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

# River Otter Component of the Oiled Mussel-Bed Study

Restoration Study Number 103-3
Final Report

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River Otter Component of the Oiled Mussel-Bed Study

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**Study History:** Restoration Study Number 103-3 was initiated as part of a detailed study plan in 1992 and was one component of group of oiled mussel bed impact assessment studies. The project effort resulted from indications in Terrestrial Mammal Study Number 3 (Assessment of Injury to River Otters in Prince William Sound, Alaska, Following the *Exxon Valdez* Oil Spill) that impacts to Prince William Sound river otters had occurred as a result of the spill.

**Abstract:** Our objective was to determine if physiological and morphological differences previously detected in river otters from oiled and nonoiled areas could be affected by mussel beds, and whether these differences would persist or subside with time. Differences in levels of blood haptoglobin and Interleukin-6 ir, which previously were elevated in river otters (*Lutra canadensis*) inhabiting oiled when compared to nonoiled areas of Prince William Sound, Alaska, were not observed in summer 1992. River otters from oiled areas continued to regain body size from levels noted in 1990; adjusted mean body mass of otters from oiled and nonoiled areas were nearly identical by summer 1992. Consequently, no adverse effects in 1992 could be attributed to oiled mussel beds from areas where river otters were captured. Likewise, oiled mussel beds may not have been a factor contributing to documented differences in these variables in 1990 and 1991. We propose that river otters may be recovering from chronic effects that we observed in 1990 and 1991 following the 1989 *Exxon Valdez* oil spill.

**Key Words:** Chronic effects, *Exxon Valdez* oil spill, *Lutra canadensis*, oiled mussel beds, Prince William Sound, river otters.

**Citation:**

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APPLIED
marine
SCIENCES

August 3, 1995

Molly McCammon
Executive Director
*Exxon Valdez* Oil Spill Trustee Council
645 G Street Ste. 402
Anchorage, AK 99501

Dear Ms. McCammon,

This report on river otters (R103) contains some excellent work by Dr. Terry Bowyer and his colleagues. However, there are still unresolved issues relating to discussion of the effects of the oil spill on river otters and possible natural pre-existing differences in the studied populations. Dr. Bowyer and the reviewers and I have not been able to find a mutually satisfactory resolution of these issues. As a result, we have agreed that this letter shall accompany his final project report.

Sincerely yours,

Robert B. Spies
Chief Scientist





2155 Las Positas Court, Suite S  Livermore, CA 94550  510.373.7142  FAX 510.373.7834

## LIST OF RELATED PUBLICATIONS

Bowyer, R. Terry, J. Ward Testa, James B. Faro, Charles C. Schwartz, and James B. Browning. 1994. Changes in diets of river otters in Prince William Sound, Alaska: effects of the *Exxon Valdez* oil spill. Canadian Journal of Zoology 72(6):970-976.

Bowyer, R. Terry, J. Ward Testa, and James B. Faro. 1995. Habitat selection and home ranges of river otters in a marine environment: effects of the *Exxon Valdez* oil spill. Journal of Mammalogy 76(1):1-11.

Duffy, Lawrence K., R. Terry Bowyer, J. Ward Testa, and James B. Faro. 1993. Differences in blood haptoglobin and length-mass relationships in river otters (*Lutra canadensis*) from oiled and unoiled areas of Prince William Sound, Alaska. Journal of Wildlife Diseases 29(2):353-359.

Duffy, Lawrence K., R. Terry Bowyer, J. Ward Testa, and James B. Faro. 1994. Chronic effects of the *Exxon Valdez* oil spill on blood and enzyme chemistry of river otters. Environmental Toxicology and Chemistry 13(4):643-647.

Duffy, Lawrence K., R. Terry Bowyer, J. Ward Testa, and James B. Faro. 1994. Evidence for recovery of body mass and haptoglobin values of river otters following the *Exxon Valdez* oil spill. Journal of Wildlife Diseases 30(3):421-425.

Testa, J. Ward, Dan F. Holleman, R. Terry Bowyer, and James B. Faro. 1994. Estimating populations of marine river otters in Prince William Sound, Alaska, using radiotracer implants. Journal of Mammalogy 75(4):1021-1032.

TABLE OF CONTENTS

## LIST OF TABLES

## LIST OF FIGURES

## EXECUTIVE SUMMARY

Differences in levels of blood haptoglobin and Interleukin-6 ir, which previously were elevated in river otters (*Lutra canadensis*) inhabiting oiled when compared to nonoiled areas of Prince William Sound, Alaska, were not observed in summer 1992. Additionally, river otters from oiled areas continued to regain body size from levels noted in 1990; adjusted mean body mass of otters from oiled and nonoiled areas were nearly identical by summer 1992. We propose that river otters may be recovering from chronic effects that we observed in 1990 and 1991 following the 1989 *Exxon Valdez* oil spill. Consequently, no adverse effects in 1992 could be attributed to oiled mussel beds from areas where river otters were captured.

## INTRODUCTION

Extensive sections of shoreline in Prince William Sound, were contaminated by oil spilled from the *Exxon Valdez* in late March 1989. River otters (*Lutra canadensis*) are sensitive to hydrocarbon pollution in the marine ecosystem (Duffy et al. 1993) perhaps because of their diet of subtidal and intratidal fishes and molluscs (Larsen, 1983; TM 3). Indeed, damage from hydrocarbon accumulation in mussels and damaged populations of fish in oiled areas of Prince William Sound have been noted (Jewett, pers. comm.). Otters also may be exposed to crude oil from grooming their pelage (Duffy et al. 1993). We reported differences in the blood chemistry of river otters (TM 3), which were related to whether they were located on oiled or nonoiled areas of Prince William Sound. Blood haptoglobin, an acute phase protein, was elevated in oiled areas in 1990 (Duffy et al., 1993). Additionally, male river otters from oiled areas had lower body mass than male otters from nonoiled areas. We reasoned that if the elevated haptoglobin levels and the reduction in body mass are evidence of chronic, oil-related effects on the health of river otters, then as the oil in the environment decreased, or became biologically unavailable, these differences in blood proteins and mass-length relations should decrease over time. Moreover, otters in oiled areas were living in close proximity to mussel beds (*Mytilus edulis*) where oil was known to be trapped in substrates, which could provide a source of continued oil contamination (Babcock et al., 1993). Our objective was to determine if physiological and morphological differences previously detected in river otters from oiled and nonoiled areas could be effected by mussel beds, and whether these differences would persist or subside with time.

## OBJECTIVES

1. To determine whether river otters captured from areas with oiled mussel beds exhibited significantly ($\alpha = 0.05$) higher levels of haptoglobin, Interleukin-6 cross-reactive protein, and lesser body mass, than otter from nonoiled areas.

## METHODS

In 1992, river otters were captured from areas containing oiled mussel beds (Appendix I) including Naked Island, Eleanor Island, and Bay of Isles; likewise, otter were live captured from Unakwik Inlet (nonoiled) using procedures detailed by Duffy et al. (1993).

River otters were immobilized, transported to a ship, weighed, measured, and a blood sample was drawn from their jugular vein; otters were released near their site of capture as soon as they fully recovered from the ketamine hydrochloride (11 mg/kg estimated body mass; Sigma, St. Louis, Missouri) used to immobilize them (Duffy et al. 1993). Animals were sexed and placed into age classes (adult, juvenile, pup) using standard procedure (Duffy et al. 1993). All methods used in this research were approved by an independent Animal Care and use Committee at the University of Alaska Fairbanks.

Blood samples collected in the field were stored in vacutainers, and sera was separated later by low speed centrifugation. Blood was stored frozen at -20°C. Gel electrophoresis of blood haptoglobin (Hp) was performed as described in detail by Duffy et al. (1993). Interleukin-6 cross reactive proteins (IL-6ir) were measured using immunochemical assay (Quantakine Elisa, R & D, Inc., 614 McLinley Place, Minneapolis, MN 55413). The accuracy of haptoglobin quantifications is based on the specificity of the hemoglobin complex formation which is separated by electrophoresis. The reproducibility of the haptoglobin assay from comparison samples in previous Hp assays of river otters was indicated by inter-assay CV of 14.8%. The inter-assay % CV for the interleukin assay was described by R and D Systems was 7.1% at the 90 pg/ml level.

Statistics procedures includes an analysis of covariance (Neter et al. 1985). A one-tailed test was used in this ANCOVA because Duffy et al. (1993) previously reported that the body mass of otters from oiled areas was significantly less than for animals captured in areas not exposed to oil, and oil was expected to affect otters. Indeed, Duffy et al. (in press) reported chronic effects from the oil spill on the blood and enzyme chemistry of otters in 1991. Because of the small number of otters captured from nonoiled areas in 1992, we combined years (1990, 1991, 1992) to have adequate sample size for this analysis. Our data met the statistical assumption of "homogeneous slopes." Sample sizes for blood values of otters on nonoiled sites in 1992 were too small for meaningful statistical analysis, and we lacked data on all blood values except Hp for all 3 years. Hence, we could not employ the same statistical protocols used for body mass. We simply plotted trends in important blood values for comparison among years.

## RESULTS

When corrected for sex, age-class, and total length (Table 1), otters from oiled areas exhibited significantly lower body mass than otters inhabiting nonoiled areas; however, a clear trend of increasing body mass through time occurred for otters living in areas exposed to crude oil (Fig. 1). Because there are no base-line data for otters in Prince William Sound, we are unsure whether weight gains in oiled otters to levels observed for nonoiled ones in 1991 and 1992 represent recovery. Body mass for oiled otters in 1992 is still below that observed for nonoiled otters in 1990 (Fig. 1). Likewise, caution should be used in suggesting complete recovery has occurred because of small sample sizes on nonoiled areas in 1992 (Fig. 1). A clear pattern exists in the reduction of blood Hp and IL-6ir in otters captured from oiled areas, all of which contained oil-contaminated mussel beds (Fig. 2).

## DISCUSSION

In 1990, adult male otters from oiled areas of Prince William Sound had significantly lower (1.13 kg) body mass than otters live-captured from nonoiled areas (Duffy et al. 1993) These otters came from two areas, each with about 80 km of shoreline (oiled, Herring Bay on northern Knight Island; nonoiled, Esther Passage). Likewise, oiled otters of both sexes differed significantly in levels of blood haptoglobins from animals living in nonoiled areas (Duffy et al. 1993). These same patterns, however, might not have occurred across broader areas of Prince William Sound. Consequently, we sampled both blood (TM 3) and other morphometrics (Table 1) for otters in 1991, and again in 1992 throughout Prince William Sound.

In 1991, we were able to predict (>86%) whether otters inhabited oiled or nonoiled areas of Prince William Sound based on only three blood values: Hp, IL-6ir, and aspartate aminotransferance (TM 3). Hp is an acute phase response protein that is induced by IL-6 following tissue injury from hydrocarbons (Henrich et al. 1990). Similarly, cell damage will cause elevated liver enzyme levels (Edqvist et al. 1991). Aspartate aminotransferase is a nonspecific leakage enzyme that elevates in serum whenever there is injury to any of a number of tissues including heart, liver, skeletal muscle, or blood cells. We initially thought that high haptoglobin levels in nonoiled otters that occurred in 1990 (Duffy et al. 1993) might have resulted from sampling otters during their mating season (TM 3). Another possibility is that fish contaminated by crude oil migrated the 40 km to our nonoiled study site, where they were eaten by otters. Sand lances (*Ammodytes hexapterus*), which are preyed upon by otters (TM 3), are an example of such a migratory fish. Whatever the cause of variation for otters on nonoiled areas, there is a clear pattern of recovery in both Hp and IL-6 ir values for otters from oiled sites (Fig. 2).

River otters were live-captured from areas with mussel beds that still possessed substantial amounts of crude oil in 1992 (Appendix I). Although otters consume mussels, blood values of otters in 1992 (Fig. 2) failed to exhibit elevated levels of Hp and IL-6 ir observed in previous years. This raises questions about the importance of mussel beds as a source of oil contamination for otters in 1990 and 1991 when Hp and IL-6 ir values were significantly higher than on nonoiled areas (Duffy et al. 1993, TM 3).

## CONCLUSIONS

Our data indicate that river otters exhibited lower body mass (Fig. 1) and elevated levels of blood values (Fig. 2) two years after the *Exxon Valdez* oil spill and following a major effort to clean oil from Prince William Sound. Such chronic effects were unexpected, and suggest that research should focus on longer-term consequences of oil spills. The apparent recovery of otters observed in 1992 is encouraging, but more data is required to conclude that there are no lingering effects from the oil spill. Because differences in body mass and blood values of otters living in oiled and nonoiled areas did not occur in 1992, we conclude that no evidence exists that oiled mussel beds affected river otters in 1992. Likewise, oiled mussel beds may not have been a factor contributing to documented differences in these variables in 1990 and 1991.

## LITERATURE CITED

Babcock, M., G. Irvine, S. Rice, P. Rounds, J. Cusick, and C.C. Brodersen. 1992. Oiled mussel beds in Prince William Sound two and three years after the *Valdez* oil spill. In: *Exxon Valdez* Oil Spill Symposium, pp. 184-185.

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Duffy, L.K., R.T. Bowyer, J.W. Testa and J.B. Faro. 1994. Chronic effects of the *Exxon Valdez* oil spill on blood and enzyme chemistry of river otters. Environmental Toxocology and Chemistry 13(4):643-647.

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Table 1. Body mass (kg) and total length (cm) of river otters from oiled and nonoiled areas of Prince William Sound, Alaska, USA. Data for 1990 are provided by Duffy et al. (1993).

| Otter sex and age class by area | 1991 | | | 1992 | | |
|---|---|---|---|---|---|---|
| | N | Body Mass X ± SD | Total X ± SD | N | Body Mass X ± SD | Total Length X ± SD |
| Oiled areas | | | | | | |
| Adult male | 3 | 9.0 ± 0.5 | 119.7 ± | 9 | 9.3 ± 0.8 | 119.7 ± 2.5 |
| Yearling male | 0 | -- | -- | 0 | -- | -- |
| Pup male | 1 | 4.5 | 92.2 | 0 | -- | -- |
| Adult female | 6 | 7.9 ± 0.6 | 113.9 ± | 1 | 8.9 | 114.6 |
| Yearling female | 0 | -- | -- | 0 | -- | -- |
| Pup female | 2 | 3.8 ± 1.4 | 87.2 ± | 0 | -- | -- |
| Nonoiled areas | | | | | | |
| Adult male | 2 | 9.4 ± 1.4 | 119.2 ± | 1 | 10.0 | 122.0 |
| Yearling male | 0 | -- | -- | 0 | -- | -- |
| Pup male | 0 | -- | -- | -- | -- | -- |
| Adult female | 6 | 8.4 ± 0.9 | 117.3 ± | 1 | 7.1 | 106.1 |
| Yearling female | 2 | 6.8 ± 0.1 | 112.0 ± | 0 | -- | -- |
| Pup female | 1 | 2.4 | 67.2 | 0 | -- | -- |

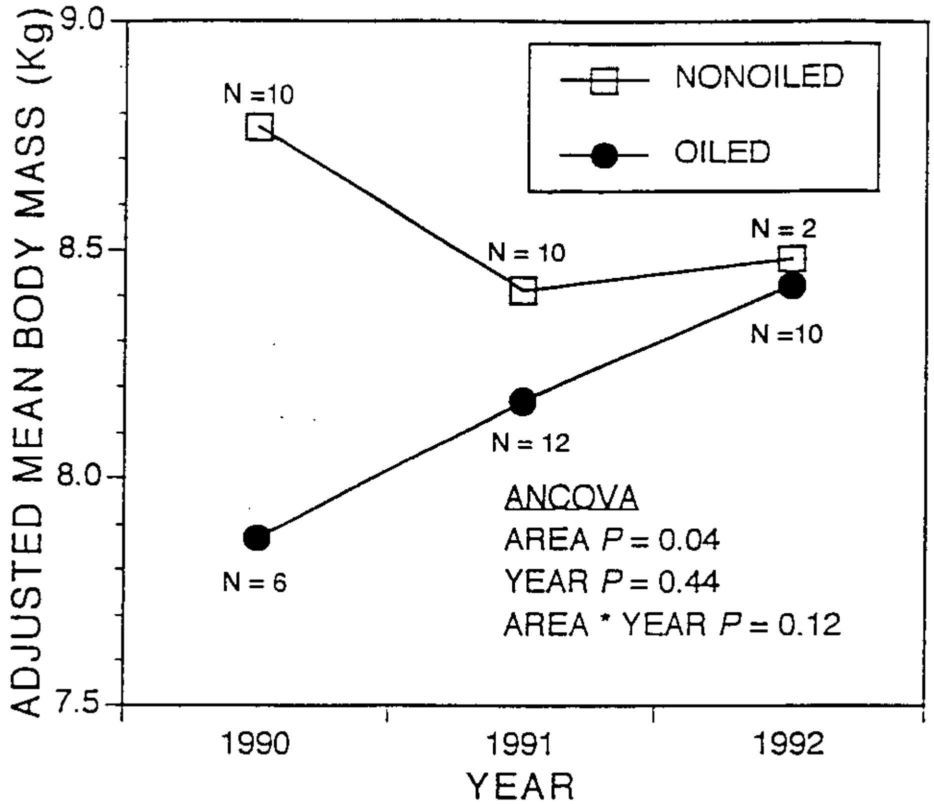


Figure 1: Yearly trends in mean body mass of river otters from oiled and nonoiled areas adjusted for sex, age-class, (pup, juvenile, adult), and total-length using a one-tailed analysis of covariance (ANCOVA), Prince William Sound, Alaska, USA. Data for 1990 are from Duffy et al. (1993).

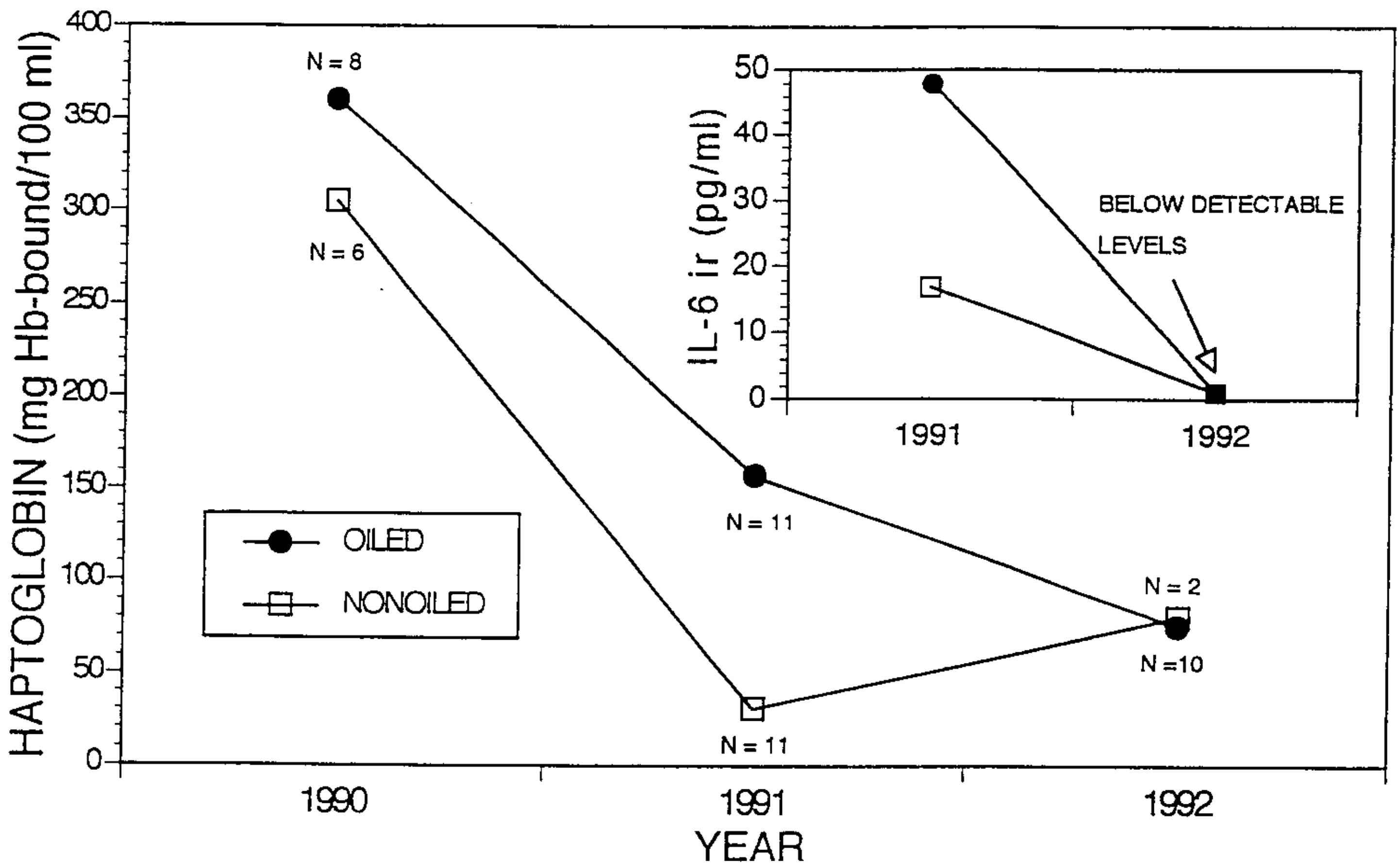


Figure 2: Yearly trends in blood haptoglobin and interleukin-6 cross reactive protein (inset) for river otters from oiled and nonoiled areas of Prince William Sound, Alaska, USA. Data for 1990 are from Duffy et al. (1993), and data for 1991 are from TM 3.

| FAST SCREENING/SEDIMENT UV DATA | | | MUSSEL BEDS: PRINCE WILLIAM SOUND 1992 | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | STATISTICS: ug/g wet weight (ppm) | | | |
| | | | | | | | DPTH | TYPE | | | oil equivalents | |
| SITE | SEG # | GENERAL LOCATION | SUBID | DATE | WHO | AGENCY | CM | SAMPLE | N | MEAN | STDEV | STDERR |
| Barnes Cove | KN575A | Knight Isl., Drier Bay | CH1B | 16-Jun-92 | ROUNDS | NMFS ABL | 0-2 | Pooled Rep | 3 | 0 | 0 | 0 |
| Bay of Isles | KN004A | Between South and West Arms | A-D | 21-Jul-92 | PATTEN | ADF&G | 10 | Pooled Rep | 3 | 18653 | 6567 | 3791 |
| Bay of Isles | KN004A | Between South and West Arms | A | 29-Aug-92 | ROUNDS | NMFS ABL | 0-2 | Pooled Rep | 3 | 1764 | 1433 | 827 |
| Bay of Isles | KN005A | Between South and West Arms | BL96-F | 25-Aug-92 | ANDRES | FWS | 0-2 | Pooled Rep | 3 | 1664 | 340 | 196 |
| Bay of Isles | KN016A | Islet Bet. So and Short Arms | BL53-F | 25-Aug-92 | ANDRES | FWS | 0-2 | Pooled Rep | 3 | 4699 | 1598 | 923 |
| Bay of Isles | KN136A | "Death Marsh" | NonStr | 16-Jun-92 | ROUNDS | NMFS ABL | 0-2 | Spot | 6 | 17359 | 7698 | 3143 |
| Bay of Isles | KN203A | So shore of West Arm | BL52-F | 25-Aug-92 | ANDRES | FWS | 0-2 | Pooled Rep | 3 | 6436 | 1162 | 671 |
| Bay of Isles | KN205B | Head of South Arm | CH1B | 18-Apr-92 | ROUNDS | NMFS ABL | 0-2 | Pooled Rep | 3 | 9 | 2 | 1 |
| Bay of Isles | KN207B | So shore/mouth of bay | | 21-Jul-92 | PATTEN | ADF&G | 10 | Pooled Rep | 3 | 21934 | 3268 | 1887 |
| Disk Island | DI059A | NE corner | | 27-Aug-92 | BABCOCK | NMFS ABL | 0-2 | Pooled Rep | 6 | 8249 | 3022 | 1234 |
| Disk Island | DI066A | West side | | 28-Aug-92 | BABCOCK | NMFS ABL | 0-2 | Pooled Rep | 6 | 11942 | 6206 | 2534 |
| Disk Island | DI067A | NW cove | A | 18-May-92 | BABCOCK | NMFS ABL | 0-2 | Pooled Rep | 3 | 14020 | 2116 | 1222 |
| Disk Island | DI067A | NW cove | ISLET-A | 22-Jul-92 | PATTEN | ADF&G | 0-30 | Pooled Rep | 3 | 4979 | 1880 | 1085 |
| Disk Island | DI067A | NW cove | ADEC | 18-Jun-92 | FREMGEN | NMFS ABL | 0-2 | Spot | 10 | 15071 | 7903 | 2499 |
| Disk Island | DI067A | NW cove | B | 22-Jul-92 | PATTEN | ADF&G | 0-30 | Pooled Rep | 3 | 6324 | 924 | 533 |
| Disk Island | DI067A | NW cove | ISLET-B | 27-Aug-92 | BABCOCK | NMFS ABL | 0-2 | Pooled Rep | 3 | 22600 | 5586 | 3225 |
| Eleanor Isl. | EL011A | NW Block Isl. | BL30-F | 25-Aug-92 | ANDRES | FWS | 0-2 | Pooled Rep | 3 | 2118 | 397 | 229 |
| Eleanor Isl. | EL013A | "Mussel Beach" tombolo | Stripped | 18-May-92 | BABCOCK | NMFS ABL | 0-2 | Spot | 5 | 7371 | 5401 | 2415 |
| Eleanor Isl. | EL015A | N of Block/S of EL013A | A | 28-Aug-92 | BABCOCK | NMFS ABL | 0-2 | Pooled Rep | 3 | 3149 | 391 | 226 |
| Eleanor Isl. | EL015A | N of Block/S of EL013A | A-D | 16-Aug-92 | PATTEN | ADF&G | | Pooled Rep | 3 | 11871 | 2559 | 1477 |
| Eleanor Isl. | EL015A | N of Block/S of EL013A | B | 16-Aug-92 | PATTEN | ADF&G | | Pooled Rep | 3 | 17179 | 692 | 400 |
| Eleanor Isl. | EL052A | NWBay/head W arm | A | 28-Aug-92 | BABCOCK | NMFS ABL | 0-2 | Pooled Rep | 3 | 198 | 57 | 33 |
| Eleanor Isl. | EL052A | NWBay/head W arm | A-D | 16-Aug-92 | PATTEN | ADF&G | 20 | Pooled Rep | 3 | 3266 | 1463 | 845 |
| Eleanor Isl. | EL052B | NWBay/head W arm | | 16-Aug-92 | PATTEN | ADF&G | | Pooled Rep | 3 | 9868 | 2010 | 1160 |
| Eleanor Isl. | EL054A | NWBay/W shore W arm | | 02-Jul-92 | BABCOCK | NMFS ABL | 0-2 | Pooled Rep | 3 | 307 | 110 | 63 |
| Ingot Island | IN031B | SW shore | | 16-Aug-92 | PATTEN | ADF&G | | Pooled Rep | 3 | 12515 | 1435 | 828 |
| Olsen Bay | Olsen | Eastern Prince William Sound | CH1B | 14-Jun-92 | ROUNDS | NMFS ABL | 0-2 | Pooled Rep | 3 | 1 | 1 | 1 |

| SUBID KEY | |
|---|---|
| Stripped, Nonstr. | Our sites where intensive sampling is occuring |
| A,B | Segments where more that 1 mussel bed sampled |
| "..."-D | Mussel bed sediment samples taken at depth (>2cm) |
| ADEC | Site of ADEC manipulation |
| CH1B | Established Coastal Habitat 1B sites (these have 4 yrs. of data) |
| BL..... | USFWS Oystercatcher project sampling |

Malin M. Babcock 2.18.93
NOAA/NMFS/Auke Bay Lab
907-789-6018

UNITED STATES DEPARTMENT OF COMME
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24 hour RAPICOM (907) 789-6094

17 February 1993

TO: Jim Faro
Alaska Department of Fish & Game

FROM: Malin M. Babcock *Malin Babcock*

RE: UV Fluorescence Sediment Data, 1992

Attached is a table of ultra-violet fluorescence (UV) data from oiled mussel beds in the general areas of Prince William Sound that we discussed yesterday.

The fast screening method is not standardized yet for tissue samples; therefore, no mussels collected from these same sites have been analyzed. Our lab is just now finishing tissue NRDA samples and is scheduled to begin Restoration samples (both sediments and mussels) soon.

UV fluorescence is semi-quantitative only, and values cannot be related directly to data produced by gas chromatography/mass spectroscopy analyses. This fast screening method utilizes the excitation and fluorescence of a particular sample at the phenanthrene wavelengths. The amount of fluorescence is then expanded to give an estimate of total oil based on the amount of phenanthrene actually present in Exxon Valdez crude oil.

The highest values in the table are from mussel beds on Disk Island and the Bay of Isles. These values equate to ~2% oil. I have included some low (background) levels to give you some idea of the range you can expect to see. The highest values we saw last summer in sediments were from oiled mussel beds on an islet in Foul Bay (over 62000 $\mu g/g$ oil equivalents) and the north end of Chenega Island (over 32000 $\mu g/g$).

I enjoyed participating in the river otter workshop on the 6th of this month. Please call me if I can be of further assistance.