Resolving allegations of oil damage to incubating pink salmon eggs in Prince William Sound

Ernest L. Brannon, Keya C.M. Collins, Lawrence L. Moulton, and Keith R. Parker

Abstract: The *Exxon Valdez* Oil Spill Trustee Council concluded that oil caused mortality of pink salmon (*Oncorhynchus gorbuscha*) eggs in Prince William Sound streams. Their conclusion was based primarily on Alaska Department of Fish and Game (ADF&G) studies which reported that mean mortality of embryos in eggs was higher in oiled than non-oiled streams when sampled shortly after spawning completion. However, developing embryos are vulnerable to shock mortality for a period of 20 days after fertilization, and the embryos in eggs from the latest spawners were still in the sensitive period at the time sampling took place. We argue that the original ADF&G analysis should have included sample timing in statistical comparisons of mortality between streams. Analysis of a subset of the ADF&G data showed that sampling shock was a major source of embryo mortality in these samples, and that source of mortality in the original survey would likely have been mistakenly interpreted as an oiling effect. Compensating for sample timing removed all statistical evidence for an oiling effect in the data subset. We conclude that the ADF&G study design confounded the ability to assess for the effect of oil exposure on pink salmon eggs.

Résumé : Le conseil des mandataires du suivi du déversement pétrolier de l'*Exxon Valdez* (*Exxon Valdez* Oil Spill Trustee Council) a conclu que le pétrole est responsable de la mortalité d'oeufs des Saumons roses (*Oncorhynchus gorbuscha*) dans les cours d'eau du détroit du Prince William. Sa conclusion se base principalement sur des études menées par le Ministère de la pêche et de la chasse de l'Alaska (Alaska Department of Fish and Game (ADF&G)) qui indiquent que la mortalité moyenne des embryons dans les oeufs était plus élevée dans les cours d'eau affectés par le pétrole que dans les cours d'eau non-affectés, lors d'un échantillonnage fait peu après la fin de la fraye. Cependant, les embryons en développement sont vulnérables à une mortalité par choc pour une période de 20 jours après la fécondation; or, les embryons dans les oeufs pondus par les poissons qui ont frayé le plus tardivement étaient encore dans leur période vulnérable au moment de l'échantillonnage. Nous croyons donc que l'analyse originale faite par le ADF&G aurait dû inclure la date d'échantillonnage dans les comparaisons statistiques de la mortalité entre les cours d'eau. L'analyse d'un sous-ensemble des données du ADF&G révèle que le choc relié à l'échantillonnage a été une cause majeure de mortalité des embryons dans ces échantillons et qu'une telle mortalité aurait vraisemblablement été interprétée dans l'inventaire original comme étant causée par le pétrole. En tenant compte de la date d'échantillonnage dans le sous-ensemble de données, on enlève toute évidence statistique d'un effet du pétrole. Il apparaît donc, en conclusion, que le plan d'analyse du ADF&G a annihilé toute possibilité de détecter les effets de l'exposition au pétrole sur les oeufs de Saumons roses.

[Traduit par la Rédaction]

Introduction

In March of 1989, the *Exxon Valdez* grounded and spilled 11 million gallons of crude oil into Prince William Sound (PWS). That spring and summer, weathered crude oil washed onshore along approximately 16% of PWS beaches and entered the intertidal zones of an estimated 30 productive pink salmon (*Oncorhynchus gorbuscha*) streams (Fig. 1). Alaska Department of Fish and Game (ADF&G) investigated the effects of oil on pink salmon eggs incubating in these streams after the spawning season in 1989. Higher embryo mortality (17.4% in oiled streams vs. 10.4% in non-oiled) was observed in oiled streams (Craig et al. 1995). Higher mortality was also reported to continue through 1993 (Bue et al. 1996) and reappeared in 1997, well after measurable oil disappeared from the streams. Higher mortality of eggs was reported in oiled streams even above high tide (Bue et al. 1996). Largely from these data, the *Exxon Valdez* Oil Spill Trustee Council (2000) concluded that the reported mortality represented long-term injury to pink salmon in PWS, and reported that patches of oil exposed by winter storms may result in new local episodes of egg mortality.

There were several problems in accepting oil as the cause of higher embryo mortality. First, the mean polynuclear aro-

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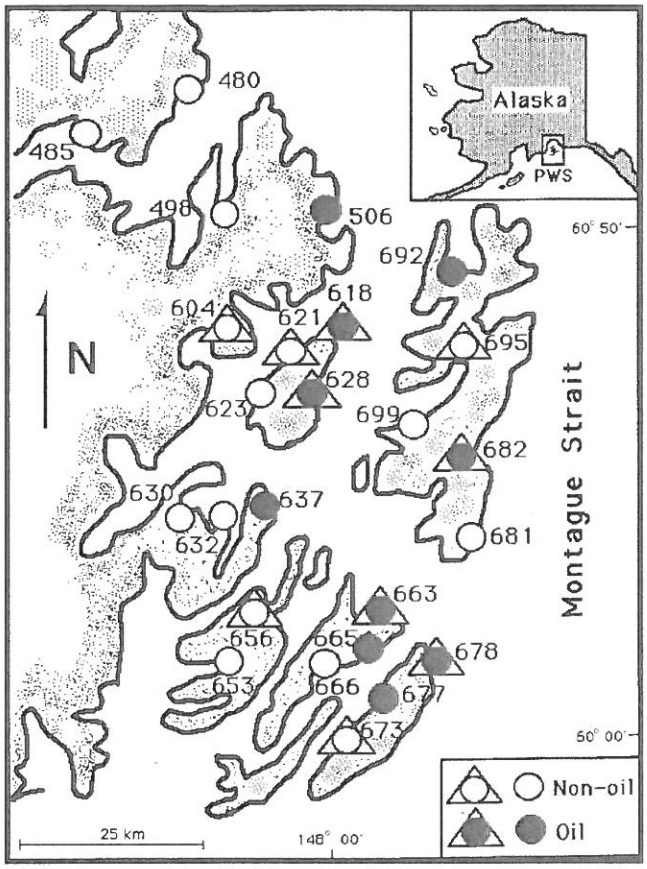
Fig. 1. Prince William Sound (PWS) beach areas where oil washed ashore following the *Exxon Valdez* oil spill. Solid circles indicate the locations of oiled streams and open circles indicate the locations of non-oiled streams. Triangles indicate the 10-stream subset used in the analysis of Alaska Department of Fish and Game data.



matic hydrocarbon (PAH) concentrations in the incubation substrate of oiled streams in 1989 ranged from only 0.5 to 267 ppb (Brannon et al. 1995), well below the 3800 ppb reported as the lethal threshold for pink salmon embryos (Murphy et al. 1999). Contrary to the ADF&G survey data (Bue et al. 1996, 1998), no measurable effects of oil were detected in pink salmon embryo survival in concurrent studies by Brannon et al. (1995), and no differences were observed in percentage of anomalies, time of emergence, or viability, as reported by Marty et al. (1997), in pink salmon embryo laboratory bioassays with the crude oil. Similarly, Moulton (1996) showed no differences in embryo mortality (3.5% in oiled streams vs. 4.9% in non-oiled) in 1989 studies of eggs from returning spawners that were artificially incubated for 2 months in oiled and non-oiled streams until the embryos reached the advanced eyed stage. Moreover, five of the streams reported in Moulton's (1996) study were streams in which ADF&G had reported higher egg mortality (16.1%) a month earlier (Craig et al. 1995). Oil as the source of egg mortality was also questioned when ADF&G reported no differences in over-winter survival of the pre-emergent fry (alevins) among the same streams in 1989 and 1990 (Bue et al. 1996), despite alevins being the life stage most vulnerable to oil toxicity (Moles et al. 1979; Rice et al. 1975).

In reviewing the ADF&G egg survey data, Brannon and Maki (1996) suggested that eggs were sampled too early after spawning and that the mortality reported was actually an artifact of sample timing. Knowledge about the sensitivity of developing salmon embryos to physical disturbance (Smirnov 1954; Jensen and Alderdice 1989; Jensen 1997) is fundamental to understanding the problem. During early development of pink salmon embryos, the delicate vitelline membrane under the chorion envelops the yolk as a barrier between the water and yolk material. Until epidermal elaboration during epiboly replaces the vitelline membrane, the pink salmon embryo is susceptible to membrane rupture from physical shock. At 6–10°C, pink salmon embryos are vulnerable to shock fatalities for a period approximating 20 days (Jensen and Alderdice 1989; Collins et al. 2000) following fertilization. We hypothesized, therefore, that the results of the survey were confounded by egg mortalities from sampling mistaken for the effects of oil.

To test and estimate the effect of sample timing on egg mortality, Collins et al. (2000) conducted a field study in 10 of the 25 streams surveyed by Bue et al. (1996), using the ADF&G hydraulic sampling methodology to assess egg mortality. Two sampling regimes were tested in each stream in 1998: the first (21–27 September) to correspond with the earliest sampling dates employed by ADF&G (Bue et al. 1996) from 1989 to 1997 and the second (16–19 October) to cover the period after embryo development had progressed beyond the point of sensitivity to physical shock. Incubation temperatures at the time of sampling ranged from 6 to10°C. The results of the field tests showed that, when sampled in September, mean mortality for all streams was 63.6% compared with 22.8% when the same streams were sampled in October. The field trials thus demonstrated the major effect that sample timing can have on mortality if hydraulic sampling is undertaken too soon after the end of spawning.

The present paper re-examines the ADF&G egg mortality survey data for potential bias caused by sample timing relative to embryo sensitivity to physical shock. We believe that, if the ADF&G sampling protocol induced bias in embryo-mortality estimates, there is a need to correct this bias, because ADF&G findings are central to the allegation that PWS pink salmon suffered long-term injury from the oil spill. This paper underscores the critical importance of the temporal element that was missing in the original analysis of the egg mortality survey data (Bue et al. 1996).

Data were analyzed to assess the effect of post-spawning incubation time on embryo mortality between oiled and non-oiled streams. The objective of these analyses was to determine whether covariate corrections of sample timing reduced the magnitude of differences in mortality between oiled and non-oiled streams and affected statistical tests on these differences. The hypothesis that differences in sample timing did not affect egg mortality between oiled and non-oiled streams was tested against the alternative that sample timing was responsible for differences in mortality.

Methods

Analysis of the 1989–1997 ADF&G egg survey data subset was

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performed by developing an analysis of covariance (ANCOVA) model to test the major factors that could affect embryo mortality. The variable of interest was sample timing and, hence, the level of embryo development among the latest-spawned eggs of each stream population relative to the end of spawning. Data defining this variable was acquired by integrating sample-timing information from ADF&G records (Craig et al. 1995, 1999) with spawning-ground adult-enumeration summaries (Moulton 1998) from 1989 to 1997. The data set was limited to those streams that were common to both studies (Fig. 1), and included 10 (five oiled and five non-oiled) of the 25 (10 oiled and 15 non-oiled) streams surveyed by Bue et al. (1996). The minimum number of post-spawning days the eggs had incubated prior to sampling was thus calculated as the difference between the estimated date of spawning completion and the date the eggs were sampled (Brannon et al. 1999), and was described as the number of days elapsed ("days") for each population. Table 1 shows statistics on egg mortality and days since spawning completion by stream and year.

Three other components of variability were included in the model. Oil was the original variable reported to affect egg mortality and was included in the model as the type ("type") of stream, representing the oiled or non-oiled classification. The variability among streams ("streams") was also included. A year ("year") effect was included to represent the change in mortality over the 9 years that resulted from differences in the post-spawning time at which sampling was executed from year to year, or from other variables such as a decreasing effect of oiling or variability in natural environmental factors that could have affected embryo mortality.

Based on days ("days") since spawning was completed and the above three factors that could have influenced the level of embryo mortality, the following ANCOVA model was used:

$$(1) \quad p = \mu + \text{type} + \text{stream(type)} + \text{days} + \text{days}^2 + \text{year} + (\text{type by year}) + \text{error}$$

The proportion (p) of dead embryos in each stream was computed as the number of dead eggs divided by the number of eggs and alevins for each year of the ADF&G surveys from 1989 to 1997. The proportion of dead embryos (p) was transformed with the arcsine of the square root to normalize residuals. The mean transformed proportion is symbolized by mu (μ). A test of stream type compares mortalities for oiled and non-oiled streams averaged over all 9 years. Stream was treated as a random nested effect, and stream(type) was used to test type. Since type compares means over all 9 years for oiled and non-oiled streams, it did not provide a very useful test for an effect of oiling, unless an effect of oiling was present in all 9 years. More useful tests, therefore, were the type-by-year interaction and contrasts. The type-by-year interaction tested the overall equality of differences in mortality for each of the 9 years between oiled and non-oiled streams. A type-by-year interaction could result from significant differences in some years and not others. In addition, separate contrasts (t tests that use the error term and degrees of freedom form the ANCOVA model) between oiled and non-oiled streams for each year were tested.

Linear and quadratic covariate effects (days and days^2, respectively) were used to model the number of days since spawning completion and embryo mortality, because exploratory analyses showed that their relationship was curvilinear. A loess plot, a nonparametric smoothing technique (Green and Silverman 1994), was used to examine embryo mortality versus days since spawning completion. The shape of the loess plot showed that a quadratic regression might be useful over the range in days sampled since spawning completion. Covariates were fit after stream and type, because the number of days since spawning completion differed for each stream (Table 1).

Equation 1 was run without and with covariates and was referred to as the uncorrected model and the corrected model, respectively. Effects in models were fit sequentially. Tests were at $\alpha = 0.05$. Data for 1993 were not available for oiled sites and thus 1993 was not included. Error was modeled as normal. Normality of residuals was tested with the Shapiro–Wilk test (Conover 1980). Covariates were fit after type and stream(type), because the length of time since spawning completion differed among streams and years. Interactions with covariates (e.g., day by year, day by type, day by year by type) were nonsignificant and dropped from the model.

Observed and corrected mortalities were compared, to demonstrate how covariates affected differences in mean mortalities between oiled and non-oiled streams. Observed mortalities were simply the means for categories of oiled and non-oiled streams for each year. Corrected mortalities were adjusted by covariate effects of days since spawning completion, and were estimated from least-squares means. Days since spawning completion were averaged for each year for oiled and non-oiled streams.

To demonstrate differences in mean sample times between oiled and non-oiled streams, the intertidal egg mortality data were also grouped separately as annual means by brood year and stream type. For each of the 9 years, mean mortalities were plotted against the mean number of days of post-spawning incubation experienced prior to the time of sampling. The regression was used to characterize the pattern of sampling between oiled and non-oiled streams, and displayed to emphasize the relationship between mean mortality and sample timing in days of post-spawning incubation by type of stream.

Results

The summary of percent mortalities and days of post-spawning incubation (Table 1) shows the wide range that occurred in each of these statistics. Differences between stream types are very apparent. Cursory examination of Table 1 shows that a pattern of higher mortality is associated with fewer post-spawning incubation days accumulated before sampling. The number of very early samples taken (<10 days, for example) is greater among oiled streams than non-oiled streams (13 vs. 2), demonstrating that the trend in higher mortality is associated with less incubation time.

Results for the uncorrected model (without covariates) show evidence that mean mortalities differed for oiled and non-oiled streams in some years (Table 2). The significant type-by-year interactions ($p = 0.048$) indicate that the relationship between oiled and non-oiled streams differed over years (1989–1997). Contrasts were significant for 1991 and 1992 ($p = 0.014$ and $p = 0.034$, respectively, df = 61); in 1991 and 1992 mortalities were higher in oiled streams by 23.7 and 18.8%, respectively (Table 3). All other contrasts were nonsignificant ($p > 0.05$). The significant year effect ($p < 0.001$) shows that mean mortalities, when computed over oiled and non-oiled streams combined, differed among years. Based on results from the Shapiro–Wilk test ($p = 0.546$, $n = 79$), normal error was appropriate.

The use of covariates (corrected model) eliminated significant effects seen for the uncorrected model (Table 2) that could be interpreted as an oiling effect. Once corrected for sample timing, the type-by-year interaction was nonsignificant ($p = 0.180$), indicating that the difference between oiled and non-oiled streams was similar over time. Contrasts for 1991 and 1992 were now also nonsignificant ($p = 0.413$ and $p = 0.098$, respectively, df = 59). The year effect was still significant, showing evidence that environmental factors unrelated

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Table 1. Percent mortality and number of post-spawning incubation days for pink salmon (*Oncorhynchus gorbuscha*) eggs in intertidal areas of five oiled and five non-oiled Prince William Sound streams from 1989 to 1997.

	% mortality								No. of post-spawning incubation days								
Stream No.	1989	1990	1991	1992	1993	1994	1995	1996	1997	1989	1990	1991	1992	1993	1994	1995	1996	1997
Oiled																		
618	18.4	29.5	34.3	66.9	11.4[a]	30.3	—[b]	4.9	45.2	16	8	8	8	19[a]	15	—[b]	25	6
628	17.0	25.3	21.2	29.9	8.2[a]	58.8	11.5	36.1	60.3	10	14	9	7	18[a]	10	16	21	2
663	16.7	12.3	65.6	21.1	24.8[a]	46.0	15.3	17.7	16.7	19	19	2	9	19[a]	17	25	21	17
678	10.3	41.3	57.0	62.7	7.7[a]	34.4	3.5	22.2	28.9	19	9	1	7	20[a]	28	29	33	10
682	17.0	30.9	57.1	30.2	22.9[a]	62.9	25.6	16.8	34.8	23	28	1	12	—[a]	18	28	33	12
Non-oiled																		
604	17.5	36.5	14.6	17.2	12.6	56.3	25.4	12.3	22.6	19	13	20	13	20	10	29	25	14
621	18.7	31.3	36.5	13.8	17.6	71.0	53.8	16.8	32.2	18	15	22	14	23	13	27	27	10
656	18.8	8.6	25.0	52.1	5.4	67.0	20.2	21.9	36.6	18	26	11	10	23	18	25	36	10
673	3.9	3.9	24.8	7.0	14.9	20.5	16.2	5.7	23.0	22	27	1	11	21	27	34	34	15
695	14.8	34.9	15.8	26.9	16.2[a]	48.5	45.5	17.2	26.0	16	13	11	12	14[a]	13	12	29	6

[a]No enumeration data available.
[b]Only 9 eggs were recovered.

Table 2. Degrees of freedom and p values for main effects, covariates, and interactions for uncorrected and corrected models; covariates effects of the number of days since spawning completion were used only in the corrected model.

	Uncorrected		Corrected	
Effect	df	p	df	p
Type	1,2	0.160	1,2	0.160
Stream (type)	2,61	0.570	2,59	0.463
Days	—	—	1,59	<0.001
Days^2	—	—	1,59	0.036
Year	7,61	<0.001	1,59	<0.001
Type by year	7,61	0.048	7,59	0.180

to oil likely changed over the years from 1989 to 1997. Both days and days^2 were significant ($p \leq 0.001$ and $p = 0.036$, respectively) (Table 2) and the coefficients describe a decreasing upward-concave relationship for these data. Based on coefficients for the intercept (0.785), day (–0.0279), and days^2 (0.00359), an estimated 79% mortality occurred at 0 days since spawning completion and 25% at 36 days since spawning completion. Examining the relationship between residuals and predicted values shows that the covariance model fit the data well. Figure 2*a* shows a loess plot of embryo mortality versus days since spawning completion. The general shape of the loess fit is typical of that described by linear and quadratic terms used in the ANCOVA model, with the relationship between embryo mortality and sample timing being negative and concave upward. Figure 2*b* shows the residuals plotted against predicted values for eq. 1. The scatter of residuals is well balanced about the zero line. Had eq. 1 not explained the curvature, residuals would have been unbalanced in numbers and magnitude of deviations from the zero line. Based on results from the Shapiro–Wilk test ($p = 0.126$, $n = 79$), normal error was appropriate.

Covariate effects of days since spawning completion reduced the difference in embryo mortalities between oiled and non-oiled streams for most years. Table 3 shows mortalities and differences between oiled and non-oiled streams. Differences for observed and corrected mortalities are tabulated. Differences (non-oiled *minus* oiled) in mean days since spawning completion are also tabulated. For 7 of the 8 years, the difference in corrected mortalities was less than that for observed mortalities. In 1995, covariates increased differences in corrected mortalities over those for observed mortalities. In all but 1994, oiled streams were sampled earlier on average than non-oiled streams. Differences in mean days since spawning completion ranged from 0.9 to 8.8 days.

The relationship between early sample timing and instances of high mean mortality levels in stream types is most effectively demonstrated when stream types are separated and identified by year in the regression of mean embryo mortality against mean post-spawning incubation time (Fig. 3). Higher mortalities are associated with the earliest sample timing, which occurred more frequently in oiled streams. It diminishes with time, as the later-spawned eggs develop and embryos become less sensitive to shock from sampling. The exception is the abnormally high mortality in 1994, which was related to exceptionally low water levels from very little

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Table 3. Comparison of observed and corrected mortalities.

	Observed mortality			Corrected mortality			Differences	
Year	Oiled	Non-oiled	Δ mortality	Oiled	Non-oiled	Δ mortality	Observed > corrected	Days
1989	0.159	0.147	0.012	0.178	0.178	<0.001	Yes	1.2
1990	0.279	0.230	0.049	0.261	0.242	0.019	Yes	3.2
1991	0.470	0.233	0.237	0.244	0.180	0.064	Yes	8.8
1992	0.422	0.234	0.188	0.293	0.170	0.123	Yes	3.4
1994	0.465	0.526	−0.061	0.489	0.527	−0.038	Yes	−1.4
1995	0.140	0.322	−0.182	0.205	0.425	−0.220	No	0.9
1996	0.195	0.148	0.047	0.299	0.277	0.022	Yes	3.6
1997	0.372	0.281	0.091	0.254	0.208	0.046	Yes	1.6

Note: Observed mortalities are mean mortalities over streams within oiling type. Corrected mortalities are from eq. 1 and were adjusted for sample timing. Differences in means between oiled and non-oiled streams were calculated for both observed and corrected mortalities. Whether or not observed mortalities were greater than corrected mortalities is tabulated. The difference in mean days since spawning completion between oiled and non-oiled streams is given in the final column.

Fig. 2. (*a*) A nonparametric smoothing fit to embryo mortality. (*b*) The residual versus predicted values for the effects adjusted by covariates of days since spawning completion, eq. 1.

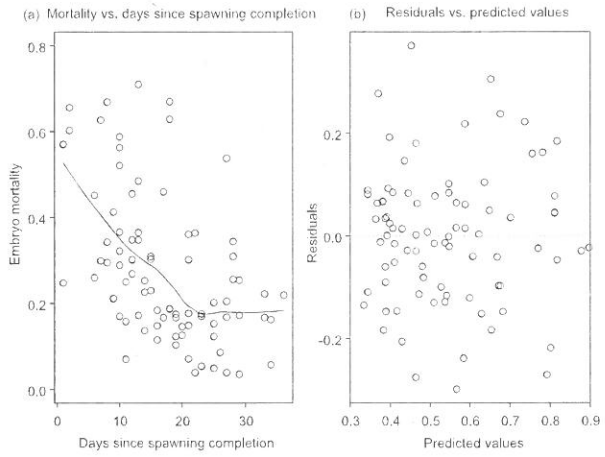
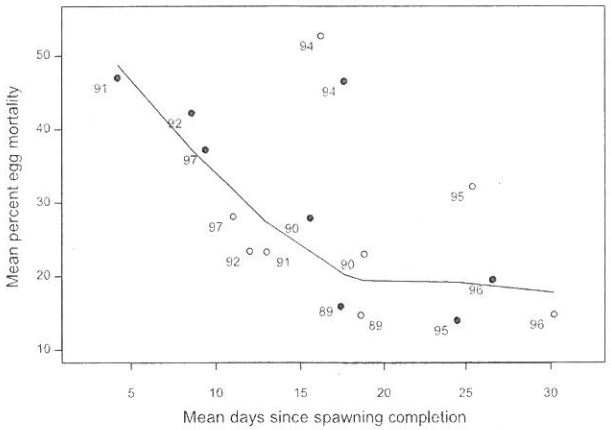


Fig. 3. Mean embryo mortality in intertidal areas of oiled (filled circles) and non-oiled (open circles) streams and mean days of post-spawning incubation at the time of sampling for 10 study streams surveyed in Prince William Sound from 1989 to 1997 (line fit by loess smooth). Mortality data are from Craig et al. (1995, 1999) and Brannon et al. (1999).



precipitation. However, it is also noteworthy that, in 1994, sampling took place within the period of embryo sensitivity but, in this case, it was the non-oiled streams that were sampled earlier and they showed the higher mortality.

Discussion

Covariate corrections of sample timing in the ADF&G data subset show that ADF&G sampling times overlapped the period of embryo vulnerability to physical shock. These analyses suggest that the mortality attributed to oil in the original ADF&G analysis may have been confused with fatalities induced by the sampling procedure. We believe that analysis of the ADF&G survey data subset has shown that sample timing likely induced substantial bias in embryo mortality estimates, owing to sampling being initiated so soon after spawning was completed. Evidence shows that correcting for sample timing was important to avoid misinterpreting the results and attributing embryo mortality to the effects of oiling when assessing for differences between oiled and non-oiled streams. Correcting for sample timing increased the magnitude of the p values to nonsignificance for differences that would have been interpreted as an oiling effect. For the uncorrected model, differences in mean mortalities between oiled and non-oiled streams differed significantly among years, as tested by the type-by-year interaction. Significant differences in mortality were eliminated by taking sample timing into account. The same was true for contrasts. Significantly higher mean mortalities were detected at oiled streams in 1991 and 1992 for the uncorrected model. Once corrected for sample timing, contrasts for 1991 and 1992 were nonsignificant. Significant interaction and contrasts would be interpreted as evidence for an effect of oiling, but such evidence disappeared when mortality was corrected for days since spawning completion. This was most apparent for years with the greatest differences in sample timing between oiled and non-oiled streams. With an 8.8-day difference in 1991, the mortality was corrected by 17.3%. When the difference was just over 3 days in 1990, 1992, and 1996, mortality was corrected by 3.0, 6.5, and 2.5%, respectively. If Bue et al. (1996) had included terms to describe the negative relationship between mortality and sample timing in their

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statistical analysis, it is likely they would not have concluded that there was an effect of oiling on embryo mortality, in which case, all studies of oiling effects (Brannon et al. 1995; Bue el al. 1996; Moulton 1996) would have agreed.

Because sample timing overlapped the period of embryo sensitivity to shock among the latest-spawned eggs, the variability in the dates when sampling commenced created a difficulty in maintaining a consistent base of reference on which to compare mortality. Sampling commenced as early as 21 September in 1992 and as late as 10 October in 1995, representing a difference of nearly 3 weeks (Craig et al. 1995, 1999). The number of post-spawning incubation days accumulated before sampling from the first to the last streams sampled ranged from 7 to 22 days over 9 years. The variation in number of post-spawning incubation days that elapsed before sampling ranged from 1 to 36 days. Such large differences in the duration of incubation among developing eggs prior to the time they were sampled grouped the eggs in dissimilar post-spawning incubation periods from year to year and complicated assessment because of differences in sensitivity.

Statistical evidence of a negative relationship between embryo mortality and days since spawning completion for pink salmon in PWS has been shown by two independent sets of data. The first, from a 1998 field study (Collins et al. 2000), demonstrated the negative relationship between days since spawning completion and embryo mortality. The second comprised the significant linear and quadratic terms in the ANCOVA model for data from 1989–1997, which show unambiguous evidence that sample timing should be included in analyses that compare mortalities between oiled and non-oiled streams. This is also apparent from data plots and nonparametric regressions, which clearly show trends over time between mortality and days since spawning completion.

Oiling in itself had no influence on embryo development rate. Although it was demonstrated under laboratory conditions that relatively high oil concentrations caused alevins to emerge prematurely with yolk stores still remaining, altered rates of development were not reported (Marty et al. 1997). In the field studies of oil effects on incubating and emerging fry, emergence timing and fry size were no different between oiled and non-oiled streams, and the index of condition (Bams 1970) indicated that emerging fry were not premature (Brannon et al. 1995).

The influence of temperature on the rate of embryo development was also considered to be a potential influence on embryo mortality. September stream temperatures varied from 6 to 10°C among streams and among years, but the extent of the variation did not exceed a mean of much more than 1°C. In years when August temperatures were higher (e.g., 1991), spawning tended to be later than in years when they were lower, which exaggerated the effect of early sampling. For 1998 data, mortality rates were regressed on water temperature and were shown to be greater in September in streams with higher temperatures, but not in October, demonstrating that higher temperatures enhanced sensitivity (Brannon et al. 1999). Therefore, while variation in temperature could account for some mortality, its primary affect would be to exaggerate mortality from early sample timing.

The results of analyzing the 10-stream subset of the 25 streams sampled by ADF&G suggest that conclusions reached about oiling effects without sample timing being included in the analysis could have been flawed by confusing fatalities from sampling with other effects on embryo mortality. The impact of sample timing is most effectively demonstrated by the 1990, 1991, 1992, and 1997 data. In each case, sampling occurred earlier in oiled streams than non-oiled streams and substantially overlapped the period of sensitivity to physical shock. The analysis of embryo mortalities by Moulton (1996), and of incubation survival by Brannon et al. (1995), avoided embryo sensitivity in eggs by sampling well after embryo vulnerability to physical shock was past. Analysis of the data subset suggests that, if there were an oil effect in ADF&G survey data, it would have been obscured by sample-timing effects.

In conclusion, the analysis of egg mortality between oiled and non-oiled streams from a subset of ADF&G stream survey data was undertaken. The assessment showed that the reported differences in mortality were likely the result of sampling protocol and unequal distribution of sampling effort over time between stream types. In the instances of higher mortality in oiled streams, eggs were sampled too early to avoid shock fatalities and, since oiled streams were more frequently sampled earlier that non-oiled streams, the increased egg mortality was misinterpreted as an oil-related mortality. The effect of shock from sampling on embryo mortality was shown by Collins et al. (2000)—mortality was nearly three times higher in early samples than in later samples taken from the same set of streams. Differences in egg mortality between stream types was not observed by Brannon et al. (1995) and Moulton (1996), because sampling was undertaken well past the period of embryo sensitivity to shock, and timing was not critical. This re-analysis helps to resolve differences in published results on the effect of oiling in PWS streams following the *Exxon Valdez* oil spill. Based on analysis of the 10-stream subset of the ADF&G 25 stream survey, we conclude that sample timing likely explains why eggs in oiled streams were perceived to have had higher mortality. It also explains why mortality was so variable in both oiled and reference streams. It explains why differences in mortality were reported to have continued after oil left the streams, and why differences occurred in those areas upstream of high tide, beyond the physical presence of oil. Induced fatalities from early sample timing also explain why differences in mortality were reported in eggs but were not observed by ADF&G in the later alevin and fry life stages at the onset of emergence.

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