

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

Assessment of Injury to Steller Sea Lions
in Prince William Sound and the Gulf of Alaska

Marine Mammal Study Number 4
Final Report

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Earl F. Becker
Terry R. Spraker
Thomas R. Loughlin

Alaska Department of Fish and Game
Division of Wildlife Conservation
333 Raspberry Road
Anchorage, Alaska 99518

October 1994

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Study History: Marine Mammal Study Number 4 was initiated in 1989 to assess the effects of the *Exxon Valdez* oil spill on the Steller sea lion population of Prince William Sound and the Gulf of Alaska. This project was carried out in two periods: The first was from April through December 1989, including field work during June and July. The second was from April through November 1990, including field work during April through July. A preliminary status report was prepared for the work during each period (dated November 27 and January 15, 1990, respectively), and the results were later integrated into a single book chapter:

Calkins, D.G., E. Becker, T.R. Spraker, and T.R. Loughlin. 1994. Impacts on Steller sea lions. Pages 119-139 in T.R. Loughlin, ed. *Marine Mammals and the Exxon Valdez*. Academic Press, San Diego.

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Abstract: This study used two approaches to measure effects of the *Exxon Valdez* oil spill on Steller sea lions. The first approach was designed to detect effects at the population level; assessment of changes in sea lion numbers and distribution began with aerial surveys of all rookeries and haulout sites from Cape St. Elias to Chowiet Island in 1989. Counts of adult and juvenile sea lions were compared with historical data using linear regression techniques. However, the statistical power to detect change was low, and no significant oil effects were documented. Data collected on premature pupping showed significantly higher premature pupping ratios at a haulout site nearer the oil spill compared to a more distant haulout site. In itself, this evidence did not demonstrate an oil-spill effect. The second approach was designed to detect effects at the individual level. Sixteen sea lions were collected and an additional 12 were found dead during response efforts. Tissue samples were taken and tested for toxicological effects through histological and hydrocarbon analyses. This study showed that sea lions were exposed to oil and that they were metabolizing and excreting metabolites of aromatic hydrocarbons into the bile. No evidence indicated damage from toxic effects of oil.

Key Words: Adult, juvenile, and pup sea lions, *Eumetopias jubatus*, *Exxon Valdez* oil spill, Gulf of Alaska, histological lesions, polycyclic aromatic hydrocarbons, Prince William Sound, Steller sea lion.

Project Data: All available data are summarized in the body of the report.

Citation:

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Chapter 7

Impacts on Steller Sea Lions

Donald G. Calkins, Earl Becker, Terry R. Spraker, and Thomas R. Loughlin

INTRODUCTION

Steller sea lions (*Eumetopias jubatus*) inhabit coastal and offshore areas in the north Pacific Ocean from southern California to Japan (Loughlin et al. 1984). They are the largest otariid and are regularly found in coastal waters and on the beaches recently affected by the *Exxon Valdez* oil spill (EVOS). Steller sea lions breed and pup at rookeries during late May to July. Rookeries are considered haulout sites if they are used at other times of the year. Generally, most haulout sites are used seasonally or throughout the year and few pups are born at these sites. Females usually produce a single pup and within 14 days *post partum* enter into estrous and breed. Fetal implantation is delayed until approximately mid-October (Pitcher and Calkins 1981). Pup dependency can be long, up to 3 years in some cases, but most pups wean by the end of their first year (Pitcher and Calkins 1981). Reproductive failures in the form of resorption in early pregnancy or late-term abortion occur at a high rate in the Gulf of Alaska (Calkins and Pitcher 1982; Calkins and Goodwin 1988).

No rookeries occur within Prince William Sound (PWS), but three haulout sites which are used on a seasonal basis and two year-round haulout sites are found there. One rookery occurs at the entrance to PWS (Seal Rocks) and three other rookeries occur along the Kenai Peninsula (Outer and Sugarloaf Islands) and in the Kodiak Island area (Marmot Island) which were in the general area affected by the EVOS. Approximately 25 additional haulout sites in the Kodiak Island and Alaska Peninsula areas were in the path of the spilled oil (Calkins and Pitcher 1982).

It is not practical to determine the total number of sea lions in a given area at a given time because an unknown number will be at sea and will not be counted. Therefore, sea lion estimates are based on counts of animals on land at rookeries and haulout sites. Counts are generally lower in winter than in summer (Calkins and Pitcher 1982).

Steller sea lion population declines have been reported throughout most of the species' range (Loughlin et al. 1992), including southern and central California, the Gulf of Alaska, the Aleutian Islands, the central Bering Sea, and Russia (National Marine Fisheries Service 1992). Populations in Oregon, Washington, British Columbia, and southeastern Alaska appear stable. Precipitous declines in the Gulf of Alaska and Aleutian Islands were detected during a 1989 range-wide survey (Loughlin et al. 1992) resulting in the National Marine Fisheries Service (NMFS) listing the species as threatened under the U.S. Endangered Species Act and depleted under the Marine Mammal Protection Act in November 1990.

Sea lion population declines first occurred in the eastern Aleutian Islands in the mid-1970s (Braham et al. 1980), spread into the Kodiak Island area in the early 1980s, into the central and western Aleutian Islands area in the mid-1980s, and into the eastern Gulf of Alaska in the late 1980s and early 1990s (Loughlin et al. 1984; Merrick et al. 1987; Byrd 1989; Loughlin et al. 1992). Counts of adult and juvenile sea lions from the Kenai Peninsula to Kiska Island in the Aleutian Islands were used as the basis for listing the species as threatened. Counts in this area declined from 89,364 adult and juvenile sea lions in 1960 to 21,737 individuals in 1991, a decline of 76% (Merrick et al. 1992). No specific cause for the decline has been identified.

Our study began in June and July 1989 with aerial surveys of rookeries and haulout sites in the area affected by the EVOS. Surveys were also conducted in the remainder of the species' range that year (Loughlin et al. 1992). Beaches were searched and animals were collected and sampled for toxicological and histological impacts from the spilled oil. Recognizing the severe decline in sea lion abundance over much of the affected area, we attempted to measure changes beyond that which could be attributed to the ongoing decline.

We used two approaches to measure effects of the spill on sea lions. The first was designed to detect effects at the population level and included assessment of sea lion numbers and their distribution. The objective was to determine if sea lion abundance on rookeries and haulout sites had changed significantly as a result of the spill.

The second approach was designed to detect effects at the individual level. Sixteen sea lions were collected and 12 were found dead during response and clean-up efforts (Zimmerman et al., Chapter 2). When practical, tissue samples were taken from these animals and tested for toxicological effects through histological and hydrocarbon analyses. The objective was to determine if sea lions were accumulating hydrocarbon contaminants in their tissues and if tissue damage had resulted. We suspected that the toxic effects of hydrocarbons could have accelerated the already high rate of premature pupping in sea lions noted by Pitcher and Calkins (1981) and Calkins and Goodwin (1988) for the Gulf of Alaska.

METHODS

Study Area

Surveys were conducted at all rookeries and haulout sites from Cape St. Elias through the north Gulf of Alaska to Chowiet Island (Fig. 7-1). Although both sea lions and oil contamination from the spill were found offshore, it was not practical to conduct our study beyond 10 km from shore.

Distribution and Abundance

Aerial surveys were conducted from 15 June to 15 July 1989, the period when sea lion counts normally are highest (Withrow 1982). Also, this is the time period for which we had the best comparable information. Note that for PWS the highest annual abundance of sea lions on shore has been recorded in March and April (Calkins and Pitcher 1982).

Aerial surveys were timed to maximize the number of animals available for counting on land (Withrow 1982; Merrick et al. 1987). Surveys were conducted between 1000 and 1600 local time, when possible. In some cases, the timing of a survey was altered due to weather or time constraints. Survey aircraft were flown at approximately 90 knots, at an altitude of between 150 to 300 m, in a vertical plane over each survey site. Replicate photographs of sea lions were taken with a hand-held, 35-mm, auto-focus, auto-exposure, SLR camera equipped with a motor-drive and a 70- to 210-mm zoom lens using high-speed (ASA 400) color transparency film. Survey time and date and environmental conditions were recorded for each site. Estimates of the number of sea lions observed were made for each survey location. Later the photographic slides were projected on a white paper screen and each adult or juvenile sea lion within the survey was counted. Some of the counts reported here were also reported by Loughlin et al. (1992).

Pups were counted from land at all rookeries from Seal Rocks to Chowiet Island in 1989 (Fig. 7-1). In 1990, all rookeries were counted except Marmot Island. Counts were timed to be completed between 25 June and 4 July, the period when most pups were born and before they were old enough to readily enter the water. Biologists landed at each site by helicopter or boat. Generally, one or two biologists walked through the rookery causing the adults to enter the water while the pups remained on land. Another biologist followed the first, counting each pup individually. In some areas a second count was conducted, but because additional disturbance may have undesirable effects, a second count was conducted only where necessary. If a second count was conducted, the two counts were averaged.

In 1989, all rookeries and haulout sites were visited and searched for aborted fetuses. Premature pupping ratios were measured by stationing two observers each at Cape St. Elias and at Chirikof Island between 5 April and 16 May. Premature pupping had been documented at that time of year at Cape St. Elias (Calkins and

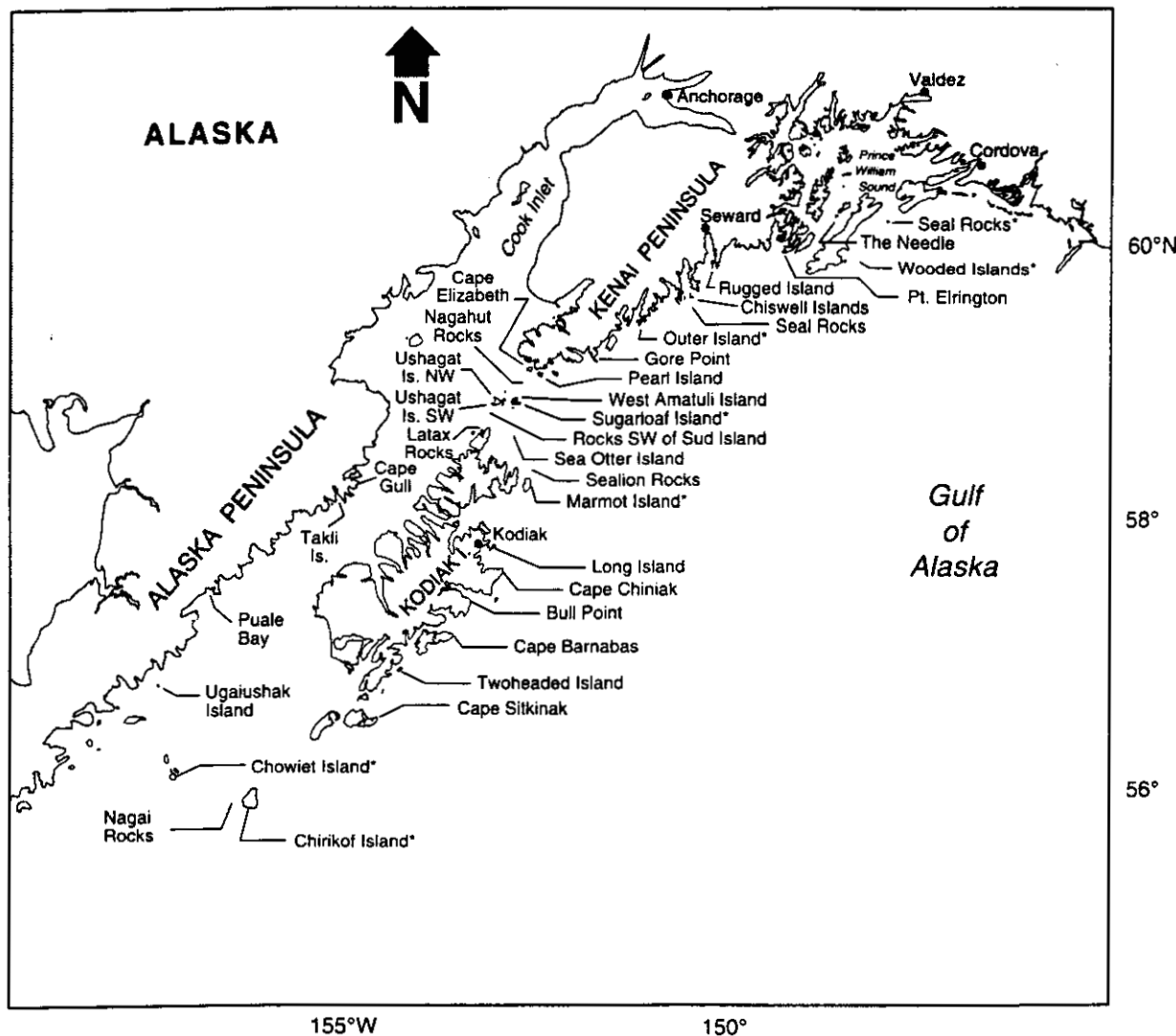


Figure 7-1. Steller sea lion study area with all haulout sites and rookeries (*) from Prince William Sound to Chowiet Island.

Pitcher 1982). In our study, observations were made for 25 days at Chirikof Island and for 34 days at Cape St. Elias. Each haulout site was searched daily using spotting scopes and binoculars. The presence of all dead, prematurely born pups was noted. Visual cues such as bird activity or activity of adult female sea lions, as well as periodic visual scans, were used to determine the presence of dead pups. Once a dead pup was located, it was retrieved, taken from the haulout site, and necropsied. Tissue samples were preserved for histological analyses according to accepted protocols (Appendix I, this volume). Adults, juveniles, and premature births were counted daily at each location.

Analyses of adult/juvenile and pup count data were accomplished by fitting simple linear regression models (Neter and Wasserman 1974) to counts conducted

prior to 1990. The regression residuals (the difference between predicted and actual counts) were analyzed to ensure an adequate model fit, and that the assumptions of normality and constant variance were met (Cook 1977). The haulout site data set was limited to only three data points because of the absence of consistent counts. As a result, it was difficult to assess model fit and the assumptions of normality and constant variance for the haulout site counts. The models were used to generate a predicted value and variance for a 1990 count without an oil spill effect. A one-sided t test (Neter and Wasserman 1974) was used to test if the observed counts were lower than the 1990 counts predicted by the model. If they were, then an oil spill effect is inferred. Power calculations were based on the one-sided t statistic, and were used to assess our ability to detect meaningful biological differences.

The adult/juvenile rookery data set used in the regression analysis was constructed by combining counts from Sugarloaf, Marmot and Chirikof Islands for the years 1976, 1978, 1979, 1985, and 1989. These rookeries were close to the spill and the most likely to be affected. Outer Island and Seal Rocks, two rookeries which were closer to the spill than the three we used, were not used in the analysis due to missing count data. The 1989 data point was included with the previous counts and treated as a nonoiled year because it seemed likely that any possible oil spill effect in 1989 would have been slight to nonexistent. Observations following the spill suggested that large numbers of sea lions were not killed in 1989. We speculated that the most likely effect would be an increase in premature birth rates which should have been detected in the 1990 counts. Also, we did not have the ability to measure premature births in 1989 because the spill occurred shortly before normal parturition.

We used the counts from 17 locations for the years 1956–1957, 1976, and 1989 for the haulout-site data set for our linear regression analysis. These 17 areas include all of the haulout sites that had at least three counts in the same years. We treated 1989 as a nonoiled year in the haulout site counts for the same reasons as the rookery counts.

Pup counts were analyzed in a similar manner to adult/juvenile counts. Previous pup counts were not adequate to analyze for an effect in 1989, so we summed and analyzed counts through 1989 to predict the count expected in 1990. The combination of pup counts from Outer, Sugarloaf, Chirikof, and Chowiet Islands for 1978, 1979, 1984, 1986, and 1989 was used as the data set for construction of the linear regression model.

To determine if premature pupping ratios were different between the two haulout sites, a Poisson model (Agresti 1990) was fitted to the number of premature pups. The model adjusted for differences in the number of adults using the natural log of

adults counted as an offset parameter and used oil treatment as an explanatory variable.

Toxicology and Histology

An extensive effort was mounted to search the beaches of PWS following the oil spill to locate and collect carcasses of animals possibly killed by toxic effects of the spill. Twelve sea lions were recovered during this effort. Detailed necropsies were performed on these animals according to established protocols. In addition, 16 animals were collected under the Natural Resources Damage Assessment (NRDA) study conducted on Steller sea lions in 1989 following the spill. Animals were collected from haulout sites in the vicinity of PWS, along the Kenai coast, and in the Barren Islands (southern tip of the Kenai Peninsula). Necropsies were begun as soon after death as possible with tissues taken and preserved according to protocol. A complete gross and histological study was done on each animal. Tissues were taken from all major organs and systems and preserved in 10% buffered formalin for histological analysis. Three complete sets of tissues were frozen in chemically clean containers for toxicological analysis. Tissues for toxicological analysis were also obtained from one sea lion taken during the subsistence harvest in Kachemak Bay in 1990.

Premature pups were collected at Cape St. Elias and Chirikof Island during April 1990. Complete gross and histological analyses were performed on the collected tissues, but toxicological analyses were not.

Analysis of bile was performed by the Environmental Conservation Division (ECD), Northwest Fisheries Science Center, NMFS, Seattle, Washington, using high-pressure liquid chromatography with fluorescence detection. Metabolites of naphthalene (D) and phenanthrene (PHN) were measured as indicators of hydrocarbon contamination (Krahn et al. 1984). Concentrations of PHN were detected at 260/380 nm and D at 290/335 nm (excitation/emission). The PHN wavelength pair was used to estimate total concentration of 3-ring aromatic compounds, and the D wavelength pair was used to estimate 2-ring aromatic compounds. Fluorescent Aromatic Compounds (FACs) values were reported in units of ng PHN (D) equivalents/gram bile. Krahn et al. (1992) have shown a high correlation between PHN and D equivalents and the summed concentrations of metabolites of phenanthrene and naphthalene/dibenzothiophene, respectively, using gas chromatography and mass spectroscopy. In the current study, results of bile analysis were compared by area with a square root transformation and one-way analysis of variance (Neter and Wasserman 1974). A Tukey Studentized Range test (Stoline 1981) was used to test all possible group differences while maintaining an overall α of 0.05.

Other tissue samples were analyzed by either the Geochemical and Environmental Research Group (GERG), Texas A&M University, College Station, Texas, or the ECD. Analysis was performed using capillary column gas chromatography



with mass spectrometry (GC/MS). Because of the large number of tissues collected in other NRDA oil spill studies, only a representative number of all tissue samples were analyzed. Thus, all tissue samples collected in our study were not analyzed.

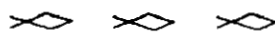
Although similar analytical methods were used (Krahn et al. 1988; Sloan et al. 1993), analyses performed by GERG were done under NRDA Technical Study No. 1 and were subject to different quality assurance and reporting requirements than the ECD analyses which were conducted as part of an ADF&G/NOAA study of subsistence foods. Aromatic compounds (ACs) consisted of the aromatic compounds naphthalene, dibenzothiophene, phenanthrene, fluorenes, and chrysenes as both parent compounds and the alkylated series. The ECD analysis consisted of and was reported in terms of the sums of lower molecular weight 2,3-ring aromatic compounds (LACs), the sums of higher molecular weight 4,7-ring aromatic compounds (HACs), and total aromatic compounds (ACs). GERG analysis was similar but in addition they analyzed and reported on aliphatic hydrocarbons *n*-C₁₀ through *n*-C₃₄, pristane, phytane, and unresolved complex mixture (UCM). This allowed calculation of a pristane/phytane ratio and a carbon preference index (Boem et al. 1987; Farrington and Tripp 1977).

Tissues for histological analysis were examined by one of us (TRS) at the Colorado State Diagnostic Laboratory, University of Colorado, Fort Collins. The tissues were imbedded in paraffin and sectioned at 5–6 μ . Tissue sections were stained with eosin and hematoxylin and mounted on slides for microscopic examination. Approximately 65 to 110 slides were prepared for each collected sea lion, including multiple slides from the following major organ systems: nervous, integument, cardiovascular, respiratory, lymphohematopoietic, digestive, urogenital, endocrine, musculoskeletal, and special senses. Because of possible hydrocarbon contaminant-related pathology found in harbor seals (Spraker et al., Chapter 17), particular attention was given to the nervous system. Tissues from sea lions found dead were in varying states of autolysis when taken and many were unsuitable for microscopic examination.

RESULTS

Distribution and Abundance

During observations of sea lions near or in oil following the EVOS, it became apparent that oil did not persist on sea lions as it did on harbor seals (Lowry et al., Chapter 12). Oil did not persist on the rookeries and haulout sites either, probably due to their steep slopes and high surf activity. However, some oil fouling was noted on Seal Rocks and Sugarloaf Island in April 1989. Insignificant amounts of oil were seen at each site during pup counts in late June 1989, but none were seen in 1990.



Adult and juvenile sea lion numbers from Cape St. Elias to Chowiet Island totaled 18,135 in 1989 (Table 7-1) which was substantially lower than the comparable 1976 count of 46,204 (Calkins and Pitcher 1982). Combined rookery counts totaled 6076 sea lions in 1989 (Table 7-2). The linear regression of the sample year (1976–1989) on the combined rookery counts resulted in a model ($R^2=0.99$) with an estimated y-intercept of 1,825,666.8 (SE=120,622.3) and an estimated year effect of -914.7 (SE=60.9). Examination of residuals suggested the model fit well and assumptions of normality and constant variance were met.

The actual count of 4243 was not significantly lower than the regression model predicted count of 5502.8 (SE=891.8) adult and juvenile sea lions in 1990 ($t=-1.41$, $df=3$, $P=0.126$) at $\alpha=0.05$. The power of the test (0.21) was low. In order to obtain a power of 85% or 95%, we would have had to count as few as 2532 and 1305 sea lions, respectively.

Pups counted at rookeries totaled 4195 in 1989 (Table 7-3). Pup numbers at these sites have fallen from 13,145 in 1979. The linear regression of year (1979–1989) on the combined pup counts resulted in a model ($R^2=0.99$) with an estimated y-intercept of 1,773,776.4 (SE=99,162.8) and an estimated year effect of -889.6 (SE=50). Examination of the residual diagnostics revealed that the model fit fairly well and that assumptions of normality and constant variance did not appear to have been violated. The 1990 count of 2952 pups was not significantly lower than the model predicted count of 3577.5 (SE=490.9, $t=-1.27$, $df=2$, $P=0.165$) at $\alpha=0.05$. Power of this test was also low at 0.12. We would have had to count 1624 or 711 sea lion pups, respectively, to obtain a power value of 85% or 95%.

Haulout site counts totaled 4782 in 1989 (Table 7-4). Comparison of the 1989 count to the 1976 count of 9277 illustrates the severity of the decline; it also suggests that the haulout sites declined at a similar rate and that redistribution from the rookeries to haulout sites did not occur. The linear regression of these data resulted in a model ($R^2=0.99$) with an estimated y-intercept of 812,914.0 (SE=2105.4) and an estimated year effect of -406.4 (SE=10.7). The model appeared to fit well. The average count was 4890.5 sea lions on haulout sites in 1990. This value was not significantly lower than the model predicted count of 4079.7 ($t=0.99$, $df=1$, $P=0.748$) at $\alpha=0.05$. Power of the test was extremely low at 0.05. A 1990 count of zero sea lions on the haulout sites would have resulted in a power value of 0.20.

One premature birth was observed at Chirikof Island and 11 were observed at Cape St. Elias in 1990 (Table 7-5). The Poisson model rejected the hypothesis of equivalent premature pupping ratios [Deviance (G^2) = 5.01, $P=0.025$, $\chi^2=4.01$, $P=0.045$]. This model estimated that if premature pupping ratios were equivalent at the two sites, we should have counted 4.33 premature pups at Chirikof Island and 7.67 premature pups at Cape St. Elias.

Table 7-1. Counts of adult and juvenile Steller sea lions at all rookeries and haulout sites from Cape St. Elias to Chowiet Island, Alaska. (--- = no data)

Location	1956-57	1973	1976	1985	1989	1990 ^a
Cape St. Elias	---	1548	1628	---	1883	948
Middleton Island	---	---	2901	---	1	---
Wooded Islands	2500	1261	878	---	1333	1232
Seal Rocks, PWS	183	1733	1709	---	2159	1471
Glacier Island	---	0	0	---	0	0
Perry Island	---	0	0	---	0	0
Point Eleanor	---	0	0	---	0	0
The Needle	130	563	537	---	668	926
Point Elrington	250	236	725	---	487	382
Rugged Island	---	---	0	---	190	25
Chiswell	2012	---	1106	---	456	408 ^b
Seal Rocks, Kenai	250	---	320	---	65	0-52 ^b
Outer Island	2989	---	3847	---	1127	589-732
Gore Point	200	---	535	---	76	63-125
Perl Island	737	---	33	---	159	97-125
Elizabeth Island	129	---	124	---	180	85-15
Nagahut Rocks	---	---	344	---	43	28-73
Sugarloaf Island	11,963	---	5226	2991	2467	1319-1513
Rks. SW Sud	---	---	670	---	93	0-111
SW Ushagat	834	---	902	---	245	441-276
NW Ushagat	---	---	106	---	4	0-14
W Amatuli Island	1576	---	57	---	0	0-0
Latax Rocks	3334	---	1164	---	354	519
Sea Otter Island	---	---	541	---	450	164
Sea Lion Rocks	343	---	432	---	46	93

(Table continues)

Table 7-1. Continued

Location	1956-57	1973	1976	1985	1989	1990 ^a
Marmot Island	4157	---	9862	4983	2331	1766
Long Island	75	---	0	---	30	93
Cape Chiniak	772	---	365	---	0	95
Gull Point	---	---	145	---	0	91
Cape Barnabas	1598	---	364	---	0	1
Twoheaded Island	2810	---	1615	---	479	268
Cape Sitkinak	343	---	120	---	204	234
Chirikof Island	1742	---	5199	2346	1278	1061
Nagai Rocks	---	---	657	---	233	196
Cape Ikokik	---	---	0	---	0	0
Cape Ugat	---	---	0	---	0	0
Cape Gull	---	---	207	---	0	0
Takli Island	---	---	1877	---	0	0
Puale Bay	---	---	1877	---	0	0
Ugaiushak Island	---	---	125	---	138	55
Sutwik Island	---	---	6	---	210	153
Chowiet Island	6323	---	---	2059	737	897
Total	45,250	5341	46,204	10,320	18,135	14,277 ^c

^a 1990 counts were conducted jointly with NMFS. Data are from Merrick et al. (1991).

^b Some areas were counted twice - first count by NMFS on 12 June, second count by ADF&G on 13 June.

^c Where two counts were available, the mean was used to calculate total numbers.

Table 7-2. Counts of adult and juvenile Steller sea lions at rookeries in the oil spill area. Only rookeries with counts conducted in the years shown are presented.

Rookery	Year					
	1976	1978	1979	1985	1989	1990
Sugarloaf Island	5226	4810	4374	2991	1467	1416
Marmot Island	9862	8506	6381	4983	2331	1766
Chirikof Island	2391	3699	5199	2346	1278	1061
Total	17,479	17,015	15,954	10,320	6076	4243

Table 7-3. Counts of Steller sea lion pups at rookeries within the oil spill area. Only those rookeries with counts for all years are shown.

Rookery	Year					
	1978	1979	1984	1986	1989	1990
Outer Island	431	888	1034	993	557	363
Sugarloaf Island	5021	5123	3114	3072	2109	1638
Chirikof Island	1573	1649	1913	1476	709	607
Chowiet Island	4670	5485	3207	1731	820	344
Total	11,695	13,145	9268	7272	4195	2952

Table 7-4. Counts of adult and juvenile Steller sea lions at haulout sites from Wooded Islands to Cape Sitkinak.

Haulout	Year			
	1956-57	1976	1989	1990 ^a
Wooded Islands	2500	878	1333	1232
The Needed	130	537	668	926
Point Elrington	250	725	487	382
Chiswell Island	2012	1106	456	408 ^b
Seal Rocks, Kenai	250	320	65	0-52 ^b
Gore Point	200	535	76	63-125
Perl Island	737	33	159	97-125
Elizabeth Island	129	124	180	85-15
SW Ushagat	834	902	245	441-276
W Amatuli Island	1576	57	0	0-0
Latax Rocks	3334	1164	354	51
Sea Lion Rocks	343	432	46	93
Long Island	75	0	30	93
Cape Chiniak	772	365	0	95
Cape Barnabas	1598	364	0	1
Twoheaded Island	2810	1615	479	268
Cape Sitkinak	343	120	204	234
Total	17,893	9277	4782	4702-5079

^a Counts were conducted jointly between ADF&G and NMFS.

^b Some areas were counted twice, the first count was by NMFS on 12 June, the second by AFD&G on 13 June.

Toxicology

Fifteen of the 16 sea lions collected under NRDA studies (Table 7-6) were tested for FACs (D and PHN) in the bile (Table 7-7). No sample was obtained from the sixteenth animal. Bile was also collected from three animals found dead but only one sample was analyzed. Bile from one sea lion (ST-SL-1; Table 7-7) taken in the subsistence harvest in Kachemak Bay was also tested.

PHN values ranged from 350 ng equivalents/g bile to 29,000 and averaged 5800. D values ranged from 4200 to 120,000 ng equivalents/g bile and averaged 35,000. Total FACs ranged from 4600 to 149,000 averaging 63,000. The mean PHN value for four sea lions collected in PWS in October 1989 (14,900 ng equivalents/g) was significantly higher than the mean for sea lions collected in PWS in June and July 1989 (3900 ng equivalents/g) or those collected in June, July, and October 1989 outside of PWS (2000 ng equivalents/g). The mean PHN values from all of the bile samples collected outside PWS in either June/July or October were relatively

Table 7-5. Counts of adult (AD), juvenile (JUV) and premature pup (PREM) Steller sea lions at Chirikof Island (CHIR) and Cape St. Elias (CSE), April and May 1990. (— = no data)

1990	AD & JUV CHIR	PREM PUPS CHIR	AD & JUV CSE	PREM PUPS CSE
5 April	400	0	—	—
7 April	681	0	—	—
8 April	796	0	—	—
9 April	631	0	—	—
10 April	797	0	—	—
11 April	669	0	—	—
12 April	765	1	—	—
13 April	665	0	732	1
14 April	736	0	637	1
15 April	748	0	609	1
17 April	690	0	300	0
18 April	670	0	297	0
19 April	799	0	838	0
20 April	859	0	814	1
21 April	759	0	724	0
22 April	628	0	908	0
23 April	919	0	1246	1
24 April	701	0	1081	0
25 April	939	0	1359	0
26 April	757	0	1344	1
27 April	804	0	1064	0
28 April	773	0	1105	0
29 April	630	0	913	0
30 April	—	—	952	0
1 May	—	—	924	1
2 May	—	—	703	1
4 May	—	—	1740	0
4 May	—	—	1139	0
5 May	—	—	1232	1
8 May	—	—	943	1
9 May	—	—	1650	0
10 May	—	—	1500	0
11 May	—	—	1107	0
12 May	—	—	933	0
13 May	—	—	670	1
14 May	—	—	638	0
15 May	—	—	921	0
16 May	—	—	744	0

Table 7-6. Steller sea lions examined for histological or toxicological analyses during 1989.

Specimen number	Date found dead (F) or killed (K)	Location	Sex	Age
AF-SL-1	F 12 May	Nuka Island	M	Adult
BD-SL-1	F 6 June	Swikshak Bay	F	Juvenile
GA-SL-1	F 23 June	Lower Cook Inlet	F	Adult
MH-SL-1	F 2 May	Kachemak Bay	F	Juvenile
MH-SL-4	F 9 June	Naked Island	F	Adult
MH-SL-5	F 9 June	Naked Island	F	Fetus of MH-SL-4
TS-SL-1	F 27 April	Sea Lion Rocks	F	Juvenile
TS-SL-2	F 13 June	Eleanor Island	M	Juvenile
TS-SL-3	F 19 June	Axel Island	M	Juvenile
TS-SL-579	K 29 June	NW Ushagat Island	F	Juvenile
TS-SL-580	K 29 June	NW Ushagat Island	F	Juvenile
TS-SL-581	K 29 June	NW Ushagat Island	M	Juvenile
TS-SL-583	K 3 July	The Needle	F	Juvenile
TS-SL-584	K 3 July	The Needle	M	Juvenile
TS-SL-585	K 3 July	The Needle	F	Juvenile
TS-SL-586	K 3 July	The Needle	F	Juvenile
TS-SL-587	K 4 July	Chiswell Islands	M	Adult
TS-SL-588	K 26 October	Sea Otter Island	F	Adult
TS-SL-589	K 28 October	Nagahut Rock	F	Adult
TS-SL-590	K 29 October	The Needle	F	Juvenile
TS-SL-591	K 29 October	The Needle	F	Adult
TS-SL-592	K 29 October	The Needle	F	Juvenile
TS-SL-593	K 30 October	The Needle	F	Juvenile
TS-SL-594	K 3 November	Chiswell Island	M	Adult

low and were not significantly different from those collected in PWS in June/July. Bile samples from sea lions taken inside PWS during June/July, with one exception, were also moderately low. The single exception was an immature female taken at the Needle on 3 July 1989 (TS-SL-586).

Selected tissues were analyzed for polycyclic aromatic hydrocarbons (PAHs) from 8 sea lions found dead, 1 sea lion taken in the subsistence harvest, and 13 sea lions that were collected (Table 7-8). These analyses were performed by ECD on liver, muscle, kidney, and blubber on sea lions TS-SL-579 through TS-SL-587. All other tissue analyses were conducted by GERG. In general, tissues analyzed by GERG were reported to have consistently higher levels of PAHs than those analyzed by ECD (in no case did both groups analyze the same tissue from the same animal). The difference in reported values between the two laboratories was probably due to the differences in detection levels and reporting requirements. Differences were also seen in harbor seal tissues analyzed by the two groups (Frost et al., Chapter 19).

Table 7-7. Levels of fluorescent aromatic hydrocarbon metabolites of phenanthrene (PHN) and naphthalene (NPH) found in the bile of Steller sea lions in 1989. Units are in ng PHN (NPH) equivalents/g bile.

Specimen Number	PHN	NPH
ST-SL-1	4600	38,000
TS-SL-579	1200	18,000
TS-SL-580	2600	14,000
TS-SL-581	2100	45,000
TS-SL-582	890	19,000
TS-SL-583	3100	29,000
TS-SL-584	3000	31,000
TS-SL-585	3700	19,000
TS-SL-586	9000	36,000
TS-SL-587	350	4200
TS-SL-588	730	16,000
TS-SL-590	15,000	54,000
TS-SL-591	6600	32,000
TS-SL-592	29,000	120,000
TS-SL-593	9900	41,000
TS-SL-594	3000	20,000
TS-SL-1	3600	58,000

The highest total PAH level reported in this study was 607 parts per billion (ppb) from the liver of an adult male sea lion found dead on Nuka Island on the Kenai Peninsula on 12 May 1989 (AF-SL-1; Table 7-8). Six percent of the tissues tested had PAH levels exceeding 200 ppb. The highest PAH levels were seen in the sea lions found dead. Fewer than 6% of the tissues tested from collected animals had PAH levels exceeding 100 ppb. PAH levels detected in all tissues from collected sea lions were from low to not detectable, regardless of location, time of collection, or laboratory conducting the analysis.

Phytane levels were generally very low in sea lion tissues (Table 7-9). The highest phytane ratio was from the liver sample of AF-SL-1. Brain tissue from some of the sea lions collected in PWS had the lowest pristane/phytane ratios (Table 7-9). The unresolved complex mixture was low for all tissues analyzed. The carbon preference index was 1 for the liver sample from TS-SL-3 and ranged higher for all other samples.

Histology

Numerous lesions were identified in adult and juvenile sea lions. The most common gross lesions were caused by parasites in the nasal cavity, stomach, and intestine. Multiple histological lesions were seen in all organ systems. No gross

Table 7-8. Polycyclic aromatic hydrocarbons (PAH) found in Steller sea lion tissues collected in 1989. LAC=lower molecular weight aromatic contaminants; HAC=higher molecular weight aromatic contaminant; nd=non detected; PAH=total polycyclic aromatic hydrocarbons. All measurements in part per billion ng/g wet weight.

Specimen number	Liver			Muscle			Kidney			Blubber			Brain		
	LAC	HAC	PAH	LAC	HAC	PAH	LAC	HAC	PAH	LAC	HAC	PAH	LAC	HAC	PAH
TS-SL-2	67.8	39.5	107.3												
TS-SL-3	120.2	89.5	209.6												
MH-SL-1	96.4	49.1	145.5							206	160	366			
MH-SL-4	82.4	38.5	120.9												
MH-SL-5	72.9	105.6	182.5							160.1	105.2	265.3			
AF-SL-1	251.4	355.1	606.5												
BC-SL-1	46.4	38.2	84.76												
GA-SL-1	75.3	28.3	103.6												
TS-SL-579	2	0.9	2.0	nd	nd	nd	nd	nd	nd	1	nd	1			
TS-SL-580	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	0.9	0.9			
TS-SL-581	5	2	7	nd	nd	nd	0.1	nd	0.1	nd	0.3	0.3			
TS-SL-582	1	1	2	nd	nd	nd	nd	nd	nd	nd	0.6	0.3	1	nd	nd
TS-SL-583	3	2	5	0.3	nd	0.3	nd	nd	nd	nd	nd	nd			
TS-SL-584	nd	0.2	0.2	0.6	nd	0.6	nd	0.3	0.3	1	nd	nd			
TS-SL-585	1	0.3	1.3	1	nd	1	nd	nd	nd	nd	0.2	0.2			
TS-SL-586	0.9	0.5	1.5	0.9	nd	0.9	nd	nd	nd	nd	0.3	0.3	40.4	33.1	73.5
TS-SL-587	nd	nd	nd	0.7	nd	0.7	nd	nd	nd	nd	0.6	0.6			
TS-SL-590	17.8	11.9	29.7							64.4	51.8	116.2	29.6	37.8	67.4
TS-SL-591	29.4	53.7	83.1							53.3	39.5	92.8	35.4	24.6	60
TS-SL-592	20.3	13.4	33.7							67	53.2	120.8	75.8	51.3	107.1
TS-SL-593	18.5	13.6	32.1							48	33.7	81.2	46.4	29.4	75.8
ST-SL-1	1	nd	1	2	0.2	2.2	4	nd	4	4	0.4	4			

Table 7-9. Pristane (PR), phytane (PH), pristane/phytane ratio, unresolved complex mixture (UCM) and carbon preference index (cpi) (Farrington and Tripp 1977) for Steller sea lion tissues in 1989. (--- = no data)

Specimen number	Liver					Blubber					Brain				
	PR	PH	ratio	UCM	cpi	PR	PH	ratio	UCM	cpi	PR	PH	ratio	UCM	cpi
GH-SL-1	1538.6	0	---	13	80										
MH-SL-4	1908	0	---	21	43	138,009	0	---	30	938					
MH-SL-5	0	0	---	18	45.6	127	0	---	46	182.8					
AF-SL-1	327,890	267	1228	5	1.2										
TS-SL-3	3428	0	---	0	1										
MH-SL-1	28,011	0	---	0	15										
TS-SL-2	848	0	---	0	42										
BC-SL-1	761	0	---	0	4										
TS-SL-586											126	24	5.3	0	3.4
TS-SL-582											37	13	2.9	1.1	170.6
TS-SL-590	29,928	46	651	6.2	1.8	47,203	29	1628	0	73.9	296	15	20	2.5	121.6
TS-SL-591	5877	27	218	12.7	2	106,563	40	2664	127	114.8	323	0	---	77.4	4.1
TS-SL-592	157	0	---	21.3	1.8	105,161	81	1298	81	31.7	45	18	2.5	46.5	143
TS-SL-593	118,679	37	505	30.4	4.3	71,681	43	1667	67.2	219.6	60	22	2.7	76.2	148.8

or histological lesions were found in premature pups. None of the lesions found were linked to exposure to hydrocarbon contamination.

DISCUSSION

Historically, sea lion abundance has been determined by aerial surveys and ground counts. (Calkins and Pitcher 1982; Loughlin et al. 1984; Merrick et al. 1987). We attempted to take advantage of the historical data base in our investigation of the effects of the EVOS on sea lions. Aerial surveys of sea lions traditionally cover broad geographical areas and are expensive, time consuming, and dangerous. Consequently, surveys have not been conducted on a regular basis and the data are incomplete. Generally, the types of data collected on sea lions in the past have been used to show trends in the population but are far less appropriate for separating and identifying sources of impact in terms of population reductions. The power calculations in the linear regression analyses from our study were consistently low because of these complications.

In the analysis of adult and juvenile counts at rookeries, 1260 (23%) fewer sea lions were counted than the model predicted. However, the adult/juvenile counts at haulout sites totaled 811 (20%) more sea lions than the model predicted. It is possible that some redistribution or even reduction in numbers of adults and juveniles did occur beyond that which was expected from the ongoing sea lion decline. This was not detected in terms of the statistical analysis because of the limitations in the data from prior counts. These limitations are illustrated by the consistently low power of the statistical tests. Power of the analysis of adult/juvenile counts at haulout sites was particularly low. It may have been difficult to statistically confirm an oil spill effect unless all of the sea lions were missing from the haulout sites. Counts at haulout sites viewed alone cannot be used for more than an assessment of redistribution.

The actual number of pups counted at rookeries was 11% lower in 1990 than the model predicted. This analysis also suffered from the same limitations as the adult/juvenile count analyses. The difference between the actual and predicted pup counts was not as large as the difference in the adult/juvenile counts at rookeries, but the power of the pup analysis was lower.

Premature pupping ratios were different at the two areas, with the highest occurring nearest the spill. However, this by itself cannot be considered an oil spill effect because overall pup abundance was not shown to have been significantly affected by the spill. Lack of historical data on Chirikof Island prevented us from separating site differences from an oil spill effect.

Sea lions that were near or contacted oil could have become contaminated with hydrocarbons internally through inhalation, contact and absorption through the skin, or ingestion either directly or by consuming contaminated prey (Engelhardt

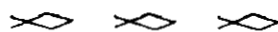
et al. 1977; Engelhardt 1987). There is little doubt that sea lions were exposed to oil inside PWS and in the northern Gulf of Alaska. Sea lions were sighted swimming in or near oil slicks, oil was seen near numerous haulout sites, and oil fouled the rookeries at Seal Rocks and Sugarloaf Island. One of the collected sea lions (TS-SL-582) was sighted at The Needle in PWS on 18 April 1989, then collected at the same location on 3 July 1989. This animal likely remained in that vicinity and should have contacted oil. However levels of contaminants measured in the bile or other tissues from this animal were very low.

Mammals have the ability to metabolize hydrocarbon contaminants to some degree (Addison et al. 1986), however, the amount of hydrocarbon contaminants that can be tolerated by marine mammals is unknown. All of the sea lions collected in PWS in October had high enough levels of metabolites of aromatic hydrocarbons in the bile to confirm exposure and active metabolism at the tissue level. These sea lions had a mean level of PHN FACs of 14,900 ng equivalents/g. Harbor seals (*Phoca vitulina*) sampled in PWS during 1989 had a mean phenanthrene level of 43,200 ng/g (Frost et al., Chapter 19), 2.9 times higher than sea lions. The highest levels of PHN FACs measured in harbor seals were from a heavily oiled female (110,000 ng/g) and a nursing pup of another heavily oiled female (215,000 ng/g). These harbor seals had PHN levels 2.8 and 7.4 times the highest PHN level measured in sea lions. Note, however, that it is difficult to make interspecies comparisons of PHN equivalents. For example, Dolly Varden and halibut injected with the same relative amount of *Exxon Valdez* oil had different proportions of AC metabolites (Krahn et al. 1992).

CONCLUSIONS

None of the data presented and analyzed provided conclusive evidence of an effect of the *Exxon Valdez* oil spill on Steller sea lions. Predicted values generated by regression models were not significantly different from actual counts. Data collected on premature pupping showed significantly higher premature pupping ratios at a haulout site nearer the oil spill compared to a haulout site farther away. However, an oil spill effect cannot be shown based on this evidence alone. This investigation was designed to detect large changes in premature pupping ratios which should have been coupled with a reduction in pup production, but numbers of premature pups were relatively small which complicated the analysis.

The data collected and analyzed in this study showed that the previously observed decline (Loughlin et al. 1984; Merrick et al. 1987; Byrd 1989; Loughlin et al. 1992) continued throughout the period. The rate of decline was as predicted by the regression models. Data from previous counts allowed only very low power statistical analysis and no significant oil spill effect was detected.



When all tissues from a single individual were compared, toxicant levels were not consistently high enough to confirm contamination. However, contamination cannot be determined by looking for aromatic compounds in tissues only, because the hepatobiliary is very efficient and eliminates the metabolized AC compounds via bile. As a result, ACs are found only at relatively low levels in other tissues. Our studies showed that some sea lions were exposed to oil and that they were metabolizing and excreting metabolites of aromatic hydrocarbons into the bile. No lesions related to hydrocarbon contamination were found during histological examinations. No evidence indicated damage caused to sea lions from toxic effects of oil.

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