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Effects of the *Exxon Valdez* Oil Spill on Pink Salmon Embryos and Preemergent Fry

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Abstract.—We examined pink salmon *Oncorhynchus gorbuscha* preemergent fry mortality, embryo mortality, and survival from embryo to preemergent fry in intertidal and upstream areas of Prince William Sound that were affected by the *Exxon Valdez* oil spill. No change in preemergent fry density or increase in numbers of dead fry was detected within 1 month of the spill. Pink salmon embryo mortality was elevated in oil-affected streams during fall of each year from 1989 through 1992 ($P < 0.023$ for all years). In 1989 increased embryo mortality was detected in the lower intertidal zones, and in 1990 elevated mortalities were observed at the highest intertidal zone. In 1991 embryo mortality was significantly higher in all intertidal zones, as well as upstream of oil contamination. The same patterns of mortalities were observed in 1992, although they were not as extreme as that observed in 1991. However, no difference in survival of embryo to preemergent fry was detected for the 1989–1991 brood years.

Wild Pacific salmon play a major role in the Prince William Sound ecosystem while also contributing to the region's commercial fisheries. Migrating salmon fry are an important spring food source for many animals. Marine mammals, fish, and birds prey on the ocean stages of salmon; terrestrial species, such as bears, river otters, eagles, and gulls depend on salmon for their summer diet. Salmon also provide a pathway for transferring nutrients from marine ecosystems to nearshore and terrestrial ecosystems. Total returns of wild pink salmon *Oncorhynchus gorbuscha* have ranged from 1.8 to 21.0 million since 1978.

Up to 75% of spawning pink salmon in Prince William Sound use intertidal areas (Helle et al. 1964), which by nature are highly susceptible to contamination from marine oil spills. Moles et al. (1987) and Rice et al. (1975) found that pink salmon embryos and preemergent fry were adversely affected by exposure to crude oil and that the effect was most acute in intertidal environments. The 24 March 1989 spill from the *Exxon Valdez* occurred just before the spring migration of salmon fry and contaminated many intertidal spawning areas in central and southwestern Prince William Sound.

We evaluated (1) the short-term effect of oil-contaminated incubation substrate on preemergent pink salmon fry, (2) the effect of intertidal oiling on pink salmon embryo mortality, and (3) the effect of intertidal oiling on the survival of pink salmon embryos to preemergent fry. This study is part of the work done by Sharr et al. (1994a; 1994b).

Materials and Methods

Study Sites

Study streams were selected using the following criteria: (1) adult salmon returns were expected to be large enough to provide a high probability of success in embryo and fry sampling, (2) embryo and fry sampling had been done in past years, and (3) streams which showed no oil contamination (reference) were selected near oil-contaminated (oiled) streams. Because pink salmon embryos and preemergent fry remain in the area of the stream where they were deposited as eggs, we were able to select oiled and reference sites in close proximity to each other, thus reducing the geographical effect on the findings.

Stream oiling was assessed through visual observations of the stream and the adjacent area. The observations were supported by photographs, observation maps, and hydrocarbon analysis of mussels (*Mytilus* sp.) collected near the stream mouths. These data were collected as part of another Natural Resource Damage Assessment study (NRDA F/S study 1).

Sample Design

The methods used for both embryo and preemergent fry sampling were similar to those described by Pirtle and McCurdy (1977). Sample sites were stratified by tide zone to account for differences in embryo mortality or overwinter survival caused by salinity, temperature, predation, and oiling gradient. Four zones, three intertidal and one above the mean high tide line, were sampled whenever possi-

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ble for each stream: 1.8–2.4 m, 2.4–3.0 m, 3.0–3.7 m above mean low water, and upstream of mean high tide (3.7 m). Zone boundaries were staked before sampling and established by elevation above mean low water using appropriate tide tables, a surveyor's level, and stadia rod. No sampling was done below the 1.8–2.4-m zone because survival was expected to be low (Helle et al. 1964). Upstream sample areas were often within the reach of extreme high tides (3.7–4.6 m) because ice and snow often limited the extent of upstream sampling.

Separate linear transects were established for each zone on the embryo and preemergent fry surveys. Although most transects were 30.5 m long, some were shorter because of steep stream gradients. Transects were placed in riffle areas where spawning was observed during escapement surveys conducted by NRDA F/S study 1. Transects ran diagonally across the river: preemergent fry survey transects started downstream against the right bank and moved upstream to the left bank, whereas embryo survey transects started downstream against the left bank and moved upstream to the right bank. This placement of embryo and fry transects reduced sampling overlap and the influence of fall embryo sampling on spring fry abundance. A map was drawn for each stream, indicating the tide zones and transect locations in relation to major landmarks. Each embryo transect was marked with surveyor's flagging and photographed to ensure the transects would be in the same immediate area.

Fourteen circular samples, each 0.186 m^2, were systematically collected at approximately equal intervals along each transect. The number of samples was a compromise between reducing variance and the practicality and expense of conducting the study. To avoid excessive sampling of the stream, fewer samples were completed on narrow stream channels. Streams that split into two or more channels within a zone were sampled either by allocating samples among channels based on spawner distribution observed during NRDA F/S study 1 or, where spawner distribution was unknown, by an equal allocation of samples among channels.

Sampling date, time, tide zone, the number of live and dead embryos, the number of live and dead preemergent fry, and an estimate of the overall yolk sac absorption percentage were collected for each transect during both embryo and fry sampling. Pink salmon embryos were separated from chum salmon *Oncorhynchus keta* and coho salmon *O. kisutch* salmon embryos by their smaller size. Pink salmon fry were differentiated from chum salmon fry by their smaller size and lack of parr marks. An embryo was considered dead if it was opaque or discolored. Sampling often killed preemergent fry (especially newly hatched fry), so fry were only considered dead if decomposition was evident.

1989 Preemergent Studies

The short-term effect of oil-contaminated incubation substrate on pink salmon preemergent fry was examined during spring 1989. The annual preemergent pink salmon fry density survey conducted by the Alaska Department of Fish and Game was underway at the time of the *Exxon Valdez* spill; consequently, 39 streams were sampled for preemergent fry density before or shortly after being oiled. An additional session of sampling was initiated approximately 2 weeks later. In this second session, 14 streams examined during the first session were reexamined to assess the immediate effect of oiling. Differences in pink salmon preemergent fry density and mortality rate between the first and second survey were tested for each stream using a fixed-effects, two-way analysis of variance. The two factors were sampling period (first and second sampling) and height in the intertidal zone (1.8–2.4 m, 2.4–3.0 m, and 3.0–3.7 m above mean low water and upstream).

Embryo Mortality

The effect of intertidal oiling on pink salmon embryo mortality was assessed by examining the number of dead and live embryos present in the stream gravel shortly before freeze-up in the fall in 10 oiled and 15 reference streams (Figure 1). Pink salmon embryo mortality rates ($\hat{M}^e_{ij}$) were estimated for each stream and zone using the following relationship:

$$\hat{M}^e_{ij} = \frac{\sum_k (\mathrm{DE}^e_{ijk} + \mathrm{DF}^e_{ijk})}{\sum_k (\mathrm{LE}^e_{ijk} + \mathrm{DE}^e_{ijk} + \mathrm{LF}^e_{ijk} + \mathrm{DF}^e_{ijk})}, \quad (1)$$

where DE^e_{ijk}, DF^e_{ijk}, LE^e_{ijk}, and LF^e_{ijk} are the number of dead embryos, dead fry, live embryos, and live fry from dig k, stream i, zone j, collected during the embryo survey, respectively.

Differences in embryo mortality rate were examined using a mixed-effects, two-factor experiment with repeated measures on one factor (Neter et al. 1990):

$$Y_{ijk} = \mu_{\ldots} + O_i + Z_j + (OZ)_{ij} + S_{k(i)} + \epsilon_{(ijk)}. \quad (2)$$

The two treatments were extent of oiling (O_i; oiled and reference) and height in the intertidal zone (Z_j;

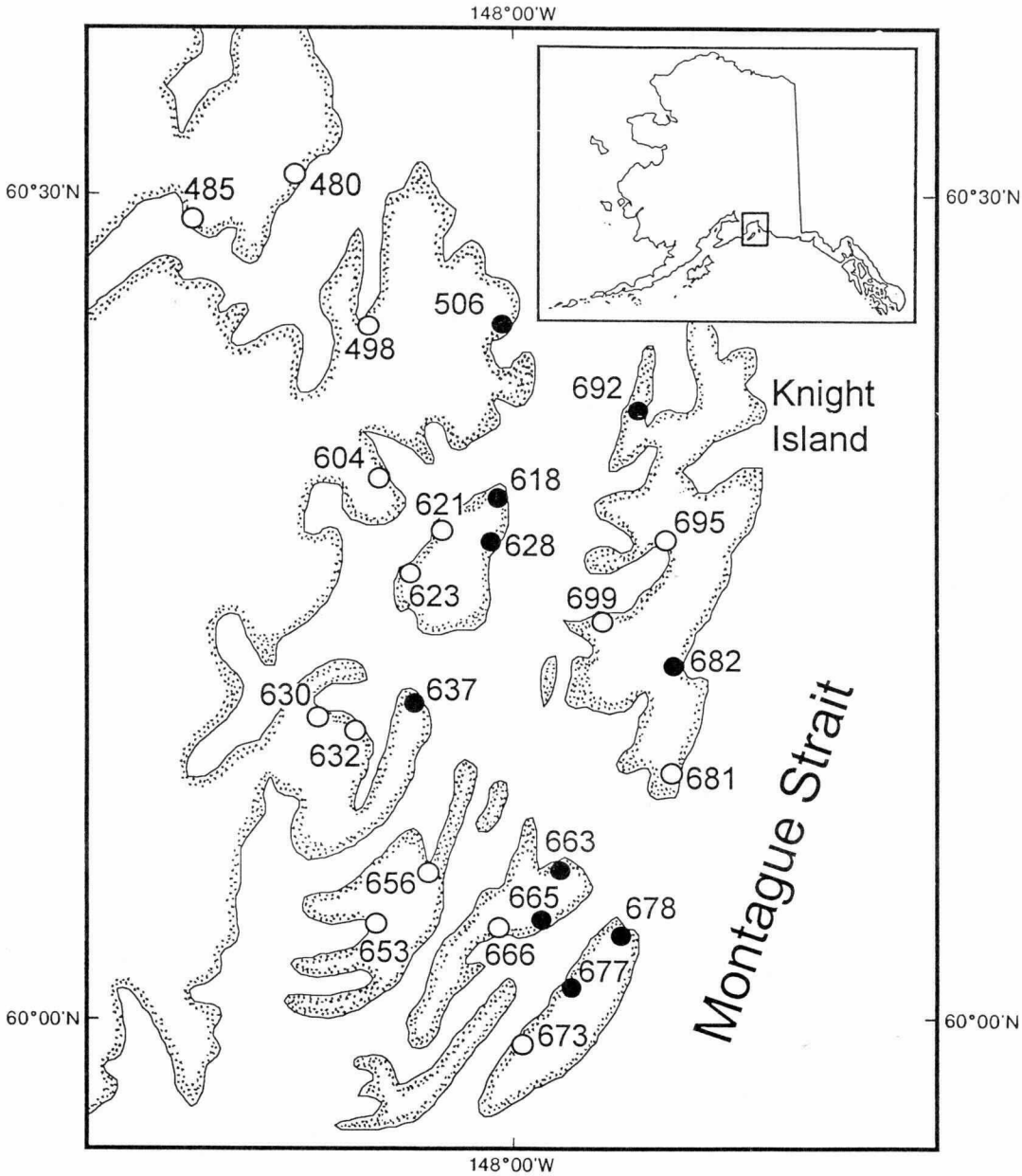

FIGURE 1.—Location of 10 oil-contaminated (solid circles) and 15 reference streams (open circles) used for assessment of pink salmon embryo mortality and survival from embryo to preemergent fry in Prince William Sound, Alaska. The number next to the stream locator is the stream identifier used by the Alaska Department of Fish and Game.

1.8–2.4 m, 2.4–3.0 m, and 3.0–3.7 m above mean low water and upstream). The data were blocked by stream ($S_{k(i)}$), a random effect that was nested within extent of oiling. The interaction of extent of oiling and height in the intertidal zone was also examined. The assumption of constant variance for error terms was tested using the F_{max}-test (Sokal and Rohlf 1969), and the normality of error term was visually assessed using scatter plots, box plots, and normal probability plots. The logit transform was used when analyzing the embryo mortality data. Assumptions relating to a valid split-plot analysis of

TABLE 1.—Results of the repeated pink salmon preemergent fry sampling during spring 1989 in Prince William Sound. Oiling (N = no, Y = yes) is based on visual observations from Natural Resource Damage Assessment F/S study 1.

Stream			Date of sample		Probability of a more extreme result (P value)					
					Live fry density			Fry mortality		
Number	Name	Oiling	First	Second	Sample[a]	Zone[b]	Interaction[c]	Sample[d]	Zone[b]	Interaction[c]
35	Koppen	N	8 Apr	24 Apr	0.33	0.00[e]	0.98	0.70	0.26	0.98
455	Paulson	N	30 Mar	14 Apr	0.73	0.01[e]	0.78	[f]	[f]	[f]
480	Mink	N	30 Mar	15 Apr	0.00[eg]	0.00[e]	0.00[e]	0.39	0.01[e]	0.32
604	Erb	N	31 Mar	16 Apr	0.66	0.00[e]	0.33	0.30	0.13	0.11
621	Totemoff	N	31 Mar	16 Apr	0.97	0.00[e]	0.51	0.14	0.41	0.61
632	Claw	N	1 Apr	17 Apr	0.36	0.00[e]	0.34	0.14	0.41	0.39
673	Falls	N	1 Apr	18 Apr	0.79	0.00[e]	0.78	0.28	0.41	0.38
677	Hayden	Y	1 Apr	18 Apr	0.32	0.30	0.81	0.12	0.13	0.13
681	Hogan	N	4 Apr	21 Apr	0.36	0.16	0.81	0.31	0.29	0.29
682	Snug	Y	3 Apr	21 Apr	0.87	0.00[e]	0.31	0.78	0.01[e]	1.00
692	Herring	Y	3 Apr	19 Apr	0.73	0.46	0.33	0.80	0.62	0.92
749	Shad	N	5 Apr	22 Apr	0.48	0.26	0.06	[f]	[f]	[f]
828	Cook	N	6 Apr	23 Apr	0.84	0.05[e]	0.20	0.21	0.32	0.32
861	Bernard	N	9 Apr	23 Apr	0.15	0.00[e]	0.46	0.55	0.99	0.60

[a]H_0: No change in fry density between the first and second sample.
[b]H_0: No difference between stream zones.
[c]H_0: No sample-by-stream zone interaction.
[d]H_0: No change in the percent dead fry between the first and second sample.
[e]Statistically significant at $\alpha = 0.05$.
[f]Low sample size because of missing cells.
[g]Statistically different, but more fry present in second sample than in first.

the repeated measures factor, zone, were also examined. Tests of homogeneity of between-treatment covariance matrices and the degree of sphericity of the pooled covariance matrix (Mauchly 1940) were effected. Four contrasts (oil versus reference for the four stream zones) and corresponding Bonferroni family confidence intervals (α = 0.10 overall) were estimated if a significant difference attributable to oiling was detected. The SAS (SAS Institute Inc. 1988) General Linear Models Procedure was used to analyze the data. The statistical power of the test for oil effects was examined if no statistical difference attributable to oil was observed. This analysis helped to evaluate whether sufficient sampling had been performed to detect a biologically meaningful difference if one existed.

Overwinter Survival

Differences in survival of pink salmon embryos to preemergent fry between the 10 oiled and 15 reference streams (Figure 1) were assessed by comparing preemergent fry densities in the spring with embryo densities observed the previous fall. Pink salmon survival rates from embryo to preemergent fry ($\hat{S}_{ij}$) were estimated by

$$\hat{S}_{ij} = \frac{\dfrac{\sum_k \mathrm{LF}^f_{ijk}}{n^f_{ij}}}{\dfrac{\sum_k (\mathrm{LE}^e_{ijk} + \mathrm{DE}^e_{ijk} + \mathrm{LF}^e_{ijk} + \mathrm{DF}^e_{ijk})}{n^e_{ij}}}, \quad (3)$$

where LF^f_{ijk} is the number of live fry from dig k, stream i, zone j, collected during the fry survey; n^e_{ij} and n^f_{ij} are the number of digs for stream i, zone j for the embryo and fry surveys, respectively. The same mixed-effects, two-factor experiment (equation 2) used for the examination of embryo mortality was employed to test for differences in embryo-to-preemergent fry survival.

Results

1989 Preemergent Studies

Few dead pink salmon fry were found during the 1989 preemergent fry surveys, although only 3 of the 14 resampled streams were observed to be oiled (Table 1). Only 9 of the 52 transects examined yielded more than five dead fry. No increase in fry mortality or decrease in fry density was detected between the first and second survey. The date of first sampling averaged 8 d after the spill (range of

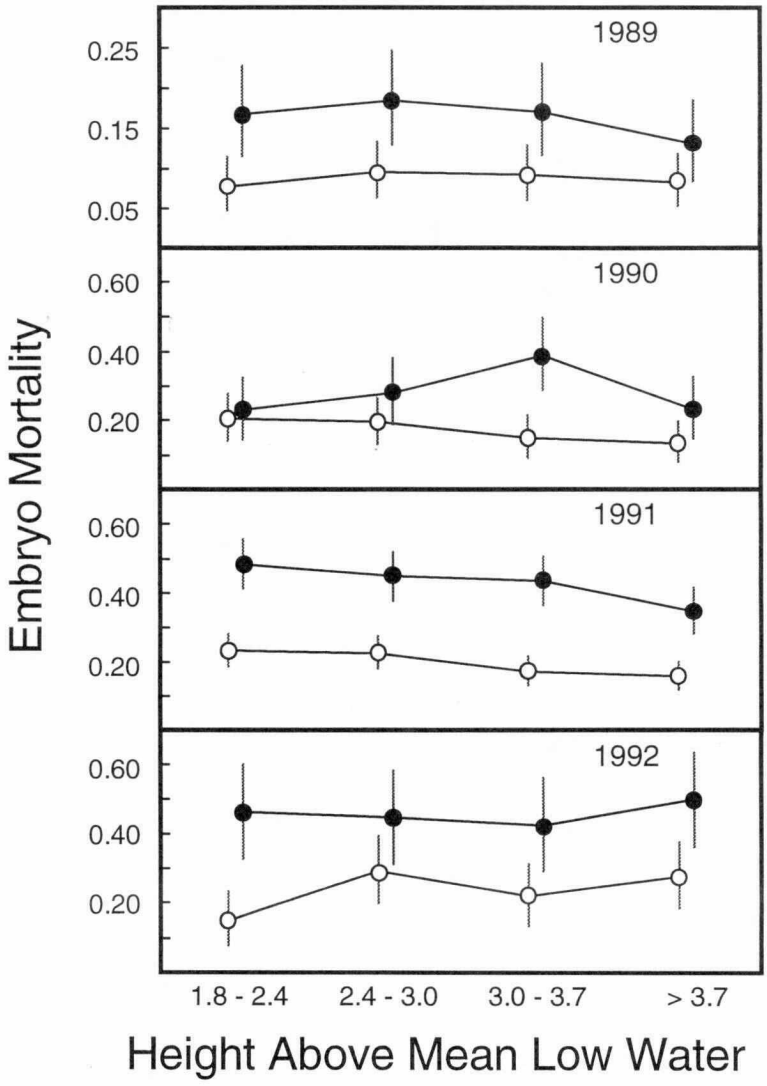

FIGURE 2.—Mean pink salmon embryo mortality (proportion) observed during field sampling in the falls of 1989–1992. Solid circles indicate data for the 10 oil-contaminated streams; open circles indicate data for the 15 reference streams. Standard error bars represent the 90% family confidence interval. Note that the scale of embryo mortality is not the same for all years. Height above mean low water is in meters.

6–16 d), and the second sampling occurred an average of 16 d after the first sampling (range of 14–18 d).

Embryo Mortality

The 1989 analysis revealed a statistically significant difference in embryo mortality ($P = 0.004$) between oiled and reference streams (Figure 2). No zone effect ($P = 0.499$) or oil-by-zone interaction ($P = 0.863$) was evident. Examination of estimated contrasts indicated the differences in mortality were in the intertidal zones. The overall mean embryo mortalities for the oiled and reference streams were 0.174 and 0.104, respectively.

The 1990 embryo mortality data also showed a statistically significant difference ($P = 0.023$) between oiled and reference streams (Figure 2). No zone effect ($P = 0.595$) or oil-by-zone interaction ($P = 0.159$) was observed. Estimated contrasts indicated that differences attributable to oiling were in the upper intertidal zone. The overall mean em-

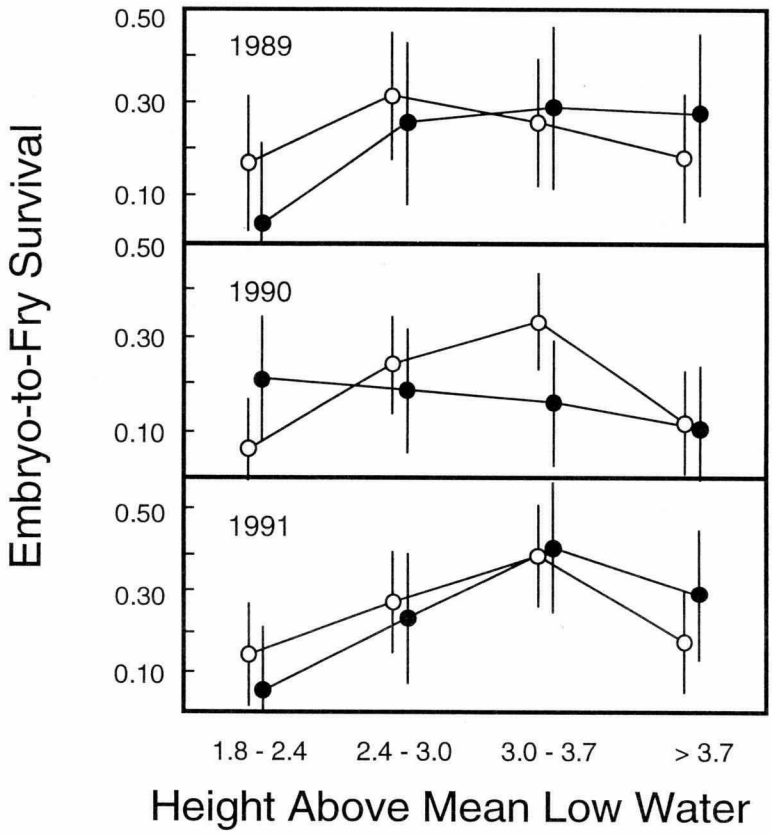

FIGURE 3.—Mean pink salmon survival from embryo to preemergent fry (proportion) observed during field sampling of the 1989–1991 brood years. Solid circles indicate data for the 10 oil-contaminated streams; open circles indicate data for the 15 reference streams. Standard error bars represent the 90% family confidence interval. Height above mean low water is in meters.

bryo mortalities for the oiled and reference streams were 0.295 and 0.195, respectively.

The 1991 embryo mortality data indicated very significant differences between the oiled and reference streams ($P = 0.003$; Figure 2). A significant zone effect ($P = 0.011$) was evident, although no oil-by-zone interaction was found ($P = 0.816$). Estimated contrasts indicated differences attributable to oiling in all three intertidal zones as well as in the upstream zone. The overall mean embryo mortalities for the oiled and reference streams were 0.433 and 0.221, respectively.

Significant differences in embryo mortality were again detected in 1992 between the oiled and reference streams ($P = 0.010$; Figure 2). No zone effect ($P = 0.167$) or oil-by-zone interaction ($P = 0.788$) was detected. Estimated contrasts indicated that the statistical differences were in two of the three intertidal zones as well as in the upstream zone. The overall mean embryo mortalities for the oiled and reference streams were 0.448 and 0.250, respectively.

Overwinter Survival

Embryo-to-preemergent fry survival data were edited before analysis to remove values greater than 2.0 (i.e., overwinter survivals greater than 200%). Only one data point in the 1990 brood year embryo-to-fry survival was removed (9,200% survival).

No significant effects attributable to oil ($P = 0.820$) were found for survival of pink salmon embryos to preemergent fry for the 1989 brood year (Figure 3). No significant zone or oil-by-zone interaction was detected ($P = 0.155$ and $P = 0.594$, respectively). Similar results were obtained for the

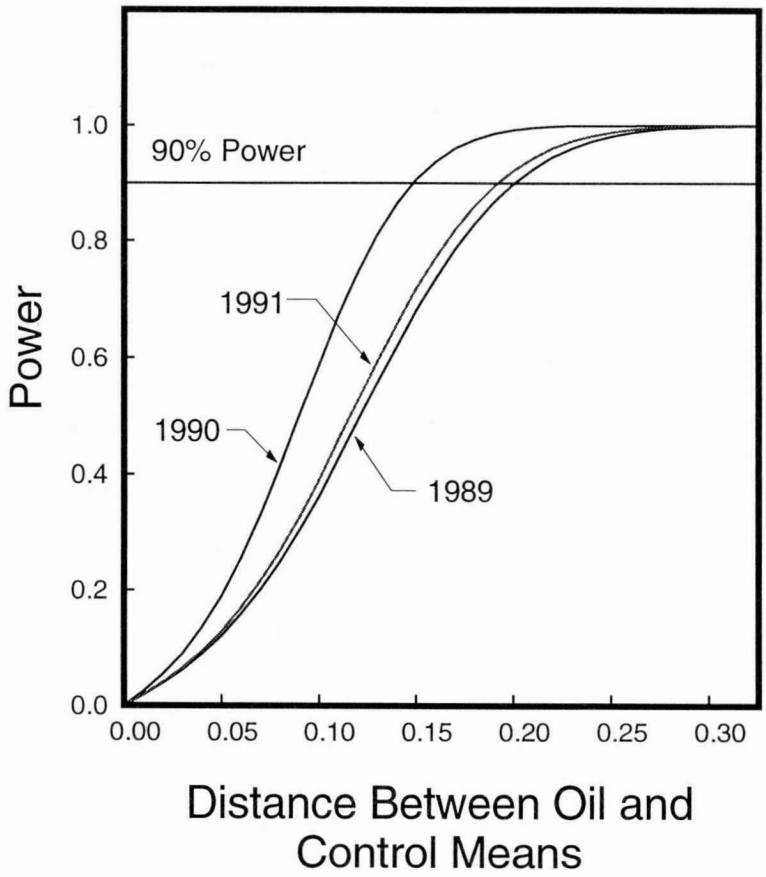

FIGURE 4.—Estimated statistical power for the 1989–1991 brood year comparisons of survival of pink salmon embryo to preemergent fry between oil-contaminated and reference streams.

survival of embryos to fry for the 1990 brood year (Figure 3). No significant oil, zone, or oil-by-zone effect was found ($P = 0.713$, $P = 0.147$, and $P = 0.112$, respectively). The 1991 brood year embryo-to-fry survival information again indicated no oil or oil-by-zone interaction ($P = 0.983$ and $P = 0.596$; Figure 3), although there was evidence for a zone effect ($P = 0.004$).

Statistical power was estimated for all 3 years of embryo-to-preemergent fry data. The test for differences in survival from embryo to preemergent fry between oiled and reference streams for the 1990 brood was more powerful than for the other 2 years (Figure 4). The test for 1990 brood year survival should have detected a difference of ± 0.15 with a probability of 0.90, whereas the 1989 and 1991 brood year tests were able to detect a difference of ± 0.20 with the same probability.

Discussion

Pink salmon embryos that incubated in oil-contaminated spawning areas in Prince William Sound in 1989–1992 appear to have been adversely affected by the *Exxon Valdez* oil spill. The elevated mortalities in 1989 and 1990 were confined to the intertidal zones whereas those detected in 1991 and 1992 were in all zones. We believe the elevated mortalities observed in 1989 and 1990 were caused by direct exposure to oil; those in 1991 and 1992 may have resulted from genetic damage sustained during early fish development. The pink salmon that spawned during fall 1991 were the fry that incubated in oil-contaminated streams during winter 1989–1990, the first winter after the spill. Likewise, the pink salmon that spawned during fall 1992 were the same fry that incubated in oiled stream

gravel during fall 1990 and spring 1991. There is a possibility that the surviving embryos sustained sublethal genetic damage that was manifested in the form of functional sterility in 1991 and 1992. Moles et al. (1987) confirmed that pink salmon embryos take up polynuclear aromatic hydrocarbons, a major component of crude oil, and McBee and Bickham (1988) showed that these compounds are capable of inducing chromosomal lesions. Also, Hose et al. (in press) found chromosome damage in Pacific herring *Clupea pallasi* exposed to *Exxon Valdez* crude oil.

An alternative to the genetic hypothesis is that the observed differences are caused by environmental variation. This study is based on observational data; as such, we were unable to randomize stream oiling to account for environmental differences between streams. We addressed this concern in our original experimental design by selecting reference streams in close proximity to oil-contaminated streams and by including as many streams as possible in the analysis. However, there is a definite oiling pattern in southwestern Prince William Sound: streams on points that faced northeast were heavily oiled, and streams that faced west and southwest were most likely not oiled. The consequences of this difference in stream orientation were not accounted for and at present are unknown.

No difference in survival from embryo to preemergent fry was detected. Survival was expected to be reduced in oil-contaminated streams because an increase in embryo mortality had already been detected. This anomaly can potentially be explained by compensation in the intergravel environment or problems in the experimental design. Geiger et al. (1996, this volume) found no evidence to suggest that compensation in the intergravel life stages is playing a role in determining the number of emerging fry for the years in the study. The power analysis for the survival from embryo-to-preemergent fry test indicated statistical power was adequate to detect a biologically meaningful difference if present. However, unexpected changes in stream characteristics may have prevented sampling the same areas for embryos in the fall and fry in the spring. This would provide unrepresentative estimates of survival because survival was estimated as the density of live fry in the spring divided by the density of total embryos and fry in the fall. Runoff from fall rains increases stream depth and width, whereas spring water levels are usually low because the majority of the winter precipitation is tied up in ice and snow. Also, stream channels in Prince William Sound are not well defined in intertidal areas. Some intertidal stream segments were found to migrate along the beach, especially if the beach is usually exposed to winter storms. The magnitude of these changes was unexpected when this study was designed and initiated.

This project is ongoing: embryo mortality is monitored annually and experiments designed to evaluate the genetic hypothesis have been initiated. We believe this study illustrates the importance of long-term damage assessment and monitoring projects.

Acknowledgments

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