were oiled based on qualitative analysis of semiquantitative lesion scores for all four sites in Prince William Sound. Subsequent principal component analysis indicated differences in oiled and unoiled sites in both 1990 and 1991. Differences were more definitively indicated in 1991 than in 1990 using this analysis.

Key Words: Demersal rockfish, Sebastes spp., histopathological analysis, lesion scores, *Exxon Valdez*, Prince William Sound, outer Kenai Peninsula.

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EXECUTIVE SUMMARY

Demersal rockfish (Sebastes spp.) in Prince William Sound were studied from 1989 through 1991 to assess injury due to the *Exxon Valdez* oil spill. Injury was assessed by establishing the exposure of rockfish to petroleum hydrocarbons and then determining if any histopathological lesions occurred with increased frequency in fish from sites with oil-exposed fish.

Dead and dying rockfish were reported from several areas in the path of the *Exxon Valdez* oil spill. Twelve dead rockfish from sites of reported fish kills were examined at collection centers in Valdez and Cordova. Five of these rockfish were fresh enough to be necropsied and exposure to crude oil was found to be the cause of death of all five. These results prompted additional testing for hydrocarbon concentrations in rockfish. The 1989 hydrocarbon analyses showed that at least 11 of the 36 rockfish tested from oiled sites had been exposed to oil within the two weeks prior to collection, while none of the 13 fish in unoiled sites were exposed to oil. This information prompted the 1990 and 1991 studies to sample rockfish for continued exposure to hydrocarbon concentrations and to include histopathological evaluations of rockfish tissues.

Tissues were collected from several species of demersal rockfish for histopathological evaluation and hydrocarbon analysis. Rockfish were collected at four sites (two oiled and two unoiled) in Prince William Sound in both 1990 and 1991. Four sites (two oiled and two unoiled) were also sampled along the outer Kenai Peninsula in 1990, but not in 1991.

The proportion of fish from oiled sites with evidence of exposure to hydrocarbons was compared to the proportion of exposed fish from unoiled sites. Evidence of exposure to hydrocarbons was indicated by elevated concentrations of phenanthrene and naphthalene equivalent compounds in the bile, in concert with chromatographic patterns characteristic of hydrocarbons. Analysis of hydrocarbon data showed that there was a significantly higher incidence of hydrocarbons in the bile of fish from oiled areas than unoiled areas in 1989 ($P=0.005$), however there were no significant differences in 1990 ($P=0.933$) or 1991 ($P=0.844$).

In 1990, nine histopathologic lesions were scored by pathologists, and in 1991, 26 different lesions were scored. Analysis of these lesion scores from rockfish in Prince William Sound in 1990 and 1991 indicated that rockfish were potentially exposed to toxic agents. There were significant differences between unoiled and oiled sites in Prince William Sound in two of the nine lesion scores, liver lipidosis ($P=0.0086$) and liver glycogen depletion ($P=0.0005$) in 1990; and two of the 26 lesion scores, liver lipidosis ($P=0.0006$) and kidney lymphocytes ($P=0.0005$) in 1991. No differences in lesion scores were seen between sites on the outer

Kenai Peninsula in 1990.

The histopathologic evaluation was conducted blind, that is, pathologists did not know if the tissues were from fish from oiled or unoiled sites. Upon completion of the histopathologic examination, the pathologists "predicted" which sites were oiled based on qualitative analysis of semiquantitative lesion scores. The speculated exposure history was accurate for all four sites in Prince William Sound. Subsequent principal component analysis indicated differences in oiled and unoiled sites in both 1990 and 1991. Differences were more definitively indicated in 1991 than in 1990 using this analysis.

INTRODUCTION

Demersal rockfish were studied as part of the Natural Resource Damage Assessment (NRDA) from 1989 through 1991 to assess potential injury due to the March 24, 1989 *Exxon Valdez* oil spill (EVOS). Evidence, documented by necropsies of dead demersal rockfish collected immediately after the spill, indicated mortality due to exposure to hydrocarbons (Moles 1990). This information prompted initiation of studies to sample rockfish for injuries resulting from the spill. Injury was assessed by determining the presence or absence of hydrocarbon concentrations and histopathological evidence of exposure in the tissues of rockfish that inhabit reefs located in oiled and unoiled sites in the study area. Additional components were added to the study to determine potential routes of hydrocarbon exposure in rockfish.

Demersal rockfish usually take up residence near a rocky reef or boulder field. The potential impact of the oil spill on various nearshore assemblages is dependent upon the location of various rockpiles relative to the route of the oil. The potential uptake of various contaminants could be related to the level of oil contamination and food web characteristics of these reefs. Of primary importance are questions of transport of oil to subsurface habitats and the potential for residual persistence of this contamination. Khan (1987) reports that crude oil can contaminate sediments and persist for long periods of time in the environment. According to Rubin (1988), in areas where fresh oil becomes trapped in sediments and is later released after subsequent disturbance, as occurred in the Amoco Cadiz spill, impacts as described below may result:

> "The ultimate fate of sunken oil depends upon the energy regime, sedimentation rates, quantities of sunken oil, bioturbation, and substrate properties. Petroleum associated with sediments persists for years or perhaps decades, undergoing slow biodegradation. Stranded petroleum is often buried, but may be introduced to the water column during seasonal erosion cycles especially on high energy beaches. In stable nearshore sediments and offshore regions, sedimented petroleum may be dispersed laterally by currents, biodegraded, and incorporated into the food chain. Eventually, a substantial amount of sedimented oil may be buried deep in stable sediments, effectively preventing further interaction with biological agents. Quantitative pathways for sunken petroleum before its ultimate isolation in deep sediments and time scale for these processes are largely unknown".

Under these conditions, the petroleum hydrocarbons can exert a broad range of effects on animals, from impaired feeding, growth, reproduction, and behavior to tissue and organ damage and changes in parasite densities (Khan 1986; Khan 1987; Kiceniuk and Khan

1986; Rice 1985; Wennekens et al. 1975; Malins et al. 1977; Rice et al. 1977; Hose et al. 1987; Gundlach et al. 1983). These possible effects are especially critical to demersal rockfish since they are long-lived, recruitment is low, and the potential for long-term stock decline due to chronic exposure to crude oil is high. This study was initiated to determine long term histopathological effects on the fish and quantify the extent to which hydrocarbons persist in the environment.

In 1989, samples were collected for analysis of hydrocarbons in various rockfish tissues. Studies were expanded in 1990 and 1991 to better document injuries through the addition of histopathological evaluation and to attempt to determine the route of hydrocarbon exposure in rockfish. To help determine the route of exposure, rockfish food (stomach contents) and surroundings (sediment and sessile invertebrates) were collected for hydrocarbon analysis. This document constitutes a final report of studies related to evaluating the effects of the EVOS on demersal rockfish and shallow reef habitats in Prince William Sound (PWS) following the third year of study.

OBJECTIVES

1. Determine the exposure of demersal rockfish to oil through hydrocarbon analysis of the bile and tissues.

2. Determine the occurrence of injury in demersal rockfish resulting from oil exposure through histopathological examination of tissues, mixed function oxidase enzyme system activity in liver tissue, and circulating erythrocyte micronuclei in blood.

3. Determine potential routes of rockfish exposure to oil through hydrocarbon analysis of stomach contents, sediments and benthic suspension feeders collected from the same sites.

4. Determine the feasibility of using microstructure of otoliths from juvenile rockfish to evaluate depressed growth as a result of oil exposure.

METHODS

The Alaska Department of Fish and Game (ADF&G) collected the samples for this project, however most analyses were conducted by contractors specializing in each specific type of analysis. The various contractors are identified as each type of analysis is discussed. This section describes the methods used to secure the samples and a description of how samples were processed by ADF&G for transfer to the various contractors. As contractors completed analyses, the results were returned for statistical analysis by the ADF&G biometrician.

In 1989, tissue samples were collected from all species of rockfish that were captured. In 1990, only demersal species were sampled in order to focus on the more bottom dwelling species which were the species found dead immediately after the spill. In 1991, only tissues from the three most common species of demersal rockfish, copper *Sebastes caurinus*, quillback *S. maliger*, and yelloweye *S. rubberimus*, were collected in order to reduce potential variation due to species. In 1989, rockfish tissues were collected for hydrocarbon analysis only. In 1990 and 1991 rockfish tissues were collected for hydrocarbon analysis and histopathological evaluation. Also, in 1990 and 1991, rockfish stomach contents, unconsolidated benthic sediments, and sessile suspension feeders were collected at each study site for analysis of hydrocarbons.

Site Selection

Samples were collected at 30 sites during the 1989 study, 16 in PWS (10 oiled and six unoiled) and 14 from the outer Kenai Peninsula (10 oiled and four unoiled)(Table 1, Figure 1). In 1990, eight sites were sampled; four in PWS (two oiled and two unoiled) and four along the outer Kenai Peninsula (two oiled and two unoiled) (Table 1, Figure 2). Sites sampled in PWS during 1991 were the same as those sampled in 1990, however no samples were collected from the outer Kenai Peninsula in 1991 based on recommendations of the peer reviewers.

Criteria for choosing the sampling sites were based on: documented exposure or lack of exposure of surface waters to oil, locations of reported kills or potential exposure of demersal rockfish, occurrence of sampling by other oil spill assessment studies related to this study, and availability of pre-spill fish community information (Rosenthal 1980).

Sample Sizes

Target sample sizes varied based on the type of analysis. Tissues from a sample size of ten fish (NMFS 1989) were collected in all years for hydrocarbon analysis. Tissue samples for histopathologic evaluation from 15 rockfish were collected in 1990 (Meyers, 1989). This was increased to 30 in 1991 in order to increase the power of the statistical tests. Species identification of adult rockfish was verified using the methods of Kramer and O'Connell (1988) and Hart (1973). A goal of fifty juvenile demersal rockfish, for examination of otolith microstructure, was set for each site. This was determined given estimated proportions of otoliths with and without stress checks of 0.6 and 0.2, where $\alpha = 0.05$ (Zar 1984). Species identification of juveniles was accomplished using methods of Matarese et al. (1989).

Sample Collection Techniques

The rockfish were collected using one of three different techniques depending upon the water conditions and relative success of each technique. Techniques used were rod and reel jigging, SCUBA divers using spears (used only in 1990 and 1991), or longlining. Jigging was the preferred technique because it was the least damaging to the fish and allowed the fish to be sampled most quickly. It was important for histopathological analysis that the samples be as fresh as possible. When a fish was on the line it was retrieved slowly to allow the air bladder to equilibrate in order to reduce the occurrence of stomach extrusion and regurgitation of its contents. When hook and line techniques did not yield results, divers tried to collect additional rockfish using spears. Longlining was used when adequate numbers of fish could not be collected using the other two techniques. Juvenile rockfish were collected using the same capture techniques as used for adult rockfish, excluding longlining. All fish caught were measured (fork length) and following dissection, both sagittal otoliths were removed and stored dry in coin envelopes labeled with location, species, date, length and a project identification number.

Hydrocarbon Procedures:

Samples for hydrocarbon analysis were collected in accordance with procedures established by the NMFS, Analytical Chemistry Group (Manen 1989) as presented in the Natural Resource Damage Assessment sample collection training sessions held in May of 1990. The following procedures were used for each sample: (1) hands and sampling gear were washed with soap and water; (2) dissection tools were rinsed in methylene chloride; (3) samples of each tissue or sediment were individually stored in certified hydrocarbon-free sampling jars; and (4) samples were frozen immediately. Samples were not touched nor was there any contact with any petrochemical product (e.g., plastic).

Ten of the rockfish collected at each site were used for hydrocarbon analysis. Bile samples were collected first by removing the whole gall bladder and emptying the bile into 0.5-oz. amber sampling jars. Approximately ten grams each of liver, muscle, and gonad tissue and stomach contents were then collected from each rockfish. All tissue samples were collected from freshly killed fish and each tissue type was stored in separate 4 oz. sampling jars and frozen immediately. (Deviations from this procedure occurred in 1989; in some instances whole gall bladders were collected rather than just the bile and some fish were not processed immediately). Tissue samples from 206 rockfish were collected for hydrocarbon analysis at thirty sites in PWS and along the outer Kenai Peninsula during four sampling trips in 1989. Tissue types collected were: gall bladder (bile), stomach, pyloric caeca, liver, and muscle. In 1990 tissues of 79 rockfish from study sites in PWS and the outer Kenai Peninsula were collected for hydrocarbon analysis (there was inadequate bile volume in nine samples). In addition, stomach content, sediment, and invertebrate samples from each site were also collected for hydrocarbon analysis. In 1991 tissue samples of 40 rockfish from study sites in PWS were collected for hydrocarbon analysis (there was inadequate bile volume in two samples). In addition, stomach content, sediment and invertebrate samples from each site were also collected for hydrocarbon analysis.

These samples were transferred to the NMFS Auke Bay Laboratory for analysis. The analysis strategy established by NMFS was to first analyze the bile, then, if hydrocarbon exposure was indicated, analyze the other tissues (Manen 1989). The hydrocarbon analysis was conducted at Texas A&M University, a subcontractor of Auke Bay Lab, using gas chromatography. Analysts at Auke Bay interpreted the concentrations of phenanthrene and naphthalene equivalent compounds in the bile in concert with chromatographic patterns characteristic of hydrocarbons and designated the status of each sample and forwarded this information to ADF&G (Appendix A). The proportion of oiled sites containing contaminated samples was

compared to the proportion of unoiled sites with contaminated samples using a two-sampled Z-test (Zar 1984), where:

$$Z = \frac{\hat{P}_c - \hat{P}_t}{\sqrt{\bar{p}\bar{q}\left(\frac{1}{N_c} + \frac{1}{N_t}\right)}} \tag{1}$$

P_c = proportion of unoiled samples with evidence of exposure,

P_t = proportion in oiled samples with evidence of exposure,

N_c = number of unoiled samples,

N_t = number of oiled samples,

$$\bar{p} = \frac{(N_c\hat{P}_c + N_t\hat{P}_t)}{(N_t + N_c)}, \text{ and:} \tag{2}$$

$\bar{q} = 1 - \bar{p}.$

Histopathology Procedures:

Tissue samples were taken from all demersal rockfish collected at each site for histopathological analysis and processed under the guidelines outlined by the Histopathology Technical Group (Meyers 1989). One cubic centimeter sections of tissue were removed, stored in 10% buffered formalin and transferred to the University of California Davis, School of Veterinarian Medicine (UCD) for examination. Tissue types collected were based on recommendations from various peer reviewers. In 1990, liver, spleen, anterior kidney, eye, gonad and gill tissues were collected. In 1991, liver, spleen, anterior kidney, heart, and gill tissues were collected. All tissues were examined for histological evidence of exposure to hydrocarbons. In addition, sub-samples of the livers were made at UCD and shipped to Woods Hole Oceanographic Institute for analysis of the mixed function oxidase enzyme system (MFO) activity. Blood samples were collected from the caudal artery or the heart using a heparinized syringe. Smears were made on microscope slides and air dried for examination for circulating erythrocyte micronuclei (Hose et al 1987). Blood samples were sent to VANTUNA Research Group, Occidental College for examination.

Rockfish tissue samples collected in 1990 and 1991 were examined for histopathological abnormalities at UCD. The histopathological evaluations were conducted blind; that is, the pathologists did not know if the samples were from oiled or unoiled sites. A semi-quantitative score was given for each lesion type based on the occurrence and severity: none (0), mild (1), moderate (2), or severe (3). Therefore, each rockfish had a score for all lesion types examined: nine types in 1990 and 26 in 1991. Details of the methods used at UCD are presented in appendices B and C. Statistical analysis was conducted by both UC Davis and ADF&G. The analysis by UC Davis (Appendices B and C) was conducted to determine which lesions explained most of the variability. The ADF&G analysis, which is discussed here, was conducted to determine differences in lesions likely to be related to oil.

Categorical data analysis was used to test for significant differences in lesion scores between rockfish tissues collected from oiled and unoiled sites. A cumulative logit model was used to take into account the ordinal nature of the dependent variable (Agresti 1990). The CATMOD procedure in SAS (SAS 1987) was used to perform the analysis. A separate statistical analysis was performed for each lesion type. The level of significance was adjusted to maintain an overall probability of a type I error at 0.10 (Zar 1984). All species and sites within a treatment group were combined. A nonparametric correlation was used to test for a relationship between the concentration of hydrocarbon in the bile and the lesion score.

Age and Length Procedures:

Otoliths from adult rockfish were used to determine age using the break and burn procedures described by Chilton and Bemish (1982) and the Pacific Coast Groundfish Aging Technicians (1984). All age and length data are presented in Appendix B.

Otoliths from juvenile rockfish were examined to determine the feasibility of using microstructure to evaluate depressed growth as a result of exposure to hydrocarbons. Measurement of growth increments was attempted to detect changes in growth which could be correlated in time with the oil spill. Otoliths were prepared for examination following methods outlined by Secor et al (1991). Otoliths were ground on 400 grit silicon carbide sandpaper until the sulcus was removed, then polished on 600 grit silicon carbide sandpaper until the nucleus was visible. They were then etched with varying concentrations of Ethylene Diamine Tetraacetate (EDTA) or hydrochloric acid (HCl) for 45 minutes to 2.5 hours. Otoliths were mounted to scanning electron microscope (SEM) tabs and examined under low (30X) and high (1500X) magnification. Photographs were taken at each appropriate magnification. The photographs were digitized and analyzed by the ADF&G Commercial Fisheries aging lab.

RESULTS

Objective 1 - Exposure to Hydrocarbons

Results of the hydrocarbon analysis provided by Auke Bay Laboratory are included for all three years in Appendix A.

1989 Samples

Analysis was conducted on bile samples from 49 fish, 36 from oiled sites and 13 from unoiled sites. These samples were from only one of the four sampling trips. The bile samples collected during the other three trips were not usable because of improper sample collection or preservation methods. A degradation pattern on the chromatogram was present on eight bile samples, seven of the oiled site samples and one of the unoiled site samples. Therefore, no determination was made regarding the oiling status of those fish. Evidence of exposure to hydrocarbon was found in eleven of 29 usable oiled site samples. No evidence of exposure to hydrocarbons was found in the 12 usable bile samples from unoiled sites. There was a significant difference between proportions of exposed samples at unoiled and oiled sites for those samples analyzed ($Z=2.82$, $P=0.005$)(Figure 3). No subsequent tissue analyses were done.

1990 Samples

In PWS, indications of hydrocarbon exposure in the bile were shown in two out of the 17 fish from unoiled sites. Both positive bile samples were from the same site, Gravina Rocks. None of the 18 fish from oiled sites in PWS were positive for hydrocarbon exposure. There was no significant difference between unoiled and oiled sites in PWS ($Z=-1.48$, $P=0.931$). One fish, collected in Pony Cove, out of 16 fish from oiled sites along the outer Kenai Peninsula was exposed to hydrocarbons. None of the 19 samples from unoiled sites on the outer Kenai Peninsula indicated exposure to hydrocarbons. There was no significant difference between unoiled and oiled sites in the outer Kenai Peninsula ($Z=1.06$, $P=0.289$) (Figure 3). Because the results of the bile analysis did not indicate exposure to oil, none of the other samples (tissue, stomach content, sediment, or invertebrate) were analyzed.

1991 Samples

The bile was analyzed for the 38 fish for which bile samples were collected. Indications of hydrocarbon exposure was shown in one out of the 21 fish from unoiled sites. The positive sample was from Zaikof Bay, which had not tested positive in previous samples. None of the 17 fish from oiled sites in PWS were positive for hydrocarbon exposure. There was no significant difference between unoiled and oiled sites ($Z=0.957$, $P=0.832$) (Figure 3). Because the results of the bile analysis indicated that rockfish were not being exposed to oil, none of the other tissue, stomach content, sediment, or invertebrate samples were analyzed.

Objective 2 - Histopathological Analysis

1990 Samples

Tissues of 121 rockfish from study sites in PWS and the outer Kenai Peninsula were collected and sent to the UCD for histopathological evaluation and MFO analysis using the planned immunohistochemical detection. MFO analyses were not done because samples were inadvertently transferred to alcohol after receipt at UCD. This rendered the samples incompatible with MFO analysis using the planned immunohistochemical detection. Results of the 1990 histopathologic examination provided by the pathologists at UCD is presented in Appendix C.

Scores from a total of nine lesions types were reported by pathologists at UCD. Statistical analysis was done comparing the scores for each lesion between unoiled and oiled sites. In PWS two of the nine lesions had significantly higher scores for samples obtained in oiled areas (adjusted α=0.011). The two lesion types were liver lipidosis (P=0.0086) and liver glycogen depletion (P=0.0005). None of the lesion scores from oiled sites on the Kenai Peninsula were significantly greater than the unoiled sites. Graphics depicting mean lesion scores are presented on pages 114-117, as evaluated by Marty, et al. (1993).

Analysis was done to determine if there were correlations between the scores for the two significant lesions in PWS and the concentration of hydrocarbons found in the bile. There was no correlation between liver lipidosis scores and the concentrations of phenanthrene and naphthalene equivalent compounds (P=0.1483 and P=0.1664 respectively) and there was no correlation between liver glycogen depletion scores and the concentrations of phenanthrene and naphthalene equivalent compounds (P=0.512 and P=0.48 respectively)

Blood samples were collected from 78 rockfish in 1990 and sent to VANTUNA Research Group at Occidental College for erythrocyte micronuclei analyses. Results of this analyses are presented in Appendix D. Mean indices for all fish at each site ranged from 0.4/1000 red blood cells (RBC) at Granite Island to 0.1/1000 RBC from Pony Cove and Day Harbor. An analysis of variance indicated that there were no differences in the counts of erythrocyte micronuclei between unoiled and oiled sites.

1991 Samples

Tissue samples from 107 rockfish from four sites in PWS were sent to UCD for histopathological evaluation. Portions or slides of the liver from each fish were sent from UCD to Woods Hole for MFO analysis. Results of the 1991 histopathologic examination provided by the pathologists at UCD are presented in Appendix E.

A total of 26 different lesions types were scored in 1991. Statistical analysis was done comparing the score of lesions between unoiled and oiled sites. A separate statistical analysis was performed on each of the 26 lesion types. Tissues obtained from fish collected in oiled areas had significantly higher scores for two of the 26 lesions (adjusted $\alpha=0.0038$). These two lesions were liver lipidosis ($P=0.0006$) and kidney lymphocytes ($P=0.0005$). Correlation analysis showed that lesion scores for liver lipidosis were positively correlated with concentrations of naphthalene equivalent compounds ($r=0.281$, $P=0.08$) but not with phenanthrene equivalent compounds ($r=0.248$, $P=0.13$). Lesion scores for kidney lymphocytes were not correlated with either naphthalene or phenanthrene equivalent compounds ($P=0.647$ and $P=0.690$ respectively). Graphics depicting mean lesion scores are presented on pages 114-117, as evaluated by Marty, et al. (1993).

Liver sections from 126 liver tissue samples were sent from UCD to Woods Hole Oceanographic Institution for mixed function oxidase enzyme system analysis. The proportion of oiled sites showing positive immunostaining for MFOs was compared to the proportion of unoiled sites with positive immunostaining samples using a two-sampled Z-test (Zar 1984). Staining was evaluated for liver hepatocytes and macrophage aggregates and positive staining did occur, however no significant differences were seen between unoiled and oiled sites ($Z=1.33$, $P=0.1836$ for liver hepatocytes and $Z=-2.32$, $P=0.98$ for macrophage aggregates). Results received from Woods Hole are presented in Appendix F, including a graphic depicting immunostaining results on page 121 and a summary of mean values on page 124, as evaluated by Stegeman (1992).

Objective 3 - Route of Exposure

The results of the bile samples indicated that rockfish were not being exposed to oil in 1990 and 1991, therefore the stomach content, sediment, and invertebrate samples collected in 1990 and 1991 were not analyzed.

Objective 4 - Otolith Microstructure

Otoliths from approximately 120 juvenile rockfish were collected during 1990-91. Eighteen of these otoliths were selected at random for initial evaluation and prepared for examination. Twelve of these were legible and scanning electron micrographs were made. The EDTA etching solution for two hours produced the best results. Various annuli, growth zones, and checks (microstructures) were visible on the micrographs, however no discernable stress checks or growth effects were noted. Digitizing and analysis of the micrographs showed the narrow bands ranged from nine to 20 microns and up to 70 microns for the wide spacing. These bands were too wide to represent daily growth events; however, it was not possible to attribute them to other specific growth events. Evaluation of daily growth increments is necessary for the level of discrimination needed to measure somatic growth. Daily increments tend to stop forming as a fish ages. Ages of these juvenile rockfish ranged from three to six years. Rockfish of this age range are juvenile with respect to reproduction and other life history functions. Results of these examinations indicate, however, that three year old rockfish are too old for evaluation of daily growth increments. Otoliths from larval or very young juvenile fish would be required for evaluating stress or changes in growth relative to a specific event.

DISCUSSION

Results from this study show that, in addition to the initial mortality that occurred immediately after the spill, continued exposure of rockfish to oil spilled from the *Exxon Valdez* occurred and injury was indicated. The evidence of exposure consists of elevated concentrations of hydrocarbon metabolites in the bile. The evidence of injury is indicated by increased incidence of certain histopathological lesions. Injury to rockfish is not unique to the EVOS. Rockfish were one of the few groups of finfish found dead after the Amoco Cadiz grounding off the coast of France in March of 1978 (Cross et al. 1978). In the days following the spill there were mortalities of "rocky bottom-dwelling finfish" reported near the wreck site (Gundlach et al. 1983).

This was not even the first time rockfish mortalities have occurred in Prince William Sound. After the 1964 earthquake, "Vast numbers of red snapper (several species of red rockfish) were exterminated in Port Valdez, Port Wells and in the area between Knight Island, Chenega Island and Evans Island... Countless thousands of these fish, which normally are bottom dwellers in deep water, were left floating on the surface." (Hansen et al. 1966). Oil was also spilled into the Sound as a result of the 1964 earthquake, but it is unknown whether this was a causal factor in the rockfish mortality. One factor that these events have in common (other than all occurring in March) is that rockfish were seen floating and other species were not. These species of rockfish are brightly colored organisms equipped with a buoyancy mechanism (air bladder) and thus float and are highly visible while other species of rockfish and other finfish groups would be less visible. The cause of mortalities from the earthquake can be explained "...possibly by turbulence or sudden upwelling associated with submarine slumping or perhaps by sudden pressure changes caused by the passage of high amplitude surface waves" (Hansen et al. 1966). However, the mechanism causing injury to rockfish in oil spills is not so obvious.

The histopathological evaluation was conducted by scoring all lesions found in the tissues rather than looking for specific predetermined lesions that would be indicative of hydrocarbon exposure. In the initial ADF&G analyses only four of the lesion types (of the nine in 1990 and 26 in 1991) were statistically tested. The four lesions tested were, liver lipidosis, liver sinusoidal fibrosis, liver karyomegaly, and kidney macrophage aggregates. These lesions were selected, based on the recommendation of the ADF&G pathologist, because they were the most likely to be caused by exposure to toxins. Exposure to a toxin may cause liver lipidosis by several metabolic mechanisms which do not affect the uptake of fatty acids by liver cells, but do prevent the ultimate release of lipoproteins. Thus, fats and fat metabolites accumulate in the liver cells. Sinusoidal fibrosis is a classic sign of chronic inflammation. This would indicate that exposure to a foreign substance or body has occurred over a significant period of time. Fibrosis without other signs of inflammation, such as macrophage and other mononuclear cell infiltration or tissue destruction, would indicate that chronic inflammation had occurred at one time but was no longer present. While none of these signs are pathognomonic for exposure to hydrocarbons, they are collectively indicative of a continuing exposure to some kind of toxin in the aquatic environment. If they occur more frequently in fish from oiled than from unoiled areas, the presumption is that the toxins to which they are still being exposed are hydrocarbons. The other five lesions scored in 1990 were not tested in the initial analysis for the following reasons. Liver glycogen depletion was not tested because this can be caused by a wide range of stress conditions and is highly variable in normal populations. Hepatic single cell necrosis and kidney vacuolar degeneration could be indicators of a wide range of toxic or pathogenic conditions and were considered too general for analysis. Liver and spleen macrophage aggregates were not examined because they would be expected to show the same trend as the kidney macrophage. The additional 22 lesions scored in 1991 were not included in the initial analysis so that tests between years would be more directly comparable. The analysis of only four, rather than the whole range of lesions, also gave more power to the statistical tests. The initial tests, using only the four selected lesions, indicated that two of the four lesions in 1990, liver lipidosis (P=0.0016) and liver sinusoidal fibrosis (P=0.0118), and one in 1991, liver lipidosis (P=0.008) were more severe in oiled areas of PWS.

The final analysis presented in the results section of this report tested all lesions scored for each year. When all lesions were tested, the level of significance was adjusted to maintain a P=0.10. In this final analysis, liver lipidosis was still significant in both years, however liver fibrosis was no longer significant. In addition, two previously untested lesions were significant: in 1990, liver glycogen depletion and in 1991, kidney lymphocytes. These results could be helpful in narrowing the field of lesions to be examined for effects of hydrocarbons. Liver lipidosis should be looked for as an indicator of hydrocarbon exposure because it showed significant difference in lesion scores under both analyses for both years and was correlated to elevated concentrations of hydrocarbons in the bile in 1991. In addition, liver sinusoidal fibrosis should be considered because it was significant when predetermined lesion types were tested.

CONCLUSIONS

The evidence from the hydrocarbon and histopathological data suggest that sublethal injuries resulted from exposure to oil from the EVOS; however, hydrocarbons and lesions were found in unoiled sites. Although there were no statistical differences, hydrocarbons were found in the bile of fish from unoiled areas in both 1990 and 1991. In addition, there were mixed function enzyme activities initiated in both unoiled and oiled sites and histopathological lesions were also found at all sites. The conclusion can be drawn from this information that the unoiled sites may have been compromised to some degree and thus were not true "controls". There are at least two explanations for the reason for this compromise. One explanation is that some sites, although they were not in the direct path of the oil spill, may have had other sources of hydrocarbon exposure. For example, both Zaikof Point and Gravina Rocks were unoiled sites which had a few fish with hydrocarbons detected in the bile. They are both, however, areas of high tanker traffic, which could be a chronic source of hydrocarbon exposure. The second explanation for the compromise of unoiled sites is that the subsurface movement of oil was more widespread than indicated by surface and beach surveys. For example, the absence of differences in lesion scores between oiled and unoiled sites along the Kenai Peninsula could be indicative of the difficulty in finding areas totally unaffected by the spill. Although unoiled sites were selected based on observations of no surface oiling, subsurface movements could have contaminated these areas. If the theory of the oil exposure being more widespread is true, then differences between oiled sites and a "true control" site would be expected to be more significant.

ACKNOWLEDGEMENTS

The authors would like to recognize a number of individuals who assisted in the development and implementation of this study. Richard Rosenthal of Alaska Coastal Research and Morgan Productions and Tony Chess from the NMFS Tiburon Lab assisted in developing and training personnel in sampling procedures and diving techniques. SCUBA divers on the project included Matt Miller, Dan Bosch, David Nees, Dave Gordon, Dave Barto, Dora Sigurdsson, Dennis Jung, David Laur, Kieran Donahue, Corrie Elms, Jerry Philips and Chris Gotschalk. Kelly Hepler was instrumental in the development, supervision and administration of the project, as well as participating as a SCUBA diver. Celia Rozen provided editing assistance on the final report.

The SEM was made available by the Anchorage office of Minerals Management Service with the assistance of Maurice Lynch and Al Powers. The photographs were sent to Pete Hagen at the ADF&G Commercial Fisheries aging lab for digitizing and analysis. Victoria O'Connell, ADF&G Commercial Fish Division in Sitka, assisted in identification several rockfish including a range extension for the vermillion rockfish.

The captain and crew of the vessels from which the field work was conducted: "Julia Breeze", Guy Piercy and Scott Higley; "Kittiwake II", Mike Yorkowski, Doug and Janet Bowen; and the "Sound Pacer", Sam Mehilich and Darrell Winchester.

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Table 1. Locations of study sites sampled in Prince William Sound and on the lower Kenai Peninsula, 1989 - 1991.

Study Sites	Latitude	Longitude	1989	1990	1991
		Sampling sites in Prince William Sound			
Unoiled					
Windy Bay	60° 34′	145° 58′	X		
Knowles Head	60° 38.73′	146° 33.95′	X		
Schooner Rocks	60° 18.55′	146° 54.00′	X		
Port Etches	60° 19′	146° 40′	X		
Gravina Rocks	60° 39.42	146° 16.60	X	X	X
Zaikof Point	60° 18.12	146° 54.42	X	X	X
Oiled					
Herring Bay	60° 27′	147° 45′	X	X	X
Squirrel Bay	60° 01′	148° 08′	X		
Lonetree Point	50° 59.13′	148° 11.38′	X		
Chenega Island	60° 23′	148° 00′	X		
Naked Island	60° 42′	147° 29′	X		
Pt. Nowell	60° 26.67′	147° 55.30′	X		
Applegate Island	60° 37.42′	148° 08.24′	X		
Northwest Bay	60° 34.18′	147° 37.00′	X		
Danger Island	59° 55.50	148° 04.20	X	X	X
Bligh Reef	60° 51′	146° 53′	X		
		Sampling sites on the lower Kenai Peninsula			
Unoiled					
Cape Puget	59° 55.80′	148° 26.70′	X		
Cape Fairfield	59° 55.00′	148° 51.50′	X		
Granite Island	59° 40.70′	149° 50.60′	X	X	
Harris Bay	59° 42.83′	149° 50.67′	X		
Day Harbor	60° 00.68′	149° 04.23′		X	
Oiled					
Driftwood Bay	59° 51.45′	149° 10.40′	X		
Aialik Cape	59° 43.90′	149° 29.80′	X		
Chiswell Islands	59° 39.13′	149° 33.70′	X		
Seal Rocks	59° 32.15′	149° 37.70′	X		
Outer Island	59° 20.30′	150° 21.40′	X		
Nuka Passage	59° 14.70′	150° 44.15′	X		
Front Point	59° 16.15′	150° 51.40′	X		
Gore Point	59° 11.95′	151° 00.10′	X		
Port Dick	59° 12.30′	151° 04.70′	X		
Aligo Point	59° 37.90′	149° 44.90′	X		
Pony Cove	59° 33.82′	149° 32.12′		X	
Morning Cove	59° 23.72′	150° 18.68′		X	

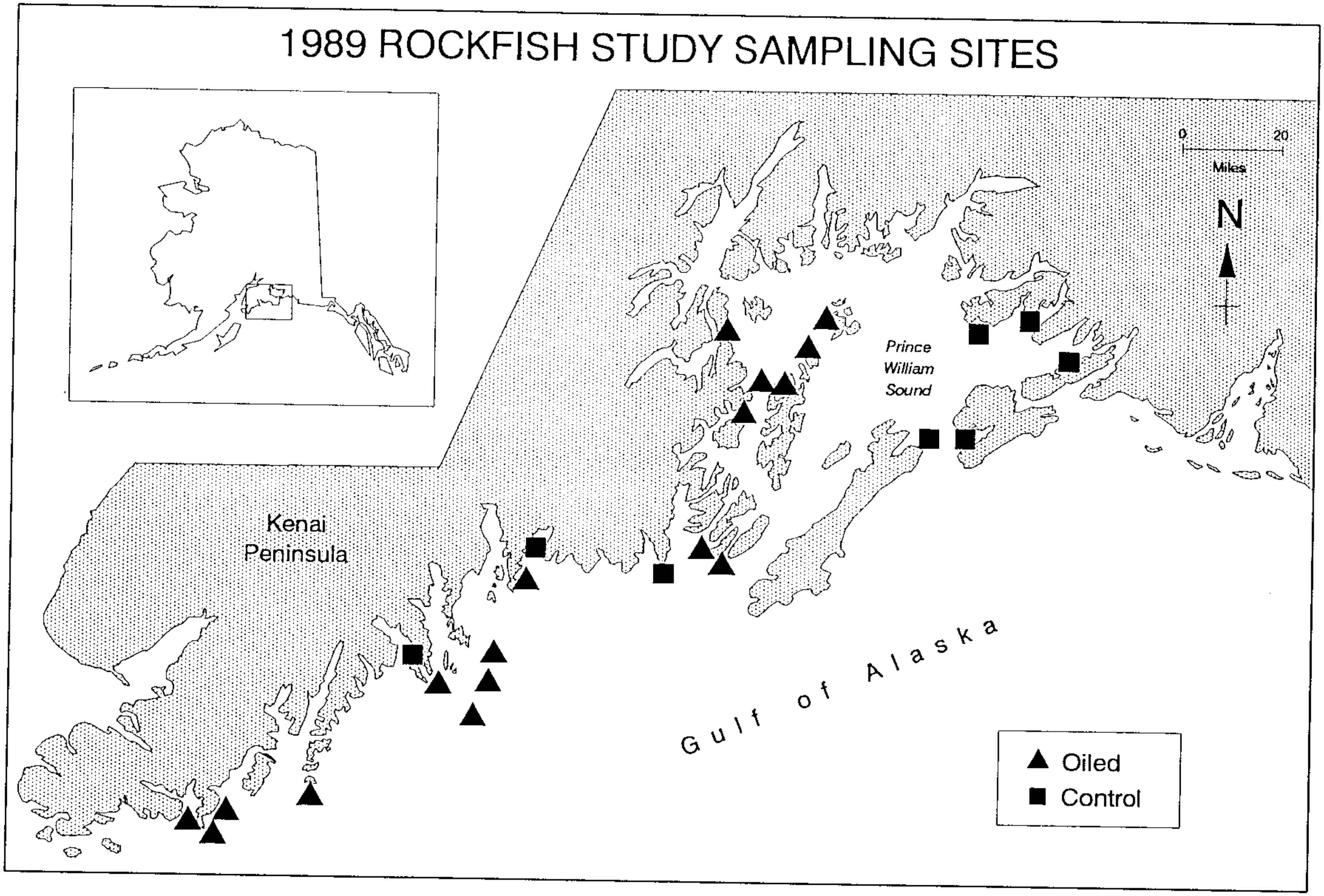


Figure 1. Map of rockfish study sampling locations in 1989.

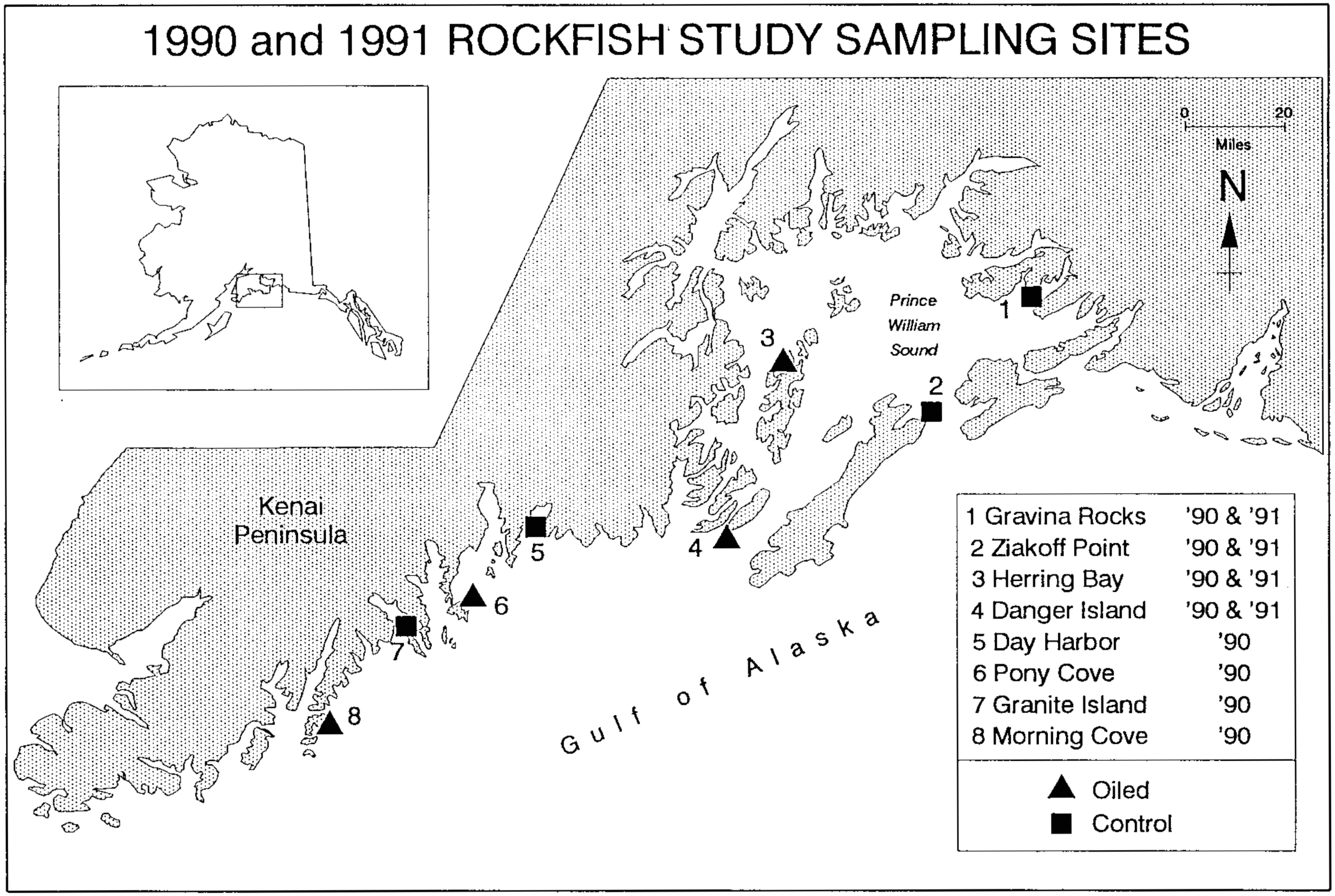


Figure 2. Map of 1990 and 1991 rockfish sampling locations.

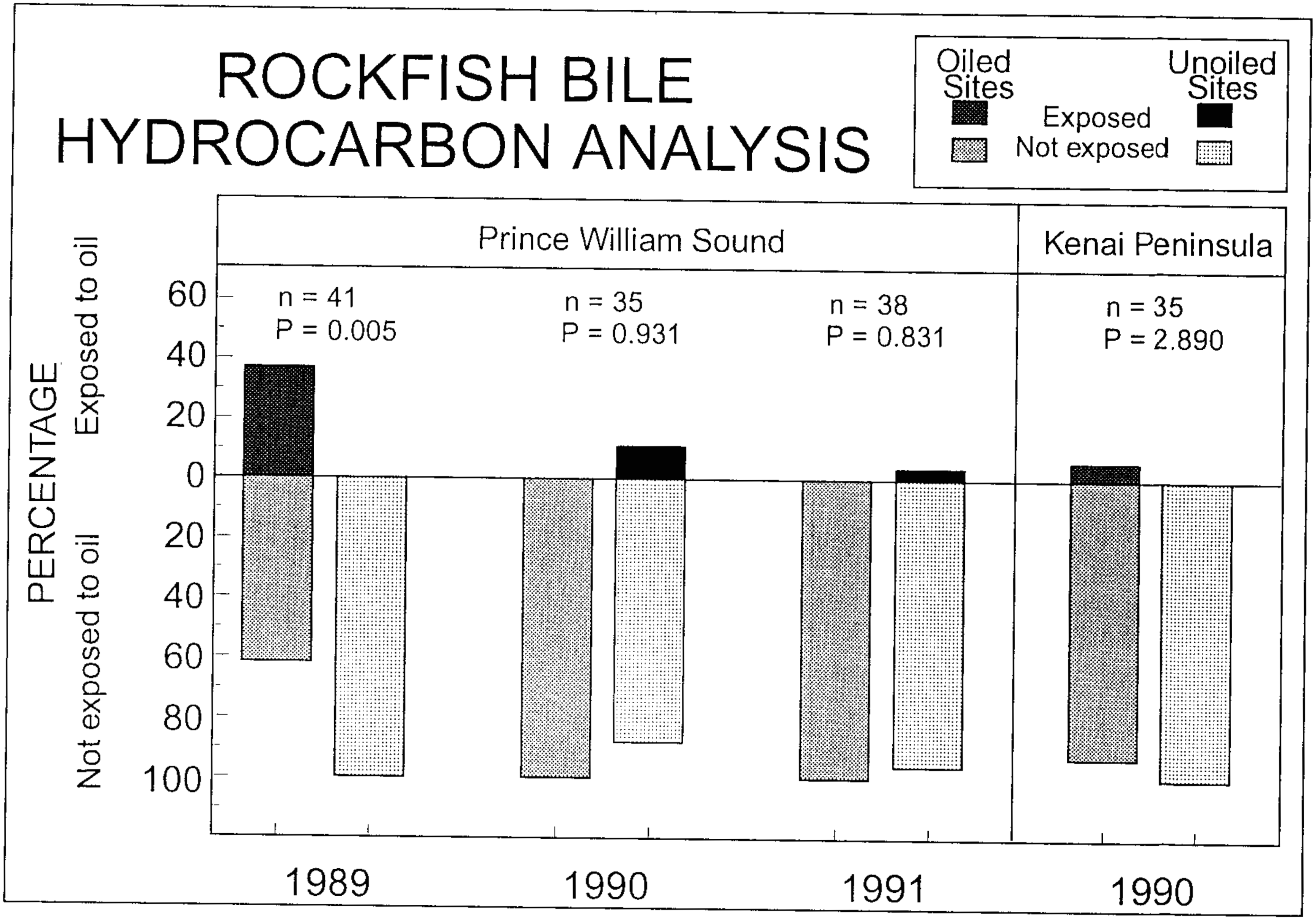


Figure 3. Summary of the hydrocarbon analysis of bile from rockfish in Prince William Sound and the Kenai Peninsula, 1989-1991.

APPENDICES

APPENDIX A

Hydrocarbon Analysis Reports
1989, 1990, and 1991
from
Auke Bay Laboratory
Auke Bay, Alaska

UNITED STATES DEPARTMENT OF COMMERCE
National Oceanic and Atmospheric Administration
National Marine Fisheries Service
Office of Environmental Assessment
and Restoration
Post Office Box 210029
Auke Bay, Alaska 99821-0029

December 28, 1989

Kelly Hepler
ADF&G, Sport Fish
333 Raspberry Rd.
Anchorage, AK 99518

Dear Kelly:

Enclosed are the data from the bile analyses for Fish and Shellfish Project #17. The summary table in the "Preliminary Report" indicates which fish were or were not possibly exposed, the data table provides more specific information on a fish by fish basis and the two chromatograms are included to demonstrate what the difference is between an "oil pattern" and a "degradation pattern". The numerical data have been incorporated into the database.

The data interpretation has been provided by the analysts, Dr. Varanasi and her group at NMFS/Seattle. Bile analysis is a semi-quantitative analytical methodology, that is, the numerical values are not absolutes. The values can be used to divide the samples into categories such as, "This fish was exposed to oil within the last two weeks before collection." or "This fish may have been exposed to oil within the last two weeks before collection." or "This fish was probably not exposed to oil within the last two weeks before collection." Because of this and the problems with sample collection and handling that we discussed, the determination as to the presence or absence of oil in these samples was based on a comparison of the chromatographic patterns as well as the numerical values for each samples and is conservative.

If you have any questions or I can help you with this in any way, please call me at (907) 789-6604.

Sincerely,

Carol-Ann Manen

enclosures

BILE SUMMARY--DAMAGE ASSESSMENT--FISH/SHELLFISH 17

Species	Site name	Sample No.	PHN equiv (ng/g)	NPH equiv (ng/g)	OCBATCH	Comments
Rockfish	Applegate Island	1780	2,500	14,000	22NOV89	no oil
		1781	700	3,000	22NOV89	no oil
		8428	*	*	20NOV89	degradation pattern
		8429	*	*	20NOV89	degradation pattern
		8430	*	*	20NOV89	degradation pattern
		8431	*	*	20NOV89	degradation pattern
		8432	260	2,400	20NOV89	no oil
		8433	320	2,800	20NOV89	no oil
			230	2,300	20NOV89	no oil
		8434	*	*	20NOV89	degradation pattern
		8435	*	*	20NOV89	degradation pattern
		8436	1,100	9,800	20NOV89	no oil
	Count for Applegate Island:		6	6		
	Average for Applegate Island:		855	5,717		
	Standard Deviation for Applegate Island:		871	4,977		
	Chenega Island	2717	150	3,800	26OCT89	no oil
			120	4,000	26OCT89	no oil
	Count for Chenega Island:		2	2		
	Average for Chenega Island:		135	3,900		
	Standard Deviation for Chenega Island:		21	141		
	Eleanor Island	1784	44,000	140,000	22NOV89	oil
			42,000	130,000	22NOV89	oil
		1785	5,700	22,000	22NOV89	?sm oil
	Count for Eleanor Island:		3	3		
	Average for Eleanor Island:		30,567	97,333		
	Standard Deviation for Eleanor Island:		21,558	65,432		
	Eshamy Bay	1792	6,800	33,000	22NOV89	no oil
		1793	460	4,200	22NOV89	no oil
		8420	1,600	6,800	21NOV89	no oil
		8421	13,000	43,000	20NOV89	sm oil
			12,000	42,000	20NOV89	sm oil
		8422	1,400	9,700	20NOV89	degradation pattern
		8423	1,100	6,700	20NOV89	no oil
		8424	5,800	15,000	20NOV89	no oil
		8425	4,400	16,000	20NOV89	no oil
		8426	6,600	17,000	20NOV89	no oil
		8427	800	3,500	20NOV89	no oil
			1,000	2,400	20NOV89	no oil
	Count for Eshamy Bay:		12	12		
	Average for Eshamy Bay:		4,580	16,608		
	Standard Deviation for Eshamy Bay:		4,387	14,718		

* not determined due to interfering degradation peak

Species	Site name	Sample No.	PHN equiv (ng/g)	NPH equiv (ng/g)	QCBATCH	Comments
Rockfish	Knowles Point	1774	4,700	13,000	22NOV89	no oil
		1775	2,300	18,000	22NOV89	no oil
		Count for Knowles Point:	2	2		
		Average for Knowles Point:	3,500	15,500		
		Standard Deviation for Knowles Point:	1,697	3,536		
	Liljegren Passage	1768	3,900	18,000	22NOV89	?sm oil
			3,500	17,000	22NOV89	?sm oil
		1769	97,000	330,000	22NOV89	lg oil
		Count for Liljegren Passage:	3	3		
		Average for Liljegren Passage:	34,800	121,667		
		Standard Deviation for Liljegren Passage:	53,867	180,423		
	Naked Island	8410	2,500	10,000	13NOV89	no oil
		8411	12,000	54,000	13NOV89	?sm oil
		8412	15,000	59,000	13NOV89	?sm oil
			14,000	58,000	13NOV89	?sm oil
		8413	13,000	65,000	16NOV89	?sm oil
		8414	15,000	51,000	16NOV89	?sm oil
		8415	11,000	26,000	16NOV89	?sm oil
		8416	15,000	42,000	16NOV89	?sm oil
		8417	2,800	10,000	16NOV89	no oil
		8418	2,800	17,000	16NOV89	no oil
			2,700	11,000	16NOV89	no oil
		8419	5,900	17,000	16NOV89	no oil
		Count for Naked Island:	12	12		
		Average for Naked Island:	9,308	35,000		
		Standard Deviation for Naked Island:	5,471	21,797		
	Windy Bay	1796	5,200	35,000	22NOV89	?degradation; no oil pattern
		1797	190	3,800	22NOV89	degradation pattern
		8400	2,500	21,000	13NOV89	?degradation; no oil pattern
		8401	100	8,800	13NOV89	no oil
		8403	4,300	33,000	13NOV89	?degradation; no oil pattern
		8404	350	12,000	13NOV89	no oil
		8405	540	8,200	13NOV89	no oil
		8406	1,300	8,400	13NOV89	no oil
			1,100	7,600	13NOV89	no oil
		8407	500	5,400	13NOV89	no oil
		8408	240	2,400	13NOV89	no oil
		8409	420	5,100	13NOV89	no oil
		Count for Windy Bay:	12	12		
		Average for Windy Bay:	1,395	12,558		

* not determined due to interfering degradation peak

Species	Site name	Sample No.	PHN equiv (ng/g)	NPH equiv (ng/g)	QCBATCH	Comments
	Standard Deviation for Windy Bay:		1,713	11,098		

* not determined due to interfering degradation peak

PRELIMINARY REPORT: FISH/SHELLFISH 17
Analyses of Bile for Fluorescent Aromatic Compounds

Environmental Conservation Division
Northwest Fisheries Center
National Oceanic and Atmospheric Administration

Bile samples from rockfish (particular species not identified) were analyzed by the methods and the quality assurance outlined in the Detailed Study Plan for the Project titled "Shellfish and Groundfish Trawl Assessment Outside Prince William Sound" submitted for State/Federal Resource Damage Assessment.

SAMPLES ANALYZED

Sample #	Total samples	Samples analyzed	Samples not analyzed	degradation[d]
1768-1797	12	12	---	2
2711-2712	3	0	3[a]	0
2717	1	1	---	0
4533-5302	69	0	69[b]	0
8400-8437	38	36	2[c]	9
8520-8596	75	0	75[a]	0
TOTALS	198	49	149	11

a Gall bladder collected; no record of collection/storage method. See Problems A1 and A2 below for explanation.

b Gall bladder collected from fish held on ice for up to 7 days. See Problems A1 and A2 below for explanation.

c Bile collected was almost solid and could not be injected onto the column.

d Degradation pattern was observed in analyzed samples. See Problem C1 below for explanation.

increasing oil contamination (see attached Table); and (2) an identifiable "pattern of hydrocarbon exposure" is present in the chromatogram when a fish has been exposed to oil (see attached example). Based on these criteria for exposure, individual fish from the following sites (see Table) can be assigned to the following categories:

Exposure level	Species	Site	n
Possibly exposed	rockfish (species not identified)	Liljegren Passage	1
		Eleanor Island	2
		Eshamy Bay	1
		Naked Island	6
Not exposed	rockfish	Applegate Island	5
		Chenega Island	1
		Eshamy Bay	9
		Naked Island	4
		Knowles Point	2
		Windy Bay	7
Degradation pattern	rockfish	Applegate Island	6
		Windy Bay	4
		Eshamy Bay	1

CONCLUDING REMARKS

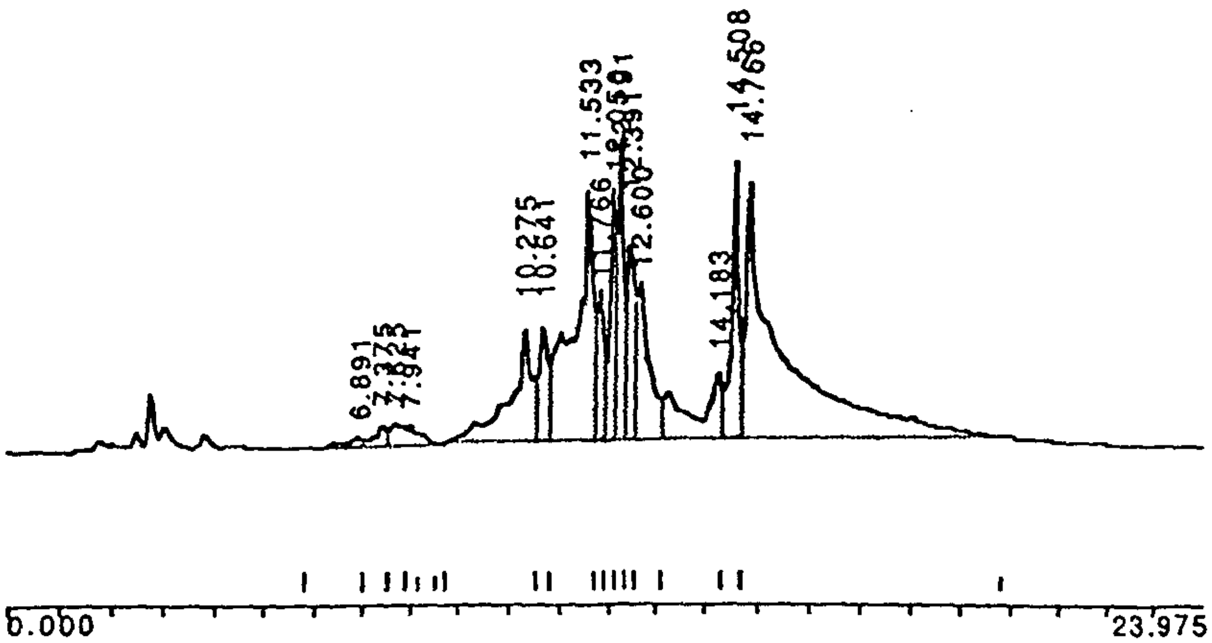
In conclusion, 10 of the rockfish collected in this study showed possible evidence of exposure to oil. However, the evidence is overshadowed by several problems with the sample collection. In particular, problems with sample handling resulting in bile degradation and with sampling design resulting in few fish of each species from some sites made it impossible to draw firm conclusions from the data.

Date: Mon, Dec 11, 1989 10:46 AM
Data: 20NOV89-039

Sample:
89.1412--PATTERN OF HYDROCARBON EXPOSURE

Processing File: bile-A-proc-10
Method: bilemeth
Sampling Int: 0.5 Seconds

Chromatogram:



Analysis: Channel A

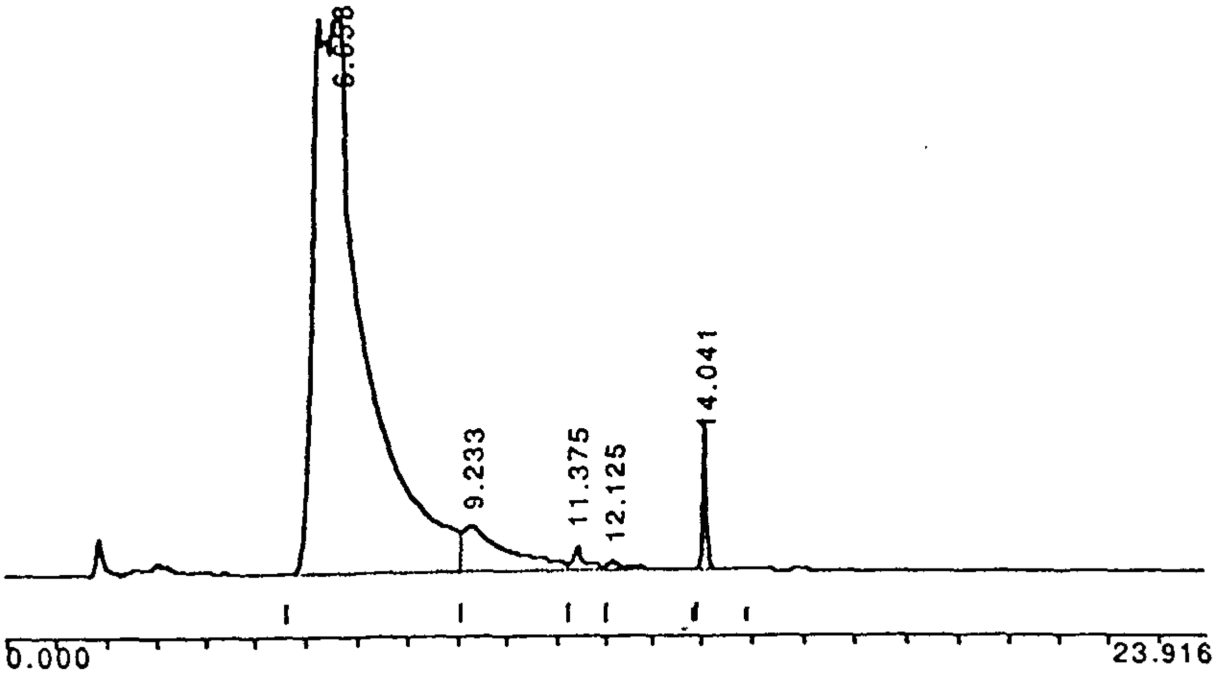
Peak No.	Time	Type	Height(µV)	Area(µV-sec)	Area%
1	6.891	*N1	411	12424	0.579
2	7.375	*N2	1088	17277	0.805
3	7.625	*T3	1138	38068	1.775
4	7.941	T4	212	1621	0.075
5	10.275	*N1	6570	167681	7.819
6	10.641	*N2	6415	74344	3.466
7	11.533	*N3	13676	353028	16.462
8	11.766	*N4	7512	81402	3.795
9	12.050	*N5	12711	118790	5.539
10	12.191	*N6	14885	121320	5.657
11	12.391	*N7	11675	125799	5.866
12	12.600	*N8	8087	138436	6.455

Date: Mon, Dec 11, 1989 10:48 AM
Data: 21NOV89-039

Sample:
8833–DEGRADATION PATTERN

Processing File: bile-A-proc-17
Method: bilemeth
Sampling Int: 0.5 Seconds

Chromatogram:



Analysis: Channel A

Peak No.	Time	Type	Height(µV)	Area(µV-sec)	Area%
1	6.658	*N1	113776	7880006	90.172
2	9.233	N2	9196	571860	6.543
3	11.375	*N3	4909	85504	0.978
4	12.125	*N4	1611	58380	0.668
5	14.041	N	20793	143293	1.639
Total Area				8739043	100.000

National Marine Fisheries Service
Office of Oil Spill Damage
Assessment and Restoration
P.O. Box 210029
Auke Bay, Alaska 99821

December 18, 1991

Andy Hoffman
ADF&G, Sport Fish
333 Raspberry Road
Anchorage, AK 99518

Dear Andy,

Enclosed are the hard copy data from the analysis of the bile collected by Fish/Shellfish 17 during 1991. Accompanying this data return is a printout of all the samples presently in the archives from Fish/Shellfish 17 and their analytical status. Data from catalog number 6678 and 6680 are included in this return. Samples included in catalog number NMFS_156 will go for analysis January 13, 1991.

At this point, review of these data do not warrant analysis of additional samples from this project. Please call me at 301/443-8655 if you wish to discuss this recommendation.

Sincerely,

Carol-Ann Manen

encl.

cc: J. Sullivan without encl.

17

HPLC BILE ANALYSIS

FILE #	SAMPLE ID	QA/QC BATCH #	FWS CAT #	ORGANISM	NAPHTHALENE NG/G WET WT	PHENANTHRENE NG/G WET WT	
N14229	102301	QAC-0257	6678	FISH	78000	13000	Y
N14231	102302	QAC-0257	6678	FISH	73000	13000	Y
N14233	102303	QAC-0257	6678	FISH	30000	3000	N
N14235	102304	QAC-0257	6678	FISH	18000	2000	N
N14237	102305	QAC-0257	6678	FISH	15000	2000	N
N14239	102306	QAC-0257	6678	FISH	25000	2700	N
N14241	102307	QAC-0257	6678	FISH	27000	3700	N
N14243	102308	QAC-0257	6678	FISH	40000	4900	N
N14245	102309	QAC-0257	6678	FISH	11000	1200	N
N14247	102310	QAC-0256	6678	FISH	22000	2500	N
N14249	102311	QAC-0256	6678	FISH	18000	2100	N
N14251	102312	QAC-0256	6678	FISH	11000	1200	N
N14253	102313	QAC-0256	6678	FISH	4500	510	N
N14255	102314	QAC-0256	6678	FISH	26000	3900	N
N14257	102315	QAC-0256	6678	FISH	30000	4500	N
N14259	102316	QAC-0256	6678	FISH	6800	960	N
N14261	102317	QAC-0256	6678	FISH	21000	2700	N
N14263	102318	QAC-0256	6678	FISH	8800	1300	N
N14265	102319	QAC-0256	6678	FISH	8800	950	N
N14267	102320	QAC-0256	6678	FISH	23000	2900	N
N14269	102321	QAC-0256	6678	FISH	51000	5800	?
N14271	102322	QAC-0256	6678	FISH	22000	2800	N
N14273	102323	QAC-0256	6678	FISH	8700	980	N
N14275	102324	QAC-0256	6678	FISH	27000	3600	N
N14277	102325	QAC-0258	6678	FISH	25000	4000	N
N14279	102328	QAC-0258	6678	FISH	26000	3500	N
N14281	102329	QAC-0258	6678	FISH	30000	3100	N
N14283	102330'	QAC-0258	6678	FISH	18000	2200	N
N14285	102333	QAC-0258	6678	FISH	19000	2000	N
N14287	102334	QAC-0258	6678	FISH	32000	4800	N
N14289	102335	QAC-0258	6678	FISH	30000	4700	N
N14291	102336	QAC-0258	6678	FISH	25000	2700	N
N14293	102337	QAC-0258	6678	FISH	51000	5300	?
N14295	102338	QAC-0258	6678	FISH	20000	2700	N

oil

N = NO
Y = YES

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HPLC BILE ANALYSIS

N14297	102339	QAC-0258	6678	FISH	35000	4700 N
N14299	102340	QAC-0258	6678	FISH	24000	3100 N
N14301	102341	QAC-0258	6678	FISH	48000	5500 N
N14303	102342	QAC-0258	6678	FISH	20000	2400 N
N14305	102343	QAC-0258	6678	FISH	20000	2700 N
N14307	102344	QAC-0259	6678	FISH	16000	2000 N
N14309	102345	QAC-0259	6678	FISH	26000	3100 N
N14311	102346	QAC-0259	6678	FISH	20000	2600 N
N14313	102349	QAC-0259	6678	FISH	21000	2100 N
N14315	102350	QAC-0259	6678	FISH	18000	2100 N
N14317	102401	QAC-0259	6678	FISH	19000	2100 N
N14319	102402	QAC-0259	6678	FISH	22000	3100 N
N14321	102403	QAC-0259	6678	FISH	31000	3200 N
N14323	102404	QAC-0259	6678	FISH	17000	2500 N

HPLC BILE ANALYSIS

QA/QC

FILE #	SAMPLE ID	QA/QC BATCH #	FWS CAT #	ORGANISM	NAPHTHALENE NG/G WET WT	PHENANTHRENE NG/G WET WT
N14265 DUP	102319	QAC-0256	6678	FISH	10000	1000
N14275 DUP	102324	QAC-0256	6678	FISH	31000	4100
N14243 DUP	102308	QAC-0257	6678	FISH	41000	4900
N14245 DUP	102309	QAC-0257	6678	FISH	11000	1200
N14285 DUP	102333	QAC-0258	6678	FISH	21000	2200
N14295 DUP	102338	QAC-0258	6678	FISH	18000	2600
N14305 DUP	102343	QAC-0258	6678	FISH	19000	2700
N14313 DUP	102349	QAC-0259	6678	FISH	21000	2000
N14323 DUP	102404	QAC-0259	6678	FISH	18000	2700
BILE REF MAT		QAC-0256		MIX	130000	54000
BILE REF MAT		QAC-0257		MIX	120000	50000
BILE REF MAT		QAC-0258		MIX	130000	52000
BILE REF MAT		QAC-0259		MIX	120000	51000

UNITED STATES DEPARTMENT OF COMMERCE
National Oceanic and Atmospheric Administration
NATIONAL OCEAN SERVICE
Office of Ocean Resources Conservation and Assessment
Rockville, Maryland 20852

May 26, 1992

Kelly Hepler
Alaska Dept. of Fish & Game
333 Raspberry Road
Anchorage, AK 99518

Dear Kelly;

Enclosed are the data from the analysis of the remaining rockfish samples. In general, all but one a quilled rockfish collected from Zaikov Bay on 08/09/91 are negative for petroleum hydrocarbons. There are several fish, particularly those collected from Danger Island where the bile metabolite values are intermediate between presence/absence of petroleum hydrocarbons. Since two of these samples (id numbers 204620 and 204629) displayed chromatograms characteristic of sample degradation, I have identified these samples as negative.

If you have any questions or wish to discuss these data and their interpretation, please call me at 301/443-8466.

Sincerely,

Carol-Ann Manen

enclosure

cc: J. Sullivan
R. Spies

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HPLC BILE ANALYSIS

0.1

FILE #	SAMPLE ID	QA/QC BATCH #	FWS CAT #	ORGANISM	NAPHTHALENE NG/G WET WT	PHENANTHRENE NG/G WET WT
N14390	204601	QAC-0314	NMFS 156	QROC	16000	2400 N
N14391	204602	QAC-0314	NMFS 156	QROC	27000	3200 N
N14392	204603	QAC-0314	NMFS 156	YROC	26000	4100 N
N14393	204604	QAC-0314	NMFS 156	QROC	11000	1500 N
N14394	204605	QAC-0314	NMFS 156	CROC	14000	2000 N
N14395	204606	QAC-0314	NMFS 156	CROC	21000	2200 N
N14396	204607	QAC-0314	NMFS 156	CROC	59000	7300 Z.
N14397	204608	QAC-0314	NMFS 156	CROC	12000	1400 N
N14398	204609	QAC-0314	NMFS 156	CROC	32000	3700 N
N14399	204610	QAC-0314	NMFS 156	YROC	28000	3500 N
N14400	204611	VIAL EMPTY	NMFS 156	BLANK	VIAL EMPTY	VIAL EMPTY
N14401	204612	QAC-0316	NMFS 156	BLANK	<500	<300
N14402	204613	QAC-0314	NMFS 156	YROC	38000	5200 Z.
N14403	204614	QAC-0314	NMFS 156	YROC	30000	3700 N
N14404	204615	QAC-0314	NMFS 156	CROC	27000	5700 N
N14405	204616	QAC-0314	NMFS 156	CROC	23000	3400 N
N14406	204617	QAC-0314	NMFS 156	QROC	29000	3400 N
N14407	204618	QAC-0319	NMFS 156	CROC	12000	1400 N
N14408	204619	QAC-0314	NMFS 156	CROC	56000	8900 Z.
N14409	204620	QAC-0315	NMFS 156	CROC	40000	6000 N
N14410	204621	QAC-0315	NMFS 156	QROC	26000	4500 N
N14411	204622	QAC-0315	NMFS 156	QROC	18000	3400 N
N14412	204623	QAC-0315	NMFS 156	QROC	14000	2200 N
N14413	204624	QAC-0315	NMFS 156	YROC	30000	3700 N
N14414	204625	QAC-0315	NMFS 156	YROC	17000	2300 N
N14415	204626	QAC-0315	NMFS 156	YROC	18000	2400 N
N14416	204627	QAC-0315	NMFS 156	YROC	21000	3300 N
N14417	204628	QAC-0315	NMFS 156	QROC	37000	4800 N
N14418	204629	QAC-0315	NMFS 156	YROC	36000	5800 N
N14419	204631	VIAL EMPTY	NMFS 156	BLANK	VIAL EMPTY	VIAL EMPTY
N14420	204632	QAC-0315	NMFS 156	YROC	34000	4800 N
N14421	204633	QAC-0315	NMFS 156	YROC	17000	2100 N
N14422	204634	QAC-0315	NMFS 156	QROC	26000	3200 N
N14423	204635	QAC-0315	NMFS 156	QROC	35000	6500 N

HPLC BILE ANALYSIS

FILE #	SAMPLE ID	QA/QC BATCH #	FWS OAT #	ORGANISM	NAPHTHALENE NG/G WET WT	PHENANTHRENE NG/G WET WT
N14424	204636	QAC-0315	NMFS 156	CROC	81000	11000 Y
N14425	204637	QAC-0315	NMFS 156	CROC	34000	4500 N
N14426	204638	QAC-0315	NMFS 156	CROC	33000	4700 N
N14427	204639	QAC-0316	NMFS 156	CROC	23000	3400 N
N14428	204640	QAC-0316	NMFS 156	CROC	34000	4300 N
N14429	204641	QAC-0316	NMFS 156	CROC	15000	2000 N
N14430	204642	QAC-0316	NMFS 156	CROC	24000	3100 N
N14431	204643	QAC-0316	NMFS 156	BLANK	<500	<300
N14432	204644	VIAL EMPTY	NMFS 156	BLANK	VIAL EMPTY	VIAL EMPTY
N14433	204630	QAC-0316	NMFS 156	BLANK	<500	<300

HPLC BILE ANALYSIS

QA/QC FILE #	SAMPLE ID	QA/QC BATCH #	FWS CAT #	ORGANISM	NAPHTHALENE NG/G WET WT	PHENANTHRENE NG/G WET WT
N14395	204606	QAC-0314	NMFS 156	CROC	20000	2300 N
N14403	204614	QAC-0314	NMFS 156	YROC	29000	3600 N
N14407	204618	QAC-0319	NMFS 156	CROC	11000	1300 N
N14414	204625	QAC-0315	NMFS 156	YROC	15000	2100 N
N14421	204633	QAC-0315	NMFS 156	YROC	17000	2100 N
N14426	204638	QAC-0315	NMFS 156	CROC	31000	4500 N
N14429	204641	QAC-0316	NMFS 156	CROC	14000	1800 N
N14431	204643	QAC-0316	NMFS 156	BLANK	<500	<300
BILE REF MAT		QAC-0314		MIX	110000	53000
BILE REF MAT		QAC-0315		MIX	100000	52000
BILE REF MAT		QAC-0316		MIX	110000	53000
BILE REF MAT		QAC-0319		MIX	99000	45000

HPLC BILE ANALYSIS

FILE #	SAMPLE ID	QC BATCH	FWS CAT#	ORGANISM	NAPHTHALENE NG/G WET WT	PHENANTHRENE NG/G WET WT
N14155	102405	QAC-0260	6680	FISH	41000	6500 Z.
N14157	102406	QAC-0260	6680	FISH	28000	2900 N
N14159	102407	QAC-0260	6680	FISH	16000	2000 N
N14161	102408	QAC-0260	6680	FISH	66000	13000 Y
N14163	102409	QAC-0260	6680	FISH	20000	2200 N
N14165	102410	QAC-0260	6680	FISH	38000	3800 N
N14167	102411	QAC-0260	6680	FISH	25000	2800 N
N14169	102412	INSUFF	6680	FISH	INSUFF SAMPLE	INSUFF SAMPLE
N14171	102413	QAC-0260	6680	FISH	23000	2600 N
N14173	102414	QAC-0260	6680	FISH	32000	4100 N
N14175	102415	QAC-0260	6680	FISH	20000	2600 N
N14177	102416	QAC-0260	6680	FISH	26000	3400 N
N14179	102417	QAC-0260	6680	FISH	26000	2900 N
N14181	102418	QAC-0260	6680	FISH	17000	1900 N
N14183	102419	QAC-0260	6680	FISH	23000	3000 N
N14185	102420	QAC-0260	6680	FISH	13000	1800 N
N14187	102421	QAC-0261	6680	FISH	23000	3100 N
N14189	102422	INSUFF	6680	FISH	INSUFF SAMPLE	INSUFF SAMPLE
N14191	102423	QAC-0261	6680	FISH	29000	4600 N
N14193	102424	QAC-0261	6680	FISH	14000	2000 N
N14195	102425	QAC-0261	6680	FISH	31000	3400 N
N14197	102426	QAC-0261	6680	FISH	28000	3800 N

APPENDIX B

Rockfish Age, Length and Weight Data

Appendix B1. Rockfish age, length and weight data for Prince William Sound, 1989 - 1991.

ID#			SITE	DATE	SPECIES	LENGTH	AGE	WEIGHT
BLACK ROCKFISH								
C284	PWS	C	SCHONER	6/28/89	BLAC	477	11	
C285	PWS	C	SCHONER	6/28/89	BLAC	395	8	
C287	PWS	C	SCHONER	6/28/89	BLAC	426	10	
C286	PWS	C	SCHONER	6/28/89	BLAC	355	6	
C266	PWS	C	SCHONER	6/28/89	BLAC	395	8	
C280	PWS	C	SCHONER	6/28/89	BLAC	524	15	
C303	PWS	C	SCHONER	6/28/89	BLAC	420	10	
C279	PWS	C	SCHONER	6/28/89	BLAC	470	15	
D153	PWS	T	DANGER	9/19/89	BLAC	511	13	
D167	PWS	T	DANGER	9/19/89	BLAC	497	13	
D162	PWS	T	DANGER	9/19/89	BLAC	455	17	
D174	PWS	T	DANGER	9/19/89	BLAC	489	14	
D166	PWS	T	DANGER	9/19/89	BLAC	493	13	
D163	PWS	T	DANGER	9/19/89	BLAC	497	13	
D164	PWS	T	DANGER	9/19/89	BLAC	449	13	
D161	PWS	T	DANGER	9/19/89	BLAC	438	14	
D152	PWS	T	DANGER	9/19/89	BLAC	498	10	
D173	PWS	T	DANGER	9/19/89	BLAC	465	14	
D160	PWS	T	DANGER	9/19/89	BLAC	472	11	
D158	PWS	T	DANGER	9/19/89	BLAC	457	11	
D159	PWS	T	DANGER	9/19/89	BLAC	468	15	
D144	PWS	T	DANGER	9/19/89	BLAC	531	15	
D172	PWS	T	DANGER	9/19/89	BLAC	470	14	
D157	PWS	T	DANGER	9/19/89	BLAC	455	8	
D175	PWS	T	DANGER	9/19/89	BLAC	510	10	
C233	PWS	T	HERRING	6/24/89	BLAC	496	13	
C328	PWS	T	LONETRE	6/25/89	BLAC	478	11	
C329	PWS	T	LONETRE	6/25/89	BLAC	465	23	
C330	PWS	T	LONETRE	6/25/89	BLAC	453	10	
C326	PWS	T	LONETRE	6/25/89	BLAC	440	10	
C322	PWS	T	LONETRE	6/25/89	BLAC	366	7	
CHINA ROCKFISH								
D150	PWS	T	DANGER	9/19/89	CHIN	335	14	
D151	PWS	T	DANGER	9/19/89	CHIN	320	14	
D154	PWS	T	DANGER	9/19/89	CHIN	308	14	
C289	PWS	T	LONETRE	6/25/89	CHIN	298	14	
6	PWS	C	GRAVINA	7/16/90	CHIN	330	21	
113	PWS	C	ZAIKOF	8/ 6/90	CHIN	220	9	275
37	PWS	T	DANGER	7/22/90	CHIN	296	14	

ID#			SITE	DATE	SPECIES	LENGTH	AGE	WEIGHT
35	PWS	T	DANGER	7/21/90	CHIN	300	15	
COPPER ROCKFISH								
C274	PWS	C	GRAVINA	6/29/89	COPP	384	15	
C278	PWS	C	GRAVINA	6/29/89	COPP	340	12	
C275	PWS	C	GRAVINA	6/29/89	COPP	385	27	
1	PWS	C	GRAVINA	7/16/90	COPP	376	37	
15	PWS	C	GRAVINA	7/18/90	COPP	371	22	
26	PWS	T	HERRING	7/19/90	COPP	290	7	
1007	PWS	C	GRAVINA	7/21/91	COPP	326	11	653
1006	PWS	C	GRAVINA	7/21/91	COPP	355	15	986
1009	PWS	C	GRAVINA	7/21/91	COPP	355	15	789
1008	PWS	C	GRAVINA	7/21/91	COPP	316	9	542
1003	PWS	C	GRAVINA	7/21/91	COPP	357	15	782
1010	PWS	C	GRAVINA	7/21/91	COPP	349	14	712
1015	PWS	C	GRAVINA	7/21/91	COPP	336	11	696
1013	PWS	C	GRAVINA	7/21/91	COPP	332	14	635
1014	PWS	C	GRAVINA	7/21/91	COPP	339	13	682
1019	PWS	C	GRAVINA	7/22/91	COPP	258	11	307
1039	PWS	T	DANGER	7/27/91	COPP	413	16	1196
1085	PWS	T	DANGER	9/ 9/91	COPP	408	18	1170
1034	PWS	T	DANGER	7/27/91	COPP	402	18	1093
1042	PWS	T	DANGER	7/27/91	COPP	420	20	1233
1038	PWS	T	DANGER	7/27/91	COPP	397	13	1214
1037	PWS	T	DANGER	7/27/91	COPP	402	15	1170
1101	PWS	T	DANGER	9/10/91	COPP	360	17	945
1036	PWS	T	DANGER	7/27/91	COPP	402	17	1195
1041	PWS	T	DANGER	7/27/91	COPP	425	18	1396
1033	PWS	T	DANGER	7/27/91	COPP	406	16	1218
DUSKY ROCKFISH								
B107	PWS	C	PORPOIS	6/17/89	DUSK	314	7	
B108	PWS	C	PORPOIS	6/17/89	DUSK	334	11	
C265	PWS	C	SCHONER	6/28/89	DUSK	342	13	
C281	PWS	C	SCHONER	6/28/89	DUSK	356	13	
C283	PWS	C	SCHONER	6/28/89	DUSK	355	11	
C282	PWS	C	SCHONER	6/28/89	DUSK	325	9	
D143	PWS	T	DANGER	9/19/89	DUSK	335	11	
D149	PWS	T	DANGER	9/19/89	DUSK	347	14	
D147	PWS	T	DANGER	9/19/89	DUSK	393	45	

ID#			SITE	DATE	SPECIES	LENGTH	AGE	WEIGHT
D171	PWS	T	DANGER	9/19/89	DUSK	395	29	
C235	PWS	T	HERRING	6/24/89	DUSK	325	13	
C234	PWS	T	HERRING	6/24/89	DUSK	419	19	
QUILLBACK ROCKFISH								
C276	PWS	C	GRAVINA	6/29/89	QUIL	395	31	
C273	PWS	C	GRAVINA	6/29/89	QUIL	392	61	
C277	PWS	C	GRAVINA	6/29/89	QUIL	380	16	
B101	PWS	C	WINDY B	6/17/89	QUIL	425	44	
B105	PWS	C	WINDY B	6/17/89	QUIL	281	9	
B103	PWS	C	WINDY B	6/17/89	QUIL	327	12	
B104	PWS	C	WINDY B	6/17/89	QUIL	357	27	
B139	PWS	T	CABIN B	6/13/89	QUIL	306	12	
B144	PWS	T	CABIN B	6/13/89	QUIL	372	28	
B145	PWS	T	CABIN B	6/13/89	QUIL	348	13	
B134	PWS	T	CABIN B	6/13/89	QUIL	395	34	
B141	PWS	T	CABIN B	6/13/89	QUIL	388	29	
B127	PWS	T	CABIN B	6/13/89	QUIL	340	14	
B143	PWS	T	CABIN B	6/13/89	QUIL	375	25	
B135	PWS	T	CABIN B	6/13/89	QUIL	355	18	
B146	PWS	T	CABIN B	6/13/89	QUIL	380	25	
B136	PWS	T	CABIN B	6/13/89	QUIL	373	27	
B140	PWS	T	CABIN B	6/13/89	QUIL	410	37	
B142	PWS	T	CABIN B	6/13/89	QUIL	390	49	
B137	PWS	T	CABIN B	6/13/89	QUIL	417	28	
B128	PWS	T	CABIN B	6/13/89	QUIL	343	20	
B138	PWS	T	CABIN B	6/13/89	QUIL	400	28	
C231	PWS	T	HERRING	6/24/89	QUIL	332	12	
C205	PWS	T	HERRING	6/24/89	QUIL	380	31	
C229	PWS	T	HERRING	6/24/89	QUIL	352	25	
C232	PWS	T	HERRING	6/24/89	QUIL	330	20	
C217	PWS	T	HERRING	6/24/89	QUIL	387	23	
C230	PWS	T	HERRING	6/24/89	QUIL	360	33	
B124	PWS	T	HERRING	6/14/89	QUIL	358	21	
C228	PWS	T	HERRING	6/24/89	QUIL	375	26	
C218	PWS	T	HERRING	6/24/89	QUIL	373	31	
C216	PWS	T	HERRING	6/24/89	QUIL	365	21	
C204	PWS	T	HERRING	6/24/89	QUIL	370	26	
13	PWS	C	GRAVINA	7/17/90	QUIL	401	51	
12	PWS	C	GRAVINA	7/17/90	QUIL	413	44	
2	PWS	C	GRAVINA	7/16/90	QUIL	229	7	
14	PWS	C	GRAVINA	7/17/90	QUIL	417	39	
3	PWS	C	GRAVINA	7/16/90	QUIL	406	30	
109	PWS	C	MIDDLEP	8/ 3/90	QUIL	385	17	1025

ID#			SITE	DATE	SPECIES	LENGTH	AGE	WEIGHT
107	PWS	C	MIDDLEP	8/ 3/90	QUIL	325	13	725
118	PWS	C	MIDDLEP	8/ 3/90	QUIL	170	6	100
119	PWS	C	MIDDLEP	8/ 3/90	QUIL	265	9	350
110	PWS	C	MIDDLEP	8/ 3/90	QUIL	345	14	830
108	PWS	C	MIDDLEP	8/ 3/90	QUIL	343	13	850
120	PWS	C	MIDDLEP	8/ 3/90	QUIL	274	10	425
127	PWS	C	ZAIKOF	8/ 5/90	QUIL	218	7	250
111	PWS	C	ZAIKOF	8/ 5/90	QUIL	261	7	430
45	PWS	T	DANGER	7/22/90	QUIL	320	10	
41	PWS	T	DANGER	7/21/90	QUIL	344	10	
21	PWS	T	HERRING	7/19/90	QUIL	420	35	
30	PWS	T	HERRING	7/20/90	QUIL	415	50	
23	PWS	T	HERRING	7/19/90	QUIL	430	16	
20	PWS	T	HERRING	7/19/90	QUIL	361	23	
28	PWS	T	HERRING	7/19/90	QUIL	356	35	
27	PWS	T	HERRING	7/19/90	QUIL	370	30	
1001	PWS	C	GRAVINA	7/21/91	QUIL	325	11	655
1002	PWS	C	GRAVINA	7/21/91	QUIL	343	14	716
1026	PWS	C	GRAVINA	7/22/91	QUIL	420	55	1259
1021	PWS	C	GRAVINA	7/22/91	QUIL	304	9	561
1029	PWS	C	GRAVINA	7/22/91	QUIL	390	32	1279
1025	PWS	C	GRAVINA	7/22/91	QUIL	434	47	1653
1005	PWS	C	GRAVINA	7/21/91	QUIL	433	30	2193
1011	PWS	C	GRAVINA	7/21/91	QUIL	360	19	909
1020	PWS	C	GRAVINA	7/22/91	QUIL	350	15	834
1028	PWS	C	GRAVINA	7/22/91	QUIL	407	17	1323
1030	PWS	C	GRAVINA	7/22/91	QUIL	369	21	909
1027	PWS	C	GRAVINA	7/22/91	QUIL	363	18	816
1012	PWS	C	GRAVINA	7/21/91	QUIL	395	33	1155
1074	PWS	C	ZAIKOF	8/ 8/91	QUIL	195	9	153
1073	PWS	C	ZAIKOF	8/ 8/91	QUIL	314	11	585
1078	PWS	C	ZAIKOF	8/10/91	QUIL	333	9	615
1077	PWS	C	ZAIKOF	8/ 9/91	QUIL	265	11	388
1103	PWS	C	ZAIKOF	9/12/91	QUIL	338	13	715
1075	PWS	C	ZAIKOF	8/ 8/91	QUIL	169	9	95
1076	PWS	C	ZAIKOF	8/ 9/91	QUIL	316	11	736
1083	PWS	C	ZAIKOF	8/12/91	QUIL	346	16	845
1081	PWS	C	ZAIKOF	8/10/91	QUIL	307	8	555
1106	PWS	C	ZAIKOF	9/13/91	QUIL	362	14	895
1079	PWS	C	ZAIKOF	8/10/91	QUIL	283	10	445
1080	PWS	C	ZAIKOF	8/10/91	QUIL	264	11	370
1084	PWS	C	ZAIKOF	8/12/91	QUIL	375	18	1040
1105	PWS	C	ZAIKOF	9/12/91	QUIL	270	9	337
1082	PWS	C	ZAIKOF	8/12/91	QUIL	251	9	300

ID#			SITE	DATE	SPECIES	LENGTH	AGE	WEIGHT
1035	PWS	T	DANGER	7/27/91	QUIL	409	24	1317
1086	PWS	T	DANGER	9/ 9/91	QUIL	378	19	975
1102	PWS	T	DANGER	9/10/91	QUIL	330	16	690
1090	PWS	T	DANGER	9/ 9/91	QUIL	410	26	1350
1040	PWS	T	DANGER	7/27/91	QUIL	400	16	1175
1087	PWS	T	DANGER	9/ 9/91	QUIL	360	18	900
1100	PWS	T	DANGER	9/ 9/91	QUIL	390	29	1130
1088	PWS	T	DANGER	9/ 9/91	QUIL	361	16	870
1064	PWS	T	HERRING	8/ 7/91	QUIL	319	11	690
1066	PWS	T	HERRING	8/ 7/91	QUIL	339	14	781
1068	PWS	T	HERRING	8/ 7/91	QUIL	339	12	650
1044	PWS	T	HERRING	8/ 6/91	QUIL	351	14	663
1065	PWS	T	HERRING	8/ 7/91	QUIL	326	11	657
1072	PWS	T	HERRING	8/ 7/91	QUIL	340	19	790
1053	PWS	T	HERRING	8/ 6/91	QUIL	276	10	405
1043	PWS	T	HERRING	8/ 6/91	QUIL	394	31	1111
1071	PWS	T	HERRING	8/ 7/91	QUIL	348	24	836
1070	PWS	T	HERRING	8/ 7/91	QUIL	365	29	886
1067	PWS	T	HERRING	8/ 7/91	QUIL	353	22	908
1049	PWS	T	HERRING	8/ 6/91	QUIL	375	34	992
YELLOWEYE ROCKFISH								
C270	PWS	C	GRAVINA	6/29/89	YELE	601	46	
C268	PWS	C	GRAVINA	6/29/89	YELE	335	11	
C272	PWS	C	GRAVINA	6/29/89	YELE	580	63	
C269	PWS	C	GRAVINA	6/29/89	YELE	460	21	
C271	PWS	C	GRAVINA	6/29/89	YELE	655	72	
B102	PWS	C	WINDY B	6/17/89	YELE	451	31	
B133	PWS	T	CABIN B	6/13/89	YELE	368	12	
B132	PWS	T	CABIN B	6/13/89	YELE	400	12	
B126	PWS	T	CABIN B	6/13/89	YELE	525	31	
D142	PWS	T	DANGER	9/19/89	YELE	560	21	
D169	PWS	T	DANGER	9/19/89	YELE	565	20	
D146	PWS	T	DANGER	9/19/89	YELE	538	23	
D145	PWS	T	DANGER	9/19/89	YELE	629	32	
D170	PWS	T	DANGER	9/19/89	YELE	468	22	
C221	PWS	T	HERRING	6/24/89	YELE	465	37	
C212	PWS	T	HERRING	6/24/89	YELE	365	14	
C215	PWS	T	HERRING	6/24/89	YELE	408	18	
B123	PWS	T	HERRING	6/14/89	YELE	425	22	
C224	PWS	T	HERRING	6/24/89	YELE	336	13	
B100	PWS	T	HERRING	6/14/89	YELE	595	63	
C203	PWS	T	HERRING	6/24/89	YELE	523	31	
C201	PWS	T	HERRING	6/24/89	YELE	505	24	
C225	PWS	T	HERRING	6/24/89	YELE	317	12	

ID#			SITE	DATE	SPECIES	LENGTH	AGE	WEIGHT
C202	PWS	T	HERRING	6/24/89	YELE	490	27	
C220	PWS	T	HERRING	6/24/89	YELE	560	61	
C227	PWS	T	HERRING	6/24/89	YELE	504	28	
C226	PWS	T	HERRING	6/24/89	YELE	350	20	
C214	PWS	T	HERRING	6/24/89	YELE	495	21	
B121	PWS	T	HERRING	6/14/89	YELE	545	60	
C200	PWS	T	HERRING	6/24/89	YELE	565	41	
C213	PWS	T	HERRING	6/24/89	YELE	521	66	
C222	PWS	T	HERRING	6/24/89	YELE	356	11	
C219	PWS	T	HERRING	6/24/89	YELE	495	36	
C209	PWS	T	HERRING	6/24/89	YELE	355	11	
B119	PWS	T	HERRING	6/14/89	YELE	405	20	
B122	PWS	T	HERRING	6/14/89	YELE	442	20	
C208	PWS	T	HERRING	6/24/89	YELE	505	30	
B120	PWS	T	HERRING	6/14/89	YELE	400	12	
C210	PWS	T	HERRING	6/24/89	YELE	500	22	
C211	PWS	T	HERRING	6/24/89	YELE	475	20	
C223	PWS	T	HERRING	6/24/89	YELE	485	31	
C207	PWS	T	HERRING	6/24/89	YELE	522	51	
C242	PWS	T	LONETRE	6/25/89	YELE	546	27	
C244	PWS	T	LONETRE	6/25/89	YELE	460	22	
C238	PWS	T	LONETRE	6/25/89	YELE	450	21	
C325	PWS	T	LONETRE	6/25/89	YELE	485	20	
C247	PWS	T	LONETRE	6/25/89	YELE	579	32	
C290	PWS	T	LONETRE	6/25/89	YELE	395	12	
C246	PWS	T	LONETRE	6/25/89	YELE	695	39	
C331	PWS	T	LONETRE	6/25/89	YELE	373	11	
C241	PWS	T	LONETRE	6/25/89	YELE	520	30	
C243	PWS	T	LONETRE	6/25/89	YELE	473	23	
C239	PWS	T	LONETRE	6/25/89	YELE	703	70	
C245	PWS	T	LONETRE	6/25/89	YELE	652	32	
C324	PWS	T	LONETRE	6/25/89	YELE	475	20	
C240	PWS	T	LONETRE	6/25/89	YELE	545	23	
B116	PWS	T	PT NOWE	6/14/89	YELE	576	70	
B118	PWS	T	PT NOWE	6/14/89	YELE	579	69	
B115	PWS	T	PT NOWE	6/14/89	YELE	520	55	
B112	PWS	T	PT NOWE	6/14/89	YELE	458	38	
B158	PWS	T	PT NOWE	6/14/89	YELE	555	58	
B159	PWS	T	PT NOWE	6/14/89	YELE	517	43	
B155	PWS	T	PT NOWE	6/14/89	YELE	470	33	
B109	PWS	T	PT NOWE	6/14/89	YELE	363	14	
B111	PWS	T	PT NOWE	6/14/89	YELE	525	43	
B117	PWS	T	PT NOWE	6/14/89	YELE	467	34	
B113	PWS	T	PT NOWE	6/14/89	YELE	431	20	
B160	PWS	T	PT NOWE	6/14/89	YELE	552	62	
B110	PWS	T	PT NOWE	6/14/89	YELE	507	37	
B157	PWS	T	PT NOWE	6/14/89	YELE	493	40	

ID#			SITE	DATE	SPECIES	LENGTH	AGE	WEIGHT
B114	PWS	T	PT NOWE	6/14/89	YELE	455	22	
B161	PWS	T	PT NOWE	6/14/89	YELE	508	27	
B156	PWS	T	PT NOWE	6/14/89	YELE	565	44	
5	PWS	C	GRAVINA	7/16/90	YELE	350	13	
10	PWS	C	GRAVINA	7/17/90	YELE	366	12	
9	PWS	C	GRAVINA	7/17/90	YELE	553	26	
4	PWS	C	GRAVINA	7/16/90	YELE	410	15	
11	PWS	C	GRAVINA	7/17/90	YELE	462	22	
8	PWS	C	GRAVINA	7/17/90	YELE	430	24	
106	PWS	C	MIDDLEP	8/ 3/90	YELE	402	12	1200
44	PWS	T	DANGER	7/22/90	YELE	626	30	
38	PWS	T	DANGER	7/22/90	YELE	546	23	
40	PWS	T	DANGER	7/22/90	YELE	520	23	
31	PWS	T	DANGER	7/21/90	YELE	611	37	
34	PWS	T	DANGER	7/21/90	YELE	614	23	
32	PWS	T	DANGER	7/21/90	YELE	620	32	
43	PWS	T	DANGER	7/22/90	YELE	510	24	
33	PWS	T	DANGER	7/21/90	YELE	490	23	
18	PWS	T	HERRING	7/19/90	YELE	540	49	
17	PWS	T	HERRING	7/19/90	YELE	400	14	
24	PWS	T	HERRING	7/20/90	YELE	417	20	
19	PWS	T	HERRING	7/19/90	YELE	625	50	
1016	PWS	C	GRAVINA	7/21/91	YELE	269	9	352
1024	PWS	C	GRAVINA	7/22/91	YELE	321	13	499
1018	PWS	C	GRAVINA	7/22/91	YELE	365	13	771
1017	PWS	C	GRAVINA	7/22/91	YELE	343	14	701
1022	PWS	C	GRAVINA	7/22/91	YELE	531	39	3275
1004	PWS	C	GRAVINA	7/21/91	YELE	566	41	
1023	PWS	C	GRAVINA	7/22/91	YELE	401	17	1222
1107	PWS	C	ZAIKOF	9/14/91	YELE	694	54	
1104	PWS	C	ZAIKOF	9/12/91	YELE	650	34	4750
1032	PWS	T	DANGER	7/26/91	YELE	440	21	1849
1095	PWS	T	DANGER	9/ 9/91	YELE	613	47	4225
1093	PWS	T	DANGER	9/ 9/91	YELE	516	26	2525
1092	PWS	T	DANGER	9/ 9/91	YELE	540	26	2750
1099	PWS	T	DANGER	9/ 9/91	YELE	570	25	3300
1098	PWS	T	DANGER	9/ 9/91	YELE	702	60	
1097	PWS	T	DANGER	9/ 9/91	YELE	680	48	5300
1031	PWS	T	DANGER	7/26/91	YELE	560	32	3910
1094	PWS	T	DANGER	9/ 9/91	YELE	585	45	3730
1096	PWS	T	DANGER	9/ 9/91	YELE	650	52	5020
1089	PWS	T	DANGER	9/ 9/91	YELE	657	36	
1091	PWS	T	DANGER	9/ 9/91	YELE	561	31	2700

ID#			SITE	DATE	SPECIES	LENGTH	AGE	WEIGHT
1069	PWS	T	HERRING	8/ 7/91	YELE	518	27	2290
1058	PWS	T	HERRING	8/ 7/91	YELE	359	16	825
1046	PWS	T	HERRING	8/ 6/91	YELE	403	15	1143
1061	PWS	T	HERRING	8/ 7/91	YELE	308	10	479
1047	PWS	T	HERRING	8/ 6/91	YELE	504	22	1967
1060	PWS	T	HERRING	8/ 7/91	YELE	286	9	359
1063	PWS	T	HERRING	8/ 7/91	YELE	343	15	714
1052	PWS	T	HERRING	8/ 6/91	YELE	500	22	2372
1056	PWS	T	HERRING	8/ 7/91	YELE	522	34	2265
1059	PWS	T	HERRING	8/ 7/91	YELE	306	10	480
1055	PWS	T	HERRING	8/ 7/91	YELE	551	37	2704
1045	PWS	T	HERRING	8/ 6/91	YELE	424	15	1282
1051	PWS	T	HERRING	8/ 6/91	YELE	370	17	489
1054	PWS	T	HERRING	8/ 7/91	YELE	405	15	1140
1057	PWS	T	HERRING	8/ 3/91	YELE	345	11	767
1048	PWS	T	HERRING	8/ 6/91	YELE	555	50	3153
1062	PWS	T	HERRING	8/ 7/91	YELE	276	8	396
1050	PWS	T	HERRING	8/ 6/91	YELE	612	58	4265
VERMILLION ROCKFISH								
112	PWS	C	ZAIKOF	8/ 5/90	VERM	325	10	900
SILVERGREY ROCKFISH								
C237	PWS	T	HERRING	6/24/89	SGRY	340	7	
25	PWS	T	HERRING	7/20/90	SGRY	378	12	
29	PWS	T	HERRING	7/20/90	SGRY	360	9	
TIGER ROCKFISH								
B125	PWS	T	HERRING	6/14/89	TIGR	352	34	
YELLOWTAIL ROCKFISH								
39	PWS	T	DANGER	7/22/90	YELT	413	10	
42	PWS	T	DANGER	7/22/90	YELT	377	8	

Appendiz B2. Rockfish age, length and weight data for the lower Kenai Peninsula, 1989 - 1991.

ID#			SITE	DATE	SPECIES	LENGTH	AGE	WEIGHT
BLACK ROCKFISH								
C315	LKP	C	CP PUGE	6/26/89	BLAC	409	11	
D072	LKP	C	GRANITE	9/17/89	BLAC	488	10	
D075	LKP	C	GRANITE	9/17/89	BLAC	478	11	
D079	LKP	C	GRANITE	9/17/89	BLAC	465	11	
D077	LKP	C	GRANITE	9/17/89	BLAC	410	11	
D078	LKP	C	GRANITE	9/17/89	BLAC	425	9	
D076	LKP	C	GRANITE	9/17/89	BLAC	515	16	
D090	LKP	T	ALIGO P	9/18/89	BLAC	436	13	
D089	LKP	T	ALIGO P	9/17/89	BLAC	450	10	
C339	LKP	T	CP AIAL	6/27/89	BLAC	484	11	
C296	LKP	T	CP AIAL	6/27/89	BLAC	525	12	
C338	LKP	T	CP AIAL	6/27/89	BLAC	454	11	
C294	LKP	T	CP AIAL	6/27/89	BLAC		10	
C307	LKP	T	DRIFTWD	6/26/89	BLAC	456	10	
C292	LKP	T	DRIFTWD	6/26/89	BLAC	530	11	
C304	LKP	T	DRIFTWD	6/26/89	BLAC	520	13	
C293	LKP	T	DRIFTWD	6/26/89	BLAC	454	15	
C306	LKP	T	DRIFTWD	6/26/89	BLAC	451	12	
C291	LKP	T	DRIFTWD	6/26/89	BLAC	498	12	
C305	LKP	T	DRIFTWD	6/26/89	BLAC	455	10	
D047	LKP	T	FRONT P	9/15/89	BLAC	432	13	
D041	LKP	T	FRONT P	9/15/89	BLAC	360	8	
D042	LKP	T	FRONT P	9/15/89	BLAC	453	17	
D043	LKP	T	FRONT P	9/15/89	BLAC	466	13	
D044	LKP	T	FRONT P	9/15/89	BLAC	456	17	
D045	LKP	T	FRONT P	9/15/89	BLAC	496	18	
D048	LKP	T	FRONT P	9/15/89	BLAC	468	23	
D046	LKP	T	FRONT P	9/15/89	BLAC	430	11	
D059	LKP	T	GORE PT	9/16/89	BLAC	484	10	
D055	LKP	T	GORE PT	9/16/89	BLAC	463	29	
D051	LKP	T	GORE PT	9/16/89	BLAC	467	10	
D056	LKP	T	GORE PT	9/16/89	BLAC	444	10	
D052	LKP	T	GORE PT	9/16/89	BLAC	495	17	
D054	LKP	T	GORE PT	9/16/89	BLAC	385	32	
D057	LKP	T	GORE PT	9/16/89	BLAC	503	14	
D053	LKP	T	GORE PT	9/16/89	BLAC	538	14	
D058	LKP	T	GORE PT	9/16/89	BLAC	482	8	
D027	LKP	T	OUTER I	9/15/89	BLAC	470	11	
D040	LKP	T	OUTER I	9/15/89	BLAC	531	32	
D024	LKP	T	OUTER I	9/15/89	BLAC	445	10	
D017	LKP	T	OUTER I	9/15/89	BLAC	541	12	
D038	LKP	T	OUTER I	9/15/89	BLAC	475	13	

ID#			SITE	DATE	SPECIES	LENGTH	AGE	WEIGHT
D019	LKP	T	OUTER I	9/15/89	BLAC	470	11	
D036	LKP	T	OUTER I	9/15/89	BLAC	510	13	
D023	LKP	T	OUTER I	9/15/89	BLAC	487	14	
D039	LKP	T	OUTER I	9/15/89	BLAC	520	41	
D025	LKP	T	OUTER I	9/15/89	BLAC	461	11	
D015	LKP	T	OUTER I	9/15/89	BLAC	521	19	
D026	LKP	T	OUTER I	9/15/89	BLAC	477	18	
D037	LKP	T	OUTER I	9/15/89	BLAC	486	14	
D022	LKP	T	OUTER I	9/15/89	BLAC	495	14	
D071	LKP	T	PORT DK	9/16/89	BLAC	427	8	
D065	LKP	T	PORT DK	9/16/89	BLAC	464	11	
D064	LKP	T	PORT DK	9/16/89	BLAC	475	10	
D069	LKP	T	PORT DK	9/16/89	BLAC	439	11	
D062	LKP	T	PORT DK	9/16/89	BLAC	466	11	
D068	LKP	T	PORT DK	9/16/89	BLAC	474	11	
D067	LKP	T	PORT DK	9/16/89	BLAC	389	8	
D063	LKP	T	PORT DK	9/16/89	BLAC	483	11	
D061	LKP	T	PORT DK	9/16/89	BLAC	441	10	
D066	LKP	T	PORT DK	9/16/89	BLAC	451	11	
D012	LKP	T	SEAL RK	9/15/89	BLAC	495	8	
D135	LKP	T	SEAL RK	9/18/89	BLAC	505	13	
D002	LKP	T	SEAL RK	9/15/89	BLAC	487	10	
D134	LKP	T	SEAL RK	9/18/89	BLAC	462	10	
D006	LKP	T	SEAL RK	9/15/89	BLAC	472	12	
D005	LKP	T	SEAL RK	9/15/89	BLAC	507	10	
D001	LKP	T	SEAL RK	9/15/89	BLAC	483	9	
D011	LKP	T	SEAL RK	9/15/89	BLAC	415	11	
D003	LKP	T	SEAL RK	9/15/89	BLAC	438	9	
D136	LKP	T	SEAL RK	9/18/89	BLAC	515	14	
D004	LKP	T	SEAL RK	9/15/89	BLAC	500	11	
CHINA ROCKFISH								
C254	LKP	C	CP PUGE	6/26/89	CHIN	313	15	
C322	LKP	C	CP PUGE	6/26/89	CHIN	319	24	
C321	LKP	C	CP PUGE	6/26/89	CHIN	281	13	
C317	LKP	C	CP PUGE	6/26/89	CHIN	338	30	
C318	LKP	C	CP PUGE	6/26/89	CHIN	308	22	
D081	LKP	C	GRANITE	9/17/89	CHIN	285	13	
D080	LKP	C	GRANITE	9/17/89	CHIN	298	13	
D096	LKP	T	ALIGO P	9/18/89	CHIN	289	20	
D093	LKP	T	ALIGO P	9/18/89	CHIN	295	14	
D095	LKP	T	ALIGO P	9/18/89	CHIN	317	30	
D111	LKP	T	ALIGO P	9/18/89	CHIN	300	21	
D109	LKP	T	ALIGO P	9/18/89	CHIN	298	14	
D108	LKP	T	ALIGO P	9/18/89	CHIN	267	11	

ID#			SITE	DATE	SPECIES	LENGTH	AGE	WEIGHT
D110	LKP	T	ALIGO P	9/18/89	CHIN	273	13	
D094	LKP	T	ALIGO P	9/18/89	CHIN	308	23	
D010	LKP	T	SEAL RK	9/15/89	CHIN	322	14	
72	LKP	C	GRANITE	7/26/90	CHIN	262	11	430
73	LKP	C	GRANITE	7/26/90	CHIN	303	14	510
62	LKP	C	GRANITE	7/26/90	CHIN	293	15	
69	LKP	C	GRANITE	7/28/90	CHIN	202	8	75
65	LKP	C	GRANITE	7/26/90	CHIN	292	15	580
74	LKP	C	GRANITE	7/26/90	CHIN	260	15	375
75	LKP	C	GRANITE	7/27/90	CHIN	325	18	700
61	LKP	C	GRANITE	7/26/90	CHIN	318	16	
67	LKP	C	GRANITE	7/27/90	CHIN	310	15	675
63	LKP	C	GRANITE	7/26/90	CHIN	297	16	
46	LKP	T	MORNING	7/24/90	CHIN	222	8	
89	LKP	T	PONYCOV	7/29/90	CHIN	298	17	525
77	LKP	T	PONYCOV	7/29/90	CHIN	270	15	450
85	LKP	T	PONYCOV	7/30/90	CHIN	335	15	650
76	LKP	T	PONYCOV	7/29/90	CHIN	292	15	570
86	LKP	T	PONYCOV	7/29/90	CHIN	277	15	500
COPPER ROCKFISH								
C316	LKP	C	CP PUGE	6/26/89	COPP	377	15	
C323	LKP	C	CP PUGE	6/26/89	COPP	390	14	
102	LKP	C	DAYHARB	8/ 1/90	COPP	285	8	375
96	LKP	C	DAYHARB	8/ 1/90	COPP	255	7	325
99	LKP	C	DAYHARB	8/ 2/90	COPP	268	7	300
94	LKP	C	DAYHARB	8/ 1/90	COPP	321	12	650
93	LKP	C	DAYHARB	8/ 1/90	COPP	402	24	1200
70	LKP	C	GRANITE	7/28/90	COPP	303	15	575
71	LKP	C	GRANITE	7/26/90	COPP	381	15	1300
58	LKP	T	MORNING	7/24/90	COPP	244	8	
52	LKP	T	MORNING	7/24/90	COPP	302	10	
54	LKP	T	MORNING	7/25/90	COPP	332	15	
55	LKP	T	MORNING	7/25/90	COPP	329	13	
60	LKP	T	MORNING	7/25/90	COPP	275	8	
50	LKP	T	MORNING	7/24/90	COPP	370	24	
87	LKP	T	PONYCOV	7/29/90	COPP	258	9	350
78	LKP	T	PONYCOV	7/29/90	COPP	278	9	450
90	LKP	T	PONYCOV	7/29/90	COPP	252	7	340
DUSKY ROCKFISH								

ID#			SITE	DATE	SPECIES	LENGTH	AGE	WEIGHT
D083	LKP	C	CRATER	9/17/89	DUSK	315	9	
D074	LKP	C	GRANITE	9/17/89	DUSK	405	20	
D073	LKP	C	GRANITE	9/17/89	DUSK	349	14	
D091	LKP	T	ALIGO P	9/18/89	DUSK	371	18	
D049	LKP	T	FRONT P	9/15/89	DUSK	415	26	
D070	LKP	T	PORT DK	9/16/89	DUSK	368	29	
D127	LKP	T	SEAL RK	9/18/89	DUSK	397	19	
D008	LKP	T	SEAL RK	9/15/89	DUSK	416	16	
D125	LKP	T	SEAL RK	9/18/89	DUSK	410	15	
D132	LKP	T	SEAL RK	9/18/89	DUSK	421	16	
D119	LKP	T	SEAL RK	9/18/89	DUSK	417	30	
D123	LKP	T	SEAL RK	9/18/89	DUSK	395	13	
D124	LKP	T	SEAL RK	9/18/89	DUSK	439	35	
D133	LKP	T	SEAL RK	9/18/89	DUSK	439	37	
D121	LKP	T	SEAL RK	9/18/89	DUSK	456	21	
D128	LKP	T	SEAL RK	9/18/89	DUSK	420	33	
D122	LKP	T	SEAL RK	9/18/89	DUSK	398	22	
D130	LKP	T	SEAL RK	9/18/89	DUSK	425	15	
D131	LKP	T	SEAL RK	9/18/89	DUSK	353	13	
D007	LKP	T	SEAL RK	9/15/89	DUSK	390	13	
D126	LKP	T	SEAL RK	9/18/89	DUSK	416	20	
D129	LKP	T	SEAL RK	9/18/89	DUSK	417	23	
D120	LKP	T	SEAL RK	9/18/89	DUSK	422	43	
QUILLBACK ROCKFISH								
D118	LKP	C	CRATER	9/18/89	QUIL	325	14	
D092	LKP	T	ALIGO P	9/18/89	QUIL	369	16	
C337	LKP	T	CP AIAL	6/27/89	QUIL	395	33	
95	LKP	C	DAYHARB	8/ 1/90	QUIL	286	8	475
104	LKP	C	DAYHARB	8/ 1/90	QUIL	259	8	330
100	LKP	C	DAYHARB	8/ 2/90	QUIL	255	7	330
101	LKP	C	DAYHARB	8/ 1/90	QUIL	278	9	425
103	LKP	C	DAYHARB	8/ 1/90	QUIL	265	8	350
105	LKP	C	DAYHARB	8/ 1/90	QUIL	246	10	310
68	LKP	C	GRANITE	7/27/90	QUIL	216	7	200
57	LKP	T	MORNING	7/24/90	QUIL	226	7	
53	LKP	T	MORNING	7/24/90	QUIL	244	8	
49	LKP	T	MORNING	7/24/90	QUIL	357	16	
56	LKP	T	MORNING	7/24/90	QUIL	217	7	
47	LKP	T	MORNING	7/24/90	QUIL	301	9	
88	LKP	T	PONYCOV	7/29/90	QUIL	247	7	

ID#			SITE	DATE	SPECIES	LENGTH	AGE	WEIGHT
YELLOWEYE ROCKFISH								
C251	LKP	C	CP PUGE	6/26/89	YELE	575	24	
C314	LKP	C	CP PUGE	6/26/89	YELE	458	21	
C319	LKP	C	CP PUGE	6/26/89	YELE	430	19	
C252	LKP	C	CP PUGE	6/26/89	YELE	583	25	
C320	LKP	C	CP PUGE	6/26/89	YELE	404	20	
C250	LKP	C	CP PUGE	6/26/89	YELE	587	29	
C249	LKP	C	CP PUGE	6/26/89	YELE	700	44	
D085	LKP	C	HARRIS	9/17/89	YELE	421	21	
D087	LKP	T	ALIGO P	9/18/89	YELE	550	37	
D088	LKP	T	ALIGO P	9/18/89	YELE	503	22	
C311	LKP	T	CHISWEL	6/27/89	YELE	498	22	
C299	LKP	T	CHISWEL	6/27/89	YELE	545	26	
C312	LKP	T	CHISWEL	6/27/89	YELE	440	19	
C297	LKP	T	CHISWEL	6/27/89	YELE	445	20	
C309	LKP	T	CHISWEL	6/27/89	YELE	522	31	
C308	LKP	T	CHISWEL	6/27/89	YELE	492	31	
C298	LKP	T	CHISWEL	6/27/89	YELE	445	19	
C300	LKP	T	CHISWEL	6/27/89	YELE	527	27	
C313	LKP	T	CHISWEL	6/27/89	YELE	490	21	
C310	LKP	T	CHISWEL	6/27/89	YELE	445	25	
C262	LKP	T	CP AIAL	6/27/89	YELE	545	29	
C335	LKP	T	CP AIAL	6/27/89	YELE	530	23	
C295	LKP	T	CP AIAL	6/27/89	YELE	455	21	
C333	LKP	T	CP AIAL	6/27/89	YELE	555	39	
C261	LKP	T	CP AIAL	6/27/89	YELE	664	42	
C263	LKP	T	CP AIAL	6/27/89	YELE	553	27	
C336	LKP	T	CP AIAL	6/27/89	YELE	515	27	
C334	LKP	T	CP AIAL	6/27/89	YELE	523	24	
C264	LKP	T	CP AIAL	6/27/89	YELE	595	60	
D060	LKP	T	GORE PT	9/16/89	YELE	578	21	
D021	LKP	T	OUTER I	9/15/89	YELE	655	31	
D020	LKP	T	OUTER I	9/15/89	YELE	674	49	
D032	LKP	T	OUTER I	9/15/89	YELE	788	59	
D018	LKP	T	OUTER I	9/15/89	YELE	680	30	
D034	LKP	T	OUTER I	9/15/89	YELE	581	24	
D016	LKP	T	OUTER I	9/15/89	YELE	687	30	
D033	LKP	T	OUTER I	9/15/89	YELE	658	32	
D014	LKP	T	OUTER I	9/15/89	YELE	715	41	
D028	LKP	T	OUTER I	9/15/89	YELE	675	38	
D035	LKP	T	OUTER I	9/15/89	YELE	445	24	
D009	LKP	T	SEAL RK	9/15/89	YELE	515	20	
D138	LKP	T	SEAL RK	9/18/89	YELE	520	22	
D137	LKP	T	SEAL RK	9/18/89	YELE	550	22	

ID#			SITE	DATE	SPECIES	LENGTH	AGE	WEIGHT
92	LKP	C	DAYHARB	8/ 1/90	YELE	239	9	275
98	LKP	C	DAYHARB	8/ 2/90	YELE	301	9	300
81	LKP	T	PONYCOV	7/30/90	YELE	542	30	2800
82	LKP	T	PONYCOV	7/30/90	YELE	499	22	410
SILVERGREY ROCKFISH								
D082	LKP	C	MNLIGHT	9/16/89	SGRY	385	8	
C301	LKP	T	CHISWEL	6/27/89	SGRY	640	47	
D050	LKP	T	FRONT P	9/15/89	SGRY	373	9	
48	LKP	T	MORNING	7/24/90	SGRY	342	9	
80	LKP	T	PONYCOV	7/30/90	SGRY	299	9	
TIGER ROCKFISH								
64	LKP	C	GRANITE	7/26/90	TIGR	278	16	
66	LKP	C	GRANITE	7/27/90	TIGR	382	20	1050
51	LKP	T	MORNING	7/24/90	TIGR	273	23	
83	LKP	T	PONYCOV	7/30/90	TIGR	362	24	800
79	LKP	T	PONYCOV	7/30/90	TIGR	335	22	700
84	LKP	T	PONYCOV	7/30/90	TIGR	327	23	660

APPENDIX C

1990 Histopathology Report
from
University of California, Davis
School of Veterinary Medicine

XI. ROCKFISH - Final Summary Report

A. 1990 Rockfish - Histopathologic Methods and Results

HISTOPATHOLOGISTS - Mark Okihiro, DVM; David Hanes, Ph.D.

Filenames: **90RF_R.PRN** or **90RF_R.WK1**

METHODS

Jars of formalin containing liver, spleen, gonad (ovary or testis), gill, and kidney from 121 rockfish (eight different species) were received [logged in on 9-17-1990 by David Hanes]. Gills were decalcified before sectioning. All fish were assigned a random number (processing code, see RF-Table 1) and all tissues were processed routinely in paraffin and stained with hematoxylin and Eosin (HE). Slides were read in ascending numerical order based on the assigned random number (i.e., blind study). Lesions were subjectively ranked using a four point scale: none (0), mild (1), moderate (2), or severe (3); tissues that were not present were designated with a period (.). Data sheets used for scoring lesions in each rockfish tissue were included in the July 3, 1991 progress report (Appendix 9, pp. 53-55). To optimize precision of results, all specimens of a given organ (e.g., all 121 livers) were read and scored before any specimens of the next organ were scored.

RESULTS

Lesion scores and basic historical/site data for each fish are listed in RF-Table 1. Gonadal lesions were minimal and are not reported in table format.

I. Quillback Rockfish - 41 quillback rockfish were examined
   A. Liver
      1. Normal histology: Normal rockfish livers had glycogen-laden hepatocytes organized into orderly tubules (RF90-Figure 1). Hepatocyte nuclei were small, round, and regular.

      2. Megalocytosis: The most striking feature of quillback rockfish livers was megalocytosis. Affected hepatocytes had marked nuclear and moderate cellular enlargement (RF90-Figures 2-5). Enlarged nuclei varied from 2 to 10X normal size, and cytomegalic hepatocytes varied from 2 to 6X normal size. Some megalocytes were multinucleated (up to five nuclei per cell; RF90-Figure 3) and enlarged nuclei were often elongate and/or irregular. Nucleoli were usually single and prominent, but some nuclei had two or three nucleoli. Some karyomegalic nuclei contained pseudoinclusions (RF90-Figure 4).

         In severely affected livers, tubular architecture was often disrupted by enlarged, irregular hepatocytes. Sinusoids were compressed and some foci of tubules appeared to lack nuclei (assumed to be due to enlargement of hepatocytes such that nuclei were out of the plane of section). Enlarged hepatocytes often contained moderate to large amounts of coarsely granular, light brown pigment which was similar to that seen in macrophages. Severe megalocytosis was usually associated with increased numbers of macrophage aggregates and scattered individually necrotic hepatocytes (apoptosis).

Interestingly, some microcytic hepatocytes were in livers that had severe megalocytosis. In a separate study in our laboratory, we are in the process of confirming differences in hepatocyte and nuclear size with morphometry of rockfish caught off the California coast.

Differences in mean megalocyte scores among sites were not significant (ANOVA, $P = 0.13$).

Comment: Megalocytosis in mammals (primarily horses) is usually associated with pyrrolizidine alkaloid toxicity, but we have occasionally seen this lesion in medaka (Oryzias latipes, a small aquarium fish) in both controls and in medaka exposed to diethylnitrosamine (DEN) (Hinton et al. 1988b). Severe megalocytosis was also observed in a group of pond-raised striped bass (Groff et al. 1992).

3. Sinusoidal fibrosis: An uncommon, but striking hepatic lesion (RF90-Figure 6). Distribution tended to be patchy, with affected sinusoids lined by variable amounts of fibrillar collagen (confirmed using Masson's Trichrome stain; RF90-Figure 7). In some areas, sinusoidal fibrosis was continuous with the connective tissue of large veins.

4. Necrosis:
    a. Coagulation necrosis: none

    b. Single cell necrosis: Individual hepatocyte necrosis or apoptosis was a common, but usually mild finding (RF90-Figure 2).

5. Inflammation:
    a. Macrophage aggregates: Macrophage aggregates were a common finding in quillback livers (RF90-Figure 8). Macrophages in these aggregates were usually vacuolated (possibly due to fat accumulation in phagolysosomes) and filled with granular brown pigment (either hemosiderin or lipofuscin).

    Comment: Macrophage aggregates were probably an indicator of previous hepatocyte degeneration and necrosis (i.e., macrophages phagocytized dead hepatocytes). The scoring scheme for macrophage aggregates in the liver was slightly different than for the spleen and kidney because the liver is not a normal terminal site for macrophages. Macrophage aggregates are being used by the EPA and NMFS as indicators of pollutant stress.

b. Lymphocytic aggregates: Small clusters of lymphocytes were occasionally in the liver.

6. Hepatocyte storage disorders
   a. Glycogen depletion: Glycogen depletion was common and characterized by loss of cell volume and increased cytoplasmic basophilia (RF90-Figure 5).

   b. Lipidosis (hepatocellular fatty change): A few fish had mild lipidosis.

   c. Eosinophilic bodies: Some hepatocytes contained refractile, eosinophilic, intracytoplasmic droplets that may represent large lysosomes (RF90-Figure 8).

7. Bile duct hyperplasia: none

8. Parasitism: minimal. A few fish had small numbers of Ichthyophonus, nematodes, and trematodes.

B. Kidney

1. Renal tubular degeneration and necrosis: Another common finding was vacuolar degeneration and necrosis of individual or small clusters of tubular epithelial cells (RF90-Figure 9). This was associated with the influx of individual macrophages into the tubular epithelium and the presence of small amounts of necrotic debris in some tubules.

   Comment: Renal tubular necrosis certainly could be related to xenobiotic exposure.

2. Glomerulonephritis: One of the most consistent renal lesions in quillback rockfish was the presence of generalized membranous glomerulonephritis (RF90-Figure 10). Affected glomeruli had mild to severe thickening of basement membranes by pale eosinophilic, acellular material. In some glomeruli, there also appeared to be mild to moderate proliferation of mesangial cells (podocytes) and mild dilation of Bowman's capsule. This lesion was, in some fish, associated with large amounts of protein droplets in proximal tubular epithelial cells.

   Comment: Membranous glomerulonephritis is a chronic renal disease which is usually associated with the deposition of immune complexes or anti-glomerular antibodies on the glomerular basement membranes. The lesion must be differentiated from amyloidosis. We do not know if the lesions could be related to oil exposure, but it seems unlikely.

3. Inflammation
   a. Macrophage aggregates: Many kidneys were massively infiltrated by macrophage aggregates (RF90-Figure 11).

Comment: The degree and number of macrophage aggregates probably reflects the amount of degeneration and necrosis of renal tubular epithelial cells.

b. Lymphoid aggregates: occasionally seen

4. Megalocytosis: In a few fish, scattered renal tubules were lined by epithelial cells with karyomegaly (RF90-Figure 12).

5. Protein: In some fish, scattered renal tubular epithelial cells were packed with eosinophilic "protein" droplets (RF90-Figure 13).

C. Spleen
   1. Inflammation:
      a. Macrophage aggregates: Macrophage aggregates were a consistent finding. Some fish had large numbers of large aggregates that replaced a considerable volume of splenic parenchyma (RF90-Figure 14).

      b. Lymphoid aggregates: occasionally seen

   2. Periarteriolar sheath hyperplasia: Periarteriolar sheaths were often hyperplastic and prominent. In some, hyaline material in the sheaths was similar to that described in renal glomeruli. The amount of pigmentation (with brown-black pigment assumed to be melanin) was highly variable.

D. Gonads:
   1. Testes: Some testes had small numbers of macrophage aggregates (RF90-Figure 15) and/or lymphoid aggregates.

   2. Ovary: A few ovaries had lymphoid aggregates, macrophage aggregates (RF90-Figure 16), and/or atretic follicles (RF90-Figure 17).

E. Gills
   1. Inflammation: The majority of fish had multifocal infiltrates of lymphocytes, and in some fish, the infiltrates were very dense and large (RF90-Figure 18).

   2. Hyperplasias: The most consistent finding was mild mucous cell hyperplasia, with affected fish having individual or small clusters of mucous cells scattered over the lamellae (RF90-Figure 19). Quantification of mucous cell numbers requires special staining (e.g. Periodic Acid-Schiff), not provided for in the original contract. A few fish also had marked squamous epithelial cell hyperplasia, involving single filaments (RF90-Figures 20 & 21).

   3. Parasites:

a. Flukes: A few fish had gill flukes (trematodes) which were sometimes associated with focal squamous epithelial hyperplasia and cartilage dysplasia (RF90-Figure 22 & 23).

b. Micro/myxosporidian: An unidentified micro or myxosporidian parasite was in a few gills (RF90-Figure 24).

II. Other rockfish species

A. Yelloweye rockfish: 26 yelloweye rockfish were examined. The most prominent lesion was sinusoidal fibrosis, with 22/25 livers examined having at least mild fibrosis. In some, fibrosis was diffuse and severe. In contrast, yelloweye rockfish had minimal megalocytosis; only 7/25 livers had megalocytosis and all seven were mild. Lipidosis was another fairly common liver lesion.

B. China rockfish: 20 China rockfish were examined. There were minimal liver lesions in these fish, but macrophage aggregates were common in both the kidney and spleen.

C. Copper rockfish: 19 copper rockfish were examined. These fish had minimal lesions in the liver. Vacuolar degeneration was fairly common in the kidney, as were macrophage aggregates in the spleen.

D. Tiger rockfish: 7 tiger rockfish were examined. Liver lesions were mild, but macrophage aggregates in the spleen were common.

E. Silvergrey rockfish: 5 silvergrey rockfish were examined; one fish had moderate hepatic megalocytosis and sinusoidal fibrosis.

F. Yellowtail rockfish: 2 yellowtail rockfish were examined. Both had mild lesions in all organs.

G. Splitnose rockfish: 1 splitnose rockfish was examined. The most prominent lesion was moderate hepatic sinusoidal fibrosis.

RF-Table 1. Summary of histopathologic findings in 1990 Rockfish adults.

Filenames: 90RF_R.PRN
or 90RF_R.WK1

Key to table symbols:

Proc. code = Random number generated by Dr. Hinton's Laboratory (= processing #)
Lesion scores = none (0), mild (1), moderate (2), severe (3), or not present "."

LIVER:
- glycogen depletion (GLY)
- lipidosis (LIP)
- macrophage aggregates (LMA)
- single cell necrosis (SCN)
- hepatocellular karyomegaly (MEG)
- sinusoidal fibrosis (FIB)

KIDNEY:
- macrophage aggregates (KMA)
- tubular epithelial vacuolar degeneration (VD)

SPLEEN (SPL):
- macrophage aggregates (SMA)

OS = oiled status; (O) oiled; (C) clean
MFO = mixed function oxidase; ranked as negative (0), very mild (1), mild (2), mod (3), or strong (4)
note: MFO values could not be determined on these samples
Hinton fish # = initial accession # used before generation of random #'s
ADF&G record # - number submitted with length and age data from Andy Hoffman (6-30-92)
ADF&G Jar # - number on jars received from ADF&G
SEX - determined from examination of gonad (M = male, F = female)

Species (in proc. code):
CH = China rockfish
CO = Copper rockfish
QB = Quillback rockfish
SG = Silvergrey rockfish
TI = Tiger rockfish
VM = Vermillion rockfish
YE = Yelloweye rockfish
YT = Yellowtail rockfish

Prince William Sound Sites:

#	Proc. Code	Liver GLY	LIP	LMA	SCN	MEG	FIB	Spl SMA	Kidney KMA	VD	SEX	OS	MFO	Collection Site	Hinton Fish #	Length (mm)	Age (yrs)	ADF&G Jar #	Date Sampled
1	CH 324	2	0	1	0	2	0	2	2	1	M	C	?	Gravina Rocks	6	330	21	UCD 111,112	7-17-90
2	CH 27	2	0	1	0	0	0	1	1	0	M	C	?	Zaikof	113	220	9	UCD 627,628	8-6-90
3	CH 55	3	0	1	1	1	1	2	2	1	F	O	?	Danger Island	35	300	15	UCD 220,221	7-21-90
4	CH 371	3	0	1	0	1	1	2	1	0	M	O	?	Danger Island	36	345	.	UCD 222,223	7-21-90
5	CH 445	2	0	1	0	1	1	1	1	1	NP	O	?	Danger Island	37	296	14	UCD 224,225	7-22-90
6	CO 264	0	0	3	0	1	0	3	3	1	M	C	?	Gravina Rocks	1	376	37	UCD 101,102	7-16-90
7	CO 266	0	0	1	0	1	0	3	2	2	M	C	?	Gravina Rocks	15	371	22	UCD 130,131	7-18-90
8	CO 404	0	0	0	0	0	0	2	1	1	M	O	?	Herring Bay	26	290	7	UCD 202,203	7-19-90

#	Proc. Code	Liver GLY	Liver LIP	Liver LMA	Liver SCN	Liver MEG	Liver FIB	Spl SMA	Kidney KMA	Kidney VD	SEX	OS	MFO	Collection Site	Hinton Fish #	Length (mm)	Age (yrs)	ADF&G Jar #	Date Sampled
9	QB 470	1	0	1	0	1	0	1	1	1	F	C	?	Gravina Rocks	2	229	7	UCD 103,104	7-16-90
10	QB 338	2	0	2	0	2	1	2	2	1	F	C	?	Gravina Rocks	3	406	30	UCD 105,106	7-16-90
11	QB 151	0	0	3	0	1	1	3	2	1	F	C	?	Gravina Rocks	7	404	.	UCD 113,114	7-17-90
12	QB 112	1	0	3	0	3	0	3	3	1	M	C	?	Gravina Rocks	12	413	44	UCD 124,125	7-17-90
13	QB 448	2	0	3	1	3	0	2	3	2	M	C	?	Gravina Rocks	13	401	51	UCD 126,127	7-17-90
14	QB 483	1	0	3	1	1	0	2	2	0	F	C	?	Gravina Rocks	14	417	39	UCD 128,129	7-17-90
15	QB 77	1	0	1	0	2	1	1	1	2	F	C	?	Zaikof	107	325	13	UCD 615,616	8-3-90
16	QB 321	3	1	1	1	2	2	1	1	1	F	C	?	Zaikof	108	343	13	UCD 617,618	8-3-90
17	QB 46	0	0	1	0	3	0	3	3	1	M	C	?	Zaikof	109	385	17	UCD 619,620	8-3-90
18	QB 481	0	0	1	0	1	0	1	2	0	M	C	?	Zaikof	110	345	14	UCD 621,622	8-3-90
19	QB 359	0	0	0	1	1	1	2	1	1	F	C	?	Zaikof	111	261	7	UCD 623,624	8-5-90
20	QB 389	0	0	0	0	1	0	1	1	2	F	C	?	Zaikof	116	166	.	UCD 633,634	8-3-90
21	QB 209	0	0	0	0	0	0	1	0	0	M	C	?	Zaikof	117	175	.	UCD 635,636	8-3-90
22	QB 440	0	0	0	0	1	0	1	0	1	F	C	?	Zaikof	118	170	6	UCD 637,638	8-3-90
23	QB 43	2	0	1	0	2	1	2	1	0	F	C	?	Zaikof	119	265	9	UCD 639,640	8-3-90
24	QB 261	2	1	1	0	2	2	1	1	1	F	C	?	Zaikof	120	274	10	UCD 641,642	8-3-90
25	QB 455	0	0	0	1	1	1	1	.	.	F	C	?	Zaikof	126	150	0	UCD 703,704	8-5-90
26	QB 283	0	0	0	0	1	1	1	1	1	M	C	?	Zaikof	127	218	7	UCD 705,706	8-5-90
27	QB 405	0	0	0	0	0	0	1	1	2	F	C	?	Zaikof	128	188	.	UCD 707,708	8-6-90
28	QB 348	2	0	2	0	3	2	1	1	1	F	O	?	Danger Island	41	344	10	UCD 232,233	7-21-90
29	QB 103	2	0	1	0	0	0	2	2	1	F	O	?	Danger Island	45	320	10	UCD 240,241	7-22-90
30	QB 138	2	1	3	0	2	1	3	2	2	M	O	?	Herring Bay	20	361	23	UCD 140,141	7-19-90
31	QB 411	3	0	3	0	2	1	2	2	1	F	O	?	Herring Bay	21	420	35	UCD 142,143	7-19-90
32	QB 248	3	0	1	0	1	1	2	1	0	F	O	?	Herring Bay	23	430	16	UCD 146,147	7-19-90
33	QB 501	1	0	1	1	3	0	3	3	2	M	O	?	Herring Bay	27	370	30	UCD 204,205	7-19-90
34	QB 9	0	0	3	0	2	1	3	3	2	M	O	?	Herring Bay	28	356	35	UCD 206,207	7-19-90
35	QB 452	1	0	3	1	2	1	3	1	1	F	O	?	Herring Bay	30	415	50	UCD 210,211	7-20-90
36	SG 259	1	1	1	0	1	0	1	1	1	F	O	?	Herring Bay	16	389	.	UCD 132,133	7-19-90
37	SG 201	1	1	1	1	2	2	1	1	1	F	O	?	Herring Bay	25	378	12	UCD 150,201	7-20-90
38	SG 409	0	0	0	0	1	1	1	1	1	F	O	?	Herring Bay	29	360	9	UCD 208,209	7-20-90
39	SN 367	0	0	1	0	0	2	1	1	1	NP	C	?	Zaikof	112	325	10	UCD 625,626	8-5-90
40	YE 194	3	1	1	0	0	1	1	1	2	NP	C	?	Gravina Rocks	4	410	15	UCD 107,108	7-17-90
41	YE 258	0	0	0	0	0	0	1	1	1	NP	C	?	Gravina Rocks	5	350	13	UCD 109,110	7-17-90
42	YE 23	3	1	1	0	1	2	1	1	1	F	C	?	Gravina Rocks	8	430	24	UCD 115,116,117	7-17-90
43	YE 62	0	0	1	0	0	0	1	1	1	M	C	?	Gravina Rocks	9	553	26	UCD 118,119	7-17-90
44	YE 75	0	0	0	0	0	0	1	0	2	F	C	?	Gravina Rocks	10	366	12	UCD 120,121	7-17-90
45	YE 22	0	0	1	0	0	1	2	1	1	F	C	?	Gravina Rocks	11	462	22	UCD 122,123	7-17-90
46	YE 257	0	0	1	0	0	1	2	1	0	M	C	?	Zaikof	106	402	12	UCD 613,614	8-3-90
47	YE 368	2	0	1	0	1	1	1	1	1	M	O	?	Danger Island	31	611	37	UCD 212,213	7-21-90
48	YE 85	3	1	1	0	1	1	2	1	0	F	O	?	Danger Island	32	620	32	UCD 214,215	7-21-90
49	YE 37	2	3	0	0	0	1	1	1	0	M	O	?	Danger Island	33	490	23	UCD 216,217	7-21-90
50	YE 185	3	3	1	0	0	3	2	1	1	M	O	?	Danger Island	34	614	23	UCD 218,219	7-21-90
51	YE 52	3	2	1	0	1	1	1	1	1	M	O	?	Danger Island	38	546	23	UCD 226,227	7-22-90

#	Proc. Code	Liver GLY	Liver LIP	Liver LMA	Liver SCN	Liver MEG	Liver FIB	Spl SMA	Kidney KMA	Kidney VD	SEX	OS	MFO	Collection Site	Hinton Fish #	Length (mm)	Age (yrs)	ADF&G Jar #	Date Sampled
52	YE 488	2	2	1	0	0	2	1	1	1	M	O	?	Danger Island	40	520	23	UCD 230,231	7-22-90
53	YE 230	3	1	1	0	0	2	1	1	1	M	O	?	Danger Island	43	510	24	UCD 236,237	7-22-90
54	YE 360	0	0	1	0	0	1	2	1	1	M	O	?	Danger Island	44	626	30	UCD 238,239	7-22-90
55	YE 210	2	1	1	0	0	1	1	1	1	M	O	?	Herring Bay	17	400	14	UCD 134,135	7-19-90
56	YE 99	3	0	2	1	1	1	3	1	1	M	O	?	Herring Bay	18	540	49	UCD 136,137	7-19-90
57	YE 499	3	1	3	2	1	1	3	1	1	F	O	?	Herring Bay	19	625	50	UCD 138,139	7-19-90
58	YE 38	2	3	1	0	0	2	1	1	1	F	O	?	Herring Bay	22	402	.	UCD 144,145	7-19-90
59	YE 449	2	1	1	0	0	2	1	1	2	M	O	?	Herring Bay	24	417	20	UCD 148,149	7-20-90
60	YT 400	0	0	1	0	0	0	1	1	0	F	O	?	Danger Island	39	413	10	UCD 228,229	7-22-90
61	YT 290	3	2	1	1	1	0	1	1	0	F	O	?	Danger Island	42	377	8	UCD 234,235	7-22-90

EMBRYO DATA - NOT PROCESSED (SAVED IN JARS)

Proc. Code	Collection Site	Hinton Fish #	ADF&G Jar #	Date Sampled
YE 398E	Gravina Rocks	8	UW 101	7-17-90
QB 353E	Gravina Rocks	14	UW 102	7-17-90
YE 14E	Herring Bay	19	UW 103	7-19-90
QB 411E	Herring Bay	21	UW 104	7-19-90
QB 248E	Herring Bay	23	UW 105	7-19-90
QB 179E	Herring Bay	30	UW 106	7-20-90
YE 85E	Danger Island	32	UW 107	7-21-90
QB 193E	Danger Island	41	UW 108	7-21-90
QB 94E	Morning Cove	49	UW 109	7-24-90
HA 383	Granite Island	562	UW 110	7-26-90
HA 26	Pony Cove	933	UW 111	7-31-90
HA 410	Pony Cove	934	UW 112	7-31-90
HA 53	Pony Cove	935	UW 113	7-31-90

Summary Statistics, Prince William Sound Rockfish only:

	Mean Lesion Scores											Mean	
	Liver						Spl	Kidney					
Species	GLY	LIP	LMA	SCN	MEG	FIB	SMA	KMA	VD	Exposure History	N	Length (mm)	Age (yrs)
CH	2	0	1	0	1	0	1.5	1.5	.5	Combined clean sites	2	275	15
CH	2.7	0	1	.33	1	1	1.7	1.3	.67	Combined oiled sites	3	313.667	14.5
CO	0	0	2	0	1	0	3	2.5	1.5	Combined clean sites	2	373.5	29.5
CO	0	0	0	0	0	0	2	1	1	Combined oiled sites	1	290	7
QB	.79	.11	1.1	.26	1.5	.58	1.6	1.4	1	Combined clean sites	19	291.316	17.8
QB	1.8	.13	2.1	.25	1.9	.88	2.4	1.9	1.3	Combined oiled sites	8	377	26.13
SG	no fish									Combined clean sites			
SG	.67	.67	.67	.33	1.3	1	1	1	1	Combined oiled sites	3	375.667	10.5
SN	0	0	1	0	0	2	1	1	1	Combined clean sites	1	325	10
SN	no fish									Combined oiled sites			
YE	.86	.29	.71	0	.14	.71	1.3	.86	1.1	Combined clean sites	7	424.714	17.71
YE	2.3	1.4	1.2	.23	.38	1.5	1.5	1	.92	Combined oiled sites	13	532.385	29
YT	no fish									Combined clean sites			
YT	1.5	1	1	.5	.5	0	1	1	0	Combined oiled sites	2	395	9

Kenai Penninsula Sites:

		Liver						Spl	Kidney										
#	Proc. Code	GLY	LIP	LMA	SCN	MEG	FIB	SMA	KMA	VD	SEX	OS	MFO	Collection Site	Hinton Fish #	Length (mm)	Age (yrs)	ADF&G Jar #	Date Sampled
62	CH 45	1	0	1	0	0	1	2	2	2	M	C	?	Granite Island	61	318	16	UCD 332,333	7-26-90
63	CH 278	1	0	1	0	0	1	2	1	1	F	C	?	Granite Island	62	293	15	UCD 334,335	7-26-90
64	CH 108	3	1	1	0	1	1	3	2	0	M	C	?	Granite Island	63	297	16	UCD 336,337	7-26-90
65	CH 233	3	0	1	0	0	1	1	1	2	M	C	?	Granite Island	65	292	15	UCD 340,341	7-26-90
66	CH 7	0	0	1	0	0	0	2	1	0	M	C	?	Granite Island	67	310	15	UCD 344,345	7-27-90
67	CH 58	3	0	1	0	1	2	1	1	0	M	C	?	Granite Island	72	262	11	UCD 405,406	7-26-90
68	CH 447	3	0	1	0	1	0	3	1	0	M	C	?	Granite Island	73	303	14	UCD 407,408	7-26-90
69	CH 355	2	0	1	0	0	1	3	2	1	F	C	?	Granite Island	74	260	15	UCD 409,410	7-26-90
70	CH 131	3	0	1	1	0	1	2	2	3	M	C	?	Granite Island	75	325	18	UCD 411,412	7-27-90
71	CH 379	2	0	0	0	1	0	1	1	1	NP	O	?	Morning Cove	46	222	8	UCD 301,302	7-24-90
72	CH 345	0	0	1	0	1	1	3	3	2	M	O	?	Pony Cove	76	292	15	UCD 501,502	7-29-90
73	CH 183	0	0	1	0	0	0	2	1	0	M	O	?	Pony Cove	77	270	15	UCD 503,504	7-29-90
74	CH 117	1	0	1	0	0	0	3	2	2	M	O	?	Pony Cove	85	335	15	UCD 519,520	7-30-90
75	CH 154	0	0	1	0	0	0	2	1	0	F	O	?	Pony Cove	86	277	15	UCD 521,522	7-29-90
76	CH 370	0	0	1	0	0	0	3	3	2	NP	O	?	Pony Cove	89	298	17	UCD 527,528	7-29-90
77	CO 49	0	0	2	0	1	1	3	2	2	F	C	?	Day Harbor	93	402	24	UCD 537,538	8-1-90
78	CO 412	1	1	1	0	0	1	1	1	1	F	C	?	Day Harbor	94	321	12	UCD 539,540	8-1-90
79	CO 186	3	1	1	0	1	1	2	1	2	F	C	?	Day Harbor	96	255	7	UCD 543,544	8-1-90
80	CO 71	0	0	1	0	1	0	3	1	2	F	C	?	Day Harbor	99	268	7	UCD 549,550	8-2-90

1990 Rockfish - Kenai Peninsula Sites

#	Proc. Code	Liver GLY	LIP	LMA	SCN	MEG	FIB	Spl SMA	Kidney KMA	VD	SEX	OS	MFO	Collection Site	Hinton Fish #	Length (mm)	Age (yrs)	ADF&G Jar #	Date Sampled
81	CO 166	3	3	1	0	1	1	1	1	0	M	C	?	Day Harbor	102	285	8	UCD 605,606	8-1-90
82	CO 123	0	0	1	0	0	0	2	1	2	F	C	?	Granite Island	70	303	15	UCD 350,401	7-28-90
83	CO 178	3	3	1	0	0	1	3	3	3	M	C	?	Granite Island	71	381	15	UCD 402,403,404	7-26-90
84	CO 302	2	3	1	0	0	1	3	1	0	M	O	?	Morning Cove	50	370	24	UCD 309,310	7-24-90
85	CO 294	0	0	1	0	0	1	2	1	1	P	O	?	Morning Cove	52	302	10	UCD 313,314	7-24-90
86	CO 242	0	0	1	0	0	0	3	2	0	NP	O	?	Morning Cove	54	332	15	UCD 317,318,331	7-25-90
87	CO 216	0	0	1	0	1	1	3	1	2	F	O	?	Morning Cove	55	329	13	UCD 319,320	7-25-90
88	CO 327	2	0	1	0	0	0	2	1	2	F	O	?	Morning Cove	58	244	8	UCD 325,326	7-24-90
89	CO 255	0	0	1	0	0	1	3	1	1	F	O	?	Morning Cove	60	275	8	UCD 329,330	7-25-90
90	CO 490	0	0	0	0	1	0	1	1	1	M	O	?	Pony Cove	78	278	9	UCD 505,506	7-29-90
91	CO 491	0	0	1	0	0	0	2	1	1	M	O	?	Pony Cove	87	258	9	UCD 523,524	7-29-90
92	CO 487	0	0	1	0	0	0	2	1	2	M	O	?	Pony Cove	90	252	7	UCD 529,530	7-29-90
93	QB 346	0	0	0	0	0	0	1	0	0	M	C	?	Day Harbor	95	286	8	UCD 541,542	8-1-90
94	QB 113	1	0	0	0	0	1	2	1	1	F	C	?	Day Harbor	100	255	7	UCD 601,602	8-2-90
95	QB 60	0	0	1	0	1	1	1	1	1	F	C	?	Day Harbor	101	278	9	UCD 603,604	8-2-90
96	QB 344	1	0	0	0	1	1	1	1	1	M	C	?	Day Harbor	103	265	8	UCD 607,608	8-1-90
97	QB 126	1	0	1	0	1	1	2	1	1	F	C	?	Day Harbor	104	259	8	UCD 609,610	8-1-90
98	QB 130	2	2	1	1	2	2	1	0	2	F	C	?	Day Harbor	105	246	10	UCD 611,612	8-1-90
99	QB 97	3	0	1	0	2	1	1	1	2	F	C	?	Granite Island	68	216	7	UCD 346,347	7-27-90
100	QB 430	2	0	0	0	3	2	2	1	0	M	C	?	Granite Island	69	202	8	UCD 348,349	7-28-90
101	QB 271	3	2	1	0	0	1	2	1	0	M	O	?	Morning Cove	47	301	9	UCD 303,304	7-24-90
102	QB 386	2	2	1	0	2	2	.	.	.	F	O	?	Morning Cove	49	357	16	UCD 307,308	7-24-90
103	QB 4	0	0	0	0	1	0	.	1	1	M	O	?	Morning Cove	53	244	8	UCD 315,316	7-24-90
104	QB 424	3	0	2	0	2	1	1	1	0	M	O	?	Morning Cove	56	217	7	UCD 321,322	7-24-90
105	QB 142	3	1	0	1	2	1	1	0	0	M	O	?	Morning Cove	57	226	7	UCD 323,324	7-24-90
106	QB 238	0	0	1	0	1	0	1	1	1	NP	O	?	Pony Cove	88	247	7	UCD 525,526	7-29-90
107	SG 381	0	0	0	0	1	1	1	1	3	NP	O	?	Morning Cove	48	342	9	UCD 305,306	7-24-90
108	SG 298	0	0	1	0	1	1	1	2	2	F	O	?	Pony Cove	80	299	9	UCD 509,510	7-30-90
109	TI 473	2	0	1	0	1	0	2	1	2	F	C	?	Granite Island	64	278	16	UCD 338,339	7-26-90
110	TI 162	2	2	1	0	1	1	2	1	1	M	C	?	Granite Island	66	382	20	UCD 342,343	7-27-90
111	TI 443	0	0	1	0	0	1	2	1	1	NP	O	?	Morning Cove	51	273	23	UCD 311,312	7-24-90
112	TI 301	0	0	1	0	0	1	3	1	1	M	O	?	Morning Cove	59	281	.	UCD 327,328	7-25-90
113	TI 3	1	2	1	0	1	0	3	2	1	M	O	?	Pony Cove	79	335	22	UCD 507,508	7-29-90
114	TI 42	0	0	1	0	0	1	3	2	1	NP	O	?	Pony Cove	83	362	24	UCD 515,516	7-30-90
115	TI 464	2	1	1	0	0	1	3	1	2	M	O	?	Pony Cove	84	327	23	UCD 517,518	7-30-90
116	YE 149	.	.	.	.	.	.	1	1	0	M	C	?	Day Harbor	91	297	.	UCD 533,534	8-1-90
117	YE 279	0	0	1	0	0	1	1	1	0	NP	C	?	Day Harbor	92	239	9	UCD 535,536	8-1-90
118	YE 182	2	1	1	0	0	2	1	2	0	NP	C	?	Day Harbor	97	247	.	UCD 545,546	8-2-90
119	YE 68	3	1	1	0	0	2	1	.	.	M	C	?	Day Harbor	98	301	9	UCD 547,548	8-2-90
120	YE 378	1	0	1	0	1	2	3	1	1	M	O	?	Pony Cove	81	542	30	UCD 511,512	7-30-90
121	YE 101	2	2	1	1	0	3	2	2	1	F	O	?	Pony Cove	82	499	22	UCD 513,514	7-30-90

Final comment on histopathologic lesions: A total of 121 rockfish (41 quillback, 26 yelloweye, 20 china, 19 copper, 7 tiger, 5 silvergrey, 2 yellowtail, and 1 splitnose) were examined. The most severe lesions were observed in the quillback rockfish, but all rockfish species had similar lesions in liver, kidney, and spleen. Evidence of both parasitism and infectious disease was minimal and the lesions are consistent with exposure to some hepatotoxic and/or nephrotoxic agent. Based on findings from the 1990-rockfish, recommendation for additional sampling were as follows:

1) Concentrate sampling on Quillback and Yelloweye rockfish
2) Equal numbers of males and females be sampled
3) Similar sized (age) fish be sampled
4) Sampled tissues to include: liver, kidney, spleen, and gill
   [Gonads could be eliminated from analysis because lesions were minimal, and confounding problems such as stage of gonad maturation and seasonal cycling, unknown to the pathologists, likely cloud the detection of lesions.]

Statistics: For general details about the types of statistical analysis used, see part III, "Statistical Analysis" on page 14.

Statistical Consultant - Neil Willits, Senior Statistician, Division of Statistics, 2116 Wickson Hall, University of California, Davis, 95616

After lesion scores were recorded in spreadsheet format (RF-Table 1) and sorted by site of origin, lesions were visually scanned for trends. Because of the mixture of species, sites, and lesions, exposure history could not be determined by a visual scan. Initial statistical analysis involved ANOVA of individual lesions scores. Using this type of analysis, we speculated on exposure history of the sites based on the analysis of glycogen depletion scores: Danger Island and Granite Island were thought to be exposed sites, and Pony Cove and Zaikof/Schooner were thought to be clean sites (RF-Table 3). As additional data sets from the Fish Histopathology Project were analyzed, and as age and exposure status for each rockfish were revealed, we reanalyzed the 1990-rockfish data using principal components analysis as described in part III, "Statistical Analysis" on page 14. The SAS statistical program was used to analyze for differences in individual scale values with MANOVA, nested for site effect and blocked for species (RF-Table 4). The program listed in RF-Table 4 also computed comparisons without nesting for site effects; results from those analyses were similar and are not reported here.

Due to missing values, only 107 of the 121 fish (88%) were used in the analysis. With principal components analysis (PCA), a correlation matrix, eigenvalues of the correlation matrix, and eigenvectors were calculated (RF-Table 5). From the proportion part of "eigenvalues of the correlation matrix," the first principal component accounted for 26% of the variability; the second principal component, 23%; the third, 12%; and the fourth, 11%. From individual scale values for the first principal component, liver, kidney, and spleen macrophages were most important (eigenvectors with the greatest absolute value contribute most to variability).

Oiled vs. clean differences were not significant for any of the first four principal components (RF-Tables 6, 7, 8, and 9). Species differences were significant for the first, second, and third principal components, whereas age differences and site (within exposure status) were significant for the first and second principal components (RF-Tables 6, 7, 8, and 9). Tests for overall effects were not significant for oiled vs. clean effects (RF-Table 10) or site (within oil status; RF-Table 11), but were significantly different for age (RF-Table 12).

RF-Table 3. Exposure history (oiled vs. clean) of rockfish sampled in 1990. Sites are labeled from Prince William Sound (PWS) or the Kenai peninsula (Kenai).

	Exposure Status	
Site	Speculated[a]	Actual[b]
Danger Island - PWS	oiled	oiled
Gravina Rocks - PWS	ND	clean
Herring Bay - PWS	ND	oiled
Zaikof/Schooner - PWS	clean	clean
Day Harbor - (Kenai)	ND	clean
Granite Island - (Kenai)	oiled	clean
Morning Cove - (Kenai)	ND	oiled
Pony Cove - (Kenai)	clean	oiled

[a]Speculated in progress report; ND = not done (lesions did not separate potential exposure history of each site)

[b]Actual exposure history revealed by Andy Hoffman on 5-18-92.

Significance of Results:

After our progress report was submitted, Pat Hansen (Alaska Dept. of fish and Game) analyzed selected lesions for frequency of occurrence and found that hepatic lipidosis and glycogen depletion occurred at a significantly greater frequency in rockfish from oiled vs. clean sites. Considering only the rockfish from Prince William Sound, and scanning the scores by species, differences in hepatic lipidosis are most evident in yelloweye rockfish (e.g., 10 of 13 yelloweye from oiled sites had hepatic lipidosis, whereas only 2 of 7 from clean sites had lipidosis).

RF-Table 4. Copy of program and output used by Neil Willits and SAS for statistical analysis of 1990 rockfish (here, 90rf_r.txt) samples.

```
data rf90;
        infile 'c:\home\t\marty\new\90rf_r.txt' firstobs=37 obs=157 lrecl=512;
        input id species $ pcode2 lgly llip lmac lscn lmeg lfib kmac kvd smac
                os $ mfo $ site $ hintonno length age;
proc princomp out=pcs prefix=scale;
NOTE: The infile 'c:\home\t\marty\new\90rf_r.txt' is file
C:\HOME\T\MARTY\NEW\90RF_R.TXT.
NOTE: 157 records were read from the infile C:\HOME\T\MARTY\NEW\90RF_R.TXT.
    The minimum record length was 0.
    The maximum record length was 224.
NOTE: The data set WORK.RF90 has 121 observations and 18 variables.
NOTE: The DATA statement used 7.00 seconds.
        var lgly--smac;
        title 'PCA on data 90rf_r.txt';
data big;
WARNING: 5 of 121 observations in data set WORK.RF90 omitted due to missing values.
NOTE: The data set WORK.PCS has 121 observations and 27 variables.
NOTE: The PROCEDURE PRINCOMP used 12.00 seconds.
        merge rf90 pcs;
proc glm data=big;
NOTE: The data set WORK.BIG has 121 observations and 27 variables.
NOTE: The DATA statement used 4.00 seconds.
        class os site species;
        model scale1-scale4 = species os age/solution;
        manova h=os age/printh printe;
        title 'initial MANOVA on 1990 rockfish data';
        title2 'blocked for species effect';
proc glm data=big;
NOTE: The PROCEDURE GLM used 17.00 seconds.
        class os site species;
        model scale1-scale4 = species os site(os) age/solution;
        lsmeans os/pdiff;
        manova h=os site(os) age/printh printe;
        title 'MANOVA on 1990 rockfish data with nested site effect';
        title2 'blocked for species effect';
```

RF-Table 5. Principal components analysis on 1990 rockfish: correlation matrix, eigenvalues of the correlation matrix, and eigenvectors (filename: 90RF_R.PRN). Important values are highlighted.

Correlation Matrix

	LGLY	LLIP	LMA	LSCN	LMEG	LFIB	KMA	KVD	SMA
LGLY	1.0000	0.4833	0.1674	0.2614	0.2080	0.4120	-.0491	-.1099	-.1071
LLIP	0.4833	1.0000	-.0375	0.1072	-.1236	0.4154	-.0794	-.0905	-.0974
LMA	0.1674	-.0375	1.0000	0.2577	0.3566	0.0508	0.4985	0.0562	0.4347
LSCN	0.2614	0.1072	0.2577	1.0000	0.2813	0.1313	0.0449	0.0359	0.0841
LMEG	0.2080	-.1236	0.3566	0.2813	1.0000	0.0624	0.2513	0.0427	0.0582
LFIB	0.4120	0.4154	0.0508	0.1313	0.0624	1.0000	-.1328	-.0135	-.1488
KMA	-.0491	-.0794	0.4985	0.0449	0.2513	-.1328	1.0000	0.2686	0.5515
KVD	-.1099	-.0905	0.0562	0.0359	0.0427	-.0135	0.2686	1.0000	0.1554
SMA	-.1071	-.0974	0.4347	0.0841	0.0582	-.1488	0.5515	0.1554	1.0000

Eigenvalues of the Correlation Matrix

	Eigenvalue	Difference	Proportion	Cumulative
SCALE1	2.29184	0.221822	**0.254649**	0.25465
SCALE2	2.07001	0.966170	**0.230002**	0.48465
SCALE3	1.10385	0.156258	**0.122649**	0.60730
SCALE4	0.94759	0.185371	**0.105288**	0.71259
SCALE5	0.76222	0.165250	0.084691	0.79728
SCALE6	0.59697	0.139814	0.066330	0.86361
SCALE7	0.45715	0.035980	0.050795	0.91440
SCALE8	0.42117	0.071966	0.046797	0.96120
SCALE9	0.34921	.	0.038801	1.00000

Eigenvectors

	SCALE1	SCALE2	SCALE3	SCALE4	SCALE5	SCALE6	SCALE 7-9
LGLY	-.013889	**0.573878**	0.018400	-.058477	-.103539	0.400661	(not shown)
LLIP	-.154218	**0.471224**	**0.453865**	-.120831	0.111367	0.361313	
LMAC	**0.504760**	0.181970	-.047674	-.233234	-.087851	-.229654	
LSCN	0.200046	0.334198	-.362854	0.201973	0.782626	-.086646	
LMEG	0.323168	0.213619	**-.564771**	0.181240	-.447549	0.168258	
LFIB	-.110746	**0.486404**	0.215230	0.172480	-.263125	-.738025	
KMAC	**0.535946**	-.047266	0.260283	-.067836	-.178537	0.198321	
KVD	0.220290	-.101380	0.342064	0.860348	0.010908	0.114210	
SMAC	**0.478283**	-.095272	0.331189	-.281763	0.234789	-.152669	

RF-Table 6. Initial MANOVA on the first principal component for 1990 rockfish, with nested site effect blocked for species effect. Significant P values ($P < 0.05$) are highlighted.

Dependent Variable: SCALE1 General Linear Models Procedure

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	15	167.13037117	11.14202474	13.57	**0.0001**
Error	91	74.69419180	0.82081529		
Corrected Total	106	241.82456297			

R-Square	C.V.	Root MSE	SCALE1 Mean
0.691122	1605.286	0.90598857	0.05643783

Source	DF	Type I SS	Mean Square	F Value	Pr > F
SPECIES	7	27.58768497	3.94109785	4.80	**0.0001**
Oiled vs. clean (OS)	1	2.85242480	2.85242480	3.48	0.0655
SITE(OS)	6	59.49751081	9.91625180	12.08	**0.0001**
AGE	1	77.19275059	77.19275059	94.04	**0.0001**

Source	DF	Type III SS	Mean Square	F Value	Pr > F
SPECIES	7	56.36519065	8.05217009	9.81	**0.0001**
Oiled vs. clean (OS)	1	0.00168069	0.00168069	0.00	0.9640
SITE(OS)	6	7.61124185	1.26854031	1.55	0.1724
AGE	1	77.19275059	77.19275059	94.04	**0.0001**

RF-Table 7. Initial MANOVA on the second principal component for 1990 rockfish, with nested site effect blocked for species effect. Significant P values (P < 0.05) are highlighted.

Dependent Variable: SCALE2 General Linear Models Procedure

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	15	64.51852605	4.30123507	2.54	**0.0034**
Error	91	154.01085355	1.69242696		
Corrected Total	106	218.52937960			

R-Square	C.V.	Root MSE	SCALE2 Mean
0.295240	4611.725	1.30093311	0.02820925

Source	DF	Type I SS	Mean Square	F Value	Pr > F
SPECIES	7	28.51571902	4.07367415	2.41	**0.0263**
Oiled vs. clean (OS)	1	1.78409706	1.78409706	1.05	0.3073
SITE(OS)	6	27.09254137	4.51542356	2.67	**0.0198**
AGE	1	7.12616860	7.12616860	4.21	**0.0430**

Source	DF	Type III SS	Mean Square	F Value	Pr > F
SPECIES	7	16.42830795	2.34690114	1.39	0.2205
Oiled vs. clean (OS)	1	0.84318030	0.84318030	0.50	0.4821
SITE(OS)	6	29.24538903	4.87423150	2.88	**0.0129**
AGE	1	7.12616860	7.12616860	4.21	**0.0430**

Parameter		Estimate	T for H0: Parameter=0	Pr > ¦T¦	Std Error of Estimate
INTERCEPT		-1.113302927 B	-1.03	0.3061	1.08162130
OS	C	0.340861485 B	0.58	0.5645	0.58938595
	O	0.000000000 B	.	.	.
SITE(OS)	Day_Harb C	0.463159510 B	0.82	0.4155	0.56625169
	Granite_ C	0.899825423 B	1.50	0.1358	0.59795109
	Gravina_ C	-0.732547476 B	-1.23	0.2217	0.59533747
	Zaikof/S C	0.000000000 B	.	.	.
	Danger_I O	1.182159148 B	2.07	**0.0412**	0.57069961
	Herring_ O	0.708508387 B	1.21	0.2283	0.58410353
	Morning_ O	0.892542537 B	1.67	0.0977	0.53334435
	Pony_Cov O	0.000000000 B	.	.	.
AGE		0.032692737	2.05	**0.0430**	0.01593229

RF-Table 8. Initial MANOVA on the third principal component for 1990 rockfish, with nested site effect blocked for species effect. Significant P values ($P < 0.05$) are highlighted.

Dependent Variable: SCALE3 General Linear Models Procedure

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	15	50.36104937	3.35740329	4.40	**0.0001**
Error	91	69.35846650	0.76218095		
Corrected Total	106	119.71951586			

R-Square	C.V.	Root MSE	SCALE3 Mean
0.420659	-6205.096	0.87302975	-0.01406956

Source	DF	Type I SS	Mean Square	F Value	Pr > F
SPECIES	7	44.61254397	6.37322057	8.36	**0.0001**
Oiled vs. clean (OS)	1	0.44185994	0.44185994	0.58	0.4484
SITE(OS)	6	5.28158814	0.88026469	1.15	0.3375
AGE	1	0.02505731	0.02505731	0.03	0.8565

Source	DF	Type III SS	Mean Square	F Value	Pr > F
SPECIES	7	30.97563237	4.42509034	5.81	**0.0001**
Oiled vs. clean (OS)	1	0.53326886	0.53326886	0.70	0.4051
SITE(OS)	6	4.88552234	0.81425372	1.07	0.3874
AGE	1	0.02505731	0.02505731	0.03	0.8565

Parameter		Estimate		T for H0: Parameter=0	Pr > |T|	Std Error of Estimate
INTERCEPT		-0.759562619	B	-1.05	0.2981	0.72585406
OS	C	-0.742642341	B	-1.88	0.0636	0.39552492
	O	0.000000000	B	.	.	.
SITE(OS)	Day_Harb C	0.341194069	B	0.90	0.3716	0.37999999
	Granite_ C	0.739764249	B	1.84	0.0685	0.40127282
	Gravina_ C	0.014951445	B	0.04	0.9702	0.39951887
	Zaikof/S C	0.000000000	B	.	.	.
	Danger_I O	-0.236901159	B	-0.62	0.5377	0.38298490
	Herring_ O	-0.558894152	B	-1.43	0.1573	0.39198000
	Morning_ O	-0.451138939	B	-1.26	0.2107	0.35791655
	Pony_Cov O	0.000000000	B	.	.	.
AGE		-0.001938610		-0.18	0.8565	0.01069184

RF-Table 9. Initial MANOVA on the fourth principal component for 1990 rockfish, with nested site effect blocked for species effect. Significant P values (P < 0.05) are highlighted.

Dependent Variable: SCALE4 General Linear Models Procedure

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	15	18.72424343	1.24828290	1.40	0.1663
Error	91	81.36953519	0.89417072		
Corrected Total	106	100.09377862			

R-Square	C.V.	Root MSE	SCALE4 Mean
0.187067	6441.056	0.94560600	0.01468091

Source	DF	Type I SS	Mean Square	F Value	Pr > F
SPECIES	7	13.76290789	1.96612970	2.20	**0.0414**
Oiled vs. clean (OS)	1	1.75802966	1.75802966	1.97	0.1643
SITE(OS)	6	1.35762635	0.22627106	0.25	0.9569
AGE	1	1.84567953	1.84567953	2.06	0.1542

Source	DF	Type III SS	Mean Square	F Value	Pr > F
SPECIES	7	12.64687082	1.80669583	2.02	0.0608
Oiled vs. clean (OS)	1	0.86341729	0.86341729	0.97	0.3284
SITE(OS)	6	1.82092790	0.30348798	0.34	0.9143
AGE	1	1.84567953	1.84567953	2.06	0.1542

Parameter			Estimate	T for H0: Parameter=0	Pr > |T|	Std Error of Estimate
INTERCEPT			-0.795699609 B	-1.01	0.3142	0.78619538
OS	C		-0.073935282 B	-0.17	0.8634	0.42840549
	O		0.000000000 B	.	.	.
SITE(OS)	Day_Harb	C	0.126969872 B	0.31	0.7584	0.41158995
	Granite_	C	0.372165695 B	0.86	0.3941	0.43463122
	Gravina_	C	0.218371756 B	0.50	0.6150	0.43273146
	Zaikof/S	C	0.000000000 B	.	.	.
	Danger_I	O	-0.051636884 B	-0.12	0.9012	0.41482300
	Herring_	O	0.057113304 B	0.13	0.8933	0.42456588
	Morning_	O	-0.382452969 B	-0.99	0.3265	0.38767068
	Pony_Cov	O	0.000000000 B	.	.	.
AGE			-0.016638010	-1.44	0.1542	0.01158066

RF-Table 10. MANOVA on all data for 1990 rockfish, to test the hypothesis of "no overall oiled vs. clean effect." Note that none of the P values are significant.

General Linear Models Procedure
Multivariate Analysis of Variance

H = Type III SS&CP Matrix for "oiled vs. clean"

	SCALE1	SCALE2	SCALE3	SCALE4
SCALE1	0.0016806941	-0.037644763	-0.029937633	0.0380938365
SCALE2	-0.037644763	0.8431802961	0.6705533503	-0.853238798
SCALE3	-0.029937633	0.6705533503	0.5332688603	-0.678552543
SCALE4	0.0380938365	-0.853238798	-0.678552543	0.8634172894

Characteristic Roots and Vectors of: E Inverse * H, where
H = Type III SS&CP Matrix for "oiled vs. clean"   E = Error SS&CP Matrix

Characteristic Root	Percent	Characteristic Vector V'EV=1 SCALE1	SCALE2	SCALE3	SCALE4
0.0253062811	100.00	0.00296108	0.03915117	0.06702359	-0.07996774
0.0000000000	0.00	0.10276002	-0.00105904	0.02553562	0.01448792
0.0000000000	0.00	-0.06608118	-0.00095623	0.09029250	0.07293066
0.0000000000	0.00	0.01230732	0.07122131	-0.04613773	0.03357945

Manova Test Criteria and Exact F Statistics for the Hypothesis of **no Overall "Oiled vs. clean" Effect**
H = Type III SS&CP Matrix for "oiled vs. clean"   E = Error SS&CP Matrix

S=1   M=1   N=43

Statistic	Value	F	Num DF	Den DF	Pr > F
Wilks' Lambda	0.97531832	0.5567	4	88	0.6946
Pillai's Trace	0.02468168	0.5567	4	88	0.6946
Hotelling-Lawley Trace	0.02530628	0.5567	4	88	0.6946
Roy's Greatest Root	0.02530628	0.5567	4	88	0.6946

RF-Table 11. MANOVA on all data for 1990 rockfish, to test the hypothesis of "no overall site (within oiled or clean site) effect." Significant P values are highlighted.

General Linear Models Procedure
Multivariate Analysis of Variance

H = Type III SS&CP Matrix for SITE(oiled or clean)

	SCALE1	SCALE2	SCALE3	SCALE4
SCALE1	7.6112418502	-10.41621264	0.2849926528	1.8190461083
SCALE2	-10.41621264	29.245389027	4.2716752388	0.6544939941
SCALE3	0.2849926528	4.2716752388	4.8855223423	1.7576479208
SCALE4	1.8190461083	0.6544939941	1.7576479208	1.8209279022

Characteristic Roots and Vectors of: E Inverse * H, where
H = Type III SS&CP Matrix for SITE(oiled or clean) E = Error SS&CP Matrix

Characteristic Root	Percent	Characteristic Vector V'EV=1 SCALE1	SCALE2	SCALE3	SCALE4
0.2547520511	69.54	-0.06274371	0.06365056	0.03431196	0.00519904
0.0732440109	19.99	0.03666467	0.00052596	0.09241401	0.03676673
0.0315970401	8.63	0.08537003	0.04753855	-0.07178953	0.01404325
0.0067394847	1.84	-0.05017438	-0.01719714	-0.02354270	0.10712220

Manova Test Criteria and F Approximations for the Hypothesis of **no Overall SITE(oiled or clean) Effect**
H = Type III SS&CP Matrix for SITE(oiled or clean) E = Error SS&CP Matrix

S=4 M=0.5 N=43

Statistic	Value	F	Num DF	Den DF	Pr > F
Wilks' Lambda	0.71501709	1.2961	24	308.2054	0.1633
Pillai's Trace	0.30859885	1.2679	24	364	0.1816
Hotelling-Lawley Trace	0.36633259	1.3203	24	346	0.1461
Roy's Greatest Root	0.25475205	3.8637	6	91	**0.0018**

NOTE: F Statistic for Roy's Greatest Root is an upper bound.

RF-Table 12. MANOVA on all data for 1990 rockfish, to test the hypothesis of "no overall age effect." Significant P values are highlighted.

General Linear Models Procedure
Multivariate Analysis of Variance

H = Type III SS&CP Matrix for AGE

	SCALE1	SCALE2	SCALE3	SCALE4
SCALE1	77.192750585	23.453966724	-1.390770571	-11.93620877
SCALE2	23.453966724	7.1261685966	-0.42256671	-3.62665459
SCALE3	-1.390770571	-0.42256671	0.0250573113	0.2150529389
SCALE4	-11.93620877	-3.62665459	0.2150529389	1.845679531

Characteristic Roots and Vectors of: E Inverse * H, where
H = Type III SS&CP Matrix for AGE   E = Error SS&CP Matrix

Characteristic Root	Percent	Characteristic Vector V'EV=1 SCALE1	SCALE2	SCALE3	SCALE4
1.3728234431	100.00	0.11944431	0.02321433	-0.03173845	-0.04066848
0.0000000000	0.00	0.00154930	-0.02065278	0.10955231	-0.04332676
0.0000000000	0.00	-0.01276648	0.06495676	0.04531022	0.03979471
0.0000000000	0.00	0.02557843	-0.03771608	0.01906827	0.08908674

Manova Test Criteria and Exact F Statistics for the Hypothesis of **no Overall AGE Effect**
H = Type III SS&CP Matrix for AGE   E = Error SS&CP Matrix

S=1   M=1   N=43

Statistic	Value	F	Num DF	Den DF	Pr > F
Wilks' Lambda	0.42143886	30.2021	4	88	**0.0001**
Pillai's Trace	0.57856114	30.2021	4	88	**0.0001**
Hotelling-Lawley Trace	1.37282344	30.2021	4	88	**0.0001**
Roy's Greatest Root	1.37282344	30.2021	4	88	**0.0001**

Figure Legends. 1990 Rockfish

Pathologist/Photographer - Mark S. Okihiro

RF90-Figure 1. Normal liver in fish QB209-90. Glycogen laden hepatocytes are organized into orderly tubules. Hepatic vein (V) is filled with blood and the bile duct (arrow head) is associated with a hepatic arteriole. HE 220X.

RF90-Figure 2. Severe megalocytosis in fish QB501-90. Note the karyomegalic nuclei (arrow heads) and single dead hepatocyte (arrow). HE 220X.

RF90-Figure 3. Hepatocyte syncytium with a large cluster of karyomegalic nuclei (arrow head) in fish QB9-90. HE 440X.

RF90-Figure 4. Megalocytosis in fish QB501-90. Note the karyomegalic nucleus with a large pseudo-inclusion (arrow head). HE 440X.

RF90-Figure 5. Megalocytosis and macrophage aggregates in fish QB209-90 (left) and normal liver in fish QB448-90. HE 220X.

RF90-Figure 6. Severe sinusoidal fibrosis in fish QB386-90. MA = macrophage aggregates. HE 220X.

RF90-Figure 7. Moderate sinusoidal fibrosis in fish QB348-90. Note the blue staining collagen fibers (arrow heads). Masson's trichrome 220X.

RF90-Figure 8. Megalocytosis, macrophage aggregates, and glycogen depletion in fish QB112-90. Note the eosinophilic "protein" droplets (arrow heads) within some hepatocytes. HE 352X.

RF90-Figure 9. Renal tubular necrosis in fish QB448-90. Note the numerous vacuolated, dead epithelial cells (arrow heads) and large macrophage aggregates (MA). HE 220X.

RF90-Figure 10. Membranous glomerulonephritis in fish QB448-90. Note the severely thickened glomerular basement membranes (arrow heads). HE 352X.

RF90-Figure 11. Macrophage aggregates (MA) in the kidney of fish QB112-90. HE 110X.

RF90-Figure 12. Megalocytosis involving the renal tubular epithelium in fish QB9-90. Note that nuclei in the affected tubule are markedly enlarged (arrow heads). HE 440X.

RF90-Figure 13. Lining epithelium of many renal tubules is packed with eosinophilic "protein" droplets in fish QB151-90. HE 220X.

RF90-Figure 14. Spleen of fish QB9-90 is packed with numerous, large, pigmented macrophage aggregates (MA). HE 44X.

RF90-Figure 15. Testicular interstitium of fish QB112-90 contains multiple macrophage aggregates (MA). HE 145X.

RF90-Figure 16. Multiple large macrophage (MA) in the ovary of fish QB411-90. HE 110X.

RF90-Figure 17. Two large atretic follicles (AF) packed with macrophages in the ovary of fish QB411-90. HE 110X.

RF90-Figure 18. Severe, dense lymphocytic infiltration into the lamellar interstitium of the gill of fish QB103-90. HE 110X.

RF90-Figure 19. Marked mucous cell hyperplasia over the gill lamellae of fish QB112-90. HE 110X.

RF90-Figure 20. Severe squamous epithelial hyperplasia involving one gill filament in fish QB321-90. HE 44X.

RF90-Figure 21. Higher magnification of figure 20. HE 110X.

RF90-Figure 22. Trematode (T) attached to the gill lamellae of fish QB483-90. HE 110X.

RF90-Figure 23. Marked filament cartilage dysplasia (CD) in the gill of fish QB452-90. There is focal squamous cell hyperplasia (SCH), and both lesions may be due to chronic attachment by a trematode (T). HE 70X.

RF90-Figure 24. Unidentified micro or myxosporidian parasite (arrow heads) in the filament interstitium of the gill of fish QB501-90. HE 440X.

APPENDIX D

Erythrocyte Micronuclei Analysis
from
JoEllen Hose

To: Andy Hoffman, ADF&G, Cordova, AK

From: Jo Ellen Hose, VANTUNA Research Group, Occidental College

Re: Rockfish Erythrocyte Micronucleus Analyses

Date: November 9, 1990

INTRODUCTION

Micronuclei (MN) are smaller, secondary nuclei formed after exposure to chromosome breaking agents (clastogens) or spindle toxins. They arise from lagging chromosomes or acentric chromosome fragments which have not fused with the parent nucleus after mitosis. MN can occur in any cell although they are easiest to detect in anucleate cells such as mammalian erythrocytes (Schmid 1976). The rodent polychromatic erythrocyte MN assay is a widely used, second tier screening test for genotoxic agents (Heddle et al. 1983). Of the 220 compounds adequately tested in the rodent system, the detection rate for carcinogens was 91% (Mavournin et al., 1990).

The method of MacGregor et al. (1980), measuring MN frequencies in circulating rodent erythrocytes, has been adapted to marine and freshwater species such as frogs, newts, mussels and fishes (Hooftman and de Raat 1982; Hose et al. 1987). However, measurement of MN in nucleated cells of these species is more difficult and nuclear changes different from the classical detached micronuclei of rodents have also been reported. These include the formation of knoblike structures extending from the nucleus. The mechanisms by which these attached micronuclei are formed are poorly understood and may be the result of exposure to membrane-active compounds rather than genotoxins.

Because the micronucleus test constitutes a potentially useful method of monitoring indigenous organisms for exposure to environmental genotoxins such as those in crude oil, it was suggested that rockfish residing within Prince William Sound be screened for micronucleus formation.

METHODS

Blood smears were prepared from rockfish collected from Prince William Sound and fixed in absolute methanol for approximately 15 min. The slides were sent to Occidental College on ice and then stained with May Grunwald-Giemsa. Blood smears were examined at 1000X using an Olympus microscope fitted with an oil immersion objective. Starting near the center of the slide where the drop of blood was applied and moving in a straight line toward the trailing edge of the smear, two replicate counts of 1000 erythrocytes each were made. Erythrocytes were examined for the presence of 1) detached micronuclei and 2) attached nuclei. The detached MN presented as circular bodies ranging in size from 1/20 to 1/10 the diameter of the parent nucleus, in the same focal plane as the parent nucleus, and with staining properties identical to those of the parent nucleus. Attached MN were knoblike structures attached to the parent nucleus by either a thin thread or a slender stalk. Measurements of detached and attached micronuclei are presented for each of the two replicate counts as well as the average score for each slide (the average count of the detached plus attached micronuclei).

REFERENCES

Heddle, JA, M Hite, B Kirkhart, K Mavournin, JT MacGregor, GW Newell and MF Salamone (1983) The induction of micronuclei as a measure of genotoxicity.

Samples: Prince William Sound Oil Spill Study
Rockfish 001-113

Analyses: Erythrocyte Micronucleus Counts

Sample	1st Count (1000 RBCs)		2nd Count (1000 RBCs)		$\bar{X}$
	Detached MN	Attached MN	Detached MN	Attached MN	(#/1000 RBC)
001A	0	0	0	0	0.0
002A	0	0	0	0	0.0
003A	1	0	0	0	0.5
004A	0	0	0	0	0.0
005A	0	0	0	0	0.0
006A	0	0	0	0	0.0
007A	0	0	0	0	0.0
008A	0	0	0	0	0.0
009A	0	0	0	1	0.5
010A	0	0	0	0	0.0
011A	0	0	0	1	0.5
016A	0	0	0	0	0.0
017A	1	2	2	0	2.5
018A	1	0	0	0	0.5
019A	0	0	0	0	0.0
020A	0	0	0	0	0.0
021A	0	0	0	0	0.0
022A	0	0	0	0	0.0
023A	0	0	0	0	0.0
024A	0	0	0	0	0.0
025A	0	0	1	0	0.5
031A	0	0	0	0	0.0
032A	0	0	0	0	0.0
033A	0	0	1	0	0.5
034A	0	0	0	0	0.0
035A	0	0	0	0	0.0
036A	0	0	0	0	0.0
037A	0	0	0	0	0.0
038A	0	0	0	0	0.0
039A	0	0	0	0	0.0
040A	0	0	0	0	0.0
046A	1	0	1	0	1.0
047A	0	0	0	0	0.0
048A	0	0	0	0	0.0
049A	0	0	0	0	0.0
050A	0	0	1	0	0.5
051A	0	0	0	0	0.0
052A	0	0	0	0	0.0
054A	0	0	0	0	0.0
055A	0	0	0	0	0.0
061A	0	0	0	0	0.0
062A	1	0	0	0	0.5
063A	0	0	0	0	0.0
064A	0	1	0	2	1.5
065A	0	0	1	0	0.5
066A	0	0	0	0	0.0
067A	0	0	0	0	0.0
068A	1	0	0	0	0.5
069A	1	1	0	0	1.0
070A	0	0	0	0	0.0

076A	0	0	1	0	0.5
077A	0	0	0	0	0.0
078A	0	0	0	0	0.0
079A	0	0	0	0	0.0
080A	0	0	0	0	0.0
081B	0	0	0	0	0.0
082B	0	0	0	0	0.0
083A	0	0	0	0	0.0
084A	0	1	0	0	0.5
085A	0	0	0	0	0.0
091A	0	0	0	0	0.0
092A	0	0	0	0	0.0
093A	0	0	0	1	0.5
094A	0	0	0	0	0.0
095B	0	0	0	0	0.0
096A	0	0	0	0	0.0
097A	0	0	0	0	0.0
098A	0	0	0	0	0.0
099A	0	0	0	1	0.5
100A	0	0	0	0	0.0
106A	0	0	0	0	0.0
107A	0	0	0	0	0.0
108A	0	1	0	0	0.5
109A	0	0	1	0	0.5
110A	0	0	0	0	0.0
111A	0	0	0	0	0.0
112A	0	0	0	0	0.0
113A	0	0	0	0	0.0

APPENDIX E

1991, Histopathology Report
from
University of California, Davis
School of Veterinary Medicine

B. **1991 Rockfish** - Histopathologic Methods and Results

PATHOLOGIST - Mark S. Okihiro, DVM

Filenames: 91RF_R.PRN or 91RF_R.WK1

Diagnoses:

Liver:
1) Hepatocellular megalocytosis
2) Sinusoidal fibrosis
3) Single hepatocyte necrosis
4) Hepatic macrophage aggregates
5) Glycogen depletion
6) Lipidosis
7) Bile duct hyperplasia

Spleen:
1) Splenic macrophage aggregates
2) Periarteriolar sheath hyperplasia

Kidney:
1) Renal macrophage aggregates
2) Vacuolar degeneration and necrosis of tubular epithelium
3) Glomerulonephritis, membranous
4) Renal tubular adenoma, multiple (one fish)

Heart:
1) Atrial and pericardial macrophage aggregates
2) Lymphocytic endocarditis and pericarditis
3) Cardiac trematodiasis

History:

Jars of formalin containing tissues from 107 adult rockfish collected in 1991 (Copper, Quillback, and Yelloweye rockfish) were received [9-23-91; logged in 10-3-91 by Gary D. Marty]. Liver, spleen, kidney, heart, and gills were received from nearly every fish. Large gills were decalcified before sectioning. All fish were assigned a random number (Hinton number, see RF-Table 13) and all tissues were processed routinely in paraffin and stained with hematoxylin and Eosin (HE). Slides were read in ascending numerical order based on the assigned random number (i.e., blind study). Lesions were subjectively ranked using a four point scale: none (0), mild (1), moderate (2), or severe (3); tissues that were not present were designated with a period (.). To optimize precision of results, all specimens of a given organ (e.g., all 107 livers) were read and scored before any specimens of the next organ were scored. Basic historical/site data and significant lesion scores are listed in RF-Table 13.

Histopathology:

I. 1991 Quillback Rockfish

A. Liver
   1. Megalocytosis: Megalocytosis was still a prominent feature of many quillback rockfish livers sampled in 1991. In contrast to the histological appearance of normal glycogen-laden livers (RF-Figures 1-3), affected livers had marked variation in size and shape of hepatocyte nuclei (RF-Figures 4-6). Nuclei were often severely enlarged and sometimes contained multiple nucleoli. Lesions were essentially identical to those described in the 1990 rockfish.

   2. Sinusoidal fibrosis: Sinusoidal fibrosis (RF-Figures 7 and 8) was prominent, and the prevalence appeared to be higher in the 1991 quillbacks than the 1990 fish. The 1991 samples had increased numbers of fish with moderate and severe (score 2 and 3) fibrosis.

   3. Necrosis:
      a. Coagulation necrosis: none

      b. Single cell necrosis: Individual hepatocyte necrosis or apoptosis was uncommon and usually was mild (RF-Figure 5).

   4. Inflammation
      a. Macrophage aggregates: Macrophage aggregates were common in 1991 quillback livers (RF-Figures 7,8).

      b. Lymphocytic aggregates: Small clusters of lymphocytes were often in the liver.

   5. Hepatocytes storage disorders
      a. Glycogen depletion: common

      b. Lipidosis: Mild to severe lipidosis was seen in a few fish.

   6. Bile duct hyperplasia: A few fish had focal reduplication of mature bile ducts (RF-Figure 9).

   7. Parasitism: none

B. Spleen
   1. Inflammation:
      a. Macrophage aggregates: Macrophage aggregates were a consistent finding. In some fish, numerous large aggregates replaced a significant volume of splenic parenchyma (RF-Figure 10).
      b. Lymphoid aggregates: occasionally seen

   2. Periarteriolar sheath hyperplasia: Periarteriolar sheaths were often prominent due to hyaline material in the sheaths.

C. Kidney

1. Inflammation
   a. Macrophage aggregates: Many kidneys were massively infiltrated by macrophage aggregates (RF-Figures 11-13).
   Comment: The degree and number of macrophage aggregates probably reflects the amount of degeneration and necrosis of renal tubular epithelial cells.

   b. Lymphoid aggregates: occasionally seen

2. Protein: A few quillback rockfish had scattered renal tubules that were lined by epithelial cells which were packed with large, intracytoplasmic, pale, eosinophilic protein droplets (RF-Figure 12).

3. Renal tubular degeneration and necrosis: Vacuolar degeneration and necrosis of individual or small clusters of tubular epithelial cells was common (RF-Figures 13,14). This was associated with the influx of individual macrophages into the tubular epithelium.

   Comment: Renal tubular necrosis certainly could be related to xenobiotic exposure.

4. Glomerulonephritis: Membranous glomerulonephritis was uncommon.

5. Neoplasms: One fish from an oiled site (Herring Bay) had multiple (3) tubular adenomas in the kidney (RF-Figure 15).

D. Heart
1. Inflammation
   a. Macrophage aggregates: Small numbers of macrophage aggregates were often in the endocardium of the atrium (RF-Figure 16) or in the pericardium.

   b. Lymphoid aggregates: Small clusters of lymphocytes were often in either the endocardium or pericardium (RF-Figure 17).

2. Parasitism: A few fish had small numbers of trematodes (flukes) within the lumen of the ventricle (RF-Figure 18). Flukes were often associated with mild lymphocytic endocardial inflammation. Some fish also had small numbers of Ichthyophonus.

E. Gills
1. Filament cartilage dysplasia: Cartilage in normal gill filaments is thin, runs the entire length of the filament, and provides structural support (RF-Figures 19 and 20). The outer edges or the entire mass of cartilage may become ossified in older fish (RF-Figure 20). Some 1991 quillback rockfish had focally severe cartilage dysplasia (RF-Figure 21) and one fish had fusion of two adjacent filaments (RF-Figure 22).

2. Inflammation: A few fish had small infiltrates of lymphocytes in the interstitium of the filament.

3. Hyperplasias:
   a. Squamous epithelial hyperplasia: A few fish had focal, moderate to severe, squamous epithelial hyperplasia, which resulted in thickening, clubbing, and occasional fusion of gill lamellae (RF-Figure 23).

   b. Mucous cell hyperplasia: Similar to the 1990 quillbacks, the most consistent finding was mild to moderate mucous cell hyperplasia with affected fish having individual or small clusters of mucous cells scattered over the lamellae (RF-Figure 24).

II. Other rockfish species

A. Yelloweye rockfish: Again in 1991, the most prominent liver lesion in yelloweye rockfish was sinusoidal fibrosis. Macrophage aggregates and lipidosis were also fairly common, while megalocytosis was uncommon and usually mild. In the gill, several yelloweye rockfish had a unique lesion of severe chloride cell hyperplasia (RF-Figures 25, 26).

B. Copper rockfish: The 1991 copper rockfish had minimal lesions in almost all organs examined, with the possible exception of the gill where mucous cell hyperplasia was fairly common.

Final comments on lesions: The lesions in the 1991 rockfish mirrored those seen in the 1990 rockfish. Again, the most severe lesions were in quillback rockfish, but all three species had somewhat similar lesions in liver, kidney, spleen, heart, and gill. Evidence of both parasitism and infectious disease was minimal and we believe that the lesions probably represent either continuing low level exposure to some hepatotoxic and nephrotic agent, or possibly the residual effects of some past exposure.

Differences in lesion scores were difficult to detect by perusal of the results, but some differences emerged. Among copper rockfish, scores for splenic macrophages and hepatic megalocytosis tended to be higher in fish from Danger Island (oiled) than from Gravina (clean). Among quillback rockfish, splenic and renal and cardiac macrophage scores tended to be higher in fish from Danger Island and Herring Bay (oiled) than from Zaikoff Schooner (clean). Among yelloweye rockfish, rockfish from Gravina tended to have lower macrophage scores (clean) than did the other groups. Although megalocytosis was prominent among many quillback rockfish, it seemed to be independent of exposure history, and might represent a "normal" finding in this species. Speculated exposure histories were correct for each sample site (RF-Table 14).

After our progress report was submitted, Pat Hansen (Alaska Dept. of fish and Game) analyzed selected lesions for frequency of occurrence and found that hepatic lipidosis and renal lymphocytes occurred at a significantly greater frequency in rockfish from oiled vs. clean sites. Scanning the scores by species, differences in hepatic lipidosis are not evident in copper rockfish, but are obvious in quillback and especially in yelloweye rockfish (e.g., 17 of 30 yelloweye from oiled sites had hepatic lipidosis, whereas 0 of 9 from clean sites had lipidosis).

RF-Table 13. Summary of histopathologic findings in 1991 Rockfish adults.

File updated: 07/06/92

Filenames: **91RF_R.PRN**
or **91RF_R.WK1**

Key to table symbols:

ADF&G NUMBER = number generated by the Alaska Dept. of Fish and Game
Sex = male (M), female (F), or unknown (U)
Hinton number = random number generated by Dr. Hinton's laboratory
rockfish species: copper (CO), quillback (QB), and yelloweye (YE)
Site = Danger Island (DI), Herring Bay (HB)
Lesion scores = none (0), mild (1), moderate (2), severe (3), or not present "."
OS = oiled status; oiled (O) or control/clean (C)
MFO = mixed function oxidase; ranked as negative (0), very mild (1), mild (2), mod (3), or strong (4)
note: MFO values in the liver were determined for hepatocytes, bile ductules, arteriolar endothelium, venular endothelium, and macrophage aggregates, but scores here are the maximum score for any of the three sites (usually hepatocytes).
ND = MFO determination was not done

LIVER:
- glycogen depletion (GLY)
- lipidosis (LIP)
- macrophage aggregates (LMA)
- single cell necrosis (SCN)
- hepatocellular karyomegaly (MEG)
- sinusoidal fibrosis (FIB)

HEART:
- lymphocytes (LY)
- granulomatous inflammation (GRI)
- macrophage aggregates (HMA)
- luminal flukes/trematodes (FLK)
- xenomas (XEN)

KIDNEY:
- lymphocytes (KLY)
- macrophage aggregates (KMA)
- tubular epithelial vacuolar degeneration (VD)
- tubular epithelial necrosis (NEC)
- glomerular basement membrane thickening (GBM)

SPLEEN (SPL):
- melanomacrophage centers (SMA)

GILL (additional comments at bottom):
- cartilaginous dysplasia (CTD)
- lymphocytes (LY)
- eosinophilic granular leukocytes (EGL)
- mucous cell hyperplasia (MCH)
- squamous cell hyperplasia (SCH)
- aneurysms (ANU)
- parasites (PAR)
- chloride cell hyperplasia (CLH)

	ADF&G	Length	Age		HINTON				SAMPLE	Liver							Spl	Kidney					Heart					Gill								HINTON
#	#	(mm)	(yrs)	Sex	NUMBER	SITE	OS	MFO	DATE	GLY	LIP	LMA	LY	SCN	MEG	FIB	SMA	KMA	KLY	VD	NEC	GBM	LY	GRI	HMA	FLK	XEN	CTD	LY	EGL	MCH	SCH	ANU	PAR	CLH	#
1	1003	357	15	F	91CO 9	GRAVINA	C	4	7-21-91	3	0	3	1	0	0	1	2	2	0	2	0	0	1	0	1	0	0	1	0	0	1	1	0	0	0	CO 9
2	1007	326	11	F	91CO 21	GRAVINA	C	4	7-21-91	0	3	0	1	0	0	0	1	1	0	1	0	0	1	0	0	0	0	0	2	0	3	1	0	0	0	CO 21
3	1014	339	13	M	91CO 40	GRAVINA	C	0	7-21-91	0	0	1	1	0	0	0	1	3	0	1	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	CO 40
4	1010	349	14	U	91CO 54	GRAVINA	C	0	7-21-91	0	0	2	1	0	0	0	3	2	0	2	1	1	1	0	0	0	0	0	0	0	2	0	0	0	0	CO 54
5	1013	332	14	F	91CO 74	GRAVINA	C	0	7-21-91	3	0	3	1	0	0	2	3	2	0	1	0	0	1	0	1	0	0	1	0	0	2	0	0	1	0	CO 74
6	1019	258	11	F	91CO 85	GRAVINA	C	3	7-22-91	3	1	1	1	0	0	1	1	1	0	0	0	0	1	0	1	0	0	1	0	0	1	0	0	1	0	CO 85
7	1015	336	11	M	91CO 92	GRAVINA	C	0	7-21-91	3	2	0	1	0	0	0	1	1	0	1	0	0	1	0	0	0	0	0	0	0	2	0	0	0	0	CO 92
8	1009	355	15	M	91CO 95	GRAVINA	C	4	7-21-91	3	0	3	1	0	0	1	1	1	1	0	0	0	1	0	1	0	0	0	0	0	1	0	1	0	0	CO 95
9	1008	316	9	M	91CO 99	GRAVINA	C	0	7-21-91	3	2	0	0	0	0	1	1	1	1	0	1	0	1	0	0	0	0	0	0	0	2	0	0	0	0	CO 99
10	1006	355	15	F	91CO 102	GRAVINA	C	0	7-21-91	3	0	2	1	0	0	1	2	1	1	0	0	0	1	0	0	0	0	0	0	0	2	0	0	0	0	CO 102
11	1034	402	18	F	91CO 13	DI	O	4	7-27-91	1	0	2	0	0	0	1	3	1	0	0	0	0	1	0	1	0	0	0	1	0	2	1	0	0	0	CO 13
12	1039	413	16	M	91CO 17	DI	O	0	7-27-91	0	3	1	0	0	0	1	3	1	0	1	0	1	1	0	1	0	0	0	0	0	2	1	0	0	0	CO 17
13	1037	402	15	M	91CO 29	DI	O	4	7-27-91	0	0	1	1	0	0	0	2	1	0	1	0	0	1	0	1	0	0	0	1	0	2	0	1	0	0	CO 29
14	1036	402	17	F	91CO 32	DI	O	3	7-27-91	3	0	2	1	0	1	1	2	2	0	0	0	0	2	0	0	0	0	0	2	0	3	0	0	0	0	CO 32
15	1038	397	13	F	91CO 37	DI	O	0	7-27-91	3	2	3	2	0	1	0	3	3	0	0	0	1	3	0	1	0	0	0	1	0	2	0	1	0	0	CO 37
16	1085	408	18	M	91CO 55	DI	O	4	9-9-91	0	0	2	0	0	0	0	3	1	0	0	0	2	1	0	1	0	0	0	0	0	1	0	0	0	0	CO 55

#	ADF&G	Length	Age		HINTON				SAMPLE	Liver						Spl		Kidney				Heart					Gill							HINTON		
#	#	(mm)	(yrs)	Sex	NUMBER	SITE	OS	MFO	DATE	GLY	LIP	LMA	LY	SCN	MEG	FIB	SMA	KMA	LY	VD	NEC	GBM	LY	GRI	HMA	FLK	XEN	CTD	LY	EGL	MCH	SCH	ANU	PAR	CLH	
17	1041	425	18	M	91CO 57	DI	0	0	7-27-91	0	0	1	1	0	1	0	3	3	0	2	1	1	1	0	0	0	0	0	0	0	1	0	0	0	0	CO 57
18	1101	360	17	M	91CO 72	DI	0	0	9-10-91	0	0	1	1	0	0	0	3	2	1	0	0	0	0	0	0	0	0	0	1	0	2	1	0	0	0	CO 72
19	1042	420	20	F	91CO 75	DI	0	0	7-27-91	0	0	1	1	0	1	1	2	1	0	0	0	2	0	0	0	0	0	0	1	0	1	0	0	0	0	CO 75
20	1033	406	16	M	91CO 89	DI	0	4	7-27-91	0	0	1	1	0	0	1	3	1	1	2	0	0	1	0	1	0	0	0	0	0	1	0	1	0	0	CO 89
21	1005	433	30	F	91QB 16	GRAVINA	C	4	7-21-91	0	0	2	1	0	1	0	3	2	0	1	1	2	0	0	1	0	0	0	0	0	1	0	0	0	0	QB 16
22	1025	434	47	M	91QB 22	GRAVINA	C	0	7-22-91	3	0	3	1	0	3	2	3	3	0	2	1	1	0	0	1	0	0	2	1	2	2	3	0	0	0	QB 22
23	1028	407	17	M	91QB 45	GRAVINA	C	0	7-22-91	0	0	1	0	0	3	0	2	3	0	2	1	0	1	0	0	0	0	1	2	1	2	3	0	0	0	QB 45
24	1030	369	21	F	91QB 46	GRAVINA	C	3	7-22-91	3	0	3	1	0	1	1	3	3	0	1	0	0	1	0	2	0	0	0	0	0	0	1	0	0	0	QB 46
25	1029	390	32	F	91QB 60	GRAVINA	C	4	7-22-91	3	0	2	1	0	2	1	3	1	1	0	0	0	1	0	1	0	0	2	0	0	2	0	0	0	0	QB 60
26	1027	363	18	M	91QB 62	GRAVINA	C	0	7-22-91	3	0	3	1	0	1	1	2	2	0	0	0	0	0	0	1	0	0	0	0	0	1	0	0	0	0	QB 62
27	1026	420	55	M	91QB 68	GRAVINA	C	4	7-22-91	3	0	3	1	0	2	1	3	3	0	1	0	0	0	0	2	0	0	0	0	0	2	0	0	0	0	QB 68
28	1012	395	33	M	91QB 78	GRAVINA	C	0	7-21-91	0	0	2	1	0	0	1	3	3	0	2	1	0	1	0	2	0	0	0	0	0	1	0	0	0	0	QB 78
29	1021	304	9 ·	F	91QB 79	GRAVINA	C	0	7-22-91	1	0	1	1	0	0	1	2	1	0	0	0	0	1	0	0	0	0	0	1	0	1	0	0	0	0	QB 79
30	1020	350	15	F	91QB 82	GRAVINA	C	0	7-22-91	0	0	0	1	0	3	2	1	1	0	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0	0	QB 82
31	1001	325	11	M	91QB 83	GRAVINA	C	4	7-21-91	2	0	1	1	0	1	2	1	2	0	2	1	0	0	0	1	0	0	0	1	0	2	0	0	0	0	QB 83
32	1002	343	14	M	91QB 87	GRAVINA	C	0	7-21-91	2	0	1	1	0	2	1	1	2	0	1	0	0	1	0	1	0	0	0	0	0	1	1	0	0	0	QB 87
33	1011	360	19	F	91QB 91	GRAVINA	C	4	7-21-91	3	0	3	1	0	2	2	2	2	0	0	0	0	1	0	1	0	0	0	0	0	2	0	0	0	0	QB 91
34	1078	333	9	F	91QB 12	ZAIKOFF	C	4	8-10-91	3	0	1	1	0	2	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	QB 12
35	1080	264	11	F	91QB 33	ZAIKOFF	C	4	8-10-91	2	0	1	1	0	1	2	1	1	0	2	0	0	1	0	0	0	0	0	1	1	1	3	0	0	0	QB 33
36	1105	270	9	F	91QB 48	ZAIKOFF	C	4	9-12-91	2	0	1	2	0	2	2	1	0	0	0	0	0	1	0	0	0	0	0	0	0	1	1	0	0	0	QB 48
37	1079	283	10	F	91QB 50	ZAIKOFF	C	4	8-10-91	2	0	0	1	0	2	2	1	0	1	1	0	0	1	0	0	0	0	2	0	0	1	1	1	1	0	QB 50
38	1083	346	16	M	91QB 51	ZAIKOFF	C	4	8-12-91	1	0	1	1	0	1	0	2	3	0	1	1	0	1	0	0	0	0	0	1	0	1	0	0	0	0	QB 51
39	1076	316	11	F	91QB 52	ZAIKOFF	C	4	8-9-91	3	1	1	1	0	2	2	2	1	1	0	0	1	1	0	0	0	0	0	1	0	1	0	0	0	0	QB 52
40	1077	265	11	M	91QB 58	ZAIKOFF	C	4	8-9-91	3	1	1	1	0	2	1	1	1	0	0	0	0	0	0	0	0	0	0	1	0	1	3	0	1	0	QB 58
41	1082	251	9	F	91QB 69	ZAIKOFF	C	4	8-12-91	3	0	0	1	0	1	1	1	1	0	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0	0	QB 69
42	1075	169	9	U	91QB 80	ZAIKOFF	C	4	8-8-91	1	0	0	1	1	2	1	2	0	0	1	0	0	2	0	0	0	0	0	0	0	1	0	0	0	0	QB 80
43	1103	338	13	F	91QB 94	ZAIKOFF	C	0	9-12-91	0	0	2	1	0	2	1	1	1	0	0	0	0	1	0	1	0	0	0	0	0	1	0	0	0	0	QB 94
44	1073	314	11	F	91QB 96	ZAIKOFF	C	3	8-8-91	1	0	1	1	0	1	1	2	2	1	0	0	0	1	0	0	0	0	0	0	0	1	1	0	0	0	QB 96
45	1081	307	8	M	91QB 103	ZAIKOFF	C	3	8-10-91	1	0	0	1	0	1	0	1	1	0	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0	0	QB 103
46	1106	362	14	F	91QB 106	ZAIKOFF	C	0	9-13-91	3	2	3	1	0	3	1	2	1	0	0	0	1	0	0	0	0	0	0	0	0	1	1	0	0	0	QB 106
47	1084	375	18	M	91QB 108	ZAIKOFF	C	0	8-12-91	0	0	1	1	0	3	1	2	3	0	0	1	0	1	0	1	1	0	0	1	0	0	1	0	2	0	QB 108
48	1074	195	9	U	91QB 116	ZAIKOFF	C	0	8-8-91	1	0	0	1	1	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	QB 116
49	1090	410	26	F	91QB 3	DI	0	4	9-9-91	2	1	2	1	1	3	2	3	2	0	2	1	1	0	0	0	0	0	0	0	0	1	0	1	0	0	QB 3
50	1087	360	18	M	91QB 10	DI	0	0	9-9-91	3	1	1	1	0	3	1	2	3	1	1	0	1	1	0	0	0	0	0	1	0	1	0	0	0	0	QB 10
51	1086	378	19	M	91QB 25	DI	0	4	9-9-91	2	1	1	1	0	2	2	3	3	1	1	0	1	1	0	1	1	0	0	1	0	1	0	0	0	0	QB 25
52	1088	361	16	M	91QB 28	DI	0	0	9-9-91	3	2	1	1	1	3	1	.	3	1	1	2	1	1	0	0	0	0	0	1	0	2	1	0	0	0	QB 28
53	1035	409	24	F	91QB 71	DI	0	3	7-27-91	3	0	3	1	1	2	3	3	2	0	0	0	0	0	0	1	0	0	0	0	0	1	1	0	0	0	QB 71
54	1040	400	16	M	91QB 76	DI	0	4	7-27-91	3	0	2	1	0	0	2	3	3	1	1	1	0	1	0	2	0	0	0	0	0	1	0	0	0	0	QB 76
55	1100	390	29	M	91QB 86	DI	0	0	9-9-91	1	3	2	1	0	3	1	3	3	0	1	0	1	0	0	1	0	0	0	1	0	1	1	2	0	0	QB 86
56	1102	330	16	M	91QB 90	DI	0	0	9-10-91	3	0	1	1	0	2	2	.	1	1	0	0	0	1	0	1	0	0	0	1	0	2	1	0	0	0	QB 90
57	1065	326	11	F	91QB 1	HB	0	4	8-7-91	0	0	1	1	0	0	0	2	1	0	1	0	0	1	0	0	0	0	.	.	.	.	.	.	.	0	QB 1
58	1068	339	12	M	91QB 19	HB	0	0	8-7-91	3	0	0	0	0	2	1	3	2	1	1	0	1	0	0	0	0	0	0	1	0	2	1	0	0	0	QB 19
59	1071	348	24	F	91QB 20	HB	0	0	8-7-91	0	0	1	1	0	3	1	3	3	0	1	0	1	0	0	1	0	1	1	0	0	2	2	1	0	0	QB 20
60	1067	353	22	F	91QB 27	HB	0	4	8-7-91	0	1	1	1	1	3	1	3	3	1	2	1	1	1	0	1	1	0	1	1	0	1	0	0	2	0	QB 27
61	1070	365	29	M	91QB 38	HB	0	4	8-7-91	0	0	1	0	0	2	1	3	3	1	2	1	0	0	0	1	0	0	.	.	.	.	.	.	.	0	QB 38
62	1064	319	11	F	91QB 39	HB	0	0	8-7-91	0	0	0	1	0	1	0	1	1	2	1	0	0	0	1	0	0	1	.	.	.	.	.	.	.	0	QB 39
63	1072	340	19	F	91QB 98	HB	0	0	8-7-91	2	0	1	0	0	1	1	3	1	0	0	0	1	0	0	1	0	0	0	0	0	1	1	2	0	0	QB 98

#	ADF&G #	Length (mm)	Age (yrs)	Sex	HINTON NUMBER	SITE	OS	MFO	SAMPLE DATE	Liver						Spl		Kidney				Heart					Gill									HINTON #
										GLY	LIP	LMA	LY	SCN	MEG	FIB	SMA	KMA	LY	VD	NEC	GBM	LY	GRI	HMA	FLK	XEN	CTD	LY	EGL	MCH	SCH	ANU	PAR	CLH	
64	1049	375	34	F	91QB 101	HB	0	0	8-6-91	1	2	3	0	0	2	1	3	2	0	0	0	0	0	0	1	0	0	1	0	0	0	1	0	0	0	QB 101
65	1043	394	31	F	91QB 105	HB	0	0	8-6-91	0	0	1	1	0	3	1	3	3	0	1	0	1	1	0	1	0	0	0	0	0	1	2	0	0	0	QB 105
66	1053	276	10	F	91QB 109	HB	0	0	8-6-91	1	0	1	1	0	2	1	1	0	0	0	0	0	1	0	0	0	0	0	0	0	1	0	1	0	0	QB 109
67	1044	351	14	F	91QB 113	HB	0	0	8-6-91	3	2	0	1	0	1	1	2	1	1	0	0	1	1	0	0	0	0	0	0	0	1	1	0	2	0	QB 113
68	1066	339	14	F	91QB 114	HB	0	0	8-7-91	1	2	1	1	1	2	1	3	1	0	0	0	0	1	0	0	0	0	2	0	0	2	1	0	0	0	QB 114
69	1023	401	17	F	91YE 4	GRAVINA	C	4	7-22-91	0	0	1	1	0	0	0	1	1	2	0	0	0	2	0	1	0	0	0	0	0	0	1	0	0	0	YE 4
70	1024	321	13	F	91YE 65	GRAVINA	C	0	7-22-91	3	0	0	1	0	0	1	0	1	1	0	0	0	1	0	0	0	0	0	1	0	0	0	0	2	0	YE 65
71	1004	566	41	M	91YE 73	GRAVINA	C	4	7-21-91	1	0	2	2	0	0	1	3	1	1	0	0	0	1	0	1	0	0	0	1	0	0	1	0	1	0	YE 73
72	1022	531	39	M	91YE 77	GRAVINA	C	0	7-22-91	0	0	0	1	0	0	0	2	1	2	0	0	0	1	0	1	0	0	0	0	0	0	1	0	0	3	YE 77
73	1016	269	9	U	91YE 84	GRAVINA	C	0	7-21-91	3	0	1	1	0	1	2	1	1	0	0	0	0	1	0	1	0	0	0	1	0	0	0	0	0	3	YE 84
74	1017	343	14	F	91YE 104	GRAVINA	C	0	7-22-91	0	0	1	1	0	0	0	1	3	1	0	0	0	1	0	2	0	0	0	0	0	0	1	0	1	3	YE 104
75	1018	365	13	F	91YE 110	GRAVINA	C	0	7-22-91	3	0	0	1	0	0	2	1	1	1	0	0	0	1	0	1	0	0	0	1	0	0	1	0	2	0	YE 110
76	1104	650	34	F	91YE 49	ZAIKOFF	C	4	9-12-91	0	0	2	1	0	1	0	3	2	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	YE 49
77	1107	694	54	F	91YE 107	ZAIKOFF	C	4	9-14-91	0	0	3	1	0	1	0	3	2	1	0	0	2	1	0	0	0	0	0	0	0	0	1	0	0	0	YE 107
78	1095	613	47	M	91YE 6	DI	0	0	9-9-91	3	2	2	1	0	1	1	3	1	1	0	0	0	0	0	1	0	0	0	0	0	0	1	0	0	0	YE 6
79	1089	657	36	M	91YE 14	DI	0	4	9-9-91	2	2	1	1	0	1	2	3	1	0	0	0	0	1	0	2	0	0	0	0	0	1	1	0	0	1	YE 14
80	1096	650	52	M	91YE 15	DI	0	4	9-9-91	2	1	2	1	0	1	1	3	.	.	.	.	.	1	0	1	0	0	0	1	0	0	1	0	0	1	YE 15
81	1093	516	26	M	91YE 24	DI	0	4	9-9-91	2	1	1	1	0	0	1	1	1	2	0	0	0	3	0	0	0	0	0	0	0	0	1	0	0	0	YE 24
82	1032	440	21	F	91YE 31	DI	0	4	7-26-91	2	1	1	1	0	1	2	1	1	2	0	0	0	1	0	1	0	0	0	0	0	0	0	0	1	0	YE 31
83	1031	560	32	F	91YE 34	DI	0	0	7-26-91	3	1	2	1	0	0	3	2	2	2	0	0	0	1	0	1	0	0	0	0	0	0	1	0	0	0	YE 34
84	1092	540	26	M	91YE 35	DI	0	0	9-9-91	3	3	1	1	0	0	1	2	1	2	2	1	0	2	0	0	0	0	0	0	0	0	1	0	1	0	YE 35
85	1098	702	60	F	91YE 36	DI	0	0	9-9-91	1	2	3	1	0	1	1	3	3	1	0	0	1	2	0	1	0	0	0	1	0	0	1	2	0	0	YE 36
86	1099	570	25	M	91YE 63	DI	0	4	9-9-91	3	0	1	1	0	0	2	2	1	2	0	0	0	0	0	0	0	0	0	1	0	0	1	2	0	0	YE 63
87	1091	561	31	F	91YE 66	DI	0	0	9-9-91	3	0	1	1	0	0	2	1	1	0	1	0	0	3	0	0	0	0	0	1	0	0	0	0	2	0	YE 66
88	1097	680	48	M	91YE 81	DI	0	4	9-9-91	3	1	3	1	0	0	1	3	2	0	0	1	1	1	0	1	0	0	0	0	0	0	0	0	0	0	YE 81
89	1094	585	45	F	91YE 88	DI	0	0	9-9-91	3	1	3	0	0	3	1	3	2	1	0	0	1	3	0	1	0	0	0	1	0	0	1	0	0	0	YE 88
90	1055	551	37	F	91YE 2	HB	0	4	8-7-91	2	3	1	1	0	1	1	2	1	2	0	0	0	1	0	1	0	0	1	1	0	0	2	0	0	3	YE 2
91	1047	504	22	F	91YE 5	HB	0	0	8-6-91	3	0	0	1	0	1	1	2	1	1	0	0	0	1	0	0	0	0	0	0	0	0	1	0	0	0	YE 5
92	1057	345	11	F	91YE 7	HB	0	4	8-7-91	0	0	0	1	0	0	0	1	1	2	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	YE 7
93	1058	359	16	F	91YE 11	HB	0	4	8-7-91	0	0	1	1	0	0	0	2	2	2	0	0	0	1	0	1	0	0	0	1	0	0	2	0	0	3	YE 11
94	1063	343	15	U	91YE 23	HB	0	4	8-7-91	2	3	1	1	0	0	0	1	2	1	0	0	0	2	0	1	0	0	.	.	.	.	.	.	.	.	YE 23
95	1059	306	10	F	91YE 30	HB	0	4	8-7-91	0	0	0	1	0	0	1	1	1	1	0	0	0	1	0	0	0	0	.	.	.	.	.	.	.	.	YE 30
96	1048	555	50	F	91YE 42	HB	0	0	8-6-91	1	2	3	1	0	1	2	2	1	1	0	0	0	1	0	1	0	0	0	0	0	0	1	0	0	1	YE 42
97	1050	612	58	F	91YE 44	HB	0	0	8-6-91	0	1	3	1	0	2	0	3	2	1	0	0	0	1	0	2	0	0	1	0	0	0	0	0	0	0	YE 44
98	1052	500	22	F	91YE 53	HB	0	0	8-6-91	1	3	0	1	0	1	1	2	1	1	0	1	0	1	0	1	0	0	0	1	0	0	1	0	0	0	YE 53
99	1062	276	8	U	91YE 56	HB	0	0	8-7-91	0	0	0	1	0	0	0	1	1	2	2	1	0	1	0	0	0	0	0	1	0	0	0	0	0	0	YE 56
100	1045	424	15	F	91YE 61	HB	0	4	8-6-91	2	3	1	2	0	0	0	1	1	1	0	1	0	1	0	1	0	0	0	1	0	0	1	0	0	0	YE 61
101	1054	405	15	M	91YE 64	HB	0	0	8-7-91	0	1	0	1	0	0	0	2	1	1	1	1	0	1	0	1	0	0	0	2	0	0	2	0	0	0	YE 64
102	1060	286	9	F	91YE 70	HB	0	0	8-7-91	1	0	0	1	0	0	0	1	1	1	0	0	0	1	0	1	0	0	0	1	0	0	0	0	0	0	YE 70
103	1046	403	15	M	91YE 97	HB	0	4	8-6-91	0	0	0	1	0	0	0	2	1	3	0	0	0	2	0	1	0	0	0	1	0	0	1	0	1	0	YE 97
104	1051	370	17	F	91YE 100	HB	0	0	8-6-91	0	0	0	0	0	0	0	1	0	2	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	YE 100
105	1056	522	34	F	91YE 111	HB	0	4	8-7-91	2	0	1	0	0	1	2	2	2	1	0	0	0	1	0	1	0	0	0	1	0	0	1	0	0	1	YE 111
106	1061	308	10	U	91YE 112	HB	0	0	8-7-91	0	0	0	1	0	0	0	1	1	2	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1	0	YE 112
107	1069	518	27	M	91YE 115	HB	0	0	8-7-91	2	2	0	1	0	0	1	1	1	2	0	0	0	2	0	1	0	0	0	0	0	0	1	0	0	0	YE 115

# #	ADF&G #	Length (mm)	Age (yrs)	Sex	HINTON NUMBER	SITE	OS	MFO	SAMPLE DATE	Liver GLY	Liver LIP	Liver LMA	Liver LY	Liver SCN	Liver MEG	Liver FIB	Spl SMA	Kidney KMA	Kidney LY	Kidney VD	Kidney NEC	Kidney GBM	Heart LY	Heart GRI	Heart HMA	Heart FLK	Gill XEN	Gill CTD	Gill LY	Gill EGL	Gill MCH	Gill SCH	Gill ANU	Gill PAR	HINTON CLH

note: the following had eggs, but they were not processed.

1036E	91C032E	DI	? 7-27-91
1031E	91YE34E	DI	? 7-26-91
1098E	91YE36E	DI	? 9-9-91
1038E	91C037E	DI	? 7-27-91
1029E	91QB60E	GRAVINA	? 7-22-91
1042E	91C075E	DI	? 7-27-91
1020E	91QB82E	GRAVINA	? 7-22-91
1009E	91C095E	GRAVINA	? 7-21-91
1006E	91C0102E	GRAVINA	? 7-21-91

Hinton #		Comments (gill lesions)
YE	104	Epitheliocystis ?
YE	97	Epitheliocystis ?
YE	112	Epitheliocystis ?
YE	110	Huge flukes, arch
YE	65	Trichodina
YE	73	Trichodina
YE	31	Trichodina
YE	35	Trichodina
YE	66	Trichodina

Mean lesion scores

SPECIES	Liver GLY	Liver LIP	Liver LMA	Liver LY	Liver SCN	Liver MEG	Liver FIB	Spl SMA	Kidney KMA	Kidney KLY	Kidney VD	Kidney NEC	Kidney GBM	Heart LY	Heart GRI	Heart HMA	Heart FLK	Heart XEN	Gill CTD	Gill LY	Gill EGL	Gill MCH	Gill SCH	Gill ANU	Gill PAR	Gill CLH
Copper rockfish																										
combined clean sites	2.1	.8	1.5	.9	0	0	.7	1.6	1.5	.3	.8	.2	.1	.9	0	.4	0	0	.3	.2	0	1.8	.2	.1	.2	0
combined oiled sites	.7	.5	1.5	.8	0	.4	.5	2.7	1.6	.2	.6	.1	.7	1.1	0	.6	0	0	0	.7	0	1.7	.3	.3	0	0
Quillback rockfish																										
combined clean sites	1.8	.14	1.4	1	.07	1.6	1.1	1.8	1.6	.18	.64	.29	.18	.75	0	.54	.04	0	.25	.36	.14	1.1	.68	.04	.14	0
combined oiled sites	1.6	.75	1.2	.8	.25	2	1.2	2.6	2.1	.55	.8	.3	.55	.55	.05	.6	.1	.1	.29	.41	0	1.2	.76	.41	.24	0
Yelloweye rockfish																										
combined clean sites	1.1	0	1.1	1.1	0	.33	.67	1.7	1.4	1	0	0	.33	1.1	0	.78	0	0	0	.44	0	0	.67	0	.67	1
combined oiled sites	1.5	1.1	1.1	.93	0	.5	.9	1.8	1.3	1.4	.21	.21	.10	1.3	0	.77	0	0	.07	.54	0	.04	.79	.14	.21	.36

RF-Table 14. Exposure history (oiled vs. clean) of rockfish sampled from Prince William Sound, Alaska, in 1991.

	Exposure Status	
Site	Speculated[a]	Actual[b]
Danger Island	oiled	oiled
Gravina Rocks	clean	clean
Herring Bay	oiled	oiled
Zaikof/Schooner	clean	clean

[a]Speculated in progress report, 7-3-91.

[b]Actual exposure history revealed by Andy Hoffman, 5-18-92.

Statistics: For general details about the types of statistical analysis used, see part III, "Statistical Analysis" on page 14.

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The SAS statistical program was used to analyze for differences in individual scale values with MANOVA, nested for site effect and blocked for species (RF-Table 15). The program listed in RF-Table 15 also computed comparisons without nesting for site effects; results from those analyses were similar and are not reported here.

Due to missing values, only 99 of 107 (93%) rockfish were used in the analysis. With principal components analysis (PCA), a correlation matrix, eigenvalues of the correlation matrix, and eigenvectors were calculated (RF-Table 16). From the proportion part of "eigenvalues of the correlation matrix," the first principal component accounted for 15% of the variability; the second principal component, 8.4%; the third, 7.8%; and the fourth, 7.8%. From individual scale values for the first principal component, liver megalocytes, and spleen and kidney macrophages were most important (eigenvectors with the greatest absolute value contribute most to variability). Hepatic lipidosis, a lesion that occurred in greater frequency in rockfish from oiled sites, contributed little to variability in the first 6 principal components, but was a major part of variability in the seventh principal component (which accounted for 5.2% of overall variability).

Oiled vs. clean differences were significant for the first principal component (RF-Table 17), but were not significantly different for the second, third, and fourth principal components (RF-Tables 18, 19, and 20). Species differences were significant for the first, third, and fourth principal components. Age differences were significant for the first, second, and fourth principal components. Finally, differences within site type (oiled or clean) were significantly different for only the first principal component. Tests for overall effects were significant for oiled vs. clean effects (RF-Table 21), not significant for site (within oil status; RF-Table 22), but were significantly different for age (RF-Table 23).

Hepatic lipidosis occurred in greater frequency in rockfish—especially yelloweye rockfish—from oiled sites (see RF-Table 13). Lipidosis contributed little to variability in the first four principal components, but was a major part of variability in the seventh principal component (which accounted for 5.2% of overall variability). To determine if oiled vs. clean differences were significant for the seventh principal component, analysis was repeated and MANOVA was done on the first seven principal components (program output not shown); in addition, overall MANOVA was computed combining the first seven principal components. Note that Neil Willits was reluctant to place much weight on these additional analyses because in all other analyses, we used only the first four principal components to determine significance. Oiled vs. clean differences were not significant for the fifth principal component (results not shown), but were highly significant for the sixth and seventh principal components (RF-Table 24). Overall MANOVA using the first seven principal components were highly significant for oil status and age (Wilks' Lambda, Pillai's Trace, et al., $P<0.01$, data not shown).

Significance of Results:

Lesions that were used to separate oiled from clean sites in the first principal component—hepatic megalocytosis, plus spleen and kidney macrophages—have been associated with oil or toxicant exposure (Haensly et al. 1982; Kent et al. 1988). Therefore, differences in lesion scores between oiled and clean sites are probably real. In the gill, epithelial hyperplasia (squamous cell hyperplasia) has been associated with oil exposure in many studies (see general literature review, section XI), but in 1991-rockfish contributed to variability primarily as a species effect (third principal component).

Additional analysis using the fifth, sixth, and seventh principal components yielded valuable results. Heart xenoma scores contributed most to variability in the sixth principal component (RF-Table 16); however, this lesions occurred only twice: in two quillback rockfish from oiled sites. It seems unlikely that the xenomas were of great biological significance. By comparison, hepatic lipidosis has been described in relation to oil exposure in several studies (Eurell and Haensly 1981; Khan and Kiceniuk 1984; McCain et al. 1978; Solangi and Overstreet 1982). Despite reservations by our statistician in using these additional analyses, hepatic lipidosis is likely a real biomarker of exposure, particularly in yelloweye rockfish.

The finding that overall oiled vs. clean effects were significant for 1991-rockfish (RF-Table 21), but not significant for 1990-rockfish (RF-Table 10), is evidence for continued deleterious effects of the *Exxon Valdez* oil spill on rockfish. Lipidosis was a useful lesion in yelloweye rockfish for separating oiled from clean sites in both 1990 and 1991. By comparison, hepatic megalocytosis was different in copper rockfish in 1991 than in 1990. In 1990, two of three copper rockfish sampled from Prince William Sound clean sites had megalocytosis, but 0 of 10 had megalocytosis from these sites in 1991. Although the presence of megalocytosis in 4 of 10 copper rockfish sampled in 1991 was used to speculate on exposure history of oiled sites, comparison with the 1990 results suggests that differences in megalocytosis were likely a result of small sample size rather than the Oil Spill [note, however, that copper rockfish were not sampled from sites in Prince William Sound in 1990]. Megalocytosis occurred in quillback rockfish regardless of exposure history in both 1990 and 1991: evidence that hepatocellular karyomegaly is normal in adult quillback rockfish.

RF-Table 15. Copy of program and output used by Neil Willits and SAS for statistical analysis of 1991 rockfish (here, 91rf_r.txt) samples.

```
 23    data rf91;
NOTE: The PROCEDURE GLM used 19.00 seconds.
 24            infile 'c:\home\t\marty\new\91rf_r.txt' firstobs=29 obs=137 lrecl=512;
 25            input id1 adfngno length age hinton1 species $ hinton3 site $ os $ mfo $
 26                    date $ lgly llip lmac lly lscn lmeg lfib smac kmac kly kvd knec
 27                    kgbm hly hgri hmac hflk hxen gctd gly gegl gmch gsch ganu gpar
 28                    gclh;
 29    proc princomp out=pcs prefix=scale;
NOTE: The infile 'c:\home\t\marty\new\91rf_r.txt' is file
C:\HOME\T\MARTY\NEW\91RF_R.TXT.
NOTE: 137 records were read from the infile C:\HOME\T\MARTY\NEW\91RF_R.TXT.
    The minimum record length was 0.
    The maximum record length was 424.
NOTE: SAS went to a new line when INPUT statement reached past the end of a line.
NOTE: The data set WORK.RF91 has 107 observations and 37 variables.
NOTE: The DATA statement used 8.00 seconds.
 30            var lgly--gclh;
 31            title 'PCA on data 91rf_r.txt';
 32    data big;
WARNING: 8 of 107 observations in data set WORK.RF91 omitted due to missing values.
NOTE: The data set WORK.PCS has 107 observations and 63 variables.
NOTE: The PROCEDURE PRINCOMP used 21.00 seconds.
 33            merge rf91 pcs;
 34    proc glm data=big;
NOTE: The data set WORK.BIG has 107 observations and 63 variables.
NOTE: The DATA statement used 5.00 seconds.
 35            class os site species;
 36            model scale1-scale4 = species os age/solution;
 37            manova h=os age/printh printe;
 38            title 'initial MANOVA on 1991 rockfish data';
 39            title2 'blocked by species';
 40    proc glm data=big;
NOTE: The PROCEDURE GLM used 16.00 seconds.
 41            class os site species;
 42            model scale1-scale4 = species os site(os) age/solution;
 43            lsmeans os/pdiff;
 44            manova h=os site(os) age/printh printe;
 45            title 'MANOVA on 1991 rockfish data with nested site effect';
 46            title2 'blocked by species';
```

RF-Table 16. Principal components analysis on 1991 rockfish: eigenvalues of the correlation matrix, and eigenvectors. Important values are highlighted. See RF-Table 13 (p. 376) for list of abbreviations.

Eigenvalues of the Correlation Matrix

	Eigenvalue	Difference	Proportion	Cumulative
SCALECALE 1	3.70276	1.59285	**0.148110**	0.14811
SCALE2	2.10990	0.15030	**0.084396**	0.23251
SCALE3	1.95961	0.00117	**0.078384**	0.31089
SCALE4	1.95844	0.20674	**0.078338**	0.38923
SCALE5	1.75170	0.31330	0.070068	0.45930
SCALE6	1.43840	0.13398	**0.057536**	0.51683
SCALE7	1.30441	0.16795	**0.052177**	0.56901
SCALE8	1.13646	0.05582	0.045459	0.61447
SCALE9	1.08064	0.10042	0.043226	0.65769
SCALE10-26:	**omit**			

Eigenvectors

	SCALE1	**SCALE2**	**SCALE3**	**SCALE4**	**SCALE5**	**SCALE6**	**SCALE7**	**SCALES 9-26** (not shown)
LGLY	0.009682	-.186853	0.360352	-.355489	0.122611	-.263421	0.212985	
LLIP	-.004832	-.032373	0.170816	0.095626	0.027230	0.079474	**0.498886**	
LMAC	0.287090	-.394891	0.106977	0.075940	0.065161	-.251015	0.028549	
LLY	-.069050	0.000632	0.069724	-.040850	0.223378	-.093848	-.017026	
LSCN	0.084970	0.074892	-.037203	-.197794	0.292399	0.279948	-.176921	
LMEG	**0.320031**	0.038504	0.141941	-.146940	0.089032	0.317407	0.018417	
LFIB	0.103601	-.150322	0.419582	-.306103	0.186651	-.030850	0.008270	
SMAC	**0.358556**	-.228337	-.035127	0.247346	0.049324	0.038258	0.093049	
KMAC	**0.350761**	-.007904	-.007439	0.303538	0.125131	-.144951	-.034912	
KLY	-.291192	0.060844	0.180542	0.278952	-.002487	0.093156	0.047037	
KVD	0.268090	0.330263	-.126172	0.004331	0.040533	-.233492	-.079108	
KNEC	0.163321	0.340769	-.116281	0.144168	0.172873	-.293560	-.000081	
KGBM	0.251962	-.079066	-.231793	0.145688	0.040035	0.219646	0.316247	
HLY	-.203141	0.038235	0.073280	0.123722	0.163348	-.109698	0.238020	
HGRI	-.000000	0.000000	-.000000	-.000000	0.000000	0.000000	-.000000	
HMAC	0.105739	-.283192	0.216000	0.382404	0.075426	-.155390	-.261161	
HFLK	0.113333	0.210266	0.057876	0.162367	0.493401	0.207700	-.036570	
HXEN	0.136043	-.025172	-.022544	0.021137	-.169920	**0.409970**	-.165565	
GCTD	0.202766	0.107540	0.263458	-.116593	-.087284	0.158845	-.260868	
GLY	0.030851	0.361360	0.128447	0.136620	-.074402	-.057233	0.387431	
GEGL	0.224477	0.310372	0.253670	-.060752	-.282824	-.188860	-.043619	
GMCH	0.260510	0.058611	-.201314	-.310829	-.144181	-.115322	0.040279	
GSCH	0.114710	0.206629	0.401255	0.132537	-.373478	0.139775	0.079735	
GANU	0.098744	-.164734	-.023983	-.004239	-.089034	0.292075	0.322986	
GPAR	-.107710	0.224302	0.215014	0.005330	0.399296	0.137925	-.028137	
GCLH	-.123580	-.049984	0.235689	0.301579	-.158492	0.059816	-.260422	

RF-Table 17. Initial MANOVA on the first principal component for 1991 rockfish, with nested site effect blocked for species effect. Significant P values ($P < 0.05$) are highlighted.

Dependent Variable: SCALE1 General Linear Models Procedure

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	6	243.27008545	40.54501424	31.19	**0.0001**
Error	92	119.60018963	1.30000206		
Corrected Total	98	362.87027508			

R-Square	C.V.	Root MSE	SCALE1 Mean
0.670405	-9999.99	1.14017633	-0.00000000

Source	DF	Type I SS	Mean Square	F Value	Pr > F
SPECIES	2	121.50314421	60.75157211	46.73	**0.0001**
Oiled vs. clean (OS)	1	14.97516303	14.97516303	11.52	**0.0010**
SITE(OS)	2	23.11920261	11.55960131	8.89	**0.0003**
AGE	1	83.67257559	83.67257559	64.36	**0.0001**

Source	DF	Type III SS	Mean Square	F Value	Pr > F
SPECIES	2	198.76283854	99.38141927	76.45	**0.0001**
Oiled vs. clean (OS)	1	9.28094651	9.28094651	7.14	**0.0089**
SITE(OS)	2	4.66624863	2.33312431	1.79	0.1719
AGE	1	83.67257559	83.67257559	64.36	**0.0001**

Parameter		Estimate	T for H0: Parameter=0	Pr > |T|	Std Error of Estimate
INTERCEPT		-3.716457956 B	-10.28	0.0001	0.36142974
SPECIES	CO	2.777501470 B	7.17	0.0001	0.38730747
	QB	3.592709925 B	12.26	0.0001	0.29294523
	YE	0.000000000 B	.	.	.
OS	C	-0.991828358 B	-2.55	**0.0125**	0.38909050
	O	0.000000000 B	.	.	.
SITE(OS)	GRAVINA C	0.715240408 B	1.89	0.0615	0.37778049
	ZAIKOFF C	0.000000000 B	.	.	.
	Danger_I O	0.116319463 B	0.33	0.7411	0.35101210
	Herring_ O	0.000000000 B	.	.	.
AGE		0.085917324	8.02	**0.0001**	0.01070930

NOTE: The X'X matrix has been found to be singular and a generalized inverse was used to solve the normal equations. Estimates followed by the letter 'B' are biased, and are not unique estimators of the parameters.

RF-Table 18. Initial MANOVA on the second principal component for 1991 rockfish, with nested site effect blocked for species effect. Significant P values (P < 0.05) are highlighted.

Dependent Variable: SCALE2 General Linear Models Procedure

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	6	36.98190121	6.16365020	3.34	**0.0051**
Error	92	169.78868014	1.84552913		
Corrected Total	98	206.77058136			

R-Square	C.V.	Root MSE	SCALE2 Mean
0.178855	-9999.99	1.35850253	-0.00000000

Source	DF	Type I SS	Mean Square	F Value	Pr > F
SPECIES	2	1.91306344	0.95653172	0.52	0.5973
Oiled vs. clean (OS)	1	1.65000668	1.65000668	0.89	0.3469
SITE(OS)	2	11.29848867	5.64924434	3.06	0.0516
AGE	1	22.12034242	22.12034242	11.99	**0.0008**

Source	DF	Type III SS	Mean Square	F Value	Pr > F
SPECIES	2	2.08570064	1.04285032	0.57	0.5703
Oiled vs. clean (OS)	1	0.37173642	0.37173642	0.20	0.6546
SITE(OS)	2	3.23774884	1.61887442	0.88	0.4194
AGE	1	22.12034242	22.12034242	11.99	**0.0008**

Parameter		Estimate	T for H0: Parameter=0	Pr > |T|	Std Error of Estimate
INTERCEPT		1.346435522 B	3.13	0.0024	0.43063797
SPECIES	CO	-0.488389744 B	-1.06	0.2927	0.46147088
	QB	-0.205006869 B	-0.59	0.5584	0.34903973
	YE	0.000000000 B	.	.	.
OS	C	-0.073530188 B	-0.16	0.8743	0.46359534
	O	0.000000000 B	.	.	.
SITE(OS)	GRAVINA C	-0.128080989 B	-0.28	0.7766	0.45011964
	ZAIKOFF C	0.000000000 B	.	.	.
	Danger_I O	-0.552274422 B	-1.32	0.1899	0.41822551
	Herring_ O	0.000000000 B	.	.	.
AGE		-0.044175868	-3.46	**0.0008**	0.01275997

NOTE: The X'X matrix has been found to be singular and a generalized inverse was used to solve the normal equations. Estimates followed by the letter 'B' are biased, and are not unique estimators of the parameters.

RF-Table 19. Initial MANOVA on the third principal component for 1991 rockfish, with nested site effect blocked for species effect. Significant P values (P < 0.05) are highlighted.

Dependent Variable: SCALE3 General Linear Models Procedure

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	6	44.98604481	7.49767414	4.69	**0.0003**
Error	92	147.05543976	1.59842869		
Corrected Total	98	192.04148457			

R-Square	C.V.	Root MSE	SCALE3 Mean
0.234252	-9999.99	1.26428980	-0.00000000

Source	DF	Type I SS	Mean Square	F Value	Pr > F
SPECIES	2	37.19984290	18.59992145	11.64	**0.0001**
Oiled vs. clean (OS)	1	0.18449049	0.18449049	0.12	0.7348
SITE(OS)	2	6.02417008	3.01208504	1.88	0.1577
AGE	1	1.57754133	1.57754133	0.99	0.3231

Source	DF	Type III SS	Mean Square	F Value	Pr > F
SPECIES	2	27.76152349	13.88076175	8.68	**0.0004**
Oiled vs. clean (OS)	1	0.01289257	0.01289257	0.01	0.9286
SITE(OS)	2	4.01380329	2.00690165	1.26	0.2898
AGE	1	1.57754133	1.57754133	0.99	0.3231

Parameter		Estimate	T for H0: Parameter=0	Pr > |T|	Std Error of Estimate
INTERCEPT		0.081216489 B	0.20	0.8399	0.40077304
SPECIES	CO	-1.760112723 B	-4.10	**0.0001**	0.42946768
	QB	-0.410936248 B	-1.27	0.2090	0.32483368
	YE	0.000000000 B	.	.	.
OS	C	-0.137619216 B	-0.32	0.7505	0.43144480
	O	0.000000000 B	.	.	.
SITE(OS)	GRAVINA C	0.621434062 B	1.48	0.1414	0.41890365
	ZAIKOFF C	0.000000000 B	.	.	.
	Danger_I O	0.294584845 B	0.76	0.4511	0.38922139
	Herring_ O	0.000000000 B	.	.	.
AGE		0.011797216	0.99	0.3231	0.01187506

NOTE: The X'X matrix has been found to be singular and a generalized inverse was used to solve the normal equations. Estimates followed by the letter 'B' are biased, and are not unique estimators of the parameters.

RF-Table 20. Initial MANOVA on the fourth principal component for 1991 rockfish, with nested site effect blocked for species effect. Significant P values (P < 0.05) are highlighted.

Dependent Variable: SCALE4 General Linear Models Procedure

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	6	66.39906963	11.06651161	8.11	**0.0001**
Error	92	125.52788392	1.36443352		
Corrected Total	98	191.92695355			

R-Square	C.V.	Root MSE	SCALE4 Mean
0.345960	9999.99	1.16808969	0.00000000

Source	DF	Type I SS	Mean Square	F Value	Pr > F
SPECIES	2	46.82459977	23.41229988	17.16	**0.0001**
Oiled vs. clean (OS)	1	5.07900842	5.07900842	3.72	0.0568
SITE(OS)	2	3.58323129	1.79161564	1.31	0.2740
AGE	1	10.91223015	10.91223015	8.00	**0.0057**

Source	DF	Type III SS	Mean Square	F Value	Pr > F
SPECIES	2	14.92153163	7.46076582	5.47	**0.0057**
Oiled vs. clean (OS)	1	4.30343467	4.30343467	3.15	0.0790
SITE(OS)	2	3.91190374	1.95595187	1.43	0.2437
AGE	1	10.91223015	10.91223015	8.00	**0.0057**

Parameter		Estimate	T for H0: Parameter=0	Pr > |T|	Std Error of Estimate
INTERCEPT		0.3337122457 B	0.90	0.3698	0.37027813
SPECIES	CO	-.5803827512 B	-1.46	0.1470	0.39678938
	QB	-.9919494156 B	-3.31	**0.0014**	0.30011701
	YE	0.0000000000 B	.	.	.
OS	C	-.8775122876 B	-2.20	**0.0302**	0.39861607
	O	0.0000000000 B	.	.	.
SITE(OS)	GRAVINA C	0.3407404192 B	0.88	0.3809	0.38702917
	ZAIKOFF C	0.0000000000 B	.	.	.
	Danger_I O	-.4713564799 B	-1.31	0.1932	0.35960544
	Herring_ O	0.0000000000 B	.	.	.
AGE		0.0310274391	2.83	**0.0057**	0.01097148

NOTE: The X'X matrix has been found to be singular and a generalized inverse was used to solve the normal equations. Estimates followed by the letter 'B' are biased, and are not unique estimators of the parameters.

RF-Table 21. MANOVA on all data for 1991 rockfish, with nested site effects, blocked by species, to test the hypothesis of "no overall oiled vs. clean effect." Significant P values are highlighted.

General Linear Models Procedure
Multivariate Analysis of Variance

H = Type III SS&CP Matrix for Oiled vs. clean

	SCALE1	SCALE2	SCALE3	SCALE4
SCALE1	9.2809465103	-1.857435287	-0.345912154	6.3198059257
SCALE2	-1.857435287	0.3717364218	0.0692288701	-1.264809631
SCALE3	-0.345912154	0.0692288701	0.0128925663	-0.235546846
SCALE4	6.3198059257	-1.264809631	-0.235546846	4.3034346652

Characteristic Roots and Vectors of: E Inverse * H, where
H = Type III SS&CP Matrix for Oiled vs. clean E = Error SS&CP Matrix

Characteristic Root	Percent	Characteristic Vector V'EV=1 SCALE1	SCALE2	SCALE3	SCALE4
0.1191874795	100.00	0.08127202	-0.03977612	0.00088285	0.03542671
0.0000000000	0.00	-0.01739342	0.01343610	0.08300061	0.03403506
0.0000000000	0.00	-0.03154229	0.05052508	-0.02003005	0.06007475
0.0000000000	0.00	0.04812972	0.04994274	-0.00889334	-0.05648912

Manova Test Criteria and Exact F Statistics for the Hypothesis of **no Overall "Oiled vs. clean" Effect**
H = Type III SS&CP Matrix for Oiled vs. clean E = Error SS&CP Matrix

S=1 M=1 N=43.5

Statistic	Value	F	Num DF	Den DF	Pr > F
Wilks' Lambda	0.89350535	2.6519	4	89	**0.0383**
Pillai's Trace	0.10649465	2.6519	4	89	**0.0383**
Hotelling-Lawley Trace	0.11918748	2.6519	4	89	**0.0383**
Roy's Greatest Root	0.11918748	2.6519	4	89	**0.0383**

RF-Table 22. MANOVA on all data for 1991 rockfish, with nested site effects, blocked by species, to test the hypothesis of "no overall site (within oiled or clean site) effect." Note that none of the values are significant.

General Linear Models Procedure
Multivariate Analysis of Variance

H = Type III SS&CP Matrix for SITE(oiled or clean)

	SCALE1	SCALE2	SCALE3	SCALE4
SCALE1	4.6662486261	-0.975364794	4.1051460953	2.0844683854
SCALE2	-0.975364794	3.2377488355	-1.962839361	2.5714856302
SCALE3	4.1051460953	-1.962839361	4.0138032923	0.7387733234
SCALE4	2.0844683854	2.5714856302	0.7387733234	3.9119037368

Characteristic Roots and Vectors of: E Inverse * H, where
H = Type III SS&CP Matrix for SITE(oiled or clean) E = Error SS&CP Matrix

Characteristic Root	Percent	Characteristic Vector V'EV=1			
		SCALE1	SCALE2	SCALE3	SCALE4
0.0830632870	68.02	0.07041202	-0.04490246	0.04721645	0.01418877
0.0390525656	31.98	-0.01063790	0.03738257	0.01238232	0.07636821
0.0000000000	0.00	-0.07142337	-0.00710634	0.06393113	0.03065592
0.0000000000	0.00	0.00683696	0.05784066	0.03000448	-0.04733101

Manova Test Criteria and F Approximations for the Hypothesis of **no Overall SITE(oiled or clean) Effect**
H = Type III SS&CP Matrix for SITE(oiled or clean) E = Error SS&CP Matrix

S=2 M=0.5 N=43.5

Statistic	Value	F	Num DF	Den DF	Pr > F
Wilks' Lambda	0.88860478	1.3535	8	178	0.2201
Pillai's Trace	0.11427770	1.3635	8	180	0.2154
Hotelling-Lawley Trace	0.12211585	1.3433	8	176	0.2250
Roy's Greatest Root	0.08306329	1.8689	4	90	0.1228

NOTE: F Statistic for Roy's Greatest Root is an upper bound.
NOTE: F Statistic for Wilks' Lambda is exact.

RF-Table 23. MANOVA on all data for 1991 rockfish, with nested site effects, blocked by species, to test the hypothesis of "no overall age effect." Significant P values are highlighted.

General Linear Models Procedure
Multivariate Analysis of Variance

H = Type III SS&CP Matrix for AGE

	SCALE1	SCALE2	SCALE3	SCALE4
SCALE1	83.672575595	-43.02169248	11.488992388	30.216790074
SCALE2	-43.02169248	22.120342423	-5.90726285	-15.53648183
SCALE3	11.488992388	-5.90726285	1.5775413289	4.1490353163
SCALE4	30.216790074	-15.53648183	4.1490353163	10.912230153

Characteristic Roots and Vectors of: E Inverse * H, where
H = Type III SS&CP Matrix for AGE E = Error SS&CP Matrix

Characteristic Root	Percent	Characteristic Vector V'EV=1 SCALE1	SCALE2	SCALE3	SCALE4
1.2242274308	100.00	0.08750288	-0.05353655	0.00674235	0.01385634
0.0000000000	0.00	0.02428914	0.05621511	0.03641300	-0.00106611
0.0000000000	0.00	-0.02695265	-0.02787724	0.07551764	0.00622998
0.0000000000	0.00	-0.03529727	0.00250761	0.01720159	0.09477071

Manova Test Criteria and Exact F Statistics for the Hypothesis of **no Overall AGE Effect**
H = Type III SS&CP Matrix for AGE E = Error SS&CP Matrix

S=1 M=1 N=43.5

Statistic	Value	F	Num DF	Den DF	Pr > F
Wilks' Lambda	0.44959431	27.2391	4	89	**0.0001**
Pillai's Trace	0.55040569	27.2391	4	89	**0.0001**
Hotelling-Lawley Trace	1.22422743	27.2391	4	89	**0.0001**
Roy's Greatest Root	1.22422743	27.2391	4	89	**0.0001**

RF-Table 24. Initial MANOVA on the sixth and seventh principal component for 1991 rockfish, blocked for species effect. Significant P values ($P < 0.05$) are highlighted.

Dependent Variable: SCALE6

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	23.86184971	5.96546243	4.79	**0.0015**
Error	94	117.10111603	1.24575655		
Corrected Total	98	140.96296574			

R-Square	C.V.	Root MSE	SCALE6 Mean
0.169277	9999.99	1.11613465	0.00000000

Source	DF	Type I SS	Mean Square	F Value	Pr > F
SPECIES	2	8.98048906	4.49024453	3.60	**0.0310**
OS	1	13.60312636	13.60312636	10.92	**0.0013**
AGE	1	1.27823429	1.27823429	1.03	0.3137

Source	DF	Type III SS	Mean Square	F Value	Pr > F
SPECIES	2	12.84188720	6.42094360	5.15	**0.0075**
OS	1	14.46608490	14.46608490	11.61	**0.0010**
AGE	1	1.27823429	1.27823429	1.03	0.3137

Parameter		Estimate	T for H0: Parameter=0	Pr > |T|	Std Error of Estimate
INTERCEPT		0.5184109024 B	1.51	0.1355	0.34425825
SPECIES	CO	-.5262111951 B	-1.55	0.1241	0.33914040
	QB	0.4457286617 B	1.59	0.1159	0.28092164
	YE	0.0000000000 B	.	.	.
OS	C	-.8268717176 B	-3.41	**0.0010**	0.24264940
	O	0.0000000000 B	.	.	.
AGE		-.0100772766	-1.01	0.3137	0.00994843

NOTE: The X'X matrix has been found to be singular and a generalized inverse was used to solve the normal equations. Estimates followed by the letter 'B' are biased, and are not unique estimators of the parameters.

Dependent Variable: SCALE7

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	17.86245487	4.46561372	3.82	**0.0064**
Error	94	109.97000627	1.16989368		
Corrected Total	98	127.83246114			

R-Square	C.V.	Root MSE	SCALE7 Mean
0.139733	-9999.99	1.08161624	-0.00000000

Source	DF	Type I SS	Mean Square	F Value	Pr > F
SPECIES	2	4.95256225	2.47628112	2.12	0.1261
OS	1	12.66874937	12.66874937	10.83	**0.0014**
AGE	1	0.24114325	0.24114325	0.21	0.6509

Source	DF	Type III SS	Mean Square	F Value	Pr > F
SPECIES	2	3.08476312	1.54238156	1.32	0.2725
OS	1	12.01313936	12.01313936	10.27	**0.0018**
AGE	1	0.24114325	0.24114325	0.21	0.6509

Parameter		Estimate	T for H0: Parameter=0	Pr > |T|	Std Error of Estimate
INTERCEPT		0.1801974309 B	0.54	0.5904	0.33361147
OS	C	-.7535132447 B	-3.20	0.0018	0.23514504
	O	0.0000000000 B	.	.	.
AGE		0.0043769895	0.45	0.6509	0.00964076

NOTE: The X'X matrix has been found to be singular and a generalized inverse was used to solve the normal equations. Estimates followed by the letter 'B' are biased, and are not unique estimators of the parameters.

Figure Legends. 1991 Rockfish

Pathologist/Photographer - Mark S. Okihiro

RF-Figure 1. Normal liver in fish QB1-91. Glycogen laden hepatocytes are organized into orderly tubules. Hepatic veins (V) contain small amounts of blood. HE 110X.

RF-Figure 2. Normal liver in fish QB1-91. Higher magnification of figure 1. HE 220X.

RF-Figure 3. Normal liver in fish QB1-91. Hepatocytes lining sinusoids (S) are glycogen laden and have small, round, regular nuclei (arrow heads). V = hepatic vein. HE 440X.

RF-Figure 4. Severe megalocytosis in fish QB28-91. Many hepatocytes contain large vacuoles (arrow heads) and karyomegalic nuclei. V = hepatic vein and BD = bile duct. HE 220X.

RF-Figure 5. Megalocytosis in fish QB28-91. Many hepatocytes have markedly enlarged nuclei (arrow heads) and scattered hepatocytes are necrotic and rounded up (arrows). HE 440X.

RF-Figure 6. Megalocytosis in fish QB28-91. Marked karyomegaly in some hepatocytes (arrow heads) and some have multiple nucleoli. HE 440X.

RF-Figure 7. Scattered macrophage aggregates (M) and sinusoidal fibrosis (arrow heads) in fish QB71-91. HE 110X.

RF-Figure 8. Marked sinusoidal fibrosis (arrow heads) and scattered macrophage aggregates (M) in fish QB71-91. HE 220X.

RF-Figure 9. Focal bile duct hyperplasia in fish QB68-91. M = macrophage aggregate. HE 220X.

RF-Figure 10. Numerous greatly enlarged macrophage aggregates (M) in the spleen of fish QB68-91. HE 44X.

RF-Figure 11. Large numbers of macrophage aggregates (M) in the kidney of fish QB78-91. HE 110X.

RF-Figure 12. Tubular degeneration. Acidophilic round bodies are likely cellular blebs which have sloughed into the lumen (arrow heads) of fish QB22-91. HE 220X.

RF-Figure 13. Vacuolar degeneration (arrow heads) and macrophage aggregates (M) in the kidney of fish QB22-91. HE 220X.

RF-Figure 14. Vacuolar degeneration and necrosis (arrow heads) in the renal tubular epithelium of fish QB22-91. HE 440X.

RF-Figure 15. Tubular adenoma (arrow heads) in the kidney of fish QB105-91 from an oiled site (Herring Bay). HE 44X.

RF-Figure 16. Endocardial macrophage aggregates (arrow heads) in the atrium (heart) of fish QB78-91. HE 110X.

RF-Figure 17. Pericardial lymphocytic inflammation (arrow heads) and fibrosis over the ventricular myocardium (heart) of fish QB1-91. HE 110X.

RF-Figure 18. Adult trematodes (flukes, F) in the ventricular lumen of fish QB27-91. There are scattered small foci of lymphocytic endocardial inflammation (arrow heads). HE 110X.

RF-Figure 19. Normal gill filaments (F) in fish YE31-91. C = filament cartilage. HE 44X.

RF-Figure 20. Normal gill filament (F) in fish YE31-91. Note the thin lamellae (L) coming off the filament.

RF-Figure 21. Severe, focal, filament cartilage (C) dysplasia in fish QB60-91. HE 44X.

RF-Figure 22. Lamellar fusion in fish QB22-91. HE 44X.

RF-Figure 23. Severe squamous epithelial hyperplasia (arrow heads) in the gill of fish QB22-91. Note the complete loss of interlamellar spaces. HE 44X.

RF-Figure 24. Mucous cell (goblet cell) hyperplasia (arrow heads) in fish QB22-91. There is mild mononuclear inflammation in the filament interstitium (I) surrounding the cartilage (C). HE 110X.

RF-Figure 25. Massive, diffuse chloride cell hyperplasia in the gill of fish YE104-91. HE 44X.

RF-Figure 26. Severe chloride cell hyperplasia in the gill of fish YE2-91. HE 220X.

C. **Significance of Lesions** in rockfish from 1990, 1991, and beyond (?).

Principal components analysis was used to identify significant differences in oiled vs. clean effects; graphical presentations included here only approximate the highlights of this analysis. Sampling from eight sites in 1990 (4 in PWS and 4 off the Kenai peninsula) included eight species of rockfish. Statistical analysis revealed significant species differences, and fish from PWS seemed more affected by EVOS. Therefore, sampling was streamlined in 1991 to include only the same four sites in PWS; in 1991, only copper, quillback, and yelloweye rockfish were sampled. Each RF-Graphic is designed to compare findings in 1990 with 1991 (i.e., only PWS). Because of the small number of fish of a given species at each site in 1990, only comparisons between Quillback and Yelloweye rockfish are relevant for most lesions. Although sex differences were not analyzed statistically, mean scores were determined for each sex for copper, quillback, and yelloweye rockfish from PWS (RF-Table 25).

RF-Graphic 1 shows that hepatic lipidosis, particularly in yelloweye rockfish, is more related to exposure status than to sex. At oiled sites in PWS, female yelloweye rockfish had greater mean lipidosis scores in 1990 whereas scores for males were higher in 1991. For quillback rockfish, mean lipidosis scores were low for both sexes in 1990, but were higher in fish (both sexes) from oiled sites in 1991.

RF-Graphics 2-5 show several trends in mean scores for liver, spleen, and kidney lesions:

1) **hepatocellular glycogen depletion (GLY)** - was more severe for all sex-species combinations from oiled sites in 1990, but differences were less in 1991—Evidence for decreasing effect from 1990 to 1991.

2) **hepatocellular lipidosis (LIP)** - was more severe for all sex-species combinations (except female quillbacks) from oiled sites in 1990. In 1991, mean lesion scores were more severe in all fish groups from oiled vs. clean sites—evidence for increasing effect from 1990 to 1991.

3) **liver macrophage aggregates (LMA)** - were more severe for most sex-species combinations from oiled sites in 1990, but not 1991—evidence for decreasing effect from 1990 to 1991.

4) **hepatocellular megalocytosis (MEG) and hepatic fibrosis (FIB)** - mean scores were elevated for all species-sex combinations from oiled sites in 1990 and for all but female quillback rockfish in 1991—evidence for continued effect from 1990 to 1991.

5) **splenic (SMA) and kidney (KMA) macrophage aggregate** - scores were more severe for all rockfish from oiled sites in 1990 except male yelloweye. In 1991, scores for fish from oiled sites were more severe for quillback but not yelloweye rockfish—evidence for continued or decreased effect from 1990 to 1991.

6) other lesions - no clear trends emerge, except possibly that single cell hepatocellular necrosis was less severe in 1991 than 1990 (evidence for decreasing effects from 1990 to 1991).

In summary, comparison of significant lesions in 1990 and 1991 reveals an increasing effect on one lesion (hepatocellular lipidosis), continued effect on four lesions (hepatocellular megalocytosis, hepatic fibrosis, splenic and kidney macrophage aggregates), and decreasing effect on one lesion (liver macrophage aggregates).

RF-Table 25. Rockfish samples from Prince William Sound in 1990 and 1991: mean length, age, and lesion scores by species and sex. Individuals for which sex was not reported/determined are not included in this table.

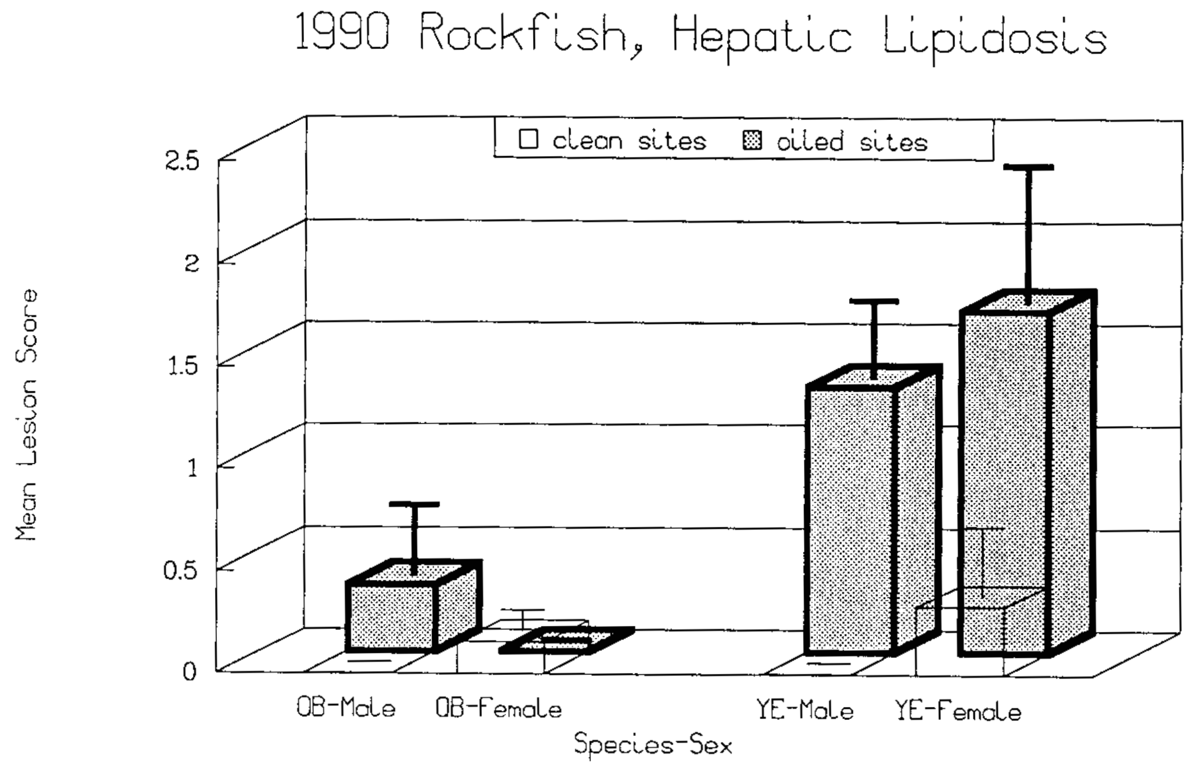
1990 Prince William Sound Rockfish:

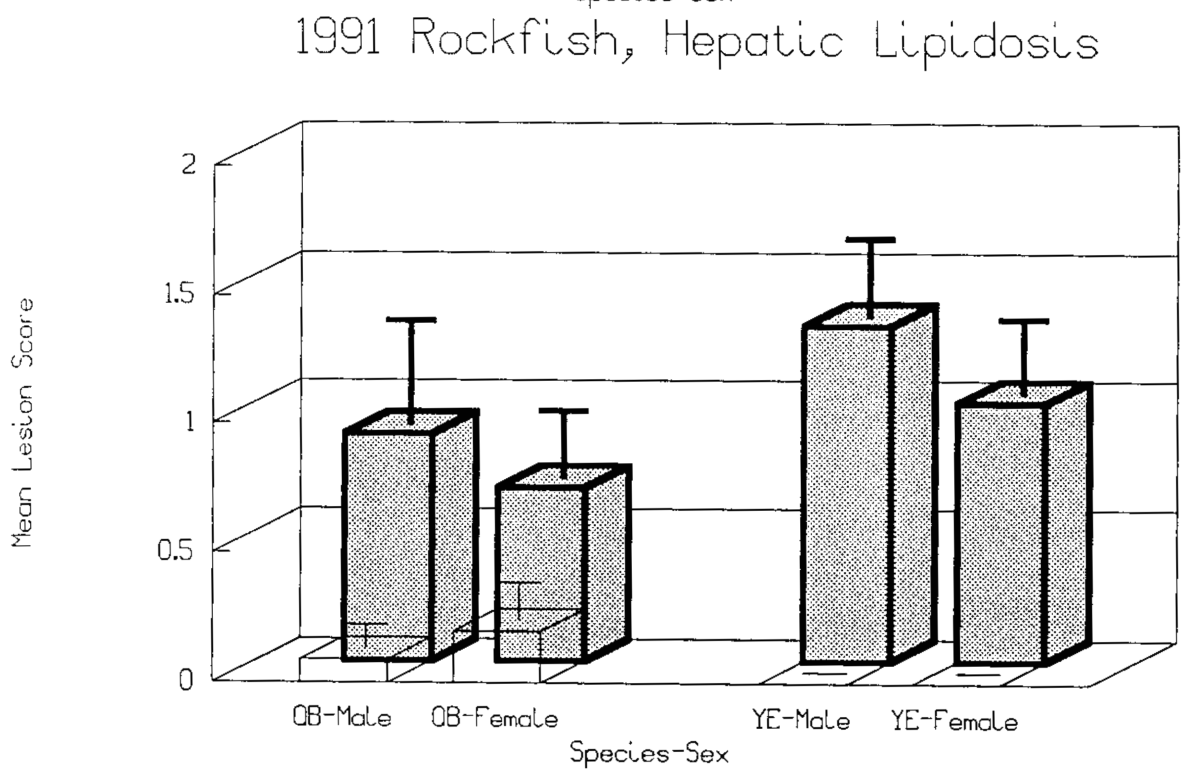
						Mean Lesion Scores								
						Liver						Spl	Kidney	
N	Length (mm)	Age (yrs)	SEX	Species	Oiled Status	GLY	LIP	LMA	SCN	MEG	FIB	SMA	KMA	VD
13	276.769	13.4	F	Quillback	clean sites	.92	.15	1	.31	1.3	.77	1.5	1.2	1.1
5	385.8	24.2	F	Quillback	oiled sites	2.2	0	2	.2	1.6	1	2	1.4	.8
6	322.833	26.6	M	Quillback	clean sites	.5	0	1.3	.17	1.8	.17	1.8	2	.83
3	362.333	29.33	M	Quillback	oiled sites	1	.33	2.3	.33	2.3	.67	3	2.7	2
3	419.333	19.33	F	Yelloweye	clean sites	1	.33	.67	0	.33	1	1.3	.67	1.3
3	549	41	F	Yelloweye	oiled sites	2.7	1.7	1.7	.67	.67	1.3	2	1	.67
2	477.5	19	M	Yelloweye	clean sites	0	0	1	0	0	.5	1.5	1	.5
10	527.4	26.6	M	Yelloweye	oiled sites	2.2	1.3	1	.1	.3	1.5	1.4	1	1

1991 Prince William Sound Rockfish:

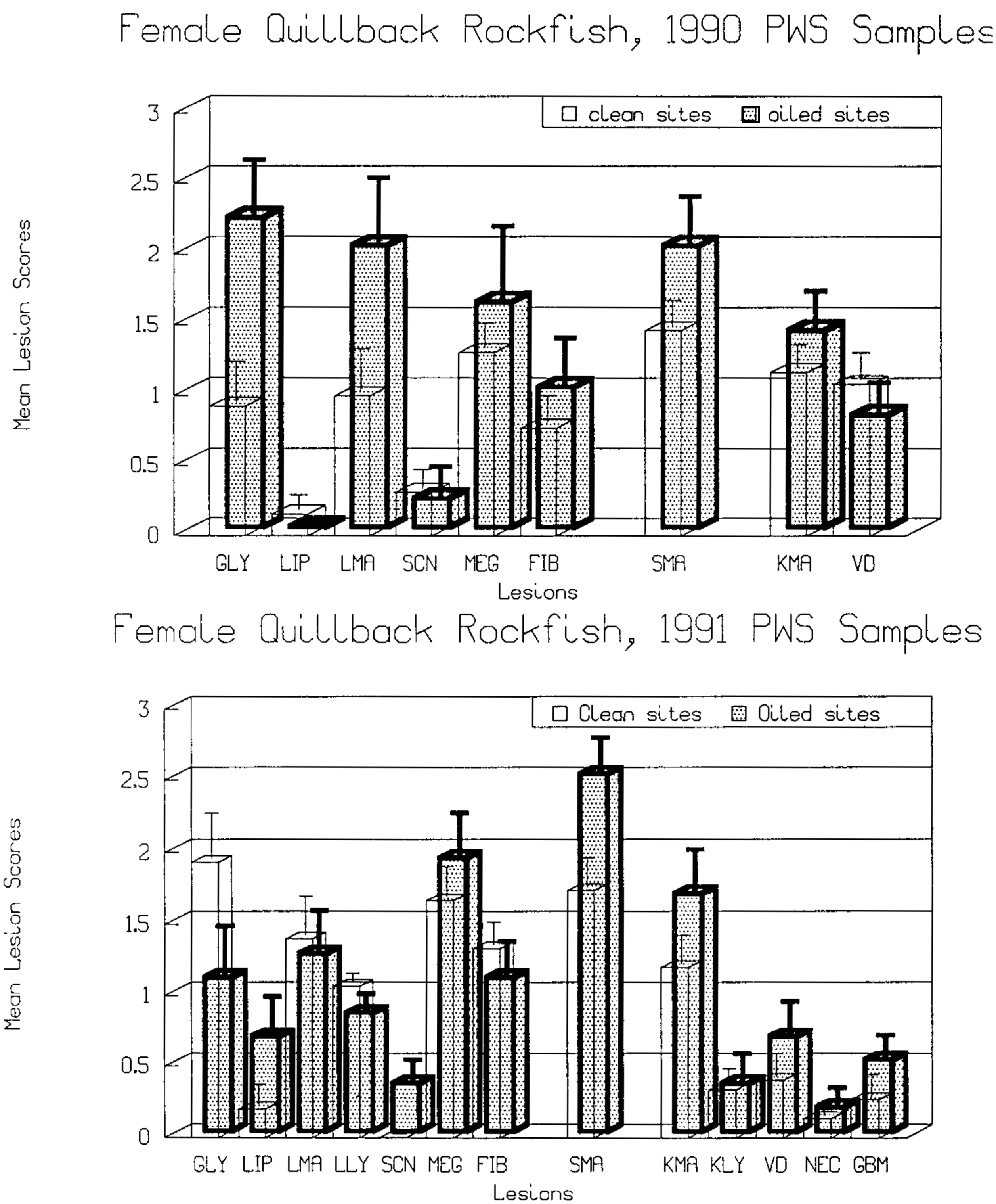
						Mean Lesion Scores																									
						Liver							Spl	Kidney					Heart					Gill							
N	Mean Length (mm)	Mean Age (yrs)	Sex	Rockfish Species	Oiled Status	GLY	LIP	LMA	LLY	SCN	MEG	FIB	SMA	KMA	KLY	VD	NEC	GBM	LY	GRI	MAC	FLK	XEN	CTD	LY	EGL	MCH	SCH	ANU	PAR	CLH
5	325.6	13.2	F	Copper	Combined clean sites	2.4	.8	1.8	1	0	0	1	1.8	1.4	.2	.8	0	0	1	0	.6	0	0	.6	.4	0	1.8	.4	0	.4	0
4	405.25	17	F	Copper	Combined oiled sites	1.8	.5	2	1	0	.75	.75	2.5	1.8	0	0	0	.75	1.5	0	.5	0	0	0	1.3	0	2	.25	.25	0	0
4	336.5	12	M	Copper	Combined clean sites	2.3	1	1	.75	0	0	.5	1	1.5	.5	.5	.25	0	.75	0	.25	0	0	0	0	0	1.8	0	.25	0	0
6	402.333	16.7	M	Copper	Combined oiled sites	0	.5	1.2	.67	0	.17	.33	2.8	1.5	.33	1	.17	.67	.83	0	.67	0	0	0	.33	0	1.5	.33	.33	0	0
15	329.133	14.9	F	Quillback	Combined clean sites	1.9	.2	1.4	1.1	0	1.7	1.3	1.7	1.2	.33	.4	.13	.27	.8	0	.4	0	0	.27	.2	.07	1.1	.53	.07	.07	0
12	353.333	20	F	Quillback	Combined oiled sites	1.1	.67	1.3	.83	.33	1.9	1.1	2.5	1.7	.33	.67	.17	.5	.5	.08	.5	.08	.17	.5	.1	0	1.1	.9	.5	.4	0
11	361.818	22.5	M	Quillback	Combined clean sites	1.6	.09	1.5	.91	0	1.7	.91	1.9	2.4	0	1	.55	.09	.55	0	.82	.09	0	.27	.64	.27	1.3	1	0	.27	0
8	365.375	19.4	M	Quillback	Combined oiled sites	2.3	.88	1.1	.75	.13	2.1	1.4	2.8	2.6	.88	1	.5	.63	.63	0	.75	.13	0	0	.86	0	1.4	.57	.29	0	0
6	462.333	24.2	F	Yelloweye	Combined clean sites	1	0	1.2	1	0	.33	.5	1.5	1.7	1	0	0	.5	1.2	0	.67	0	0	0	.33	0	0	.67	0	.83	.5
17	481.294	28.8	F	Yelloweye	Combined oiled sites	1.4	1	1.2	.88	0	.71	1	1.8	1.4	1.3	.06	.12	.12	1.3	0	.82	0	0	.13	.56	0	0	.75	.13	.19	.5
2	548.5	40	M	Yelloweye	Combined clean sites	.5	0	1	1.5	0	0	.5	2.5	1	1.5	0	0	0	1	0	1	0	0	0	.5	0	0	1	0	.5	1.5
10	555.2	31.7	M	Yelloweye	Combined oiled sites	2	1.3	1.1	1	0	.3	1	2.2	1.1	1.4	.33	.33	.11	1.3	0	.8	0	0	0	.5	0	.1	1	.2	.2	.2

RF-Graphic 1. Comparison of sex and exposure differences in mean hepatic lipidosis scores (±SE) for rockfish species collected in Prince William Sound (PWS) in 1990 and 1991. See RF-Table 25 for mean age and number of fish per group.

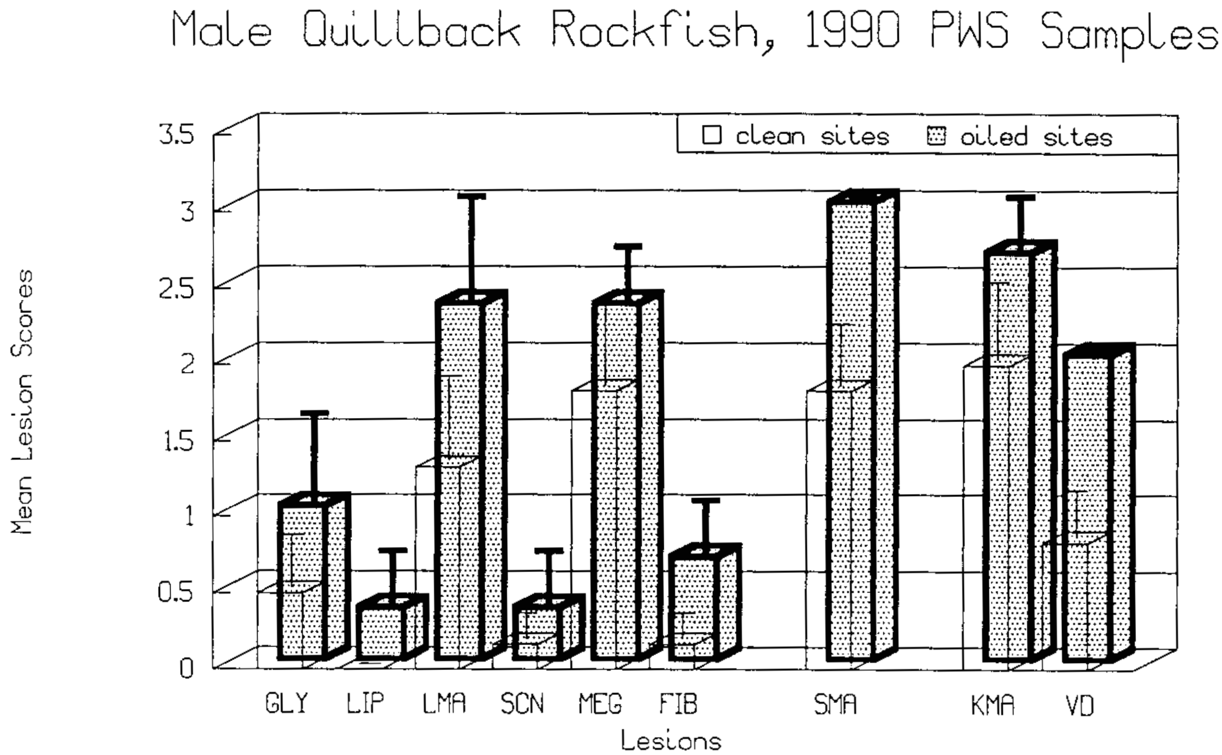


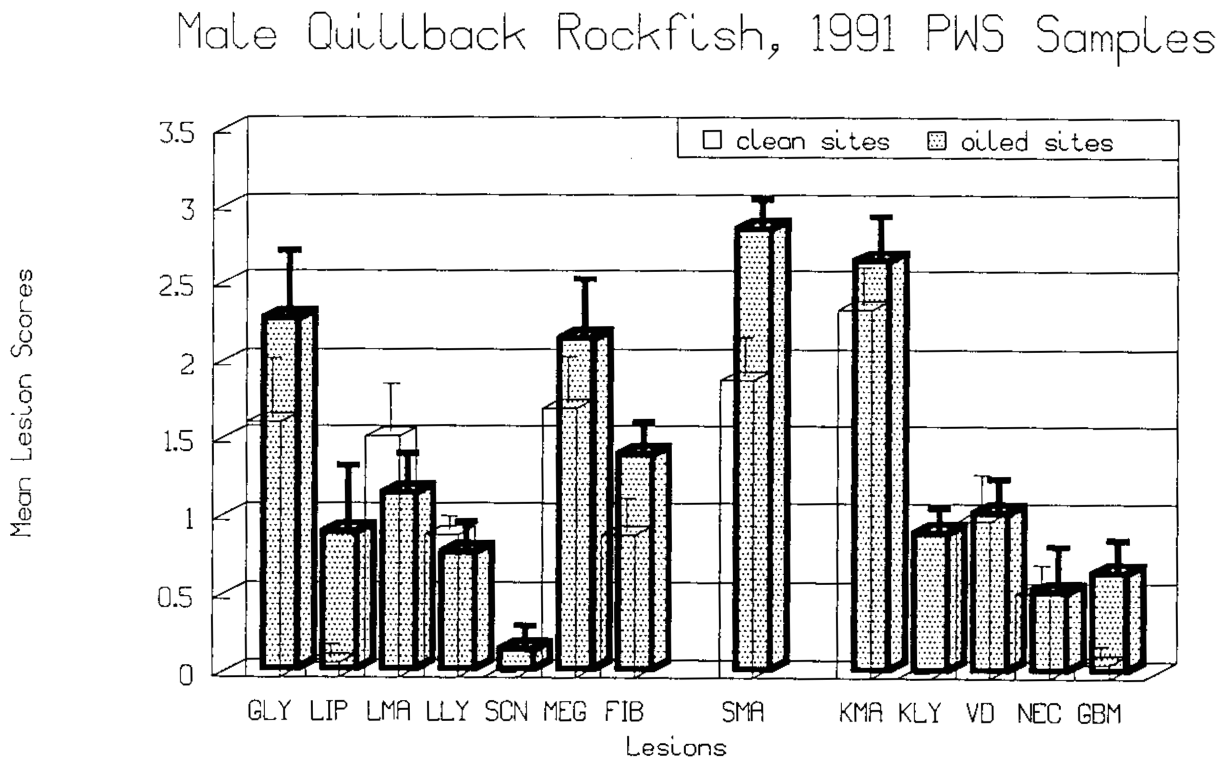


RF-Graphic 2. Comparison of mean lesion scores (±SE) for female quillback rockfish collected from clean vs. oiled sites in Prince William Sound (PWS) in 1990 and 1991. See RF-Table 25 for mean age and number of fish per group, and RF-Table 13 (p. 376) for lesion abbreviations.

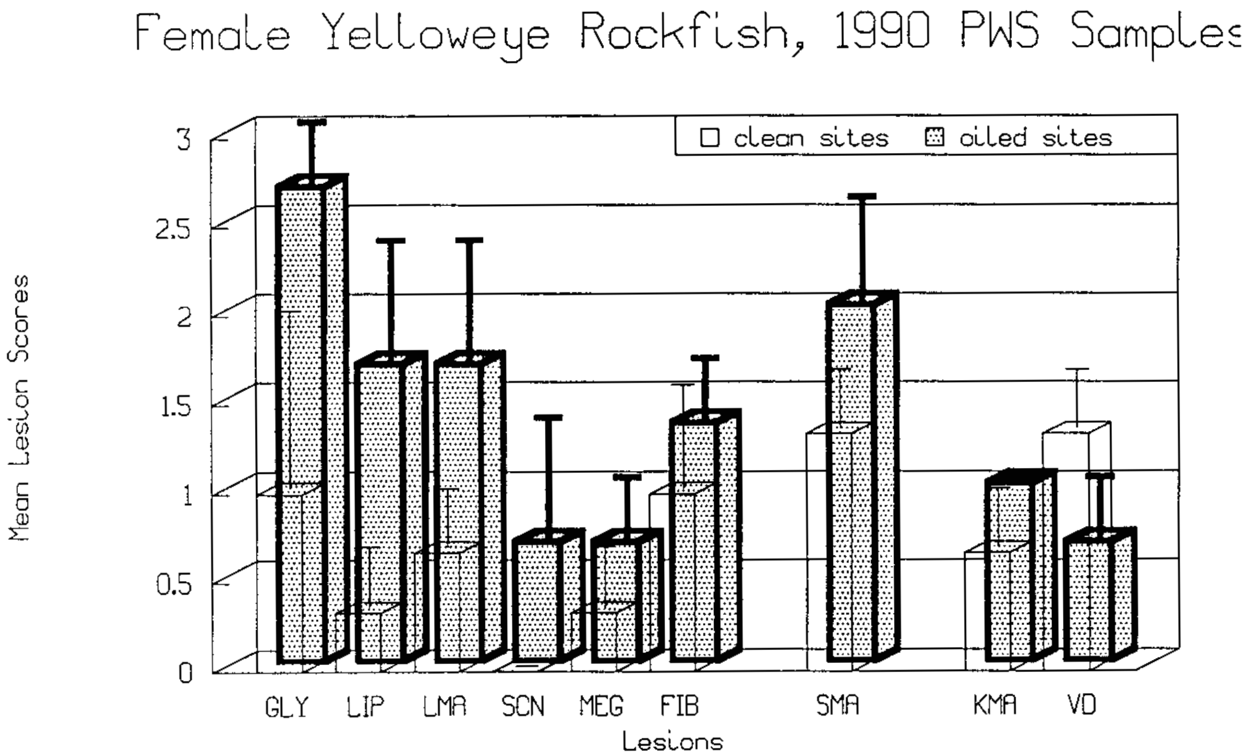


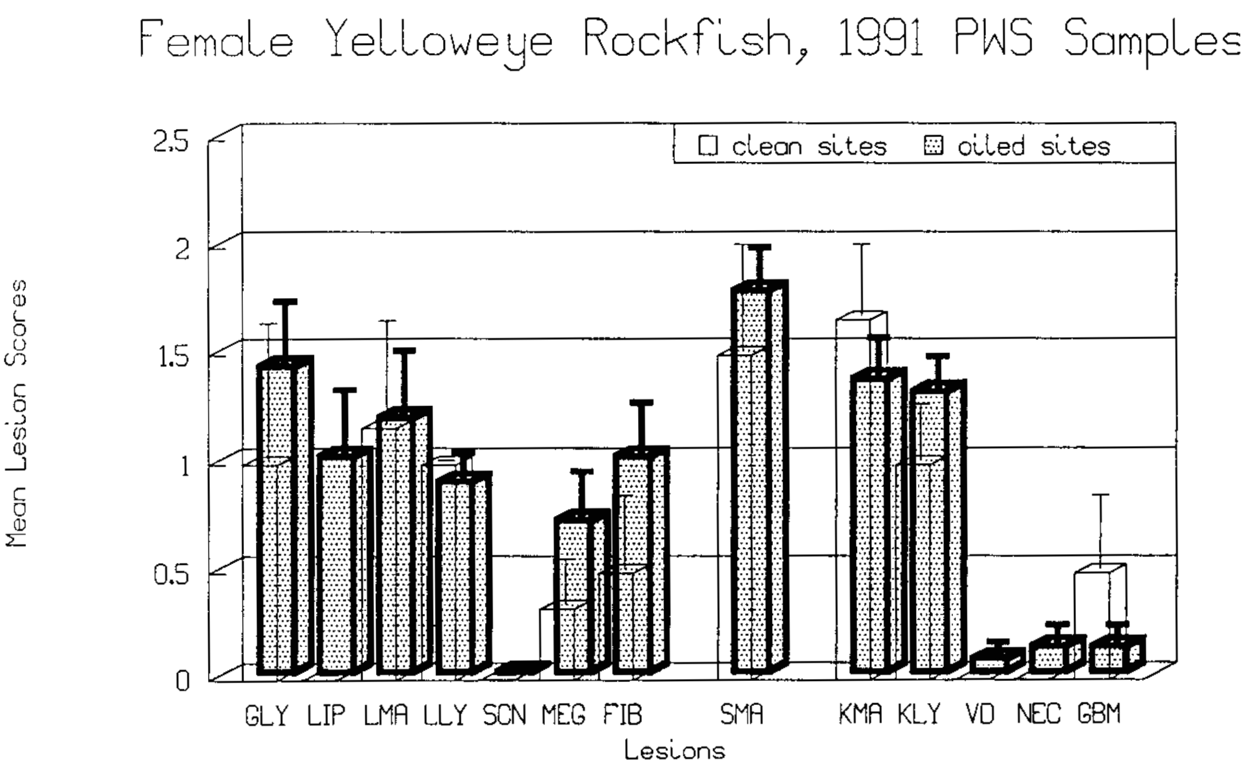
RF-Graphic 3. Comparison of mean lesion scores (±SE) for male quillback rockfish collected from clean vs. oiled sites in Prince William Sound (PWS) in 1990 and 1991. See RF-Table 25 for mean age and number of fish per group, and RF-Table 13 (p. 376) for lesion abbreviations.



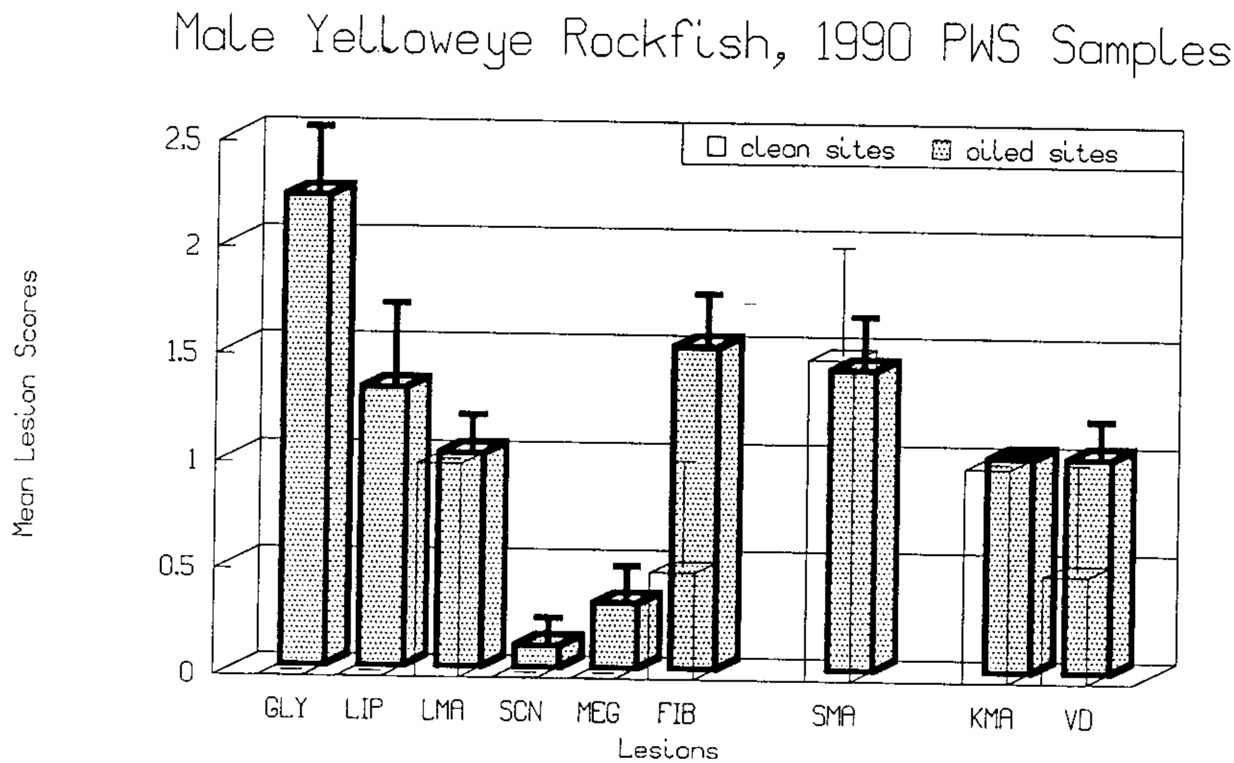


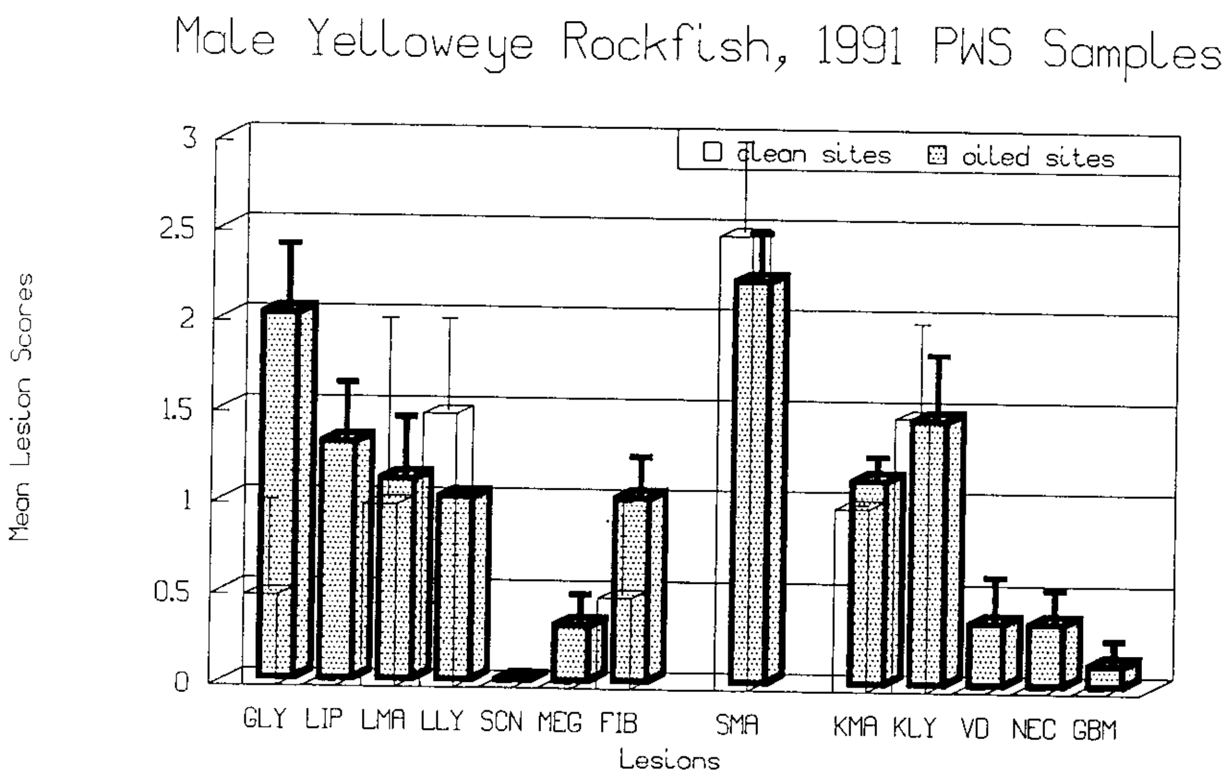
RF-Graphic 4. Comparison of mean lesion scores (±SE) for female yelloweye rockfish collected from clean vs. oiled sites in Prince William Sound (PWS) in 1990 and 1991. See RF-Table 25 for mean age and number of fish per group, and RF-Table 13 (p. 376) for lesion abbreviations.





RF-Graphic 5. Comparison of mean lesion scores (±SE) for male yelloweye rockfish collected from clean vs. oiled sites in Prince William Sound (PWS) in 1990 and 1991. See RF-Table 25 for mean age and number of fish per group, and RF-Table 13 (p. 376) for lesion abbreviations.





D. **Potential publication** involving rockfish histopathology:

TITLE- Histopathology and cytochrome-P450 induction of Rockfish (*Sebastes* spp.) in Prince William Sound, Alaska: chronic effects of the *Exxon Valdez* oil spill.

AUTHORS- Okihiro, Marty, Hinton, Smolowitz?, Stegeman, Hepler, Hoffman, others?

JOURNAL- Science ? [not without study in 1993]

E. **Recommended Future Work** with rockfish:

1) Differences between rockfish from oiled and clean sites in 1991 were more pronounced than in 1990. We recommended sampling again in 1992; funding was denied. Although funding was approved for continued study in 1993, it was later rescinded.

2) A weak link in our study of oil spill effects on rockfish is lack of histopathologic data from acutely exposed fish in 1989. According to Andy Hoffman (ADF&G), the NMFS Auke Bay Laboratory sampled rockfish in 1989 for hydrocarbon analysis, and livers from many of the fish were frozen. Histopathologic examination of these livers is warranted. If the livers are allowed to melt while immersed in 10% neutral buffered formalin, architecture will likely be sufficient to analyze for necrosis (the major lesion in 1989-sampled herring), macrophage aggregates and megalocytosis (common lesions in 1990- and 1991-sampled rockfish. Cytoplasmic vacuolation (e.g., lipidosis or hydropic degeneration), potentially an important lesion, might not be able to be evaluated in these previously frozen tissues.

APPENDIX F

Mixed Function Oxidase Analysis
from
Woods Hole Oceanographic Institution

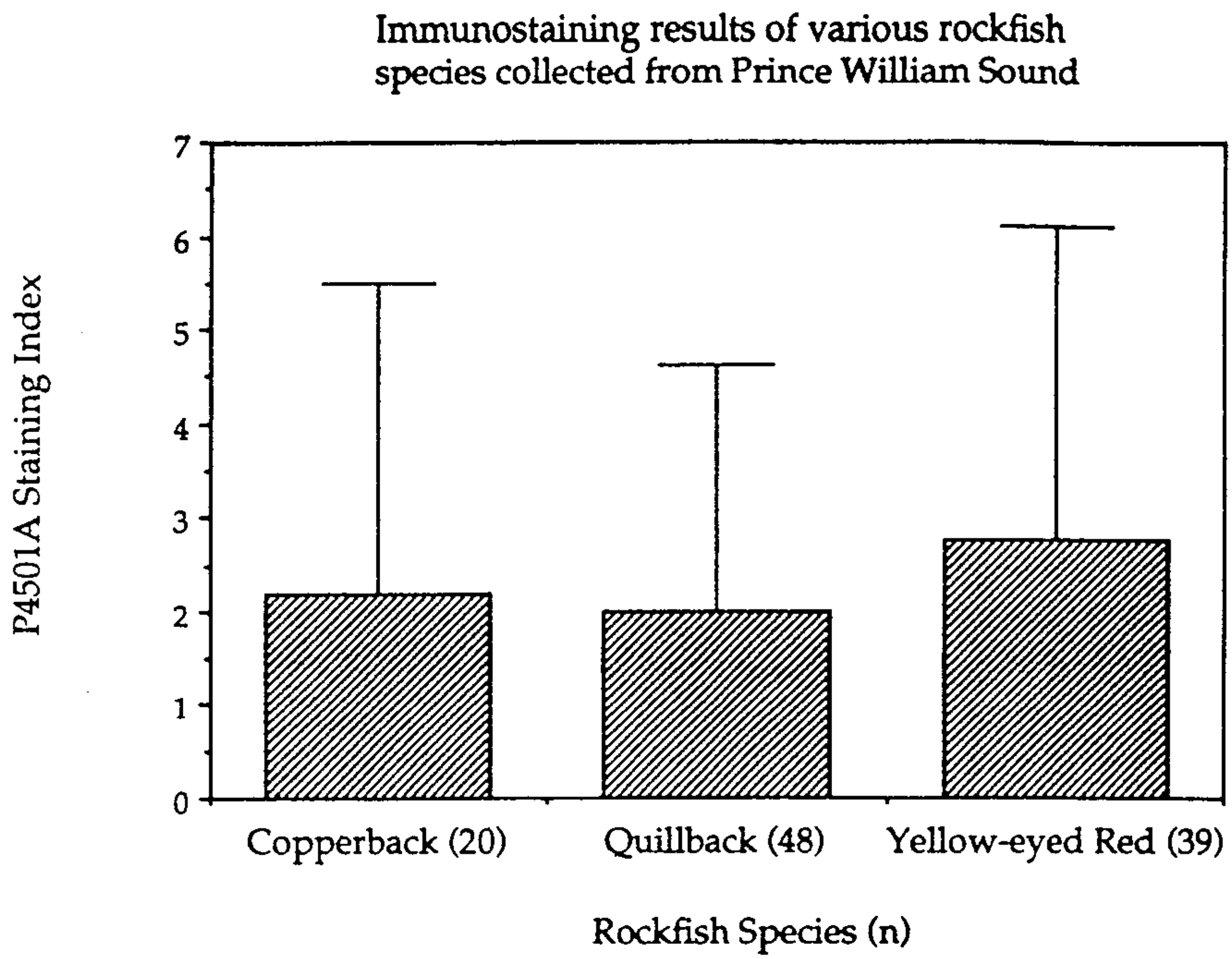
Key

Occurrance of the stain

x = tissue not present in section examined
0 = no staining
1 = rare cells are staining (rare)
2 = many cells are staining (multifocal)
3 = all cells are staining (confluent, diffuse)

Intensity of the stain when does occurr.

blank space = none
1 = neg/ very mild
2 = mild
3 = moderate
4 = strong
5 = very strong


Immunostaining results of various rockfish
species collected from Prince William Sound
P4501A Staining Index
0
1
2
3
4
5
6
7
Copperback (20)
Quillback (48)
Yellow-eyed Red (39)
Rockfish Species (n)

Rockfish immunostaining results

ADF&G No.	Hinton No.	sample date	species	hepatocyte			bile ducts	ven. endo	art. endo	mac ags		
				Int.	Occ.	O X I	Occ.	Occ.	Occ.	Int.	Occ.	O X I
1006	91-CO-102	21-Jul	copperback		0	0	0	0	0		0	0
1034	91-CO-13	27-Jul	copperback	2	4	8	0	0	0		0	0
1039	91-CO-17	27-Jul	copperback		0	0	0	0	0		0	0
1007	91-CO-21	21-Jul	copperback	1	4	4	0	0	0		0	0
1037	91-CO-29	27-Jul	copperback	1	4	4	0	0	0		0	0
1036	91-CO-32	27-Jul	copperback		0	0	0	0	0	3	3	9
1038	91-CO-37	27-Jul	copperback		0	0	0	0	0		0	0
1014	91-CO-40	21-Jul	copperback		0	0	0	0	0		0	0
1010	91-CO-54	21-Jul	copperback		0	0	x	0	0		0	0
1085	91-CO-55	9-Sep	copperback	1	4	4	0	0	0		0	0
1041	91-CO-57	21-Jul	copperback		0	0	0	0	0		0	0
1101	91-CO-72	10-Sep	copperback		0	0	x	0	0		0	0
1013	91-CO-74	21-Jul	copperback		0	0	0	0	0		0	0
1042	91-Co-75	27-Jul	copperback		0	0	0	0	0		0	0
1019	91-CO-85	22-Jul	copperback		0	0	0	0	0	3	3	9
1033	91-CO-89	27-Jul	copperback	2	4	8	0	0	0		0	0
1003	91-CO-9	21-Jul	copperback	2	4	8	0	0	0	3	4	12
1015	91-CO-92	21-Jul	copperback		0	0	0	0	0			0
1009	91-CO-95	21-Jul	copperback	2	4	8	0	0	0	3	3	9
1008	91-CO-99	21-Jul	copperback		0	0	0	0	x		0	0
1087	91-OB-10	9-Sep	quillback		0	0	x	0	x		0	0
1090	91-OB-3	9-Sep	quillback	2	4	8	0	0	0	3	3	9
1065	91-QB-1	7-Aug	quillback	2	4	8	0	0	x	3	3	9
1049	91-QB-101	6-Aug	quillback		0	0	0	0	0		0	0
1081	91-QB-103	10-Aug	quillback		0	0	0	0	0	3	3	9
1043	91-QB-105	6-Aug	quillback		0	0	0	0	0		0	0
1106	91-QB-106	13-Sep	quillback		0	0	0	0	0		0	0
1084	91-QB-108	12-Aug	quillback		0	0	0	0	0		0	0
1053	91-QB-109	6-Aug	quillback		0	0	0	0	x		0	0
1044	91-QB-113	6-Aug	quillback		0	0	0	0	0		0	0
1066	91-QB-114	7-Aug	quillback		0	0	x	0	x		0	0
1074	91-QB-116	8-Aug	quillback		0	0	0		x		0	0
1078	91-QB-12	10-Aug	quillback	1	4	4	0	0	0		0	0
1005	91-QB-16	21-Jul	quillback	1	4	4	0	0	0	3	3	9
1068	91-QB-19	7-Aug	quillback		0	0	0	0	0		0	0
1071	91-QB-20	7-Aug	quillback		0	0	0	0	0		0	0
1025	91-QB-22	22-Jul	quillback		0	0	0	0	0		0	0
1086	91-QB-25	9-Sep	quillback	1	4	4	0	0	0	3	3	9
1067	91-QB-27	7-Aug	quillback	1	4	4	0	0	0		0	0
1088	91-QB-28	9-Sep	quillback		0	0	0	0	0		0	0
1080	91-QB-33	10-Aug	quillback	2	4	8	0	0	0		0	0
1070	91-QB-38	7-Aug	quillback	1	4	4	0	0	0		0	0
1064	91-QB-39	7-Aug	quillback		0	0	0	0	0		0	0
1028	91-QB-45	22-Jul	quillback		0	0	0	0	0		0	0
1030	91-QB-46	22-Jul	quillback		0	0	0	0	0	3	2	6
1105	91-QB-48	12-Sep	quillback	1	4	4	0	0	0		0	0
1079	91-QB-50	10-Aug	quillback	1	4	4	x	0	0		0	0
1083	91-QB-51	12-Aug	quillback	1	4	4	0	0	x	3	3	9
1076	91-QB-52	9-Aug	quillback	1	4	4	0	0	0	3	3	9
1077	91-QB-58	9-Aug	quillback	1	4	4	0	0	x		0	0
1029	91-QB-60	22-Jul	quillback	1	4	4	0	0	0	2	3	6
1027	91-QB-62	22-Jul	quillback		0	0	0	0	x		0	0
1026	91-QB-68	22-Jul	quillback	1	4	4	0	0	0		0	0
1082	91-QB-69	12-Aug	quillback	2	4	8	0	0	0		0	0
1035	91-QB-71	27-Jul	quillback		0	0	0	0	0	3	3	9
1040	91-QB-76	27-Jul	quillback	1	4	4	0	0	0	3	2	6
1012	91-QB-78	21-Jul	quillback		0	0	0	0	0	3	3	9
1021	91-QB-79	22-Jul	quillback		0	0	0	0	0		0	0
1075	91-QB-80	8-Aug	quillback	1	4	4	0	0	0		0	0
1020	91-QB-82	22-Jul	quillback		0	0	x	0	x		0	0

Rockfish immunostaining results

ADF&G No.	Hinton No.	sample date	species	hepatocyte			bile ducts	ven. endo.	art. endo	mac age		
				Int.	Occ.	O X I	Occ.	Occ.	Occ.	Int.	Occ.	O X I
1001	91-QB-83	21-Jul	quillback	1	4	4	0	0	0	3	2	6
1100	91-QB-86	9-Sep	quillback		0	0	0	0	0		0	0
1002	91-QB-87	21-Jul	quillback		0	0	0	0	x		0	0
1102	91-QB-90	10-Sep	quillback		0	0	0	0	x		0	0
1011	91-QB-91	21-Jul	quillback	1	4	4	0	0	0	3	3	9
1103	91-QB-94	12-Sep	quillback		0	0	0	0	0		0	0
1073	91-QB-96	8-Aug	quillback		0	0	0	0	x	2	3	6
1072	91-QB-98	7-Aug	quillback		0	0	0	0	x		0	0
1051	91-YE-100	6-Aug	yellow-eyed red		0	0	0	0	0		0	0
1017	91-YE-104	22-Jul	yellow-eyed red		0	0	0	0	0		0	0
1107	91-YE-107	14-Sep	yellow-eyed red	1	4	4	0	0	0	3	2	6
1058	91-YE-11	7-Aug	yellow-eyed red	2	4	8	0	0	0		0	0
1018	91-YE-110	22-Jul	yellow-eyed red		0	0	0	0	0		0	0
1056	91-YE-111	7-Aug	yellow-eyed red	1	4	4	0	0	0		0	0
1061	91-YE-112	7-Aug	yellow-eyed red		0	0	0	0	0		0	0
1069	91-YE-115	7-Aug	yellow-eyed red		0	0	0	0	0		0	0
1089	91-YE-14	9-Sep	yellow-eyed red	2	4	8	0	0	0		0	0
1096	91-YE-15	9-Sep	yellow-eyed red	2	4	8	0	0	0		0	0
1055	91-YE-2	7-Aug	yellow-eyed red	1	4	4	0	0	0		0	0
1063	91-YE-23	7-Aug	yellow-eyed red	1	4	4	0	0	0		0	0
1093	91-YE-24	9-Sep	yellow-eyed red	2	4	8	0	0	0		0	0
1059	91-YE-30	7-Aug	yellow-eyed red	2	4	8	0	0	0		0	0
1032	91-YE-31	26-Jul	yellow-eyed red	2	4	8	0	0	0		0	0
1031	91-YE-34	26-Jul	yellow-eyed red		0	0	0	0	0		0	0
1092	91-YE-35	9-Sep	yellow-eyed red		0	0	0	0	0			0
1098	91-YE-36	9-Sep	yellow-eyed red		0	0	0	0	0		0	0
1023	91-YE-4	22-Jul	yellow-eyed red	2	4	8	0	0	0		0	0
1048	91-YE-42	6-Aug	yellow-eyed red		0	0	0	0	0		0	0
1050	91-YE-44	6-Aug	yellow-eyed red		0	0	x	0	x		0	0
1104	91-YE-49	12-Sep	yellow-eyed red	1	4	4	0	0	0		0	0
1047	91-YE-5	6-Aug	yellow-eyed red		0	0	0	0	0		0	0
1052	91-YE-53	6-Aug	yellow-eyed red		0	0	x	0	x		0	0
1062	91-YE-56	7-Aug	yellow-eyed red		0	0	0	0	0		0	0
1095	91-YE-6	9-Sep	yellow-eyed red		0	0	0	0	0		0	0
1045	91-YE-61	6-Aug	yellow-eyed red	1	4	4	0	0	0		0	0
1099	91-YE-63	9-Sep	yellow-eyed red	1	4	4	0	0	0			0
1054	91-YE-64	7-Aug	yellow-eyed red		0	0	0	0	0		0	0
1024	91-YE-65	22-Jul	yellow-eyed red		0	0	x	0	x		0	0
1091	91-YE-66	9-Sep	yellow-eyed red		0	0	0	0	0		0	0
1057	91-YE-7	7-Aug	yellow-eyed red	2	4	8	0	0	0		0	0
1060	91-YE-70	7-Aug	yellow-eyed red		0	0	0	0	0		0	0
1004	91-YE-73	21-Jul	yellow-eyed red	1	4	4	0		0		0	0
1022	91-YE-77	22-Jul	yellow-eyed red		0	0	0	0	0		0	0
1097	91-YE-81	9-Sep	yellow-eyed red	2	4	8	0	0	0		0	0
1016	91-YE-84	21-Jul	yellow-eyed red		0	0	x	0	x		0	0
1094	91-YE-88	9-Sep	yellow-eyed red		0	0	0	0	0		0	0
1046	91-YE-97	8-Aug	yellow-eyed red	1	4	4	0	0	0		0	0

P4501A response in various species of rockfish collected in Princ William Sound

One Factor ANOVA X 1: Rockfish species Y 1: Scaled P4501a response

Analysis of Variance Table

Source:	DF:	Sum Squares:	Mean Square:	F-test:
Between groups	2	13.0732	6.5366	.717
Within groups	104	948.1231	9.1166	p = .4906
Total	106	961.1963		

Model II estimate of between component variance = -.0764

One Factor ANOVA X 1: Rockfish species Y 1: Scaled P4501a response

Group:	Count:	Mean:	Std. Dev.:	Std. Error:
copperback	20	2.2	3.3023	.7384
quillback	48	2	2.6093	.3766
yellow-eyed red	39	2.7692	3.3282	.5329

One Factor ANOVA X 1: Rockfish species Y 1: Scaled P4501a response

Comparison:	Mean Diff.:	Fisher PLSD:	Scheffe F-test:	Dunnett t:
copperback vs. quillback	.2	1.5937	.031	.2489
copperback vs. yellow-ey...	-.5692	1.6469	.2349	.6855
quillback vs. yellow-eyed...	-.7692	1.2909	.6983	1.1818